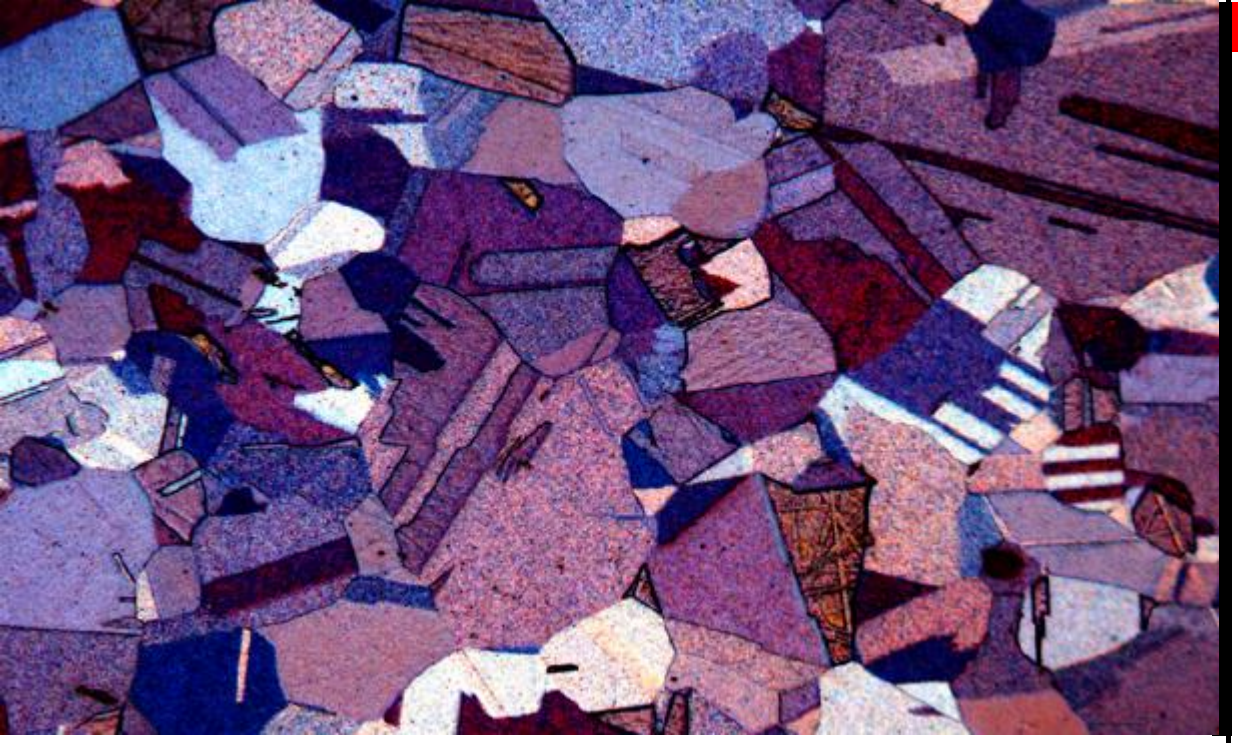


Leica Application Suite

LAS User Manual

Living up to Life

Leica
MICROSYSTEMS

LAS User Manual

All reasonable steps have been taken to ensure that this publication is correct and complete, but should any user be in doubt about any detail, clarification may be sought from Leica Microsystems (Switzerland) Ltd., or their accredited representative. The information in this document is subject to change without notice and should not be construed as a commitment by Leica Microsystems (Switzerland) Ltd.. Leica Microsystems (Switzerland) Ltd.accepts no responsibility for any errors that may appear in this document.

Date: September 2013

Software Version: LAS V4.4

Leica Microsystems (Switzerland) Ltd.
Stereo and Macroscope Systems
CH 9435 Heerbrugg
Switzerland

Telephone: +44 1223 411411
FAX +44 1223 210692
Hotline: +44 1223 401824
di.support@leica-microsystems.com
www.microscopy-imaging.com

Contents

Introduction 11

The Core 12

Optional Modules 13

Getting Started 14

Documentation 15

Documents Available 16

General Guidance and Advice for LAS Users 17

Starting LAS 18

Hardware Setup 19

Administrators: Reaching the Setup Dialog 20

All Users: Select a Configuration 30

The User Interface 31

Main Areas 32

The Side Tool Bar 33

Context Menus 34

Shortcut Keys: General 35

Shortcut Keys: General (cont.) 36

Live and Interactive Measurements Shortcuts 37

Extended Annotation Shortcuts 38

Image Analysis Shortcuts 39

Montage 3D Viewer Shortcuts 40

Power Mosaic Shortcuts 41

Dual Monitors 42

Swap Monitors 43

Enabling and Disabling 44

Primary Monitor 45

Secondary Monitor 46

Side Bar Tools 47

Image Options 48

The Core 49

Functions Widely Available 50

Import/Export LAS Settings 51

Preferences 55

Scale Bar Introduction 77

Update Calibration 85

Image Comparison: Dual Viewer 95

Export Images 100

Printing 107

Reporting 118

Gallery Docking 143
The Setup Workflow 144
Image Explorer and LAS Archive 145
Stereo Microscope Setup 146
DM Microscope Setup 168
The Acquire Workflow 196
Acquire User Interface 197
Starting Acquire 198
The Side Tool Bar 199
Stereo Microscope Operation 212
DM Microscope Operation 238
FS Microscope Operation 264
Camera 270
The Browse Workflow 348
User Interface 349
Launching Browse 350
Navigator Panel: Image Explorer 351
The Side Tool Bar 369
Full Screen & Second Monitor 388
Image Options 389
The Gallery 390
The Data Form 392
The Grid 396
Device Settings: Store & Recall 397
The Process Workflow 398
Basic Annotation 399
Enhance 408
The Analysis Workflow 419
Optional Modules 420
Demo Licence and Installing Optional Modules 421
The Demo Licence: Install & Start 422
Starting Optional Module Demo Period 423
End of the 60-day Demo 424
Excel Reports with Optional Modules 425
Tags 426
Links 431
Measurements: User Data 435
Grain Expert: User Data 440
Phase Expert: User Data 450
Image Analysis: User Data 458
LAS Archives: Basic and Standard 470
Data Organisation 471
Archive Structure 472

The Example Archive 474
Archive Editions: Basic 475
Archive Editions: Standard 476
Navigator Panel: LAS Archives 477
Select Image Explorer or LAS Archives 478
Browse with LAS Archive 483
LAS Archives Basic Edition 488
LAS Archives Standard Edition 518
Leica Image Manager 531
Extended Annotations 549
Launching Extended Annotation 550
Features 551
Drawing and Control Tools 552
Lines: Thickness and Colour 559
Shapes: Settings 562
Delete, Undo and Redo 567
Image Comparison 568
Zoom Region 574
Templates 575
Show Grid 581
Merge All 582
Annotation Techniques 583
MultiTime 585
TimeLapse 586
Movie 606
MultiFocus 632
Enable MultiFocus 633
Select Image Format 634
Select or Create Capture Folder 635
Options 636
Configuration 638
Define Z-Stack: Automatic Microscopes 639
Set Z-Stack: Manual Microscopes 645
Show Image Set Slide Show 651
Montage Multifocus 652
Launch and Operating Steps 653
Select the Z Stack 654
Stack Images 655
Source Image Selection 656
Create Multifocus Image 657
Enhance 659
Depth Map 664
Confidence Map 665
Anaglyph 666

Stereo Pair 667
Colour Relief 668
3D Model 670
Montage Edit 672
Measure Tools Position Readout 675
Appendix: Essentials & Reflected Light 679
Leica Map 683
Installation and dongle 684
Select the File Format 685
Select the Layer Type 686
Montage Results 687
Launch Leica Map 688
The Template Option 689
The Default Template 690
No Template 691
Operators 692
Studies 693
Image Overlay 694
Enable Image Overlay 695
Setup Tools 696
Channel Dialog 698
Adjust Channel Exposure 700
Snapshot and Working Gallery 702
Auto Create Sequence Record 703
Auto Create Overlay 705
Auto Overlay Without Auto Folder 706
Auto Overlay With Delete Channels 707
Recall Configurations and Manual Acquire 708
Creating and Viewing Overlays 709
Colour Control 711
Fine Tuning 714
Converting a Folder of Images 715
MultiStep 716
MultiStep Operating Modes 717
MultiStep Preferences 718
Control Panels 719
Easy UI mode 722
Define MultiStep Sequence mode 723
Acquire the MultiStep Sequence 734
Browse MultiStep Images 735
Live XY Builder 739
Stages, Specimens & Terminology 740
Features and Workflow 743
Planning and Launching 751

Start Building 756
Live Z Builder 762
Terminology, Focus and Lighting 763
Features and Workflow 765
Launch Live Z Builder 773
Start Building 776
Live XYZ Builder 780
Terminology, Concepts and Fast Track 781
Launch Live XYZ Builder 782
Start Building 785
Power Mosaic and Power Mosaic Plus 793
Power Mosaic Features 794
Operating Steps 797
Starting 801
Imaging Setup 807
Select Scan Pattern 820
Focus Methods 829
Create Pattern Grid 835
Save Configuration 839
Scan 840
Z-Stacks 841
Pattern Navigator 845
Browse: Save & Display 876
Leica ISO Pro Stage 881
Live and Interactive Measurements 887
User Interface 888
Main Features 889
Fast Track 890
Calibration and Preferences 891
Launching the Modules 895
Control Tools 896
Measurement Tools 903
Classes 954
Configurations 963
Templates 967
Select Results 999
Create Report 1006
Acquire and Merging 1019
Appendix: Summary Definitions 1022
Image Analysis 1023
Getting Started 1024
The User Interface 1029
Processing Sequence 1035
Main Steps 1040

Runner with Applications 1149
Starting Applications 1150
Using Runner with LAS Macros 1151
Phase Expert 1152
Step by Step 1153
Set up the Phases 1155
Select Images to Measure 1159
Set the Measure Frame 1161
Adjust Threshold 1162
Binary Processing Pre-Filters 1168
Binary Image Editing 1169
Results and Histogram: Phase 1170
Reference Data 1177
Create Report 1178
Configurations 1181
Grain Expert 1183
Step by Step 1184
Select Images to Measure 1185
Identify the Grain Boundaries 1187
Select the Boundary Colour 1190
Detect the Boundaries 1191
Binary Image Editing 1195
Select Standard and Method 1196
Results and Histogram: Grain 1199
Reference Data 1205
Create Report 1206
Layer Thickness Expert 1211
Layer Thickness Analysis 1212
User Interface 1213
Getting Started 1214
Advice and Prerequisites 1215
Before Starting a Leica Expert Application 1216
Start Leica Layer Thickness Expert 1217
Operating Leica Layer Thickness Expert 1219
Cast Iron Expert 1251
Cast Iron Analysis 1252
User Interface 1253
Getting Started 1254
Advice and Prerequisites 1255
Before Starting a Leica Expert Application 1256
Start Leica Cast Iron Expert 1257
Cast Iron Start-up Screen 1258
Operating Leica Cast Iron Expert 1259
Dendrite Arms Expert 1290

Dendrite Arms Analysis 1291
User Interface 1292
Getting Started 1293
Advice and Prerequisites 1294
Before Starting a Leica Expert Application 1295
Start Leica Dendrite Arms Expert 1296
Operating Leica Dendrite Arms Expert 1298
Decarburisation Expert 1332
Decarburisation Analysis 1333
User Interface 1335
Getting Started 1336
Advice and Prerequisites 1337
Before Starting a Leica Expert Application 1338
Start Leica Decarburisation Expert 1339
Operating Leica Decarburisation Expert 1341
Reticule 1370
Reticule Fast Track 1371
Reticule Library 1372
Available Reticules 1373
Reticule Types: Fixed 1374
Reticule Types: Scalable 1375
Reticule Settings 1376
Apply Reticule 1377
Using Reticules 1378
Creating Reticules 1379
Creating Reticules: Continued 1380
Web Sharing 1381
The Launch Panel 1382
Cursor and Scale Bar 1383
Camera Settings 1384
Sharing Stored Images 1385
Select Image Size and Enable 1388
Client Check Network Connection 1389
Client Software 1390
Client Connection Status 1391
Client Restart and Acquire Image 1392
Client Acquire Image: Continued 1393
Multi-User Package 1394
Users and Controls 1395
Reload and Delete a Profile 1396
The Default Profile 1397
Profile Preview 1398
Copying a Profile 1399
Administrator Publishing 1400

Introduction

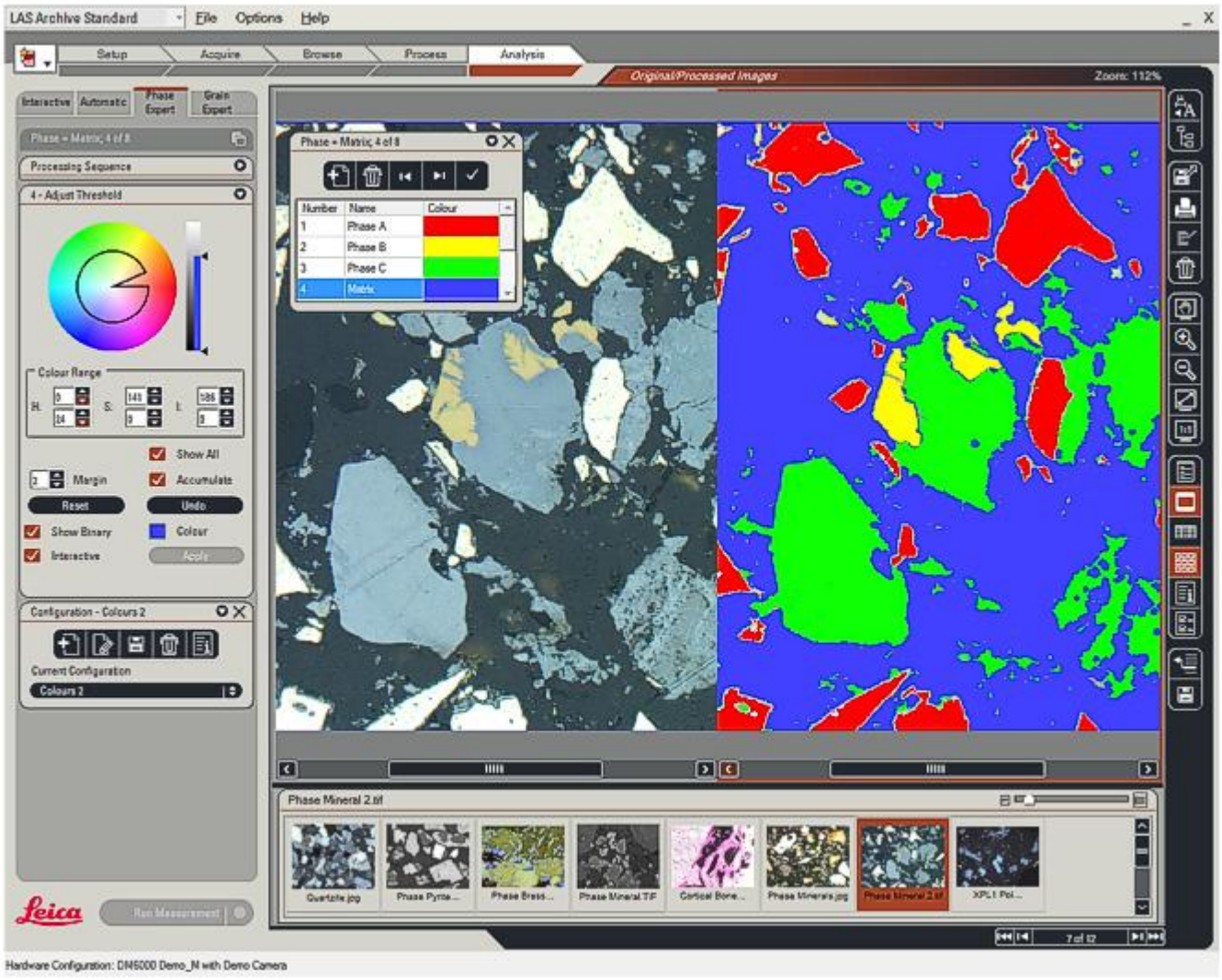
The *Leica Application Suite* (LAS) comprises:

- **The Core:** The basic part of the Suite which includes the *Framework* and the *Setup* [144], *Acquire* [196], *Browse* [348] and *Process* [398] *Workflows.* These are the essential tools needed to refine, capture and present images from the microscope. The *Core* utilities are always available and do not have to be licensed.
- **Optional Modules:** Powerful,dedicated capabilities that perform specific functions to enhance, augment and extend the *Core*. *Optional Modules* are provided free for an evaluation period of 60 days. After that, they have to be purchased and licensed to continue to work.

When the Leica Application Suite is first installed on the computer, all of the *Optional Modules* [420] are also installed but not enabled. Selecting *Demo* mode will allow each one to be enabled ready for evaluation.

Some of the modules may not be appropriate to the tasks the microscope will be required to perform, so they need not be enabled initially. Instead, they can be turned on at a later date to start the 60-day evaluation [423]. However, once evaluation is started the 60 days will start to run and cannot be turned off, even if the module itself is disabled.

LAS installation is described separately in the *Installation Guide.pdf* on the LAS DVD.

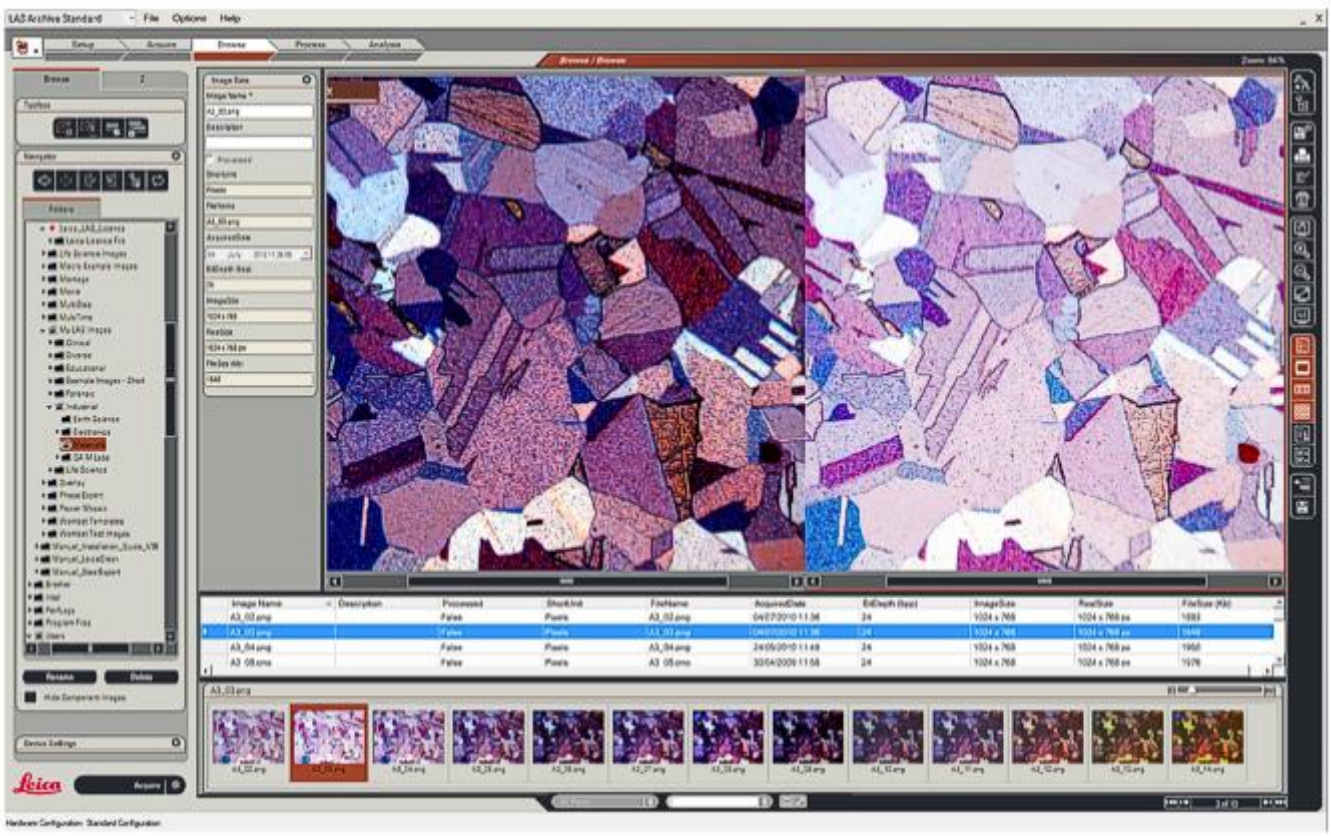


The Core

The *Core* provides the basic software for configuration and control of the selected microscope as well as for the acquisition, review and processing of high quality digital images. The *Core* components include:

- *Microscope* and *Digital Camera* configuration and control - all fully integrated.
- *Auto* and *Manual Exposure* adjustments to allow optimised imaging.
- *Image Calibration* based on data read directly from Leica microscopes and cameras.
- *Scale Bar* displayed on the live image to indicate image size.
- *Digital Image Acquisition* into the familiar *Image Explorer* tree and folder structure. There is an *Optional Module* with extended *Archive* capability.
- *Thumbnail Gallery* of acquired images for quick and easy review.
- *Text, Scale Bar* and *Distance Tools* for straightforward image annotation.

Link to the Core 49



Optional Modules

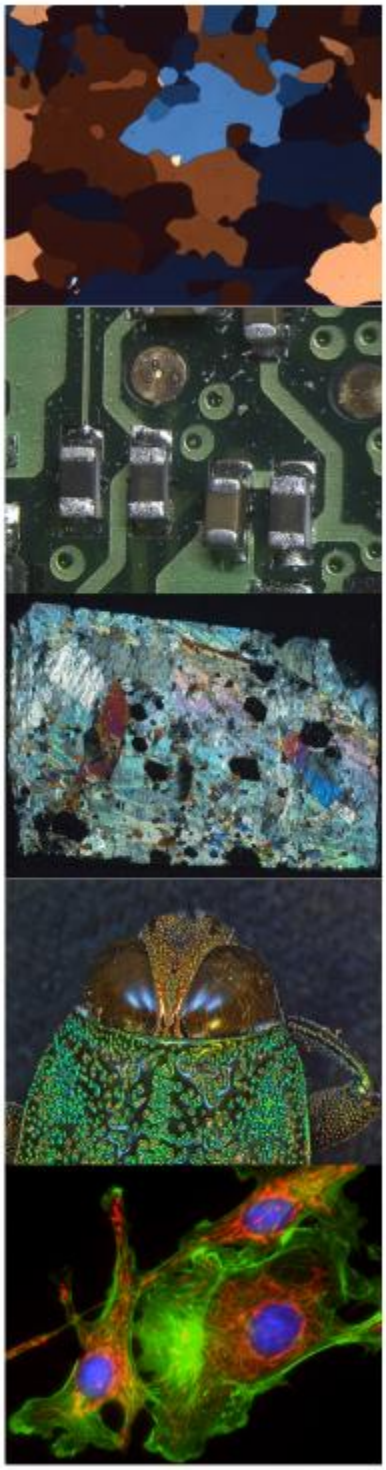
The powerful features of the *Core* can be enhanced with a range of advanced *Optional Modules*, each providing the flexibility to tailor a system to suit individual needs.

The range of *Optional Modules* includes:

- LAS Archives: Basic and Standard.
- Image Analysis
- Macro Programming
- Live Image Builder XY, Z and XYZ
- Extended Depth of Focus
- Movie Recording
- Image Measurements
- Autofocus
- Image Overlay
- Power Mosaic

Optional Modules 420

Optional Module 60-day evaluation 421



Getting Started

The following topics describe how to make effective use of LAS when you first start to use the software after installation. The following topics are covered:

Documentation:

Please read the Release Notes for details of new features and restrictions in use. Run the PC performance checker and ensure you are using supported hardware as described in the System Requirements [15]

Configure the Hardware:

When LAS is first used, specify the microscope and camera hardware [19] being used

Starting LAS:

Once the system is configured, this is the usual starting procedure [18]

The User Interface:

Get familiar with the concept and terminology of the user interface [31]

Short-cut Keys:

These help to improve productivity [35]

Dual Monitors:

If the hardware has dual monitors [42], this topic shows how to make best use of them

Documentation

Documentation comprises this User Help and other documents about the installation and operation of Leica Application Suite - LAS. Please read them before using the software.

Before installation all the information can be found on the installation DVD by running it and choosing *Documentation* from the startup menu.

Once installed, all of the documents can be found by:

1: Click on Windows *Start.* Click on the *All Programs* arrow...

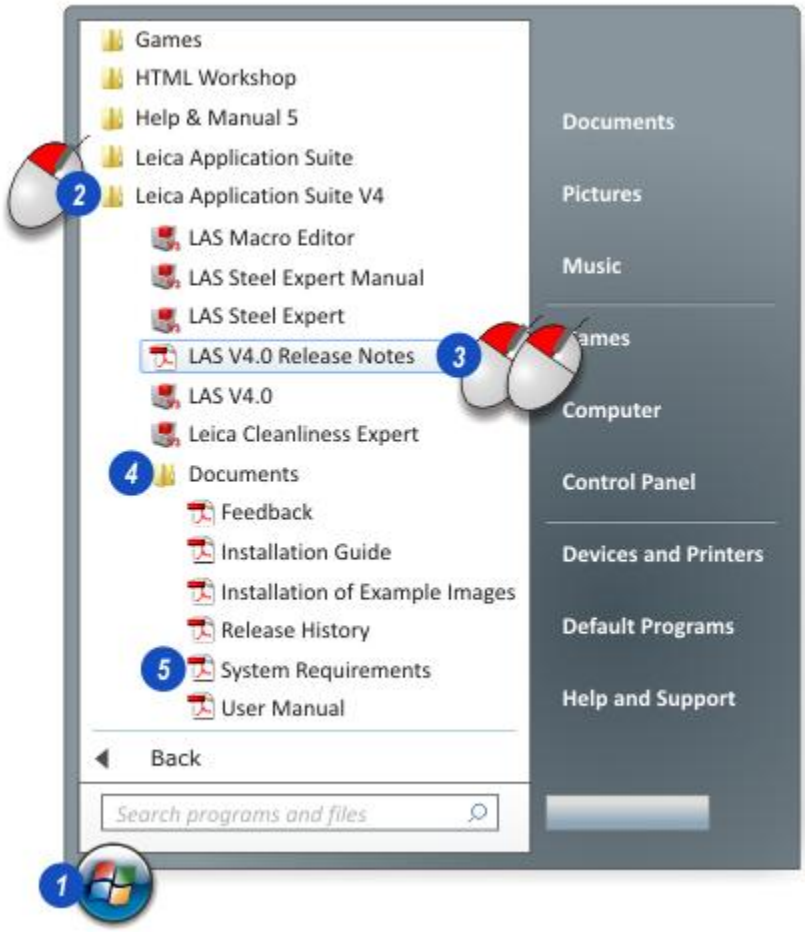
2: ...and on *Leica Application Suite V4*.

3: For the *Release Notes*, double click on the entry and the default pdf reader will be launched and display the notes.

4: More information is listed by clicking the *Documents* folder and then...

5: ...double-clicking on the required document.

Continued 16



System Requirements:

The supported hardware - the microscopes, macroscopes and cameras that can be used with LAS - is described in the document *System Requirements.pdf.*

This document also describes the appropriate computer specification. Please ensure that your computer specification corresponds to the recommendations made. Other factors that influence the performance of LAS are also noted in the same document.

For Leica DM microscopes, refer to the operator's manual supplied with the microscope for detailed guidance on configuring and operating the microscope.

Install Guide:

A detailed description of the installation procedure for Leica Application Suite is in the *LAS Install Guide.pdf.*

Release Notes:

Recent information about the version of LAS is described in the document *LAS Release Notes.pdf.* This describes features of the software that have recently changed, operational limitations and other technical information.

General Guidance and Advice for LAS Users

Users should read these general notes before using Leica Application Suite for the first time, and as a source of ongoing information to get the best performance from LAS software.

- The computer, camera(s), microscope and peripheral controls like *SmartMove* must be connected before starting LAS.
- LAS performance may be limited if there is less than 400 MB of free RAM available.
- Other running programs may use processor time and limit LAS performance so it is recommended that when possible other programs are closed.
- The user must have write access to the folders where images and data are stored.
- Data should be stored to a local drive and not to a mapped network location which can be very slow due to the network speed. The archive images and metadata (and sequences with administrator permission) can be stored on a mapped network drive if the speed is acceptable. Unmapped network locations cannot be accessed by LAS.
- A back-up device and a back-up strategy for archives, images and data is essential. The user must use regular and reliable back-up.
- LAS might fail to start, or it might display errors when capturing images, if the *Windows Management Instrumentation Service* is not running or *The Desktop*, *My Documents* and similar folders use a network drive that is not mapped.
- The gallery and other image handling in LAS is designed for use with images of the size generated by the various Leica DFC Cameras. Larger images, up to 400 MB - those created by LAS *MultiStep* and *Power Mosaic* for example - can be stored but are not displayed or processed, so that a reasonable operating speed is maintained.
- The image size is defined as the number of bytes of memory used by the uncompressed image. For example a 48-bit image has a size equal to 6 bytes per pixel multiplied by the number of pixels in X and Y. The image file size may be considerably smaller if the image is stored in a compressed format such as *JPEG* or *PNG*.
- If a *Windows Standard User* (not *Administrator* or *Power User*) is using LAS some folders are not available. For example, *Shading Correction* files are stored in the *All User* folder and cannot be accessed by a *Standard User* and will need to be granted write privileges by an *Administrator*. Similar issues can arise with annotation and measurement files and must be resolved by a *Windows Administrator*.
- LAS does not support Windows *Guest Users* due to limitations with folder access. Please log on as a *Standard User.*
- LAS may only be used by one logged-on user at a time. Multiple copies of LAS cannot get access to the microscope and camera hardware.
- When LAS is closed it takes a short time for the microscope to shut down. Wait a few seconds before re-starting LAS.
- LAS uses named *Hardware Configurations* 19 to access different arrangements of camera, microscopes and accessories.
- *Leica QWin, MW, Steel Expert, Cleanliness Expert* and *Oasis Drivers* use the *Standard Hardware Configuration* only. Make sure it is selected before starting the application.
- If the *Twain* window is opened within LAS and then another Windows programs become active, although the main LAS window appears on the taskbar it will not become active because *Twain* is blocking it. Use *Alt+Tab* and select the *Twain* window.
- Camera hot-swapping while LAS is running is not supported. Close LAS, change the camera and re-start LAS.
- In the unlikely event that LAS 'hangs' simply find the LAS desktop icon and Re-start LAS. You may get a message that mentions that LAS is already running, this instance will be closed and LAS started again.
- If both LAS and LAS EZ are installed and LAS EZ is used, LAS may not start completely the next time it runs. Exit LAS, re-start the computer and LAS.

In most cases all users can start Leica Application Suite by double-clicking on the desktop icon:

To start in Administrator mode, right-click on the icon, and from the context menu click *Run as Administrator*.

Alternatively, for all users:

1: Click the *Start* button on the *Windows Task Bar* (bottom left).

2: From the popup click on *All Programs* and from the list locate and click *Leica Application Suite V4.*

3: Double-click on *LAS V4* from the options. LAS will load and run.

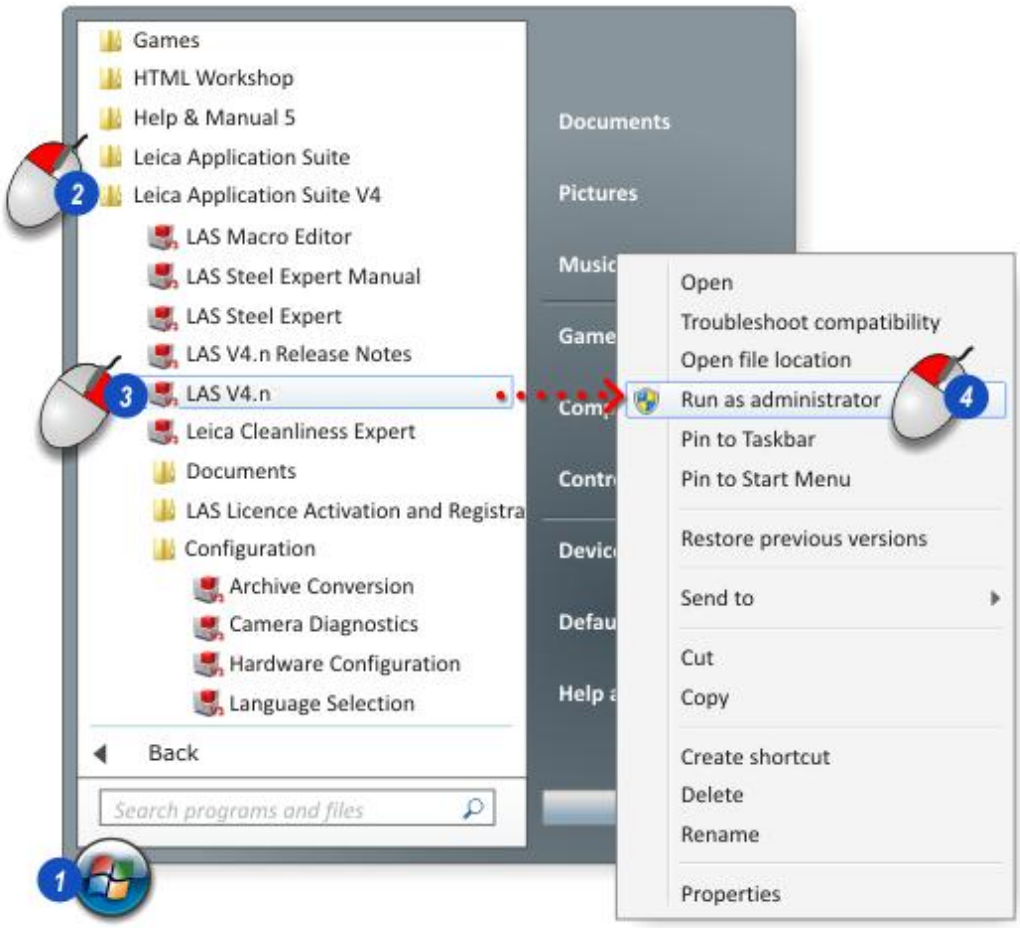
Administrators can launch LAS with their privileges:

1: Click the *Start* button on the *Windows Task Bar*.

2: From the popup click on *All Programs* and from the list locate and click *Leica Application Suite V4*.

3: Right-click on *LAS V4.n* from the options.

4: From the context menu click on the *Run as administrator* option. LAS will launch and run.



Hardware Setup

The *Hardware Setup* facility allows Administrators to create configurations for either a single microscope and camera combination or multiple combinations that are sharing the same personal computer.

Although several microscope/camera combinations can be plugged into the computer, only one can be active and connected to Leica Application Suite. *Hardware Configurations* allow a specific combination to be selected and connected.

As well as the microscope and camera combination, the *Hardware Configuration* saves the last used camera settings and the calibration values so when the *Hardware Configuration* is recalled all of the settings are automatically applied.

An overwrite is available that will apply the settings established by the Administrator rather than recall those last used by the current User.

Only computer Administrators can create *Hardware Configurations*, but both Administrators and Users can select and use a *Hardware Configuration*.

- *Administrators*: Hardware Setup: Creating configurations 26
- *All Users*: Selecting and loading configurations 30

Administrators Only:

Hardware Setup Dialog

Choose a Microscope Stand

Choose a Camera Type

Create and Save a New Configuration

Administrators and Users:

Select a Configuration as Active

Done

Option: Override settings such as Camera *Exposure* and *Calibration* that are stored with the *Configuration*, and use the current settings instead.

Option: For a single Microscope/Camera combination use the *Standard Configuration* instead of creating a new one.

Only Administrators can setup hardware and create a *Configuration.*

Note: If LAS is already running, choose *File* > *Exit*.

Administrators reach the Hardware Setup:

1: Click the Windows *Start* button.

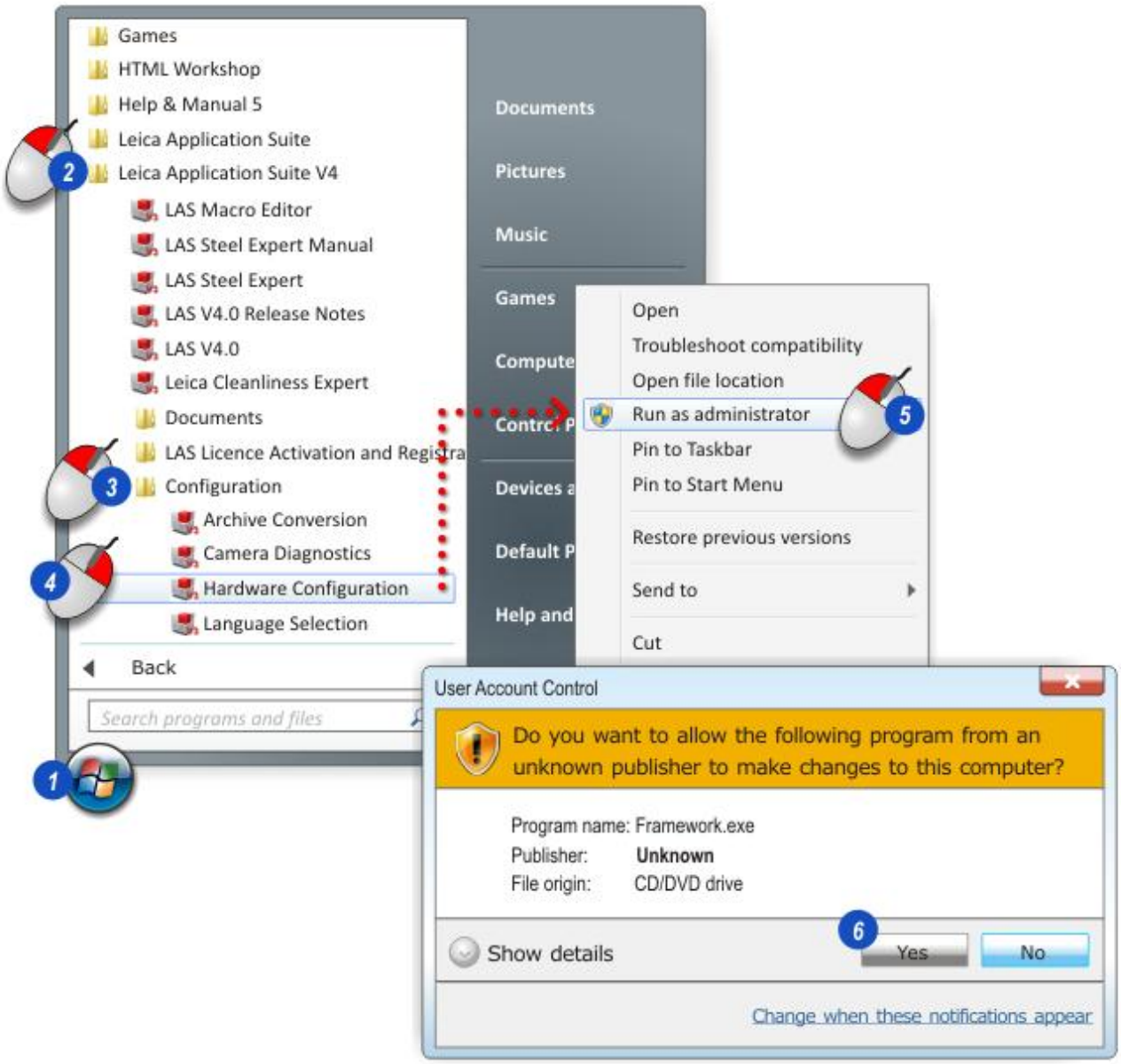
2: From the *All Programs* list, click *Leica Application Suite V4.*

3: Left click the *Configuration* option.

4: Right-click *Hardware Configuration.*

5: Left-click *Run as administrator.*

6: Click *Yes* to allow LAS *Framework* to run.



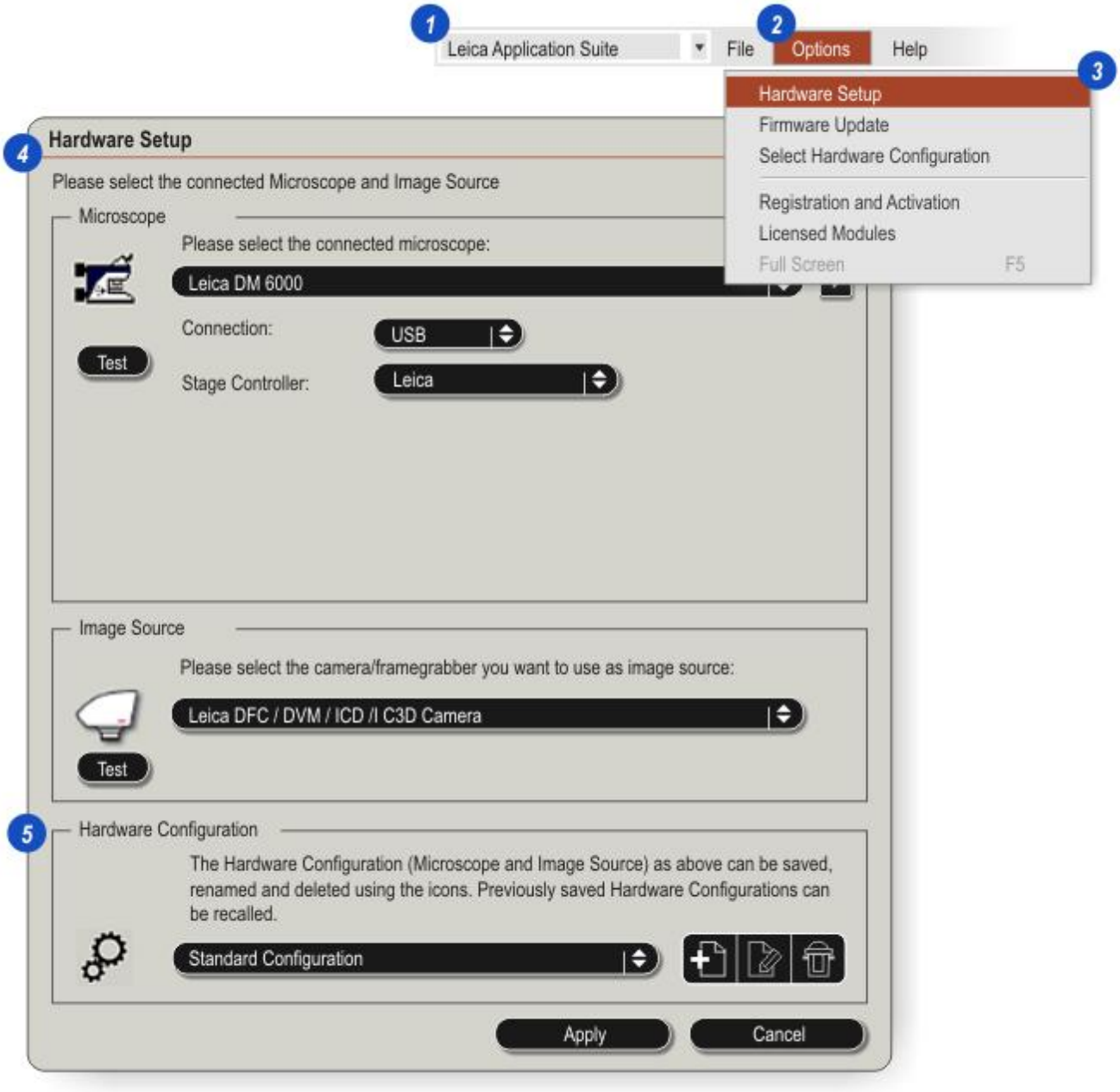
1: The LAS *Framework* is displayed.

2: Click to select *Options*.

3: Choose *Hardware Setup* from the drop-down menu.

4: The *Hardware Setup* dialog appears with the microscope and camera selections made during the first run of LAS.

5: There is an additional part of the dialog - *Hardware Configuration* - that allows different combinations of microscope and camera to be saved and recalled.



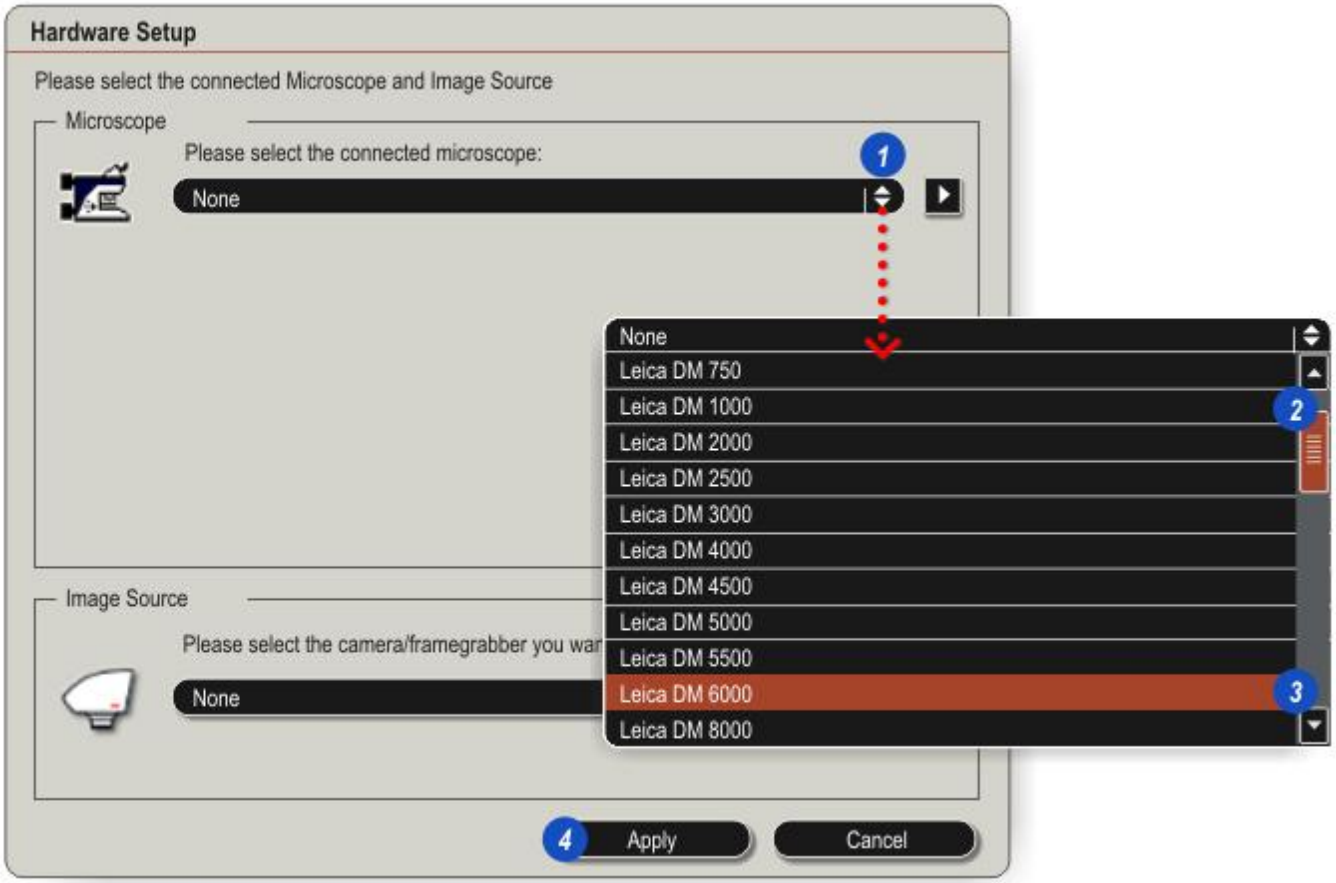
On the *Hardware Setup* dialog:

1: Click on the arrows to the right of the *Microscope* window. The microscope selection list appears.

2: Use the up/down arrows and slider to scroll through the list.

3: Click on the required microscope.The list will close and the microscope selected will appear in the window.

4: Click *Apply*.



Depending upon the microscope selected, options will be available to choose the connection type and peripherals.

To select a connection type:

1: Click on the small arrows to the right of the *Connection* drop-down header.

2: From the list, click to select the connection type. If a microscope is not present it can be simulated by selecting either of the *Demo* options - *DEMO-B* or *DEMO-M. (For a demo stereo you just select the M205 (Demo) directly.*

3: Test the connection by clicking the *Test* button.

Selecting Peripherals (If fitted):

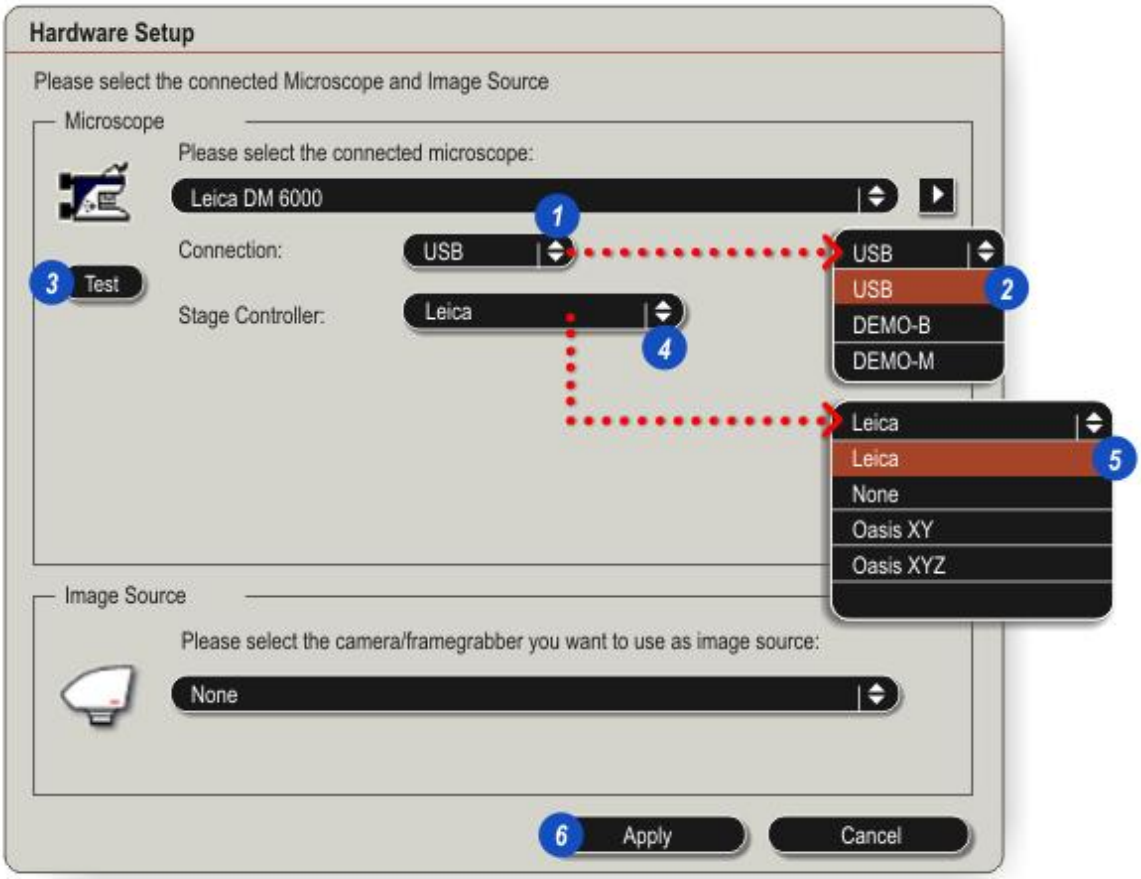
The available accessories will depend upon the selected microscope and the appropriate list will be displayed. This example shows stage controller selection:

4: Click on the small arrows to the right of the *Stage Controller* drop-down header.

5: Click to select the fitted stage controller name from the list.

6: Click the *Apply* button.

Note - Oasis software is installed and configured external to LAS.



To select the fitted camera:

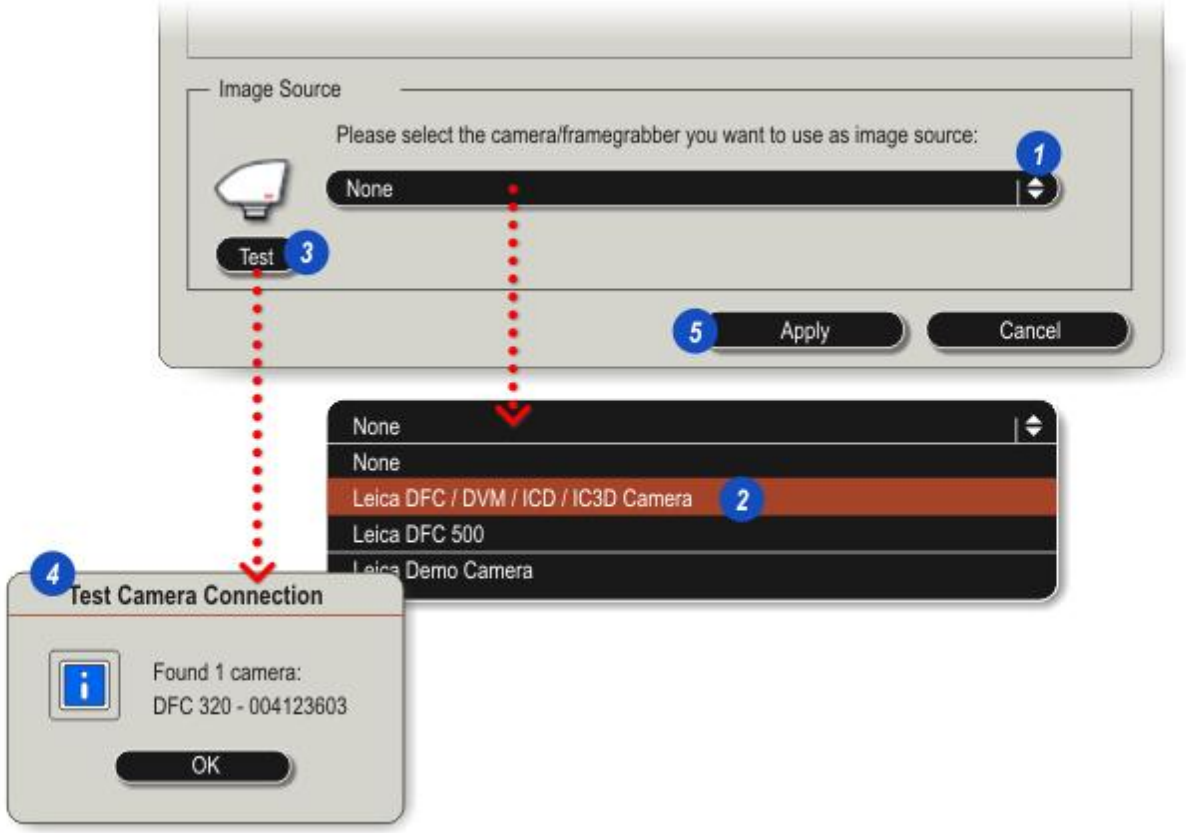
1: Click on the arrows to the right of the *Image Source* text box and...

2: ...from the drop-down list click to select the fitted camera type.

3: Test the connection by clicking the *Test* button.

4: If the camera is found, the type and serial number are reported. If the camera is not found a *'Data source not found'* message appears that indicates either the wrong camera type has been selected or there is a fault with the camera hardware or physical connection.

5: Click *Apply*.



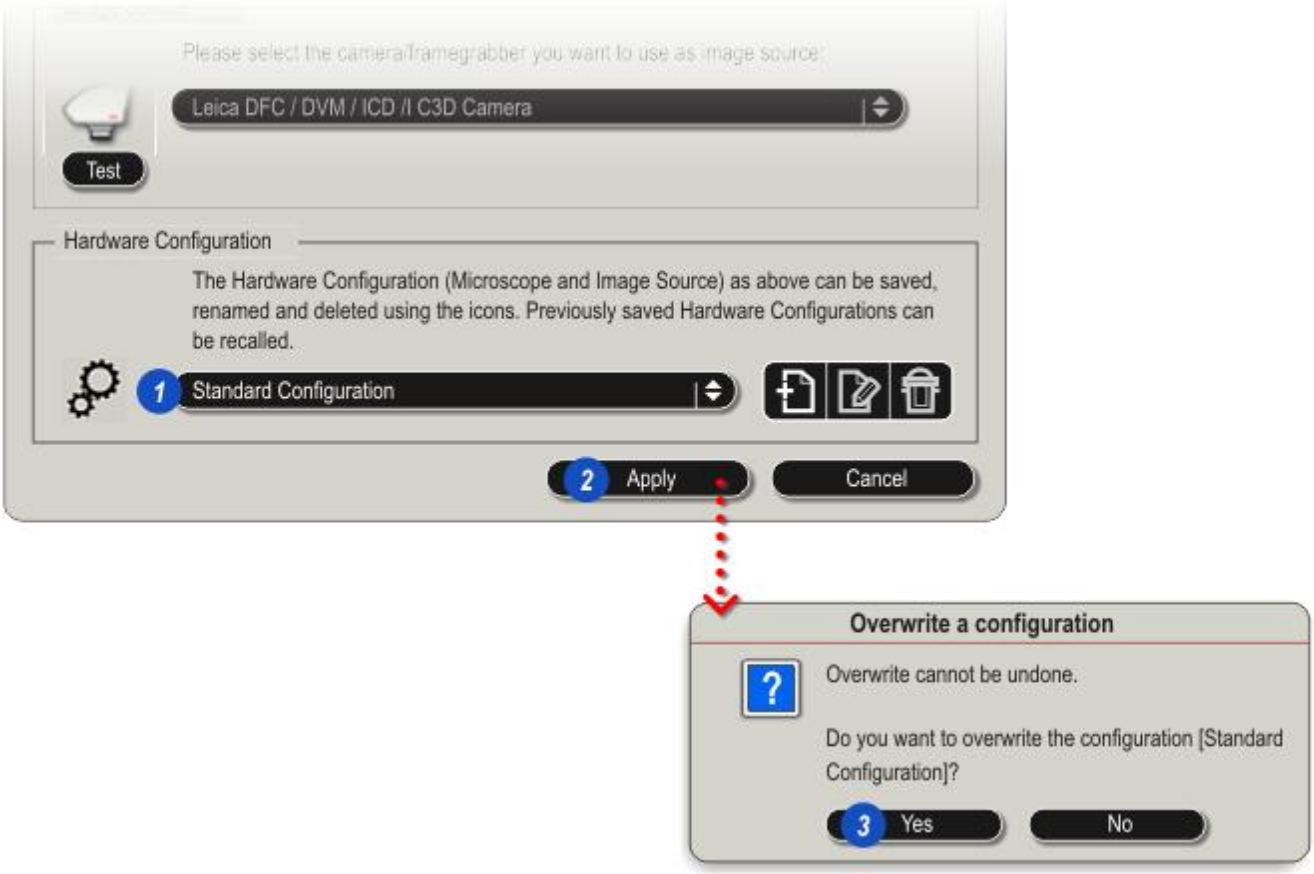
The fitted camera and microscope combination can be saved as a *Hardware Configuration* that can be retrieved and applied at any time. This feature is especially useful for several users working on the same microscope but with different cameras.

However, for a single microscope/camera installation the default *Standard Configuration* can be overwritten with the new microscope and camera models and automatically loaded when LAS is started.

1: *Standard Configuration* is displayed on the *Hardware* panel if no other configuration has been created and used.

2: Click on the *Apply* button.

3: The *Overwrite Configuration* warning appears. Click *Yes* and the microscope/camera setup is saved.



Configurations are especially useful for several users working on the same microscope but having different cameras.

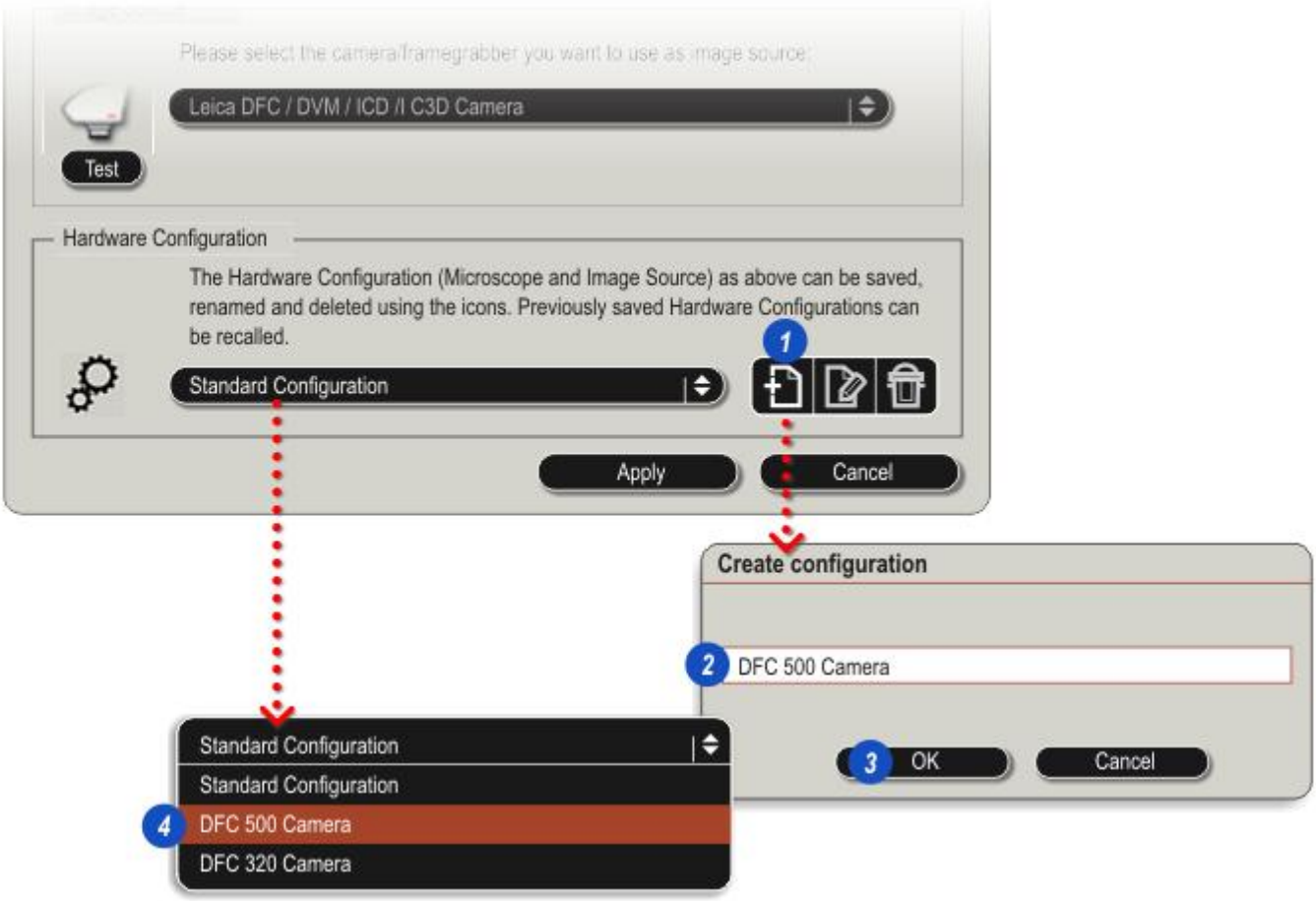
Create a new configuration by:

1: Click on the *Create* icon.

2: On the *Create Configuration* dialog, click inside the text box and type a unique name for the configuration.

3: Click *OK*.

4: The configuration is added to the list.



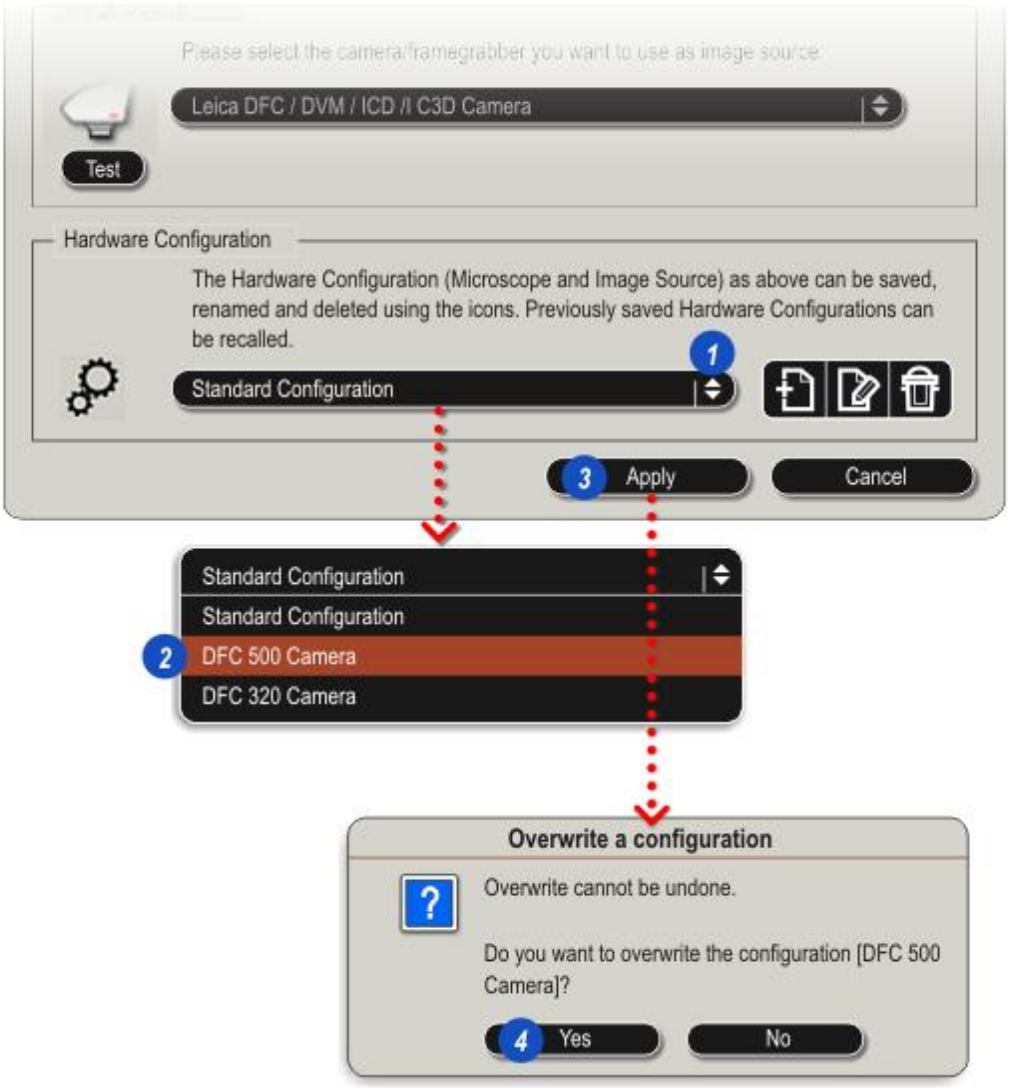
Changing an existing configuration but keeping the name: With a new microscope and/or camera already selected, change an existing configuration by:

1: Click on the arrows to the right of the *Configuration Menu* header to reveal all configurations.

2: Click to select the configuration to be changed.

3: Click the *Apply* button.

4: Confirm the change by clicking *Yes* and the configuration is updated.



Delete a Configuration

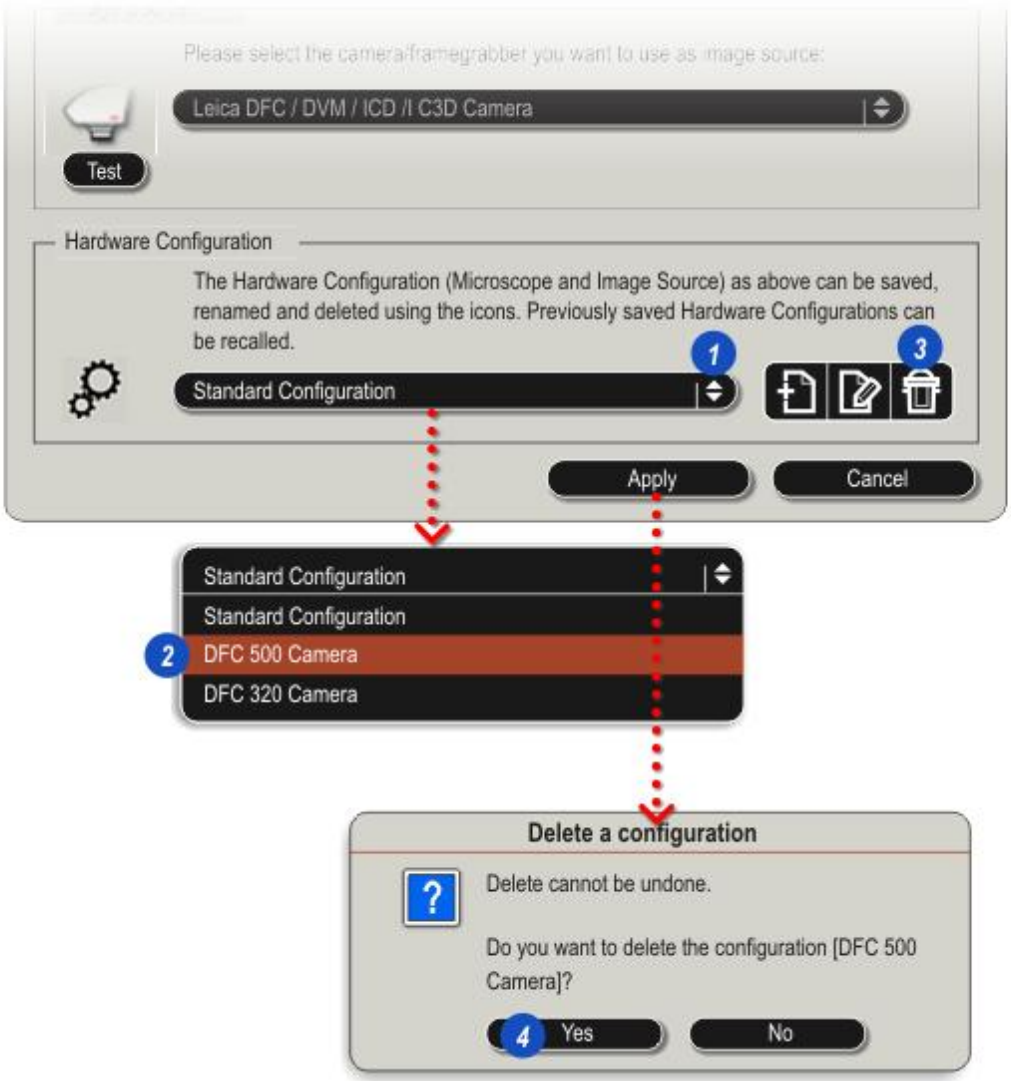
Configurations can be deleted but administrators should be aware that the process cannot be reversed:

1: Click on the arrows to the right of the *Configuration Menu* header to reveal all configurations.

2: Click to select the configuration to be deleted.

3: Click the *Delete (Trash Can)* button.

4: Confirm the change by clicking *Yes* and the configuration is deleted.



Change a Configuration Name

Change the name of an existing configuration by:

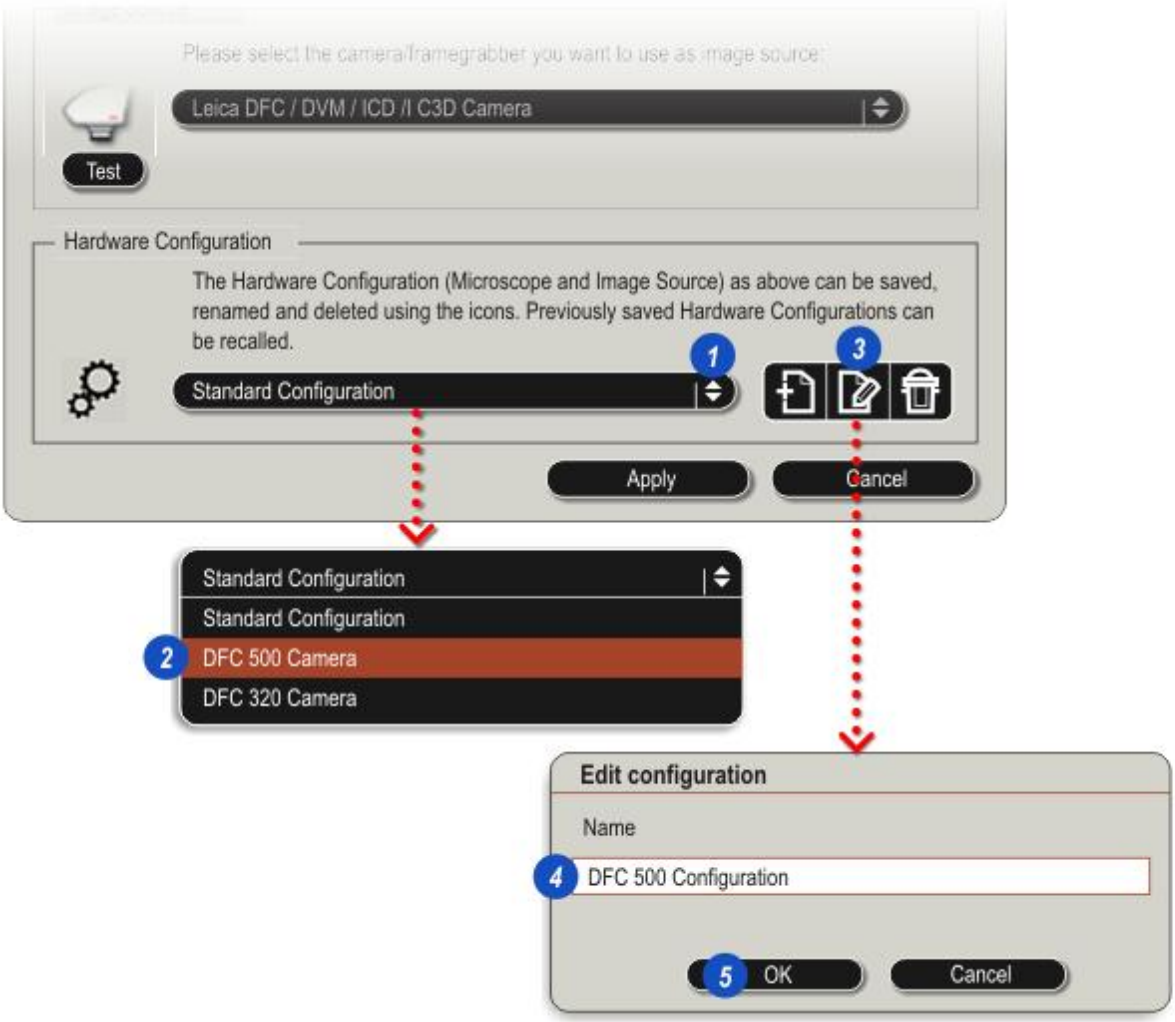
1: Click on the arrows to the right of the *Configuration Menu* header to reveal all configurations.

2: Click to select the configuration name to be changed.

3: Click the *Edit* button.

4: On the *Edit* Configuration dialog, click inside the *Name* text box and type a new name.

5: Click *OK* and the configuration name is changed.



All users can select a *Hardware Configuration* to load into LAS.

Note: If LAS is already running, choose *File* > *Exit*.

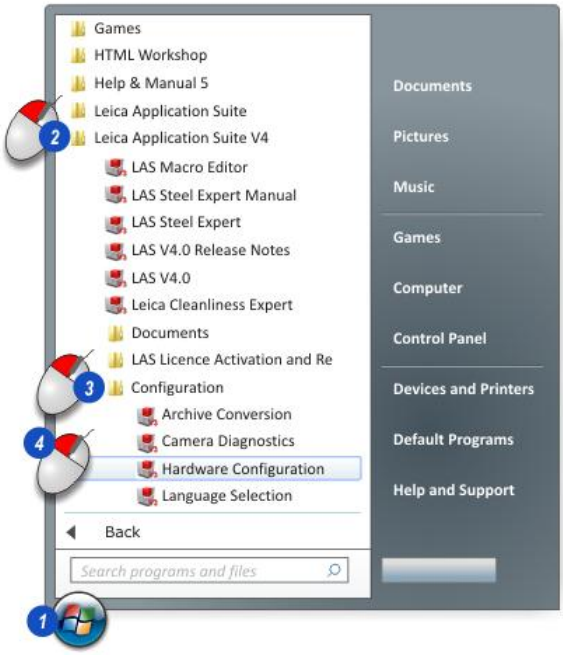
To reach the LAS *Framework* and the *Select Hardware Configuration* option:

1: Click on the *Windows Start* button and select *All Programs*.

2: Click *Leica Application Suite V4.*

3: Click *Configuration*.

4: Click *Hardware Configuration*.



When the *Framework* appears:

1: Click on the *Options* entry on the *Main* tool bar.

2: Choose *Select Hardware Configuration* from the drop down menu.

Administrators already working in *Hardware Setup* can go directly to *Options* > *Select Hardware Configuration* because the *Framework* is already active.

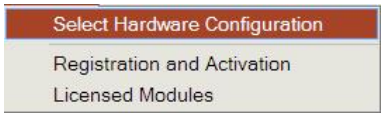
3: On the *Select Hardware Configuration* dialog, click on the *Hardware Configurations* drop-down menu.

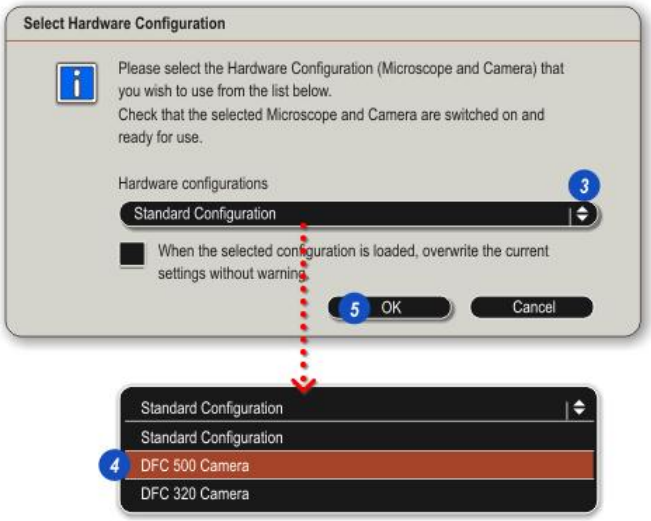
4: Select the configuration to load from the drop-down list.

5: Click *OK.*

Admin User menu

Standard User menu





The illustration is a graphical representation of the LAS display showing the principal features:

1: *Main Tool Bar*: Come here for File, Options and Help menus.

2: *Workflows*: Select Setup, Acquire, Browse, Process and Analysis here.

3: *Module Launcher:* Click to reveal the installed sequence modules and launch them if they are enabled.

4: *Control Panels:* All of the tools for the selected *Workflow*.

5: *Tabbed Control Panels:* Click tabs to select additional tools for the *Workflow* and running module.

6: *Image Data Form:* Displays and edits selected data for the current image.

7: *The Image Viewer:* Display and working area for the current image: Press keyboard F5 to show full screen.

8: *Side Tool Bar:* Common working tools control all aspects of the display and tasks.

9: *The Grid:* Displays the current folder image data in a scrollable grid format. Available only with LAS *Archives*.

10: *The Gallery:* Displays a thumbnail of all the images in the selected folder.

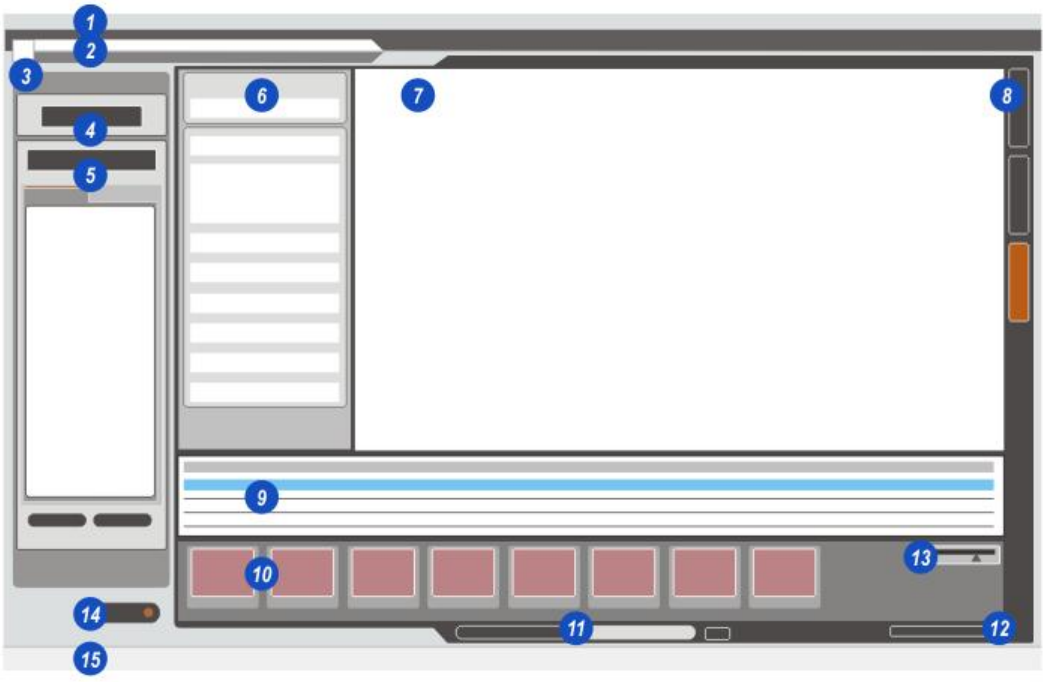
11: *Fast Search Controls*: Create filters and fast search for images. (*Archive* Option dependant).

12: Gallery *Browser*: Locate rapidly thumbnails and display in the Viewer.

13: *Gallery Thumbnail Scaler*: Click and drag to re-size the thumbnails.

14: *Acquire*: Universal capture button.

15: *Status Bar:* Displays *Hardware Configuration, RGB Intensity, Stage Position* and *Magnification* data.



The Leica Application Suite *User Interface* is divided into 5 main areas.

1: *Sequence Module Selector:* Click here to show the available modules and click to select the required one.

2: *Menu bar:* Select items here to access administrative options and *Preferences*

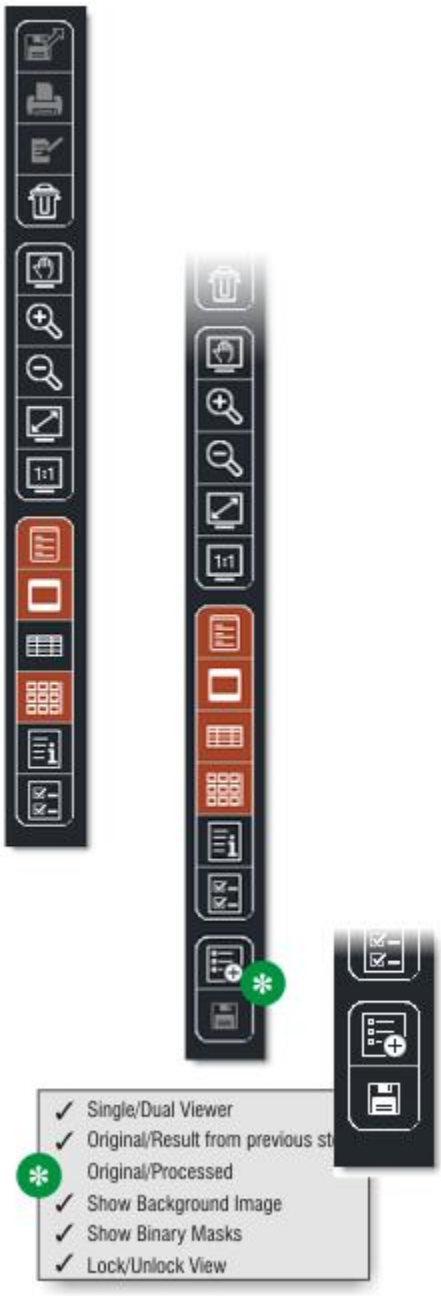
3*: Workflow bar*: The *Workflow* creates the appropriate panels and controls for the selected application. Click on a *Workflow* to open it.

4: *Control Panels:* The programme controls are displayed on tabbed panels. Click on the tab to display the panel.

5: *Image Viewer* and *Gallery:* The remaining part of the screen application is devoted to the *Image Viewer*, *Grid* and *Gallery.* The thumbnail images in the *Gallery* can be re-sized by clicking on the slider **(6)** and dragging it up to increase the size or down to decrease it.

The *Side Tool Bar* is situated on the right-hand edge of the *Viewer* and provides the essential working tools for many of the tasks concerned with managing images - *Export, Delete* and *Print* - and customising the environment - *Hide* and *Reveal* display features, *Fit to Screen* - and so on.

If optional modules are installed, where appropriate the *Side Tool Bar* changes to reflect the additional functions that the modules provide (*). In the illustration the additional tools are loaded with module *Image Analysis.*



From LAS 4.2 onwards, small red triangles on some panels indicate that a *Context Menu* with further options is available.

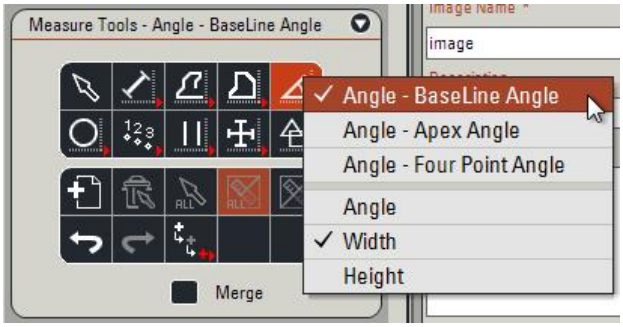
A good example is the *Measure Tools - Selection* panel in the *Acquire* module, where you can right-click to select a tool type and its parameter:

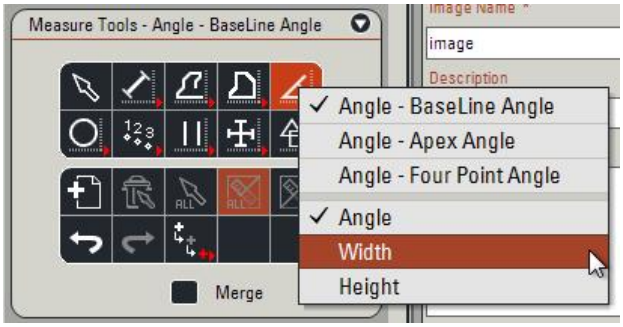
1: Right-click on a tool button, such as the *Angle Tool*.

2: Left-click on the *Context Menu* to select the tool type (e.g. *Angle - Baseline Angle*).

3: Right-click on the tool button again.

4: Left-click on the *Context Menu* to select the parameter (e.g. *Width*).





Shortcut Keys: General

Leica Application Suite has a wide range of Keyboard Keys and Mouse combinations to simplify actions for the user and so speed throughput and improve productivity. Some shortcuts are applicable only to particular optional modules and if those modules are not installed the shortcuts will not function.

Shift + *Left click* LAS desktop icon: Start LAS framework only so hardware configuration and firmware download, licensing can be used. *Note: Requires administrator privileges.*

Control + *Left click* LAS desktop icon: Show Hardware Configuration dialog when LAS starts.

Show help files for the module currently active.

Acquire single image / current mode image sequence.

Enable and Cancel Cinema Mode - Show image full size without controls - live image on Acquire only.

Enable and Cancel Full Screen Mode - Show image full screen size with controls.

Enable and Cancel Dual Monitor mode - switch image to second monitor with controls.

If Measurements is licensed show a zoom region around the mouse position. For example, when setting calibration end points or drawing in *Live Measurements.*

When the *Gallery* or *Grid* have focus: Selects all images in *Gallery*.

When the *Gallery* or *Grid* have focus: Copy the selected image and its metadata to the clipboard so it can be pasted back to LAS. To copy an image to another program, use Copy to Clipboard found by right clicking on the image.

Paste the selected image and its metadata to the folders indicated in the navigator. Note the folder must be a folder that the user can access and also not a sequence folder.

Control Left click on an image in the *Gallery* or *Grid* entry: Add this image to the selected images.

Shift Left click on an image in *Gallery* or *Grid* entry: Select all the images between a previously selected image and the clicked image.

Right click on an image in *Gallery* or *Grid* entry: Show pop-up context menu with *Open*, *Open with*, *Send to* and *Export* options.

Shortcut Keys: General (cont.) 36

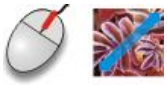
Shortcut Keys: General (cont.)

Left click and drag on live image: Used to set Region of Interest for spot exposure, zoom focus, etc.

Show camera refresh rate top right corner of image.

Show *RGB* values of image at mouse pointer.

Show *Preferences* dialog.



Mouse wheel on image: Image zoom in and out.

Slider control is selected: Mouse wheel adjusts slider is single steps - for example in *Exposure*.

Right click on an image in *Browse*: Show pop-up context menu with *Open, Open with, Send to, Export, Print, Copy, Zoom* and *Pan window* options.

Select all data in the *Record Details* table.

Select and individual record in the *Record Details* table.

Select all the records between a previously selected record in the *Record Details* table and the clicked one.

Copy all of the records in the *Record Details* table to the clipboard. They can then be pasted into another application, *Word* for example.

Live and Interactive Measurements Shortcuts

Used for node nudging. For the selected node or nodes of a drawing, move by 1 pixel in the direction indicated. With the Ctrl key held down at the same time, move by 10 pixels - super nudge.

Alt Gr held down + draw measurement: Hide the labels so that it is easier to see where the measurement is drawn

Shift held down: Show a zoom region around the mouse position.

Space bar held down: Temporarily switch to *Edit* mode after drawing

With the *Select* tool 'on', click and drag rectangle around measurements: Selected measurements in the rectangle can be grouped. Or moved at the same time.

Double click on Parameter name in Grid: Label all measurements with this *Parameter* if possible. *Note: Some measurements don't have all parameters.*

Click on Grid header near small arrow: Re-orders the results in increasing or decreasing values.

Selects all of the measurements in the *Grid*.

Selects an individual measurement in the *Grid*.

Selects all of the measurements between and including the currently selected measurement in the *Grid* and this one.

Copies the selected measurements in the *Grid* so they can be pasted into another application such as *Word* or *Notepad*.

Extended Annotation Shortcuts

When drawing a line, if the Ctrl key is held down at the same time, the line will be drawn either horizontally or vertically, an ellipse will be drawn as a circle and a rectangle drawn as a square.

Start a new line in a *Text Box* or *Rectangle.*

If Measurements is licensed, toggles On/Off a zoom region around the mouse position.

With an annotation selected, copy the annotation to the clipboard.

Paste the contents of the clipboard into a *Word* document.

Select all of the annotations in the *Grid*.

Select an individual annotation in the *Grid*.

Selects all of the annotations between and including the currently selected annotation in the *Grid* and this one.

Copy the selected annotations to the clipboard so that they can be pasted into another application such as *Word* or *Notepad*.

For the selected node or nodes of a drawing, move by 1 pixel in the direction indicated. With the Ctrl key held down at the same time, move by 10 pixels.

When in the Adjust Threshold step, click and drag to set the threshold.

When in the Adjust Threshold step, right-click on the image to hide the overlay.

Double click on Parameter name in *Grid*: Label features with this parameter where possible.

Click on Grid header near small arrow: Re-orders the results in increasing or decreasing values

Shift held down: Show a zoom region around the mouse pointer whilst in the *Binary Edit* step.

Click on a feature in the *Measurement* step: Select the feature and move it to the top of the *Grid*.

Click on a row on the *Grid*: Selects and highlights the feature on the image.

Whilst in *Measurement,* the features selected in the grid are deleted.

Selects all measurements.

Selects an individual measurement.

Selects a range of measurements between and including the currently selected measurement and this one.

Copies the selected measurement(s) to the clipboard so that they can be pasted into another application such as *Word* or *Notepad*.

Montage 3D Viewer Shortcuts

Drag the model to a different viewing angle: *Rotate* and *tilt*.

Drag the model to a new location: *Pan*.

Zoom the model.

Scale the model height.

Mouse Interactive (on side tool bar) enabled: Double click Mouse Wheel: Return directly from a zoom to show mosaic in *'Fit to Window'* mode.

Move Pattern (on side tool bar) enabled: Hold down *Control*: Click and drag Mouse on the mosaic *Pattern* to rotate it.

Move Pattern (on side tool bar) enabled: Hold down *Shift*: Halt mosaic *Pattern* rotation.

Mouse Interactive (on side tool bar) enabled: Right Mouse click on *Map* to display Map Properties dialog.

Power Mosaic Plus Shortcuts:

Mouse Interactive (on side tool bar) enabled: Right click on *Workspace* to display Workspace properties.

Move Pattern (on side tool bar) and *Pattern Navigator* enabled: Click and drag Workspace or Pattern.

Dual Monitors

With the necessary *Dual Screen Video* card and software installed (*), Leica Application Suite can be configured quickly and easily to show all of the usual controls - including optional modules - on one monitor (*Primary*) whilst using the entire viewable area of the other (*Secondary*) for the image - live or captured.

The extra-large image area provides greater precision and ease of working for capture, analysis and measurement, whilst the greater area given to *Gallery Thumbnails* means many more can be displayed at once or individuals enlarged to examine fine detail.

The *Side Tool Bar* is automatically displayed on the appropriate monitor and *Dual Monitor Mode* can be enabled or disabled with a single keystroke.

(*) Use of dual monitors requires that the graphics card in the PC supports this option. Some graphics cards lack performance that slows down the movement of the mouse cursor. If this occurs, the drivers of the graphics card may need updating or it may not have the necessary performance.

Swap Monitors

The conventional layout for dual monitors is primary monitor (displaying Windows controls) in front of the user, and the secondary monitor to the right. This is initially setup in the video driver software, but can be changed in Windows so that the secondary monitor is moved to the left without affecting the smooth transition of the cursor from one screen to the other.

- LAS users may find swapping the monitors a convenient way of reducing the distance the cursor has to be moved to place it on the image.

1: Right-click on the *Desktop*.

2: On the menu, for Windows XP users, click on *Properties*. For Vista users click on *Personalise*.

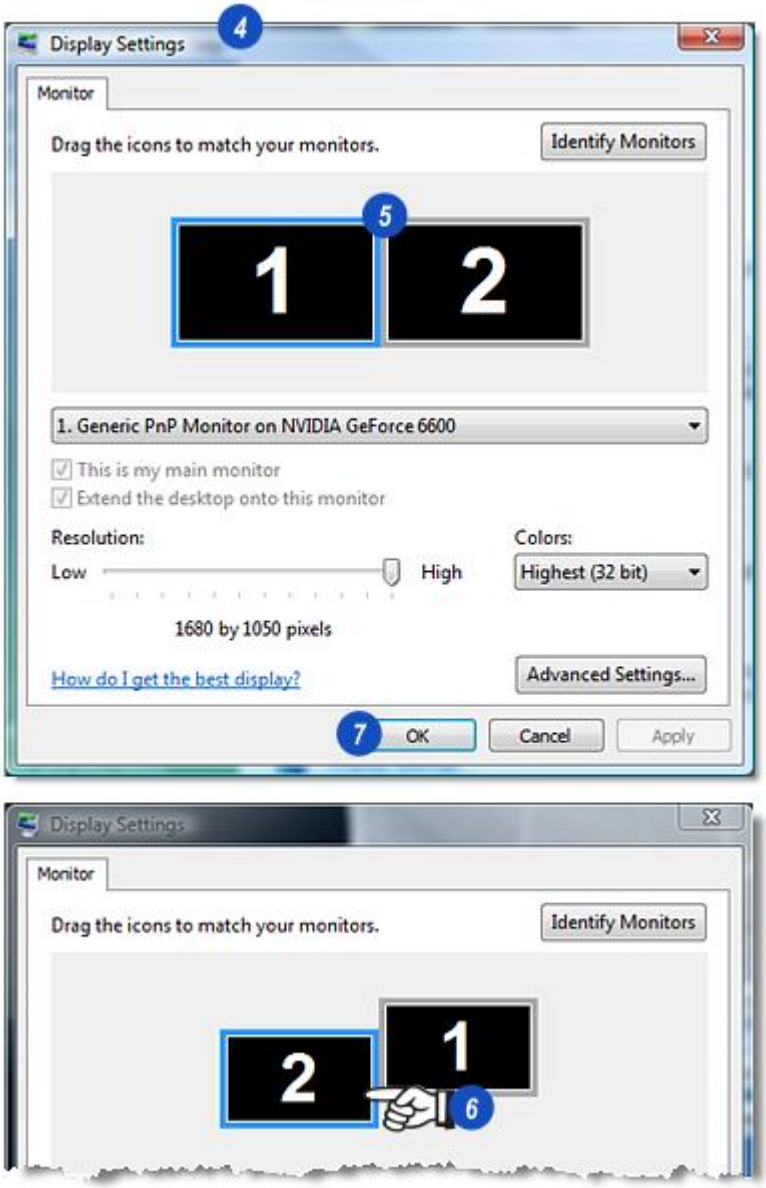
3: On the dialog, Windows XP users click on the *Settings* tab and Vista users the *Display Settings* option.

4: The illustration shows the Vista dialog but the Windows XP version is very similar.

5: The two monitors are shown as icons – *1* being the primary display and *2* the secondary.

6: Click and drag the secondary display to the left of the primary display and release the mouse button.

7: Click *OK*.

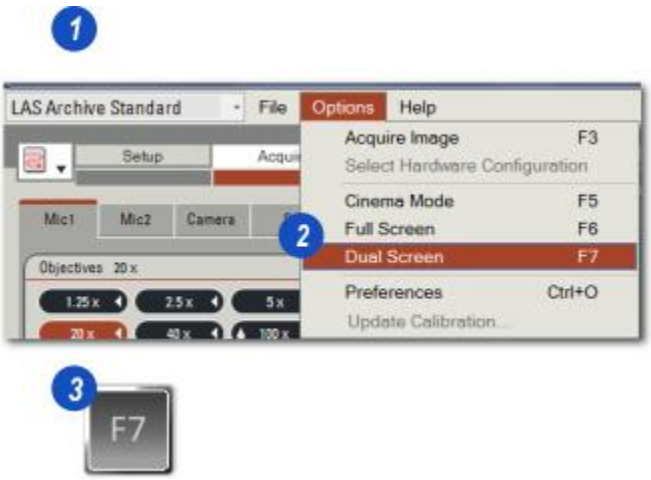


The secondary monitor is turned on or off either from the *Options* menu or by pressing keyboard function key *F7.*

1: Click on *Options* on the main tool bar.

2: On the drop down menu, click to enable the check box to the left of the *Use Second Monitor* option. Clicking the checkbox again will disable the second monitor and move the *Image Viewer* back to the primary monitor – the usual LAS layout.

3: Function key *F7* performs the same operation with fewer mouse clicks. Press *F7* to enable the second monitor and press again to disable it.

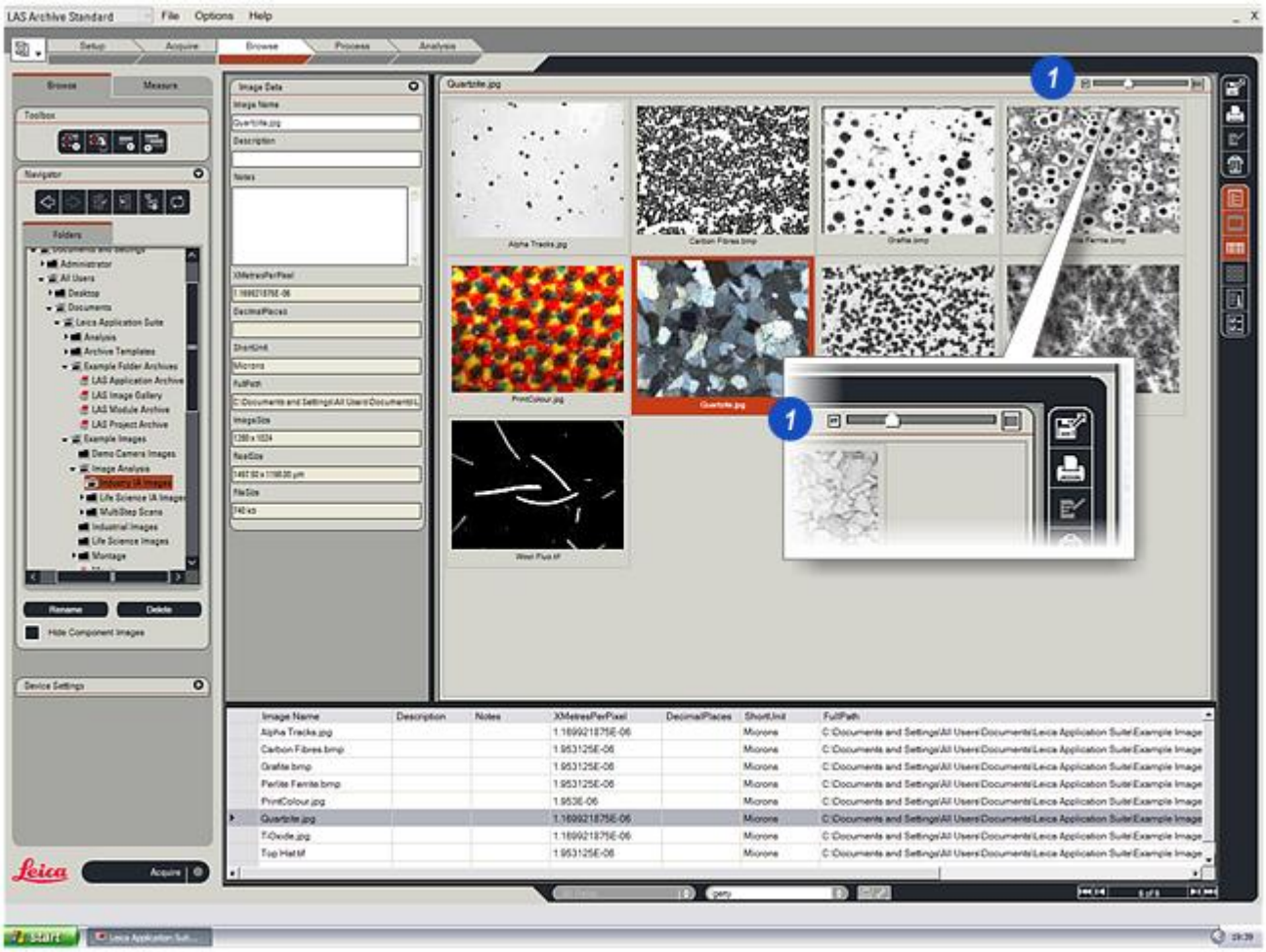


With the *Viewer* moved to the *Secondary Monitor*, all of the *Primary Viewer* area can be occupied by the *Gallery Thumbnails* which means they can be larger and clearer.

The smooth *Thumbnail Sizing* slider has been moved to the top edge - click and drag to the left for smaller, more-on-view *Thumbnails*, or to the right for larger, fewer images.

All of the tools (except image zoom and sizing) are available on the *Side Bar* to show and hide the *Record Form, Record Information, Data Grid* and *Data Items* to *Display.*

Export, Print, Reporting (with some images) and *Delete* are also present. *See Side Bar Tools:* *Go there...* 47

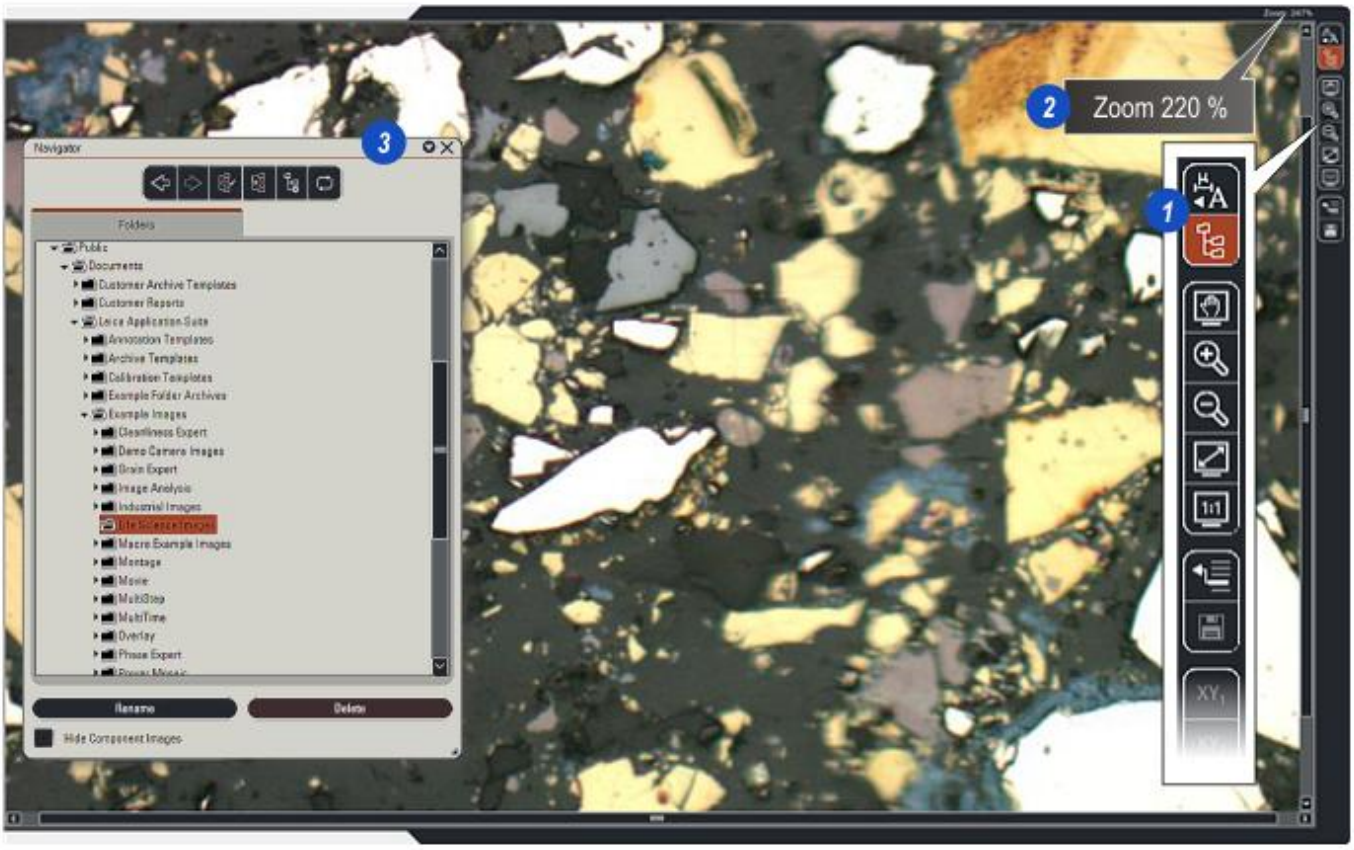


The *Secondary Monitor* is dedicated totally to the *Viewer* so that images can fill the entire screen. Taking measurements or analysing the image is easy and precise with such a large working area.

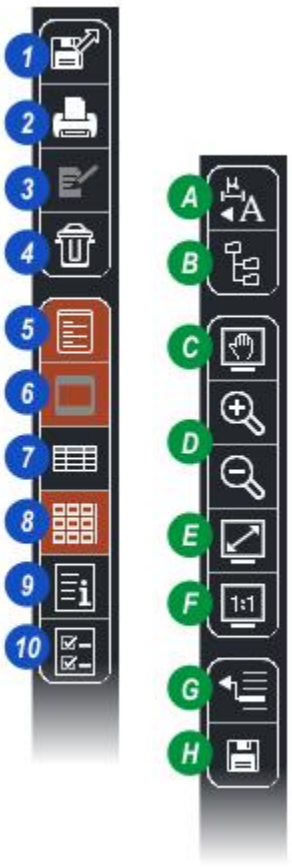
1: The *Image Control Tools* - detailed on the following page - are automatically moved to the *Side Tool Bar*.

2: A *Zoom Level* readout on the *Viewer* top edge.

3: The monitor can also display the *Floating Navigator* which allows users to move between folders without having to return to the *Browse Workflow.*



With the second monitor enabled and displaying the image, the right-hand *Tool Bar* is divided appropriately between the two displays.



On the *primary* monitor displaying the LAS controls:

1: Export image.

2: Print image.

3: Prepare Report depending upon the image type.

4: Delete image (Trash Can).

5: Show/hide the Record form.

6: Show/Hide the image, disabled in Dual Monitor mode.

7: Show/hide the Grid.

8: Show/hide the Gallery Thumbnails, disabled in Dual Monitor mode.

9: Show Record Details.

10: Show Select Visible Fields dialog.

On the *secondary* monitor displaying the Image Viewer:

A: Show the Annotations Options.

B: Open/Close the Floating Navigator (Browse).

C: Pan the Image. The Pan Window appears on the primary monitor.

D: Zoom Into/Out of image.

E: Image to Fill Viewer.

F: Display Image at Actual Size.

G: Viewer Options (Dual Viewer etc).

H: Save the Output Image.

A wide range of options is available by right-clicking the image in the *Viewer* or its *Thumbnail* in the *Gallery*. The options vary depending upon which item was clicked, the operating system and the software installed on the computer.

1: Right-click the *Thumbnail* for the context menu of basic options and...

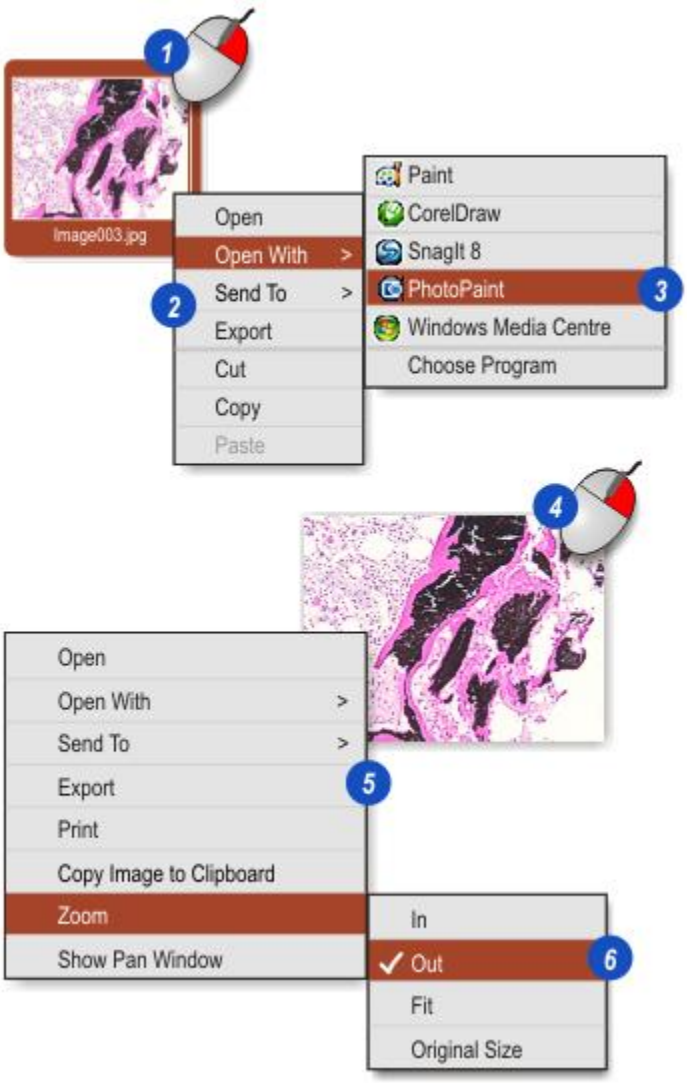
2: ...click it to select.

3: Some options have additional possibilities displayed as a sub-menu.

4: Right-clicking the image in the *Viewer* displays a different context menu.

5: Additional functions are available some of which will also have sub-menus **(6)**.

For detailed help on the available options, see the *Browse Workflow* 348



This chapter describes the *Workflow* organization of the unique Leica Application Suite user interface and its basic capabilities.

Additionally, the functions that are common to the various Workflows are described in *Functions Widely Available* 50.

LAS *Workflow* describes the order and grouping of tasks for image documentation and analysis. While the *Workflow* suggests an order for the tasks, versatility is retained so that the operation of the software is not constrained to fixed steps. Grouping tasks into related operations makes working with LAS intuitive and easy.

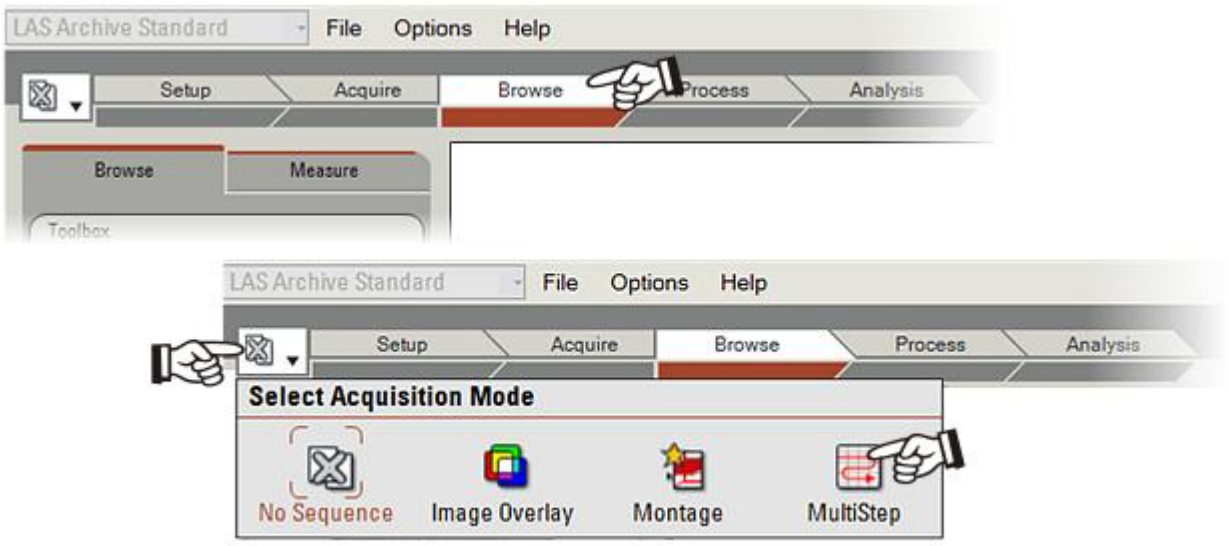
Each is selected by clicking on the *Workflow* bar.

Selecting a *Workflow* displays the appropriate controls arranged on one or more panels that allow the user to perform the selected action.

Because the *Workflow* arrangement is so versatile, in contrast to many Windows programs, LAS does not employ a menu bar for the main operation of the software.

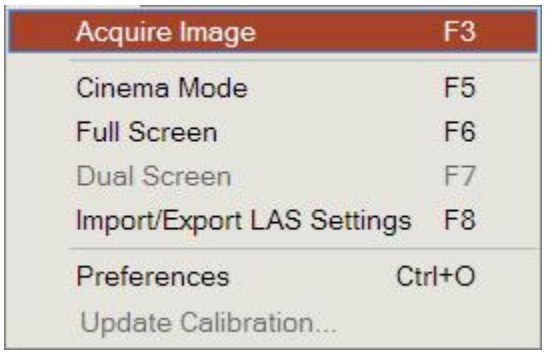
Installed and enabled *Optional Modules* are listed on a menu revealed by clicking the *Select Acquisition Mode* icon to the left of the *Workflows*.

- *Setup* 144
- *Acquire* 196
- *Browse* 348
- *Process* 398
- *Analysis* 419



Functions Widely Available

Some functions within Leica Application Suite apply to several Workflows, or have a more global application. These are available from the *Options* drop-down menu.



Choosing *Acquire Image* captures an image, and is the equivalent to pressing the function key F3 on the keyboard or clicking the *Acquire* button on the *Acquire* or *Browse* Workflows.

Cinema Mode (F5) displays the image full screen without any controls; to cancel *Cinema Mode*, click the cross icon in the top right of the screen (or press F5 again).

Full Screen mode: Show image full screen size with *Side Tool Bar* controls. Click *Exit Full Screen* to return to normal view.

Dual Screen (F7) displays the image on the second monitor (if fitted). This works on all Workflows.

Import/Export LAS Settings 51: Individual LAS modules (such as *Live* and *Interactive Measurements*) already allow you to save *Configuration* files and settings. Now you can save and import global settings files, and specify exactly what these settings files contain.

Additionally the *Scale Bar* can be accessed from nearly all steps and the *Export Images* feature is also widely available so both are included in this section:

- *Setting Preferences* 55
- *Scale Bar* 77
- *Update Calibration* 85
- *Image Comparison* 95
- *Export Images* 100
- *Printing* 107
- *Gallery Docking* 143

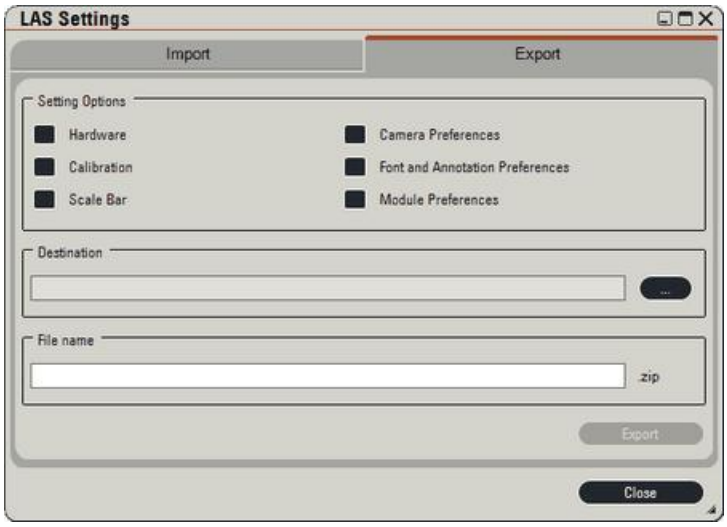
Import/Export LAS Settings

Individual LAS modules (such as Live and Interactive Measurements) already allow you to save Configuration files and settings. Now you can save and import global settings files, and specify exactly what these settings files contain.

To display the Import/Export dialog:

1. Choose **Options > Import/Export LAS Settings** from the main menu.

2. Display the appropriate tab on the *LAS Settings* dialog.



See:

Export Configuration Settings 52

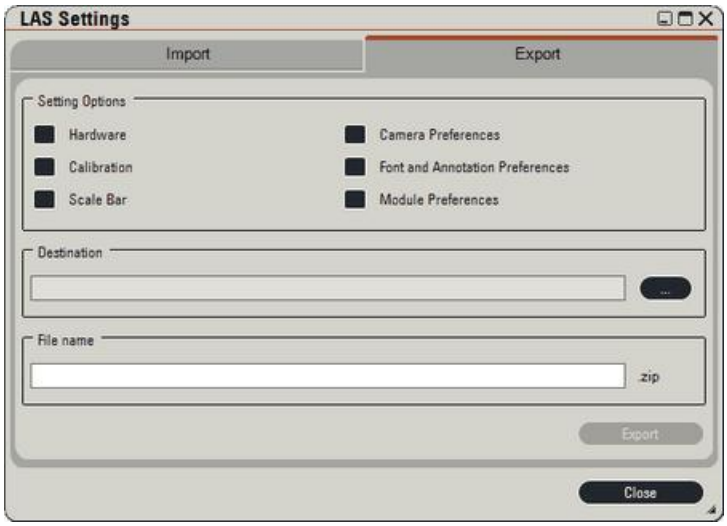
Import Configuration Settings 53

Restore Configuration Settings 54

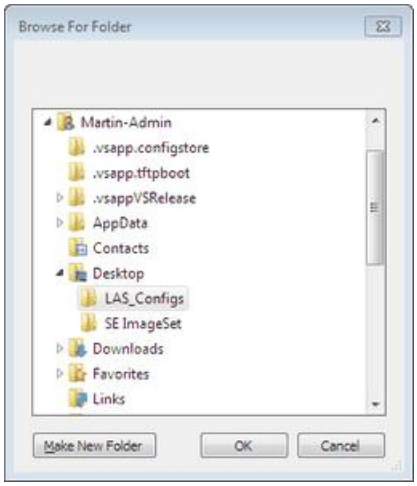
Export Configuration Settings

To export current LAS settings as a global Configuration file:

1. On the *LAS Settings* dialog, display the *Export* tab.



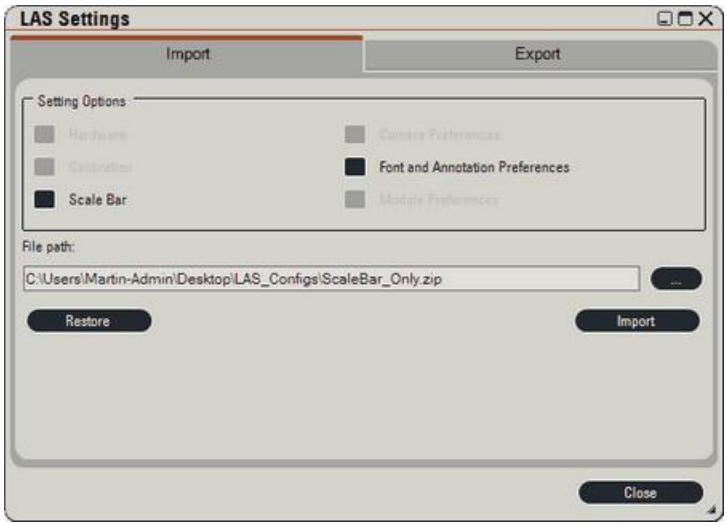
2. Click to enable the *Setting Options* that you want to include in this Configuration.
3. Click the *Destination* browse button.
4. Navigate to the folder where you want to save the *Configuration* file.
5. If necessary, click *Make New Folder* and enter a name.



6. Click *OK* to set this as your chosen folder.
7. Enter a *File name* for for the *Configuration* (the .zip extension will be added automatically).
8. Click *Export*.

To import LAS settings from a saved Configuration file:

1. On the *LAS Settings* dialog, display the *Import* tab.
2. Click the *File path* browse button.
3. Navigate to the folder containing your saved *Configuration* files.
4. Select the appropriate .zip file and click *Open*. The file path will be displayed in the *Import* tab.
5. Click to enable the *Setting Options* that you want to import.
   Note: Only the settings that were originally exported to that file are available. Unavailable options are greyed out.

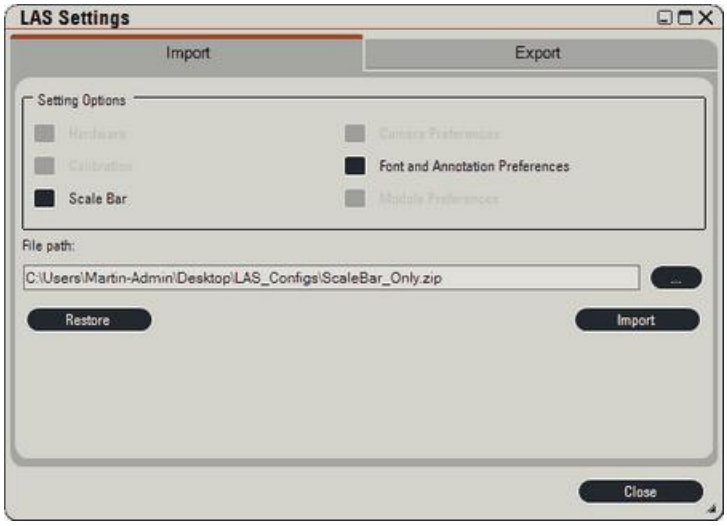


6. Click *Import*.
7. The *Restart LAS* dialog will be displayed. Do one of the following:
   - Click *Yes* to restart LAS immediately
   - Click *No* if you want to continue working with the current settings for now (but note that the new settings will take effect the next time you start LAS).
8. When LAS restarts, click *OK* to acknowledge that new settings have been imported.

If LAS starts and you do not want the new imported settings to take effect (for example, if another user imported a Configuration file and opted to restart later), you can restore LAS to its previous state (i.e. before the settings file was imported):

1. When LAS starts, you have to click *Yes* acknowledge that new LAS Settings have been imported.

2. Choose **Options > Import/Export LAS Settings** and display the *Import* tab.
3. Click *Restore*.



4. The *Restart LAS* dialog will be displayed. Click *Yes* to restart LAS immediately.
5. When LAS restarts, click *OK* to acknowledge that the old LAS settings have been restored.

The *Preferences* dialog allows the user to select preferred options for Leica Application Suite and most of the *Optional Modules* to reflect the fastest and most convenient working methods.

As *Optional Modules* are added to Leica Application Suite, tabs on the *Preferences* dialog are also added to include settings that are specific to the modules.

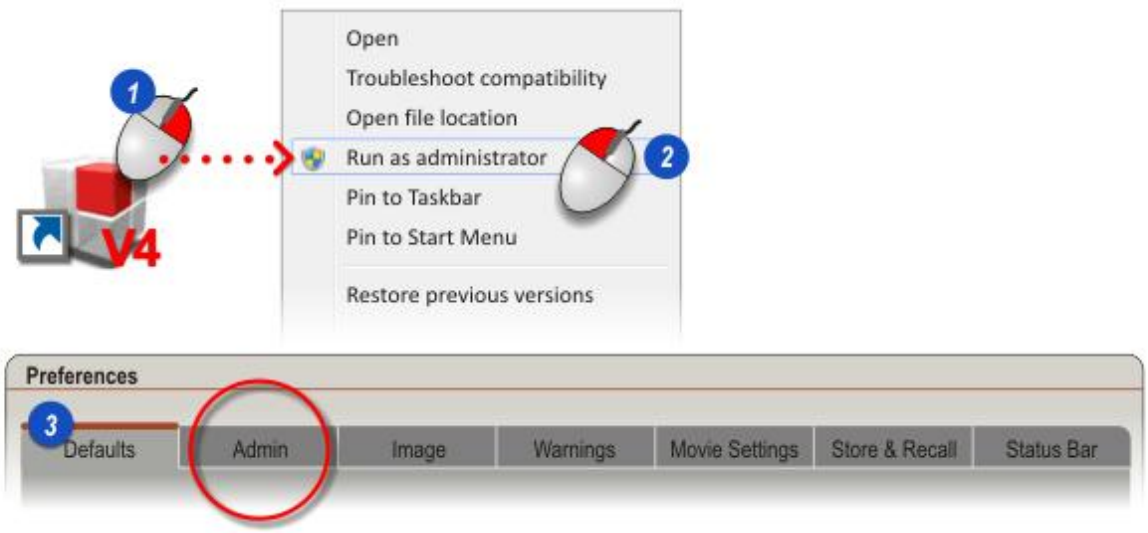
Preferences may be altered at any time – even while a module is running.

When LAS is launched by an administrator, the tabs will vary to include options that only administrators are permitted to set:

1: Administrators launch LAS by right-clicking the desktop icon and...

2: ...left-clicking the *Run as administrator* option on the context menu.

3: When LAS starts and the *Preferences* dialog is opened, the *Admin* tab is present. This is not available to other users.



Preferences may be altered at any time – even while a module is running.

1: On the *Main Toolbar*, click on the *Options* label.

2: From the drop down options, click to select *Preferences.*

3: The *Preferences* dialog appears. Click on a tab along the top of the dialog to reveal the options and settings required.

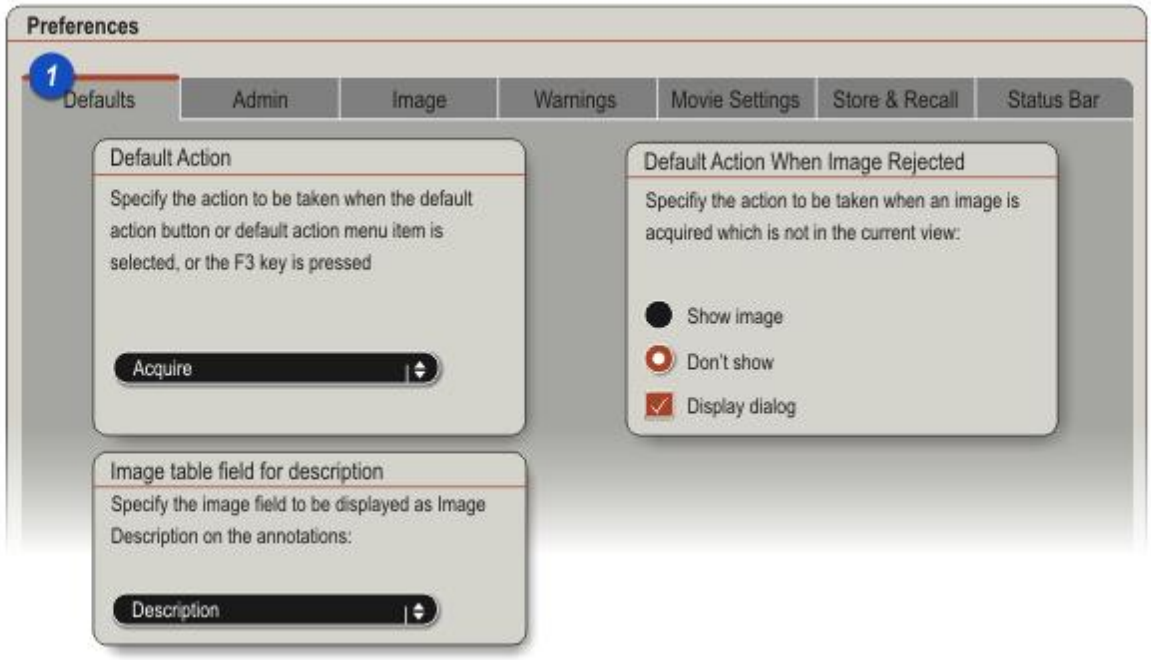
4: When changes have been made, click on the *OK* button to save them or…

5: …click on the *Cancel* button to keep existing settings.

Quick links to the Preferences tabs:

- *Defaults:* What happens after image capture.
- *Admin:* Settings that only administrators can change.
- *Image:* Formats, captions and other image settings.
- *Warnings:* Turns individual warnings on or off.
- *Movie Settings:* Controls the size of movies.
- *Store & Recall:* Save and retrieve image data.
- *Status Bar:* Display helpful information and calibrate monitors.

Click a tab on the illustration for more information.



The *Defaults* tab has three panels that specify the actions that will occur in various situations. The *Default* actions are user defined:

1: Click on the *Defaults* tab to reveal the panels.

- *Default Action*: What should happen when an image is captured.
- *Image Table Field for Description*: Specify the data field to display as the annotation on the image.
- *Default Action When Image Rejected*: The capture location is not currently active so specify the options.

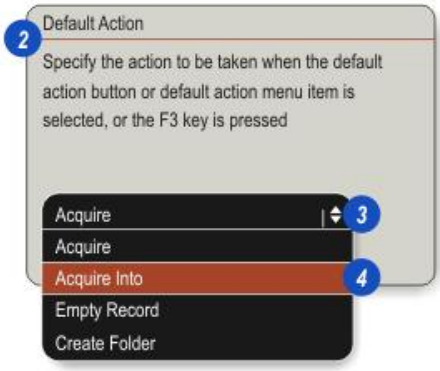
Click on a panel on the illustration for more information:

1: Click on the *Defaults* tab to reveal the panels.

2: *Default Action* that determines the action when the shortcut key *F3* is pressed with an archive selected.

3: Click on the arrows to the right of the *Default Action* drop-down header.

4: Click to select the required option that will automatically occur when key *F3* is pressed.



Field for Image Description & Image Rejected

1: Click on the *Defaults* tab to reveal the panels.

2: An archive field from the *Image Table* can be used as part of the image description.

3: Click on the small arrows to the right of the header and...

4: ...from the drop-down menu click to select the field to use with the image. Use the scroll slider to reveal the entire list.

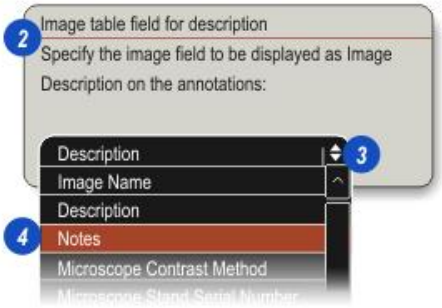
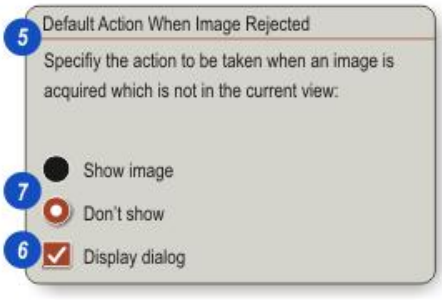


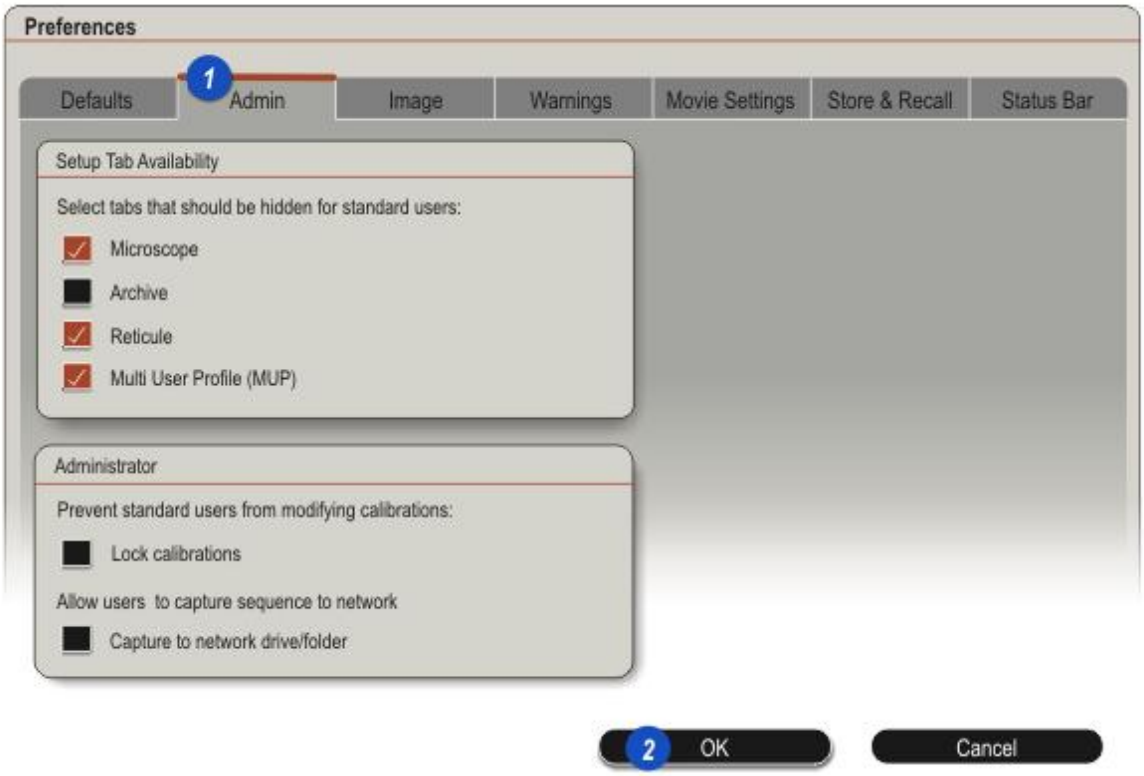
Image Rejected:

5: If an image cannot be saved in the current folder - usually because it has not been nominated as the fixed capture location - chose a default action as follows:

6: Clicking to enable the *Display dialog* check box (Tick mark visible) displays a *Capture Options* dialog to the user. It is recommended that this check box is enabled.

7: Choose to either *Show* or *Not Show* the image by clicking the appropriate radio button.





- Administrators can restrict access to panels on the *Setup Workflow* to prevent unauthorised changes being made to essential settings by standard users.
- Once a microscope has been calibrated, administrators can prevent further changes by standard users can be prevented by locking the calibrations.
- Users can be allowed to save images to a network drive as well as the local computer.

1: Click on the *Admin* tab to reveal the *Setup Tab Availability* and *Administrator panels.*

2: When changes have been made click the *OK* button.

Click on a panel for more information.

Hide Setup Workflow Tabs

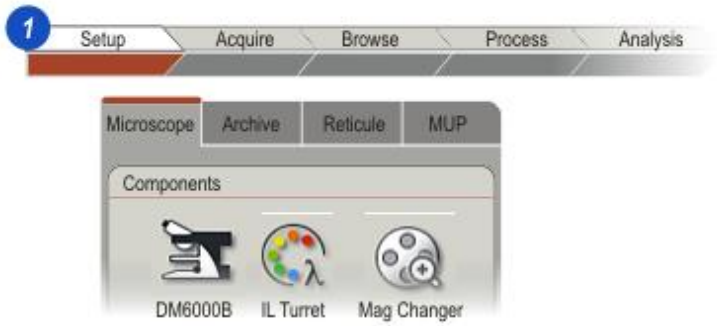
Administrators can restrict access to panels on the *Setup Workflow* to prevent unauthorised changes being made to essential settings by Standard Users.

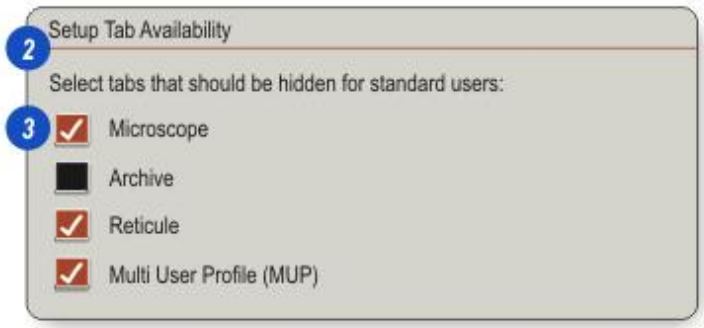
Each of the available tabs on *Setup* can be hidden by checking the check box and so prevent display and access.

1: Click on the *Admin* tab on the *Preferences* dialog.

2: On the *Setup (Workflow) Tab Availability* dialog...

3: ...click the required check box to hide (checked) or reveal (un-checked) a tab. In the example only the *Archive* tab will be displayed in the *Setup Workflow; Microscope, Reticule* and *MUP* will be hidden and their panels inaccessible.

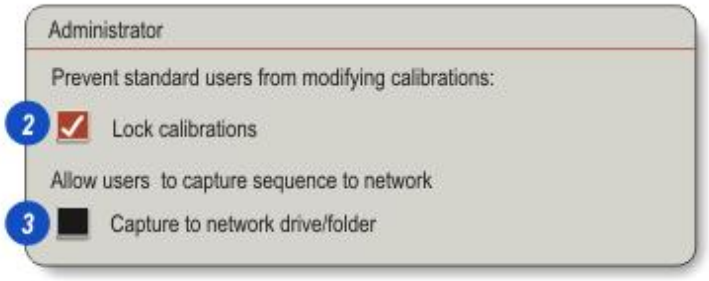




1: Click on the *Admin* tab to reveal the *Administrator* panel.

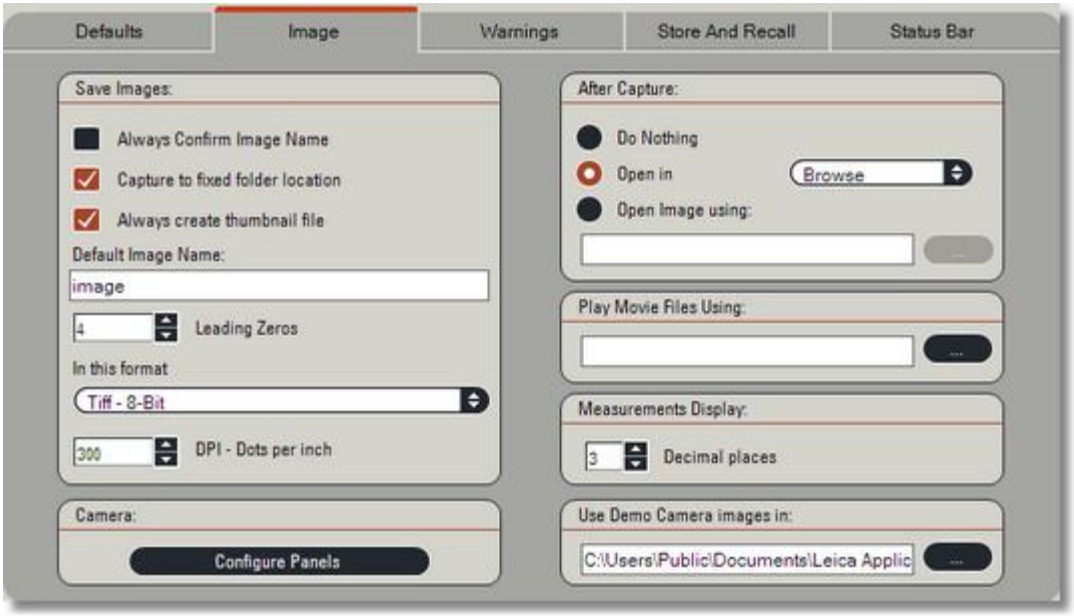
2: Once a microscope has been calibrated, administrators can prevent further changes by standard users can be prevented by checking (tick mark visible) the *Lock calibrations* check box.

3: Allow standard users to save images to a networked drive or folder as well as a local computer by clicking to enable the *Capture to network drive/folder* check box.



Points to consider before enabling *Capture to network...*

- Is the network accessible.
- Is it fast enough to capture extensive sequences like time-lapse.
- Movies cannot be saved using the network.
- A slow network will slow down LAS startup.



The controls on the *Image* tab determine the details of the image capture and what should happen after capture.

Administrators can also set up the *Camera* panels both to avoid modification and avoid clutter.

Click on the *Image* tab **(1)** to reveal the dialog. The tab has 5 individual panels:

- *Save Images* 64 which determines the names and format of saved images.
- Application to launch *After Image Capture* 66.
- The application to launch for the *Movie File* 67 player.
- *Measurement Displays* 67 sets the number of decimal places for some measurement labels.
- *Camera* 65 panel configuration. Determines those controls that are displayed or hidden on the *Acquire* > *Camera* panel.
- *Use Demo Camera images in*. Determines the default folder used to store demo camera images, as used on the *Acquire* > *Camera* > *Input Options* 284 panel.

Click *OK* to save the settings and close the *Preferences* dialog.

1: Click to check and get a prompt for an image name before saving a captured image.

2: Check the *Fixed Capture Location* checkbox to have images saved in the folder selected as 'fixed' on the Browse Workflow.

3: With the *Always Create Thumbnail File* checkbox enabled, when the image is captured a separate thumbnail file with the extension *.thb* will also be created. Whilst this does occupy additional disk space, thumbnails are loaded to the *Gallery* far quicker. Applicable to all folders.

4: The *Default Image Name* is a prefix for each image saved. Click and swipe on the text box and type a new name appropriate to the work in hand.

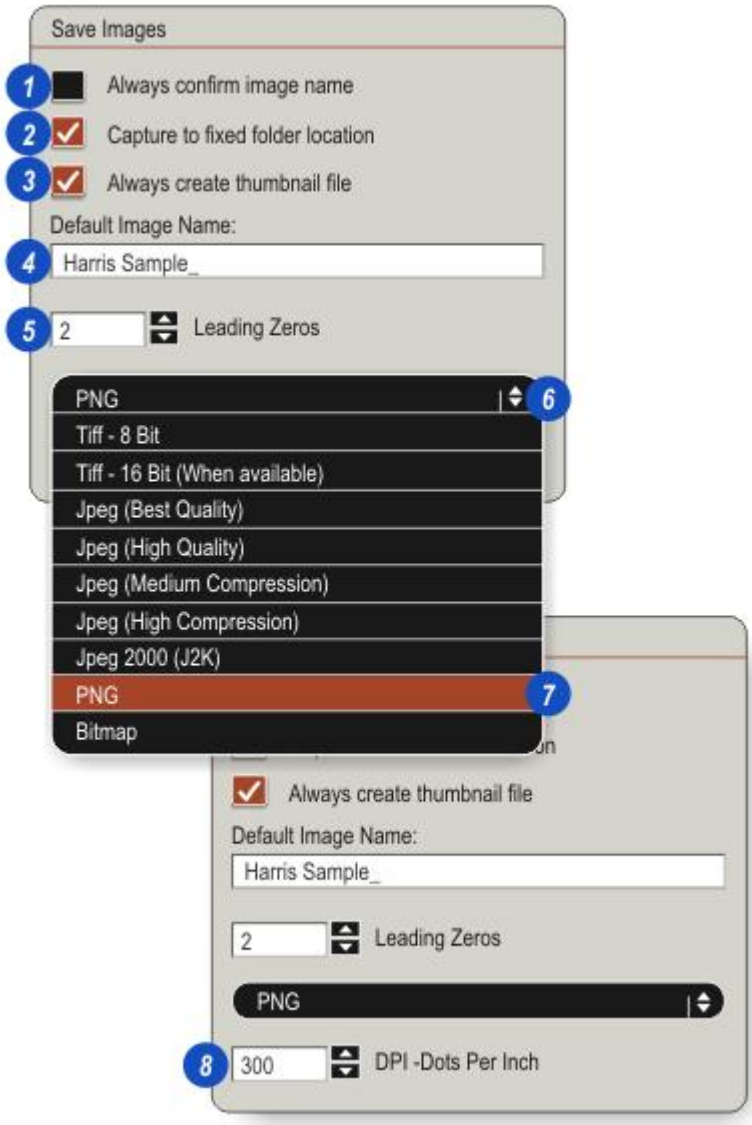
5: *Leading Zeroes* automatically pads the *Image Name* sequence with zeroes so that all image names are the same length. Use the up/down arrows to the right of the *Leading Zeroes* to set the value.

6: To select the *Image Format* and compression, click on the arrows to the right of the *In this Format* header and...

7: ...from the drop down menu click to select the format required.

8: Image resolution measured in *Dots Per Inch (DPI)*, can be set using the up/down arrows to the right of the *DPI* box. This function is particularly useful to ensure that 3rd party applications such as *Word* or *PowerPoint®,* display images correctly.

The *DPI* setting does not affect the capture format or the capture resolution set up in *Acquire > Camera*.



The *Acquire* > *Camera* panel has a wealth of tools and controls but for some users who do not require all of the available facilities, it can become 'cluttered' especially if *Optional Modules* are added. The number of panels on display can be reduced by setting them as hidden on the *Configure* camera *Panels* dialog. The controls are not lost only hidden.

Any user can hide or reveal a panel but only an Administrator or member of the LAS Administrator Group can lock or unlock it. If a panel is locked it cannot be hidden or revealed - its state when the lock was applied remains in force until the lock is released.

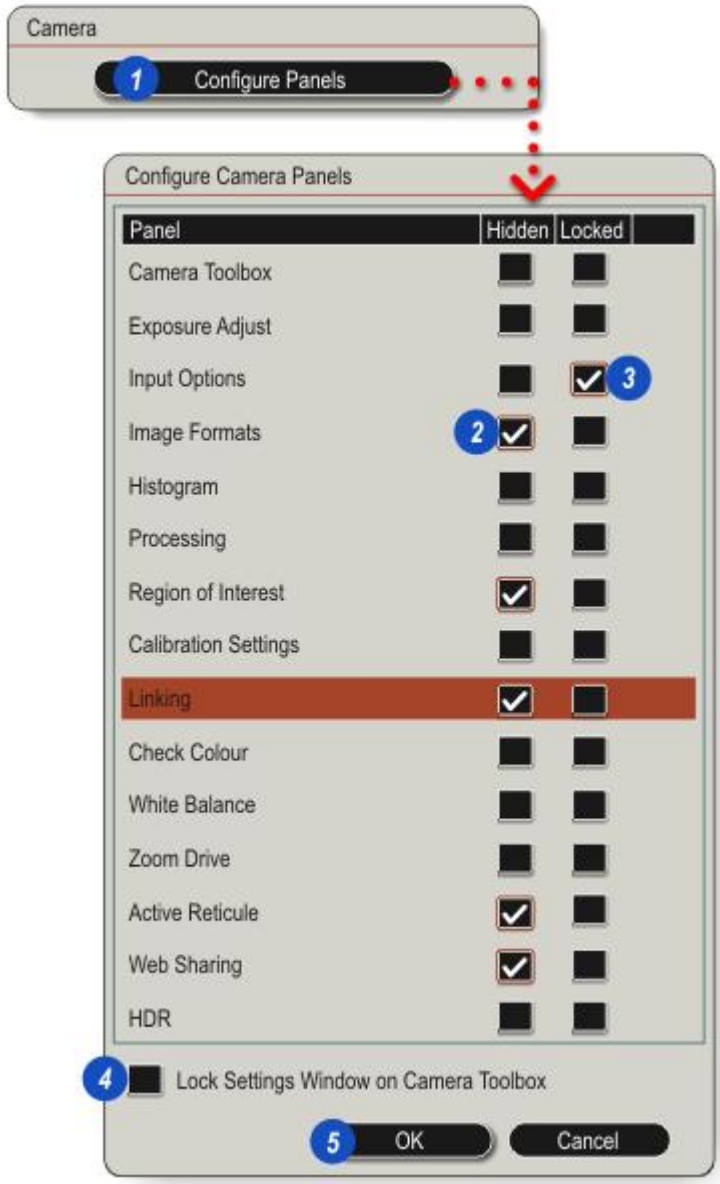
1: Click on the *Camera* > *Configure Panels* button.

2: Control panels on the *Camera* tab can be *Hidden* - the selected panel will not appear - and...

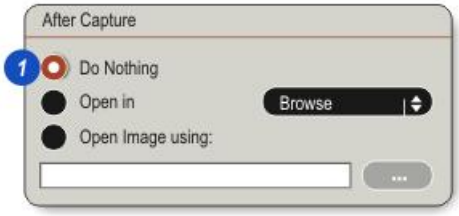
3: ...also *Locked* which locks the selected panel in position on the panel sequence. Click on a check box to the right of the selected panel to enable hiding and locking.

4: Enabling the *Lock Settings Window* will prevent the camera setting dialog from being displayed or altered.

5: Click *OK* to save the settings and close the dialog.



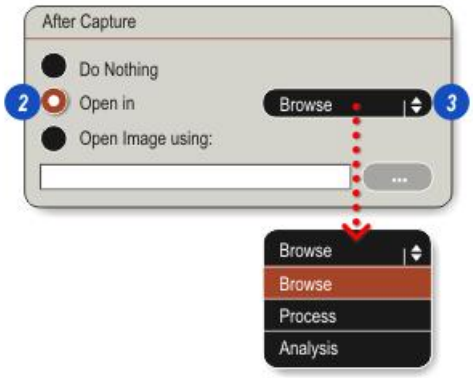
After an image is captured there are three possible automatic options that can aid speed and efficiency especially if the image required post-capture work:



1: *Do Nothing*. The image and data will be saved and the current *Workflow* will remain unaltered. Click on the radio button if a number of images need to be captured in quick succession without any intervening editing process.

2: *Open In* will automatically divert to the selected *Workflow*:

3: Click on the arrows to the right of the window and from the drop down click to select the required *Workflow*.

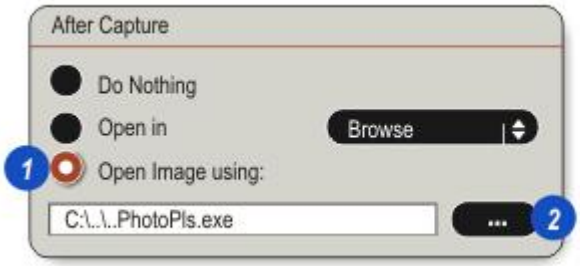


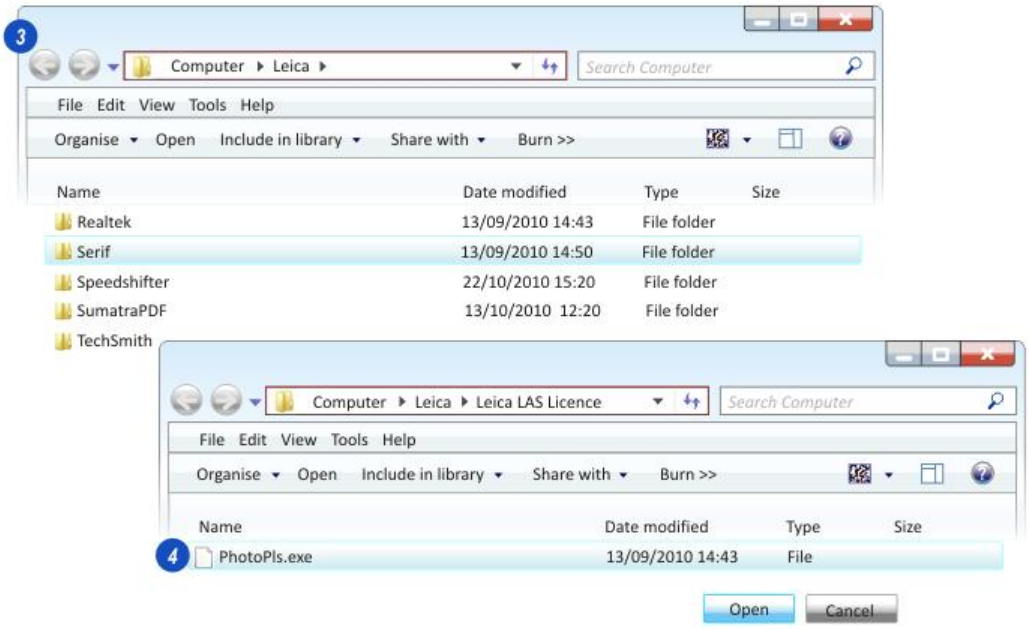
1: *Open Image Using* allows a (usually) third-party application to be launched to display and/or edit the image. Click on the radio button and then...

2: ... on the *Browse* button to reveal…

3: …the *Windows Navigator*.

4: Navigate to and select the application required and click *Open*. The selected application will appear in the *Open Image Using* text box.





1: *Measurement Display* sets the number of decimal places that will be displayed on some of the *Workflows* and *Optional Modules:*

2: Click on the *Up/Down* arrows to the right of the *Measurements Display* widow to increase/decrease the number of decimal places.

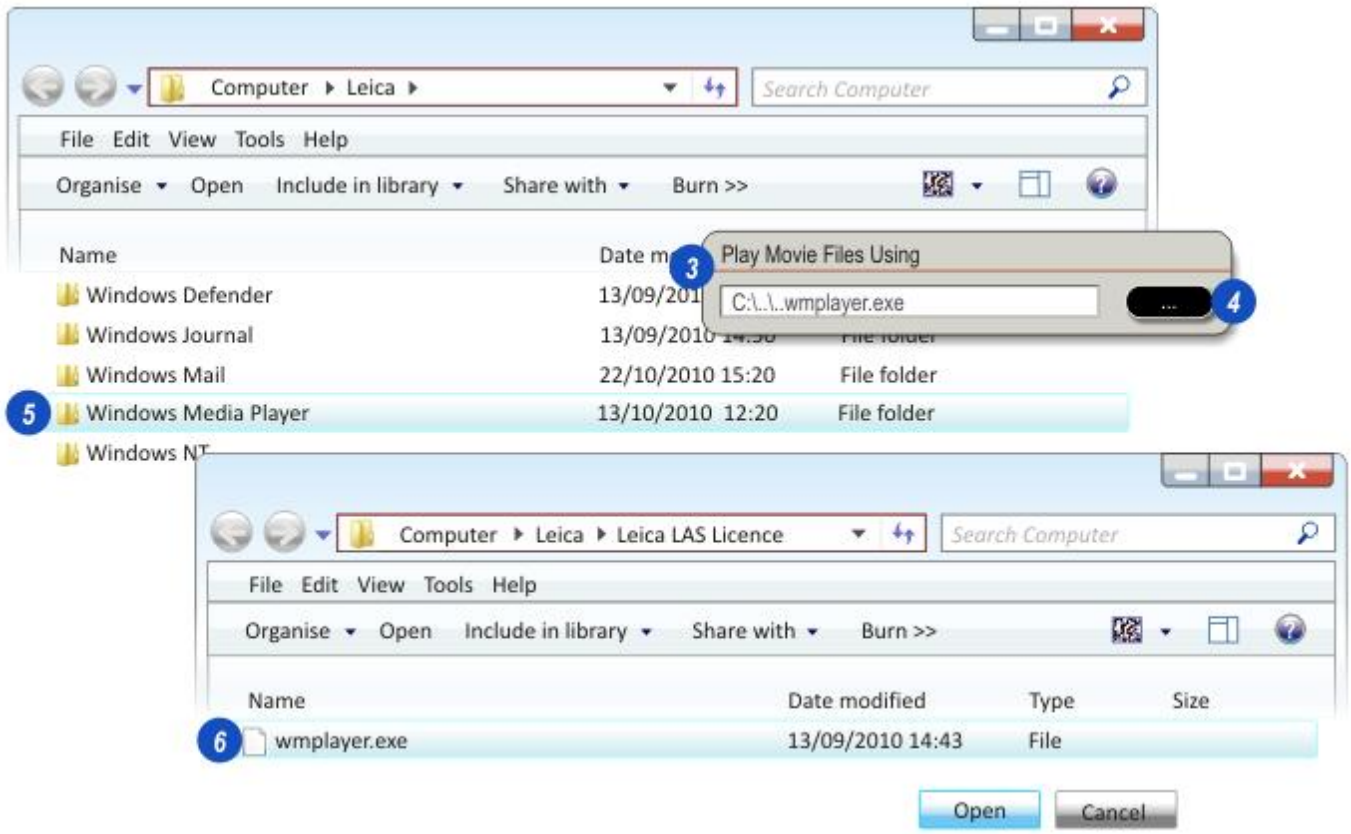
Play Movie Using...

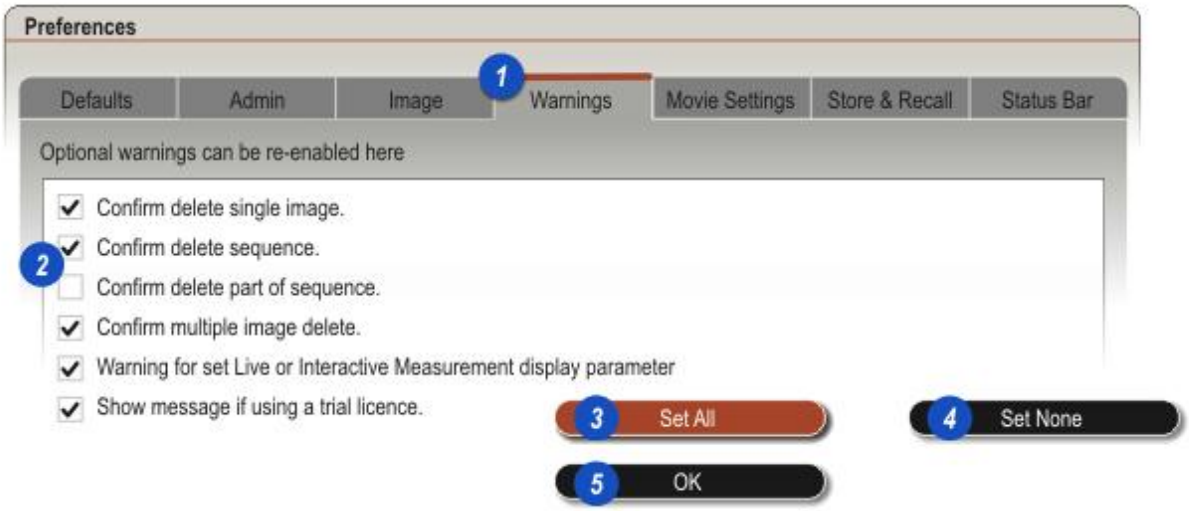
3: After a movie has been created, it may be played using a nominated application.

4: Click on the *Browse* button to the right of the Play Movie Files text box and...

5: ... from the *Windows Navigator* navigate to...

6: .. and select the application required. Click on the *Open* button and the application name will appear in the *Play Movie Files Using* text box.





The major confirmation and warning messages can be turned on or off on this tab.

1: Click on the *Warnings* tab to reveal the options panel.

2: Click to check (message on) or un-check (message off) the check box to the left of each warning.

3: The *Set All* button will check all of the messages and...

4: ...the *Set None* button will clear them all.

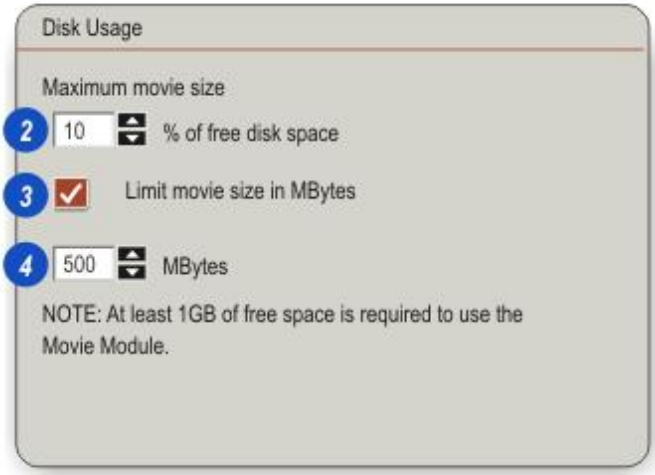
5: Click *OK*.

Because movies can be disk-space 'hungry', the *Movie Settings* tab provides two ways of limiting movie size:

- *Maximum Movie Size* limits file size in terms of free disk space whereas:
- *Limit Movie Size* prevents files exceeding a physical size measured in Megabytes (MBytes).

If *Limit Movie Size* is enabled, both features will run together to control movie size.

1: Click on the *Movie Settings* tab to reveal the *Disk Usage* panel.



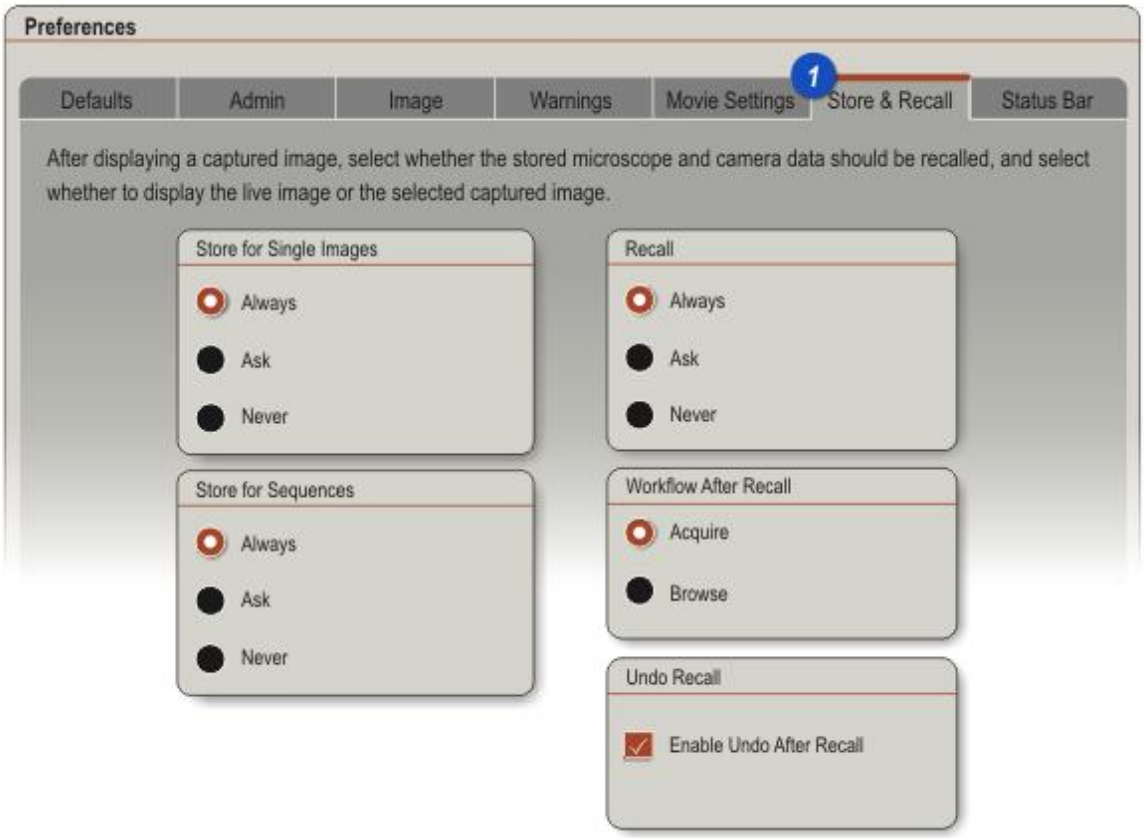
Limit the size of movies as disk space:

2: Click on the up/down arrows to the right of the *Maximum Movie Size* window to increase/decrease the percentage of disk space that can be allocated to movies.
Note that at least 1 Gigabyte (GByte) of free disk space is required simply to run the movie application.

Limit movie files to a specific size:

3: Click on the *Limit Movie Size* checkbox to enable size limiting. The checkbox will become red with a white tick.

4: Click on the up/down arrows to the right of the *Limit Movie Size* window to increase/decrease the maximum file size. Each click is a 1MByte step.



The *Store and Recall* optional module allows the microscope and camera setting to be stored with an image so that precisely the same conditions may be repeated at a later date. It can also provide consistency across a range of different specimens.

Fully automated microscopes can automatically adjust to the settings; The settings display can be used to adjust manual models.

Click on a panel on the illustration for more information.

Of the five functions available:

- *Store Single Images,*
- *Store Sequences,*
- *Recall and*
- *Workflow After Recall...*

...each have options selected by clicking a button. The buttons are mutually exclusive - only one may be active within a function.

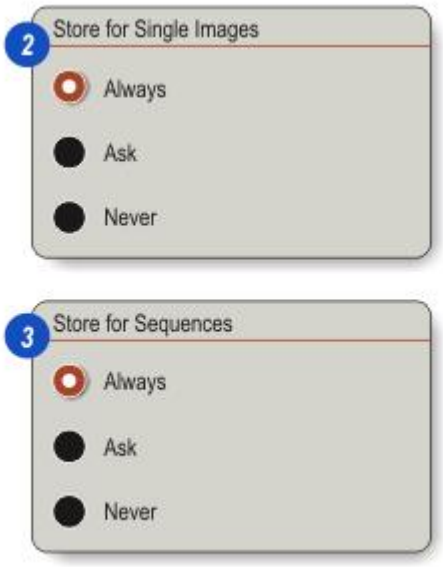
- *Undo Recall....*

...is switched on or off by clicking a checkbox. This is a toggle action.

1: To reveal the *Store and Recall* options, click on the tab.

2: There is a settings panel for storing *Single Images* and...

3: ...another for storing *Sequences*.



Both panels have the same options:

- *Always* will always store the settings.
- *Ask* will prompt you to store or not when the image is saved.
- *Never* switches off settings store. The store facility adds a very short delay to storing images and the files are longer.

Click a button to select the option.

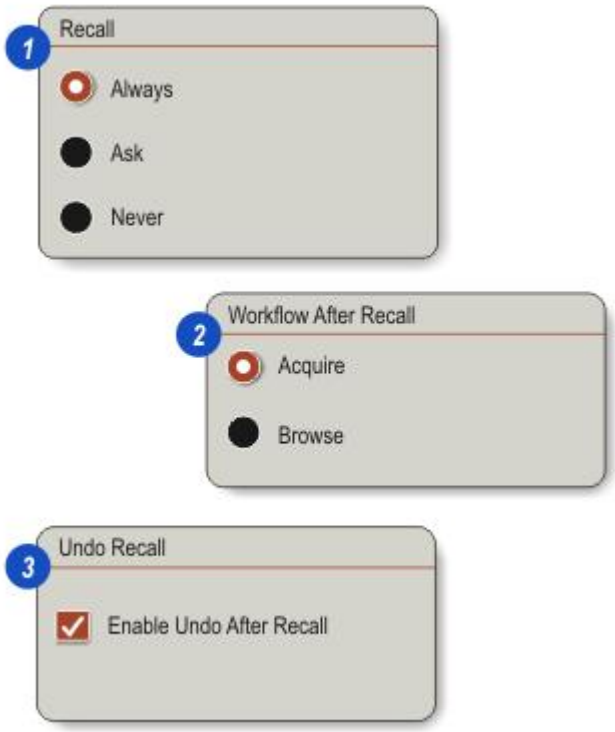
1: *Recall* determines if stored settings are also recalled when an image is retrieved from disk.

- *Always* will always recall any settings stored with the image. Automated microscopes will adopt the settings.
- *Ask* prompts if the settings should be retrieved or not.
- *Never* switches off settings recall.

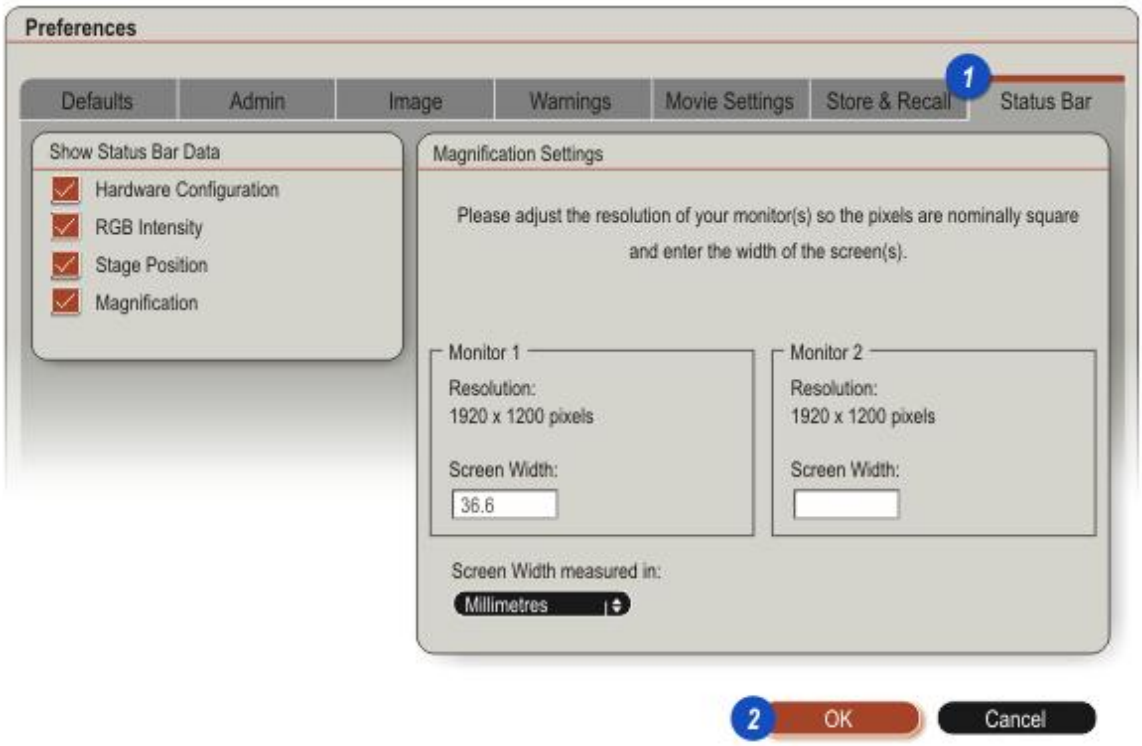
2: When a stored image is selected two options are available on the *Workflow After Recall* panel:

- *Acquire* will switch to the Acquire Workflow and display the live image from the microscope, whilst...
- *Browse* will remain in the Browse Workflow and display the selected image in the *Viewer*.

3: If *Undo Recall* > *Enable Undo After Recall* is checked, the current microscope and camera settings are saved before any recalled settings are applied to the microscope and camera. Reverting to the current settings is then possible.



Status Bar: Data Display



The *Status Bar* is located along the bottom edge of the *Viewer* and displays data relating to:

- The current *Hardware Configuration*.
- The intensity of the image *RGB* (Red, Green, Blue) components beneath the mouse.
- *Stage Position* is the *X/Y* co-ordinates when the image was captured.
- *Viewer Magnification*.

The *Status Bar* panel also allows the user to set up the resolution both for the PC monitor and a second monitor allowing the software to calculate the magnification.

To reveal the *Status Bar* panels:

1: Click on the *Status Bar* tab.

2: Save settings by clicking the *OK* button.

Click on a panel on the illustration for more information.

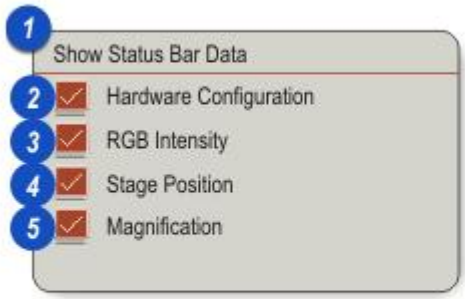
1: The *Show Status Bar Data* panel determines the items to be displayed along the *Status Bar.*

2 to 5: Enable a data item by clicking the associated check box. A tick mark indicates the item display is enabled. Click again to turn off the display.

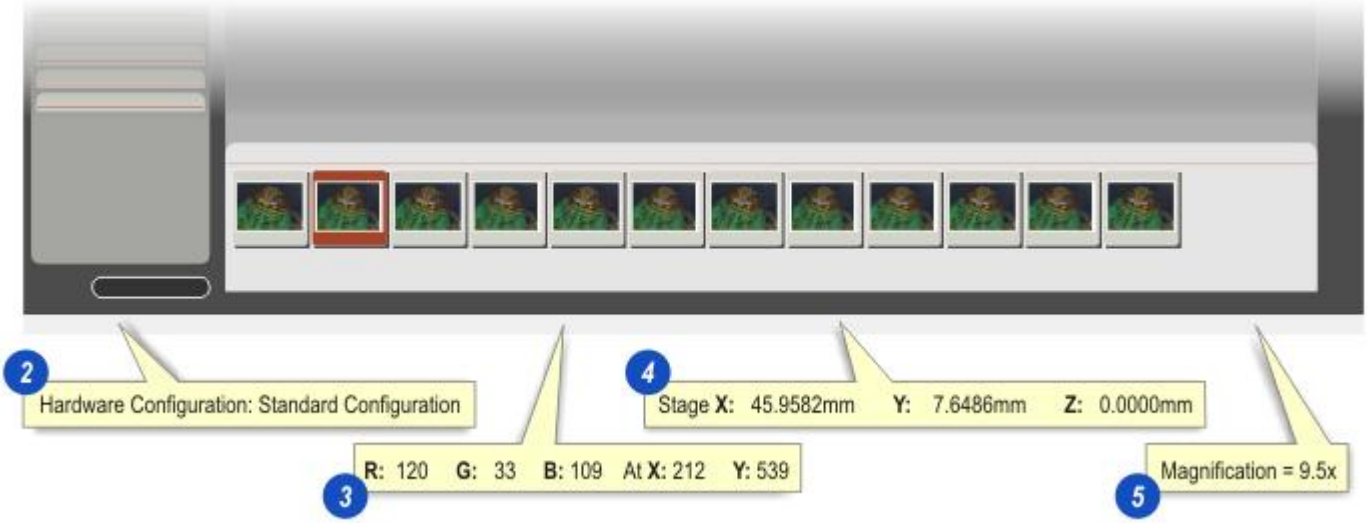
2: *Hardware Configuration* shows the currently selected and active configuration.

3: *RGB Intensity* displays the *Red(R), Green(G)* and *Blue (B)* values of the image pixel directly below the mouse. The mouse position is indicated by the X/Y co-ordinates. Greyscale images are represented as a single value - Intensity.

4: *Stage Position* is the *X, Y* and *Z* stage co-ordinates when the selected image was captured or current values for the live image. The values are stored with the image when it is captured.



5: *Magnification* represents the monitor display magnification - not the microscope magnification. As the user clicks the *Zoom* buttons on the side tool bar the magnification value changes. The value will only display if the monitor[75] has been set up.



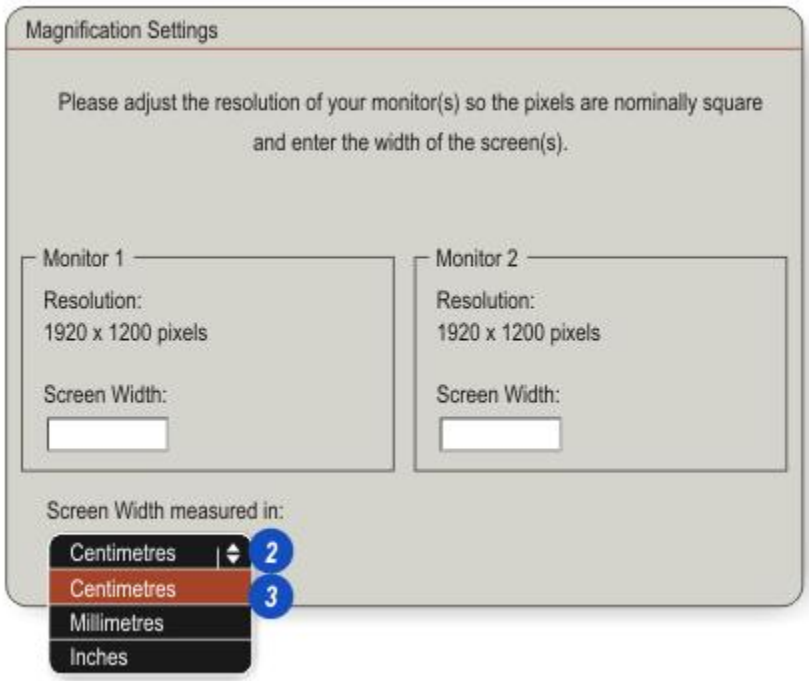
The microscope magnification can be read from the microscope or from *Leica Application Suite*, but the magnification on the computer monitor(s) is different because *LAS* scales the image to the user's demands and the monitor's capabilities.

LAS can indicate the image magnification by calculation from the live or acquired image pixel size and that of the monitor. The image calibration is established as described in the section *Camera > Calibration*.

1: Click on the *Status Bar* tab.

2: If necessary, change the measurement type – *millimetres, centimetres* or *inches* – by clicking on the arrows to the right of the *Screen Width* header and...

3: ...click to select the measurement type required



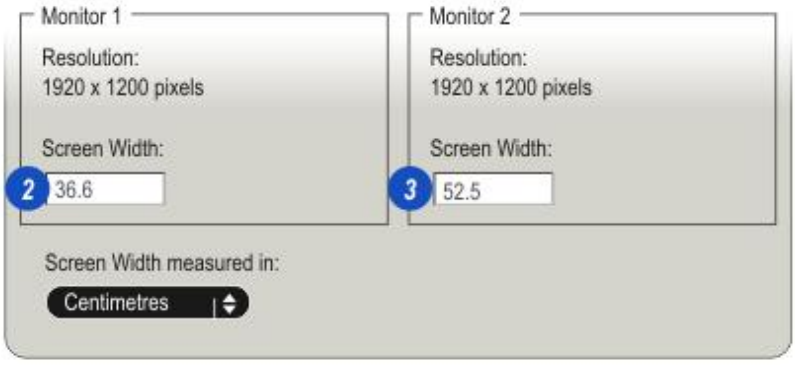
Continued 76

1: Measure the monitor(s) horizontally across the entire viewable area of the screen (not just the displayed image) with a ruler using the measurement type selected.

2: Click in the *Monitor 1* text box and type the monitor width.

3: Repeat the process if a second monitor is fitted, in the *Monitor 2* text box. Click *OK*.

The magnification factor is shown bottom right on the screen. It will change as the microscope objective zoom or the image zoom - either on the *Side Tool Bar* or mouse wheel - is changed. It will also update if the *Viewer* available display area is resized by opening, closing or re-sizing the *Grid* or *Gallery*.



- The magnification value is not displayed if a monitor size has not been entered or if the *Viewer* is in *Dual View* mode.

The *Scale Bar* is available on *Acquire, Browse, Process* and *Analysis* Workflows.

1: Clicking the *Show Annotations* button on the *Side Tool Bar...*

2: ...displays the *Annotations and Scale Bar Quick Launch* menu.

3: Click to select the *Scale Bar* option.

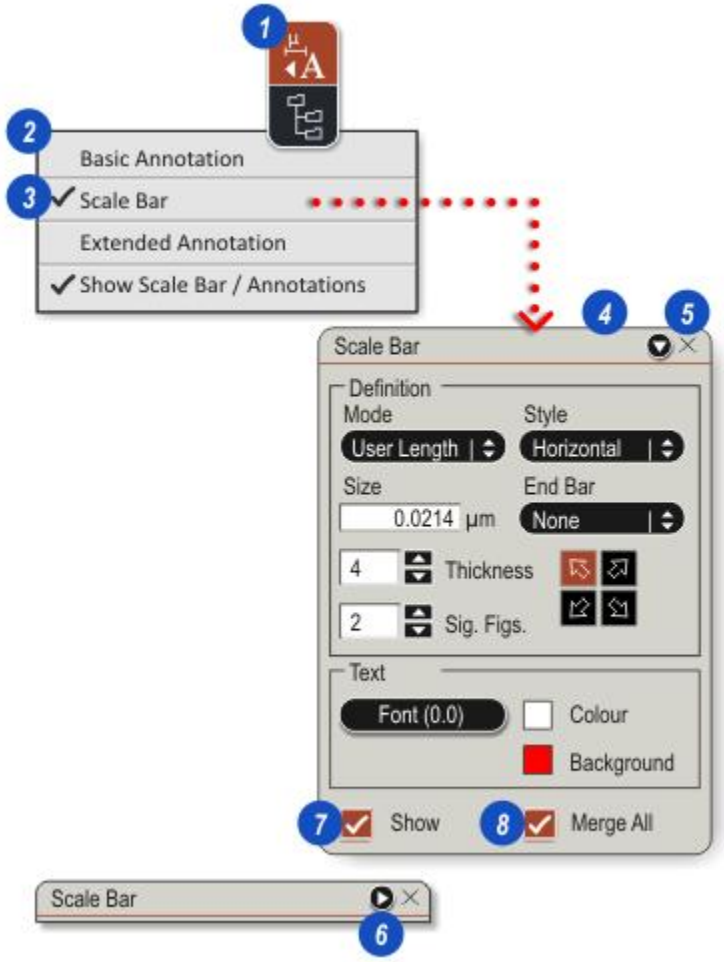
4: The *Scale Bar* dialog can be dragged by the header and 'parked' on any part of the *Viewer*.

5: Close the dialog and return it to the Workflow by clicking the 'X' on the dialog caption.

6: If the dialog is obscuring the image or controls, collapse it by clicking on the small arrow to the right of the dialog caption. Expand it by clicking the arrow again.

7: The *Scale Bar* is revealed or hidden by clicking the *Show* check box...

8: ... and can be *Merged* so that it is a permanent part of a captured image.



! The *Scale Bar* dialog will be active only when the Show check box is enabled.

Scale Bar Features:

- *Mode Selection* 78
- *Style Selection* 79
- *End Bars, Thickness, Digits* 80
- *Placement and Font Change* 81
- *Scale Bar and Font Colour* 82
- *Background Colour* 83
- *Merging* 84

Three *Scale Bar* Modes are available to determine how it behaves on the image.

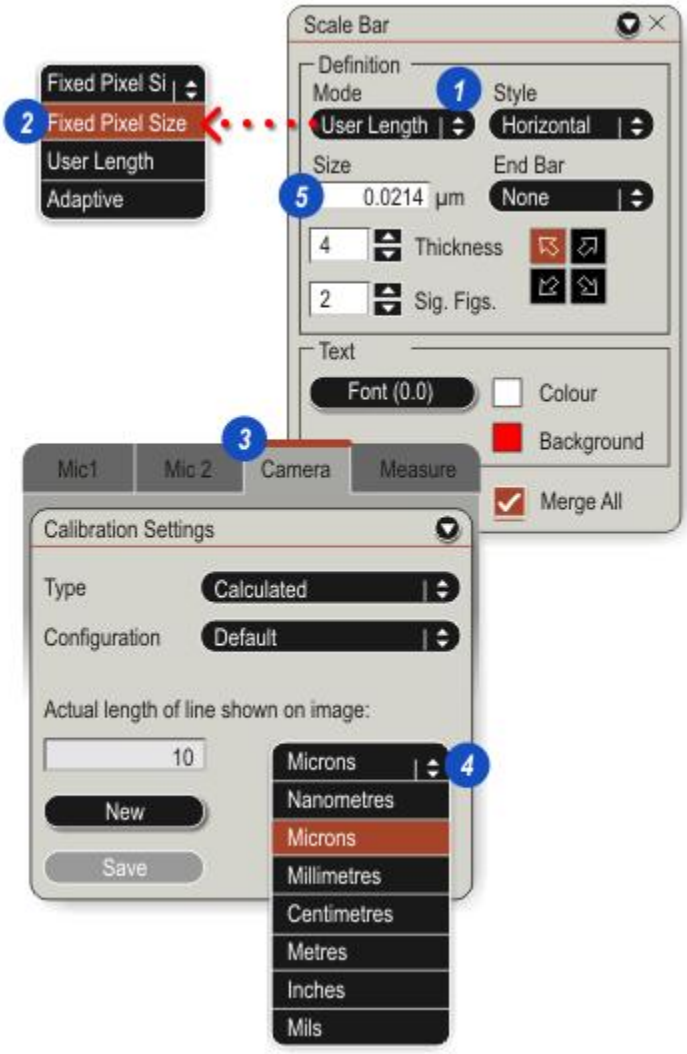
Fixed Pixel Size: Acts as a known distance 'ruler'. As the image is zoomed the *Scale Bar* length remains constant whilst the distance value changes to reflect the zoom level.

User Length: In contrast to the *Fixed Pixel*, the numeric value of the *User Length* option remains unchanged as the zoom is changed, but the line length on the display is adjusted. Values typed in to the *Size* text box will change to '*Undersize*' if too small or revert to the overall image width/height if too large.

Adaptive: Displays a 'true' calibrated *Scale Bar* at about 20% of the image width or height depending upon the bar orientation and as the zoom is changed, the numeric distance and the pixel size of the scale bar both change to maintain a 'reasonable' size on the image.

1: Click on the small arrow to the right of the *Mode* header and…

2: …click to select the required option.



Change the measurement units for *Fixed Pixel Size* and *User Length* modes*:*

3: On the *Camera* tab...

4: ... in *Calibration Settings*.

The *Adaptive* mode 'inherits' the last measurement units used.

5: To enter a distance in either *Fixed Pixel Size* or *User Length* modes, click in the *Size* text box and type a value.

The orientation and shape of the *Scale Bar* is set on the *Style* menu.

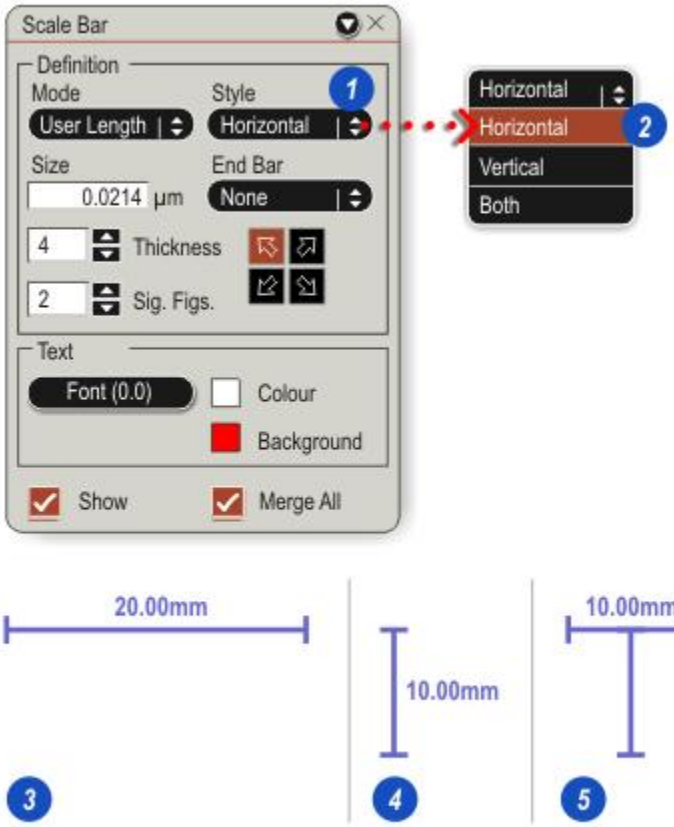
1: Click on the small arrows to the right of the *Style* header and…

2: …click to choose the required style from the drop down list.

3: Conventional *Horizontal* style.

4: *Vertical* style.

5: Combined *Horizontal* and *Vertical* styles (*Both*). Although only one dimension is shown both 'legs' represent the same distance.



There are four options for the type of *Scale Bar* ends:

1: Click on the small arrows to the right of the *End Bar* header and…

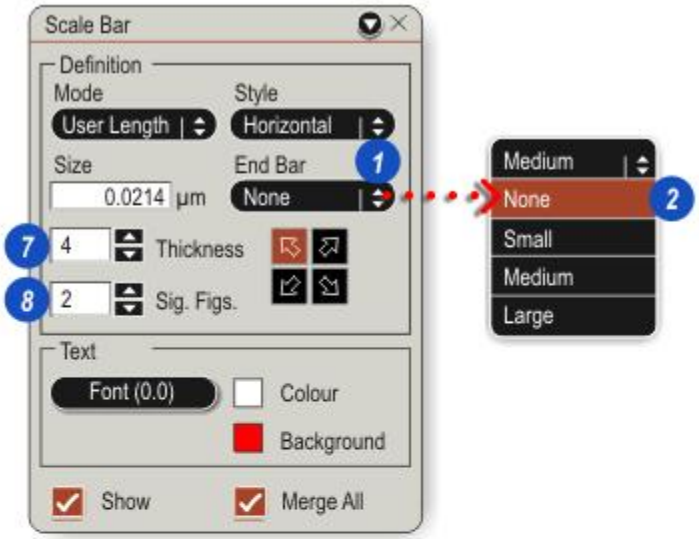
2: …from the drop down list click to select the type required:

3: *None*,

4: *Small*,

5: *Medium* or

6: *Large*.



Scale Bar Thickness:

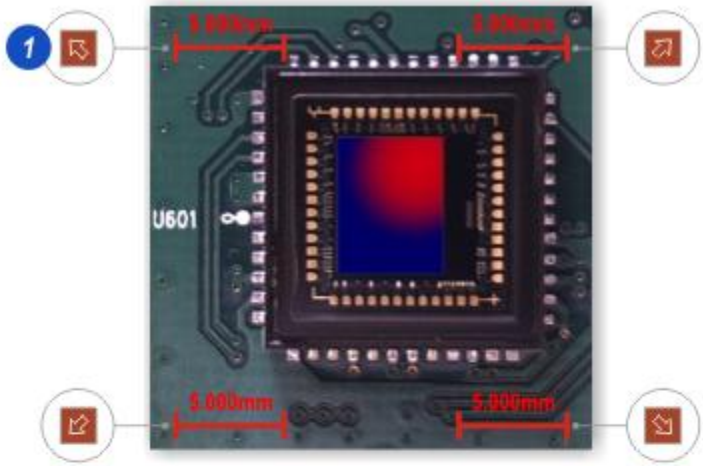
7: Click on the *Up/Down* arrows to the right of the *Thickness* text box to increase/ decrease the Bar thickness. The End Bars are not affected.

Significant Digits:

8: Increase or decrease the number of digits displayed by clicking the *Up/Down* arrows to the right of the *Sig Fig* text box. The value determines the total number of digits displayed not just those after the decimal point. So, in Figure **(6)** the *Sig Fig* value would be 5. Whole numbers before the decimal point are not affected.

The *Scale Bar* can be placed quickly and precisely at the four corners of the image by using the *Placement* buttons.

1: The *Placement* buttons will position the *Scale Bar* at top-left, top-right, bottom-left or bottom-right of the image, live or captured, accurately and precisely simply by clicking the appropriate button. The *Scale Bar* can still be clicked and dragged if needed.

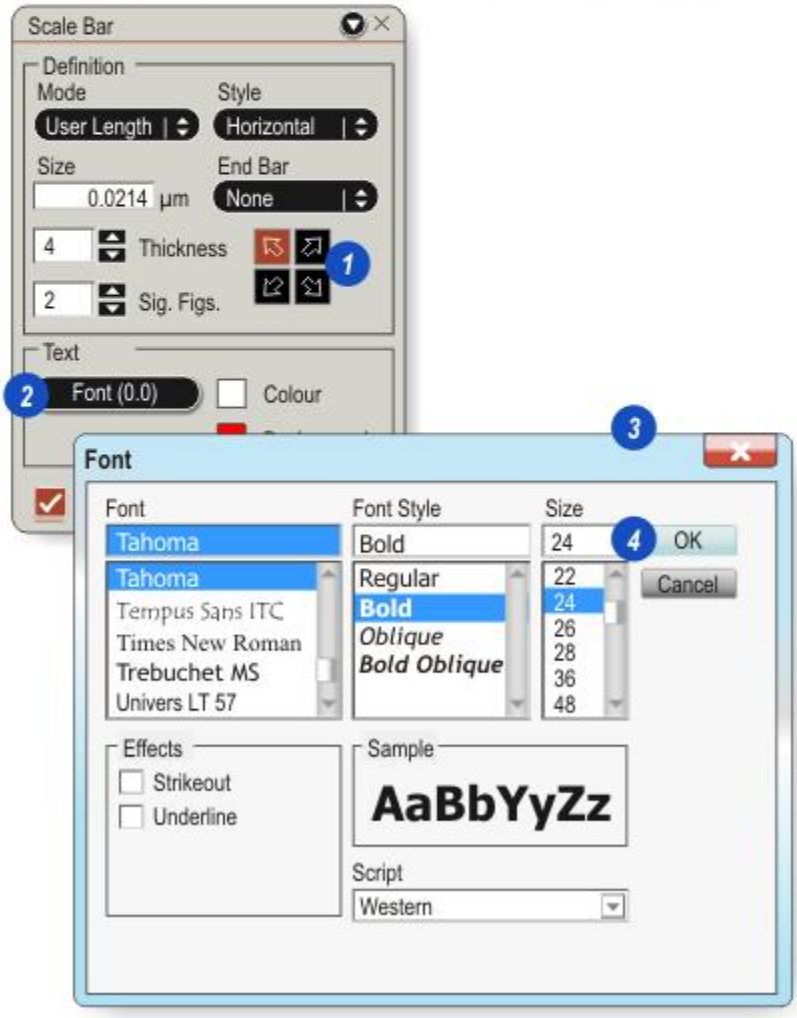


Changing the Font:

2: Click on the *Font* button.

3: On the *Font* dialog select the *Font*, *Font Style* and *Size* as required and…

4: …click *OK*.



Change the *Scale Bar* and *Font* colour by:

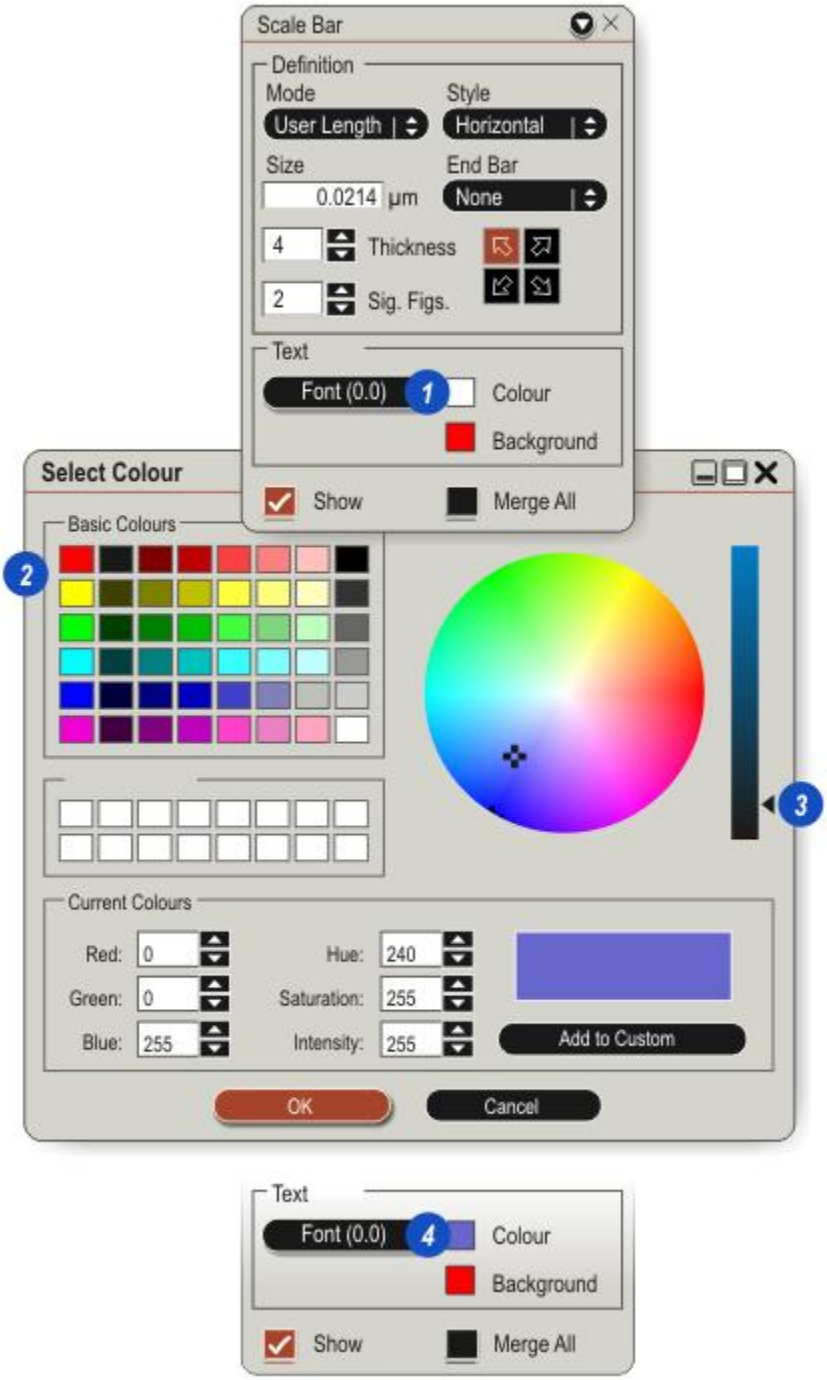
1: Click on the *Colour* button and…

2: …on the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes and type the *Red*, *Green* and *Blue* values.

3: Adjust the colour intensity by clicking and dragging the slider on the *Intensity* bar.

4: Click *OK*.

5: The new colour is shown on the *Colour* button.



The *Scale Bar* is displayed against a small panel the colour and transparency of which may be changed to suit the image:

1: On the *Scale Bar* dialog click on the *Background* button. The *Select Colour* dialog appears with additional controls to change the transparency of the background.

2: Choose a new background colour by clicking a *Basic Colour Swatch* or…

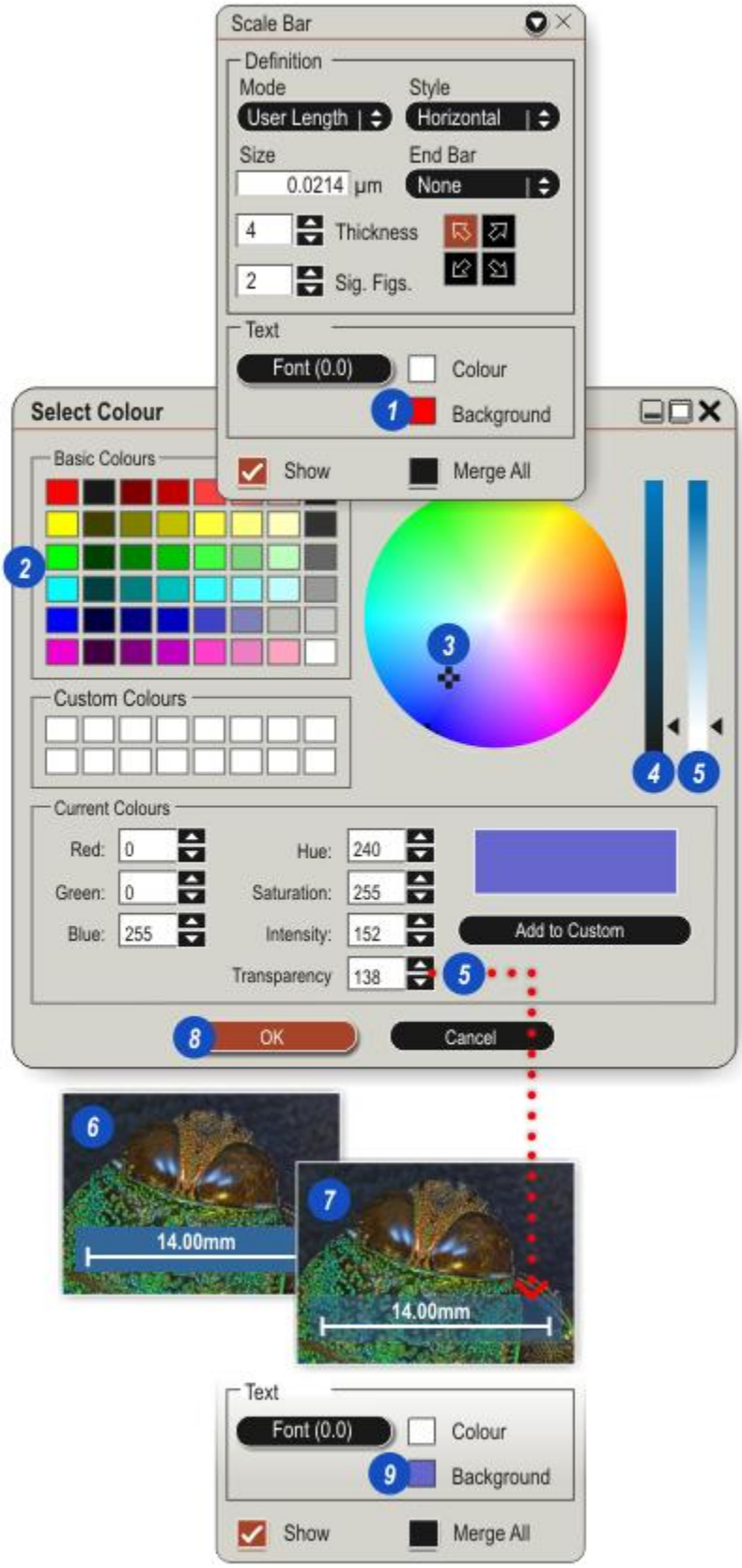
3: …clicking and dragging the 'target' on the *Colour Wheel*.

4: Adjust the colour intensity by clicking and dragging the slider on the *Colour Bar* or typing new values for *Red, Green* and *Blue* or *Hue, Saturation* and *Intensity* in the appropriate text boxes.

Background Transparency:

5: The *Scale Bar* background transparency can be altered by clicking and dragging the slider or typing a value in the *Transparency* text box. A value of 255 results in a solid colour **(6)** and a value of 0 makes the background panel disappear. The illustration **(7)** shows the result of a value of 138.

8: Click *OK*. The selected colour appears in the *Background* check box **(9).**



Merging is the process of combining the *Scale Bar* and its caption with the saved image.

Once merged, the image will always appear with the *Scale Bar* that cannot be altered.

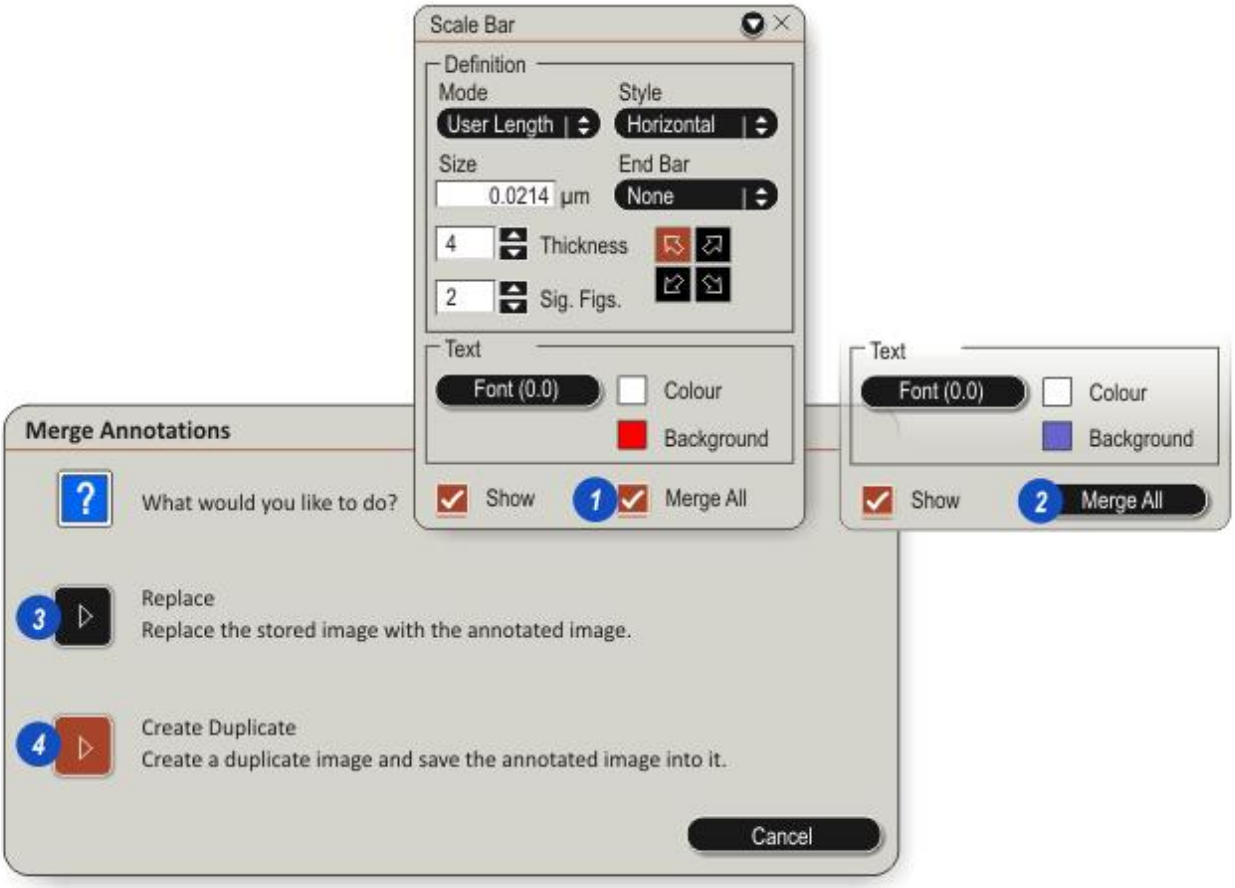
1: For live images *Merge* is a check box – click to enable merging when the image is captured.

2: For previously captured images *Merge* is a conventional LAS button – click to merge the *Scale Bar* with the image. The *Merge Annotations* dialog appears.

3: Two options are available: *Replace* the captured image including the *Scale Bar* with it, or …

4: *…Duplicate* which makes a copy of the existing captured image and merges the *Scale Bar with the copy.*

Click the required option button.



Update Calibration

If the system calibration has changed or images are being used that do not reflect the current calibration values, *Update Calibration* provides a simple and quick way to bring images up-to-date.

Four options for the calibration source are available:

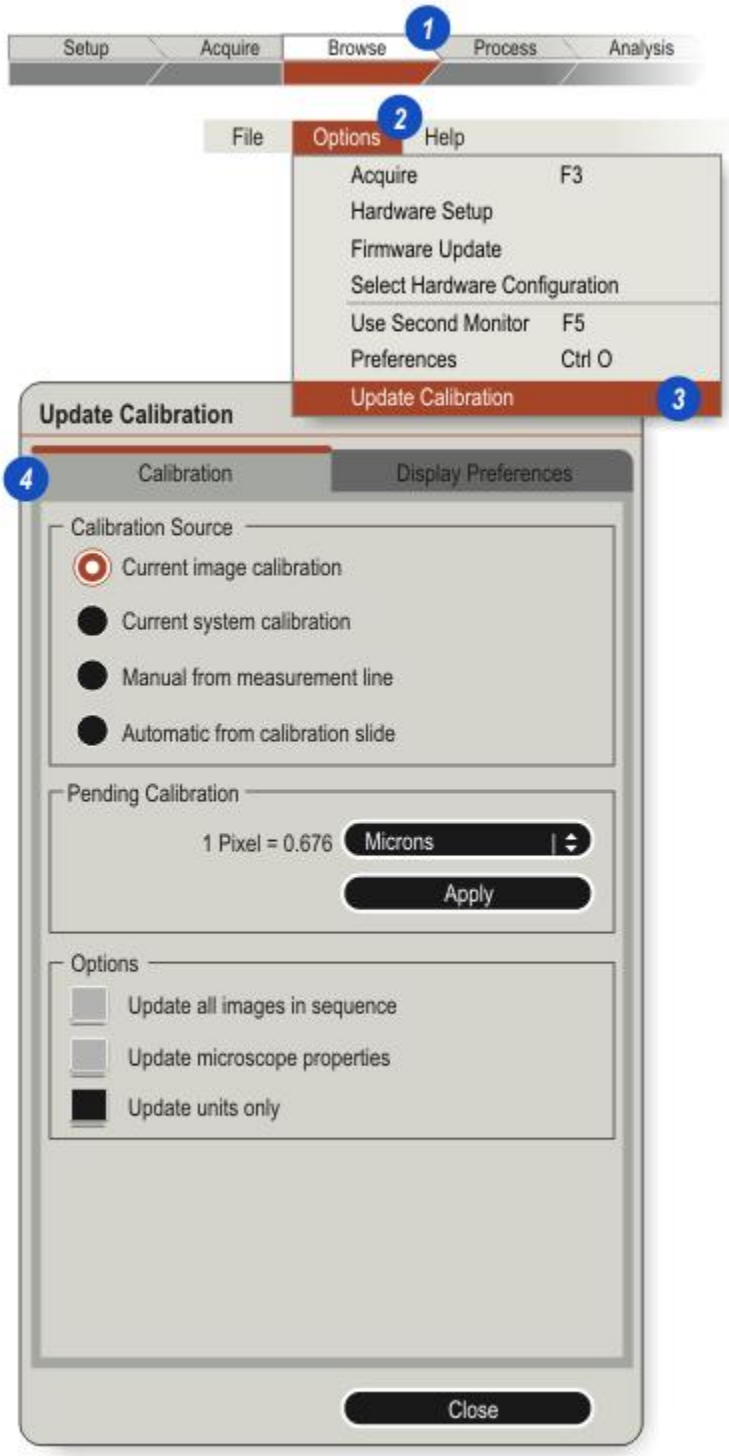
- *Use the current image* [87] Uses the calibration values of the selected image..
- Use system calibration [88] Uses the prevailing calibration settings.
- *Manual from the measurement line* [89] Allows new calibration values to set up directly from a known distance on the displayed image.
- *Automatic from Calibration Slide* [90] The calibration is calculated automatically from an image of a calibration slide captured at the same time as those to be updated.

Update Calibration is used on captured images including those that have a *Scale Bar* 'burnt in' (merged) but in those cases the merged displayed value will not change.

1: Click to select the *Browse Workflow.*

2: Click on *Options* on the main tool bar and from the drop-down menu,...

3: ... click to select *Update Calibration.* The *Update Calibration* dialog **(4)** appears.



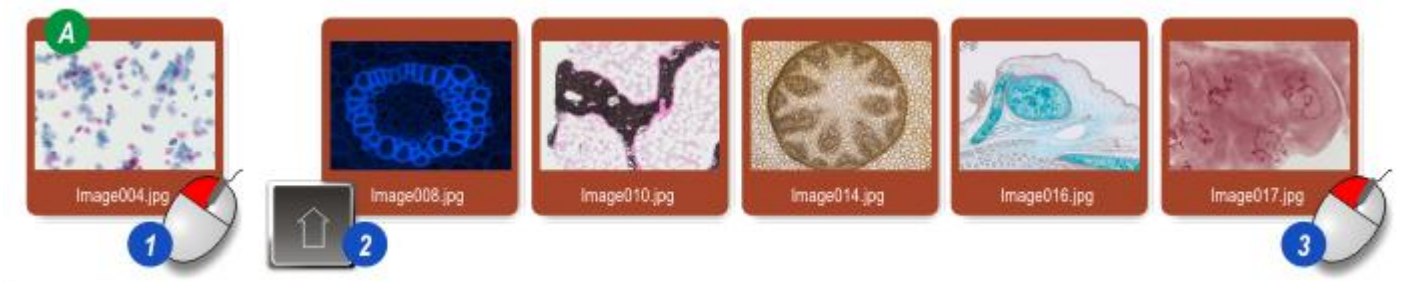
There are several ways to select images that will updated:

A: Range of images:

1: Click on the first image to be selected.

2: Press and hold down the *Shift* key.

3: Click on the image from which the calibration will be copied. All of the images between the two will also be selected.

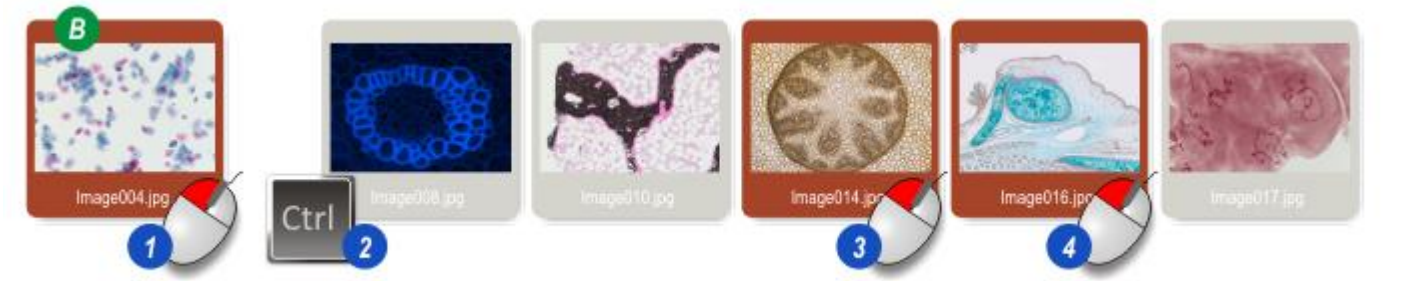


B: Individual Images:

1: Click on the first image to be selected.

2: Press and hold down the *Ctrl* key.

3: Click individually on all the other images to be included in the selection. The last image will be used as the calibration source.

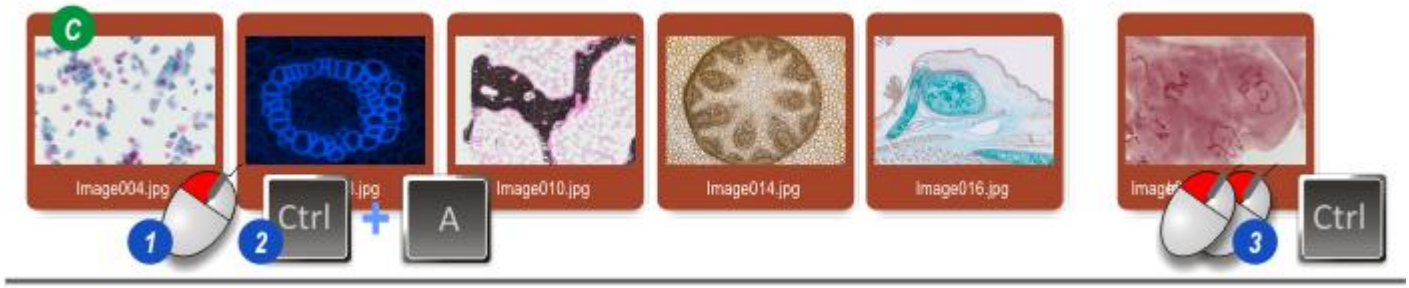


C: All of the images in the Gallery:

1: Click on the first image.

2: Press and hold down the *Ctrl* key and then press and release the *'A'* key (All).

3: To select the calibration source, still holding down the *Ctrl* key double-click the source image.



To use an image as the calibration source for all of the other selected images, choose the images to be updated making sure that the calibration source is the last to be selected.
More information 86

1: Click the *Current image calibration* button.

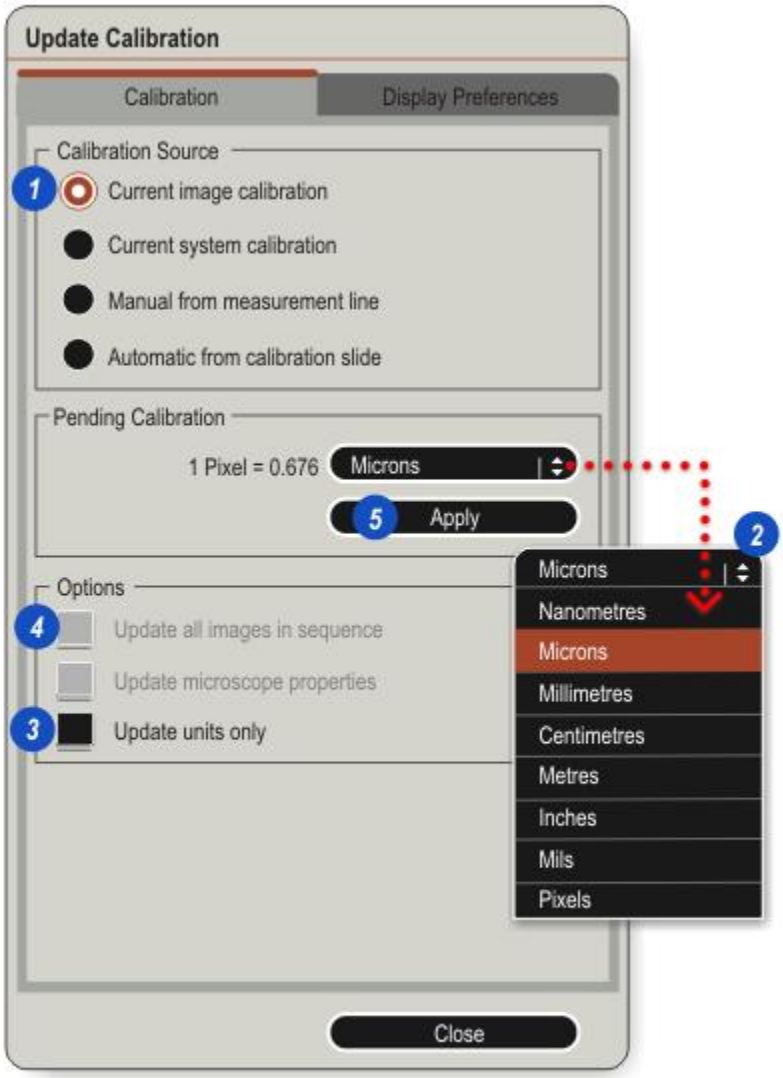
2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the calibration of the selected source image. The reference to Pixel represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: To update the *measurement units* for the selected image but **not** to change their calibration values, click to enable (tick mark visible) the *Update units only* check box.

4: If the selected images are part of a sequence the *Update all images in sequence* check box becomes available. Click to enable it and update all of the sequence images.

5: Click the *Apply* button.
Click the *Close* button to exit *Update Calibration*.



Apply the current System Calibration settings to an image or range of images by selecting the images to be updated. More information 86

1: Click to select *Current system calibration.*

2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

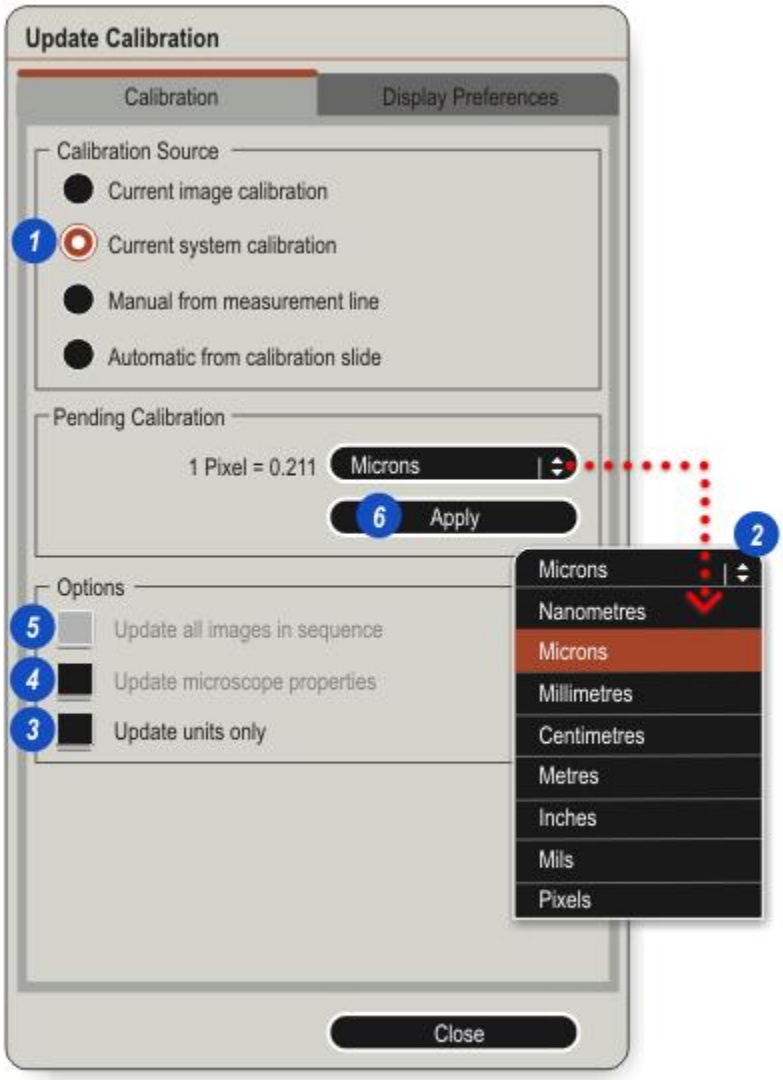
! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the system calibration. The reference to *Pixel* represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: To update the *measurement units* for the selected image but **not** to change their calibration values, click to enable (tick mark visible) the *Update units only* check box.

4: Update the image microscope properties to the current system settings by clicking to enable the check box.

5: If the selected images are part of a sequence the *Update all images in sequence* check box becomes available. Click to enable it and update all of the sequence images.

6: Click the *Apply* button.
Click the *Close* button to exit *Update Calibration*



A new calibration value can be set by using a line extended across a feature with a known dimension on the image. Best accuracy is achieved by using a Calibration Slide.

Select the images to be updated. More information 86

1: Place a specimen with a known and precise dimension on the stage. A clean *Calibration Slide* is the best option. Focus the specimen or slide.

2: If necessary, change the *measurement units* by clicking the small arrows to the right of the units header and from the drop-down list, clicking to select the units.

! In the *Pending Calibration* panel the 1 *Pixel* = nnn value refers to the system calibration. The reference to *Pixel* represents the unit used to store the image not the *Camera* or the *Monitor* pixels.

3: Click to select the *Manual from measurement line* option.

4: The measurement line appears. Click on the centre of the line and drag it so that the left end stroke aligns with the edge of the specimen or slide.

- Hold down the *Shift* key to display the magnifier, moving it with the mouse to assist alignment.

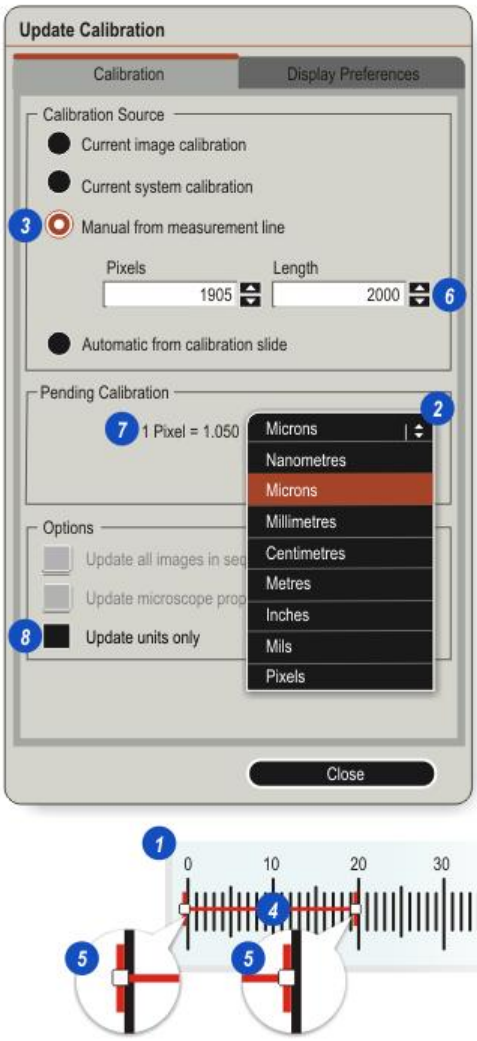
5: Aim to place the end stroke on the outside of the left edge. Click and drag the right 'handle' so that the right stroke aligns with the inside of the right edge.

6: Click inside the *Length* text box and type the known length of the line in the chosen measurement units.

7: The calibration value is automatically calculated.

8: Enable the Update units only check box to apply changes in the measurement units only.

Click *Apply* to update the selected images.



Automatic from Calibration Slide

Automatic calibration offers a fast method of applying precise calibration to selected images. It uses an image of a calibration slide that was captured using the same microscope settings as the images to be updated.

The software can accurately and automatically detect the slide image and calculate the calibration from a known interval between the divisions, providing the image is sharp and the division lines clearly defined.

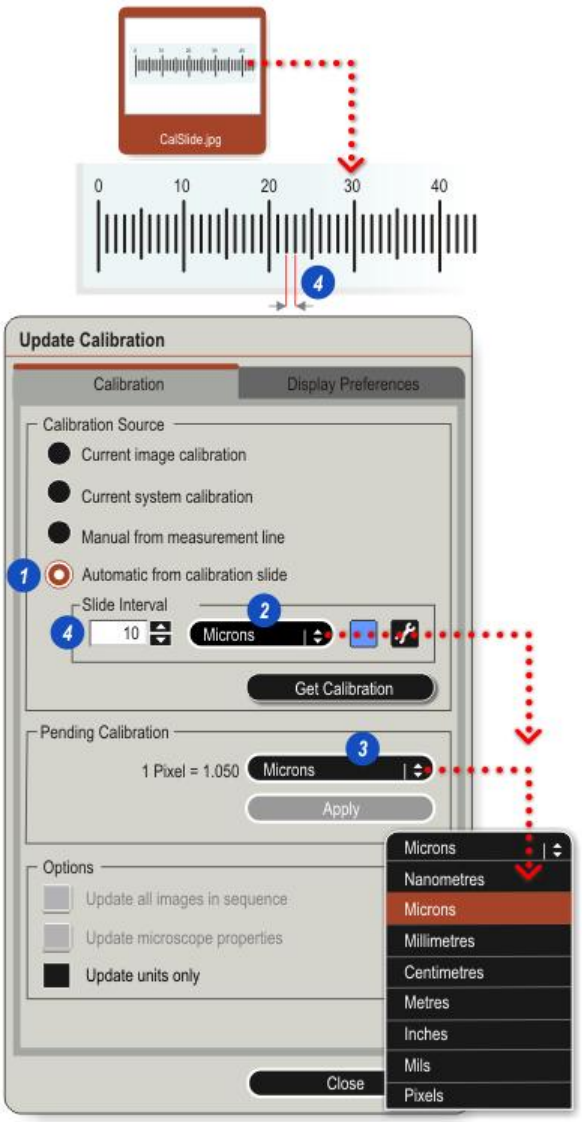
Select the images to be updated making sure that the calibration slide image is the last selected and is displayed in the *Viewer.*
More information 86

1: Click to select *Automatic from calibration slide.*

2 & 3: If necessary, change the measurement units for both the calibration slide and the calibration value by clicking the arrows to the right of the headers and selecting from the drop-down list.

- The measurement units do not have to match.
- The calibration slide image does **not** have to be perfectly horizontal or vertical, but the closer it is then the faster the detection.

4: Click inside the *Slide Interval* text box and type the interval value of the calibration slide - that is the distance between two adjacent divisions in the selected measurement units.



The software will now try to find a calibration slide and verify that it is precise and suitable as a calibration source:

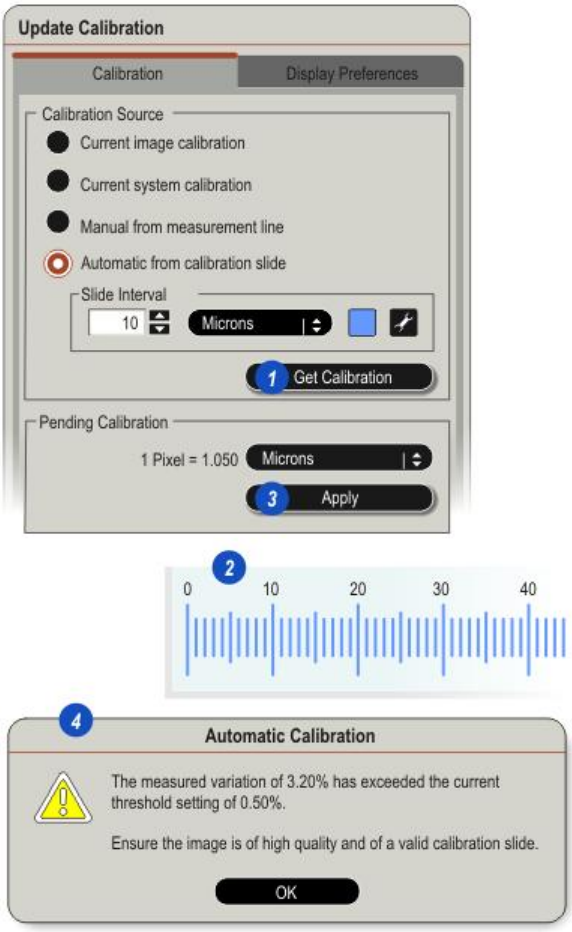
1: Click on the *Get Calibration* button.

2: If a calibration scale is found and verified, the colour of the division strokes on the scale will change, a new calibration value is automatically calculated and...

3: ...the *Apply* button becomes active. Click it to apply the new calibration value to the selected images.

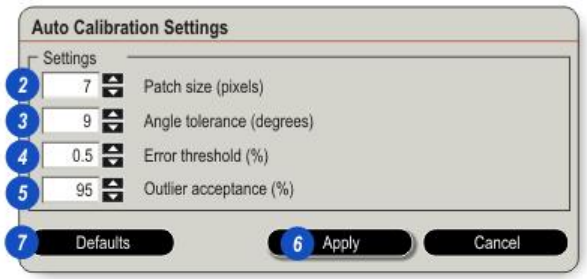
4: If a scale is not detected or does not conform to the verification parameters, an error message appears. The message changes to reflect the error.

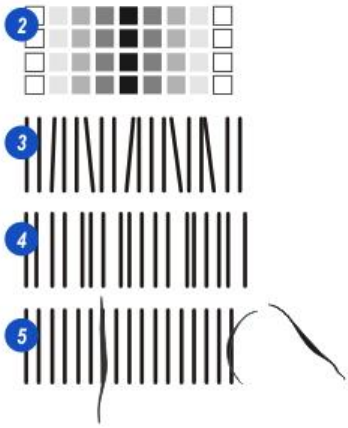
- Calibration slide[92] scale verification parameters can be changed
- The calibration scale detected colour[93] can be changed



The verification parameters check that a calibration slide image is sufficiently accurate to be used as a calibration source. For example, random fibres on an calibration slide image could be interpreted as part of the scale and have to be 'filtered' out.

Users can change the settings but should be aware that significant changes can result in compromised calibration accuracy. If in doubt revert to the factory optimised defaults.





1: Click on the 'spanner' icon to reveal the *Auto Calibration Settings* dialog.

2: The *Patch size* refers to the spread of pixels leading to a discernible edge. In the illustration there are several pixels ranging from white to dark grey before the black central 'edge' appears. Increasing the patch size could 'create' spurious edges. Keep the patch size as small as possible.

3: *Angle tolerance* determines the how much the angle between two adjacent scale strokes can vary from the parallel. The software is looking for a series of parallel strokes at a consistent 'pitch' or interval.

4: The interval of the scale strokes must be close to the value entered by the user. The *Error threshold* determines how much it can be allowed to vary.

5: Outliers are scratches and debris that may be present on the image and could be interpreted as part of the scale. The *Outlier acceptance* sets the % level at which the interval *mean* (a central 'average' for all of the detected intervals) can vary. Strokes falling below the *Outlier acceptance* are removed.

6: If changes are made to the settings click the *Apply* button to save them.

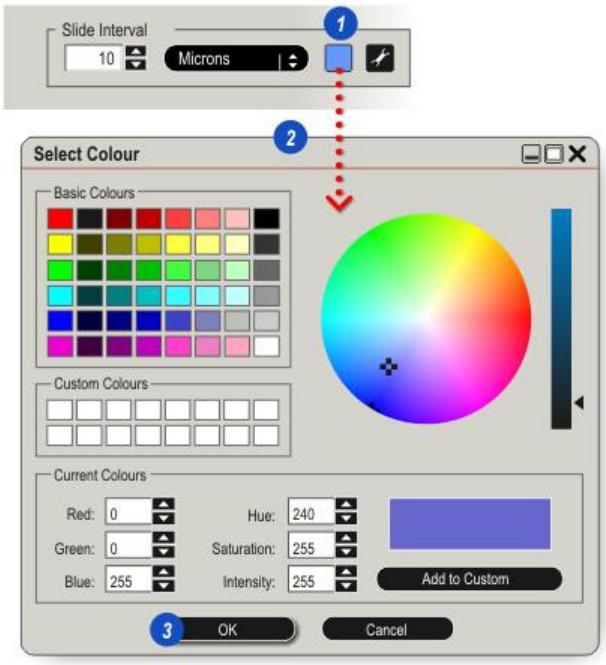
7: To restore the factory settings click the *Defaults* button.

Change the colour of a detected calibration slide image by:

1: Click on the *Colour* button on the *Slide Interval* panel.

2: From the *Windows Colour* dialog choose a colour by clicking a swatch, dragging the crosshairs on the wheel or typing Red, Green and Blue (RGB) values.

3: Click *OK*.



The number of decimal places displayed in *Interactive Measurements* and *Image Analysis*, can be set by:

1: Click on the *Display Preferences* tab.

2: Using the Up/Down arrows, increase or decrease the number of digits to be displayed after the decimal place.

3: Click *Apply*.

Return to the calibration options by clicking the *Calibration* tab.

The *Dual Viewer* option is available in the *Acquire, Browse* and *Process Workflows*. The *Viewer* area can be split to show two images simultaneously:

- In *Acquire* the current live image usually appears in the left pane, with a previously captured image selected from the *Gallery* in the right pane.
- In *Browse* and *Process* two previously captured images selected from the *Gallery* appear left and right.

1: On the *Side Tool Bar* click the *Viewer Options* button.

2: Click to enable the *Dual Viewer* option. The *Viewer* will then divide into two panes.

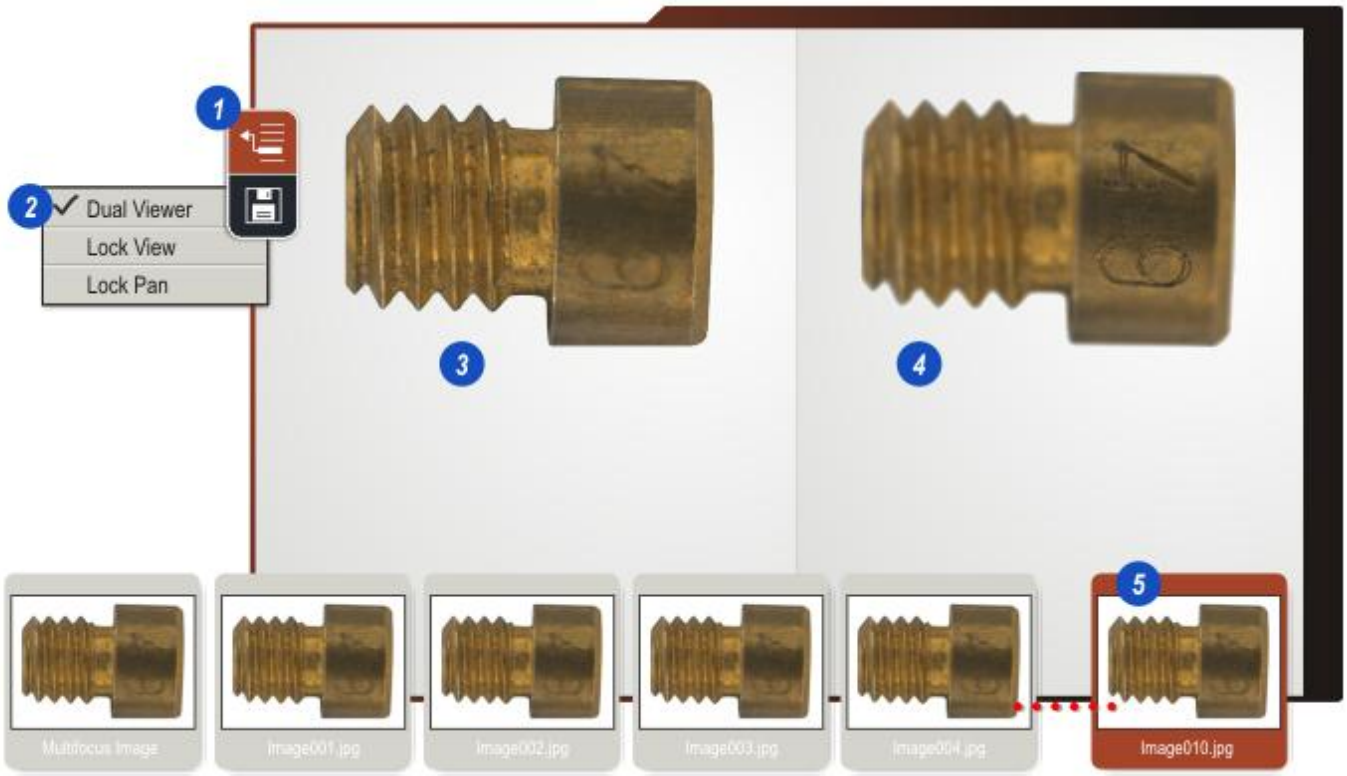
3: In *Acquire* the image currently being viewed will usually appear in the left-hand pane.

4: To display a captured image in either pane, click anywhere in the pane and then...

5: ...click a thumbnail in the *Gallery*.

Dual Viewer features and quick links:

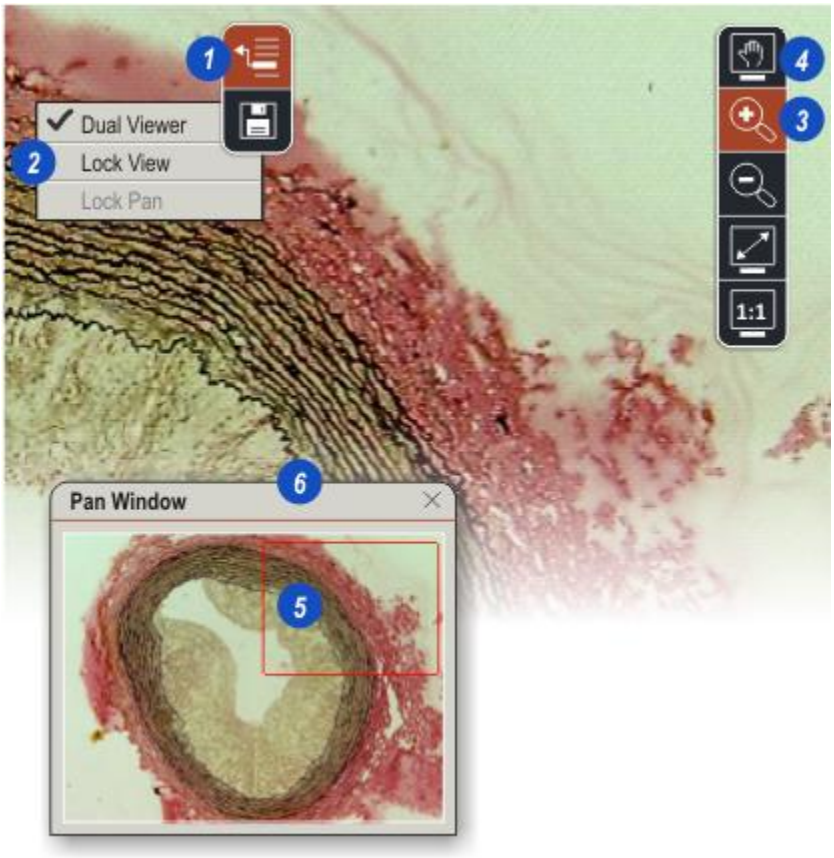
- Unlock and Lock views 97
- Pan Window 96
- Comparing Features 99



Pan Window

The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

Dual Viewer will work in either locked or unlocked modes when *Pan* is being used.



Pan with Lock View disabled: Only one of the images is panned

1: Click the *View Options* button on the *Side Tool Bar*.

2: From the context menu click to disable *Lock View* – no tick mark visible. The images are now independent.

3: Click on the image to pan and, using the *Zoom In* button enlarge it.

4: Click on the *Pan Window* button and the window appears with...

5: ... a red-outlined *Pan Area*. Click inside the *Pan Area* and drag it to the required position. The selected image tracks the movement

.

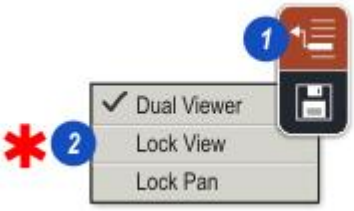
6: The *Pan Window* can be moved to any convenient position in the *Viewer* by clicking and dragging its header bar.
Either image can be displayed in the *Pan Window* simply by clicking on it.

Lock View Disabled:

With the *Lock View* option disabled, both images can be scaled independently:

1: Click on the *View Options* button on the *Side Tool Bar* and…

2: …from the context menu click to disable *Lock View*.



3/4: Click to select the image in either pane.

5: Use the *Side Tool Bar* buttons to *Zoom In* or *Zoom Out,*

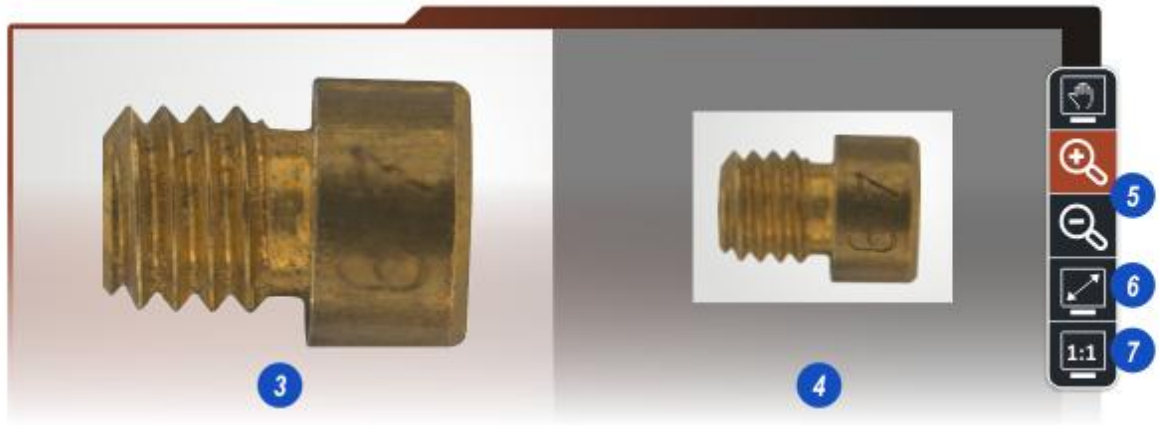
6: …*Fit to Screen* or…

7: …display at *Original Size.*

Lock View enabled:

Follow steps **(1)** and **(2)** above but click to *enable Lock View*.

Both images automatically scale to the smaller view.No need to click on an image because both are now synchronised – all of the *Side Tool Bar* options affect both images simultaneously.

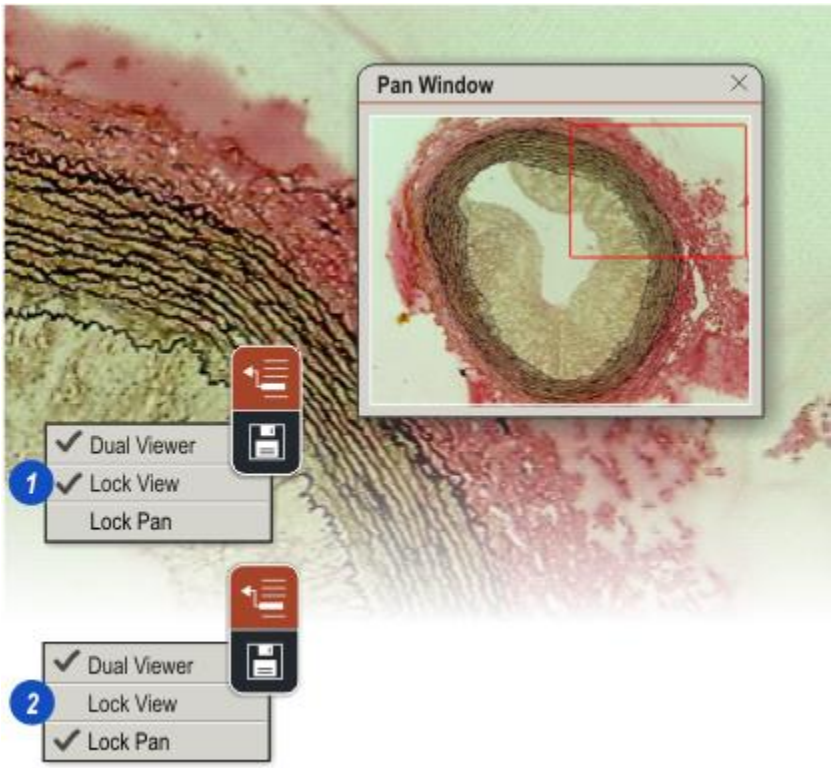


Pan with Lock View enabled:

1: Follow the lock/unlock[97] sequence to enable *Lock View*. Both images are automatically scaled to the same size and both pan in unison.

Pan with Lock Pan enabled:

2: Follow the lock/unlock[97] sequence to enable *Lock Pan*. The images can be displayed at different sizes but will pan in unison as the *Pan Area* is dragged.



To compare both live and captured images:

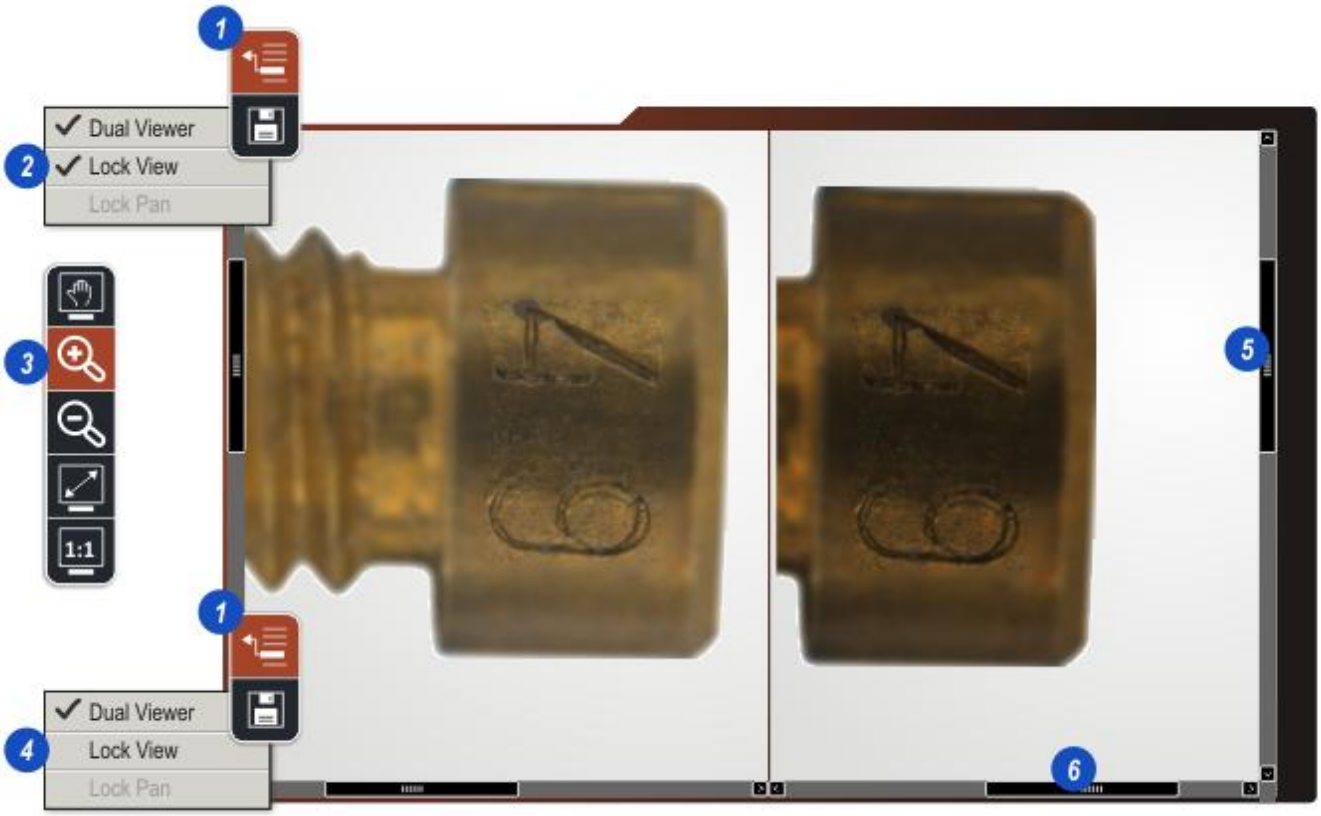
1: Click on the *View Options* button on the *Side Tool Bar* and…

2: …from the menu click to *enable* the *Lock View* option – tick mark visible.

3: Enlarge the images (now scaling in unison) to the desired size. In the illustration the two bolt heads are being compared and so the images were enlarged to view them easily.

4: Click on the *View Options* button again **(1)** and this time click to *disable* the *Lock View.*

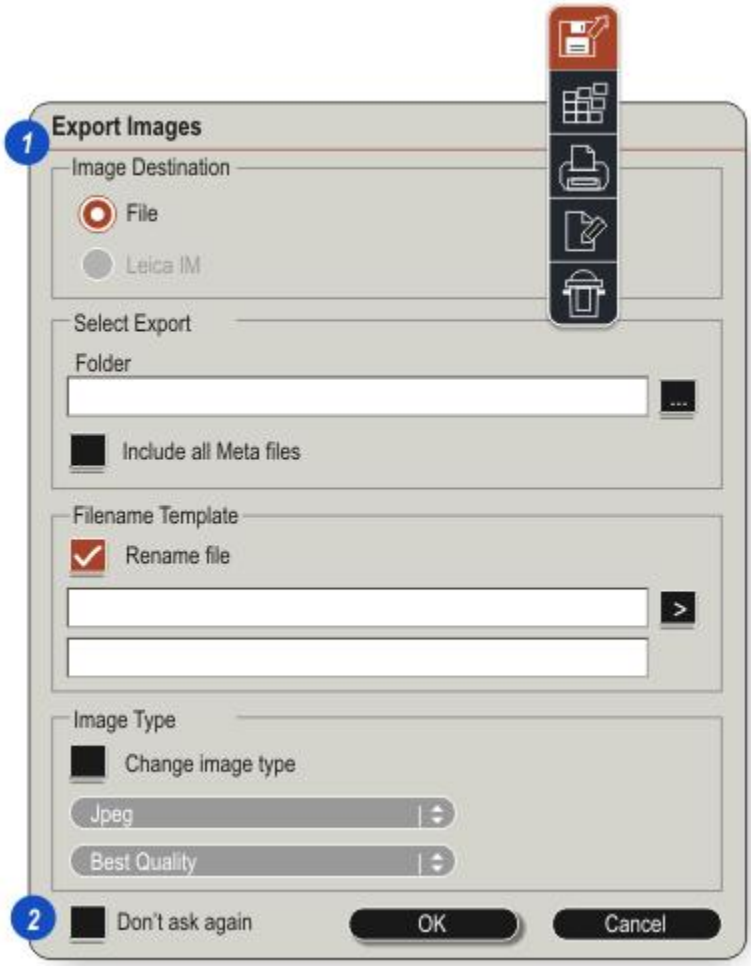
5 & 6: Use the *Scroll Bars* to independently move the images so that the features are close enough to be compared.



The *Export* function copies a selected image or multiple images to a location of the users choice.

Options allow complete meta data files or individual fields of data to be attached to, and exported with the image(s). Meta data contains the settings for the *Scale Bar* and annotations so is useful only for other LAS installations.

Additionally, the image type - *jpg, png* etc - can be changed during the export process. Image names can be changed as well or image sequences exported with the same name but with an automatically applied incremental suffix.



1: On the *Export Images* dialog, there is a...

2: ...*Don't ask again* checkbox which, when enabled (a tick mark visible) will skip the dialog options. This is very useful if a large number of images are to be exported and continually completing the dialog becomes a chore.

Enabling the checkbox clears the *Show Export Options* in *Preferences,* so to display the *Export* dialog again the option has to be re-enabled in *Preferences* 101.

Enable Show Export Options

Enabling the *'Don't ask again'* checkbox clears the *Show Export Options* in *Preferences*, so to display the *Export* dialog again:

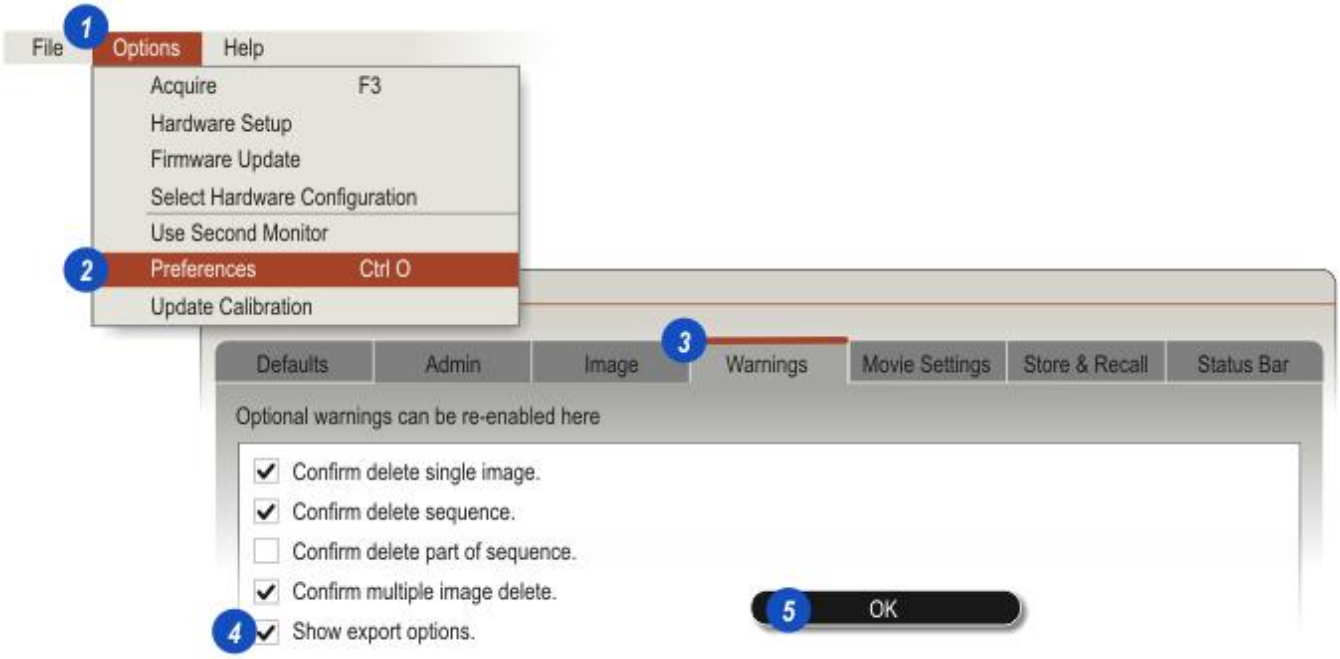
1: Click on *Options* on the *Main Menu.*

2: Click to select *Preferences*.

3: On the *Preferences* panel click the *Warnings* tab and...

4: ...click to enable the *Show Export Options* check box.

5: Click *OK* and the next time *Export* is invoked the dialog will re-appear.



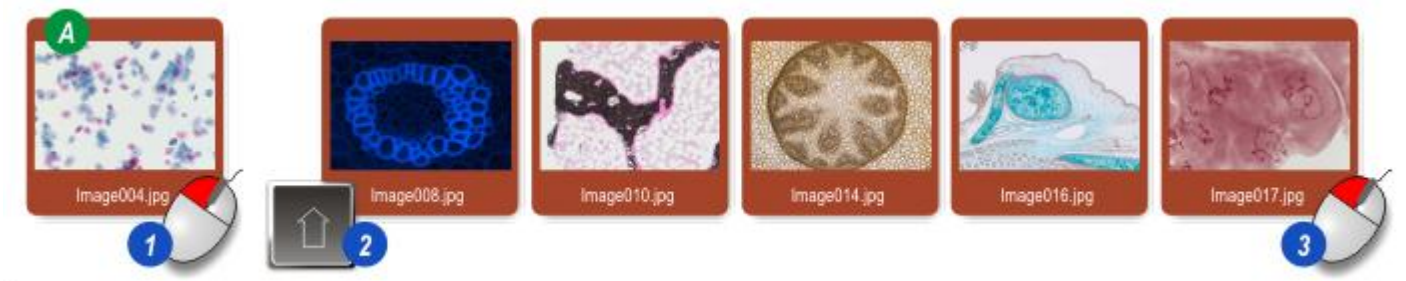
There are several ways to select images that will updated:

A: Range of images:

1: Click on the first image to be selected.

2: Press and hold down the *Shift* key.

3: Click on the image from which the calibration will be copied. All of the images between the two will also be selected.

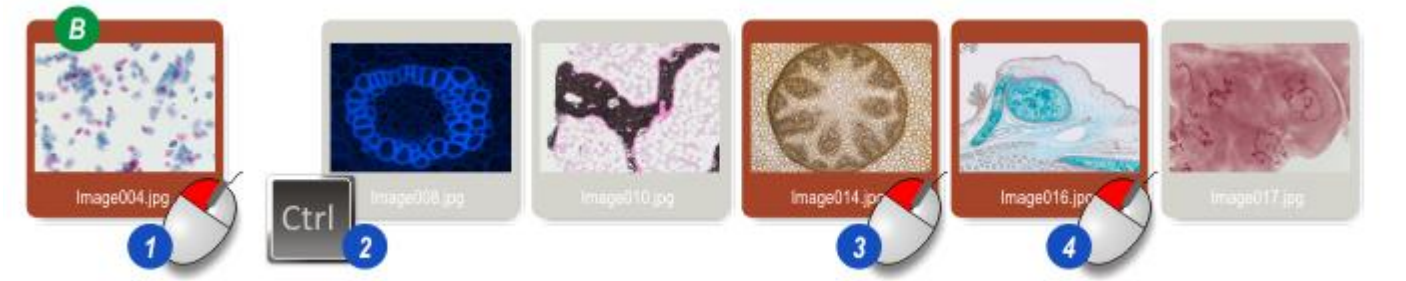


B: Individual Images:

1: Click on the first image to be selected.

2: Press and hold down the *Ctrl* key.

3: Click individually on all the other images to be included in the selection. The last image will be used as the calibration source.

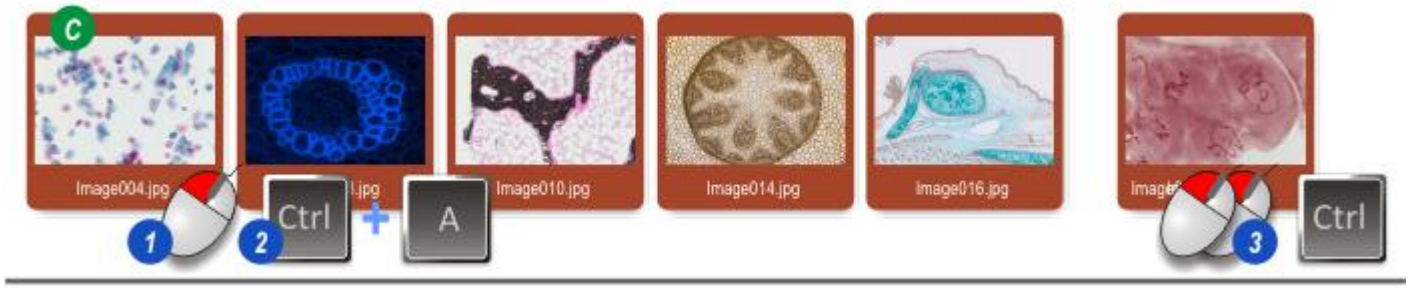


C: All of the images in the Gallery:

1: Click on the first image.

2: Press and hold down the *Ctrl* key and then press and release the *'A'* key (All).

3: To select the calibration source, still holding down the *Ctrl* key double-click the source image.



Select the Destination Folder

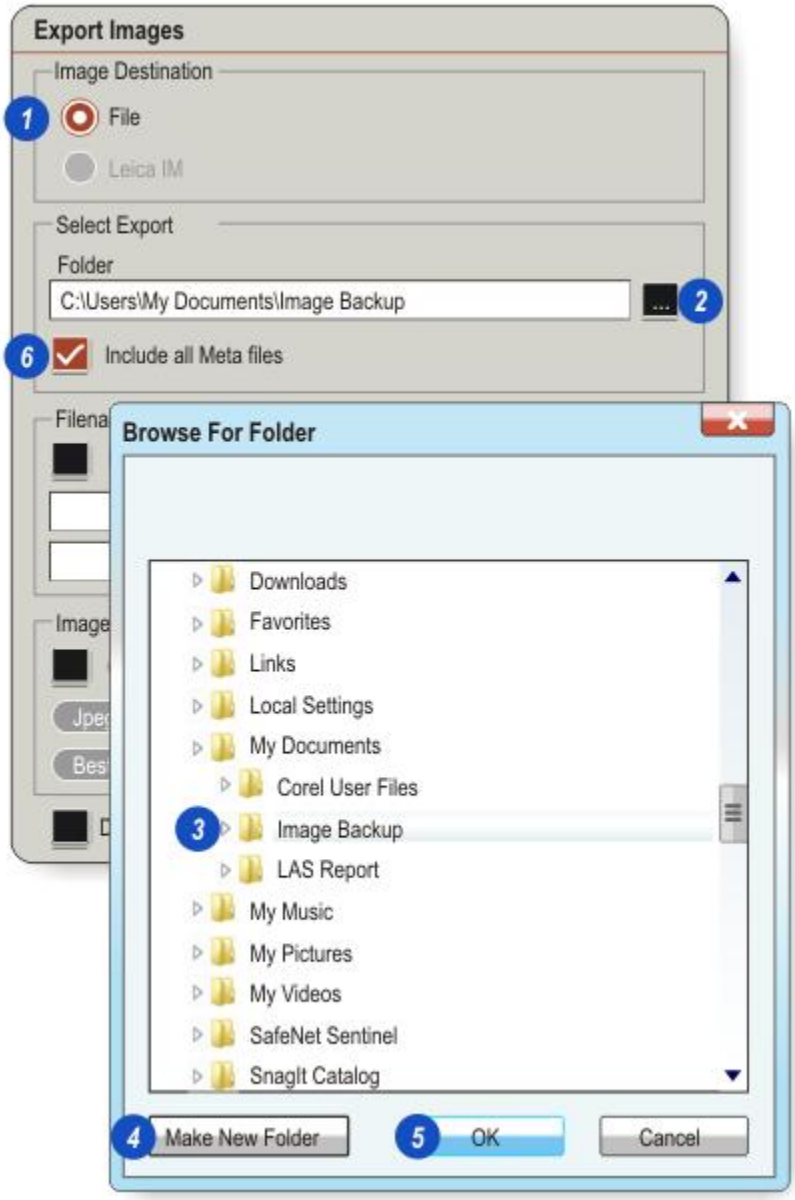
1: If necessary, click to select the *File* option on the *Image Destination* panel.

2: To change the destination folder, click on the *Browse for Folder* button and...

3: ...navigate to the destination folder. Create a new folder if required **(4).**

5: Click the *OK* button.

6: To include all of the *Meta D*ata with the image, click to enable the *Include all meta files* check box.



The images can be renamed before they are stored in the destination folder:

1: Click to enable the *Rename File* check box.

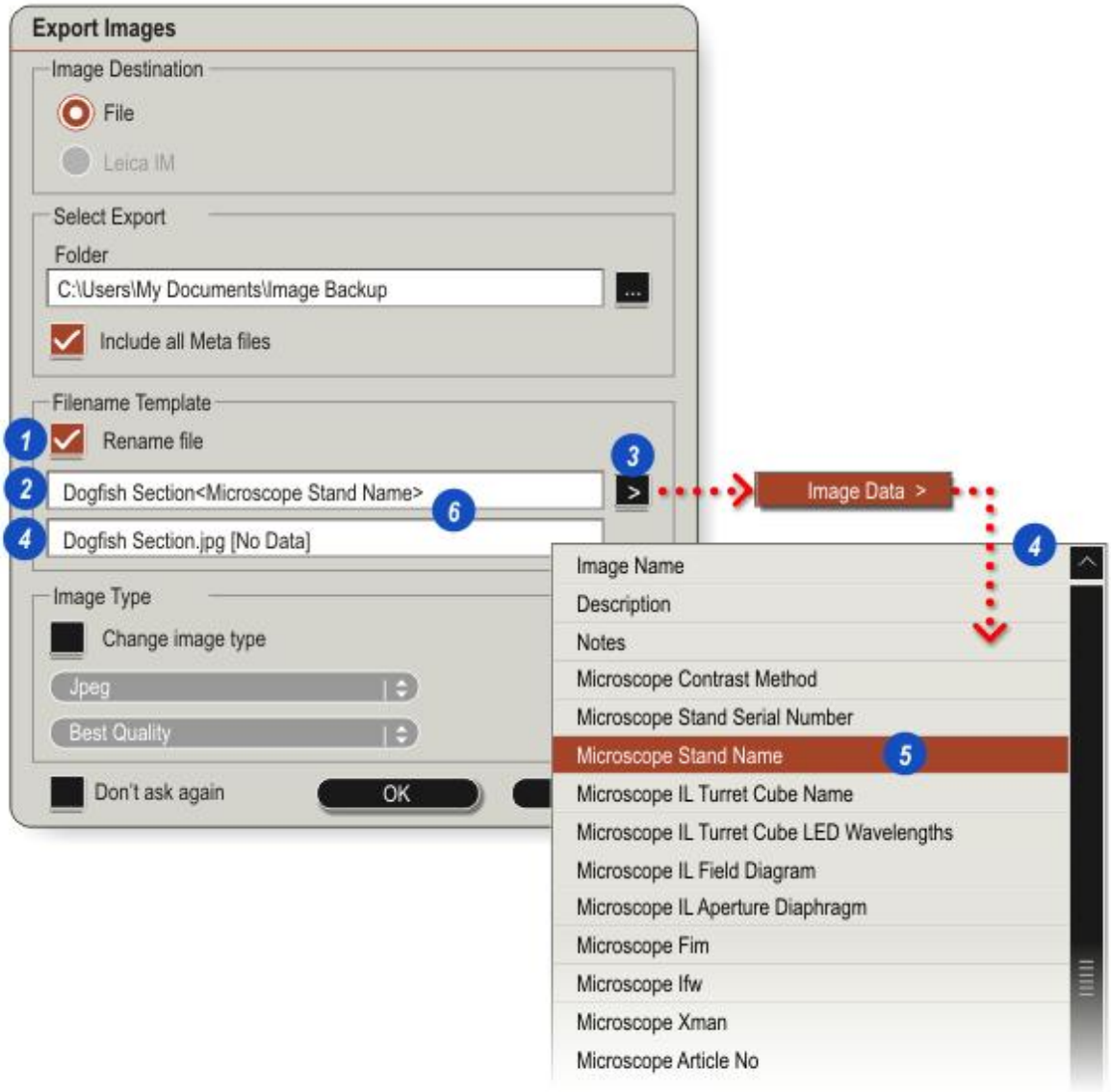
2: Click in the text box and type a new name for the image(s).

3: To include selected *Data Fields* with the image, click on the right-facing arrow and on the *Image Data* prompt.

4: The new name is displayed in the lower text box and the *Image Data* panel opens on the right.

5: To include a field and its data, click to select the *Field Name.*

6: The *Field Name* is added to the image name in the upper text box contained in tag marks <>, and relevant data to the lower text box within brackets [].



Change Image Type

The image type can be changed before it is saved:

1: Click to enable the *Change image type* check box.

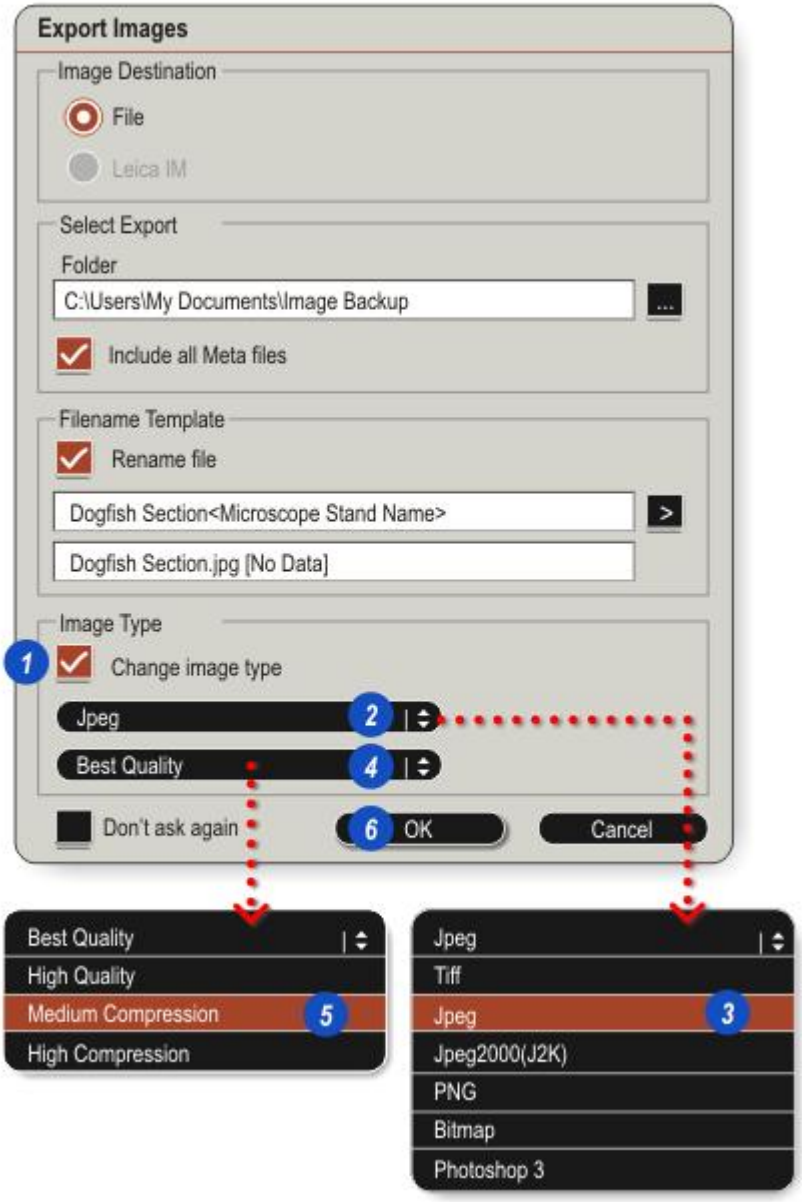
2: Select the new type by clicking on the small arrows to the right of the *Image Type* header and from the drop down menu...

3: ...click to select the *Image Type* required.

4: When the *jpeg* option is selected the quality menu is enabled. Click on the small arrows to the right of the header and...

5: ...click to select the required quality or compression.

6: Click the *OK* button.



If an image with the same name already exists in the destination folder, a warning will appear. There a three options:

1: Overwrite the existing with the new export. The existing image is lost.

2: Abort the export - *Don't copy.*

3: The *Copy but keep both files* option exports the image but adds a numeric suffix to the new copy to distinguish it. This is the option to use if multiple images are being exported and a common name has been chosen for them.

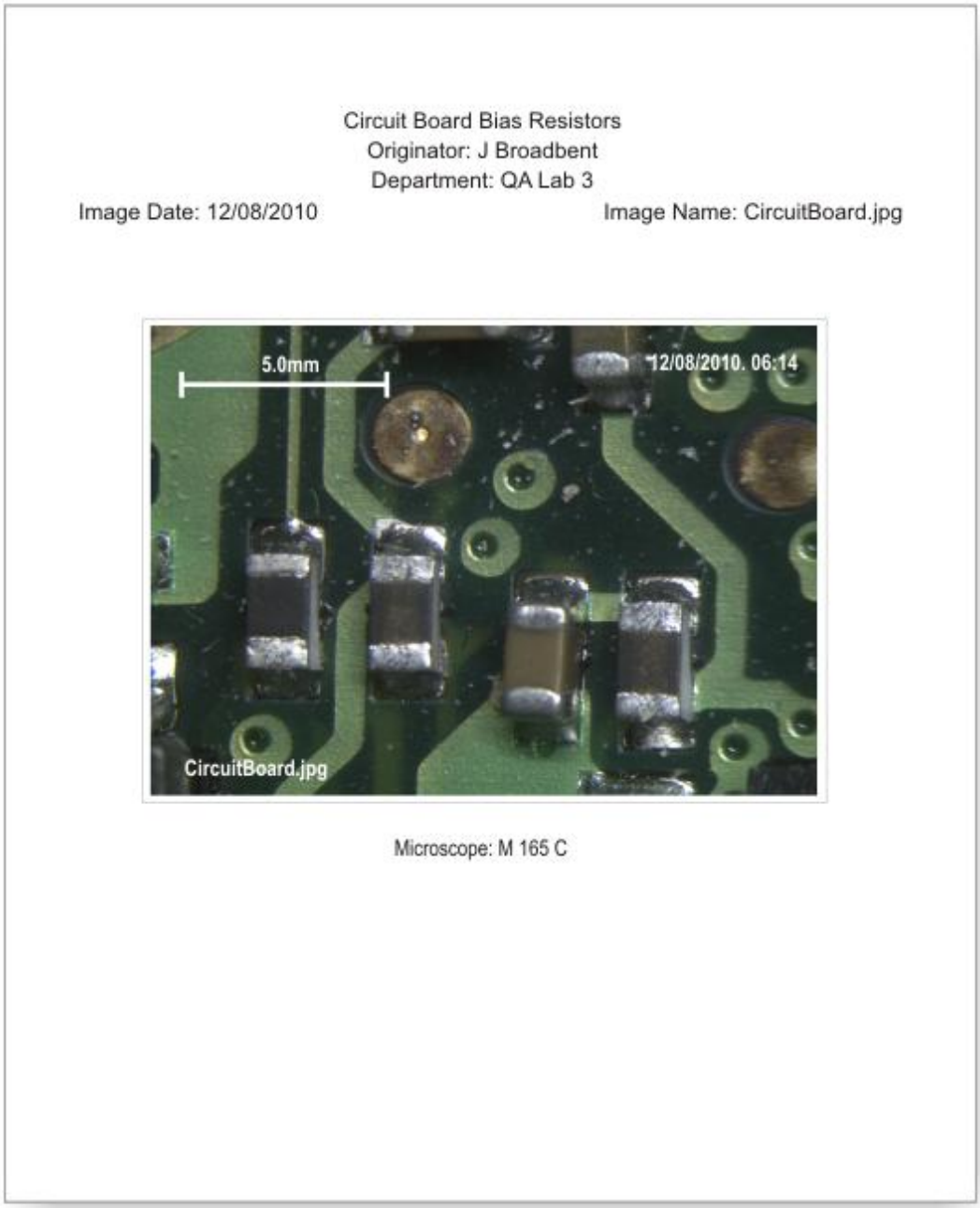
4: Click to enable the *Do this...* check box to automatically employ the chosen option in the future.

The *Printing* facility allows the user to print individual images quickly and simply. The built-in formatting provides flexible styling resulting in professional-looking documents.

Printing's most powerful feature is direct access to the image data, including all of the microscope settings, that can be included with just a mouse click to display alongside the users own text.

Printing features and quick links:

- Print LAS images in high resolution colour..
- Include *Annotations and Scale Bar* [116] on the image
- Include *Measurement Drawings* on the image.
- Include multiple *Header and Footer* [115] comments.
- Print landscape for maximum image size [111] or portrait for extra comment space
- Automatically [114] insert image information - no need to type it in
- Choose the *Font, Style and Size* [112] to personalise the report
- Comments can be re-used with other images - no need to re-type.
- See a full-screen preview [116] before printing



To print a single image together with a wide range of data:

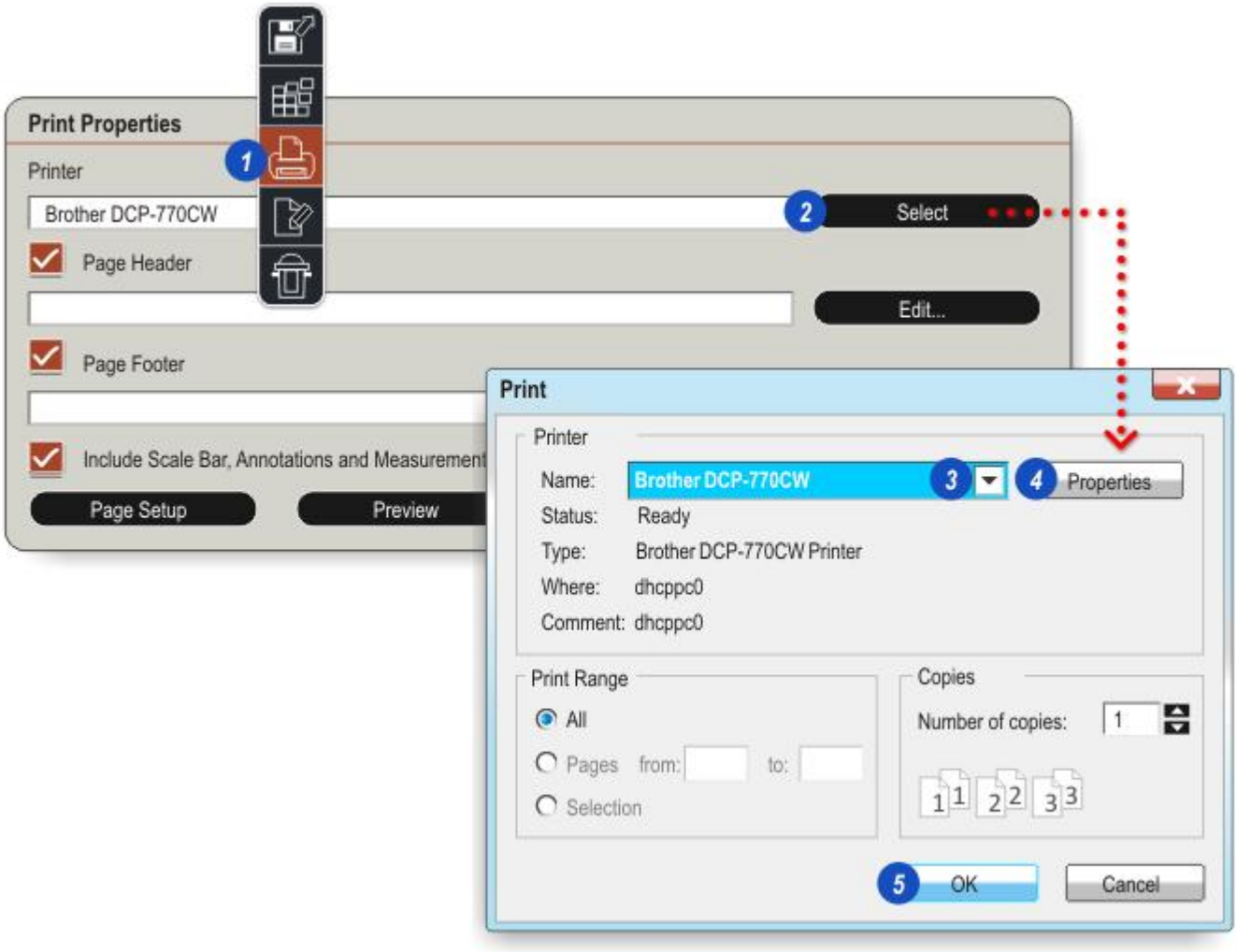
1: Click on the *Print* icon. The *Print Properties* dialog appears.

2: The currently selected printer (if there is more than one connected to the machine or network) is displayed in the *Printer* window. To change the printer, click on the *Select* button and the *Windows Printer* dialog appears.

3: Click on the arrow to the right of the *Name* text box and from the drop down list click to select and alternative printer.

4: Change the print properties by clicking on the *Properties* button.

5: Click *OK* to apply the new printer and its properties.

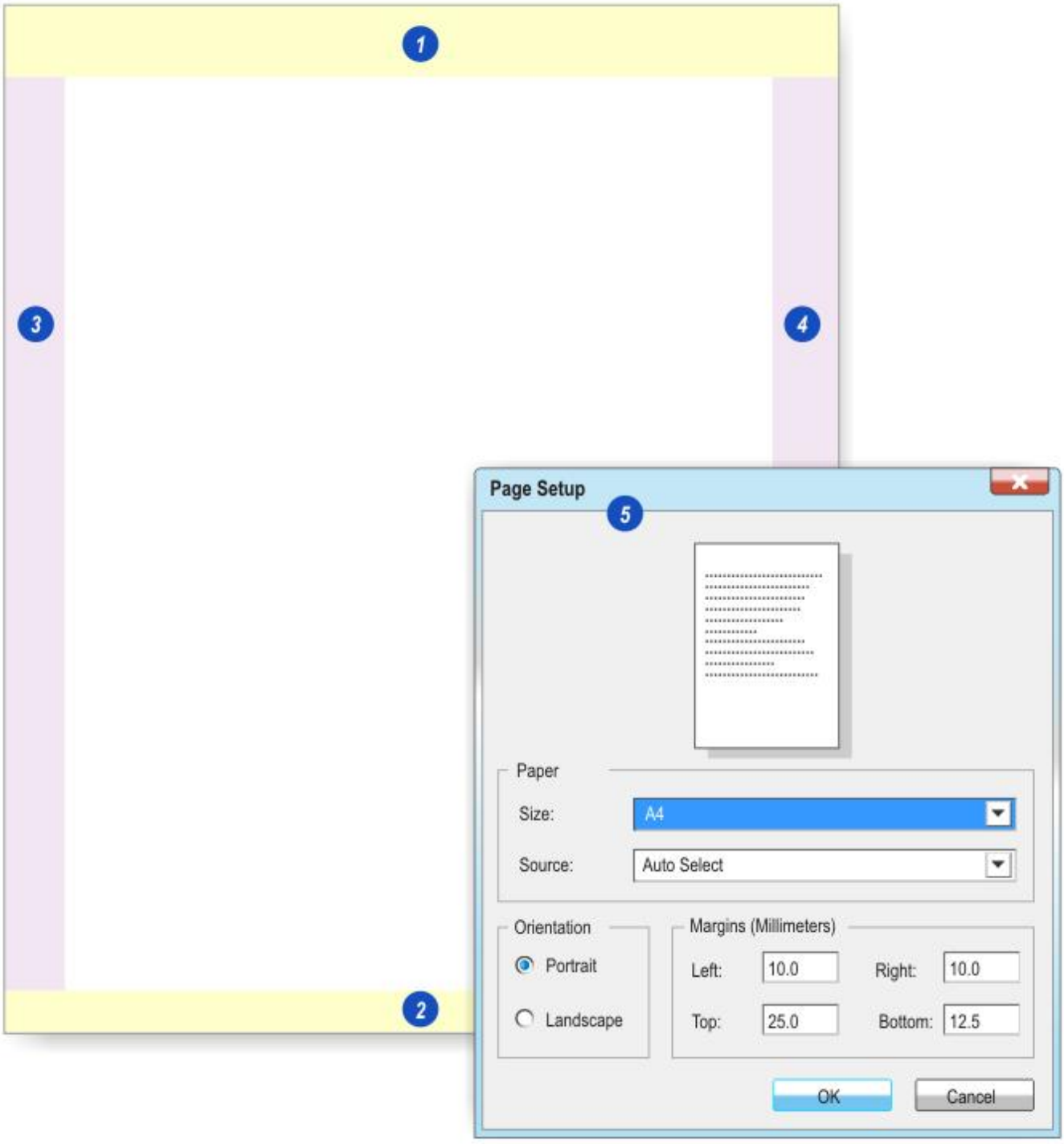


The print page is structured to provide the user with a quick and simple way of adding text and data to an image. The layout has been designed to provide maximum flexibility and a professional presentation.

There are 4 margins that can be setup by the user to accommodate different printers and bindings:

1, 2, 3 & 4: Top, bottom, left and right margins can all be set independently using the *Page Setup* dialog **(5).**

The dialog also allows the page *Size* and *Orientation* to be set.



The printable area inside the margins is divided into 3 columns each of which can contain justified text:

1: The left-hand column contains left-aligned text.

2: The centre column displays centred text, and...

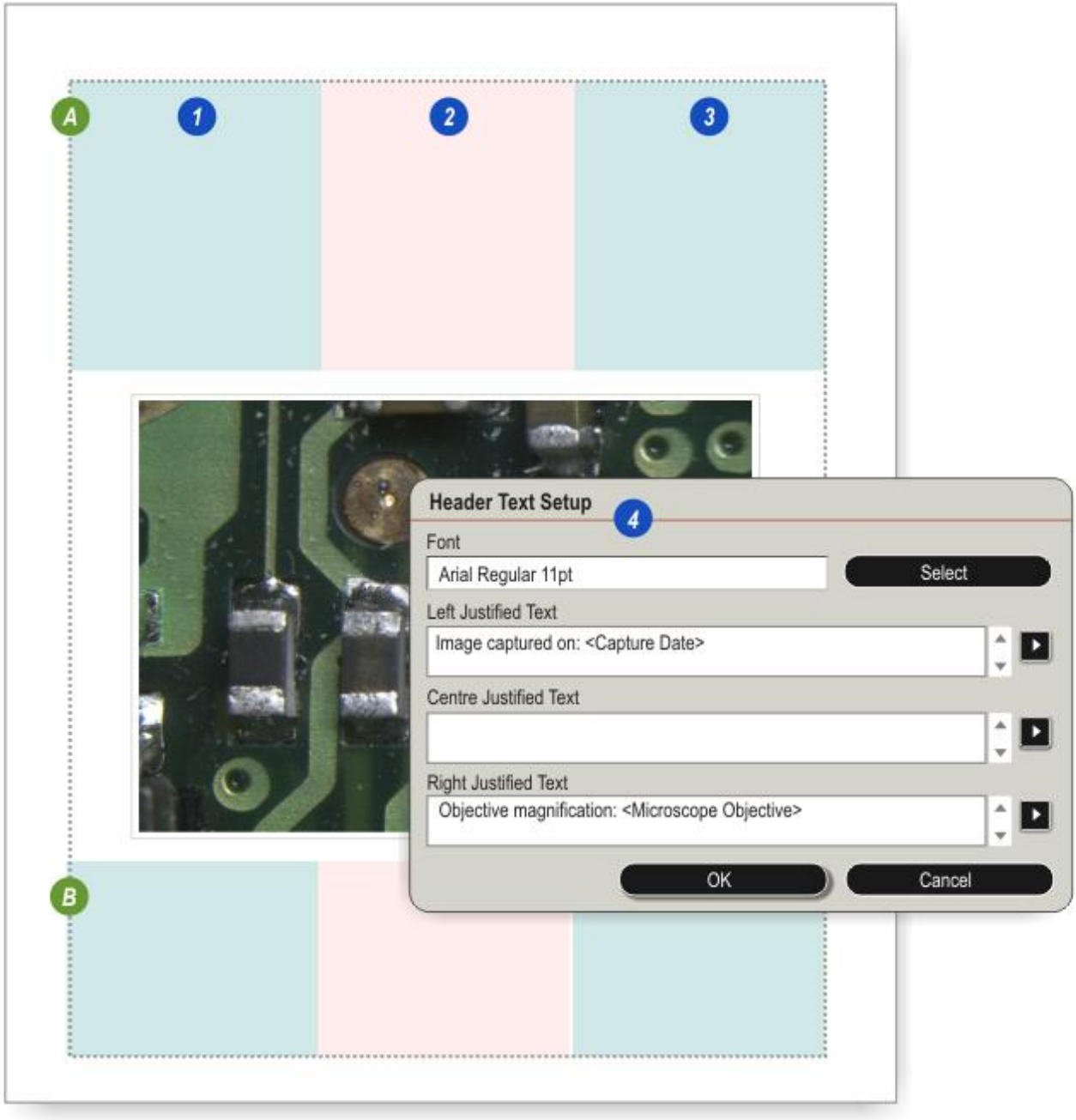
3: ...the right-hand column right-aligned text.

The columns are further divided:

A: Text appearing above the image called *Header Text*, and...

B: ...text below the image referred to as *Footer Text*.

The fonts are selected and the text entered using two dialogs - *Header Text* and *Footer Text* setups **(4)**.



To change the page size, margins and the orientation:

1: From the *Print Properties* dialog click on the *Page Setup* button.

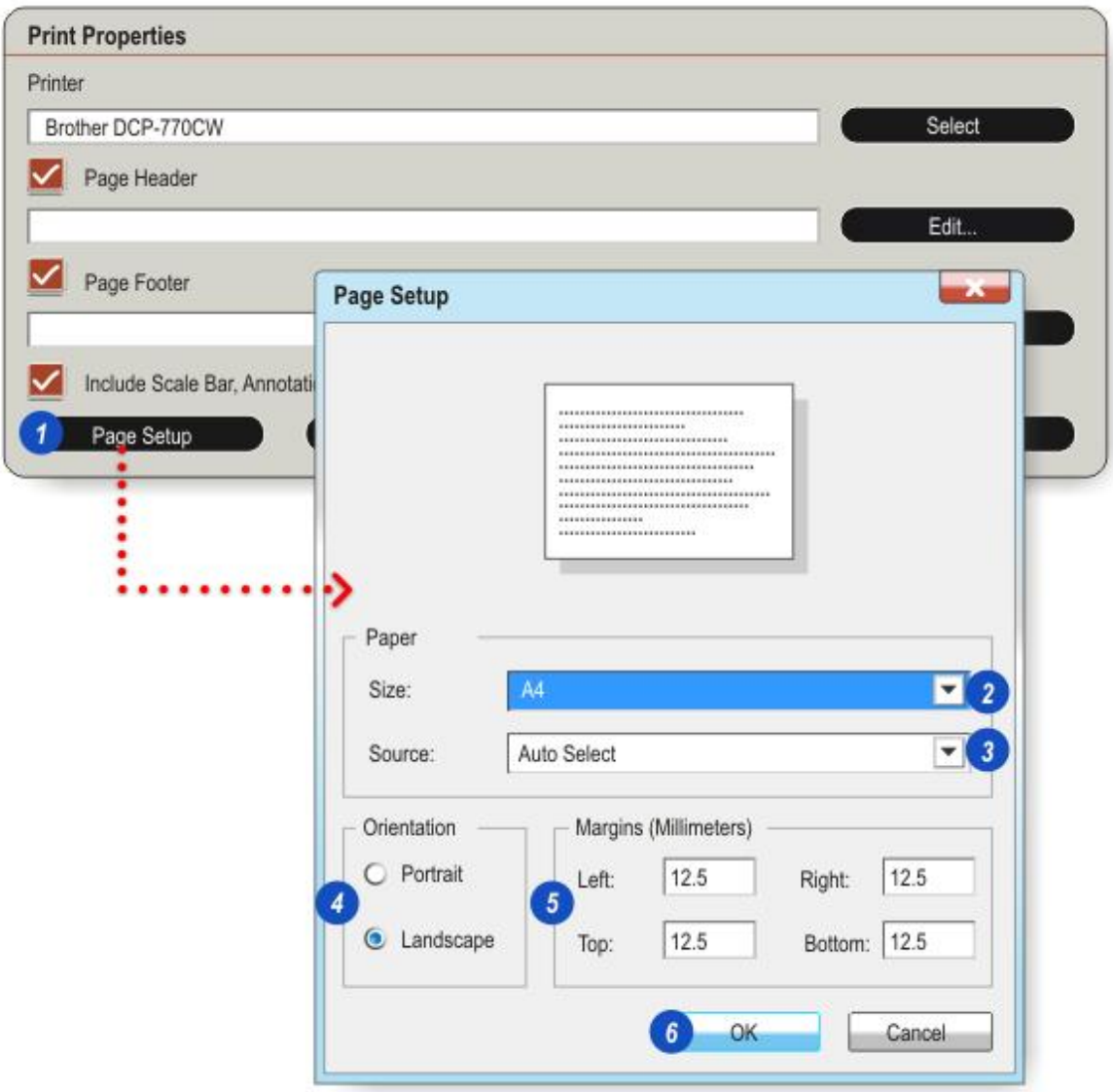
2: On the *Page Setup* dialog, if required change the page *Size* by clicking the arrow to the right of the *Size* header and selecting from the drop-down menu.

3: If the printer software allows, the printer *Paper Source* can be set by clicking the arrow to the right of the header and selecting from the menu.

4: Click to select the page *Orientation* - portrait or landscape.

5: Set the margins by clicking inside the appropriate text box and typing a value.

6: Click *OK.*



Different *Fonts, Style* and *Type Size* can be used for *Header* and *Footer* text but cannot be mixed across the columns.

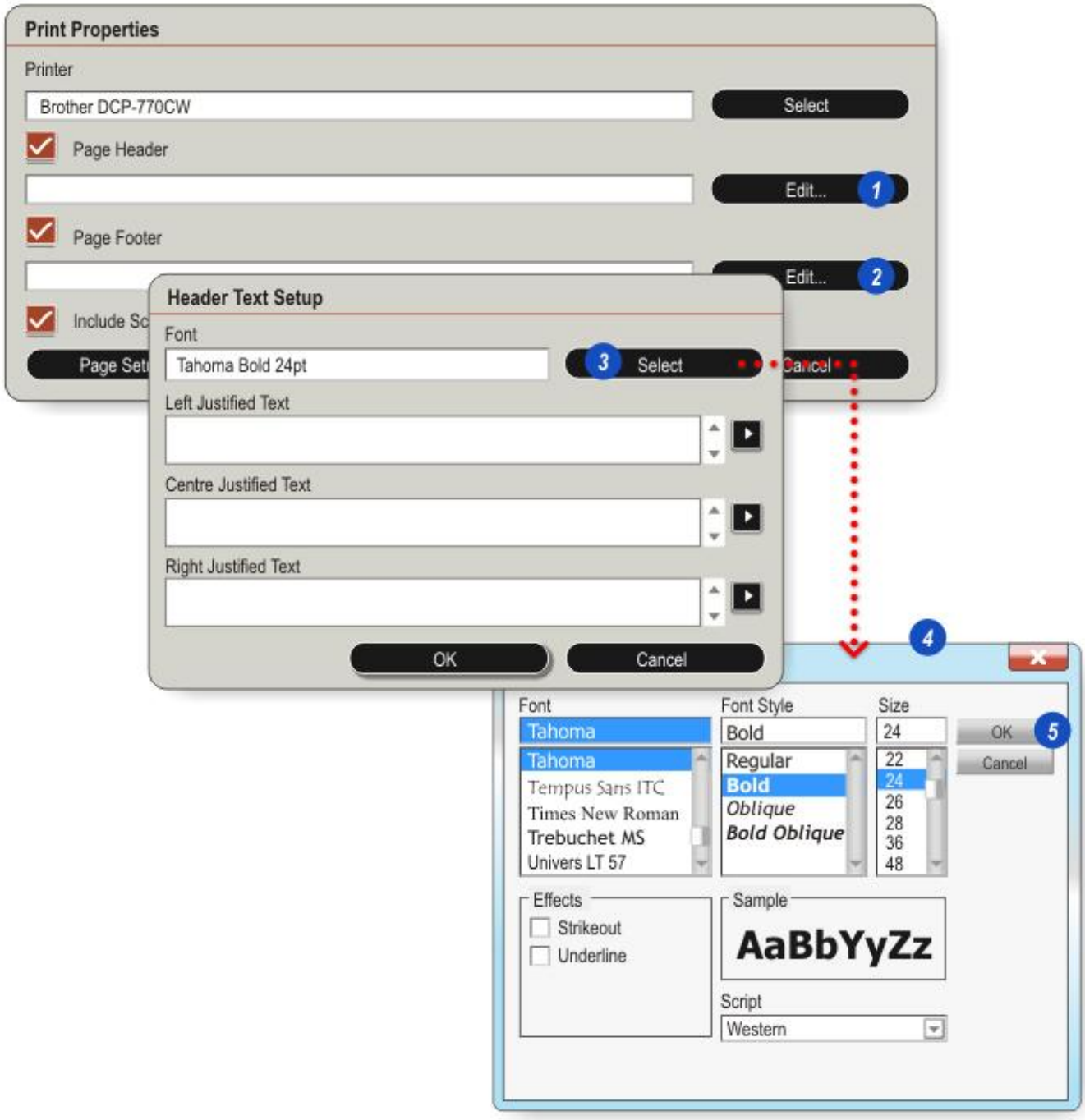
To select a *Font, Style* and *Size*:

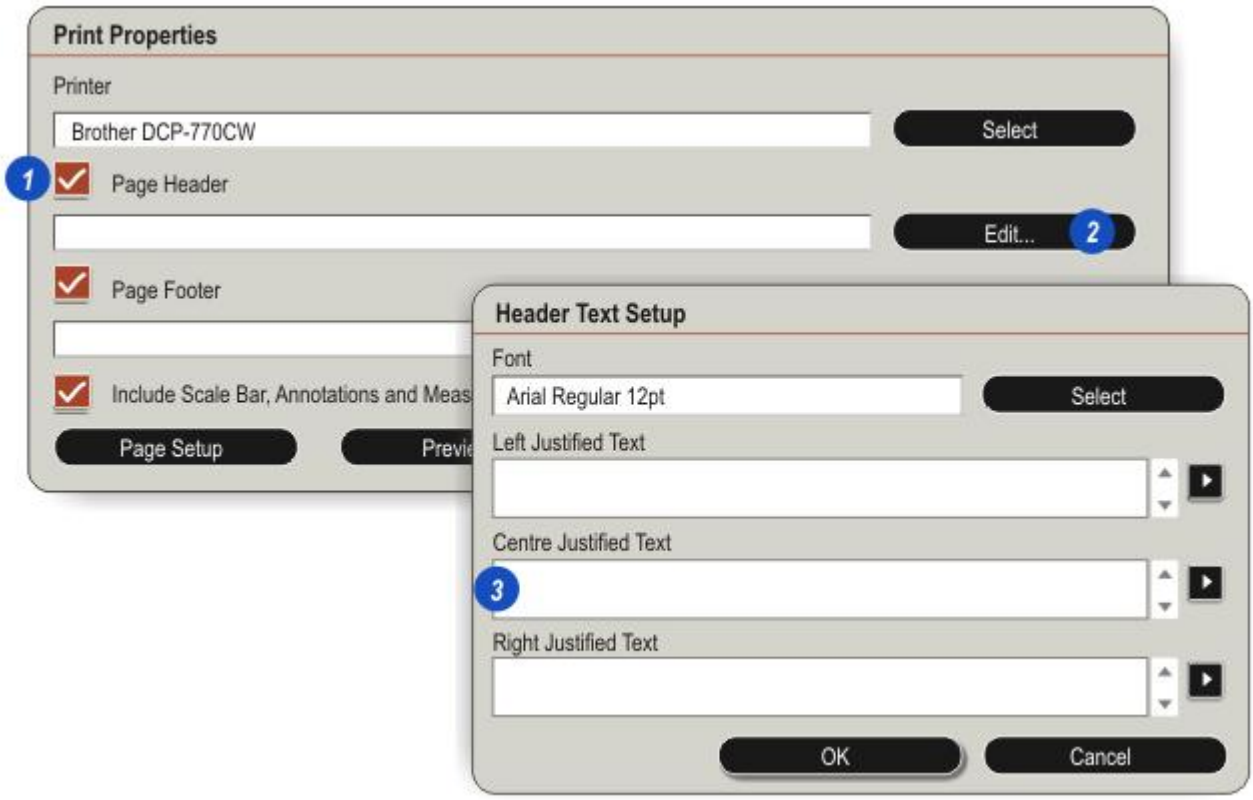
1 & 2: On the *Print Properties* dialog click the appropriate *Edit* button - either *Header* or *Footer* text.

3: On the *Header* or *Footer Text* dialog, click the *Select* button to the right of the *Font* text box.

4: From the *Windows Font* dialog, select the *Font, Style* and *Size* from the lists.

5: Click *OK*.





In this example the document title, the originator and the department are to be displayed in the centre column above the image. It will look like this:

1: On the *Print Properties* dialog click the *Page Header* check box to display a tick mark. This will ensure the header text prints.

2: Click the *Page Header Edit* button.

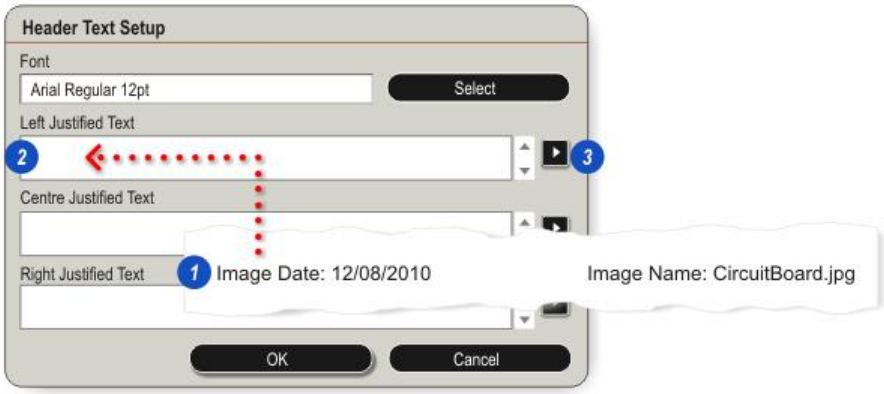
3: On the *Header Text Setup* dialog click inside the *Centre Justified Text* box.

4: The document title is typed...

5: ...followed by the key combination *Ctrl* + *Enter*: Press and hold down the *Ctrl* key and then press the *Enter* key. This will insert a new line break.

6: The originator text is added, again followed by the new line break combination *Ctrl* + *Enter.*

7: Finally, the department text is added in the same way.



LAS stores a wealth of data about an image. Individual data items can be selected from a list and added automatically to the page.

The date of the image capture and the image name are to be added to the left and right *Header Text* respectively **(1)**. The captions will be typed into the 'Justified' text boxes but the actual values are imported from the image data.

To add the image capture date:

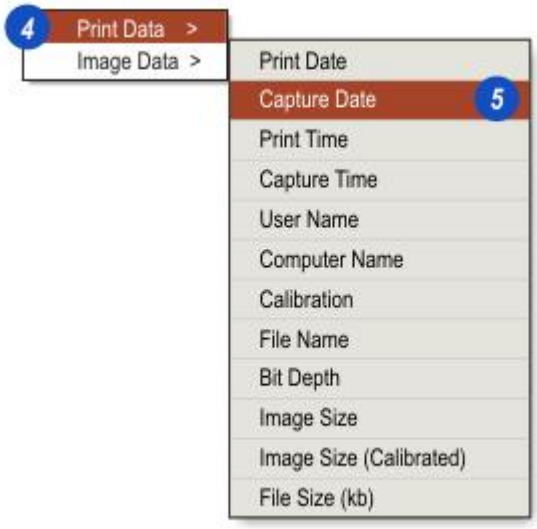
2: On the *Header Text Setup* dialog click inside the *Left Justified Text* box. In this example the text *'Image Date: '* is typed. Do not add a new line break.

3: The image *Capture Date* is going to be added to this text. Click on the arrow to the right of the *Left Justified Text* box.

4: Click to select *Print* from the data menu, and...

5: ...from the drop-down list, click to select the data item required - in this case *Capture Date*. Data items are represented in the text box enclosed in tags:

Capture Date: <Capture Date>

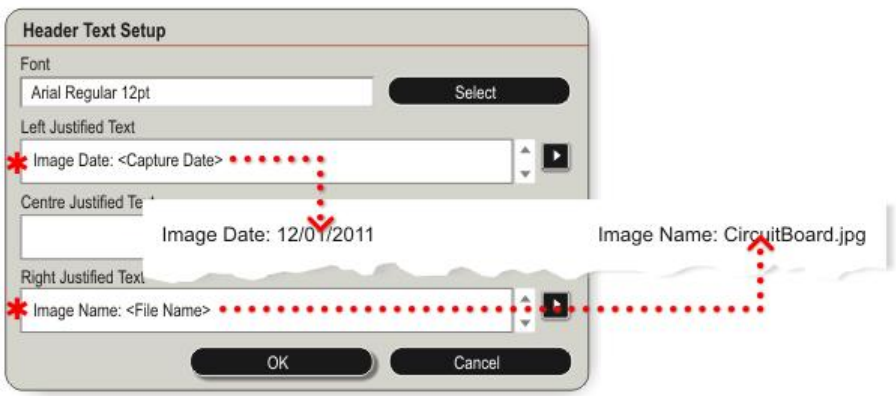


The *Image Name* is displayed in the same way but using the *Right Justified Text* box instead and selecting *File Name* from the list. It will appear as:

Image Name: <File Name>

The illustration shows the *Header Text Setup* dialog as it would appear from the example on the previous page.

When the page is printed the data inside the tags (<>) is retrieved and formatted. When this image was captured it was given the name *CircuitBoard.jpg.*



Footers - text appearing below the image - is entered in the same way as described for the *Headers* with left, centre and right text boxes.

Image data - information about the hardware, exposure and illumination - is also included using the same method as for print data.

In the example the final piece of information is the microscope name which will be centred below the image. On the *Print Properties* dialog click the *Footer Edit* button and...

1: ...the *Footer Text Setup* dialog appears.

2: For centred text click inside the *Centre Justified Text* box and type - in this example the word *'Microscope*: '.

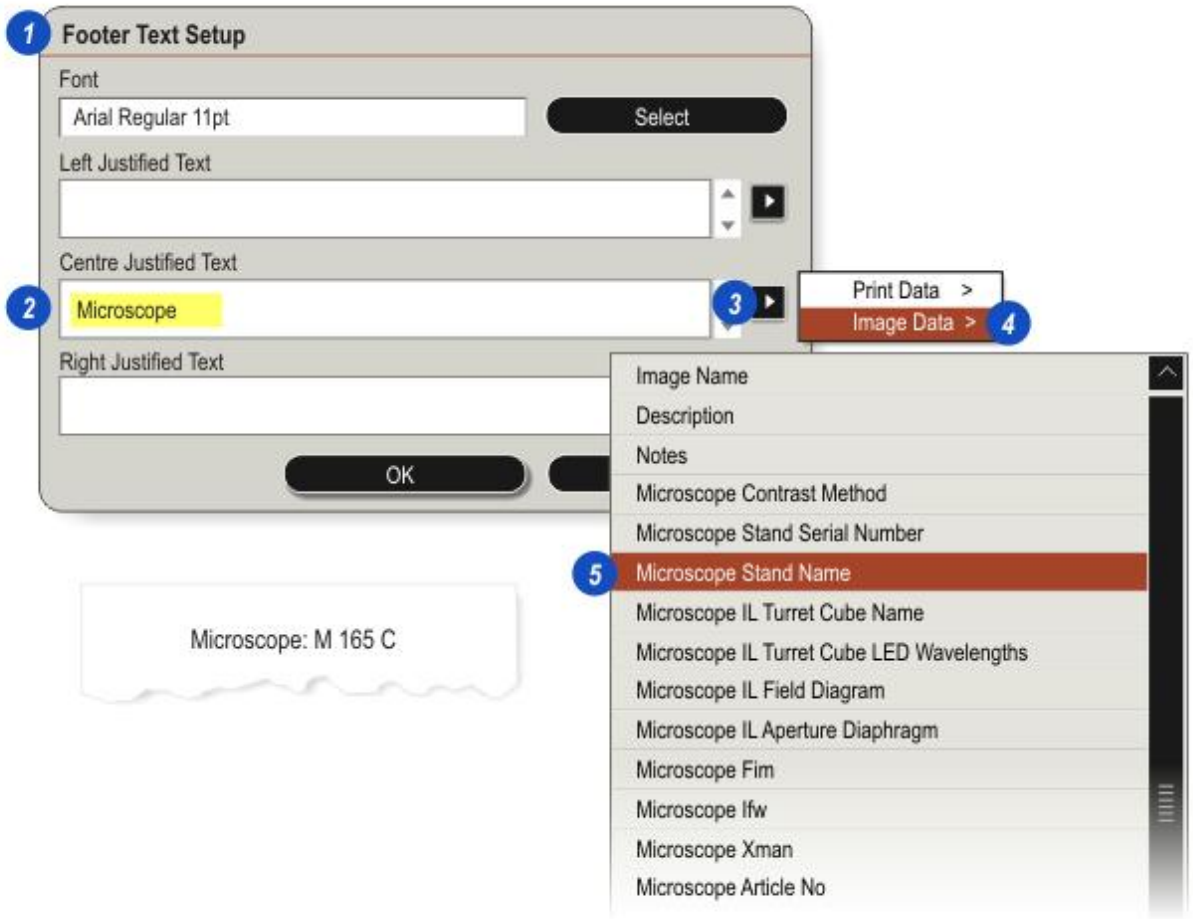
3: To add an *Image Data* item, click on the arrow to the right of the text box and...

4: ...select *Image Data* from the options.

5: Click to select the required item from the drop-down list. In this example the text would appear as:

Microscope: <Image Data.Microscope Stand Name>

Any number of data items can be added to a printout providing space allows.



1: To preview how the page will look when printed, on the *Print Properties* dialog click the *Preview* button.

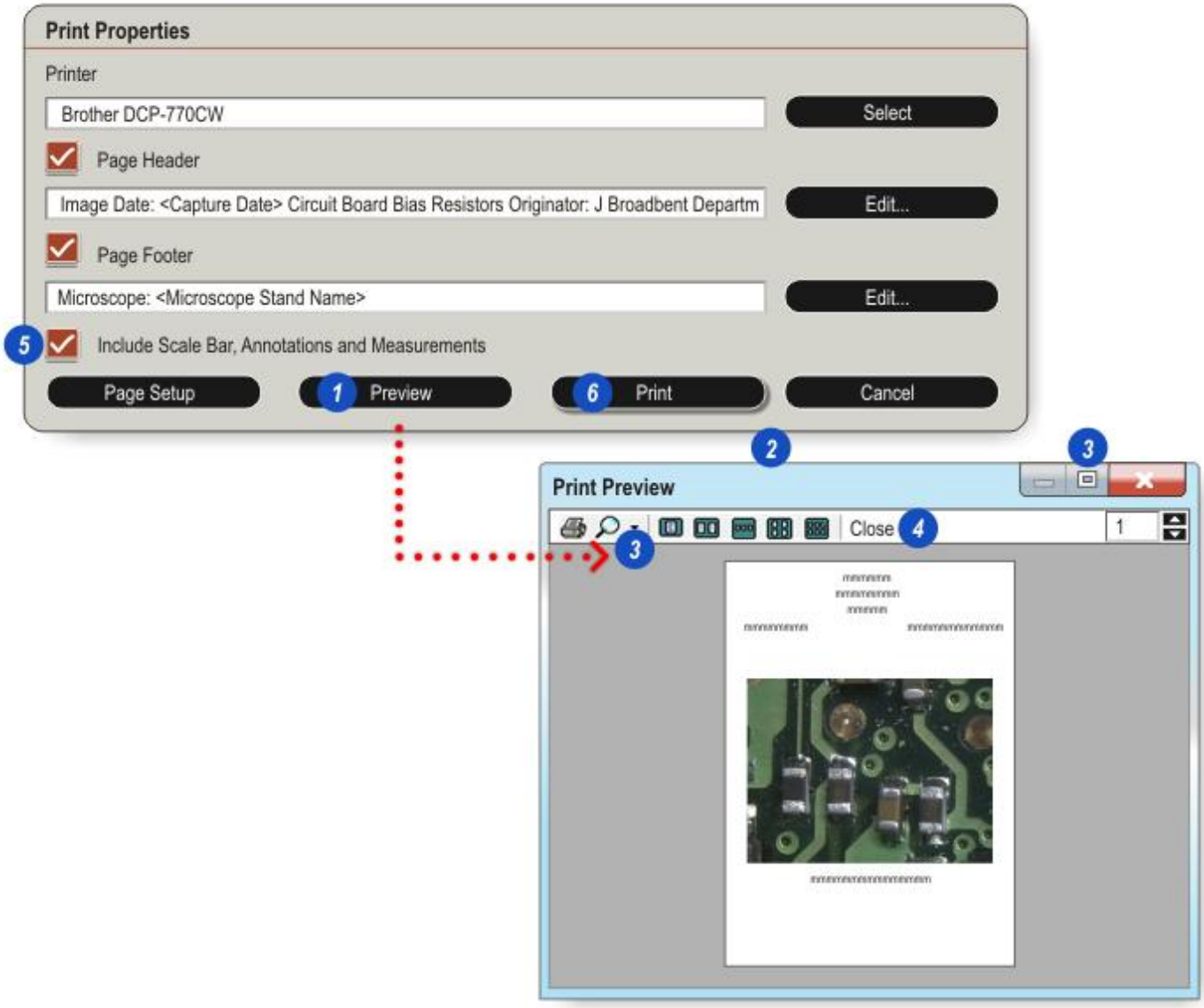
2: The *Print Preview* window appears with a scaled down representation of the page with the *Header*, *Footer* and image in relative positions.

3: Use the *Preview Zoom* to enlarge or reduce the preview or display it full-screen.

4: Close the *Preview* by clicking the *Close button.*

5: If a *Scale Bar* and *Annotations* have been added to the image but not merged, they can be included on the printout by clicking to enable the *Include Scale Bar...* check box.

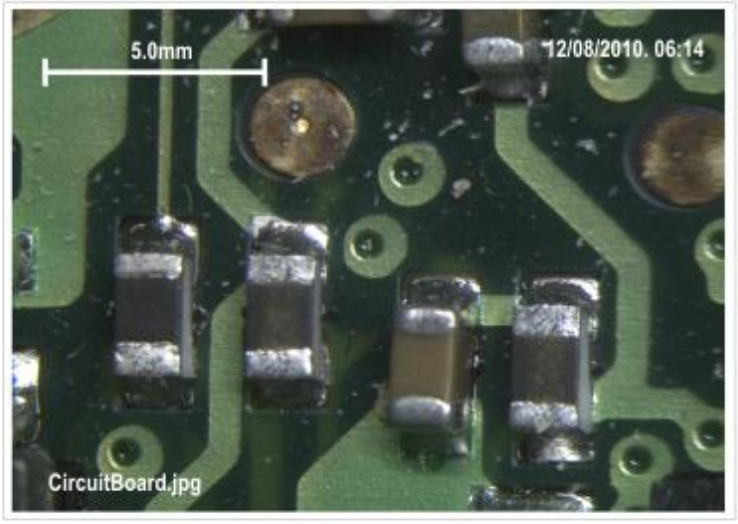
6: Click the *Print* button on the *Print Properties* dialog to print the page(s).



This is the printed result of the example described in the text.

Circuit Board Bias Resistors
Originator: J Broadbent
Department: QA Lab 3

Image Date: 12/01/2011 Image Name: CircuitBoard.jpg



Microscope: M 165 C

A report contains one or more images together with associated data. Standard templates[120] determine how many images are displayed on a page and what information is included. Images and text are automatically scaled to fit the page.

Browse mode: LAS Archives or LAS Image Explorer?

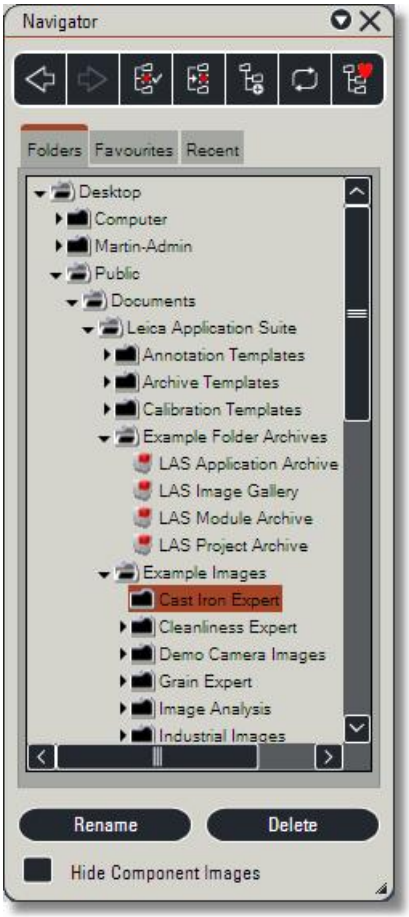
Reporting works within LAS Core and LAS Archive Standard Edition. The types of report you can generate, and the data they contain, depend on which browse mode you are currently using.

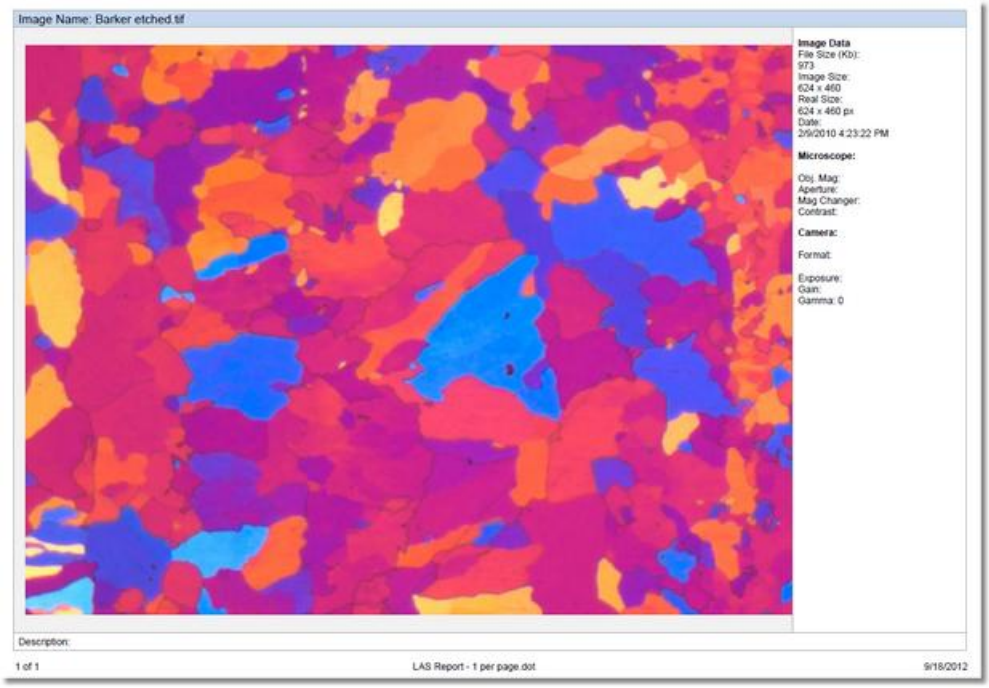
LAS Image Explorer: Available in both LAS Core and LAS Archive Standard Edition; Reports contain images, camera and microscope details, and summary data (i.e. information that is stored automatically with the image in the LAS database)

LAS Archive: Only available if LAS Archive Standard Edition is installed. In addition to images and data available to LAS Core, extra reporting information may be available from the archive (i.e. extra user-entered information) that you can include in the reports, such as customer and project details

See here[483] for details of swapping between these two browse modes. The basic process of creating reports is the same in both cases; only the information available in the reports changes.

Navigator, showing archives and standard image folders:





The basic procedure to create a report using one of the supplied templates is very straightforward:

1: In the *Browse Workflow,* navigate to an image folder.

2: Use the *Navigator* to select an image folder or LAS Archive.

3: Select the images for your report in the *Gallery* or *Grid*. Use the keyboard *Ctrl* and *Shift* keys to select multiple images.

Note: A warning is given if you select more than 10 images to include in a report. You need to consider if the file size is reasonable (large files can quickly fill up your hard drive, and processing them in LAS requires plenty of RAM).

4: Click on the *Create a Report* icon in the *Side Tool Bar*.

5: From the drop-down, select which standard template group (*DM core*, *SM core* or *LAS Archive*) to use. The default is *DM core*. See Templates and File Formats 120 for descriptions.

6: Select one of the standard templates that suits your requirements. See Choosing a Template 121.

7: Click *Settings* and select the report File Format 120 from the drop-down menu.

You can also choose to embed images 122 (in a Word report) and display the magnification factor 123 as a label.

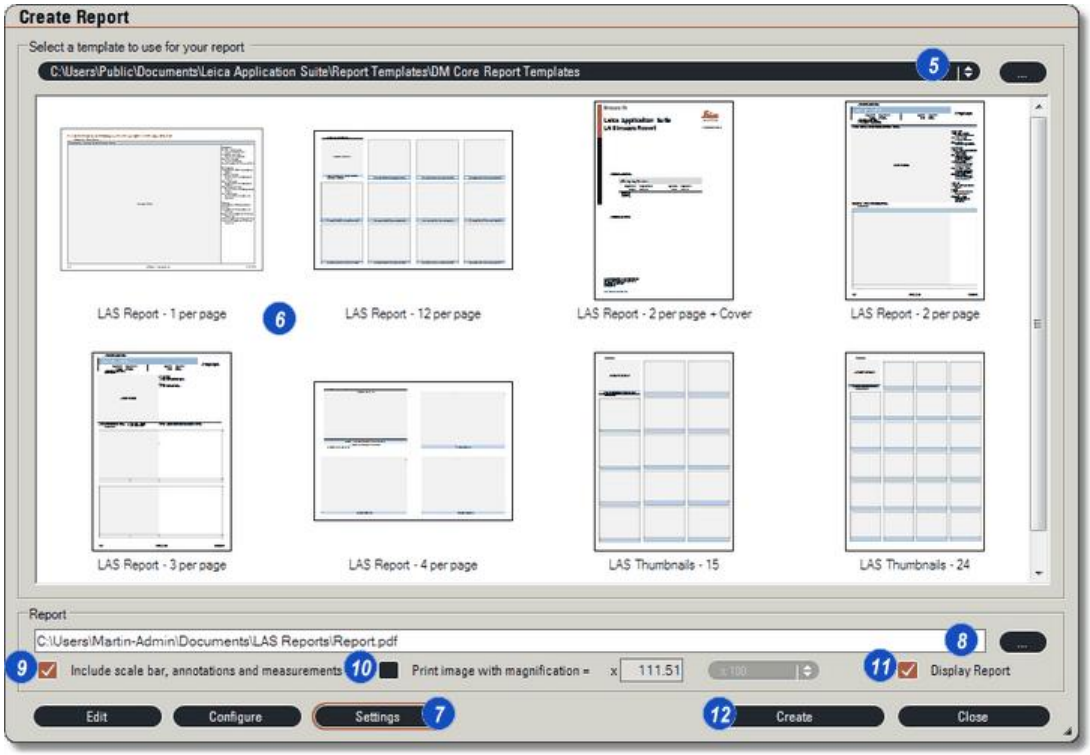
8: If necessary, browse to the folder where you want the report to be saved, and enter a name. The extension should match the *File Format* you chose above.

9: Choose whether to *Include the scale bar, annotations and measurements*; these are superimposed on images in the report. This process may take some time.

10: Choose whether to print images with a specific magnification factor (enable the option, enter a value in the text box, or choose a value from the drop-down menu).

11: Choose whether to *Display the Report* after it has been generated.

12: Click *Create*. Your report will be generated (but note that for some report templates there is an extra step 124).



Report Templates

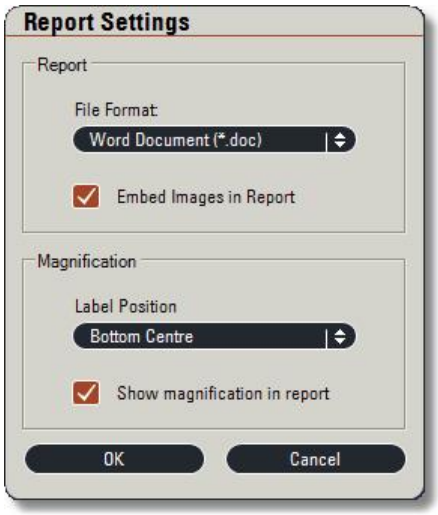
LAS provides the following standard template types:

- *DM Core*: Optimised for creating reports for images acquired by compound microscopes
- *SM Core*: Optimised for creating reports for images acquired by Stereo and Macro microscopes
- *LAS Archive:* Optimised for creating reports for images stored in a LAS Archive; extra information is often available for such images.

You won't normally need to edit the template source files, but if you do, please see here.

Report File Formats

1: To select a report file format, click *Settings* on the main *Create Report* window and select a format from the drop-down.



You can generate reports in the following formats:

- Microsoft Word (.doc, .docx or .xml)
- Web Page (.html)
- Adobe Acrobat PDF

Template groups

Choosing a Template

When choosing which template to use, bear in mind the following:

- If you are including many images in your report, obviously it makes sense to choose one of the templates that displays more than one image per page, especially if the report will be printed.
- The template thumbnails in the *Create Report* dialog give you some idea as to the amount of detail that will be included with any images.
- Some templates allow for extra Header or Summary information to be included. For example, the report template with the word *Cover* in the title will generate a cover page with summary information that you supply relevant to all images in the report (for example, if they are all part of the same experiment). See here 126 for details on configuring this extra information.

Some report thumbnails in the Create Report dialog

Embedding Images (Word-style reports)

Reports created using *Microsoft Word* can include images in the following ways:

- Image Embedding: Copies the image into the report so that it becomes an intrinsic part of it. Embedding can result in very large files but they are always integrated - text and images cannot be separated.

- Image Linking: A path is established between the report and the image source; the image is included only when the report is displayed, printed or exported - by e-mail for example. Linking creates small, compact files. However, if the image source is moved or changed, the link is lost and the images will not appear.

To embed images in a Word-style report:

1: Click *Settings* on the main *Create Report* screen.

2: Enable the *Embed Images in Report* box.

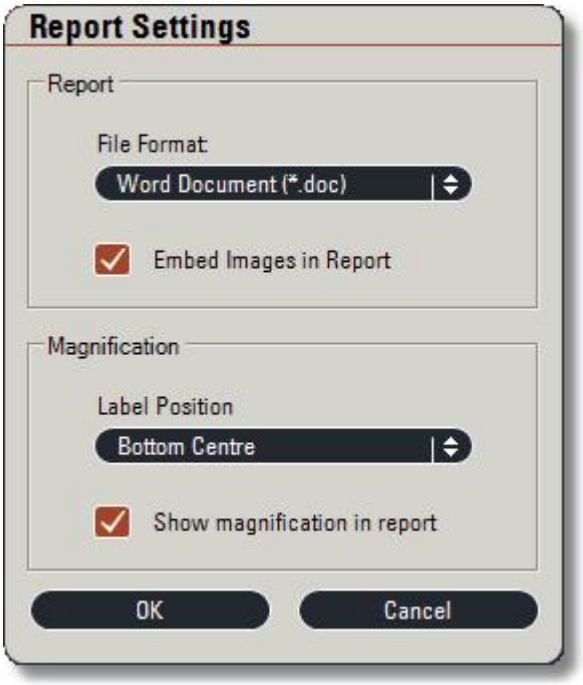
3: Click *OK*.

Note: You can only disable this option for Word-style reports; for all others it is locked ON.

Images can be very large and occasionally – especially in reports that have many images – there is insufficient machine memory and a *Report Generation Error* occurs.

To cure the problem, do one of the following:

- Reduce the number of images
- Acquire smaller images by using a more compressed file format and lower resolution
- Resample the images to a lower resolution
- Use a report template that allows the use of LAS high quality thumbnails rather than full-size images



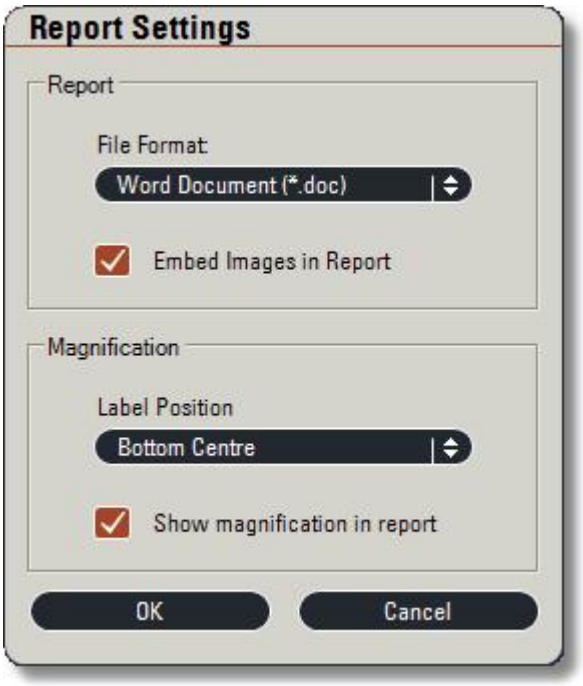
To include magnification details in your report:

1: Click *Settings* on the main *Create Report* screen.

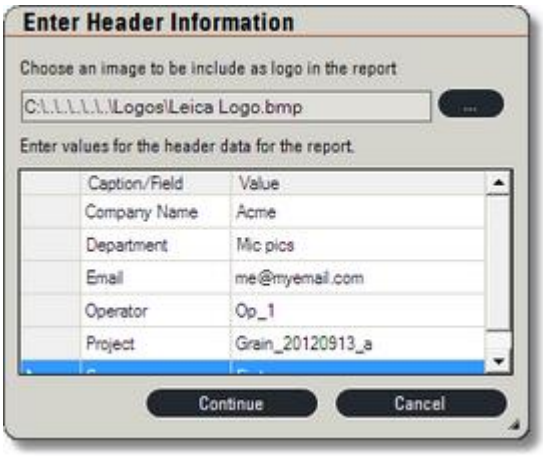
2: Enable the *Show magnification in report* box.

3: Select a Label Position from the drop-down menu.

4: Click *OK*.



If you chose a report template that contains extra information, such as Summary information for a Cover page, there is an extra step after you click *Create Report*:



1: If you want your company logo to appear on the report, click the browse button, navigate to and select the logo and click *Open*.

2: Click in a *Value* field and type in the information relevant to that field.

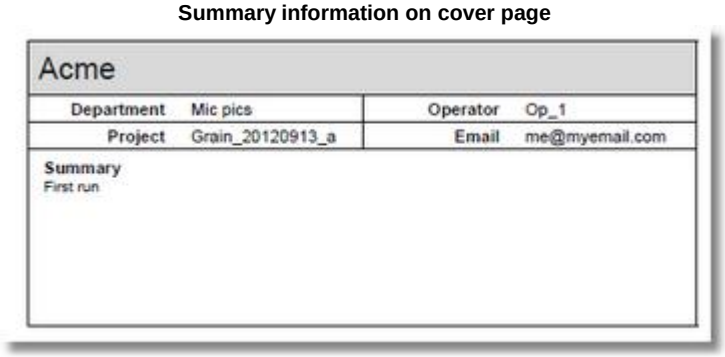
Note: You can edit the information that appears in this dialog as described here 126.

3: Repeat for all other fields that you want to appear in the report (if you leave a field blank, the title will still appear in the report, just with no data).

4: Click *Continue*.

Some reports only display this extra information on the cover page, others display it in the header of every page in the report.

Summary information on cover page



You can modify the existing Standard Templates supplied with LAS to suit your own requirement. The page layout - margins and indents - can be altered, as well as new image data fields added and fonts changed.

1: On the *Create Report* dialog select the template to be changed.

2: Click *Edit*.

3: Word is launched and the selected template opened. Refer to the steps in *Create a New Template* 129 to make the changes.

! Users should consider copying a Standard Template, saving it under a different name and making changes to the copy to preserve the original.

Configure Report Details

You can configure the default data that appears in a given report template, along with any user-entered summary data, by editing the *Fields* and *Header* information for that template.

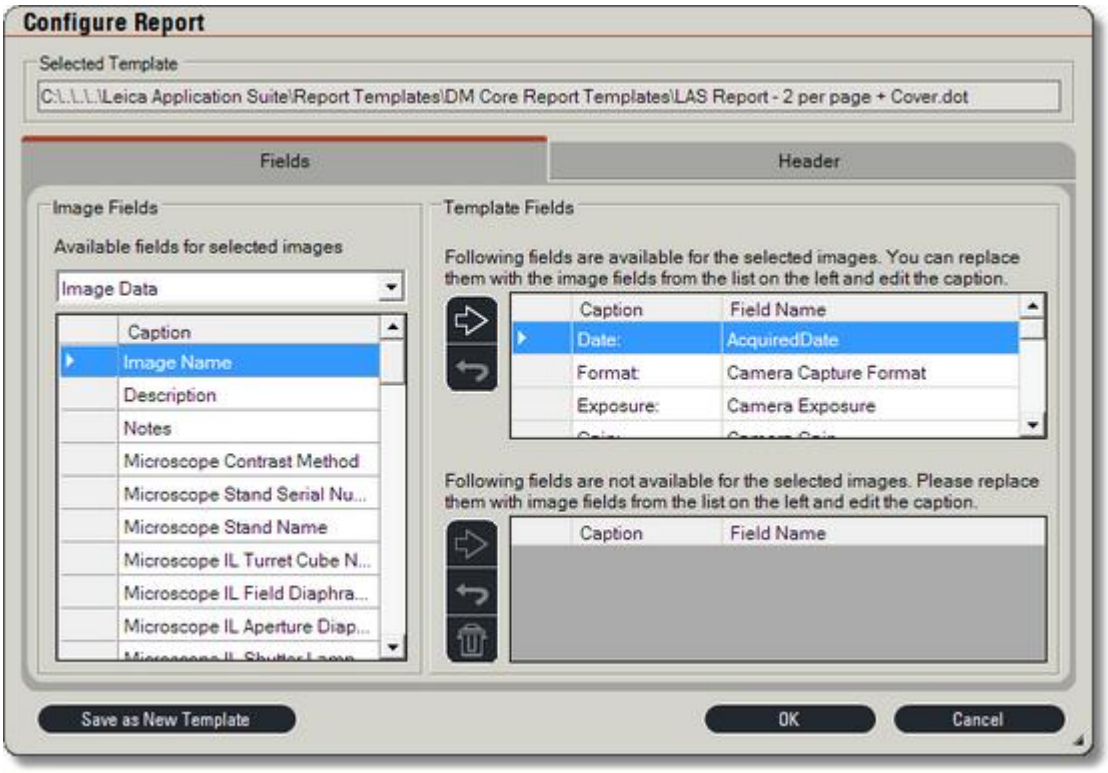
- Each image in a LAS database has data stored with it (image details, camera and microscope details). This data is captured and saved automatically as part of the acquisition process.
- In addition, images stored as part of a LAS Archive have extra data available, such as customer and project details. This data is usually entered manually by the operator at capture time.
- When you come to generate a report, you can add more details (in up to 5 fields) as Summary or Header information.

1: Display the *Create Report* dialog.

2: Select a template and click *Configure*.

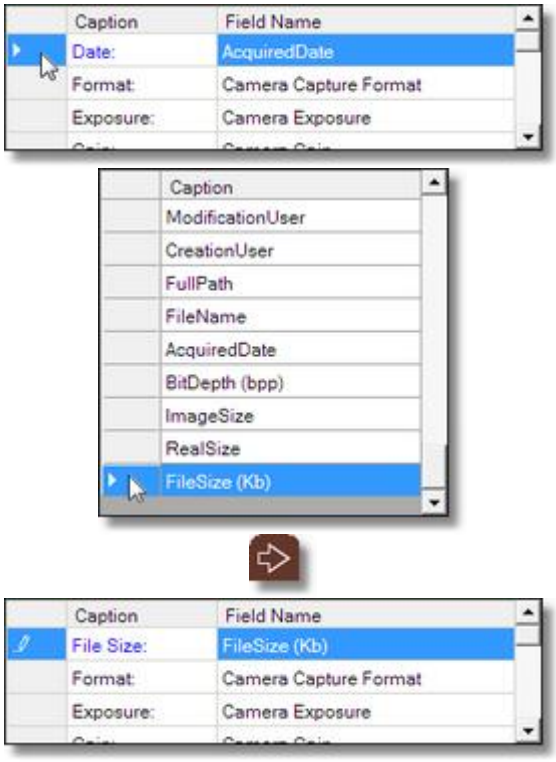
3: The *Configure Report* dialog appears.

Continued: See Configure Fields 127 and Configure Headings 128



A report template allows for data from a number of fields to be displayed. The number is defined in the template, but you have control over which of those fields are displayed.

The *Image Fields* panel contains a list of fields available in the image. The *Template Fields* panel is a list of fields defined in the template that are available for the selected image. The table at the bottom right contains list of fields that are defined in the template but not available in the image. You can remove or replace the fields in the bottom right list with those available for the image. You can also replace fields in the *Template Fields* list with fields available in the image.



To change a Template Field:

1: In the *Template Fields* panel, select the row containing the field you want to change.

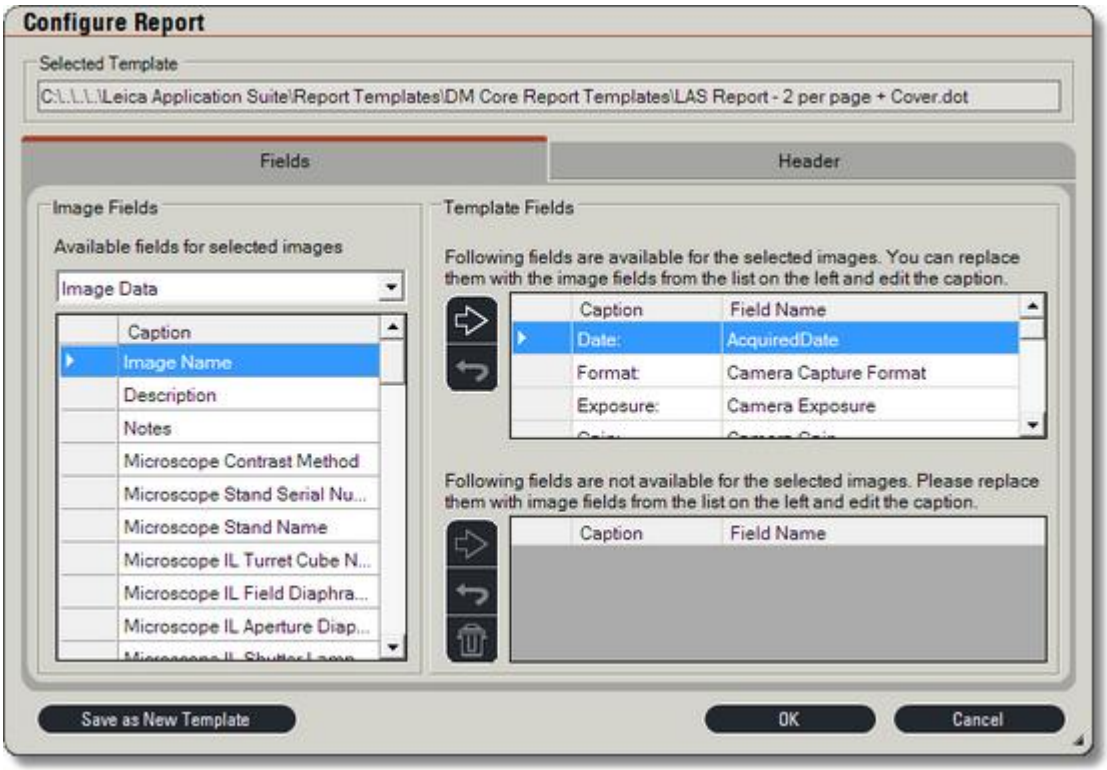
2: In the *Image Fields* panel, select the field you want to use instead.

3: Click the arrow to make the replacement.

4: Back in the *Template Fields* panel, click in the appropriate *Caption* field and enter a suitable caption.

5: Click *OK*.

Some reports only display this extra information on the cover page, others display it in the header of every page in the report.



Some report templates allow for extra Summary/Header fields to be displayed. Header fields are data items that are not part of image meta data (or fields).

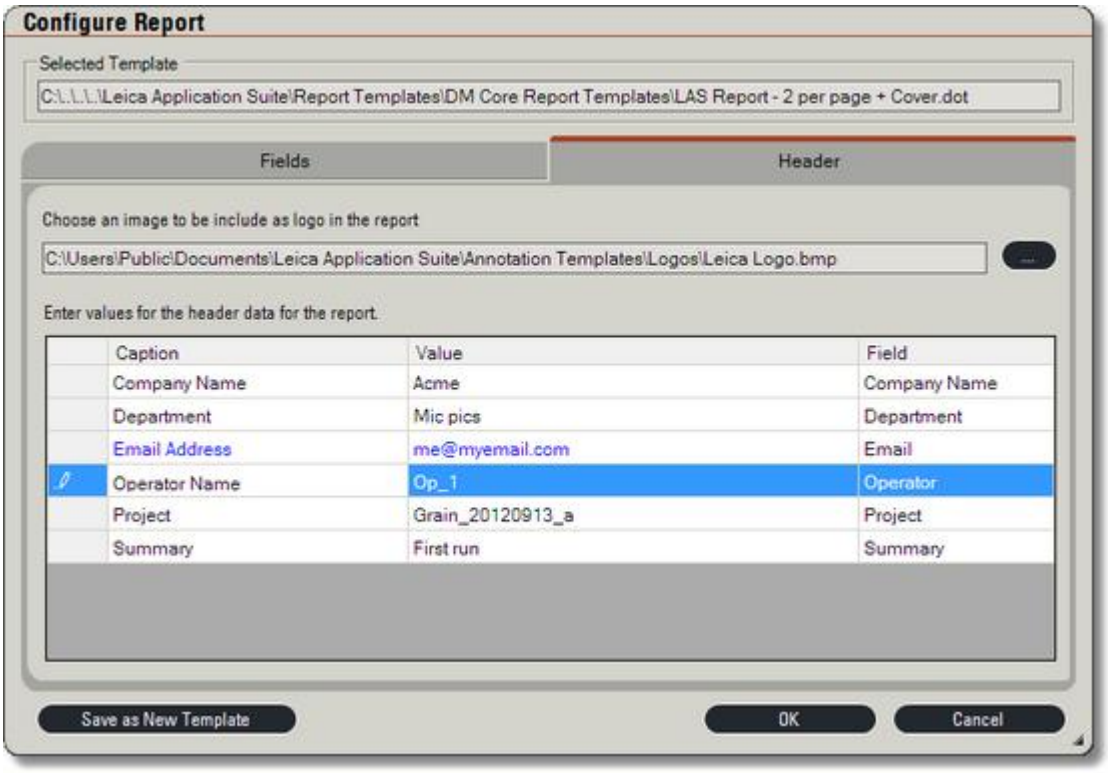
To change a Header/Summary field:

1: If you want a particular logo displayed, click the browse button, navigate to and select your logo file in the resulting Windows dialog and click *Open*.

2: Edit the text in the *Caption* and *Value* fields as appropriate.

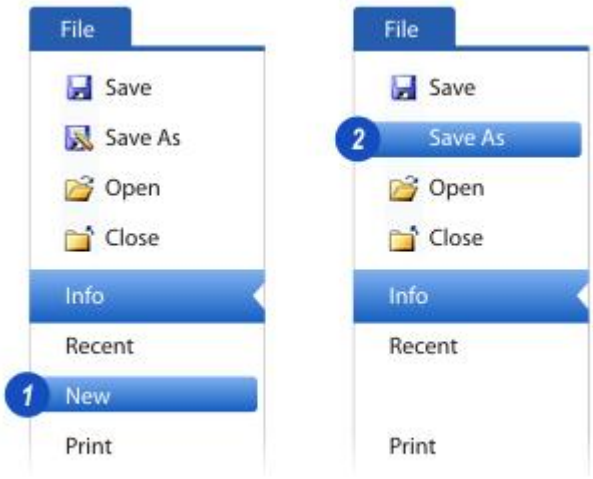
3: Click *OK*.

Some reports only display this extra information on the cover page, others display it in the header of every page in the report.



LAS Standard Report Templates can be modified to either remove fields or include others, but there are occasions when a new, bespoke report is a more appropriate solution.

Both LAS and Word must be active. The screen images included here have been created using Word 2010; Earlier versions may have slightly different dialogs but the process is the same.



1: Open *Word* and create a new document.

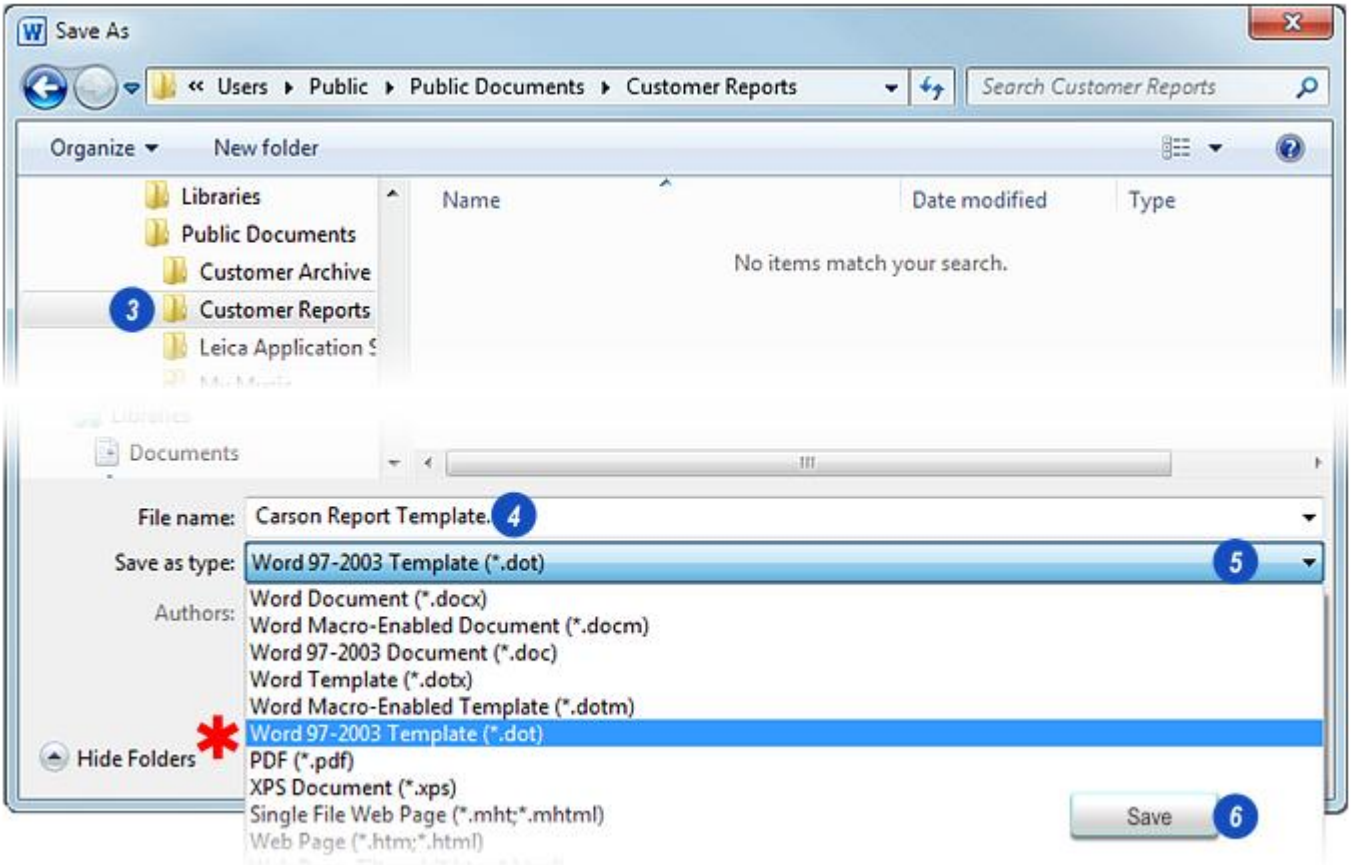
2: Click *Save As* and...

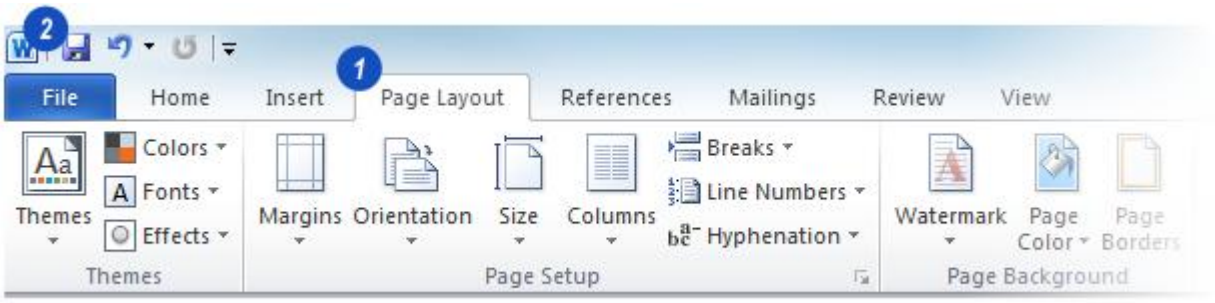
3: ...navigate to a folder of choice,...

4: ...give the document an appropriate name and...

5: ...from the *Save as type* drop-down menu, click to select *Word 97-2003 Template (*.dot).* This ensures compatibility across different versions of Word.

6: Click *Save*.





1: Click on the Word *Page Layout* tab and:

- Select a Margin: Use standard layout or set up new ones.
- Choose Portrait or Landscape: From the page *Orientation* options.
- Select the page Size. Format A4 is widely used but regions will have their own requirements.
- Select the number of Columns: The new template can use columns which is useful if there are a number of small images that need to fitted on to a page.
- *The Watermark, Page Colour and Border* features found in the *Page Background* section can be used in a template.

2: When the page format is complete, click the *Save* button.

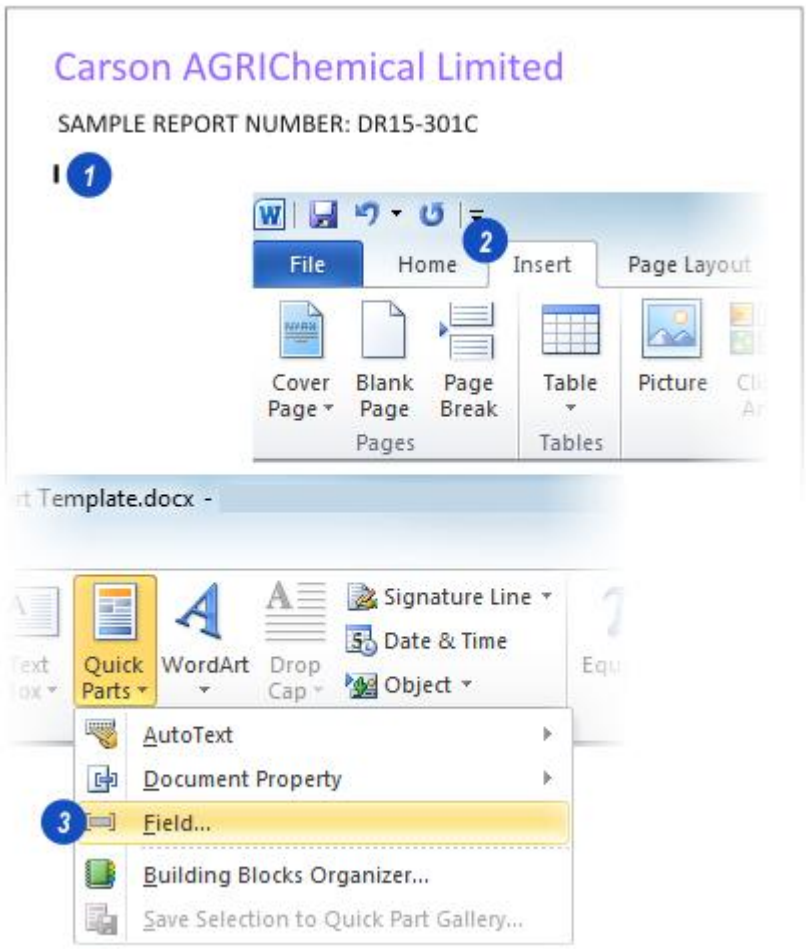
The report template can have as many lines of 'static' text - headings, corporate disclaimers, terms of business etc. - as required. These are items that remain unchanged unless specifically altered before the template is used to create a report.

In the example a heading and sub-heading have been added to the template.

Simply type a heading or any other line(s) of text and if required, format it with the usual Word facilities. In the example the font size has been increased to 11 point, the style changed to bold and the text filled with blue.

The template is a link between Leica Application Suite and the report itself. Special template fields - easily recognised by the « and » characters that enclose them - mark where image data from LAS is to be inserted into the report. Any of the LAS data items can be used.

The part of the template that contains the fields is marked with a top and a bottom 'boundary' fields; They are called *TableStart* and *TableEnd* respectively - no space between the words. The *TableStart* field must appear before any other fields and data fields cannot occur after *TableEnd*.



Like all other fields, *TableStart* and *TableEnd* are enclosed with the « and » markers and are placed on the template using the Word *Field Insert* procedure as follows:

1: On the new template move the cursor to where the data field is to be inserted.

2: Click on the Word *Insert* tab

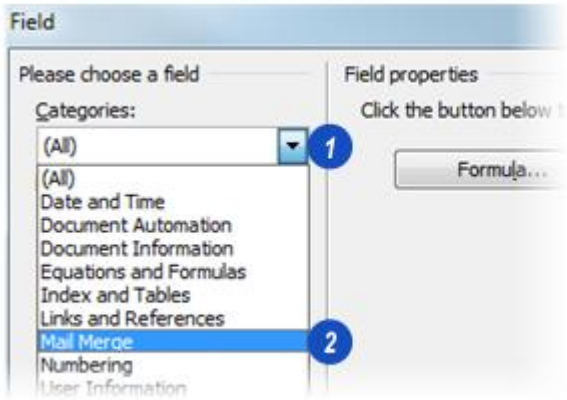
3: Locate the *Field Insert* feature - it varies with the Word version - and select it.

Select Mail Merge

This step sets up the *Field Insert* feature to use the *Mail Merge* option. This setting is retained whenever *Field Insert* is selected unless changed by the user:

1: On the *Field* dialog, click on the small arrow to the right of the *Categories* header.

2: From the drop-down menu click to select the *Mail Merge* option.



When the Word *Field* dialog appears:

1: On the *Mail Merge* menu, click to select *Merge Field*.

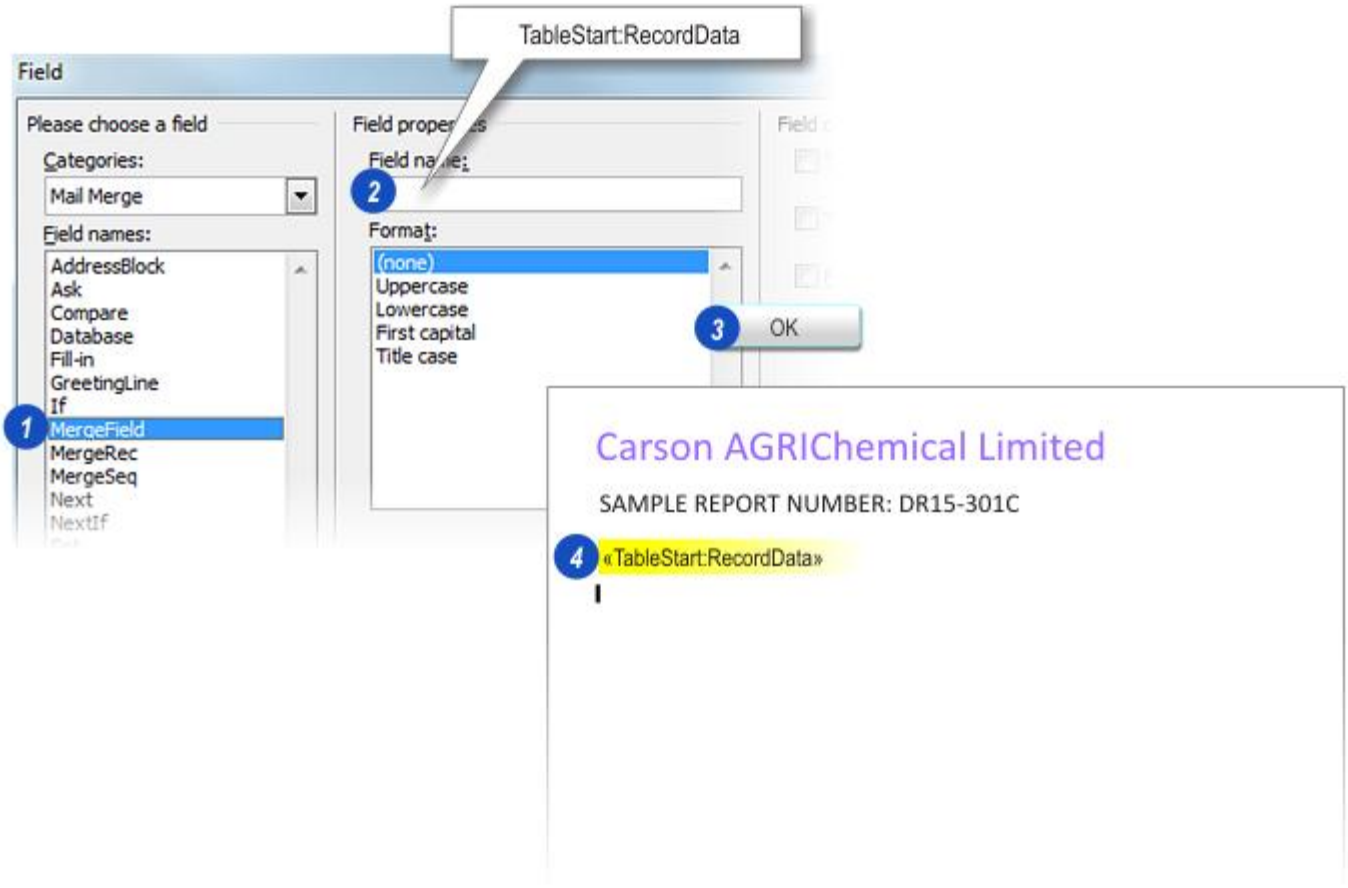
2: Click inside the *Field properties* text box and type:

TableStart:RecordData

- There are no spaces between the words,
- A colon between *TableStart* and *RecordData* and
- No need to type the « and » markers.

3: Click *OK.*

4: The *TableStart* field appears at the cursor on the template with the markers inserted.



Again, the Word *Field Insert* facility is used to create fields that will display the data imported from an *LAS Archive.*

It is a 2-step process:

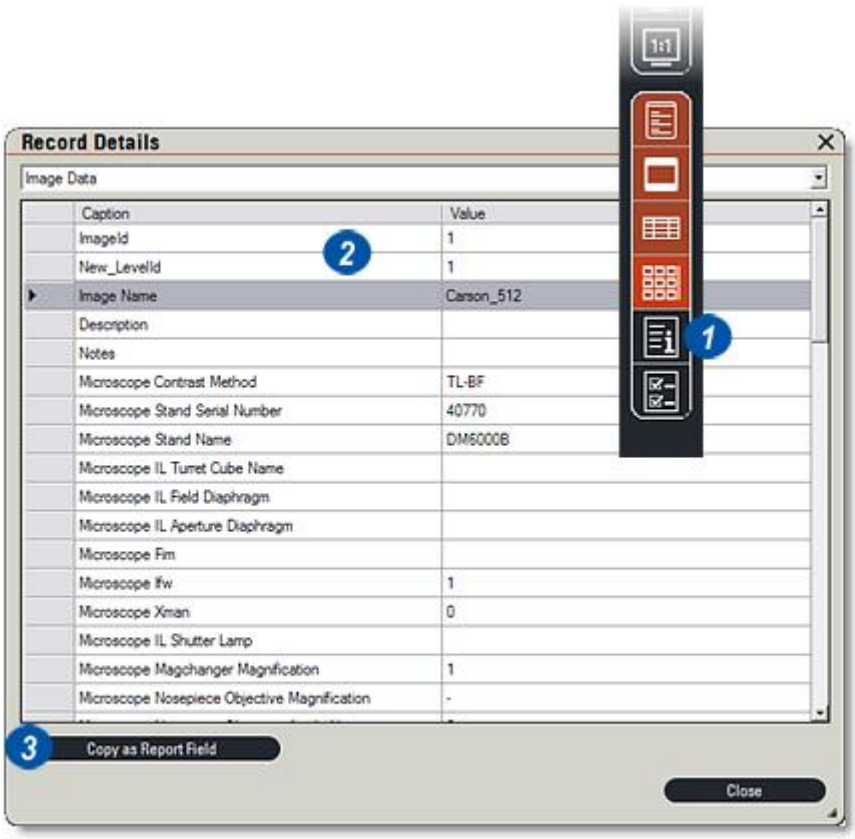
- *Copy* the field name from LAS.
- *Create* a field on the template and link it to the LAS Archive field by pasting the LAS field name into it.

LAS must be running in the *Browse Workflow* with the required archive selected.

1: On *LAS > Browse* click on the *Field Information* button on the *Side Toolbar.*

2: The *Record Details* dialog appears which lists all of the fields available for that archive. In the example the Image Name field is selected to be copied to the new template in Word.

3: *LAS Field Names* are not the same as the captions displayed on the *Record Details* dialog. – they can only be retrieved by using the *Copy as Report Field* function which copies the real field name to the *Windows Clipboard*. From there it is available to Word.



This step copies the LAS Field Data link from the clipboard and places it on the new template:

1: Position the cursor on the new template where the filed data is to appear.

2: Click to select the *Merge Field* option on the *Field Names* menu.

3: Click inside the *Field name* text box and...

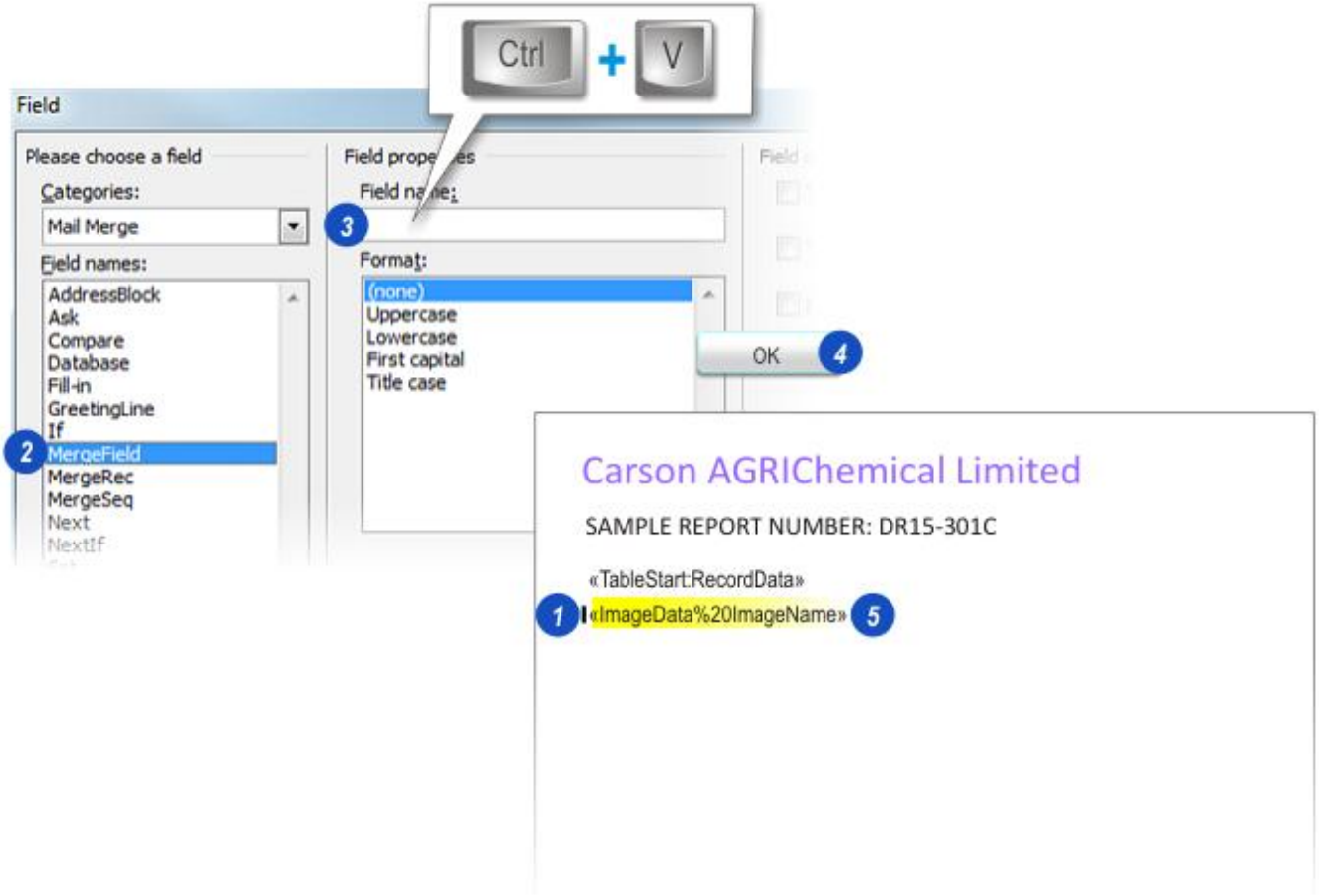
- Press and hold down the Ctrl key.
- Press the V key

Some versions of Word may have a paste button available that can be used instead of the Ctrl+V combination.

The LAS Field Data link appears in the text box.

4: Click *OK*.

5: The LAS Field Data link complete with markers appears on the new template.



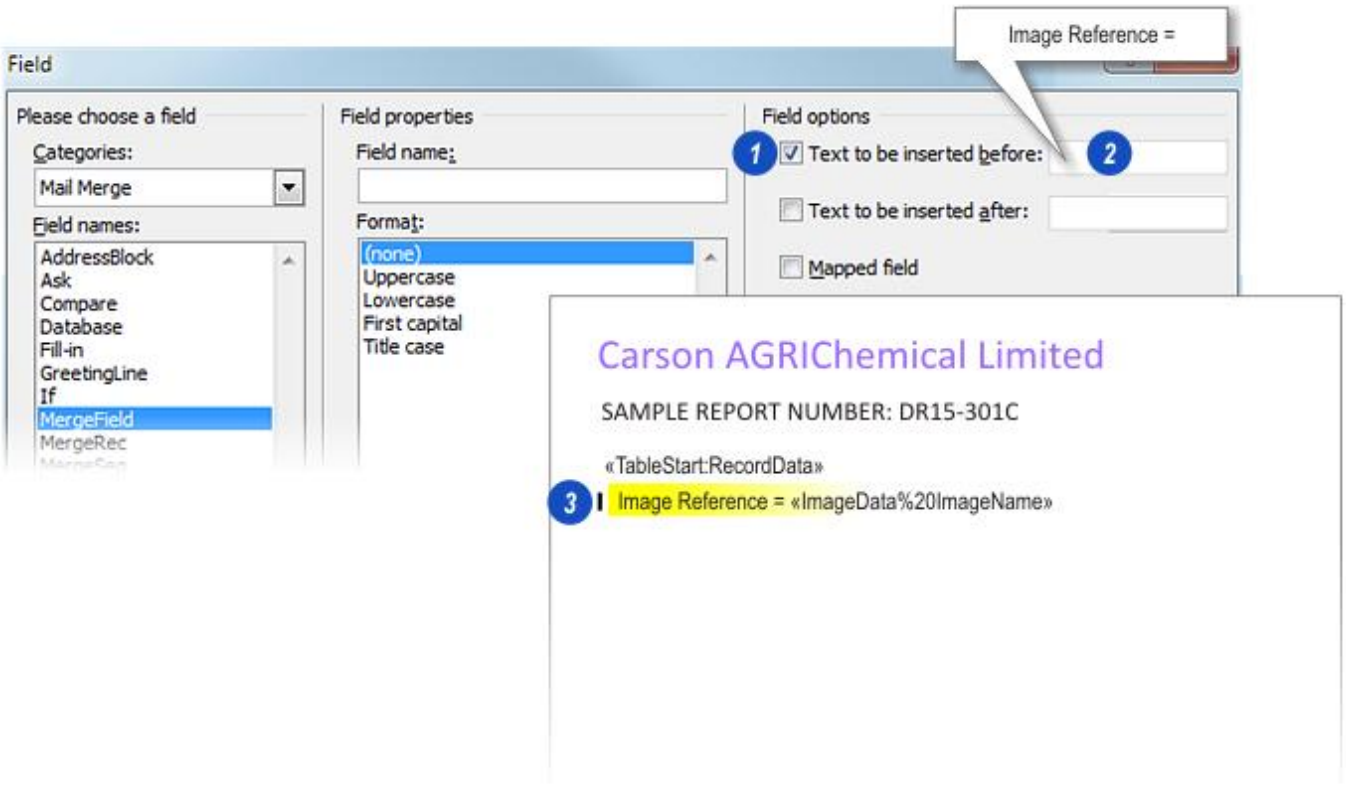
Insert a Field Prefix

A prefix can be added before the Field Data as a descriptor that will appear on the display and printout:

1: Click to check the *Field Options* > *Text* to be inserted check box.

2: Click inside the text box and type the prefix - in this case the words *Image Reference =.*

3: When the *Field Data* is inserted into the template the prefix appears before the data.



The Word *Date Field* is dynamic - the date automatically displays the date today when the report is printed or displayed:

On the *Insert* > *Field* dialog:

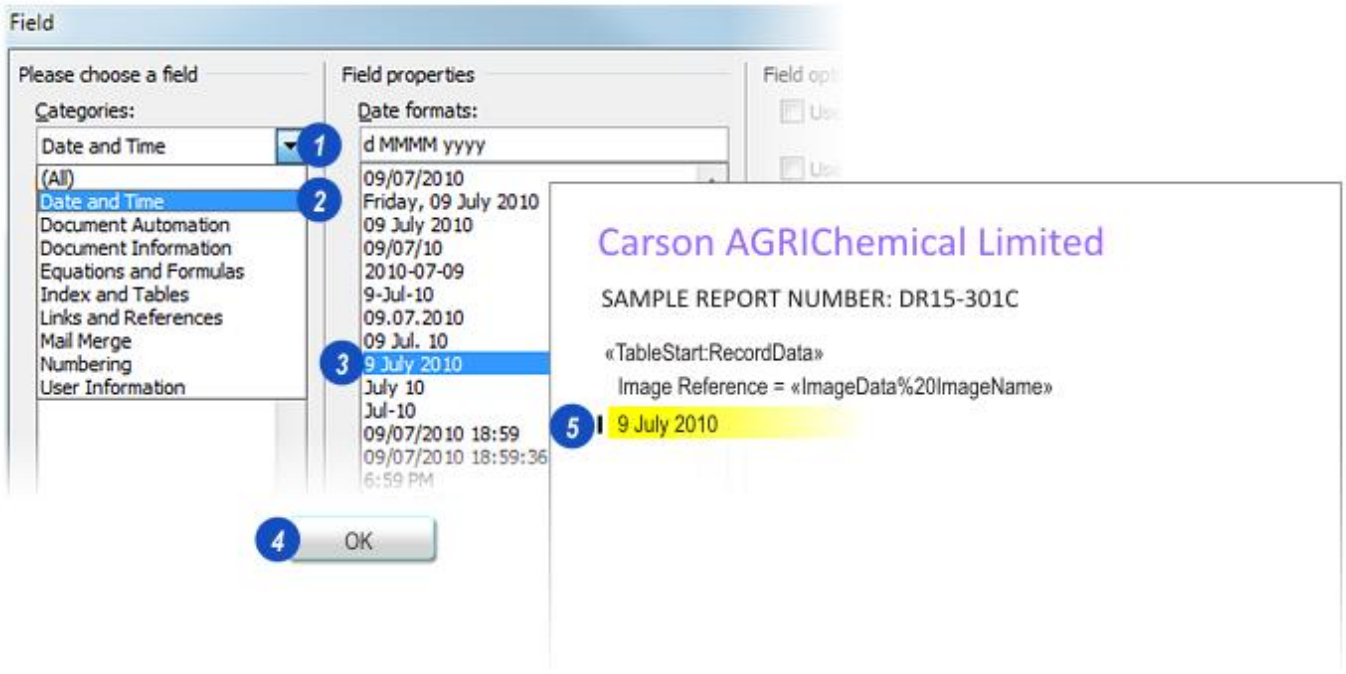
1: Click the small arrow to the right of the *Categories* header and...

2: ...from the drop-down menu click to select *Date and time.*

3: The *Date formats* menu appears; Click to select the format required.

4: Click *OK*.

5: The date appears on the template.



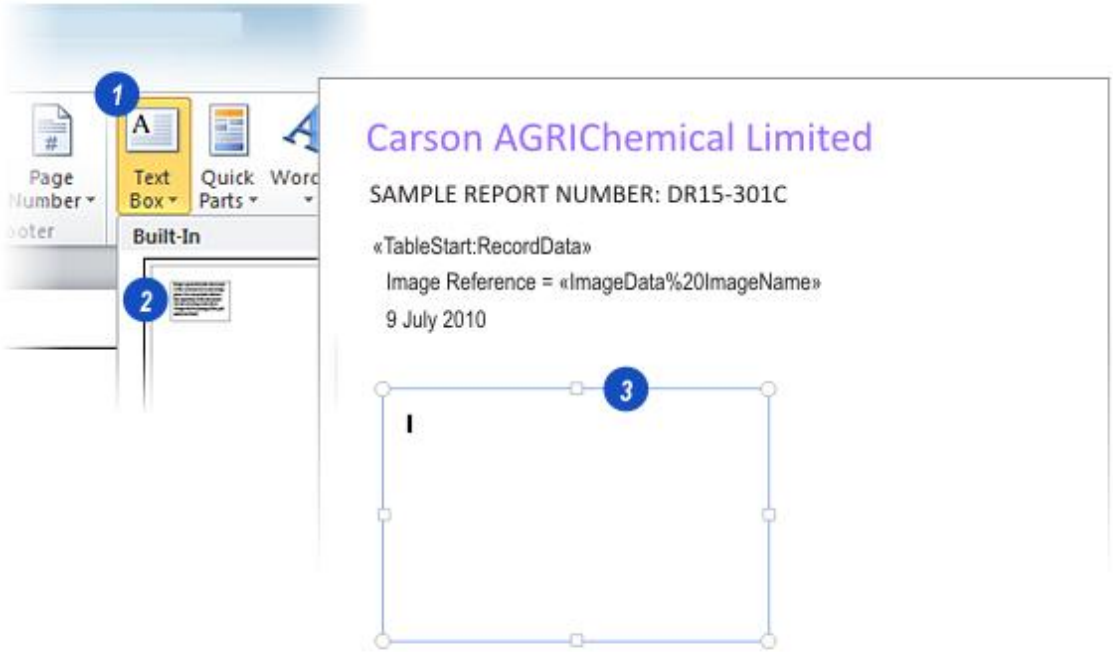
Add an Image

Report images are contained with Word *Text Boxes*. The first step is to create a text box:

1: On the Word *Insert* tab, click to select *Text Box.*

2: If text box styles are available in the Word version, click to select *Simple Text Box.*

3: Using the handles that surround the text box, re-position and re-shape the text box to contain and limit the extent of the image. Images are automatically scaled to fit inside the text box.



Next, a field that draws the image is created within the *Text Box*.

1: Click inside the *Text Box* and on the *Insert* > *Field* dialog...

2: ...click to select *MergeField* from the *Field names* list.

3: Click inside the *Field name* text box and type:

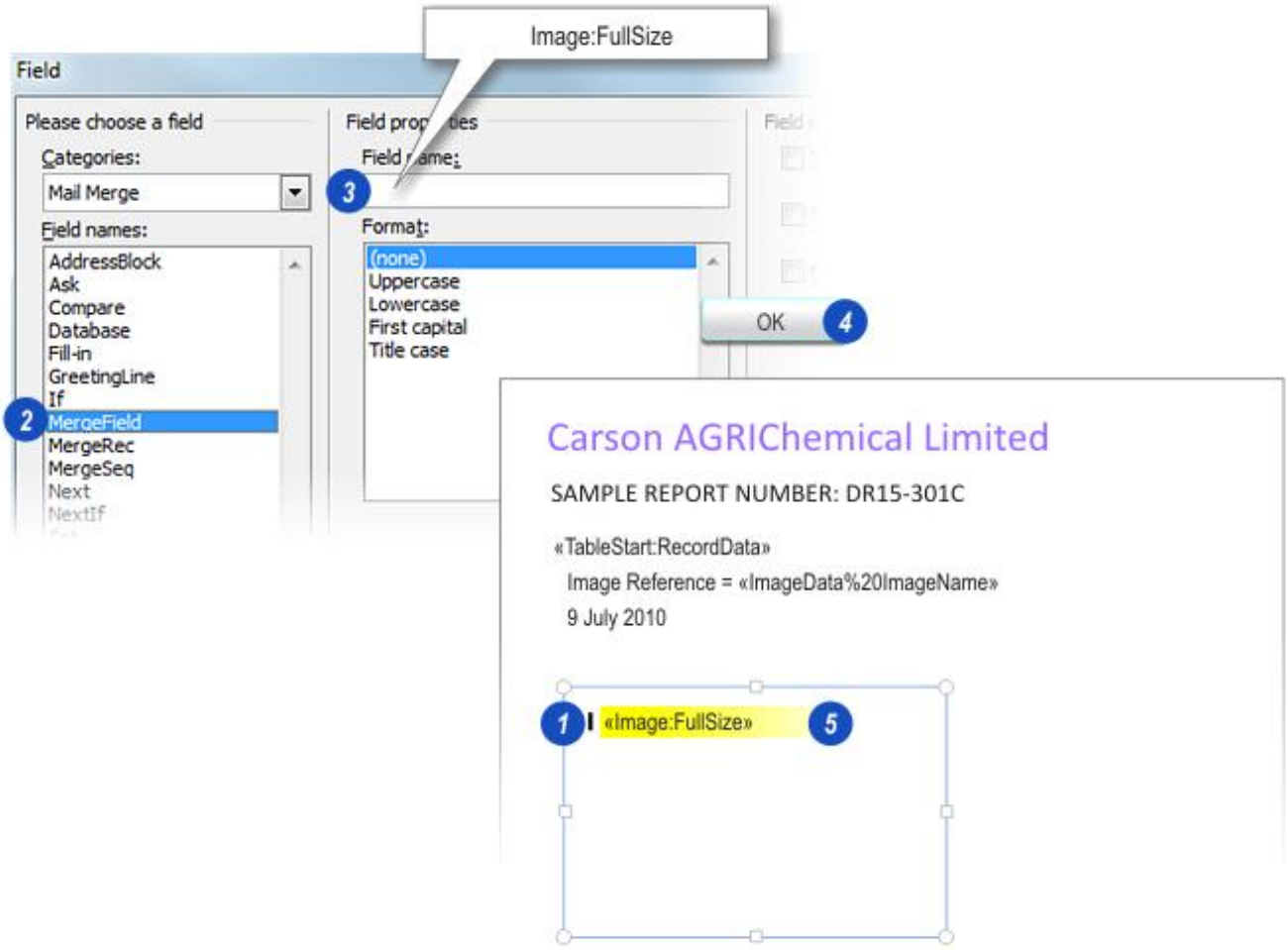
Image:FullSize

- A colon between *Image* and *FullSize*.
- No space between *Full* and *Size*.
- No need to add the « and » markers.

4: Click *OK*.

5: The field appears inside the text box.

Only a single image text box is required on the new template even if it is required to show multiple images. LAS will export all of the image selected in the Gallery, drawing and placing them in sequence on the report. New report pages are created automatically as they are required.



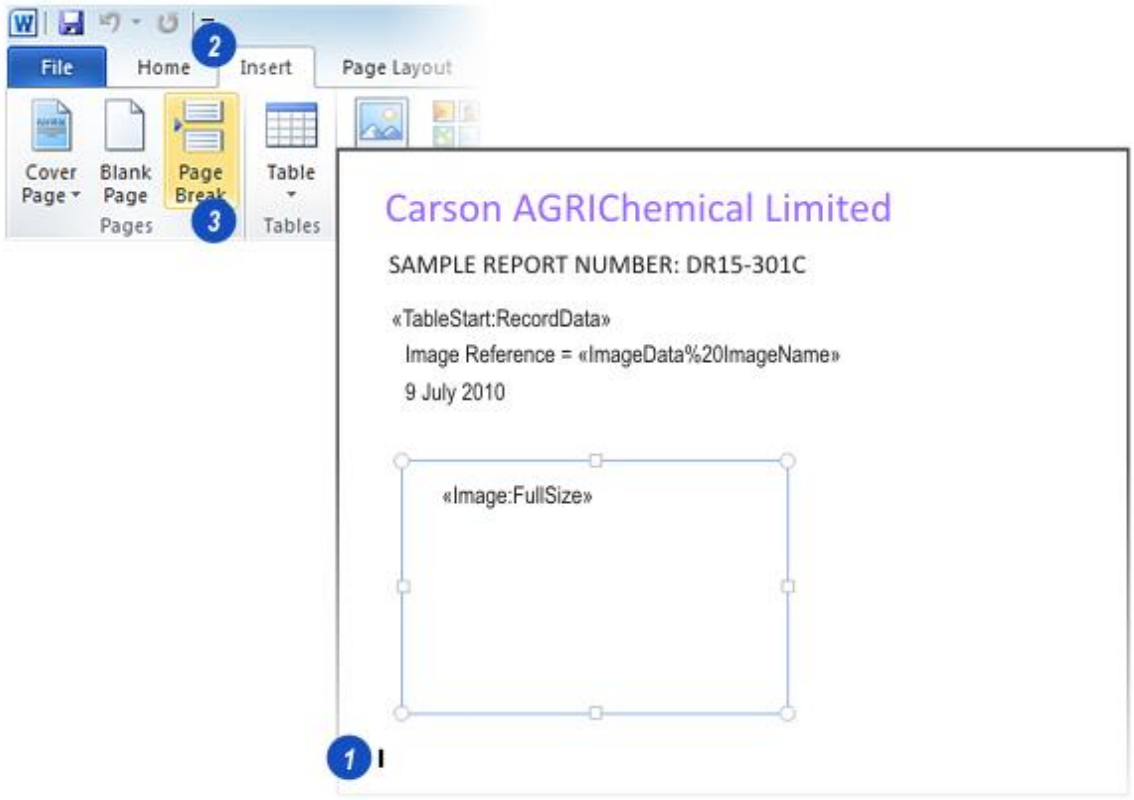
Insert a Page Break

If only a single image is to be displayed on each report page, a *Page Break* can be inserted after the fields and image text box to force a new page for the next image.

1: After the fields and image text box, click to mark where the *Page Break* is to occur.

2: Click on the Word *Insert* tab.

3: Click the *Page Break* option. This will vary with different versions of Word.



The final step in creating a new template is to indicate the end of the data. *Field* Insert is used again:

On the Word *Field* dialog:

1: On the *Mail Merge* menu, click to select *MergeField*.

2: Click inside the *Field properties* text box and type:

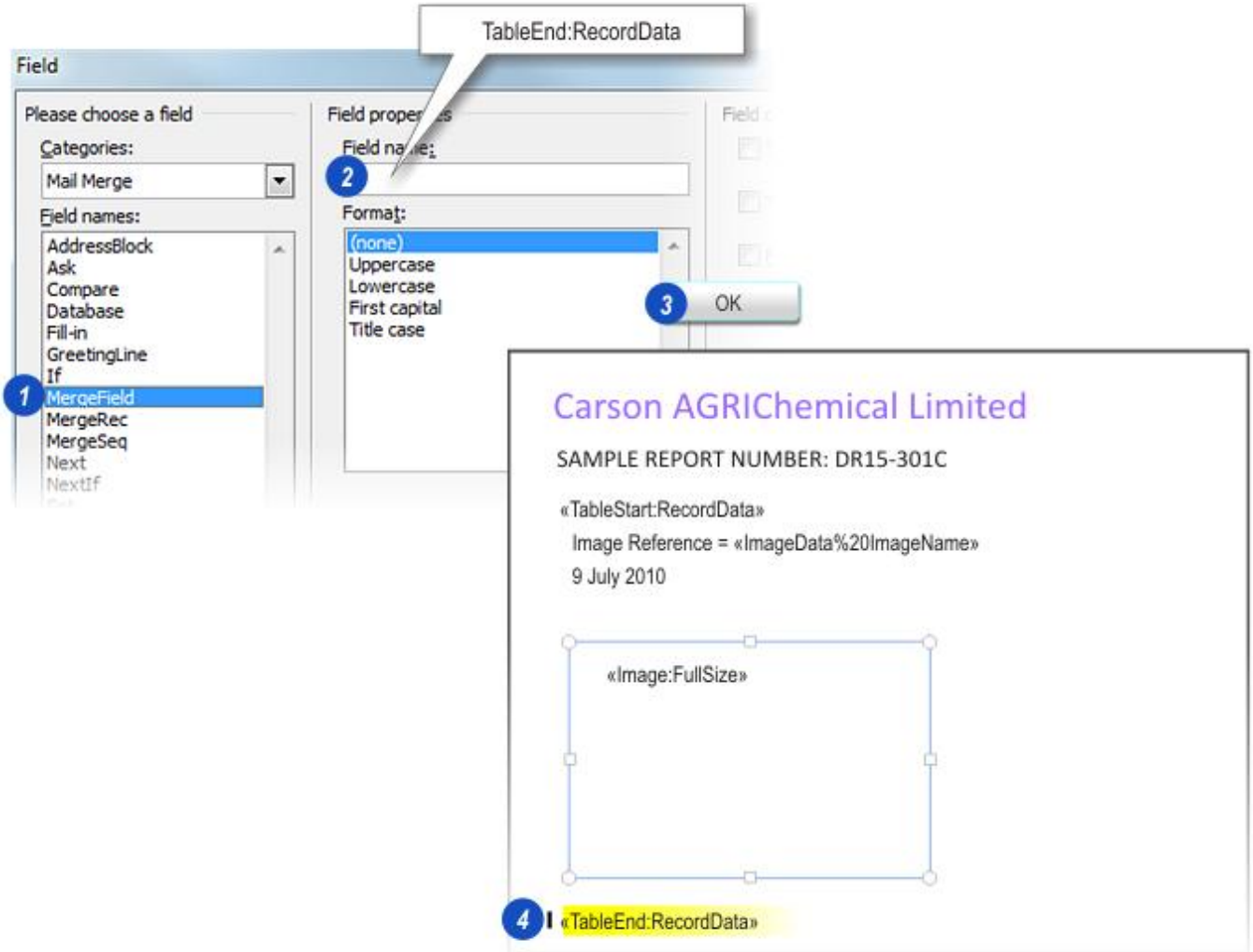
TableEnd:RecordData

- There are no spaces between the words,
- A colon between *TableEnd* and *RecordData* and
- No need to type the « and » markers.

3: Click *OK.*

4: The *TableEnd* field appears at the cursor on the template with the markers inserted.

Finally, save the new template, close Word and test it: *Go there...*



Available in *Acquire, Browse* and *the Process* Workflows, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

The action toggles between horizontal and vertical docking.

Scroll bars, if required, are placed automatically.

The Setup Workflow

The *Setup Workflow* provides the method of specifying the Leica microscope components connected to Leica Application Suite and, when the optional LAS Archive modules are used, creating archives to capture images and data.

Components such as objective types and filter descriptions can be readily selected, saved and subsequently recalled.

In addition, ‘fine tuning’ of some of the microscope features can be performed such as parfocality, setting the focus step size for each lens.

Upon leaving the *Setup Workflow*, the new items/settings are permanently stored.

The major types of Leica microscope that are used with LAS are described in separate sections.

- *Image Explorer and LAS Archive* 145
- *Stereo Microscope Setup* 146
- *DM Microscope Setup* 168

If you are an LAS *Core* user without optional *LAS Archive* modules installed, please ignore this topic.

If LAS optional module *Archive* – either *Basic* or *Standard* – is installed, the *Archive* tab appears on the *Setup Workflow.*

1: If necessary click on the tab to reveal the control panels.

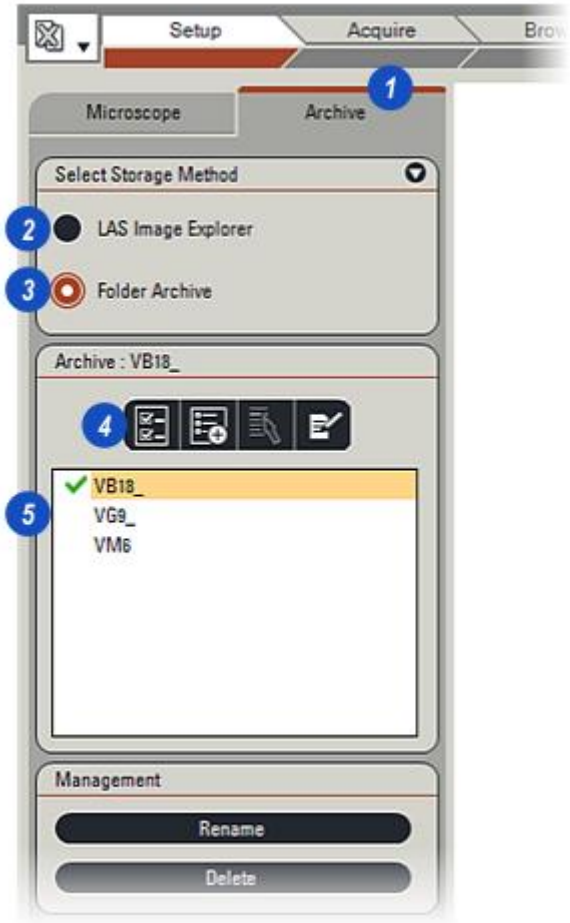
2: Because two image storage methods are available (Image Explorer and LAS Archive) the *Select Storage Method* facility allows either to be selected. The buttons are mutually exclusive so it is not possible to use both methods at the same time.

Click *LAS Image Explorer* to use tree-and-folders navigation or…

3: …click *Folder Archive* to use archives.

4: The *Archive Toolbar* and…

5: …the *Archive List* window are active only when Archive is selected.

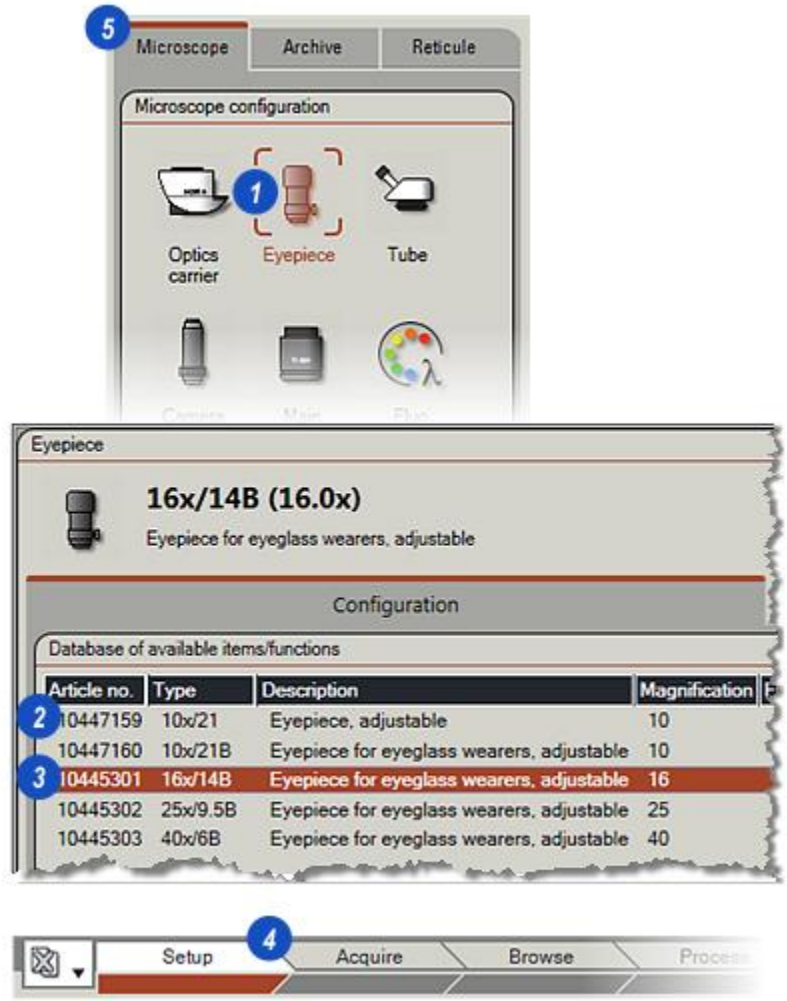


Stereo Microscope setup and configuration is a fast and simple exercise due to the intuitive interface and concise option lists.

The setup program is part of Leica Application Suite that is able to 'read' data from the microscope and sort and display setup options that are available and appropriate.

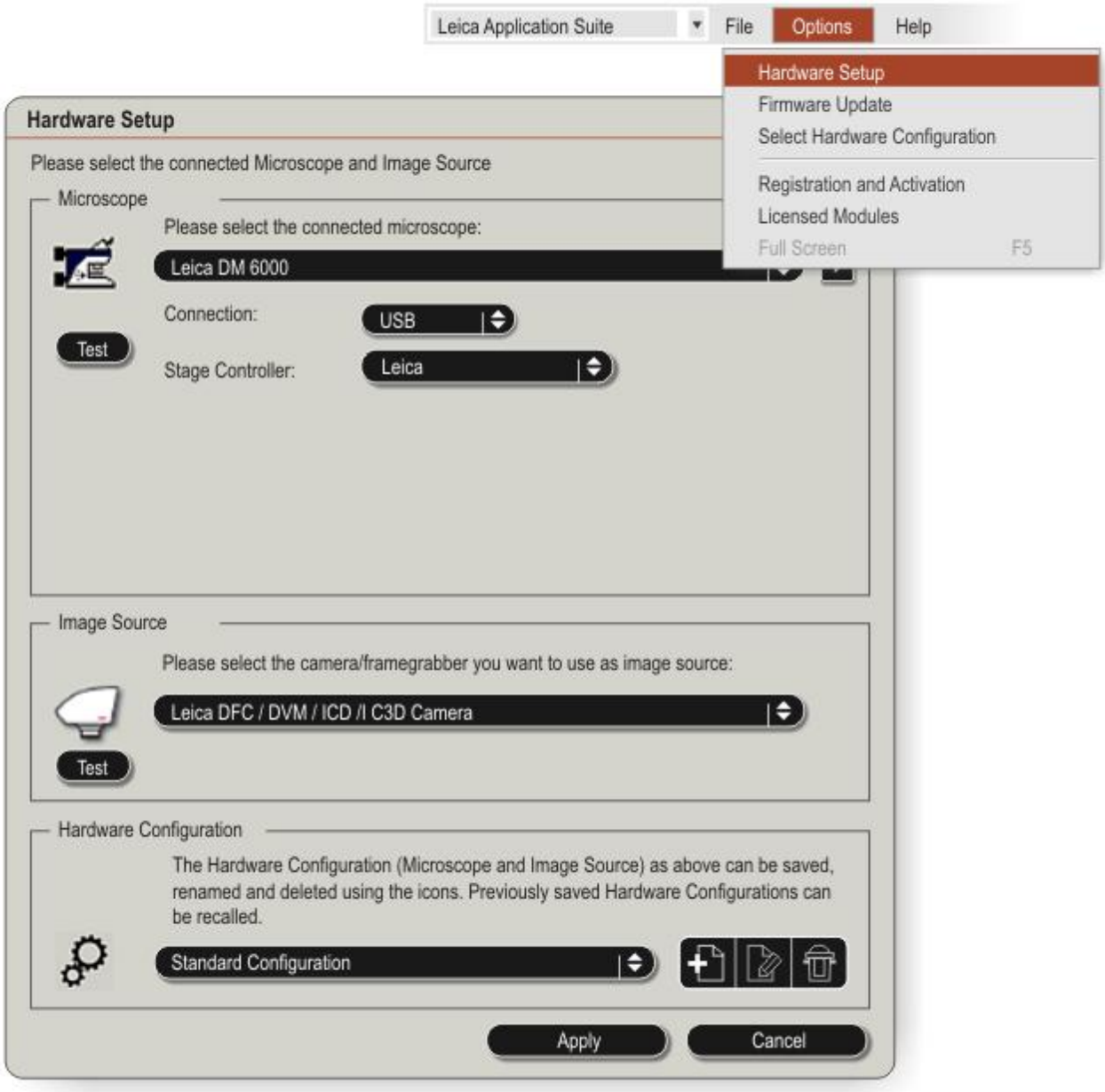
Each setup parameter is displayed as an icon on the *Microscope Configuration* panel **(1)** - clicking it reveals the options **(2)** and clicking an entry on the list **(3)** assigns all of the chosen attributes to the parameter.

The *Stereo Microscope Setup* dialog is reached by clicking on the *Setup Workflow* **(4)** and then on the *Microscope* tab **(5)**.



Before starting the *Stereo Microscope Setup*, the stand and camera have to be configured for use with Leica Application Suite and optionally, a new *Hardware Configuration* created.

Go there... 26



There are two selection panel styles in the *Stereo Microscope Setup*:

Single panel in which all of the options are displayed as a list and simply clicking on the highlighted entry selects it, and

Dual panel in which a range of controls are shown on the upper panel (**A** on the illustration) and depending upon the selection, a list of options is displayed in the lower panel (**B** on the illustration.).

The example shows the setup screen for the SmartTouch. The controls - keys, touch screen and Dual Rotary Actuator are listed in the upper panel with references to the image on the right - and the various functions that can be applied to the selected control are shown in the lower panel.

1: Click a control to select and highlight it.

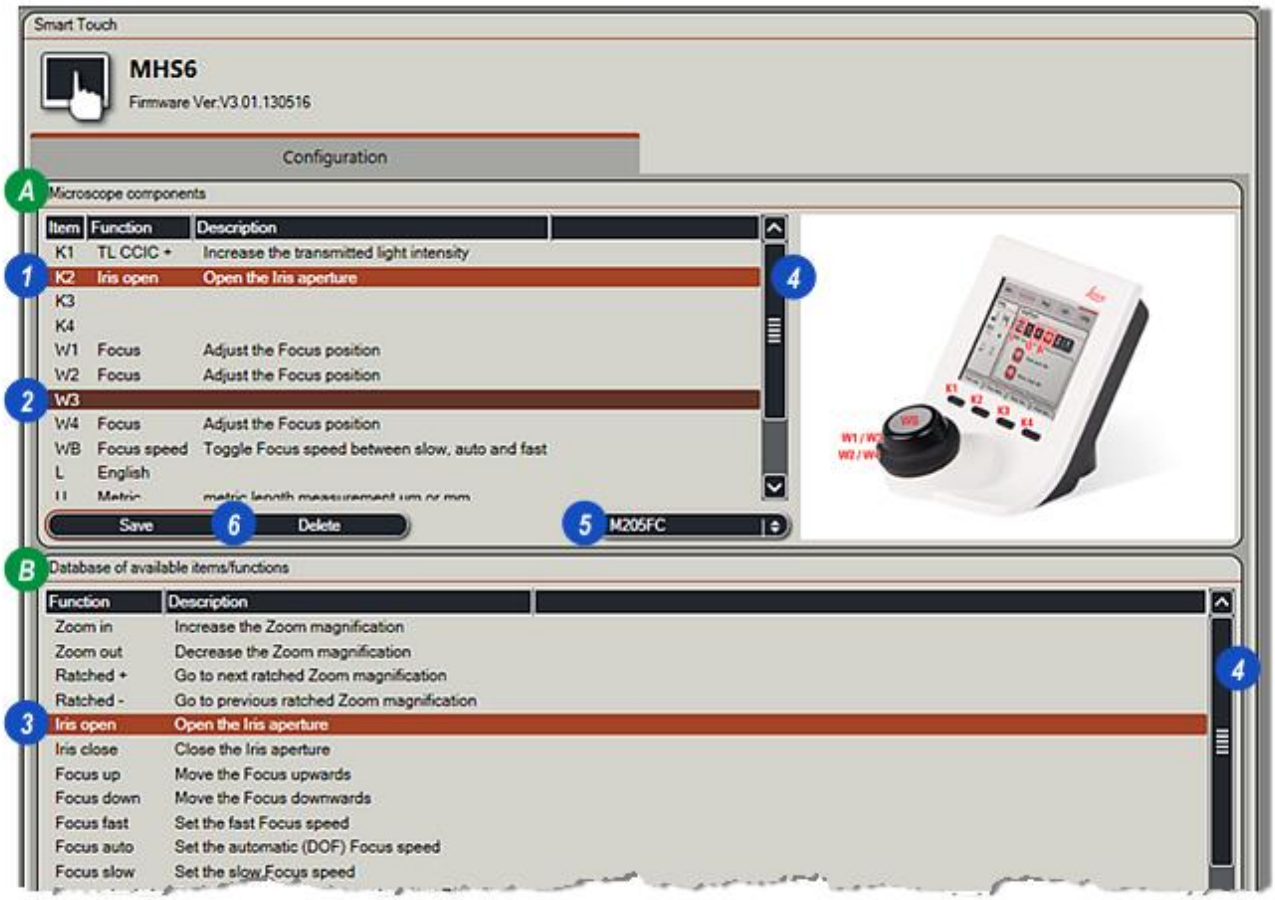
2: As the mouse moves across the items in the list they are highlighted in dark brown.

3: Clicking on an option in the lower panel assigns the function to the control in the upper panel. In the illustration, *Hardware Key K2* is selected in the upper panel and when pressed, it will *Open the Iris*. It could have been assigned any of the options in the lower panel to suit the user.

4: Long option or control lists automatically display a *Scroll Button*. Click on the button and drag it downward to view more list entries.

5: Different users will have different preferences for the way in which controls operate. Each user can store his preferences in a *Configuration* that can be recalled and loaded every time he uses the equipment.

6: Configurations are saved and if necessary, deleted using the buttons.



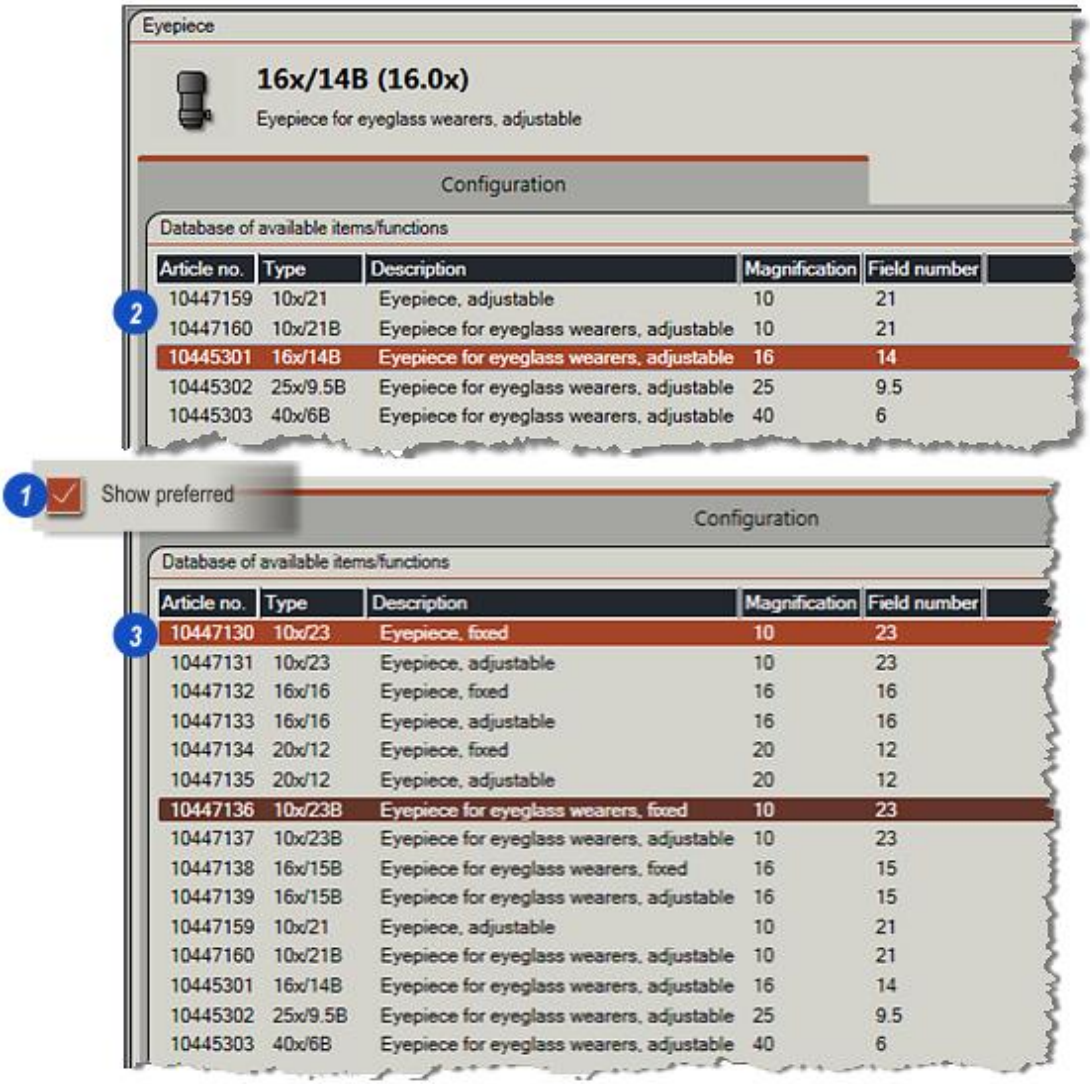
The available options for some microscope features are grouped as *preferred* - those recommended as better suited to the setup - and a wide range of additional options that will not be preferred.

1: A checkbox appears bottom-left of the options panel...

2: ...toggles between displaying only the *preferred* options - checkbox enabled - and...

3: ...all of the additional options - checkbox disabled.

Click on the checkbox to toggle enable/disable.



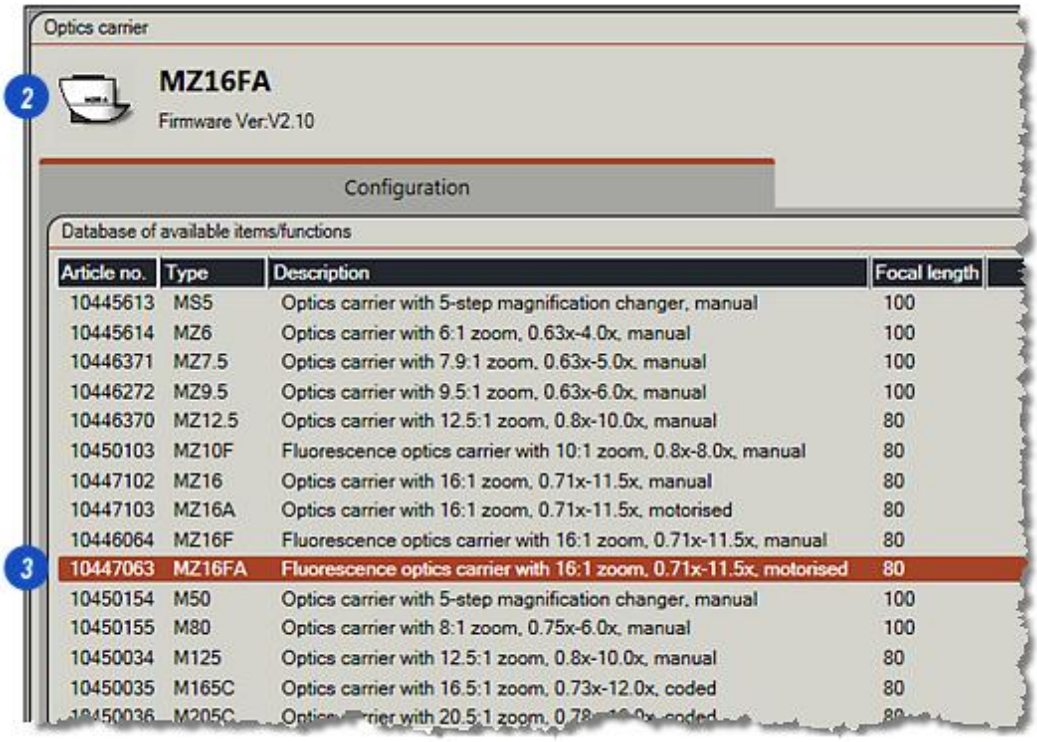
In most cases, the *Optics Carrier* type is detected by the software, highlighted on the database list but cannot be changed.

For microscopes on which assignment is possible:

1: Click on the *Optics Carrier* icon on the configuration panel.

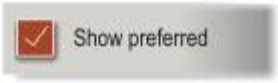
2: The current setting is displayed on the header.

3: Click to select a new setting from the list.



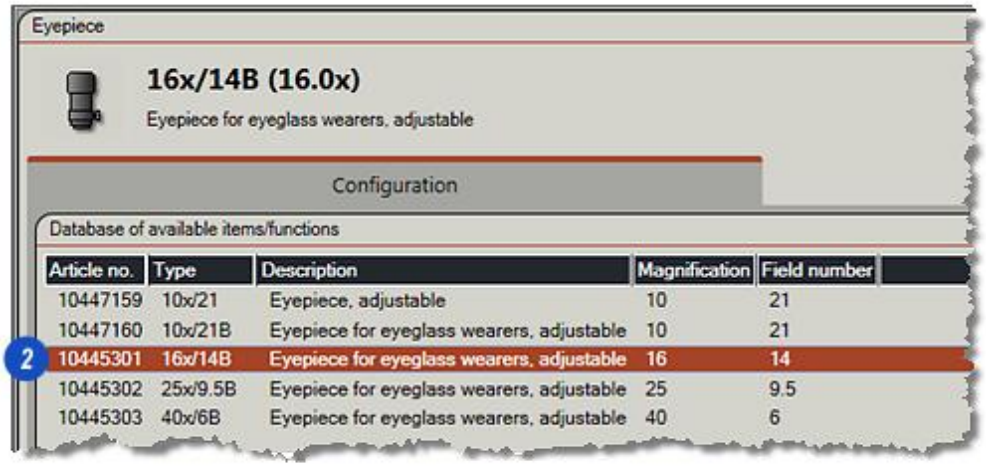
Article no.	Type	Description	Focal length
10445613	MS5	Optics carrier with 5-step magnification changer, manual	100
10445614	MZ6	Optics carrier with 6:1 zoom, 0.63x-4.0x, manual	100
10446371	MZ7.5	Optics carrier with 7.9:1 zoom, 0.63x-5.0x, manual	100
10446272	MZ9.5	Optics carrier with 9.5:1 zoom, 0.63x-6.0x, manual	100
10446370	MZ12.5	Optics carrier with 12.5:1 zoom, 0.8x-10.0x, manual	80
10450103	MZ10F	Fluorescence optics carrier with 10:1 zoom, 0.8x-8.0x, manual	80
10447102	MZ16	Optics carrier with 16:1 zoom, 0.71x-11.5x, manual	80
10447103	MZ16A	Optics carrier with 16:1 zoom, 0.71x-11.5x, motorised	80
10446064	MZ16F	Fluorescence optics carrier with 16:1 zoom, 0.71x-11.5x, manual	80
10447063	MZ16FA	Fluorescence optics carrier with 16:1 zoom, 0.71x-11.5x, motorised	80
10450154	M50	Optics carrier with 5-step magnification changer, manual	100
10450155	M80	Optics carrier with 8:1 zoom, 0.75x-6.0x, manual	100
10450034	M125	Optics carrier with 12.5:1 zoom, 0.8x-10.0x, manual	80
10450035	M165C	Optics carrier with 16.5:1 zoom, 0.73x-12.0x, coded	80
10450036	M205C	Optics carrier with 20.5:1 zoom, 0.78x-12.0x, coded	80

Eyepiece selection has a single list of options that will show either the preferred (checked) or additional options (unchecked):

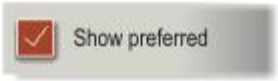


1: Click on the *Eyepiece* icon on the configuration panel.

2: Scroll to the required setting and click to select it.

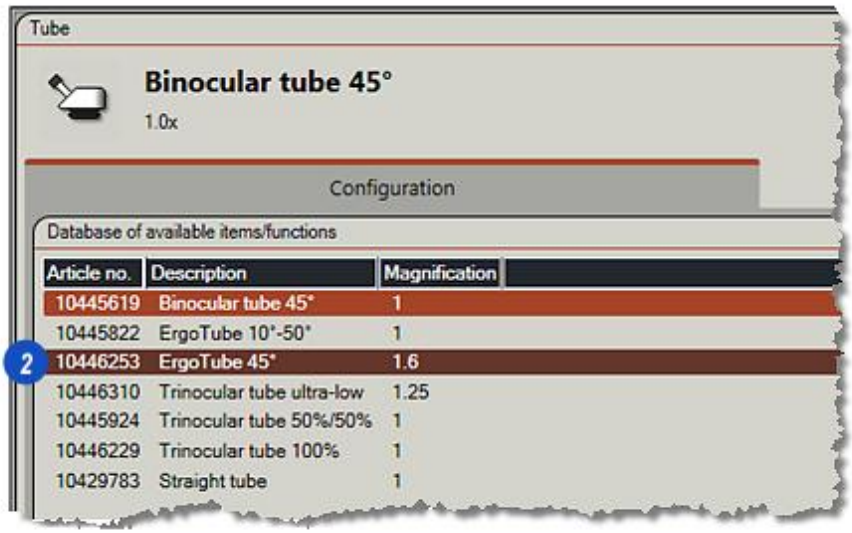


Tube Selection has a single list of options that will show either the preferred (checked) or additional options (unchecked):

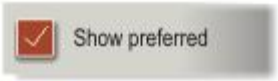


1: Click on the *Tube* icon on the configuration panel.

2: Scroll to the required setting and click to select it.

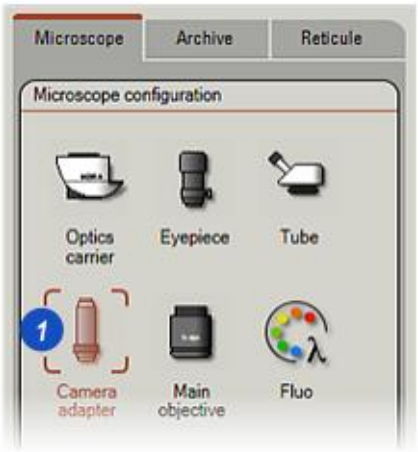


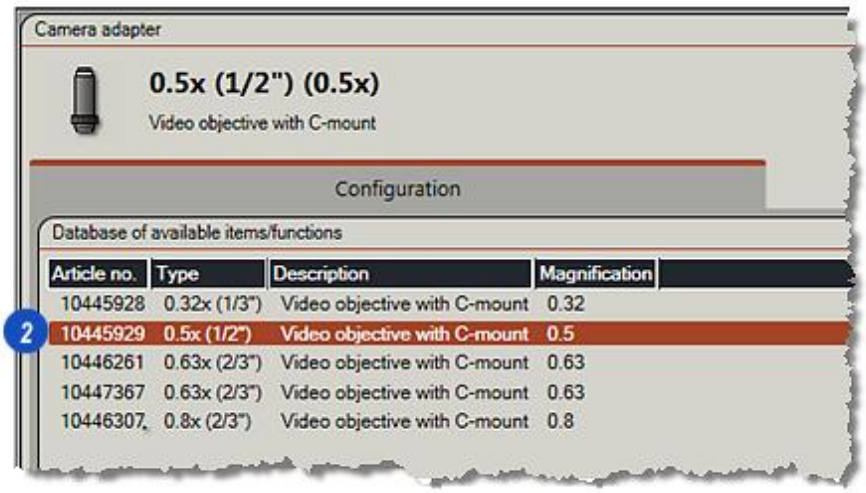
The *Camera Adapter* has a single list of options that will show either the preferred (checked) or additional options (unchecked):



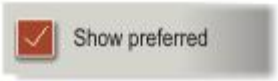
1: Click on the *Camera Adapter* icon on the configuration panel.

2: Scroll to the required setting and click to select it.



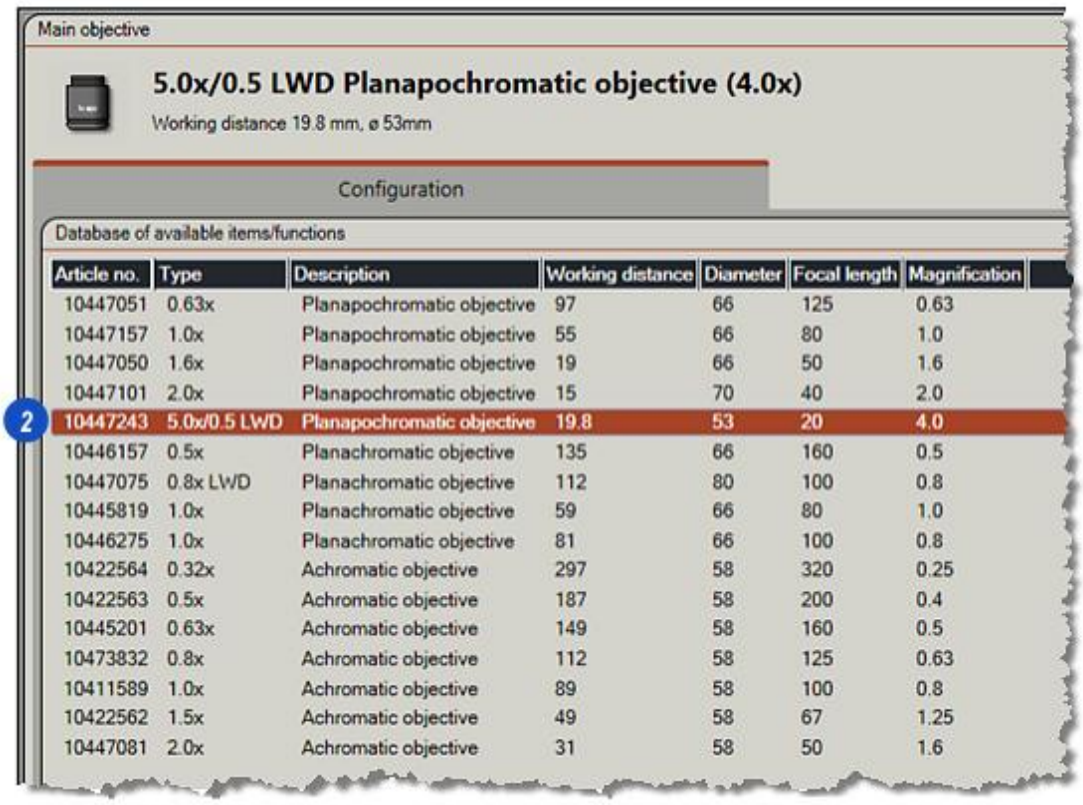


The *Main Objective* has a single list of options that will show either the preferred (checked) or additional options (unchecked):



1: Click on the *Main Objective* icon on the configuration panel.

2: Scroll to the required setting and click to select it.



Article no.	Type	Description	Working distance	Diameter	Focal length	Magnification
10447051	0.63x	Planapochromatic objective	97	66	125	0.63
10447157	1.0x	Planapochromatic objective	55	66	80	1.0
10447050	1.6x	Planapochromatic objective	19	66	50	1.6
10447101	2.0x	Planapochromatic objective	15	70	40	2.0
10447243	5.0x/0.5 LWD	Planapochromatic objective	19.8	53	20	4.0
10446157	0.5x	Planachromatic objective	135	66	160	0.5
10447075	0.8x LWD	Planachromatic objective	112	80	100	0.8
10445819	1.0x	Planachromatic objective	59	66	80	1.0
10446275	1.0x	Planachromatic objective	81	66	100	0.8
10422564	0.32x	Achromatic objective	297	58	320	0.25
10422563	0.5x	Achromatic objective	187	58	200	0.4
10445201	0.63x	Achromatic objective	149	58	160	0.5
10473832	0.8x	Achromatic objective	112	58	125	0.63
10411589	1.0x	Achromatic objective	89	58	100	0.8
10422562	1.5x	Achromatic objective	49	58	67	1.25
10447081	2.0x	Achromatic objective	31	58	50	1.6

Leica Application Suite can read settings from the microscope and will detect if a turret is fitted. If there is, then all objectives have to be selected and assigned:

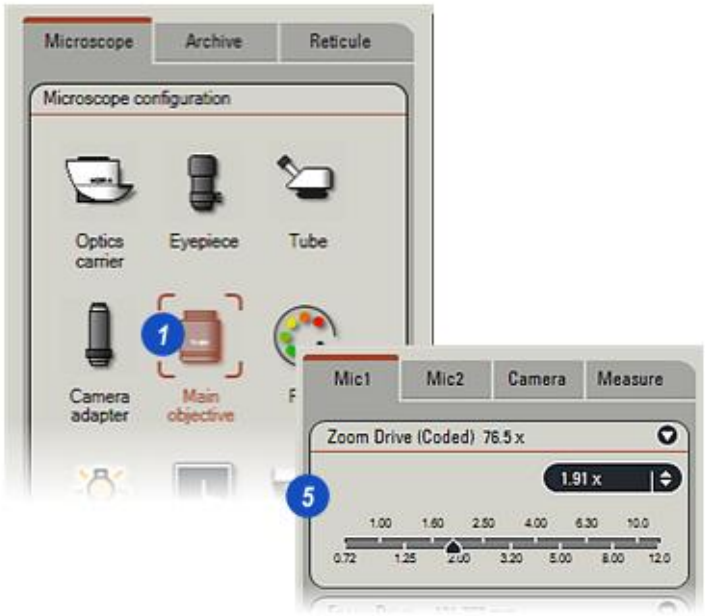
1: Click on the *Main Objective* icon on the configuration panel.

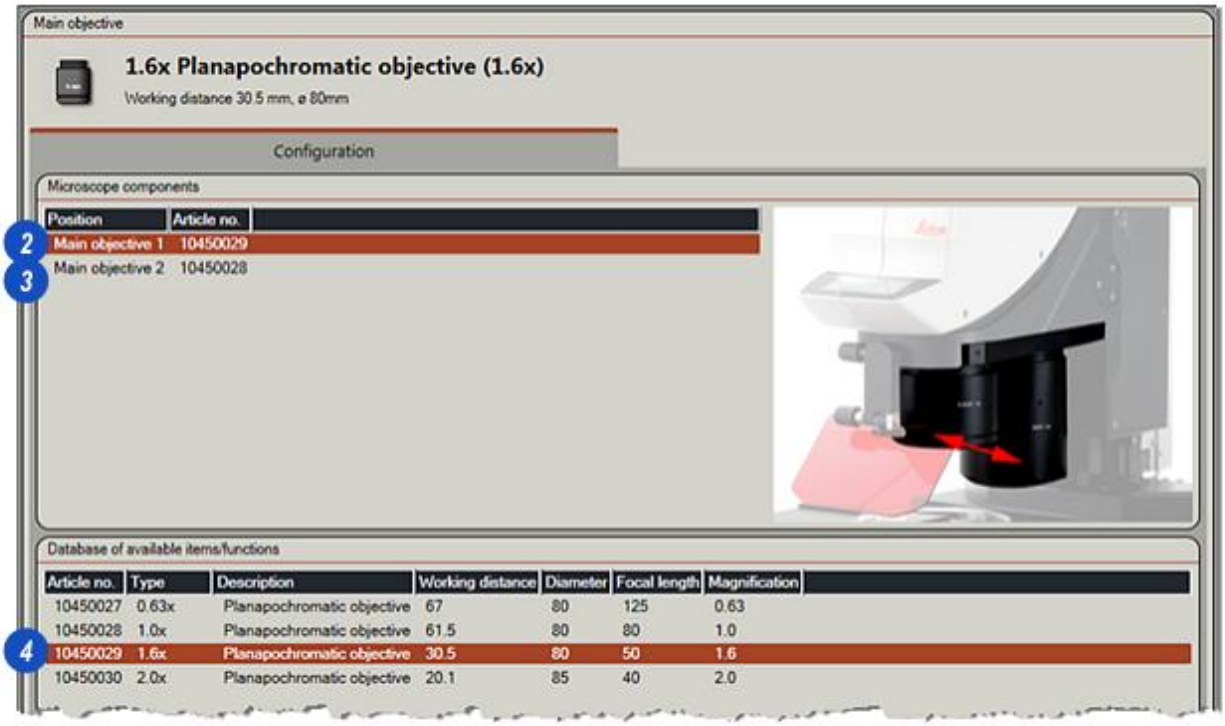
2 & 3: The upper panel lists the objectives to be selected. Click on an entry to select it and...

4: ...click to assign the objective type from the lower panel. Repeat for the other objective.

Users with coded objective revolvers will need to manually rotate the revolver to both positions before selecting the assignment.

5: The objective magnification is displayed on the *Zoom* panel in the *Acquire Workflow*.

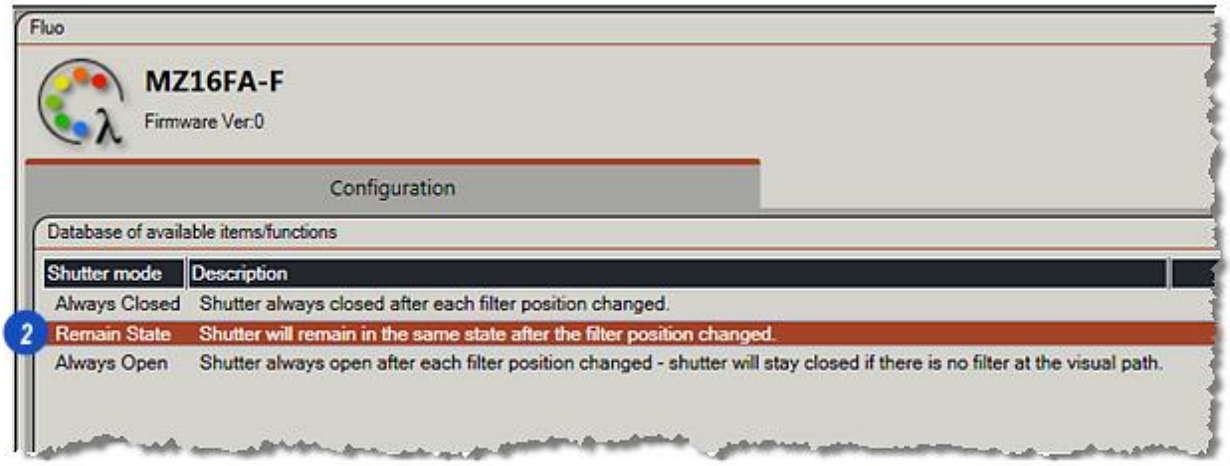




The *Fluo* setting determines how the fluo shutter will behave as filters are changed:

1: Click on the *Fluo* icon on the configuration panel.

2: Click to select the required behaviour.

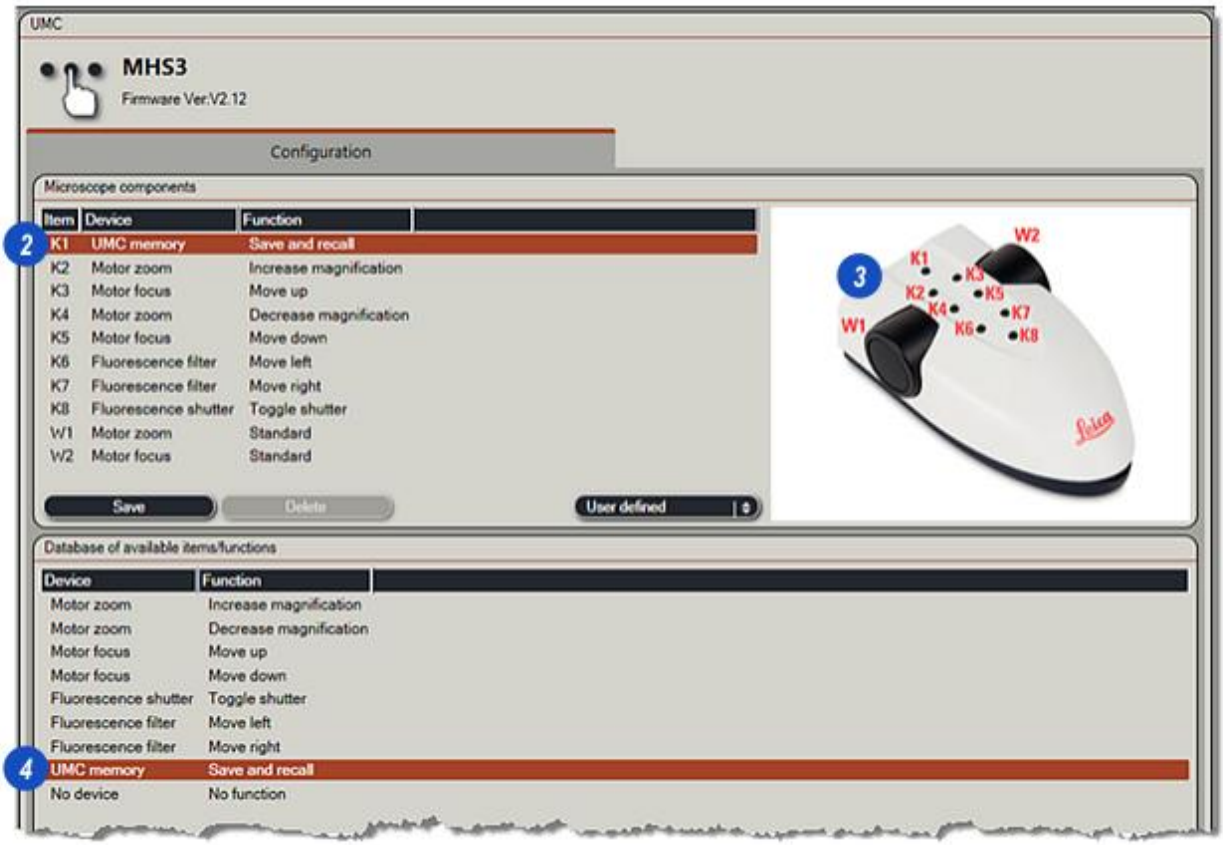


The button and wheel functions on the *Universal Manual Control (UMC)* can be changed to suit individual users and saved as configurations that can be recalled whenever a user operates the microscope.

1: Click on the *UMC* icon on the configuration panel. The icon appears only if a *UMC* is connected.

2: On the upper panel click to select the control - button or wheel - that is to be assigned a function. The image of the *UMC* **(3)** is captioned with the control references. Functions applicable to the selected control are listed on the lower panel.

4: Scroll through and click to select the function to assign to the control.



UMC Configuration (cont.) 158

To save the *UMC* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save UMC Configuration* dialog appears.

4: Click inside the *UMC Configuration* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

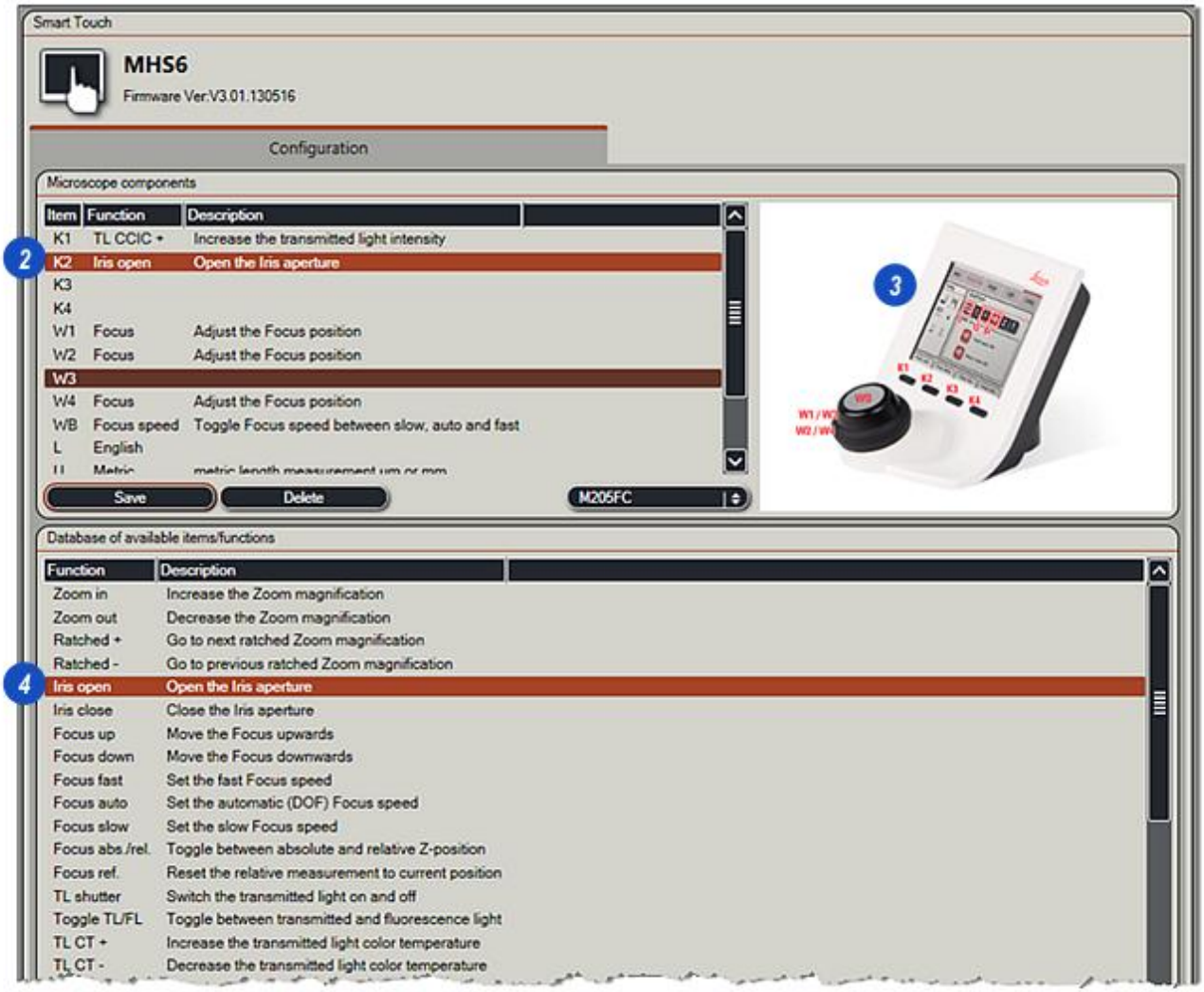
Two *Default* settings - *Right-handed* and *Left-handed* users - that revert to the factory settings, can be loaded by clicking on the entry on the drop-down list.

The *Hardware Keys, Double Rotary Actuator, Toggle Button* and *Touch Screen* functions on the *SmartTouch* control can be changed to suit individual users and saved as configurations that can be recalled whenever a user operates the microscope.

1: Click on the *SmartTouch* icon on the configuration panel. The icon appears only if a *SmartTouch* is connected.

2: On the upper panel click to select the control - *Hardware Key, Rotary Actuator, Toggle Button* or *Touch Screen* icon - that is to be assigned a function. The image of a *SmartTouch* **(3)** is captioned with the control references. Functions applicable to the selected control are listed on the lower panel.

4: Scroll through and click to select the function to assign to the control.



To save the *SmartTouch* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save Smart Touch Configuration* dialog appears.

4: Click inside the *Smart Touch* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

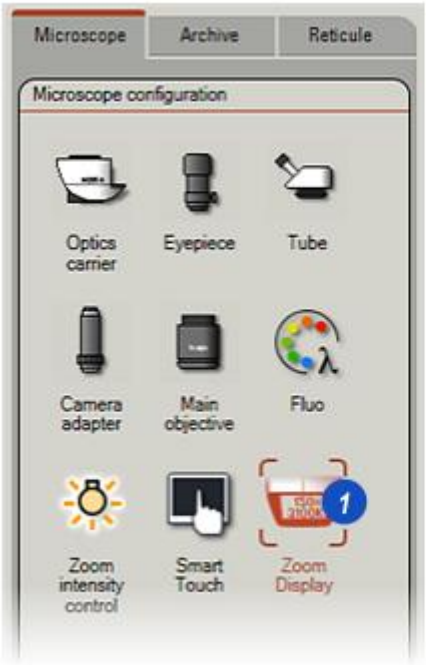
Clicking to select the *Default* option from the drop-down list, will revert to the factory settings.

The items displayed in the Zoom Display window on the front of the microscope, can be changed to suit the user and then saved as a configuration that can be recalled whenever the user operates the microscope.

1: Click on the *Zoom Display* icon on the configuration panel.

2: On the upper panel click to select the display element that is to be assigned a feature. The image of the *Zoom Display* window **(3)** is captioned with the element references.

4: Scroll through the list in the lower panel and click to select the feature to assign to the element.



To save the *Zoom Display* settings as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header.

2: From the drop-down list, click to select *User Defined.*

3: Click on the *Save* button and the *Save Zoom Display Configuration* dialog appears.

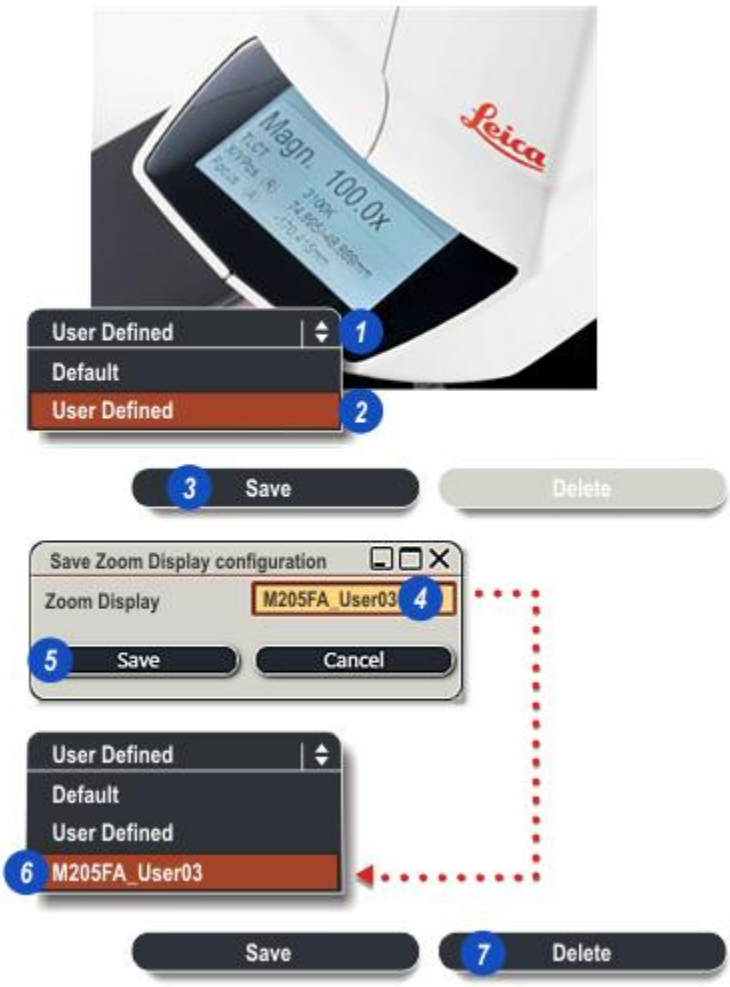
4: Click inside the *Zoom Display* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.

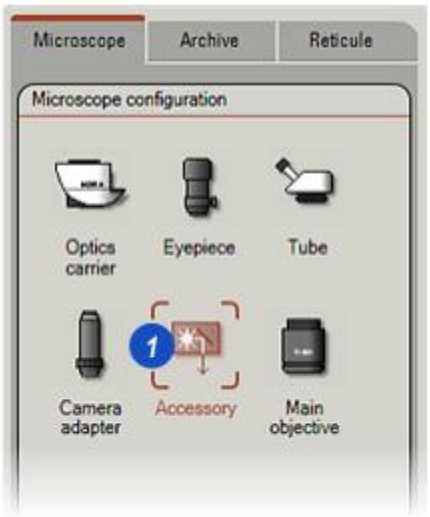
Clicking to select the *Default* option from the drop-down list, will revert to the factory settings for the *Zoom Display*.

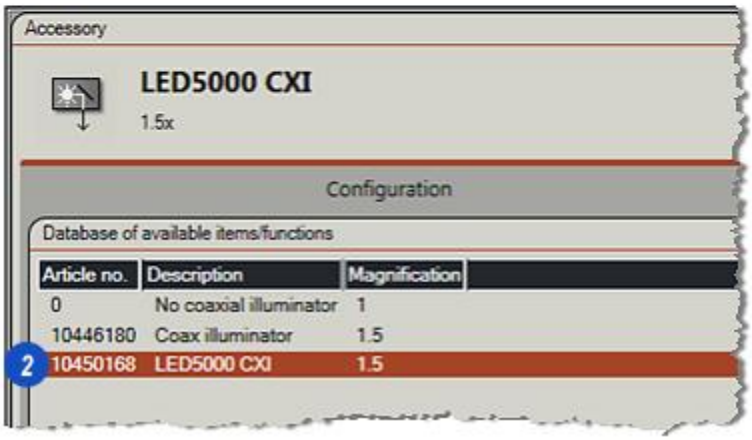


Accessories available for the current microscope are shown in a single list. When an accessory is fitted:

1: Click on the *Accessory* icon on the configuration panel. The icon is always available whether an accessory is fitted or not.

2: From the list, click to select the fitted accessory and enable its function and features.





The *Footswitch* actions can be configured to suit the user and then saved as a configuration that can be recalled whenever the user operates the microscope.

1: Click on the *Footswitch* icon on the configuration panel.

2: On the upper panel click to select the pedal that is to be assigned a feature. The image in the *Footswitch* window **(3)** is captioned with the pedal 1 *(P1)* and pedal 2 *(P2).*

4: Scroll through the list in the lower panel and click to select the feature to assign to the selected pedal.

To save the *Footswitch* setup as a *Configuration* that can be retrieved and loaded at any time:

1: Click on the small arrows to the right of the *Configuration* header and...

2: ...click to select *User Defined.*

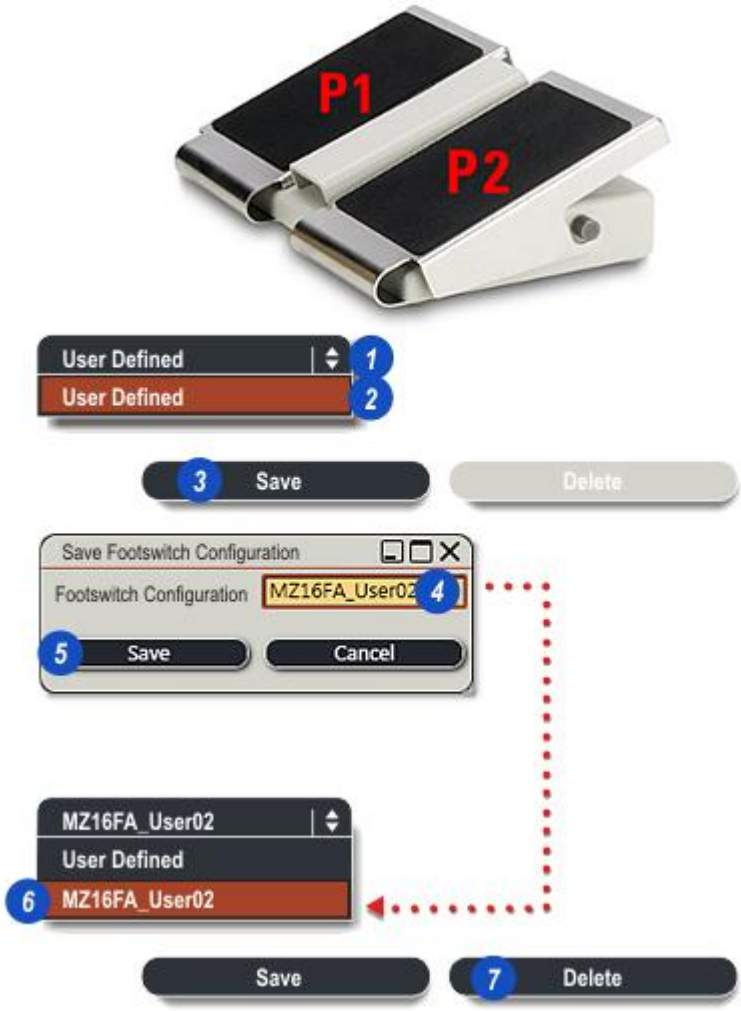
3: Click on the *Save* button and the *Save Footswitch Configuration* dialog appears.

4: Click inside the *Footswitch Configuration* text box and type a unique name for the new *Configuration*.

5: Click the *Save* button.

6: The new *Configuration* name appears in the *Configuration* list and can be loaded at any time simply by clicking to select it.

7: Delete a *Configuration* by clicking to select it from the drop-down list and then clicking the *Delete* button. This action cannot be undone.



The *Transmitted Light Reflectivity Control* (TLRC) can be enabled and disabled on the *Configuration* tab of the *TL CCIC* panel:

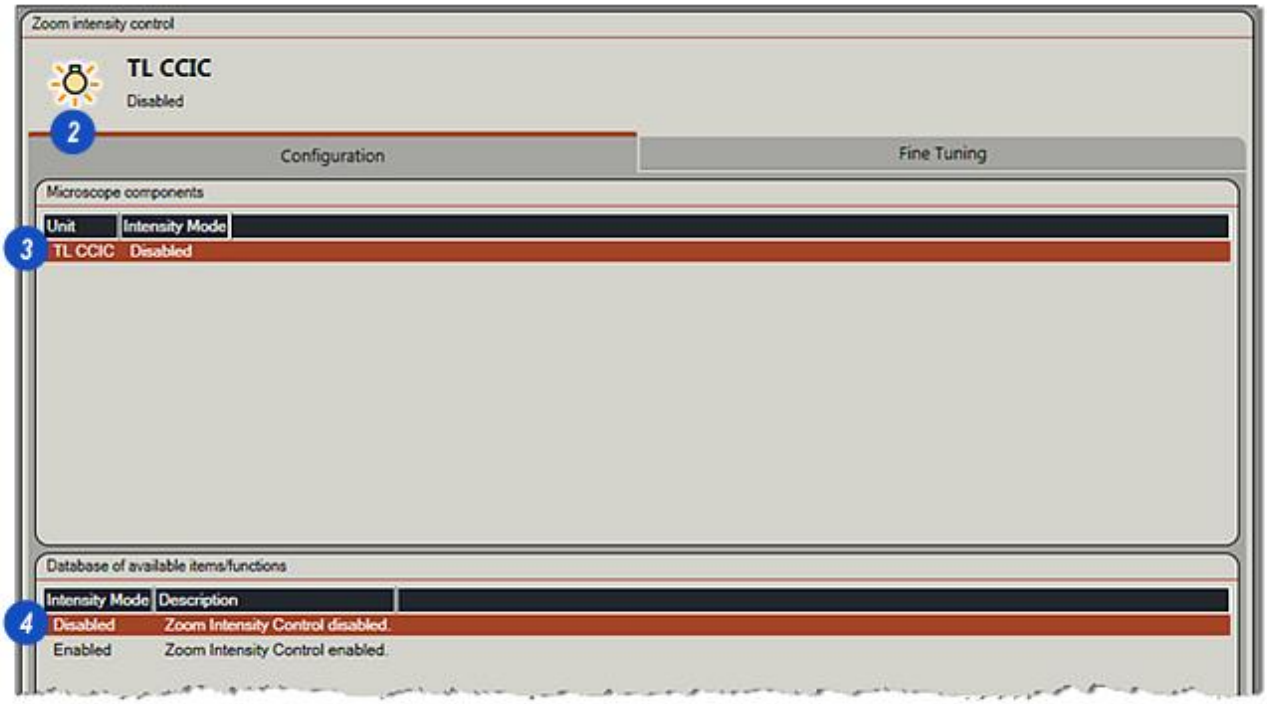
1: Click on the *Zoom Intensity Contro*l icon on the *Configuration* panel.

2: On the *TL CCIC* panel *Configuration* tab...

3: ...click the *TL CCIC* entry.

4: Toggle between enabled and disabled by clicking on the required option on the Database panel.

5: If the control has not been calibrated, the warning appears.



1: Click on the *Fine Tuning* tab.

2: Follow the list of setup instructions on screen.

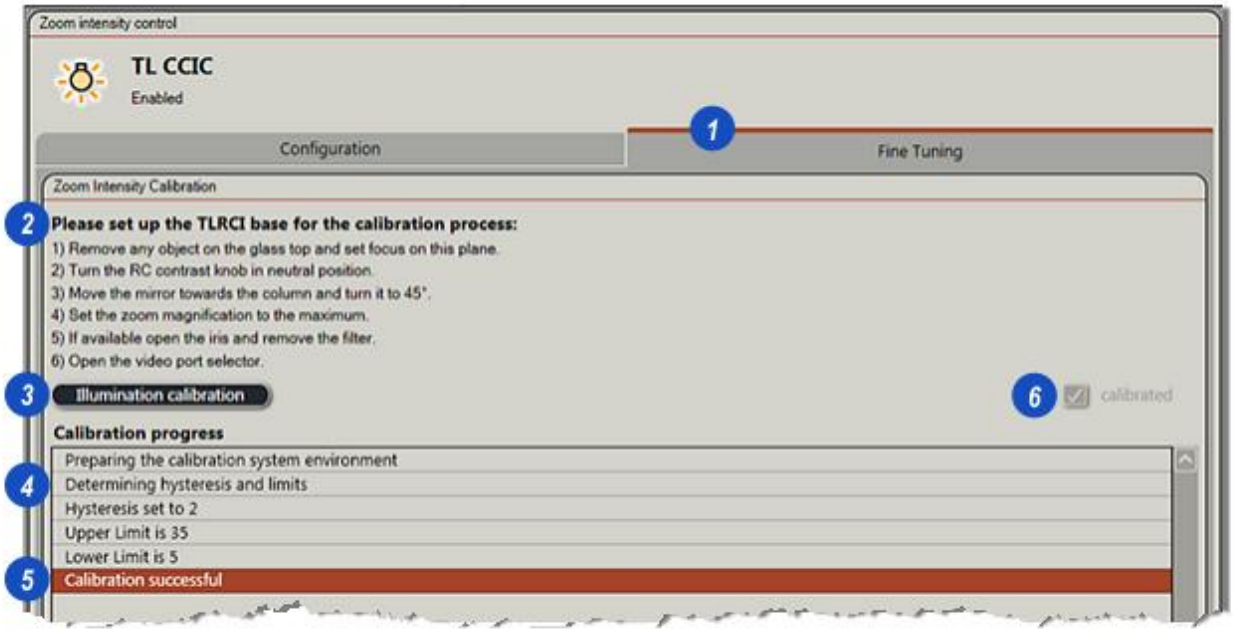
3: Click the *Illumination Calibration* button.

4: The calibration process begins with the results from each step displayed...

5: ...until the *Calibration successful* message appears...

6: ...and the *Calibrated* check box is enabled.

Return to the previous page (*Go there...* 166) to enable the TL CCIC.



The *DM Series Microscope Setup* has been arranged so that configuring the microscope is both fast and simple.

Each setup feature is displayed on the *Components* panel - clicking an icon will take the user directly to the appropriate setup dialogs.

The feature icons displayed will depend upon the microscope model and fitted accessories.

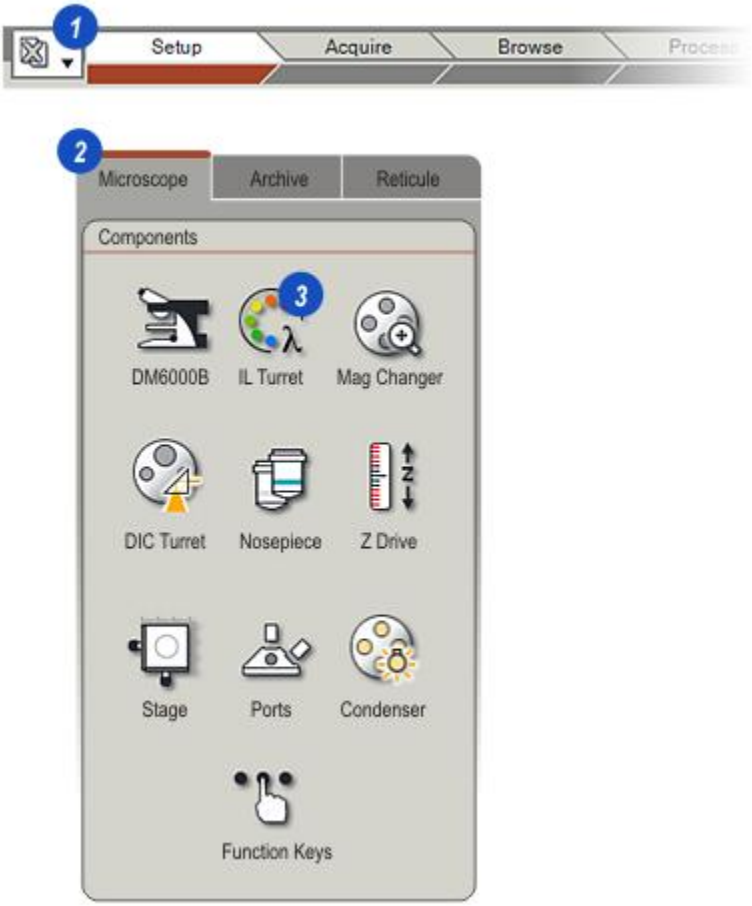
Generally, all of the setup options for each feature are listed in a database and all that is required is a double-click on the entry to assign it to the selected feature.

1: Click on the *Setup Workflow.*

2: Click on the *Microscope* tab.

3: Click on a feature icon as described on the following pages.

C-Mount Selection 188



Many of the dialogs have features designed to help user navigation. For example:

1: Click on the border around either the top or bottom panels and drag it up or down to re-size it. This can be used to display more database items.

2: To change the width of the database columns and so reveal more of the item descriptions, click on the vertical bar between two columns and drag to the left or right.

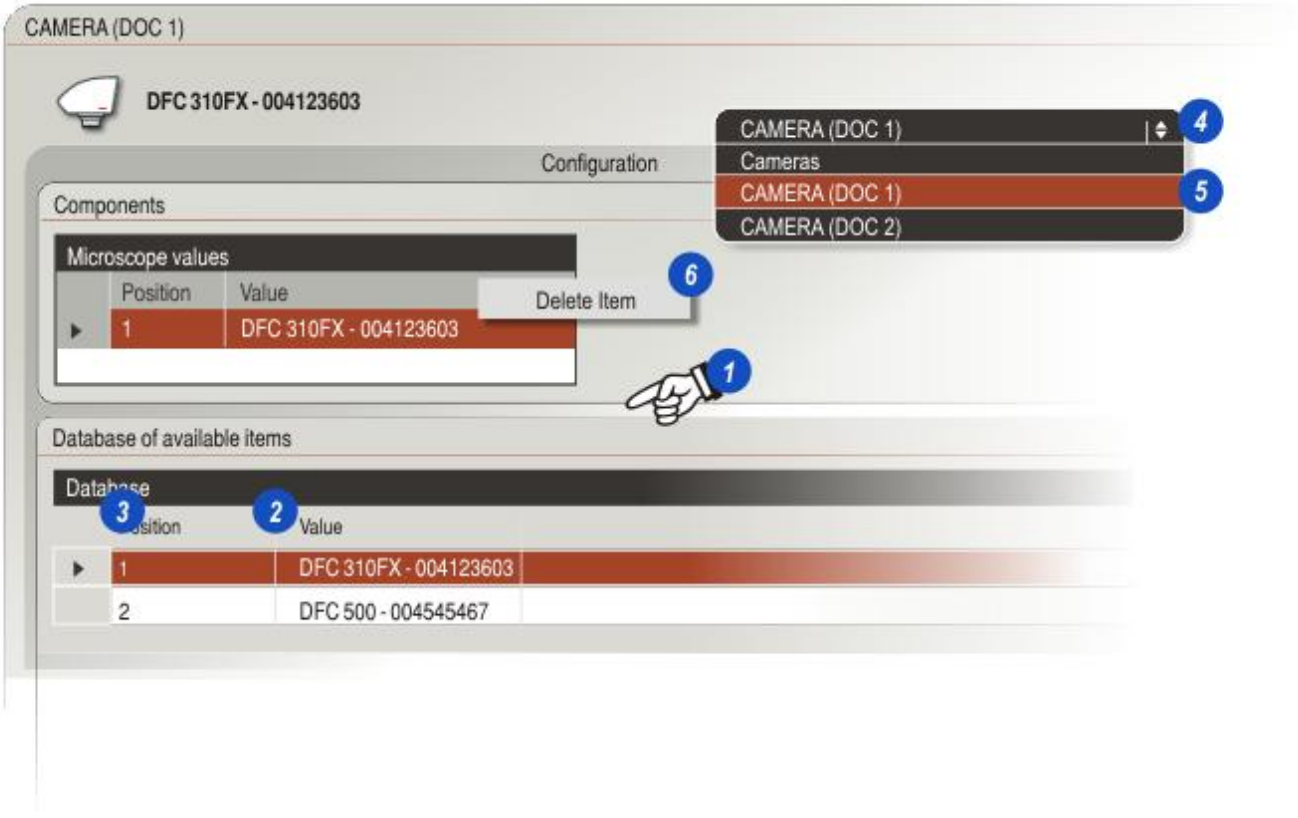
3: By default the database items are listed in ascending order - lowest numeric value at the top of the list. Change to descending order by double-clicking on the column header.

4: Some dialogs have drop-down menus and, depending on the selection the database listings will change.

To reveal the drop-down options click on the small arrows to the right of the menu header and...

5: ...click to select the required option.

6: To remove a selected database item from the *Microscope Values* list, right-click on the item and left-click on the pop-up *Delete Item* button.



To view the microscope data:

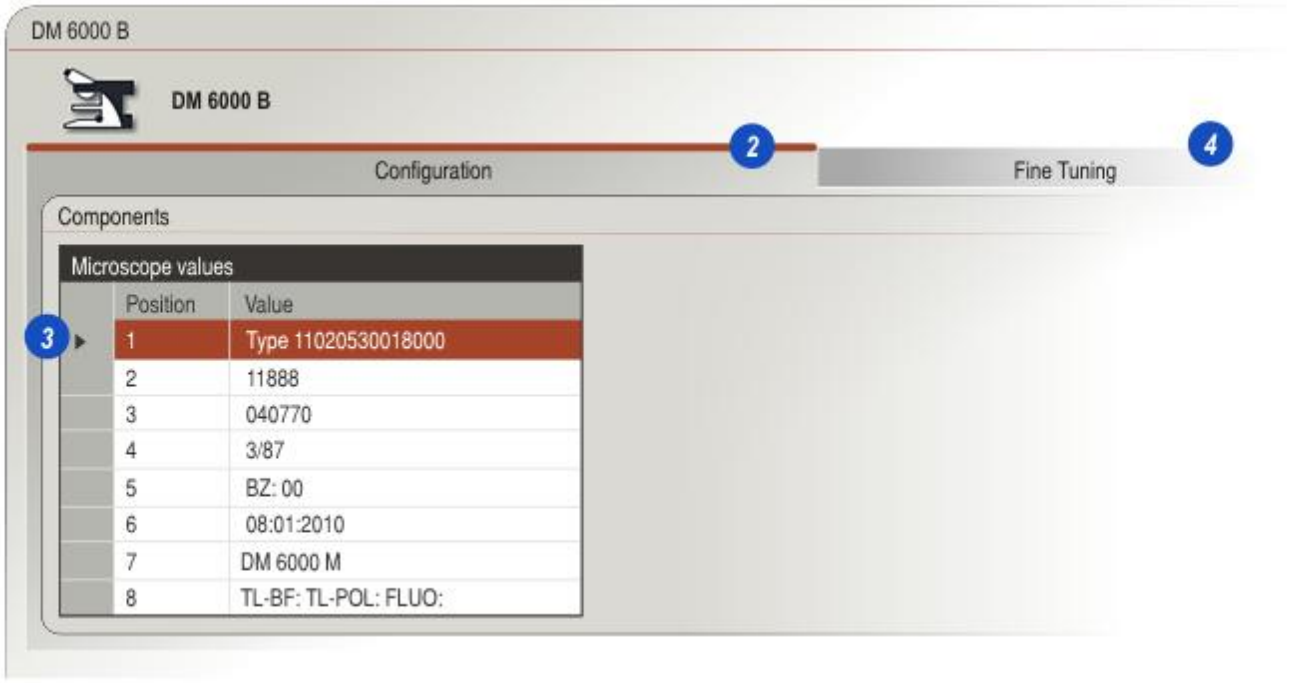
1: Click on the microscope icon on the *Microscope* > *Components* panel. The microscope stand type is retrieved from the Hardware Configuration and displayed beneath the icon.

2: Click on the *Configuration* tab and...

3: ...the data is displayed.

4: Click on the Fine Tuning tab to display all of the setup features in a single list. The setup can be achieved from here or by going to the required section:

- *IL Turret Selection* 171
- *Mag Changer Setup* 172
- *DIC Turret Setup* 173
- *Nosepiece Setup and Fine Tuning* 174
- *Z-Drive Setup* 192
- *Stage Selection & Initialisation* 190
- *Port Selection* 186
- *Camera Selection* 189
- *Condenser Selection* 194
- *Function Key Assignment* 195



To set up the *IL Turret Filter Cubes:*

1: Click on the *IL Turret* icon on the *Microscope* > *Components* panel.

2: The *IL Turret* dialog appears with the number of turret positions indicated as dark circles on the graphic.

3: Click on the turret position to select it.

4: Double-click on the required database item to assign it to the turret position.

5: The database item details are displayed in the turret position and...

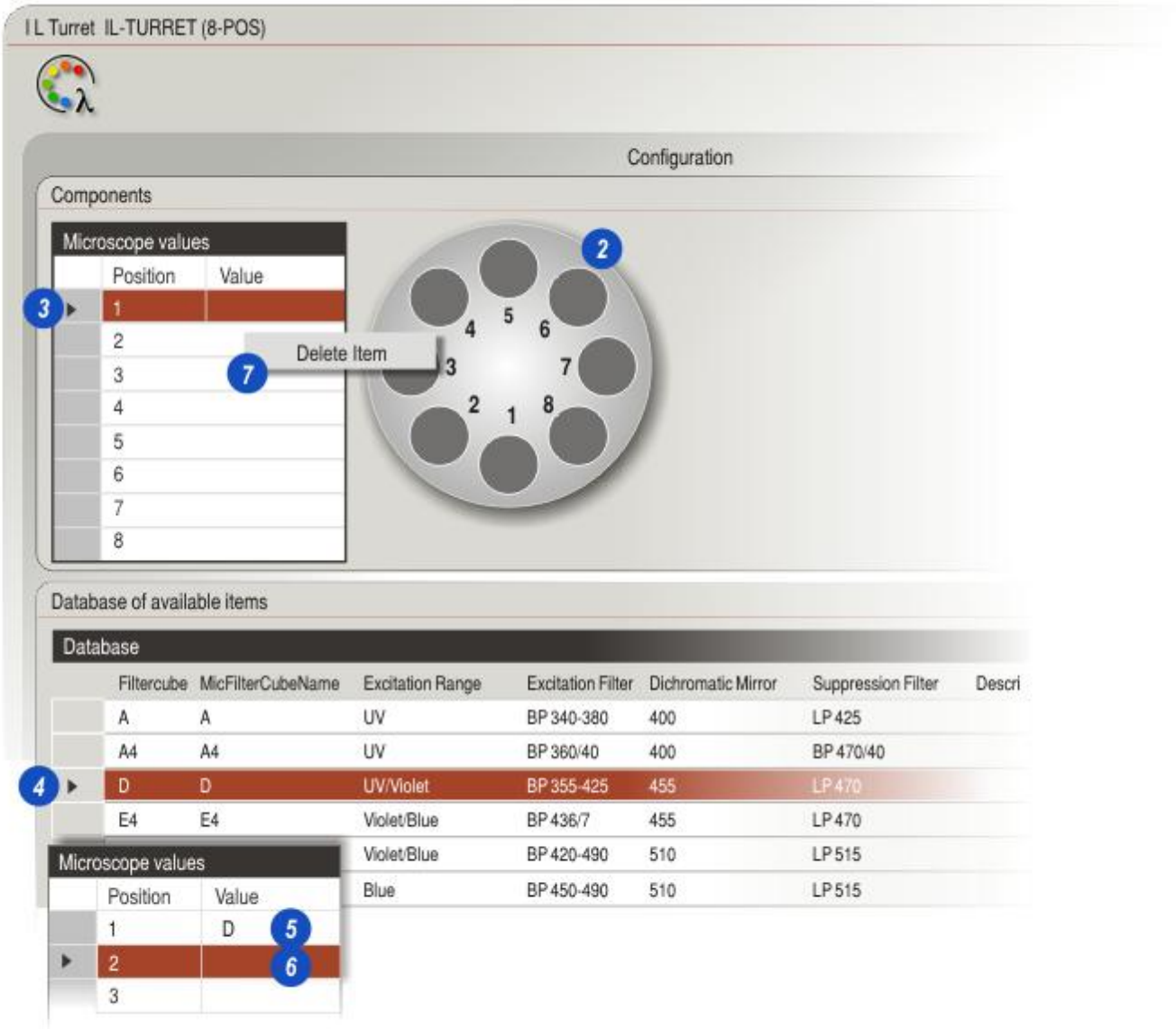
6: ...if there is another position to be filled, it is automatically selected.

7: To remove an item from the turret list, right-click the entry and left-click the Delete Item pop-up button.

Note: At least one position of the fluo turret has to be learned in as *'EMPTY'* to perform bright field contrast (*BF*) in transmitted light axis.

EMP-BF has to be selected in case the stand is capable of a fully motorized *IC* contrast in transmitted light axis. If the microscope is capable of a partial manual *IC* contrast, the *EMP-DIC* has to be selected.

Alternatively in case an *A* cube is used:
Select *A-TL* to perform contrast methods of the transmitted light axis with this fluo cube.



To set up the Mag Changer:

1: Click on the *Magnification Changer* icon on the *Microscope* > *Components* panel.

2: The *Mag Changer* dialog appears. There is a single position value that assigns the *Mag Changer* component.

3: Click on the position to select it.

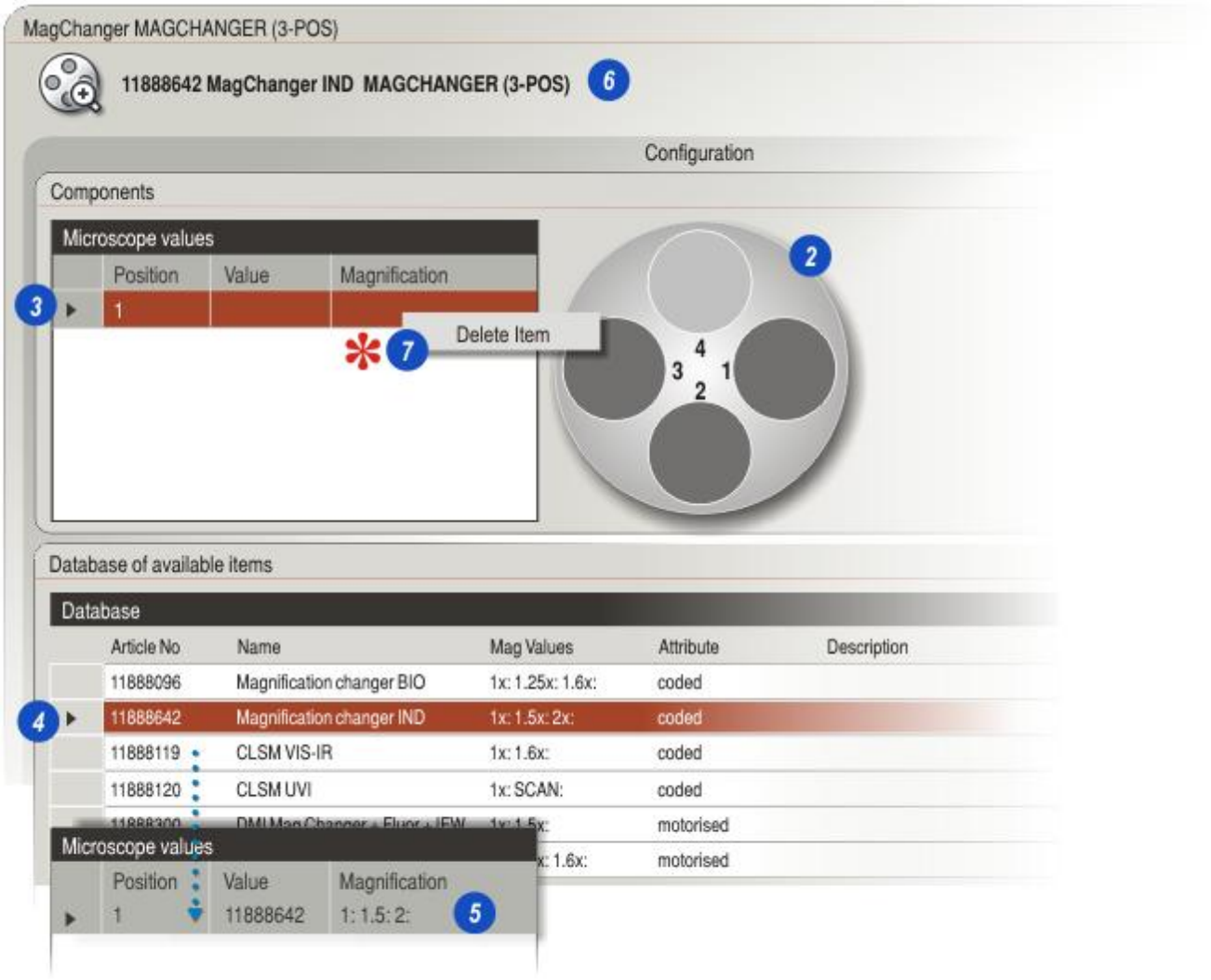
4: Double-click on the required database item to assign it to the *Mag Changer*.

5: The database item details are displayed in the changer position and...

6: ... on the header.

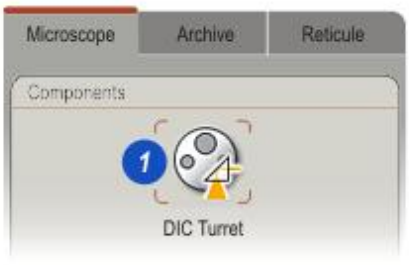
No Mag Changer Setup: *

7: If a *No Mag Changer* setting is needed, right-click the position entry and left-click the *Delete Item* pop-up button. The *Microscope Value* will appear as 1,1,1 which indicates *No Changer.*



To set up the *DIC (Differential Interference Contrast) Turret* components:

1: Click on the *DIC Turret* icon on the *Microscope* > *Components* panel.



2: The *DIC Turret* dialog appears with the number of positions indicated as dark circles on the graphic.

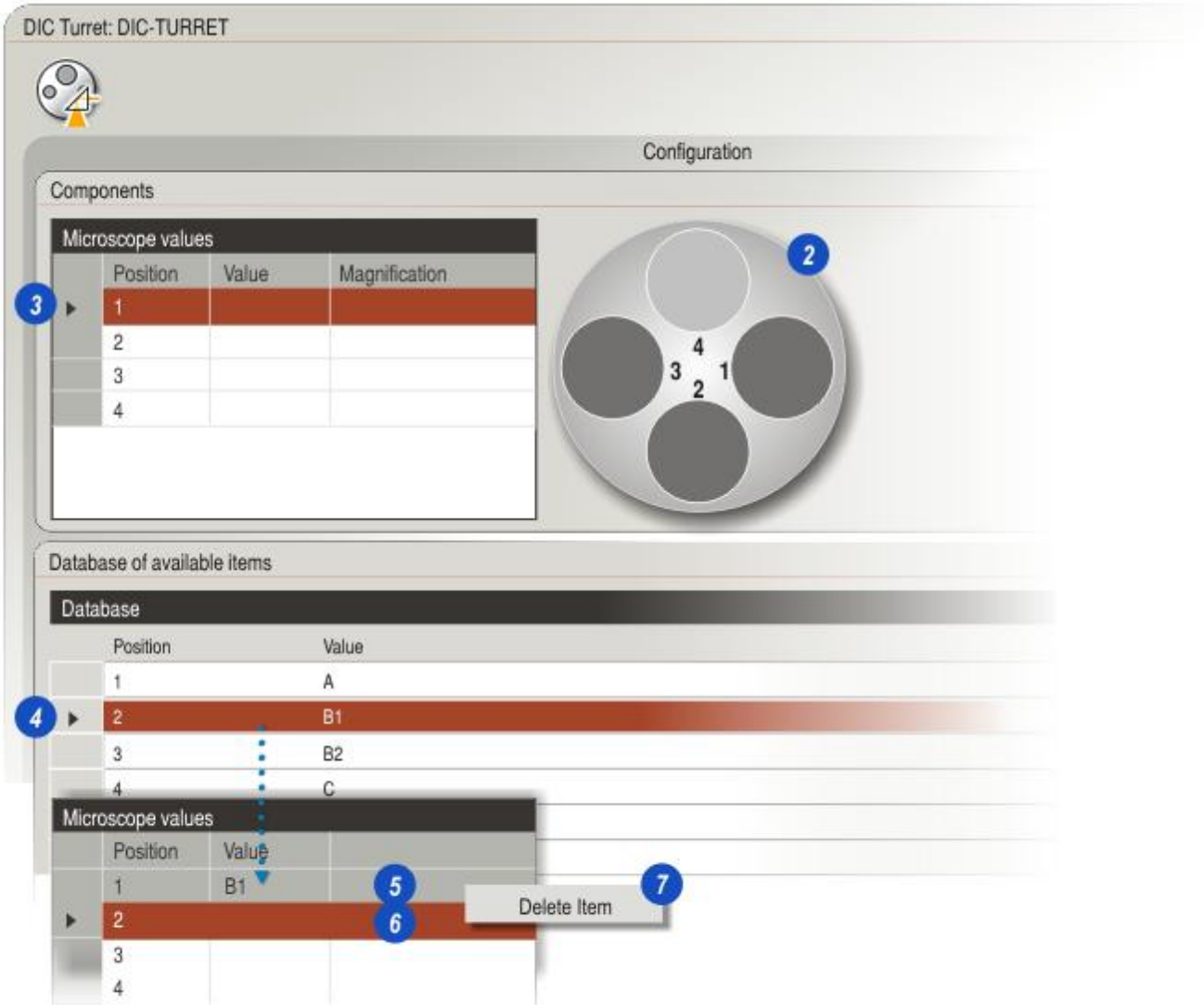
3: Click on the turret position to select it.

4: Double-click on the required database item to assign it to the turret position.

5: The database item details are displayed in the turret position and...

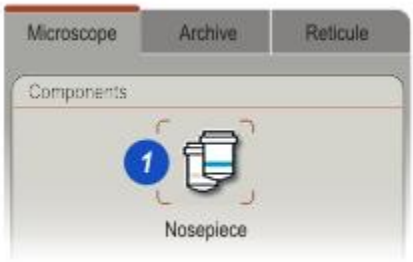
6: ...if there is another position to be filled, it is automatically selected.

7: To remove an item from the turret list, right-click the entry and left-click the *Delete Item* pop-up button.



Setting up the *Nosepiece* is a two-step process:

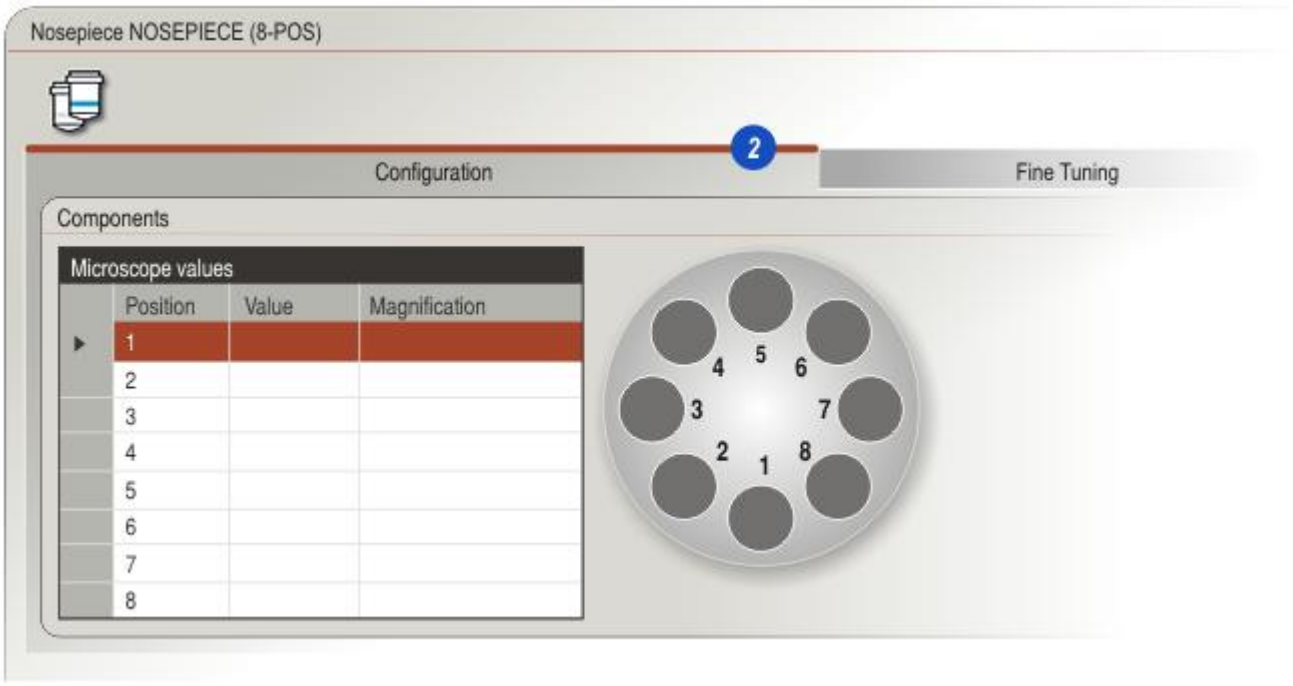
- Select and assign an *Objective* to the *Nosepiece* positions.
- Fine-tune the *Objective* setup.



To assign an *Objective* to a *Nosepiece* position:

1: Click on the *Nosepiece* icon on the *Control Panel*.

2: Click on the *Configuration* tab on the *Nosepiece* dialog.



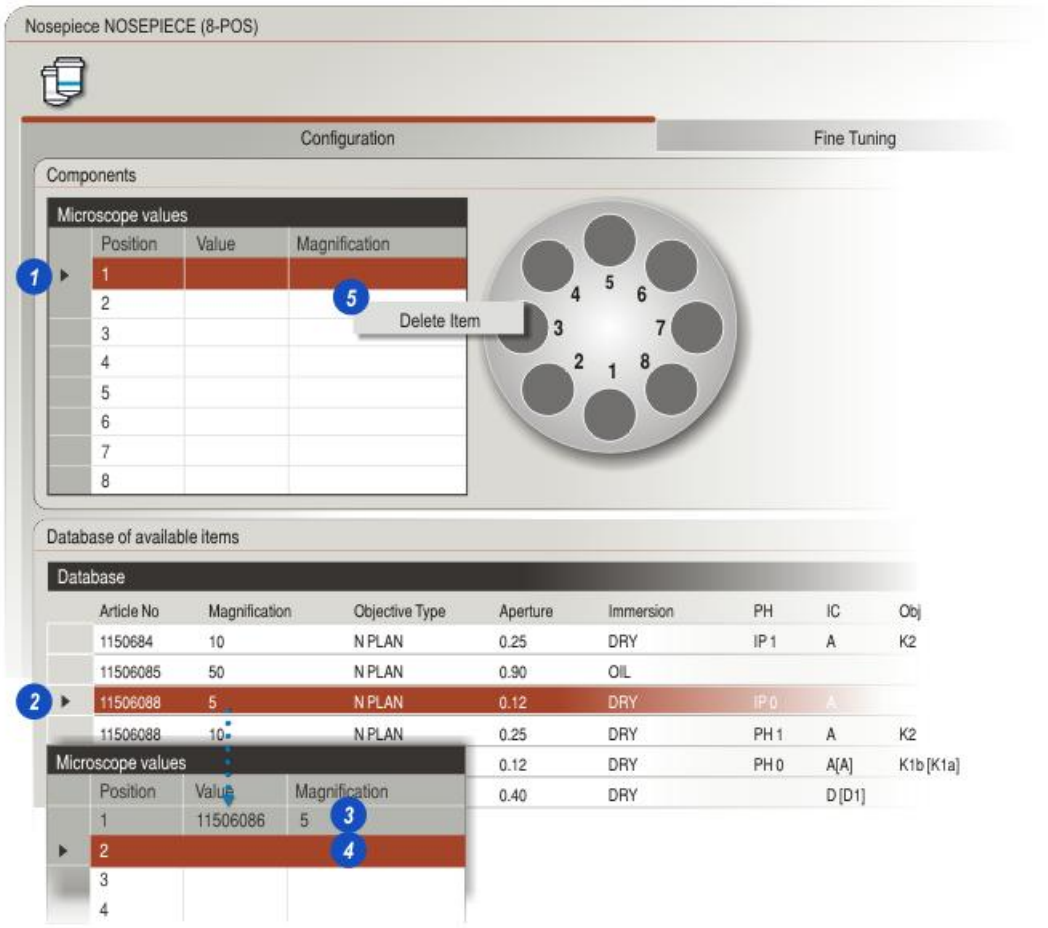
1: Click on the *Nosepiece* position.

2: In the database, double-click on the required *Objective*.

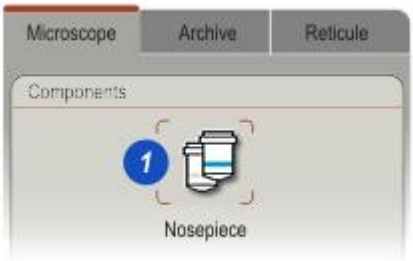
3: The *Objective* details are displayed in the *Nosepiece* position and...

4: ...if another position remains to be assigned, it is automatically selected. Repeat Step **(3)**.

5: To remove an *Objective* from a position, right-click on the *Nosepiece* position and left-click on the *Delete Item* pop-up button.



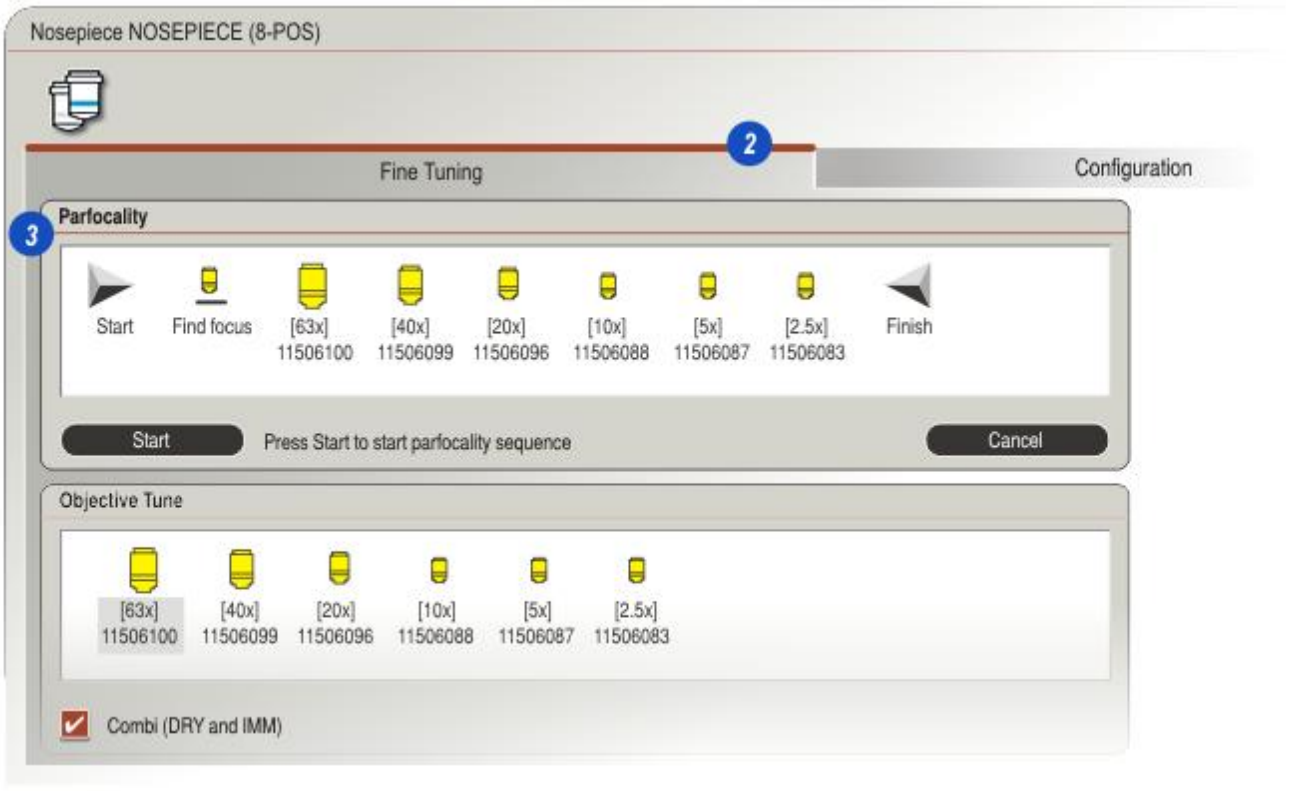
1: Click on the *Nosepiece* icon on the *Control* panel.



2: Click on the *Fine Tuning* tab.

3: There are individual dialogs for each of the *Objective* fine-tuning features:

- Parfocality[177]
- Objective Combination tag[179]
- Stage and Z-Stepsize[180]
- Auto Stage Positition[191]
- Parcentricity Enable[184]
- Parcentricity Adjustment[183]
- Nosepiece Rotation[185]



Even with closely matched objective sets, *Parfocality* - maintaining sharp focus when an objective is changed - may need to be fine-tuned. A Leica motorised focus axis must be fitted.

The process involves initially focussing on a specimen using the objective with the greatest magnification, and then checking each of the remaining objectives in turn and, where necessary adjusting the focus to maintain a clear, sharp image.

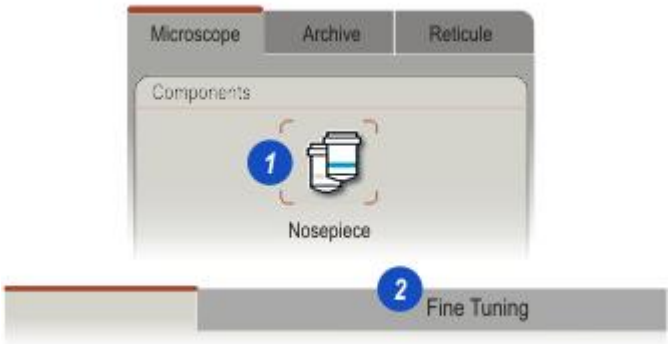
A focus factor is created for each objective, stored and retrieved when the objective is selected to drive the stage to the precise Z-position to maintain focus.

There are two options for setting the *Parfocality:*

- From this setup dialog with focus being checked through the eyepieces - the *Viewer* is not available here - and...
- From the *Acquire Workflow* with the specimen displayed on the *Viewer.*

For both options the dialogs are the same but are accessed differently.

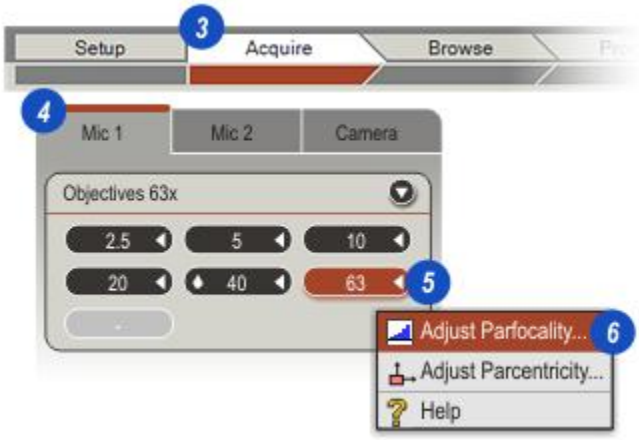
Set Parfocality from DM Setup (Here):



1: Click on the *Nosepiece* icon on the *Microscope* > *Component* panel.

2: Click on the *Fine Tuning* tab and then click to select the *Parfocality* dialog.

Set Parfocality from Acquire Workflow:



3: Click on the *Acquire Workflow* and then...

4: ...on the *Mic 1* tab.

5: Right-click on one of the objective buttons and...

6: ...left click to select *Adjust Parfocality* on the context menu.

Parfocality (cont.) 178

1: Click on the *Parfocality* dialog and...

2: ... the *Start* button. The *Find Focus* option is selected. Navigate the stage to and adjust for the sharpest focus on the specimen.

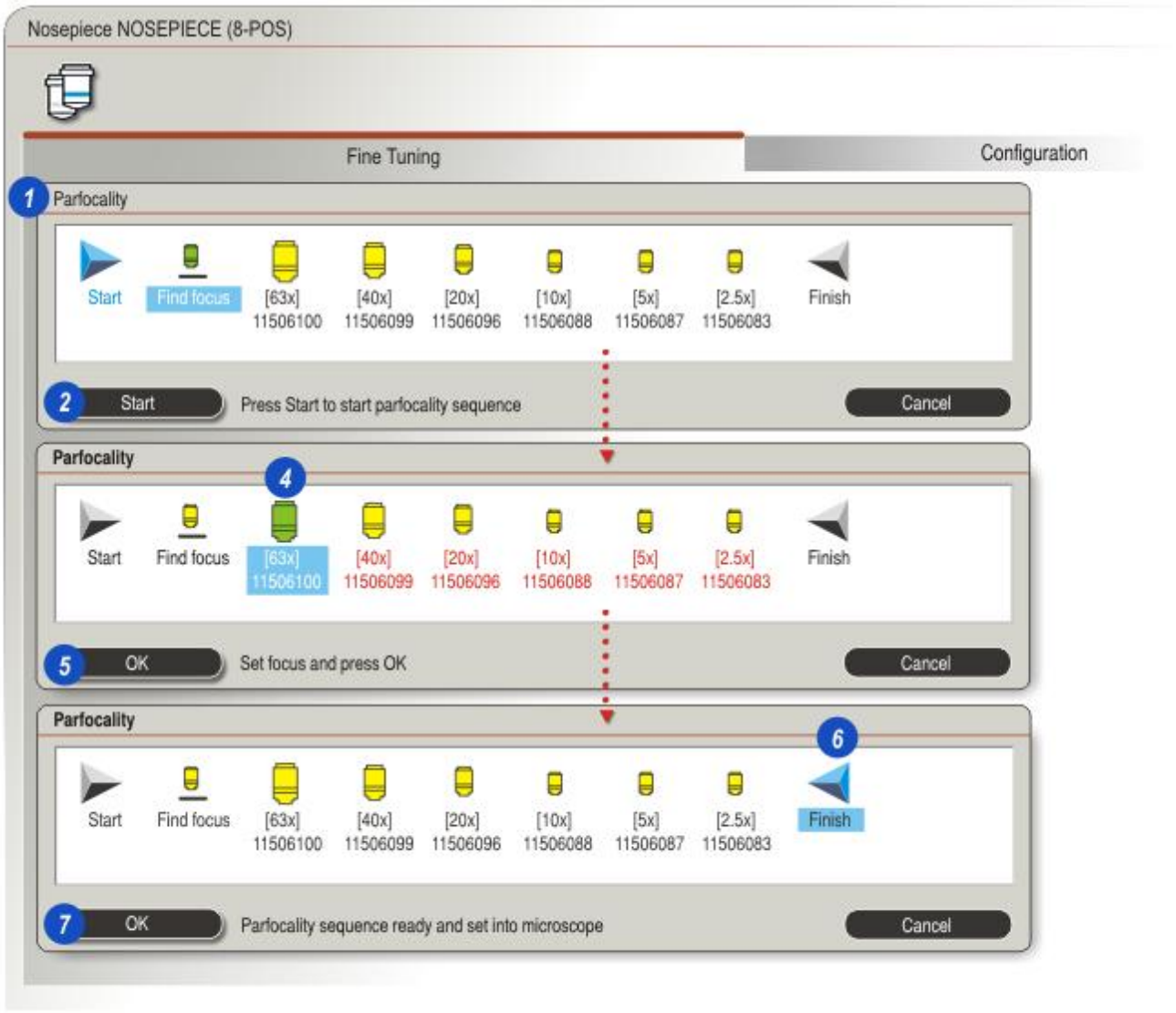
3: Click *OK*.

4: The objective with the greatest magnification is automatically chosen first. Focus the specimen and...

5: ...click *OK*. The dialog will advance to the next objective.

6: When all objectives have been fine-tuned the *Finish* icon is highlighted.

7: Click *OK.*



Objective Combination Tagging

Some objectives can be used both in air and also immersed in water or oil. These so-called *Combi* (Combination) objectives can be tagged so that when they are selected the user is given a warning that immersion can be an option.

To Set / Clear Combi Tagging:

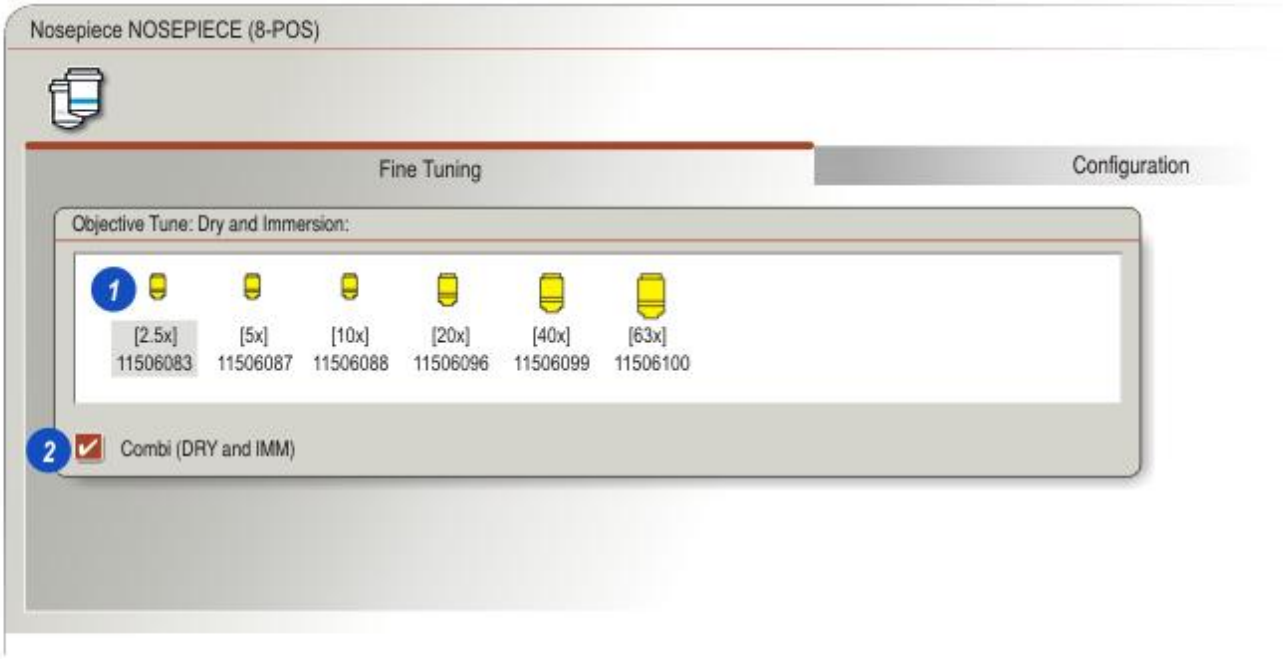
1: Click on the *Objective* Icon to select it.

2: Click the check box to enable (Tick mark visible) or disable (No Tick Mark) the *Combi* tag.

If either a tagged *Combi* or *Immersion Only* objective is selected on the *Acquire* > *Mic 1* tab, the button will flash to warn the user.

3: *Immersion Only* objectives are marked with a 'filled' teardrop icon whereas...

4: ...*Combi* objectives are marked with an outlined teardrop.



Only available when a Leica Stage Controller is fitted, it allows a stage speed - *X, Y* and *Z* - to be matched to an objective based upon its magnification. Optimising the stage speed improves efficiency.

Higher stage speeds are used with low magnification objectives and low stage speeds with high magnification objectives, both with the aim of maintaining the best navigation speed without 'losing' the image region of interest.

There are five step speeds ranging from:

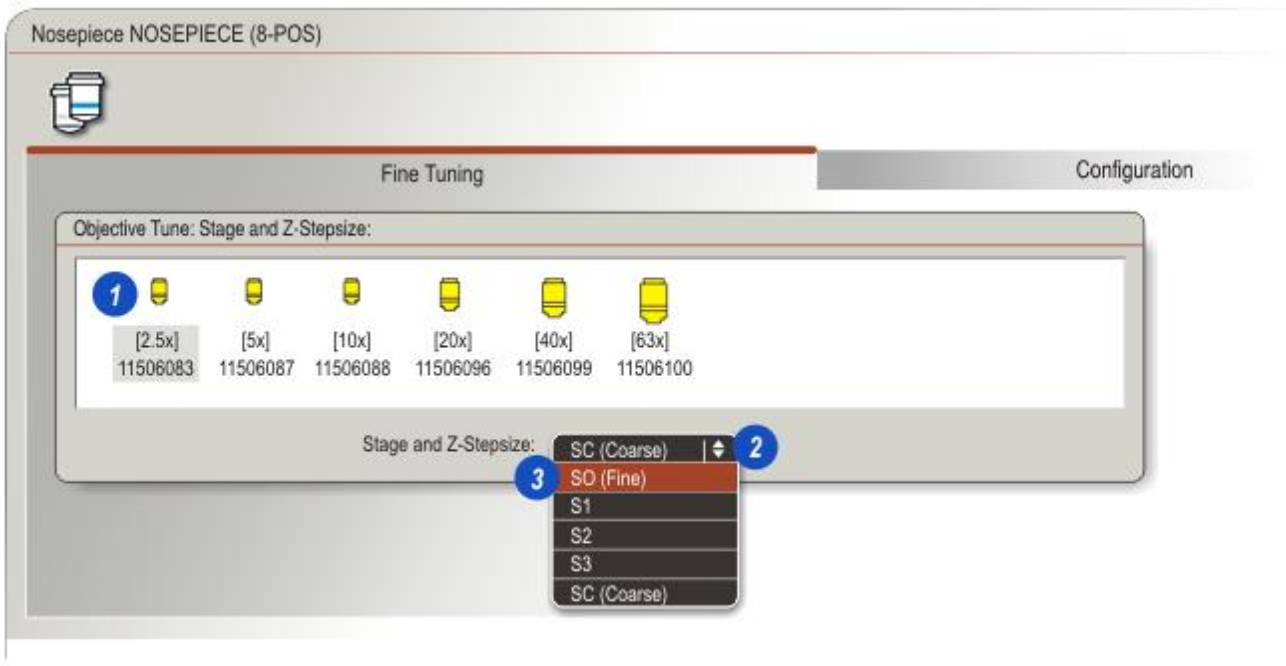
- *SC* - fast and coarse to...
- *S0* - slow and fine.

Any one of the five speeds can be assigned to an objective so that when it is selected the appropriate stage speed is engaged automatically:

1: Click on the *Objective* Icon to select it.

2: Click on the small arrows to the right of the *Stepsize* menu header and...

3: ...click to assign the appropriate *Stage* speed.



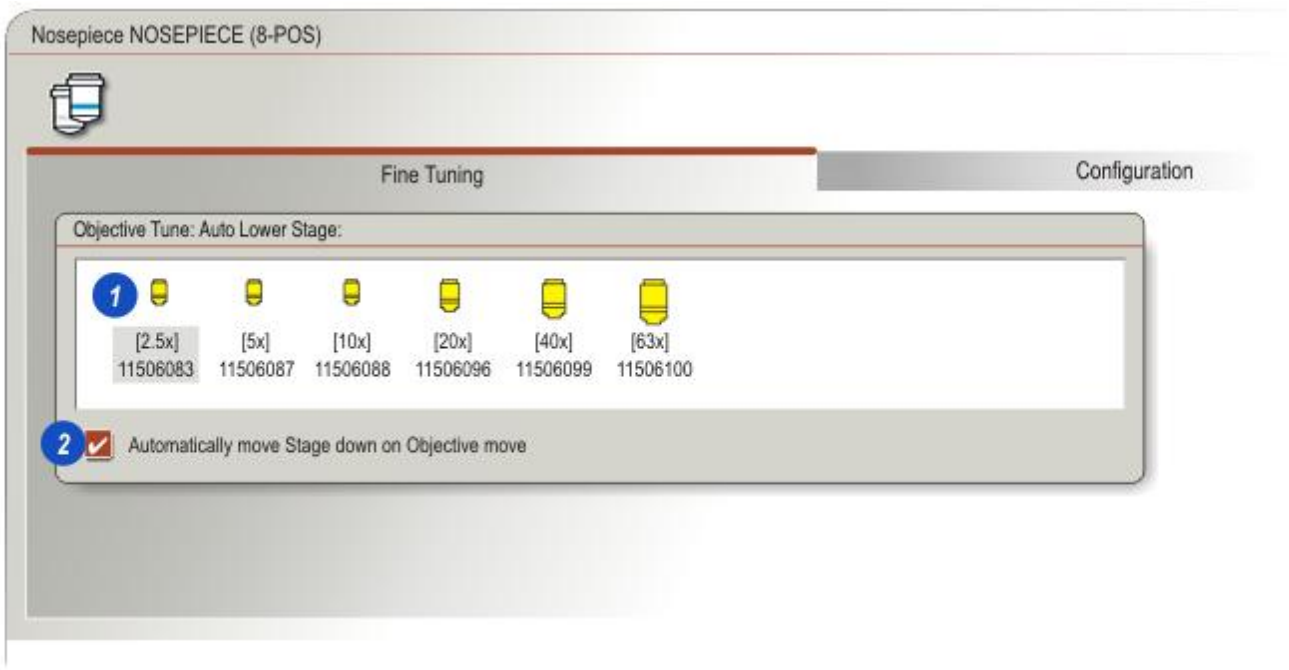
Automatic Stage Lowering

To avoid an objective colliding with the specimen, the stage can be automatically lowered whenever the objective is selected.

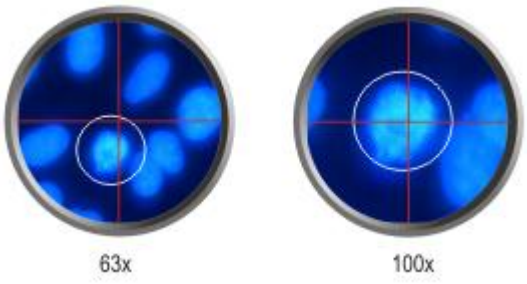
To assign Automatic Stage lowering to an objective:

1: Click on the *Objective* Icon to select it.

2: Click on the check box to enable (Tick Mark visible) or disable *Stage Down* (Lowering) for that objective.



Adjusting *Parcentricity* aims to maintain the specimen feature of interest as close to the centre of the field of view regardless of the objective magnification.



The process is straightforward. A feature on a specimen is focussed and moved as close as possible to the centre of the field of view using the stage navigation controls. The stage *X* and *Y* positions are stored as an 'offset factor' for the selected objective.

The objective is then changed and the stage adjusted so that the same feature is again close to the centre and again the *X/Y* offset stored. The process is repeated for all of the objectives.

Parcentricity improves if the feature position is finely adjusted for each objective.

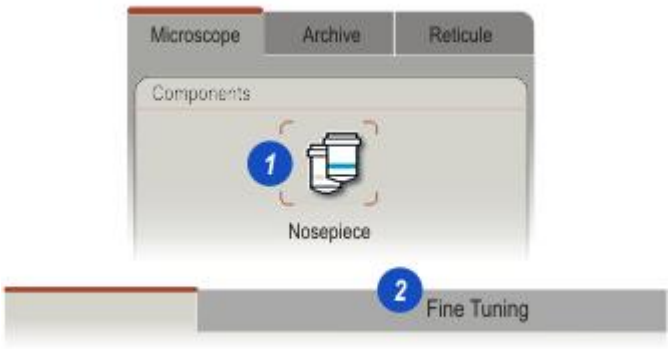
In the future when an objective is selected, the offset factor is automatically applied to the stage drivers to maintain *Parcentricity.*

There are two options for adjusting the *Parcentricity:*

- From this setup dialog with position being checked through the eyepieces - the *Viewer* is not available here - and...
- From the *Acquire Workflow* with the specimen feature displayed on the *Viewer*.

For both options the dialogs are the same but are accessed differently:

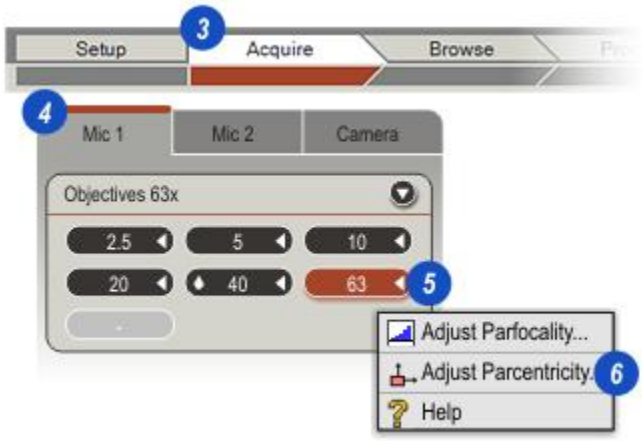
Set Parcentricity from DM Setup (Here):



1: Click on the *Nosepiece* icon on the *Microscope* > *Component* panel.

2: Click on the *Fine Tuning* tab and then click to select the *Parcentricity* dialog. Continued 183

Set Parcentricity from Acquire Workflow:



3: Click on the *Acquire Workflow* and then...

4: ...on the *Mic 1* tab.

5: Right-click on one of the objective buttons and...

6: ...left click to select *Adjust Parcentricity* on the context menu.

1: Click on the *Parcentricity* dialog and...

2: ... the *Start* button. The *Find Focus* option is selected. Navigate the stage to and adjust for the sharpest focus on a recognisable feature on the specimen.

3: Click *OK.*

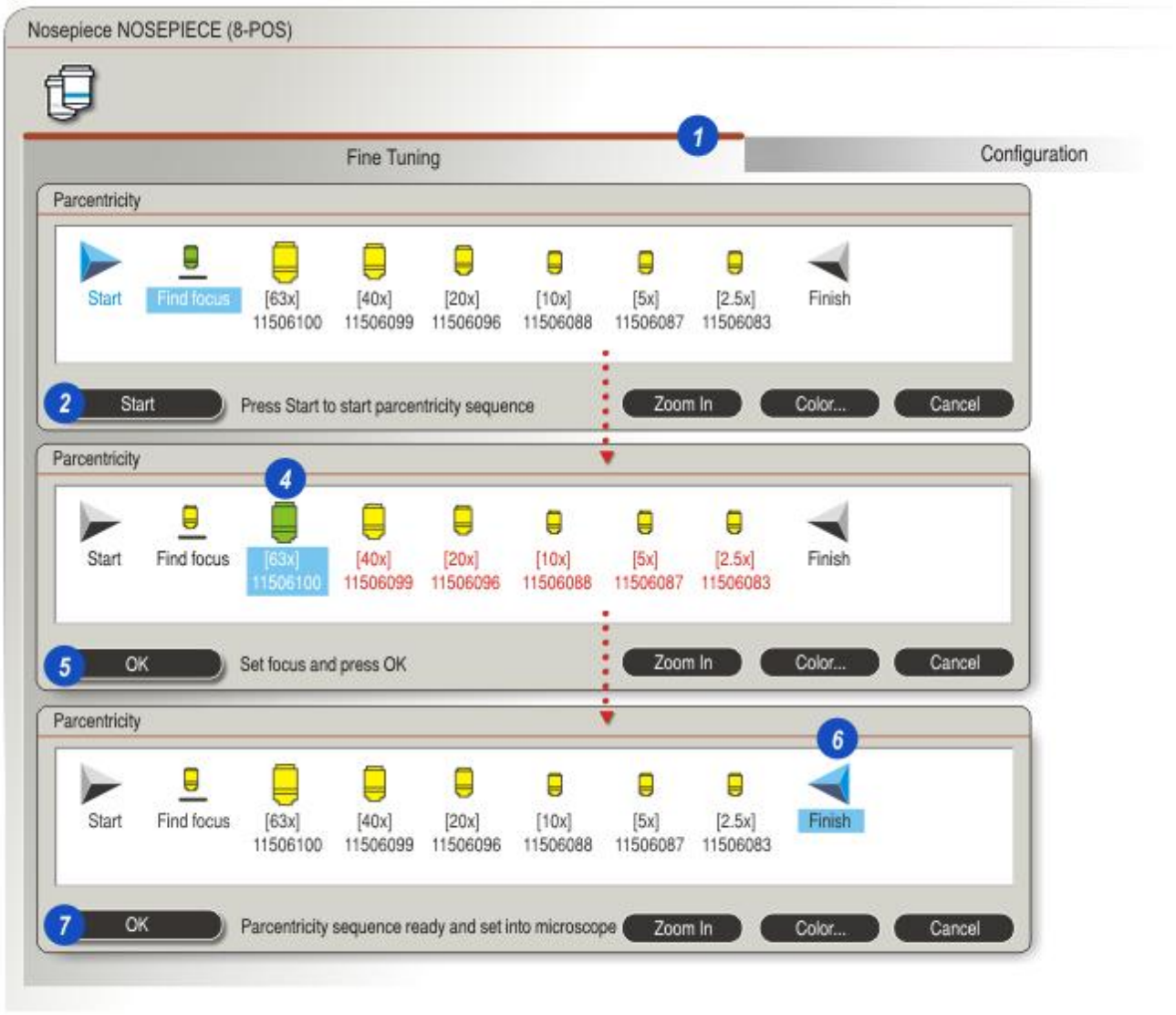
4: The objective with the greatest magnification is automatically chosen first. If necessary, focus the specimen, move the stage so that the feature is in the centre of the field of view and...

5: ...click *OK*. The dialog will advance to the next objective.

Re-focus if necessary and again centralise the feature in the field. Repeat the process for all of the objectives.

6: When all objectives have been fine-tuned the *Finish* icon is highlighted.

7: Click *OK.*

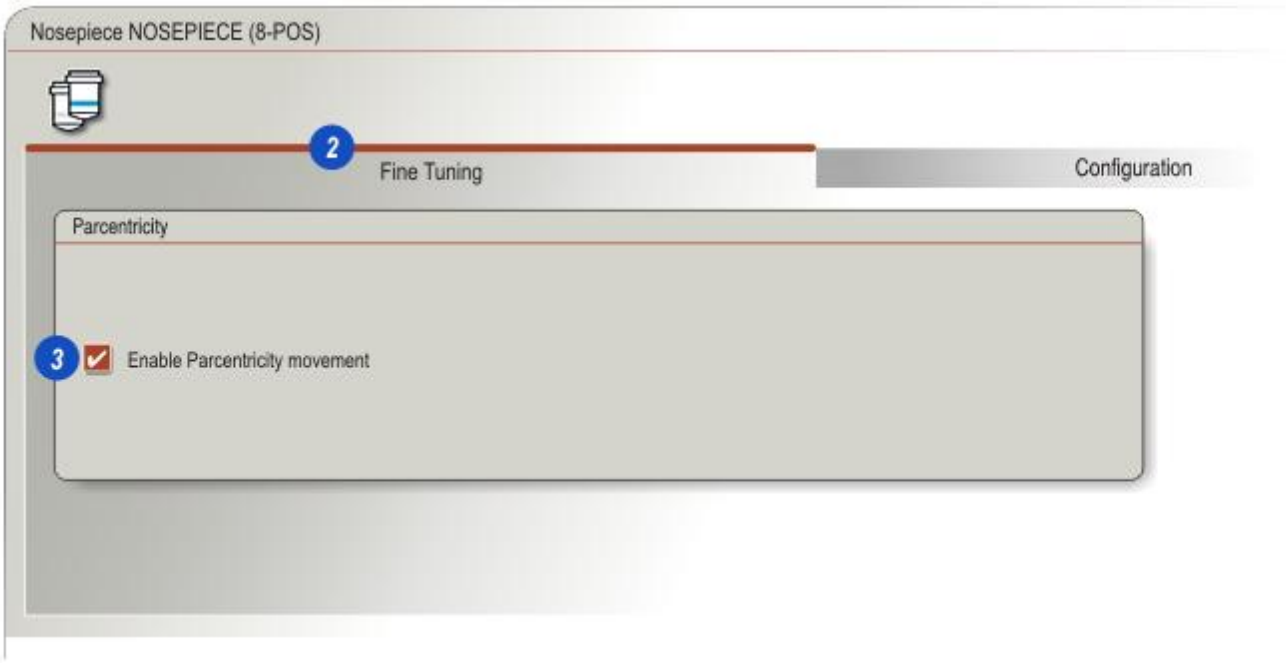


Automatic *Parcentricity* adjustment can be turned on and off on the *Enable* panel:

1: Click on the *Nosepiece* icon on the *Microscope* > *Components* panel.

2: Click on the *Fine Tuning* tab.

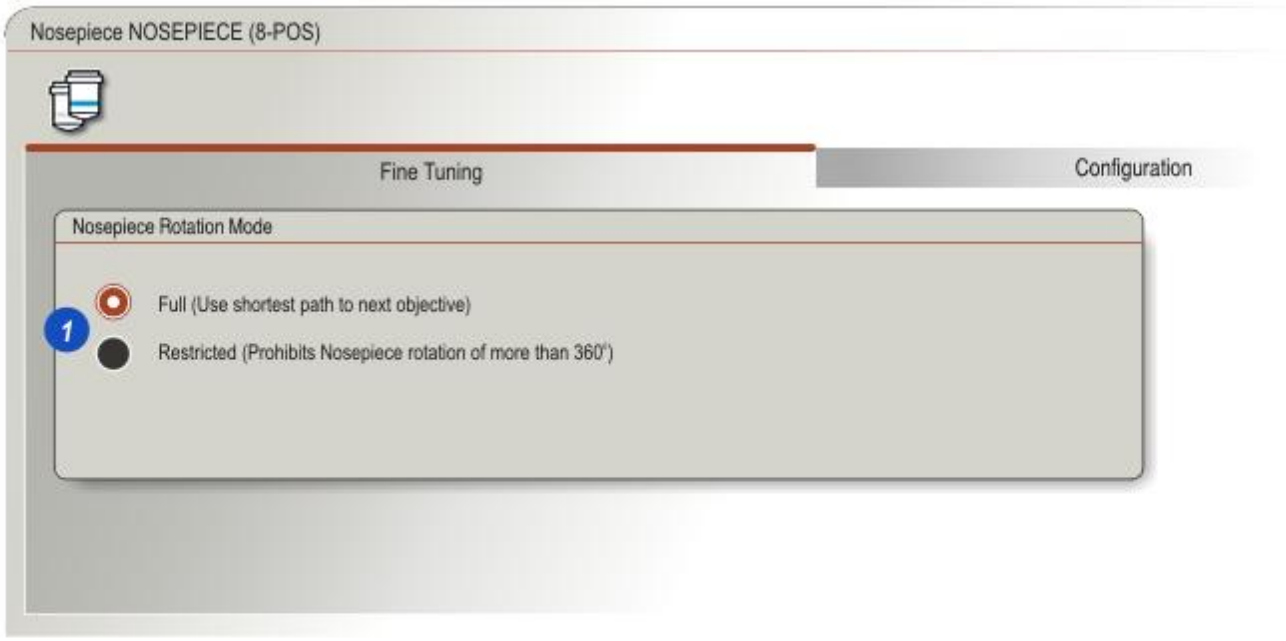
3: On the *Parcentricity* panel, click the check box to enable (Tick Mark visible) or disable automatic *Parcentricity*.



There are two options for determining how the *Nosepiece* rotation behaves when an objective is selected:

1: Click to select the required mode:

- Full allows the software to determine the shortest path - clockwise or counter-clockwise between the current and selected objective, or...
- Restricted allows the software to prevent the Nosepiece from ever rotating more than 360° even if that means taking the longest path between the current and selected objectives.



Select the tube *Eyepiece* as follows:

1: Click on the *Ports* icon on the *Microscope* > *Components* panel.

2: Click on the small arrows to the right of the *Port Options* drop-down menu header.

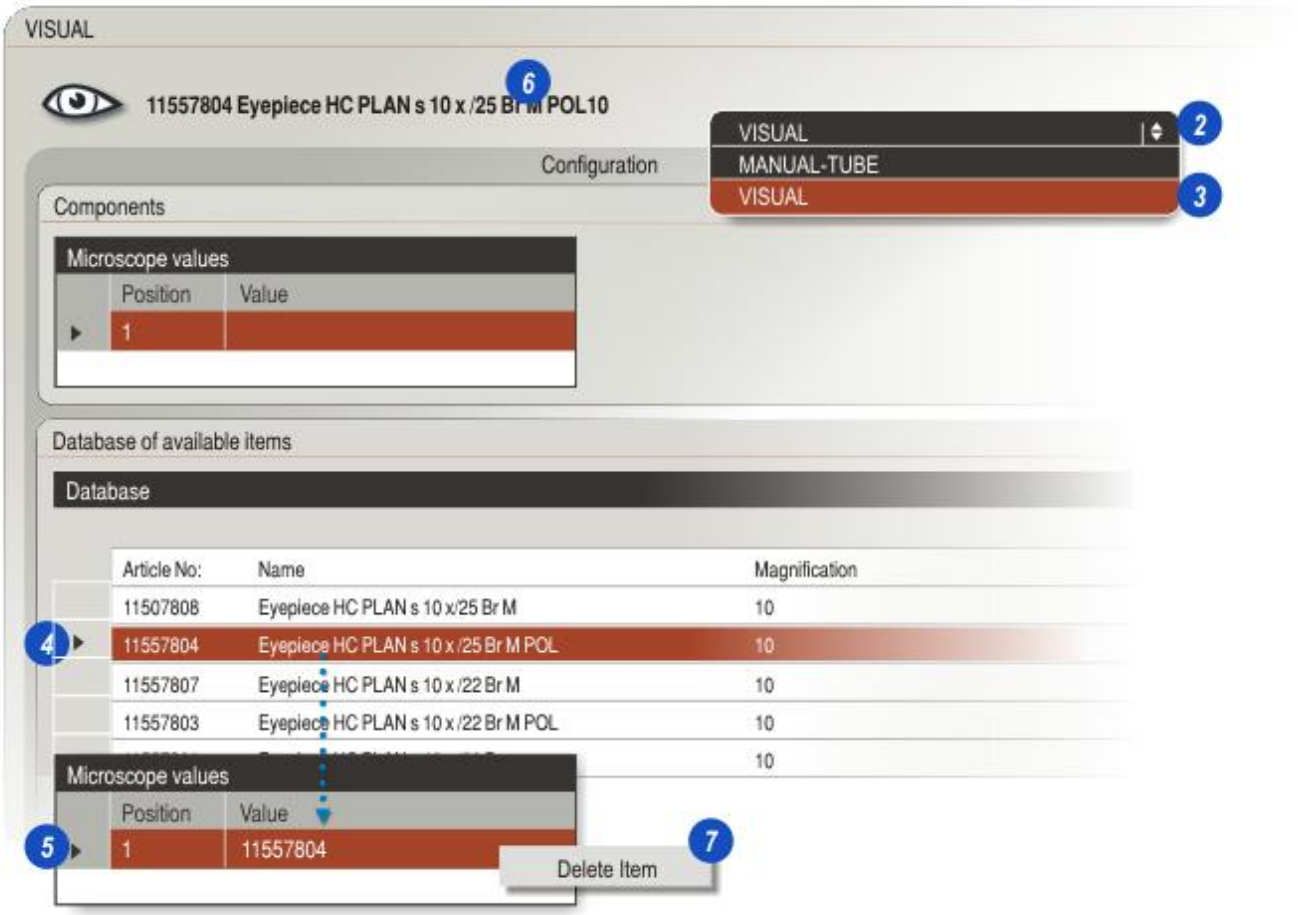
3: From the drop-down list, **c**lick to select *Visual* tube. The list of *Eyepiece* options appears on the *Database* panel.

4: Double-click on the *Database* entry to select it.

5: The item number appears in the *Microscope Values* list with...

6: ...the number and name displayed in the header.

7: Change a selection by right-clicking the value and then left-clicking the *Delete Item* pop-up button to remove the selection. Then proceed as describe to make a new selection.



The number of *Camera (DOC) Ports* is determined by the fitted hardware and the configuration selected here in setup.

There a three possible setups:

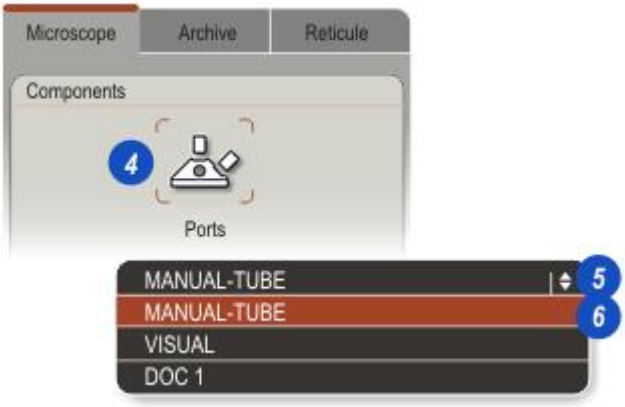
1: No Camera Port - the DOC option is missing.

2: A single Camera Port - *DOC 1* and...

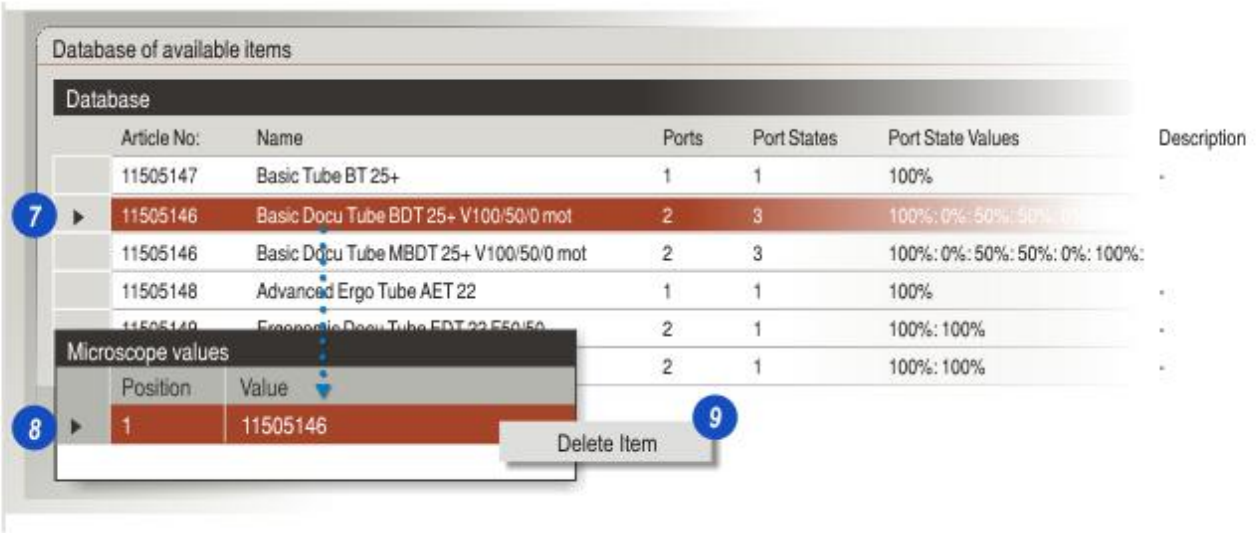
3: ...dual Camera Port - *DOC 1* and *DOC 2.*

The *Camera Port(s)* must be configured before camera(s) can be assigned by:

4: Clicking on the *Ports* icon on the *Microscope* > *Components* panel.



5: Click on the small arrows to the right of the *Port Options* header.



6: Click on the *Manual-Tube* option.

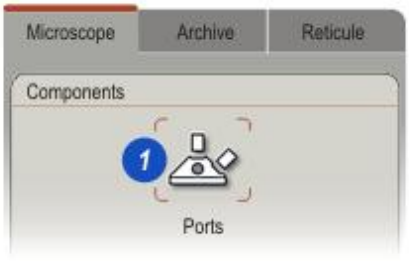
7: On the *Database* panel scroll to the required configuration and double-click to...

8: ...save it as the port setting. Repeat Step **(5)** and check that the required number of *Camera Ports* is available from the drop-down options.

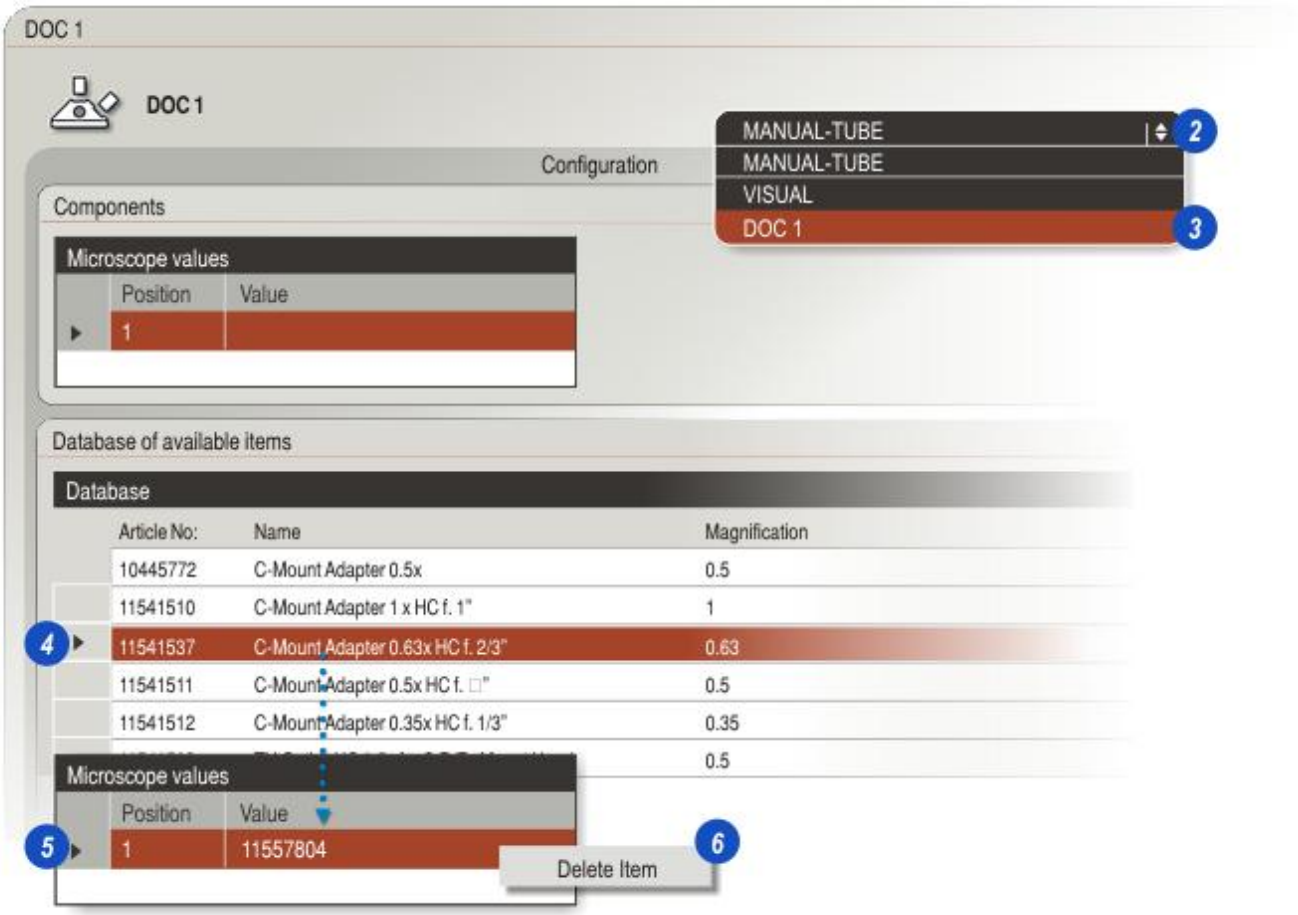
9: Remove the configuration by right-clicking it in the *Microscope Values* panel and then left-clicking the pop-up *Delete Item* button.

With the *Camera Ports* configuration selected, the *Camera Adapter* has to be selected for each port.

1: Click on the *Ports* icon on the *Microscope* > *Components* panel.



2: Click on the small arrows to the right of the *Port Options* header and from the drop-down...



3: ... click to select the *Camera Port* to which the adapter will be assigned - in the example *DOC 1*.

4: On the *Database*, double-click to select the name of the fitted *Adapter*.

5: The selection appears in the *Microscope Values* panel.

6: To remove *a selection, right-click on the* Microscope Values entry and left-click the *Delete Item* pop-up button.

Repeat the process for the other *Camera Port* if fitted.

The *Camera Ports* [187] and *Mounts* must be set up before the camera(s) can be assigned to them:

Fit and connect the camera(s) so that they can be detected by the software.

1: Click on the *Camera* icon on the *Microscope* > *Components* panel.

2: Click on the small arrows to the right of the *Camera Options* header.

3: From the list, click to select *Camera (DOC 1)*. This is *Camera Port DOC 1* to which a camera is about to be assigned.

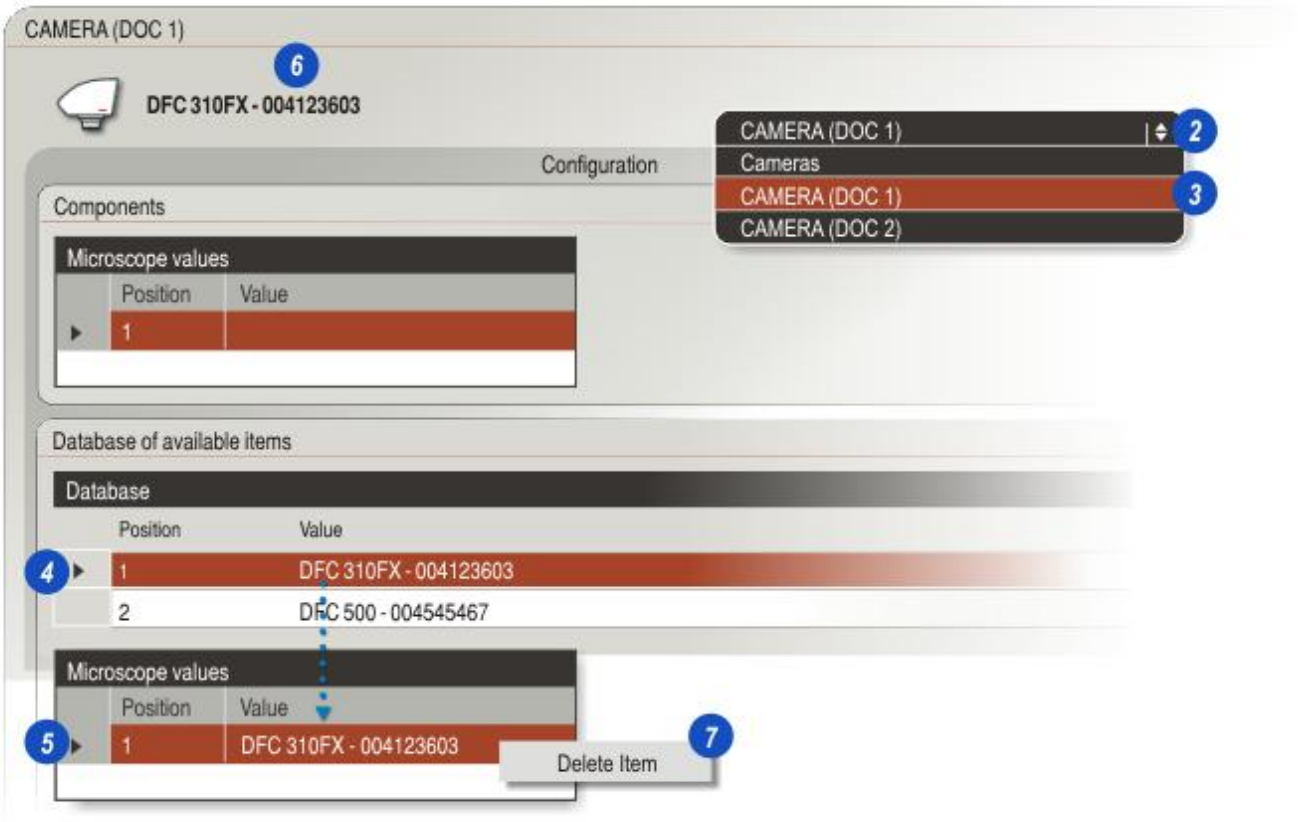
4: The *Database* lists the fitted cameras - for the majority of systems there will be just one *Camera Port* and therefore one *Camera* type in the list. Double-click on the *Database* entry and...

5: ...the *Camera* type is assigned to the *DOC 1* port and appears in the *Microscope Values* list.

6: The *Camera* and its serial number are also displayed on the header.

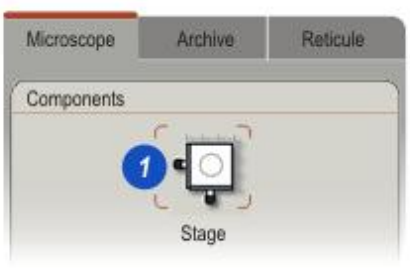
7: To remove a *Camera* from the *Microscope Value*, right-click on the entry and then left-click the pop-up *Delete Item* button.

Repeat the process if a second camera is fitted selecting the *DOC 2* port.

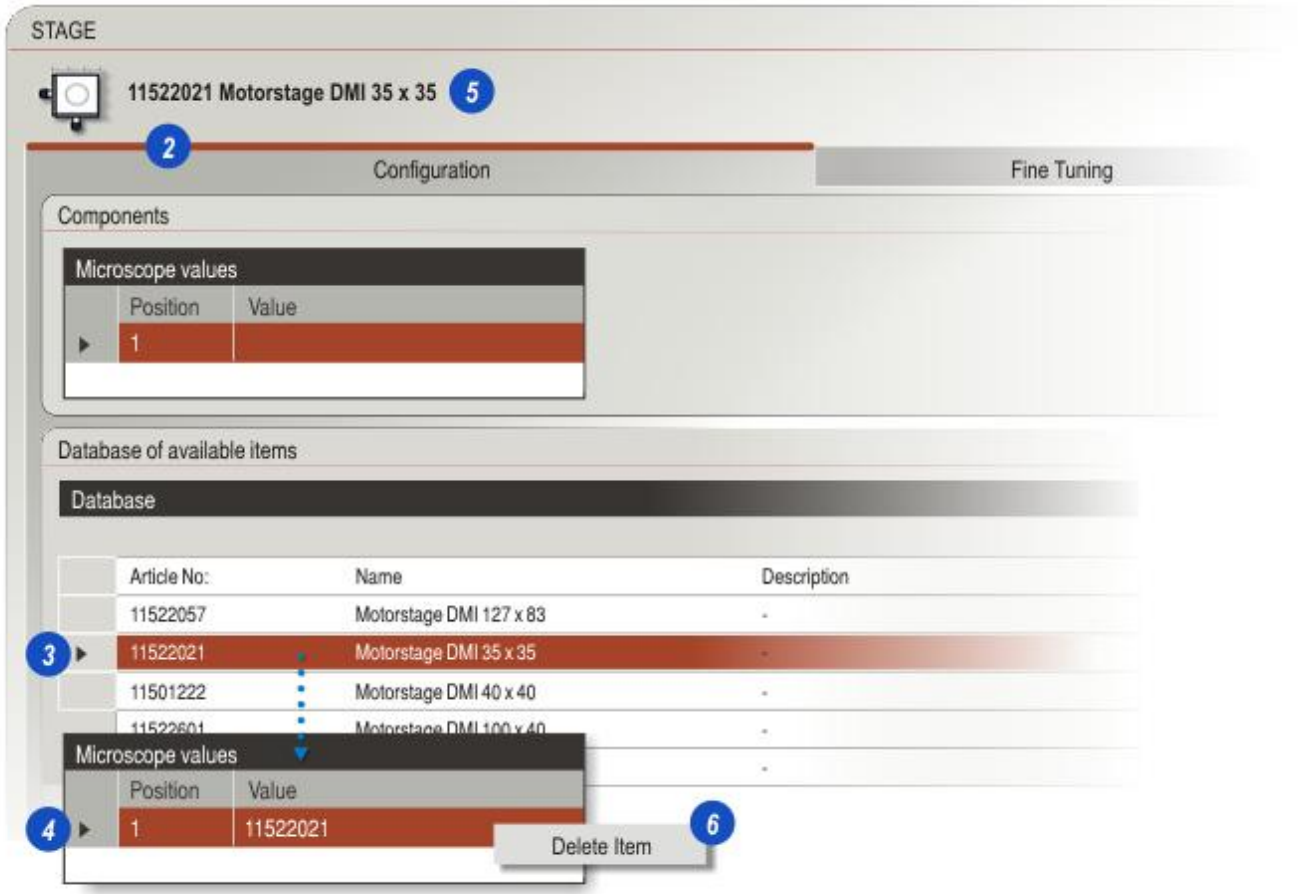


Stage Selection and *Initialisation* are both carried out from the same panel.

1: Click on the *Stage* icon on the *Microscope* > *Components* panel.



2: To select the stage type click on the *Configuration* tab.



3: Move to the fitted stage name and type on the *Database* and double-click it.

4: The stage *Article Number* appears in the value list with...

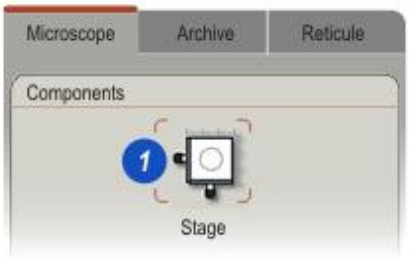
5: ...the description displayed on the header.

6: To change the selection, right-click on the selection in the values list and then left-click the *Delete Item* pop-up button.

Repeat the process described above to make a new selection.

Stage Selection and *Initialisation* are both carried out from the same panel.

1: Click on the *Stage* icon on the *Microscope* > *Components* panel. For initialisation the stage must be lowered sufficiently to avoid collision with specimens or objectives.

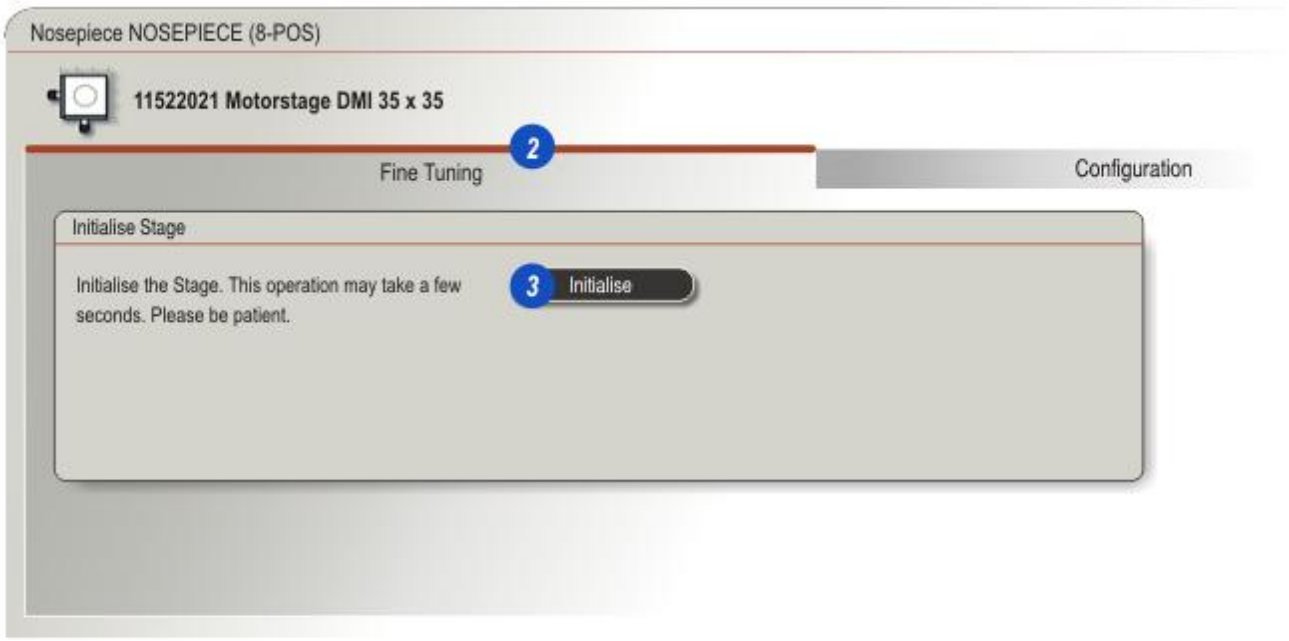


2: To initialise the stage, click on the *Fine Tuning* tab and...

3: ...click the *Initialise* button to drive the stage to its limits and set the starting position to top-left (X = 0/ Y = 0).

During initialisation the stage can move very quickly and the drive mechanism is powerful enough to severely damage specimens and objectives.

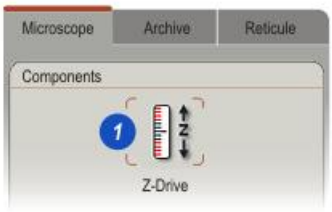
Also ensure that there are no cables that are likely to become entangled.



The *Z-Drive* dialog is divided into 2 panels:

- Focus Position and...
- Initialisation.

1: Click on the *Z-Drive* icon on the *Microscope* > *Components* panel.



2: Set the preferred initial *Handwheel Speed* by clicking either *Fine* or *Coarse*. The selection is highlighted.

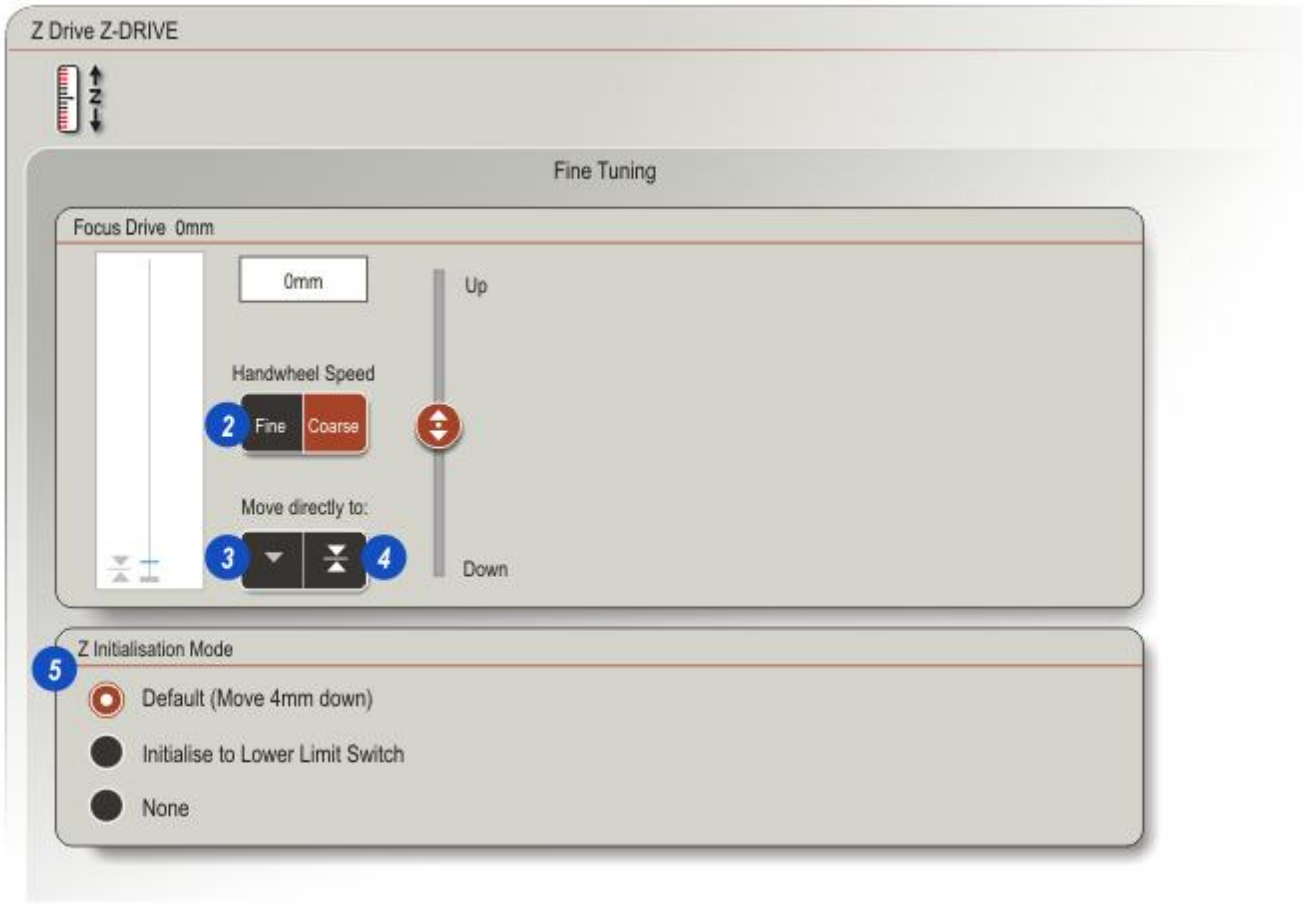
3: Drive immediately to the *Lower Limit Switch* or...

4: ...to the selected *Lower Threshold.* Please see here [193]

5: The *Initialisation Mode* can be set to one of the following:

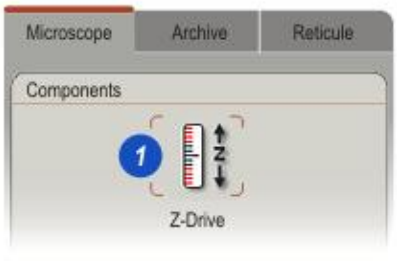
- *Default*: Factory setting for 4mm lowering.
- *Lower Limit Switch*: Drives the stage to its lowest position.
- *None:* The stage remains in the focus position last set.

See also *Stage Initialisation* [191]



The current *Z* position can be saved either as a repeatable focus or as the lower threshold for initialisation.

1: Click on the *Z-Drive* icon on the *Microscope* > *Components* panel.



2: To set the focus or lower threshold position, click and hold down the *Focus Drive Slider* and move it to the required position. The distance is displayed in the window.

3: Right-click on the *Slider* to reveal the *Settings* and *Help* options. Click on *Settings* and...

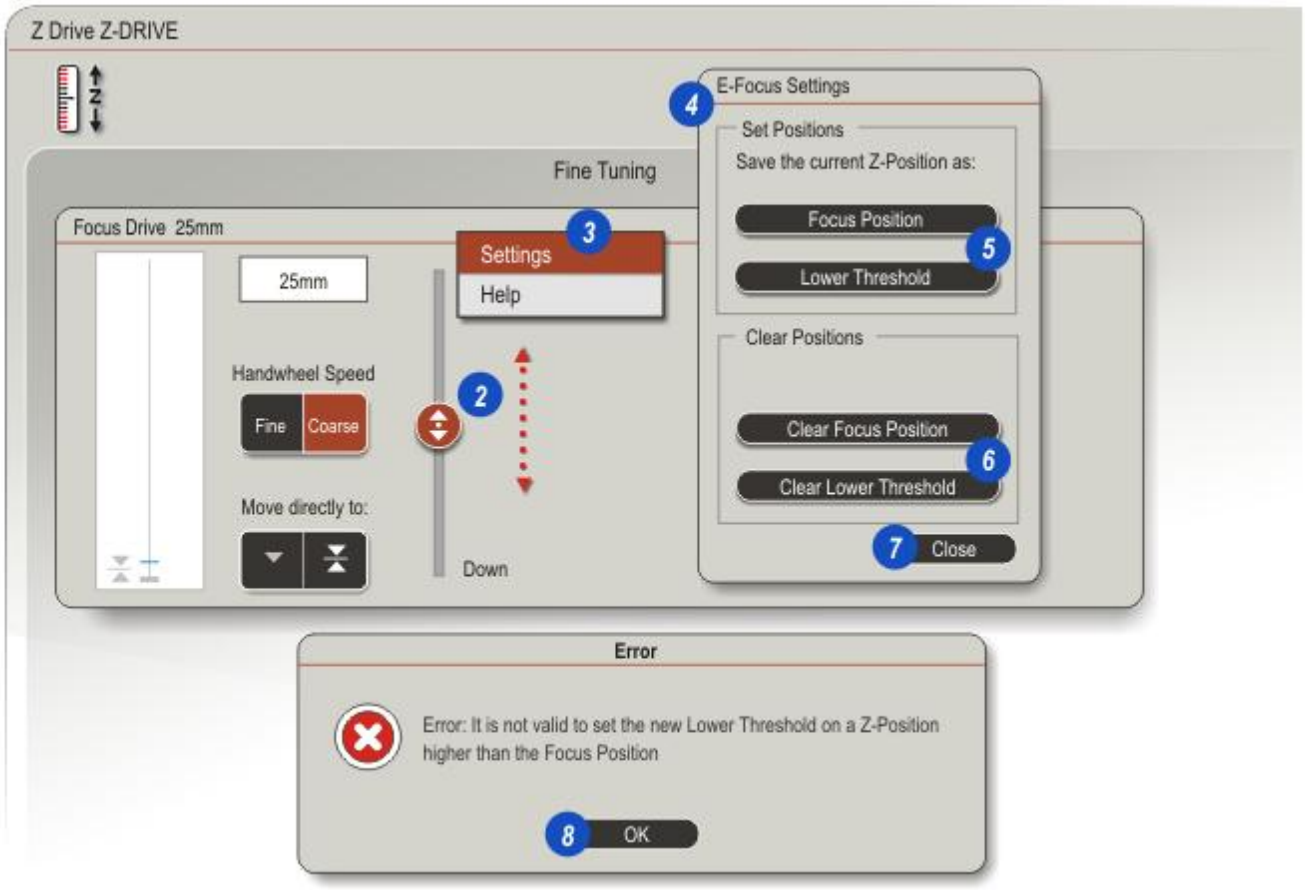
4: ... the *Focus Settings* dialog appears.

5: Save the current *Z* position as either the repeatable *Focus* or the *Lower Threshold* by clicking the appropriate button.

6: Clear either setting by clicking the appropriate clear button and...

7: ...click *OK.*

8: It is not possible to save a *Focus Position* that is lower than the *Lower Threshold* - the warning message appears.



To select a single condenser or assign different condensers to turret positions:

1: Click on the *Condenser* icon on the *Microscope* > *Components* panel.

2: Click on the small arrows to the right of the *Condenser* options drop-down menu and...

3: ...click to select either a single condenser or the turret.

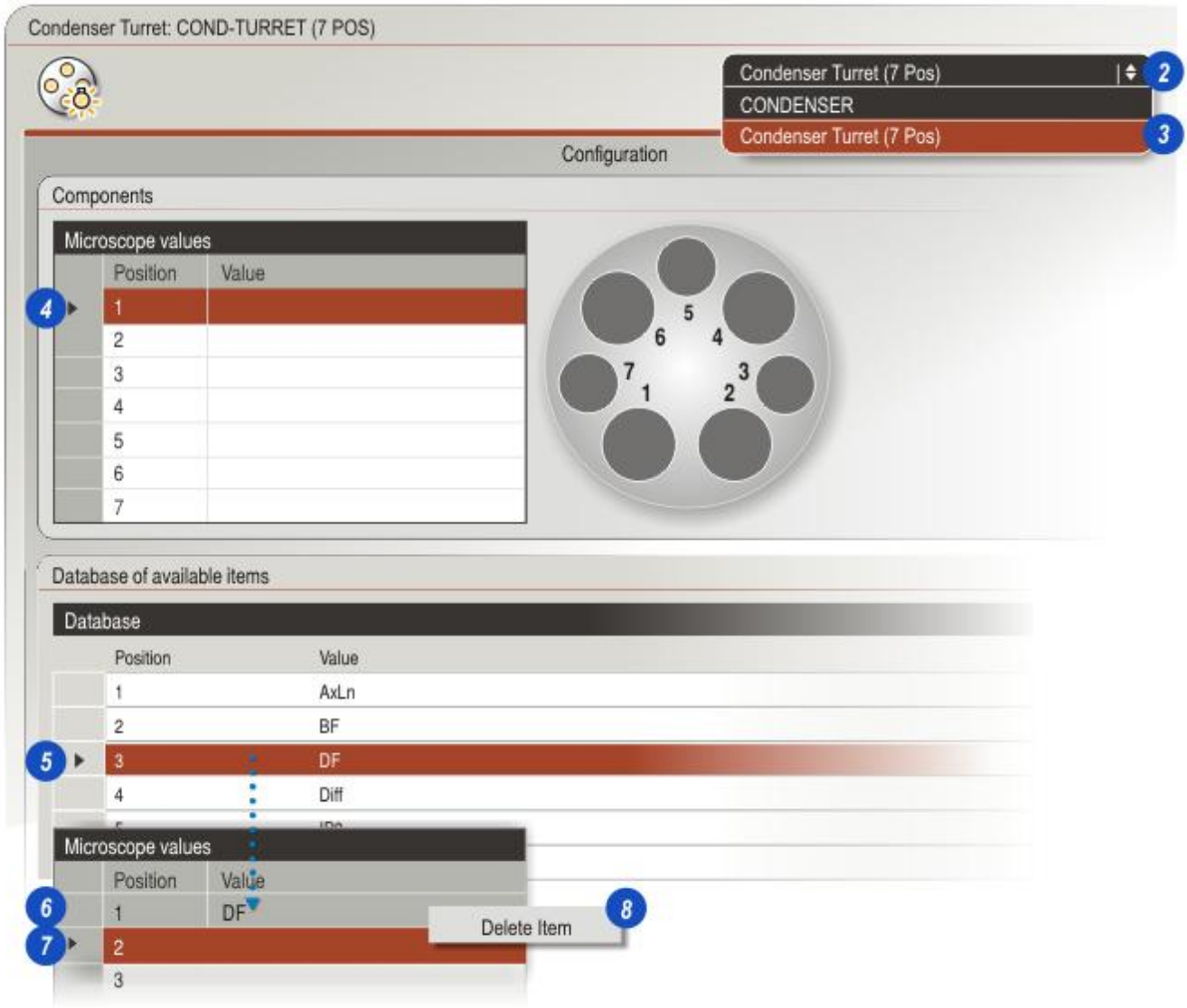
4: Either a single position or the number of turret positions is listed under *Microscope Values*. Click on the position to be assigned.

5: From the *Database*, double-click the type to be assigned to the position.

6: The selected type is displayed in the chosen position and...

7: ...the next position (if the turret option is selected) is automatically highlighted.

8: The remove an assigned condenser type, right-click on the position and left-click the *Delete Item* pop-up button,



The microscope push-button controls can be assigned to functions that suit the user.

1: Click on the *Function Keys* icon on the *Microscope* > *Components* panel.

2: The *Configuration* dialog appears with the push-buttons displayed as a graphic 'map', each with a number.

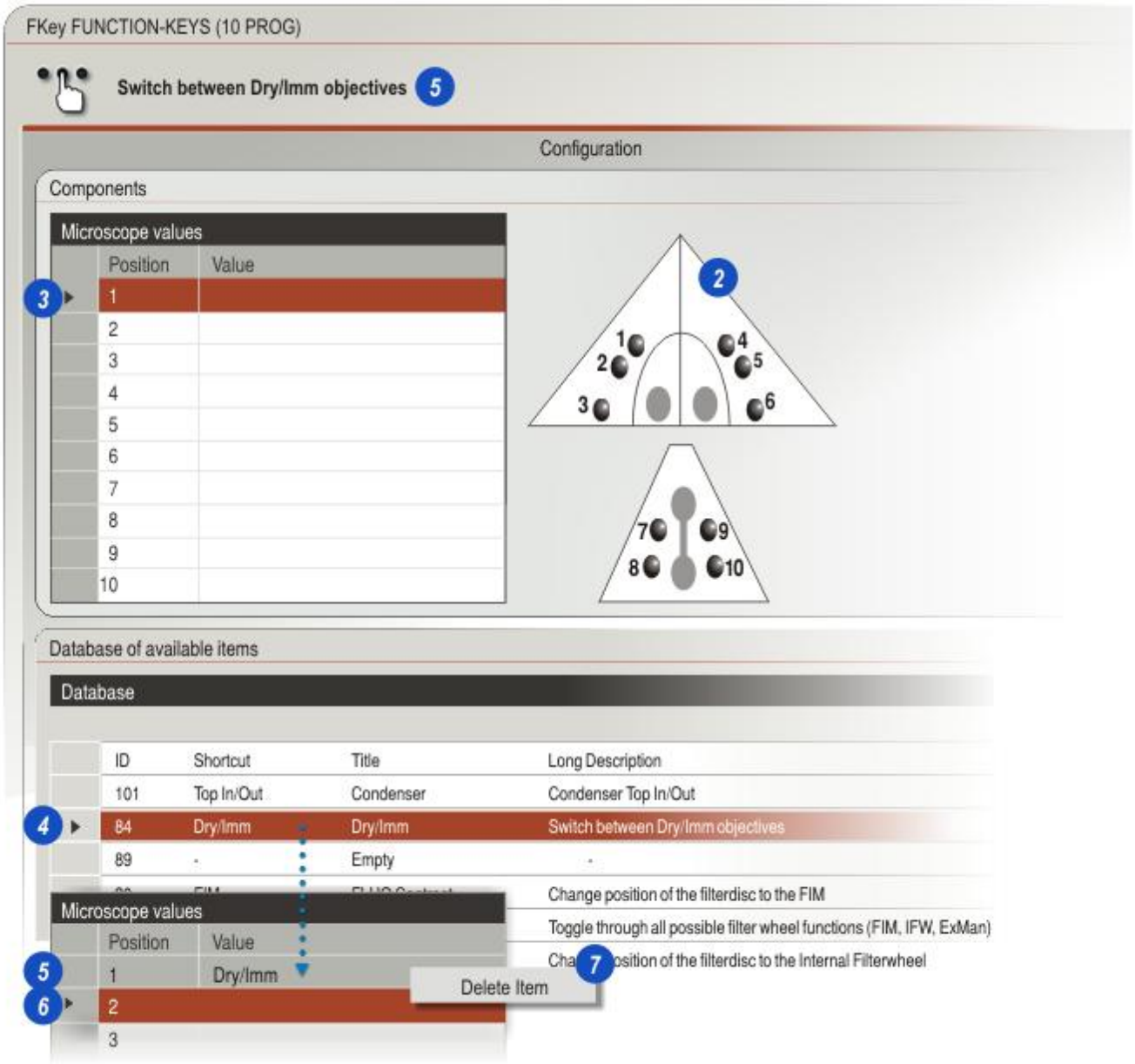
3: The push-button numbers are listed under the *Microscope Values*. Click a position to select the push-button.

4: Double-click the function on the *Database* list to assign it to the button.

5: The function is displayed on the *Microscope Values* list and on the header.

6: The next push-button is automatically selected.

7: Right-click on an entry and then left-click the *Delete Item* pop-up button to clear an assignment.



The *Acquire Workflow* provides control for the attached microscope and camera and also displays a live image on the *Viewer*.

Images are acquired, stored and displayed in the *Gallery* as thumbnails.

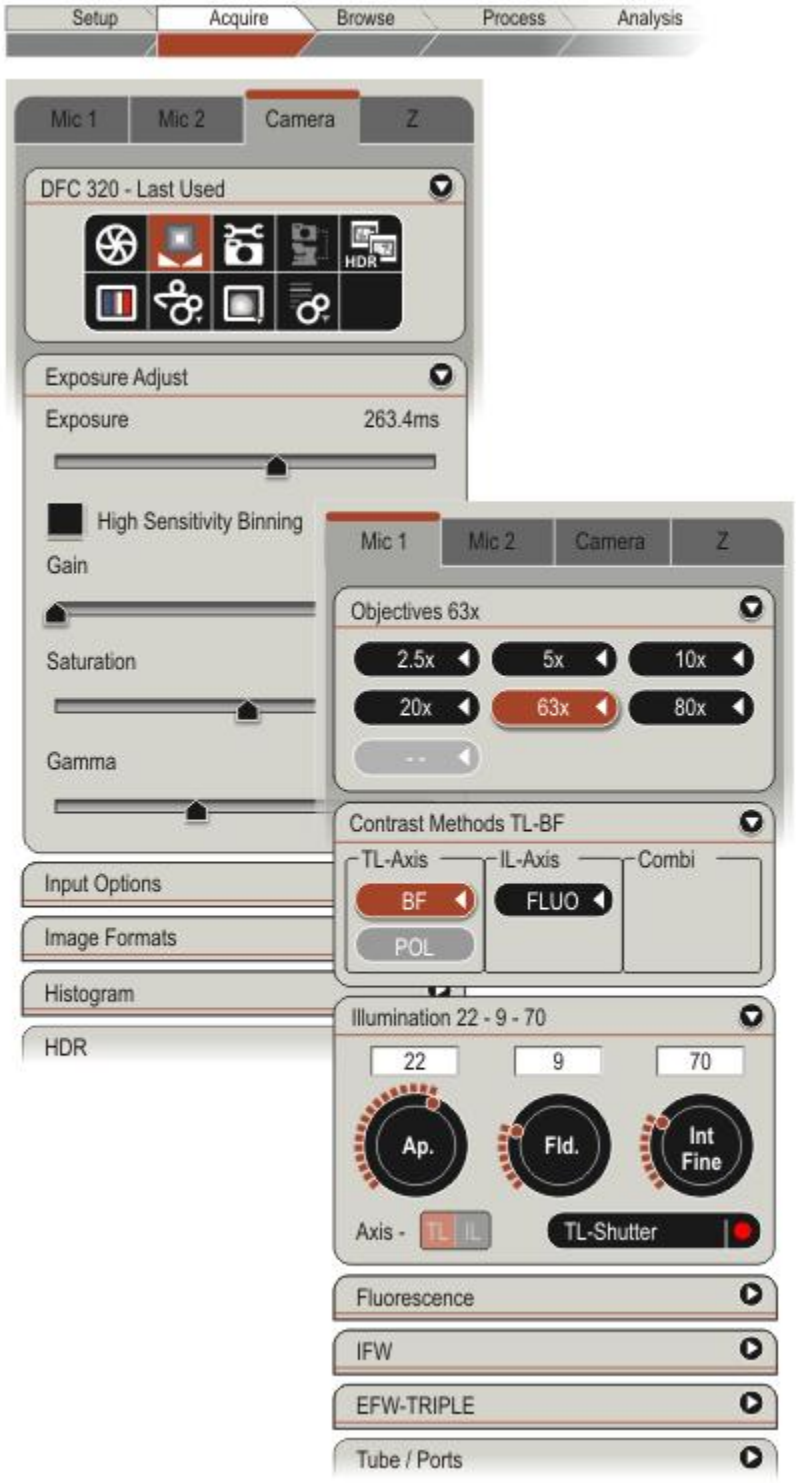
Within *Acquire* all microscope and camera controls can be set to individual requirements ranging from objective magnification, contrast method, exposure, gain and gamma.

The following optional modules provide more specialised acquisition options:

- Montage MultiFocus.
- Measurements
- Image Overlay
- MultiStep
- MultiTime Timelapse.
- Image Analysis
- Power Mosaic
- Phase Expert

The optional module *LAS Archive* provides the speed and power of a database for storing images and data. The following variants are available:

- *Archive Basic:* Essential *Archive* tools with versatile structuring and storage options
- *Archive Standard:* Taking *Archive* to the very highest levels of reporting and display.



The illustration is a graphical representation of the LAS display and Acquire user interface showing the principal features and quick links:

1: *Workflows*[198]*:* Click the *Acquire Workflow* to launch the microscope and camera controls.

2: *Tabs:* Select microscope, camera or specialised acquisition functions.

3: *Tools*[273]*:* Toolbox when *Camera* is selected.

4: Control Panels for:

Stereo Microscope[212]

DM Microscope[238]

FS Microscope[264]

Camera[270]

5: *Image Data Form*[210]*:* Displays and edits selected data for the current image.

6: *The Grid*[209]*:* Displays data for all of the images in the selected folder.

7: *The Gallery*[207]*:* Displays thumbnails of all the images in the selected folder.

8: *Acquire Button*[211]*:* Click to grab and image from the microscope camera.

9: *Side Toolbar*[199]*:* Working tools for image sizing, printing and deleting as well as switches for the *Gallery* and *Grid*.

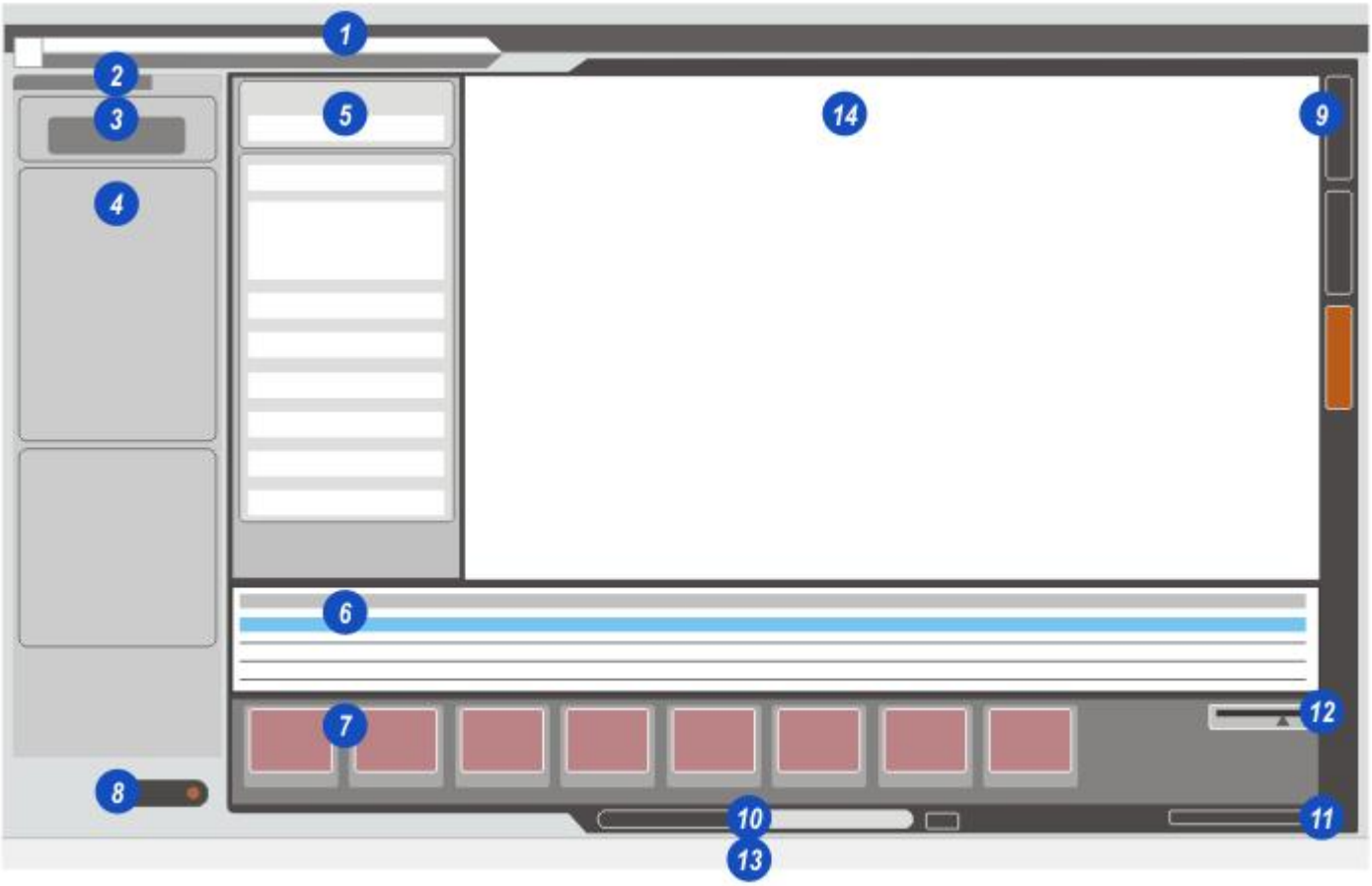
10: *The Search Controls*[508]*:* Available only with *LAS Archives.*

11: *Gallery Navigation Browser*[207]*:* Rapidly find thumbnails in the *Gallery*.

12: *Thumbnail Scaler*[207]*:* Slider adjusts the size of the thumbnails in the *Gallery*.

13: *Status Bar*[73] : Displays Hardware Configuration, RGB Intensity, Stage Position and Magnification data.

14: *The Image Viewer*[206]*:* Display and working area for the current image: Press keyboard *F5* to show full screen.



1: Click on the *Acquire Workflow*.

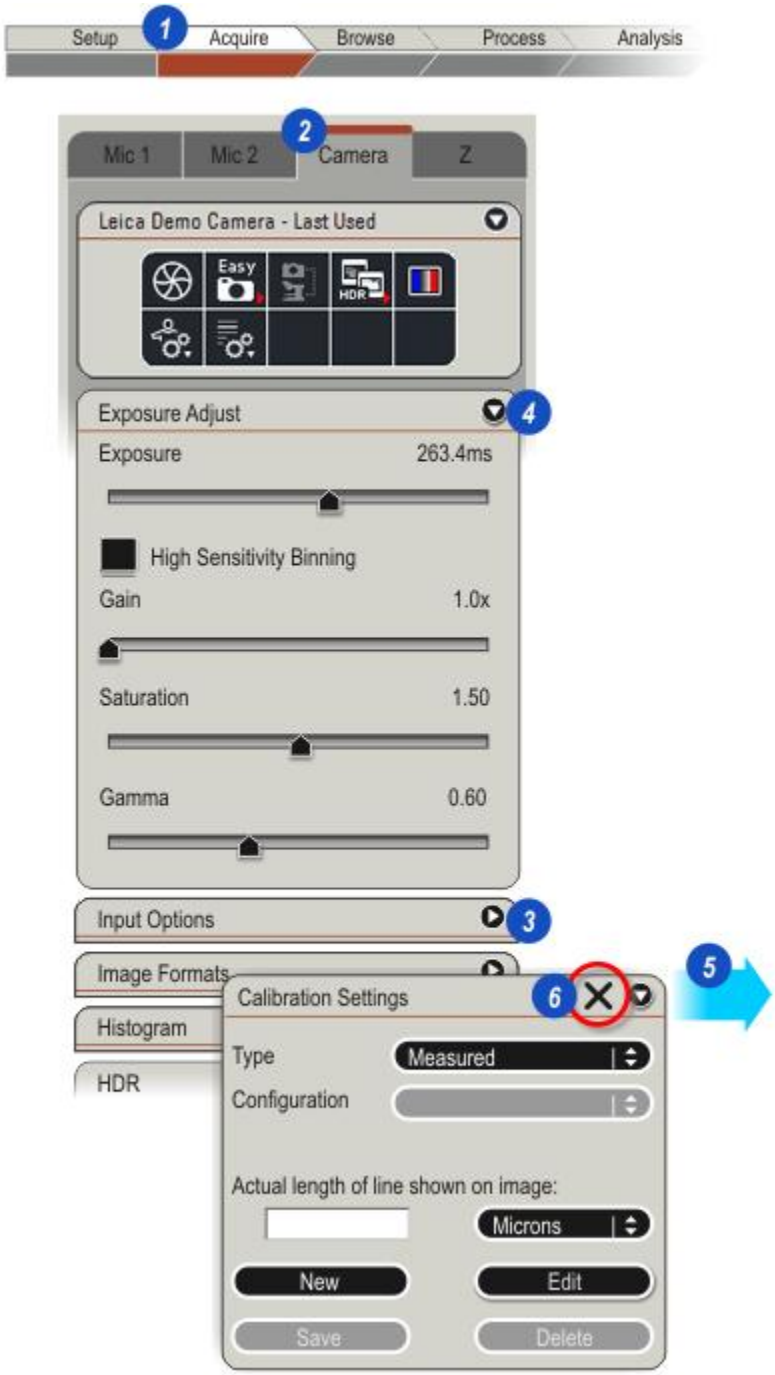
2: Click on a tab to select microscope, camera, the selected sequence module or *Live Measurement.*

3: Some control panels are collapsed to avoid cluttering the working area.

4: Expand a control panel by clicking on the arrow to the right of the panel header.

5: Most panels can be moved to any part of the *Viewer* and docked by clicking on the panel header and dragging it to the preferred location.

6: When a panel is moved a 'snap back' symbol (X) appears on the header. Click it to return the panel to its usual position on the tab.



This section describes the *Side Tool Bar* tools that are common to most of the Acquire features although the tool range may change with a selected feature.
Click on a *Tool Bar* button for more information:

Scale Bar and Annotations: Run Annotations and Scale Bar without leaving Browse.

Floating Navigator: Click to enable the *Floating Navigator* and click again to dock it.

Panning: Examine areas of images that extend beyond the *Viewer* edges into the display area.

Zoom in and...

Zoom Out.

Fit the image to the Viewer area.

Display at Original Size: Displays the image at its captured size.

Hide and Reveal the Record Panels.

Hide and Reveal the Viewer.

Hide and Reveal the Data Grid: Only available with LAS Archives.

Hide and Reveal the Thumbnail Gallery.

View the image Record Details: Not available in Acquire.

Select the Form Details to display: Not available in Acquire.

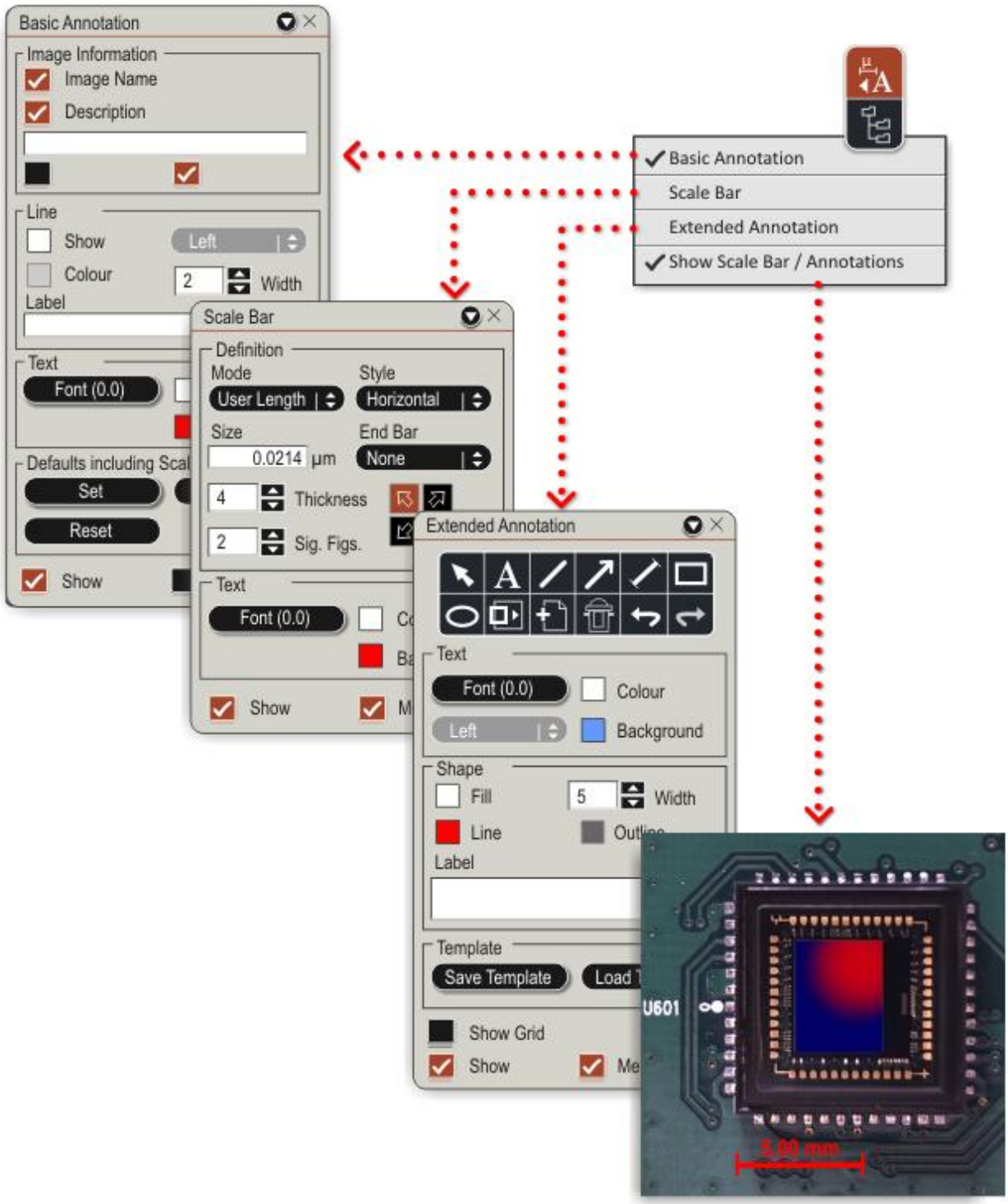
Viewer Options: Select Dual Viewer, Lock the Views and *Lock the Pan View.*

Copy Output Image: Not available in Acquire.

Clicking the *Show Annotations* button displays the *Annotations* and *Scale Bar Quick Launch* menu.

On the live image *Basic Annotation, Extended Annotation* and the *Scale Bar* setup can be launched without leaving *Acquire* by checking (a tick mark is displayed) the required function. All of the function tools are available.

The *Scale Bar* display can be turned on and off by clicking the *Show Scale Bar / Annotations* option. Turned on and the annotation tools are available.



The *Browse Navigator* panel can be displayed and docked on the *Acquire Viewer* by:

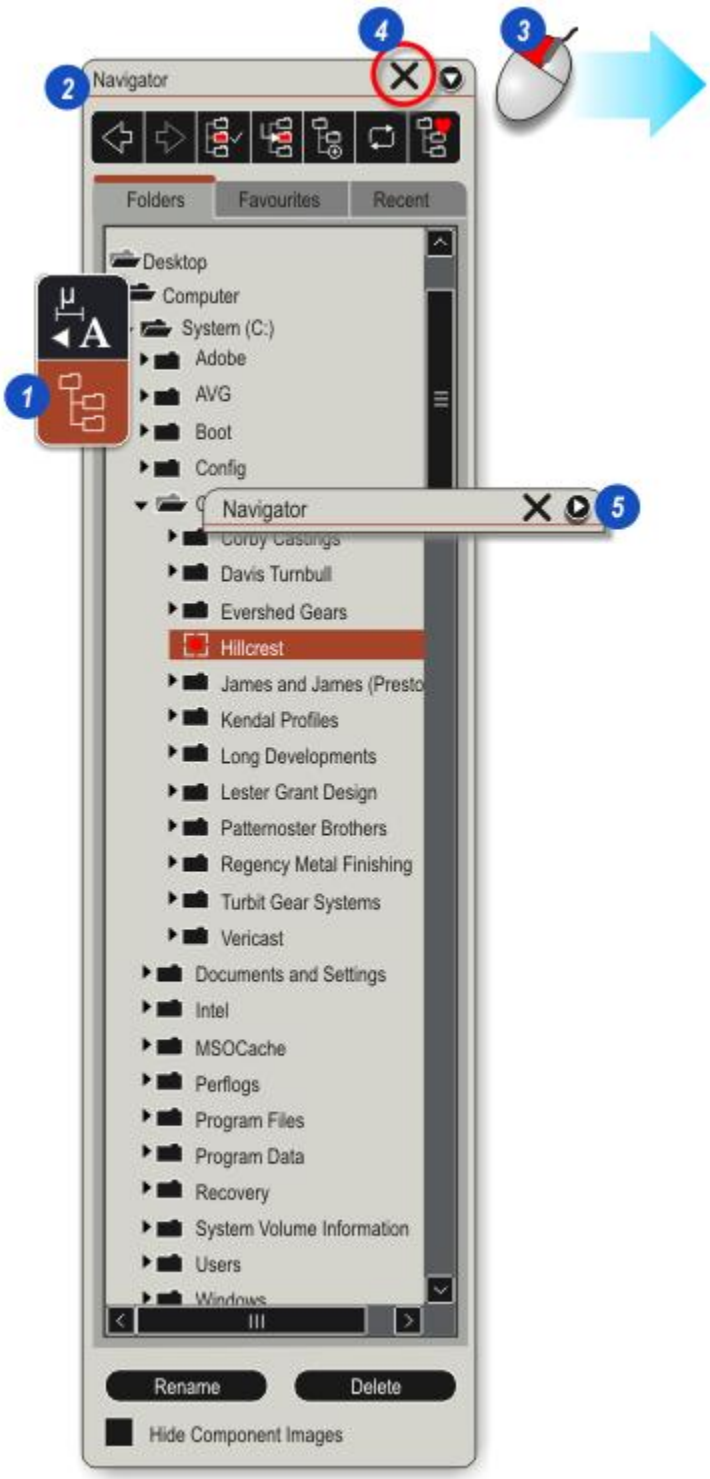
1: Click on the *Floating Navigator* button on the *Side Tool Bar.*

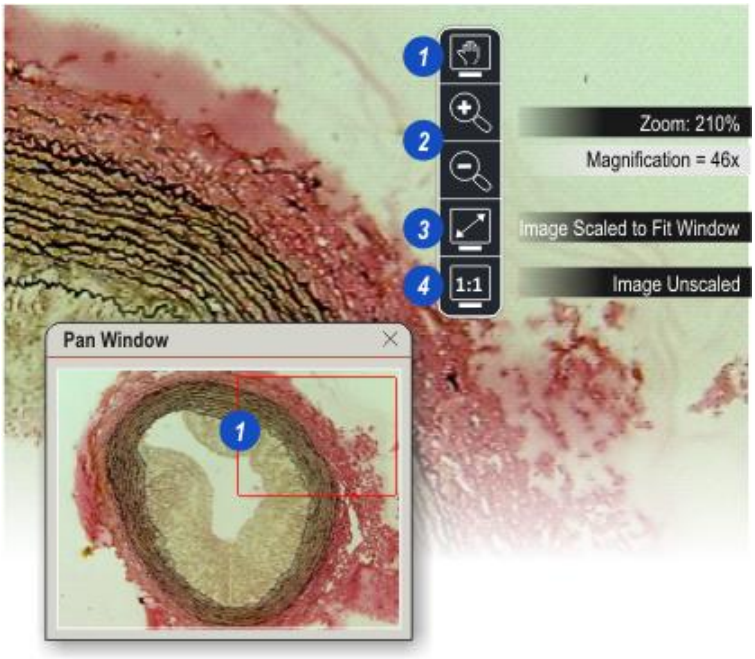
2: The *Navigator* panel appears and can be used in the same way as it would be from the *Browse Workflow* except the *Toolbox* is not available.

3: Move and dock the *Navigator* by clicking on the header bar and, holding down the mouse button, drag the panel to a new position.

4: Click on the 'snap-back' button (X) to close the *Navigator*.

5: Collapse the *Navigator* without closing it completely by clicking on the arrow to the right of the header. Click again to restore the panel.





1: *Panning:* The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

On the *Pan Window* viewer, click and hold in the outlined rectangle and drag it to the area to be examined. The selected area is displayed in the main *Viewer*.

To move the *Pan Window* away from the main *Viewer*, click and hold the header bar and drag it to another part of the screen.

Click the *Pan* tool to close the *Window*.

2: *Zoom:* Click on the (+) to zoom in to the image or (-) to zoom out. The zoom level as a percentage, is displayed top right of the the *Viewer* border.

If the monitor *Magnification Settings* have been set in *Preferences* 55, the image *Magnification* value appears bottom right of the *Viewer* border.

3: *Fit to Viewer:* Click to fit the image to the available *Viewer* area regardless of the original size of the image. The *Image Scaled to Fit Window* message appears top right of the *Viewer* border.

4: *Display at Original Size:* Click to display the image at its original size. The image may appear smaller or larger than the *Viewer* area. The *Image Unscaled* message appears top right of the *Viewer* border.

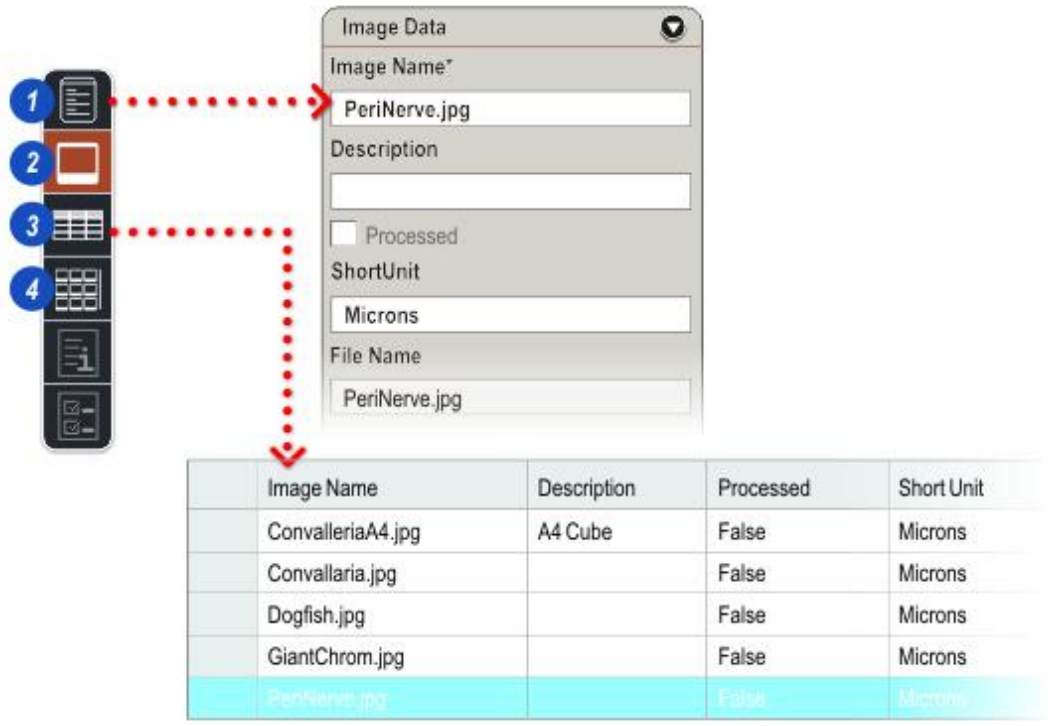
The various screen areas - *Viewer, Gallery, Report* and *Grid* (where applicable), may be revealed or hidden to create the best working environment for the user. Some tools are toggles – click once to reveal the area, click again to hide it:

1: *Hide/reveal the Record panels:* The *Image Viewer* expands to fill the vacant space.

2: *Hide/reveal the Viewer:* The *Record* panels expand to occupy the *Viewer* width.

3: *Hide/reveal the Data Grid:* The *Viewer* will expand to cover some of the vacated space. The *Grid* is only available if an *LAS Archive* is installed.

4: *Hide/reveal the Thumbnail Gallery:* The *Gallery* is hidden and the *Viewer* expands to include the *Gallery* space.



The *Viewer* area can be split to show two images simultaneously.

1: Click the *Viewer Options* button.

2: Click to enable (tick mark visible to the left) the *Dual Viewer* option. The *Viewer* will then divide into two panes.

3: The live image currently being viewed will appear in the left-hand pane.

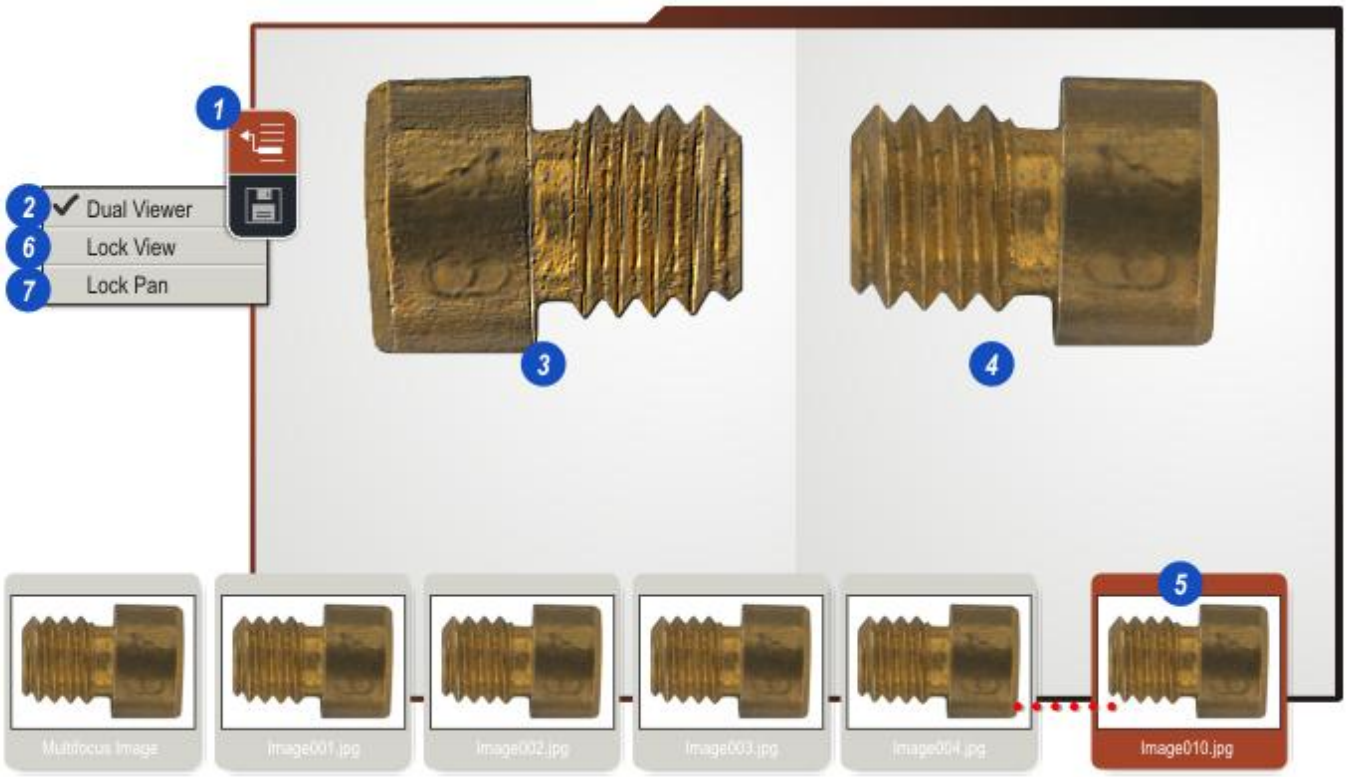
4: Display an image in the right-hand pane by clicking the pane and...

5: ...the thumbnail of the required image.

6: To synchronise the panes and enlarge or reduce the images as the zoom and fit tools are used, click to enable the *Lock View* option.

7: Enabling *Lock Pan* will synchronise the images as the *Pan* tool is used. Click the pane to pan and then on the *Panning* tool. Both images will automatically move to and display the image segment shown in the *Pan* window.

Dual Viewer *more information* 95



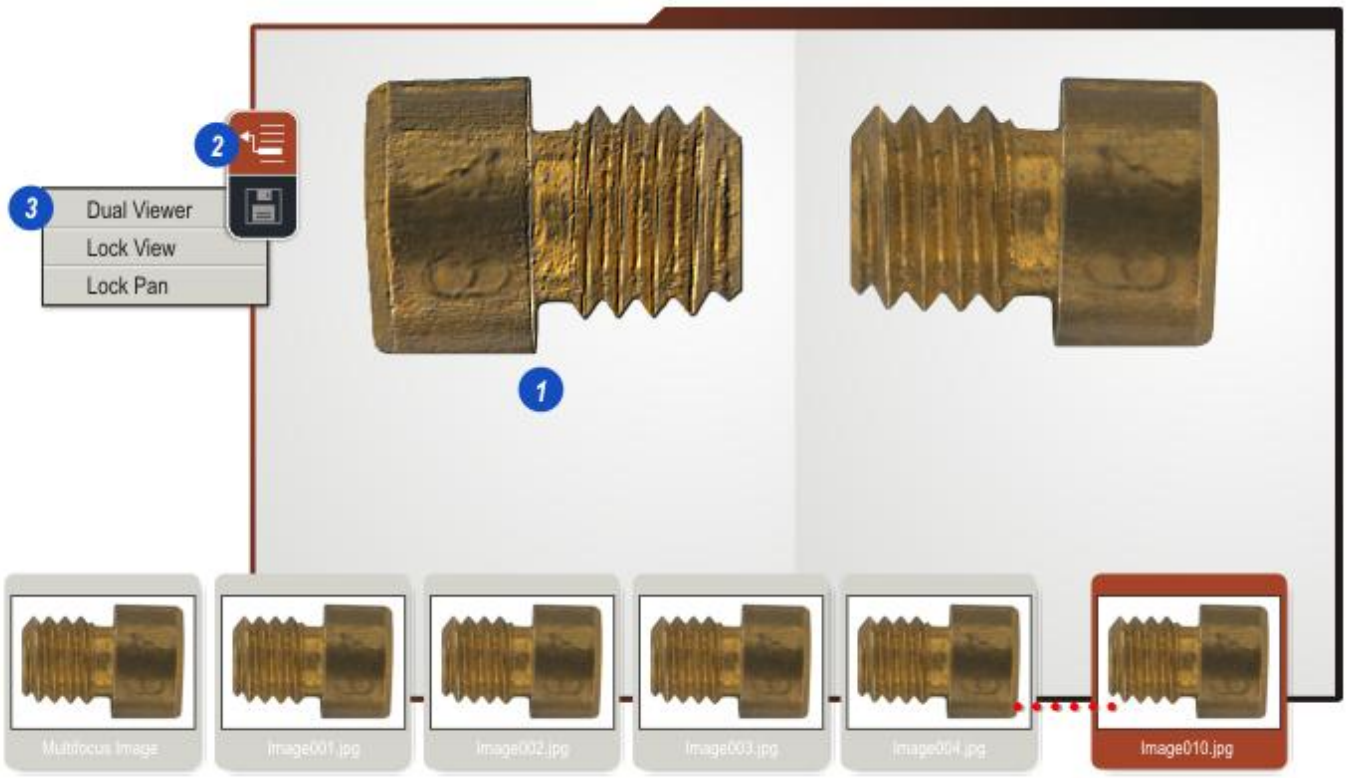
Turn Off Dual Viewer

To revert to the live image occupying the entire *Viewer*:

1: Click on the left-hand *Viewer* pane - the current live image.

2: Click on the *Dual Viewer* button.

3: Click to disable *Dual Viewer* - the tick mark disappears - and the live image expands to fill the *Viewer*.

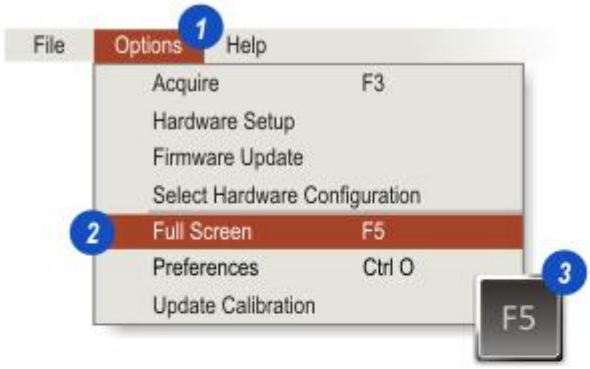


Full Screen Mode: Single Monitor:

1: The *Viewer* area can be expanded to fill almost the entire screen by either clicking *Options* on the main menu and...

2: ...selecting *Full Screen* from the drop down menu, or...

3: Pressing *Key F5*. Press *F5* again to return to the normal display.



Second Monitor:

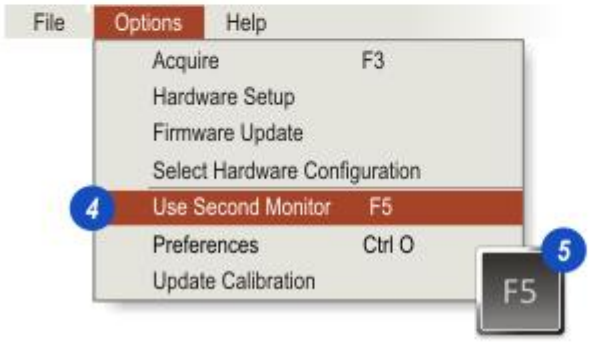
The software detects a second monitor and changes the Options drop-down menu to:

4: ... *Use Second Monitor*. Click the option to use both monitors.

The *Viewer* and image occupy all of the second monitor whilst the *Gallery* and *Thumbnails* together with the controls remain on the primary monitor.

The *Side Tool Bar* buttons are appropriately shared between the two monitors.

5: Alternatively, press *Key F5* to move between using both monitors and returning to single monitor.



The *Gallery* is a thumbnail display of images in the current folder in both *Image Explorer* or *LAS Archive.*

The *Gallery* can be hidden or revealed using the *Side Tool Bar* tools.

1: Clicking on a thumbnail will immediately display the full-sized image in the *Viewer* and the data associated with it in the *Record* and *Grid* (If LAS Archives is installed).

2: A *Slider* is automatically displayed for multiple rows of thumbnails - click and drag it to scroll the *Gallery*.

3: The *Navigation Bar* (bottom right of the screen) provides a way of moving through the thumbnails quickly and is especially useful with large galleries of thumbnails. Click on the arrows to move a single image **(3)** ...

4: ...or go to the extreme ends of the *Gallery*.

5: The thumbnails can be re-sized by clicking and dragging the *Scaling Slider* - slide left to reduce the thumbnail size and right to increase it.

6: Move the mouse over a thumbnail to reveal basic data bout the image.

7: Right-click a thumbnail to show the *Context Menu*. Left-click to select an option.

- Gallery Docking Position 208

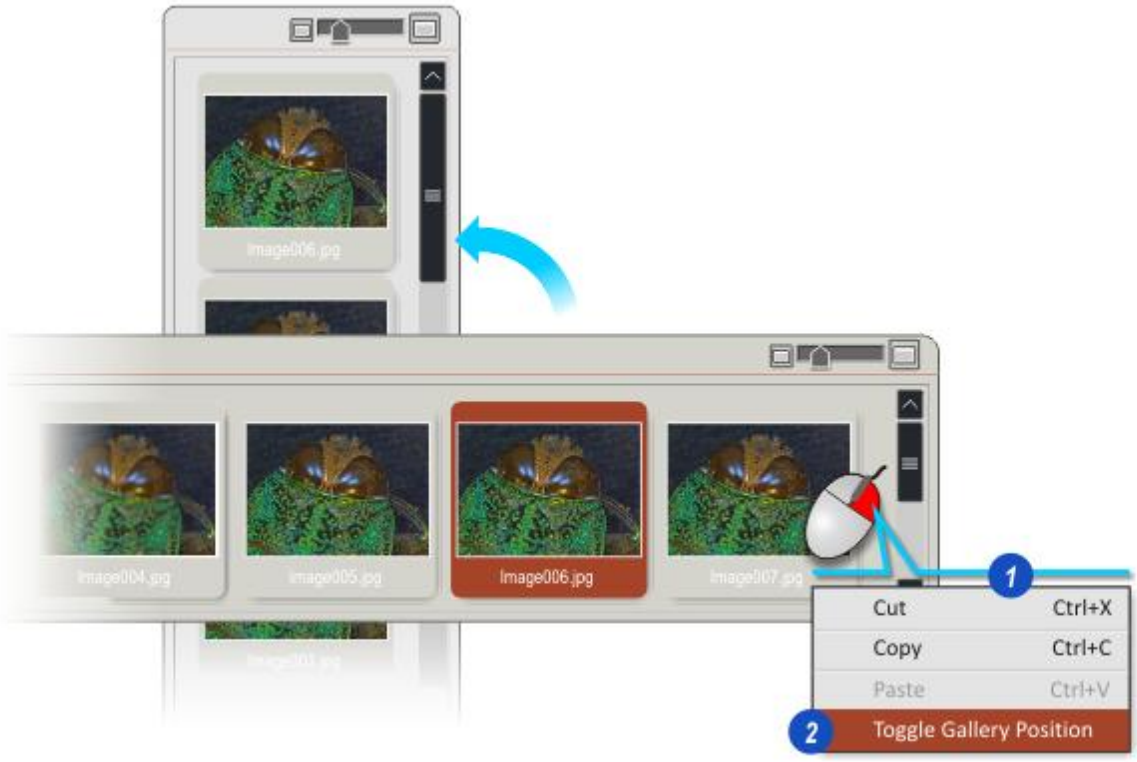
Available in *Acquire* and the *Process* Workflows as well as *Browse*, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

The action toggles between horizontal and vertical docking.

Scroll bars, if required, are placed automatically.



The *Grid* displays data for all of the captured images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

In Acquire captured images are displayed in the right-hand pane when the Dual Viewer is enabled.

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

2: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* right-hand pane and also highlight the thumbnail.

3: Header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

4: Column widths can be changed by clicking and dragging the vertical bars that separate the columns.

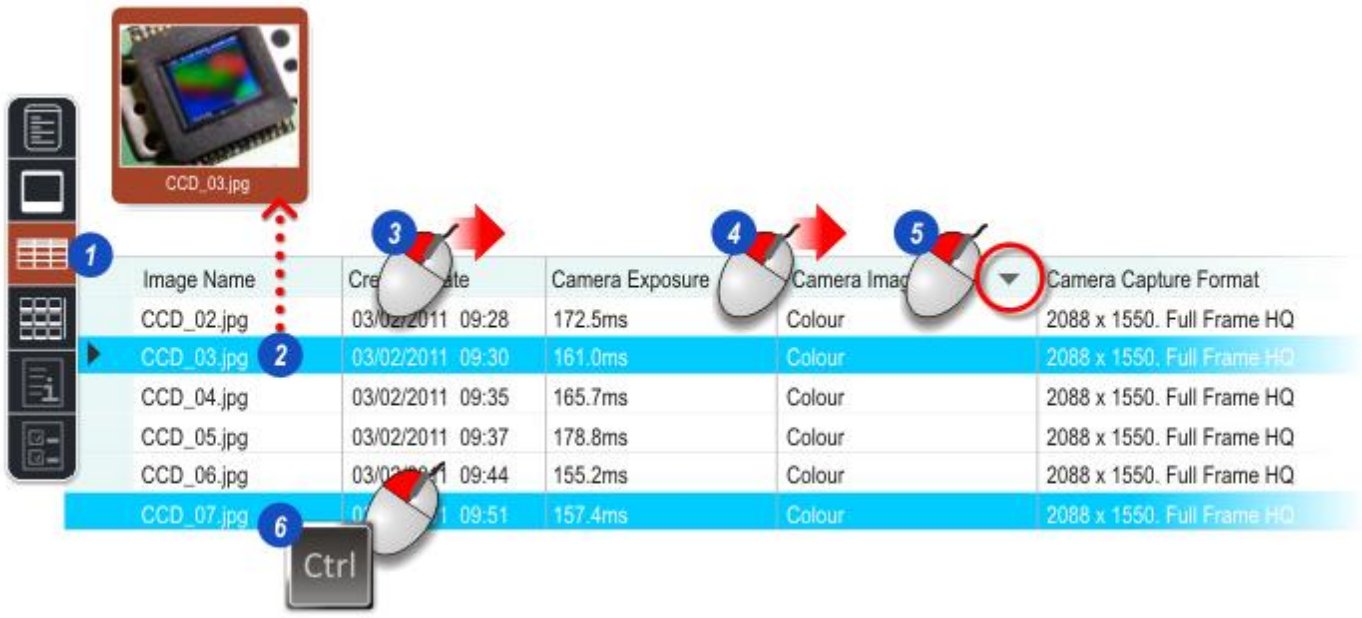
5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: To make multiple selections prior to cutting or exporting, hold down the keyboard *Ctrl* key whilst clicking individual thumbnails.

Keyboard combination:
Ctrl + *A* will select all of the image data.

Ctrl + *C* will copy all the selected image data to the clipboard.

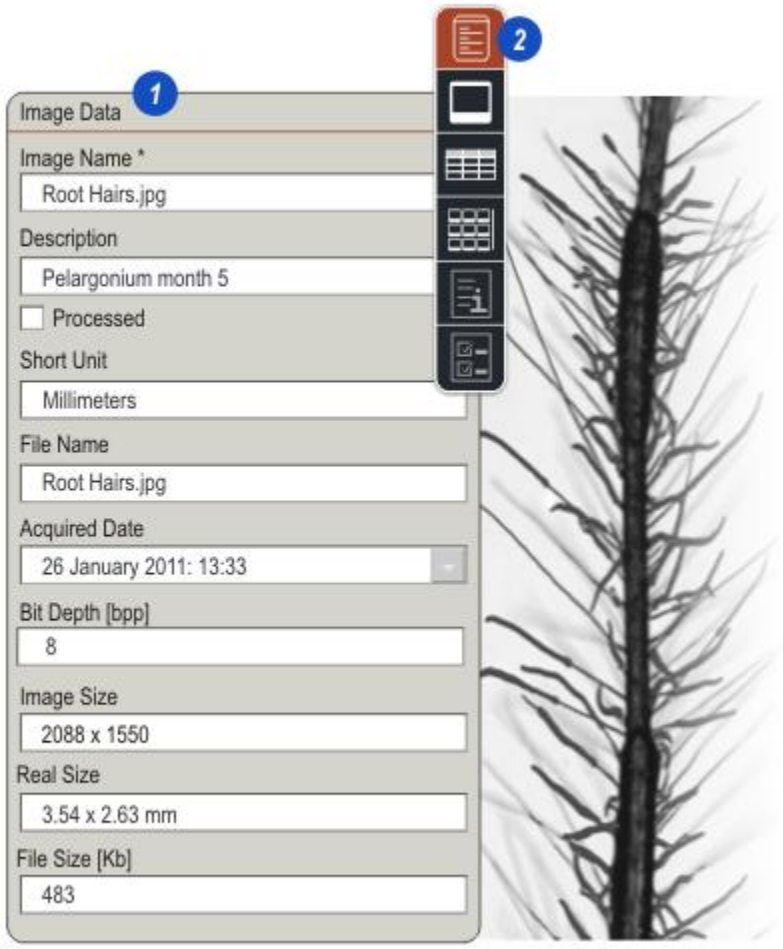
Ctrl + *V* will paste into another application.



1: The *Data Form* displays selected data associated with the image.

All of the data about the camera, microscope, exposure, creation date and so on, are actually stored and all can be displayed, but the Form can be configured to display only the more pertinent items.

2: The *Data Form* can be hidden by clicking the *Form* button on the *Side Tool Bar*. This is a toggle action - click again to reveal the *Form*.



The are three methods for starting image capture:

1: Click on the *Acquire Image* button at the bottom of the *Acquire Workflow*.

2: Press the keyboard *F3* function key.

3: Click on *Options* on the *Main Menu* and click the *Acquire Image* option **(4)**.

Stereo- and Macroscope Systems (SMS) is available on Leica Application Suite if an automated micro- or macroscope is connected to the computer.

SMS provides control of the micro- or macroscope from the computer and, if a digital camera is also fitted to the microscope will be especially helpful in optimising specimen images.

SMS can control:

- Motorised focus, 219
- Motorised zoom, 217
- X and Y stage positioning, 224
- Filter wheel, 216
- Zoom iris aperture, 221
- Zoom objective changer. 218
- Internal light source (TL), 223
- External light source (IL) and 222
- CCIC and Fluorescence shutters. 215

Five memory locations allow settings to be saved and recalled, precisely replicating the microscope setup.

Before using SMS, please check that the motorised focus cable is fitted, that the security clamp is properly fitted and in a position to prevent collision with the specimen and that all cables have sufficient slack to allow the carrier to travel to the top of the stand.

The following motorised/coded microscope devices are currently supported by LAS:

M 165 C and M 205 C
M 205A, M 205FA, M 165FC
MZ 16A microscope (10 447 103)
MZ 16FA microscope (10 447 063)
Z 6 APOA microscope (10 446 368)
Z 16 APOA microscope (10 446 369)
Motorised focus drives 300mm and 500mm (10 446 176 & 10 447 041)
Motorised focus drives 420mm and 620mm
TL RCI Internal light source (10 446 352)
TL4000 RCI Internal light source (10 450 126)
TL5000 Ergo Internal light source (10 450 541)
SmartTouch
UMC, Universal Manual Control (10 447 080)
Foot switch (10 447 398)
IsoPro, motorised X/Y stage
ITK Hydra XY
CLS150 LED
CLS150 XD and CLS150 LS (30 111 480 & 30 110 481)
KL1500 LED+
KL2500 LED
KL2500 LCD (31 250 200 & 31 250 201)

The following devices are automatically detected:

LED3000 RL, LED3000 SLI, LED3000 NVI
LED5000 RL, LED5000 SLI, LED5000 CXI, LED5000 HDI, LED5000 MCI

The following functions are automatically detected and can be controlled from SMS:

Light source intensity, On and Off
CCIC shutter Open and Close
Zoom magnification (Objective changer)
Motorised focus position
Fine focus position
Filter wheel position
Fluorescence shutter Open and Close
Zoom iris aperture.

The following manual microscopes and devices have reduced support in SMS:

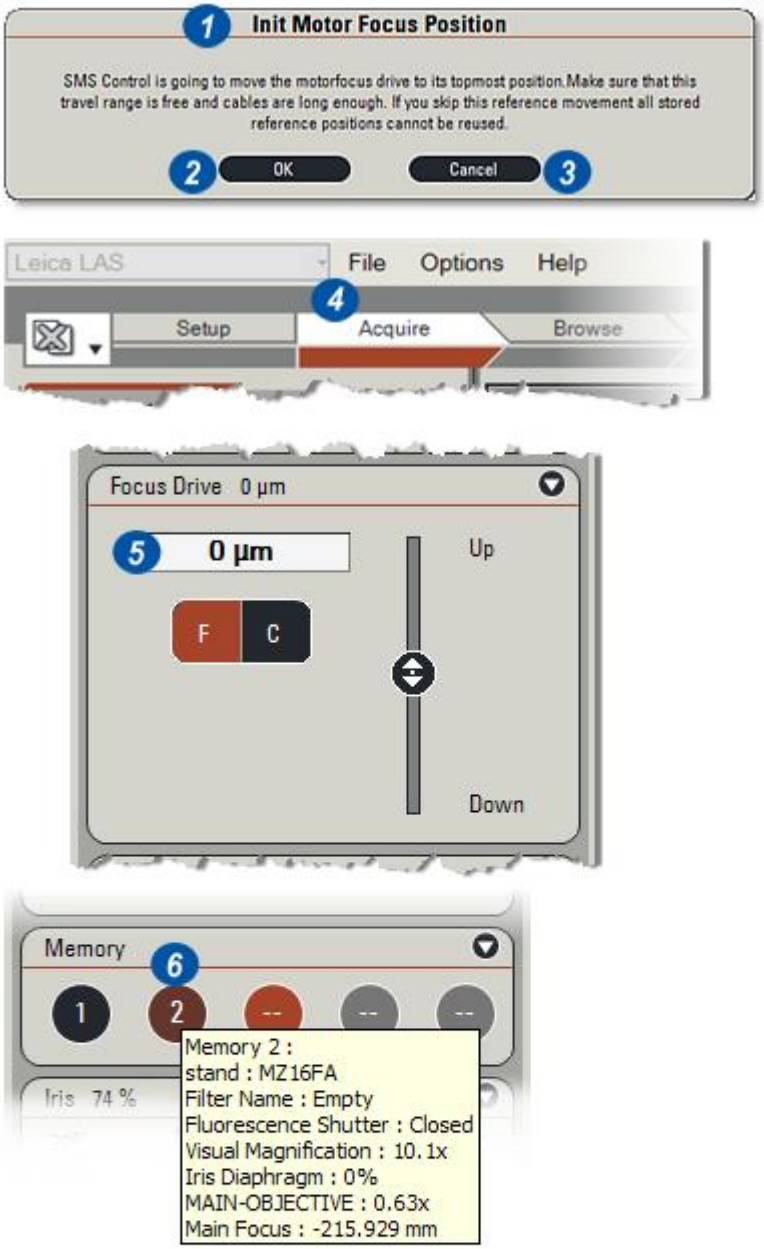
MZ 16F (10 447 064)
MZ 16 (10 447102)
MZ 12.5 (10 446 370)
MZ10 F
MZ 9.5 (10 446 272)
MZ 7.5 (10 446 371)
MZ 6 (10 445 614)
MS 5 (10 445 613)
M 125, M80, M60, M50
S6D (10 446 297)
S8 APO (10 810 038)
Z16 APO (10 447 173)
Z6 APO (10 447 174)
EZ 4 D, EZ 4 HD
Macrofluo
Fluocombi

If, at start-up, LAS detects a motorised focus, it will ask to initialise the focus position by displaying the message (**1**).

2: Click *OK* to send the carrier to the top of the stand and automatically initialise the focus position.

3: Click *Cancel* to skip the initialisation. If initialisation is skipped the exact focus position may not be reliable and will not be saved with other settings.

4: Click the *Acquire Workflow* to reveal the SMS control panels.
If focus was initialised the Focus Drive position (**5)** will display the current focus position.
If focus was NOT initialised, the value will be '0'.
Previous settings that were saved may be recalled and loaded by clicking the appropriate *Memory* button (**6**).

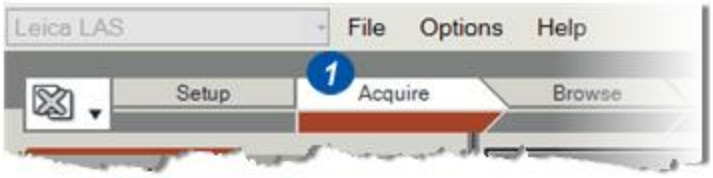


The Stereo- and Macroscope controls in the Leica Application Suite are revealed by:

1: Click on the *Acquire Workflow.*

2: Click on the *Mic1* tab. Depending upon the microscope and the functions available, the controls may be displayed on additional tabs named 'n' sequence - *Mic2* or *Mic3.*

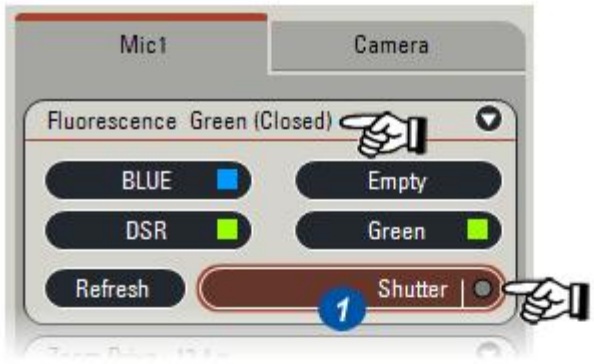
The components of the Stereo- and Macroscopes must be setup in the Setup Workflow before the Acquire:Mic tabs can be used.



The Shutter button on the fluorescence panel opens and closes the shutter. Close the shutter when the microscope is not in use to protect delicate specimens.

1: Click on the *Shutter* button. This is a toggle action, the Shutter opening and closing on successive clicks.
The Shutter status (Opened or Closed) is shown on the Fluorescence panel header bar and when it is open a red dot appears on the button.

2: If an *Empty* filter wheel position is selected, the Shutter control is not available(**3**) and remains closed.



1: Place the mouse cursor over a *Filter* button to reveal its specification (**2**). Click the button and the *Filter Wheel* will rotate to select the filter.

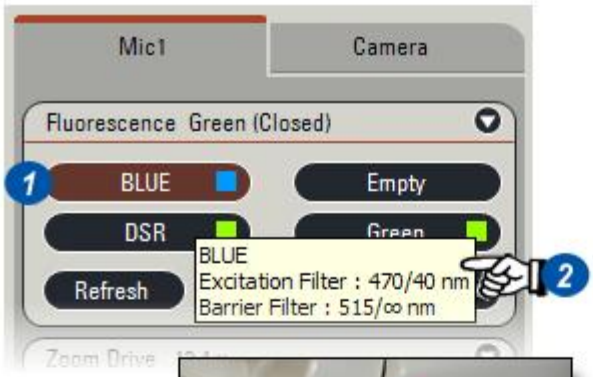
To change a filter:

3: Manually turn the *Filter Wheel* to the required position. Each filter has an identifying tag on its rim. Carefully slide out the filter and insert the new one.

After filters have been changed:

4: Click on the *Refresh* button. All of the Filter button labels will clear, the Filter Wheel will turn and the fitted filters identified.

5: The button labels will be displayed with the correct filter data.



There are five control options for the Motorised Zoom:

Drag and drop:

1: Click and hold the *Scale Indicator* and drag it to the required zoom position. The shadow indicator (**2**) remains in the starting position until the mouse button is released.

Click on the Scale:

3: Click on the *Scale Bar* at the desired zoom position. The *Scale Indicator* will move to the selected position.

Type a value:

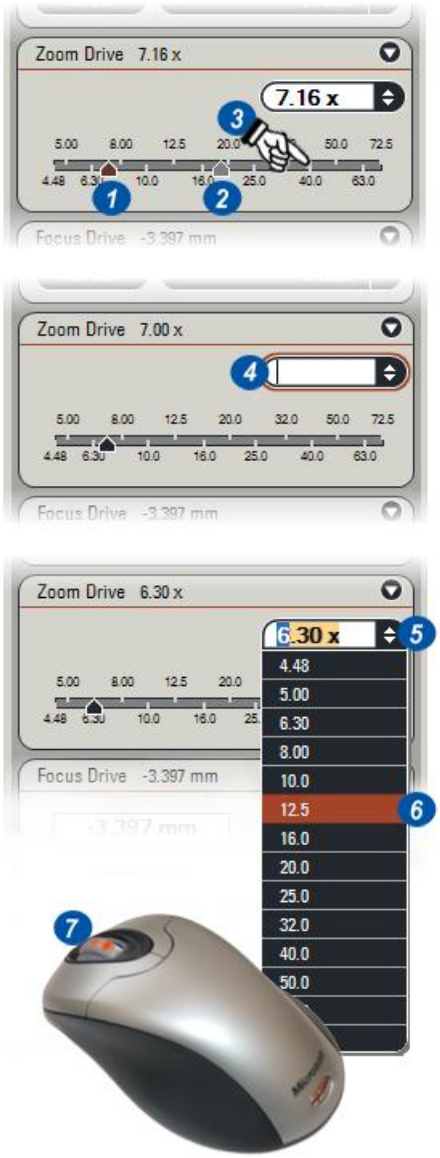
4: Click in the *Zoom Drive* text box and type the required zoom position. Values larger or smaller than the zoom limits will be ignored.

Preset positions:

5: Click on the arrows to the right of the *Zoom Drive* text box and from the drop down list click to select a preset position (**6**).

Fine adjustment:

7: Use the *mouse wheel* (if fitted) to move the zoom in small steps.



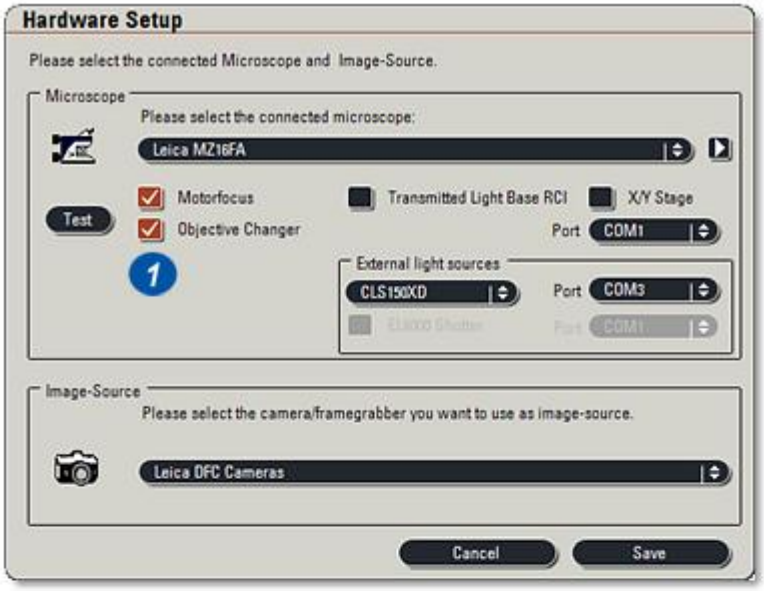
1: If the *Objective Changer* feature is fitted and is enabled on the Hardware Setup panel, High and Low magnification levels are available.

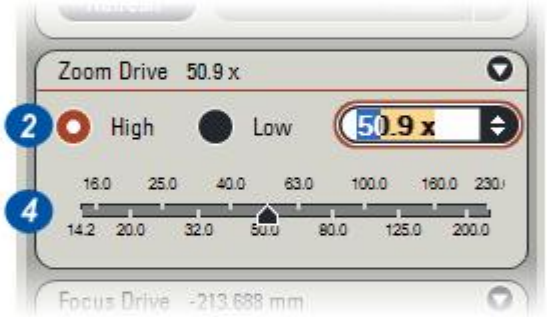
2: Click to enable *High* magnification or…

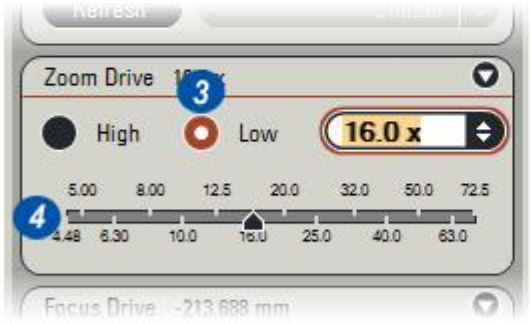
3: Click the *Low* magnification option.

4: The *Zoom Drive Scale* changes to reflect the magnification level selected.

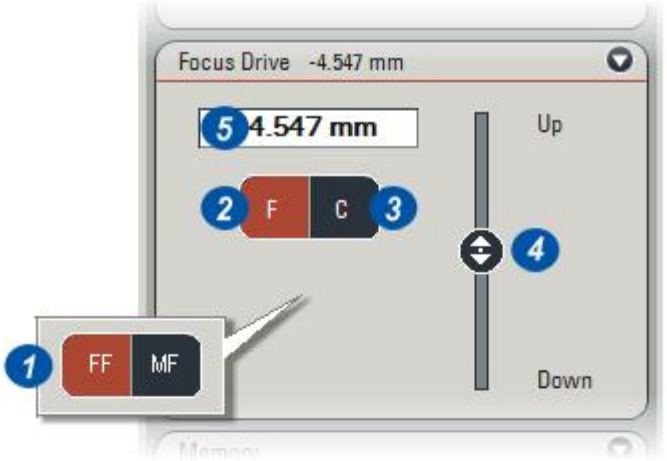
See: Installation and Licensing Help for selecting hardware and the Hardware Setup panel.







There are three options for driving the Motorised Focus:



Focus Control:

1: Fine focus (FF) and motor focus (MF) buttons are available with MF designated microscopes and Z6APO(A) and Z16APO (A) macroscopes.

2: Click on fine (F or FF) to move the focus in small increments.

3: Click on coarse (C or MF) to move the focus in large increments.

4: Click, hold and drag the *Focus Control* up or down. Release the mouse button when the desired focus is reached. Generally, start with coarse (C or MF) selected to get close to optimum focus and then select fine (F or FF) to 'tune' the focus position.

Type a position:

5: Click in the *Focus Position* text box and press the keyboard Delete key to clear the existing entry. Type a new value and press the keyboard *Enter* key.
The default units are micrometers (µm) but to move in millimeters type 'mm' after the value.

Mouse Wheel:

4: Click on the *Focus Control.*

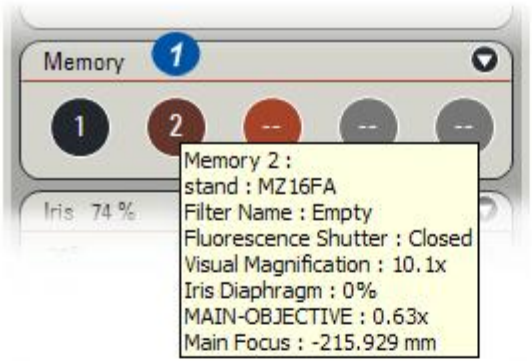
6: Rotate the *mouse wheel* to move the motorised focus up or down. The focus step for each indent of the mouse wheel depends on the depth of focus and the zoom magnification.

Current microscope settings may be saved in any one of five separate memory locations represented by a button and numbered 1 to 5.

1: Place the *mouse cursor* over a numbered memory button to reveal the microscope settings:
Motorised focus position,
Zoom position,
Filter selected,
Iris diaphragm setting,
Internal and external light source settings and
Motorised stage X and Y values.

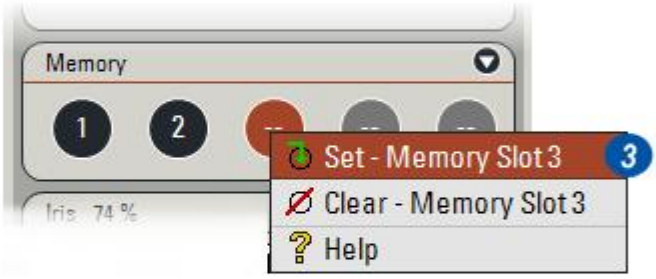
Left click on the button to drive the microscope to the memorised values.

2: Empty *memory locations* are denoted by (--) on the button.

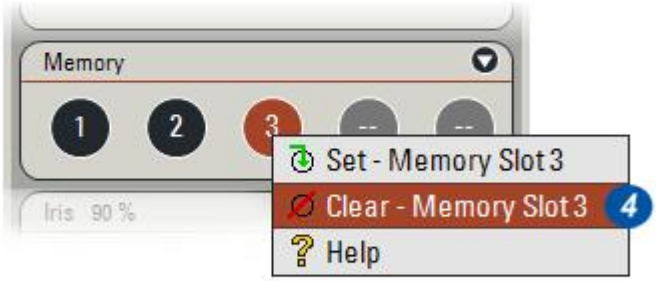


To save the current microscope settings:

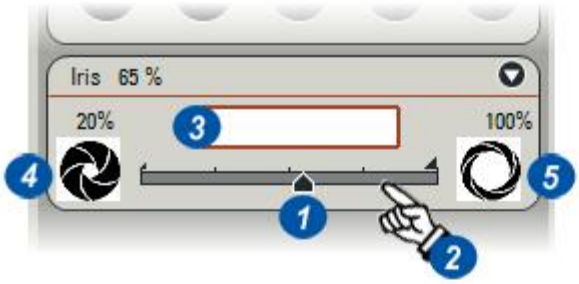
3: Select a memory location - either Set or Clear - and right-click the button.
From the drop down menu, click on *Set Memory*. The button will display the location number to indicate that the settings have been saved.



4: To clear a memory location, right-click on the button and from the drop down menu, click to select *Clear Memory.* The button number will be replaced with the empty (--) symbol.



There are three methods for changing the Zoom Iris aperture:



1: Click, hold and drag the *Slider* to the left to close the iris, and right to open it. The aperture value is shown as a percentage (%) of fully open on the header bar. The minimum value is limited to 20%.

2: Click on the *Slider Bar* and the Slider will track to the selected position.

3: Click in the *Iris Setting* text box to select the existing value and press the keyboard *Delete* key to clear it:
Type a new aperture value and press the *Enter* key on the keyboard. The maximum value is 100 and the minimum value 20.

4: Click on the *'closed iris'* icon to close the iris to 20% of full, or...

5: Click on the *'open iris'* to fully open the aperture.

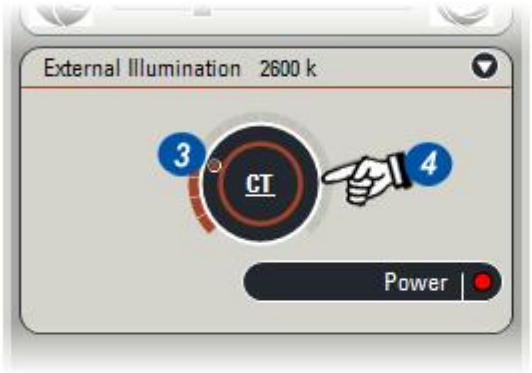
The CLS 150XD and 150LS external light sources may be controlled remotely from SMS:

1: The *Brightness Control* may be rotated to increase or decrease brightness.

2: The light source may be turned on or off by clicking the *Power* button. A red dot indicates that the source is on.

3: Click, hold and drag the red 'handle' on the rotary control, clockwise to increase brightness, or anti-clockwise to decrease it. The light output value is displayed on the header bar.

4: Alternatively, click on the *outer rim* of the Brightness Control which will rotate to the selected position.



There are three controls for the Internal Transmitted Light Base:

- *CT (Colour Temperature)* which controls the lamp average voltage and therefore its brightness. This also affects the light colour.
- *CCIC (Constant Colour Intensity Control)* which acts like a window blind, reducing the amount of light reaching the specimen without affecting the colour.
- *Shutter* is a toggle action button which stops light reaching the specimen altogether.

The CT (1) and CCIC (2) controls work in combination so for some procedures it will be necessary to adjust the light colour using the CT control, and then adjust the brightness with the CCI control.

Both controls are adjusted in the same way:

1: Click and hold on the *red dot* on the periphery of the control. Drag either clockwise or anticlockwise to the desired position and release the mouse button. Or...

2: Click on the *outline of the control* and it will rotate to the selected position. The CT light colour (k) value and the CCIC opening (%) are displayed on the panel header.

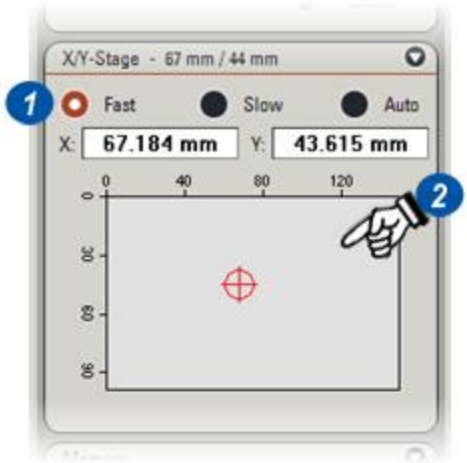
3: Click on the *Shutter* to open it as indicated by the red dot, or…

4: Click again to close it and the red dot disappears.

The Motorised Stage is represented on the control panel as a rectangle with rulers along the top and left side. Actual X and Y co-ordinates are displayed in two text boxes, and the stage initial traverse speed is selected using one of the three buttons:

- ***Fast***
- ***Slow***
- ***Auto*** for precise positioning within the field of view.

The traverse speed changes automatically as the stage nears the required position.



To move the stage to target:

1: Select the desired speed by clicking on the appropriate button. Choose *Fast* for longer distances.

2: Double-click *within the rectangle* approximately on the X/Y position required. The *Target Marker* will move to the selected position and the stage will start to track toward it. As the stage approaches the Target the traverse speed will drop to *Slow* and within the Target, *Auto* (precise) will be selected.
Click in either the X or Y text box and then use the *Mouse Wheel* (5) to 'fine tune' the position in 2µm increments.

To move the stage interactively:

3: Click and hold *within the stage rectangle.* The *4-Way Arrow* appears.

4: Drag in the required direction. The Arrow changes to reflect the direction. The stage will follow the mouse position until the button is released.
Stage traverse speed automatically slows as the mouse position is approached.

5: Click in either the X or Y text box and then use the *Mouse Wheel* to 'fine tune' the position in 2ìm increments.

To move the stage to entered co-ordinates: To be used when actual X/Y co-ordinate values are known. Both co-ordinates are entered in the same way:

1: Click on the *X* or *Y* text box and type a new value.

2: For positions measured in *millimetres,* type '*mm*' after the value otherwise the units will default to µm.

3: Press the *Enter* key on the keyboard. The stage will go to the entered positions.

4: If necessary 'fine tune' the position in 2µm increments with the Mouse Wheel after clicking in either the X or Y text boxes.

5: Optional accessories such as the Joystick, SmartMove or UMC controls may be used together with the SMS controls.

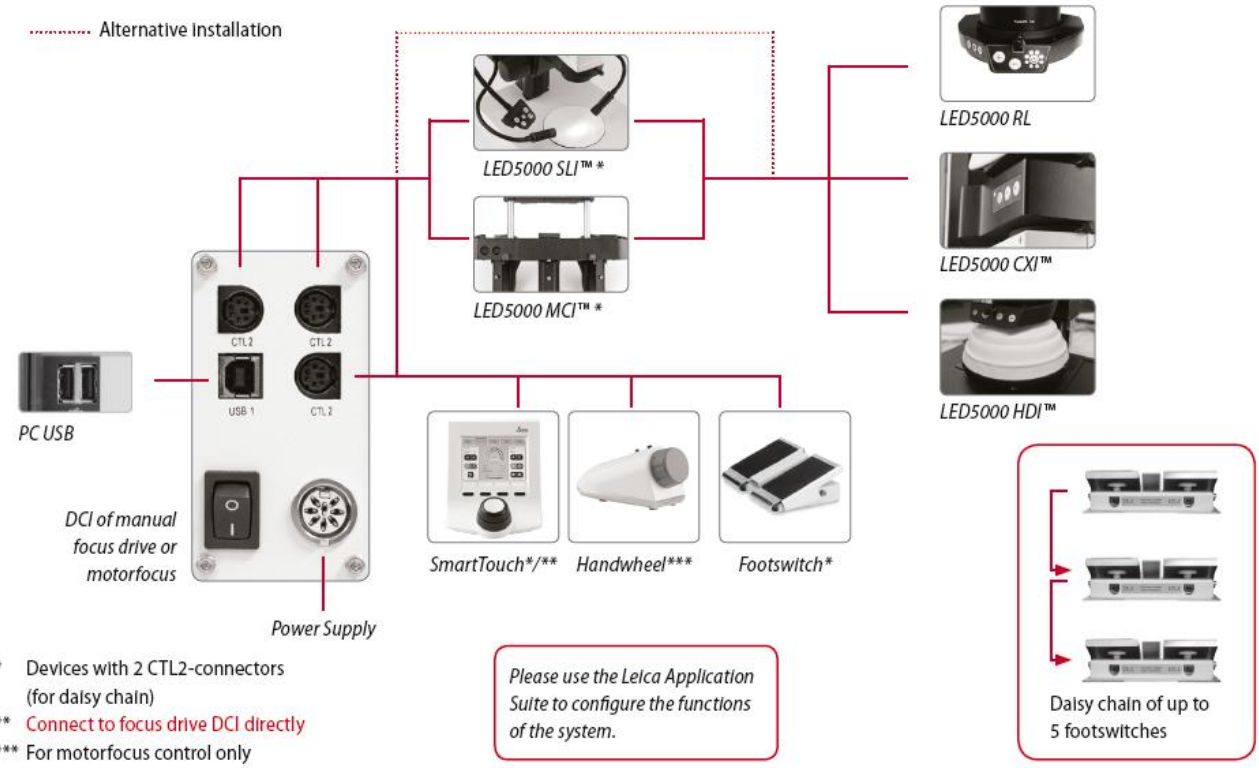
Wiring Diagrams

The following topics show current Leica wiring diagrams:

- M-Series Incident Light Base [227]
- M-Series TL4000 [228]
- M-Series TL4000 RCI IsoPro [229]
- M-Series Incident IsoPro [230]
- M-Series TL5000 Ergo [231]

Note: Wiring diagrams for earlier systems are shown here [235].

Wiring Diagram 1 – Typical Configuration with Incident Light Base

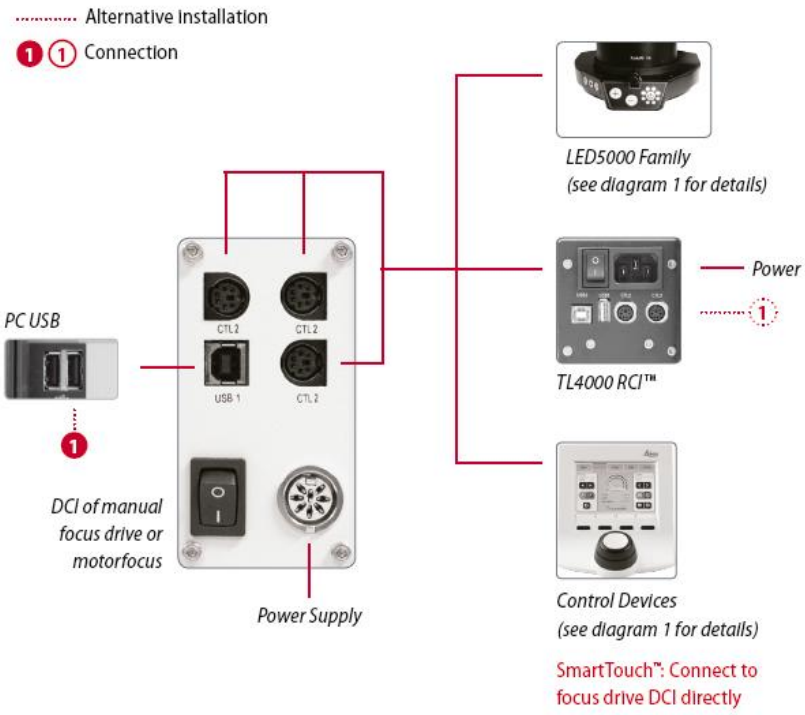


* Devices with 2 CTL2-connectors (for daisy chain)
** Connect to focus drive DCI directly
*** For motorfocus control only

Please use the Leica Application Suite to configure the functions of the system.

Leica M-series | Wiring Diagram | 1

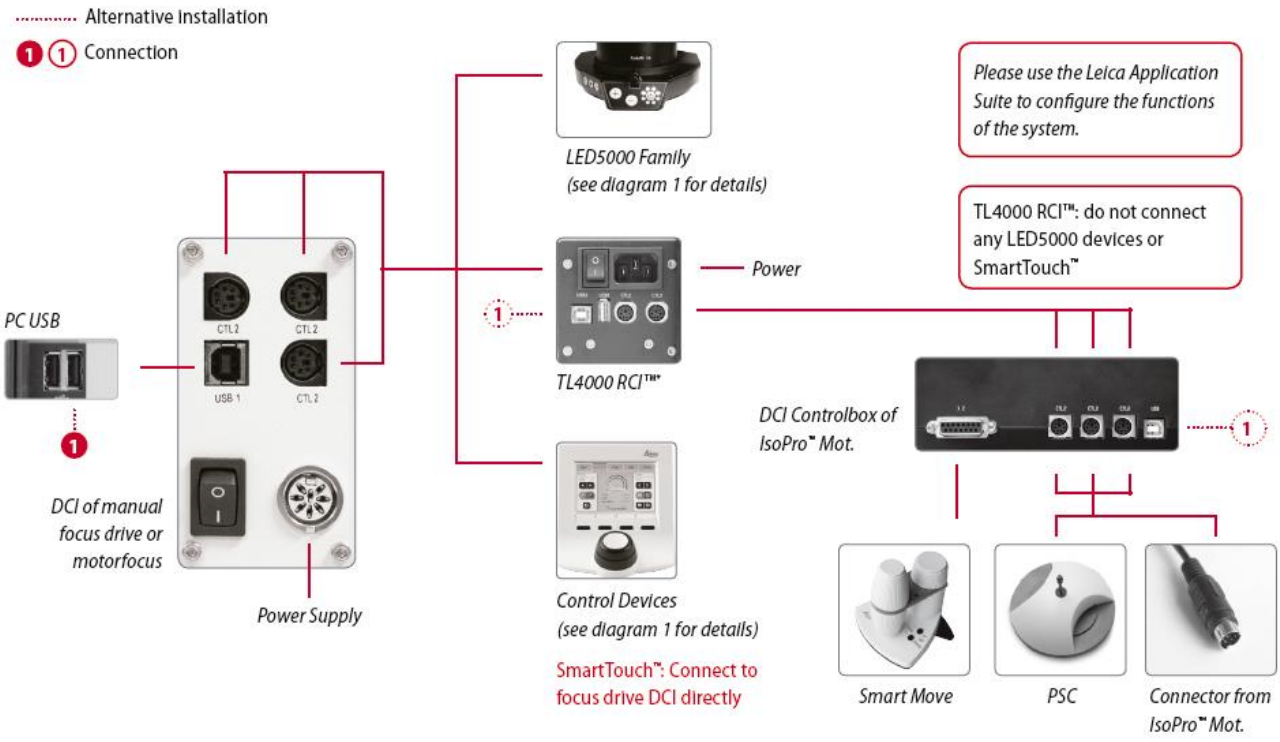
Wiring Diagram 2 – Typical Configuration with TL4000 RCI™ Base



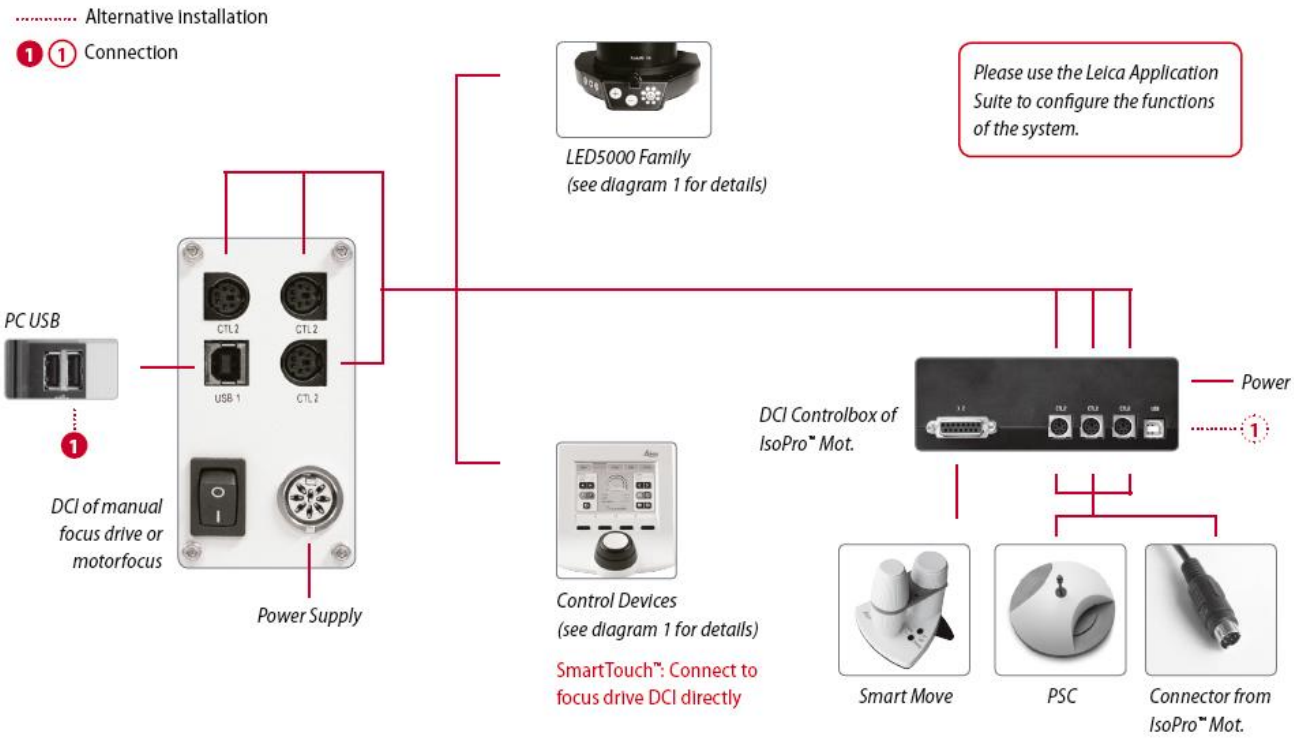
Please use the Leica Application Suite to configure the functions of the system.

TL4000 RCI™: do not connect any LED5000 devices or SmartTouch™

Wiring Diagram 3 – Typical Configuration with TL4000 RCI™ Base and IsoPro™ Stage



Wiring Diagram 4 – Typical Configuration with Incident Light Base and IsoPro™ Stage

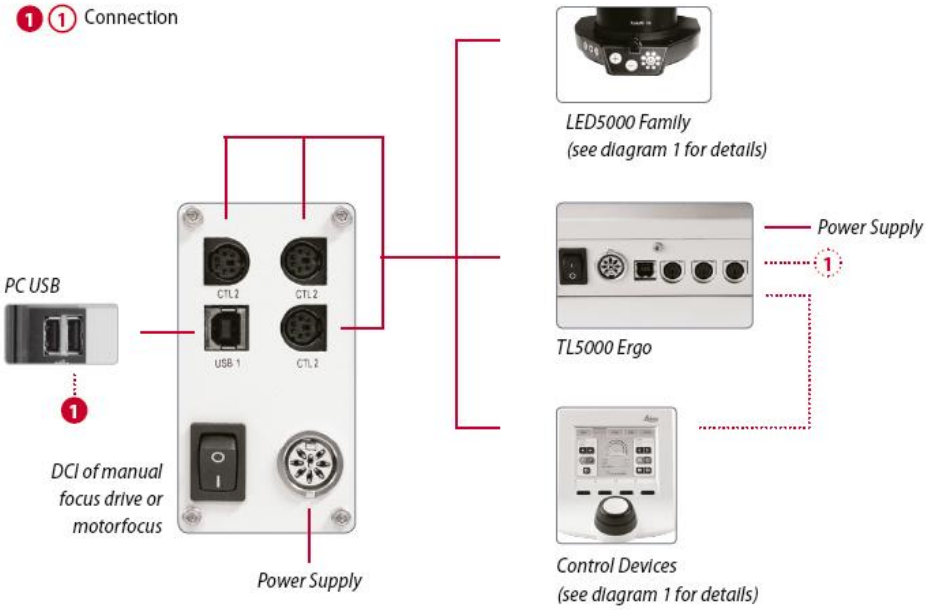


Leica M-series Wiring Diagram 4

Wiring Diagram 5 – Typical Configuration with TL5000 Ergo Base

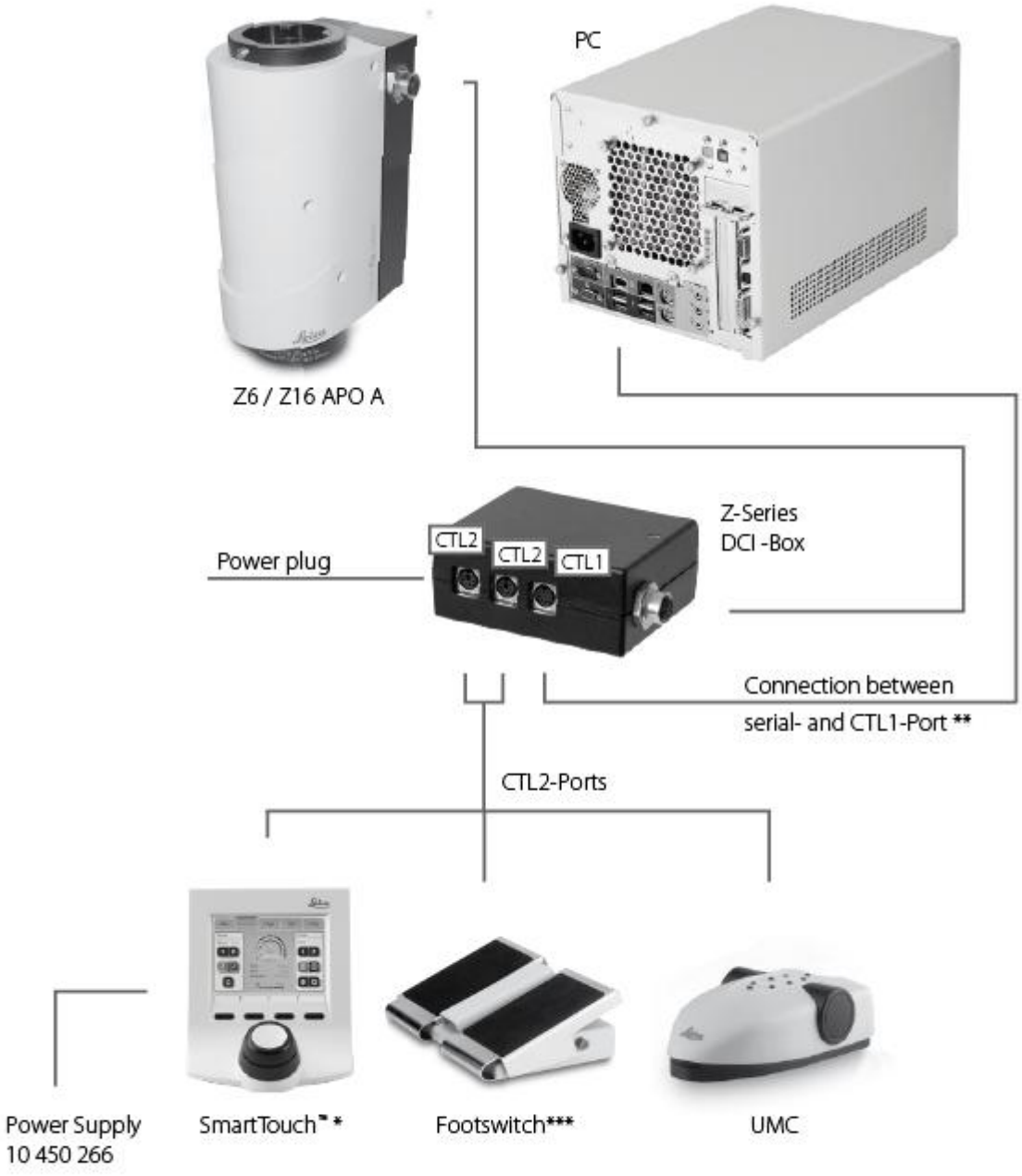
Alternative Installation

1 (1) Connection



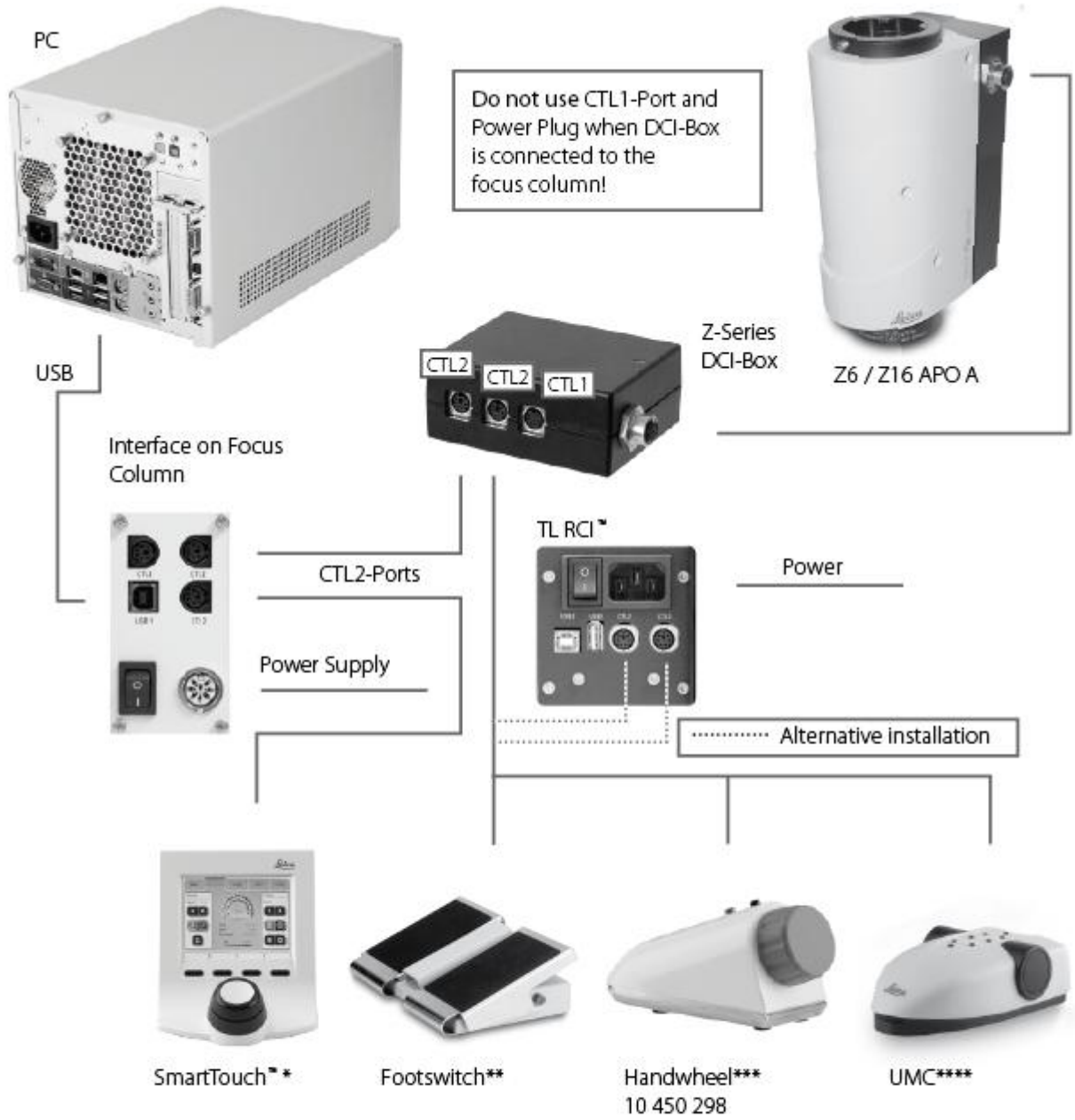
Please use the Leica Application Suite to configure the functions of the system.

Z6 / Z16, APO & APO A with Z-Series DCI-Box



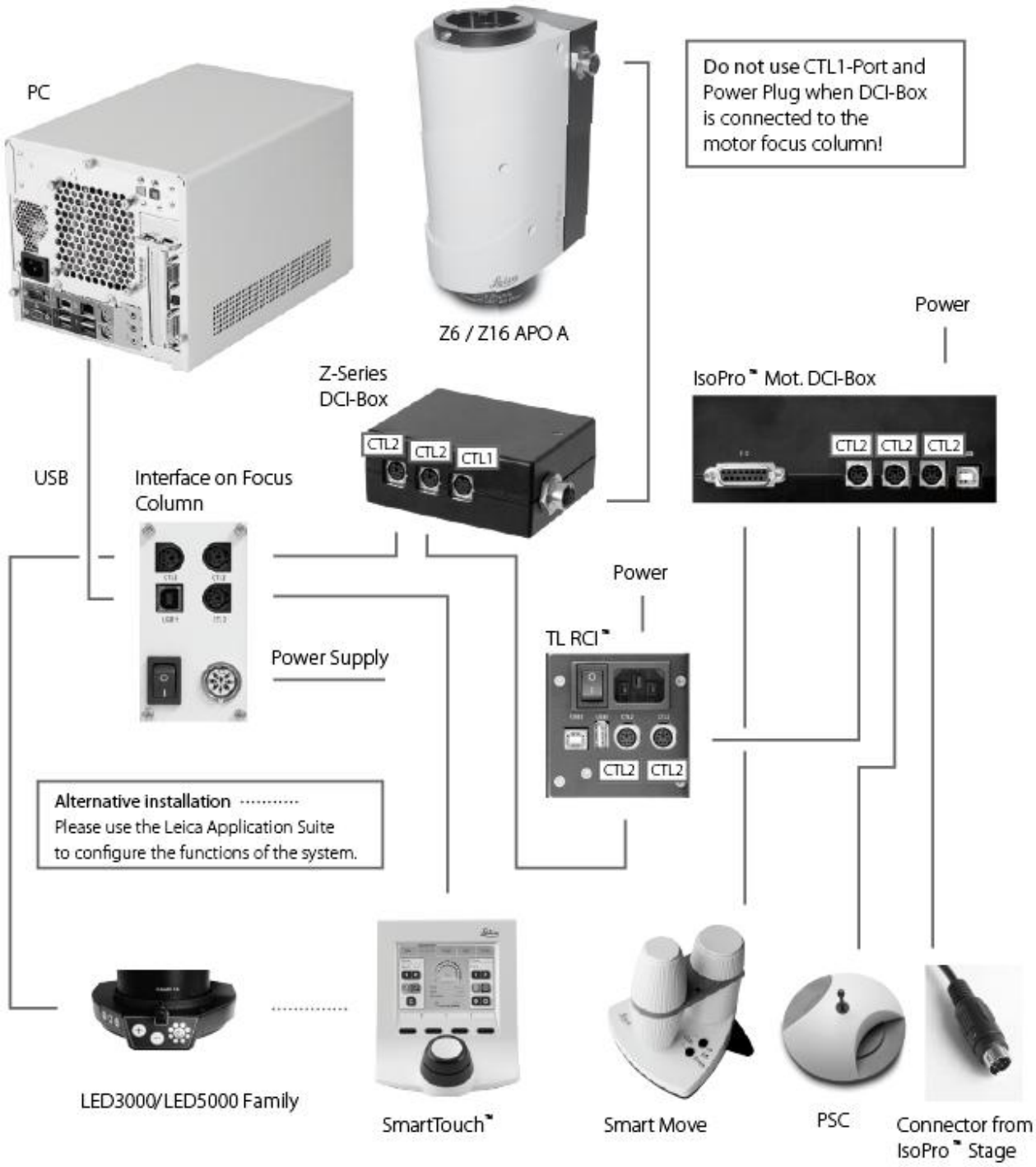
* Daisy chain possible
** Adapter-cable 10 447401 needed
*** Daisy chain of up to 5 footswitches possible

Z6 / Z16, APO & APO A with interface on High Performance M-Series Column



* Daisy chain possible
** Daisy chain of up to 5 footswitches possible
*** For motorfocus only
**** No support of motorfocus M-series

Z6 / Z16, APO & APO A with TL RCI™ Base and IsoPro™ Stage

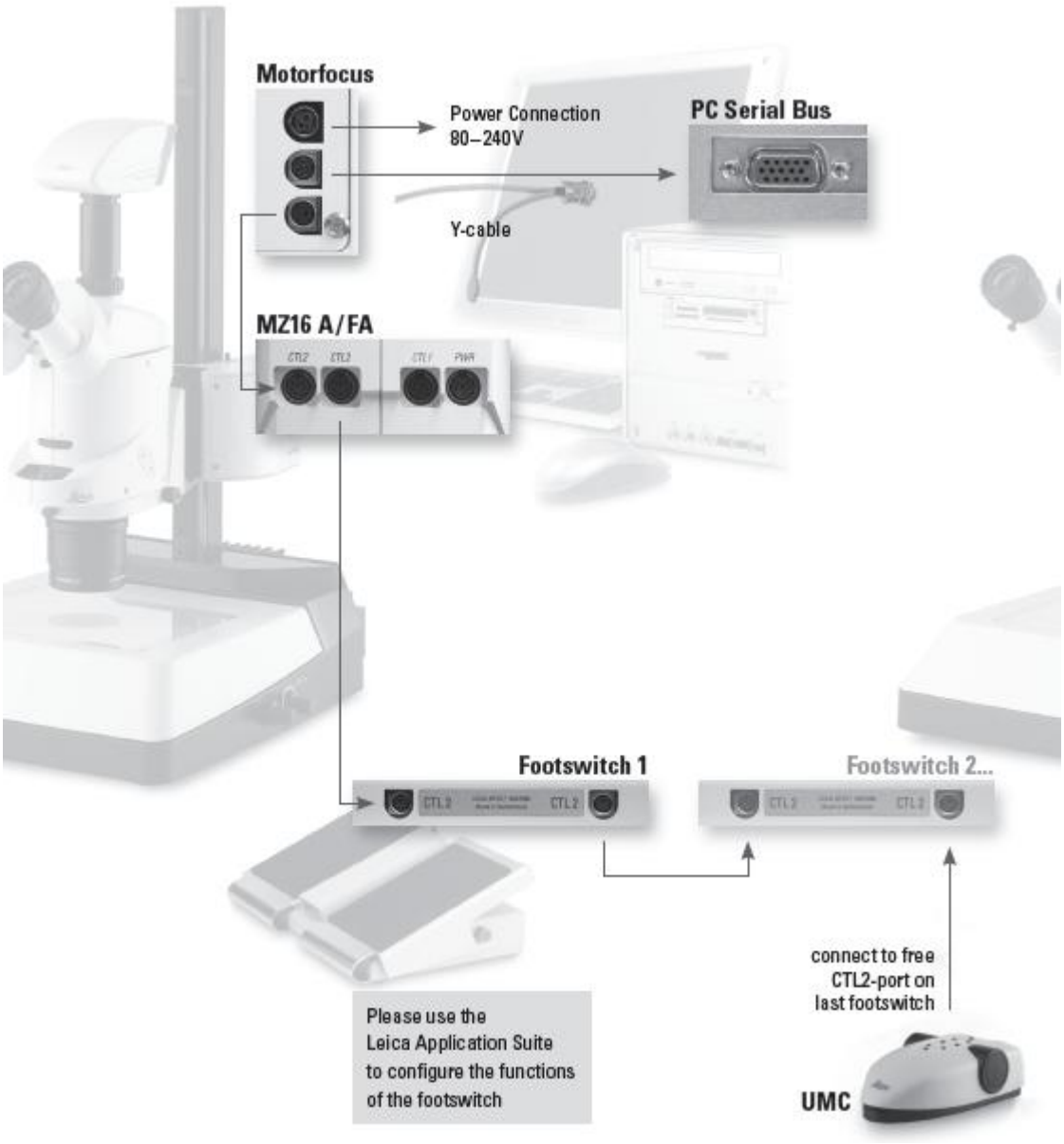


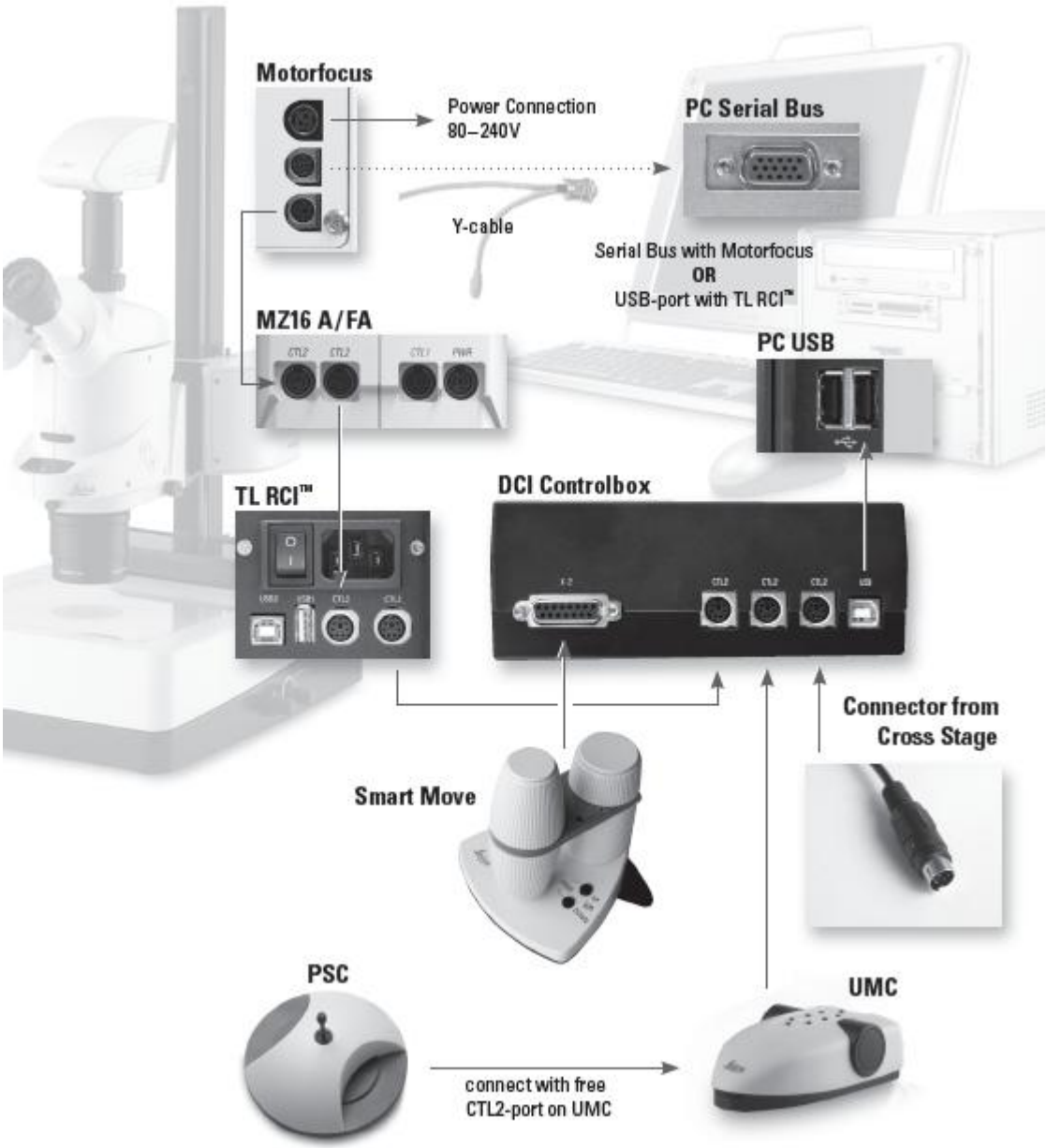
Wiring Diagrams: Earlier Systems

Please see here[226] for current wiring diagrams.

The following topics show wiring diagrams for earlier Leica systems:

- Leica MZ16 A/FA with Motorfocus[236]
- Leica MZ16 A/FA with Motorfocus, TL RCI and IsoPro[237]


Motorfocus
Power Connection
80–240V
PC Serial Bus
Y-cable
MZ16 A/FA
Footswitch 1
Footswitch 2...
connect to free
CTL2-port on
last footswitch
UMC
Please use the
Leica Application Suite
to configure the functions
of the footswitch



DM Control provides remote control for all motorized functions of the Leica DM microscope series.

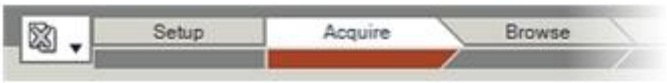
If a camera is attached to the microscope, the camera can be controlled at the same time.

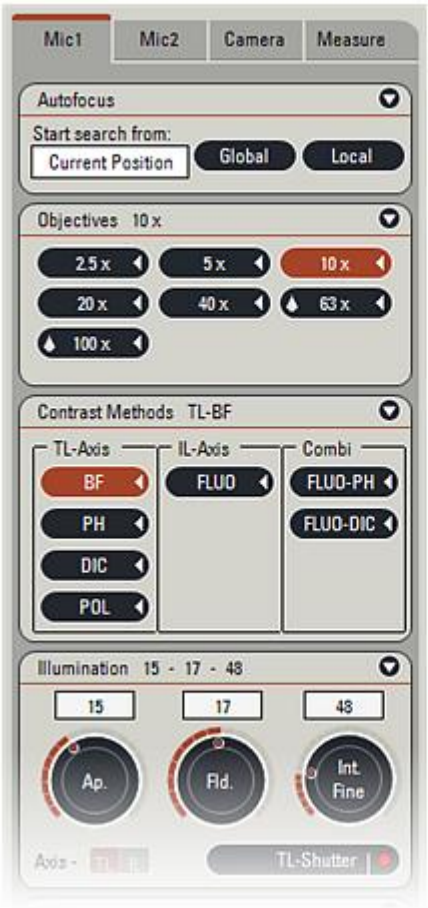
DM Control consists of the following control control windows depending on the connected microscope:

- *Contrast Methods Control* 239
- *Fluorescence Control* 240
- *Illumination Control* 242
- *Objective Nosepiece Control* 243
- *Magnification-Changer Control* 244
- *Focusdrive Control* 245
- *Stage Control* 249
- *Motorised Tube Control* 246
- *Autofocus Control (optional module)* 248

The Multi-User Package has been designed especially for DM microscope users. It allows users to create profiles that store all of the hardware settings for a specific task, retrieve them later and automatically re-configure the microscope.

More about the Multi-User Package: Go there...



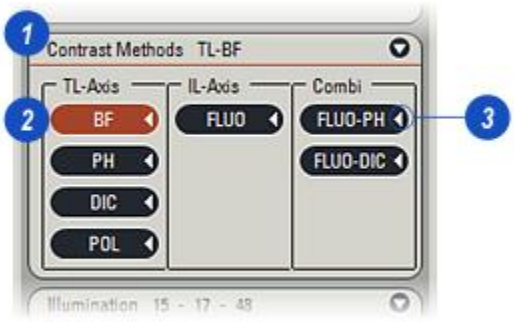


1: All available contrast methods for transmitted and incident light axis are displayed in the control window.

2: The current contrast method is highlighted on the control. Each contrast method can be selected by a left mouse click.

3: Appropriate contrast methods for the current objective in the light path are marked with a triangle ◀.

If the selected contrast method is not valid 'Pseudo Bright Field' is applied.



Light rings, dark stops, DIC prisms, polarizer (mot.) and analyzer (mot.) are addressed automatically if necessary. Mechanical polarizer and analyser have to be inserted manually.

Note: For Combi-Contrast FLUO-DIC a manual analyser has to be inserted in the appropriate slot on the upper left side of the stand.

1: All learned in *Fluo-Cubes* are listed in the control window.

2: The current fluo cube in the light path is highlighted on the control. *Fluo-Cubes* can be selected with a left mouse click.

3: The *IL-Shutter* can be closed to protect the sample from bleaching.

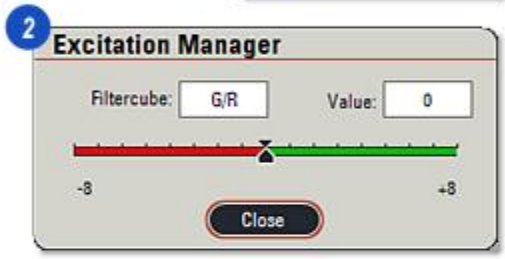
To learn in a new *Fluo-Cube*, please go to the *Setup Workflow.*

Motorized Excitation Manager (ExMan):

Allows the balancing of different fluorochrome intensities. Suitable for the following Leica dual and triple filter cube systems: *G/R; BFP/GFP; CFP/YFP; B/G/R, C/Y/R*

1: For access to the balancing slider select/ activate the appropriate dual or triple cube in *'Acquire'* and use right mouse key to open the *Excitation Manager C*ontrol Window **(2).**

Note: This function is only available if the microscope is equipped with the appropriate fluo axis.

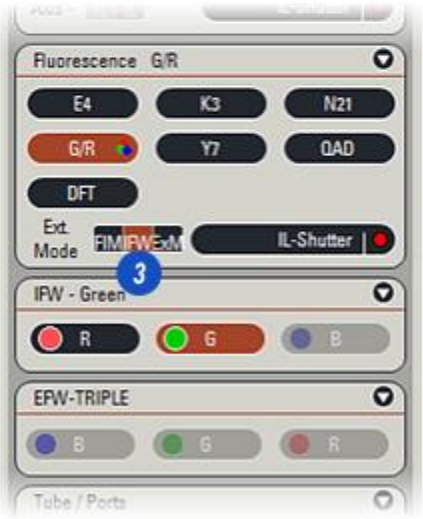


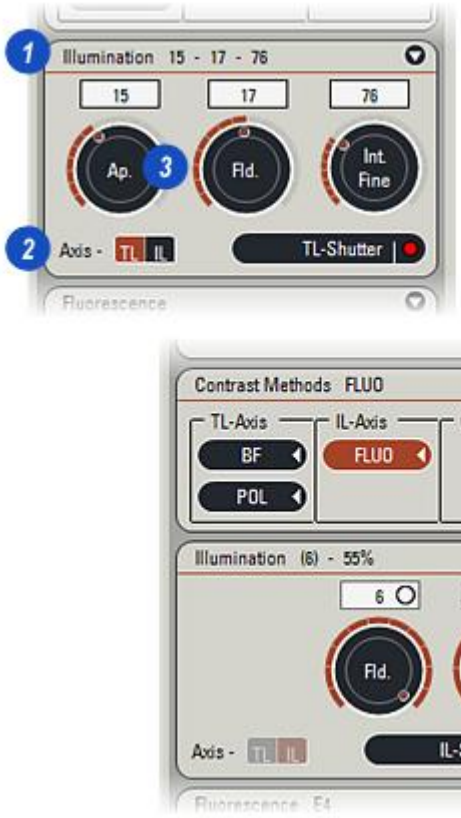
Internal Fast Filterwheel (IFW):

For fast excitation in red, blue, or green. Suitable for the following Leica dual and triple filter cube systems: *G/R; BFP/GFP; CFP/YFP; B/G/R, C/Y/R*

3: For access select/ activate the appropriate dual or triple cube in *'Acquire'*. The filter wheel positions are then directly accessible.

Note:This function is only available if the microscope is equipped with the appropriate fluo axis.





1: The current settings for the active light-axis are displayed in the control.

2: Depending on which light-axis is activated *(TL or IL)*, light settings can be modified.

3: Use left mouse button, the mouse wheel or the cursor buttons to change the values for light *Intensity (Int)*, *Field Diaphragm (Fld)*, and *Aperture Diaphragm (Ap)* respectively. For precise adjustment of the light intensity, the Fine mode is automatically selected.

4: *FIM (Fluorescence Intensity Manager*) in *FLUO* mode: the intensity of the excitation light can be reduced in 5 steps to protect the sample from bleaching.

For *COMBI* mode *(FLUO/DIC or FLUO/Phase,*: use the tabs to switch between the control panel of *TL* and *IL* axis.

Mirrorhouse

To change the fluorescence illumination with the Mirrorhouse, move the mouse into the Illumination Window of LAS 'Acquire':

Press: Right Mouse Key.
Select: Mirrorhouse
Select: Light Path

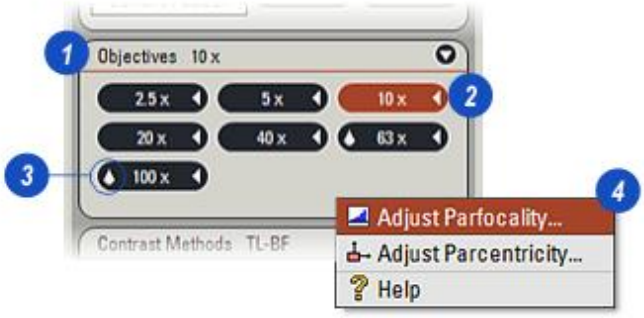
Note: Modifications in the light settings are stored and automatically recalled when the microscope in turned on.

1: All learned in objectives are displayed in the control window.

2: The current objective in the light path is highlighted on the control.

3: Objectives which are valid for the selected contrast method are marked with a triangle.
Immersion objectives are marked with a black drop.

Objectives which have been learned in as combi-objectives (module 'Fine tuning') are marked with a clear drop.



The selected objective blinks if you are changing the mode from *DRY* to *IMMersion* and vice versa. The stage is lowered and you have to confirm the change of mode with an additional mouse-click.

4: *Parfocality* can be adjusted using the context menu of the *right* mouse button. This will start the *Parfocality* wizard **(5)**. It is recommended that the *Parfocality* of all listed objectives is adjusted if new objectives are learned in.

To learn in new objectives please go to the *Workflow Setup.*

Each objective button shows small status icons:

Marks an objective, if it is valid for the currently selected contrast-method.

Marks Immersion-Objectives (Oil, Water, Glycerine).

Marks Combi-Objectives (for use in both modes, Immersion- and Dry-Mode).

Starts the Parfocality Wizard

1: The current value of *Magnification Changer* is highlighted in the control window.

Since the *Magnification-Changer* is not motorized remote control is not possible

Note: The values of the Magnification-Changer are used for the calculation of the total magnification (see LeicaScreen).

1: The current position of the Z-Focus is displayed in the control window.

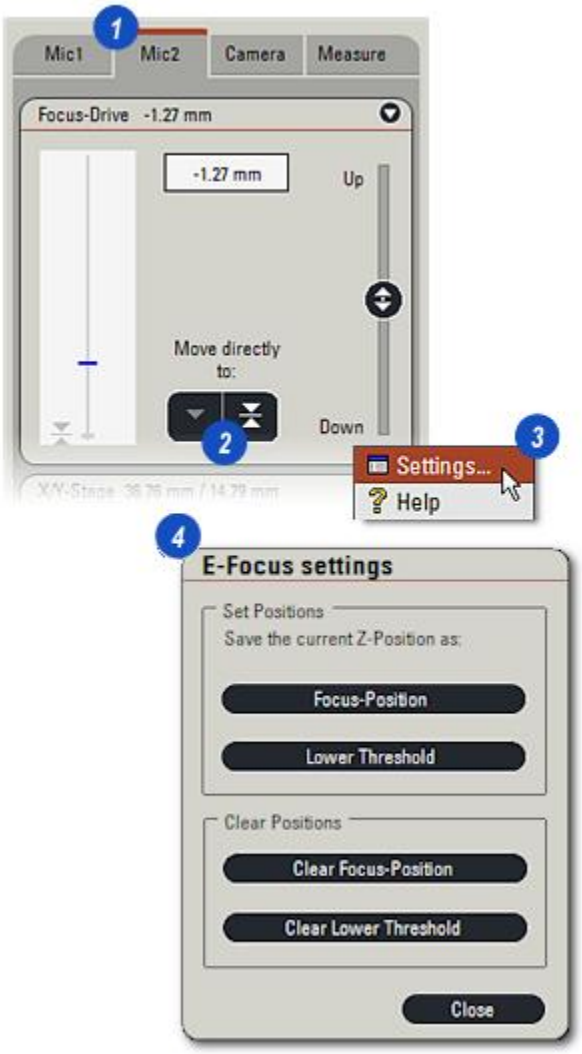
2: Lower threshold and focus position are displayed as icons in the control window and can be recalled. Press the appropriate button until the threshold or focus point is reached.
The control window uses the following status icons:

Focus-Position
Lower-Threshold

3: Please use the right mouse-button to get a context-menu for additional functions:

Advanced Settings, define/clear the lower threshold and the focus-position

4: Lower threshold and focus position can be deleted or set in the *Advanced Settings*.



1: The current beam-ratio is displayed is highlighted in the control window. The beam-ratio for a motorized tube can be selected.

Each button on the control shows a small status icon:

 *The light path is set to 100 % visual exit (eyepieces).*

 *The light path is set to 100 % camera port.*

 *The light path is set to 50% / 50%.*

Note: After turning on the microscope the light path is automatically set to 100% visual exit (eyepieces).

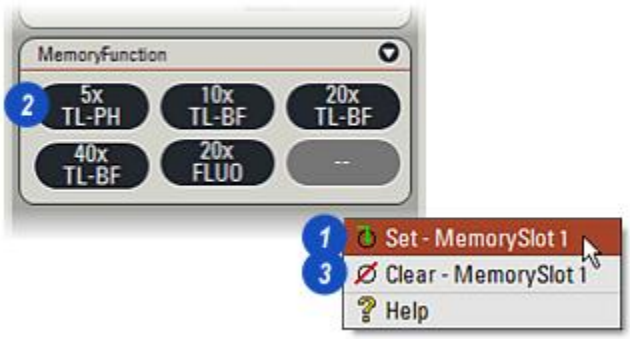
Only available for DM6000.

Allows to store the current combination of objective and contrasting method (e.g. 20x /D).

1: Using the *right* mouse key ('settings') the current combination can be stored...

2: ...available combinations recalled from the memory.

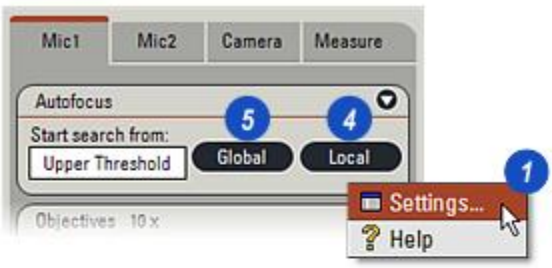
3: Clear the data on the selected memory position.

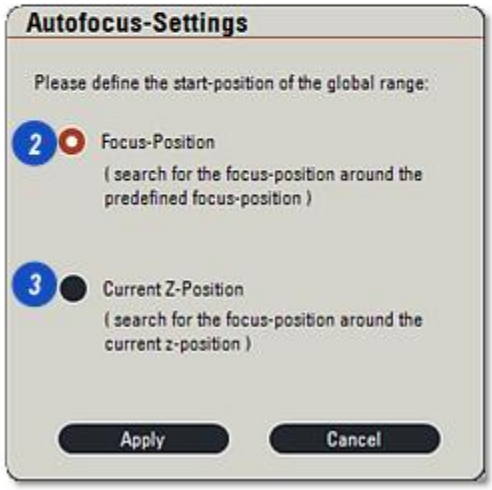


This is an optional module and needs to be licensed before use.

The cameras supported by digital *Autofocus* are listed in the document 'Systems Requirements'.

By calculation the digital *Autofocus* finds a reasonable focus plane and adjusts the Z-focus level automatically. For proper functionality please adjust the *Parfocality* values for each objective.





1: Depending on the samples two appropriate modes can be selected:

2: Search starts from pre-selected focus: suitable for sample with approximately the same thickness. Adjustment of *Parfocality* is a prerequisite for this mode.

3: Search starts from the current Z-position: suitable for samples with variable thickness.

Within each mode the span of search can be selected:

4: Near: Search of focus position considers only the near proximity.

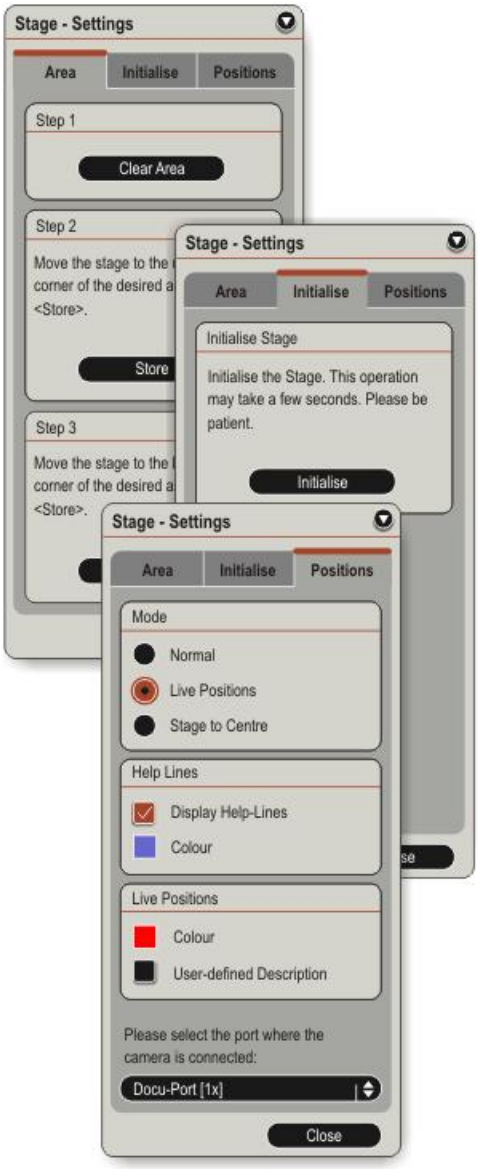
5: Global: Range of search is extended.

Note: For correct operation please install the newest camera driver. It is highly recommended to adjust the exposure time of the camera to values < 10 msec to ensure fast response time.

The Stage Settings panel contains three tabbed dialogs that allow the user to initialise and configure the stage working features.

The tabbed panels are:

- *Initialise:* Sets the top-left (X = 0 and Y = 0) and bottom-right co-ordinates of the stage limits. It will not drive beyond these co-ordinates and all of the other positioning features are measured from them. *Go there.* 250 .. 250
- *Area:* Allows the user to setup a defined working area within the stage limits. The stage will not be driven beyond the boundaries of the user *Area*. *Go there.* 251 ..
- *Positions:* Provides the *Mode* - *Live* and *Stage to Centre* - settings that define how the stage will be driven to specific points; Controls the colour and visibility of the *Help Lines* - guides that show the stage centre; If *Live Positions* will be displayed with a number or a user description and their colour, and the nominated camera port for microscopes with several port options. *Go there.* 253 ..



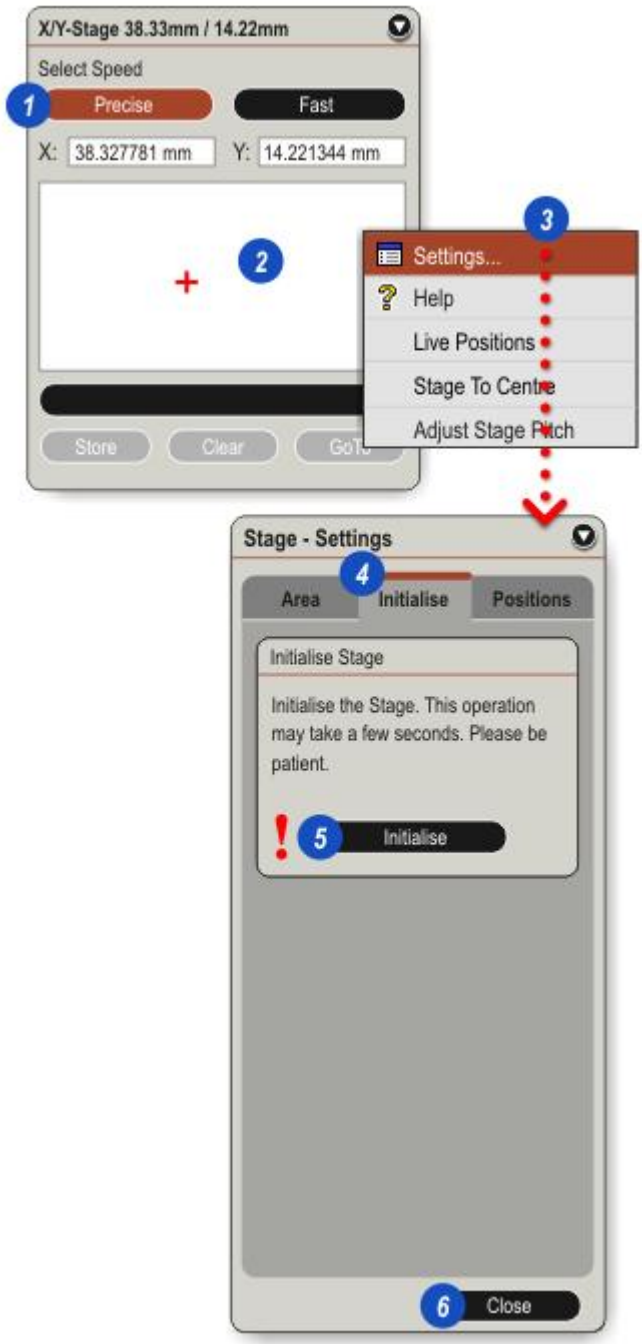
1: The speed of the stage can be modified by clicking the control buttons *'Fast'* or *'Precise'*. The *Fast* option uses the settings for pitch entered in:

Setup Workflow > *Stage* > *Fine Tuning Tab*

The *Precise* option drives the stage more slowly to avoid 'overshoot' and maintain positioning accuracy.

2: The Stage Settings are accessed by right-clicking the stage area and...

3: ...clicking to select the *Settings* option on the context menu.



Initialise the Stage:

4: Click to select the *Initialise* tab.

! Check that the stage is clear of specimens and slides. Make sure the objectives or magnifiers will not collide with the moving stage and that the connecting cables have sufficient slack to allow the stage unrestricted movement.

5: Click the *Initialise* button. The stage will drive to the top-left corner limit switches to establish *X = 0* and *Y = 0*. It will then travel to the bottom-right to set *X Max* and *Y Max* travel.

6: Click *Close*.

1: The *Stage Settings* are accessed by right-clicking the stage area and...

2: ...clicking to select the *Settings* option on the context menu.

Area:

Within the extreme limits of the stage movement, an area that (usually) contains the image, can be user-defined - the stage will not drive beyond the boundaries of the setup area.
To create a user area:

3: Click to select the *Area* tab.

4: Click the *Clear Area* button to clear any existing user settings.

5: Drive the stage using the stage manual controls or *SmartMove*, to the *upper-left corner* of the user area. This is usually set with reference to the image. Click on the *Step 2 > Store* button to save the X/Y position in memory.

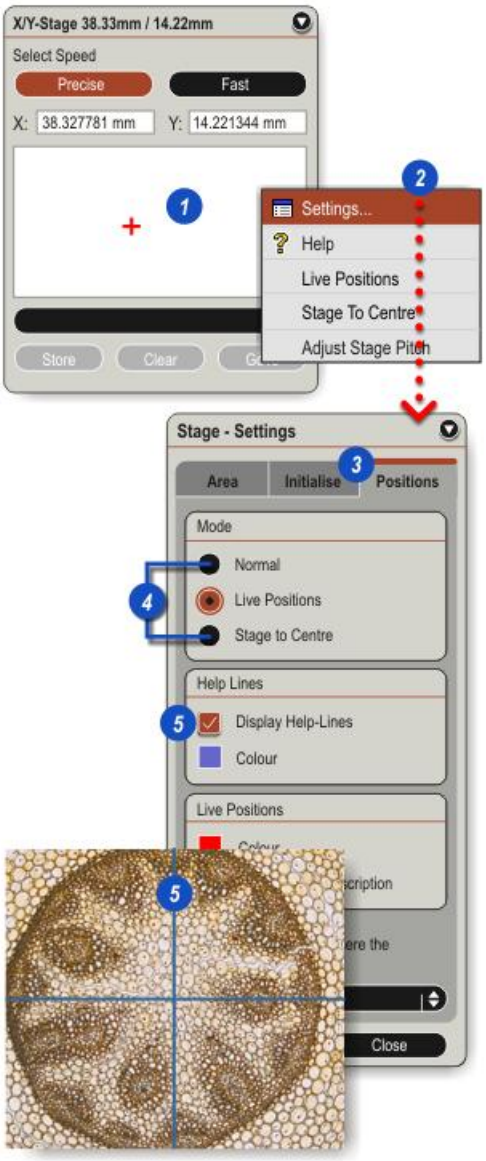
6: Repeat the process but this time driving the stage to the *bottom-right corner* of the user area and click *Step 3 > Store*.

7: Click the *Close* button.

Note: The Area boundary co-ordinates are not stored permanently and are lost when the microscope is switched off.

1: The *Stage Settings* are accessed by right-clicking the stage area and...

2: ...clicking to select the *Settings* option on the context menu.



Positions:

The *Positions* tab displays the controls for setting up the *Help Lines (Crosshairs)* and *Live Position* markers as well as selecting the current mode:

3: Click to select the *Positions* tab.

4: There are three *Mode* options selected by clicking the appropriate button:

- *Normal:* Disables both Live Positions and Stage To Centre and hides the Help Lines. This is normal stage operation.
- *Live Positions:* Allows up to 15 individually selected positions to be marked on the image. The stage can be driven directly to a selected position. The Help Lines are displayed if they are enabled.
- *Stage To Centre:* Drives the stage centre directly to a point indicated on the image by a double-left click. If Help Lines are enabled they are displayed and positioned above the point.

  Live Positions and *Stage To Centre* are mutually exclusive - only one can be enabled and active. They can also be selected from the context menu **(2)** by clicking the required option.

5: The *Help Lines* - crosshairs that indicate the centre of the stage - can be turned on or off by clicking the checkbox. This is a toggle action with a tick mark displayed when the lines are enabled and visible.

1: The colour of the *Help Lines* and...

2: ...that of the *Live Position* markers can be changed to suit the user by clicking on the *Colour* box and...

3: ...from the *Select Colour* dialog choosing a colour from the *Basic Colours* or...

4: ...dragging the 'target' to the required position on the colour wheel.

5: Adjust the selected colour intensity by dragging the slider along the graduated bar.

6: Click *OK* to save the colour.

7: To display an appropriate description next to a *Live Position* marker, click to enable the *User-defined Description* check box. This is a toggle action - click again to turn off the description display.

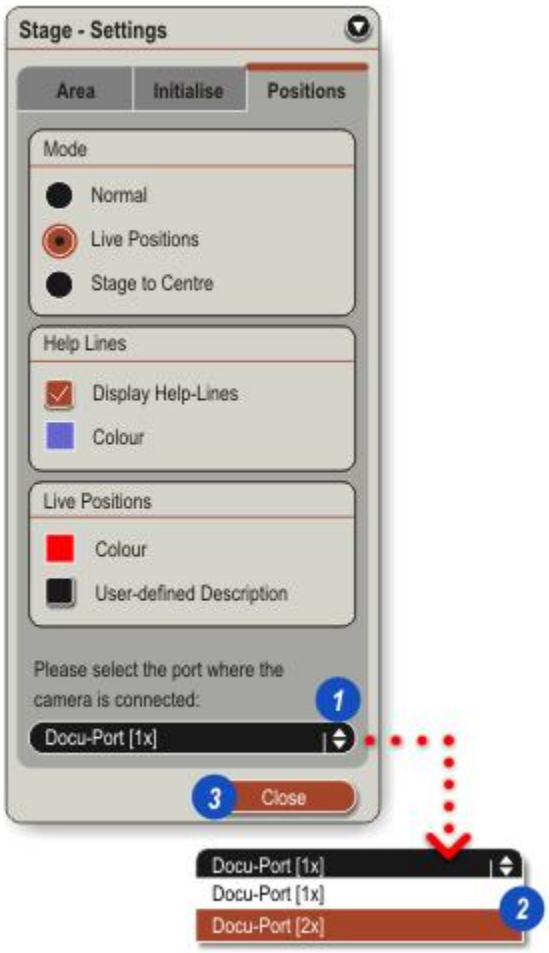
The active port option is for microscope models that have more than one port and allows the user to select the port that has the active camera attached.

To select an active port:

1: Click on the small arrows to the right of the *Port* header.

2: From the drop-down menu click to select the port that has the active camera attached.

3: Click the *Close* button to close the *Settings* dialog.



The *Live Positions* feature allows the user to select up to 15 individual points on the image storing each either as a set of *X/Y* co-ordinates or as a *User Description* on a *Position List*. The *Description* option is enabled under *Stage Settings* (*Go there...* 253). The stage can then be driven directly to a point by selecting it on the *Position List* and clicking the *GoTo* button.

1: If the *Live Positions* option is not already selected on the *Stage Settings* > *Position* > *Mode* panel,

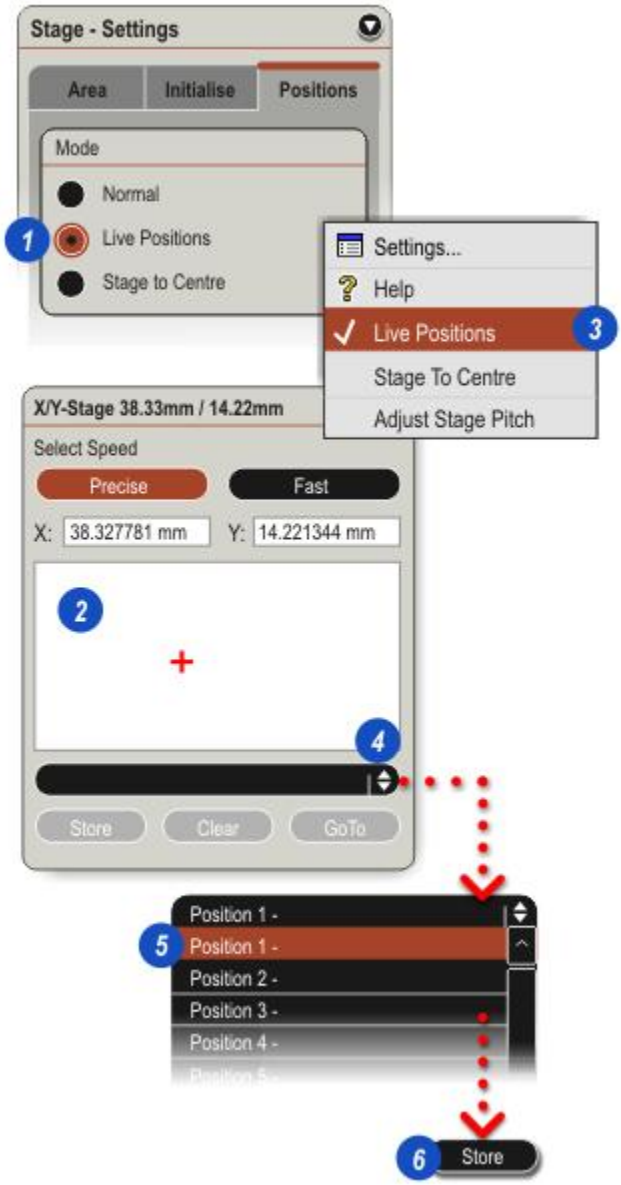
2: ...right-click inside the stage panel and...

3: ...from the context menu click to select *Live Positions*. A tick mark will appear along side the option.

4: Click on the small arrows to the right of the *Positions* header.

5: On the drop-down list, click to select an empty position. If necessary use the scroll bars to extend the list. Positions cannot be overwritten - they have to be cleared first (*Go there...* 259).

6: The *Store* button becomes enabled. Do not click it yet.



1: If *User-defined Description* has been enabled on the *Stage Settings* dialog *(Go there..* 253 *.)...*

2: ...move the stage so that the point of interest on the image is directly beneath the *Help Lines* intersection*. (The green square on the illustration is for indication only and does not appear in use).*

3: Click the *Store* button.

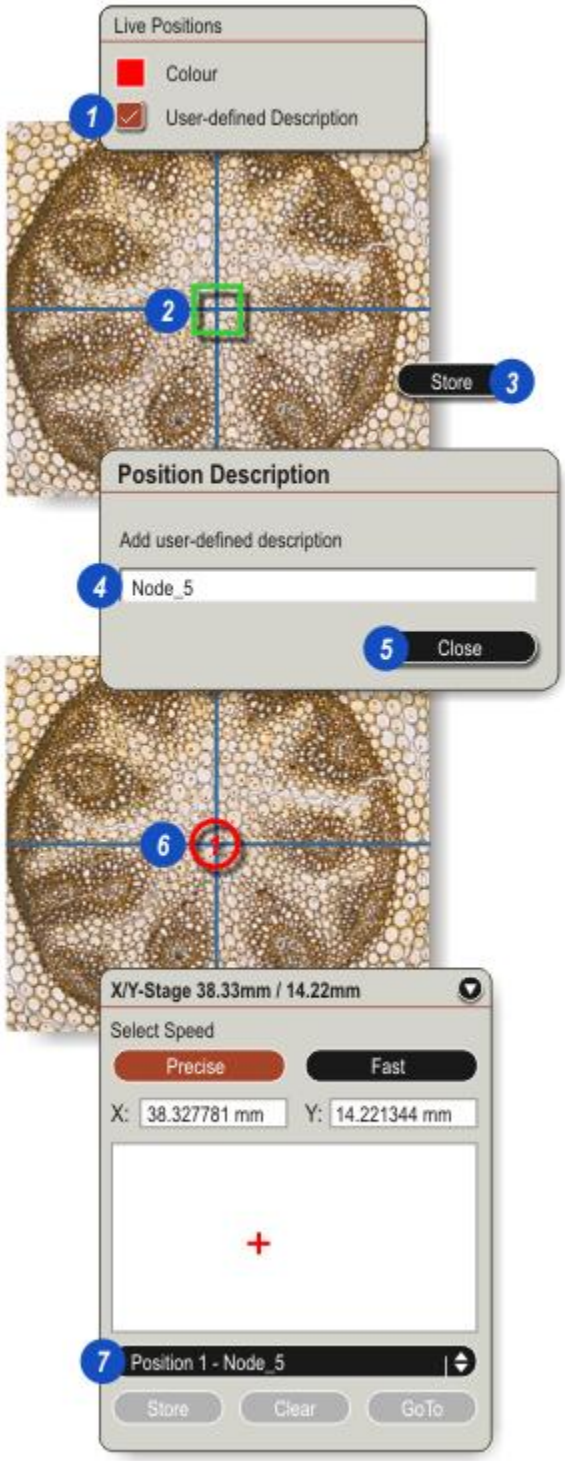
4: The *Position Description* entry dialog appears. Click in the text box and type a unique name for the for the point.

5: Click the *Close* button and...

6: ...the *Point Number* appears over the *Help Lines* and therefore, the point of interest, and...

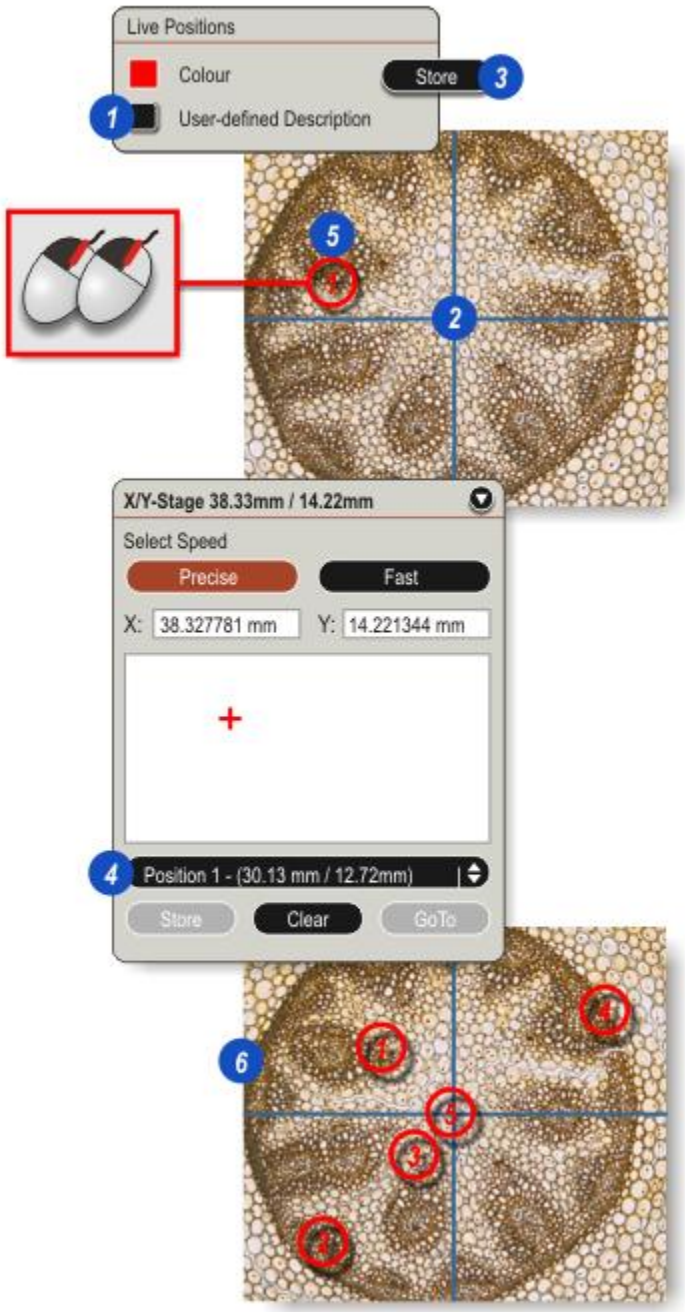
7: ...the *Position Description* appears in the *Position List.*

Note: The positions are not stored permanently and are lost when the microscope is switched off.



1: This sequence applies when the *User-defined Description* is disabled.
Two options are available:

- *Navigate to and store a position, and...*
- *Double-click to store a position.*



Navigate and Store:

2: Drive the stage to the point of interest so that it lies directly beneath the *Help Lines* - the centre of the stage.

3: Single-click the *Store* button. The marker is placed over the *Help Line* intersection and...

4: ... the position is stored as co-ordinates on the *List.*

Double-click to Store:

5: Double-left click on the point of interest on the image - no need to drive the stage to the *Help Line* intersection. A marker with the position number is placed over the point and its co-ordinates are displayed on the *Position List* ***(4)***. Empty list positions can be selected and filled automatically by double-clicking again over other points on the image.

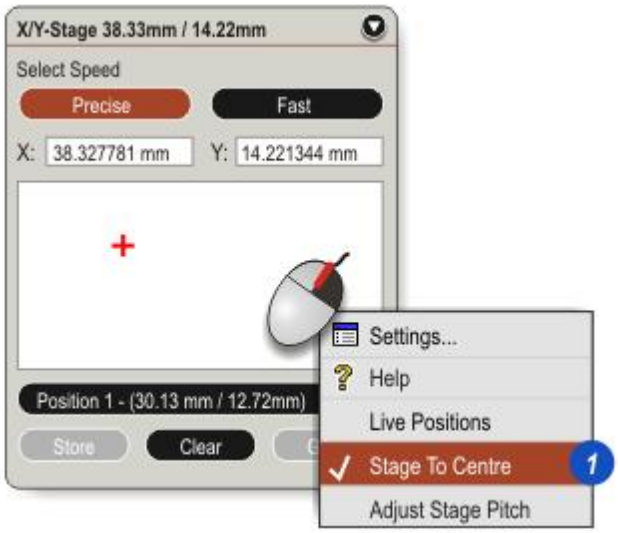
6: Up to 15 separate points can be selected on an image with each being stored on the *Position List.*

More (Stage To Centre 258)

Note: The positions are not stored permanently and are lost when the microscope is switched off.

Stage to Centre is a convenient method for examining selected points of interest on the specimen quickly. It drives the stage immediately to a point indicated by a double-left click on the image so that it moves to the centre of the field of view:

1: Right-click inside the stage area and from the context menu click to select and enable the *Stage To Centre* option.



2: Double-left click a point of interest on the image...

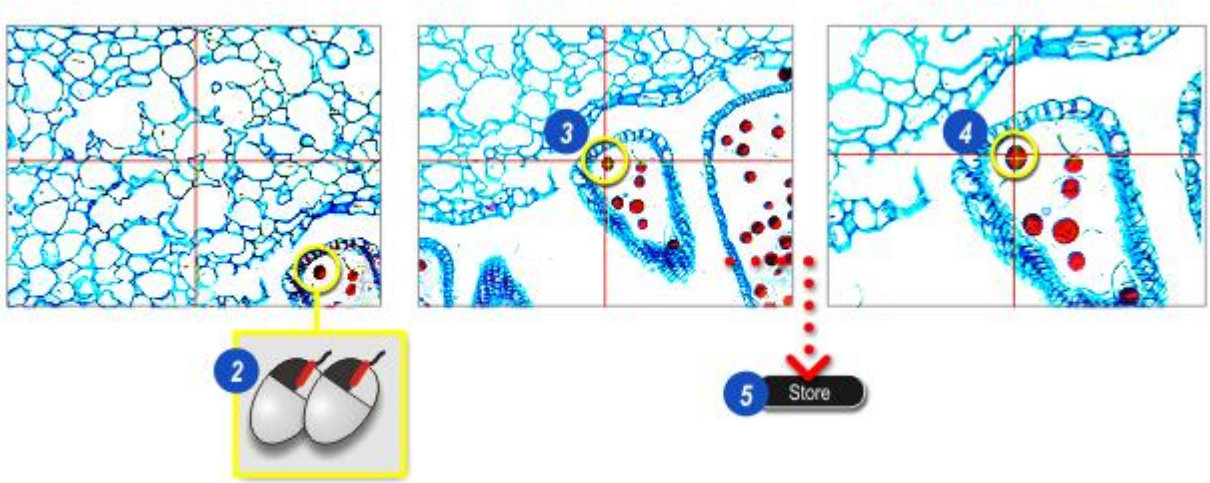
3: ...and the stage will move immediately to the centre of the field of view.

4: If required, higher magnification can be selected to examine the point of interest in greater detail which will stay in focus.

Continue to do this whilst *Stage To Centre* is enabled to effectively 'browse' the image.

5: If an empty position on the list has been selected, the *Store* button becomes available. Click it to store the position co-ordinates and return to it later using the *GoTo* feature.

To change *Stage to Centre* settings: *Go There..* 252.



GoTo:

The stage can be driven directly to any of the set positions by:

1: Click on the small arrows to the right of the *Position List* header.

2: From the drop-down *Position List*, click to select the one required.

3: Click the *GoTo* button.

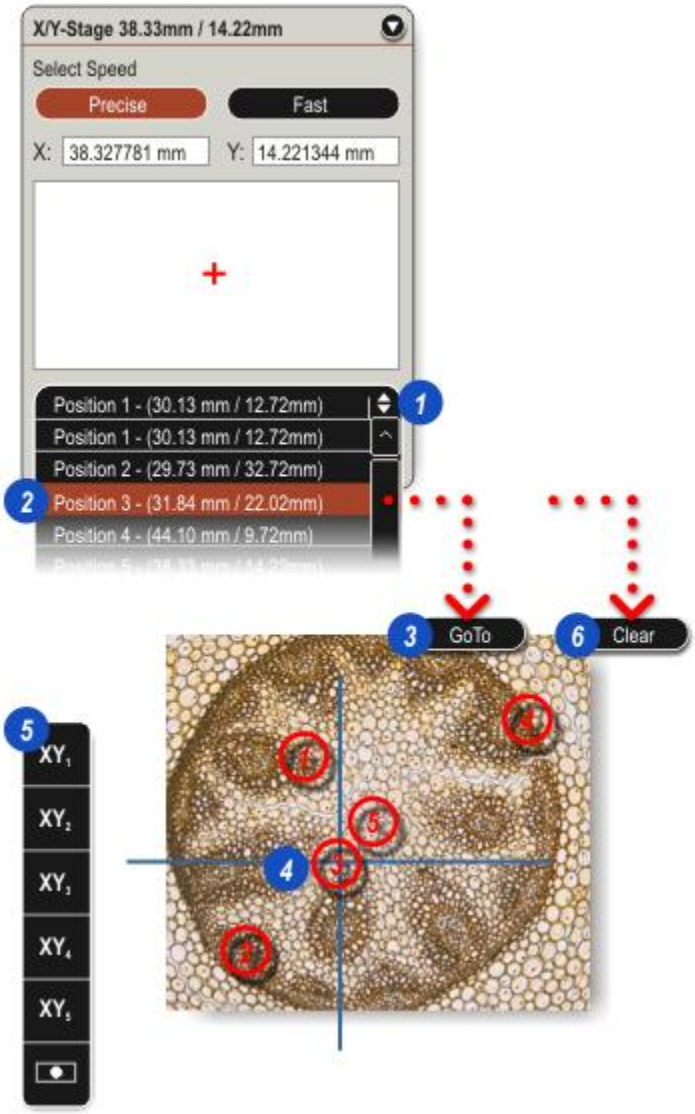
4: The stage drives to the position locating it beneath the *Help Line* intersection - stage centre.

5: A fast alternative to driving to a set position is to click one of the *Side Tool Bar* buttons. However, these only respond to the first 5 positions.

Clear:

With a position selected from the drop-down *Position List:*

6: Click on the *Clear* button to remove the position co-ordinates or point name. The image marker is also removed. A list entry must be cleared before it can be used for another position.



Stage movement and accurate positioning relies upon the *Leica Application Suite* software 'knowing' just how far the stage will travel for a single revolution of a lead screw or gear train. If tiny manufacturing tolerances make the travel distance slightly larger or smaller, then precise alignment of individual montage images can be compromised.

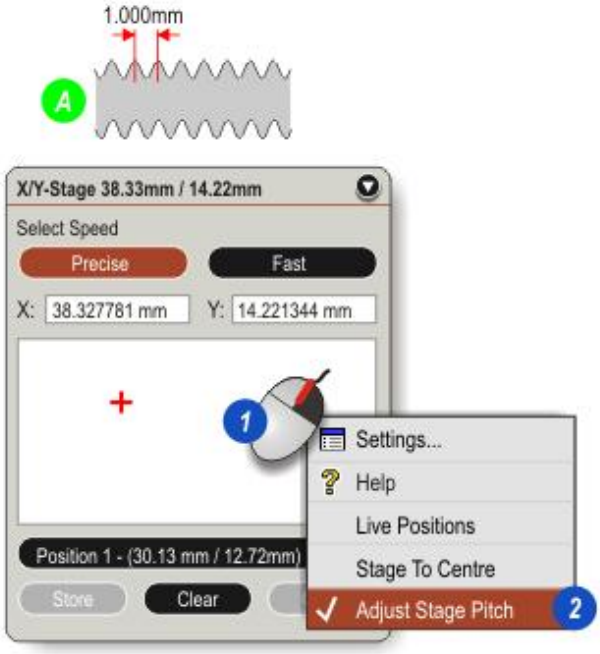
The *Adjust Stage Pitch* feature allows the users to manually position the stage at the ends of a known distance - usually a calibration slide - and from the measurement determine exactly how far the stage travels for a single revolution of the drive. The value is stored and used for subsequent stage positioning.

It is a two-step process - first for the *X* axis and then for the *Y* axis. The measurements are specific to the fitted stage and must be repeated if it is changed or swapped.

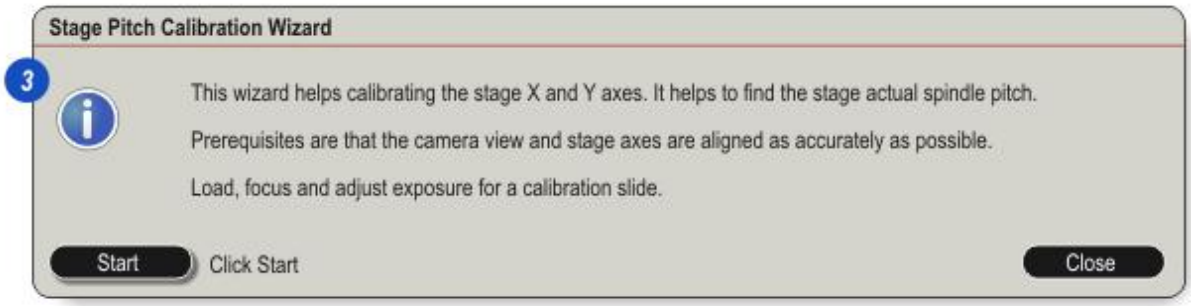
1: Right-click within the graphic stage area and...

2: ...click to select the *Adjust Stage Pitch* option from the drop-down menu.

3: The *Stage Pitch Calibration Wizard* appears.



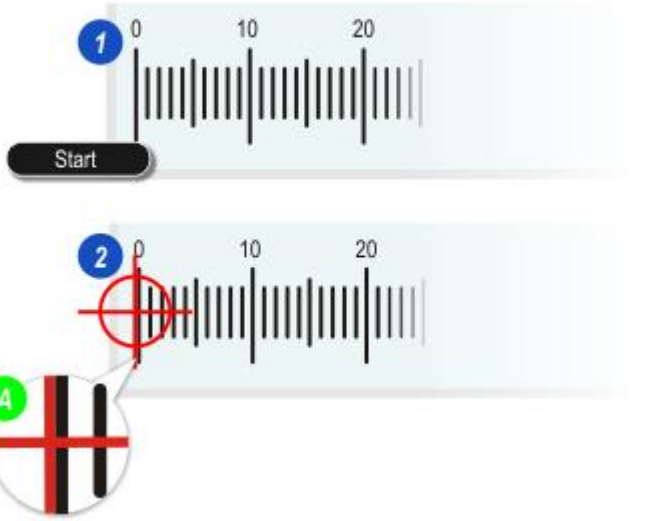
A: Section through a stage lead screw. The manufacturer states a 1.000mm screw pitch - the stage will move 1mm for each turn of the screw. If the tolerance is ± 0.002mm then for 25 turns the stage may have moved between 24.95 and 25.05mm and perhaps not the expected 25.00mm.



1: Place a calibration slide on the stage with the '0' mark to the left and bring it into sharp focus. Click the Wizard *Start* button. A 'sight' mark appears in the field of view.

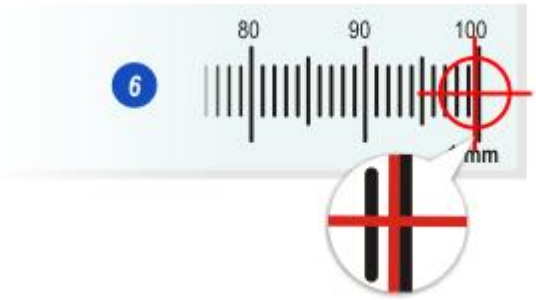
2: Drive the stage so that the '0' mark outer edge perfectly aligns with the sight mark inner edge **(A)**.
To aid alignment, click the *Zoom In* button **(4)** for a magnified view. Change the sight mark colour to improve contrast by clicking the *Colour...* button **(5)** and selecting a preferred colour from the dialog.

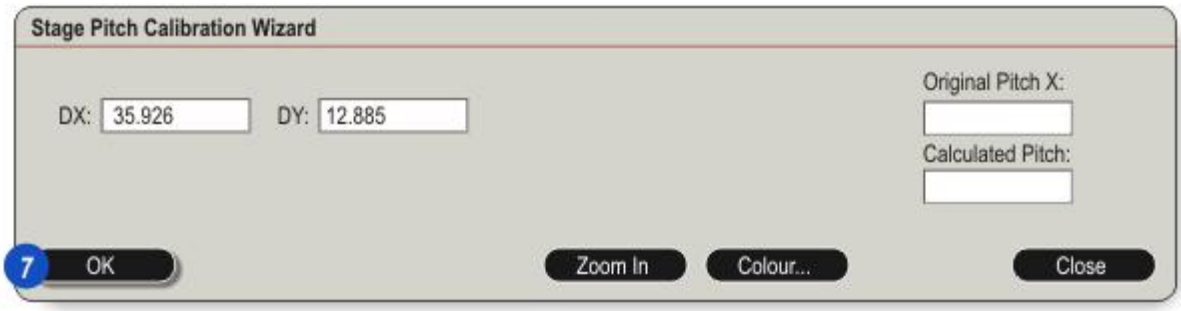
3: Click the *OK* button to capture the stage position.



6: Drive the stage to the extreme right-hand end of the calibration slide this time positioning the inner edge of the calibration mark with the outer edge of the calibration mark.

7: Click the *OK* button.

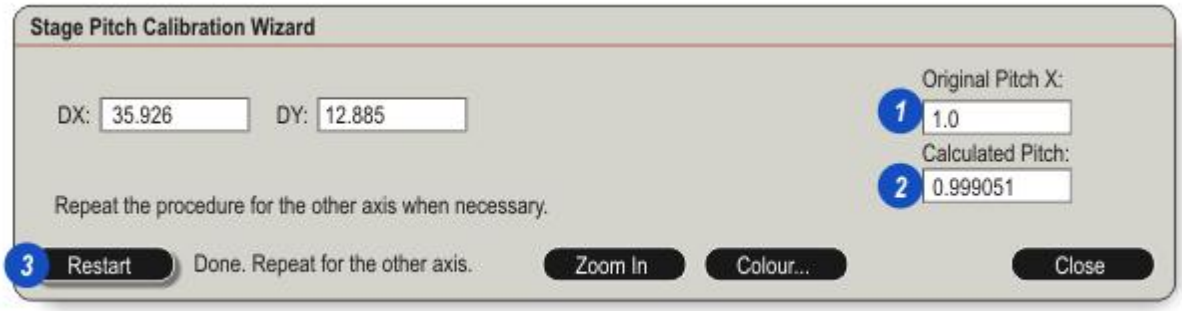




1: Click inside the *Original Pitch X* text box and type the calibration slide distance - in the example 1.0mm.

2: The *X* axis calibration is calculated and stored.

3: The *'OK'* button has been replaced with the *Restart* button ready to calibrate the *Y* axis.



Follow the same procedure to calibrate the *Y* axis drive train:

1: Turn the calibration slide through 90º and check for position and focus.

2: Click the *Restart* button.

3: Drive the stage to the calibration slide *'0'* position carefully aligning it with the sight mark.

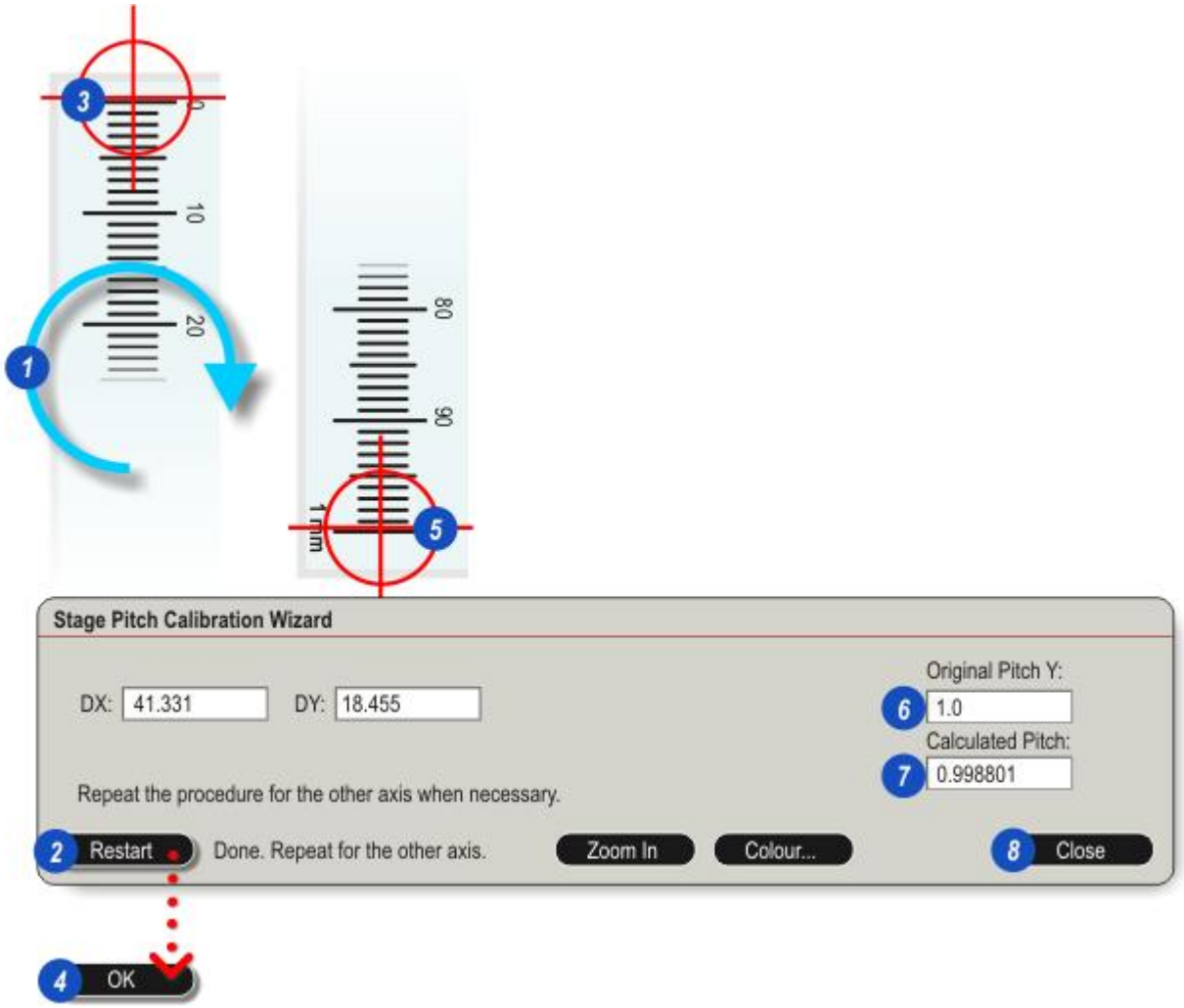
4: Click *OK*.

5: Drive the stage to the end of the calibration slide and again, carefully align it with the sight mark. Click *OK* **(4).**

6: Click inside the *Original Pitch Y* text box and type the calibration slide distance - in the example 1.0mm.

7: The *Y* axis calibration is calculated and stored.

8: Click *Close*.



This module serves as remote control and status display of all motorized functions of the FS C and FS CB .
If a camera is attached to the microscope, it can be controlled simultaneously.
The module can consist of the following control plug-in windows: (depends on the connected hardware (FS C or FS Comparison Bridge only):

Objective Magnifications 265

Comparison Bridge Control 266

Illumination Intensity (Cold light sources) 267

Magnification Changer 268

Tube and Photo Port 269

X/Y Stage

Focus Drive

Objective Magnifications:

- This window shows the status of the objectives available and teached-in for both the left and right hand revolver turret of the FSC. Switching over between left and right takes place by clicking the corresponding button L / R.
- The currently used objective magnification is indicated in red. This is a display function only.
- The turret of the FSC is coded but not motorized!

Comparison Bridge Control:
This module enables the direct control of the bridge modes as well as the position and width of the dividing line. It displays the current status for the a.m. functions and indicates whether the bridge is in the calibrated (LED = green) or Zoom-mode (LED = red). Four direct control keys are available that switch the comparison bridge into one of the following modes:

- L = full left image (right side = not used)
- R = full right image (left side = not used)
- LR = Split center image. The dividing line will be positioned to its default position and default width previously
- teached-in in DM-control. Later adjustments of either line-position and or line-width result in the switch-off of
- the LED in the LR-key. This mode is then called “split image” and no longer “split center”.
- MIX = Superimposed image of both, the full left and full right image mode.

The rotary button “Pos.” controls the dividing line position that is used to introduce more or less of the left and right half-image. In its minimum and maximum settings a full left or full right image and all intermediate positions can be achieved.
The rotary button “Size” controls the width of the dividing line and can be set from a very thin (almost invisible) line to a full superimposed image by using the full potential of this function knob. This line or superimposed strip, can be moved across the image or positioned to any location in the image with the “Pos” key.
The LED “Magnification calibration” indicates two conditions of the comparison bridge:

- LED = green, the comparison bridge has identical magnifications left and right taking all optics and objectives
- into account. The specified accuracy is less than 1 per mille.
- LED = red, the comparison bridge can have different magnifications of the right and left imaging paths by as much as +/-5%.

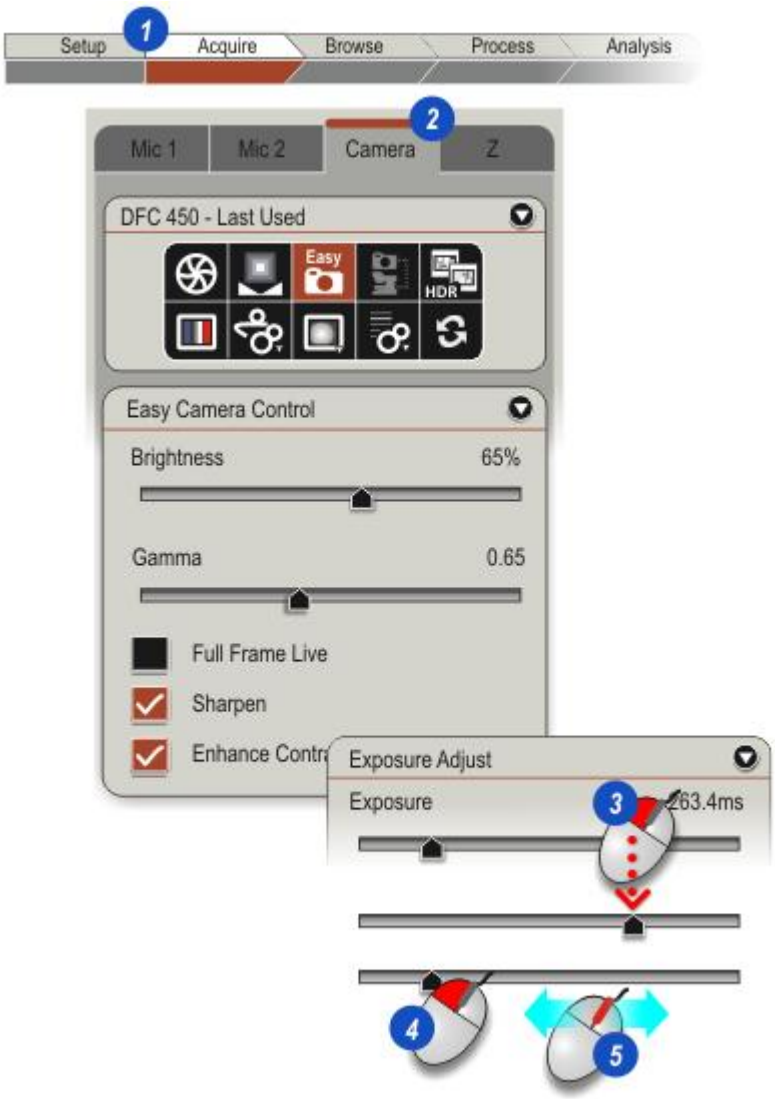
Illumination Intensity of the cold light sources:
This window allows for the control of two independent cold light sources and displays the illumination intensity in degree Kelvin. The allocation of the light sources (L & R) depends on the connectors on the rear of the FSC.

Magnification Changer:
The Mag. Changer window allows for the direct control of the 1.5x additional magnification factor to be introduced at any time with any objective magnification. It acts on both, the eyepieces and the photo port. After clicking on the desired factor (1x or 1.5x) the current status is indicated in red. It will further be correlated into the total magnification with the auto-calibration function.

Tube & Photo port:
This is a display only function. The beam splitter has a fixed factor of 50%/50%.

The *Camera* panel provides convenient control over the functions of a Leica DFC digital camera ranging from colour balance to histogram black and white levels.

- Comprehensive image controls allow users to select the Easy option for straight-forward specimens or Advanced for those requiring extensive camera control and exposure tools.
- Images can be acquired in a variety of sizes, colour depths and file formats to provide even more flexibility. Setting the sharpening option and shading reference acquires images of the highest quality so that further processing requirements are minimal.
- Leica Application Suite also allows a focusing region to be defined on a live image so that areas of significance can be easily identified and focused more rapidly.
- All parameters and configurations can be saved and recalled at a later date.



To reveal the Camera controls:

1: Click on the *Acquire Workflow*.

2: If necessary display the *Camera* tab.

Using the Slider Controls:

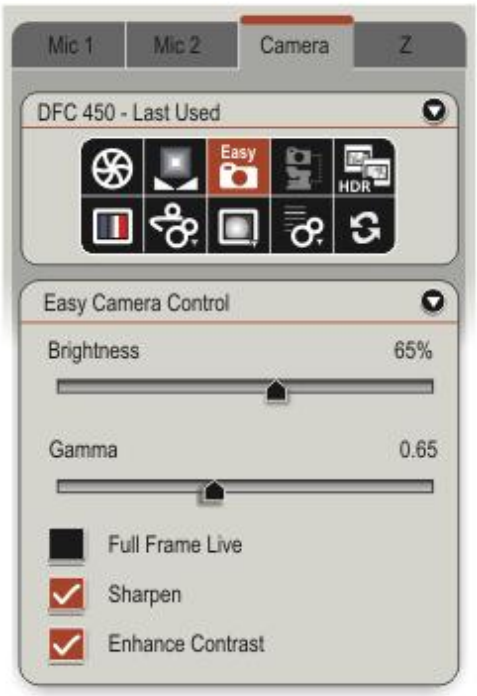
3: Drive a slider control to an approximate position by hovering the mouse cursor over the slider - the pointer moves there - and then using the mouse wheel (if fitted) to fine-tune the control value. If you don't have a mouse wheel, use click and drag.

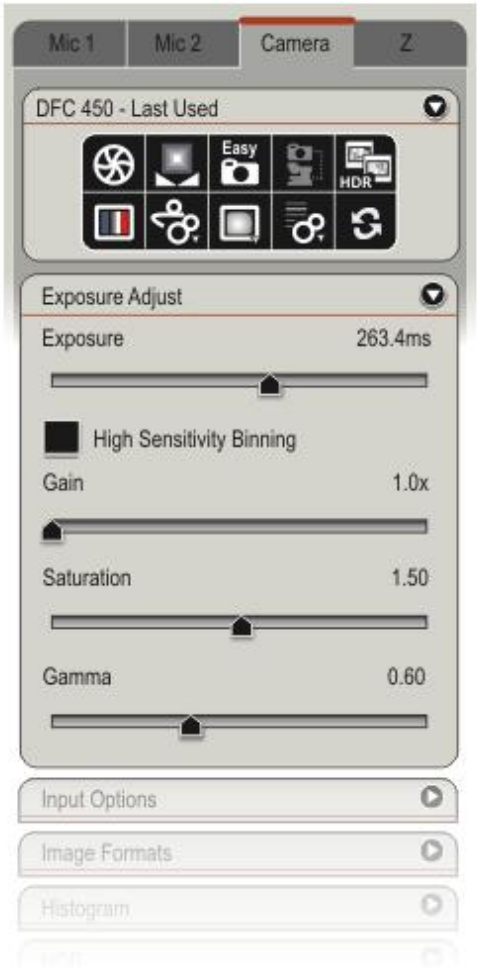
4: Move a slider in large increments by clicking and dragging on the slider pointer.

5: Once the slider is selected the mouse wheel (if fitted) can be used to fine-tune the control value.

Quick Links: Easy and Advanced Exposure Controls

Quick links to the *Easy* (Left) and *Advanced* (Right) camera controls. Click on a panel to go directly to the topic:





As well as the Easy and Advanced Exposure Controls, Leica Application Suite has a wide range of powerful image optimisation tools on the Camera tab.

Link directly to a tool description by clicking the appropriate panel header on the right.

The panels to be displayed or concealed can be set up in *Preferences* 55.

Camera Toolbox

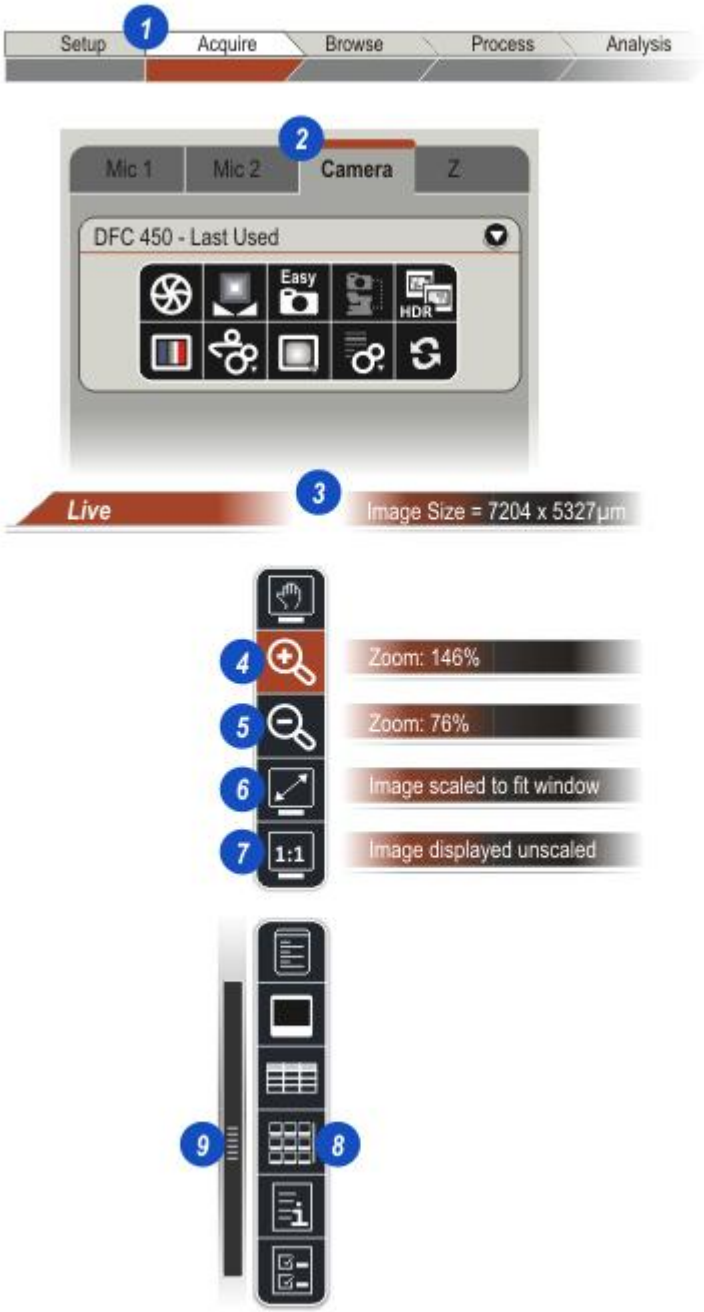
Each of the *Camera Toolbox* buttons links to an image control or camera feature.

- Automatic and Manual Exposure 281
- Automatic White Balance 281
- Easy Exposure 277
- Camera and Microscope Linking 338
- High Dynamic Range / Averaging enable 299
- Show Under/Over Exposure 296
- Camera Configurations 287
- Shading Configurations 309
- Pre-defined Camera Setups 285
- DFC Twain tools 288 (Right click)
- Reset Camera

Reset Camera provides a quick way of restoring the camera to factory default settings (if, for example, you made some changes and ended up with a very poor image).

Live Image Display Controls

Allowing closer and more detailed examination of the live image before deciding to capture it, *Live Image Display Controls* also include an extended *Side Tool Bar* and *Zoom Level* display.



1: Click on the *Acquire Workflow .*

2: If necessary click on the *Camera* tab to reveal the controls.

3: Image information is displayed in the *Live Bar* that appears along the top edge of the *Viewer*. When *Acquire* is selected the size of the *Live Image* is displayed.

4: On the *Side Tool Bar*, clicking the **(+)** button will zoom into the image.

5: Zoom out by clicking **(-)**.

6: *Zoom to Fit* displays the image fitted into the *Viewer* window. The image is scaled so the largest dimension will be fitted to the *Viewer*.

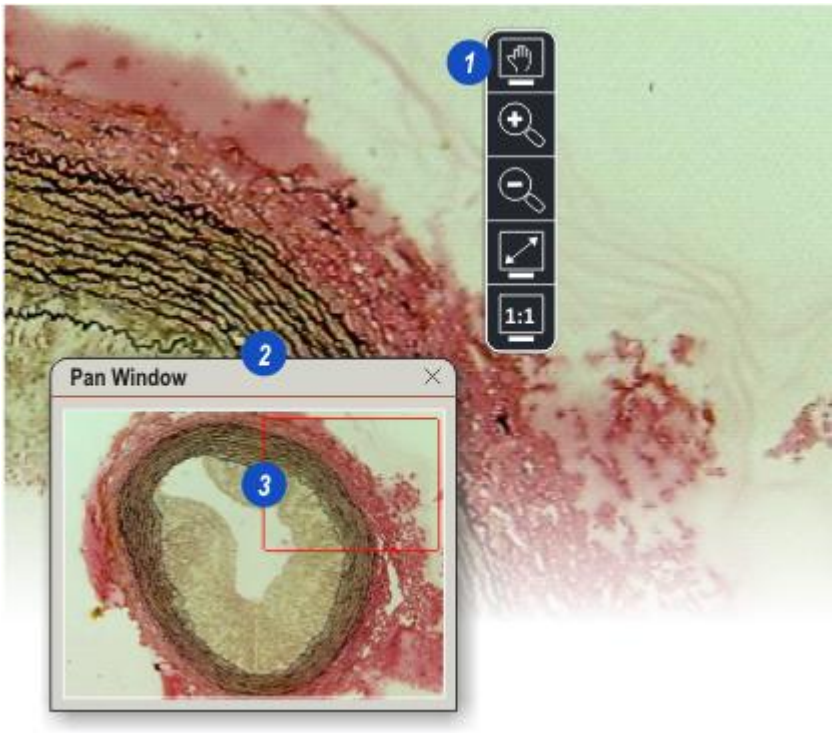
7: *Same Size* displays the image at its actual size - one camera pixel is represented by one display pixel. For high resolution images only part will be displayed and for low resolution it could appear small and centred in the *Viewer*.

8: The *Gallery* thumbnails are hidden or revealed in a toggle action if images have been captured to the current folder.

9: Images zoomed beyond the boundaries of the *Viewer* are automatically displayed with *scroll bars* top and bottom as necessary. See also Pan Live Image 275

Pan Live Image

The *Pan* feature has also been included in the *Live Image Display Controls*. It works at any display level that leaves part of the image concealed beyond the boundaries of the *Viewer*. The 'hidden' area can be brought into the *Viewer* by manipulating the *Pan* window.



1: Click on the *Pan* button.

2: The *Pan Window* appears. Click on the header bar and drag the window to any convenient area on the display.

3: A *red outline* shows the part of the image currently displayed on the *Viewer*. Click inside the outline and drag it to another location in the window and the *Viewer* display will change to reflect the new position.

Click on the *Pan* button **(1)** again to hide the *Pan Window.*

The are three methods for starting image capture:

1: Click on the *Acquire Image* button at the bottom of the *Acquire Workflow*.

2: Press the keyboard *F3* function key.

3: Click on *Options* on the *Main Menu* and click the *Acquire Image* option **(5)**.

If the specimen is evenly and well lit, focus and contrast are acceptable, then the *Easy Camera Control* tool allows users to 'fine tune' the image and achieve even better results.

With *Easy* selected some of the other tools are still active and available. For example:

Automatic Exposure ON: Proper exposure is maintained but the user can adjust the brightness and any hardware changes that could affect the exposure are compensated for automatically.

Automatic Exposure OFF: Users can still change the brightness of the presented image - not necessarily a good exposure that *will* be affected by hardware changes.

Easy provides only the essential controls for image improvement so is quick and very easy to use:

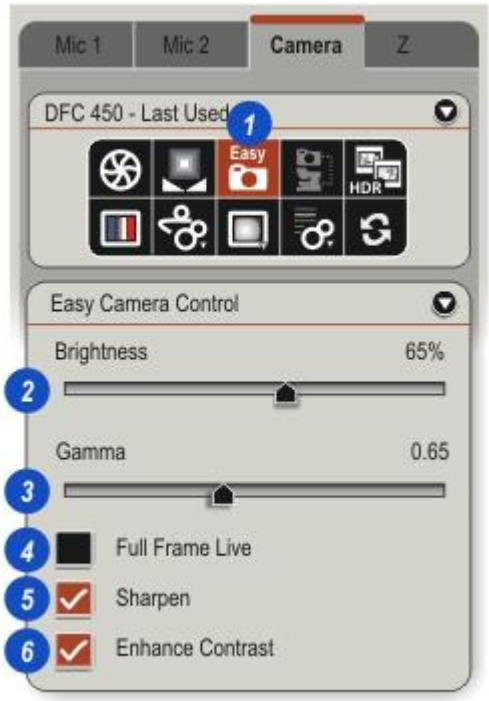
1: Click on the *Easy* button. The *Advanced* LAS panel headers are replaced with a single panel and 5 controls:

2: *Brightness*: Click and drag the slider. A measure of how light or how dark each colour in the image is. It can swing from solid black to solid white.

3: *Gamma*: Click and drag the slider. Low gamma reduces contrast while high gamma increased contrast. The user should adjust gamma so the image displayed matches the image viewed on the microscope.

The remaining controls are all check boxes - enabled by clicking - so image adjustments are made completely in software. However, users should be aware that the *Frame Rate* - the number of images that can be captured and saved in a second - may be reduced.

4: *Full Frame Live:* When checked (tick mark visible) the *Image Formats*[290] *> Live Image Format* will be overridden and *Full Frame* automatically selected to give the user the highest resolution the display will allow. The *Captured Image* format is not affected.



5: *Sharpen:* Software improves edges without introducing artefacts. The equivalent of setting *Processing* > *Sharpening*[310] to medium.

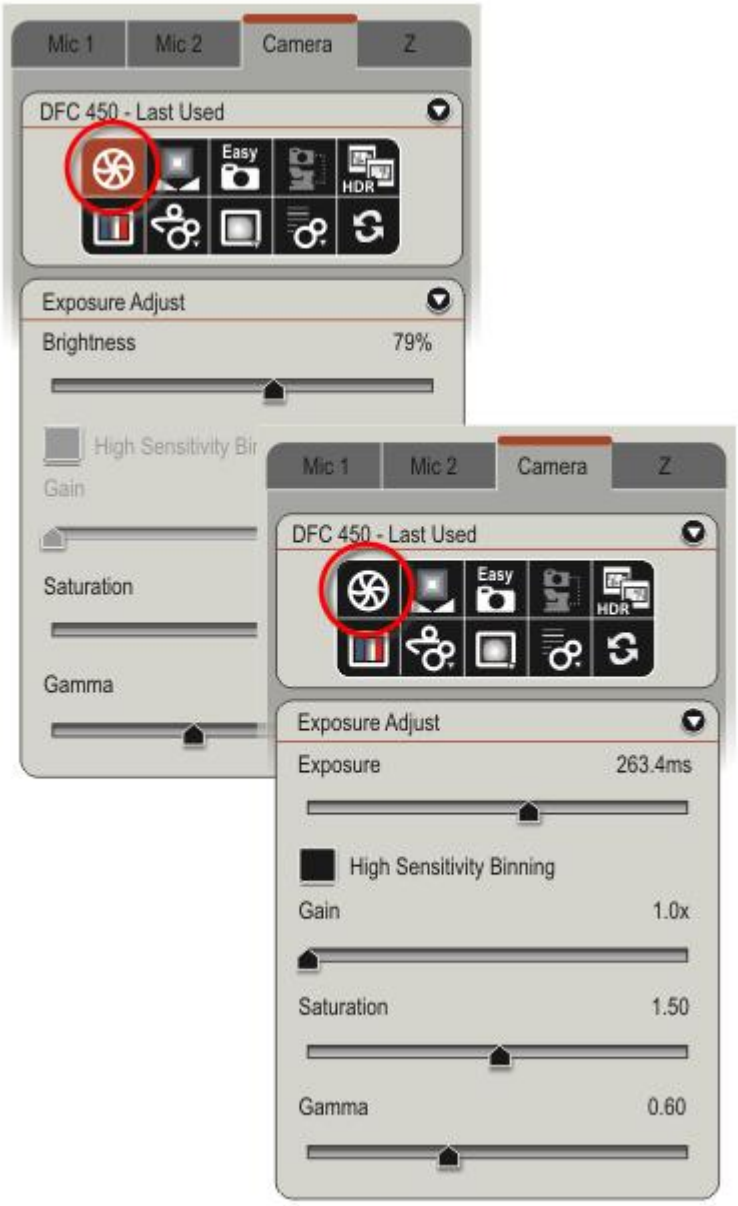
6: *Enhance Contrast:* Increases or decreases colour values to subtly enhance the differences between adjacent areas of the image - differences both with respect to each other and also the white levels - to improve clarity.

Click the *Easy* button (1) again to return to the *Advanced* LAS panel layout.

There are two options for adjusting the exposure:

- *Automatic* [280] with some fine-tuning
- *Manual* [282] with a range of precision controls.

Before starting work on a live image, using *Automatic Exposure* is a good option because combined with *Automatic White Balance* it could produce a perfectly acceptable image very quickly.



Often a very acceptable image can be achieved in seconds by making basic settings to the exposure using *Auto Exposure*, and then fine-tuning the result with white balance.

Click the *Easy* button to turn on the *Advanced* tool panels.

The following applies to a typical brightfield, colour image.

Make the basic exposure settings:

1: Set the *Saturation* to 1.75.

2: Set the *Gamma* to 0.60.

On the Histogram:

3: Set the black level to 0.

4: Set the white level to *255*.

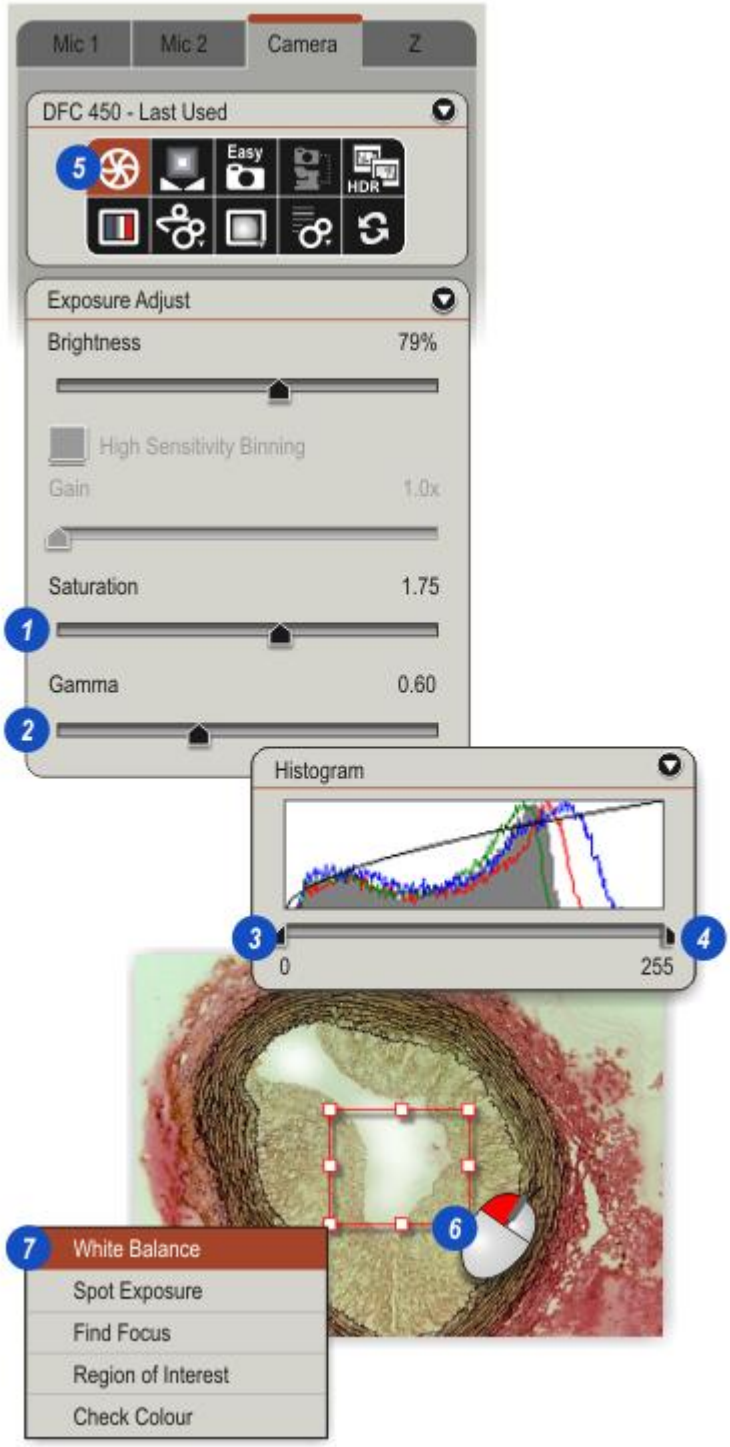
Run Auto Exposure:

5: Click the *Auto Exposure* button and then click again to turn off *Auto Exposure*.

Set the white balance:

6: Click and drag a *Region of Interest* around a white area.

7: Select *White Balance* from the menu.

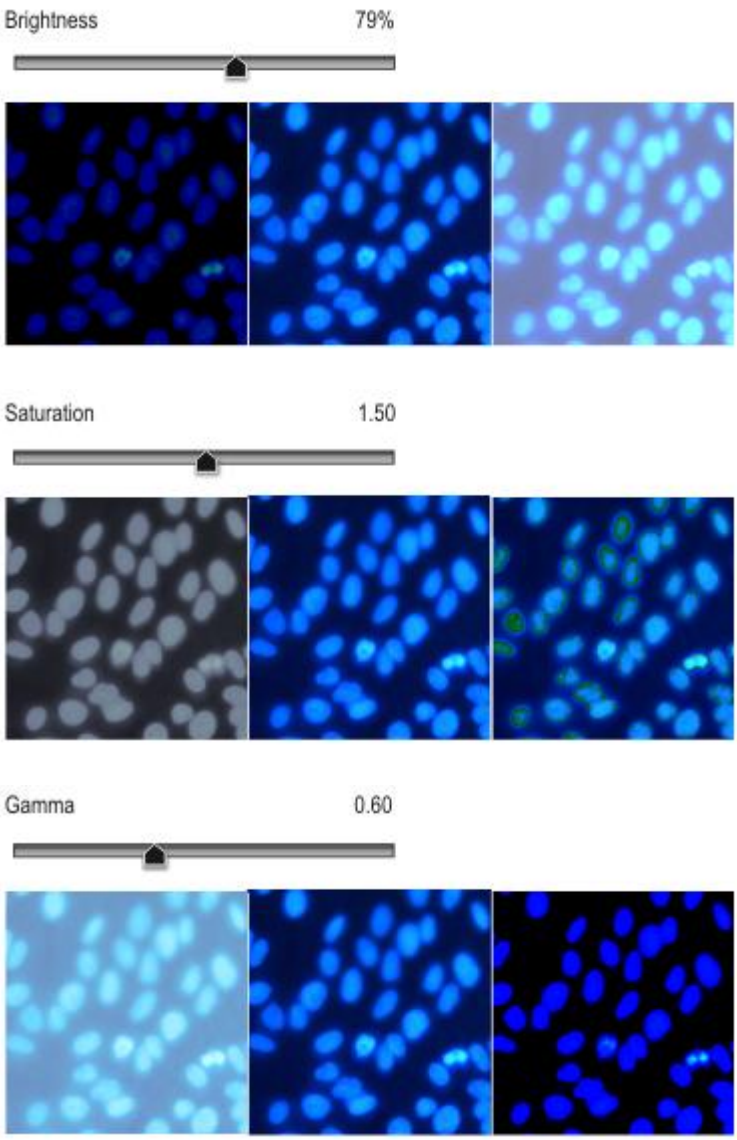


The software uses current light levels to establish best values for *Brightness*, *Saturation* and *Gamma*:

Brightness: A measure of how light or how dark each colour in the image is. It can swing from solid black to solid white.
Use small increases in brightness to help differentiate between colours; too much and detail begins to disappear.

Saturation: Determines the amount of each colour that is present. At the highest setting, each colour will be at its most vibrant - right hand image - and the colours cannot be more prominent without combining to make white.
Use *Saturation* to achieve colour subtlety in the image.
Reducing *Saturation* is a convenient way of turning a colour image into a monochrome image - essentially just shades of grey - without losing detail or becoming a black solid.

Gamma: A value applied to colour levels to compensate for different ways in which the image is viewed. Liquid crystal displays (LCDs) have a specific *Gamma* setting, monitors will have another and printers yet another.
Changes in Gamma are applied automatically so for example, when an image is printed the printer software will make adjustments before the printing.
Very small changes in *Gamma* can have dramatic effects; the examples show a range of *0.35* to *1.50* with the original in the centre. Use *Gamma* to achieve a contrast 'match' to the specimen



To apply *Automatic Exposure:*

1: If necessary reveal the *Exposure Adjust* panel by clicking on the arrow right of the header bar.

2: Click on the *Automatic Exposure* button.

3: Adjust the *Brightness, Saturation and Gamma* controls as necessary to achieve the required image. See Automatic Exposure Tools 280.

Automatic White Balance:

When *Automatic White Balance* is applied, all of the neutral tones - white through grey to black - are adjusted to remove any 'colour' content to maintain a clean, well-defined image.

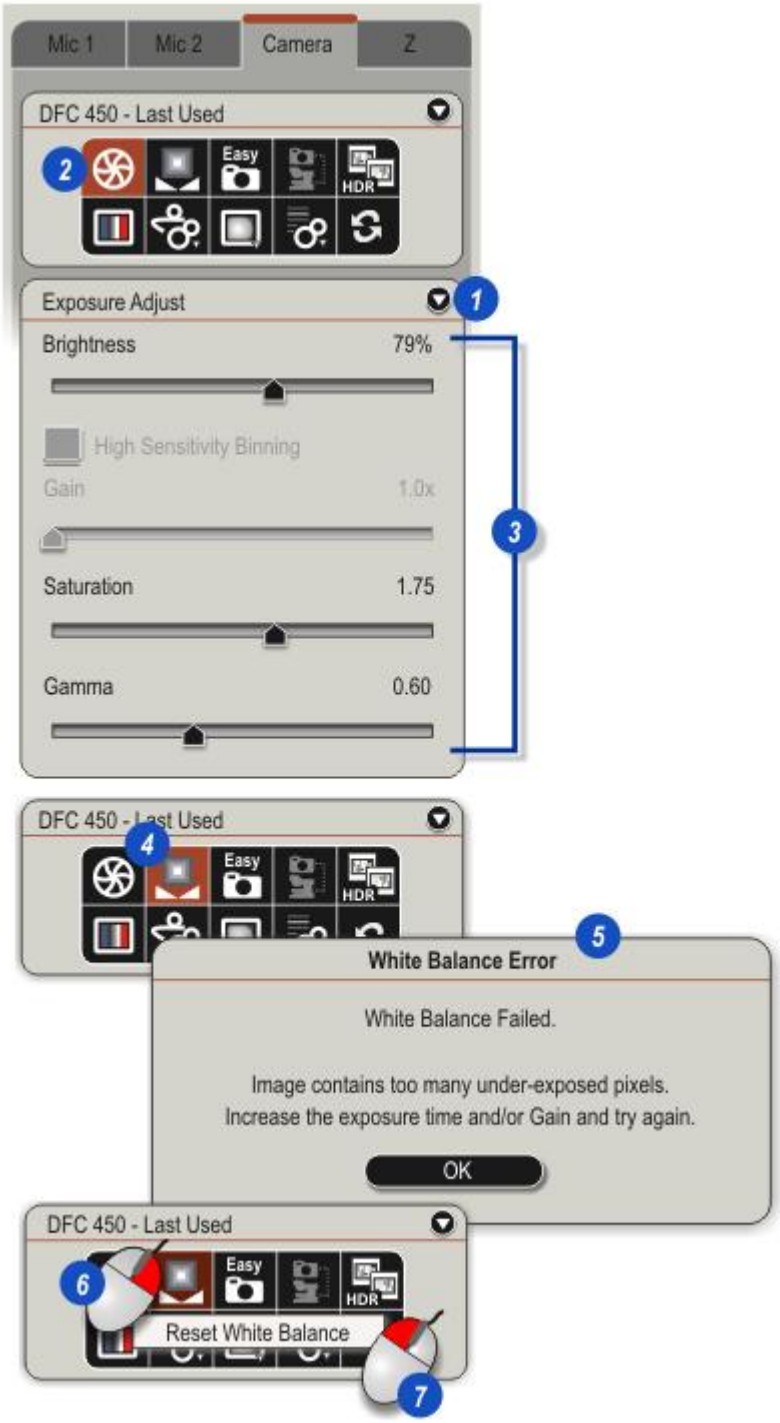
4: Click on the *Automatic White Balance* button. *White Balance* is applied to the entire image.

5: If the image is too dark or too light *Automatic White Balance* may fail and an error message displayed. It may be possible to lighten or darken the image with the *Exposure Adjust* controls or change the lighting conditions at the microscope.

To undo *Automatic White Balance:*

6: Right-click on the *White Balance* button.

7: Left click the *Reset White Balance* label.



The image controls change when *Exposure Adjust* is in manual mode - *Brightness* is replaced by *Exposure*: *Saturation* and *Gamma* remain but another control - *Gain* - is added.

If necessary, click the *Automatic Exposure* button to ensure it is disabled.

Exposure: Controls the time that the camera sensing elements are exposed to the specimen. It is sometimes called the scanning rate.

At the start of the exposure time period, all of the camera sensing elements are reset - they have no usable image information at all. Then they are exposed to the specimen and each begins to 'charge' to a value that numerically represents the light falling upon it. Individual elements are designed to respond to one of the three colours - Red, Green or Blue (RGB).

At the end of the exposure period, each element is 'read' and its value used in combination to create a pixel on the *Viewer*.

For any image, there will be an optimum period of exposure for the elements to reach values that truly represents the image **(B)**. Too short an exposure and the elements will not have sufficient time to reach the proper value - the image will be dark and muddy, under-exposed **(A)**. Too long and the image will be 'washed out' and lacking detail, over-exposed **(C)**.

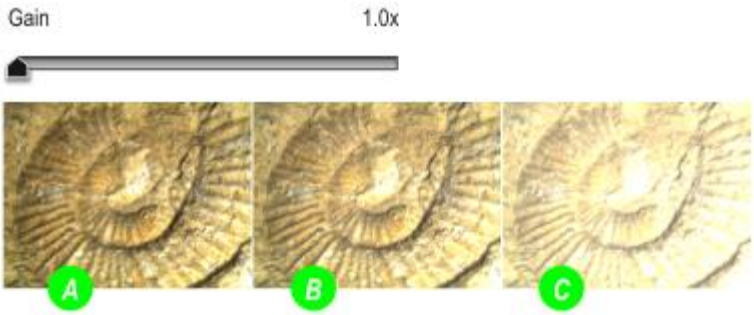
The time scale for exposure will depend upon the camera and may be measured in either microseconds(µs), or milliseconds (ms).

Gain: A function for changing the brilliance of an image without changing the exposure. The examples shown are:

Gain = 1.0 ***(A)****,*

Gain = 1.5 ***(B)*** and

Gain = 3.0 ***(C****).*



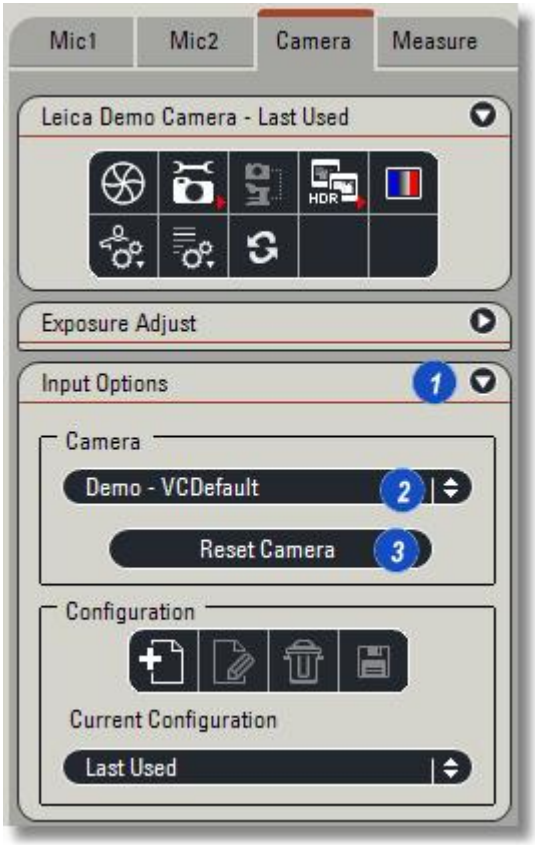
High levels of *Gain* can make an image look noisy and badly defined, so as a general rule leave the setting at 1.0 unless light levels are particularly low.

Start with a *Gain* value of 1.0 and gradually increase the value. Too high a *Gain* setting will 'bleach' the image, cause a loss of fine detail and may introduce 'noise'.

Saturation and Gamma information 280

Input Options: Camera Selection and Reset

Input settings cover the camera setup. The settings are established using several of the *Camera* control panels and *Preferences*.



You can:

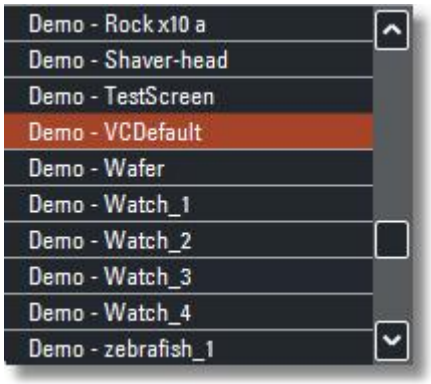
- Select the Active Camera[284]
- Choose and load a Pre-defined Camera Configuration[285]
- Save[286] a User Camera Configuration
- Load[287] a User Camera Configuration
- Access the Twain[288] User Interface.

Occasionally, more than one camera, each with its own *FireWire* connection, may be connected to the computer. They appear in a drop-down menu with model names and serial numbers. The current active camera appears at the top of the list.

If you have no camera connected, you can select a demo camera and an associated image. This image will be displayed in the Live Image window. Images can be acquired as if a camera were connected:

1: Expand *Input Options* panel.

2: Display the *Camera* drop-down menu and select a camera.



See here[63] for details on specifying the folder where the default demo camera images are stored.

To restore a temporarily lost camera connection:

3: Click *Reset Camera*.

Pre-defined Camera Setups

Pre-defined Camera Setups are settings for the most common microscope contrast methods that you can quickly select and use.

Pre-defined Setups automatically configure LAS for image exposure and processing corresponding to the selected technique.

To select the required technique:

1: Click on the *Pre-defined Setups* button.

2: From the drop down list, click to select and apply the required configuration.

3: *Automatic Exposure* is automatically launched to allow you to make adjustments if necessary.

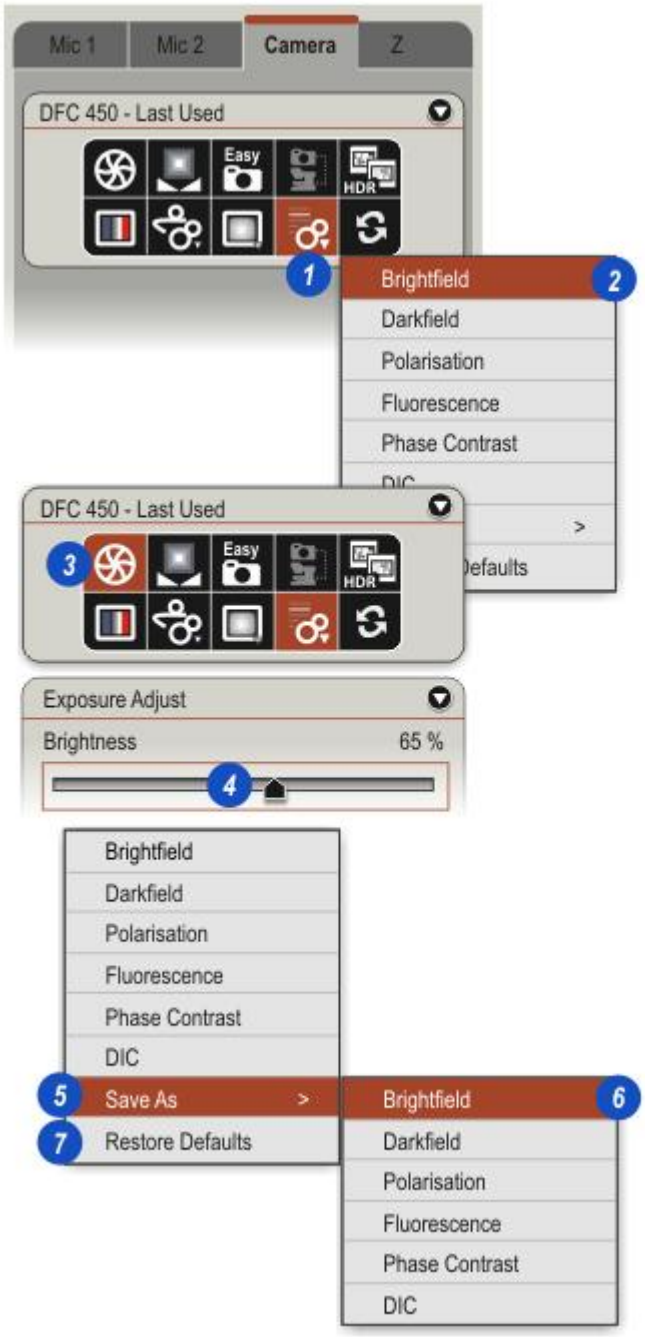
4: Make adjustments to a *Pre-defined Setup.*

To save as an update:

5: Click on the *Save As* option.

6: Select the original setup from the drop-down menu.

7: To restore *Setups* to their original settings, click on the *Restore Defaults* options.



Save and Delete a User Camera Configuration

With the camera setup complete, you can save the settings and use them on another occasion to perfectly replicate current values.

1: Click on the small arrow to the right of the *Input Options* header to reveal the panel.

2: Click on the *New Configuration* button.

3: On the *Save Current Configuration* dialog, click inside the text box and type a new, unique name for the configuration.

4: Click *OK.*

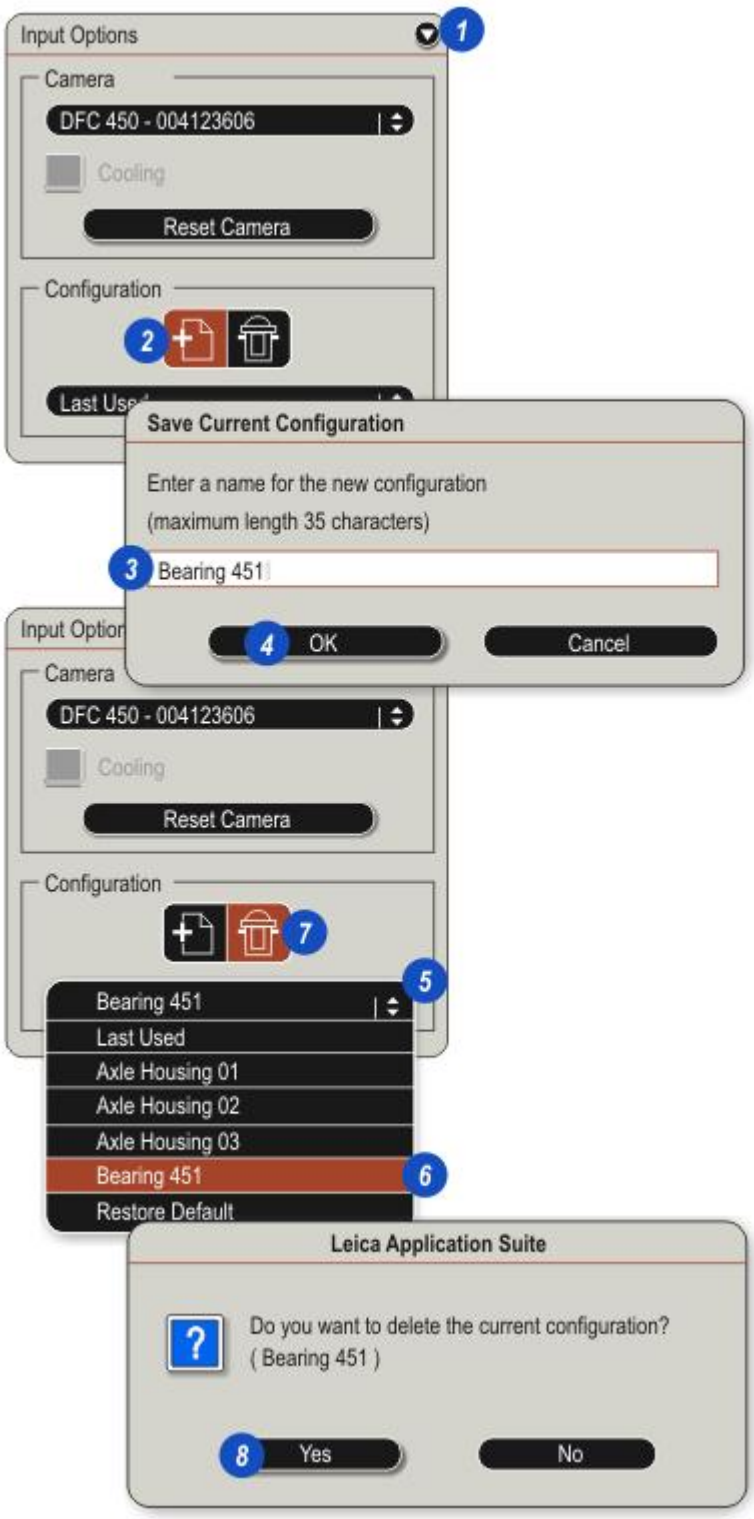
To delete the current Configuration:

5: Click on the arrows to the right of the of the *Configuration* header bar.

6: From the drop down menu click to select the configuration to be deleted.

7: Click on the *Trash Can* (Delete) button.

8: Click *Yes* to confirm the deletion.



you can save Input settings and use them on another occasion to perfectly replicate current values.

When the *Viewer* opens, the configuration defaults to the *Last Used* settings.

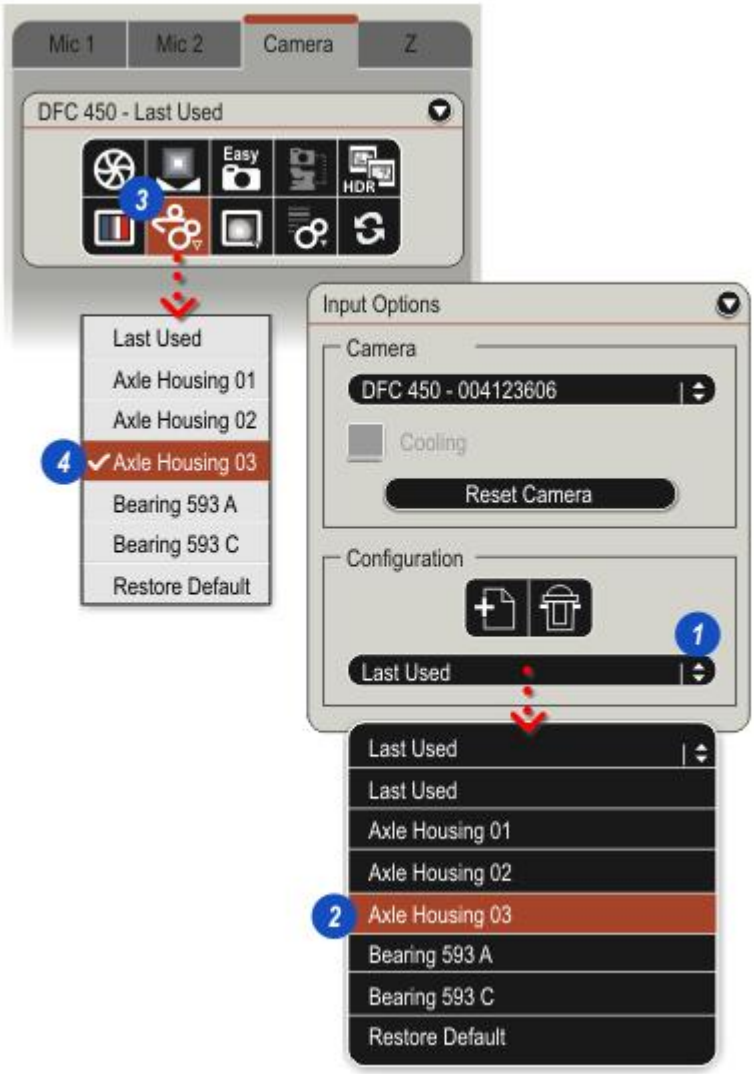
To Select and Load a previously saved User Camera Configuration:

1: Click on the arrows to the right of the *Configuration* window.

2: From the drop down menu click to select a saved configuration, or...

3: Click on the *Select camera configuration* button in the toolbox.

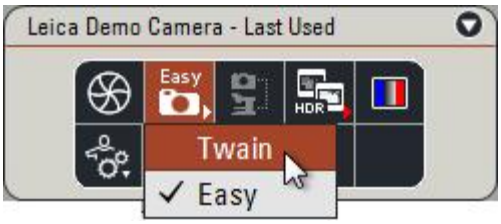
4: Select and load a saved configuration from the drop down list.



The Twain Interface

The *Twain* User Interface displays settings and tools associated with the active camera.

Use the *Twain* Interface to view the camera data and make basic exposure settings on a single, compact display.

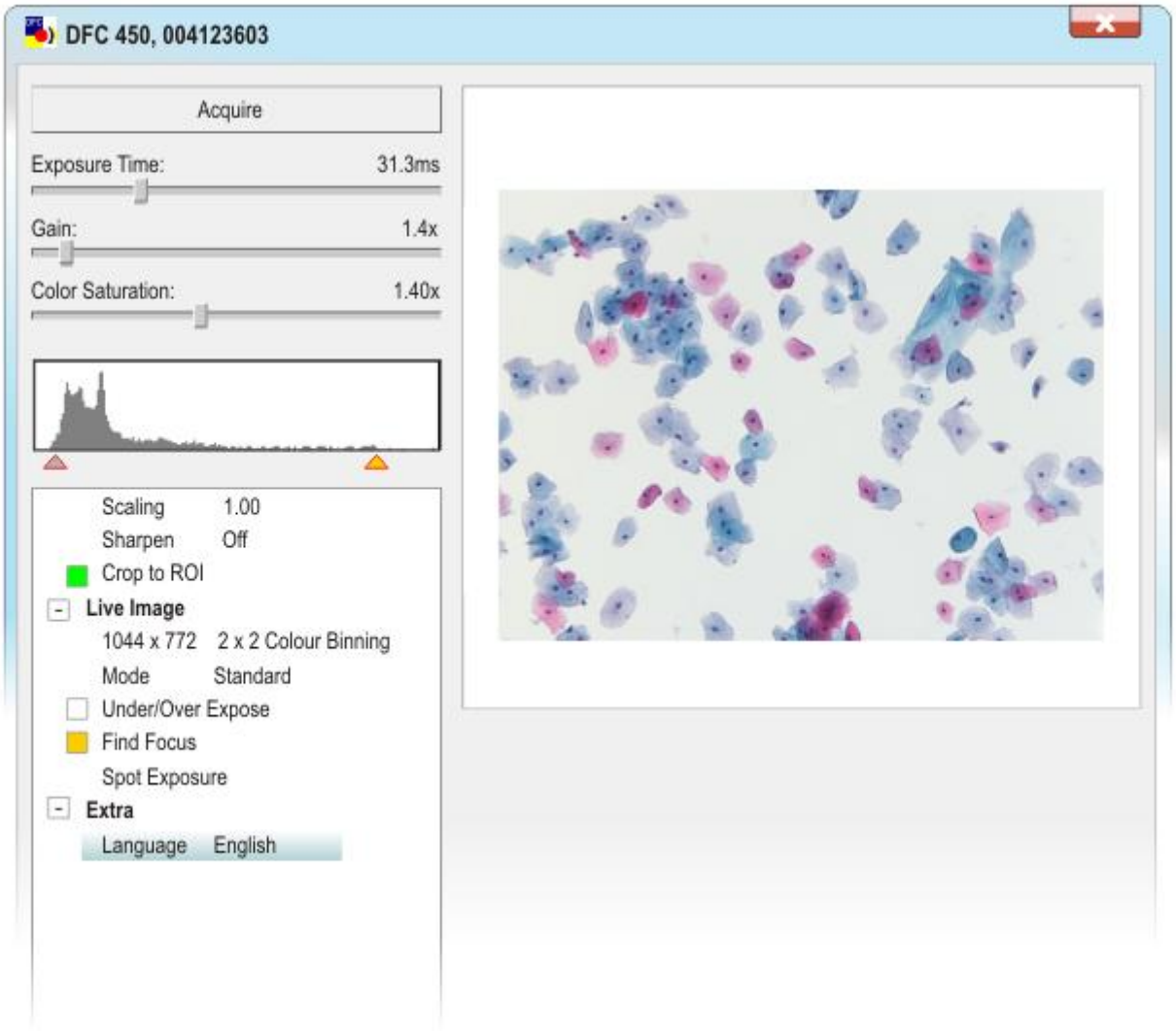


To display the Twain interface:

1: Right-click on the *Easy Camera Controls* button.

2: Select *Twain* from the drop-down menu.

3: The button icon changes to a spanner and camera, and the Twain interface is displayed.



See also *Finding Twain Documentation* 289.

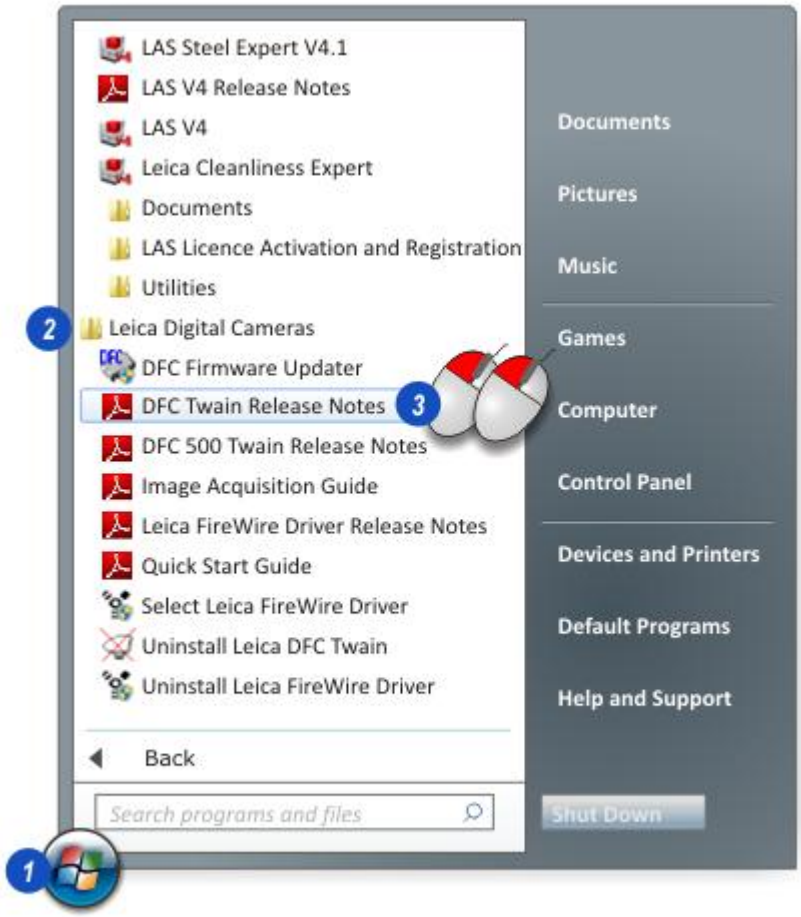
Finding Twain Documentation

For a detailed description of *Twain* and how it is used refer to the *Leica DFC Twain Guides* as follows:

1: Click on Windows *Start > All Programs.*

2: From the list of applications click on the *Leica Digital Cameras* folder icon.

3: Double-click *DFC Twain* or *DFC 500 Twain Release Notes*.

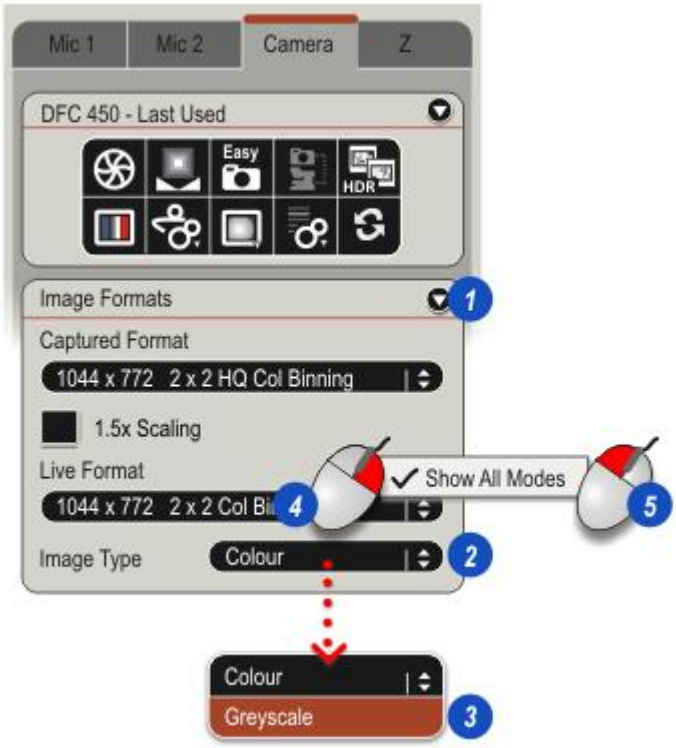


On the *Image Formats* panel you can:

- Select a Colour or Grayscale image.
- Select Live and Captured image formats [292].

Also in this section, how to:

- Choose the Bit depth [293].
- Enable/disable High Sensitivity Binning [294]



If a colour camera is active, both colour and greyscale (monochrome) options are available.

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.

2: Click on the arrows to the right of the *Image Type* header bar.

3: From the drop-down menu select the type required.

Live Format:

Live Format determines the quality and resolution of the displayed image in the*Viewer*. The active camera will determine the extent of the format options available.

To ensure that all possible options are available:

4: Right-click on the *Live Format* text box.

5: Click on the *Show All Modes* label to check it.

Depending upon the active camera, there will be a range of formats available for both live and captured images.

- *Progressive VGA* produces the lowest resolution images but is suitable for very fast exposure rates.
- *Colour Binning* is a process of grouping adjacent camera element pixels to create 'super' pixels. Each group is then used to 'drive' a single display pixel. The feature improves overall sensitivity and speed.
- Available binning options depend upon the camera being used; Three formats - *2 x 2, 3 x 3* and *4 x 4* - are available, each with a HQ (High Quality) option. The format numbers describe how the pixel values are grouped: *2 x 2* = 4 pixel group: *3 x 3* = 9 pixel group and *4 x 4* = 16 pixel group.

  As a guide and depending upon the camera model, the *2 x 2 Colour Binning 1044 x 772* format is a good choice for most situations.
- *Full Frame* options display each of the camera element pixels individually. Resolution and quality is very high especially if a *HQ* (High Quality) option is selected.
- *Progressive Red, Blue or Green* use only the value of the selected colour. The *Viewer* displays a grayscale image representing the intensity of the chosen colour. Even if the *Image Type* is set to colour, the image will appear monochrome.

Avoid saving images in 16-bit format. They will be slow to expose and process and, if captured and saved in the same format, may be unusable in third-party image processing applications.

To select the Live Image Format:

1: Click on the arrows to the right of the *Live Format* header bar.

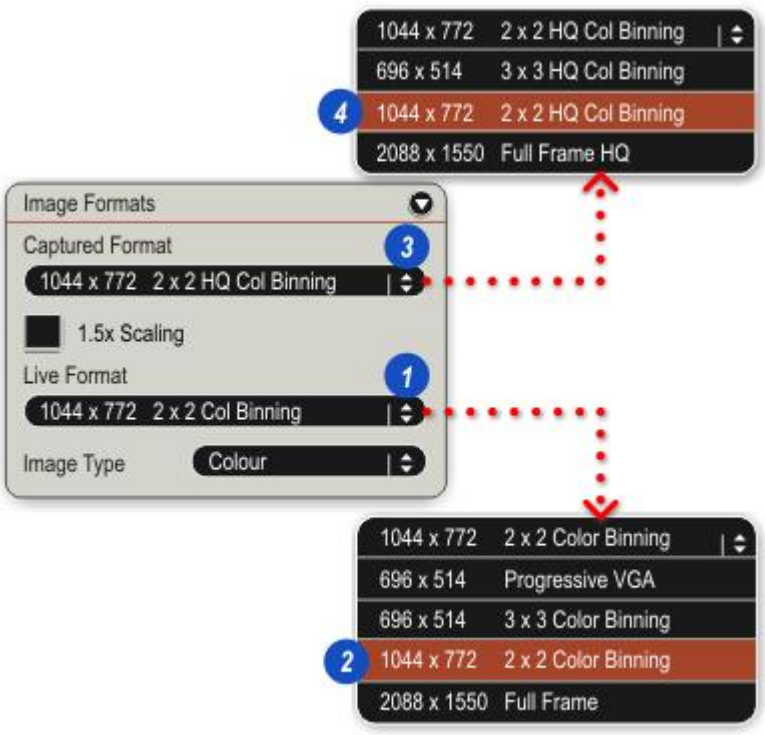
2: Select a format from the drop-down menu. If the camera supports a wide range of formats, small *Scrolling Arrows* will appear at the bottom of the drop down list. Click to scroll down.

To select the Captured Format:

3: Click on the arrows to the right of the *Captured Format* header bar.

4: Select a format from the drop-down menu. To save an image with 16-bit colour depth, you must select a *HQ (High Quality)* option.

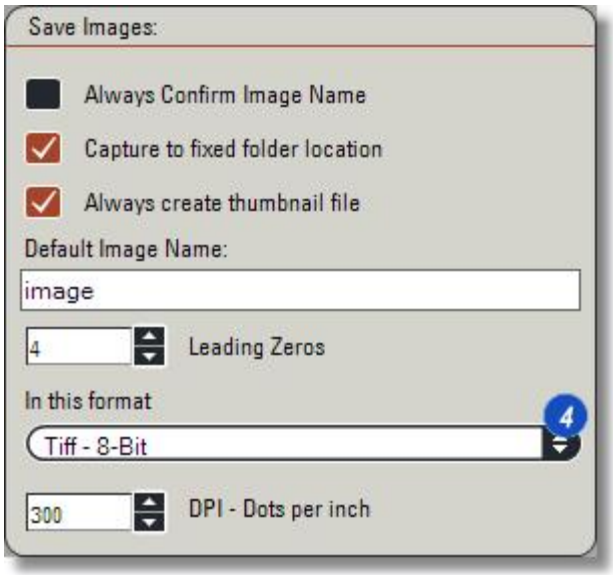
Captured Format determines how the image is finally captured and saved. In many cases, the *Captured Format* will be the same as the *Live Format* so, providing the image is saved as a bitmap on a bit-by-bit basis without compression, when the image is retrieved it will be identical to the original.



Bit Depth is a digital value which determines the colour range and precision of a saved image. A value of 8 bits gives 256 separate colours (2^8); 16 bits gives 65536 colours (2^{16}).

Greyscale images are captured and saved as either 8 or 16 bits per pixel. Colour images require three times the number of bits per pixel to store the three primary colours - *red, green* and *blue*. So, colour images are stored either as *3 x 8* bits or *3 x 16* bits (High Quality).

The *Bit Depth* setting has a considerable effect on the disc file size so generally 8 bit should be chosen unless more colour subtlety and variation are needed. The 16-bit option is not always compatible with some third-party software.



The Bit Depth setting in Preferences:

The *Image Format* setting made in *Preferences* > *Save Images* sets the image compression and, in some case the *Bit Depth*. They override those made here in *Camera*.

To check or select the Preferences settings:

1: Click *Options* on the *Main Header.*

2: Select *Preferences*.

3: On the *Preferences* dialog, display the *Image* tab.

4: Click on the arrows to the right of *In this format.*

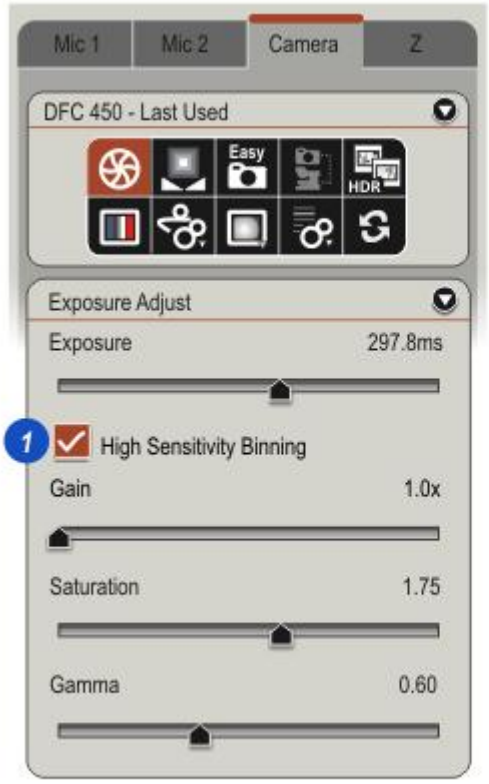
5: Select the *Compression* type and associated *Bit Depth*.

6: Click *OK.*

If binning is used for *Live Format* but not for the *Captured Format*, when the image is retrieved it will appear darker than expected.

To maintain binned live image brightness on the captured image:

1: Click to enable the *High Sensitivity Binning* check box on the *Exposure Adjust* panel.



Histogram

The *Histogram* is a graphical display of the colour values in the image represented as 256 points ranging from 0 (Black) through to 255 (White).

In this section:

- Histogram display options.
- Checking Under- and Over-Exposure[296].
- Automatically cropping[297] Under- and Over-Exposed areas.
- Setting the Gamma[298] Level automatically.

To set the display detail:

1: Click on the small arrow to the right of the *Histogram* header to reveal the *Histogram* panel.

2: Right-click on the histogram display window.

3: From the drop down menu,click on *Histogram Options* and...

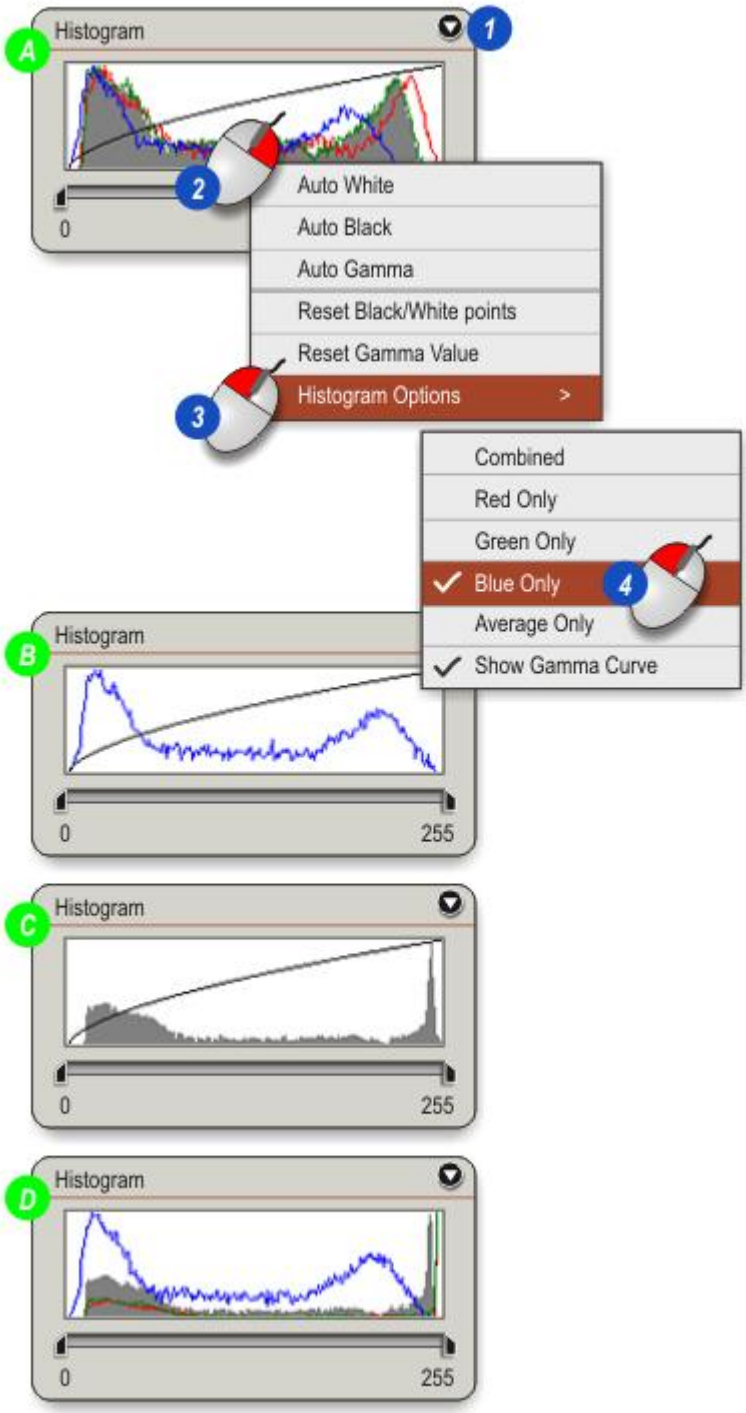
4: Select an option:

Combined ***(A)****:* Shows all of the colours and the average.

Red, Blue or Green ***(B)****:* Show that colour level only.

Average ***(C)****:* Displays the average of all of the Red, Green and Blue (RGB) values.

Show Gamma Curve ***(D)****:* Click to display or hide the *Gamma Curve*.



Over and Under-Exposure

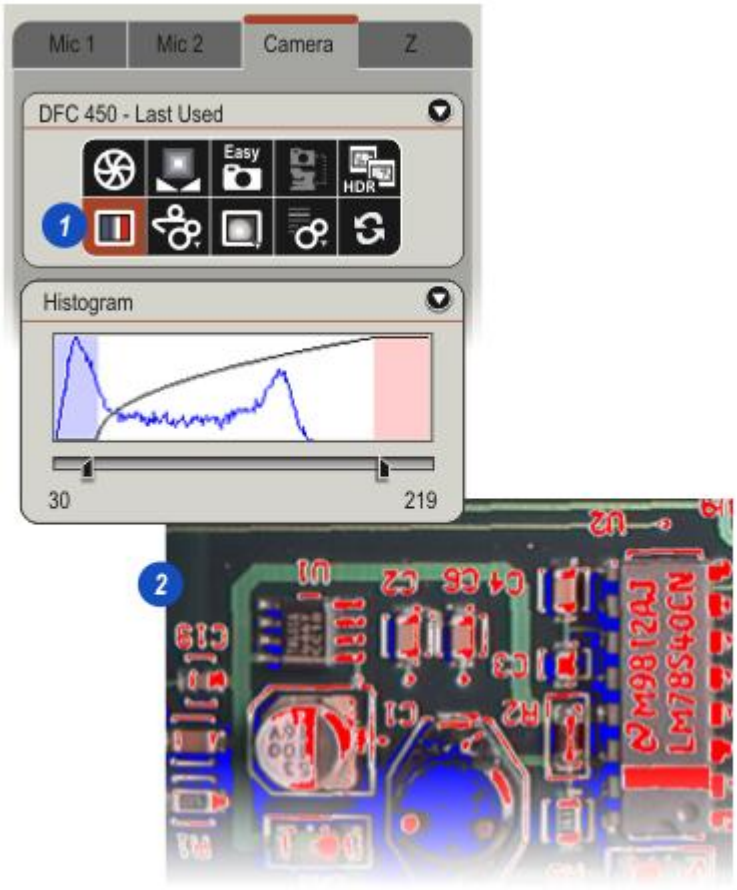
The ‘one-click’ *Show Under/Over Exposure* feature gives a fast indication of those areas of the image that are not exposed properly - and probably not adding to the image quality.

To check exposure:

1: Click on the *Show Under/Over Exposed* button.

On the *Histogram* window, the blue region indicates under-exposure at the 0 (Black) end; the red region indicates over-exposure at the 255 (White) end.

2: Under- and over-exposed pixels are highlighted in blue and red on the *Live Image*.



Auto Black and Auto White

Under- and over-exposed areas of the image do not contain useful detail, so you can safely ignore them.

The *Auto White* and *Auto Black* commands automatically adjust the black and white points of the image.

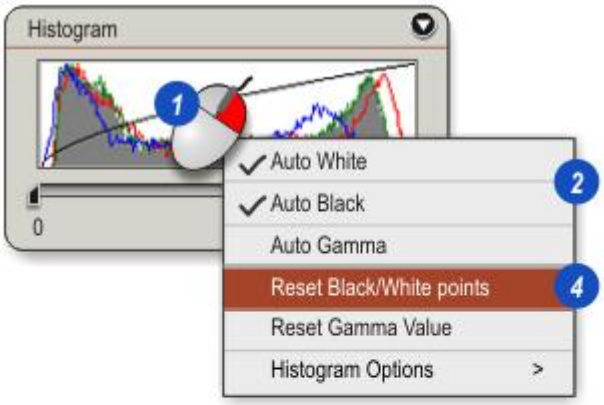
1: Right click on the *Histogram* window.

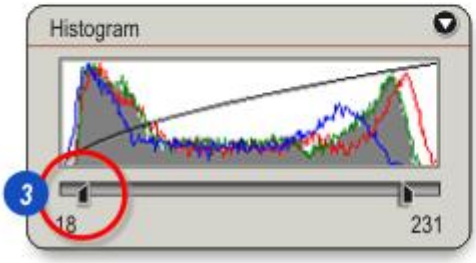
2: From the drop down menu, click to enable the *Auto White* or *Auto Black* option. Click again to disable the option.

3: Beneath the *Histogram* window, the slider corresponding to the white or black option moves to reflect the ignored light levels.

4: To reset the black and white values, right-click on the *Histogram* window and select *Reset Black/White Points*.

Auto White and Auto *Black* are disabled by default.





The *Auto Gamma* option sets the gamma level based upon the active light levels. Under- and over-exposed levels are not included if they have been 'cropped' either manually or automatically.

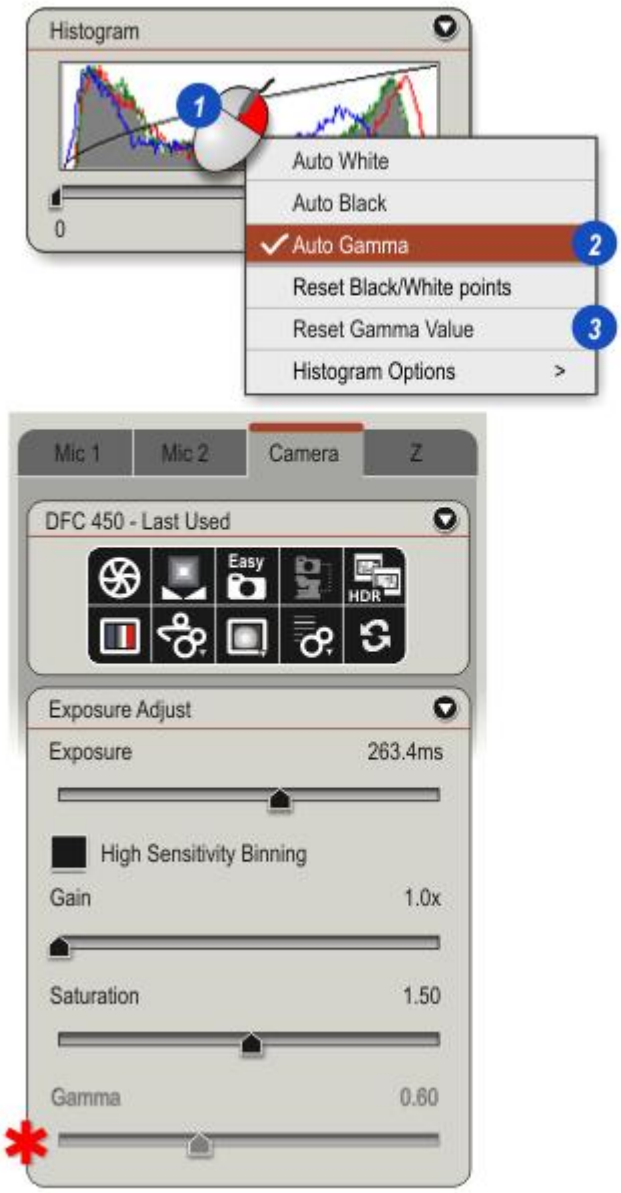
1: Right click on the *Histogram* window.

2: From the drop down menu, click to enable the *Auto Gamma* option. Select again to disable the option.

3: To reset the gamma value, right-click on the *Histogram* window and select *Reset Gamma Value*.

While *Auto Gamma* is enabled, the *Gamma* control on the *Exposure Adjust* panel is disabled.

Auto Gamma is disabled by default.

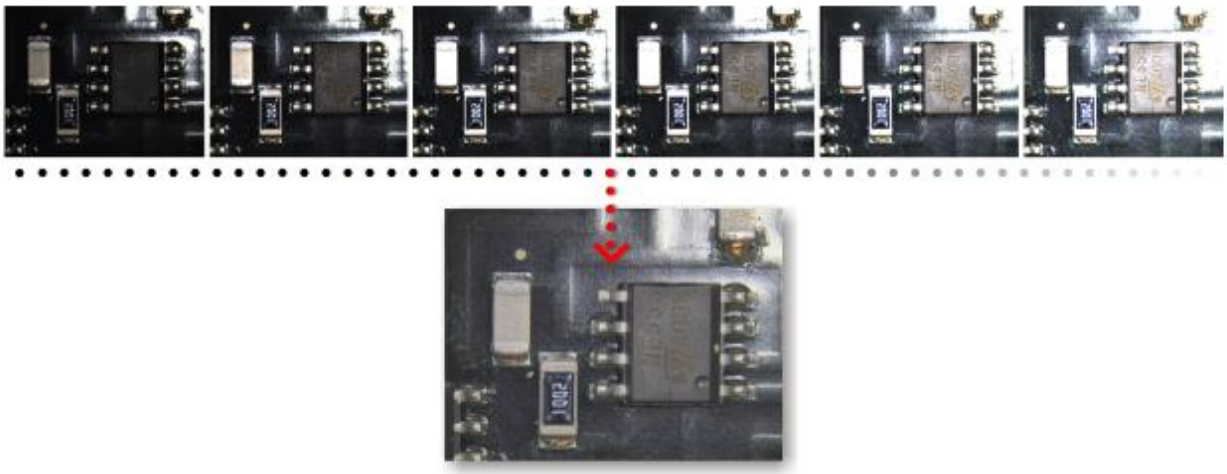


High-contrast images cause problems in digital imaging: if you expose for the shadows,you may lose information in the highlights; expose for the highlights and the shadow detail is compromised.

Leica High Dynamic Range (HDR) provides a fast solution by automatically capturing a number of images each at a different exposure, and then combining them digitally into a single image that balances the contrast range. Detail across the entire image is retained and clear.

You can choose between automatic *HDR* that handles the processing and saving in a simple, fast single step, or a manual operation that allows some fine-tuning before the image is saved.

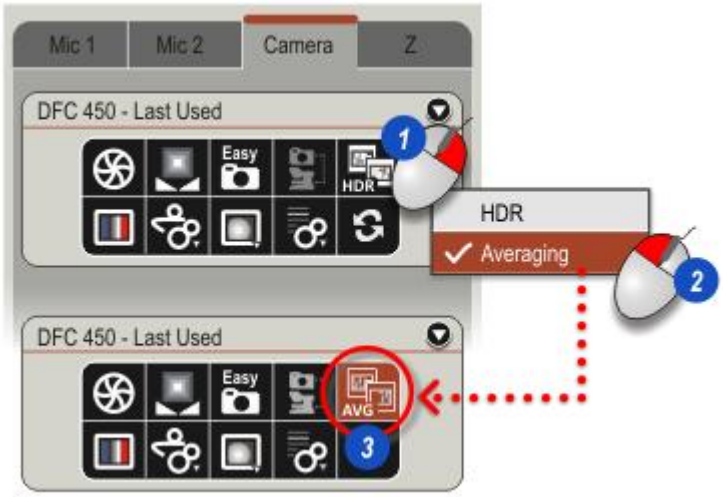
Additionally, an *Averaging* feature is available that can reduce the amount of noise in an image. Noise reduction can enhance the final image, perhaps making it more suitable for analysis.



Setup for HDR and Averaging

Before using *HDR* or *Averaging*:

- Set the capture details in *Preferences* 64.
- Select a capture folder in *Browse* 389.
- Check that required *HDR* function is enabled.



To enable *HDR*:

1: Right-click on the *HDR* button.

2: From the drop-down menu, select *HDR* or *Averaging*.

3: The button caption displays the selected function - *HDR* or *AVG (Averaging).*

- Adjust the specimen illumination so that it is even, reduces highlights, reaches into crevices and does not cast shadows.
- Use the *Fast Track Exposure* 279 sequence and aim for a sharp and reasonably exposed image.

See also:

Automatic HDR 301

Manual HDR with Preview 302

Averaging 305

The *Automatic HDR* feature captures a series of images to memory, creates the *HDR* image and saves it in the set capture folder.

All the settings are controlled by the software - you cannot select the *Sample Contrast* or adjust the *Brightness* before the *HDR* image is saved, but you can use the *Enhance* controls on the *Process Workflow* to make adjustments to it after capture.

Previous settings made using the *Preview* function are ignored.

1: If necessary, click to disable *Automatic Exposure.*

2: Click on the *HDR* button.

3: On the *HDR / Averaging* control panel, click to enable *Automatic*.

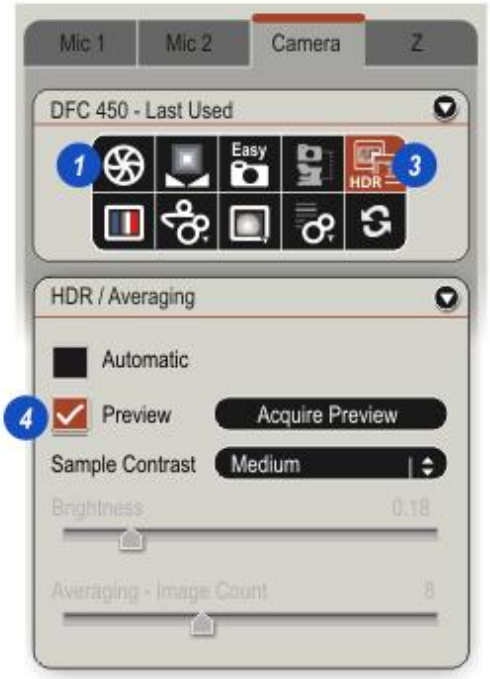
4: Click the *Acquire Image HDR* button or function key *F3*.

5: The *Acquisition Progress* bar appears while the images are being captured to memory and the *HDR* image created.

6: The final *HDR* image is stored in the capture folder on the hard drive.

7: A thumbnail is created in the *Gallery*.

In manual mode, the Preview facility is used to optimise the appearance of the image by interactively adjusting the contrast range and brightness of the HDR image. These settings are then used for actual image acquisition while HDR is in manual mode.



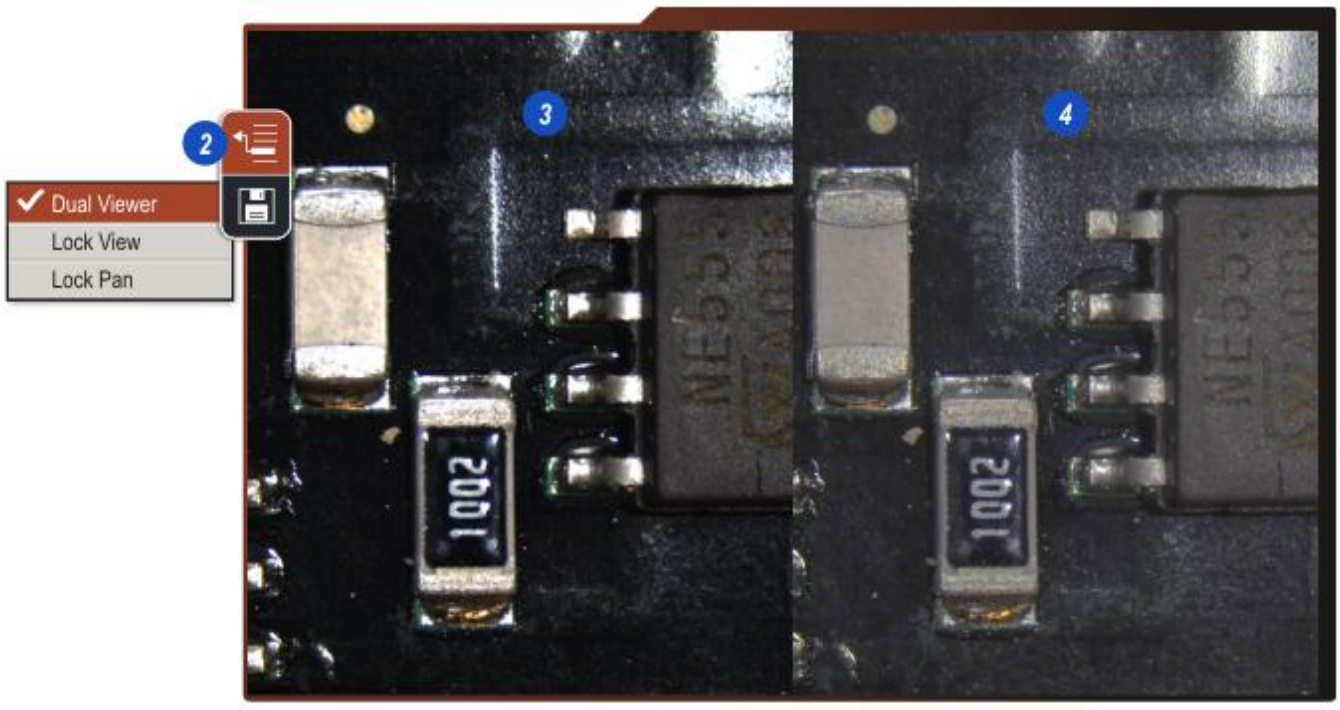
1: If necessary, click to disable *Automatic Exposure* and...

2: On the *Side Tool Bar* turn on *Dual Viewer* so that the live image can be compared with the *HDR* processed image. The live image is on the left and the *Preview* image on the right.

Users will find it convenient to select the *After Capture Do Nothing* option in *Preferences* 66 to remain on the *Acquire Workflow* after an image is captured.

3: Click on the *HDR* button.

4: On the *HDR / Averaging* control panel, click to enable (tick mark visible) the *Preview* check box.



Sample Contrast

Sample Contrast allows you to select the exposure values either side of a fixed exposure point.

The additional exposure times are determined by the *Sample Contrast* setting - *Low, Medium, High* and *Very High*. Each option applies different factors as follows:

Low Contrast: -1 | Centre | 1

Medium Contrast: -1.5 | Centre | 1.5

High Contrast: -2 | Centre | 2

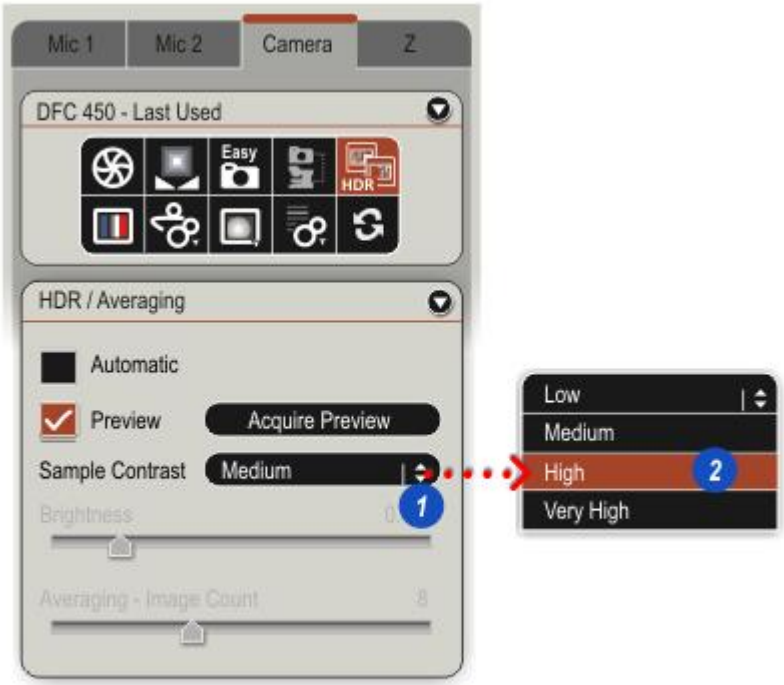
Very High Contrast: -3 | -1.5 | Centre | 1.5 | 3

The *Very High Contrast* option captures 2 additional images either side of the centre exposure.

You should experiment to find the best level for the image.

1: Click on the arrows to the right of the *Sample Contrast* header.

2: Select the required contrast level.



1: Click the *Acquire Preview* button.

2: The *Acquisition Progress* bar appears while the images are captured at different exposures.

3: The composite *HDR* image is displayed with balanced contrast in the *Viewer* right-hand pane.

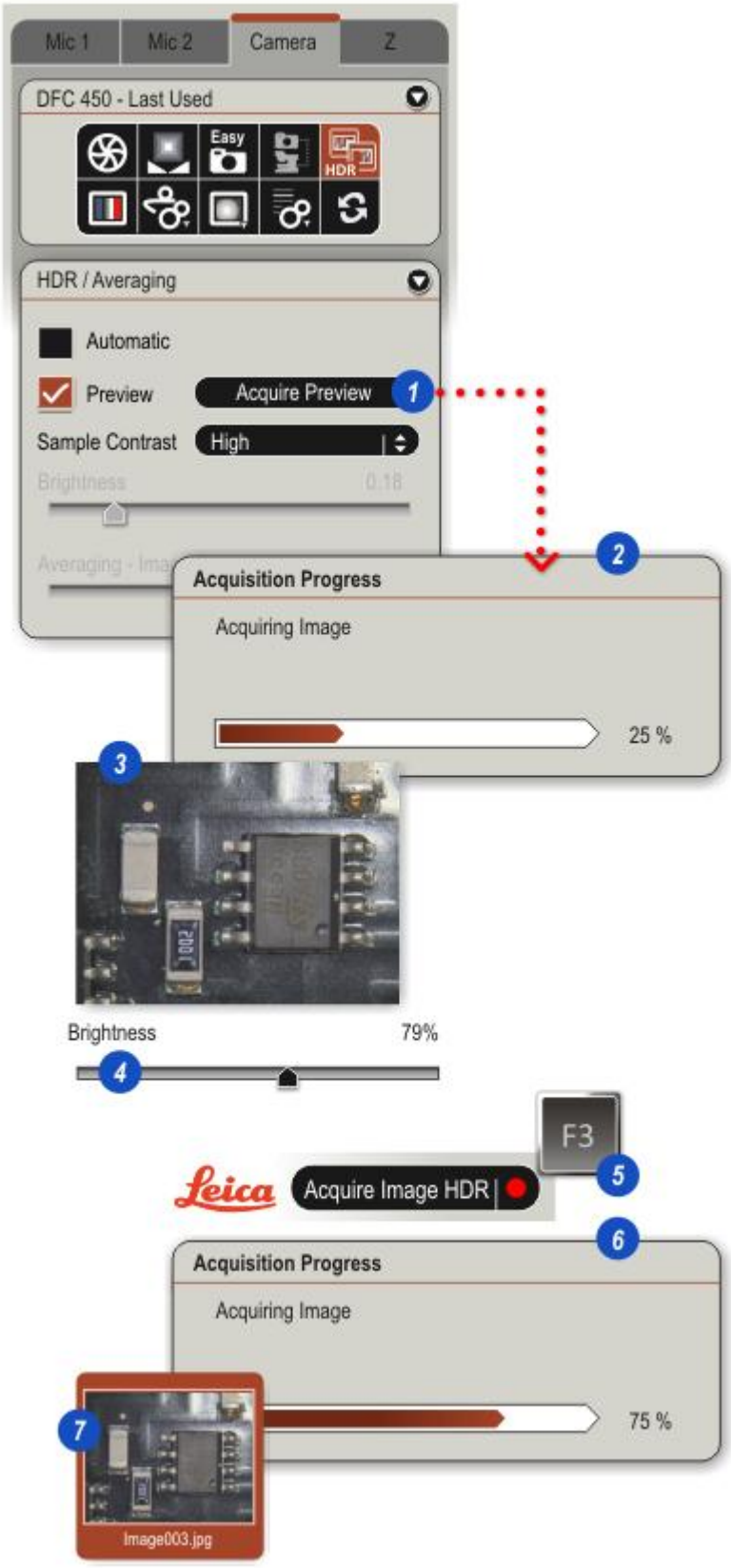
4: If necessary, fine-tune the image brightness.

5: To save the image, click *Acquire Image (HDR*) or press *F3*.

6: The *HDR* image is saved to the capture folder.

7: A thumbnail is displayed in the *Gallery*.

The *HDR* settings derived from *Preview* remain active for any further captures unless changes are made to *Sample Contrast* or *Brightness.*

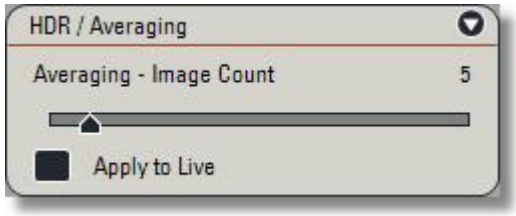


Averaging

Noise can compromise the level of detail in digital images, particularly in regions where the amount of light is low or a higher gain setting has been used. Reducing noise can enhance the final image either making it look better or making it more suitable for analysis. Some techniques to reduce or remove noise soften the image as well.

The *HDR* panel includes a technique that averages multiple exposures to reduce noise. Averaging can reduce noise without reducing detail, because it actually increases the Signal-to-Noise Ratio (SNR) of the image and may retain detail by increasing the bit depth of the image. Images captured using *Averaging* tend to take a little longer to acquire.

Image averaging works on the assumption that the noise in the image is truly random and fluctuations above and below actual image data will gradually even out as more and more images are averaged.



1: If necessary, click to disable *Automatic Exposure.*

2: Click the *AVG* [300] button.

3: Drag the *Average - Image Count* slider to change the number of exposures to be tested.

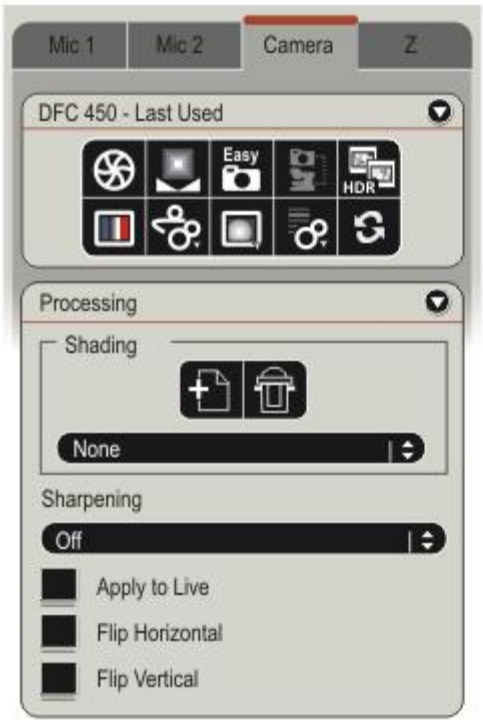
4: If required, enable *Apply to live*, to support averaging for the live image.

5: Click *Acquire Image HDR* or press *F3*.

6: The *Acquisition Progress* bar appears as the range of exposures is checked.

7: The exposure with the best signal-to-noise ratio (i.e. least noise) is saved to the capture folder and a thumbnail appears in the *Gallery*.

The *Processing* panel provides tools to improve quality and orientation primarily for a captured image but may be used for live images as well.



- *Shading*[307]: Corrects light level variations that often occur due to bright spots caused by the microscope light source and the optics.
- *Sharpening*[310]: Enhances the edges of indistinct features on the image making is clearer and crisper.
- *Flip*[311] *Horizontal* and *Flip Vertical:* Flips the image top-to-bottom or side-to-side.

Shading

Shading is the name given to variations in the background light level across an image.

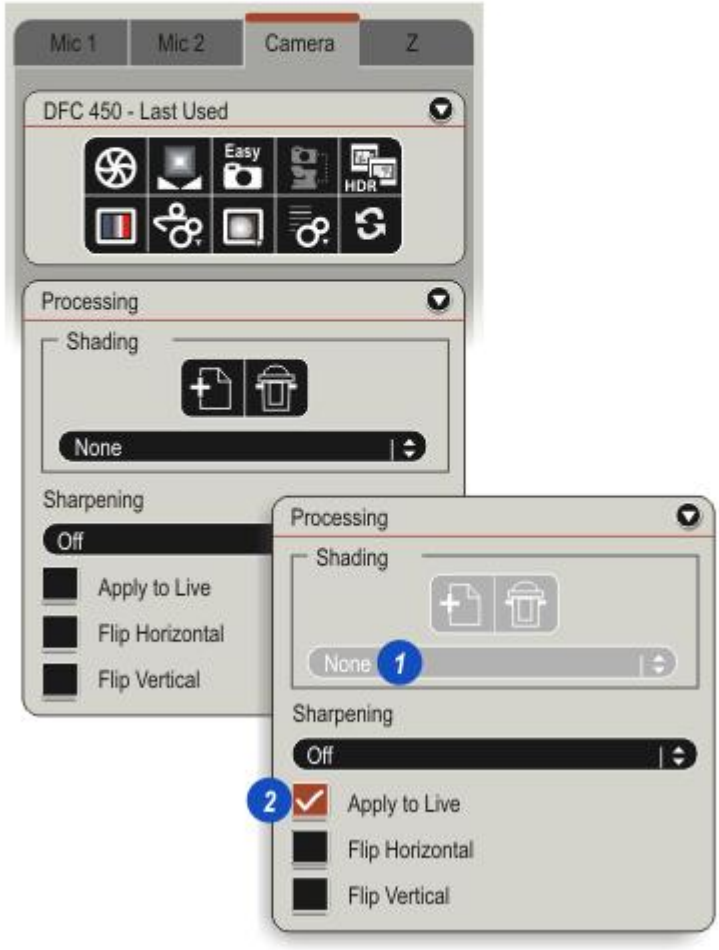
The examples show transmitted light through a microscope. On the left, the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even illumination on live images can be achieved in software by applying a ‘blank area’ value to the entire image area. The effect is shown in the image on the right.

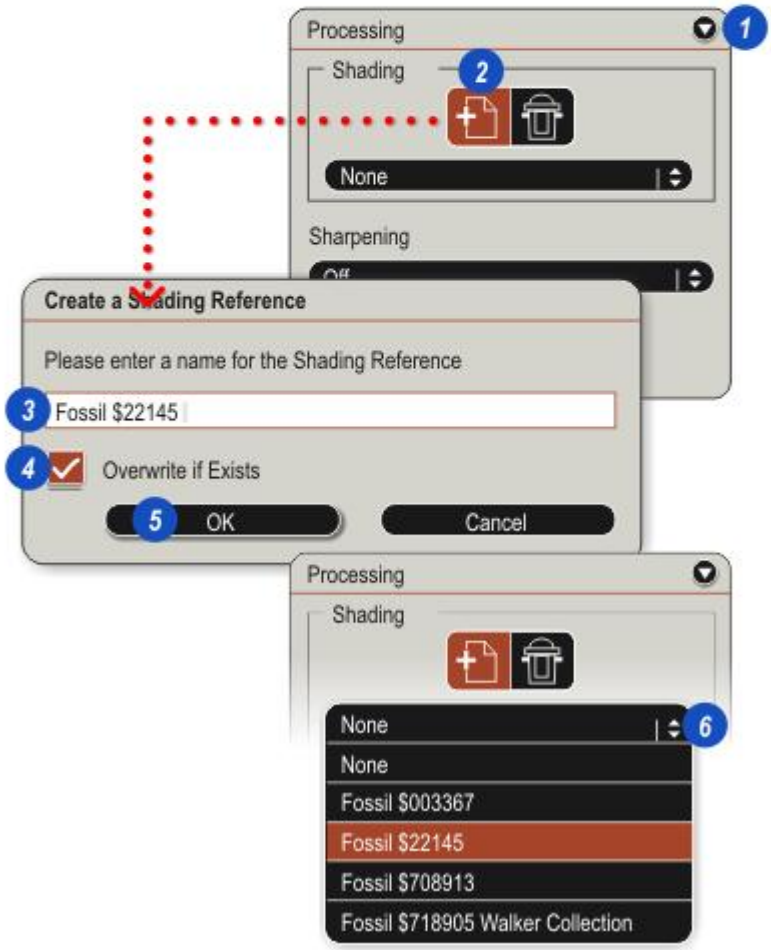
Different light sources and optical elements will produce different shading levels, so each microscope element combination should have its own shading setting.

1: If *Linking* > *Shading* is enabled it takes precedence and *Processing* > *Shading* is disabled on the *Processing* panel

2: The *Apply to Live* check box is available to allow all current settings to be applied to the live image.



- Make sure the image is sharp and well lit.
- Move the stage to a clear, unblemished area of the image – introduce a very small amount of de-focus if the chosen area is not completely clear.
- Over-exposing slightly can also help to 'remove' blemishes.
- Use the *Show Over/Under Exposed* control **(A)** on the toolbox and adjust the *Exposure* **(B)** until just a few red pixels are visible or...
- Replace the specimen slide with a plain slide (and cover slip if used) of the same type.



1: If necessary, expand the *Processing* panel.

2: Click on the *New Shading* button.

3: Type a unique name for the shading reference in the text box.

4: Enable *Overwrite if Exists* to replace an existing shading reference.

5: Click *OK.*

6: The new shading reference is available in the drop-down list.

Select and Load a Shading Reference

There are two methods for selecting and loading a *Shading Reference.*

Method 1

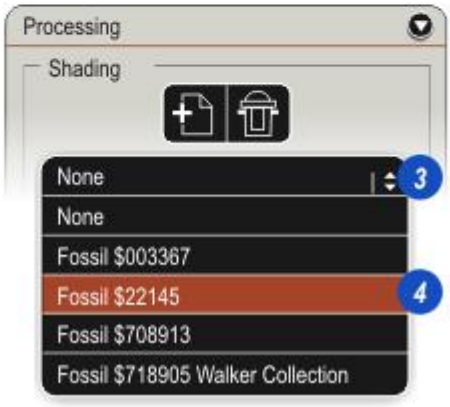
1: Click on the *Toolbox Shading* button.

2: Click to select and load a reference from the drop-down list.

Method 2

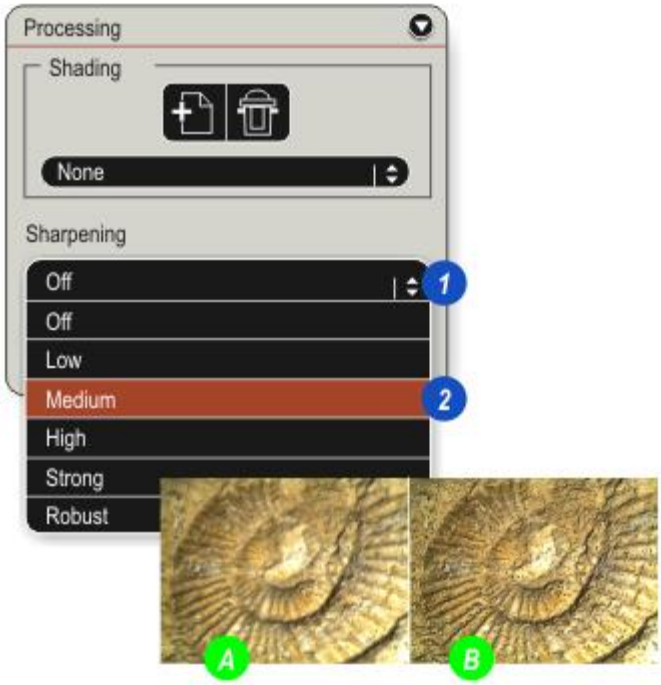
3: On the *Processing* panel, click to display the *Shading* drop-down list of references.

4: Click to select and load a reference.



Sharpening enhances the boundaries between tonal values. Use it to improve image clarity.

The level of enhancement is selectable between *Low* and *Robust* (very high). However, too much sharpening can make the image appear grainy and speckled. It is a fast process so the best approach is to start with the *Low* setting and work gradually towards *Robust* in small steps.



1: Click to display the *Sharpening* drop-down menu.

2: Select the level of sharpening required.

3: Select another level if the result is not suitable.

The illustrations show the original image **(A)** and *Medium* sharpening **(B)**.

Flip

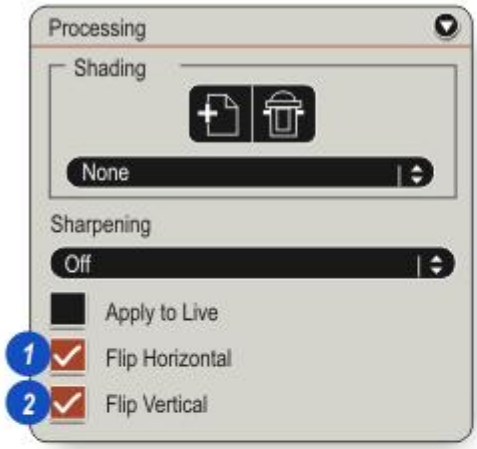
Flipping is often used to emulate the view through the eyepieces.

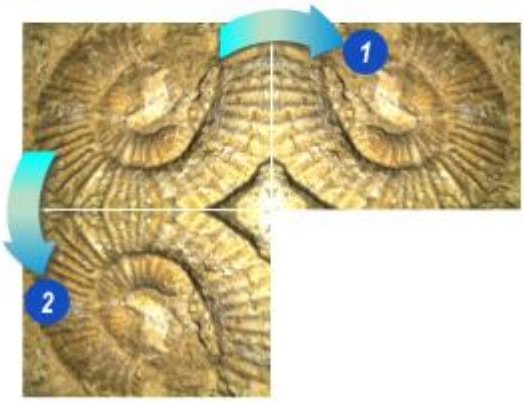
To flip the image from side-to-side:

1: Click on the *Flip Horizontal* check box. Click again to return it to its original position.

To flip the image from top-to-bottom:

2: Click on the *Flip Vertical* check box. Click again to return it to its original position.





Create a *Region of Interest* (ROI) by drawing a rectangle on an image.

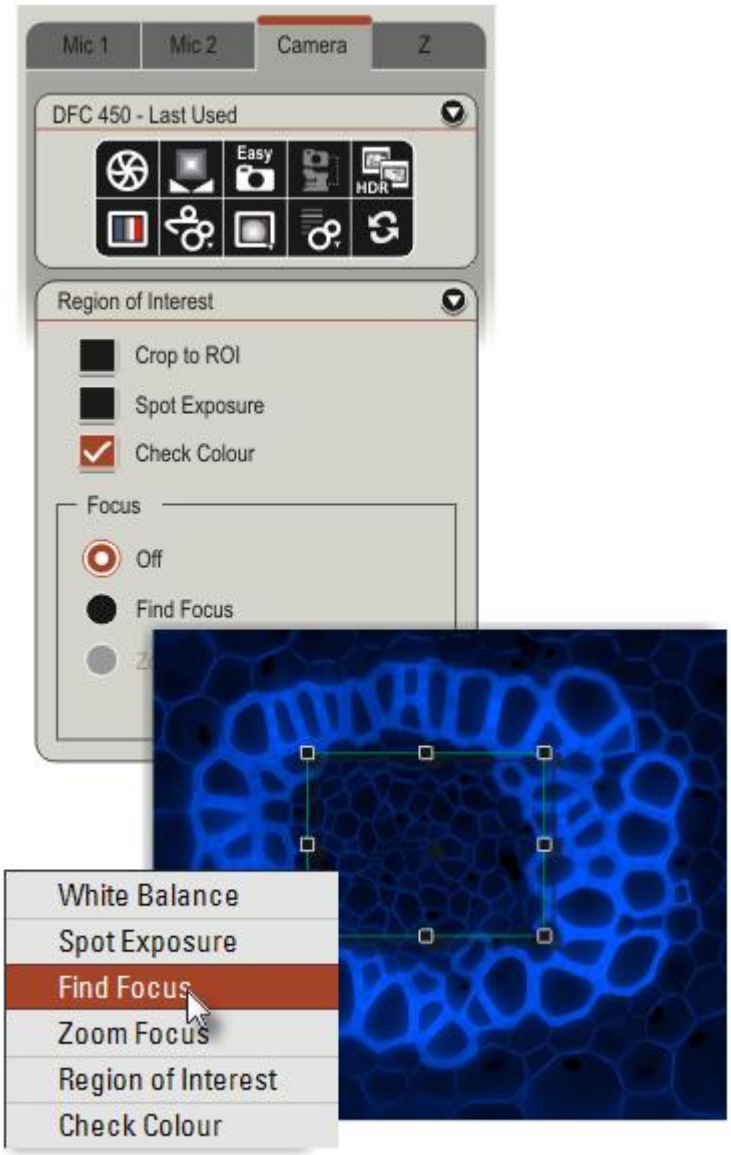
You can apply the following functions to a *Region of Interest:*

- *White Balance*: Displays all the neutral tones as shades of black and white but only within the *Region of Interest.*
- *Spot Exposure*[315]*:* Automatic exposure is applied to the entire image but using only the values contained within the *ROI*.
- *Find Focus*[316]: Defines a moveable *Region of Interest* in which to focus.
- *Region of Interest*[314]*:* When the image is acquired, only the part within the *ROI* is captured; the rest is discarded.
- *Check Colour*[317]: Used to adjust the overall image colour balance by using a small region as the reference for both *Hue* and *Saturation.*

You can define a different region for each of these functions. With the exception of *White Balance,* you can recall them by enabling the check boxes on the *Region of Interest* control panel. Each *ROI* has a different identifying outline colour.

Create a new region for any of the options at any time to overwrite the existing one.

White Balance has an immediate effect as soon as it is selected. You must create a new region each time you use it.



Creating a Region of Interest

To create a *Region of Interest*:

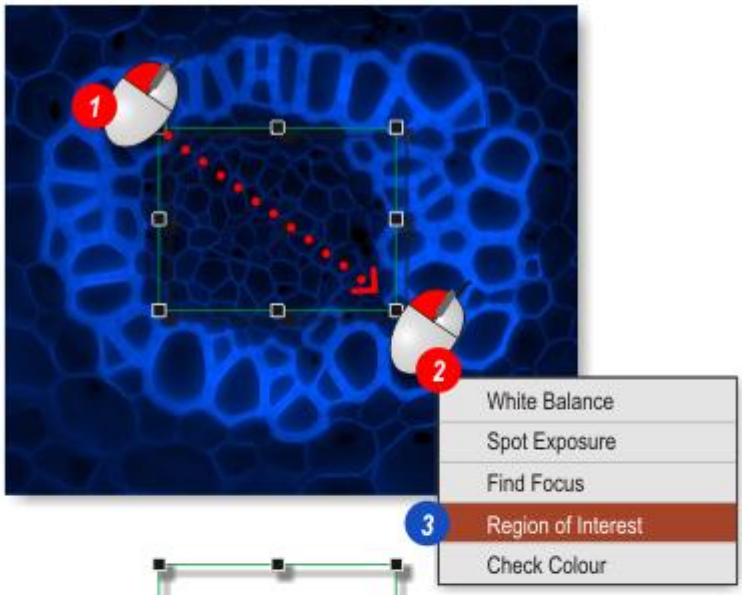
1: Click on a corner of the region.

2: Drag to the opposite corner. (Later, you can move the region, or resize it using the scaling handles.)

3: Release the mouse button and the select a region type from the contextual menu.

If none of the options is chosen the region will default to the *Region of Interest* which represents the capture area.

You can create a separate *ROI* for each type. The location and dimensions are stored.



Move the cursor near a region to display the scaling handles.

4: To stretch the region either vertically or horizontally, click on a handle. The cursor changes to a double-ended arrow. Holding down the mouse button drag the handle to re-size the region.

5: Scale the region proportionally by clicking on a corner handle - the cursor changes to a double-ended arrow - and holding down the button dragging it diagonally.

6: Move the entire region by positioning the mouse over an edge. The cursor changes to a hand. Click and holding down the cursor button drag the region to a new position.

Crop to Region of Interest

This feature captures only that part of the image within the *Region of Interest.* The rest of the image is discarded.

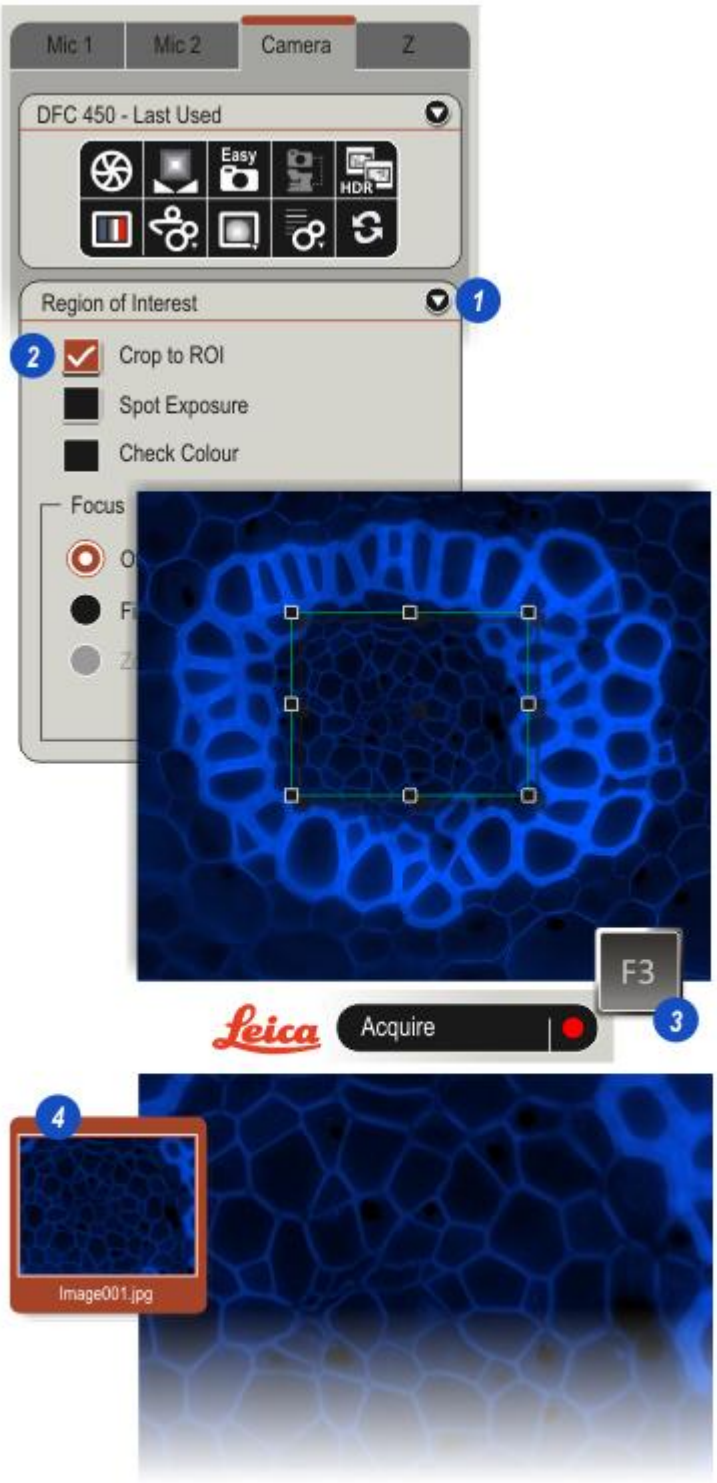
1: Expand the *Region of Interest* control panel.

2: If necessary, enable *Crop to ROI*. The *Region of Interest* appears green on the image.

3: Click *Acquire Image* or press *F3*.

4: Only the area of the image within the *ROI* is captured and scaled to suit the *Viewer*.

A thumbnail is displayed in the *Gallery*. Depending on the *After Capture* setting in *Preferences* 66 the program will move to another *Workflow* or remain where it is.



Spot Exposure

Spot Exposure automatically adjusts the exposure of the entire image using only the area within the *Region of Interest.*

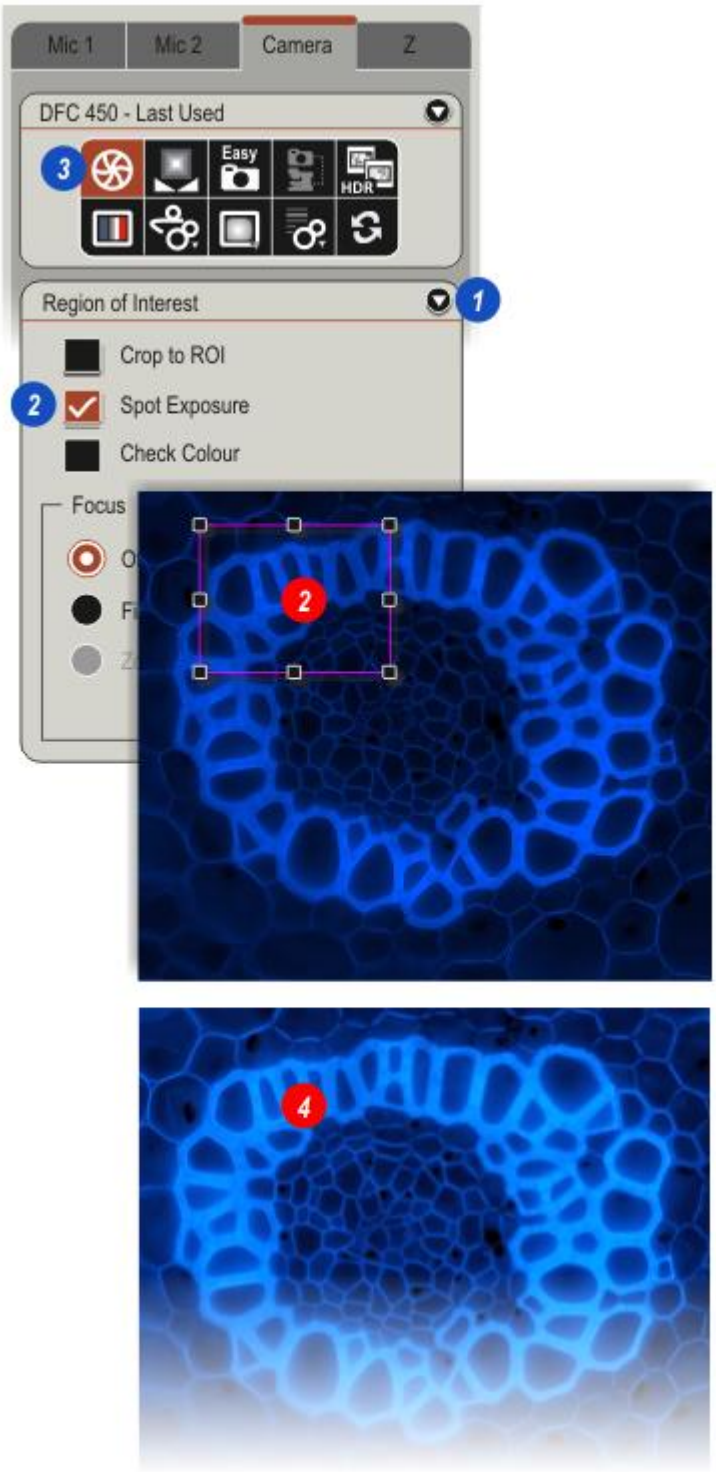
You can move the *ROI* around the image to compare different exposure results.

1: Expand the *Region of Interest* control panel.

2: Click to enable *Spot Exposure*. A *Region of Interest* is drawn with a magenta outline. Re-size or move the region as required.

3: The Toolbox *Auto Exposure* button is automatically enabled.

4: The image exposure is adjusted using the values within the *ROI.*



Find Focus

This feature displays the focus precision for the area of the live image contained within the *Region of Interest*.

You can move the region around the image to compare different areas with a focus bar indicating the best level achieved.

1: Expand the *Region of Interest* control panel.

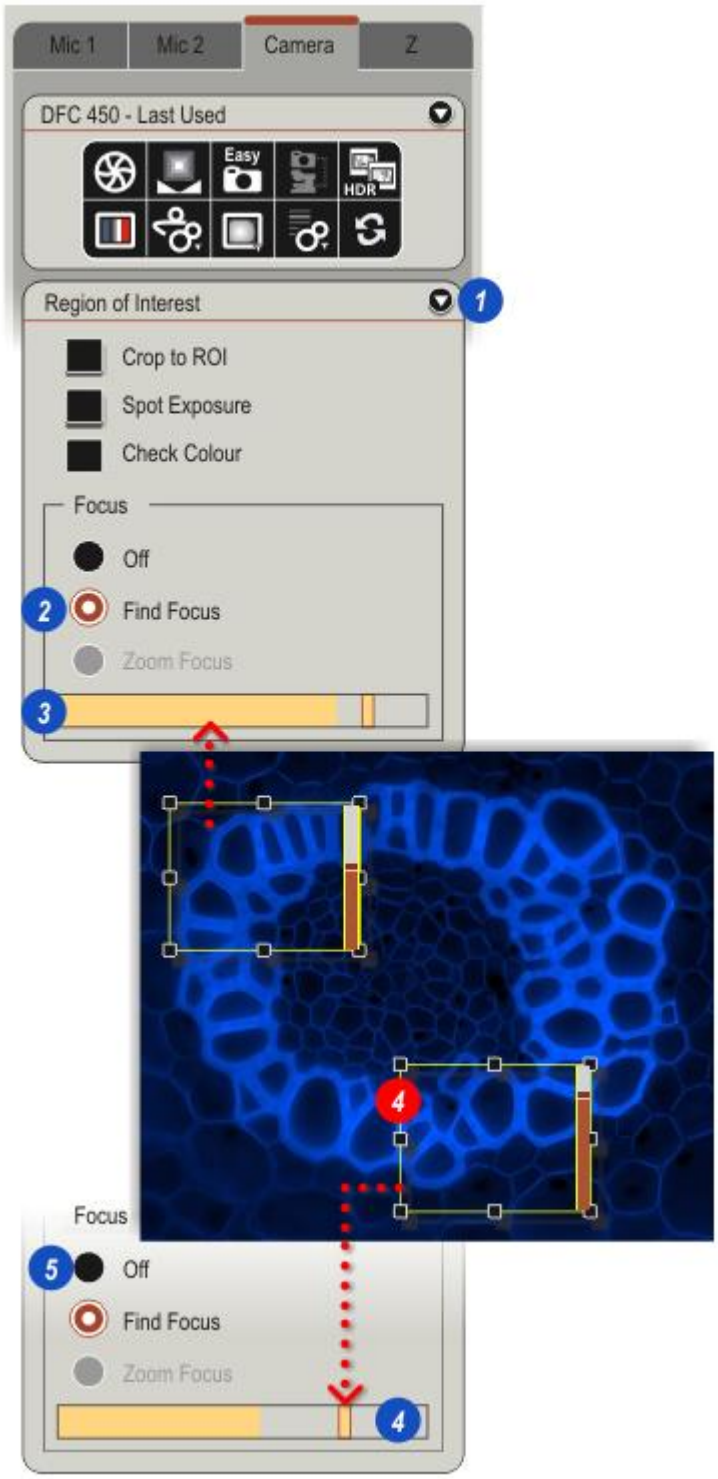
2: Click to enable *Find Focus*. A *Region of Interest* is drawn with a yellow outline. Re-size or move the region as required.

3: The level bar indicates the focus precision. The further the bar is to the right, the better the focus.

Note that a level bar also appears on the right of the ROI on the Live Image.

4: To check the focus at another point on the image, drag the yellow ROI outline (not a handle) to a new position.

5: Turn off *Find Focus* by clicking the *Off* button. Click the *Find Focus* button **(2)** to reveal the *Region of Interest* at its last size and position.



Check Colour

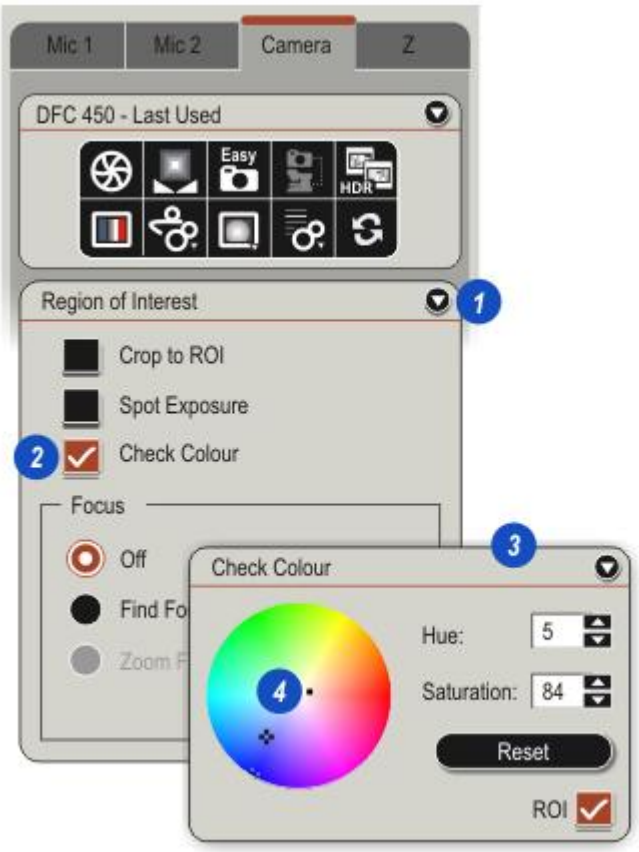
The *Check Colour* function on the *Region of Interest* panel is a convenient control for creating a region that can be used with the *Check Colour* feature.

1: Expand the *Region of Interest* control panel.

2: Click to enable *Check Colour* and drag to create a *Region of Interest*. A region is drawn with a blue outline. Re-size or move the region as necessary.

3: With *Check Colour* enabled and the *Check Colour* 346 feature launched, the region is represented by...

4: ...a small dot on the colour wheel which represents the *Hue* and *Saturation* levels inside it.

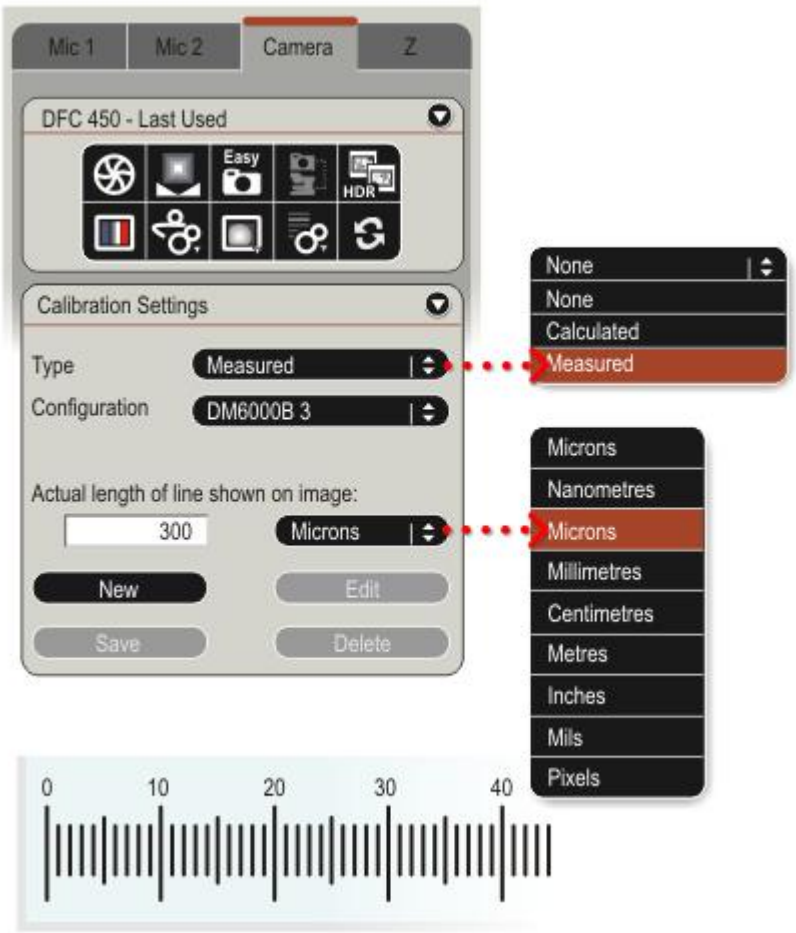


Calibration Settings

Calibration ensures that measurements indicated by the software are given in real world units taking into account the selected optical magnification of the microscope and the size of pixels of the digital camera.

The following calibration options are available depending upon the level of precision required:

- *None*[319]*:* Calibration not required - distances are measured in pixels..
- *Calculated Default Configuration*[320]*:* Based upon the software's 'knowledge' of the microscope optical components and the pixel size of the digital camera being used. This is the quickest way of establishing a reasonable but approximate calibration as it does not make any checks against a calibration slide.
- *Calculated User Configuration*[321]*:* Using a calibration slide on the stage the calibration of a single, greatest magnification objective is measured and from this all the other objective/mag changer combinations are derived. This is more accurate than simply using the *Configuration Default*.
- *Measured Calibration*[324]*:* Again, a calibration slide placed on the stage is used, but all objectives in combination with all Mag changer settings are measured. A 'Wizard' helps to speed the process and the result can be a high level of calibration precision.
- *Automatic Calibration*[329]*:* Speeds the *Measured Calibration* process by using the software to detect and measure the calibration slide. You do not have to make any measurements.



No Calibration

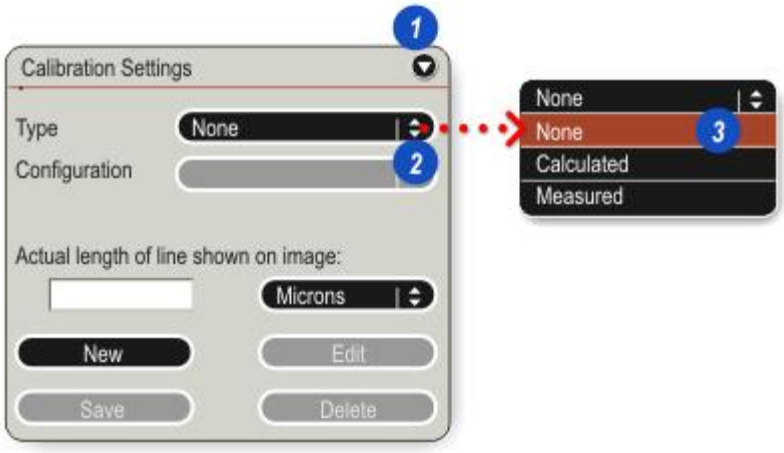
If calibration is not required then one display pixel = one camera pixel.

All measurements are reported in pixels:

1: Expand the *Calibration Settings* panel.

2: Display the *Type* drop-down menu.

3: Select *None*.



Calculated Calibration with the *Default Configuration* uses the microscope optical data and camera pixel size to create a calibration value. This is used to return measurements in the type selected – *Microns, Millimetres* etc. It is a very fast and moderately accurate method of setting the calibration.

1: Display the *Type* drop-down menu.

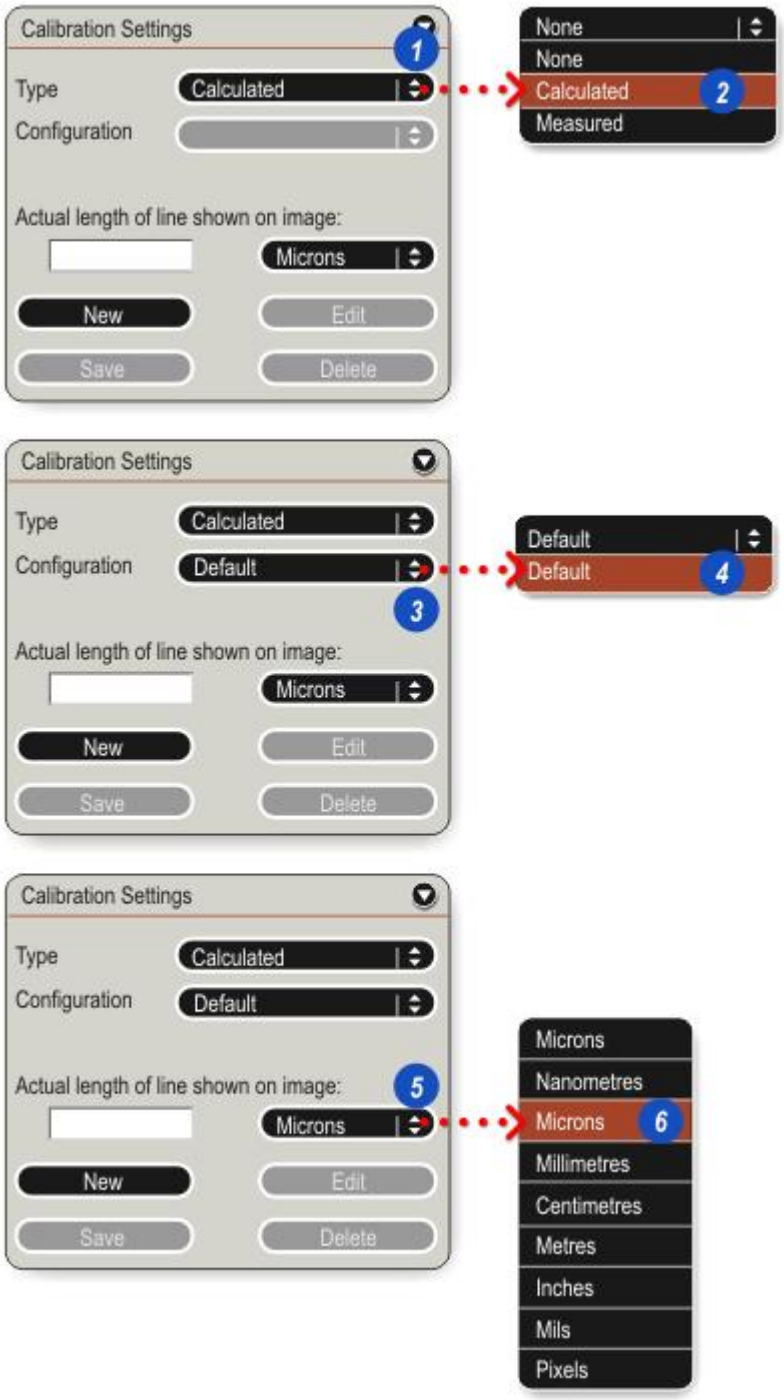
2: Select *Calculated*.

3: Display the *Configuration* drop-down menu.

4: Select *Default*.

5: Display the units drop-down menu.

6: Select the measurement units required.

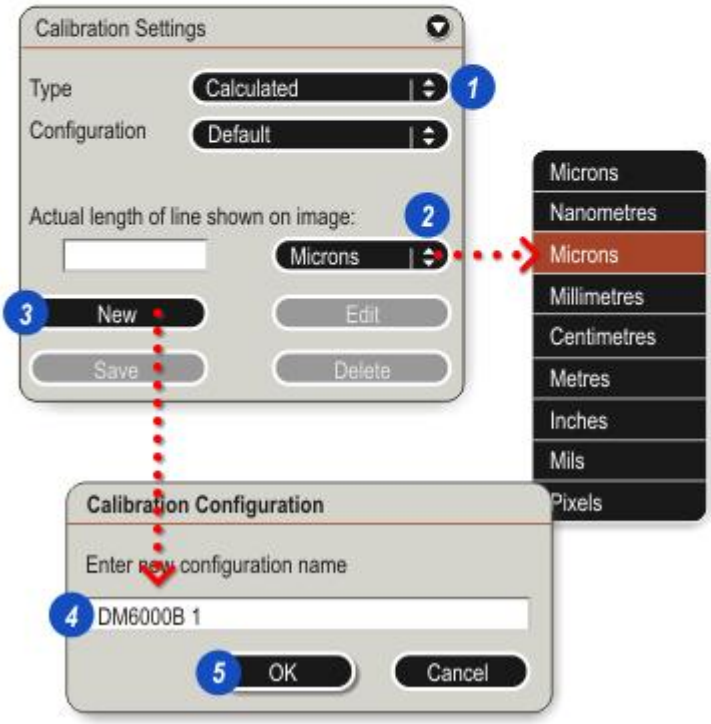


Calculated Calibration User Configuration

User Calibration calculates calibration values by making an on-screen measurement across a *Calibration Slide* placed on the microscope stage.

Only one calibration is taken with a single objective/mag combination. The resulting value is then applied automatically to all other combinations using the microscope optical data.

- Select the objective with the greatest magnification and set the Mag at 1.
- For Stereo- and Macroscopes ensure that that the zoom position is selected at one of the click stops listed in the zoom magnification values.
- Make sure the *Calibration Slide* is properly focused and corresponds to the measurement units selected in Step **(2)**.



1: Display the *Type* drop-down menu and select *Calculated*.

2: Select the *Calibration Slide* measurement units.

3: Click *New*.

4: On the *Calibration Configuration* dialog, type an appropriate name for the calibration.

5: Click *OK*.

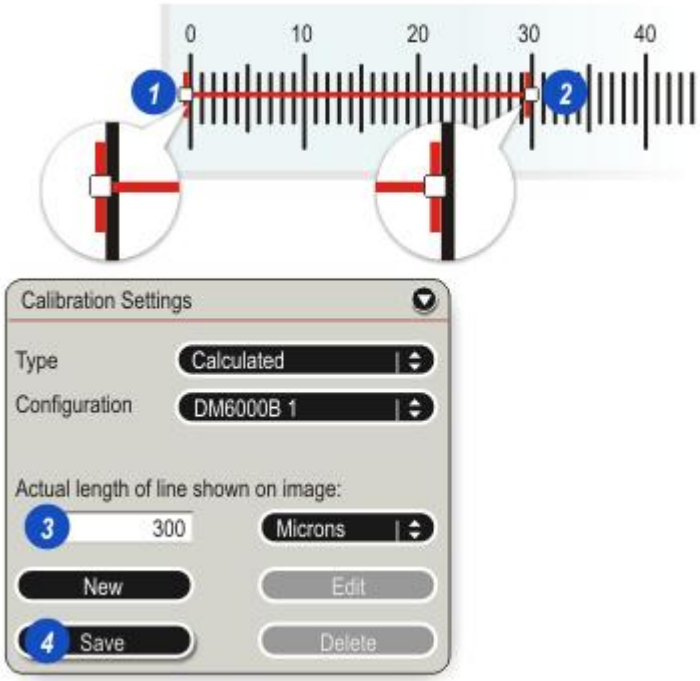
1 & 2: Click on the red measurement line that appears in the *Viewer* and drag it to sit over the calibration slide.

Click on the handles at either end of the measurement line and drag them so that they precisely align with selected marks on the slide.

Greater accuracy is achieved if both of the line end strokes are aligned either to the left or right of the marks. In the illustration the measurement line represents a distance of 300 microns.

3: Type the length of the measurement line into the *Actual length of line* text box – in this case 300 microns.

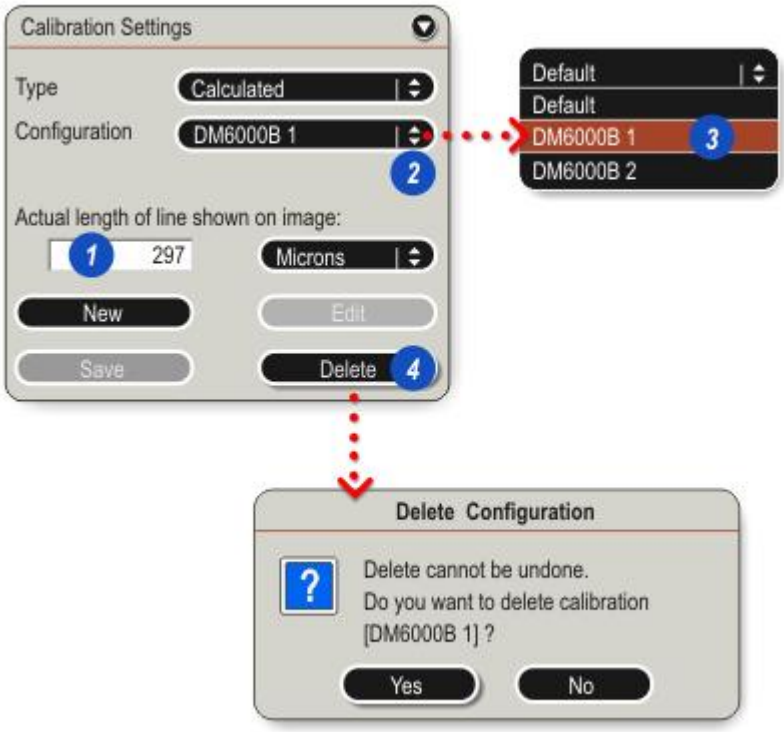
4: Click *Save*.



1: When you click *Save,* the value in the *Actual length of line* text box may change to reflect settings made on the *Acquire* > *Scale Bar* panel. This does not affect the calibration.

2&3: You can retrieve and apply calibration settings using the *Configuration* drop-down list.

4: Delete a configuration by selecting it (as above) and then clicking *Delete*. Click *Yes* to confirm.



Measured Calibration provides the most precise results because every objective/Mag combination is individually calibrated using a calibration slide. A *Wizard* automatically steps through the combinations.

Place a calibration slide on the stage. It is important that the slide is clean and correctly focussed.

1: Display the *Type* drop-down menu.

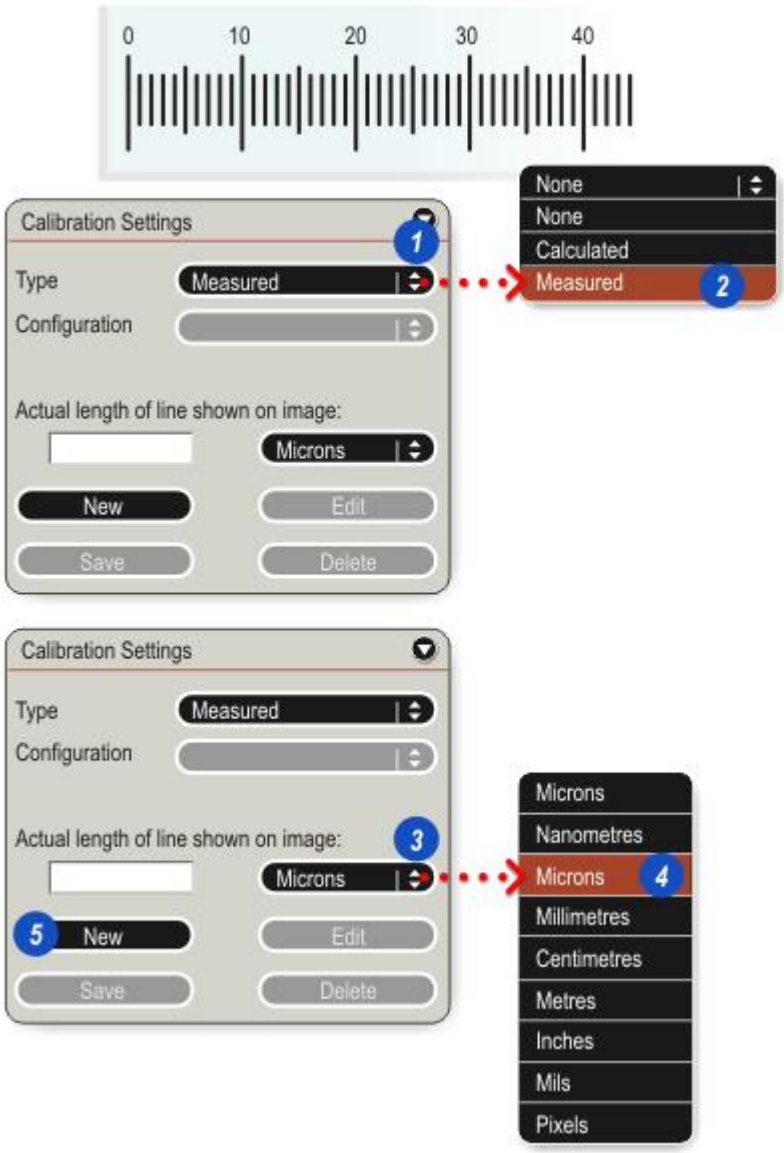
2: Select *Measured*.

3&4: Select the *measurement units.* This must correspond with your chosen calibration slide.

5: Click *New*.

The following options are available on the *Calibration Wizard* dialog:

- *Manual*[325]: The user is responsible for placing a measurement line across a known distance on a calibration slide..
- *Automatic*[329]: The software detects and measures the *Calibration Slide* and automatically applies the calibration values.



On the *Measured Calibration Wizard* dialog:

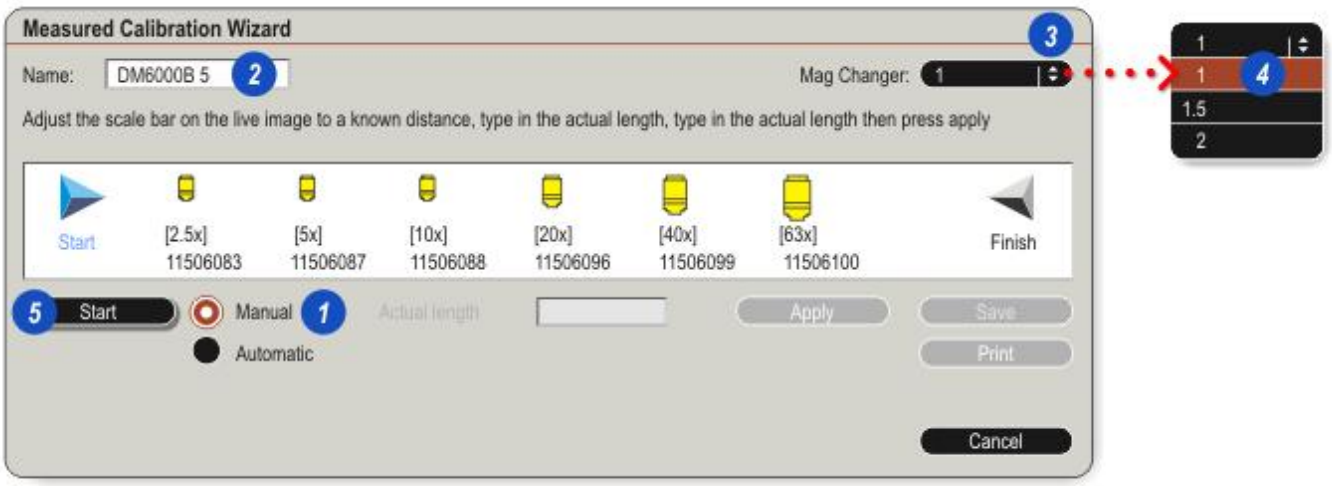
1: Select *Manual*.

2: Type an appropriate name for the calibration in the *Name* text box. The calibration values will be stored in a separate file using this name.

3: Display the *Mag Changer* drop-down.

4: Select *1*. For the first pass, every objective will be calibrated using the *1 Mag Changer*. Subsequent passes use the next incremental *Mag Changer* value with all of the objectives.

5: Click *Start*.



On the *Measured Calibration Wizard* dialog:

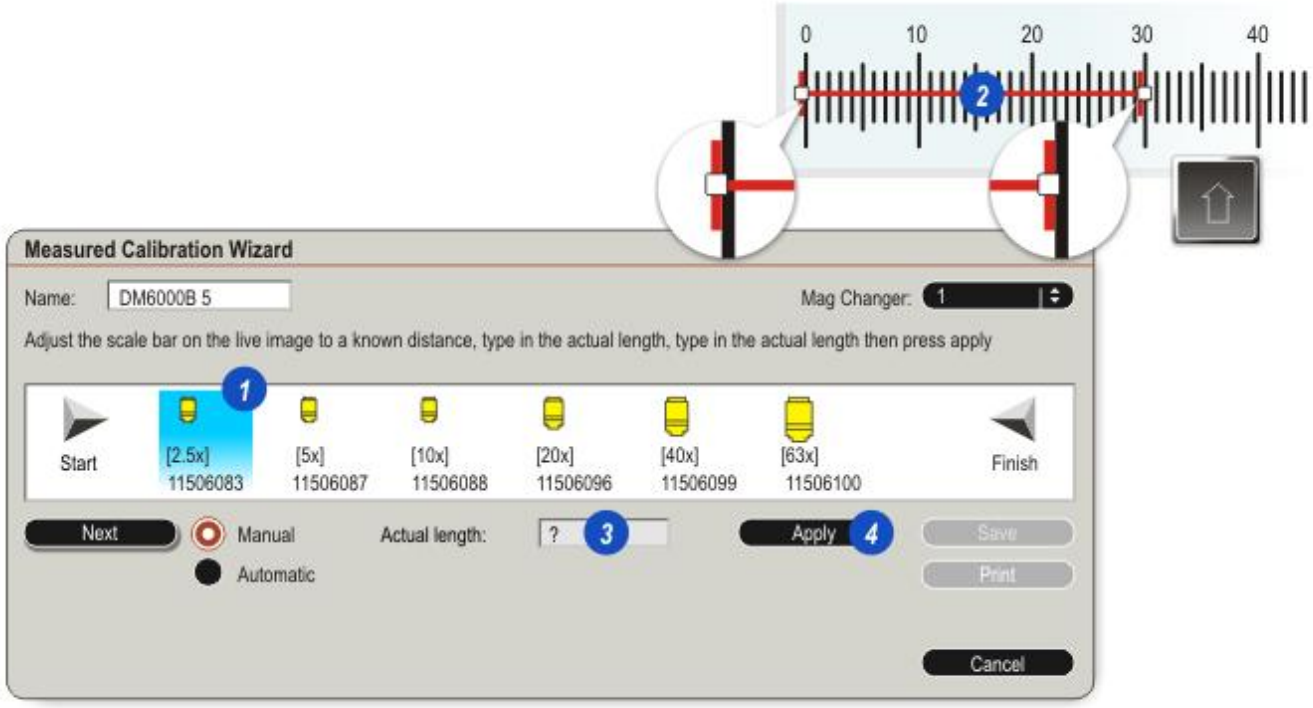
1: The first objective is automatically selected.

2: A measurement line is displayed on the *Viewer*: Drag it so that it is positioned over the calibration slide. Drag the small handles at each end of the line so that it extends to the distance required – in this case 300 microns. Position both the end stokes to either the left or right of the division marks – this is easier than trying to align exactly with the centre of the mark.

Hold down the *Shift* key to display the *Magnifier*. Drag it over the calibration slide to help with the alignment.

3: Type in the distance measured in the *Actual length* text box (e.g. 300).

4: Click *Apply*.



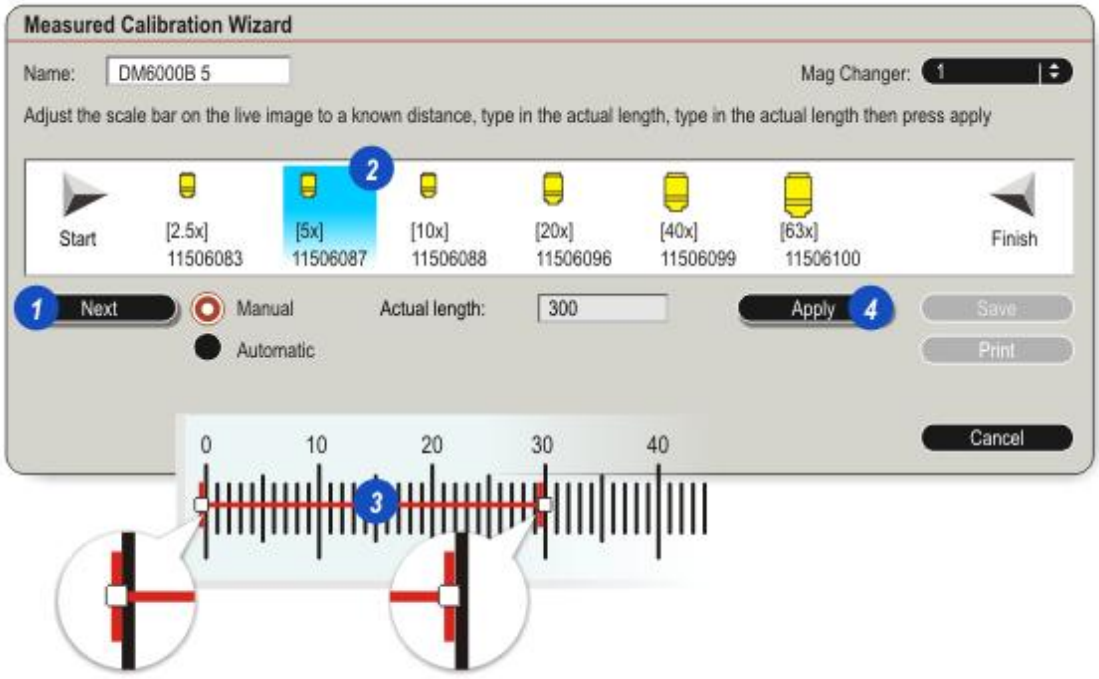
1: Click *Next*.

2: The next objective is selected.

3: Repeat the measurement line procedure described here[326].

4: Click *Apply*.

Repeat this procedure for each of the objectives.



With the last objective in combination with the *Mag 1* complete, click *Next*.

1: Display the *Mag Changer* drop-down menu.

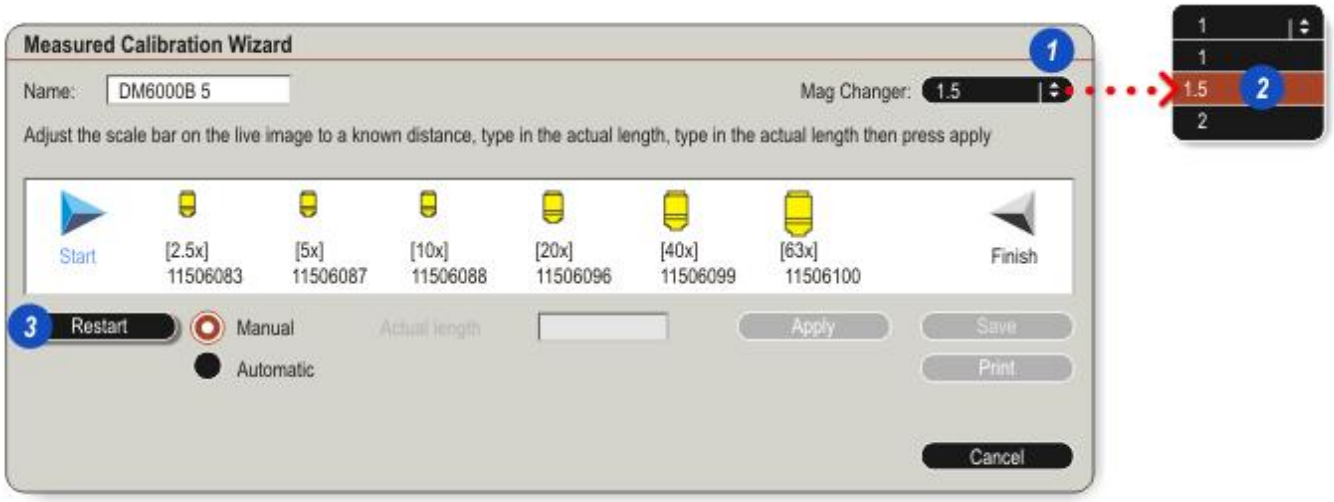
2: Select the next *Mag Changer* value – in the example *1.5.*

3: Click *Restart* and the first objective will be selected again.

Repeat the calibration sequence described in the previous topics.

Move through all the *Mag Changer* values in the same way until you have calibrated all the combinations.

Next: Save 335 the calibrations.



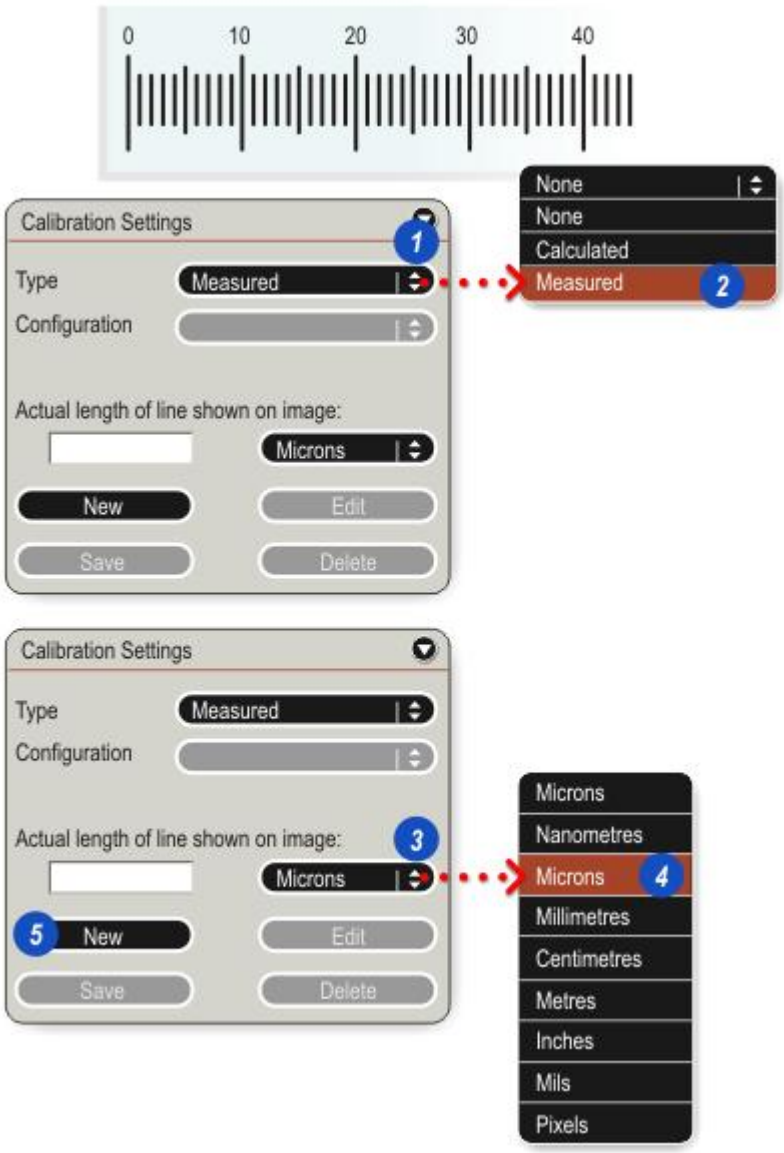
Automatic Measured Calibration

Automatic Measured Calibration offers a fast, precise method using a calibration slide and allowing the software to detect it and apply the values. You are not involved in any measurements, but an active and licensed option measurement module must be installed.

The software can accurately and automatically detect the slide image and calculate the calibration from a known interval between the divisions, providing the image is sharp and the division lines clearly defined.

Place a calibration slide on the stage. For the calibration process to be successful, it is important that the slide is sharply focussed with distinct divisions visible, clean and clear of debris.

- Select high resolution for the camera capture format[291].
- Choose an objectives greater than x2.5 on a compound microscope if using a 10µm/ division calibration slide.



1: Display the *Type* menu.

2: Select *Measured*.

3&4: Select the *measurement units*. This must correspond with the calibration slide that is going to be used.

5: Click *New*.

On the *Measured Calibration Wizard:*

1: Select *Automatic.*

2: If necessary, change the measurement units for the calibration slide.

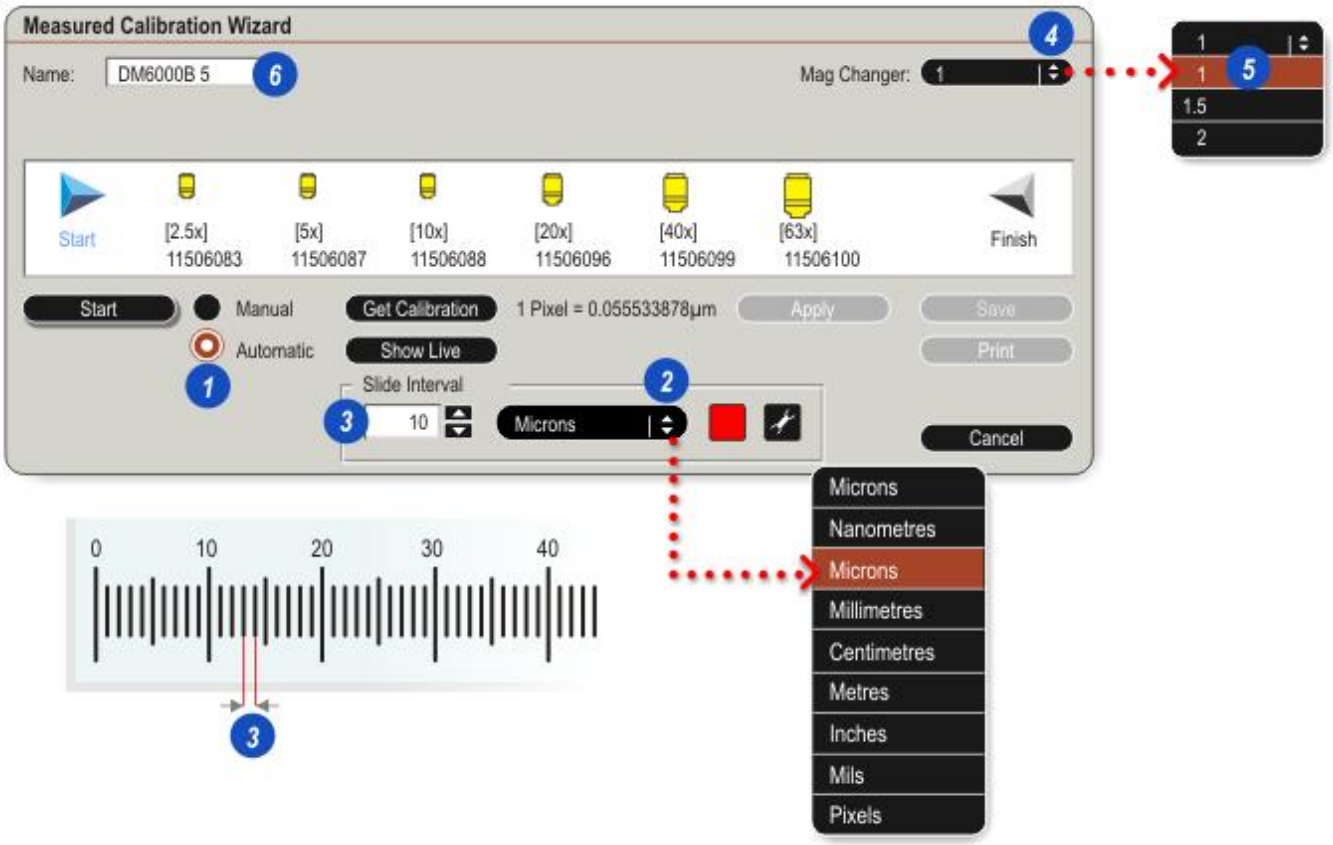
- The measurement units for the slide and calibration do not have to match.
- The calibration slide does **not** have to be perfectly horizontal or vertical, but the closer it is then the faster the detection.

3: Type the interval value of the calibration slide (i.e. the distance between two adjacent divisions in the selected measurement units). Typically, this will be 10µm for a compound slide and 100µm for a stereo calibration slide.

4: Display the *Mag Changer* menu.

5: Select *1*.

6: Type a unique name for the calibration configuration.



1: Click *Start*

2: **T**he first objective is selected.

3: Click *Get Calibration*.

The software will now try to find a calibration slide and verify that it is precise and suitable as a calibration source:

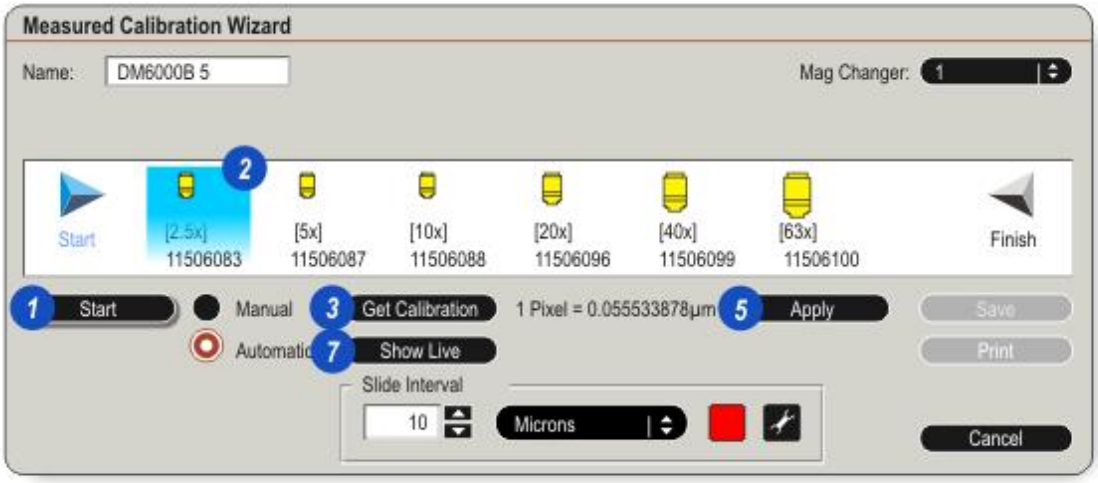
4: If a calibration scale is found and verified, a coloured overlay is applied to the division strokes on the scale, a new calibration value is automatically calculated.

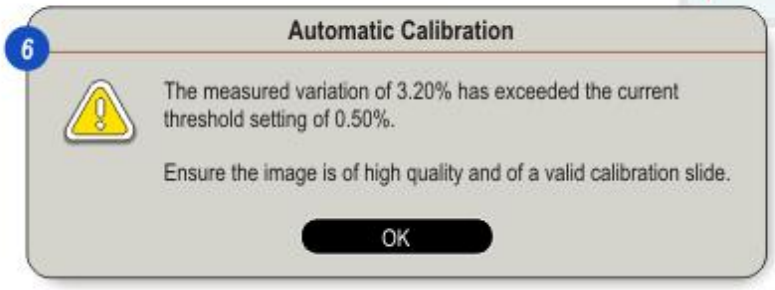
5: The *Apply* button becomes active. Check that the calibration intervals have been properly detected and click *Apply* to apply the new calibration value for the mag/objective combination.

6: If a scale is not detected or does not conform to the verification parameters, an error message appears. The message changes to reflect the error.

7: Sometimes detection can be improved by re-focussing. Click *Show Live* to view the live image and adjust the focus.

- You can change calibration slide scale verification parameters[333].
- You can change the calibration scale detected colour[334].





With the calibration complete for the selected mag/objective combination:

1: Click *Next*.

2: The next objective is selected.

3: Click *Get Calibration* and the detection process will begin again.

4: Click *Apply* if the detection is successful.

Continue this way until all of the objectives have been calibrated. Then:

5: Display the *Mag Changer* menu and select the next magnification.

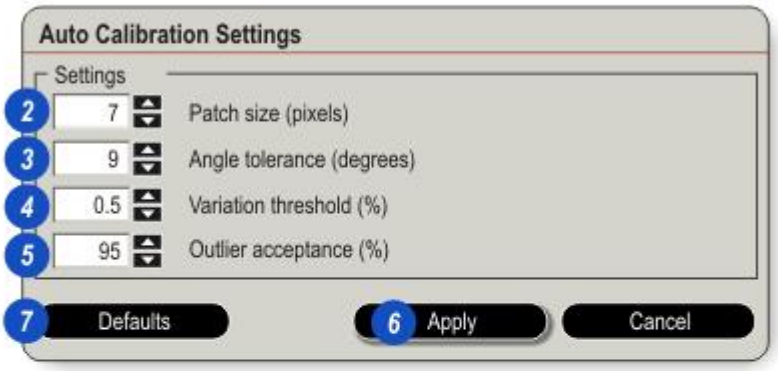
6: Click *Restart* and the process will begin again for the new mag value.

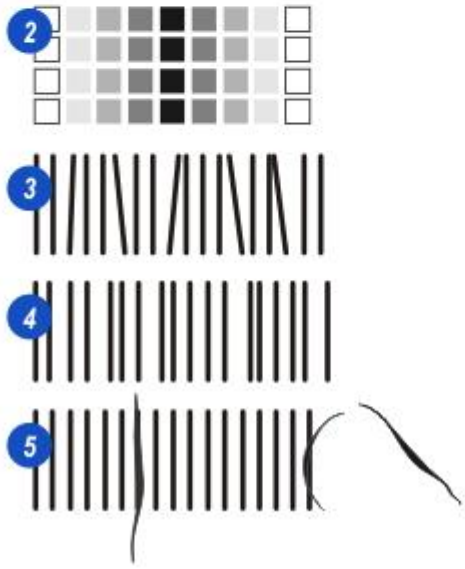
7: When all of the mag/objective combinations have been calibrated, save[335] the configuration and values.

- You can edit[336] a calibration configuration to adjust individual combinations.
- You can print[337] the Mag/objective combinations together with the calibration settings.

The verification parameters check that a calibration slide image is sufficiently accurate to be used as a calibration source. For example, random fibres on an calibration slide image could be interpreted as part of the scale and have to be 'filtered' out.

You can change the settings, but be aware that significant changes can result in compromised calibration accuracy. If in doubt, revert to the factory optimised defaults.





1: On the *Measured Calibration Wizard* click on the 'spanner' icon to reveal the *Auto Calibration Settings* dialog.

2: The *Patch size* refers to the spread of pixels leading to a discernible edge. In the illustration there are several pixels ranging from white to dark grey before the black central 'edge' appears. Increasing the patch size could 'create' spurious edges. Keep the patch size as small as possible.

3: *Angle tolerance* determines how much the angle between two adjacent scale strokes can vary from the parallel. The software is looking for a series of parallel strokes at a consistent 'pitch' or interval.

4: The interval of the scale strokes must be close to the value entered. The *Variation threshold* determines how much it can be allowed to vary.

5: *Outliers* are scratches and debris that may be present on the image and could be interpreted as part of the scale. The *Outlier acceptance* sets the % level at which the interval *mean* (a central 'average' for all of the detected intervals) can vary. Strokes falling below the *Outlier acceptance* are removed.

6: If you make any changes to the settings, click *Apply* to save them.

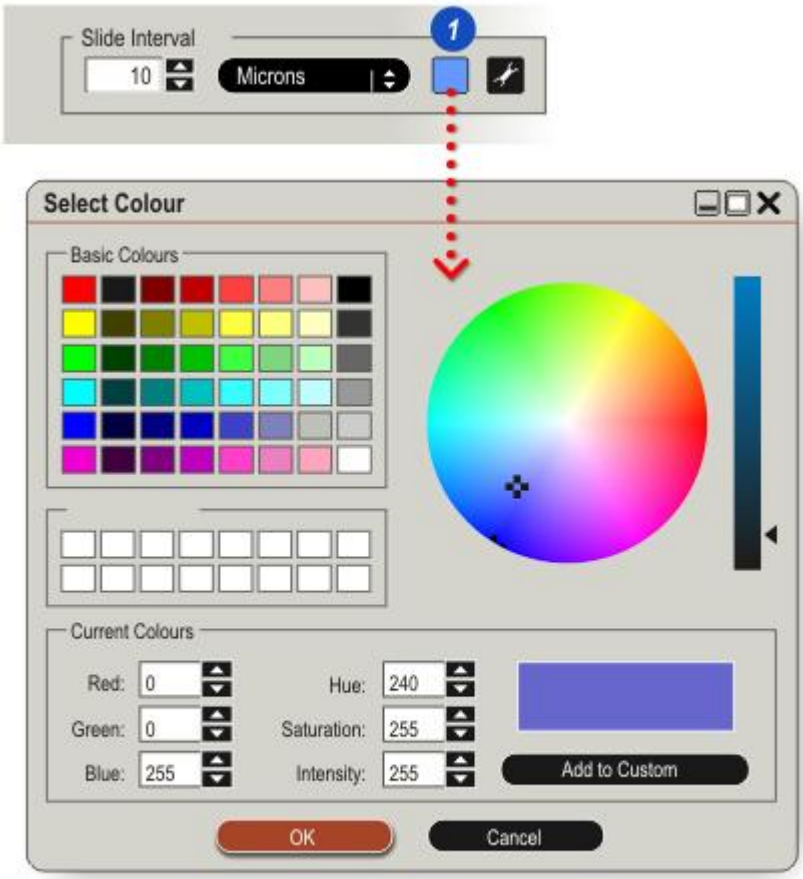
7: Click *Defaults* to restore the factory settings.

To change the colour of a detected calibration slide image:

1: Click *Colour* on the *Slide Interval* panel.

2: From the *Windows Colour* dialog choose a colour by clicking a swatch, dragging the crosshairs on the wheel or typing RGB values.

3: Click *OK*.

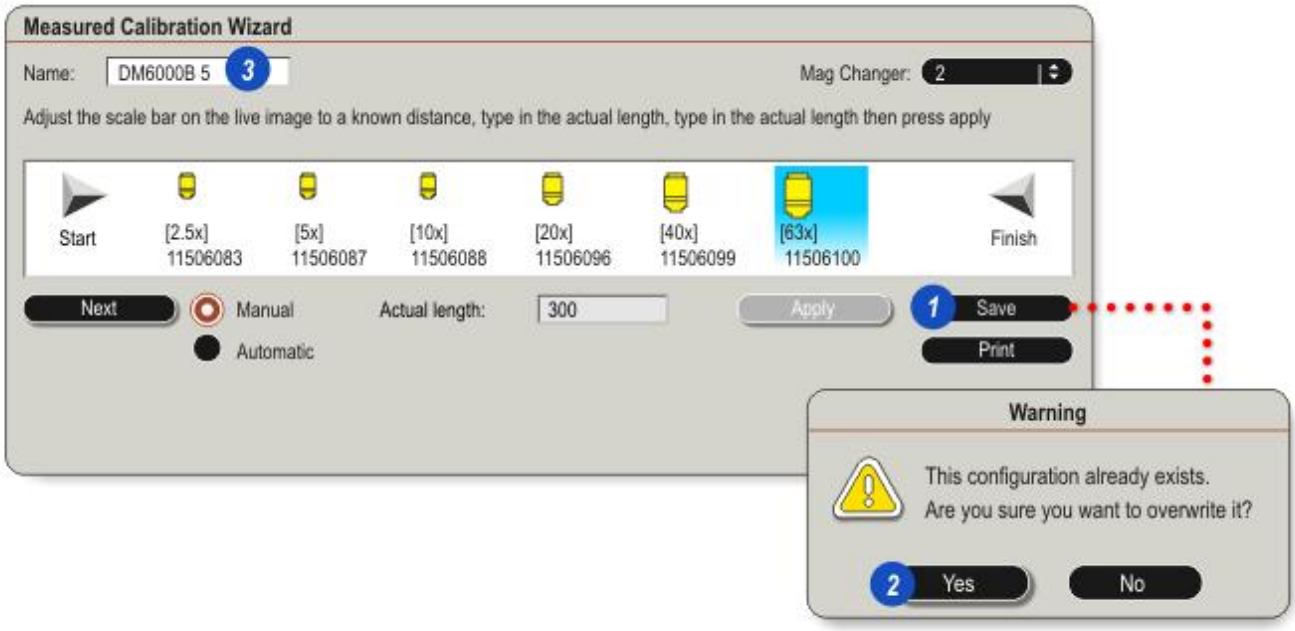


Save Calibrations

1: When all objective/Mag combination calibrations are complete, click *Save* to store the calibrations.

2: If a configuration file already exists, click Yes to overwrite the existing calibration file.

3: Alternatively, type a new, unique name and click *Save* again.

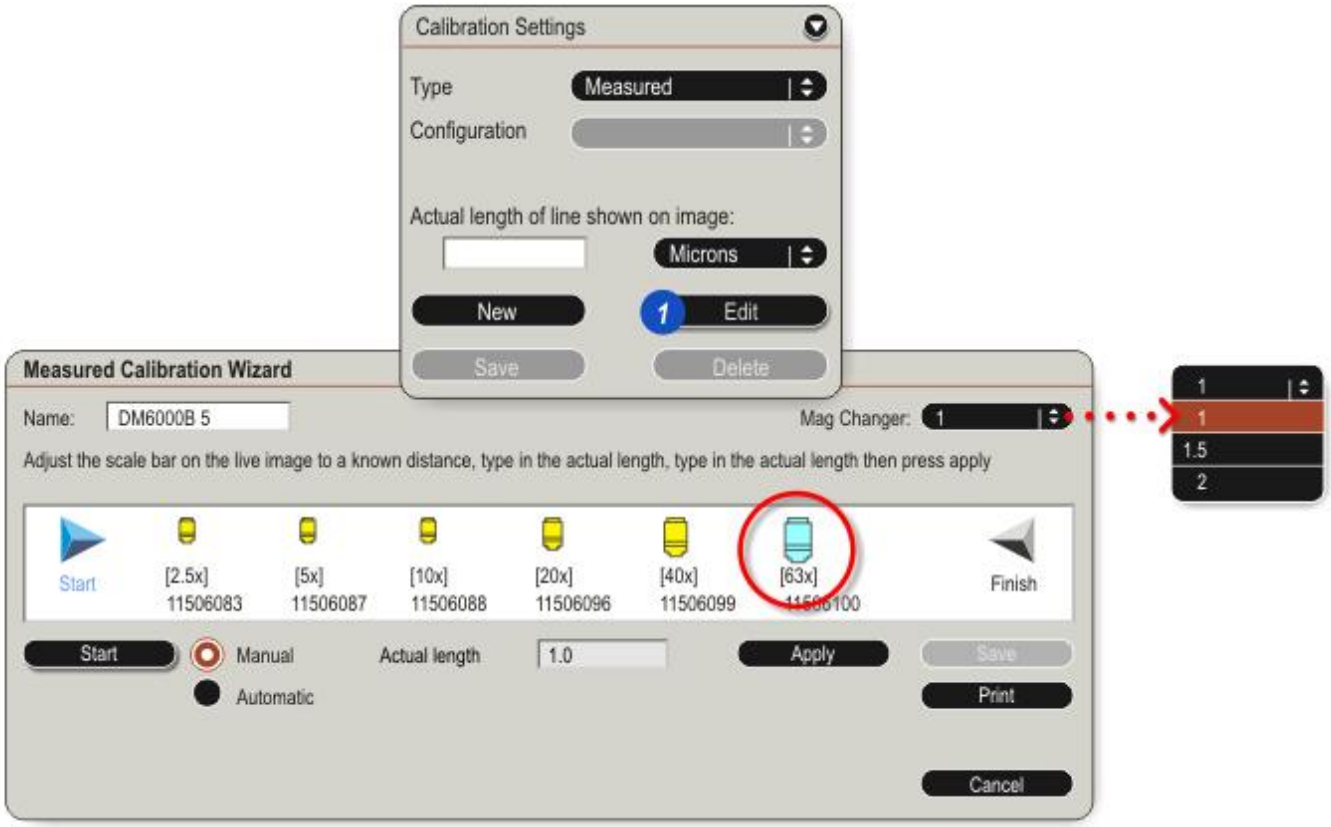


The *Edit* feature allows selected Mag/objective combinations to be calibrated without having to go through the entire Wizard sequence. This is especially useful if an objective is changed.

1: Click *Edit* and the *Measured Calibration Wizard* appears with un-calibrated objective(s) highlighted and the *Actual length* value reset to 1.00.

To calibrate a Mag/objective combination, use the procedure already described:

- Select a *Mag Changer* value.
- Click to select an objective.
- Extend the measurement line over the *Calibration Slide* or allow the software to detect it (Automatic calibration only).
- Type the actual value.
- Click *Apply*.
- Save and exit the calibration Wizard at any time.



Printing Calibration Settings

To display and print a complete list of all objective/mag combination calibrations:

1: Click *Print*. The *Calibration Sheet* is displayed on the computer's default browser.

Use the browser's print button to make a hard copy.

Leica Digital Microscopes

Calibration Sheet

Mag Changer	Objective	Calibration State	Calibration Value	Adjustment
1	1.25	Measured	0.5747 µm	0.1041
1	2	Measured	0.5747 µm	0.1666
1	4	Measured	0.5747 µm	0.3332
1	5	Measured	0.5747 µm	0.4165
1	10	Measured	0.5747 µm	0.8329
2	5	Measured	0.5747 µm	0.8329
2	10	Measured	0.5747 µm	1.6658
2	16	Measured	0.5747 µm	2.6653
2	20	Measured	0.5747 µm	3.3317

Exposure Linking associates a microscope setup with a specific camera setup, whereas *Shading Linking* associates a microscope setup with a specific image shading level.

- *Exposure Linking* 338
- *Shading Linking* 341

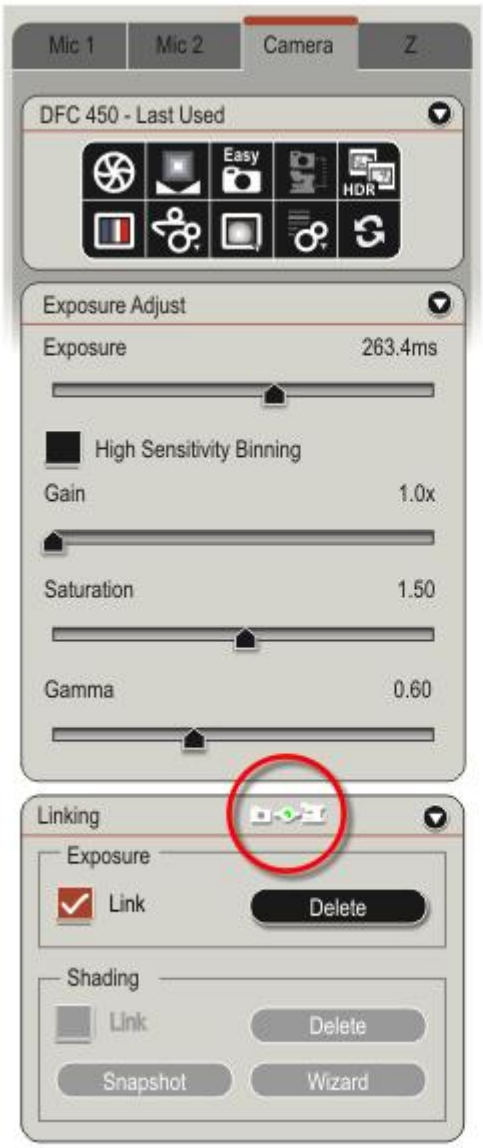
With *Exposure Linking* enabled, the Leica Application Suite automatically checks the major microscope settings against a previously stored *Linking list.* If there is a match, LAS will retrieve and load the camera settings associated with it.

You create the *Linking* list - it is not a preset part of the Leica Application Suite.

The microscope settings checked are:

- Objective or zoom level for stereo microscopes
- Mag changer
- Camera and port
- Filter
- Contrast method.

You can create a *Link* for all of these items in any combination, making it a really powerful tool for precise repetition.



Exposure: Create a Link

To create an *Exposure Link* the microscope must be setup for the image required.

1: Click to enable the *Link* check box.

2: The *Status Display* on the header bar will show RED.

This indicates there is no stored camera information for the current microscope settings.

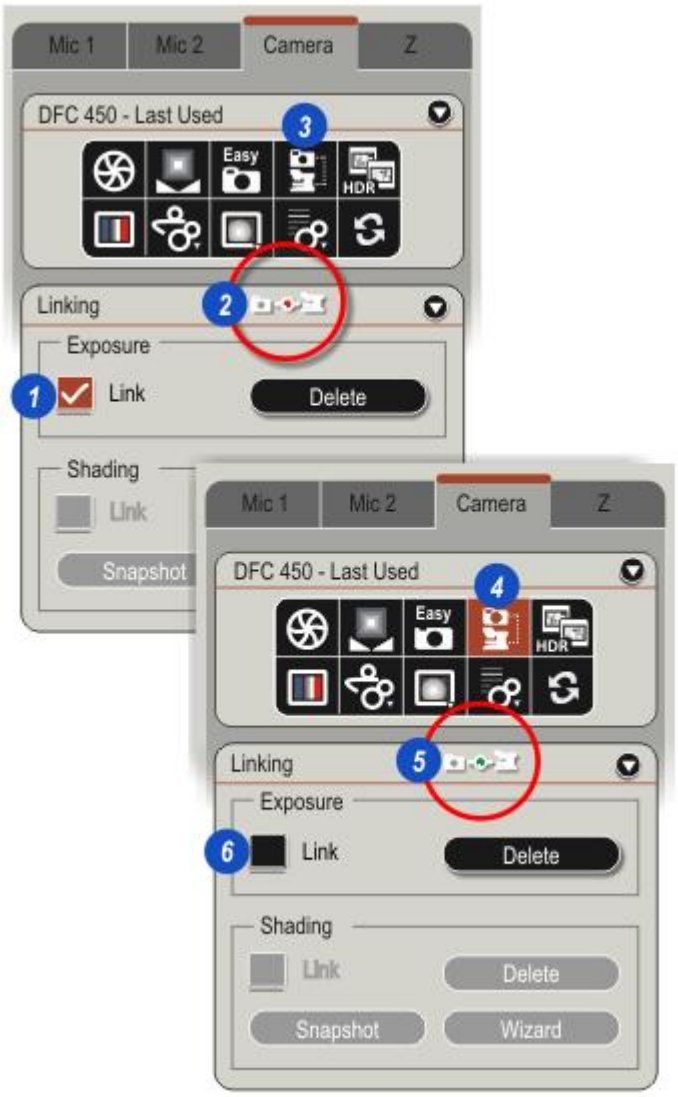
3: The *Linking* button on the*Toolbox* will be enabled.

4: To create a link, click on the *Linking* button.

5: The *Status Display* will turn GREEN to indicate an established link.

To turn *Exposure Linking* on or off:

6: Click to disable the *Link* check box.



If you make changes to the camera settings:

1: The *Status Display* will change colour to YELLOW.

2: The *Linking* button also becomes enabled again. Click it to update the link.

If you change the *Exposure* setting before the link is updated, you can retrieve the original exposure as follows:

3: Right-click the *Linking* icon and then left-click on the *Revert* tag.

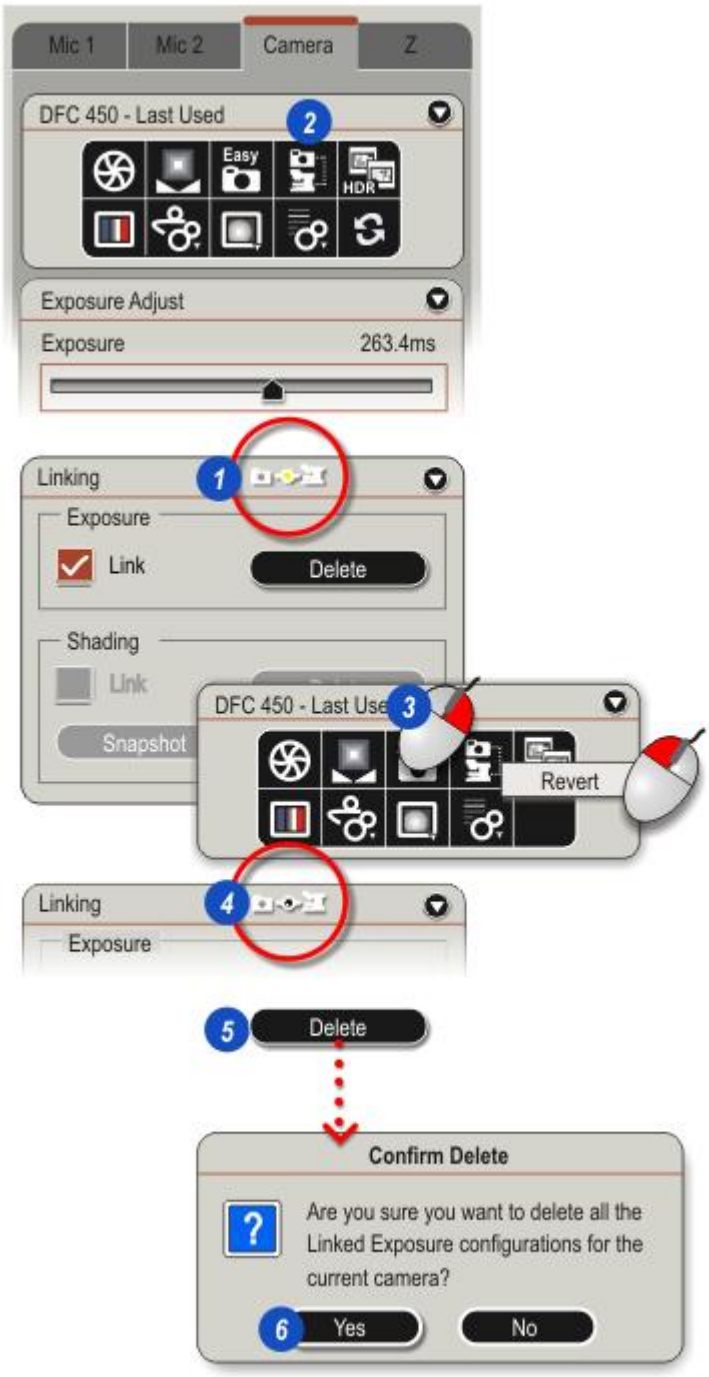
On stereo microscopes an additional warning may occur - the *Status Display* on the header bar changes to BLACK **(4)** indicating that the zoom level has changed but is being ignored. All other settings are correct. Update the link to include the revised zoom by clicking the *Linking* button **(2).**

To delete all the links for the current camera:

5: Click *Delete*.

6: Click *Yes* to confirm.

This is not reversible: Use with care.



Shading Linking associates a specific microscope setup with a specific shading level.

Shading is the name given to variations in the background light level across an image. In the example, the left-hand image represents transmitted light through a microscope. The light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even illumination can be achieved in software by applying a 'blank area' value to the entire image area. The effect is shown in the image on the right.

Different light sources and optical elements will produce different shading levels, so each microscope element combination should have its own shading setting.

When enabled, *Shading Linking* checks the following:

- Objective or zoom level (for stereo microscopes)
- Mag changer
- Camera and port
- Contrast method.

LAS automatically tries to find and apply a matching *Shading Link*. If a match is found the status icon on the *Linking* header bar will be GREEN. Without a match it will be RED and no shading settings applied.

Shading Links are not supplied as presets - you must create your own.

If no Shading Link exists, the icon on the Linking header bar is RED. To create an immediate *Shading Link*:

1: Click to enable the *Shading Link* check box.

2: Click *Snapshot*.

3: When the *Linked Shading* prompt appears, make sure the microscope and camera exposure settings are suitable. Then:

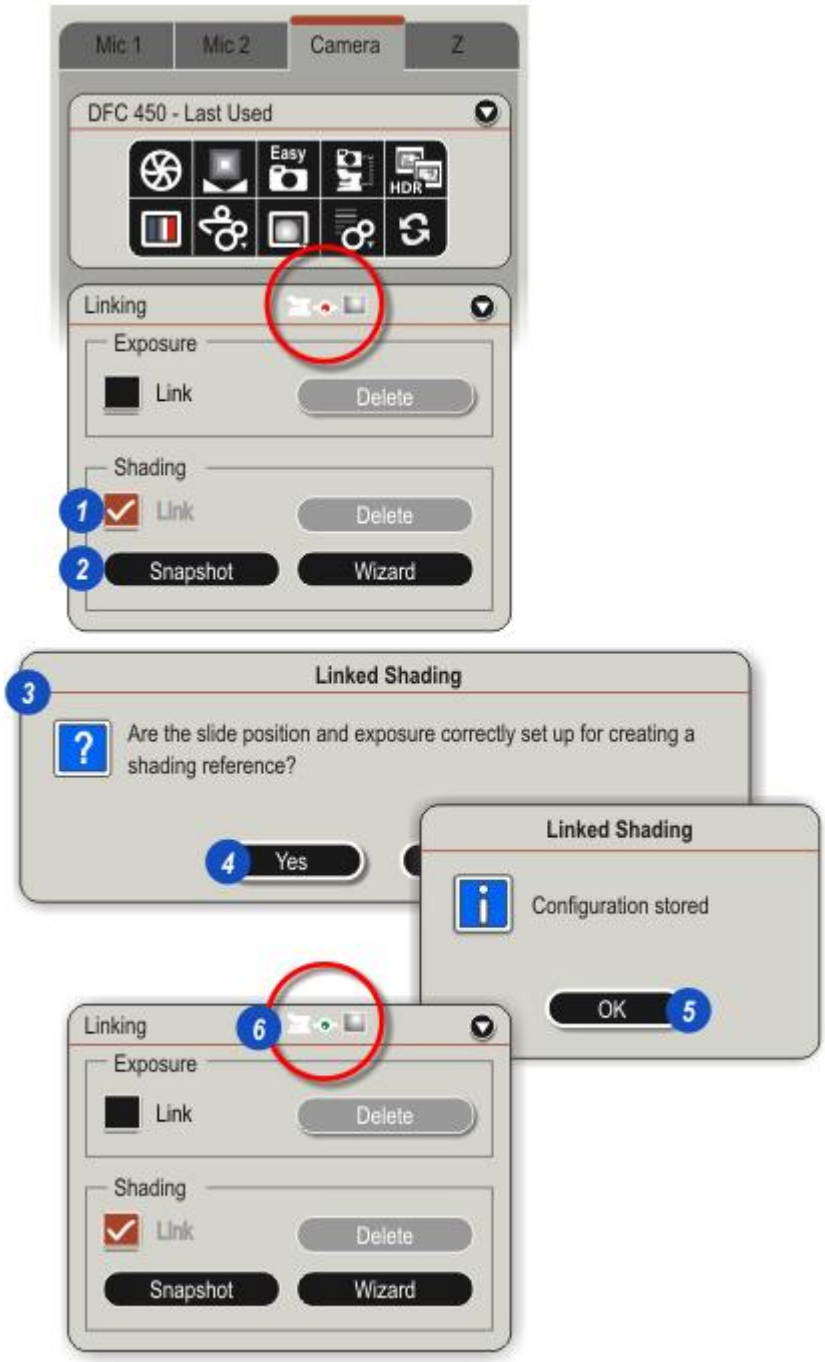
- Either move the stage to view an empty field on the specimen, or remove the specimen slide and replace it with a slide (and cover slip if necessary) of the same type and quality.
- A very small amount of microscope de-focus may be helpful to prevent contaminants affecting the *Shading* reference.

4: When prompted on the *Linked Shading* dialog, click *Yes*.

A shading link will be created, and when complete the *Configuration Stored* message will appear.

5: Click *OK.*

6: **T**he header bar icon becomes GREEN.



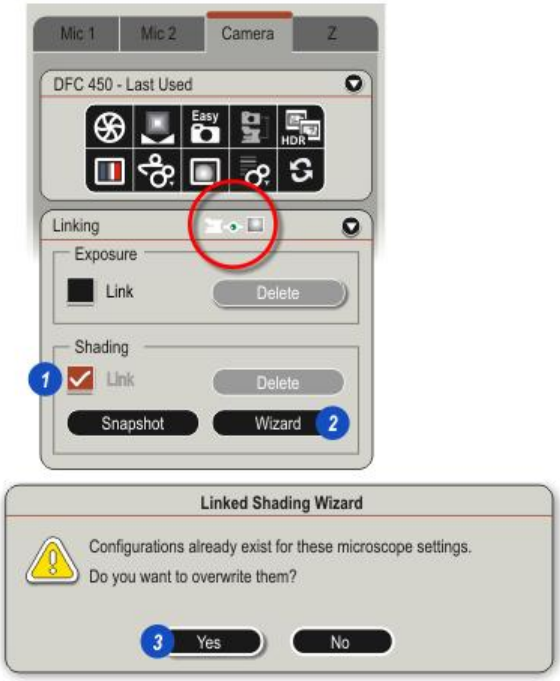
The *Shading Wizard* creates a *Shading Link* for each microscope objective in turn, starting with the one selected. Having completed the first, it automatically moves to the next. The Mag changer, camera and contrast method remain the same for each link.

Because it can be stopped after completing an objective, groups rather than all the objectives can be processed.

For a single objective use *Snapshot* 342.

Before starting the *Wizard*, the specimen image must be properly exposed and focused.

- Either move the stage to view an empty field on the specimen, or remove the specimen slide and replace it with a slide (and cover slip if necessary) of the same type and quality.
- A very small amount of microscope de-focus may be helpful to prevent contaminants affecting the *Shading* reference.



1: Click to enable the *Shading Link* check box.

2: Click *Wizard*.

3: If shading links have already been created for the current microscope settings, a warning will appear.
Click *Yes* to overwrite the existing link or *No* to cancel the *Wizard*.

If necessary, carefully adjust *Exposure* and *Gain* on the empty field or blank specimen slide to achieve a very small amount of over-exposure. Refer to *Histogram* 295 for details of how to turn on over/under-exposure indication.

On the Wizard dialog:

1: Click *Start*.

2: Select the objective to be processed.

The first objective is selected automatically for motorised microscopes; you can select a different one if required.

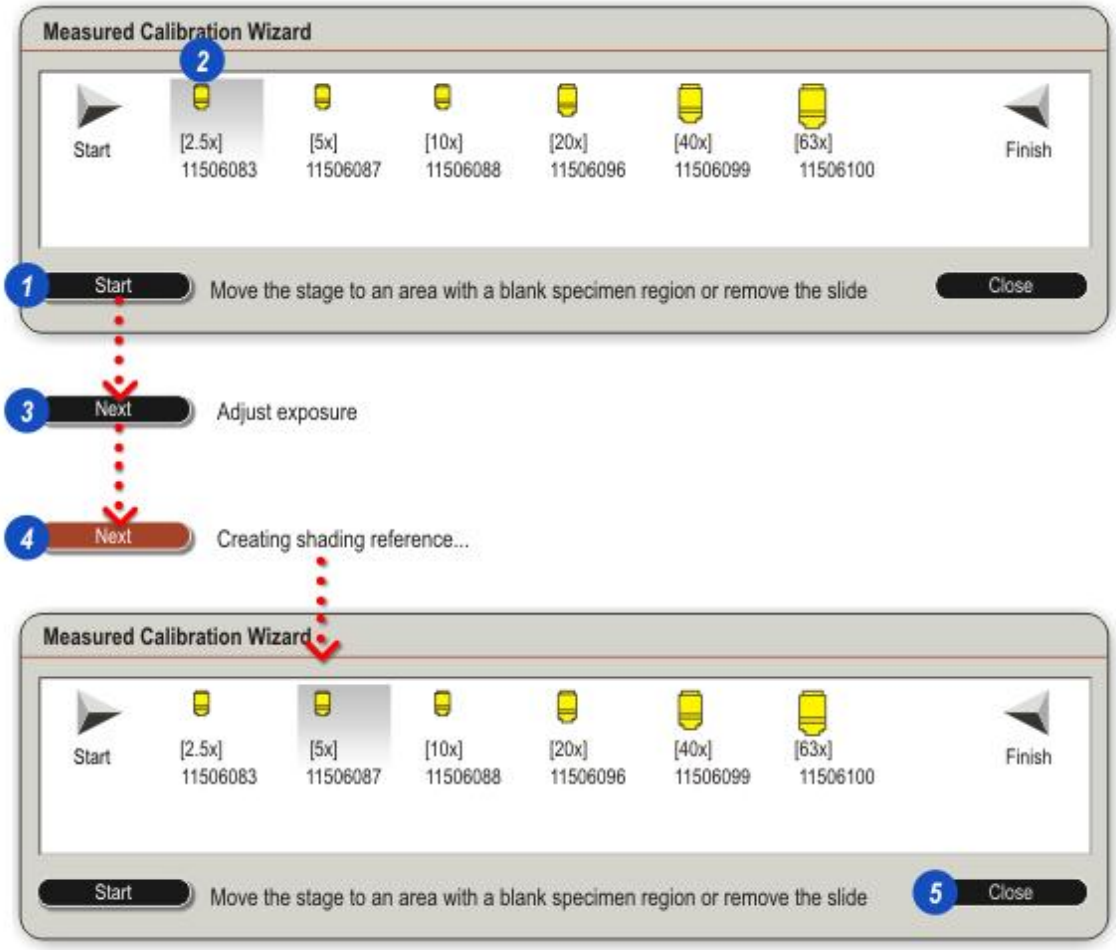
To prevent contamination affecting the shading, a very small amount of de-focus may be used.

3: Click *Next*.

4: The *Wizard* will create the *Shading Link* for the current objective.

When complete, it will automatically select the next objective (change the objective manually for non-auto microscopes) and return to Step **(2)**.

5: When a *Shading Link* for all of the required objectives has been created, click *Close*.



Shading Link: Delete

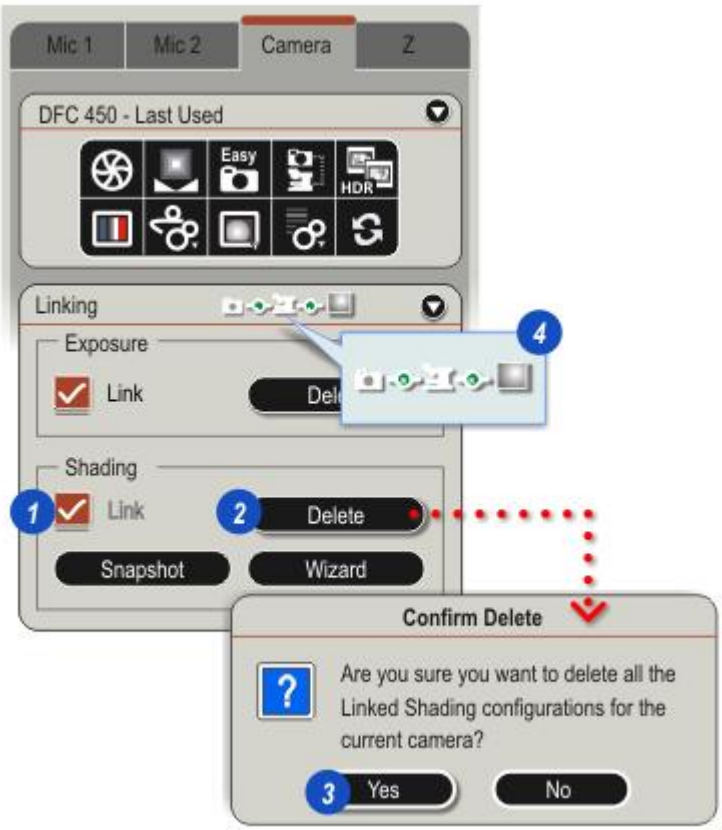
1: Turn *Shading Linking* on or off by clicking the *Link* button.

2: *Shading* links can be deleted by clicking on the *Delete* button and...

3: ...confirming the deletion by clicking the *Yes* button.

Use with care: This will delete ALL of the *Shading Links*.

4: Both *Exposure* and *Shading Linking* can be enabled together. In this case the status icons on the *Linking* header bar will appear combined.



Check Colour

You can adjust the overall image colour. This is sometimes necessary to compensate for colour shift due to small variations in the specimen lighting and camera characteristics.

Use this feature for fine-tuning colour after carrying out a *White Balance* 281.

1: Expand the *Check Colour* panel.

2: Draw a rectangle around the required area to create a *Region of Interest (ROI).*

3: Release the mose button and select *Check Colour* from the context menu that appears.

4: A small dot on the *Colour Wheel* represents the *Hue* and *Saturation* values within the region.

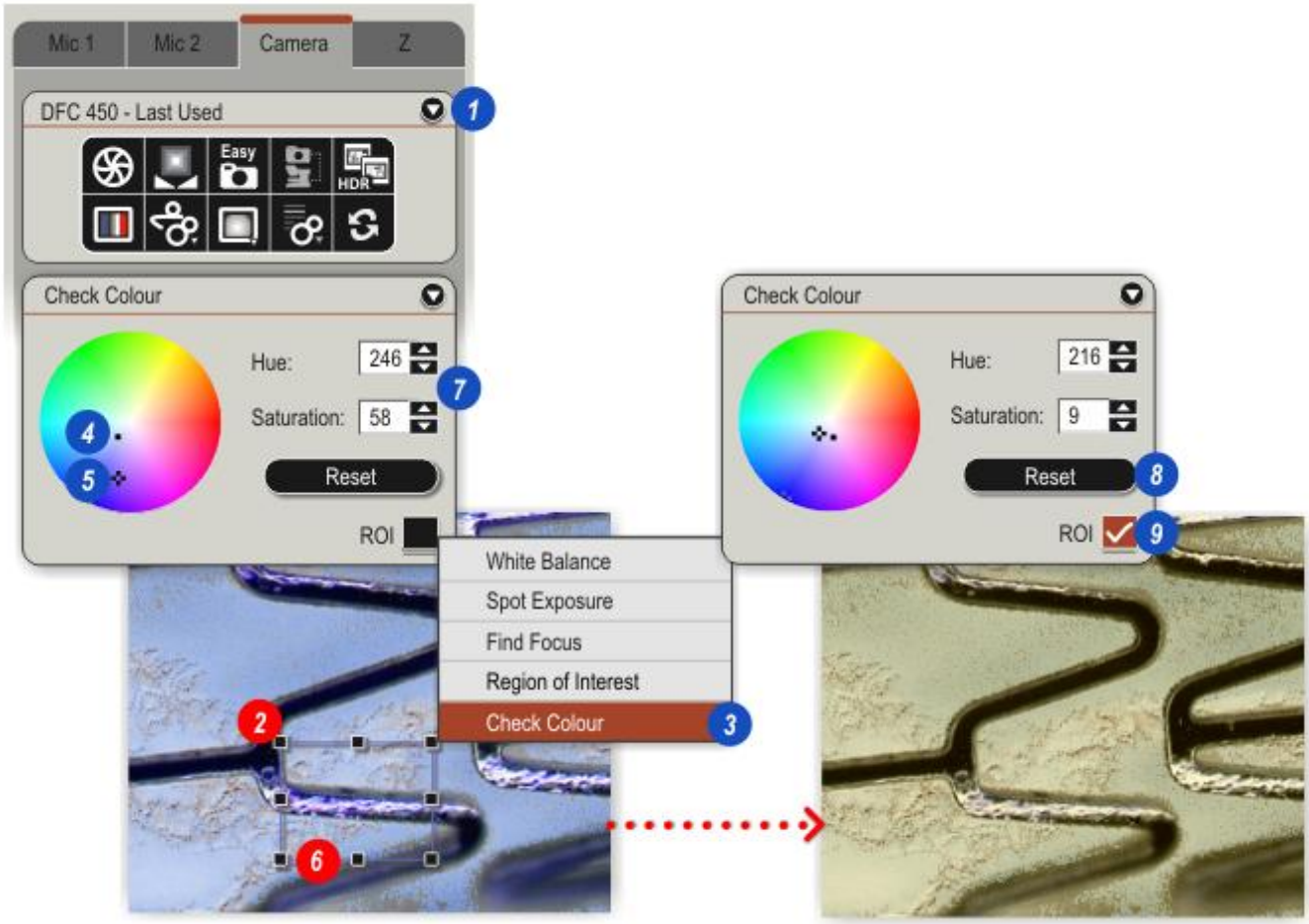
5: To apply the *Hue* and *Saturation* values to the entire image, click on the 'target' mark and drag it towards the dot.

6: Select another area of the image by clicking on the region outline (not the handles) and dragging it to the new location.

7: The *Hue* and *Saturation* windows show the values at the current target location. Fine tune the values by using the up and down arrows or by typing a new value.

8: Hide or reveal the *Region of Interest* using the *ROI* check box.

9: Reset any adjustments by clicking *Reset*.

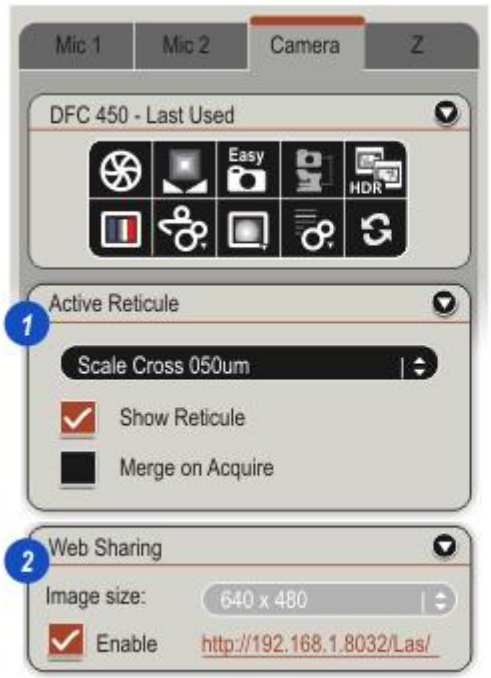


Reticule and *Web Sharing* are optional modules that have to be installed and licensed. Once installed, the appropriate control panel will appear in *Camera*.

See:

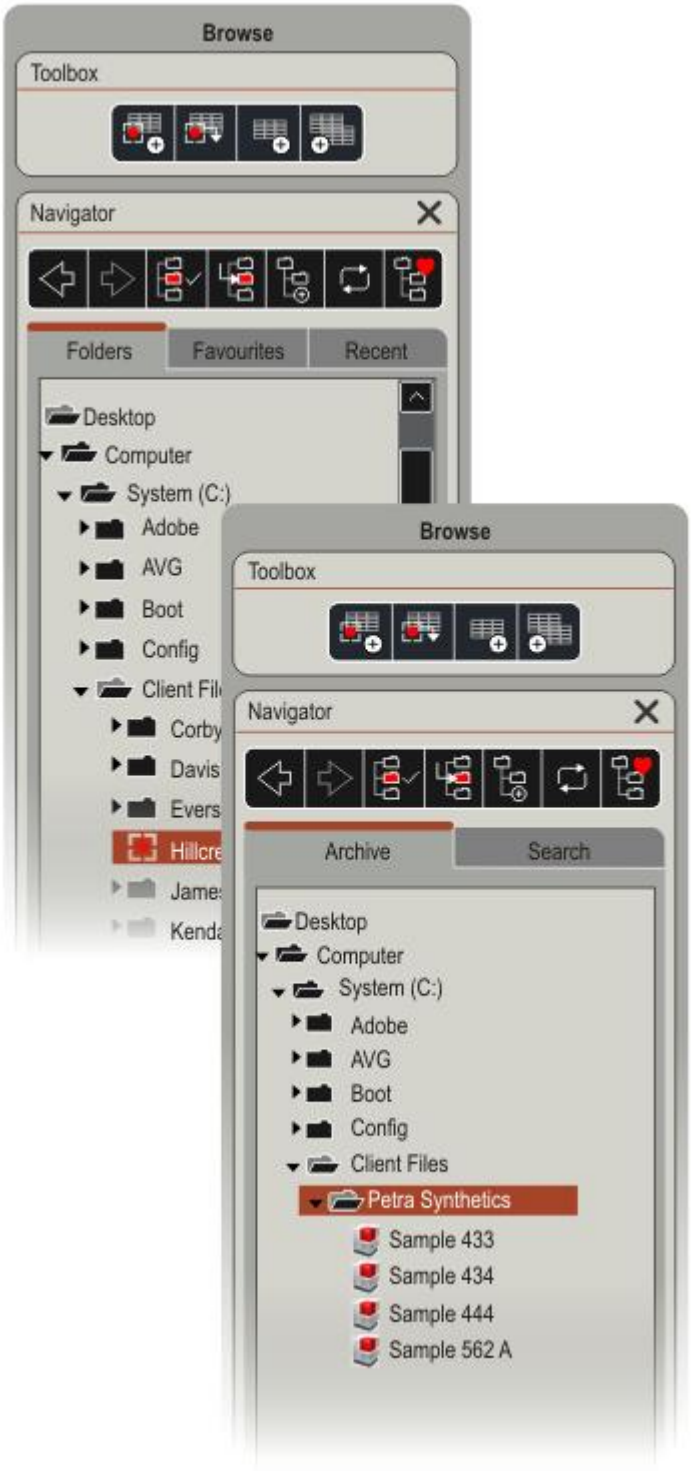
Reticule 1370

Web Sharing 1381



Browse provides access to stored images and their data, such as the time of acquisition, bit depth and calibration. The fast *Image Explorer* was developed especially for Leica Application Suite, and uses the familiar tree and folder structure.

As well as *Image Explorer,* you can install and use the optional *LAS Archive* [470] module. You can switch seamlessly between the two storage methods. With *LAS Archive*, you can import images captured before LAS V3.3.



Browse features and quick links:

- *Quick navigation* [359] between folders with a single click.
- Create new folders [360] without having to leave LAS.
- Set a folder [357] as the capture location and immediately begin to grab images.
- Naming images automatically [354] with meaningful names and auto-incremented sequence numbers.
- Microscope and camera data [392] saved with the image: Users can add their own comments and observations
- A scalable Thumbnail Gallery [390] to show all of the images in a folder and, with a single click select and display an individual.
- A wide range of tools [353] for image fast storage and access.

The illustration is a graphical representation of the LAS display and *Browse* interface showing the principal features and quick links:

1: *Workflows*[350]*:* Click the *Browse Workflow* to open the navigator.

2: *Toolbox*[353]*:* Common tools for both *Image Explorer* and *LAS Archives.*

3: *Navigator Window*[351] Tab shows *Folders* for *Image Explorer* or *Archive* and *Search for Archives*.

4: *Image Data Form*[392] Displays and edits selected data for the current image.

5: *The Image Viewer*[388]*:* Display and working area for the current image: Press keyboard *F5* to show full screen.

6: *The Grid*[396]*:* Displays data for all of the images in the selected folder.

7: *The Gallery*[390]*:* Displays thumbnails of all the images in the selected folder.

8: *Acquire Button*[354]*:* Click to grab and image from the microscope camera.

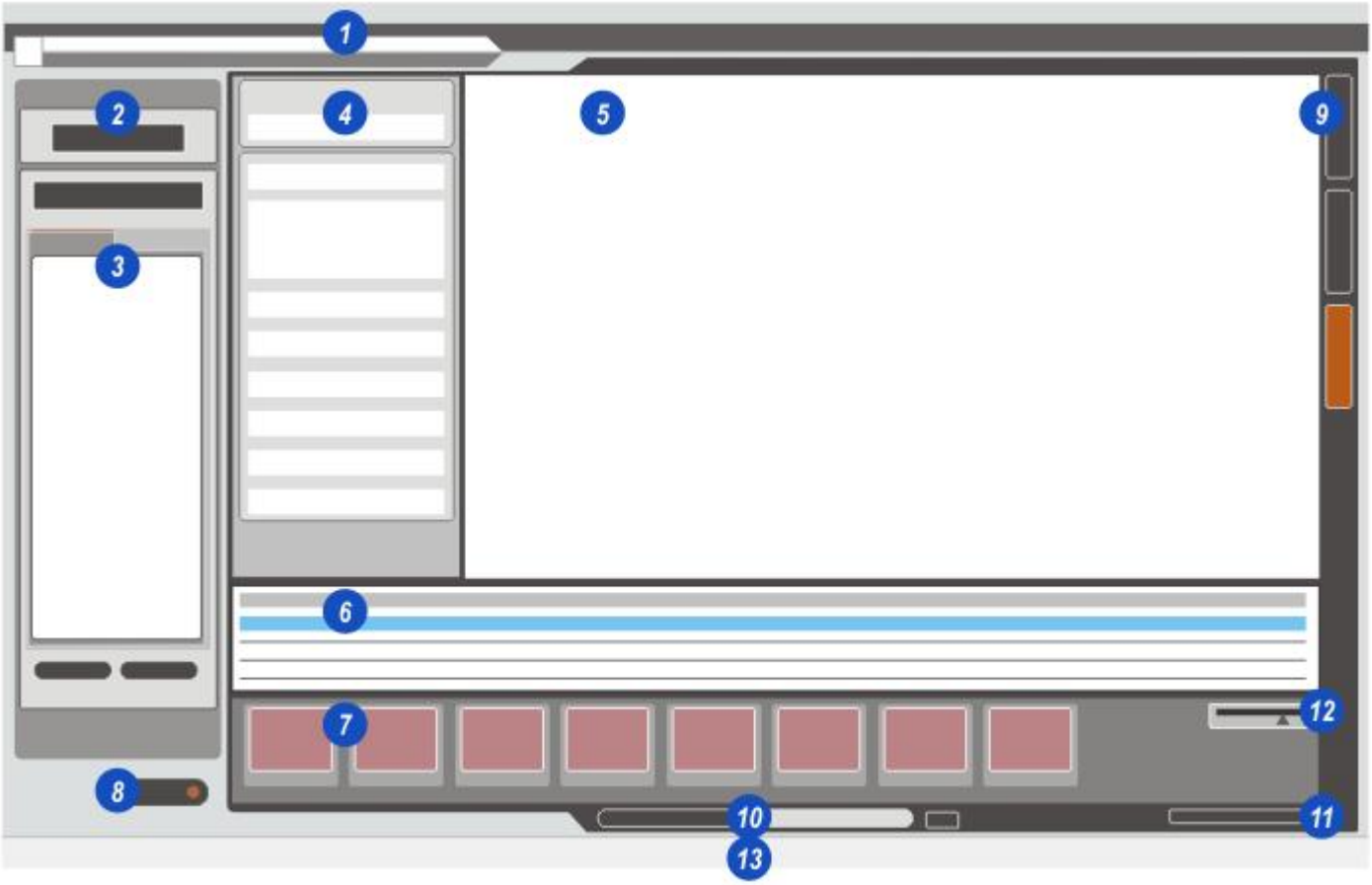
9: *Side Tool Bar*[369]*:* Working tools for image sizing, printing and deleting as well as switches for the *Gallery* and *Grid*.

10: *The Search Controls*[508]*:* Available only with *LAS Archives.*

11: *Gallery Navigation Browser*[390]*:* Rapidly find thumbnails in the *Gallery*.

12: *Thumbnail Scaler*[390]*:* Slider adjusts the size of the thumbnails in the *Gallery*.

13: *Status Bar*[73]: Displays Hardware Configuration, RGB Intensity, Stage Position and Magnification data.



1: Click on the *Browse Workflow* and if necessary…

2: …on the *Browse* tab to reveal the main panel.

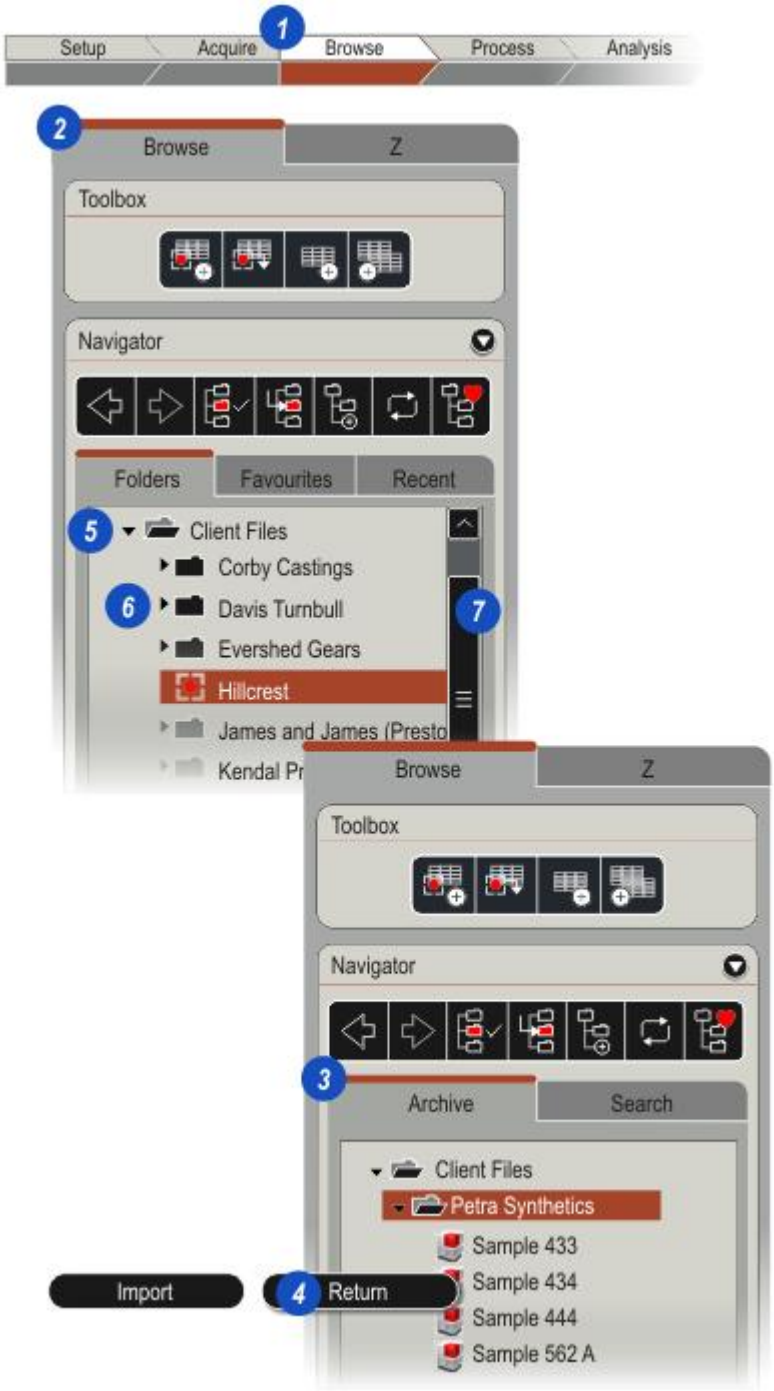
3: If *LAS Archive* is installed and has been previously selected...

4: ...click on the *Return* button to switch to *Image Explorer*.

5: Click on the small arrows to the left of the folders to expand them.

6: Click on a folder to reveal the contents.

7: The *Scrollbars* to the right and bottom of the navigation window are displayed automatically if necessary. Click and drag a *Scrollbar* to move the explorer tree within the window.



The main panel layout and controls changes depending upon the storage system being used - either *Image Explorer* or *LAS Archives.*

Image Explorer is the default navigator supplied as part of *LAS Core*.

LAS Archives are optional modules designed especially for those users who need a fast database for image storage. More about *LAS Archives*[531] .

The illustration shows a typical panel with *Image Explorer* active. The control tab label displays the word *Folders*.

The principle features and quick links:

- *Floating Navigator*[352]*:* Drag and drop the *Navigator* to a location that suits the user, even on to a second monitor..
- *Toolbox*[353]*:* Image grabbing and record creation.
- *Navigation Buttons*[357]*:* Move between folders and set the capture folder. *Favourites* creates a fast navigation list.
- *Create a new Folder*[360].
- *Rename a Folder*[363].
- *Delete a Folder*[365].
- *Image Sequence Move and Copy*[367].
- *Hide Component Images*[366].

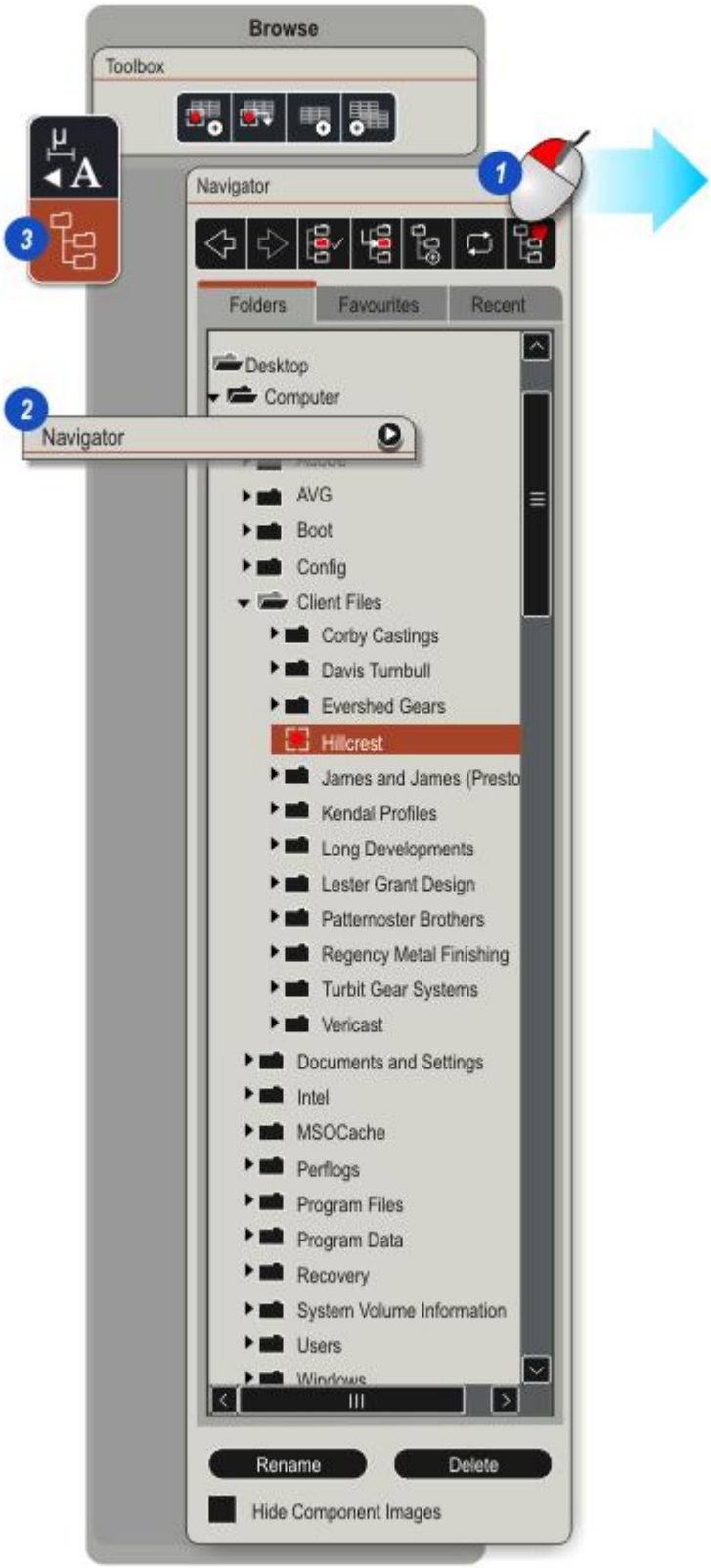
The *Navigator* panel can be detached from its 'dock' and parked anywhere on the *Viewer* - or even on another monitor - to provide access to folders and archives immediately from any workflow or Optional Module without returning to *Browse*.

To move the *Navigator*:

1: On the *Browse Workflow*, click on the *Navigator* header and, holding down the mouse button drag the panel to the required docking position. All of the *Navigator* features are available in the new position...

2: ... including the minimise button that will close the *Navigator* leaving only the header on display. Click the minimise button to reveal the *Navigator* again.

3: The *Show Navigator* button works as a toggle. If the *Floating Navigator* has been moved the first click will return it to the side panel. The second click will move it back to the user's docking position.



The *Toolbox* buttons are grouped on a small panel at the top of the *Browse* tab **(1)**. Click a *Toolbox* button for more information.

Acquire Image: click to grab an image: Has the same function as the *Acquire* button at the bottom of the screen.

Acquire in the Current Image: Grabs an image and overwrites the current selected image.

Create an Empty Record: Click to create a record without an image – an image can be captured into it at a later date.

Duplicate Current Record: Duplicates the current record and image.

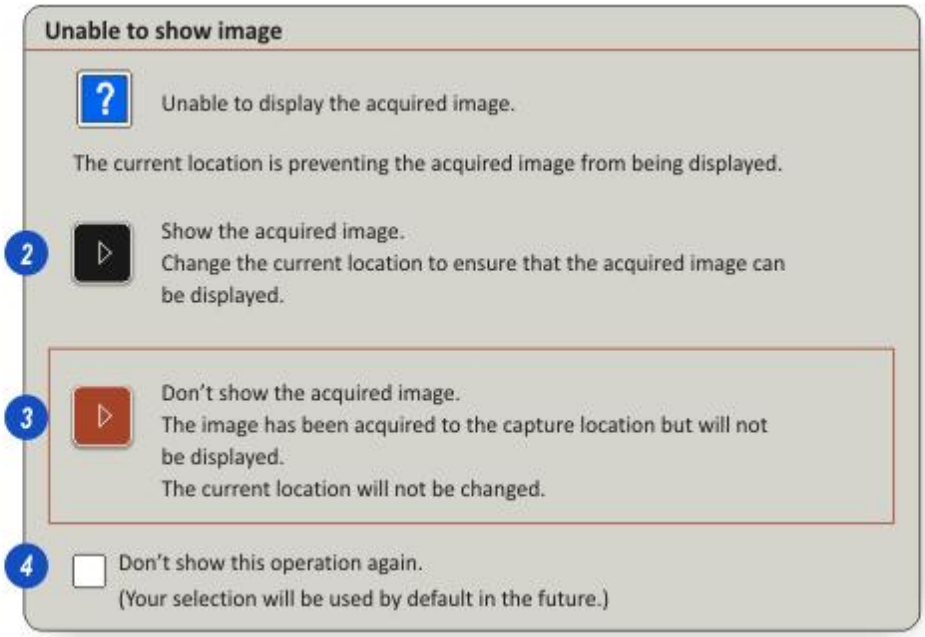
1: Click the *Acquire New Image* button. If *Capture to Fixed Location* [64] is enabled in *Preferences*, and the folder (indicated with a red dot) is selected in the Navigator window, the image will be captured to that folder.
The *Acquire* button at the bottom of the screen performs the same function.

If a folder other than that chosen as a 'fixed location' is selected, the *Unable to Show Image* dialog appears. The options are:

2: Show the acquired image and move to the *Fixed Capture Location* to display it or...

3: Don't show the acquired image but do save it to the *Fixed Capture Location* anyway.

4: To make your choice the future default action, click to enable the *Don't show this operation again* check box



1: *Acquire into the Current Record* replaces the selected image with a new one. Where necessary, the data is updated but the *Image ID* and *Image Name* remain the same.

2: *Create an Empty Record* does not capture an image and stores only essential microscope data, but it does give the record and *Image ID* and an *Image Name.*

The image and data may then be captured later by clicking to select the empty record and then using the *Acquire into Current Record (above).* This provides a convenient method of 'loading' images later.

3: The *Empty Record* is represented as a Leica Cube in the *Gallery* thumbnails.

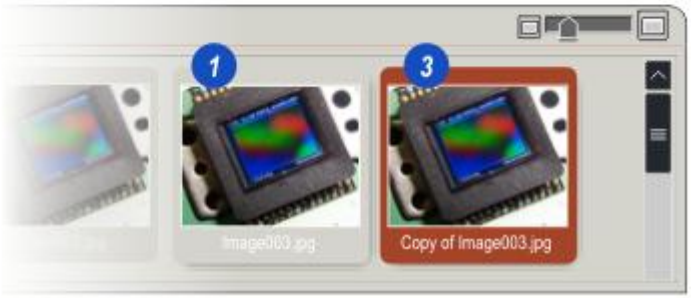
Duplicate Current Record is a simple way of copying an image and its data.

Some data such as notes and descriptions may then be changed to avoid having to type in the description again, the image and the microscope data remain the same.

1: In the *Gallery*, click on the thumbnail of the image to be duplicated.

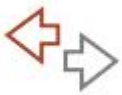
2: In the *Toolbox* click the *Duplicate Current Record* button.

3: The duplicate appears as a thumbnail in the *Gallery* with the name *Copy of…* followed by the original *Image Name*. Change data on the *Form* as required.



The *Browse Navigation Buttons* allow the user to move back and forth between folders and files, nominate a folder into which images should be captured, create a child of an existing folder and to store 'favourite' folder locations for speedy retrieval.

Click a *Navigator* button for more information:



Step Back: Click to move to the previous folder (left arrow) and return (right arrow).

Set Capture Folder: Makes the selected folder the capture target – grabbed images will be stored here.

Move to Capture Folder: Returns to the *Capture Folder* from anywhere on the tree.

Create a New Folder: Creates a folder as a child of the currently selected folder.

Refresh the Current Folder: Re-displays the *Gallery* and selected image in the *Viewer.*

Favourites: Stores and links to frequently used folders and files.

There are a further three controls below the *Navigator* window:

2: *Rename*[363] Allows the user to rename a folder.

3: *Delete*[365] Deletes the selected folder and all of its contents.

4: *Hide Component Images*[366] When enabled displays only the 'analytical' images in the *Gallery.*

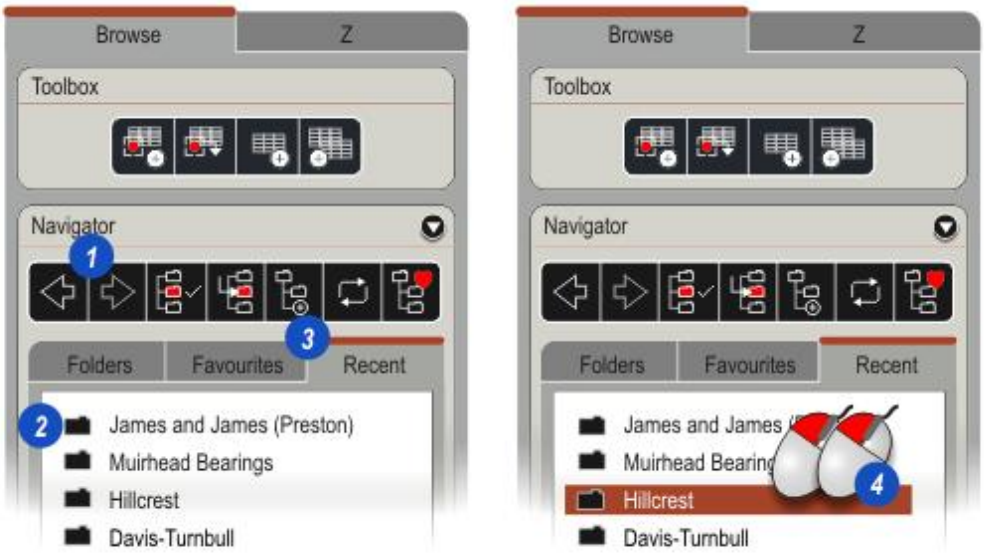
The software keeps track of the navigation by the step buttons **(1)** and allows the user to go directly to a folder without having to make multiple clicks on the step buttons.

The *Recent* feature is only available in *Folders*.

2: The list of visited folders is displayed when...

3: ...the *Recent* tab is clicked.

4: Return directly to a folder by double-clicking on it in the list.



Set and Move to Capture Folder

Users can nominate a folder into which captured images will be automatically saved. The nominated folder can be changed at any time:

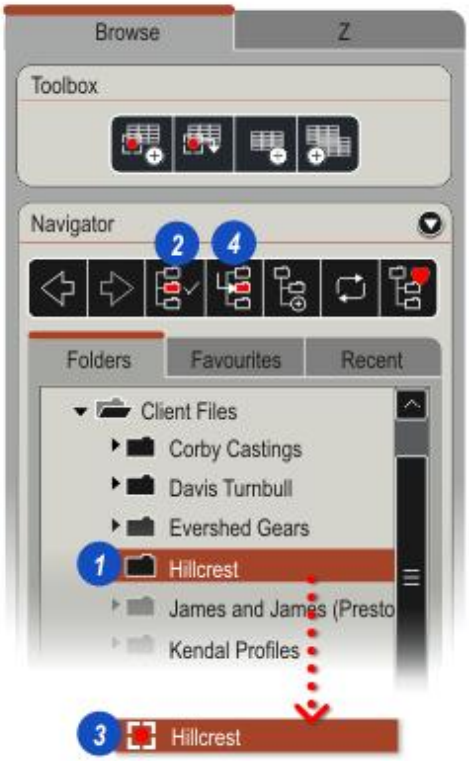
1: Click to select the *Capture Folder*.

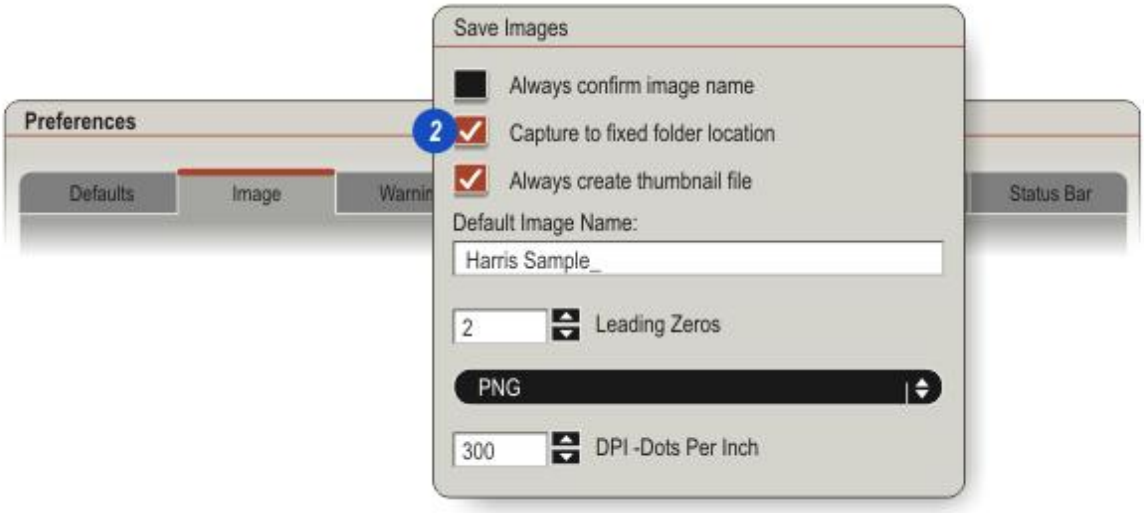
2: Providing that the *Capture to fixed folder location* option is enabled in *Preferences* 64, clicking the *Set Capture Location* button will make the selected folder the saved image location.

3: The *Capture Folder* is indicated by the red dot to the left.

To Move to the Capture Folder:

4: If, having navigated away from the *Capture Folder* a user needs to return to it, clicking the *Move to Capture Folder* button will go directly there.





Create a New Folder

A new folder is created as a child of the selected folder that does not have to be the *Current Capture* folder.

1: Click on the folder that is to be the parent of the new folder.

2: Click on the *Create New Folder* button.

3: The new folder appears with the default name *New Folder*.

4: With the new folder selected and highlighted, click on the *Rename* button and...

5: ...type a new name. Press the *Enter* key on the keyboard and the new folder has the new name.

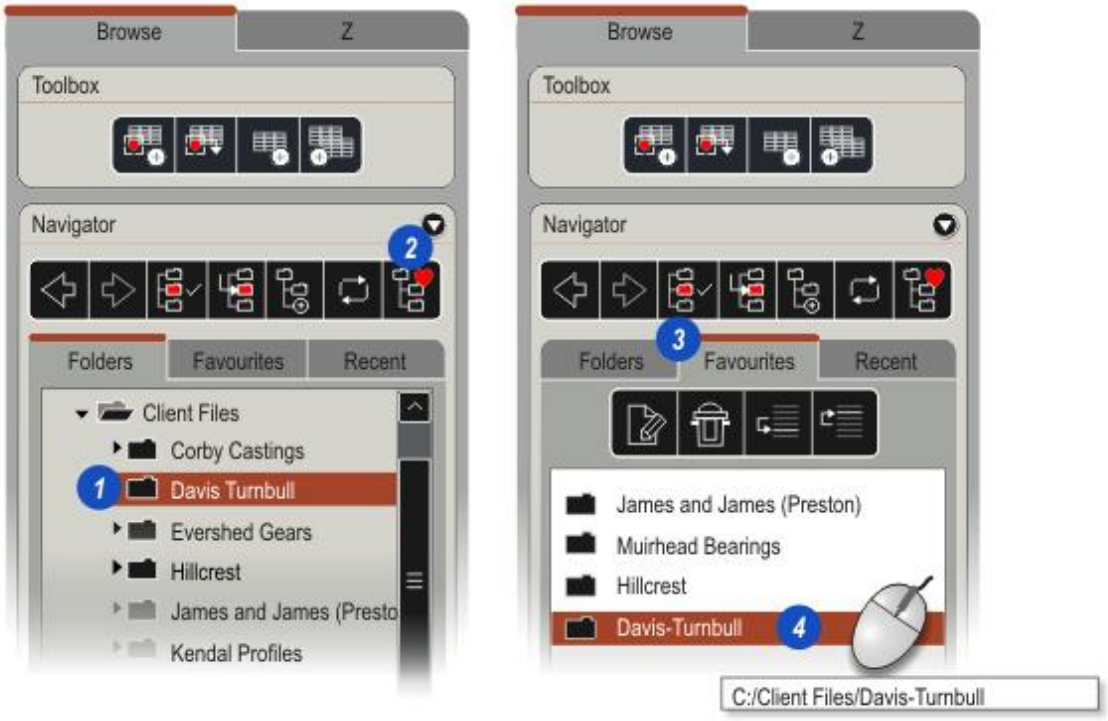
Users can store links to folders that they often use with the *Favourites* button. They can then return directly to a listed folder simply by double-clicking its link. This is a very useful feature for 'trees' with many folders and saves a considerable amount of time. The *Favourites* feature is only available in *Folders*.

1: Click to select the folder to add to *Favourites*.

2: Click the *Add Favourite* button to create a link to the folder.

3: The list of links can be seen by clicking to *Favourites* tab...

4: ...with the newly created link highlighted. Move the cursor over the link to reveal the complete path.

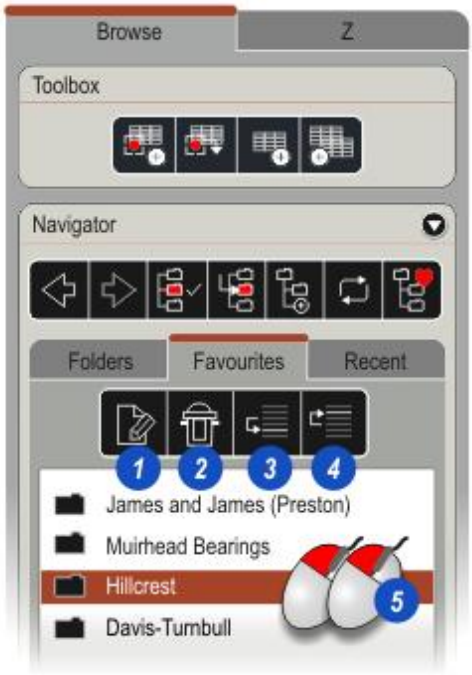


1: To change the name of a link click the *Edit* button and enter the new name.

2: Delete a link from the list by clicking to select the link and then clicking the *Delete (Trash Can)* button.

3 & 4: Move a link up or down in the list by clicking to select the link and then either the *Move Up* or *Move Down* button.

5: Return to a chosen folder by double-clicking its link. The *Folders* tab opens automatically and the selected folder is highlighted.



Rename a Folder or Image

Rename Folder

1: In the Navigator, click on the folder to be re-named.

2: Click on the *Rename* button or press the *F2* function key.

3: Click in the folder text box and type the new folder name finishing by pressing the *Enter* key on the keyboard.

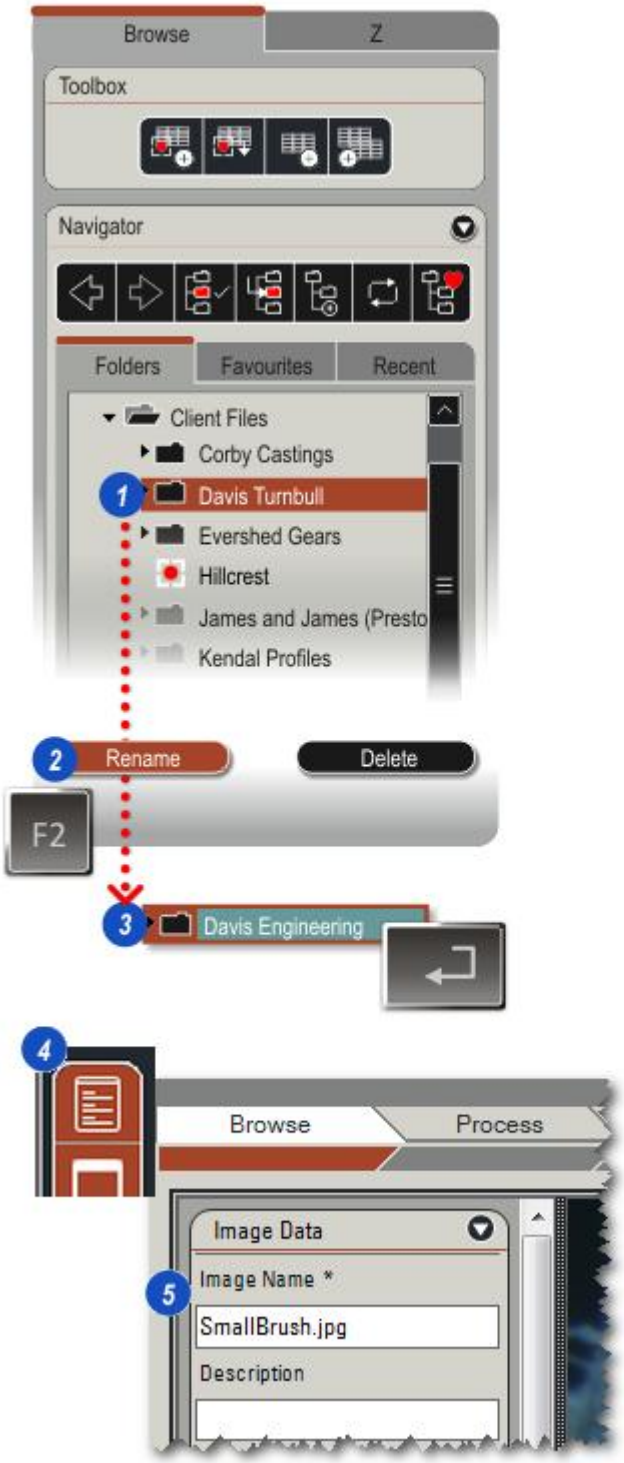
Rename Image File (method 1)

4: Click on the show Image Data form icon on the right-side toolbar to show the Image Data form.

5: Type in your new name in the text box. There is no need to type the image extension.

Press the Tab key on the keyboard or click on another field in the Image Data panel. The new name will be applied and copied to all metadata. The file type extension will be added.

Note: you cannot change the image file format by typing in a different extension. To change the file format you can use the Export icon on the Side Tool Bar, or right-click on the image in the *Gallery* and click on the *Export* context menu.



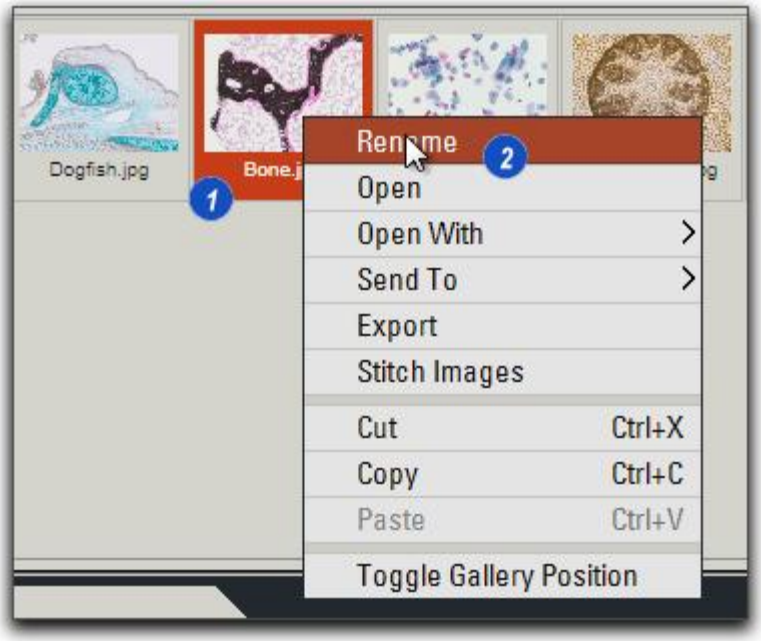
Rename Image File (method 2)

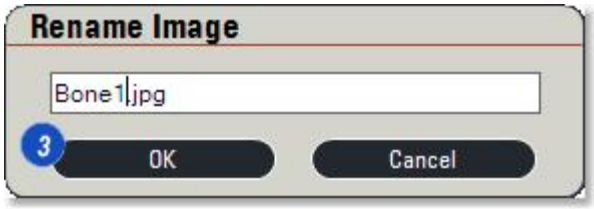
1: Right-click on the image in the *Image Viewer* or the *Gallery*.

(If necessary, click on the *Show gallery* icon on the Side Tool Bar to show the *Gallery*.)

2: Click on *Rename* in the context menu.

3: Enter a new name in the *Rename Image* dialog and click on *OK*.





Delete Folder

Use with caution: *Folder Delete* cannot be undone.

Trying to delete a folder that contains images results in the *Unable to Delete* warning **(1)**. All of the images must be removed by:

2: Click on the thumbnail of the first image and...

3: ...holding down the keyboard *Shift* key, click on the thumbnail of the last image. All of the thumbnails and images are selected.

4: Click on the *Delete (Trash Can)* button on the *Side Tool Bar*.

5: Confirm the deletion.

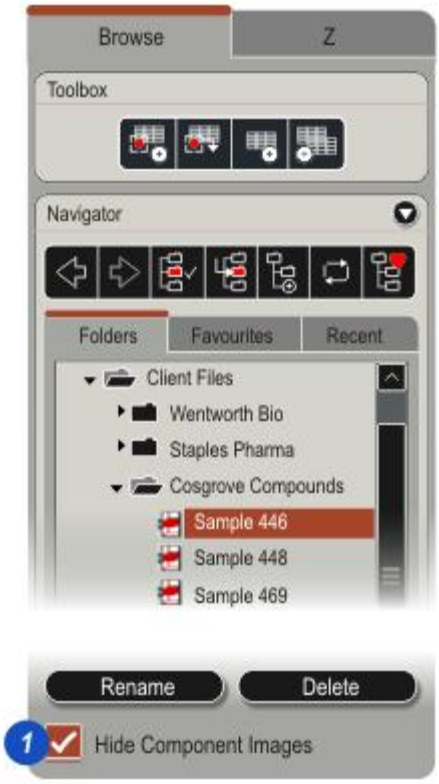
6: Click the *Navigator* > *Delete* button.

7: Confirm the deletion and the folder will be removed.

The *Gallery* when displaying sequences **(A)** can become very cluttered with the individual component images - the important results may be scattered and time-consuming to find.

The component images can be easily hidden leaving only the 'analytical' images on view in the *Gallery* **(B)** by enabling (tick mark displayed) the *Hide Component Images* check box **(1)**.

The individual images are not deleted, only hidden and can be revealed by disabling *Hide Component Images* by clicking it again.



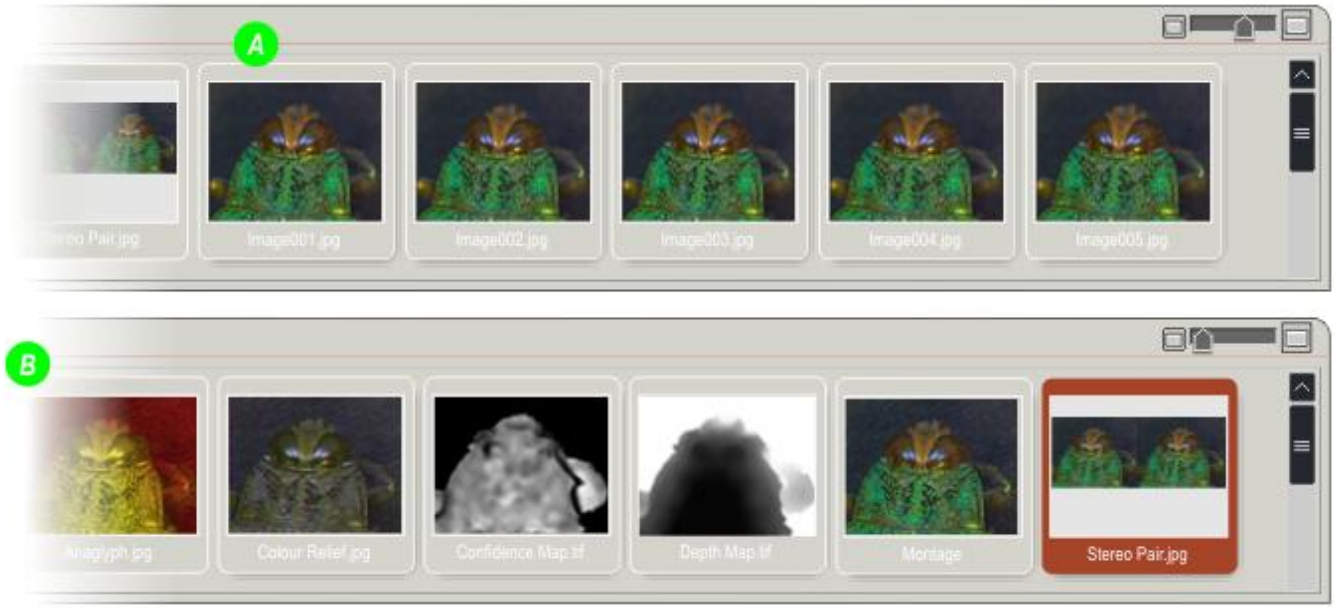


Image sequences - montage and multistep for example - can be copied or moved in a single step using mouse drag and drop:

To move a sequence:

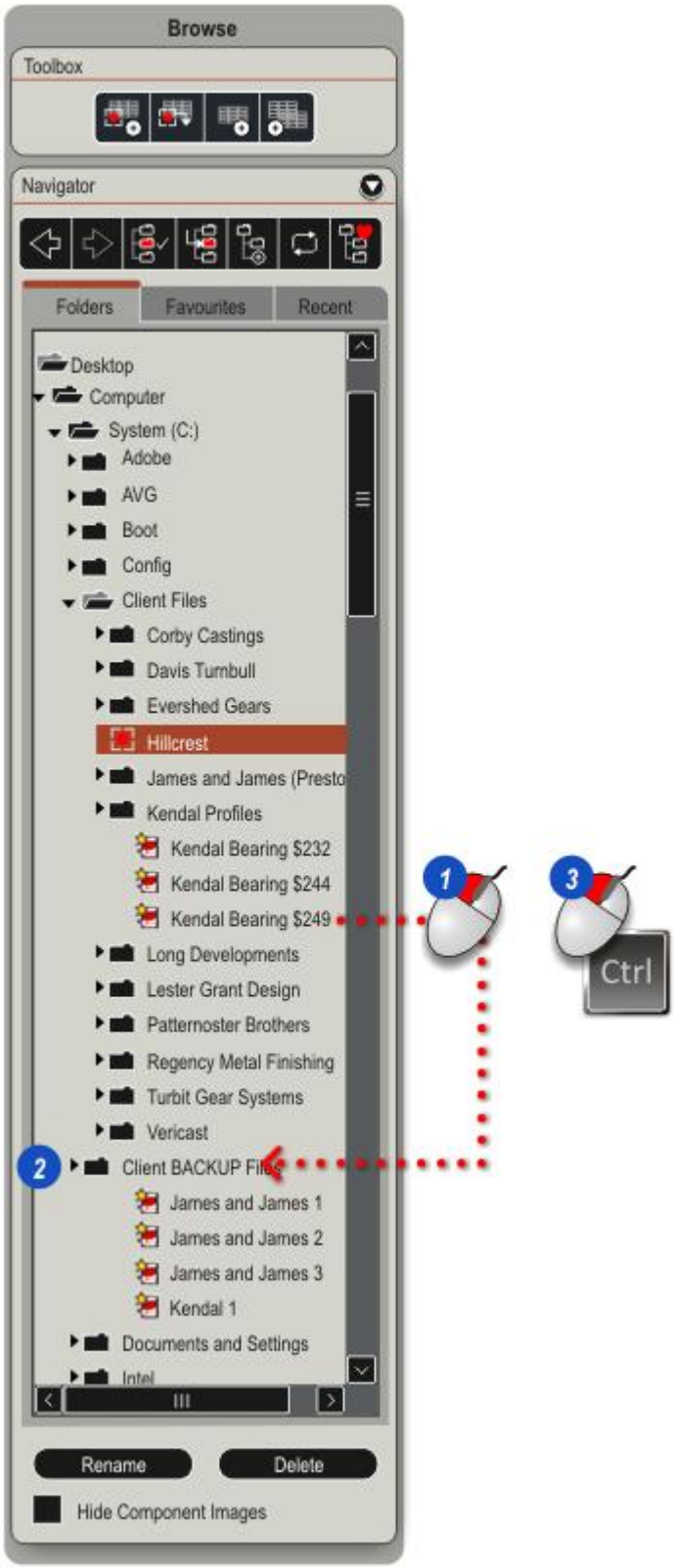
1: Left click on the sequence name and, holding down the mouse button...

2: ...drag to the target folder and release the mouse button. The sequence is moved from the source folder to the target folder.

To copy a sequence:

3: Press and hold down the keyboard *Ctrl* key, left-click and also hold down the mouse button on the sequence to be copied. Drag the mouse to the target folder and release the mouse. Release the *Ctrl* key.

The sequence is duplicated in the target folder.



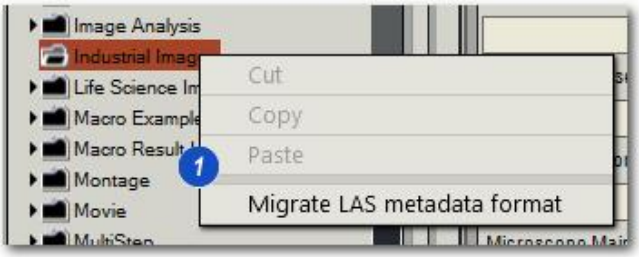
LAS saves data associated with images in files that are known as Metadata files. To maintain maximum flexibility these small files are dedicated to a particular function such as defining the Scale Bar or the measurements.

Normally these files are set to be hidden files so you don't see them when you browse to a folder with Windows Explorer. Because these files contain useful information about the images, they should always be copied when an image is copied. This is done by using the LAS copy, paste, move and export functions. Do not move your images in file browser programs external to LAS if you want to continue using them with LAS as the Metadata file may become separated.

Some example of metafiles are:

*.cal.xml	Stores image calibration, microscope and camera information.
*.snr	Used for Store and Recall
*.eax	Extended Annotation
*.thb	Image thumbnail
*.sbx	Scalebar
*.anx	Annotation
*.lmd	Interactive measurements

These files are stored in folder called .Metadata that is in the same folder as the images.



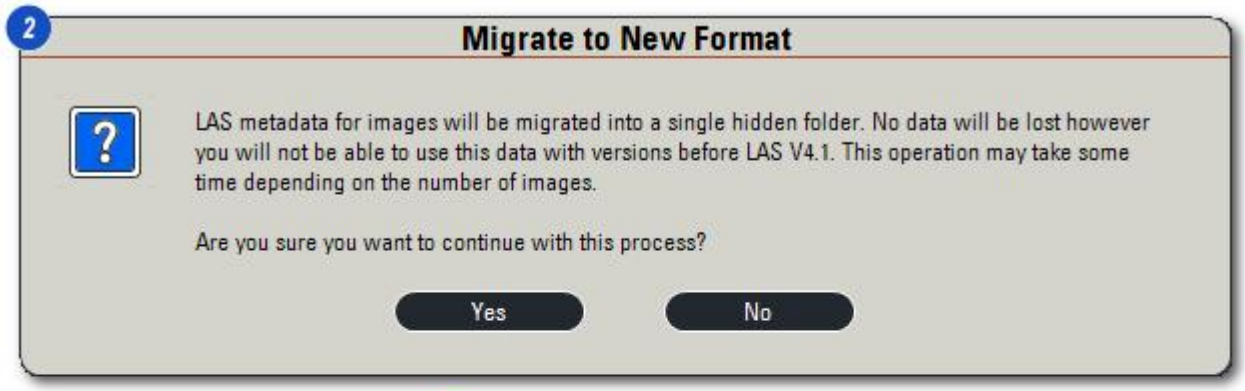
In LAS versions before V4.1, the metafiles were stored in the same folder as the images. These files can still be used with LAS V4.1, however any new images will store their metadata in the .Metadata folder. Keeping the metadata separate from the images, means that it is easier to locate your image when browsing external to LAS. To migrate all your data to the new organisation used by LAS V4.1:

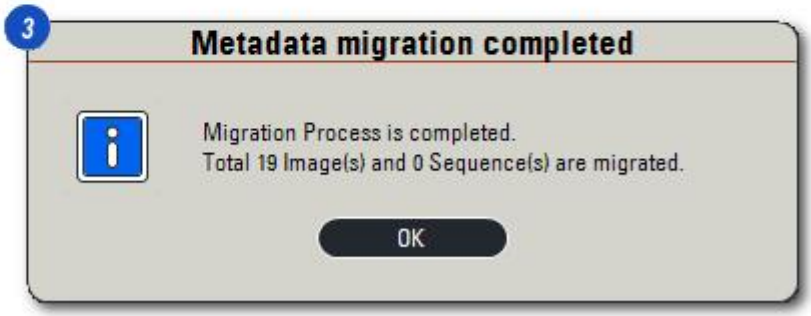
1: Use the LAS Navigator to find the folder you wish to migrate and right-click on this folder. From the pop-up menu select *Migrate LAS metadata format*.

2: This message will appear asking you to confirm this migration.

3: On the completion of the migration you will be informed how many images and sequences have been moved.

It is not possible to revert to the format used by LAS earlier than V4.1. This means that if you are using LAS on multiple PCs, they must all be updated to use LAS V4.1 if images are to be shared.





This section describes the *Side Tool Bar* tools that are common to both *Image Explorer* and *LAS Archive.* Click on a *Tool Bar* button for more information:

Scale Bar and Annotations: Run Annotations and Scale Bar without leaving Browse.

Floating Navigator: Click to enable the *Floating Navigator* and click again to 'park' it.

Export: Export the current image or image selection to a location of the users choice.

Image Stitching: Combines a set of overlapping images into a single, aligned overview image.

Print: Print the selected image together with headers, footers and a wide range of formatted data.

Create Report: Produces a printable report. Appears only when an archive is being displayed.

Delete the selected Image(s): Deletes the images and their associated data.

Panning: Examine areas of images that extend beyond the *Viewer* edges into the display area.

Zoom in and...

Zoom Out.

Fit the image to the Viewer area.

Display at Original Size: Displays the image at its captured size.

Hide and Reveal the Record Panels.

Hide and Reveal the Viewer.

Hide and Reveal the Data Grid: Only available with LAS Archives.

Hide and Reveal the Thumbnail Gallery.

View the image Record Details.

Select the Form Details to display: Allows the user to add or remove image details from the Form.

Viewer Options: Select Dual Viewer, Lock the Views and *Lock the Pan View.*

Save the Output File: Click to save an image of the output file currently displayed in the *Dual Viewer.*

Clicking the *Show Annotations* button displays the *Annotations* and *Scale Bar Quick Launch* menu.

Basic Annotation, Extended Annotation and the *Scale Bar* setup can be launched without leaving *Browse* by checking (a tick mark is displayed) the required function. All of the function tools are available.

Additionally, any annotations applied to the current image as well as the *Scale Bar* can be displayed.

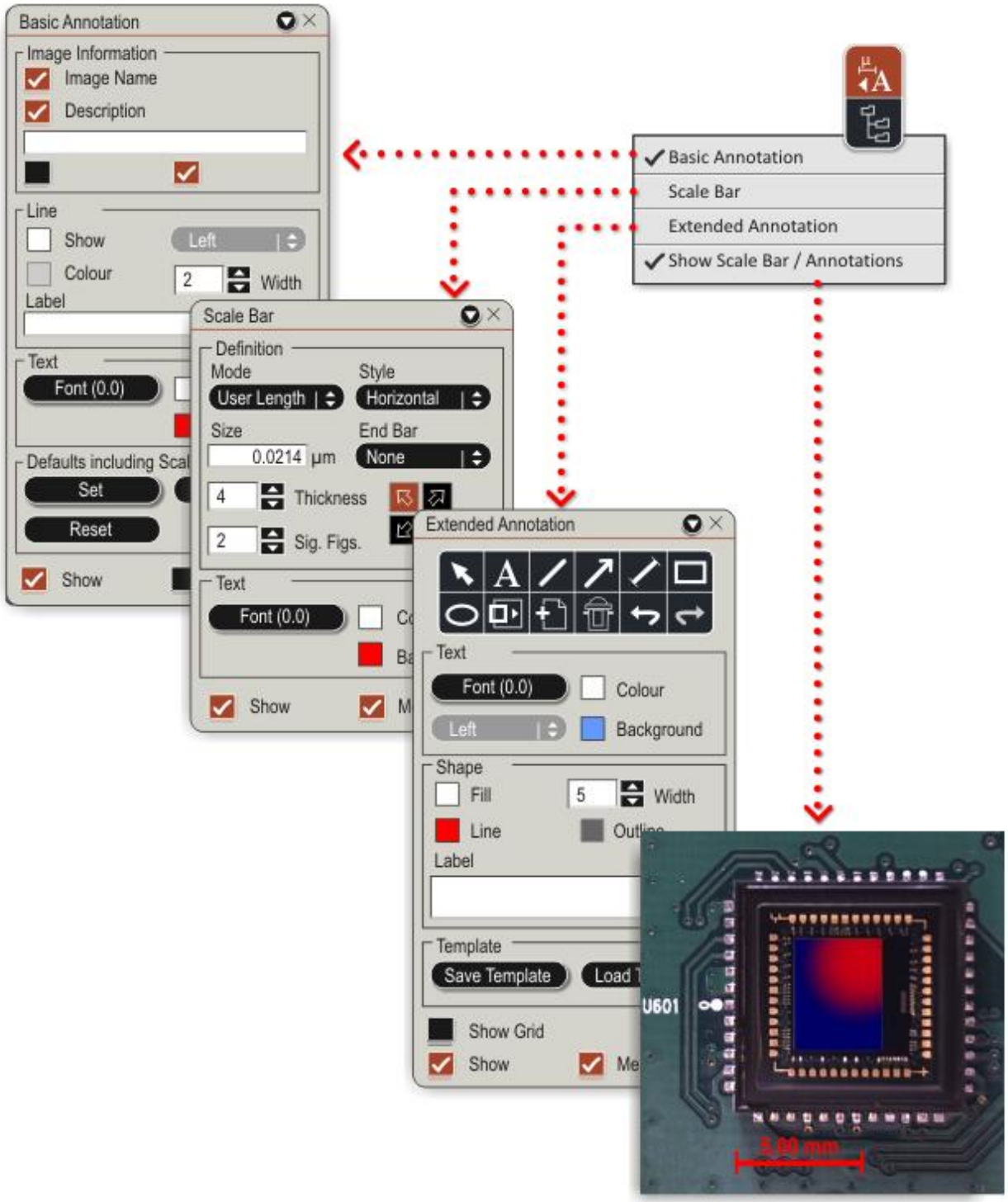


Image Stitching

LAS *Image Stitching* software has been designed to create a composite image of a large specimen from a sequence of individual 'tiles' captured on a manual stage.

LAS *Image Stitching* is ideal for small to moderate tile counts. It is fast and flexible and is part of the LAS Core and so available to all users.

Features:

- Designed especially for image capture using manual stages - demanding precision is not required.
- Suitable for a wide range of specimens - colour or greyscale.
- Areas of interest can be chosen from a tile sequence - there is no need to stitch an entire sequence.
- The number of tiles and image size are not fixed but the recommendation is for no more than 50 tiles or an image no larger 500MB.
- Background colour is user selectable.
- Smooth blending feature helps to correct uneven lighting effects or incorrect shading.
- Automatic image scaling to reduce the final image size.
- Users to set result image names with automatic suffix increments.

Using Image Stitching:

Tile Capture Guide 372.
Advanced Settings 378 - Blend and Background Colour.
Image Reduction Factor 379.
Select and Stitch all Tiles in a Sequence 377.
Stitch Selected Tiles 382.

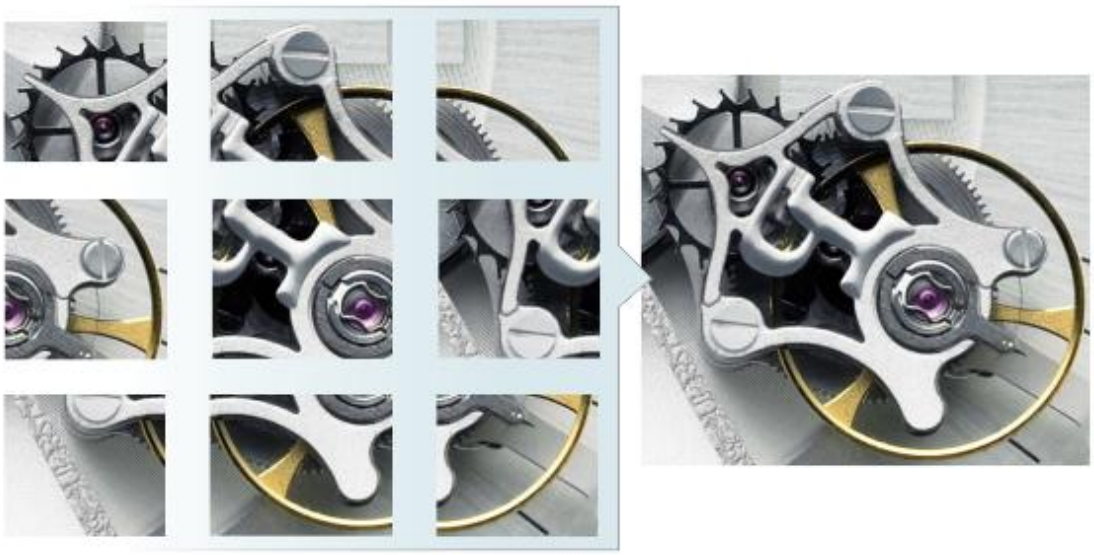


Image and Tile Requirements:

- *Image Stitching* works best with images that have complex, random detail. Avoid repetitive, grid-like detail.
- Tile overlap[373] is essential. Detailed images do not require as much overlap as images with sparse features.
- All tiles must have the same magnification and be as sharp as possible. The minimum tile size is 512 x 512 pixels.
- Tiles must have the same *Capture Format*[374] - jpg etc.
- All tiles must have same *Calibration*[375] value and must not be rotated with respect to each other.
- *Shading*[376] correction is not essential but is recommended to avoid 'striping' in the result image.
- The image can be colour or greyscale.

This is an image of a watch escapement magnified x5:

The field of view extends to just one corner...

...which means that at least 9 individual tiles are required to represent the entire image:

Because the individual tiles are captured using a manual stage, the edges are unlikely to represent precise fits to those of their neighbours. Therefore, the stitching process compares each tile with all of the others in the selection looking for matches in pixel groups that will constitute an edge and to achieve this there must be a *guaranteed* overlap between adjacent tiles.

As a guide, users should aim for about 25% tile area overlap although for images with random, non-repetitive detail this can be lower.

The illustration shows by using different colours, how the nine tiles overlap on all internal edges.

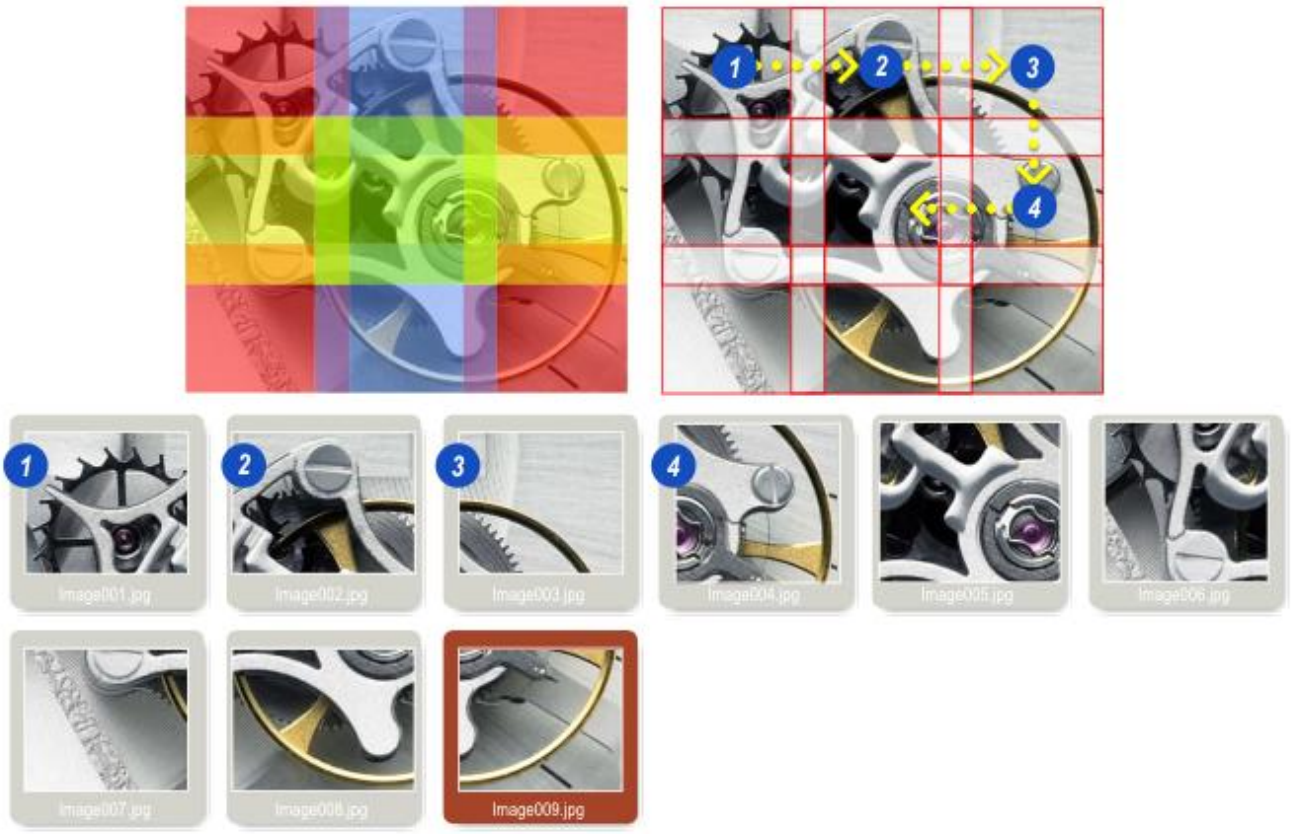
Users who have stages fitted with a Vernier scale can make some simple calculations to make sure there is sufficient overlap and use the scale to position the stage rather than just relying upon a visual estimate.

1: Capture commenced at the top-left corner.

2: The stage was them moved along the X axis to the second tile making sure there was a good overlap with the first tile.

3: This was repeated for the third tile, again ensuring a good overlap with the second.

4: Then the stage was moved along the Y axis to start the second row, checking there was adequate row-to-row overlap - and so on until the entire image had been captured.



The specimen capture settings and made in both *Preferences* > *Image* and *Acquire* > *Camera.*

1: Set the *Image Format* and compression by launching *Preferences*[63] and clicking the Image tab.

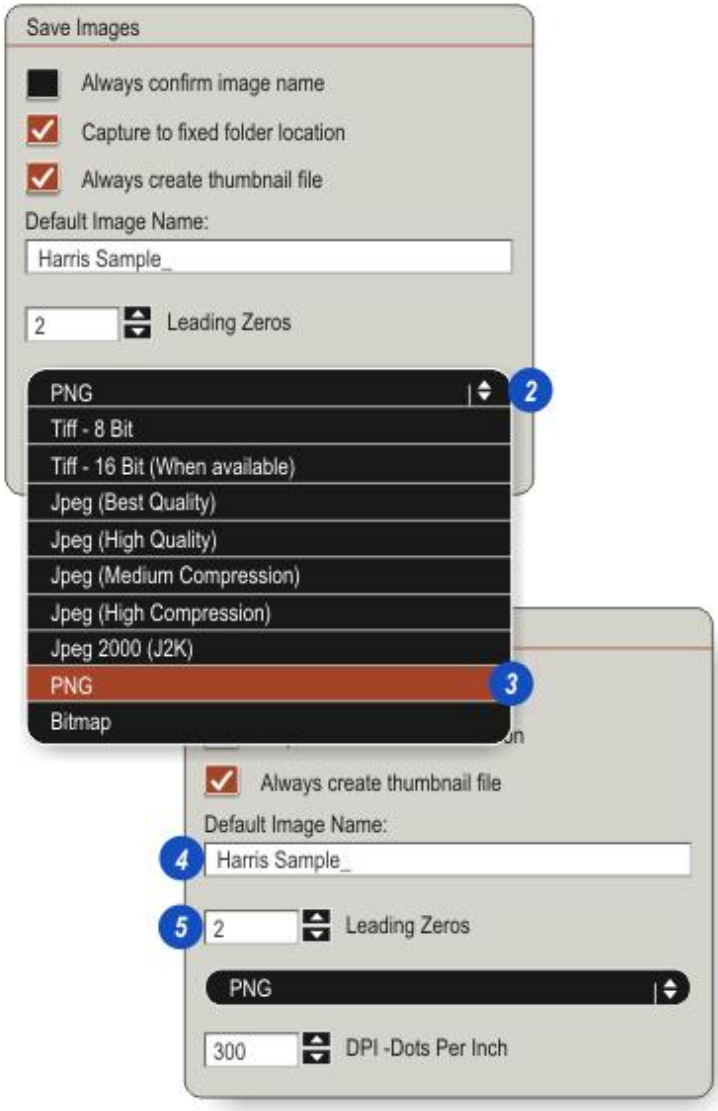
2: On the *Save Images* panel, click on the arrows to the right of the *Image Format* header and...

3: ...click to select the required format.

! Jpeg 2000 *(J2K)* is not suitable for *Image Stitching*.

4: Whilst on the *Save Images* panel, users may like to give the tiles an appropriate name by clicking inside the *Default Image Name* text box and typing a new name, and...

5: ...setting the number of zeros to be displayed in the suffix following the image name. In the example the individual tiles would be named: Harris Sample_00, 01, 02...10, 11 and so on.



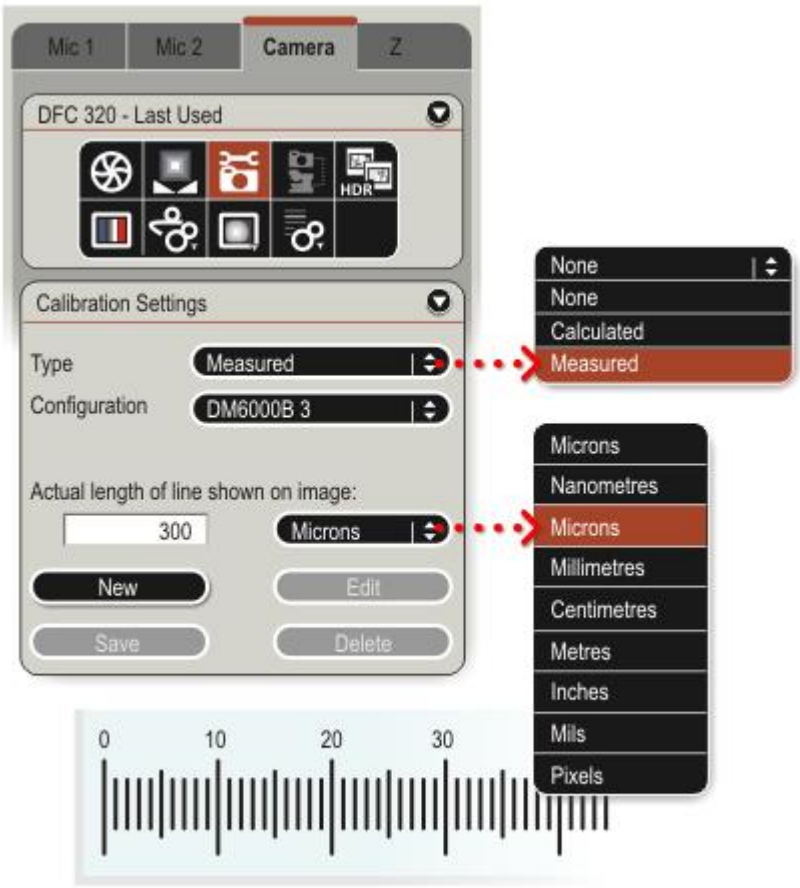
Calibration ensures that measurements displayed by the software are given in 'real world' - millimetres, inches etc - units taking into account the selected optical magnification of the microscope and the size of the camera pixels.

For initial system calibration users should refer to:

- *Acquire > Camera > Calibration* 318 .

If system calibration has changed and the tiles pre-dates the change, they can be updated to ensure they are all consistent by using *Update Calibration* found in:

- *Functions Widely Available > Update Calibration* 85 .

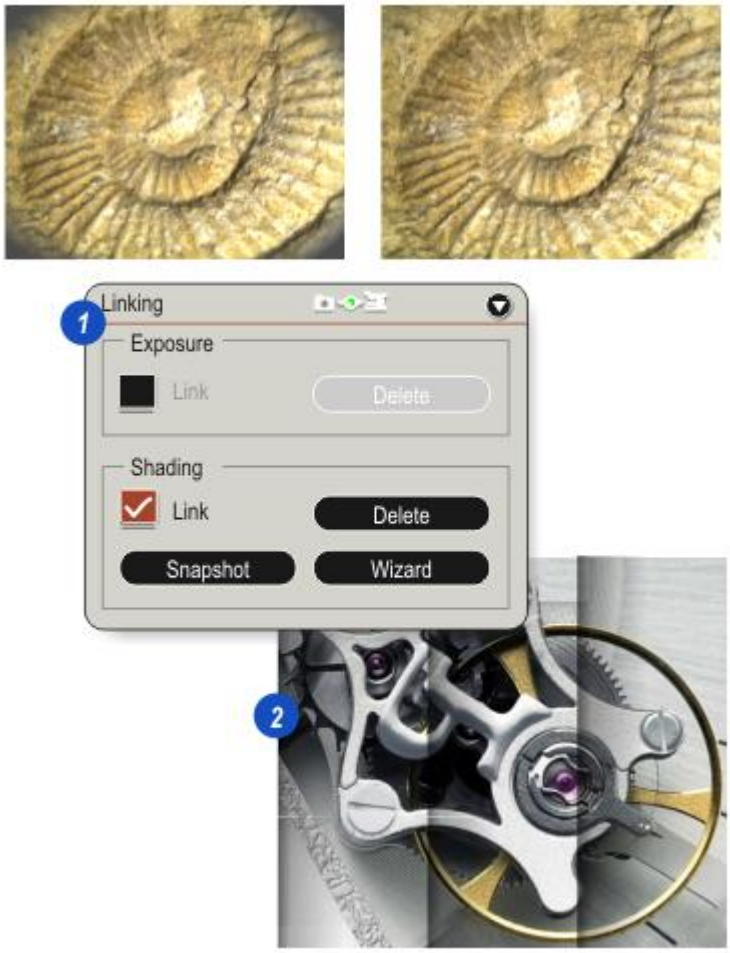


Shading is the name given to variations in the background light level across an image.

In the example, the image on the left shows the effects of shading - the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

A more evenly 'illuminated' image can be achieved by applying a digital 'correction' based upon the brighter part of the image. This is shown on the image on the right.

Because shading is caused by variations in the optics, each objective has to have the level of 'correction' applied individually.



1: Shading is described in detail and applied in *Acquire > Camera > Shading* 342.

2: With the *Shading* facility turned off, the stitched image has a 'corrugated' look caused by the dark edges.

Check the *Blend* option in *Advanced Settings* 378 to help minimise the effects of uneven lighting.

Select and Stitch all Tiles

Start *Image Stitching* by selecting the tiles to be used - in this example all of them:

1: In the *Gallery*, left click on the first image to select it and...

2: ...holding down the keyboard *Shift* key, left-click the last tile in the collection. All of the tiles between the two selections are automatically selected - the thumbnail frame is coloured brown.

3: On the *Side Tool Bar* click the Image *Stitching* button. Alternatively, right-click on one of the selected tiles and choose *Stitch Images* from the context menu.

4: The *Image Stitching* dialog appears showing the number of tiles that have been selected.

5: The stitched image is given a name preferred by the user. Click inside the *Result image name* text box and type a name.

6: Click the *Show advanced settings* 378 button to set the *Blend, Background Colour* and *Reduction Factor*:

7: The *Further Information* 380 button when clicked, provides a reminder of the image requirements.

1: After *Image Stitching* is launched and the *Advanced Settings* button is clicked, the dialog expands to reveal the *Blend, Background Colour* and *Reduction Factor* options:

Blend images where they overlap:

2: The *Blend* option smoothes the tonal transition between adjacent tile edges. It especially useful if *Shading* was not enabled or incorrectly set when the tiles were captured.

Click the check box to enable (tick mark visible) the *Blend* option. Click again to turn it off. The default is enabled.

Result image background colour:

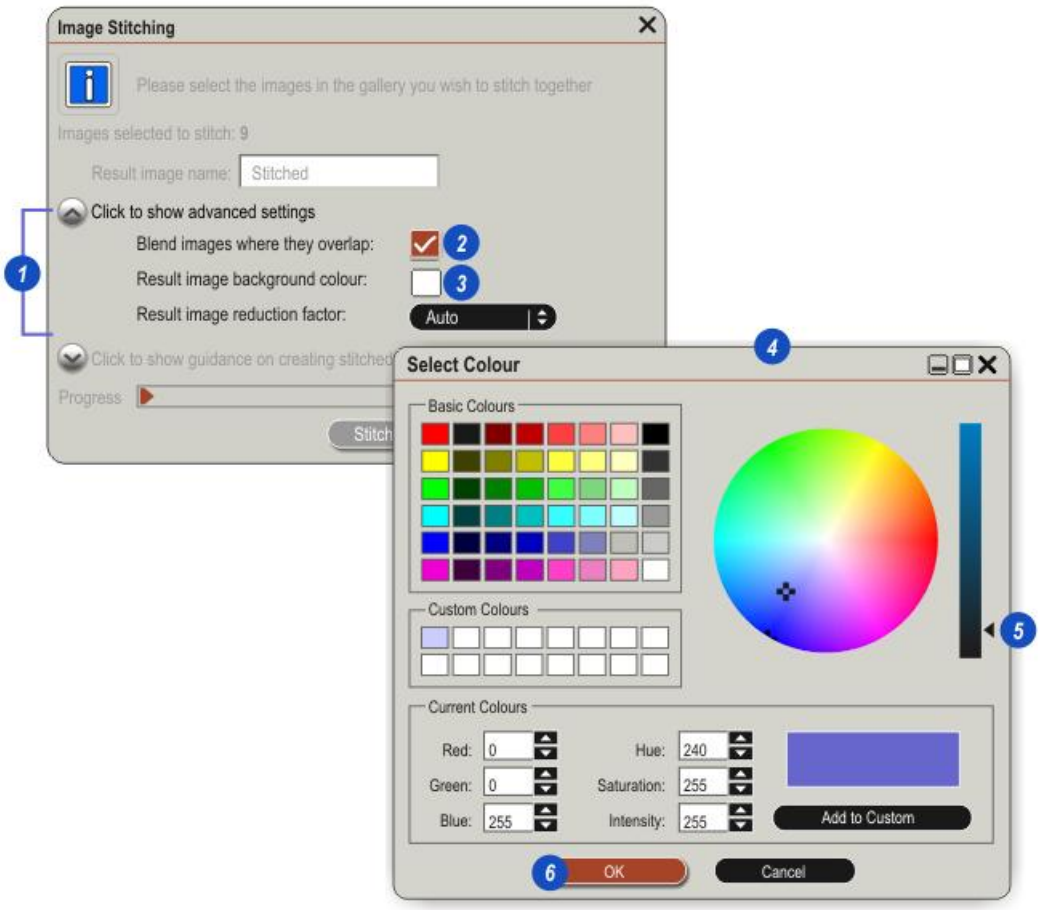
3: If unimportant tiles are not selected and therefore not included in the stitching[382], there will be a gap in the composite image. Users can select a colour to appear in the gap(s).

Click in the *Background colour* box and...

4: ...on the *Select Colour* dialog choose a background colour from the swatches, by dragging the 'target' on the wheel or typing values for red, green and blue (RGB) in the text boxes.

5: Adjust the colour intensity by clicking and dragging the slider.

6: Click *OK.* The selected colour appears in the box.



Some stitched images can be large and unwieldy making them difficult to include in reports or share digitally. The *Image reduction factor* automatically scales the result image to reduce its size.

1: Click on the small arrows to the right of the *Result image reduction factor* and...

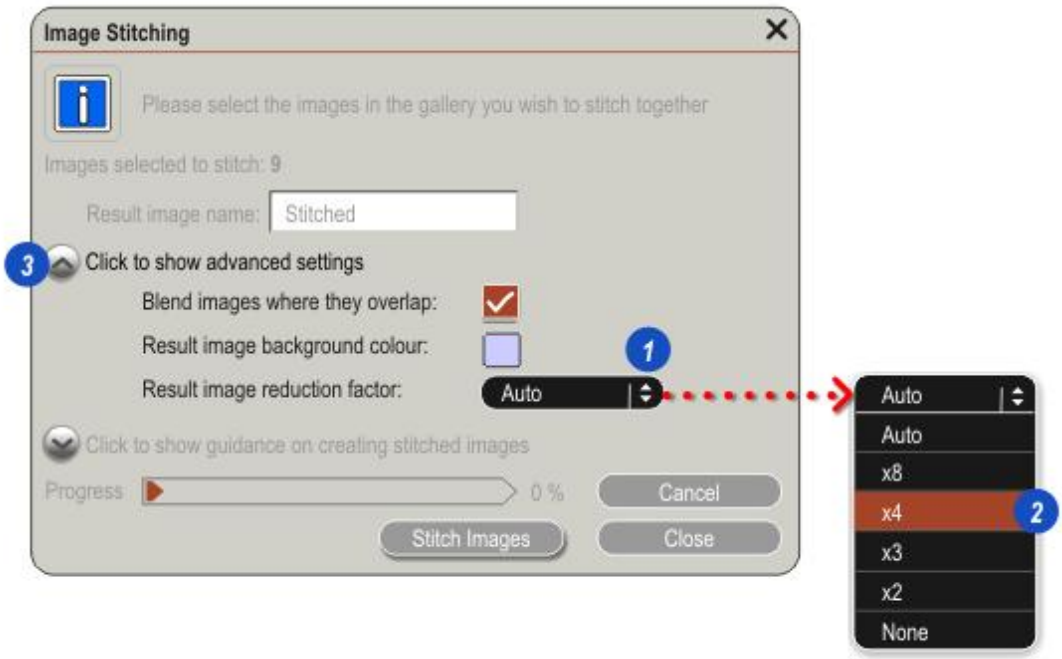
2: ...from the drop-down menu click to select the reduction factor required.

Larger values: Result in a smaller result image.

Auto: The reduction factor is based upon the pixel count of the individual tiles.

None: The result image is not scaled at all.

3: Click on the *Advanced Settings* button to close the settings.

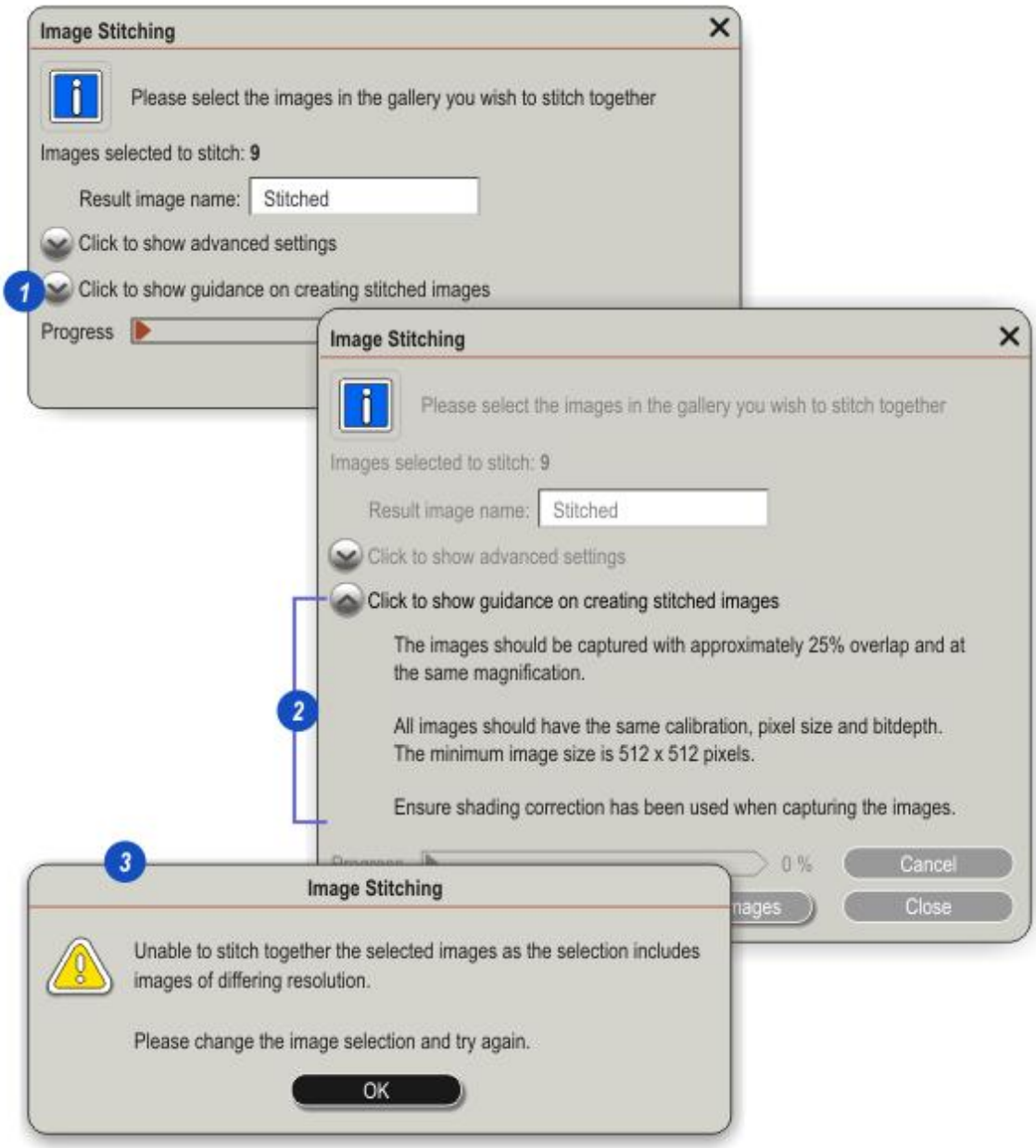


1: Click the *Show guidance...* button to...

2: ...display a reminder regarding the essential parameters for tile capture.

Click the button again to hide the dialog.

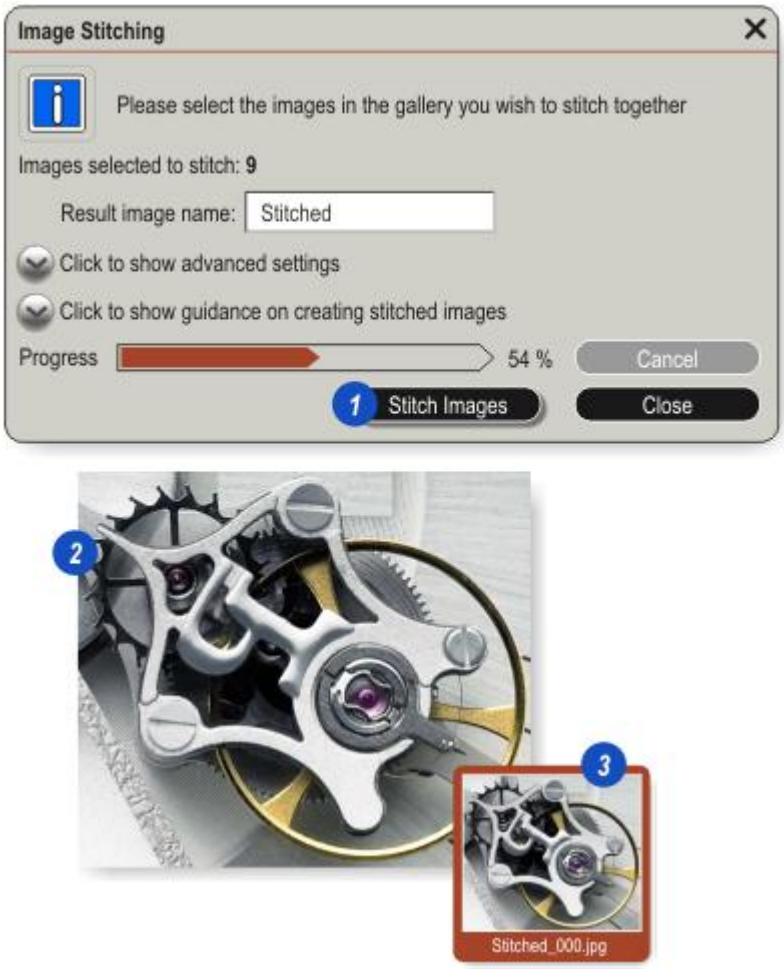
3: The *Image Stitching* program provides a range of warnings to help the user capture and select tiles that will help ensure the best possible result.



1: Start the stitching process by clicking the *Stitch Images* button.

2: The completed composite image appears in the *Viewer* together with...

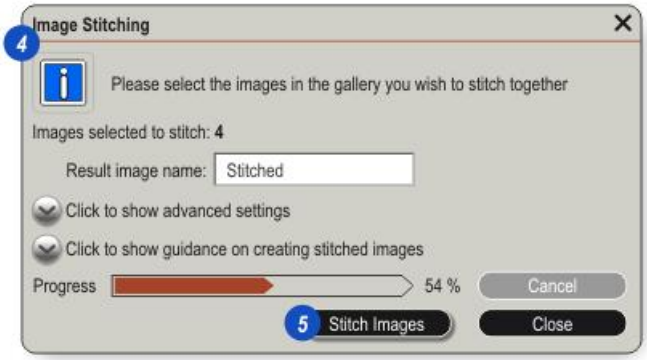
3: ...a thumbnail in the *Gallery*. The caption comprises the users preferred name together with an automatically incremented numeric suffix.



The tile selection prior to stitching does not have to include the entire sequence - tiles that represent an area of the image that is not important or needed can be omitted from the selection provided the remainder represent a contiguous area.

However, if the selected tiles do not stitch properly, include some of the omitted tiles and run *Image Stitching* again.

In this example only the main bearing is of interest and so only those tiles that are part of it are selected:



1: In the *Gallery*, left click on the first image to select it and...

2: ...holding down the keyboard *Ctrl* key, left-click the other tiles required individually. The illustration shows just the tiles that together make up the bearing.

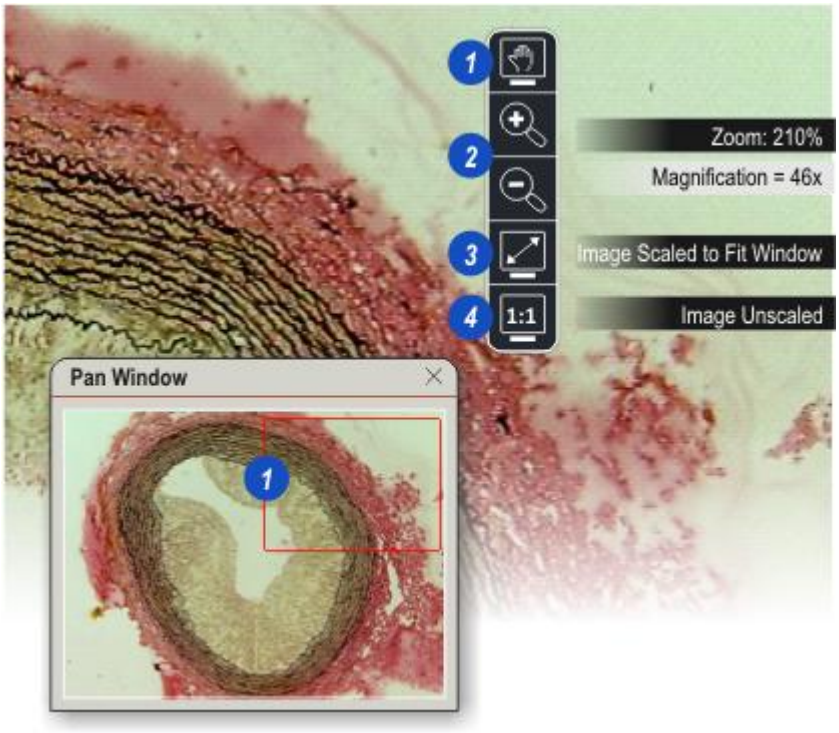
3: On the *Side Tool Bar* click the Image *Stitching* button. Alternatively, right-click on one of the selected tiles and choose *Stitch Images* from the context menu.

4: The *Image Stitching* dialog appears showing the number of tiles that have been selected.

5: Click the *Stitch Images* button.

6: The finished composite image with all of the tiles stitched together...

7: ...represented by a thumbnail in the *Gallery*. The final image can now be used like any other.



1: *Pan:* The *Pan* tool allows detailed areas of an image that exceeds the visible area of the *Viewer* to be examined. It will not work if *Fit to Viewer* is enabled because all of the image is being displayed.

On the *Pan Window* viewer, click and hold in the outlined rectangle and drag it to the area to be examined. The selected area is displayed in the main *Viewer*.

To move the *Pan Window* away from the main *Viewer*, click and hold the header bar and drag it to another part of the screen.

Click the *Pan* tool to close the *Window*.

2: *Zoom:* Click on the (+) to zoom in to the image or (-) to zoom out. The zoom level as a percentage, is displayed top right of the the *Viewer* border.

If the monitor *Magnification Settings* have been set in *Preferences* 73, the image *Magnification* value appears bottom right of the *Viewer* border.

3: *Fit to Viewer:* Click to fit the image to the available *Viewer* area regardless of the original size of the image. The *Image Scaled to Fit Window* message appears top right of the *Viewer* border.

4: *Display at Original Size:* Click to display the image at its original size. The image may appear smaller or larger than the *Viewer* area. The *Image Unscaled* message appears top right of the *Viewer* border.

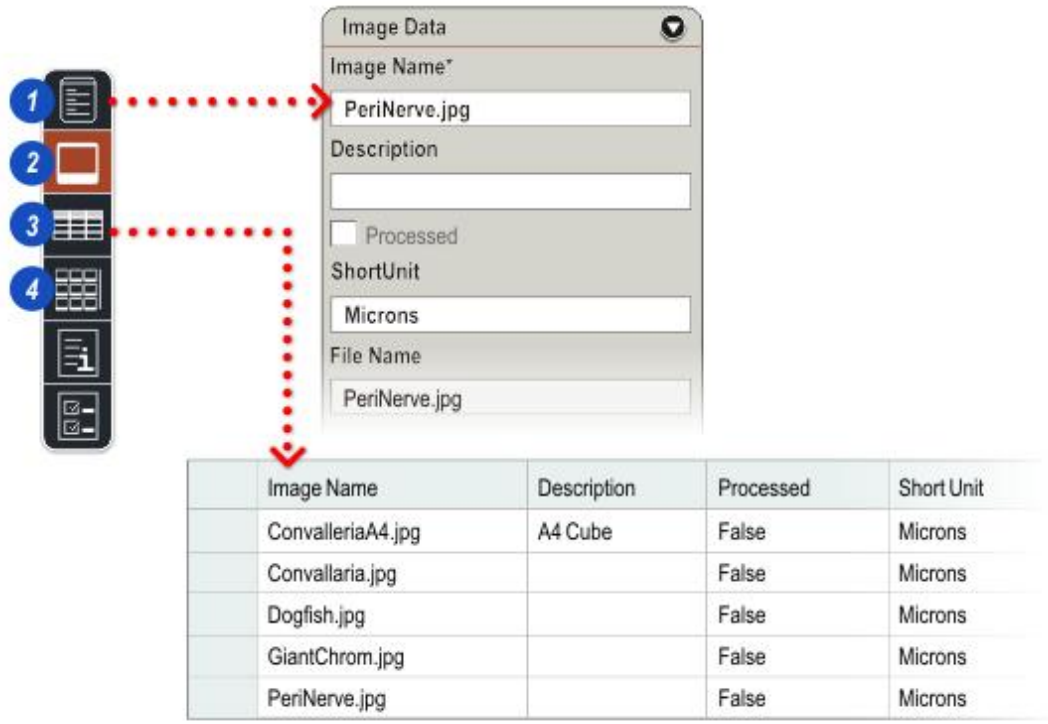
The various screen areas - *Viewer, Gallery, Report* and *Grid* (where applicable), may be revealed or hidden to create the best working environment for the user. Some tools are toggles – click once to reveal the area, click again to hide it:

1: *Hide/reveal the Record panels:* The *Image Viewer* expands to fill the vacant space.

2: *Hide/reveal the Viewer:* The *Record* panels expand to occupy the *Viewer* width.

3: *Hide/reveal the Data Grid:* The *Viewer* will expand to cover some of the vacated space. The *Grid* is only available if an *LAS Archive* is installed.

4: *Hide/reveal the Thumbnail Gallery:* The *Gallery* is hidden and the *Viewer* expands to include the *Gallery* space.



Delete a single image by clicking its thumbnail and then the *Delete (Trash Can)* button.

Alternatively, delete a group of images by:

1: Hold down the *Crtl* key and click on a thumbnail. Do this for all of the images to be deleted, or...

2: ...hold down the *Shift* key and click on the first thumbnail. Do the same for the last thumbnail in the group to be deleted and all of the images between are selected.

3: Click the *Delete (Trash Can)* button.

4: Confirm that the images are to be deleted and cannot be recovered and the images and their data will be removed from the folder.

The *Viewer* area can be split to show two captured images simultaneously.

1: Click the *Viewer Options* button.

2: Click to enable (tick mark visible to the left) the *Dual Viewer* option. The *Viewer* will then divide into two panes.

3: The image currently being viewed will usually appear in the left-hand pane. If it does not or the image needs to be changed, click on the left-hand pane and...

4: ...click a thumbnail in the *Gallery*.

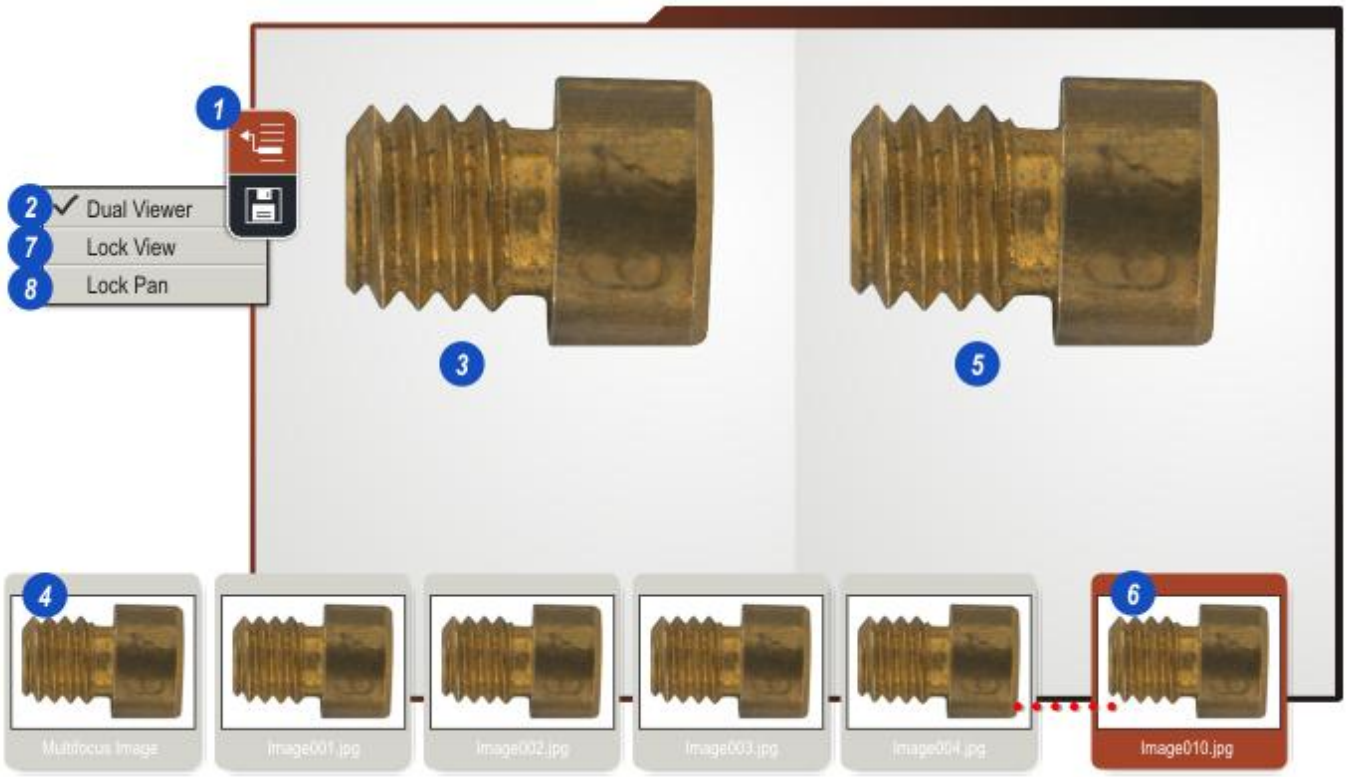
5: Display an image in the right-hand pane by clicking the pane and...

6: ...the thumbnail of the required image.

7: To synchronise the panes and enlarge or reduce the images as the zoom and fit tools are used, click to enable the *Lock View* option.

8: Enabling *Lock Pan* will synchronise the images as the *Pan* tool is used. Click the pane to pan and then on the *Panning* tool. Both images will automatically move to and display the image segment shown in the *Pan* window.

Dual Viewer more information 95

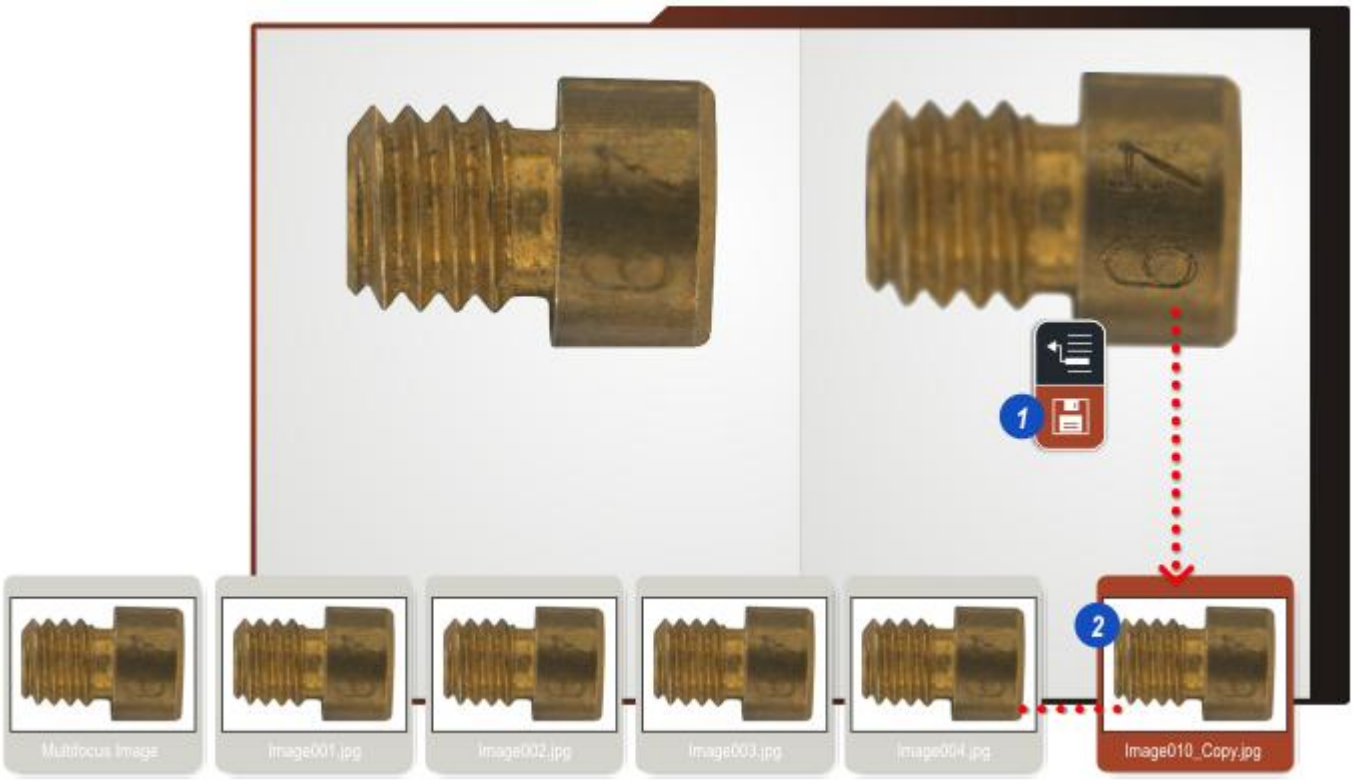


Copy Output Image

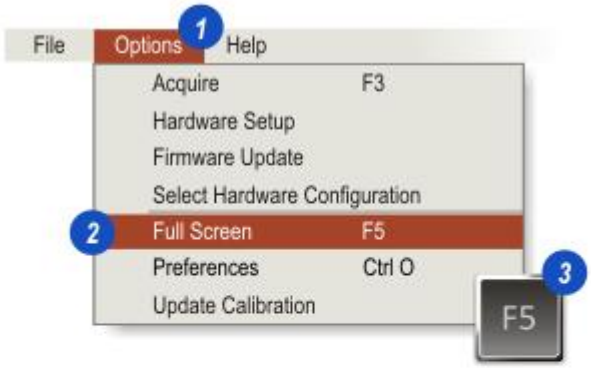
The output image can be copied (duplicated) by:

1: Click the *Copy Output Image* button.

2: The image is copied to the current folder with the output image name plus the suffix '_Copy'.



Full Screen Mode: Single Monitor:



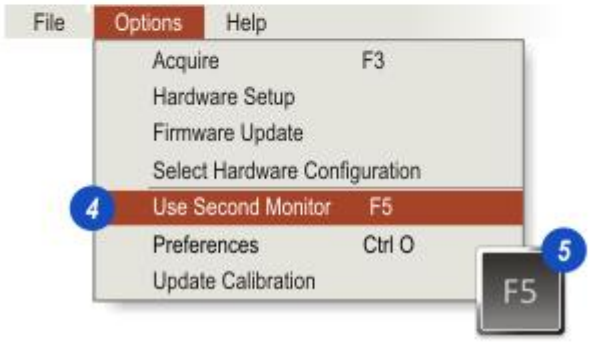
1: The *Viewer* area can be expanded to fill almost the entire screen by either clicking *Options* on the main menu and...

2: ...selecting *Full Screen* from the drop down menu, or...

3: Pressing *Key F5*. Press *F5* again to return to the normal display.

Second Monitor:

The software detects a second monitor and changes the *Options* drop-down menu to:



4: ... *Use Second Monitor*. Click the option to use both monitors.

The *Viewer* and image occupy all of the second monitor whilst the *Gallery* and *Thumbnails* together with the controls remain on the primary monitor.

The *Side Tool Bar* buttons are appropriately shared between the two monitors.

5: Alternatively, press *Key F5* to move between using both monitors and returning to single monitor.

A wide range of options is available by right-clicking the image in the *Viewer* or its *Thumbnail* in the *Gallery*. The options vary depending upon which item was clicked, the operating system and the software installed on the computer.

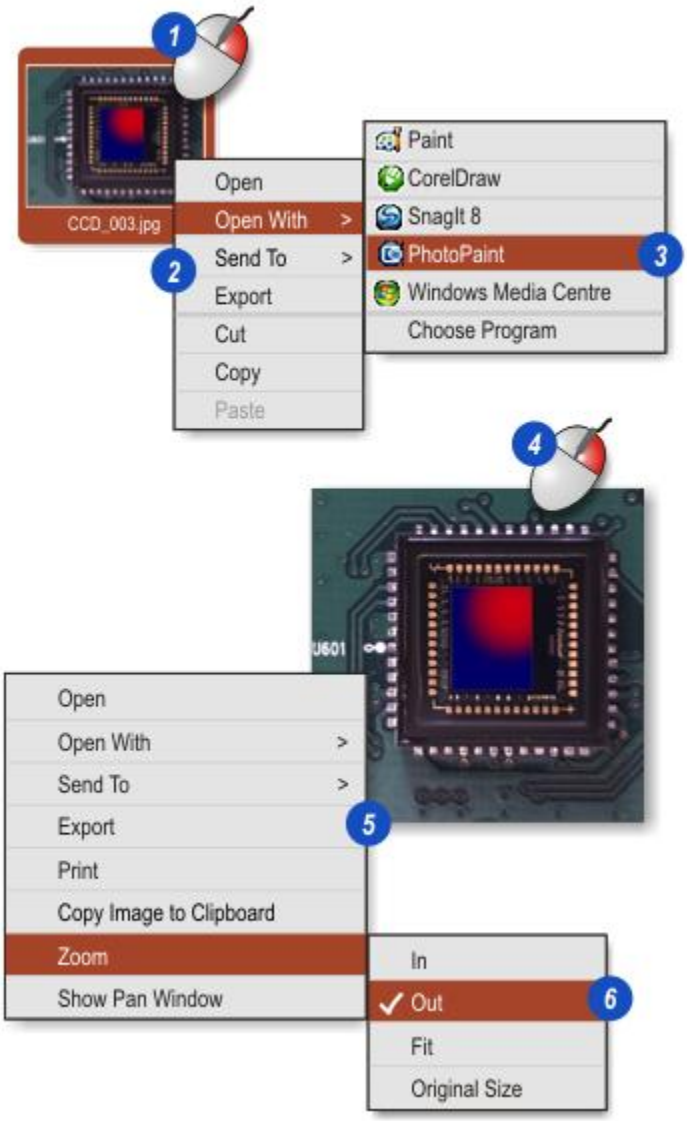
1: Right-click the *Thumbnail* for the context menu of basic options and...

2: ...click it to select.

3: Some options have additional possibilities displayed as a sub-menu.

4: Right-clicking the image in the *Viewer* displays a different context menu.

5: Additional functions are available some of which will also have sub-menus **(6)**.



The *Gallery* is a thumbnail display of images in the current folder in both *Image Explorer* or *LAS Archive.*

The *Gallery* can be hidden or revealed using the *Side Tool Bar* tools.

1: Clicking on a thumbnail will immediately display the full-sized image in the *Viewer* and the data associated with it in the *Record* and *Grid* (If LAS Archives is installed).

2: A *Slider* is automatically displayed for multiple rows of thumbnails - click and drag it to scroll the *Gallery*.

3: The *Navigation Bar* (bottom right of the screen) provides a way of moving through the thumbnails quickly and is especially useful with large galleries of thumbnails. Click on the arrows to move a single image **(3)** ...

4: ...or go to the extreme ends of the *Gallery*.

5: The thumbnails can be re-sized by clicking and dragging the *Scaling Slider* - slide left to reduce the thumbnail size and right to increase it.

6: Move the mouse over a thumbnail to reveal basic data about the image.

7: Right-click a thumbnail to show the *Context Menu*. Left-click to select an option.

- *Gallery Docking Position* 391

Docking Position

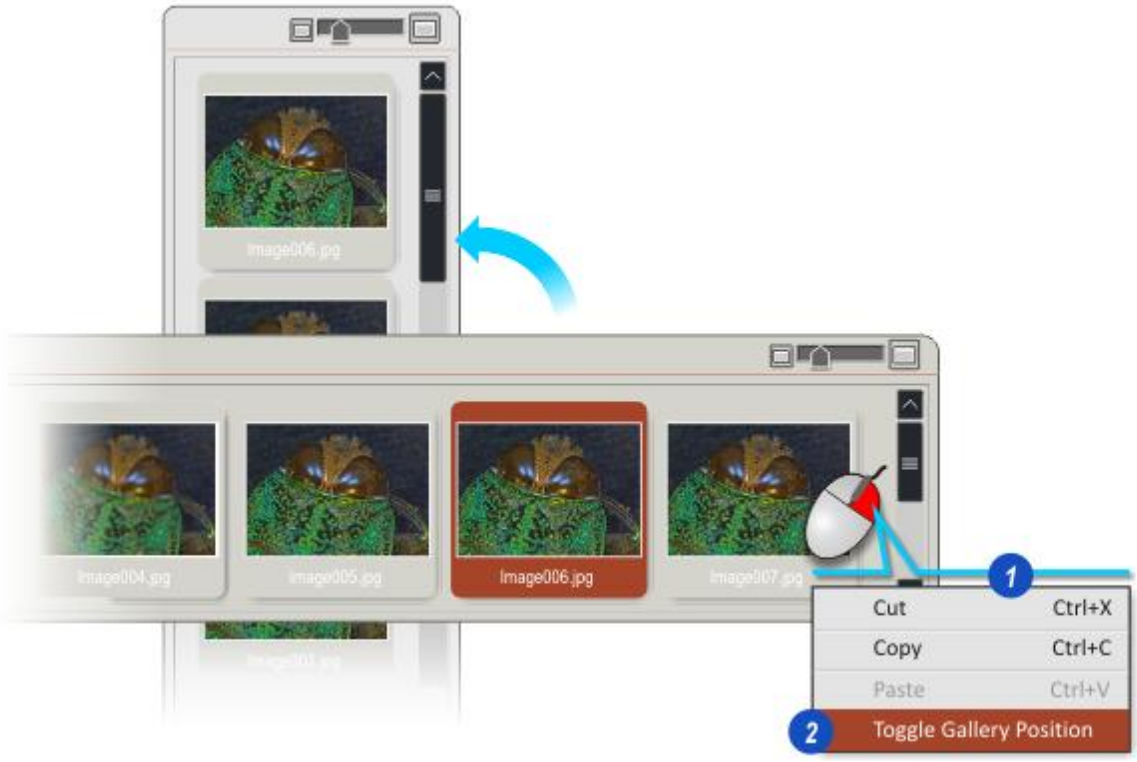
Available in *Acquire* and the *Process* Workflows as well as *Browse*, the thumbnail *Gallery* can be 'docked' either horizontally - along the bottom edge of the *Viewer* - or vertically - along the left-hand edge of the *Viewer* - to suit the user.

1: Right-click on a thumbnail or on the spaces around the thumbnails and...

2: ...from the drop-down menu, left-click to select the *Toggle Gallery Position* option.

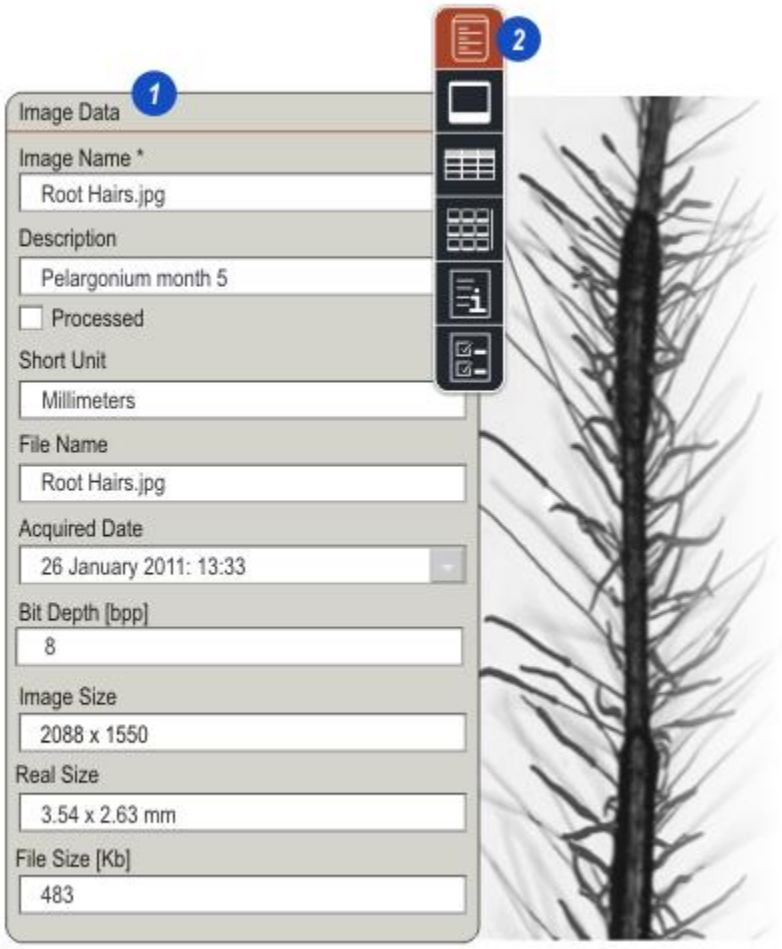
The action toggles between horizontal and vertical docking.

Scroll bars, if required, are placed automatically.



1: The *Data Form* displays selected data associated with the image. All of the data about the camera, microscope, exposure, creation date and so on, are actually stored and all can be displayed, but the *Form* can be configured to display only the more pertinent items.

2: The *Data Form* can be hidden by clicking the *Form* button on the *Side Tool Bar*. This is a toggle action - click again to reveal the *Form*.



Select Visible Fields

To select the data (Fields) to be displayed on both the *Data Form* and on the *Grid (*if an *LAS Archive* is installed):

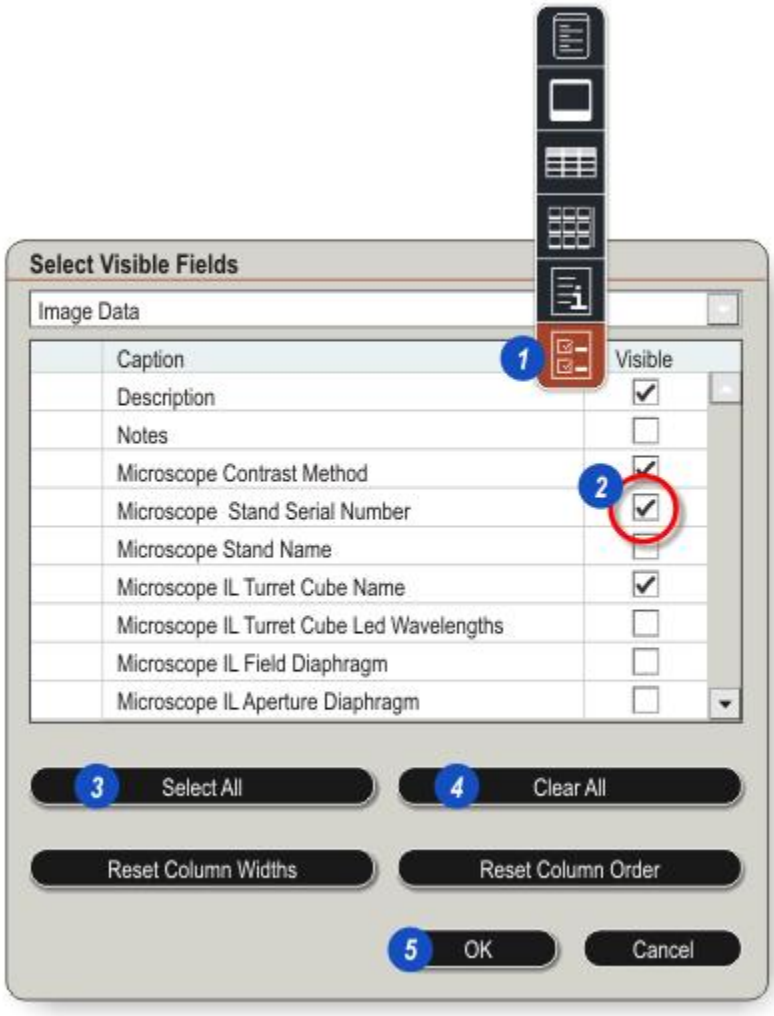
1: Click on the *Visible Fields* button on the *Side Tool Bar.*

2: To display a data field, click in the check box to the right of the field name. This is a toggle action - click again to clear the check box and hide the field.

3: Display all of the data fields on the *Data Form* by clicking the *Select All* button. Because there will now be too many items to display simultaneously, a *Scroll Bar* is automatically positioned to the right of the *Data Form.*

4: The *Clear All* button will hide all of the data fields and is useful before making a new selection range.

5: Click *OK* to close the *Select Visible Fields* dialog. The chosen data fields are immediately displayed on the *Data Form*.



1: The data fields displayed on the *Data Form* are also displayed on the *Grid* (if an LAS Archive is installed) - click the *Grid* button on the *Side Tool Bar* to reveal it.

The order of columns on the *Grid* can be changed by clicking on the column heading to be moved, holding down the mouse button and dragging the column to a new location.

In a similar manner, column widths can be changed by dragging the column *Dividing Bar* to the required width.

Reset the column widths by:

2: Click on the *Visible Fields* button on the *Side Tool Bar.*

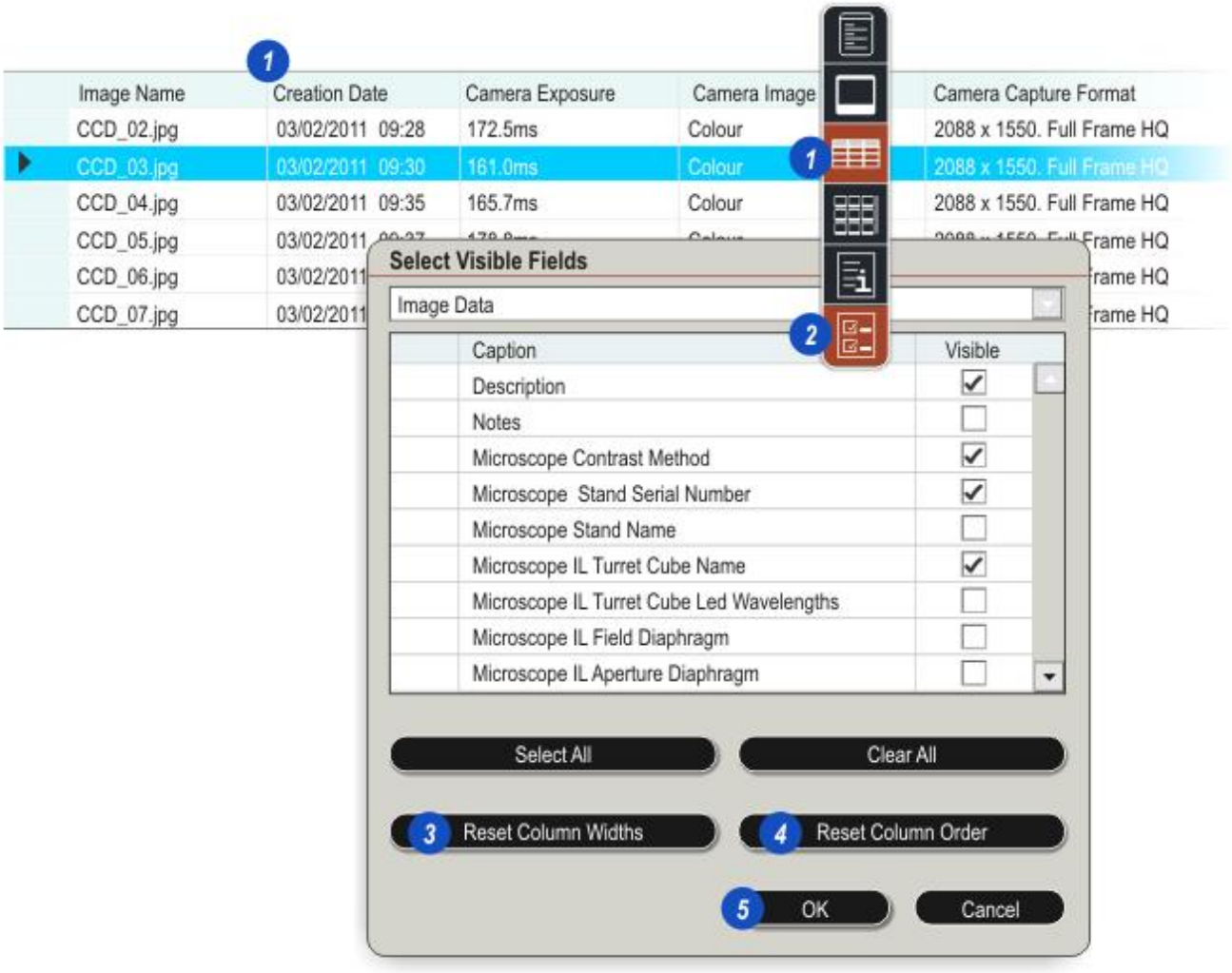
3: Click on the *Reset Column Widths* button.

Reset the column order to match the *Data Form* by:

2: Click on the *Visible Fields* button on the *Side Tool Bar.*

4: Click on the *Reset Column Order* button.

5: Click *OK*.



To view all of the available data and if LAS Archive is installed, select them for use in a report:

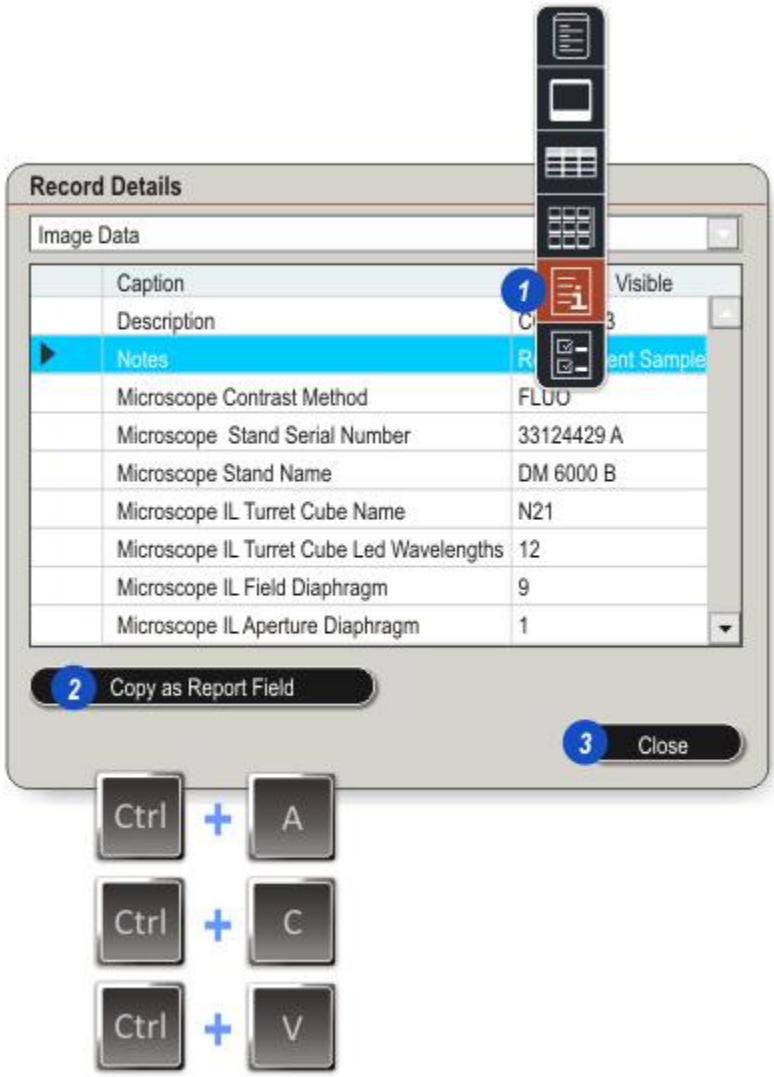
1: Click on the *View All Details* button on the *Side Tool Bar.*

The *Record Details* dialog displays all of the data fields available for the chosen image and the values that have been captured for them. Use the *Scroll Bar* on the right of the window to scroll through the list.

2: To use a field in a report, click on the required item to highlight it and then on the *Copy as Report Field* button. The field description is copied and pasted into the report template.

Use the keyboard shortcut keys to:
Select all fields: Ctrl + A
Copy all fields: Ctrl + C
Paste copied fields: Ctrl + V

3: Click the *Close* button to close the dialog.



The *Grid* displays data for all of the images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

2: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* and also highlight the thumbnail.

3: Header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

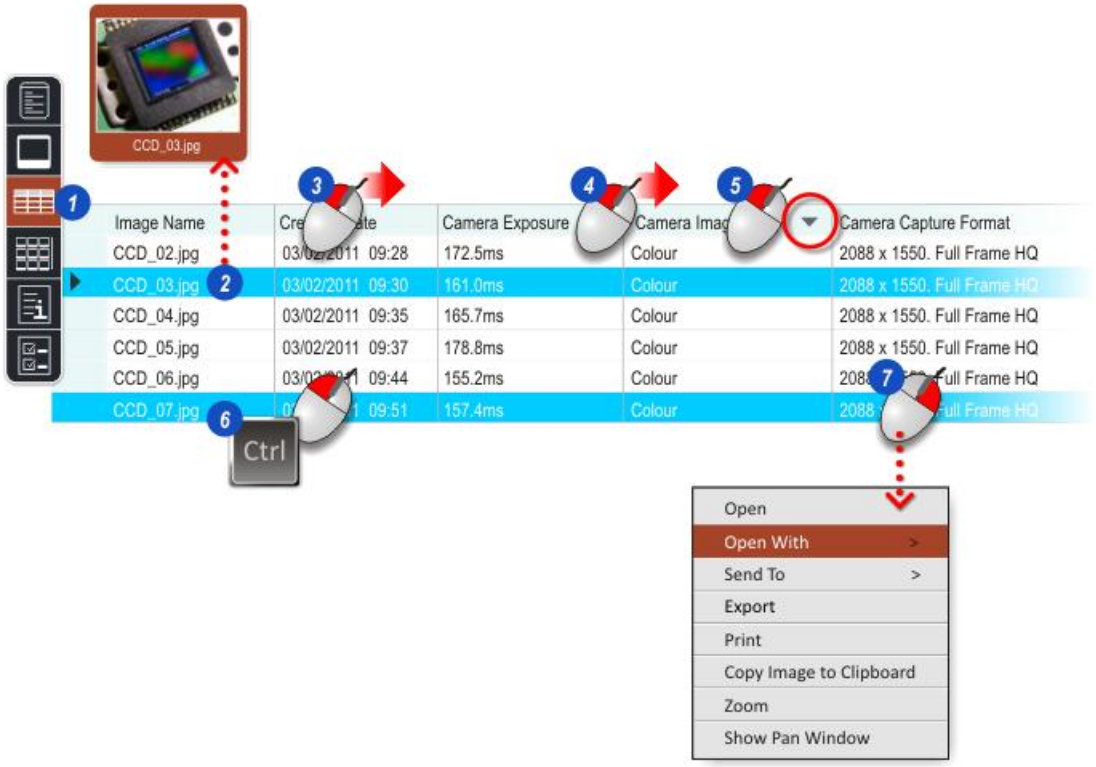
4: Column widths can be changed by clicking and dragging the vertical bars that separate the columns.

5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: To make multiple selections prior to deleting or exporting, hold down the keyboard *Ctrl* key whilst clicking individual thumbnails.

Keyboard combination *Ctrl* + *A* will select all of the image data.
Ctrl + *C* will copy all the selected image data to the clipboard.
Ctrl + *V* will paste into another application.

7: The *Grid* data can be exported to a range of other applications by right-clicking on the *Grid* then navigating to and clicking to select an application.



Providing the *Store and Recall* module is registered and enabled, the *Store and Recall Settings* panel details the microscope data - camera and microscope setup - relevant to the displayed image.

1: Click on the expand/collapse arrow to the right of the header bar to display the settings.

2: Click the *Recall* button and...

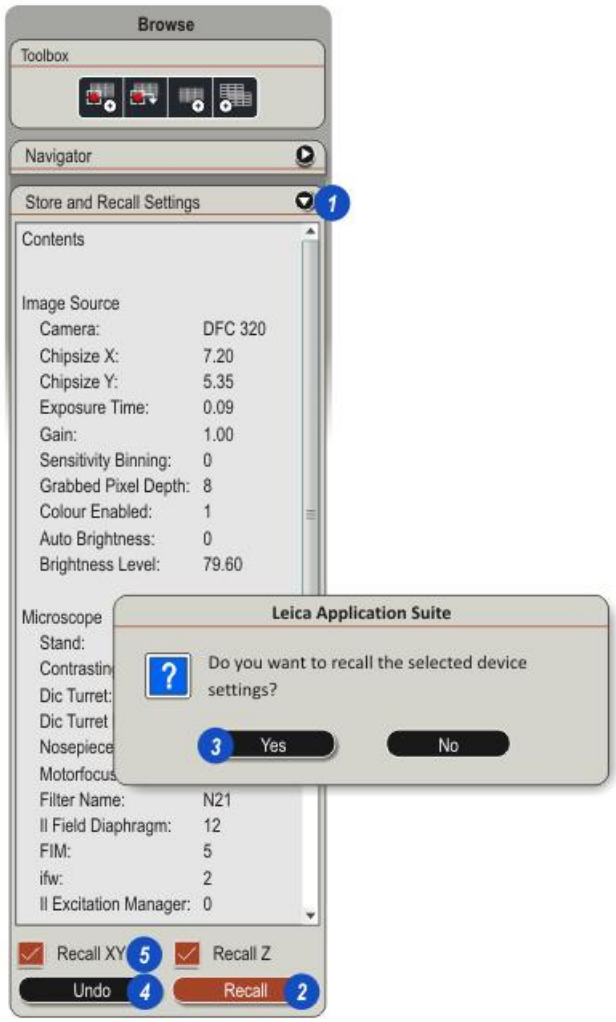
3: ...the *Recall Confirm* dialog appears. Click *Yes* to confirm.

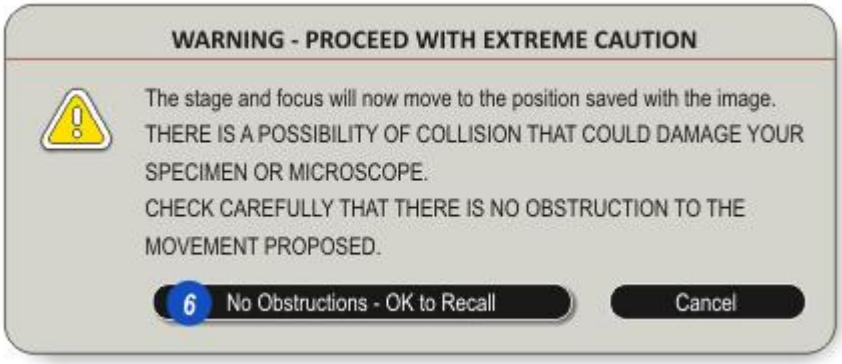
If the system has an automated microscope attached, it will automatically return the microscope and camera to the settings that were used to acquire the image.

4: To cancel the restore and return to the previous settings, click the *Undo* button.

5: If the image *XY* and/or *Z Stage* positions are required, click to enable (tick mark visible) the appropriate check box and...

6: ...when the Recall button is clicked the stage movement warning appears. Check that the stage and objective are clear of any possible obstructions and click the *No Obstructions - OK to Recall* button and the stage will move to the original capture positions.





By using the *Process Workflow* with its *Basic Annotations* and *Enhance* features, images can be used to convey a wealth of added information and detail.

Annotations can either be saved with the image so that they can be edited at any time, or when the user is satisfied with the results merged with it so that the data is still visible when the image is exported.

- *Basic Annotations* to add simple pointers, labels and captions to your image.
- *Enhance* has the tools to brighten, adjust saturation, gamma and contrast as well as rotate and crop an image.

Digital images can be annotated with graphics and text to provide information or to indicate features of interest.

The annotations are stored as a file with the image but separate from it and recalled whenever the image is displayed. They can be positioned or modified if required. However, they can also be merged with the image to produce a single, integrated image that can be exported to other applications with the annotations intact.

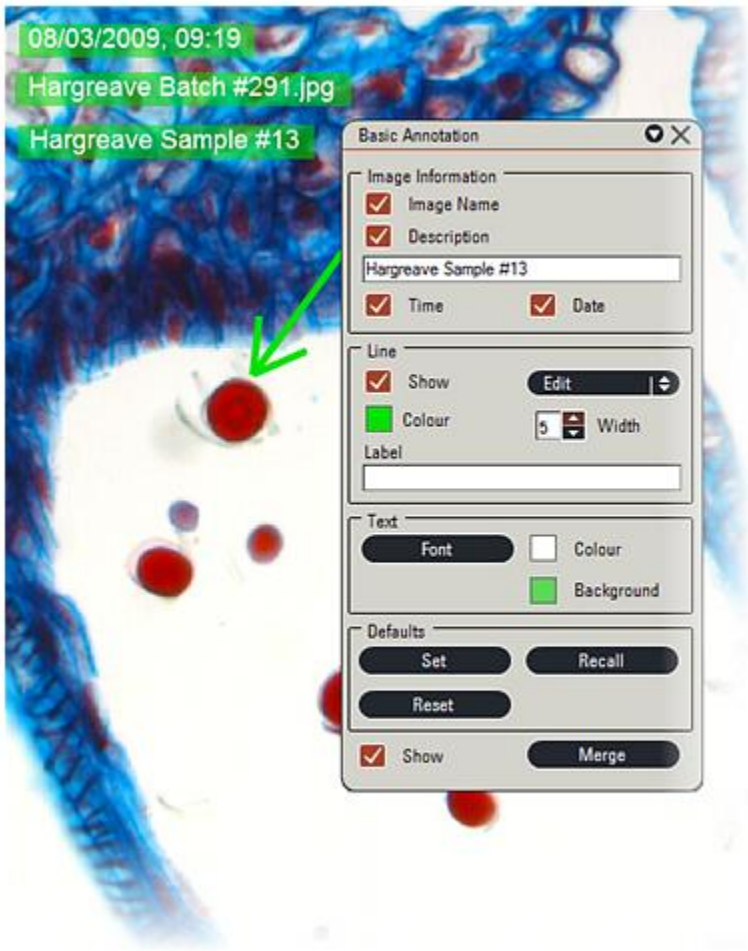
The essential tools allow automatic annotation with an Image Name, Description, Time and Date simply by enabling appropriate check boxes. A line can be drawn at any angle formatted as a Arrow with a label, a simple Line or to indicate a point-to-point Distance.

Annotations appear in colour overlaid on an image regardless of its colour depth - colour or monochrome.

Where images are larger than the window and it is possible to scroll the image, the annotations scroll to retain their positions. If the image is zoomed or reduced the annotations are scaled accordingly but limited to a readable size.

Basic Annotations normally reside on the Process Workflow but can be invoked from all of the Workflows (except Setup) using the Side Tool Bar icon.

The Annotation tools are designed to be quick and easy to use; For greater power and functionality the optional module Extended Annotation module can be added to the suite.



Basic Annotation features:

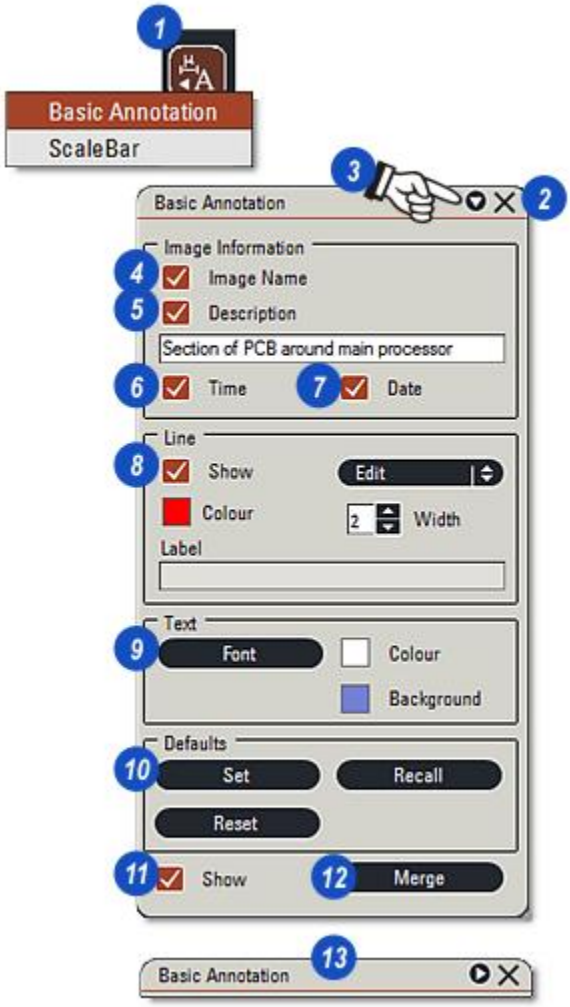
- The Control Panel [400]
- Display and Image Name and Description [401]
- Display the Time and Date [402]
- Draw a Line with a Caption Label [403]
- Selecting the Font [405]
- Changing the Font and Background colours [406]
- Defaults and Merging [407]

To launch the *Basic Annotation Control Panel*:

1: Click on the *Basic Annotation* icon on the *Tool Side Bar* and select the *Basic Annotation* option.

2: The Control Panel can be closed and re-docked in the *Process Workflow* by clicking the '*X*' to the right of the dialog caption.

3: Collapse the panel if it is obscuring the *Viewer* or other controls by clicking the small arrow to the right of the dialog caption **(13)**. Click and drag the Control Panel dialog caption to move it to another part of the screen.



The *Basic Annotation* features are:

4: Display Image Name 401

5: Display a Description 401

6: Show the Time 402 and…

7: …the Date 402

8: Draw an Annotation Line 403

9: Set the Font and Colour 405

10: Set and Reset the Defaults 407

11: Show/Hide the Control Panel 407

12: Merge

Display Image Name and Description

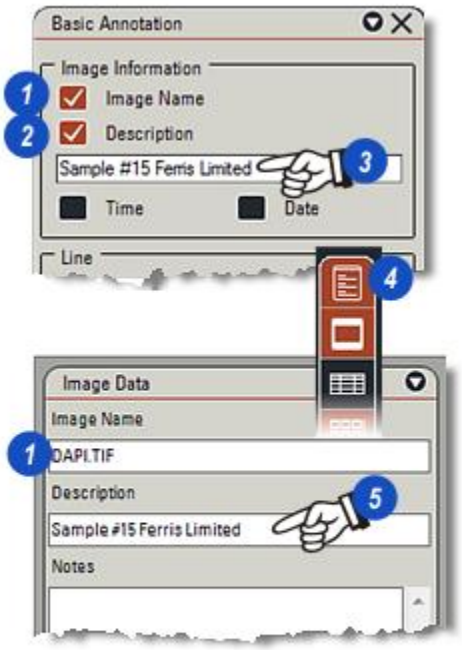
1: Click to enable the *Image Name* check box and the name by which the image was saved appears bottom left of the image **(6).** Click and drag it to another position if required. Whilst working on a live image the *Image Name* is not yet available but will be displayed after the image is captured.

2: To display a description on the image, click to enable the *Description* check box. The text is displayed bottom right of the image **(7)** but can be moved to another location by clicking and dragging.

3: Description text can be typed in the text box beneath the *Description* check box or, for a captured image…

4: …clicking the *Form* icon to display the Image *Data Form* and…

5: …clicking in the *Description* text box and typing a description.



1: Click the *Time* check box to display the current time in the top right corner of the image. It can be clicked and dragged to another location. The time format (12 or 24 hour) is determined by the computer settings.

2: To display the date in the regional format set in the computer, click to enable the *Date* check box. If both *Time* and *Date* are enabled they are displayed as a single line.

The illustration shows *Date* and *Time* together with the *Image Name* and *Description* dragged to a new location.

1: Click the *Show* check box to enable line drawing.

2: Click on the Up/Down arrows to the right of the *Size* text box to Increase/Decrease the width of the line. The size of distance line ends and arrow heads scales with the line thickness.

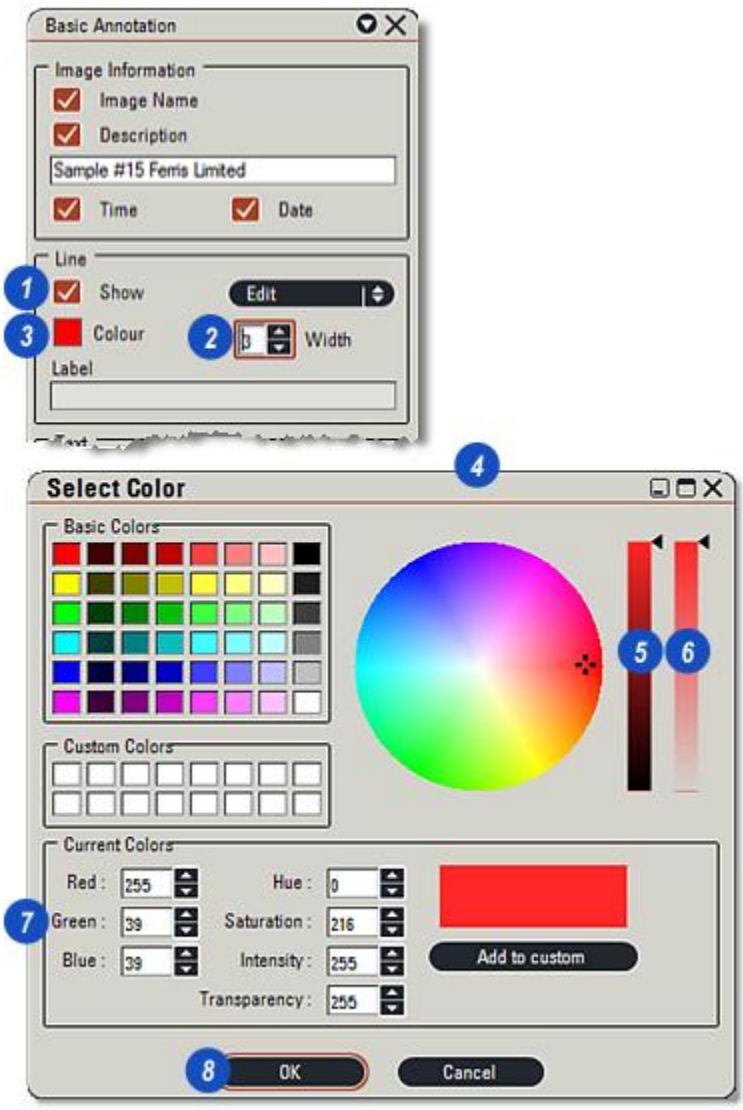
3: Click on the *Colour* button and…

4: …on the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes **(7)** and type the Red, Green and Blue values.

5: Adjust the colour by clicking and dragging the *Colour Shade* slider.

6: Adjust the colour transparency if required by clicking and dragging the slider on the *Transparency* bar.

8: Click *OK*. The new colour is shown on the *Colour* button.



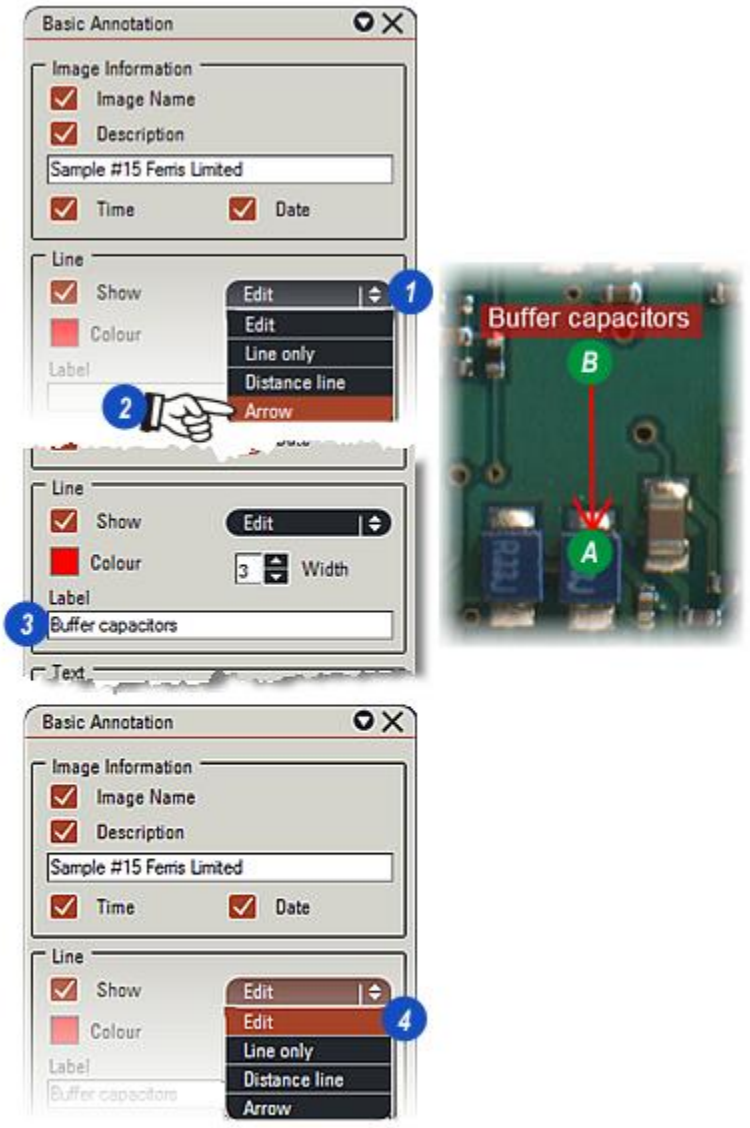
1: Choose the *Line Style* by clicking on the small arrows to the right of the *Style* drop down menu and…

2: …click to select the required style. The *Line Only* option draws a plain line; *Distance Line* draws a line with bars at each end; *Arrow* draws a line with an arrow head.

A: To draw the line, click and hold down the left mouse button at the start point, drag to the end point **(B)** and release the mouse button. Whilst drawing, the line can be dragged in any direction and at any angle.

3: To add a *Caption Label*, click in the *Label* text box and type the caption. It can be clicked and dragged to re-position it on the image.

4: *Line Width, Colour, Style* and the *Label* can be changed at any time (before merging) by selecting the *Edit* option from the *Style* menu, clicking on the item to be changed and altering the settings as detailed above.

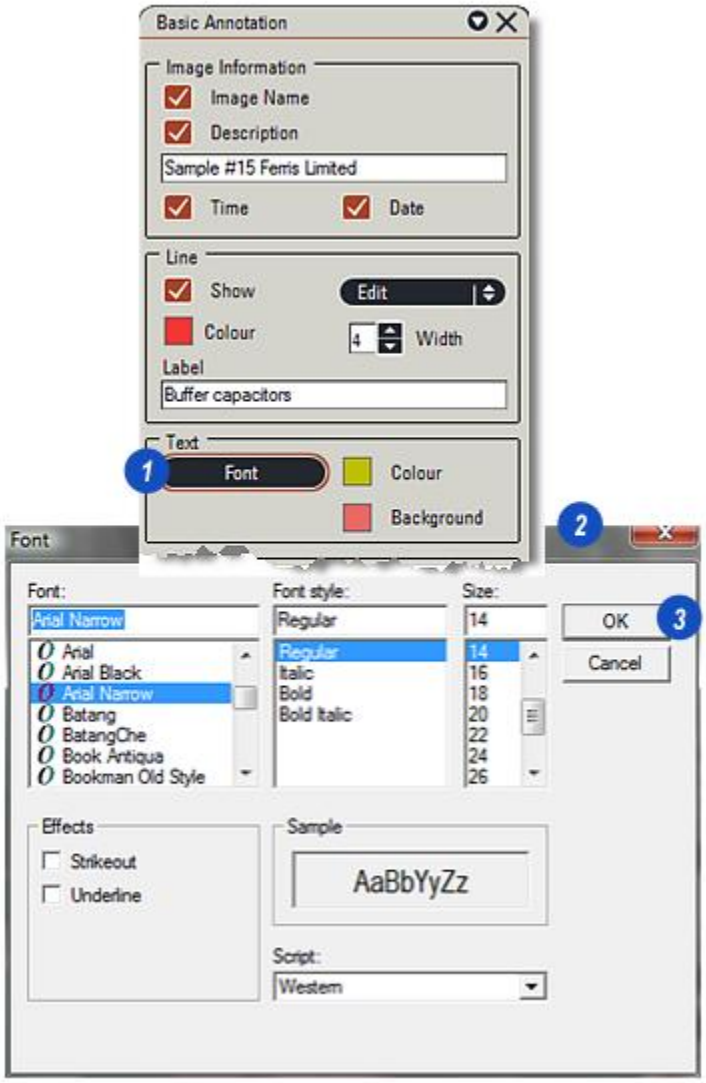


To change the *Font* settings for all of the *Basic Annotation* labels:

1: Click on the *Font* button.

2: On the *Font* dialog, select the *Font Type Face*, *Style* and *Size* as required and…

3: …click *OK*.



The *Font* colour and the *Label Background* colours are both selected in the same way:

1: Click on the (Font) *Colour* button or …

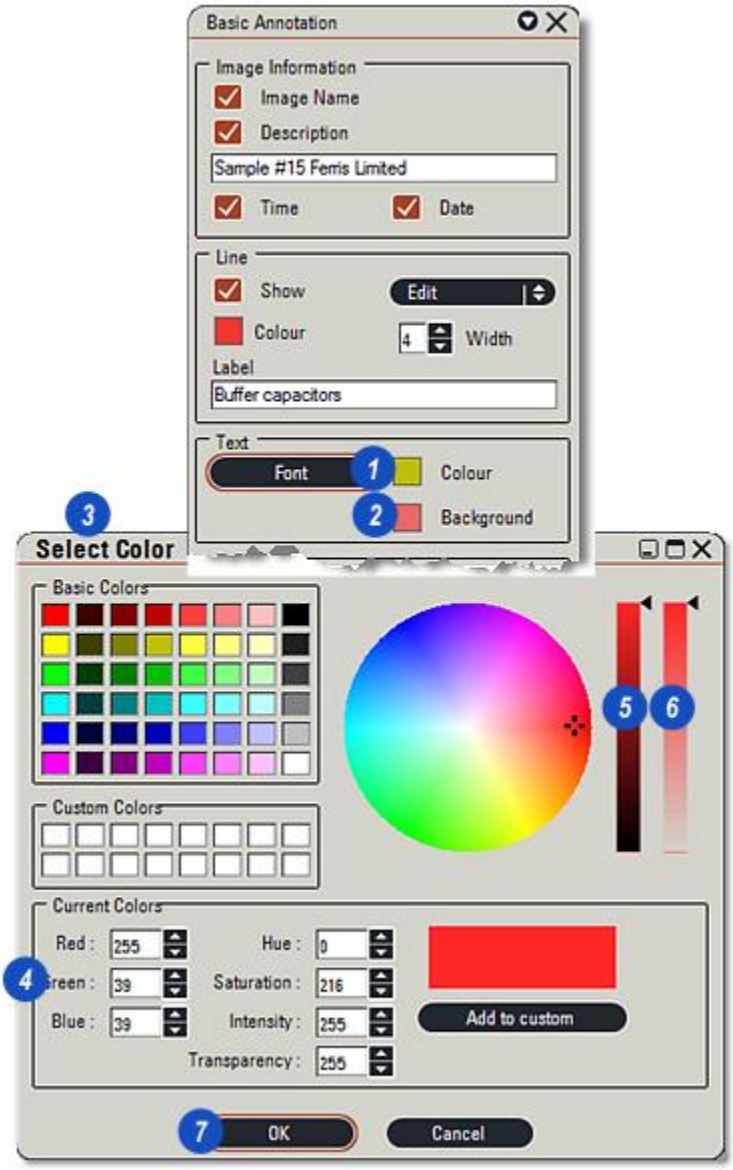
2: …the *Background* button.

3: On the *Select Colour* dialog choose a new colour from the swatches or from the colour wheel. Alternatively, click in the *Current Colours* text boxes **(4)** and type the Red, Green and Blue values.

5: Adjust the colour value by clicking and dragging the *Colour Shade* slider.

6: If required, adjust the colour transparency by clicking and dragging the slider on the *Transparency* bar.

7: Click *OK*. The new colour is shown on the appropriate button.

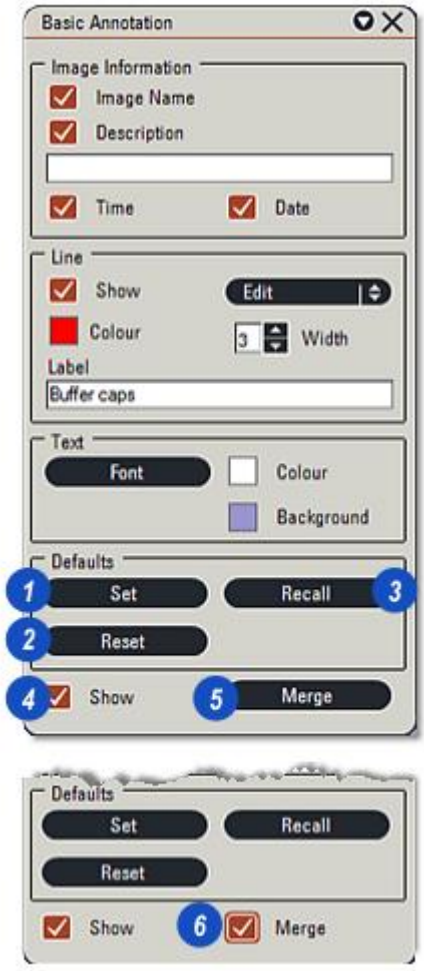


1: Clicking the *Set* button will save all of the current Basic Annotation settings for future image captures. However, any of the settings can be adjusted on individual images before merging.

2: Click the *Reset* button to return the settings to the original installation defaults.

3: *Recall*.

4: The *Show* check box when enabled displays the *Basic Annotation* control panel and activates the tools.



Merging:

When *Merging* is enabled all of the Labels, Captions and Lines drawn on the image will be permanently included as part of the image and cannot be altered.

5: Merge on a captured image is a ‘one shot’ button and only merges annotations with the current image.

6: Merge on live images is a check box setting and whilst it remains enabled will merge annotations with all captured images.

Enhance provides a range of easy to use yet powerful controls for image enhancement.

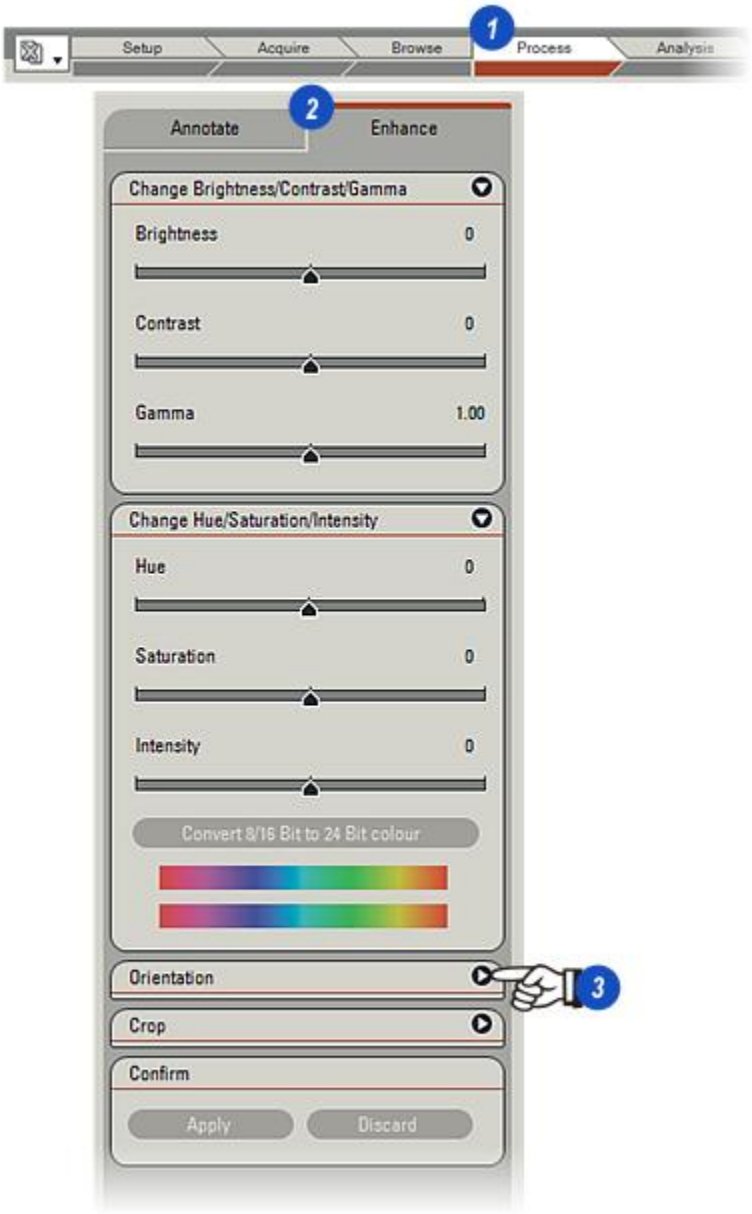
The controls work post-capture to change Brightness, Contrast, Gamma, Hue, Saturation and Intensity. The image can be Flipped, Rotated or Cropped and saved as a new image or to replace the original.

To reach the Enhance control Panels:

1: Click on the *Process Workflow.*

2: Click on the *Enhance* tab.

3: Some *Control Panels* are minimised - expand them by clicking on the small arrow to the right of the panel.



Crop: Defining an Area

Cropping is the process of removing unwanted parts of the image leaving only the area of interest.

1: This step is not mandatory but strongly recommended. Right click on the image. The *Viewer* menu appears.

2: Select *Send To* and then select a drive to save a copy of the image - just in case the cropping or colour adjustment go wrong, this is a pristine backup.

3: Click the *Fit To Screen* icon on the *Side Bar* to display the entire image.

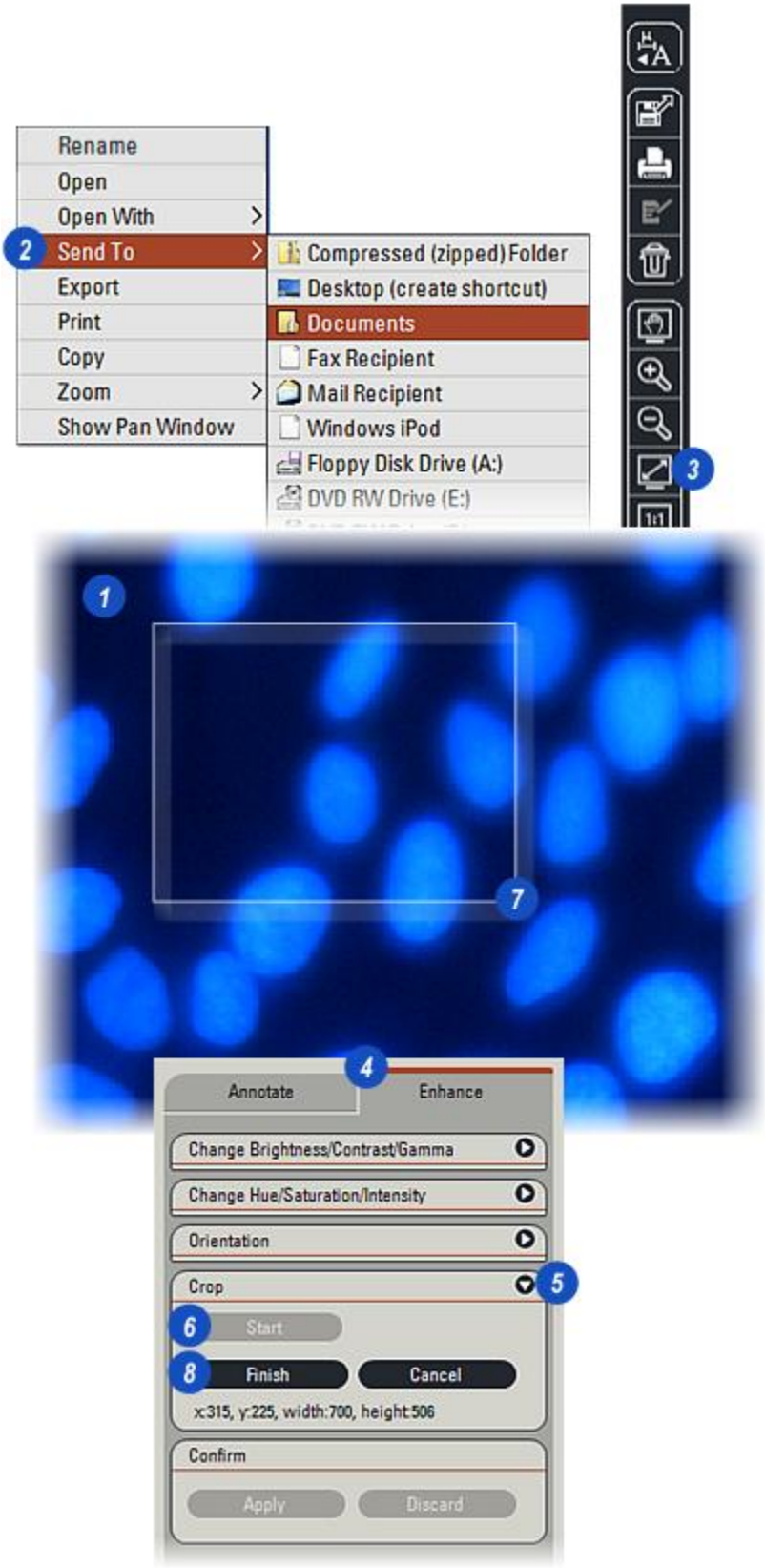
4: Click on the *Enhance* tab.

5: Click on the arrow to the right of the *Crop* header to reveal the panel.

6: Click *Start* on the *Crop* panel.

7: Click-and-hold on a point on the image where the top left-hand corner of the crop mask will be. Drag to the right and down. The mask appears as an outlined rectangle. When the area of interest is enclosed by the mask, release the mouse button.
The position and size of the mask (in pixels) is displayed in the *Crop* panel as the mask is being drawn.

8: Either click the *Finish* button if the mask is satisfactory, or click *Cancel* to clear the mask and start again.

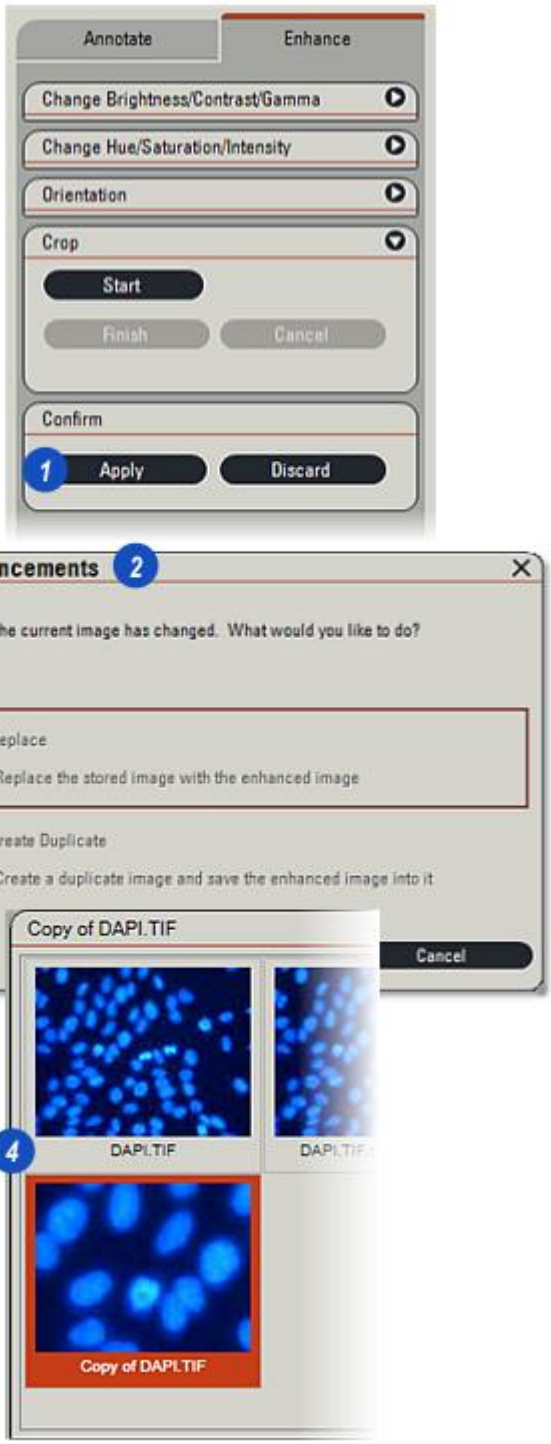


1: To keep the masked area, click *Apply* on the *Confirm* panel or click *Discard* to start again.

2: If *Apply* is clicked the *Save Changes* prompt appears:

3: Click *Replace* to replace the original image with the cropped area.

4: Click *Create Duplicate* to keep the original image intact with the same image name and also create an new image of just the cropped area with the same image name prefixed by *Copy of...*



Orientation: Flipping and Rotating

The image may be flipped - top to bottom or side to side and rotated about its central axis.

1: Click on the arrow to the right of the Orientation bar to reveal it.

To flip the image top to bottom:

2: Click on the *Flip Vertical* button.

To flip the image side to side:

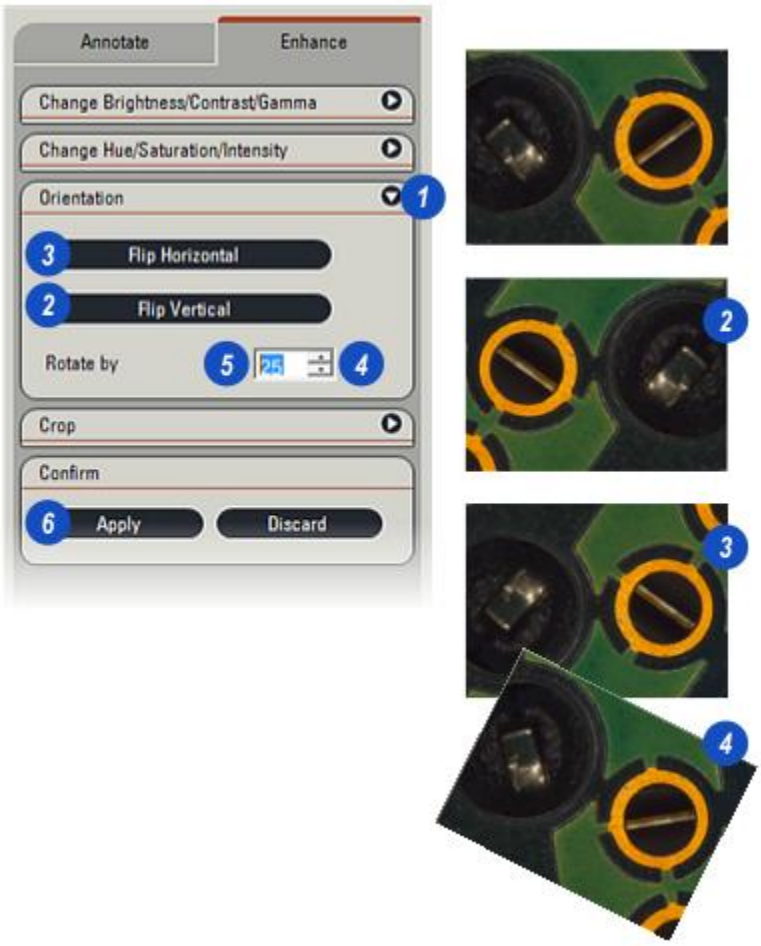
3: Click on the Flip Horizontal button.

To rotate image about its central axis:

4: Click on the *Rotate Up* arrow to rotate clockwise by one degree increments or on the *Rotate Down* arrow to rotate anti-clockwise by one degree increments, or...

5: Double-click the *Rotate* by window to highlight the existing value. Type the number of degrees to rotate and press the *Enter* key on the keyboard. The image will rotate to the required position clockwise.
To rotate anti-clockwise, precede the number with the negative (-) sign.

6: On the *Confirm* panel, click *Apply* to keep the new orientation or *Discard* to start again.

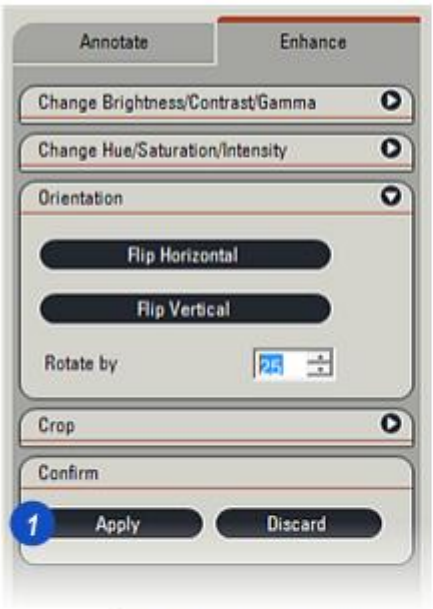


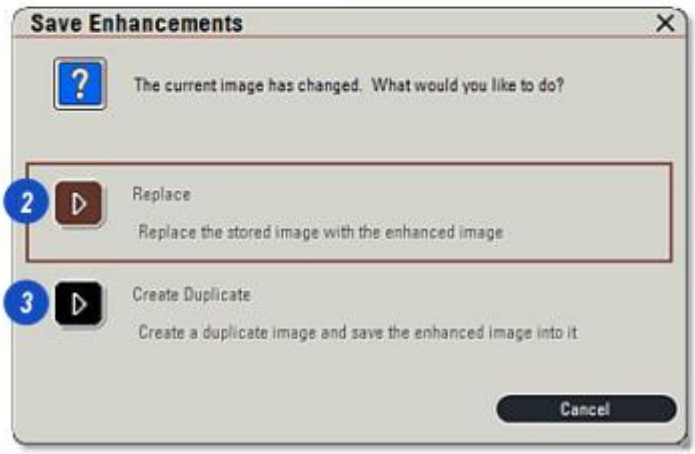
1: On the *Confirm* panel, click *Apply* to keep the new orientation or *Discard* to start again.

2: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.

3: *Create Duplicate* to save the cropped image with the original name prefixed with *Copy of...* The original image remains intact. Click *Cancel* to restore the image and start again.

Before proceeding to colour adjustment, it is good practice to make a copy of the re-orientated image as a backup.





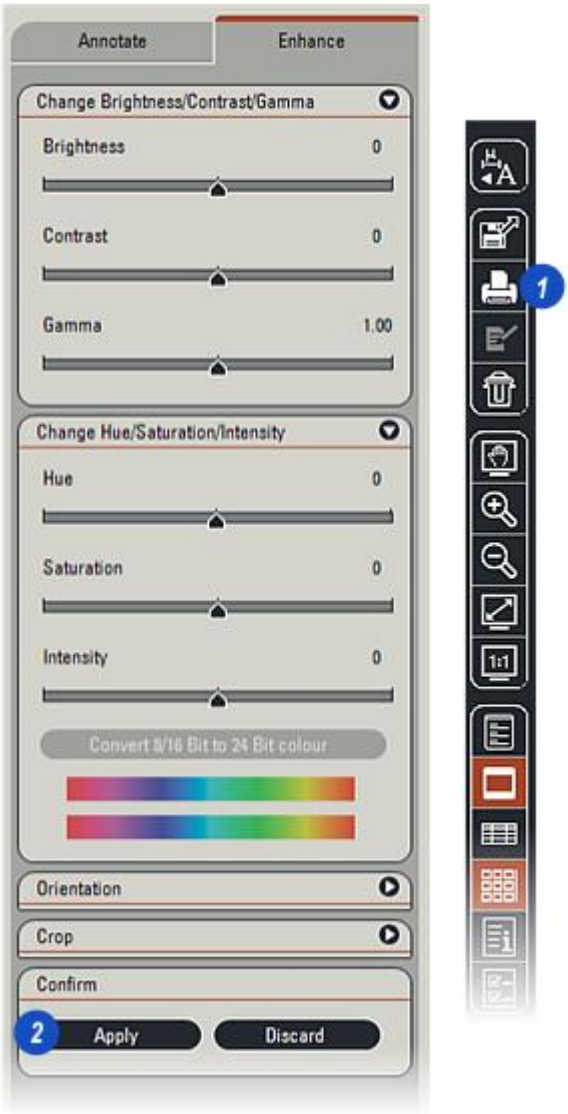
Two panels provide a range of powerful tools to adjust and modify the image colours - *Brightness: Contrast: Gamma (BCG)* and *Hue: Saturation: Intensity (HSI).* To reveal the panels click on the arrow to the right of the bar.

While the panels use complex mathematics to manipulate the image, ultimately colour is simply a matter of perception - what our eyes and our brains 'see'- and if what we see suits our purpose, then the colour is 'correct'.

Before adjusting the image colour, consider how it is going to be presented. For archiving or electronic transmission, perhaps very little adjustment will be necessary; for projection - in a Powerpoint presentation for example - *Saturation* may need to be increased to maintain colour vibrancy on the screen; for paper printing, *Gamma* and *Intensity* may need increasing to keep colours 'pure' and clean.

1: Print it to check how it looks and...

2: ...if the image is satisfactory, only then *Apply* and keep it.

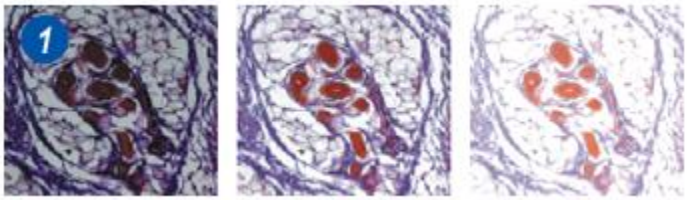


The image in the *Viewer* comprises tiny, individual ‘dots’ called pixels. Each pixel is a mixture of three primary colours - red, green and blue (RGB) and each colour is represented by a value. For 8 bit colour, the values are in the range 0 to 255.

All of the colour controls manipulate the values of the three colours to produce a certain effect.

If all three colour values are set to ‘0’(0:0:0) then black is the result. If they are all set to ‘255’(255:255:255) white is the result.

On both the *Brightness/ Contrast/ Gamma* and *Hue/ Saturation/ Intensity* colour panels, click and hold the slider and move it to the left to decrease or right to increase values. The displayed numbers are not a reflection of the colour byte values but rather a scale associated with the parameter being changed.

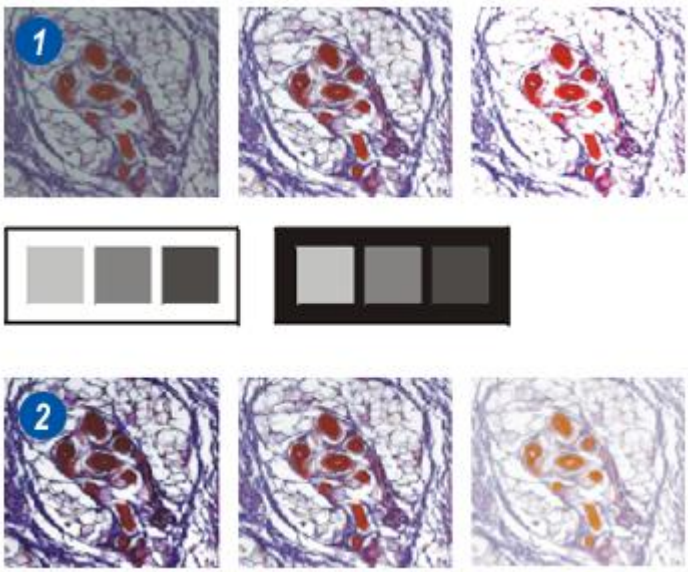


1: *Brightness:* increases or decreases the value of all three colours simultaneously. The illustrations show (left to right) the result to the image of a swing of -300 to +300 with the middle image representing the original captured value - ‘0’on the brightness scale. A maximum negative value will produce a black image and a maximum positive value a white image.

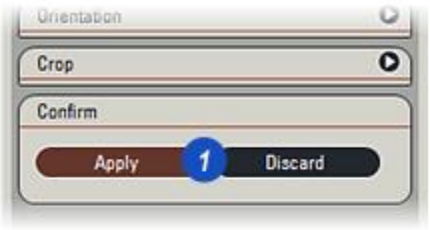
See Contrast and Gamma 415

1: *Contrast* increases or decreases the colour values individually both with respect to each other and also white levels. It is a proportional adjustment. The three illustrations represent a swing of 1000 in both directions with the original image in the centre.
The perception of contrast depends upon the ambient light levels. The three small squares opposite are identical in both illustrations and yet those surrounded by black are perceived as having a lower contrast - they look closer to each other in terms of colour - than those surrounded by white. If your image is to be projected in dim lighting conditions, consider increasing the contrast to compensate.

2: *Gamma* is a value applied to colour levels to compensate for different ways in which the image is viewed. Liquid crystal displays (LCDs) have a specific Gamma setting, cathode ray tube (CRT) monitors will have another and printers yet another. Changes in Gamma are applied automatically so, when an image is printed for example, the printer software will make adjustments before the printing takes place.
Very small changes in Gamma can have dramatic effects; the examples show a range of 0.35 to 1.50 with the original in the centre. Generally, avoid altering the Gamma settings unless really necessary.

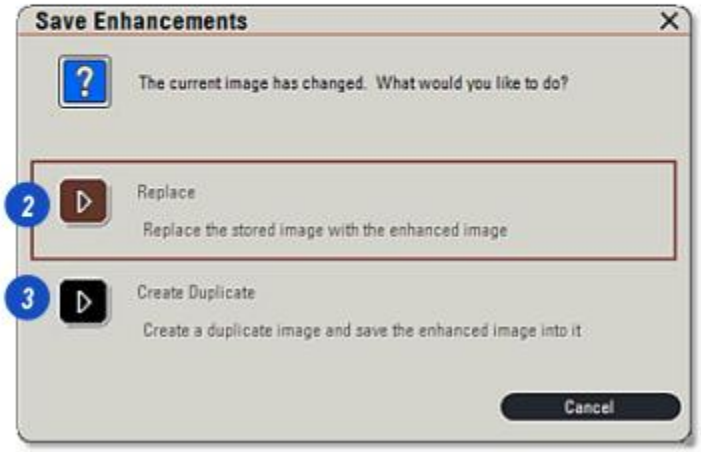


1: All three controls may be applied to the image simultaneously.
To reset the changed values and start with the original image, click *Discard* on the *Confirm* panel. To keep the changes, click *Apply*.



2: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.

3: *Create Duplicate* to save the cropped image with the original name prefixed with Copy of... The original image remains intact. Click *Cancel* to restore the image and start again.

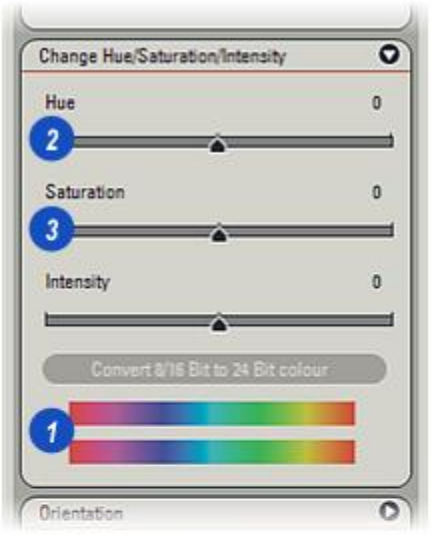


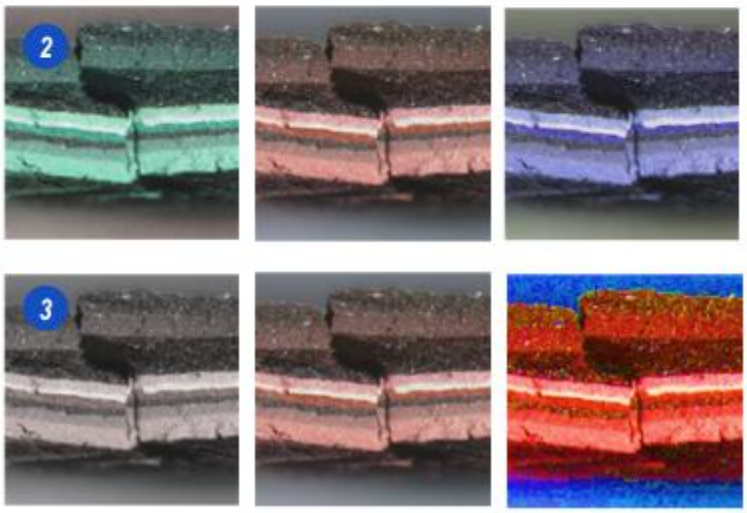
Brightness 414
Hue and Saturation 417

Hue, Saturation and *Intensity* control the actual colours, the amount of colour and the vibrancy. As each is adjusted, the lower of two *Spectrum Bars* **(1)** changes as a comparison to the static spectrum above it.

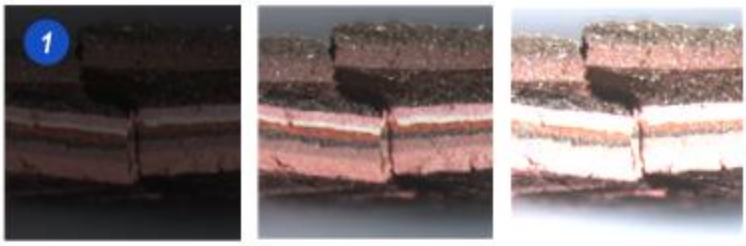
2: *Hue* is another word for colour. As the slider is moved, the colours shift from the dominant red in the middle illustration which is the original, toward green on the left or blue on the right. The *Spectrum Bar* shifts to reflect the change in dominance.
Use *Hue* to correct any perceived colour imbalance, especially on printed images.

3: *Saturation* determines the amount of each colour that is present. At the highest setting, each colour will be at its most vibrant. The right hand illustration is the high setting and the colours cannot be more prominent without combining to make white. Use *Saturation* to make powerful (if a little bit 'unnatural') images. Reducing *Saturation* is a convenient way of turning a colour image into a monochrome image - essentially just shades of grey - without losing detail or becoming a black solid.





1: *Intensity* is close to *Brightness* in the way it affects the image. It is a measure of the 'strength' of each colour swinging from solid black to solid white. Use small increases in Intensity to help differentiate between colours; too much and detail begins to disappear.

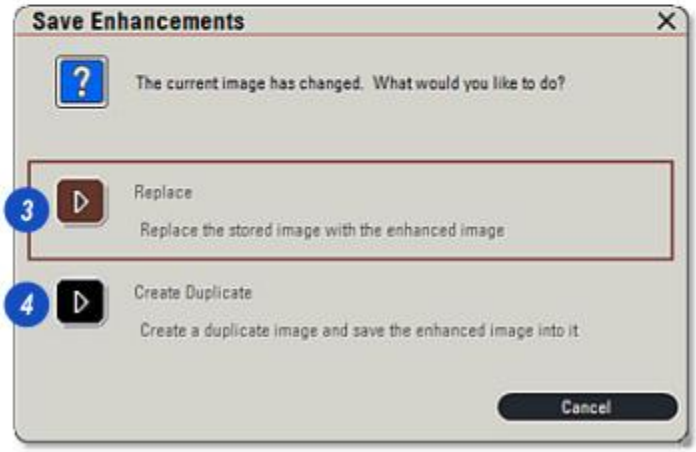


All three controls – *Hue, Saturation* and *Intensity* - may be used together to achieve a desired effect and they may be combined with *Brightness, Contrast* and *Gamma* to 'fine tune' an image.

2: *Discard*, return to the original, or *Apply* changes on the Confirm panel.

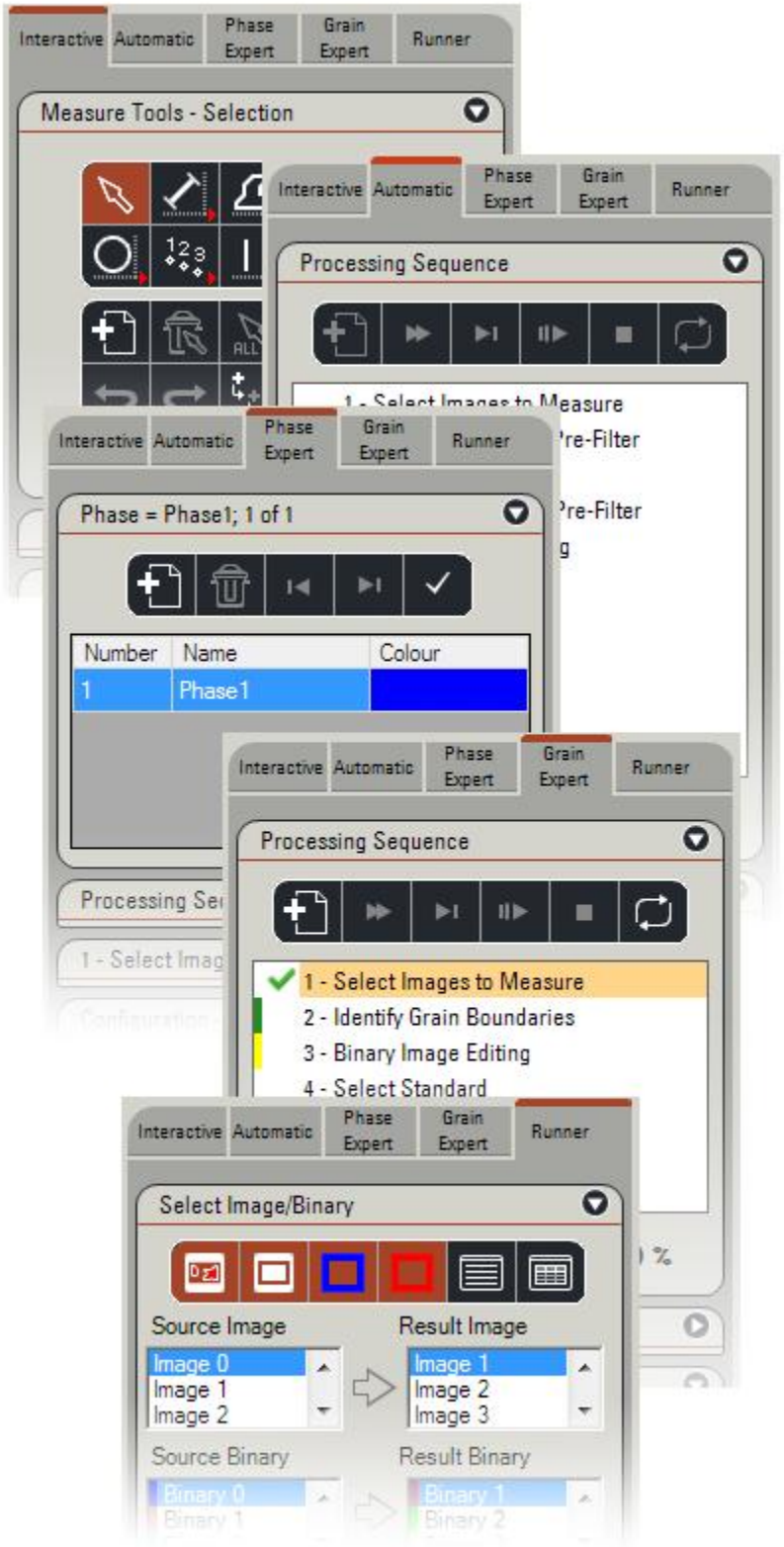
3: If *Apply* is clicked the *Save Enhances* prompt appear. Click *Replace* to save the cropped image overwriting the original.

4: *Create Duplicate* to save the cropped image with the original name prefixed with Copy of... The original image remains intact. Click *Cancel* to restore the image and start again.



The *Analysis Workflow* appears when the optional analysis modules are installed.
In this version of the Leica Application Suite, the *Interactive Measurements* or *Automatic Measurements* modules may appear here:

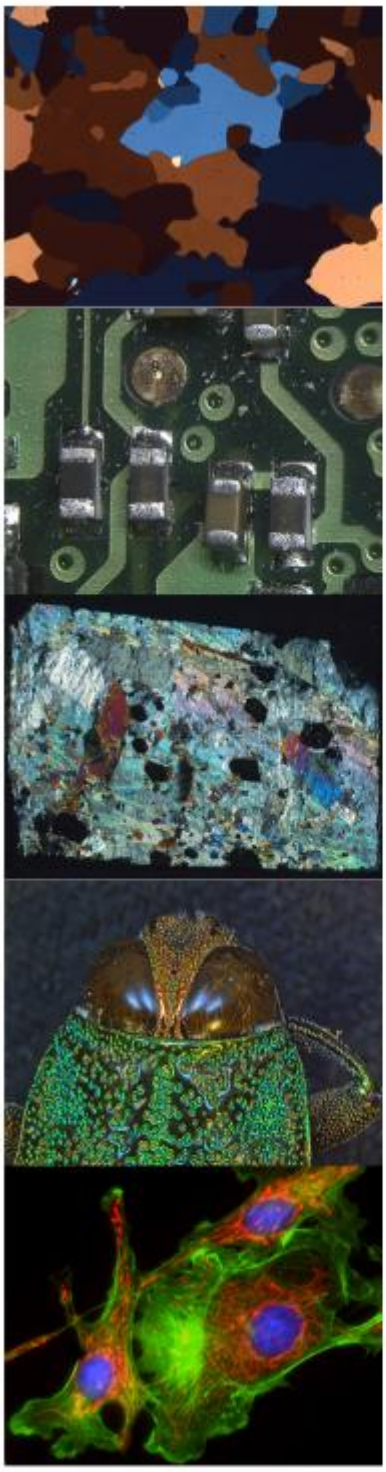
- *Interactive Measurements* [887]
- *Automatic Measurements (also known as Image Analysis* [1023]*)*
- *Phase Expert* [1152]
- *Grain Expert* [1183]
- *Runner* [1149]



Optional Modules

The underlying capabilities of the *Core* functions can be enhanced with a range of advanced modules and applications.

Each LAS module provides the flexibility to tailor a system solution to fulfill individual needs with upgrade options available for future requirements.



Optional Modules include:

- LAS Archive Basic.
- LAS Archive Standard
- Extended depth of focus
- Movie recording
- Image measurements
- Macro Programming
- Image Overlay
- Power Mosaic
- Image Analysis
- Web sharing of images
- Grain Expert
- Phase Expert
- LAS Macro

...and many others, described in this manual and help.

Link to installing the *Demo Licence* 422 and *Enabling Optional Modules* 421.

Use of the optional *LAS Macro* capability is described the *LAS Macro Editor* help file.

Demo Licence and Installing Optional Modules

This section is for users who want to install the 60-day *Demo* evaluation licence. Users who intend to apply online for a licence or those who already have a licence file or dongle should consult the *Installation Guide* for the next steps.

To reach the LAS *Framework* and select *Registration and Activation:*

1: Click on the *Windows Start* button and select the *All Programs* option.

2: Click *Leica Application Suite V4* and...

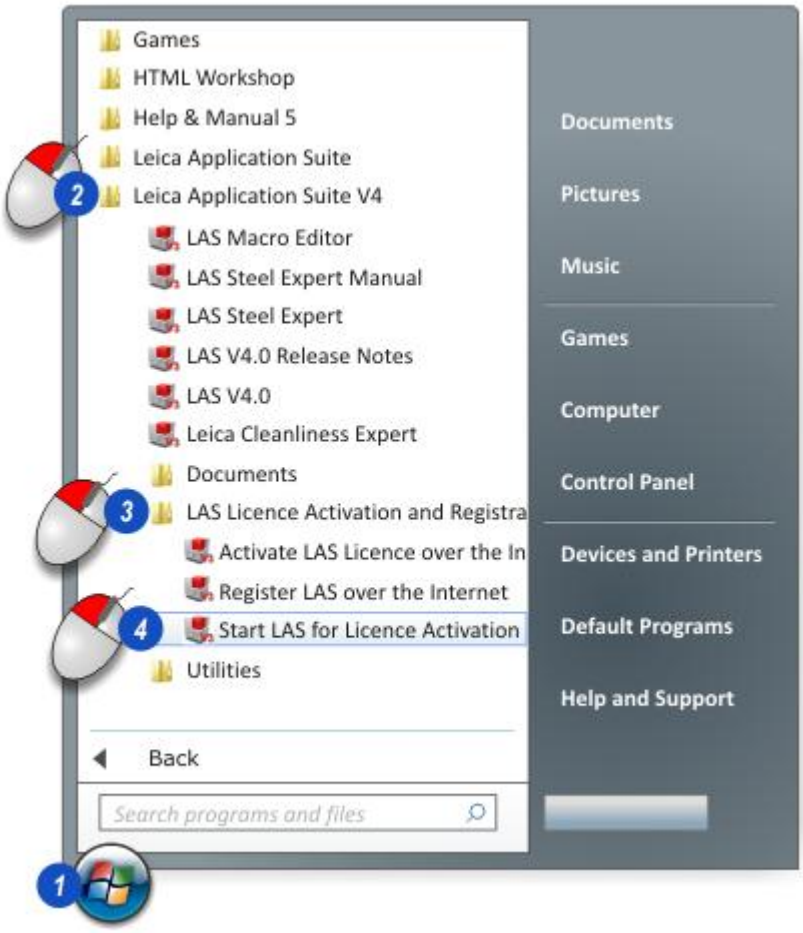
3: ...*LAS Licence Activation and Registration.*

4: From the option list, left-click to select *Start LAS for Licence Activation.*

When the LAS Framework appears:

5: Click on *Options* on the *Main Tool Bar.*

6: From the drop-down click on the *Registration and Activation* option.



! Before installing the *Demo Licence*, make sure that the personal computer clock is set to the correct region, date and time.

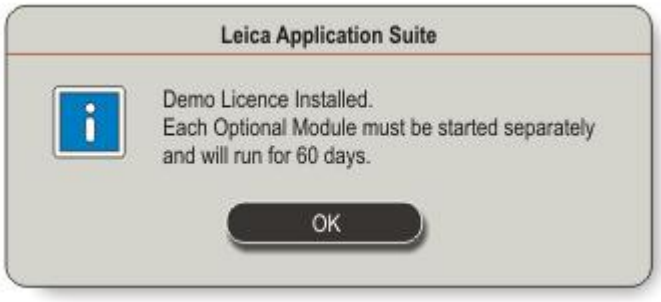
If the clock settings are changed after the *Demo Licence* is installed the *Optional Modules* will become inaccessible

On the *Licence Activation and Registration* dialog:

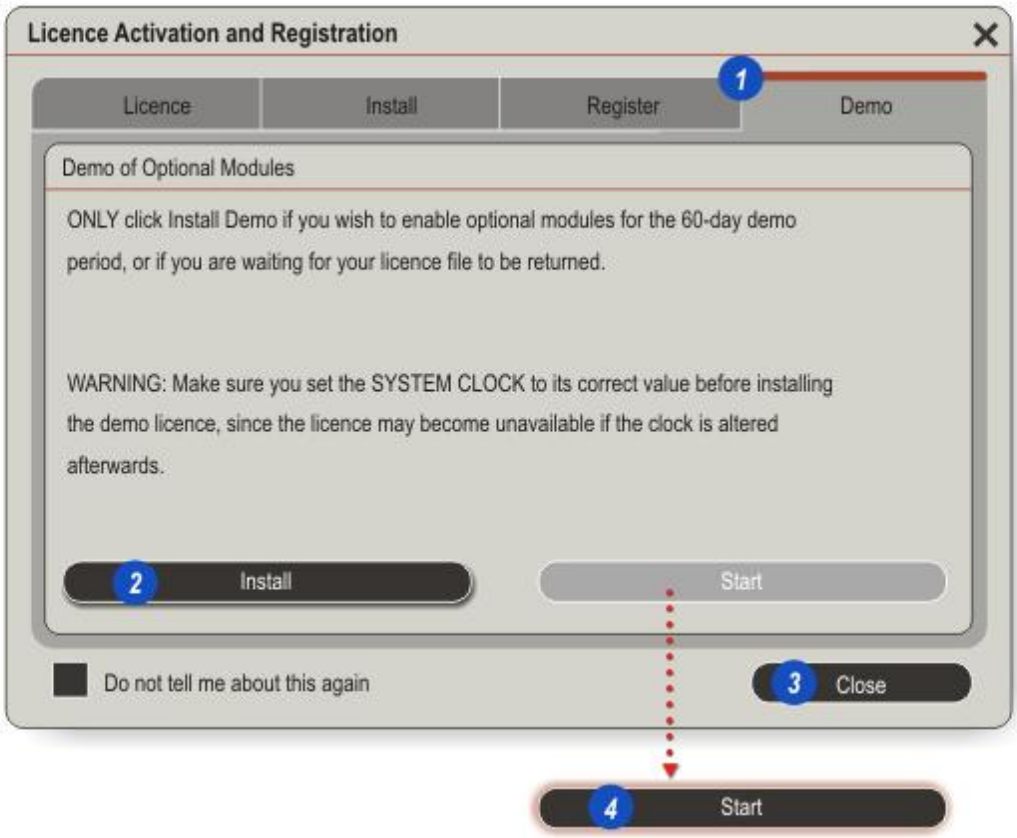
1: Click on the *Demo* tab.

2: Click on the *Install* button.

3: Click the *Close* button. The *Demo Licence Installed* advice panel appears. Click *OK.*



4: Each Optional Module must be enabled and started separately for the 60-day evaluation. Click the *Start* button to go to the *Optional Module Status* dialog.



The results for Optional Modules licensing status can be filtered using the drop-down menu situated at the top of the panel. For initial enabling set the filter to *'All'* by:

1: Clicking on the small arrows to the right of he menu header and...

2: ...from the list, clicking to select the *'All'* option.

There are 2 steps in making an Optional Module available during the *Demo* period:

- *Starting the module* and...
- *Enabling it*.

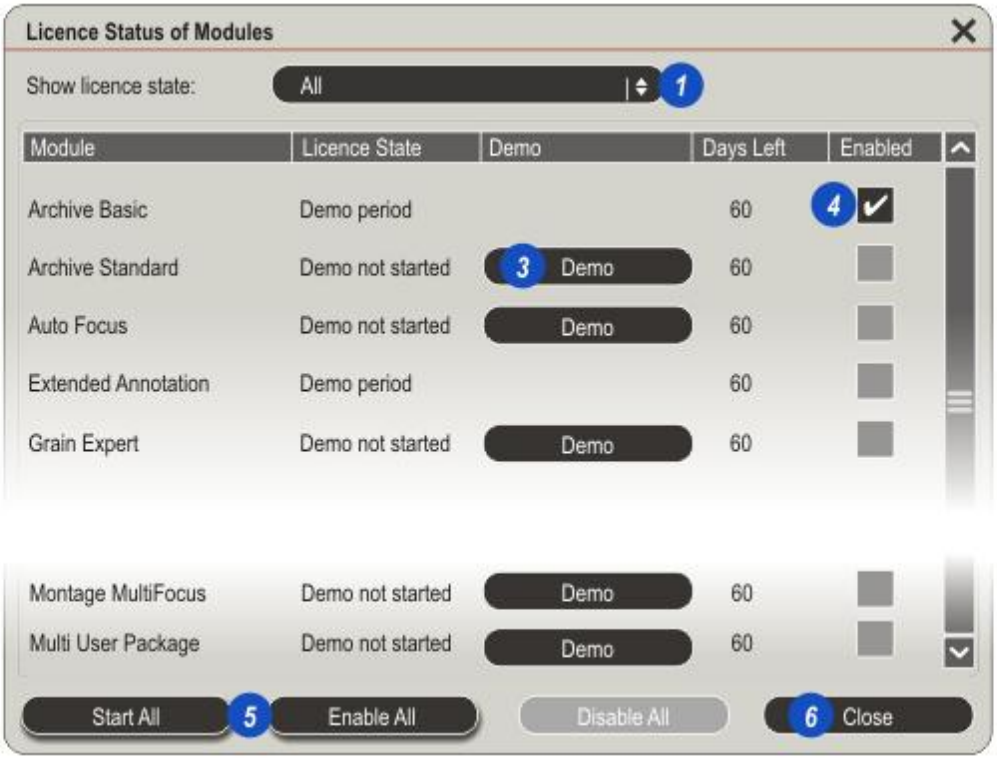
Once the *Demo* period is started for a module the 60-day clock begins to run and cannot be stopped. Disabling a module will will just make it unavailable to LAS - it will not stop the *Demo* period clock.

3: Click on the *Demo* button to the right of the module to be started. The *Licence State* changes to *'Demo period'*.

4: Click on the associated checkbox to enable the module - a small tick mark is displayed.

5: The *Start All* and *Enable All* buttons will do just that - start the *Demo* period for all of the Optional Modules and enable them. The *Disable All* button will make all of the modules unavailable but will not stop the *Demo* clock.

6: Click the *Close* button.



Optional Modules have to be purchased and fully licensed and the licence activated before the end of the 60-day *Demo* evaluation period. If they are not they will no longer be available to LAS and only the *Core* features will be usable.

When the 60 days have elapsed the *LAS Expired Demo* message appears:

1: Users that only require the *Core* features or are postponing purchase and licensing can click the *OK* button and the *Core* will continue to function normally.

Additionally, they can click to enable the *'Don't tell me...*' check box **(3)** so that the *Expired Demo* message does not appear in the future.

2: If the Optional Modules have been purchased but are yet to be licensed, click the *Registration* button to go directly to the *LAS Registration* sequence.

Excel Reports with Optional Modules

LAS Measurement, Analysis, Expert and Macro Modules create reports based upon *Microsoft Excel* using Excel templates automatically installed with Leica Application Suite.

In some cases these templates will be adequate for users' needs, but others will want to customize them to reflect their own or corporate preferences. With this in mind, LAS has based the installed templates on *Tags,* an arrangement that gives users complete flexibility.

Customizing always begins with two basic steps:

- *Create a Report:* In the LAS Optional Module create a simple report with all display options enabled. This provides a 'map' to the data contained in the Excel cells.
- *Copy the Pre-Installed Template:* Saving it under a new, appropriate name. This retains the original just in case customizing does not go to plan.

  Copying the pre-installed template also copies the Excel *Macros* - small programs that carry out specific tasks - associated with it. Excel macros are necessary for the *Context Menu* feature to function but if users are going to type or copy and paste tags, then they will not be required and the new template can be copied without Excel macros enabled.

  Some versions of Excel will warn that macros are included in the template and switch them off for security reasons. Click the option to switch them on again.

Create a Report:

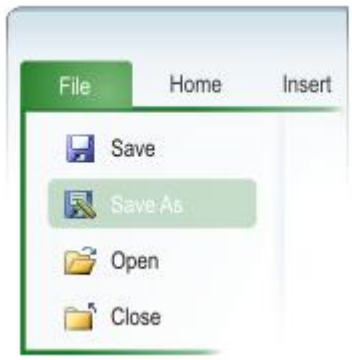
- *Enable all of the measurement parameters:* This includes results and images to be included in the report. These options vary depending upon the Optional Module.
- *Make a simple measurement:* Again, this will depend upon the module being used. *Live* and *Interactive Measurements* will require at least a simple drawn line; *Image Analysis* and the *Expert* modules will need an image to be selected and processed, but tools and accuracy are not important .
- *Create a Report:* Using the pre-installed template and save it from Excel.

Copy the Template: In Excel:

- *Open the Pre-Installed Template:* The default location for Excel templates is:

  C:/Users/Public/Public Documents/Leica Application Suite/Excel Templates/

  ...followed by a list of folders that contain the templates for Optional Modules. In some versions of Excel it may be necessary to right-click the template and select *Open With* to open it as a template.



- *Save the Template:* Use the *Save As* option giving the template a suitable name. Save in either the default folder as above, or a location of choice.

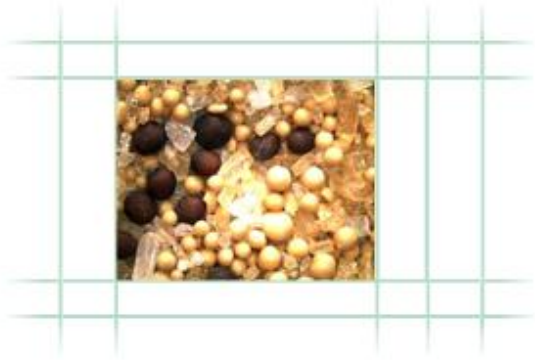
  If the *Context Menu* method for inserting tags is to be used, make sure that the template is saved in a *Macros Enabled* format.

- Now the template can be customized.

Tags are simple lines of text in a pre-defined format. The text is always enclosed within angle brackets < > and instructs Excel to import data or images and display them in a set layout. For example, the following tag is an instruction to display the *original* image at a *width* of 256 pixels:

<*LAS AM Image* ***Width:256 Type:Original***>

The column containing the tag is automatically expanded to 256 pixels wide and the row height scaled to fit the original image:



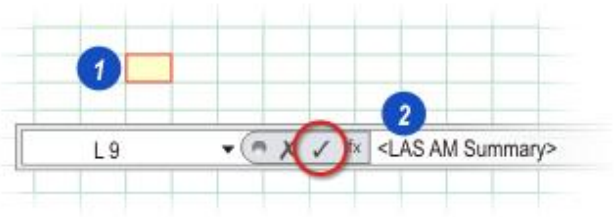
Tags can be copied into cells within an Excel template in several ways:

- *Click and Type:* Click the cell to select it and type the tag into the formula bar. Does not require Excel macros - a simple workbook saved as a template is sufficient .
- *Copy and Paste:* Click a cell that already contains the tag then *copy* or *cut* it. Click in the new target cell and *paste* the tag. Does not require Excel macros - a simple workbook saved as a template is sufficient .
- *Context Menu*, the preferred method of copying a tag because it avoids typing mistakes and provides an immediate source for all tags. This option requires a template that has Excel macros enabled.

Click and Type:

1: Click on the cell to contain the tag.

2: On the *Formula Bar*, type the tag and click the tick mark to save it to the cell.



Copy and Paste:

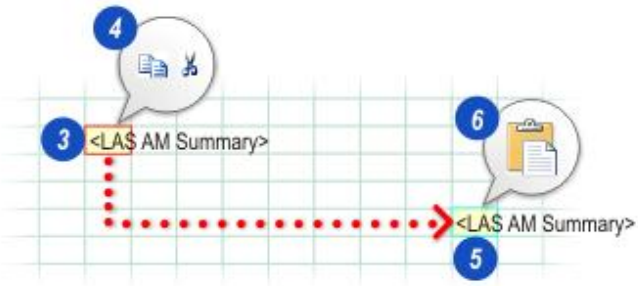
Copies or moves an exiting tag to the same or a different sheet.

3: Click on the cell containing the tag - the source.

4: Click the *Copy* icon to simply copy the tag to the clipboard, or the *Cut* icon to copy the tag but remove it from the cell.

5: Click on the target cell and...

6: ...then the *Paste* icon.



Save the modified template.

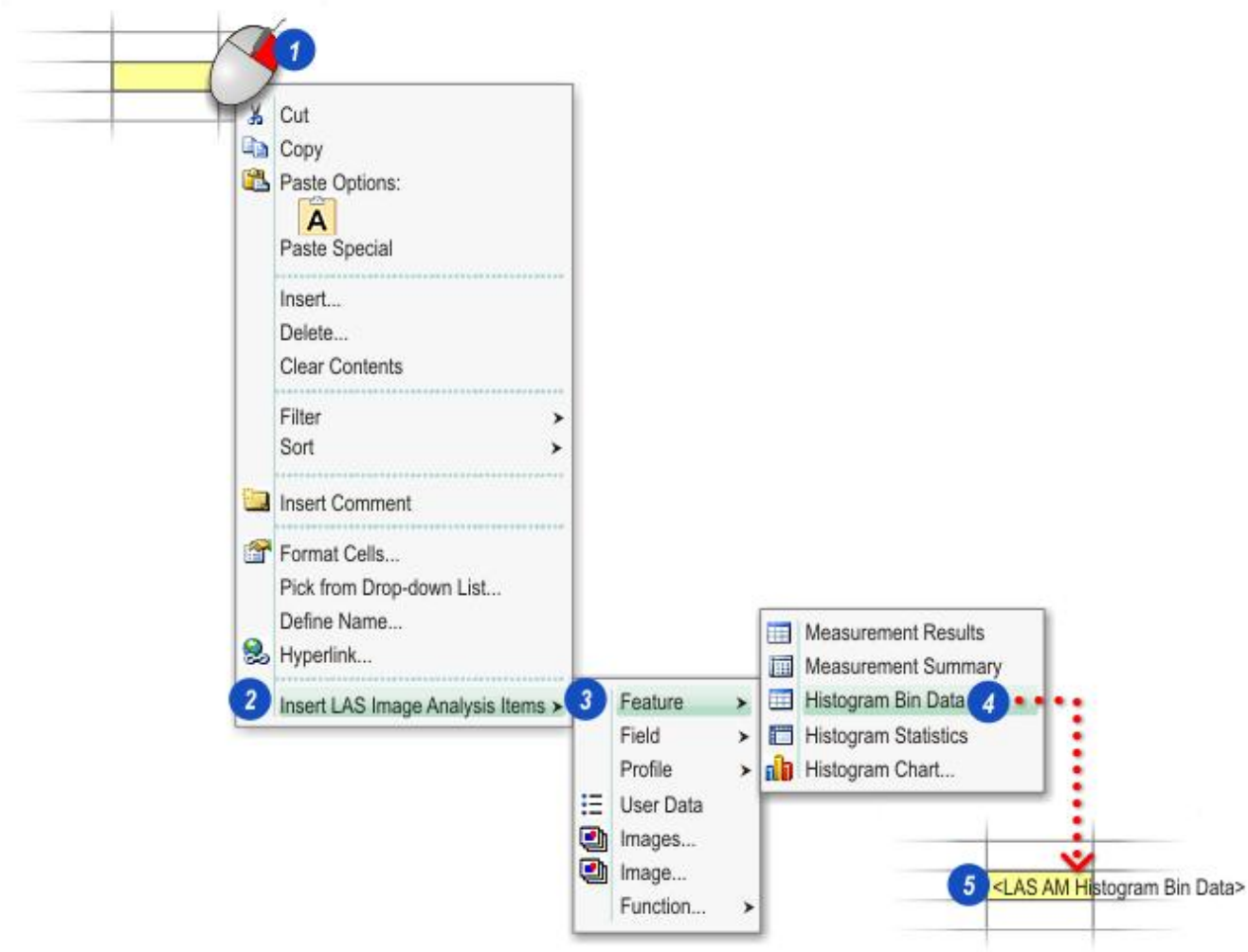
1: Requires a template with Excel macros enabled. Right-click on the Excel cell. This reveals...

2: ...the *Context Menu*. Click on the *Insert LAS Image Analysis Items* option.

3: On the sub-menu, click to select the required tag type - *Feature*, *Images* etc. Those options with an arrow (➤) display another menu...

4: ...with the tag descriptions. Click to select the required tag.

5: In some cases the tag is copied into the cell immediately. For others, a dialog appears providing alternative display options.



Some tag types have an additional dialog (1) that give users options - to resize or scale an image or display all or part of the results.

In the illustration the option *Full Column* List determines whether all of the results summary will be included or only those appropriate to the features. For example, including the greyscale summary for a colour image would just result in empty columns so *Full Column List* would be disabled.

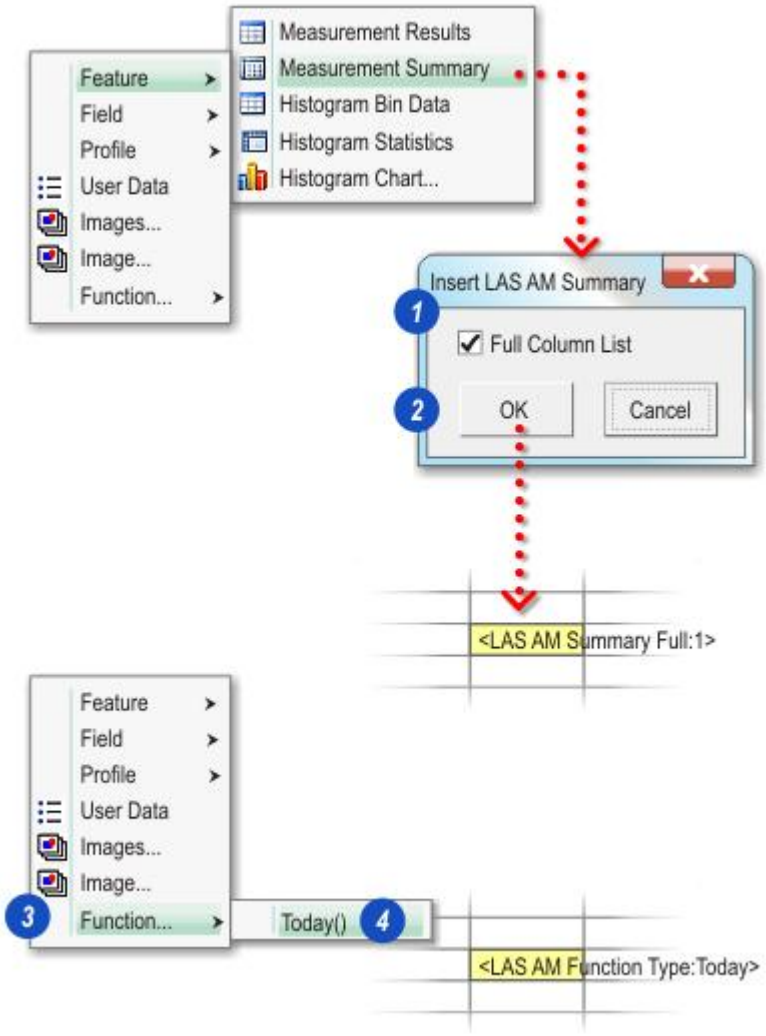
Click *OK* (2) and the appropriate tag is copied to the cell.

Functions:

Small formulas that produce a specific result are called *Functions* and are available on the sub-menu (3).

Useful and frequently used is the *Today* function that automatically inserts the current *Date* and *Time* into the cell (4).

<LAS AM Function ***Type:Today****>*



The *Context Menu Image(s)* and *Histogram Charts* options offer the user dialogs for selecting, scaling and resizing images.

1: The *Images* option displays all of the images associated with the report - providing they are marked as included on the Optional Module - but with the opportunity to scale or resize them.

2: The *Image* option allows the user to select an individual image type and to scale or resize it by...

3: ...clicking the *Customize* option and...

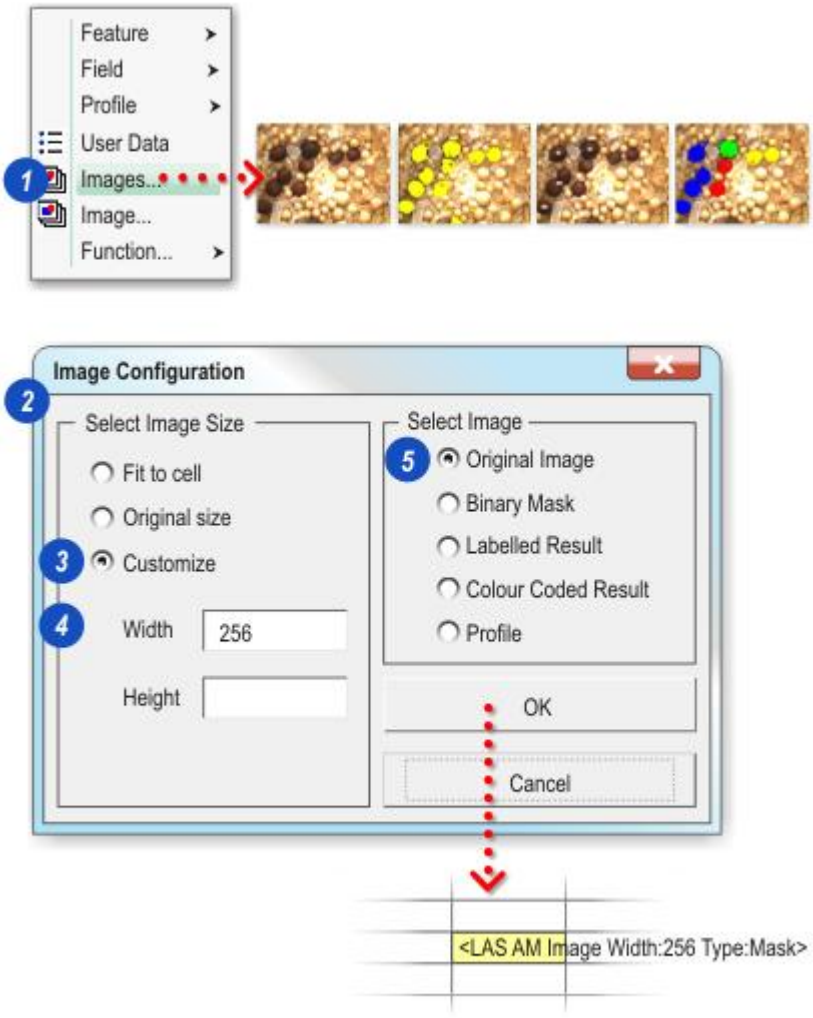
4: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

5: Click to choose the image to display.

Click the *OK* button to copy the tag with the dimension and type parameters set up.



Tag text is contained within angle brackets < > and will begin with either *<LAS AM for Image Analysis, Grain* and *Phase Experts,* or *<LAS LM* for *Live and Interactive Measurements.* Both styles perform in a similar way.

Following this is the name of the result group - *Summary, Results, Images* etc.

In some cases this is then followed by an *Indicator* of the data extent. For example:

<LAS AM Summary ***Fixed:1****>*

The keyword *Fixed* followed by *1* causes all of the *Summary* results to be display. In contrast:

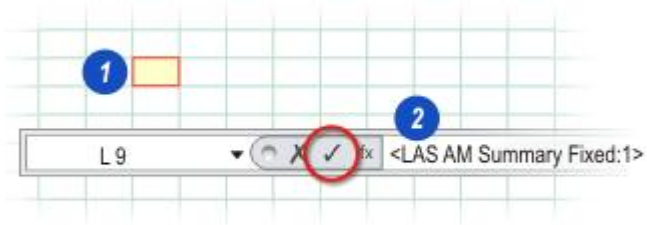
<LAS AM Summary> or
<LAS AM Summary Fixed:0>

...only displays an extract of the data.

Users can change the Indicator settings by:

1: Clicking the cell containing a tag.

2: On the *Formula Bar* typing an Indicator or new value.

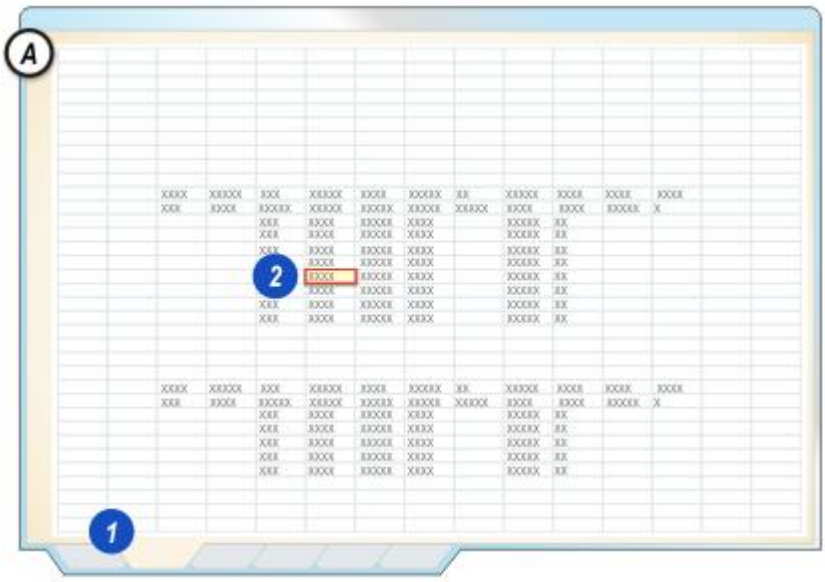


Links to Optional Module Tag Details:

- *Live Measurements* 435
- *Interactive Measurements* 435
- *Grain Expert* 440
- *Phase Expert* 450
- *Image Analysis* 458

In customising templates, users may want to display selected cell data on several sheets in the workbook. The front page of the installed templates for example, links to cells on several other sheets.

The links are dynamic - the data is automatically updated every time a report is created so that it is exactly the same in every place. Links can be typed or copied.



Typing a Link:

A: *Create a Report:* When it opens in Excel locate the data *source* cell by:

1: Selecting the appropriate *Tab* (sheet) and...

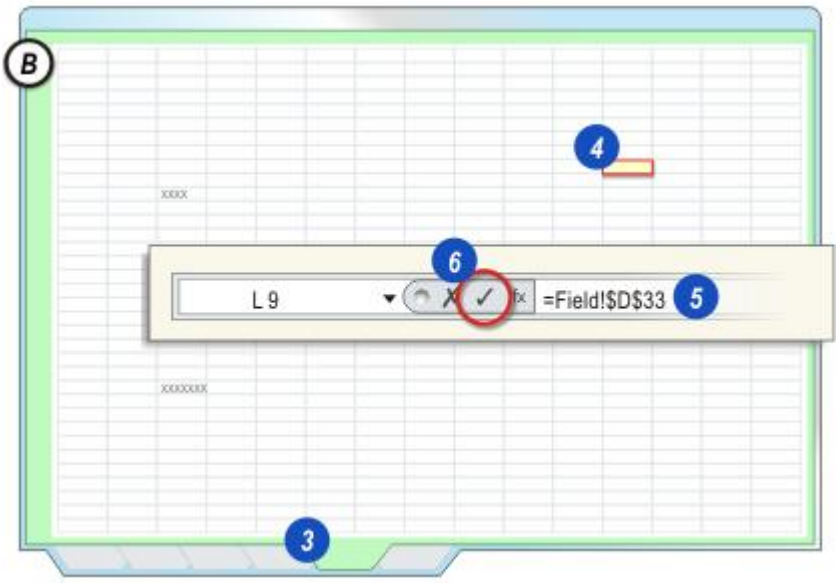
2: ...clicking the *Cell*.

Its *Column* and *Row* address appears top-left on the sheet - *D33* for example. Make a note of it together with the tab name.

B: *Open the Template*. The default location for Excel templates is:

C:/Users/Public/Public Documents/Leica Application Suite/Excel Templates/

...followed by a list of folders that contain the templates for Optional Modules. In some versions of Excel it may be necessary to double-click the template to open it as a template.



Select the target cell - where the data will be displayed by:

3: Clicking the target tab and...

4: ...clicking to select the target cell.

5: On the *Formula Bar* type the link in this format:

- =
- Name of the source cell Tab: For example *Field*
- !
- $
- Source address *Column* letter.
- $
- Source address *Row* number.

6: Click the tick mark to load the link to the cell.

The final link will look something like:

=Field!D33

Use the *$* symbols if the source address is to be absolute - never changing. Omit them if it will be relative - moving as the volume of data changes but still linking to the same data item.

Save and test the template.

Copying a Link

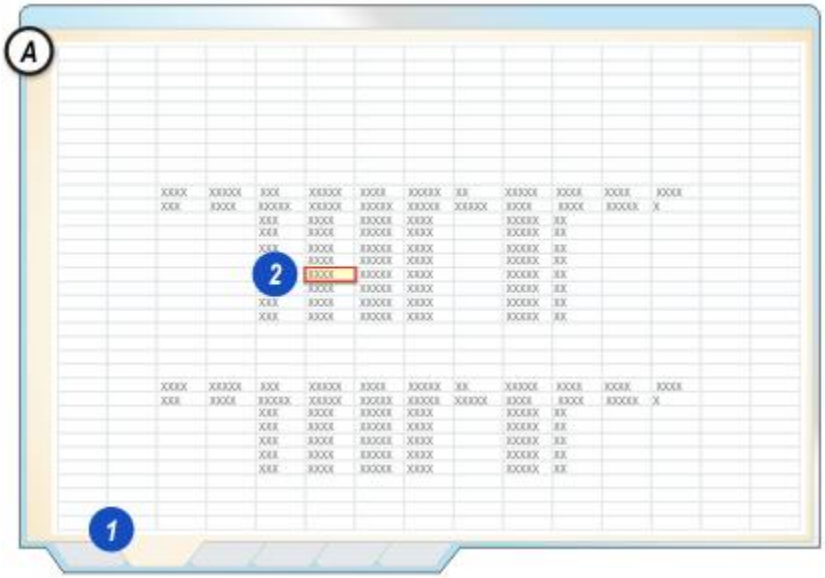
Copying a link requires an extra step - pasting - but it is fast and avoids typing mistakes

A: *Create a Report:* When it opens in Excel locate the source cell by:

1: Selecting the appropriate *Tab* (sheet) and...

2: ...clicking the *Cell*.

Its *Column* and *Row* address - *D33* for example - appears top-left on the sheet. Make a note of it together with the tab name.



B: *Open the Template*. The default location for Excel templates is:

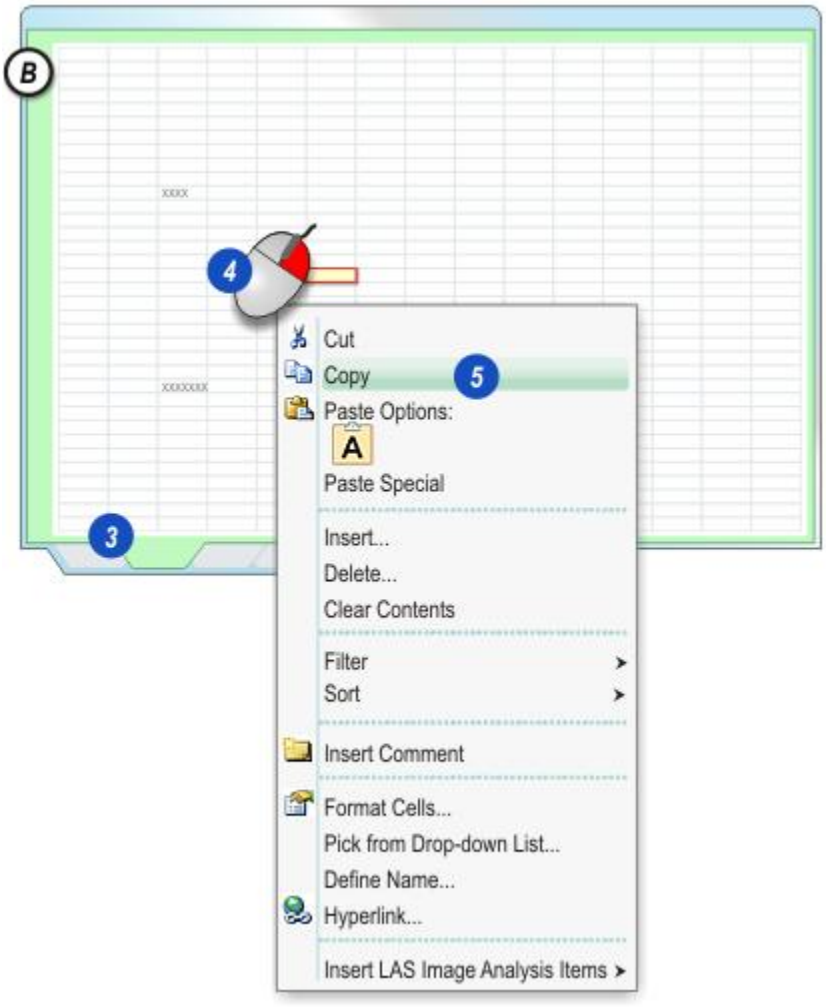
C:/Users/Public/Public Documents/Leica Application Suite/Excel Templates/

...followed by a list of folders that contain the templates for Optional Modules. In some versions of Excel it may be necessary to right-click the template to open it as a template.

3: Select the same *Tab* noted on the *Report* and...

4: ...right-click in the same cell, even though it is probably empty.

5: On the *Context Menu* left-click the *Copy* option.



C: On the Template:

1: Click on the *Tab* of the *Target* sheet.

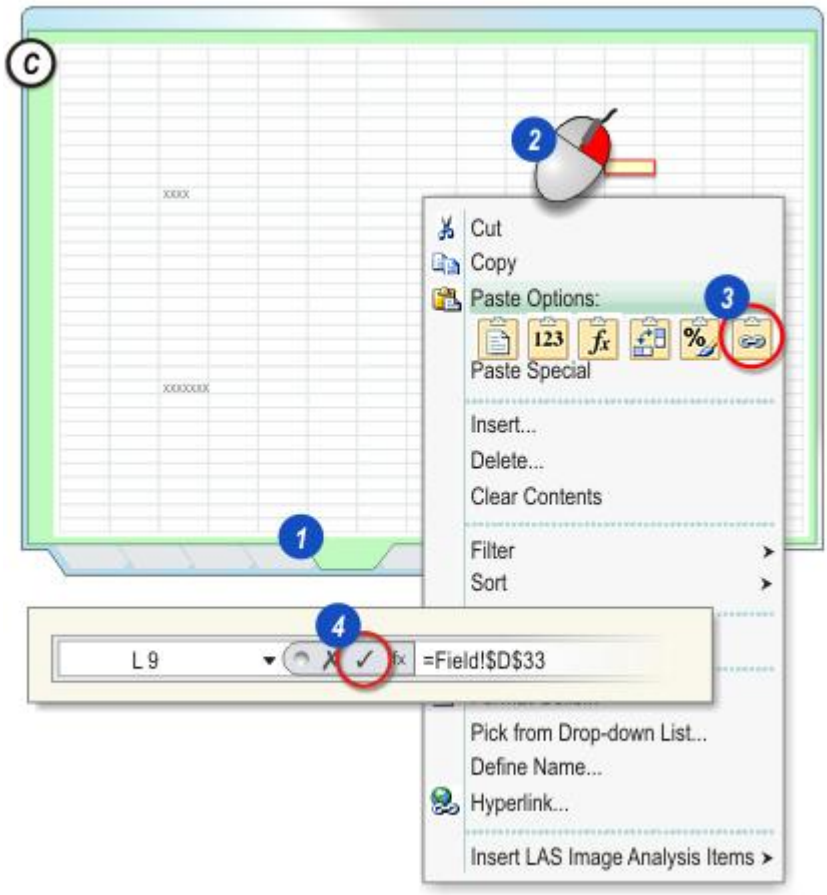
2: Right-click the target cell and...

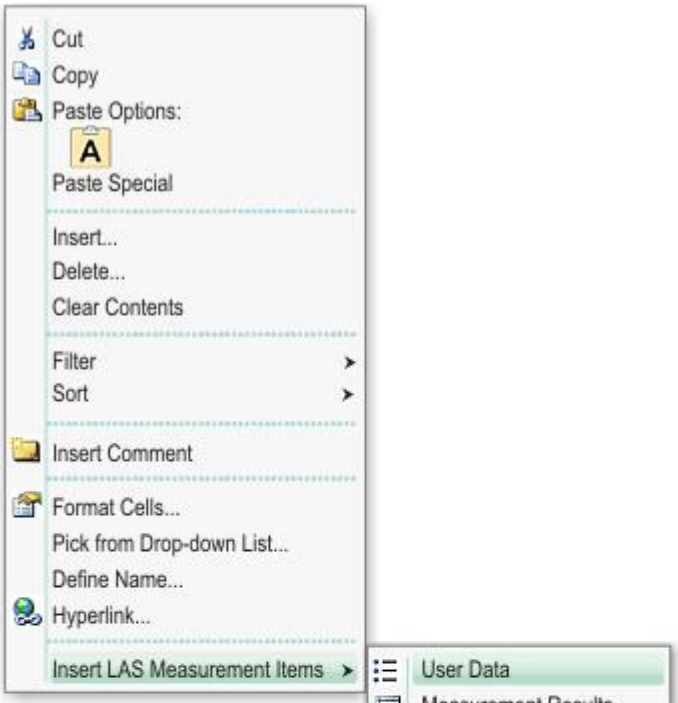
3: ...on the *Context Menu* left-click the *Paste Link* icon.

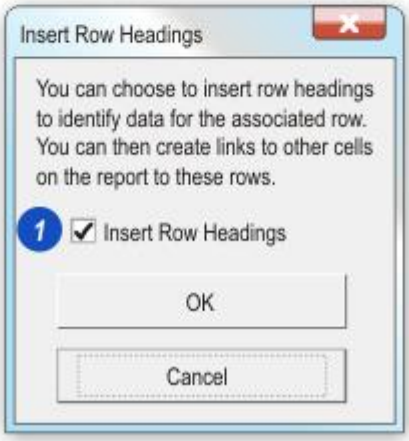
4: The address of the source sheet and cell appear on the *Formula Bar*. Click the tick mark to complete the paste.

Links are pasted as absolute addresses - never changing - with *$* symbols preceding the column letter and row number. Click on the link on the *Formula Bar* and delete them if the link is to be relative - moving as the volume of data changes but still linking to the same data item.

Save and test the template.







User Data is taken from the image file and from the *Create Report* panel text boxes:

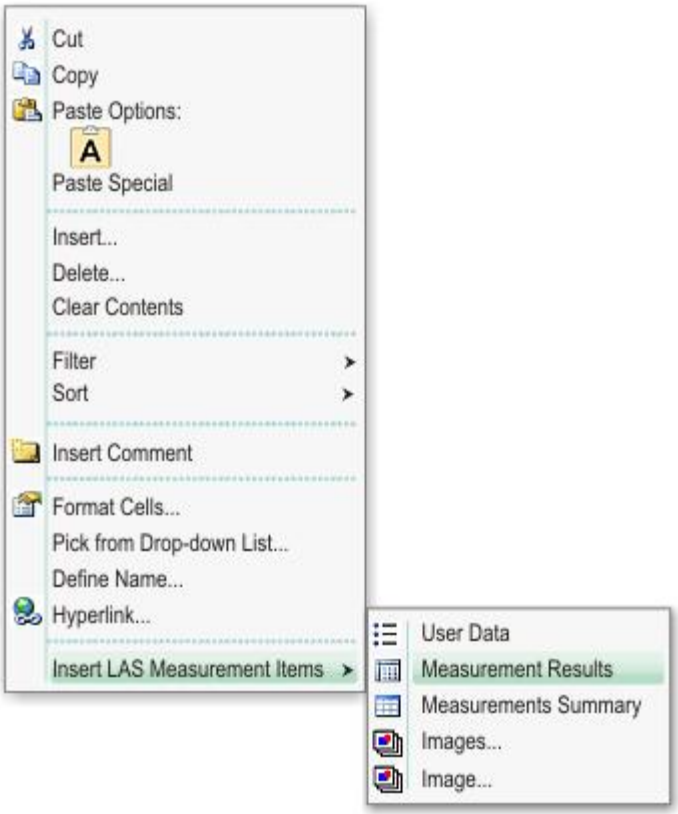
- *Image Name*.
- *Specimen*
- *Description*
- *Observations*
- *Calibration*

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS LM User Data Fixed:1>

When disabled:

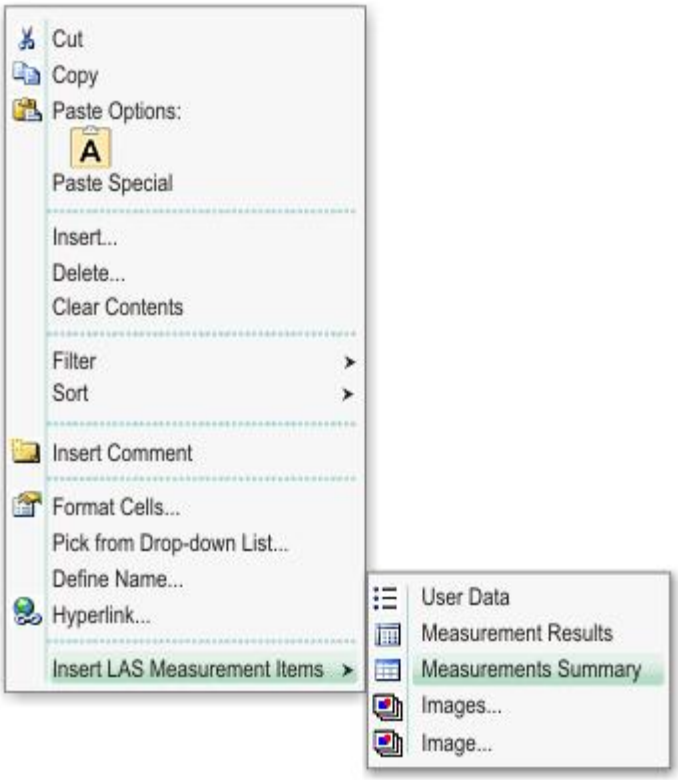
<LAS LM User Data>



<*LAS LM Results*> No user options

For *Measurement Results* the complete list of measurement parameters is:

- *Measurement: Number* .
- *Image Name:* Undefined in Live Measurements
- *Tool: List of tools used*
- *Group*
- *Comments*
- *Line Length (mm)*
- *Width (mm)*
- *Width2 (mm): Refers Dual Circle*
- *Height (mm)*
- *Height2 (mm)*
- *Diameter (mm)*
- *Diameter2 (mm)*
- *Major Axis (mm)*
- *Minor Axis (mm)*
- *Radius (mm)*
- *Radius2 (mm)*
- *Area (mm²)* .
- *Area2 (mm²)*
- *Perimeter (mm)*
- *Perimeter2 (mm)*
- *Centre X*
- *Centre Y*
- *Centre2 X*
- *Centre2 Y*
- *Angle (°)*
- *Angle2 (°)*
- *Angle3 (°)*
- *Side Length (mm)*
- *Side Length2 (mm)*
- *Side Length3 (mm)*
- *End Distance (mm)*
- *Distance (mm)*
- *Distance2 (mm)*
- *Count*
- *Distance Between Centres (mm)*
- *Closest Approach (mm)*
- *Class*
- *Difference Between Area (mm²)*
- *Difference between Radius (mm)*
- *Difference Between Diameter (mm)*
- *Angle Apex Point X*
- *Angle Apex Point Y*
- *X (mm)*
- *Y (mm)*
- *Stage X (mm)*
- *Stage Y (mm)*
- *Stage Z (mm)*
- *Red*
- *Green*
- *Blue*
- *Intensity*



The *Measurement Summary* lists all of the measurement parameters summarised as:

- *Total* .
- *Mean*
- *Mode*
- *Median*
- *Maximum*
- *Minimum*
- *Standard Deviation*
- *Standard Error*
- *Confidence Interval Lower*
- *Confidence Interval Upper*
- *Total Count*
- *Image Area*

<LAS LM Summary> No user options

The *Images* tag display the *Original Image* together with all of the processed image - Merged Image - and image data:

- *Image Name:* Original Image .
- *Pixel Size*
- *Calibrated Size*
- *Calibration:* Pixel = mm
- *Created: Date:* dd/mm/yyyy *Time*: hh:mm:ss
- *File Size:* Kb

The *Images* dialog has two radio buttons (1) that allow the user to:

- Display at the *Original Size* or .
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

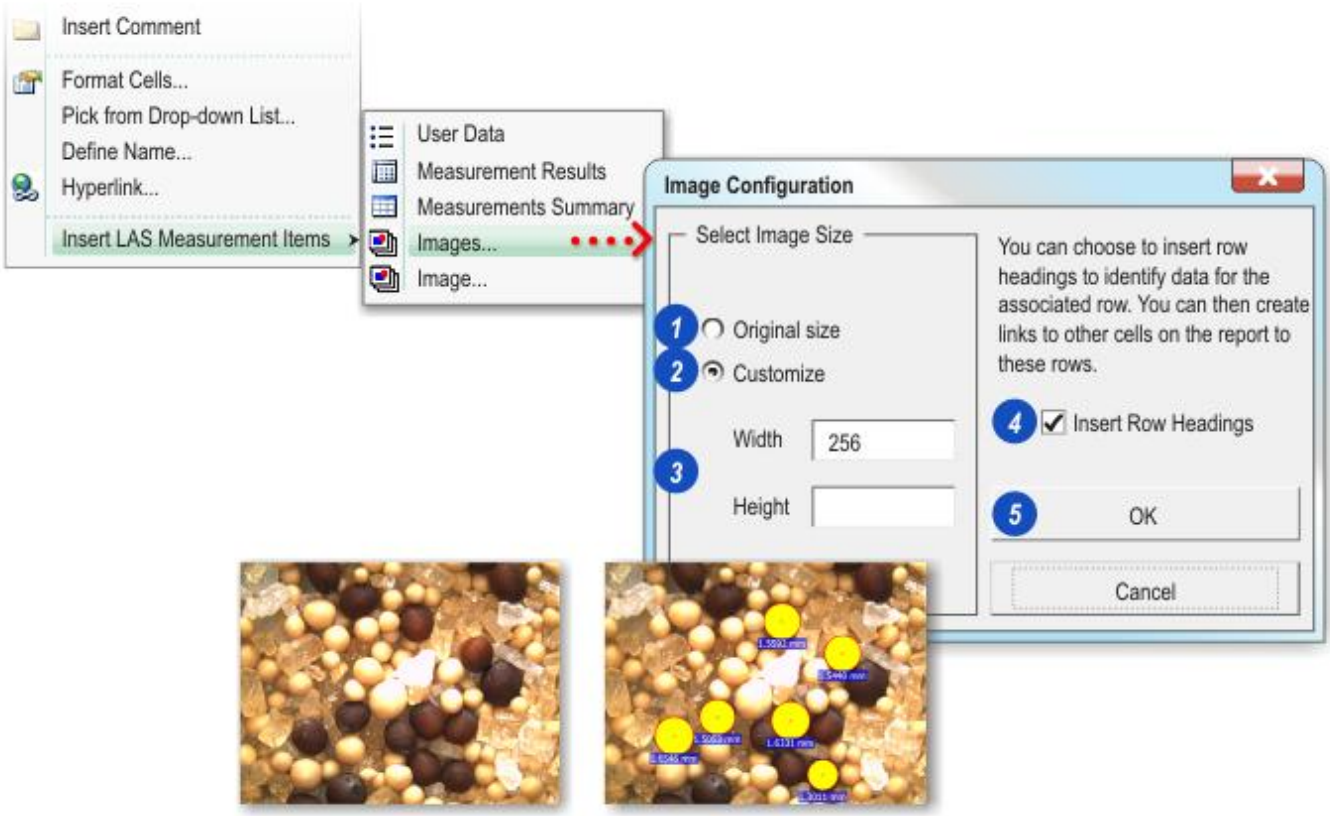
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

Additionally, there is an option to display the image data row headings on the template as a guide to data cell location:

4: Click to enable (tick mark visible) to display the row headings on the template.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - and template display option set up. For example:

<LAS LM Images ***Width:300 Fixed:1****>*



The *Image* tag displays a single, user-selected image at a scale or size also determined by the user:

The dialog has three radio buttons (1) that allow the user to:

- *Fit the Image* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

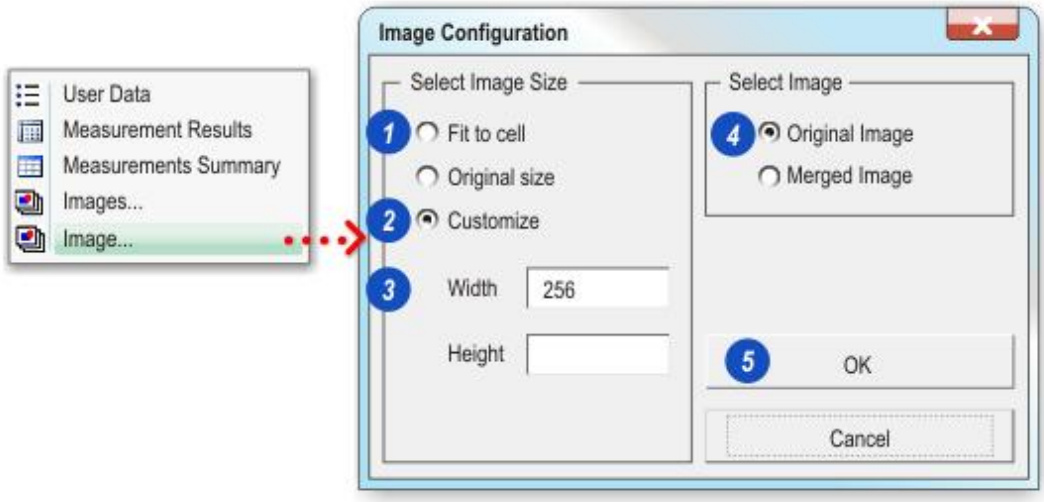
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

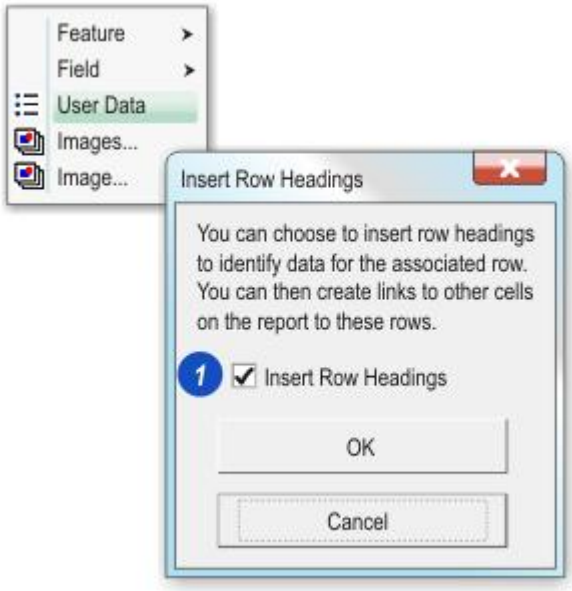
There are 2 additional radio buttons - *Original Image* and *Merged Image* - used to select the image to be displayed:

4: Click the required button.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS LM Image ***Width:256 Type:Original****>*





User Data is taken from the *Reference Data* panel in *Grain Expert.* The row headings are:

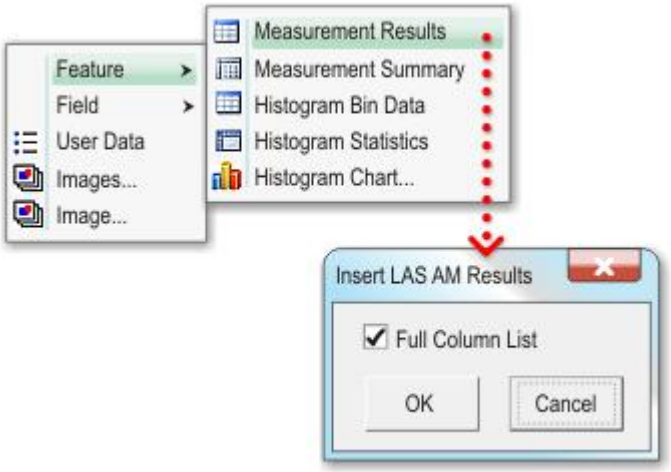
- *Project* .
- *Specimen*
- *Technologist*
- *Keywords*
- *Preparation*
- *Observation*
- *Result*

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM User Data Fixed:1>

When disabled:

<LAS AM User Data>



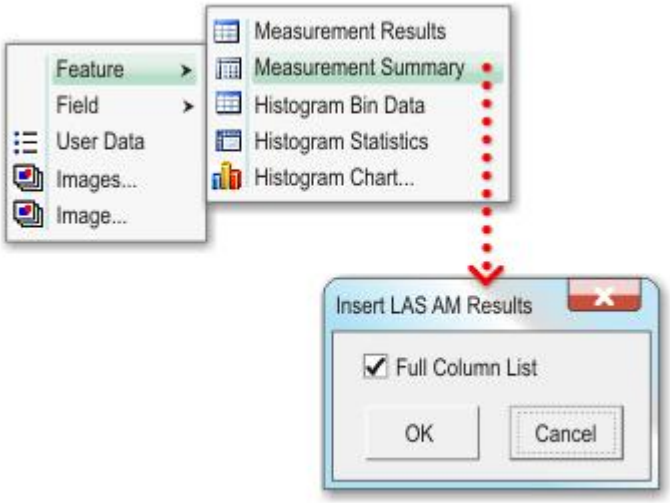
All selected features are listed as:

- *Number: Feature number* .
- *Accepted: Within limits*
- *Area (mm²)*
- *Perimeter*
- *Conv Perim (mm)*
- *V Projection (mm)*
- *H Projection (mm)*
- *Length (mm)*
- *Breadth (mm)*
- *Orientation*
- *X FCP*
- *Y FCP*
- *Roundness*
- *Aspect Ratio*
- *Equiv Circ Diam (mm)*
- *X Centroid*
- *Y Centroid*
- *Grain Number*

On the dialog, users have the option of displaying the row headings on the template as a guide to data position - enable *Full Column List*:

*<LAS AM Results **Full:1**>*

...with the *Full:1* keyword added.



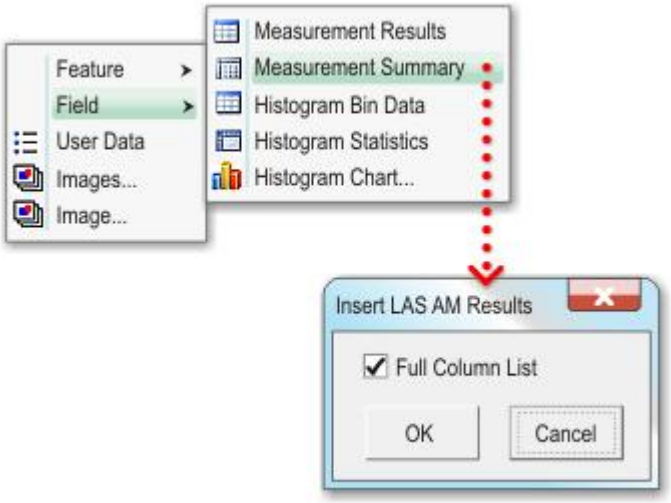
The *Feature Summary* displays statistics derived from all the possible measurement parameters:

<LAS AM Summary Full:1>

...with the keyword *Full:1* added to the tag if the *Full Column List* check box is enabled to display the row headings on the template.

The statistic row headings are:

- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*



The *Field Summary* column headings are:

- *Grain Number* .
- *Mean Grain Area (mm²)*
- *Mean Linear Intercept (mm)*
- *Grain Specific Surface (mm-1)*
- *Phase Percentage (%)*
- *ALA Grain Size*
- *Minimum Grain Size*

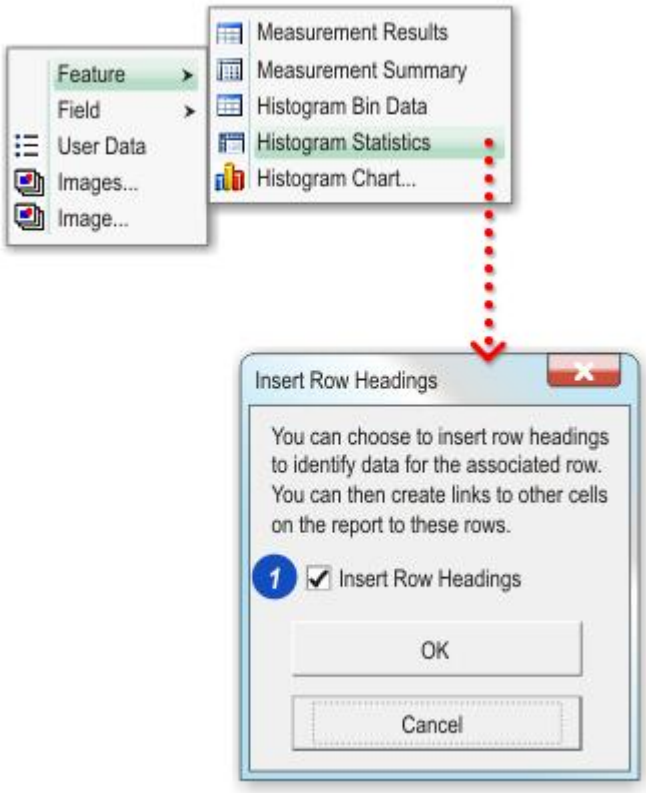
The statistic row headings are:

- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*
- *95% CI*
- *Relative Accuracy*

On the dialog, users have the option of displaying the row headings on the template as a guide to data position - enable *Full Column List*:

<*LAS AM Field Summary* ***Full:1***>

...with the *Full:1* keyword added.



The *Histogram Statistics* are presented in two row sections:

Histogram *Y* axis parameter (***) - for example *Undersize Count*, as:

- *Undersize* *** (mm)
- *Total* *** (mm)
- *Oversize* *** (mm)
- *Normalised* ***
- *Percent (%)* ***

General Statistics:

- *Total*
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*
- *Median*
- *Mode*
- *Skewness*
- *Kurtosis*
- *Features*
- *Specimen Area* (mm^2)

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM Histogram Statistics Fixed:1>

When disabled:

<LAS AM Histogram Statistics>

The *Feature Histogram Bin Data* is arranged as a bin for each image - *Image Number* along the *X* axis - with the bin value representing the *Y* axis parameter:

- *Bin: Number* ,
- *Image Number Lower*
- *Image Number*
- *Count Of images*
- *Y Axis Parameter*

There are no user options.

Histogram Chart:

Displays the *Histogram* as a graphic with the style chosen by the user on the *Results & Histogram* panel. The *Histogram Chart* option has three radio buttons (1) that allow the user to:

- *Fit the Histogram* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or ,
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

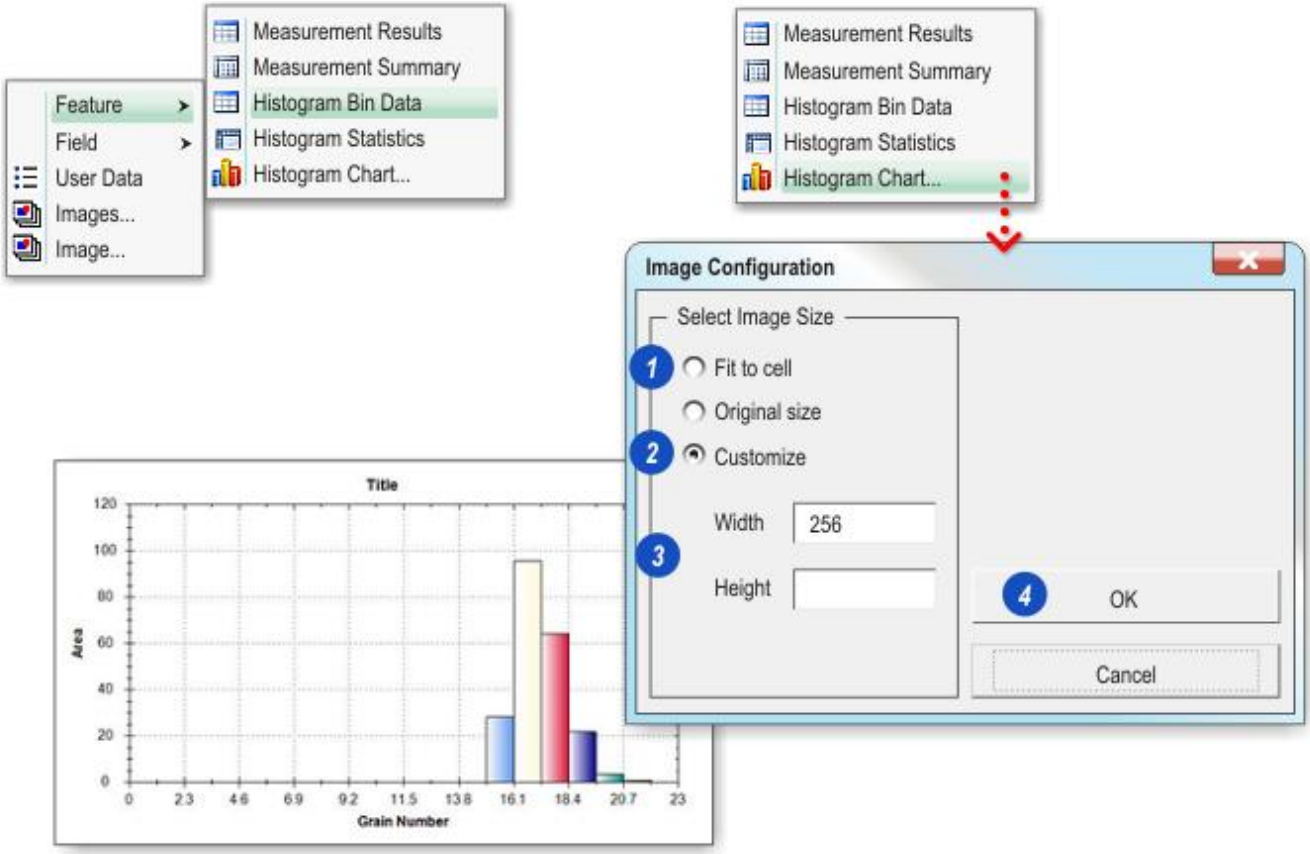
3: ...typing dimensions into the *Width* and *Height* text boxes.

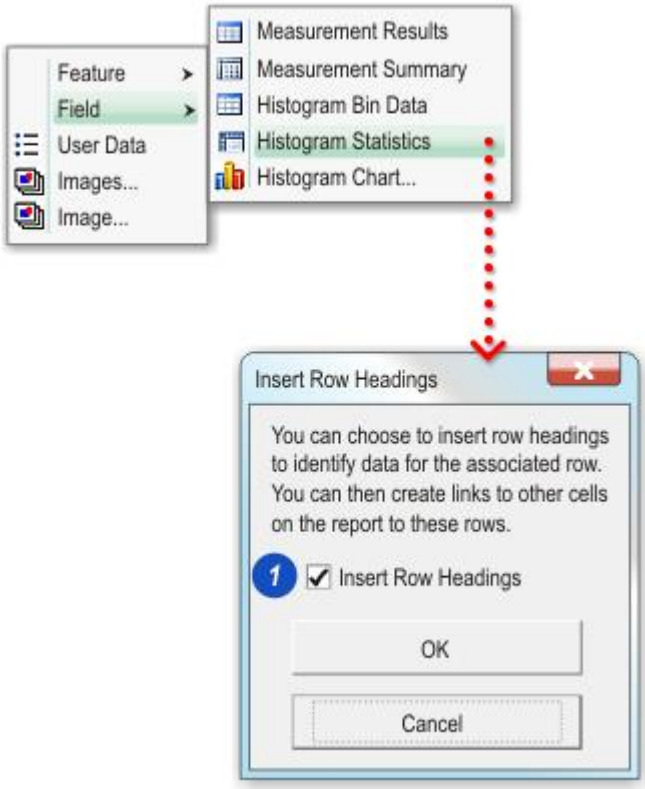
To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

4: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Histogram Chart ***Width:256****>*





The *Field Histogram Statistics* row headings are:

- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*

On the dialog, users have the option of displaying the row headings on the template as a guide to data position - enable *Insert Row Headings (1):*

*<LAS AM Field Summary **Fixed:1**>*

...with the *Fixed:1* keyword added.

The *Field Histogram Bin Data* is arranged as a bin for each image - *Image Number* along the *X* axis - with the bin value representing the *Y* axis parameter:

- *Bin: Number* ,
- *Image Number Lower*
- *Image Number*
- *Count Of images*
- *Y Axis Parameter*

<LAS AM Field Bin Data>

There are no user options.

Histogram Chart:

Displays the *Histogram* as a graphic with the style chosen by the user on the *Results & Histogram* panel. The *Histogram Chart* option has three radio buttons (1) that allow the user to:

- *Fit the Histogram* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or ,
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

4: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Histogram Chart ***Width:256****>*

The *Images* tag display the *Original Image* together with all of the processed images - *Binary Mask, Labels, Colour coded* and *Profile* - and image data:

- *Image Name:* Original Image .
- *Pixel Size*
- *Calibrated Size*
- *Calibration:* Pixel = mm
- *Calibration Value*
- *Created: Date:* dd/mm/yyyy *Time*: hh:mm:ss
- *File Size:* Kb

The *Images* dialog has two radio buttons (1) that allow the user to:

- Display at the *Original Size* or .
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

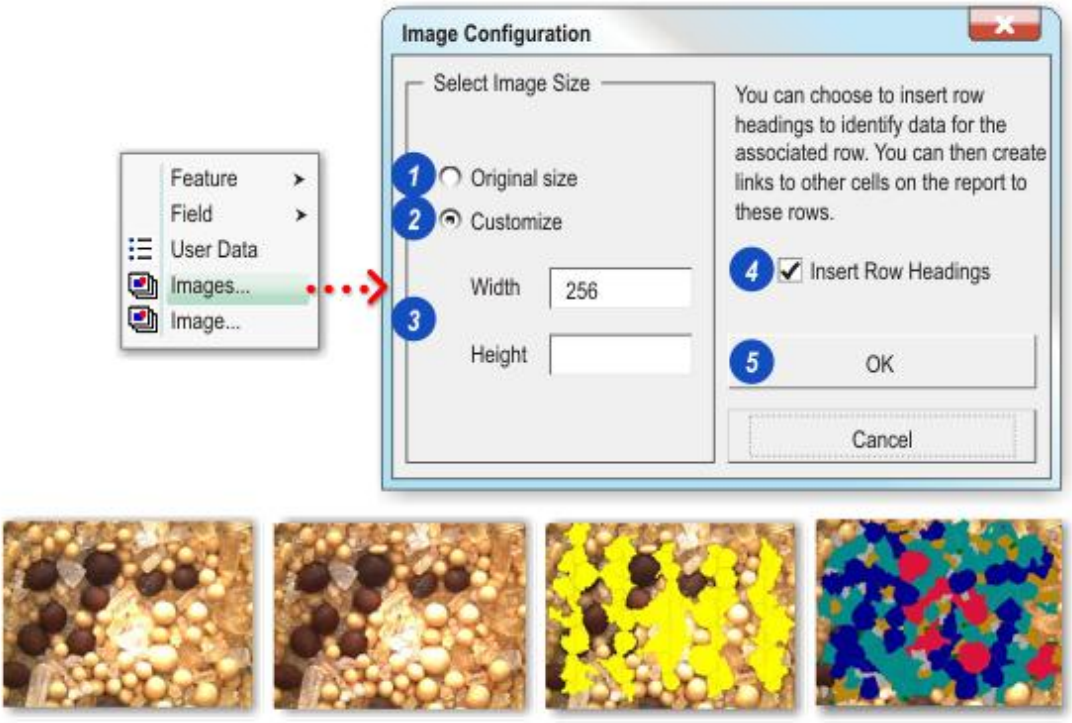
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

Additionally, there is an option to display the image data row headings on the template as a guide to data cell location:

4: Click to enable (tick mark visible) to display the row headings on the template.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - and template display option set up. For example:

<LAS AM Images ***Width:300 Fixed:1****>*



The *Image* tag displays a single, user-selected image at a scale or size also determined by the user:

The dialog has three radio buttons (1) that allow the user to:

- *Fit the Image* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

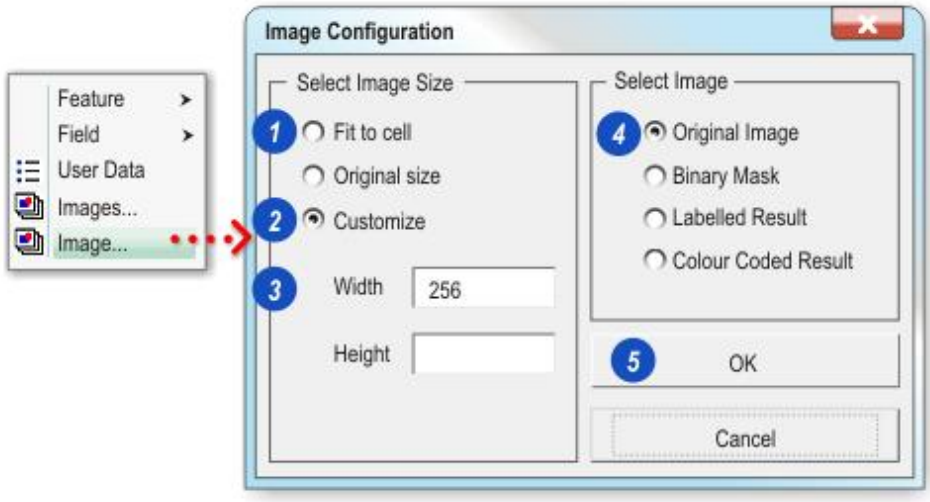
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

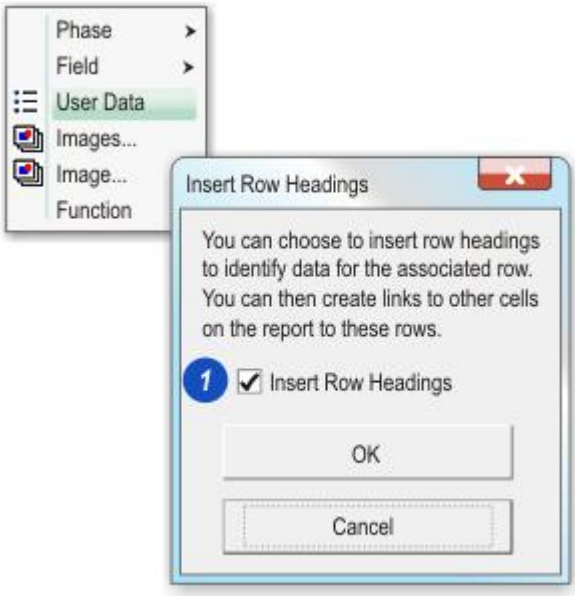
There are 4 additional radio buttons - *Original Image, Binary Mask, Labels* and *Colour Coded* - used to select the image to be displayed:

4: Click the required button.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Image ***Width:256 Type:Original****>*





User Data is taken from the *Reference Data* panel in *Grain Expert.* The row headings are:

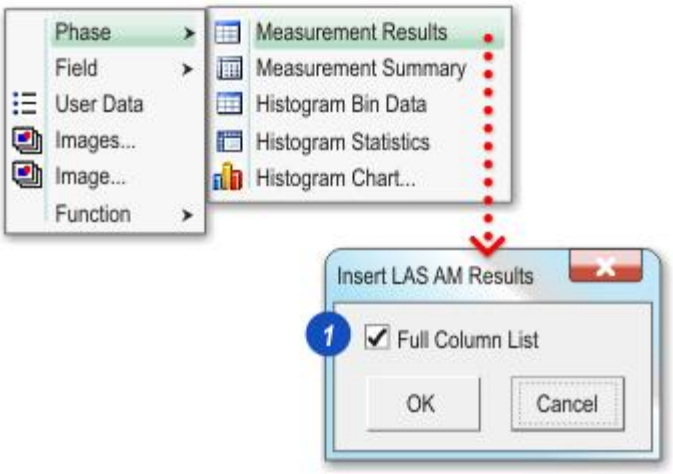
- *Project*
- *Specimen*
- *Result*
- *Observation*
- *Technologist*
- *Preparation*
- *Keywords*

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM User Data Fixed:1>

When disabled:

<LAS AM User Data>



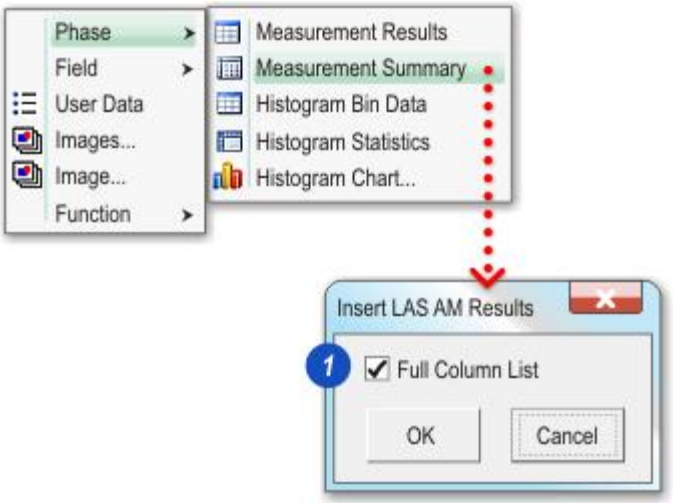
All selected phases are listed under parameters:

- *Number* .
- *Images*
- *Phase Name*
- *Area Percent (%)*
- *Area (mm²)*
- *Area Fraction*
- *Area Fill*
- *Perimeter (mm)*
- *Mean Chord (mm)*
- *Intercept H (mm)*
- *Intercept V (mm)*
- *Aninotropy*
- *Count*
- *Count Per Area (mm²)*
- *Absolute Specific Surface (mm-1)*
- *Relative Specific Surface (mm-1)*
- *Frame Area (mm²)*

On the dialog, users have the option of displaying the row headings on the template as a guide to data position - enable *Full Column List* (1):

*<LAS AM Phase Results **Full:1**>*

...with the *Full:1* keyword added.



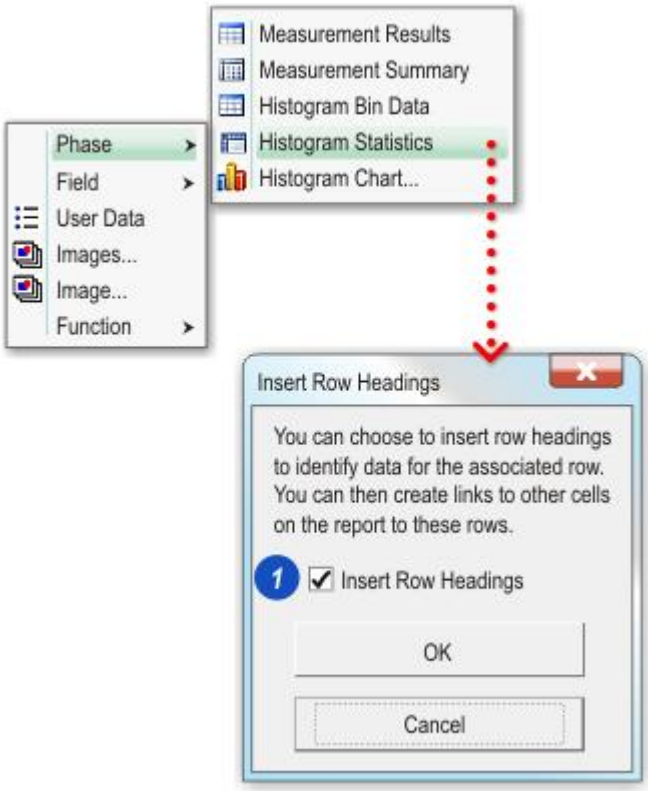
The *Phase Summary* displays statistics derived from all the possible measurement parameters:

<LAS AM Phase Summary Full:1>

...with the keyword *Full:1* added to the tag if the *Full Column List* check box (1) is enabled to display the row headings on the template.

The statistic row headings are:

- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*
- *95% CI*
- *Relative Accuracy*



The *Phase Histogram Statistics* are presented as:

- *Total*
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*
- *95% CI*
- *Number of Images*
- *Specimen Area* (mm²)

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM Phase Histogram Statistics Fixed:1>

When disabled:

<LAS AM Phase Histogram Statistics>

The *Phase Histogram Bin Data* is arranged as a bin for each image - *Image Number* along the *X* axis - with the bin value representing the *Y* axis parameter:

- *Bin: Number* ,
- *Phase Number Lower*
- *Phase Number*
- *Phase Name*
- *Y Axis Parameter*
- *Percent of Total (Y Axis Parameter)*

There are no user options.

Histogram Chart:

Displays the *Histogram* as a graphic with the style chosen by the user on the *Results & Histogram* panel. The *Histogram Chart* option has three radio buttons (1) that allow the user to:

- *Fit the Histogram* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or ,
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

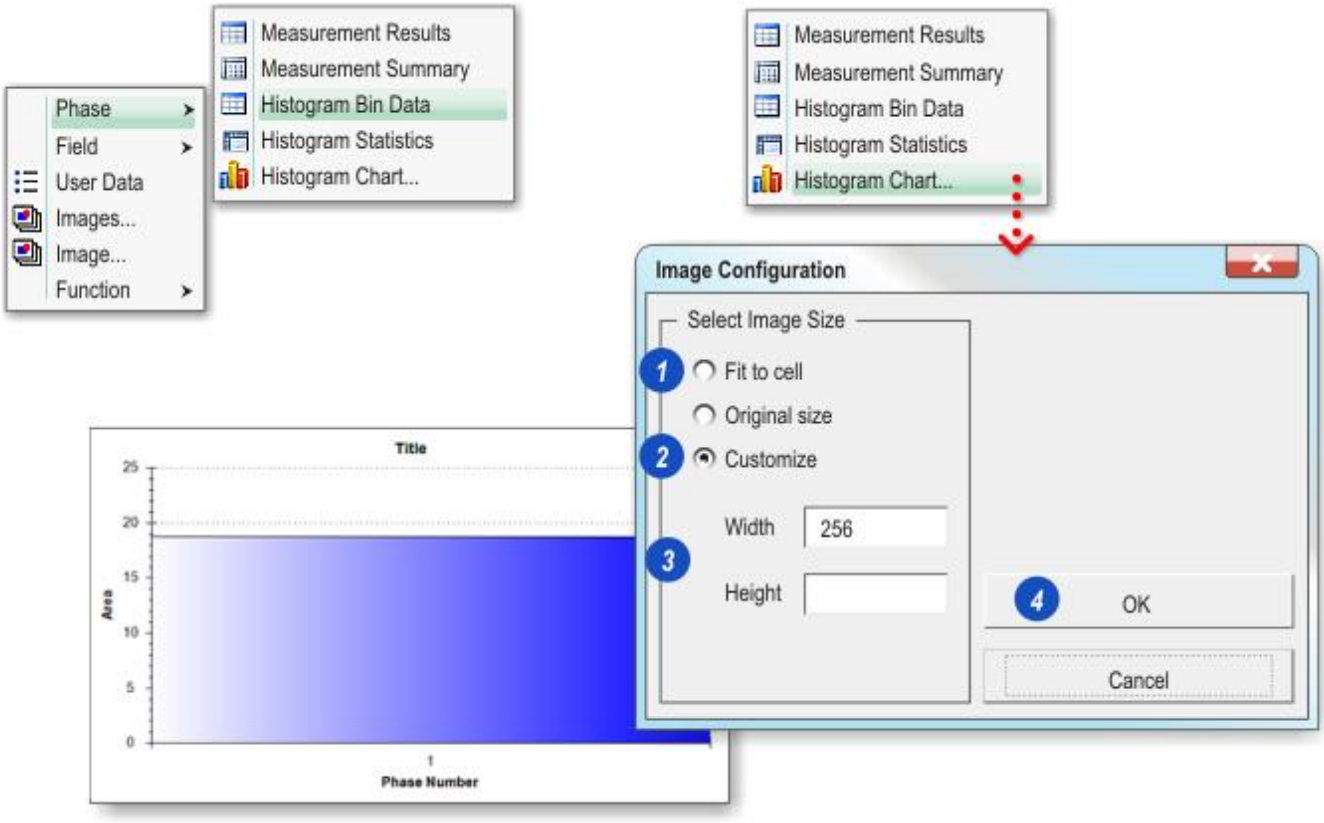
3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

4: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Phase Histogram Chart ***Width:256****>*



The *Images* tag display the *Original Image* together with all of the processed images - *Binary Mask, Labels, Colour coded* and *Profile* - and image data:

- *Image Name:* Original Image .
- *Pixel Size*
- *Calibrated Size*
- *Calibration:* Pixel = mm
- *Calibration Value*
- *Created: Date:* dd/mm/yyyy *Time*: hh:mm:ss
- *File Size:* Kb

The *Images* dialog has two radio buttons (1) that allow the user to:

- Display at the *Original Size* or .
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

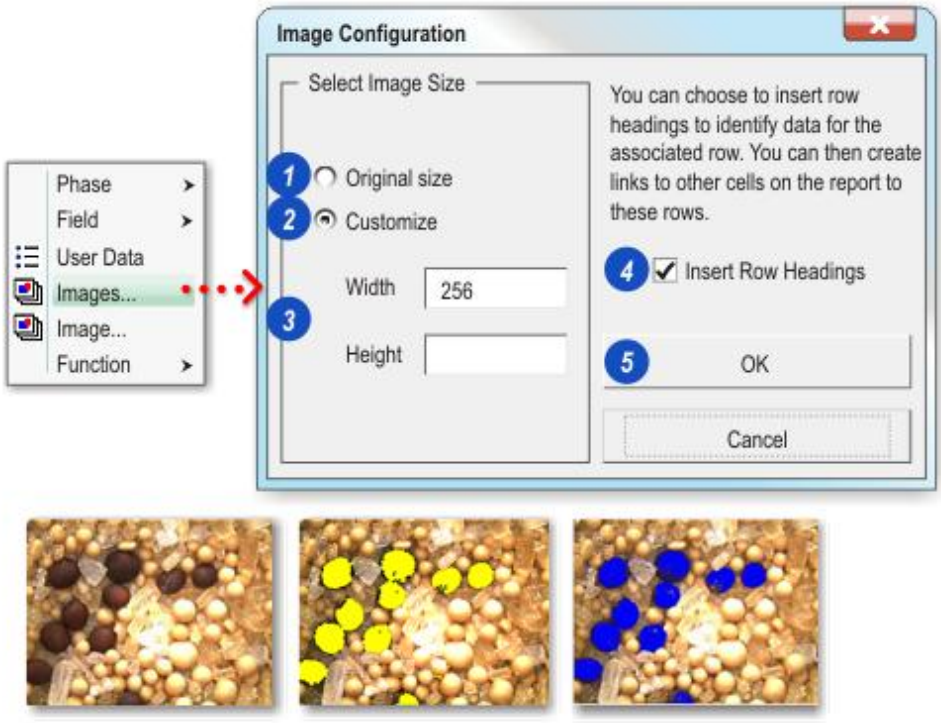
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

Additionally, there is an option to display the image data row headings on the template as a guide to data cell location:

4: Click to enable (tick mark visible) to display the row headings on the template.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - and template display option set up. For example:

<LAS AM Images ***Width:300 Fixed:1****>*



The *Image* tag displays a single, user-selected image at a scale or size also determined by the user:

The dialog has three radio buttons (1) that allow the user to:

- *Fit the Image* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

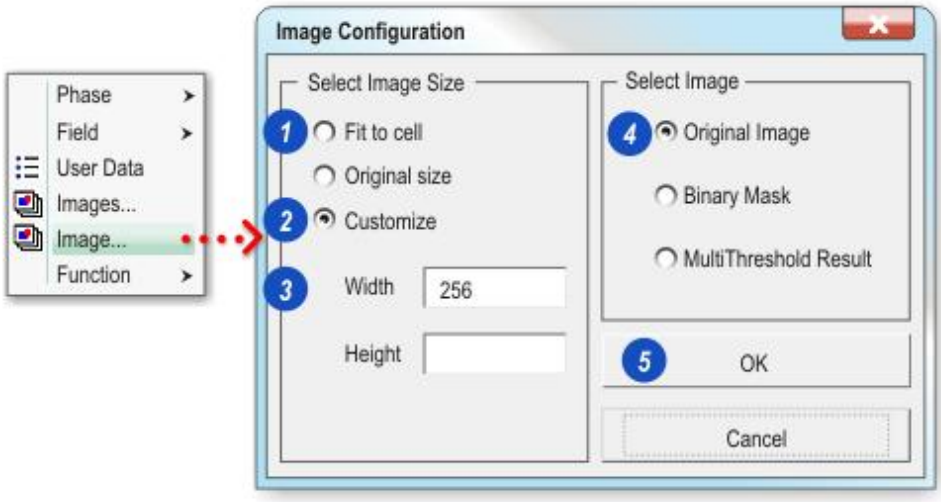
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

There are 4 additional radio buttons - *Original Image, Binary Mask* and *MultiThreshold* (a combination of all the Binary images) - used to select the image to be displayed:

4: Click the required button.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Image ***Width:256 Type:Original****>*

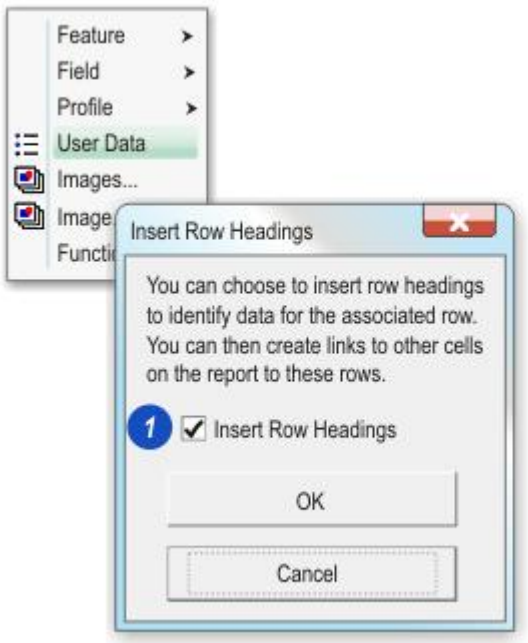


Small formulas that produce a specific result are called *Functions.*

The *Today* function that automatically inserts the current *Date* and *Time* in the format:

- *Date*: dd/mm/yyyy and..
- *Time*: hh:mm:ss into the cell.

<*LAS AM Function* ***Type:Today***>



User Data is taken from the *Reference Data* panel in *Image Analysis.* The row headings are:

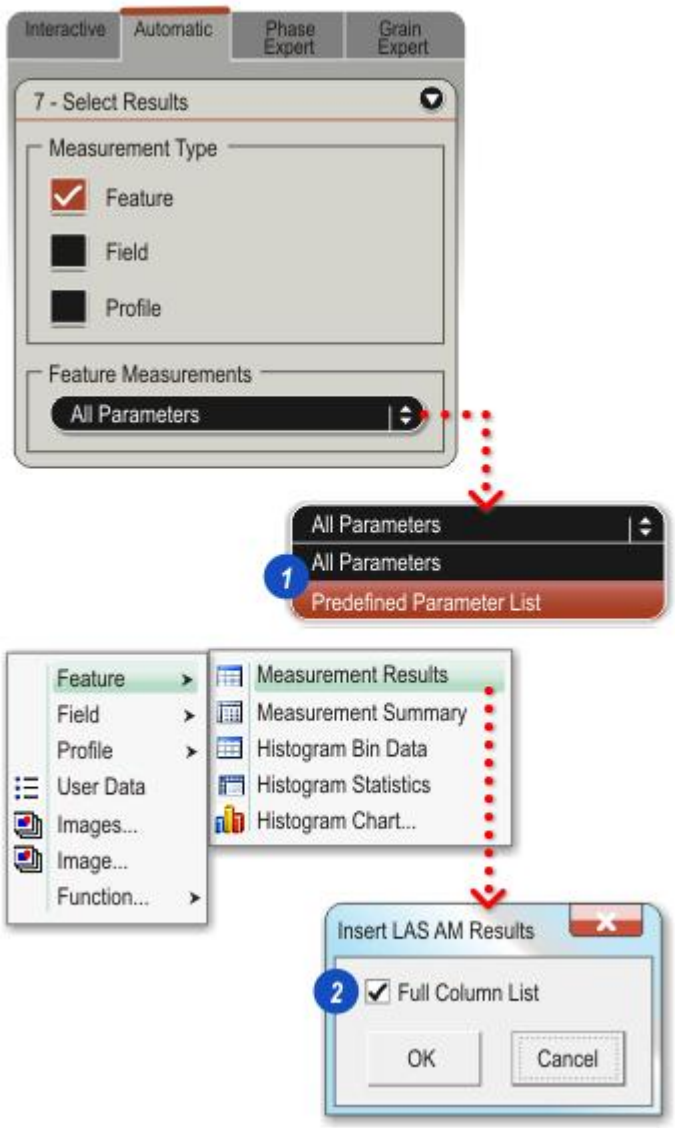
- *Project* .
- *Preparation*
- *Specimen*
- *Keywords*
- *Result*
- *Observation*
- *Technologist*

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM User Data Fixed:1>

When disabled:

<LAS AM User Data>



In *Image Analysis* users can choose between making measurements for all parameters or a reduced predefined selection (1). The *Results* tag can be configured to display these options.

On the dialog (2) enable the *Full Column List* check box to displays all of the parameters:

<LAS AM Results Full:1> *(see here[460])*

...with the keyword *Full:1* added to the tag.

With the check box disabled only the *Predefined Parameters* are displayed:

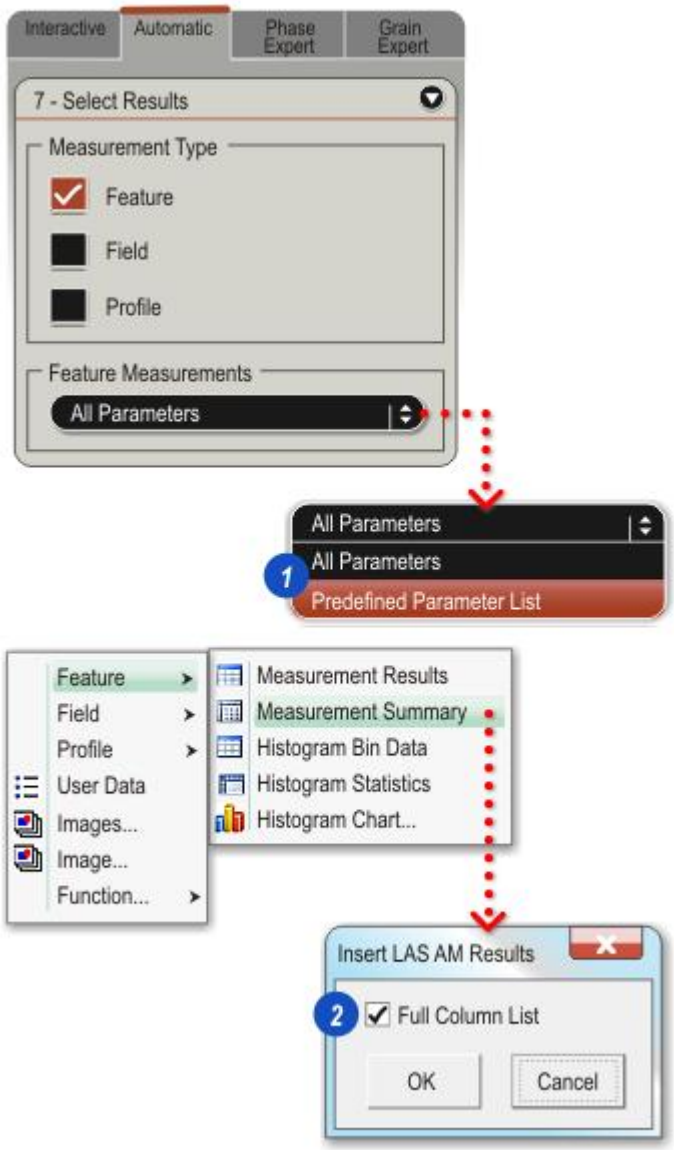
<LAS AM Results> *(see here[464])*

- *Number*: Feature Number .
- *Images*: Image Names
- *Accepted*: Features within the result parameters
- *Area* (mm2)
- *X-FCP*
- *Y-FCP*
- *Feret 0*
- *Feret 90*
- *V Projection* (Vertical Projection)
- *H Projection* (Horizontal Projection)
- *Length*
- *Breadth*
- *Orthl Feret* (Orthogonal Feret)
- *Orientation*
- *Perimeter*
- *Conv Perim* (Convex Perimeter)
- *Roundness*
- *Compactness*
- *Y Max*
- *X Max*
- *Y Min*
- *X Min*
- *X Centroid*
- *Y Centroid*
- *Derived Orientation* .
- *Aspect Ratio*
- *Equiv Circ Diam* (Equivalent Circle Diameter)
- *Curve Length*
- *Curve Ratio*
- *Convex Area*
- *Fullness Ratio*
- *Forks*
- *Joins*
- *Tops*
- *Ends*
- *Integrated Grey*
- *Grey Mean*
- *Grey Variance*
- *Integrated Red*
- *Red Mean*
- *Red Variance*
- *Integrated Green*
- *Green Mean*
- *Green Variance*
- *Integrated Blue*
- *Blue Mean*
- *Blue Variance*

** Greyscale images only*

Predefined Parameter List:

- *Number*: Feature Number .
- *Images*: Image Name
- *Accepted*
- *Area* (mm2)
- *X-FCP*
- *Y-FCP*
- *Length* (mm) .
- *Perimeter* (mm)
- *Roundness*
- *X Centroid*
- *Y Centroid*
- *Equiv Circle Diam* (Equivalent Circle Diameter mm)



The *Summary* displays either statistics derived from either all the possible measurement parameters:

<LAS AM Summary Full:1>

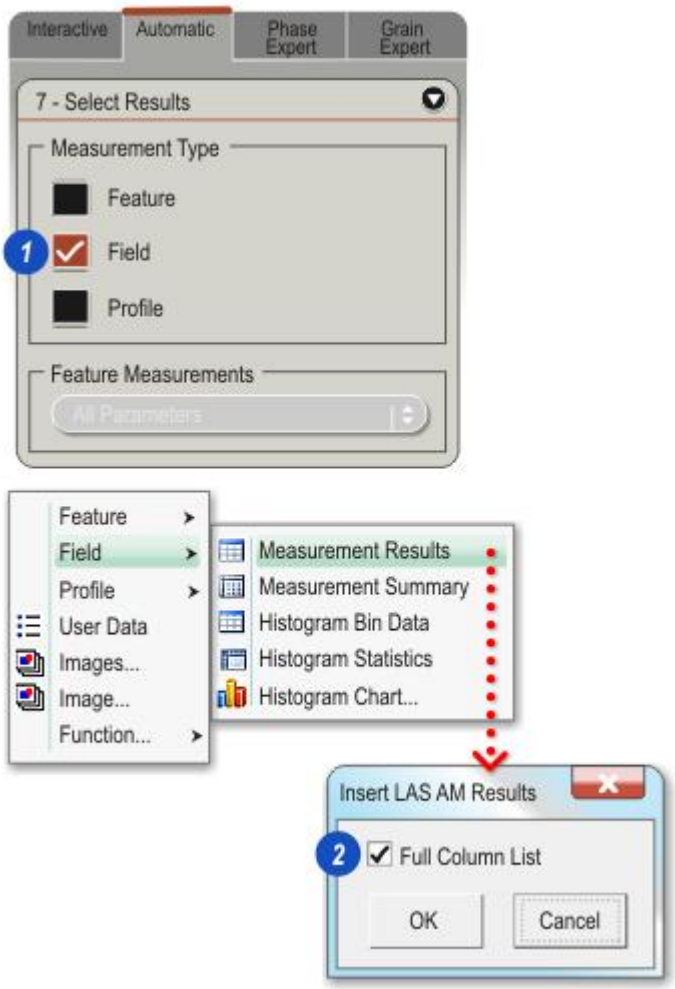
...with the keyword *Full:1* added to the tag, or statistics based only upon the *Predefined Parameters* (1):

<LAS AM Summary>

The option is chosen by enabling/disabling the *Full Column List* check box on the dialog (2).

The statistic row headings are the same for both options.

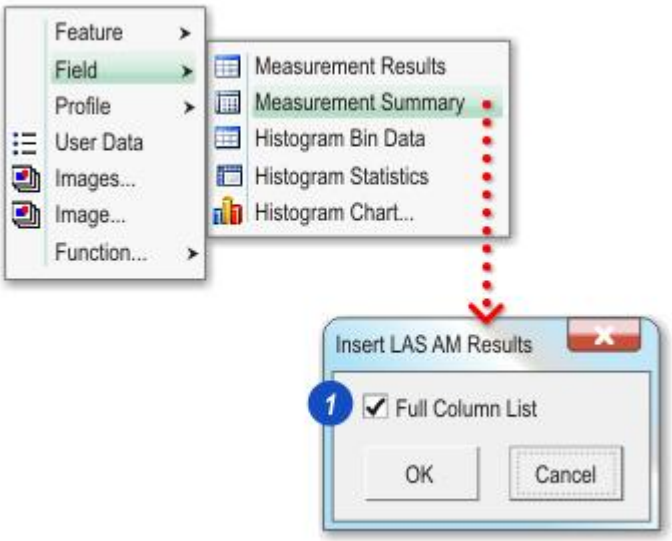
- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*



With the *Field* check box enabled on *Image Analysis Select Results* panel (1), the column parameters are:

- *Number:* Field included .
- *Images:* Image name
- *Area Percent (%)*
- *Area (mm²)*
- *Area Fraction*
- *Area Fill*
- *Perimeter*
- *Mean Chord (mm)*
- *Intercept H (mm)*
- *Intercept V (mm)*
- *Anisotropy*
- *Count*
- *Count per Area (mm^{-2})*
- *Absolute Specific Surface (mm^{-1})*
- *Relative Specific Surface (mm^{-1})*
- *Frame Area (mm^2)*

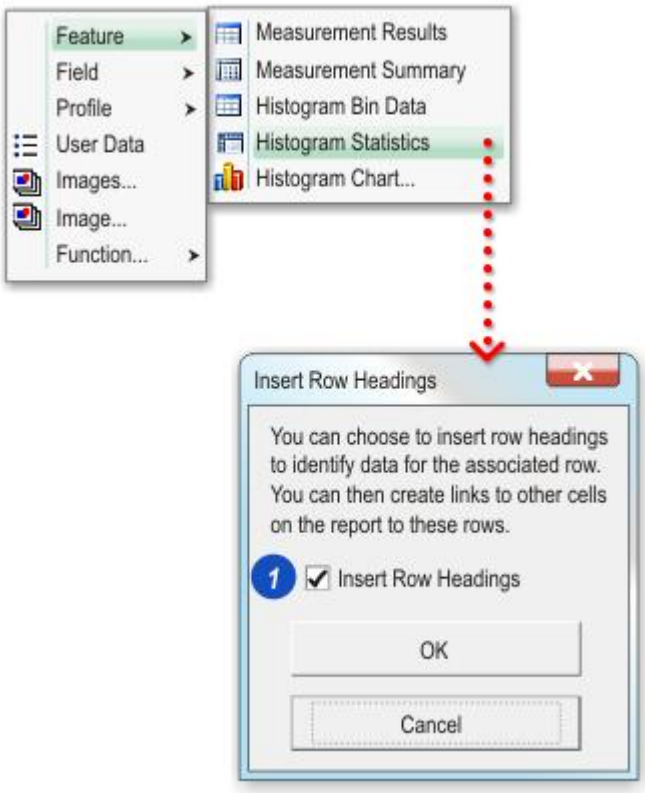
On the dialog, if the *Full Column List* check box is enabled (2) all results are displayed regardless of the *Select Field Statistics* settings.



All of the *Field* parameters are analysed as:

- *Total* .
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*

On the dialog, if the *Full Column List* check box is enabled (1) all results are summarised regardless of the *Select Field Statistics* settings.



Feature Histogram Statistics are presented in two row sections:

Histogram *Y* axis parameter (***) for example *Total Length*, as:

- *Undersize* *** (mm)
- *Total* *** (mm)
- *Oversize* *** (mm)
- *Normalised* ***
- *Percent (%)* ***

General Statistics:

- *Total*
- *Mean*
- *Std Dev* (Standard Deviation)
- *Standard Error*
- *Maximum*
- *Minimum*
- *2-S Range*
- *Median*
- *Mode*
- *Skewness*
- *Kurtosis*
- *Features*
- *Specimen Area* (mm^2)

The dialog provides the option to include these row headings on the template - *Insert Row Headings* (1) enabled - and adds the keyword *Fixed:1* to the tag:

<LAS AM Histogram Statistics Fixed:1>

When disabled:

<LAS AM Histogram Statistics>

The *Histogram Bin Data* is displayed under row headings reflecting the *X* and *Y* axis parameters.

For example, if the *X* axis parameter is *Breadth* the first column would be:

Breadth (mm) Lower.

If the Y axis parameter is *Length* then the *Percent of* column would be:

Percent of Total Length.

Headings:

- *Bin Number*.
- X Parameter *Lower*
- X Parameter *Upper*
- *Count:* Of total features in bin.
- Y Parameter
- *Percent of Total* Y Parameter

There are no options for *Histogram Bin Data.*

Histogram Chart:

Displays the *Histogram* as a graphic with the style chosen by the user on the *Image Analysis Histogram* panel. The *Histogram Chart* option has three radio buttons (1) that allow the user to:

- *Fit the Histogram* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

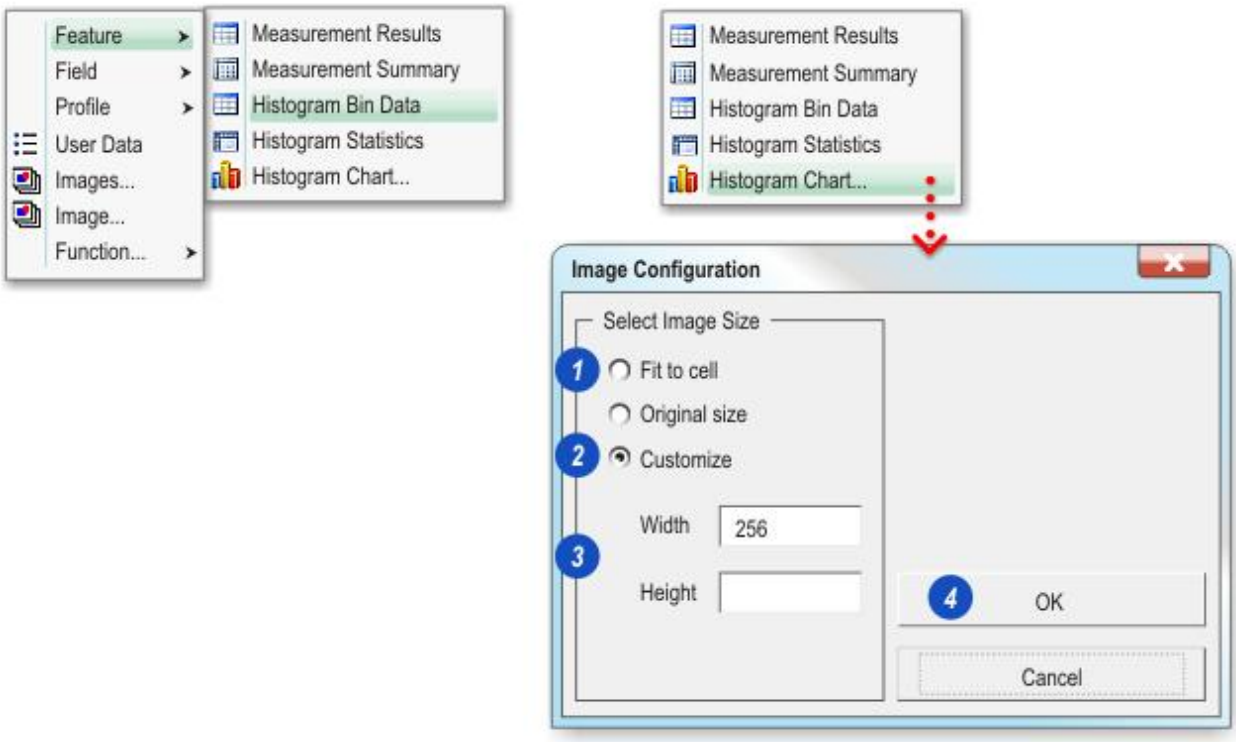
3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

4: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Histogram Chart ***Width:256****>*



Profile Results list all of the colour values - *Red, Green* and *Blue* - for every pixel across the width of the *Profile Measure Frame:*

<LAS AM Profile Results>

- *Distance*: In pixels from the left of the frame .
- *Red*: Count
- *Blue*: Count
- *Green*: Count

Profile Bin Data:

Profile Bin Data list all of the colour values - *Red, Green* and *Blue* - for every pixel across the width of the *Profile Measure Frame*, each pixel representing a *Histogram Bin*:

<LAS AM Profile Bin Data>

- *Histogram Bin*: In pixels from the left of the frame .
- *Red*: Count
- *Blue*: Count
- *Green*: Count

Histogram Chart:

Displays the *Histogram* as a graphic with the style chosen by the user on the *Image Analysis Histogram* panel. The *Histogram Chart* option has three radio buttons (1) that allow the user to:

- *Fit the Histogram* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

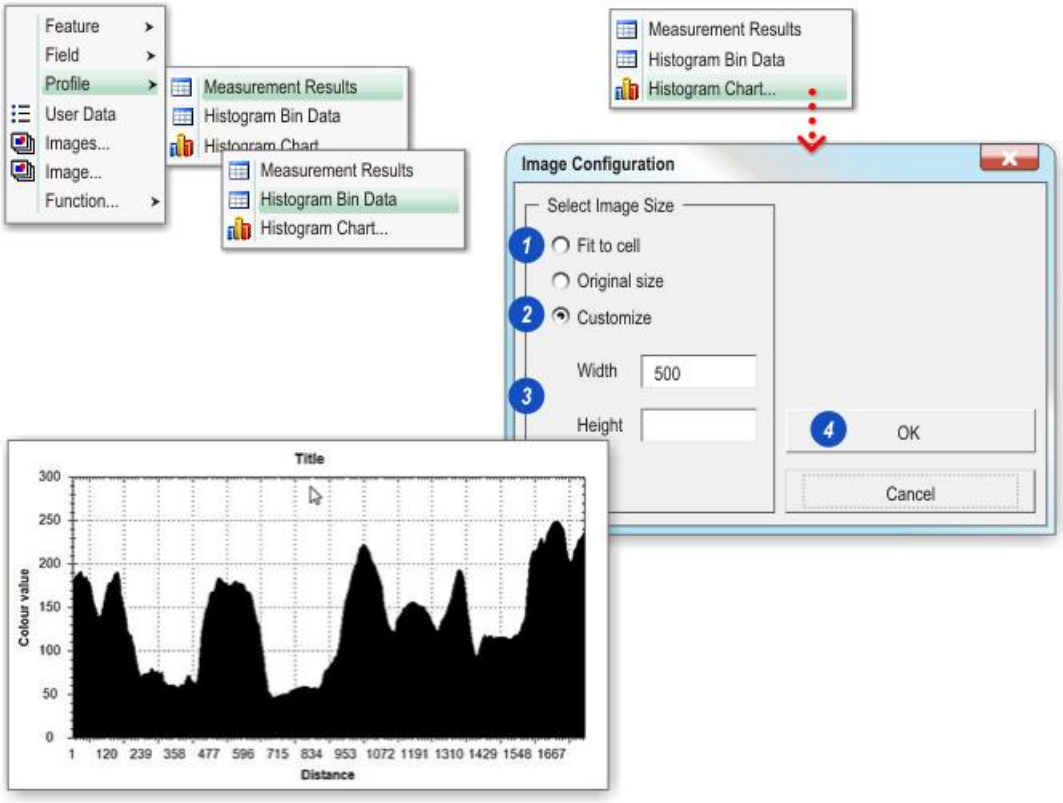
3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

4: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Histogram Chart ***Width:500****>*



The *Images* tag display the *Original Image* together with all of the processed images - *Binary Mask, Labels, Colour coded* and *Profile* - and image data:

- *Image Name:* Original Image .
- *Pixel Size*
- *Calibrated Size*
- *Calibration:* Pixel = mm
- *Calibration Value*
- *Created: Date:* dd/mm/yyyy *Time*: hh:mm:ss
- *File Size:* Kb

The *Images* dialog has two radio buttons (1) that allow the user to:

- Display at the *Original Size* or .
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

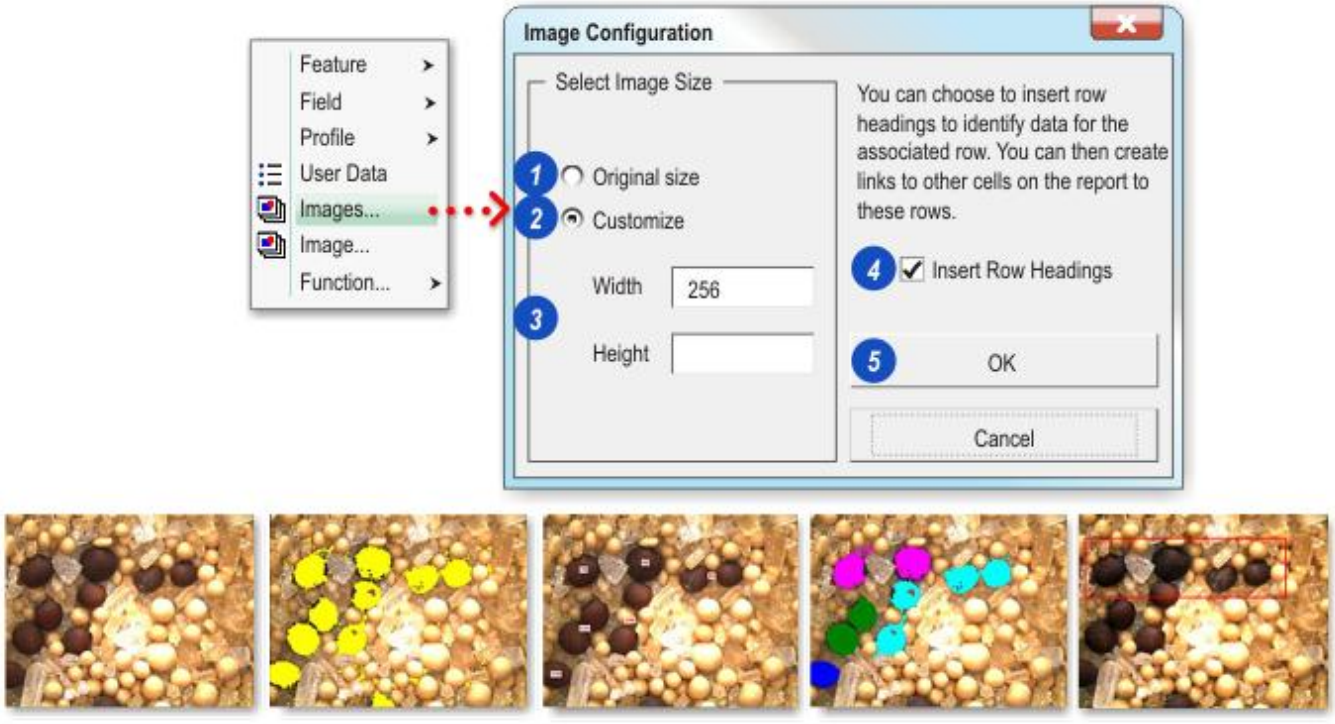
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

Additionally, there is an option to display the image data row headings on the template as a guide to data cell location:

4: Click to enable (tick mark visible) to display the row headings on the template.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - and template display option set up. For example:

<LAS AM Images ***Width:300 Fixed:1****>*



The Image tag displays a single, user-selected image at a scale or size also determined by the user:

The dialog has three radio buttons (1) that allow the user to:

- *Fit the Image* into the selected cell - the user is responsible for sizing the cell to reasonable dimensions - or,
- *Display at the Original Size* regardless of the selected cell dimensions or
- *Customize* - Scale or resize by...

2: ...clicking the *Customize* option and...

3: ...typing dimensions into the *Width* and *Height* text boxes.

To scale the image, enter only a *Width* or *Height* dimension.

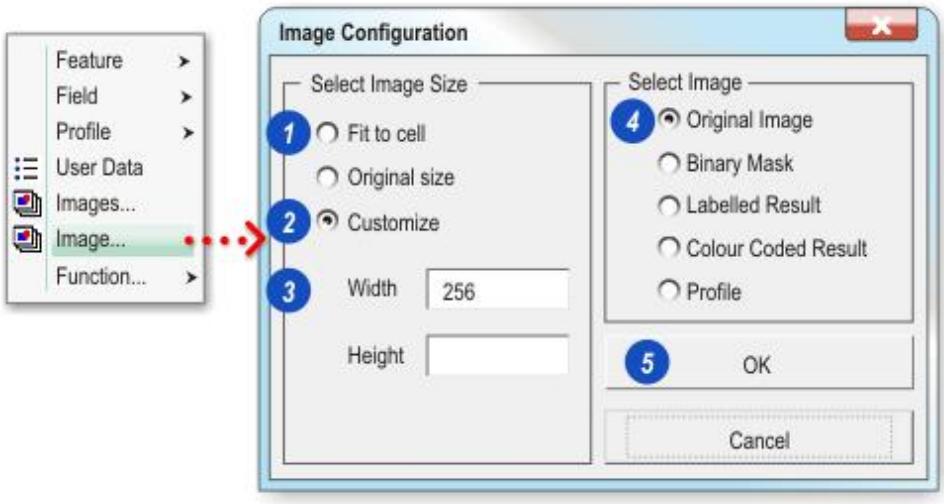
To resize the image type a value in both boxes and the image will be stretched or shrunk accordingly.

There are 5 additional radio buttons - *Original Image, Binary Mask, Labels, Colour Coded* and *Profile* - used to select the image to be displayed:

4: Click the required button.

5: Click the *OK* button to copy the tag with the dimension - *Width* and *Height* - set up. For example:

<LAS AM Image ***Width:256 Type:Labels****>*



Small formulas that produce a specific result are called *Functions.*

The *Today* function that automatically inserts the current *Date* and *Time* in the format:

- *Date*: dd/mm/yyyy and..
- *Time*: hh:mm:ss into the cell.

<*LAS AM Function* ***Type:Today***>

LAS Archives: Basic and Standard

LAS operates in 2 distinct modes depending how the user wishes to manage the acquired images. These are:

LAS Image Explorer and
LAS Folder Archive.

LAS Image Explorer is used when images are stored to the hard drive in an informal manner. The user can save the images anywhere on the hard drive in any folder and organizes the images as convenient. While this method has the benefit of simplicity, there is a chance that over time the images will become harder to find.

LAS Folder Archives are used where a more disciplined approach to the organization of data is demanded. This might occur where it is mandatory to save additional user defined fields with the images or where several users need to add data to images in a systematic manner. This also means that images are saved in locations where other users will be able to find them. In this case it is a distinct advantage to restrict the locations that the images are saved. We call these '*Folder Archives*' because the images are still saved to the Windows file system but only in predefined locations.

Implementation Overview
Images are combined with text and numeric data, microscope information and camera parameters in individual records of a database that you can tailor to the specific needs of the application.

The content of a record is defined by use of the Archive Design tool on the Setup Workflow. The Archive Designer allows you define hierarchical levels by which data is grouped (*e.g. Lab Name >Procedure >Customer Name > Experiment >Specimen Number >Result*). There is virtually no limit to the number of different fields or the volume of information stored. The original high-resolution images are held external to the database. New fields can be added to an existing database or redundant fields removed without difficulty. System information, including operator name, data and time are added automatically, while all the microscope and camera parameters are included by default for both Basic and Standard editions.

LAS keeps the metadata in a file associated with the image and in the same location although this file is normally hidden. If you do make changes to these files you do so at your peril! For the purposes of managing your disk space, you can choose where the database and associated files are kept if you want to Because the archive uses the normal Windows file system, you can use standard back-up tools.

Once the Archive is created, it remains in the location on the hard drive that was originally specified. When the Archive is created, the location of the images and metadata is also specified. However, the images can easily take up many GB of space and may fill your hard drive. If this happens, the entire archive can be moved using normal Windows tools to an alternative location. This location can be on a remote drive but please be aware that this will affect the performance of recall for large images and be dependent on the network speed.

When more than one user accesses the same data, there is inevitably some security needed. This may be a matter of preventing less experienced users from accidentally damaging or destroying valuable data. LAS supports the Windows user log-on so that if you wish you can create databases with restricted access.

Each image in the database has an image name. Software in LAS ensures that image names are unique to a folder by appending an incrementing number where possible and by providing numbered sequences for the sequence modules.
Clearly it is vital that you have easy access to the images that are in the database and LAS provides several feature to expedite this.

If you need to export image files directly to external media or to a personal file space, you should use the export tool. This will ensure that a copy of the image is made and you will not then be likely to damage or destroy vital data in the database. The exported images from a sequence will each use the image name as part of the file name. This ensures that sequence of images will be readily recognizable by you once exported.

Summary
In order to achieve a fast, reliable and scalable tool for microscope data capture and processing, it is important to use modern database technology and to enforce some simple rules about how the user interacts with that data. This may impose some limitations on the ways in which you work with your data and may mean that you have to change some of the procedures you operate in your work. In creating LAS Archive, we have taken great trouble, however, to provide new features that will allow you to find new working practices to achieve your workload and in a way that will allow traceability, scalability and security for the future.

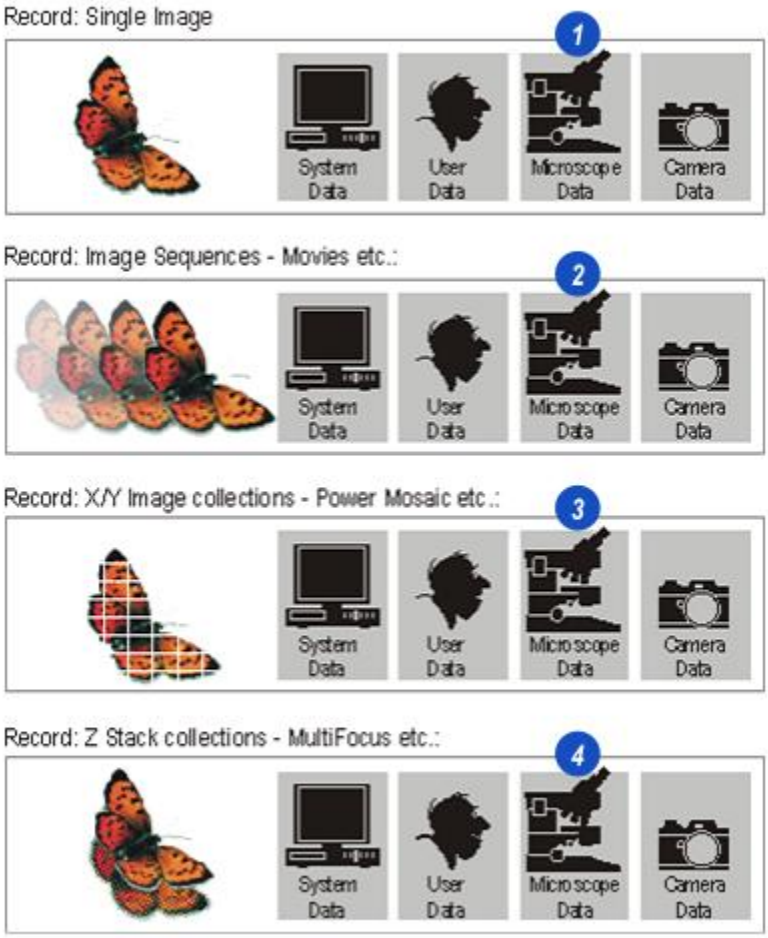
Every single image or collection of images is associated with a Record. It is a self-contained entity storing not only the actual image data but a wealth of information associated with it. Images may be:

1: Single images.

2: Image sequences such as time lapse, size only being constrained by the hardware and preferences.

3: Multiple images like those from Montage.

4: Image groups produced as Z-Stacks…
…or any created and modified within Leica Application Suite.



Depending upon the hardware, each image has data automatically stored with it:

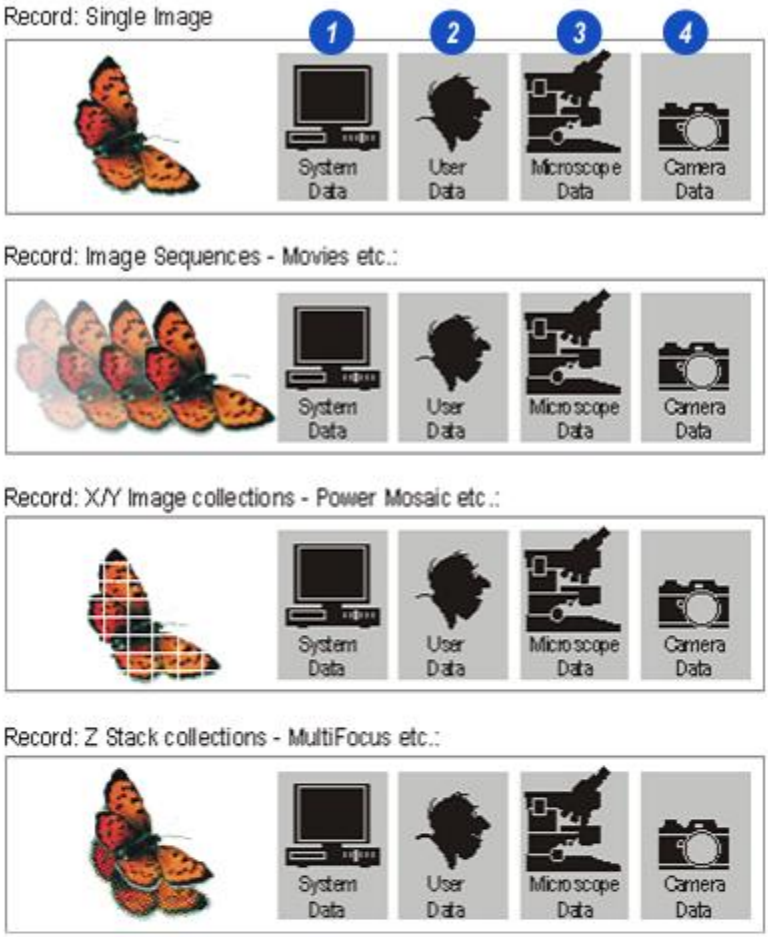
1: *System Data* such as image bit depth, size and sequence.

2: *User Data* like image name, creation date and time.

3: *Microscope Data* that will allow the conditions to be quickly replicated – model name, mag-changer, nosepiece and so on.

4: *Camera Data* relating to exposure, gamma, brightness and all of the essential settings.

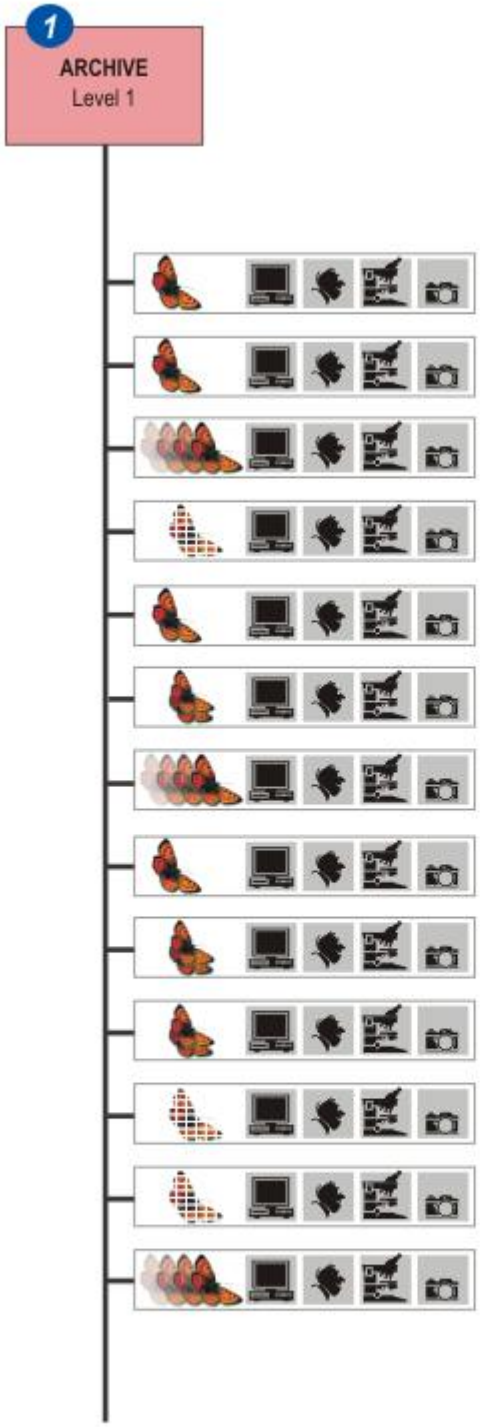
The data is stored in *Fields* within the *Record.* All of the essential Fields appropriate to the hardware setup have been pre-defined by Leica – it is only necessary to click *Acquire Image* – but for more versatility users can add their own Fields and definitions with the LAS Basic and Standard Editions.



LAS Archive has a pre-defined and pre-loaded archive called *Example Archive,* which is an archive structure only; it does not contain data. This is what you see immediately after *LAS Archive Basic Edition* is installed and started for the first time.

Example Archive is a Single-Level Archive **(1)**. This means that all of the records are directly associated with *Example Archive* – or whatever a structural copy is named – it cannot have Level 2 record groups.

Example Archive is ready to use immediately. It is the fastest way - just a few minutes - of getting into production, especially for a single-station user.



There are two editions of *LAS Archive* both purchased as optional modules:

- *Basic Edition* 488 *:* Essential functionality and tools for LAS Archives.
- *Standard Edition* 518 *:* Builds upon the facilities available in the *Basic Edition* and additionally allows the creation of multi-level archives with many field types in addition to text fields.

Basic Edition:
Provides for 1 or 2-Level archives. This means that image records can be associated directly with the *Primary Archive* **(1)** or indirectly by grouping shown on the illustration as **(2)** and **(3)** – *Record Group A* and *Record Group B.*

The *Primary Archive* is Level 1 and the *Record Groups* are Level 2. With *Basic Edition* as many *Primary Archives* as required can be created, each with a different name. In turn, they can contain as many *Record Groups* as needed also with unique names. However, *Record Groups* can have the same name providing they reside in different *Primary Archives*.

Basic Edition also features:

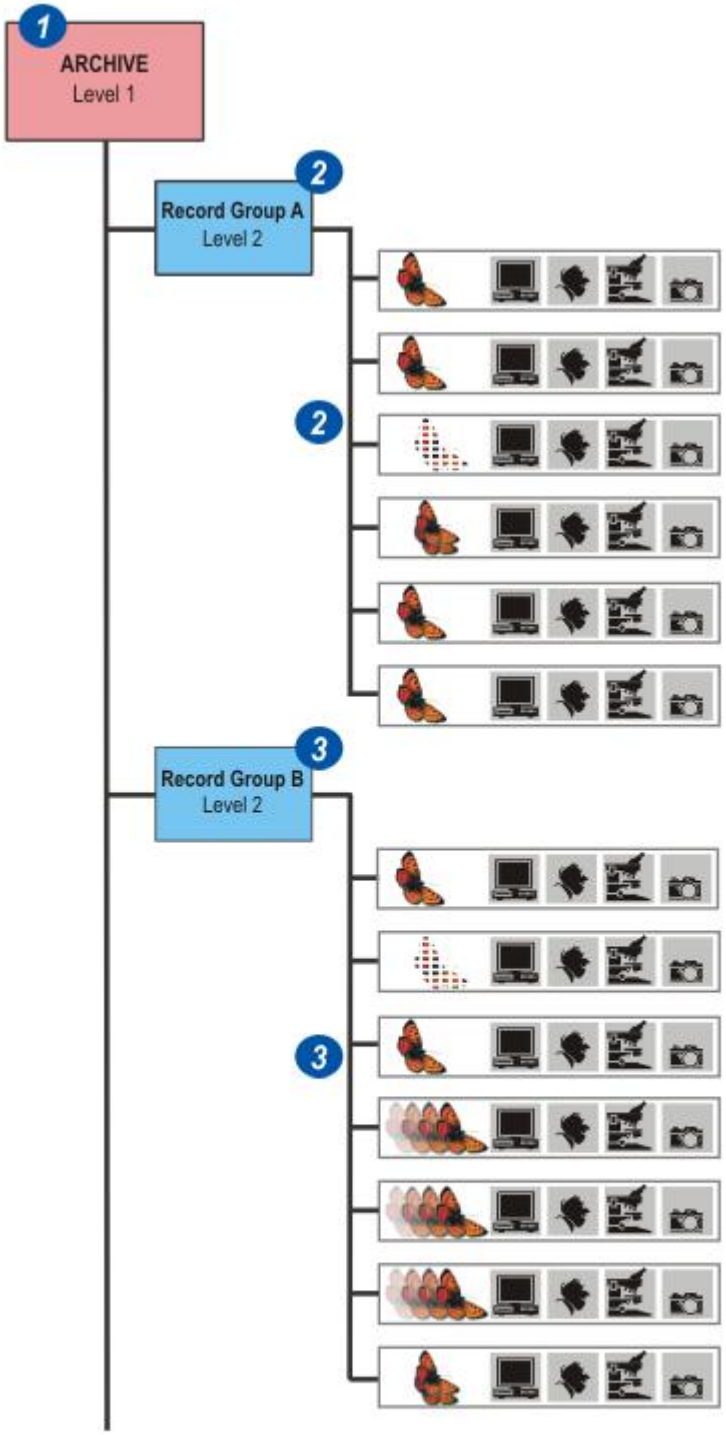
Microscope and camera data captured automatically with the image.

Fast Archive Search with detailed filtering to locate specific images and data.

User Field Selection for Form display.

Attaching documents to image in any format - not just text.

Audio recording inclusion with image. Add Multiple Text Boxes to the Archive.



Optional module *Standard Edition Archive* extends the power and flexibility of the Basic Edition to provide:

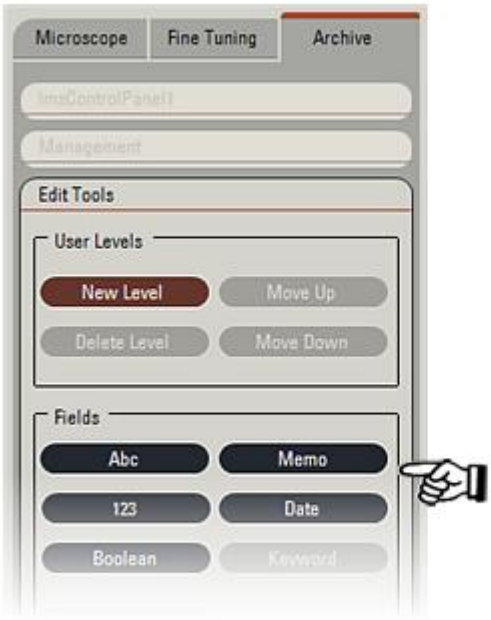
Multiple archive levels.

Wide choice of Named Archive Fields - Memo, Boolean, Numeric, Date and Keywords.

Form Layout design and control.

Highly Detailed Reports to include scaled images. Export reports to Adobe Acrobat (pdf) and Browser (html) facility.

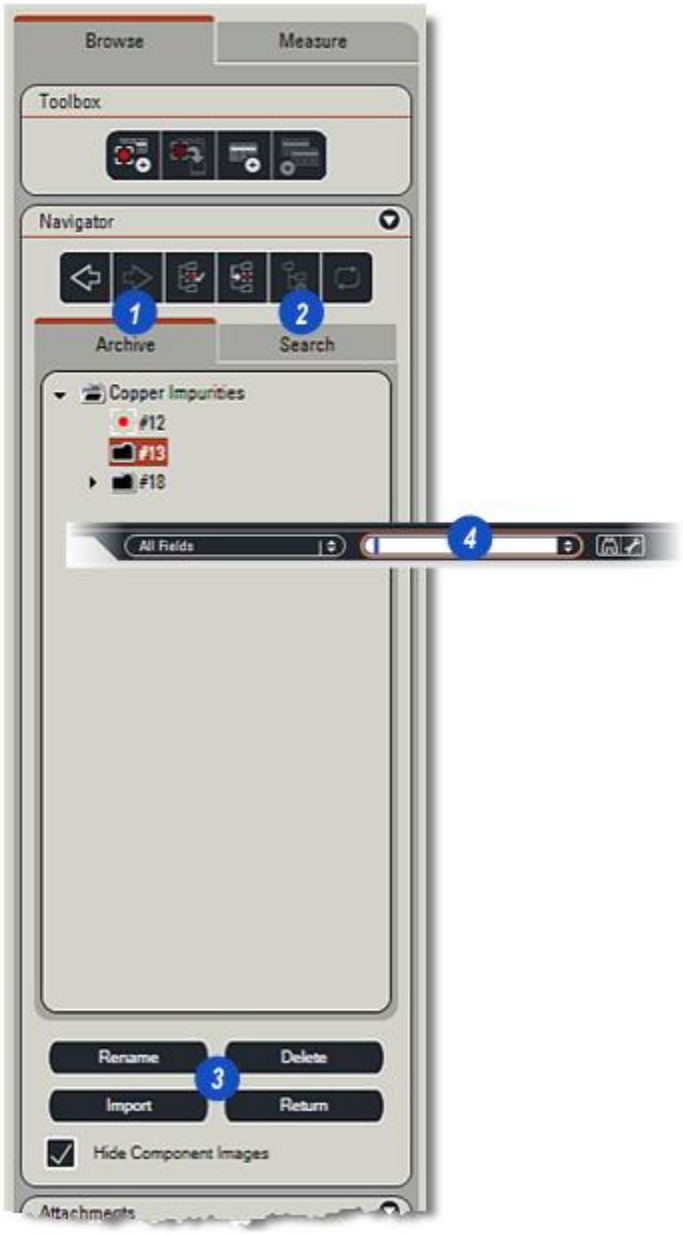
Increased flexibility and security by its use of specialised fields - fields into which only pre-determined data can be loaded.



The illustration shows a typical *Browse Navigator Panel* with an archive selected. *LAS Archives* are optional modules that can run on the same computer as *Image Explorer* although not at the same time. *See Selecting the Storage method:* *Go there…* 470

The panel layout with *LAS Archive* running displays the *Archive* tab **(1)** and the *Search* tab **(2)**. The rapid *Search* facility **(4)** is also available.

LAS Archives has addition function buttons **(3)** compared to *Image Explorer.*



Select Image Explorer or LAS Archives

The image storage method – Image *Explorer* or *LAS Archive* – is selected on the *Setup Workflow.*

1: Click on the *Setup Workflow.*

2: If necessary, open the *Archive* panel by clicking the tab.

3: To select and use *Image Explorer*, click the *LAS Image Explorer* button. *Image Explorer* and *LAS Archive* cannot run simultaneously so clicking a button cancels the other. Return immediately to *Browse* by…

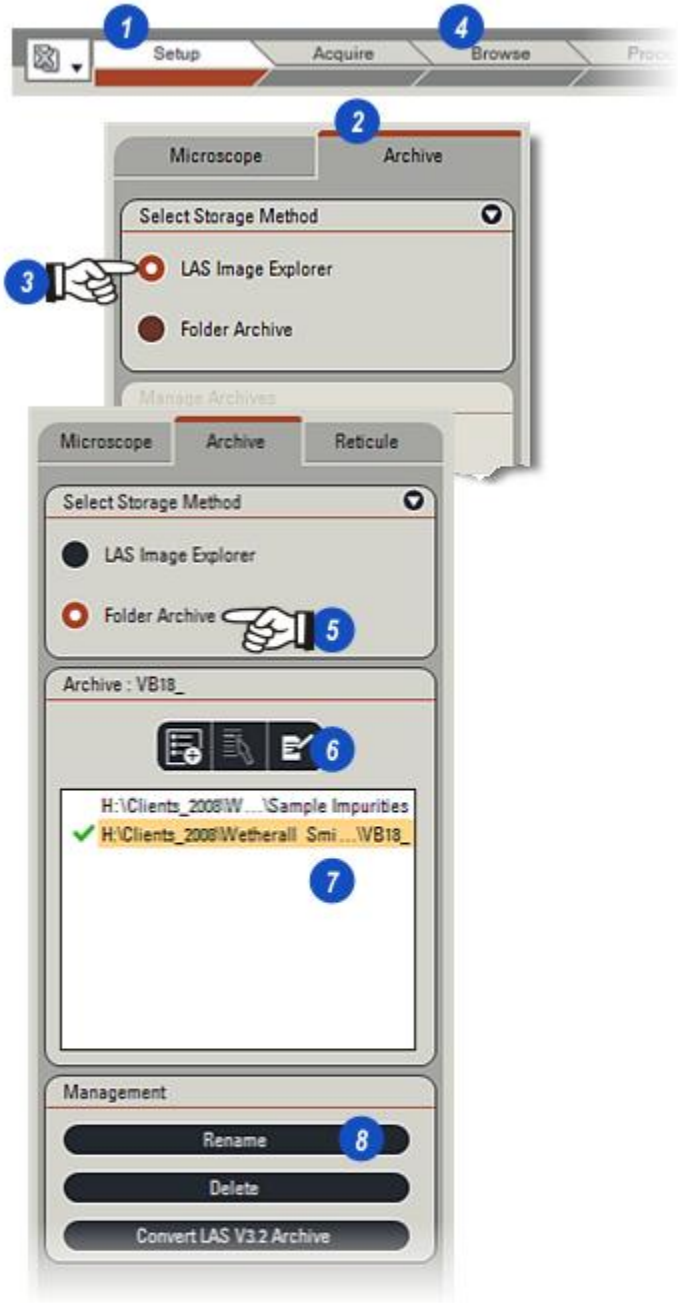
4: …clicking the *Workflow*.

5: Select *LAS Archive* by clicking the button.

6: The *Archive* toolbar,…

7: …the *Archive List Window* and…

8: …the *Management* controls immediately become active.

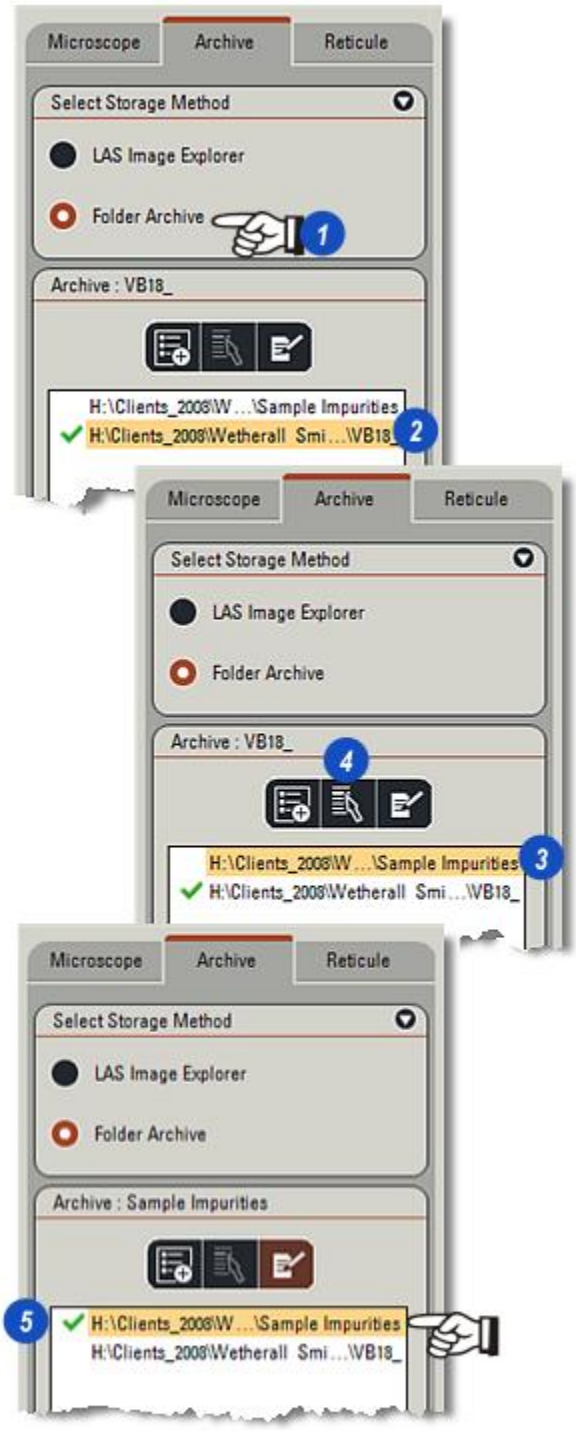


1: With *Archive* selected…

2: …a previously selected archive (if any) will be highlighted and checked – a green tick mark to the left – will be displayed in the *Archive List Window*.

3: To change to another archive either double-click it or click it once and then click the *Set As Current* (Active) Archive button **(4)**.

5: The selected archive is activated and the green tick mark appears to the left. Return to *Browse* by clicking on the *Workflow*.



To rename an existing archive, ensure that *LAS Folder Archive* is selected and...

1: Click on the *Rename* button.

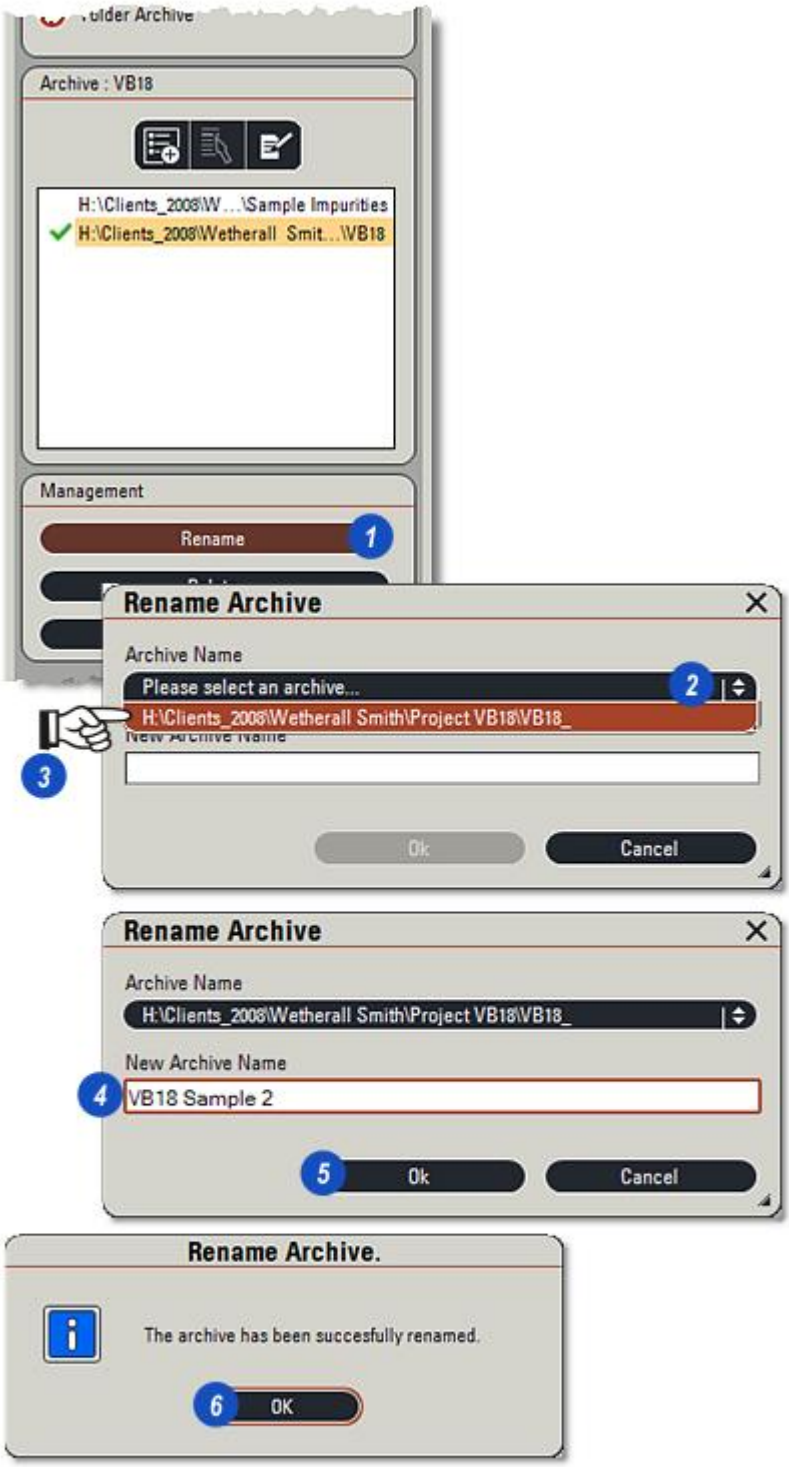
2: On the *Rename* dialog, click on the small arrows to the right of the *Archive Name* header and from the list of archives...

3: ...click to select the one to be renamed.

4: Click in the *New Archive Name* text box and type a new name.

5: Click *OK*.

6: Click *OK* on the *Rename Archive Confirmed* dialog.



Delete an Archive

Use with caution as a deletion cannot be reversed.

1: An archive cannot be deleted if it is currently active.

2: …click on the *Delete* button.

3: On the *Delete Archive dialog* click on the arrows to the right of the *Archive Name* header and from the drop down list...

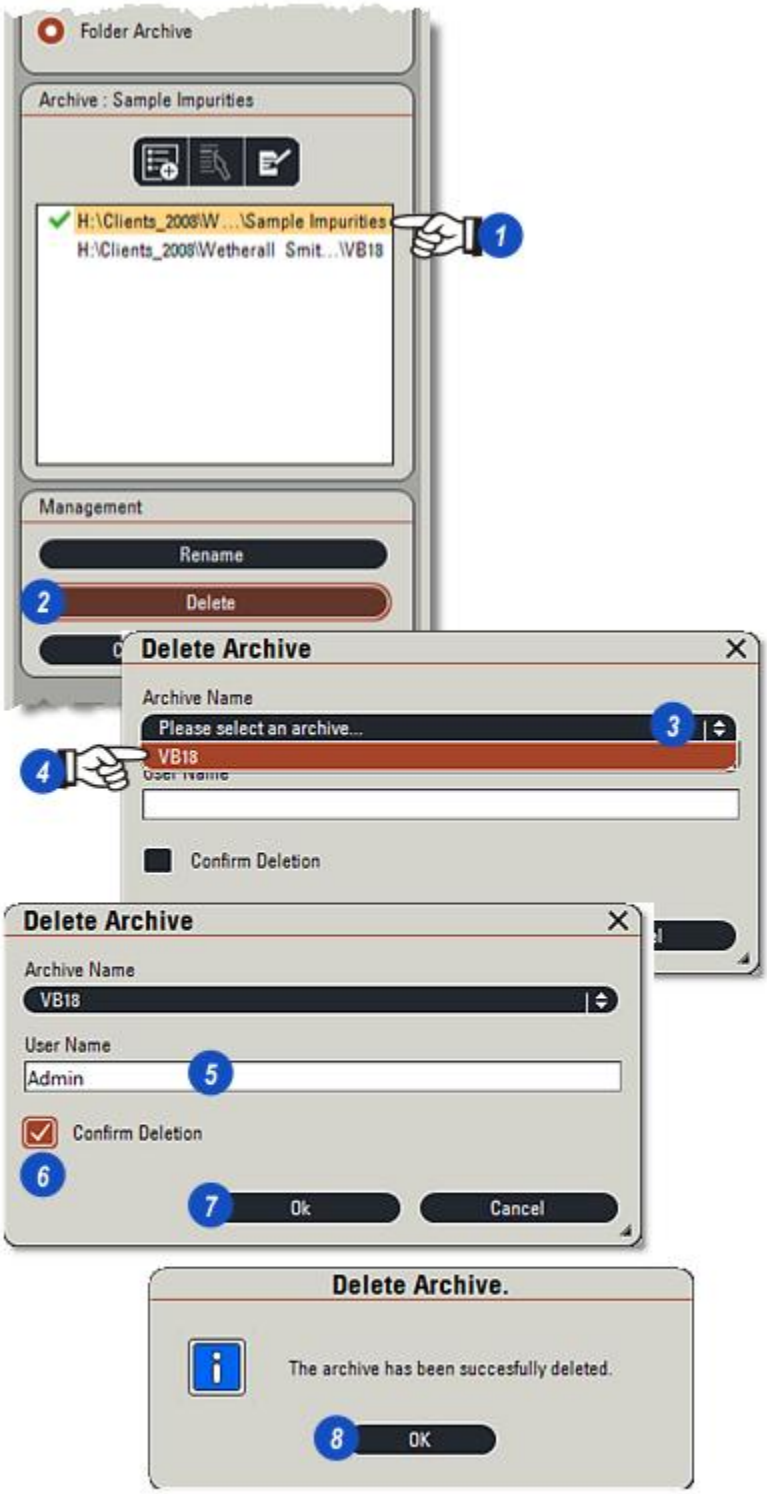
4: ... click to select the archive name.

5: Type the name of the user that installed Leica Application Suite in the *User Name* text box. Most often this will be *Admin.*

6: Click to check the *Confirm Deletion* check box.

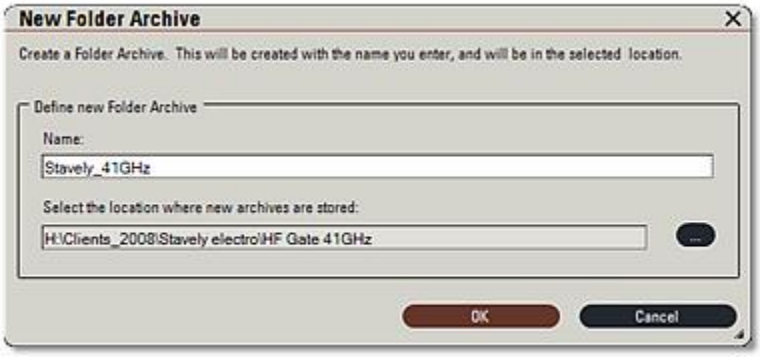
7: Click *OK*.

8: On the confirmation dialog click *OK* and the archive is deleted.



Create a New Archive

Concise details for creating different types of archive are described in *Optional Modules > LAS Archive > Basic Edition:* *Go there...*[507]



A complete list of available archives is displayed by:

1: Clicking on the *Setup Workflow.*

2: Clicking to select *LAS Folder Archive.*

3: The list appears in the *Archive* window with the currently selected archive (if any) having a green tick mark to the left. An archive can be made active by double-clicking on it.

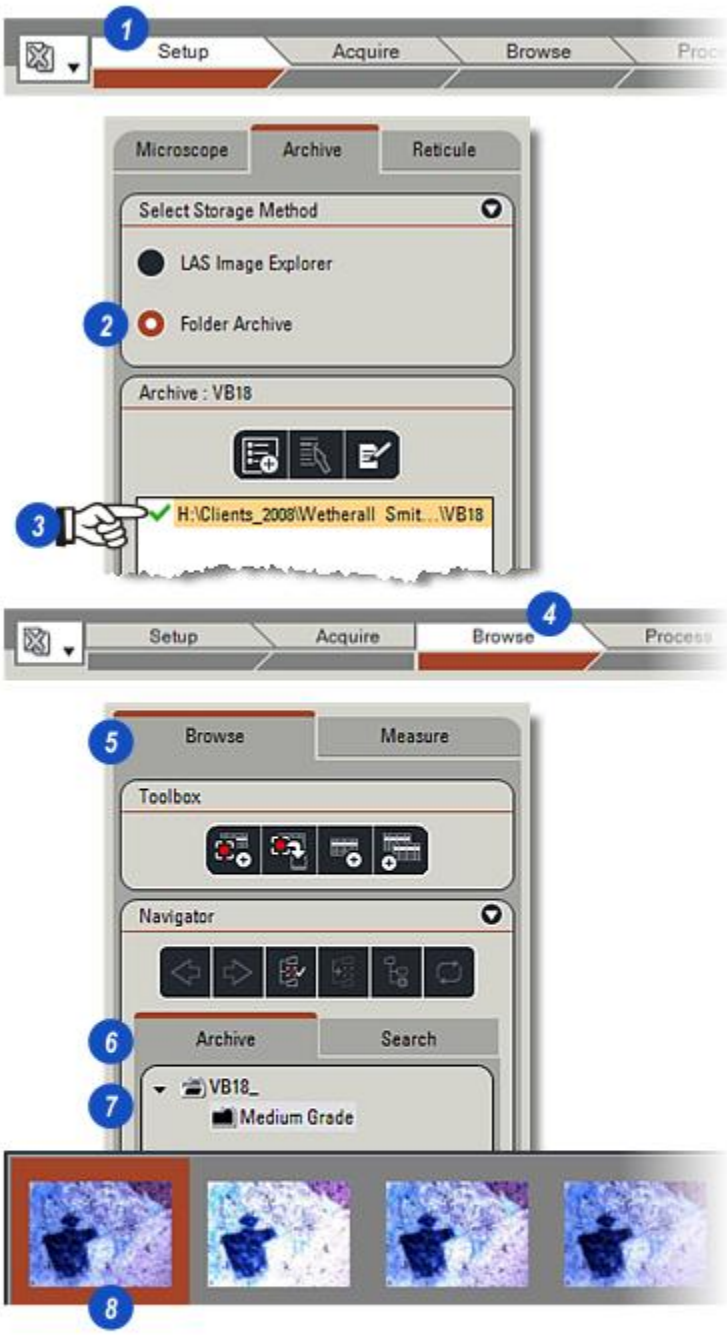
4: With an archive selected and active in Setup, when the *Browse Workflow* is selected...

5: ...and the *Browse* tab visible...

6: ...the *Archive* and *Search* tabs are revealed and...

7: ...the active archive displayed in the *Navigator* window.

8: If the *Gallery* is enabled the archive thumbnails will be displayed and the first image and its data present on the *Viewer*.



Swapping in Navigator between the conventional folders of Image Explorer and LAS Archive is as simple as a mouse click.

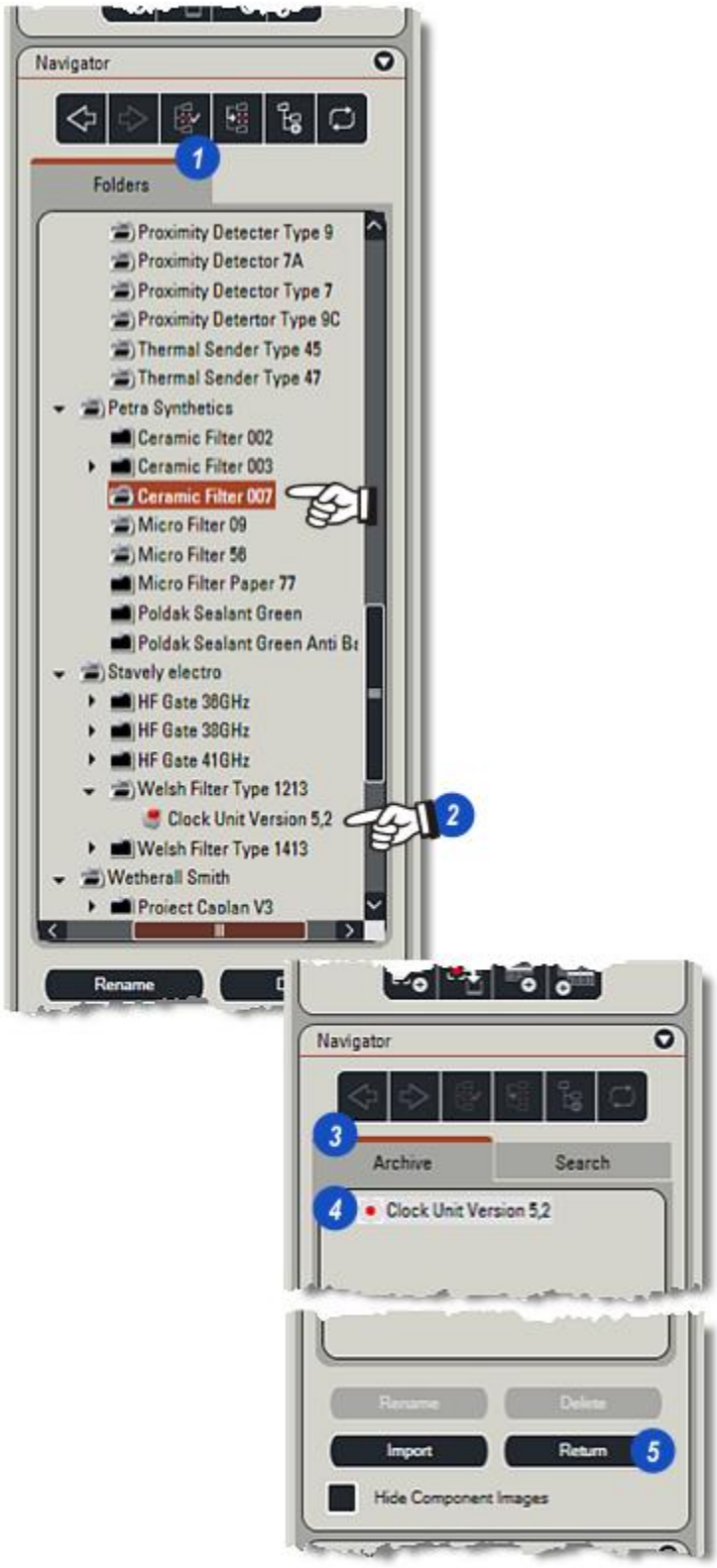
In the illustration, an Image *Explorer* folder is currently selected - the *Folder* tab **(1)** is visible and the selected folder is highlighted.

An archive name is displayed further down the tree **(2)** and is indicated by the Leica Cube icon to the left of the name.

Simply double-clicking on the archive name will change the storage mode from *Image Explorer to LAS Archive* - the *Archive* tab **(3)** appears...

...and opens and loads the images *(4)*.

Go back to browsing in *Image Explorer Folder* mode by clicking on the *Return* button **(5).**



Renaming an Archive

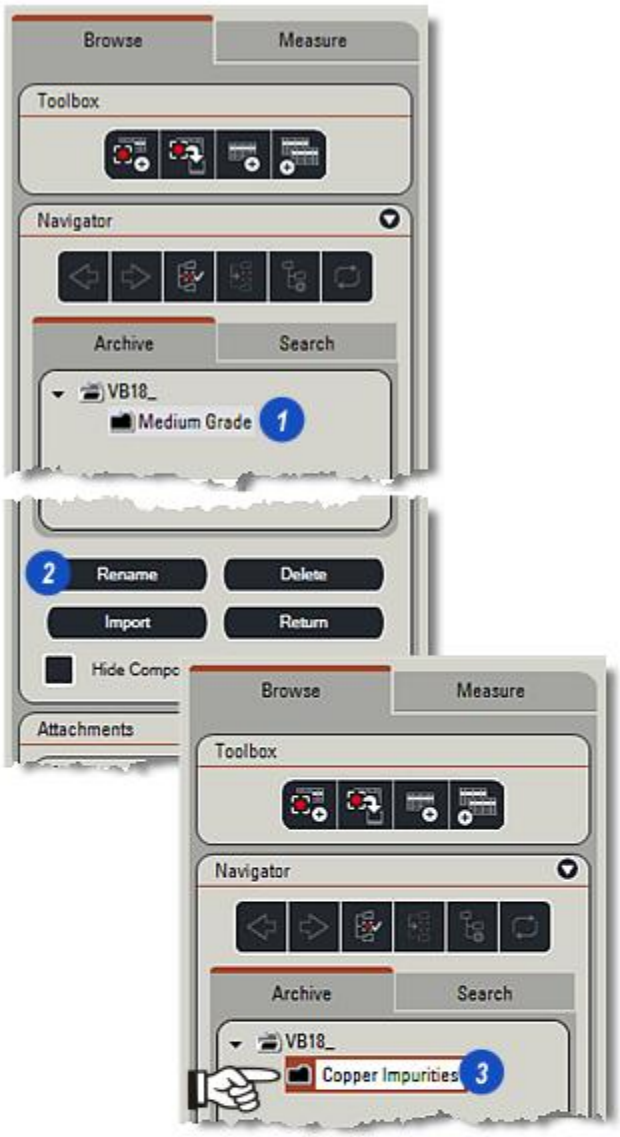
Rename an Archive by:

1: Click on the *Archive* to select it.

2: Click on the *Rename* button.

3: The *Archive Name* in the *Navigator* window changes to a highlighted outline. Type the new name and press *Enter* on the keyboard.

The Archive now appears under its new name in the Navigator.



Delete an Archive

To Delete an Archive:

1: Click on the archive to be deleted.

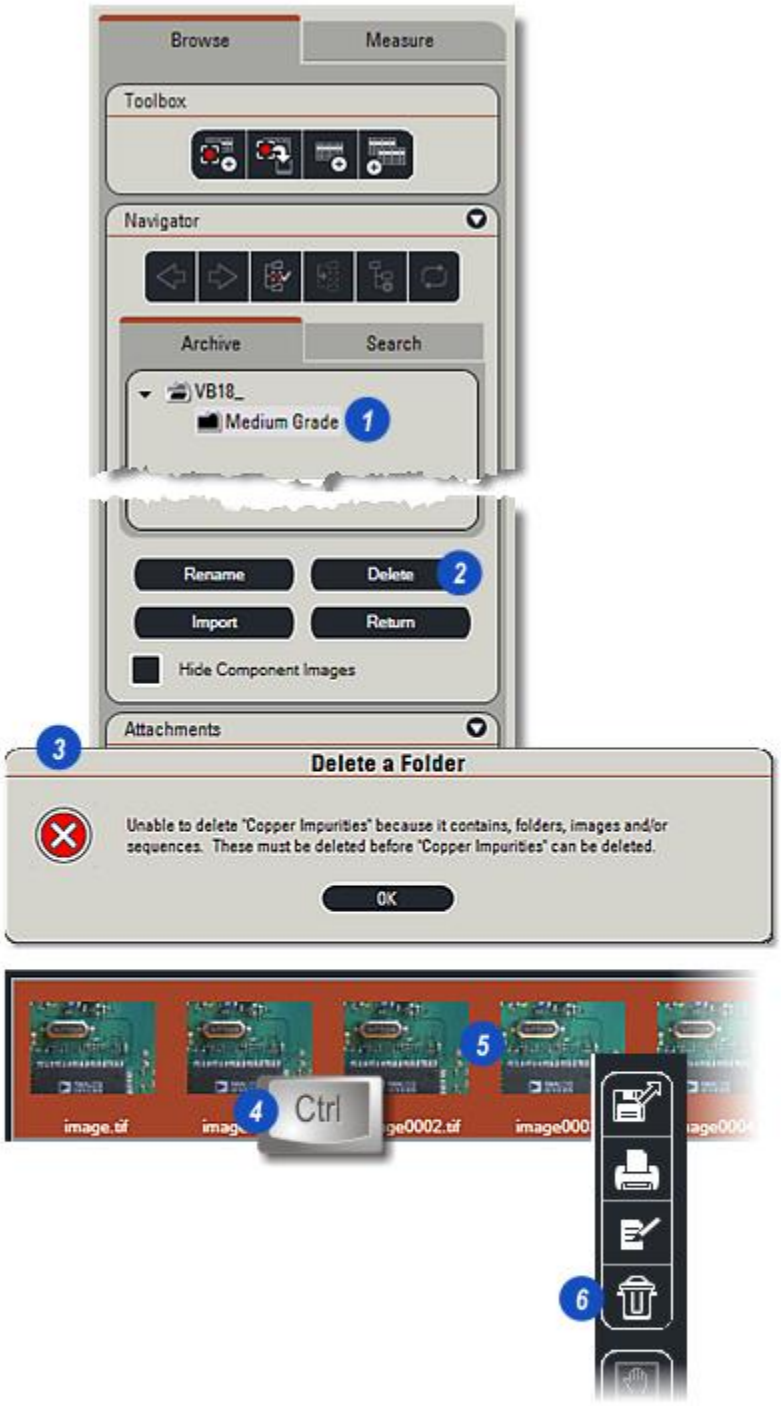
2: Click on the *Delete* button.

3: For security reasons, an archive that contains images and data cannot be deleted - they have to be removed first by...

4: ...holding down the keyboard *Ctrl* key and...

5: ...clicking on the individual *thumbnails* in the *Gallery*.

6: Click on the *Delete* (Trash Can) button on the side tool bar.

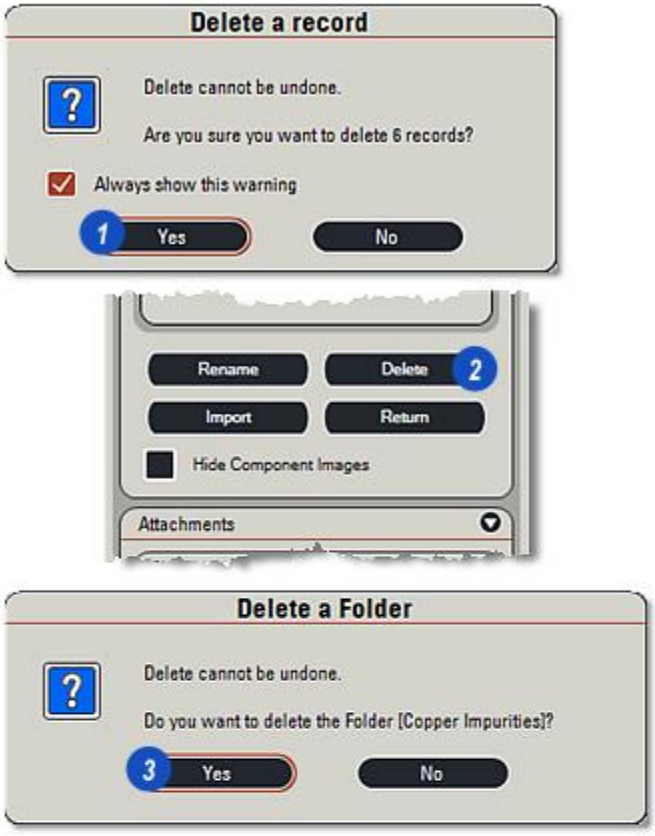


Continued from previous page:

1: Confirm that the images and data (Records) should be deleted by clicking the *Yes* button.

2: To delete the empty archive click on the *Delete* button and...

3: ...confirm the deletion on the *Delete a Folder* dialog by clicking the *Yes* button.



With the optional Basic Edition Archive installed, all of the features and tools needed to produce powerful and versatile Archives are immediately available to create:

- User designed Single Level Archives.
- User designed 2-Level Archives with almost unlimited Record Groups (Folders).
- Archives using existing structures either from User designs or the comprehensive library of templates supplied as part of the module.
- As well as User defined data fields, microscope and camera data is captured automatically with the image.
- Elegant and detailed Form display for every image.
- Forms can be configured to display the data that the User requires and hide everything else.

Basic Edition Archive also includes features to bring even more sophistication to archiving:

- Fast Archive Search with detailed filtering to locate specific images and data.
- Attaching documents of any format - not just text.
- Audio recording.
- Adding Multiple Text Boxes to the archive.

Create a New Archive: Introduction

This section describes setting up both a simple single level archive and a comprehensive 2-Level archive.

Three options are available when creating a new archive:

From New: Starting from a completely blank structure and configuring precisely to the user requirements.

From Template: Creating an archive based upon a template that can be either supplied in the Leica Template Library or one created by the user previously.

From Archive: Copies the structure and fields of an existing archive.

Archives are created in the Setup Workflow:

1: Click on the *Setup Workflow* and if necessary...

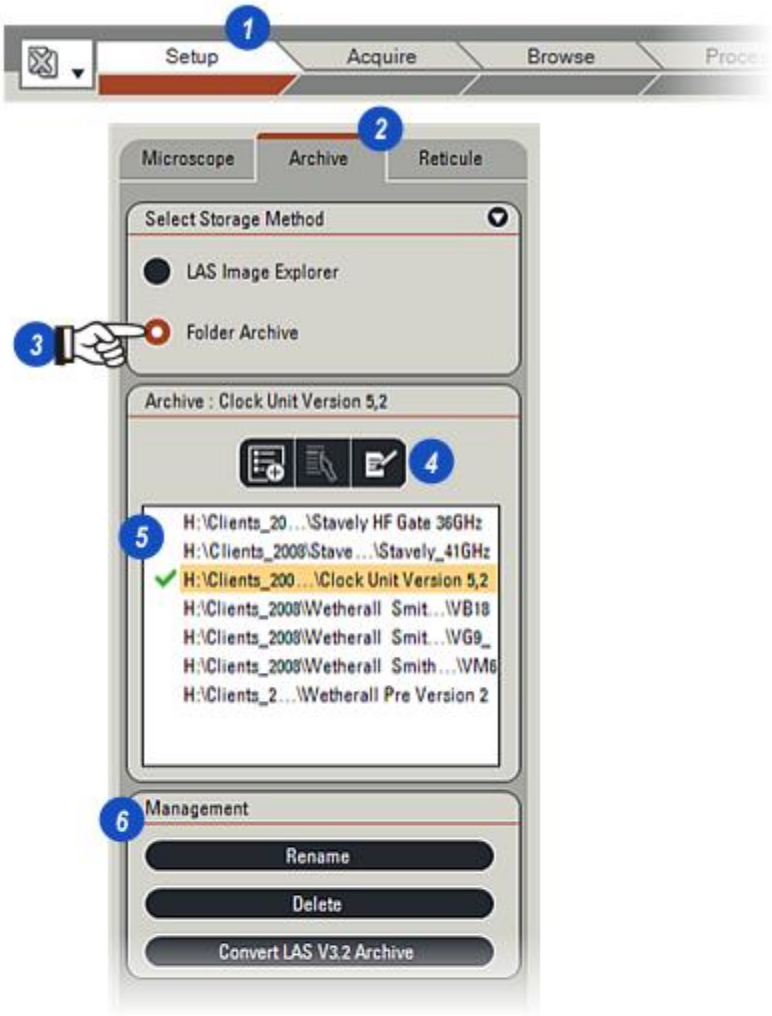
2: ...click on the *Archive* tab.

3: On the *Storage Method* panel click *Folder Archive.*

4: The *Archive Toolbar* and...

5: ...the *Archive* window together with...

6: ...the *Management* tools become active.



To create a completely new archive that can be structured precisely to the users needs:

1: Click on the *Create Archive* button on the toolbar. The *New Folder Archive* dialog appears.

2: On the *Source* panel click the *New* button. The three Source options are mutually exclusive - clicking one automatically de-selects any other.

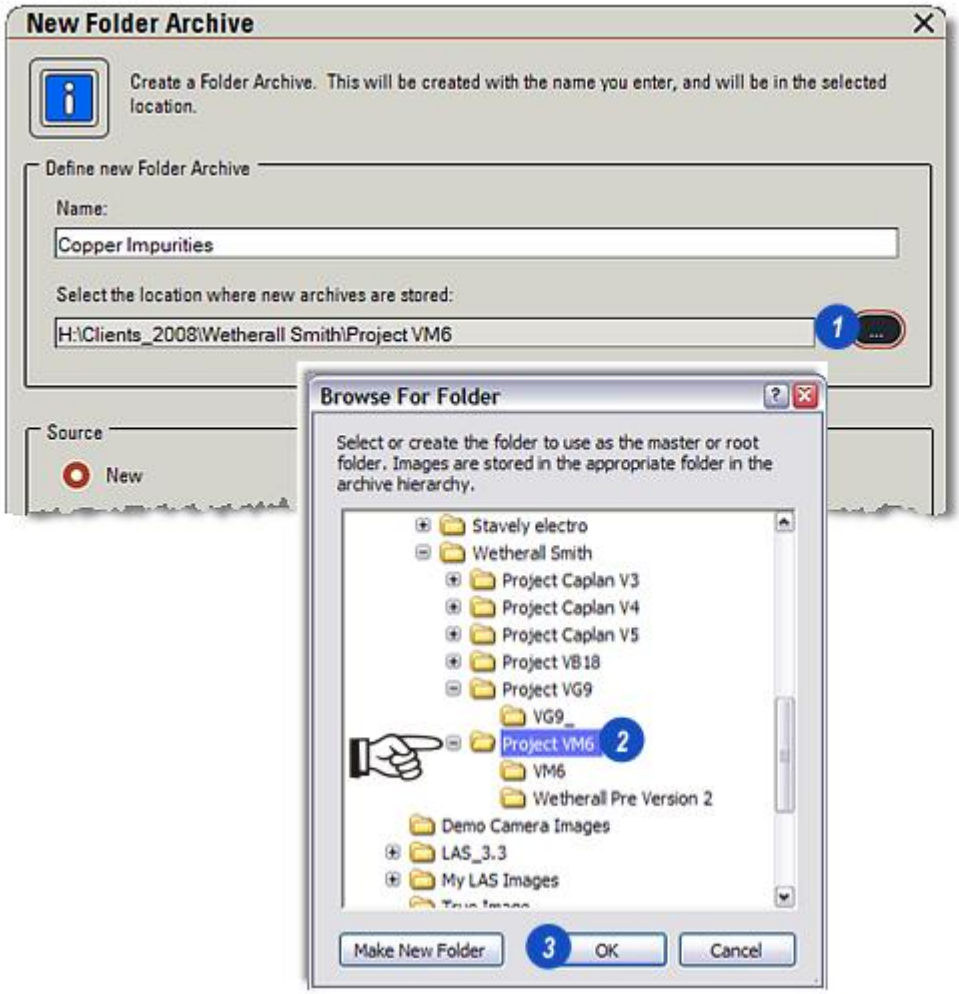
3: On the *Define Folder Archive* panel click in the Name text box and type a unique name for the new archive.

Select Storage Location

1: Click on the browse button to the right of the *Select Location* text box.

2: On the *Windows Navigation Browse for Folder* dialog, navigate to the location where the new archive is to be stored.

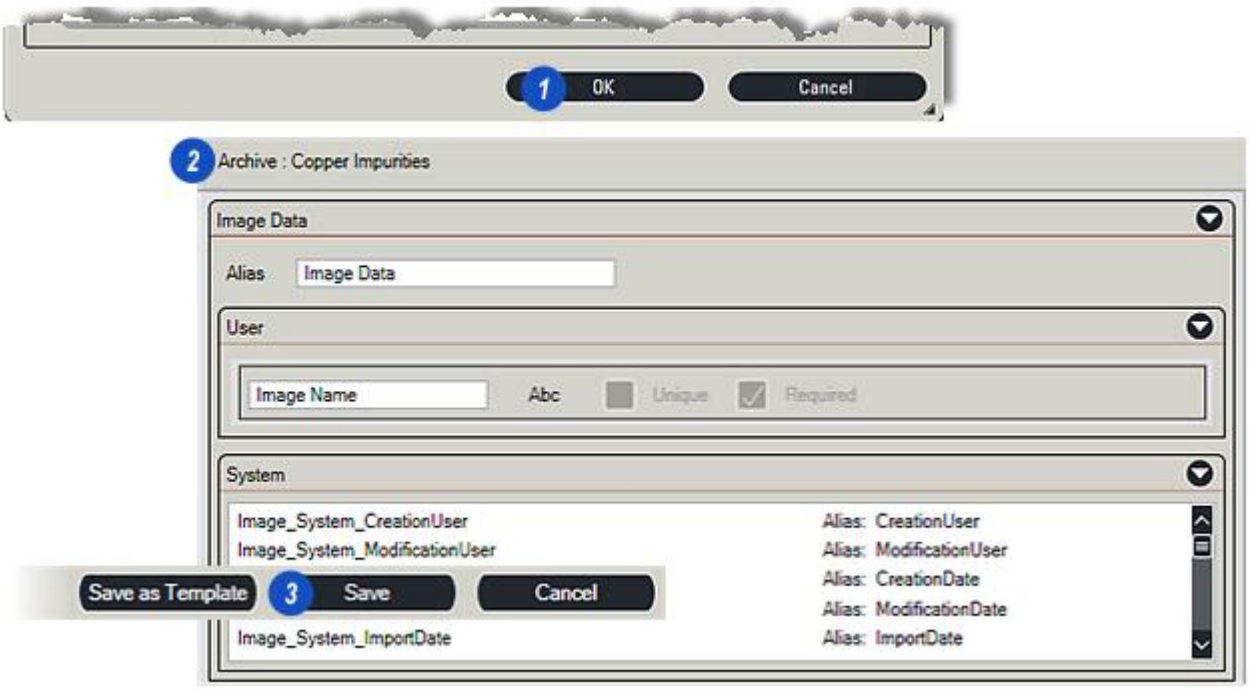
3: Click *OK*. The new storage location appears in the *Select Location* text box.



1: Click on the *OK* button.

2: The *Single Level Archive Structure* appears. This will be the basis for the Record Form for each captured image. To create a 2-Level Archive: Go there...

3: Users that require just a simple archive should just click the *Save* button (Bottom right-hand of the Viewer). There is no need to change any of the data field names.



With the new archive saved:

1: The new archive name appears in the *Archive List* and is set as current and active.

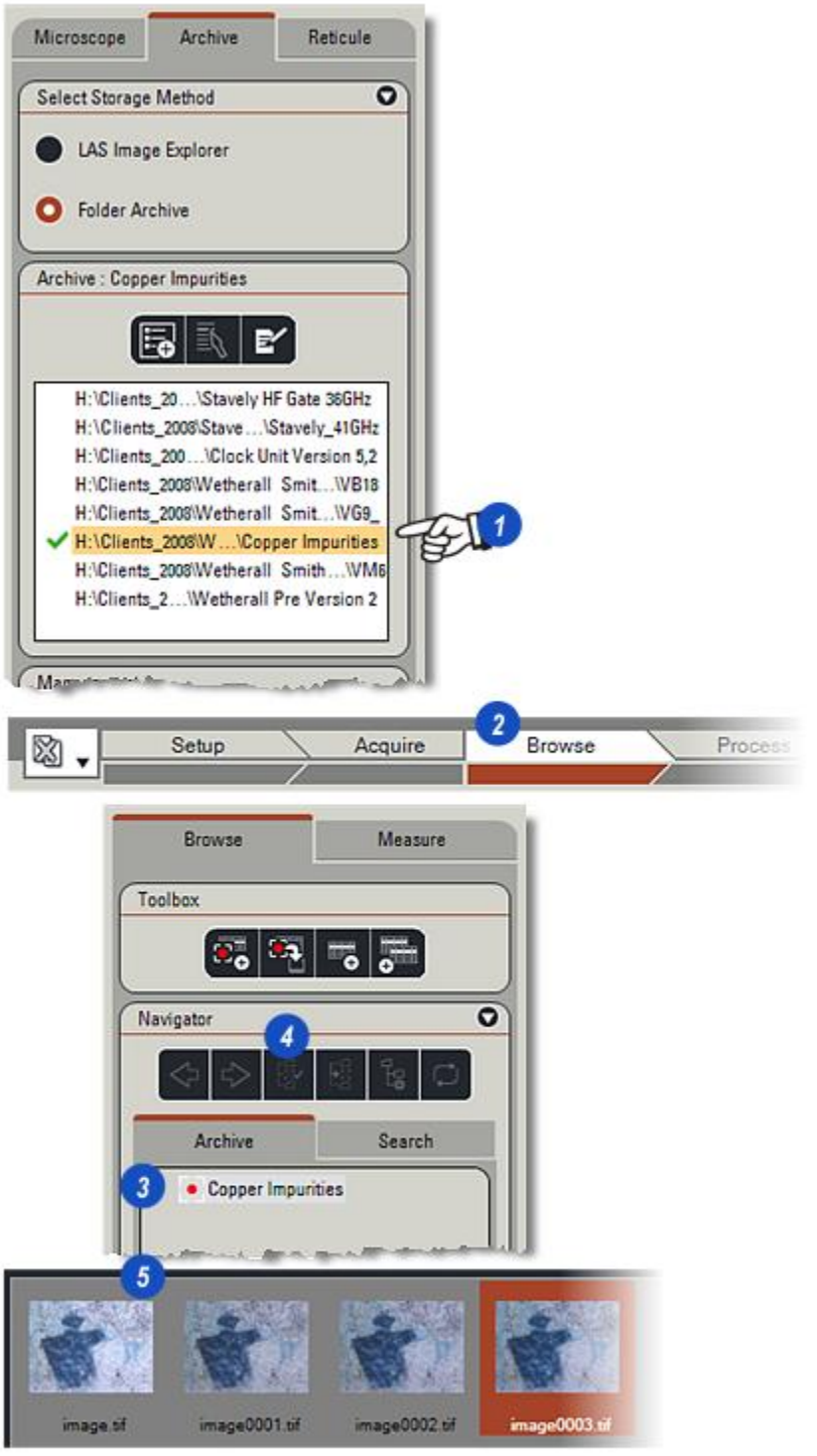
2: To start capturing images click on the *Browse Workflow* and...

3: ...click on the new archive.

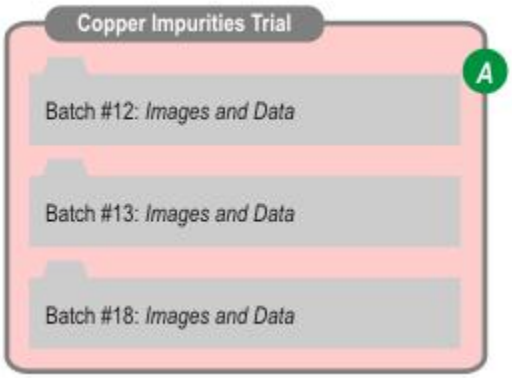
4: Click on the *Set as Capture Location* button to ensure that all images are saved in the new archive and...

5: ...start capturing images either in *Browse* or in the *Acquire Workflow*.

Acquire: Camera setup and image capture: Go there... 270



1: A new archive called Copper Impurities has been created as previously described. This is intended to be a 2-Level archive which means that within the archive separate collections of images will be captured and stored as Record Groups, each having a unique name. There will be three Record Groups in the archive - *Batch #12, Batch #13* and *Batch #18* - chosen to reflect the job in hand. Figure **(A)** illustrates the structure.



2: Every image within the archive, no matter what Record Group it is stored in will have all of the System Data Items - capture date, exposure settings, microscope stand and so on - saved with it. This data is displayed as Fields on the Form associated with each image. The default name for the Form is *Image Data* but can be changed to something more appropriate. Click in the *Alias* text box and type a new name. In the example, the new Form Name is Copper Impurities Results.

3: The default reference for the image is Image Name but this can also be changed by clicking in the User text box and type a new, more appropriate name, in this example is is called Sample. The *Abc* to the right of the text box indicates that the new name can comprise letters and numbers.

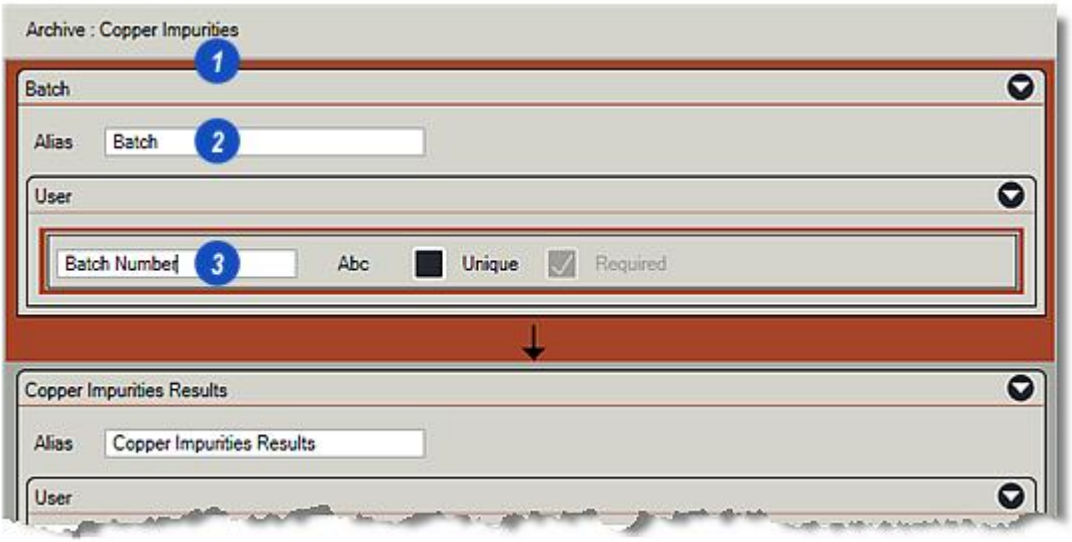
4: The next step is to create the Record Groups. Click on the *New Level* button.

Continued from the previous page:

1: After the *New Level* button is clicked a dialog appears. This represents the structure of any Record Groups added to the archive.

2: The three Record Groups that re going to be added will be called *Batch #12, Batch #13* and *Batch #18* so in this example the structure will be given the name Batch. Click in the *Alias* text box (this is pre-loaded with the example text *New Folder*) and type an appropriate name - Batch in the example.

3: In this example each batch will be given a reference number - *#12, #13* and *#18* - and that will appear as the first User Field (pre-loaded with the example text Folder Name). Click in the first *User Field* text box and type an appropriate name - Batch Number in the example.



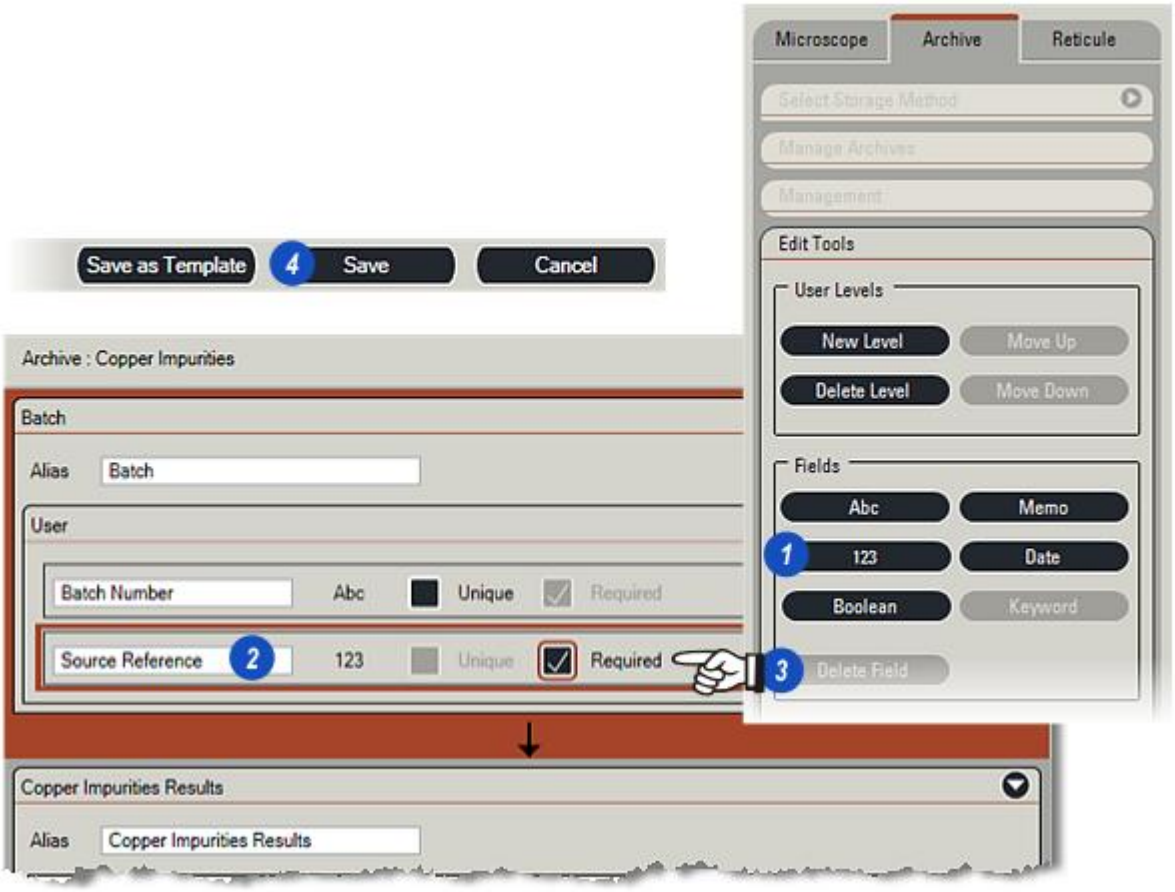
Continued from the previous page:

1: In this project each Record Group is to have an additional field to identify the source of the sample batch. A new field to contain the Source Reference is added by clicking the appropriate Fields button - in this example it is a numeric code so the *123* (Numbers only allowed in this field) button is clicked.

2: The new field is added to the Record Group with the selected field type to the right - in this case *123*. Click in the *Field* text box and type an appropriate name - in this example Source Reference.

3: The Source Reference is a vital and required piece of information so the *Required* check box is enabled which means that an image can only be acquired if this field is complete.
Almost any number of User Specific Fields can be added in this way; This example requires only two described so...

4: ...click the *Save* button.

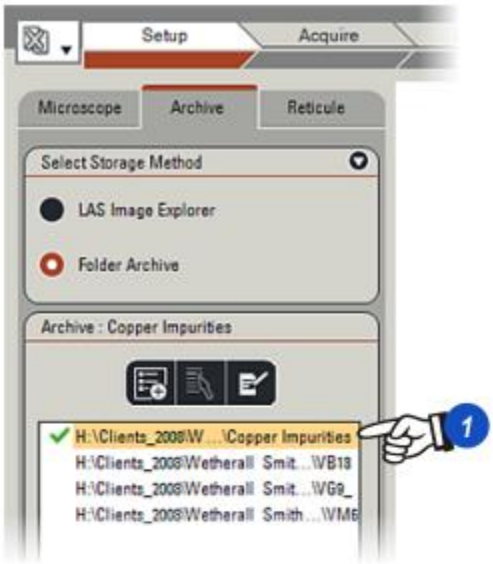


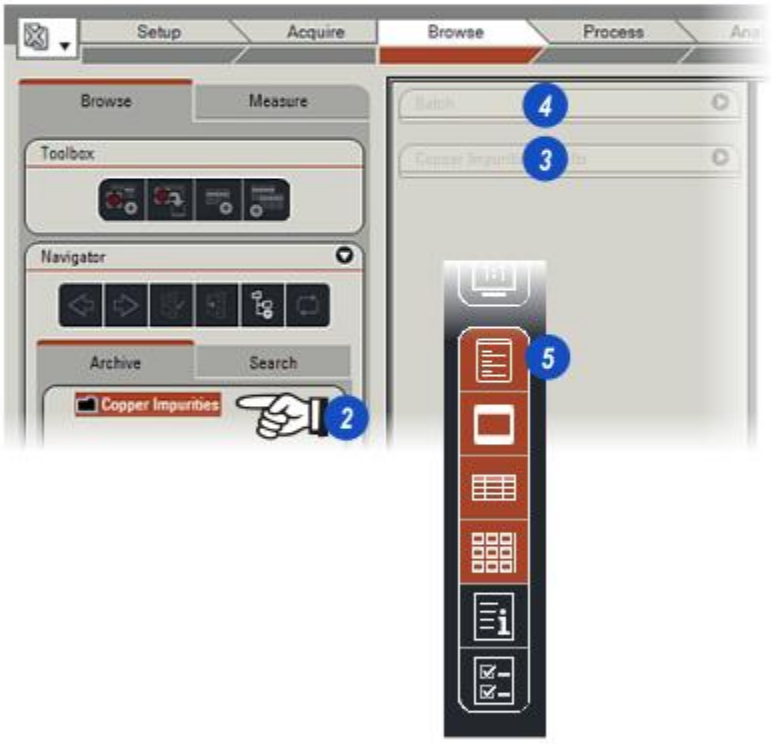
1: The new 2-Level archive now appears in the *Setup Workflow* > *Archive* window and is selected and ready to be used.

2: In the *Browse Workflow* it appears on the *Archive* tab with...

3: ...the *Form Name (Copper Impurities Results)* and ...

4: the *Record Group (Batch)* both ghosted in the *Viewer*. These will only be displayed if the *Hide/Show Form* button on the *Side Tool Bar* is enabled **(5).**





1: Click on the archive to select it.

2: Click on the *Create New (Batch)* button.

3: A new folder icon appears to represent the *Record Group* with the (*Batch*) name against it.

4: Rename the new *Record Group* - in this example it will be called *#12* - by clicking on the *Rename* button.

5: Type a new name for the *Record Group* and press *Enter* on the keyboard.

6: The new name appears on the *Form*.

Set the Capture Location

1: Before images can be captured to the correct *Record Group*, it has to be set as the *Capture Location*. Click on the *Record Group* and...

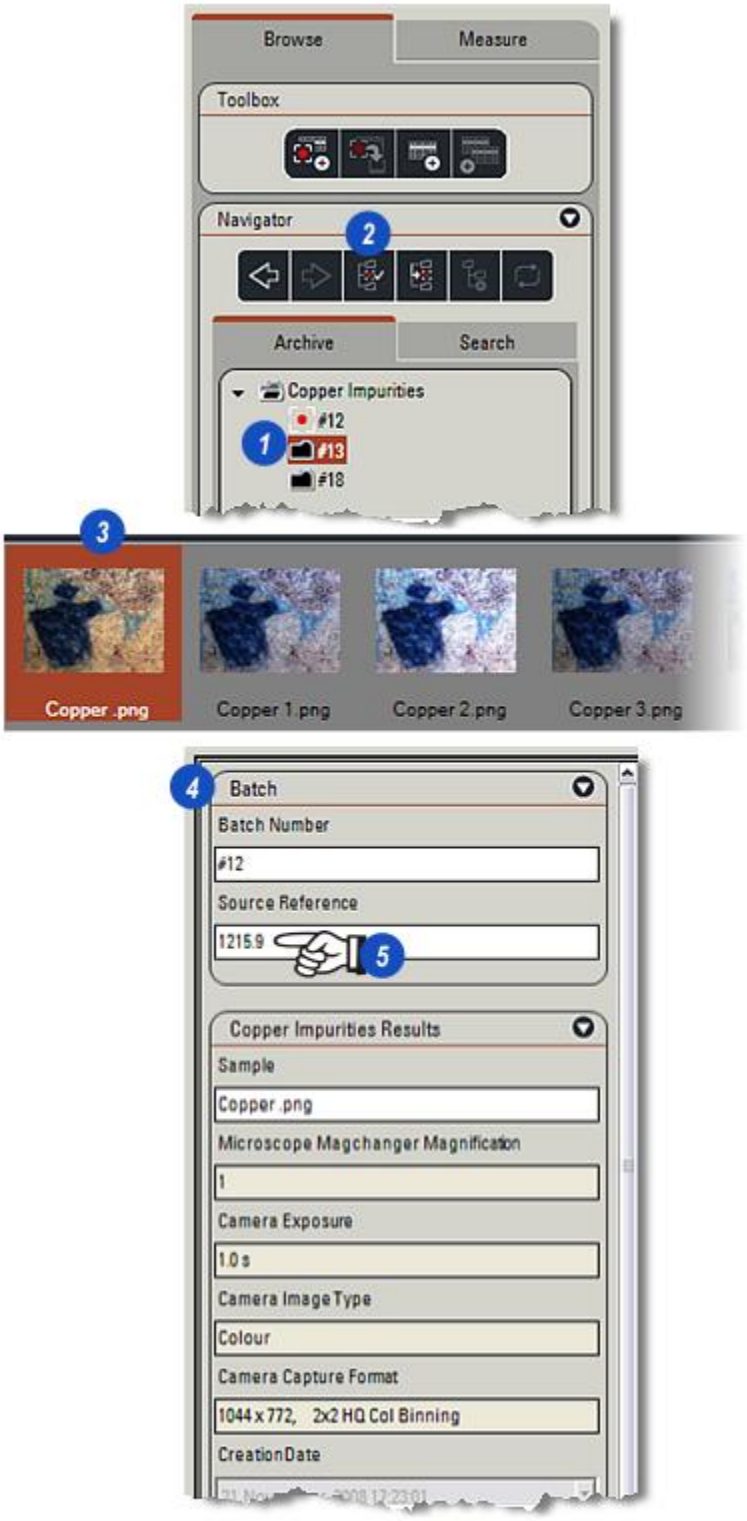
2: ...click on the *Set Capture Location* button. A red dot appears to the left of the *Record Group* to indicate it is set.

3: Capture images in *Browse* or in *Acquire*.

4: As images are captured the *System Data* is displayed on the *Form*.

5: Because in this example *Source Reference* is a required field, it must be completed before an image can be saved.

For details of Deleting and Importing an archive, see Browse: Go there...



It is a simple and very fast matter to copy an archive structure - all of the pre-defined fields but no data - give it a meaningful name that reflects the tasks in hand and then start capturing images and data into it.

1: Click on the *Setup Workflow.*

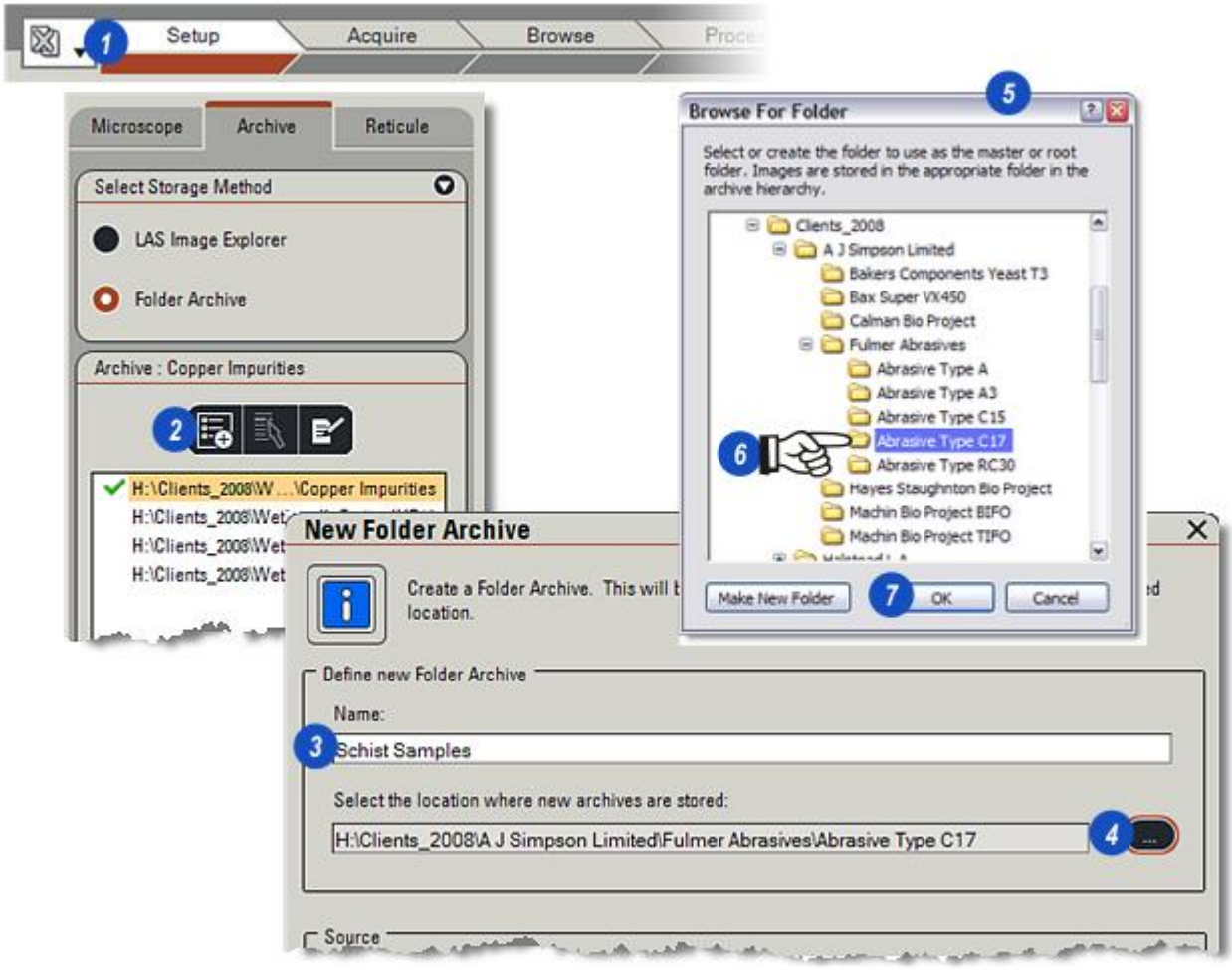
2: Click on the *Create Archive* tab and the *New Folder Archive* dialog appears.

3: Click in the *Name* text box and type a name for the new archive.

4: Click on the *Browse* button to the right of the *Select Location* text box and...

5: ...on the *Browse for Folder* dialog, navigate to the folder **(6)** in which to save the new archive and...

7: Click *OK*. The path of the new location appears in the *Select Location* text box.



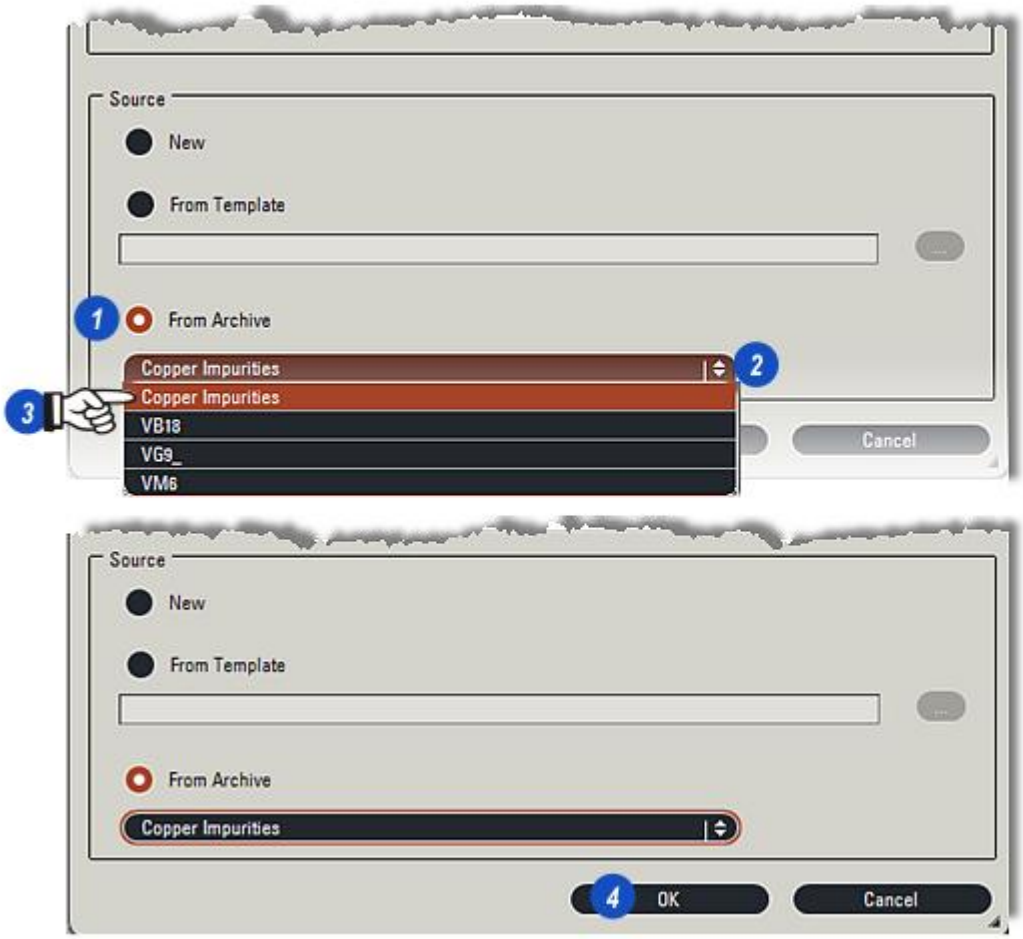
Set the Source Archive

1: Click on the *From Archive* button.

2: Click on the arrows to the right of the *From Archive* header to reveal a list of archives available for copying and...

3: ...click to select the required archive

4: Click *OK*.

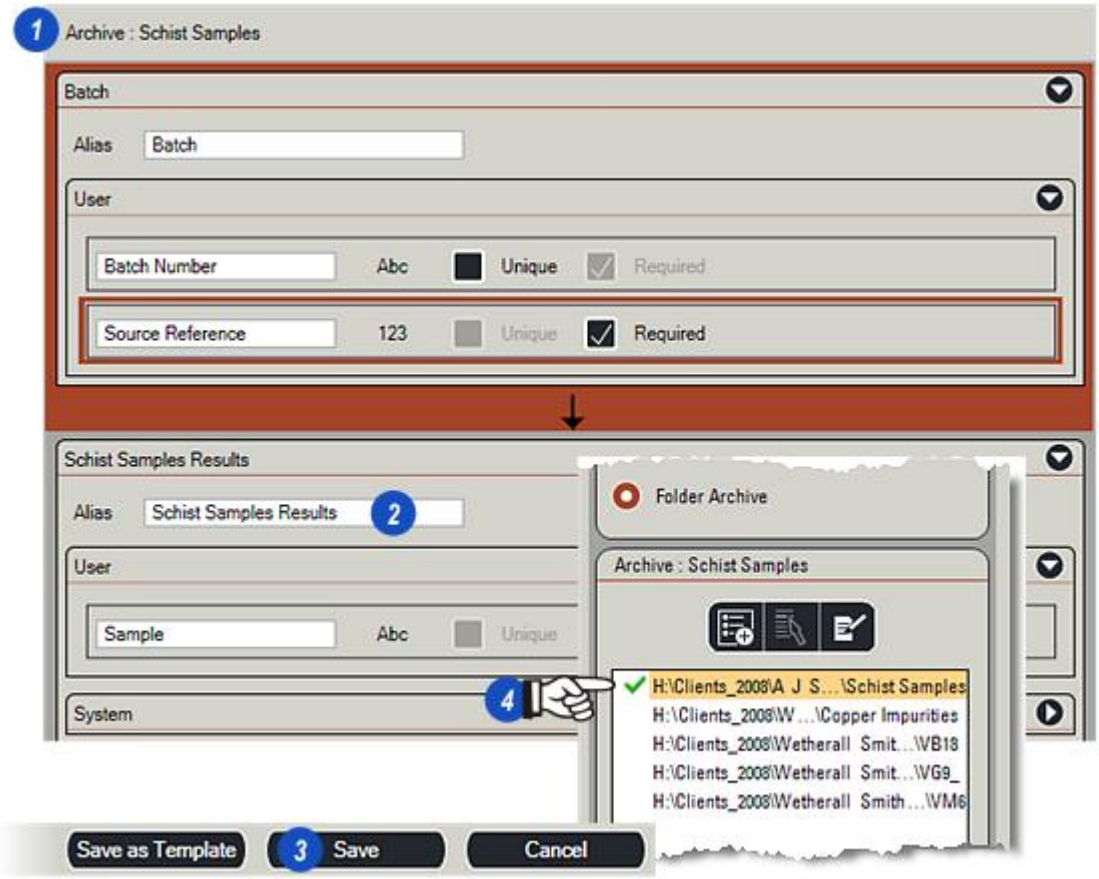


1: A new archive with a new name appears with all of the fields and their properties based upon the original archive.

2: The Field Names can be changed as required by clicking in the text boxes and typing a new, appropriate name. The field type cannot be changed.

3: Click *Save* to save the new archive.

4: It appears in the Archive List, is selected and active and ready to be used just like its 'parent'.



Any archive may be used as a template for future archive creation, especially useful if a range of archives have to share the same corporate or end-user style.

Only the structure and field names of the source archive are replicated – not the actual data or images.

1: Click to select the Setup Workflow.

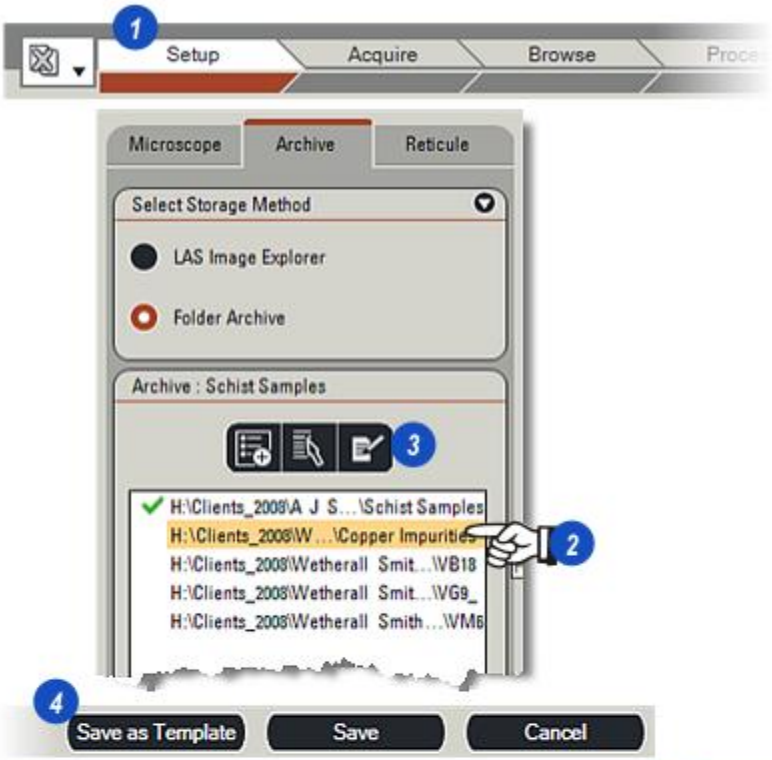
2: Click to select the archive to be used as a source for the template.

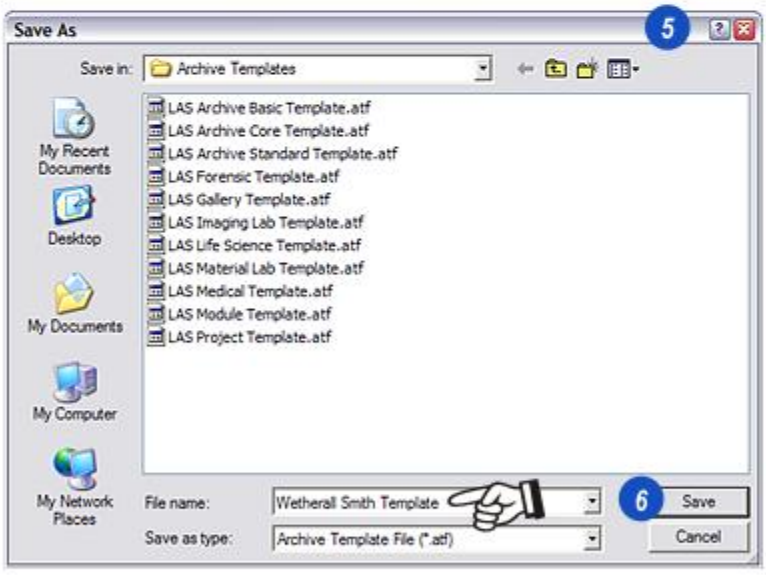
3: Click the *Edit* button. The archive fields will appear.

4: Click on the *Save as Template* button.

5: On the Windows dialog navigate to the folder in which to save the template.
Leica Application Suite has a default folder in which to store templates at:
C:\Documents and Settings\All Users \Documents\Leica Application Suite\Archive Templates
…and it is recommended that this be used wherever possible.
Give the template a new and unique name.

6: Click *Save* and the template will be created.





A new archive can be created quickly and easily using either a template saved by the user or from the range of pre-configured templates provided by Leica and designed to suite a wide range of applications and disciplines.

1: Click to select the *Setup Workflow.*

2: Click the *Create New* button.

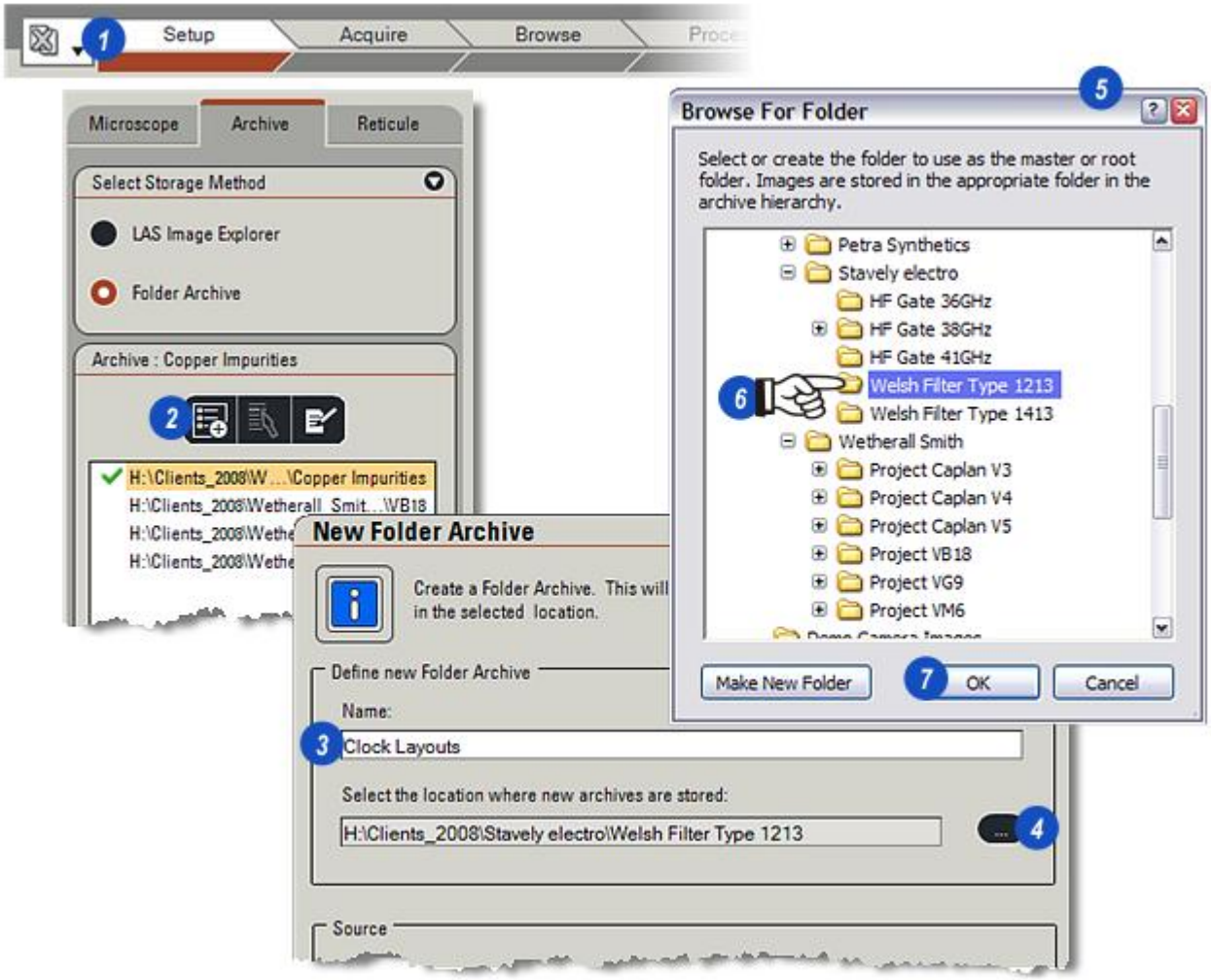
3: On the *New Folder Archive* dialog, click in the *Name* text box and type a name for the new archive.

4: Click on the *Browse* button to the right of the *Select Location* text box and...

5: ...on the *Browse for Folder* dialog...

6: ...select the folder in which the new archive will be saved.

7: Click *OK*.



Select the Source Template

1: On the *New Folder Archive* dialog click the *From Template* button.

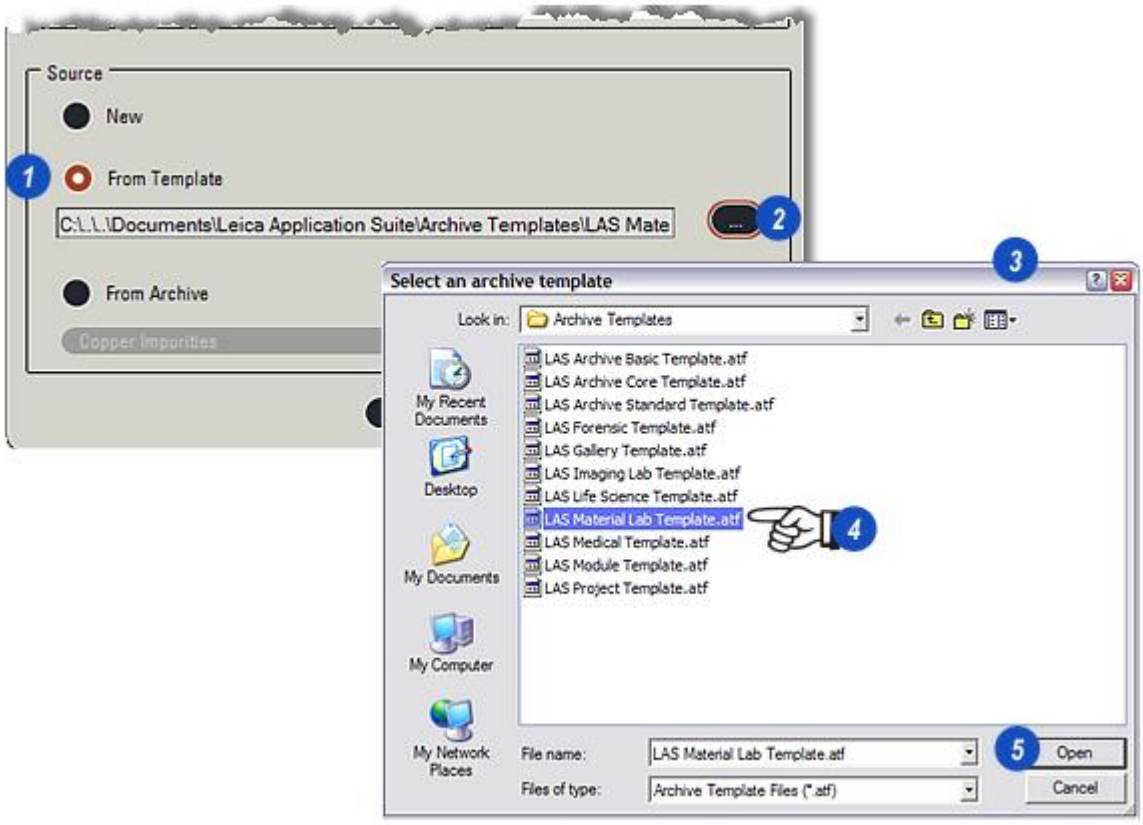
2: Click on the *Browse* button to the right of the *From Template* text box.

3: The pre-configured templates supplied by Leica are stored in a reserved location:

C:\Documents and Settings\All Users\Documents\Leica Application Suite\Archive Templates

4: Click to select the template style required. The name appears in the *File name* text box.

5: Click *Open*.

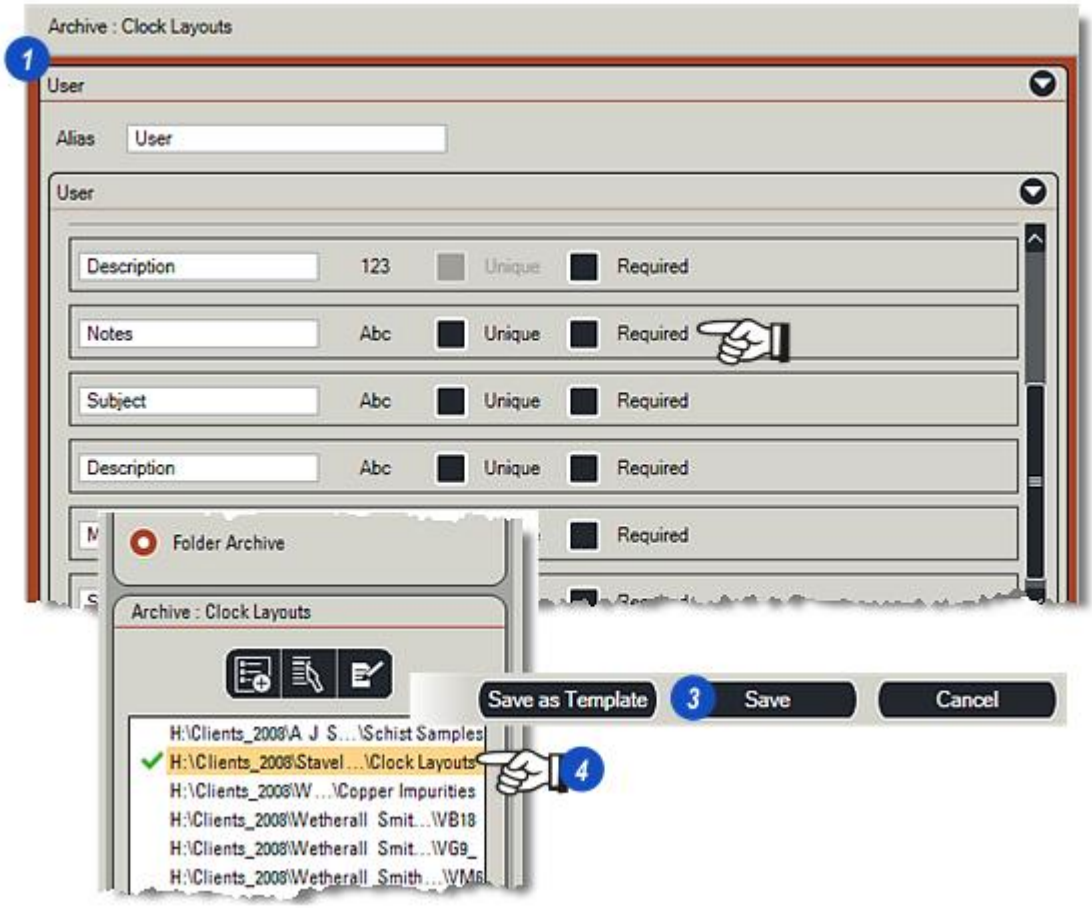


1: The new archive created from the template appears with all of its pre-defined fields.

2: Field names can be changed by clicking in the Field text box and typing an appropriate name. The Field Properties can be set or cleared but the Field style cannot.
The *Unique* property when set means the value in the field cannot be the same as any other.
The *Required* property when enabled means that the image will not be saved until there is some valid data entered in the field.
To set/clear (enable/disable) a property click on the check box to the left.

3: Click on the *Save* button to save the new archive.

4: The new archive appears in the *Setup* > *Archive* window,



The *Grid* displays data for all of the images in a folder in a tabular structure. The image names are listed on the left and the data items as headers across the top.

1: The *Grid* is revealed and hidden by clicking on the *Side Tool Bar* button. This is a toggle – click to reveal, click again to hide.

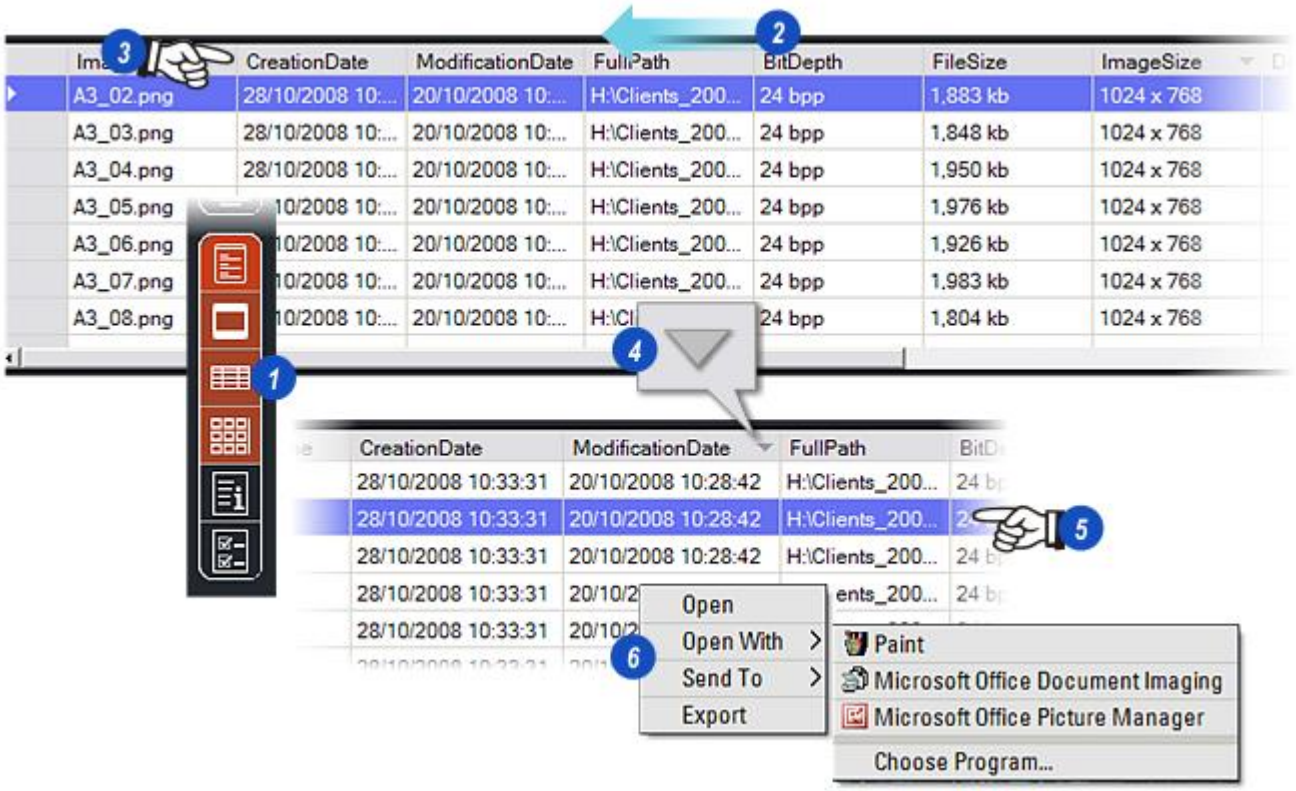
2: The header positions can be changed by clicking and holding the left mouse button on the header to be moved, dragging it to the new position and releasing the mouse button.

3: The column widths can be changed by clicking and dragging the vertical bars that separate the columns.

4: Clicking on an entry in the *Grid* will immediately display that image in the *Viewer* and also highlight the thumbnail.
Hold down the *keyboard Ctrl key* whilst clicking to make multiple selections prior to deleting or exporting. Keyboard combination *Ctrl + A* will select all of the image data: *Ctrl + C* will copy all the selected image data to the clipboard and *Ctrl + V* will copy into another application.

5: A small arrow is revealed when a header is clicked. This allows the image data to be sorted – high-to-low or low-to-high – by successive clicks on it.

6: The *Grid* data can be exported to a range of other applications by right-clicking on the *Grid* then navigating to and clicking to select an application.



The *Search* option provided with optional *Basic* and *Standard* editions, is both fast and flexible. It is possible to search on all fields within a record group or on specific selected fields. An editor is available to create search configurations which can be used at any time simply by clicking them.

Rapid Search:
Locating specific items which have known names or text strings, is achieved quickly by:

1: Select *All Fields* in the Search Toolbar field selector - bottom right of the Viewer.

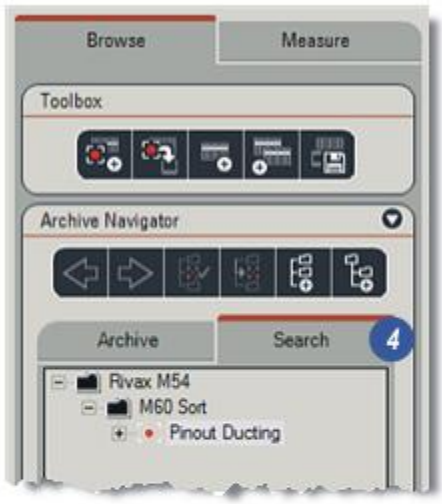
2: Type the name or text string into the *Search For* window.

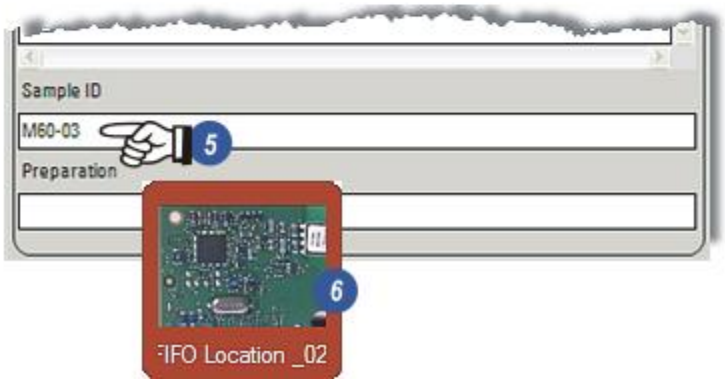
3: Click on the *Search* button.

4: If a match is found the *Search* tab is automatically selected showing the Archives in which the search was made, and...

5: ...the appropriate record is populated - if there are more than one that satisfies the search criteria, the first is selected.

6: The image *Thumbnail(s*) appear in the *Gallery.*



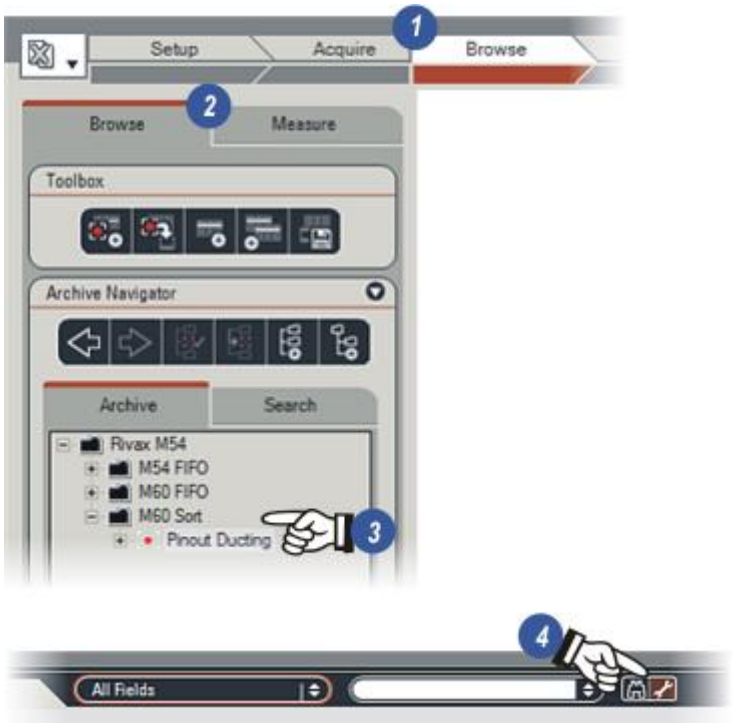


Search Configuration Editor

A *Search Configuration* is a file that contains all of the parameters - such as fields and search strings - needed to carry out a fast, repetitive search.

Search Configurations are stored under unique names and can be retrieved and used for fast searching, or modified to reflect changing search requirements - for example the search fields could be extended and search strings altered.

Mixing field types in a single configuration has made LAS Archive Search even more powerful. Now, up to 10 fields of different types - text, Boolean, date and so on, - can be mixed and each setup for different search criteria. And Boolean limits can be set to ensure that the search is narrow and very precise; Use the AND command to ensure that only those images that conform to every field search string are returned; Alternatively, the OR command will only retrieve images that fulfill at least one search parameter.



Create a New Search Configuration:

1: Click on the *Browse Workflow*.

2: Click to select the *Browse* tab and...

3: ...check that the correct archive is displayed in the *Archive* window.

4: Click on the *Configuration Editor* launch button on the *Search Toolbar*.

1: On the *Search Configuration Editor* dialog, click the *New* button to create a new configuration.

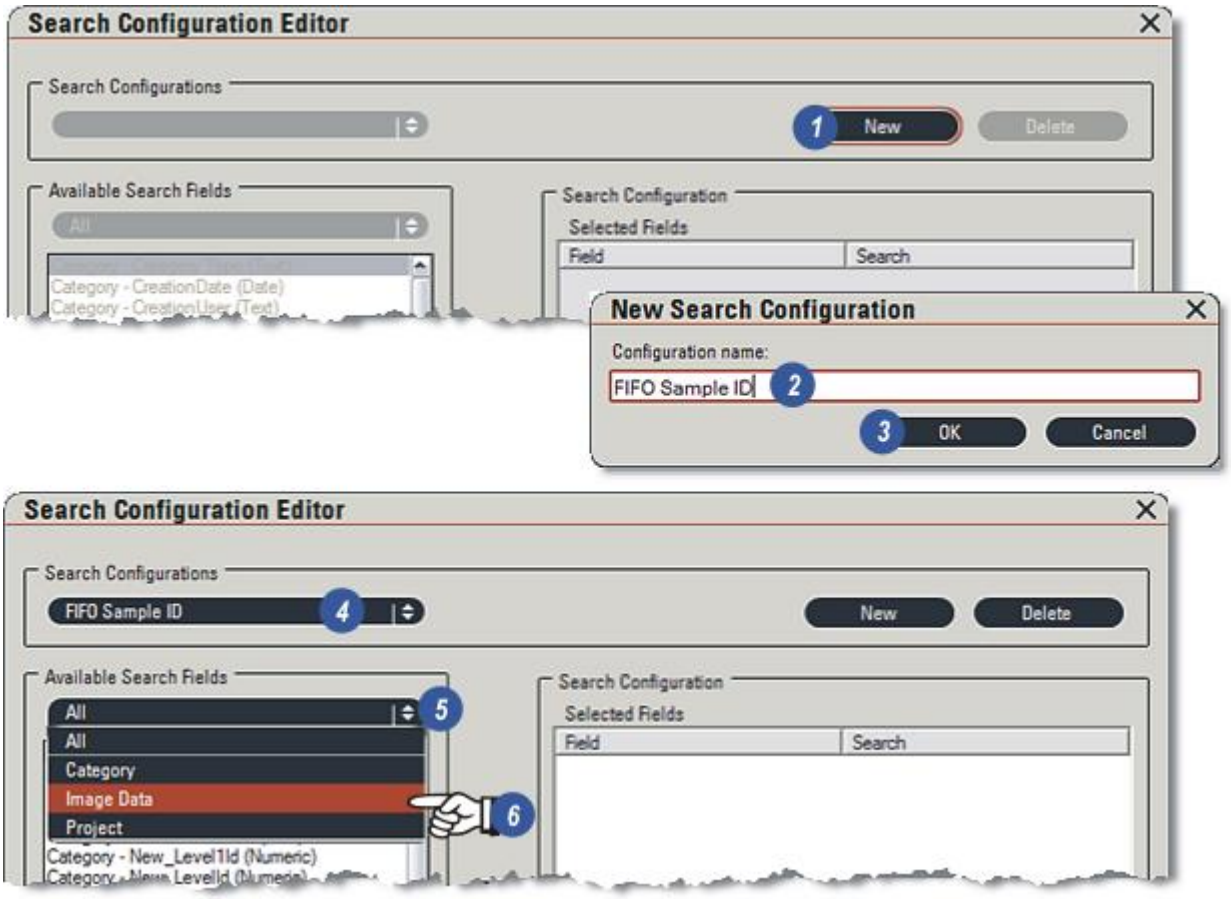
2: Give the new configuration a unique name by clicking in the *Configuration Name* text box and typing.

3: Click *OK.*

4: The new name appears in the *Search Configuration* list.

5: The fields to search can be set to *All* - the entire record set - or narrowed to include only specific groups by clicking on the arrows to the right of the header and...

6: ...clicking to select the group to be searched.



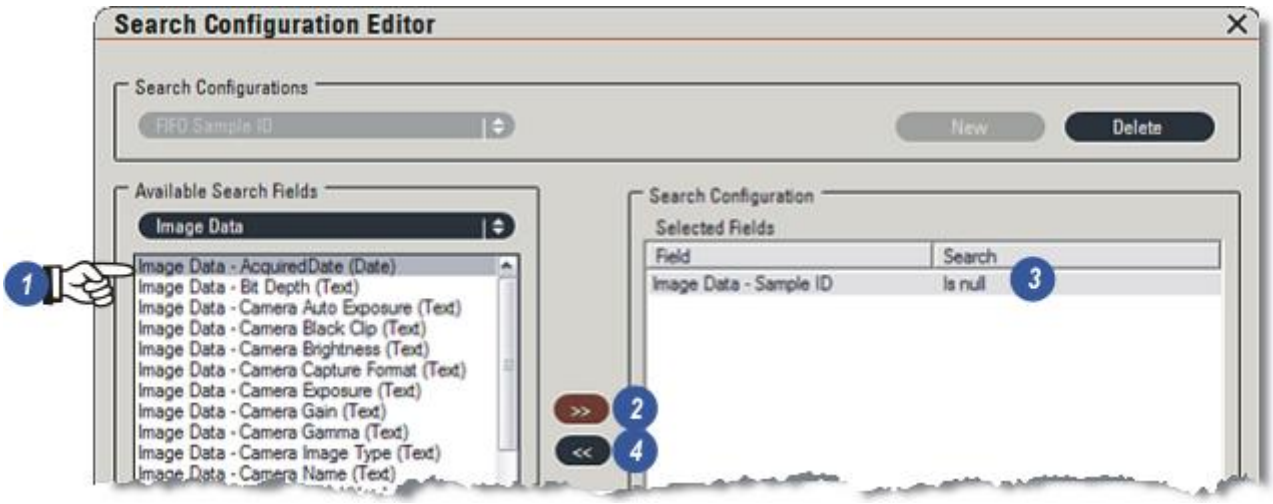
All of the available fields are shown on the left pane of the Search Configuration Editor. To include a field in the search:

1: Click on the field to select it.

2: Click on the *Select* button and...

3: ...the field name appears in the *Selected Fields* pane. Any number or any type of field can be selected.

4: To remove a field from the configuration, click to select the field in the *Selected Fields* pane and click on the *De-select* button.



The search format for a field depends upon the field type - Text and Memo fields will be searched for character strings, Numeric for numbers - and so on. The appropriate format options are automatically displayed for the selected field and are explained on the following pages (Go there...). The illustrations show a text field called 'Sample ID'.

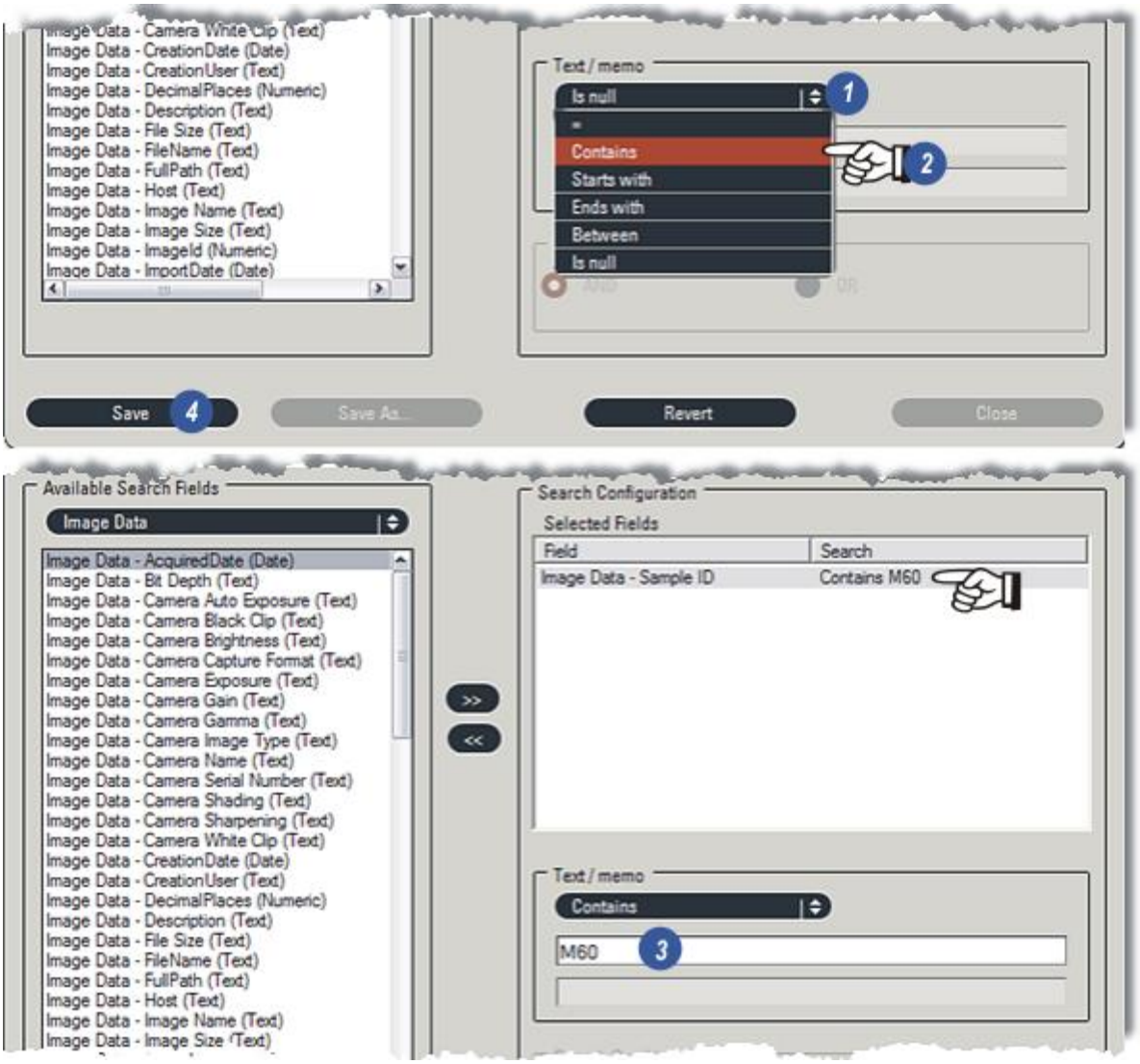
The search format is selected by:

1: Click on the arrows to the right of the format header.

2: From the drop down menu click to select the desired format. In the illustration the '*Contains'* format has been chosen.

3: Now the actual search string has to be entered. Click in the window and type the required string to search for. In the example ***'M60'*** has been entered so any image with a Sample ID field containing the characters ***'M60'*** will be returned as fulfilling the search criteria. The *'Contains'* option means that text such as *'Sample **M60'*** or *'Batch **M60** Local'* will satisfy the criteria.
The search string appears in the *Selected Fields* pane to the right of the *Field* name.

4: Click the *Save* button to save the configuration.



Search Field Options

Each field type has a range of search string options associated with it which are displayed automatically. The types and options are:

Text and Memo options: Accepts numbers and characters in the search string:

1: Click on the arrows to the right of the header and click to select from the drop down.

=: The field data must match exactly the search string.

Contains: The search string can appear anywhere within the field data.

Starts with: The field data must begin with the search string.

Ends with: The field data must end with the search string.

Between: Two search strings are entered and each computed as an ASCII string value - converted to a number. The field data, also converted to an ASCII value, must lay between the two.

Is Null: The field must be empty (nothing).

Date options:

2: Selected from the drop down with additional options available if the down arrow **(6)** is clicked.

The *Date Picker* provides a simple way of moving between Years and Months (click the arrows (4), and by clicking on the required day (5).

On: Date in the field corresponds exactly with the date selected.

Before: The field date must occur before the selected date.

After: The field date must occur after the chosen date.

Between: Two date windows **(3)** open and a date is selected for both. The field date must occur between the two.

Is Null: No date in the field: Nothing.

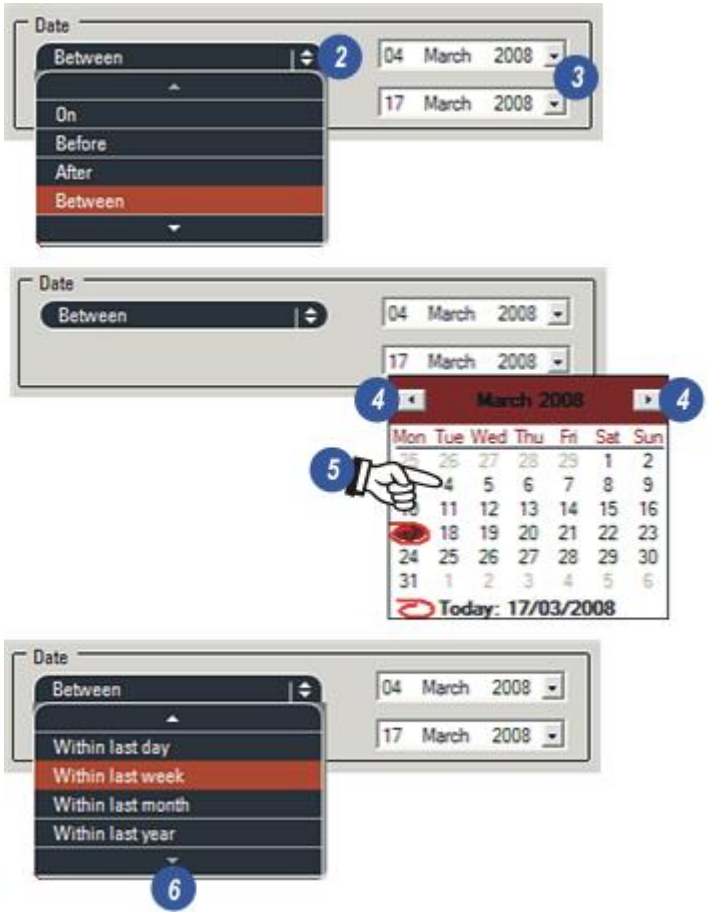
Within options:

Last Day: During the last 24 hours.

Last Week: During the last 7 days. If it is 10am on Tuesday all records that have a date and time equal or later that 10am on the previous Tuesday will be found.

Last Month: If the current date is 17 March then all records from and including 17 February of the same year are found. Leap years are automatically accommodated but the time of day is ignored.

Last Year: All images with a date on or later than the same date during the past year will be found. Leap years are accommodated automatically but the time of day is ignored.



Numeric options:

1: Click on the arrows to the right of the header.

2: Values are entered in the text boxes. The option chosen will determine if 1 or 2 text boxes open.

=: The field value must match the search value exactly.

<: The field value must be smaller than the search value.

>: The field value must be greater than the search value.

Between: Two values are entered for the search value limits and the field value must lay between the two.

Is Null: No value in the field: Nothing, not even zero (0).

Boolean options:

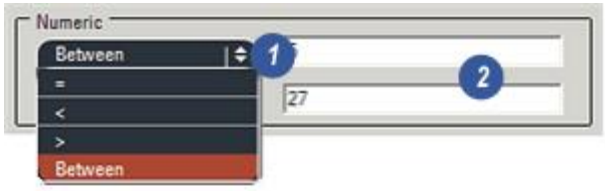
3: Select either *True* or *False*. The field setting must match.

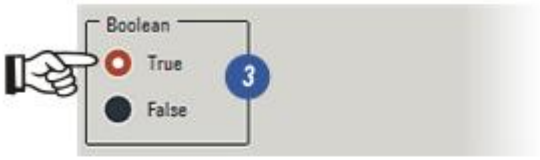
Multiple Field Search:

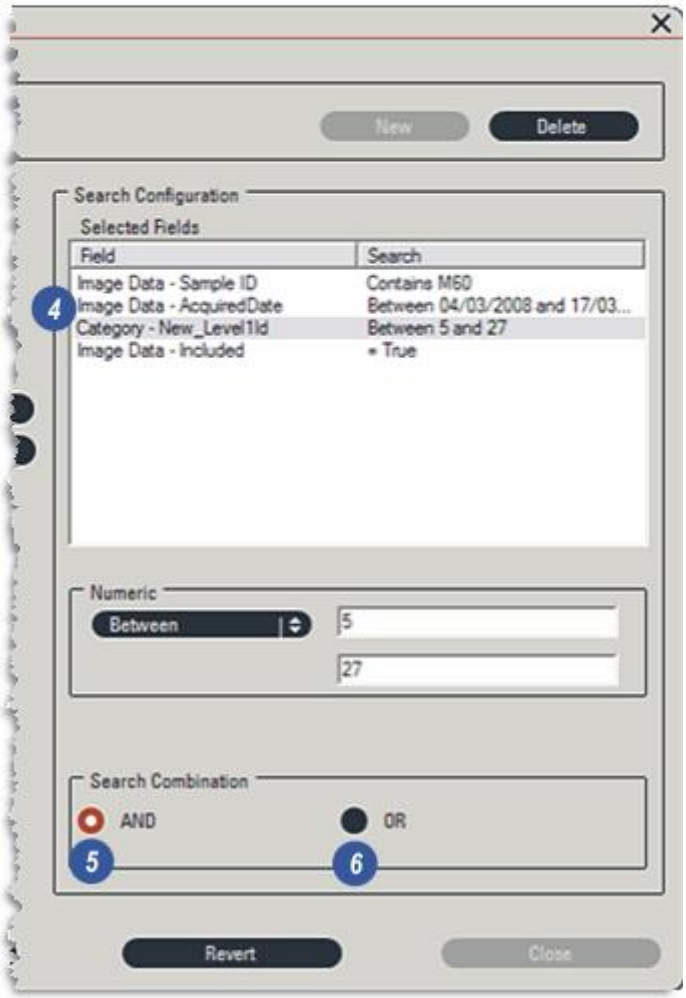
4: Up to 10 fields of any type 'mix' of can be selected for a search and in this case the *Search Combination* is enabled. It is based upon two Boolean parameters...

5: ...*AND* determines that ***all*** fields must conform to the search strings to return an image.

6: *OR* means that ***one or more*** fields must satisfy the search conditions to return the image.







Delete a Search Configuration:

1: Click on the arrows to the right of the *Search Configurations* menu and click to select the configuration to be deleted.

2: Click on the *Delete* button.

3: Confirm the deletion. Deleted configurations cannot be recovered.

Save a Configuration As...

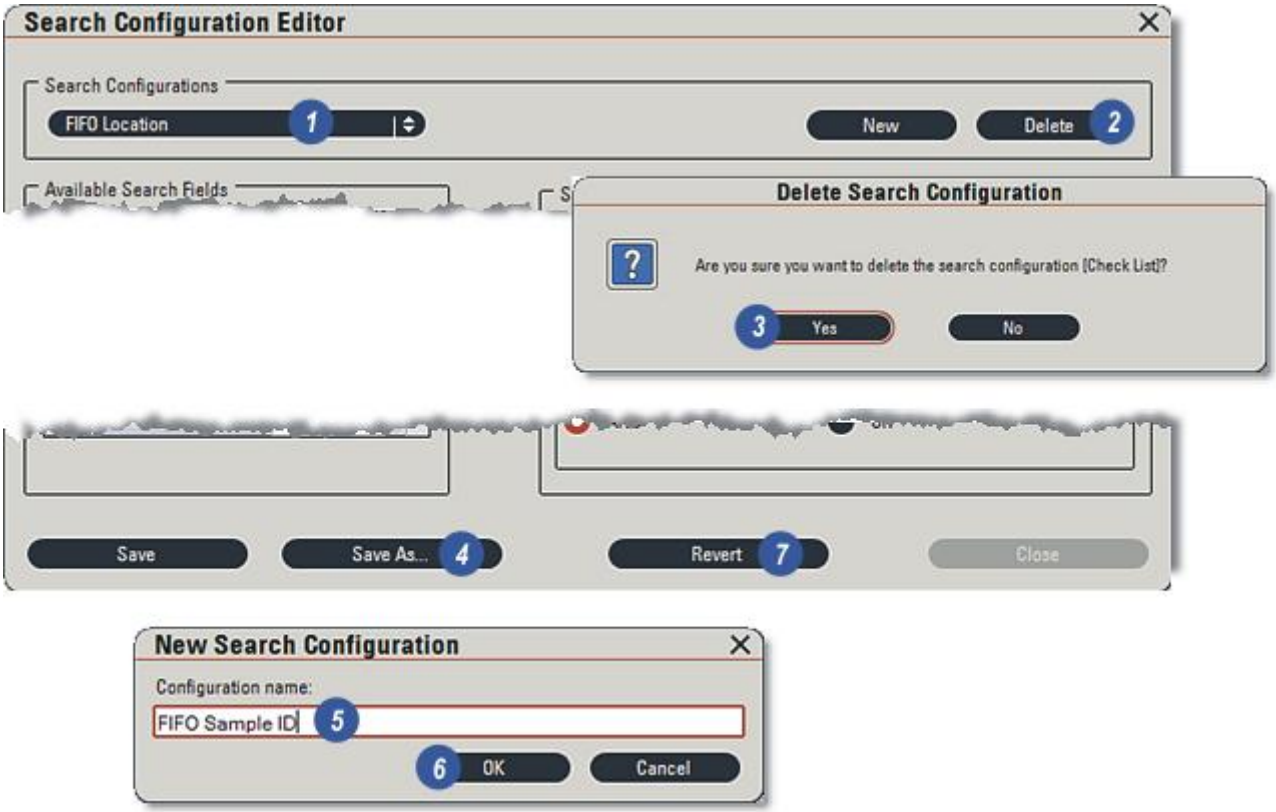
4: Clicking the *Save As* button will...

5: ...reveal the *Search Configuration* dialog. Click in the text box and type a new name for the configuration.

6: Click *OK* to save the search configuration.

Revert:

7: The *Revert* button will set the configuration to the last one saved. Any changes made since will be lost.



If a new configuration has just been created, all that is necessary is to click on the *Search* button **(5)** to run the search.

Search using an existing Configuration:
To run a search using a previously created configuration:

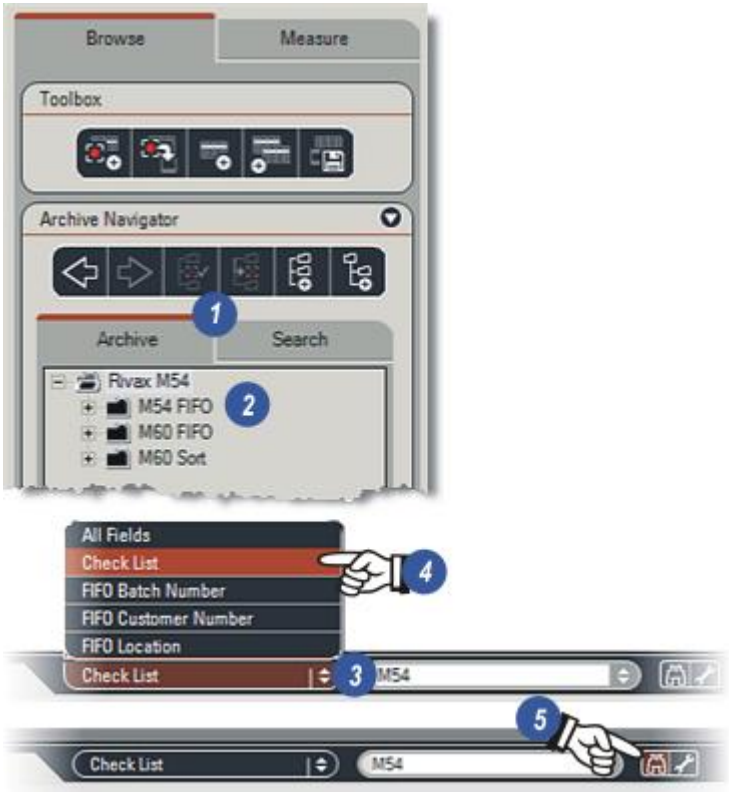
1: Click on the *Archive* tab and...

2: ...check that the required archive is selected and active.

3: Click on the arrows to the right of the *Search Configurations* header on the *Search* bar.

4: From the pop-up menu, click to select the configuration required.

5: Click on the *Search* button.

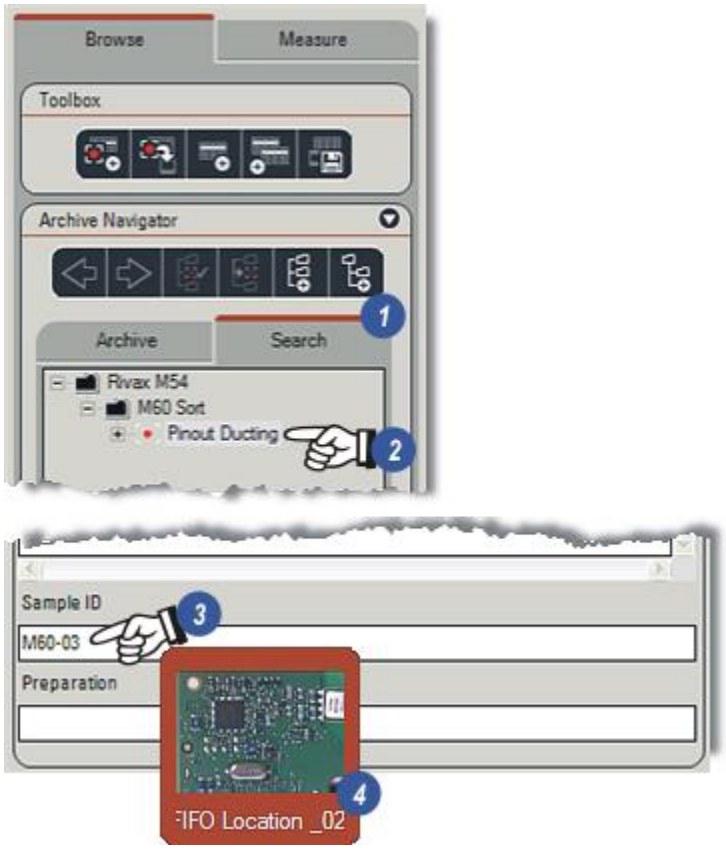


1: If a match is found the *Search* tab is automatically selected...

2: ...showing the Archives in which the search was made, and...

3: ...the appropriate record is populated - if there are more than one that satisfies the search criteria, the first is selected.

4: The image *Thumbnail(s*) appear in the *Gallery.*



The Standard Edition Archive extends the power and flexibility of the Basic Editions to provide:

- Multiple archive levels.
- Wider choice of Named Archive Fields - Memo, Boolean, Numeric, Date.
- The Keywords feature establishes preferred field descriptions.
- (Note: Reporting[118] is now handled by the Core.).

The *Standard Archive* option provides a further 4 field types:

1: *Numeric***:** 32-bit floating point numbers.

2: *Memo:* Alpha-numeric with over 32,000 characters. Line returns are added automatically as the text is entered.

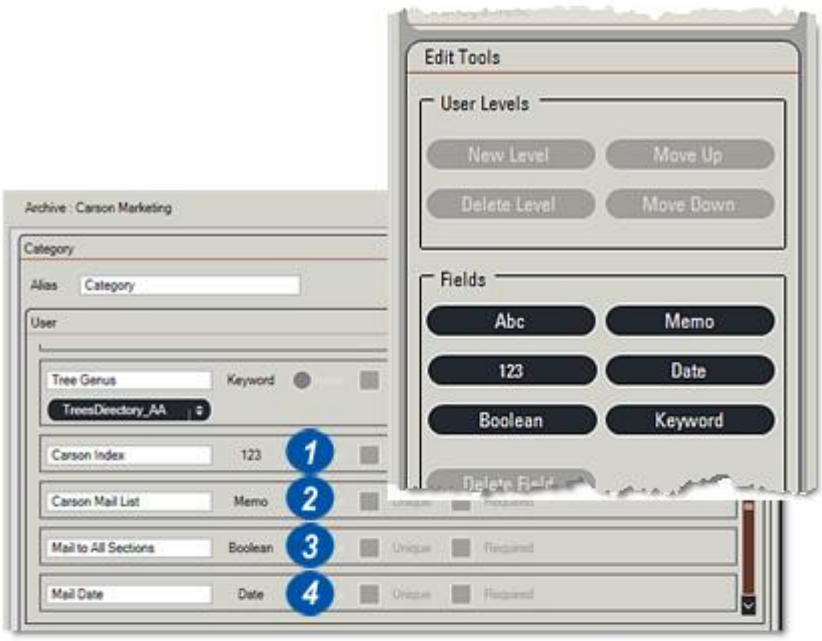
3: *Boolean:* Yes/No: True/False. On the Report this appears as a check box which, when enabled equates to true (Yes).

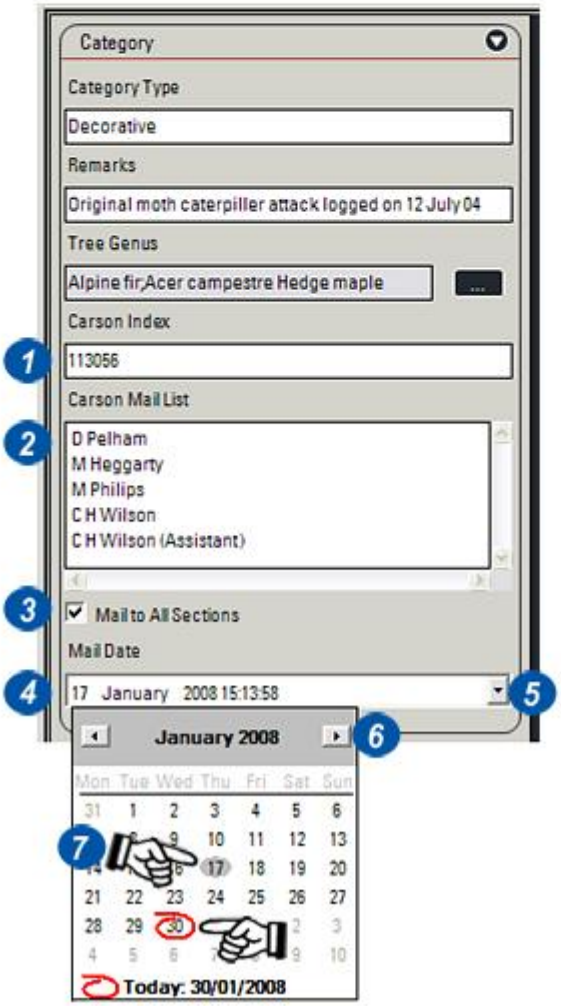
4: *Date:* On the report this is selected using the *Date Picker* by...

5: Clicking on the arrow to the right of the date text box.

6: Using the left and right pointing arrows to scroll through months and years, either forward or backward.

7: Clicking to select the date required. Today's date is circled in red.





To add a new field to an archive:
With the archive active:

- Select Edit mode.
- Click to select either User or Image field groups.
- Click on the required field button and the new field will appear.
- The name can be altered by clicking in the field window and typing a new name.
- Click the Save button to save the amended archive.

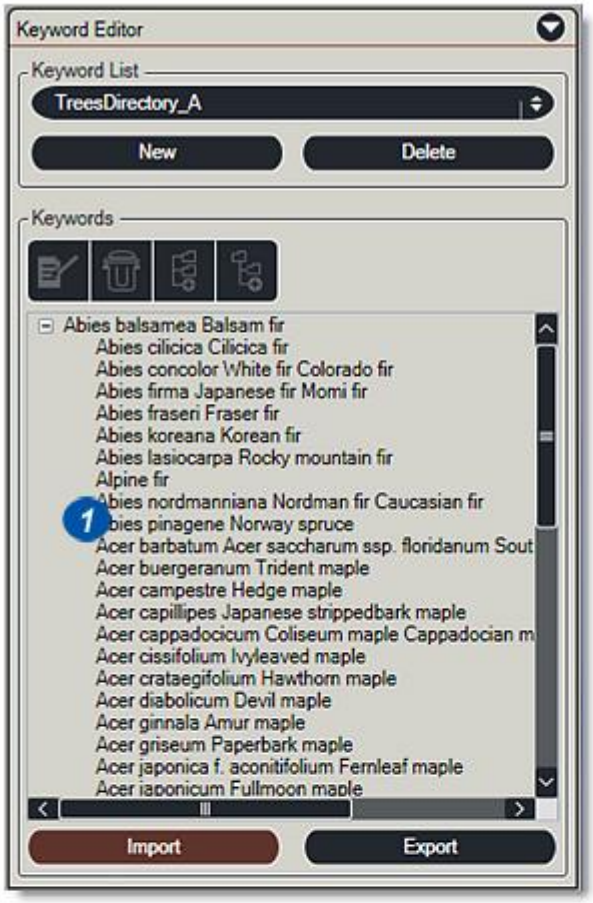
The *Keyword* feature can create lists of words and associate them with archives so that they can be used to populate selected fields.

This means that spellings – especially for complex subjects, possibly in another language – only have to be captured once and after that can be used indefinitely, confident that spelling and 'cases' are consistent **(1)**

So, *Keywords* are fast and accurate and can ensure that field searches will be precise.

Keyword lists can be shared across any number of archives with any changes to the list be reflected in all of the archives. This is because each archive maintains a link to the *Keyword* list rather than copying it.

New lists can be created by typing, by cutting and pasting words or phrases from an existing document or by importing a complete existing list.

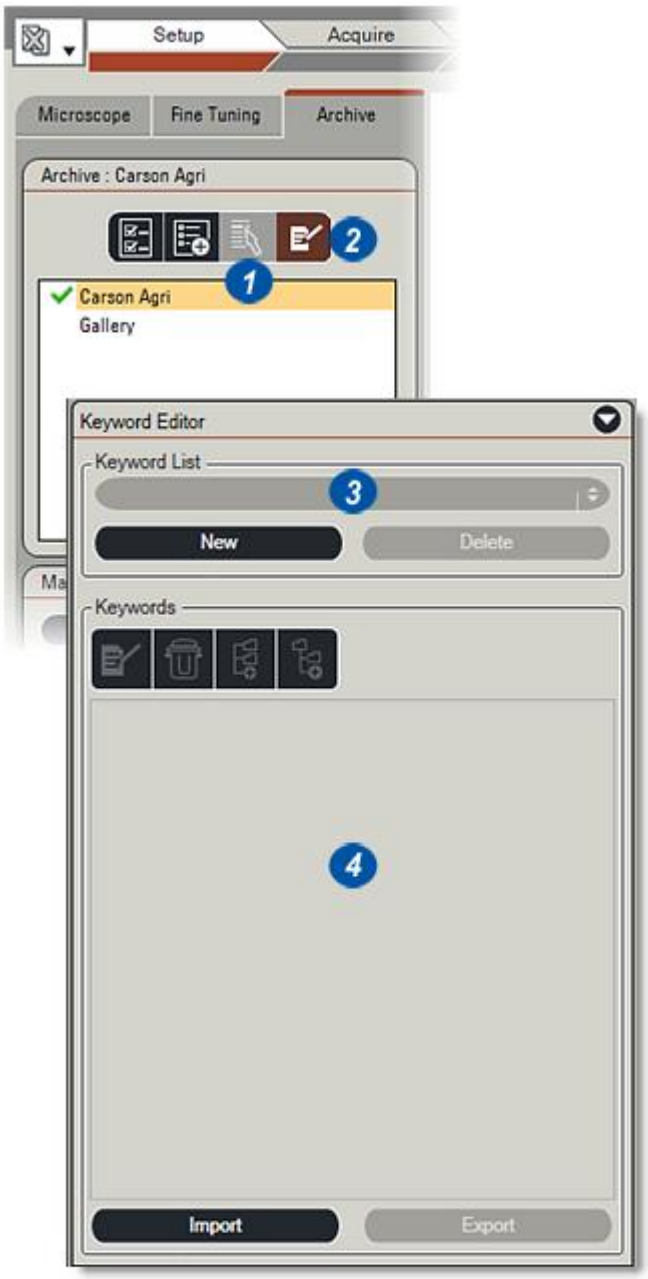


Create a New Keyword List

When a new archive is created the Keyword dialog automatically appears on screen ready for a new list to be created or imported. For existing archives:

1: Select the archive and if necessary make it active current by double-clicking it or clicking the *Set Archive as Current* button.

2: Click on the *Edit* button. The field structure and the Keyword dialog will appear. If there is a Keyword List already associated with the archive, its name will be displayed in the *Keyword List* window **(3)** and its contents in the main window **(4).**



1: On the *Keyword* dialog click on the *New* button.

2: When the *New Keyword List* dialog appears, type a name for the new list and click *OK*.

3: The name appears in the *Keyword List* window and the other controls become active.

4: Change the name and start again by clicking the *Delete* button and returning to step **(1).**

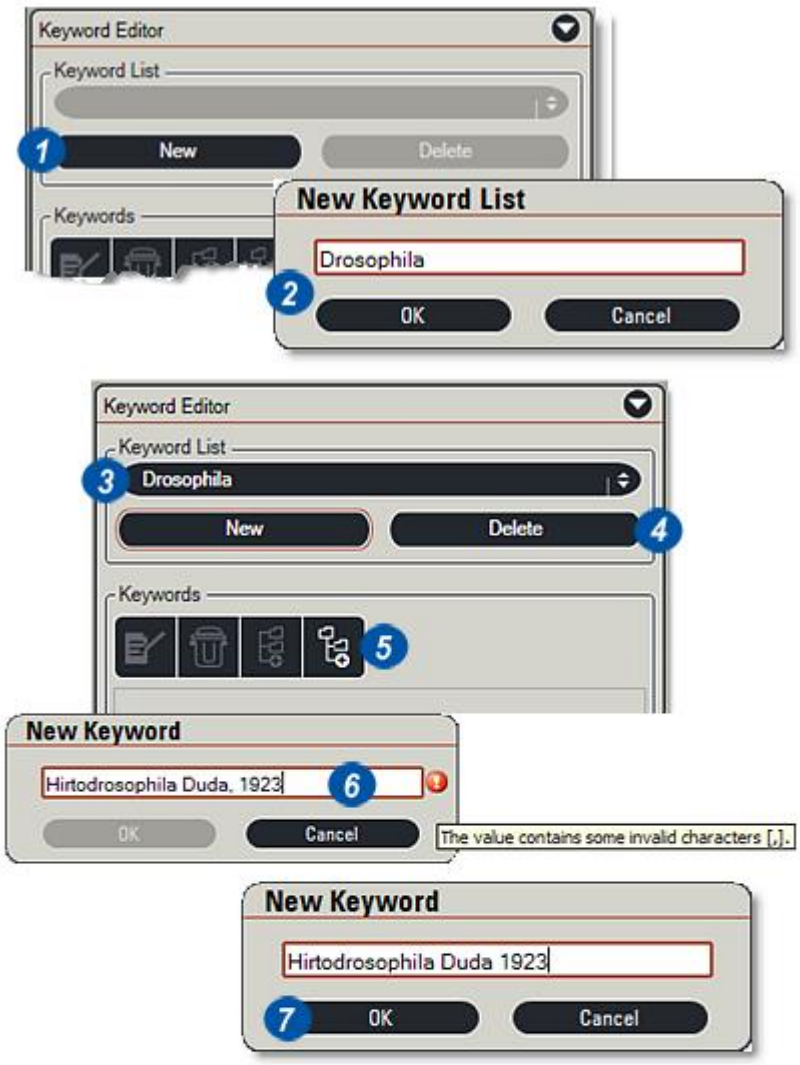
Typing the Keyword List:

5: The first word or phrase must be preceded by clicking the *Add a Child* button. The *New Keyword* dialog appears.

6: Type the first word or phrase. Words can be copied from other open documents – a text editor or internet browser for example – and pasted into the New Keyword text box using the *Ctrl+C (Copy)* and *Ctrl+V (Paste*) keyboard combinations.

Generally, only letters and numbers are allowed in keywords; If an invalid character is typed a red **(!)** appears to the left of the entry text box and the flyout prompt itemises the invalid character. In the example it is the comma between *Duda* and *1923* shown as **[,].** Delete the invalid character and…

7: …click *OK*.



1: The first word or phrase appears in the *Keyword Editor* main window.

2: To add more words click the *Add New Word at this Level* button and when the *New Word* dialog appears…

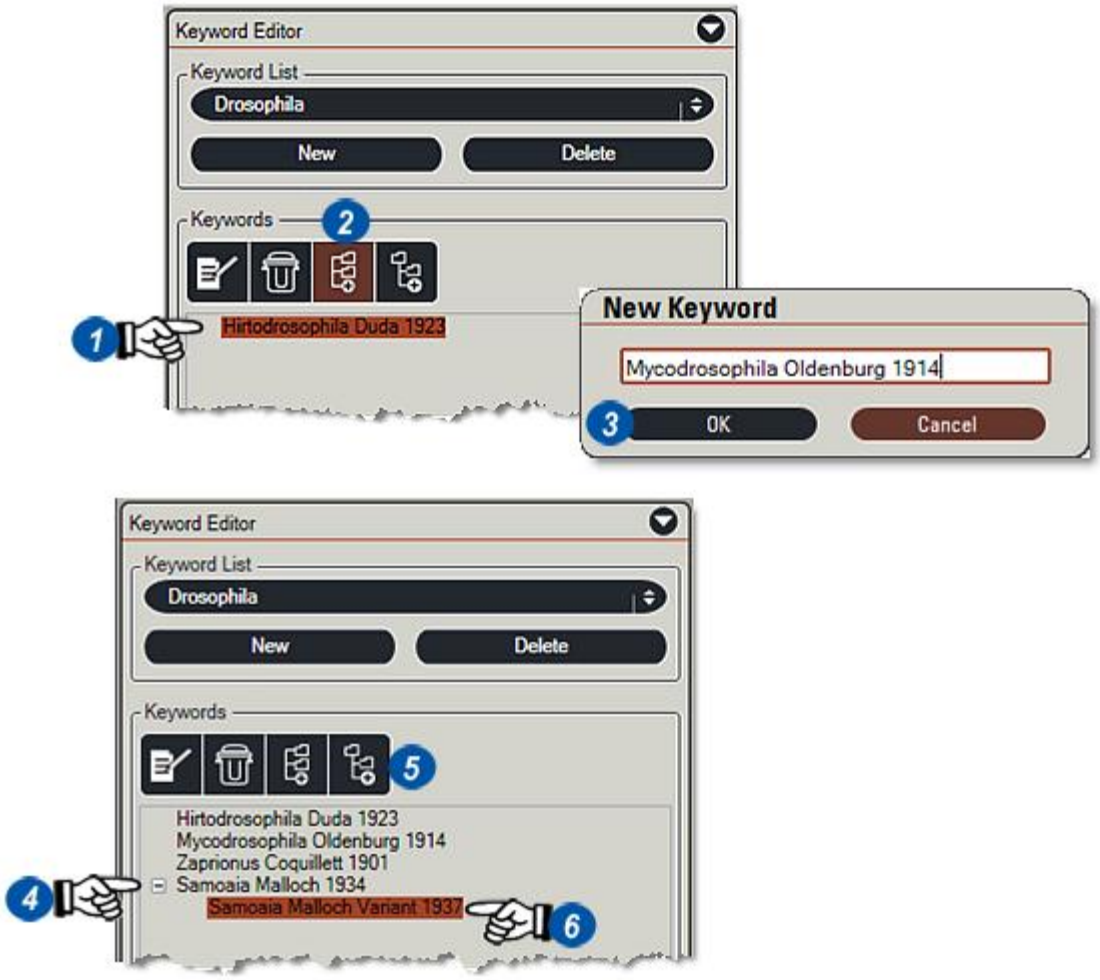
3: …type a word or phrase and click *OK*.

Keyword Grouping:
To make long lists of keyword easier to read or to group related entries, the list can be indented by:

4: Clicking on the entry that is to 'head' the group.

5: Click on the *Add a Child* button and type the word or phrase to be indented **(3).**

6: The word will appear indented in the main window. The last word or phrase added becomes the selected item automatically, so to continue to indent use the *Add New Word at this Level* button.
To revert to the original level, click to select the entry that 'headed' the group and use the *Add New Word* at this Level button.
Several indent levels are permissible.



To edit a word or phrase:

7: Click to select the word and…

8: …click the *Edit* button.

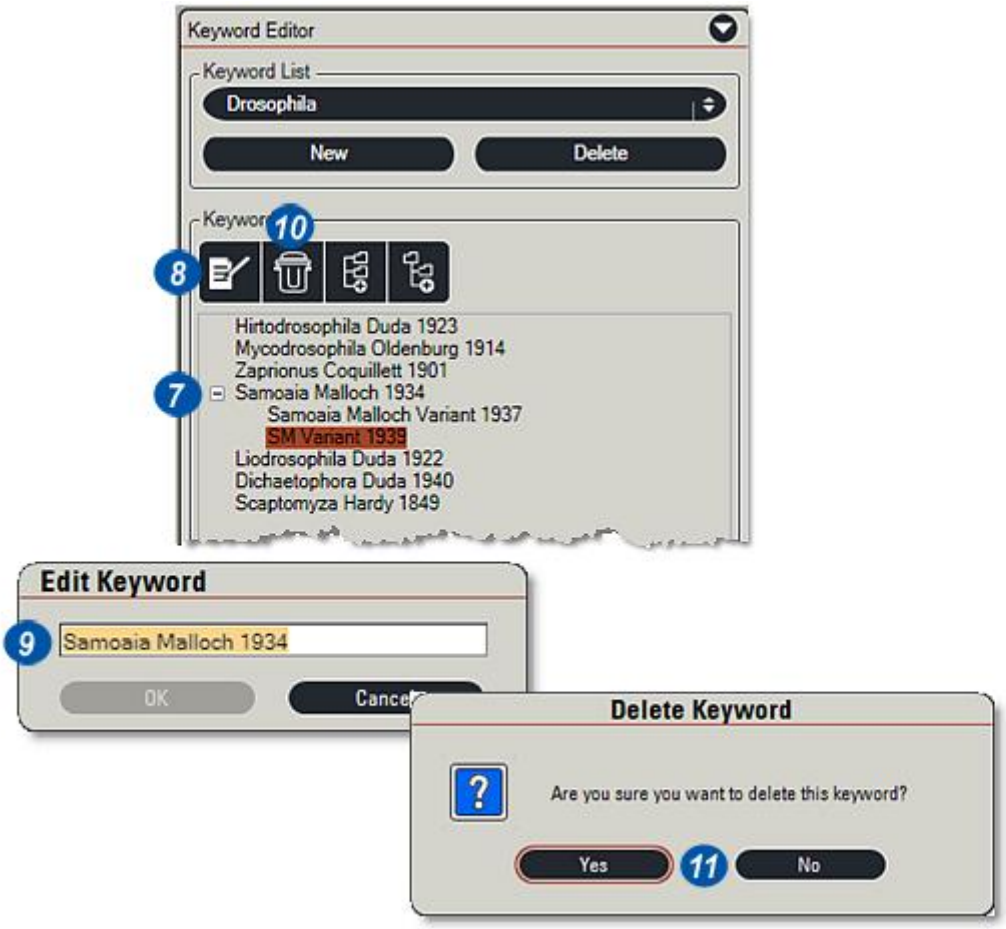
9: The word appears in the *Edit Keyword* dialog. Make the changes and click *OK*.

Delete a Keyword:

7: Click to select the word and…

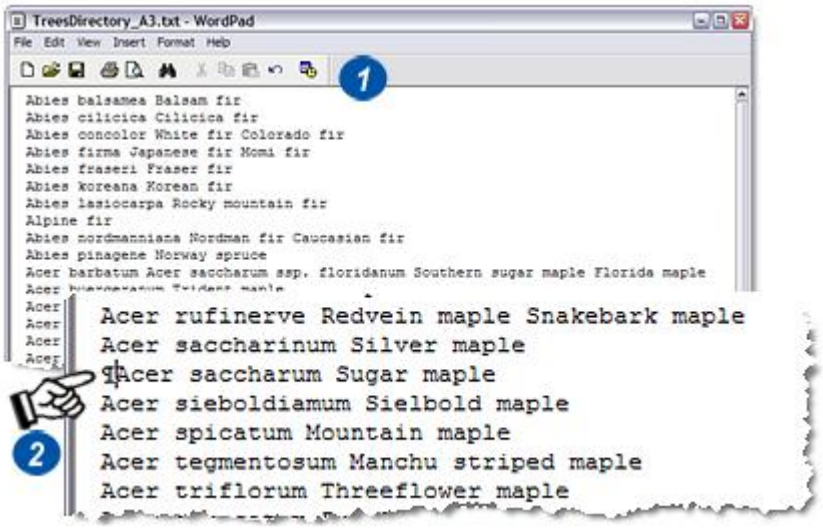
10: …click the *Trashcan* (Delete) button.

11: Confirm (or cancel) the deletion.



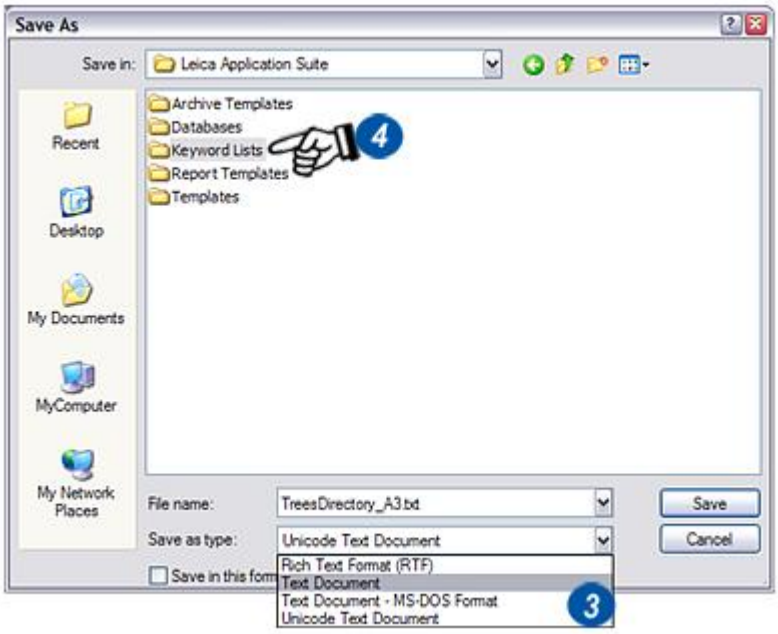
Create a List with Cut and Paste

New lists can also be created from existing documents and imported as Keywords.
The list is created in a simple test editor such as *Wordpad* or *Notepad* – **DO NOT** use Microsoft Word to create the list, but individual words or phrases can be cut from Word and pasted into Wordpad for example.



1: Either type directly into the text editor or cut and paste from another source.
Avoid punctuation characters especially commas *(Keyword Lists* are comma-delimited), using mainly letters and numbers.

2: To create an indented entry or block, insert the *Paragraph* (sometimes called Pilcrow) character at the beginning of the line. These will create an indent when the list is imported into the *Keyword Editor.*
The *Paragraph* character can be inserted by holding down the keyboard Alt key and typing 0182 on the *Numeric Keypad* usually situated to the right of the main keyboard.
The character can be copied (highlight and use *Ctrl+C*) and then pasted (*Ctrl+V*) if there are many entries to indent.
Do not use tabs or additional spaces to make an indent.



3: Save the file as a .txt document either as *Text (ASCII), MS-DOS Text (ASCII)* or *Unicode (UTF-8)* but not as Rich Text Format (RTF) which, like Microsoft Word includes extensive formatting.

4: Although *Keyword Lists* can be saved to any folder on the hard drive, Leica Application Suite has a default location:

C:\Documents and Settings\All Users \Shared Documents\Applications\Leica Application Suite\Keyword Lists

…which is recommended.

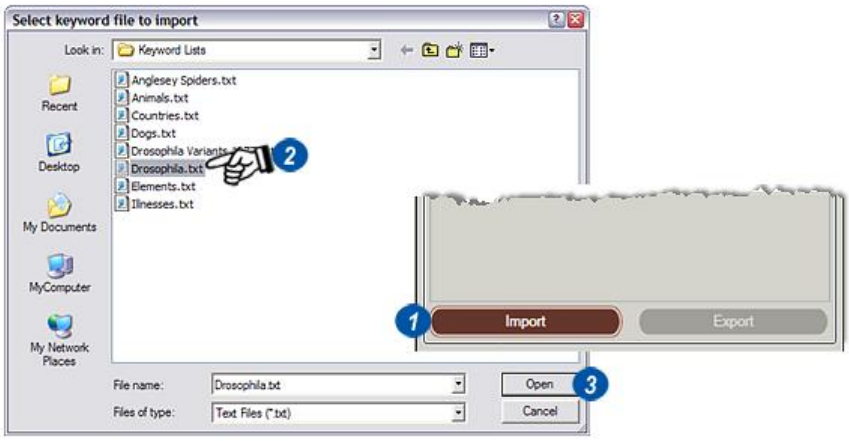
With a new *Keyword List* created in a text editor and saved as a text (.txt) file, it can now be 'imported' into an archive. The import process actually establishes a link between archive and *Keyword List* so that any changes made to the list will be reflected in the archive.

To make major changes in he list and **NOT** have the archive affected, save the text file under a different name and use it as a completely separate list.

1: Click on the *Keyword Editor Import* button.

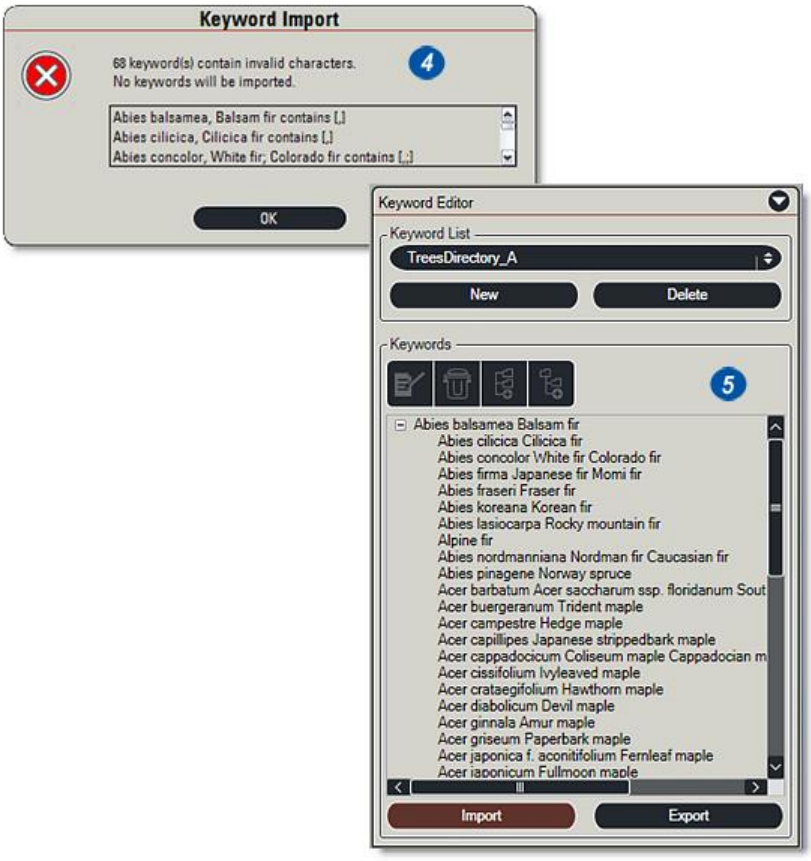
2: On the Windows dialog, navigate to the folder in which the new *Keyword List* is stored. The example shows the LAS default folder, *Folder Lists*. Click to select the list and…

3: …click *Open*.



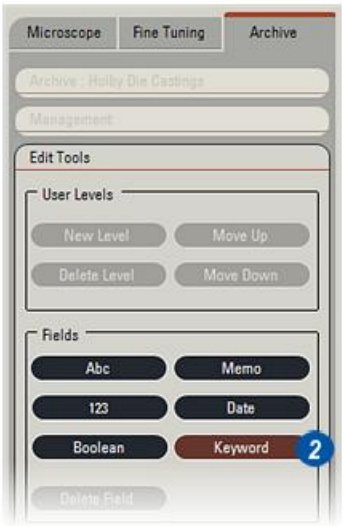
4: If there are invalid characters in the List a warning appears indicating the problem characters and where they are located. The list will have to be corrected and saved with the text editor.

5: When import succeeds the list appears in the *Keyword Editor* main window. Long and wide lists automatically have scroll bars to the right and bottom.



Keywords can only be copied to archive fields that has been designated as *Keyword Fields*.

When a *Keyword List* (or lists) is associated with an archive, the *Keyword* button on the *Archive* panel becomes active:

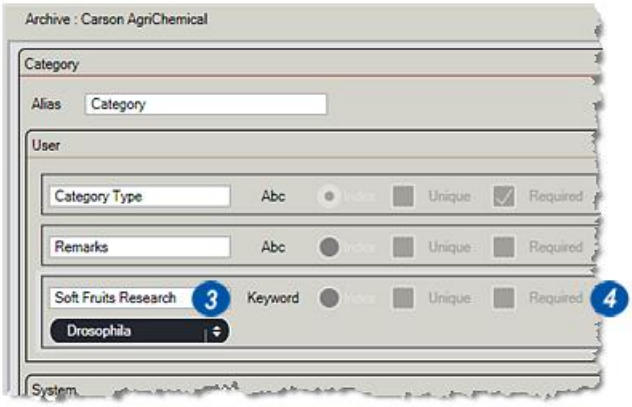


1: Click to select either the *Category (Record Group)* or *Image* panel. Keywords in the Category will appear on every image captured within that group; Keywords associated with the image will only appear if they are selected for that image.

2: Click on the *Keyword* button. A keyword text box opens on the selected panel.

3: Click in the *Keyword* text box and type a meaningful name for the Keyword Field.

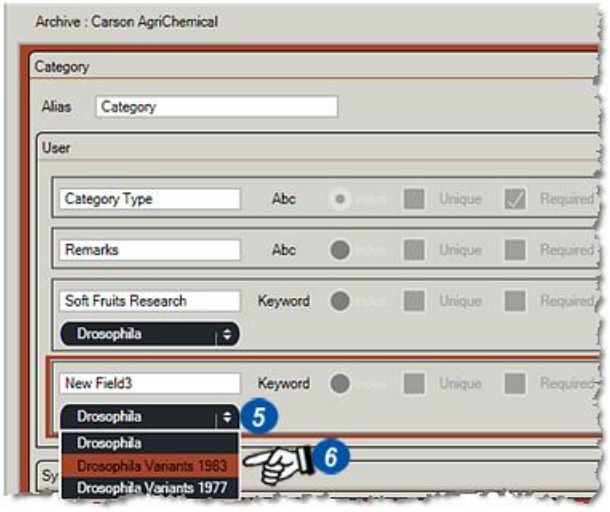
4: If it important that a keyword always appears in the field, click to enable the *Required* check box. With Required enabled, a user will be forced to select a keyword from the List before an image can be saved.



5: In cases where several *Keyword Lists* are associated with an archive, select the appropriate list from the drop down menu by clicking on the arrows to the right of the *Keyword List* name and…

6: …clicking to select the list required.

Several *Keyword Fields* may be added to the *Record Group* or Image parts of the archive and this, together with the facility to associate a range of *Keyword Lists* with the archive, makes *Keywords* a powerful and fast method of gathering data. And if, for example, a list comprises the cost centres within an organisation and is set as *Required*, every image without fail could become the property of a particular department.



7: Click *Save*.

Keywords are copied to keyword fields in the *Browse Workflow*. For *Required* keywords in the *Category* (Record Group), these are selected when the group is created as follows:

1: Click on the *Create a New Category* button.

2: Type a name for the new category.

3: If the keyword is *Required*, a red **(!)** will flash to the right of the *Archive Navigator*. This means that the new category cannot be saved until a keyword has been selected.

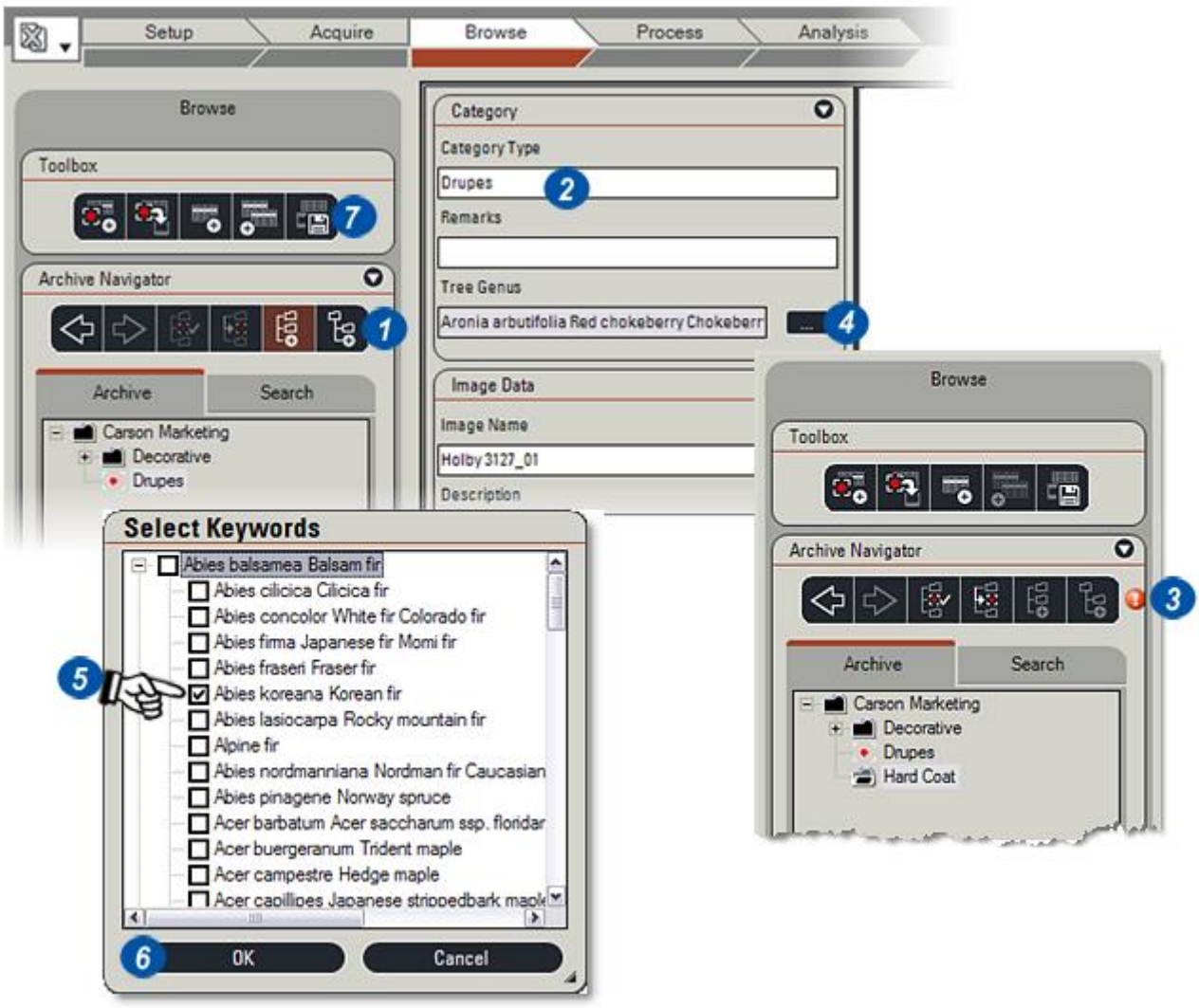
Use this process any time a keyword is required:

4: Click on the button to the right of the *Keyword Field* – this could be in *Category* or in Image. The *Select Keyword* dialog appears.

5: Scroll through the *Keyword List* to the required entry and then click in the check box to the left of it. A tick mark should appear. Clicking the entry itself is not sufficient.

6: Click *OK* and the keyword will be copied to the field.

7: Click on the *Save* button.



Exporting a Keyword List

Keyword Lists may be exported to any folder on the computer or to an external storage device such as a memory stick. The *Export* feature makes a copy of the *Keyword List* at the new location; The original remains intact.

If there are several lists associated with an archive:

1: Click on the arrows to the left of the *Keyword List* on the Keyword Editor and from the menu…

2: …click to select the List to be exported.

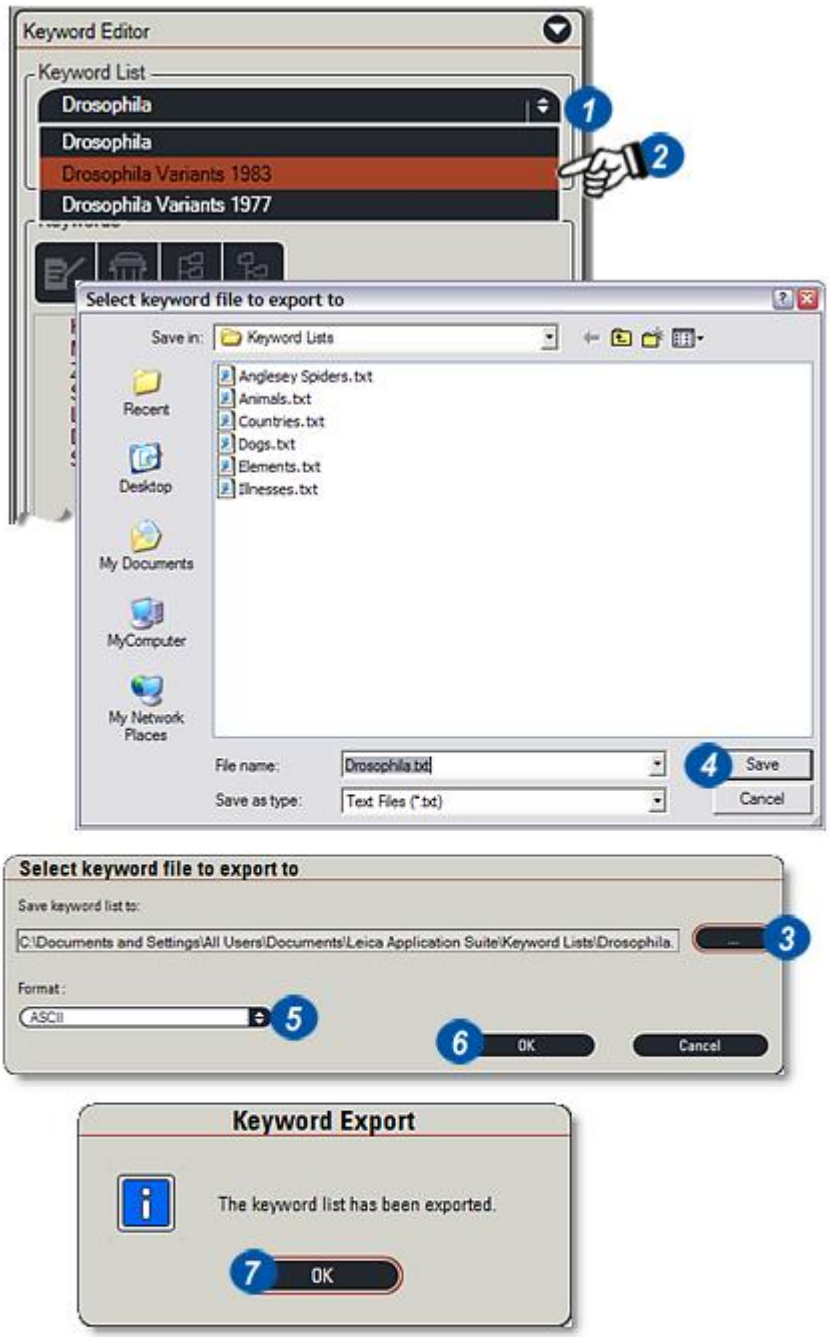
3: Navigate to the export target folder by clicking on the browse button to the right of the *Save Keyword List to* window, and…

4: …selecting it on the Windows dialog. Click *Save*.

5: Click on the arrows to the right of the *Format* text box and from the menu click to select the character coding – *ASCII* is the default and generally preferred.

6: Click *OK*.

7: A message indicates that the Export is complete. Click *OK*.



A *Keyword List* may be deleted – disassociated with the archive – at any time BEFORE a *Keyword Field* has been created. After that, clicking the *Delete* button will result in the *Cannot Delete* message **(1).**

If you wish to simply transfer an image from an LAS Archive to a memory stick then it is simple to use the Export Image facility.

See Export from LAS Archive to File System 532

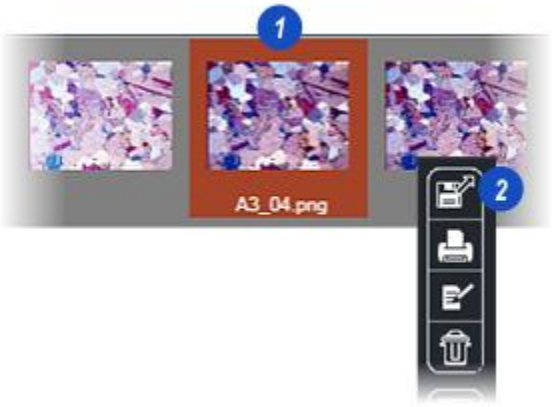
For those organisations using Leica *Image Manager (IM)* as a networked archive store and organiser, features are provided in LAS that allow images from workstations running under *Leica Application Suite* to be sent to Leica IM (from version 5 onward) and managed like any other IM image.

See Sending Images from LAS into Leica IM V5 533

Images stored in an IM archive can also be transferred into LAS.

See Receiving Images into LAS from Leica IM V5 544

The *Side Tool Bar* is displayed on the right hand edge of the *Image Viewer*. The *Export* button is situated in the top group.

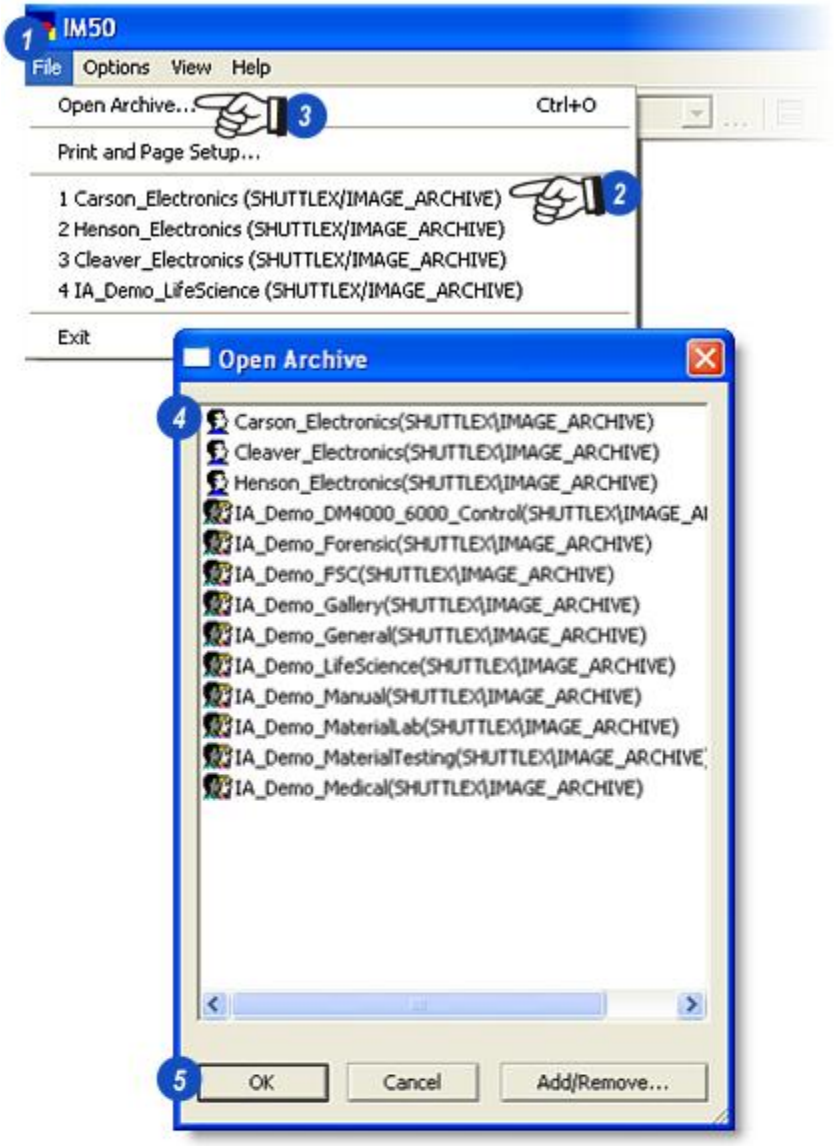


To *Export* the image being displayed using *LAS Archive* to a selected destination folder:

1: Click a thumbnail to select and display the image to export.

2: Click on the *Export* button.
For detailed help on the *Export* procedure look in *Functions Widely Available:* *Go there...* 100

To be able to use this feature, the Leica IM Image Data Read-in module must be licensed.



Leica Image Manager can store all of the data fields captured with the image in LAS providing they are nominated but it cannot itself display them – they are stored in a special file associated with the image. However, when the image is retrieved from Leica Image Manager by LAS, the data will be displayed in the *Browse* and *Process Workflows* in the usual way.

Setting up Image Manager to receive LAS images and data:
Images and data from LAS can be sent to either an existing or a new archive in Leica Image Manager. Run Leica Image Manager and to use an existing archive:

1: Click on *File* on the main menu and from the drop down menu…

2: …click to select an archive from the *Recent* list or…

3: …click the *Open Archive* option. This will reveal the *Open Archive* dialog.

4: Click to select an archive from the list.

5: Click *OK*.

Create a New Archive

To create a New Archive:

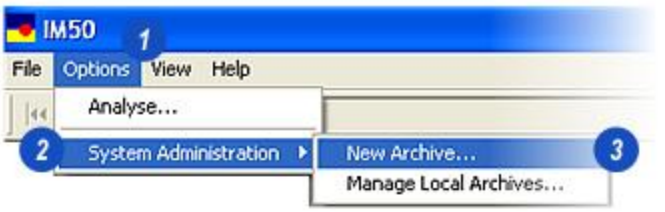
1: Click on *Options* on the main menu.

2: Click on *System Administration* and…

3: …click to select *New Archive*. The *New Archive Wizard* will appear. Follow the instructions.

A new archive need not have a range of fields – these will be selected later and the data for them will be supplied by LAS.

However, a new archive MUST have a *Text* field for every level and that must be chosen as the *Identifier (Key)*, as well as at least one *Image* field at the lowest level. If either are missing the archive will not be created.



Setup AutoSave in Image Manager

The *AutoSave* option in Leica Image Manager must be enabled so that the incoming images (from LAS) are directed to the selected archive and all of the data is correctly stored.

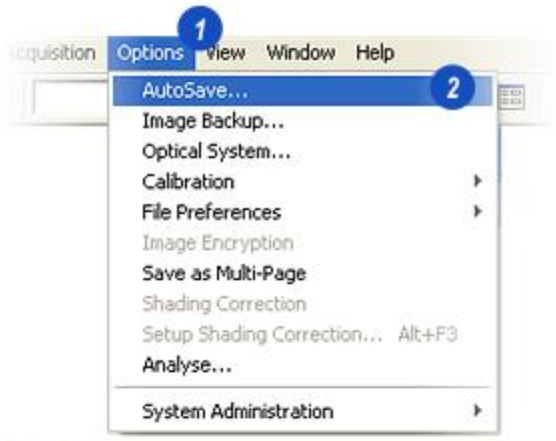
1: Select *Options* on the main menu…

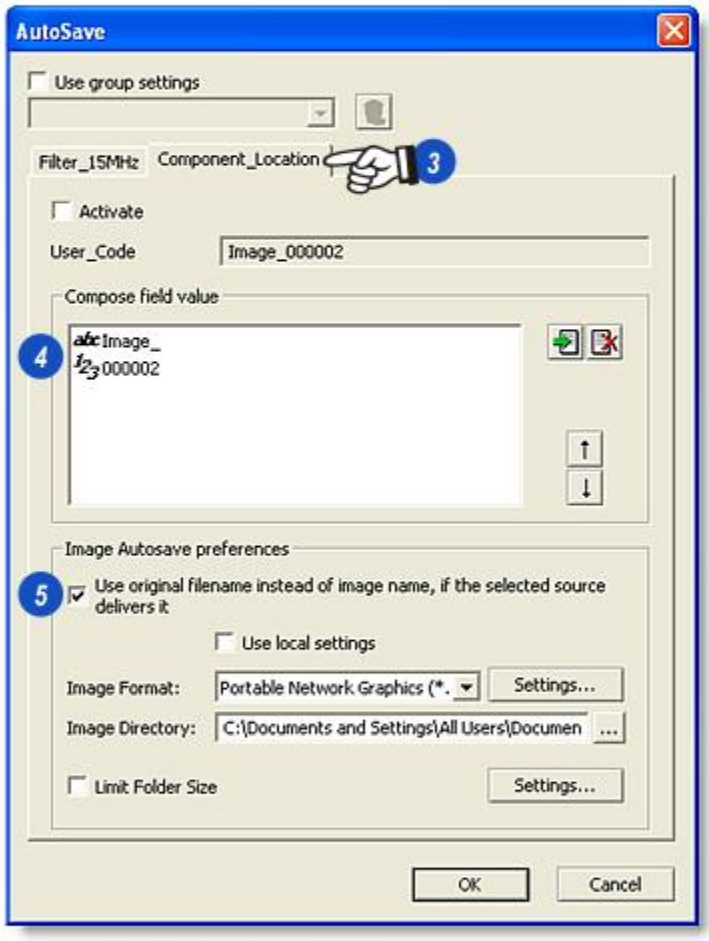
2: …and click *AutoSave*.

3: On the *AutoSave* dialog, click the right-hand tab that represents the new data sheet – the label on our example is *Component_Location.*

4: The fields existing in the chosen archive are displayed in the *Compose* window.

5: If it is intended that the *LAS Image Names* are used to refer to them within Leica Image Manager, click to enable *Use original filename…*





The image storage format can be changed as it is imported into Leica Image Manager. Jpeg compression is recommended because images are more compact and large archives will import quicker.

To change the image format:

1: Click on the small arrows to the right of the Image *Format* header and from the drop down list…

2: …click to select the required format.

3: The *Image Directory* (in Image Manager) will be set to that of the selected archive but can be changed by clicking on the browse button to the right of the *Image Directory* header and…

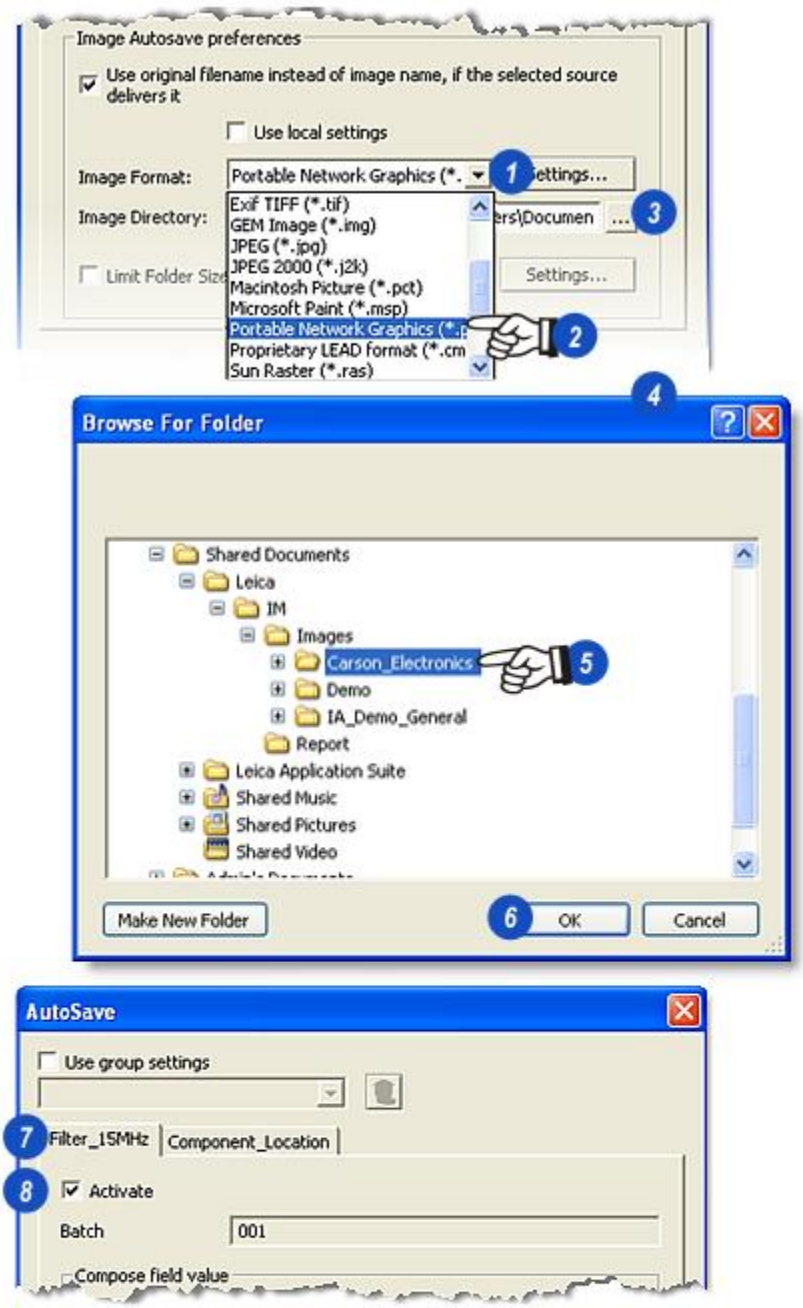
4: …using *Windows Browse for Folder* dialog to…

5: …navigate to the required directory.

6: Click *OK* to close the dialog.

7: Click on the left-hand tab and also…

8: …on the *Activate* checkbox to enable AutoSave.



Link the Import Plug-in

When Leica Application Suite is installed it makes available to Leica Image Manager an *Import Plug-in*, a small software application that will 'instructs' Leica IM how to store the data fields that will be sent from LAS.

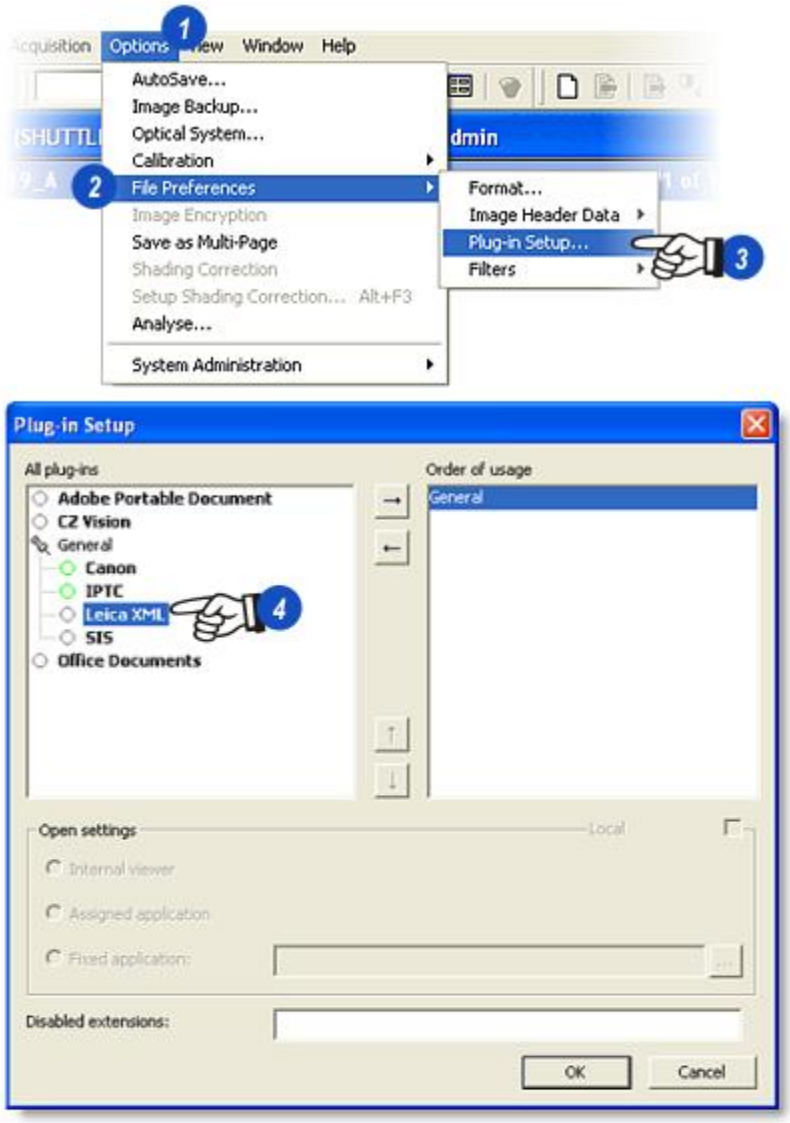
The next step is to link Leica Image Manager to the *Plug-in:*

1: Click *Options* on the main menu.

2: From the drop down list, click on *File Preferences* and then…

3: …on *Plug-in Setup.*

4: On the *Plug-in* dialog, click to select *Leica XML*.



With the *Leica XML Plug-in* selected:

1: Click on the *Include* button.

2: The *Leica XML Plug-in* is listed in the *Order of Usage* window and…

3: …the *Plug-in* is marked with a pin in the *All Plug-ins* window.

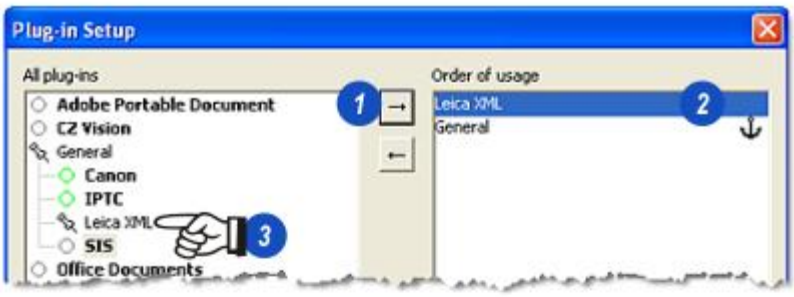
4: Check that the *Fixed Application* check box is enabled and…

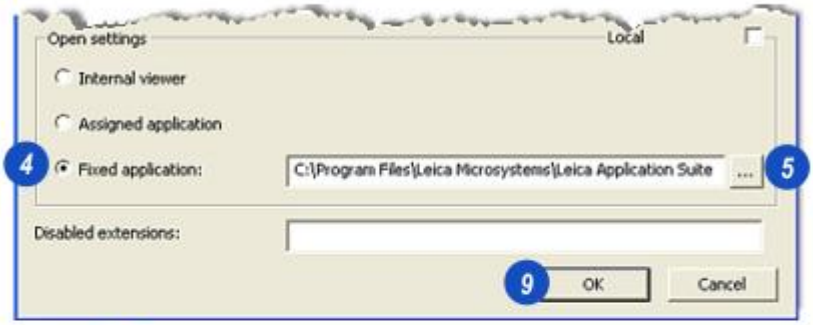
5: …the application being pointed to is *ImagePackageImporter.exe.* If it is not, click on the browse button to the right of the *Fixed Application* window and…

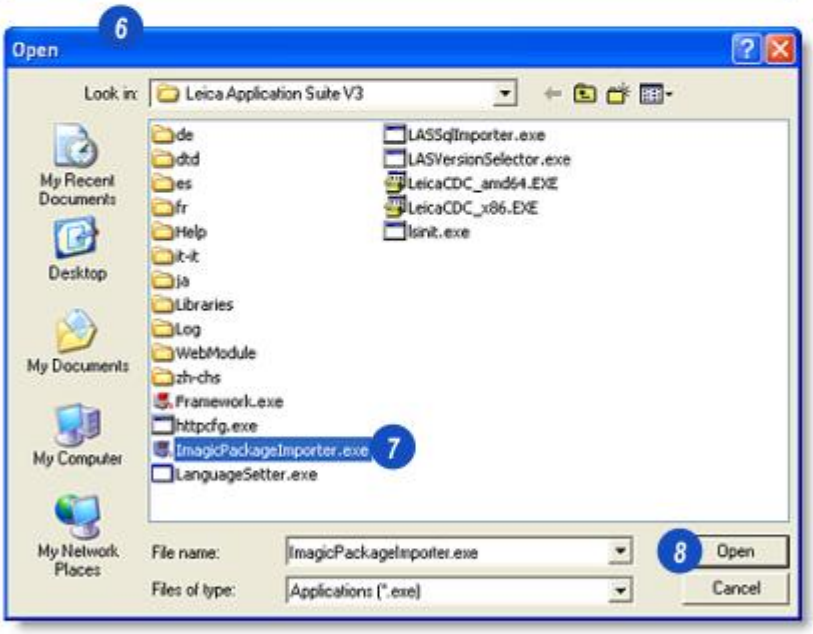
6: …on the *Browse (Open)* dialog, navigate to the *Leica Application Suite* directory and select the *ImagePackageImporter* application **(7).**

8: Click *Open*.

9: Click *OK* on the *Plug-in Setup* dialog.







Select the LAS Fields

With the *Leica XML Plug-in* linked to Image Manager all of the LAS data fields are available to Leica IM. Now they can be displayed and selected for inclusion with the imported images.

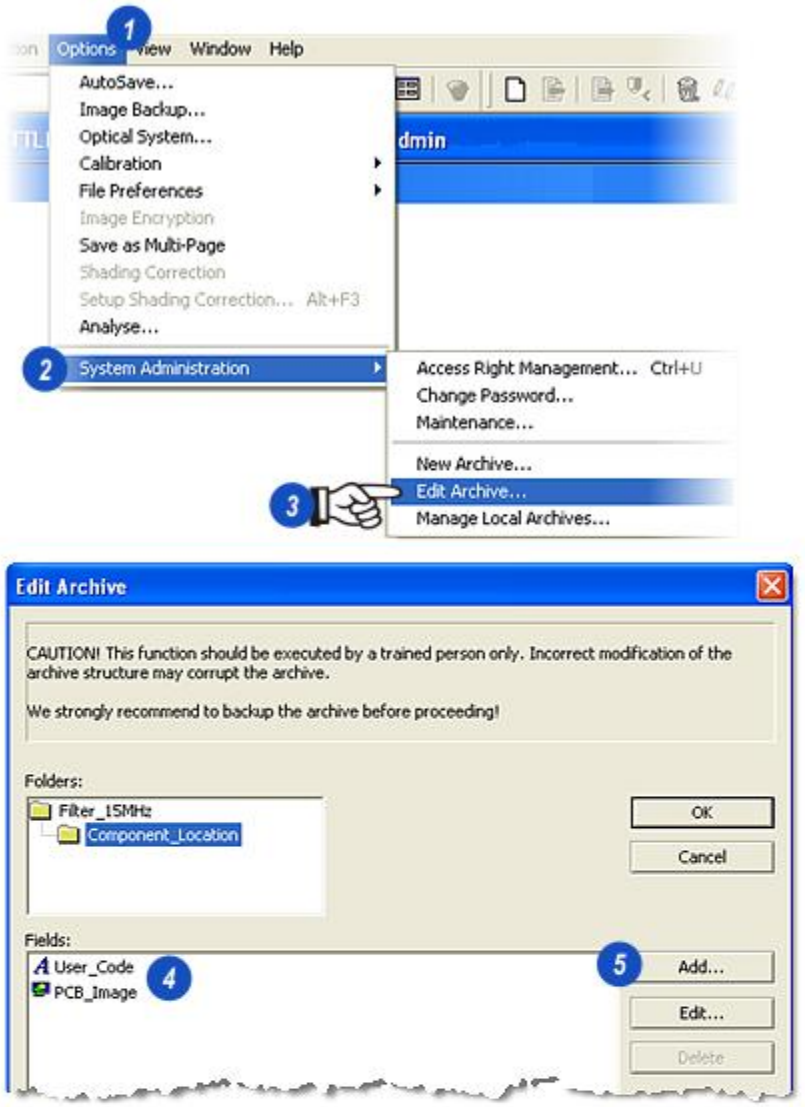
1: Click *Options* on the main menu.

2: From the drop down list click to select *System Administration* and…

3: …click to sleect *Edit Archive.*

4: The *Edit Archive* dialog appears with the current *Image Manager* archive displayed including the Leica IM fields: Shown on the illustration are the minimum that would be allowed – one *Text* field as the key (Indicator) and one *Image* field.

5: Click on the *Add* button.



On the Field Properties dialog:

1: Click on the small arrow to the right of the *Type* header.

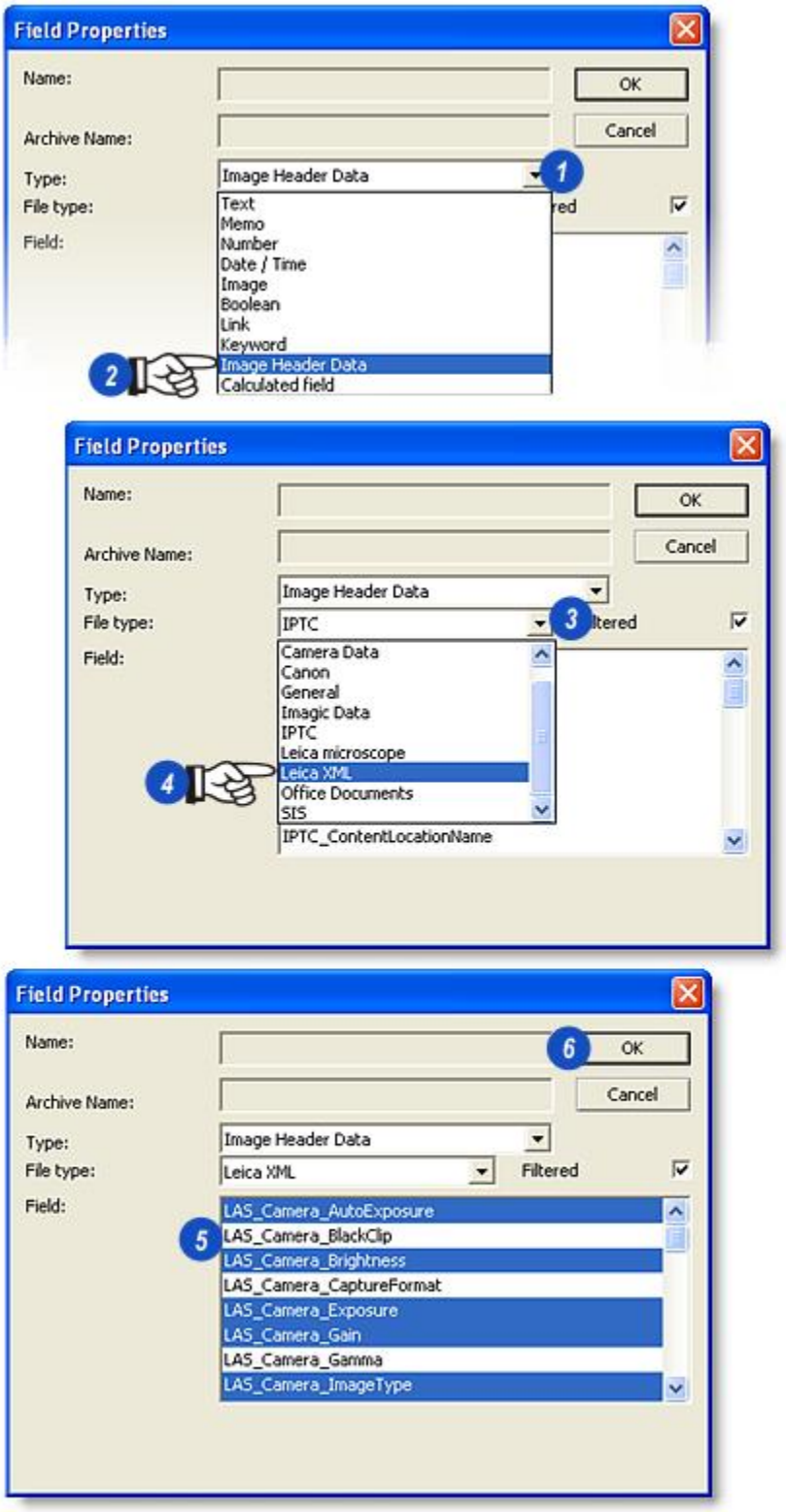
2: From the drop down list click to select *Image Header Data.*

3: Click on the arrow to the right of the *File Type* and from the drop down…

4: …click to select *Leica XML.*

5: A list of all the available LAS data fields now appears in the *Field* window. Not all will be appropriate to the imported images so click to select only those required. The selected data will be imported into Image Manager but will be packaged in a separate file that is associated with the image. The fields are not available to Leica Image Manager but will be retrieved and displayed by LAS along with image.

6: Click *OK*.



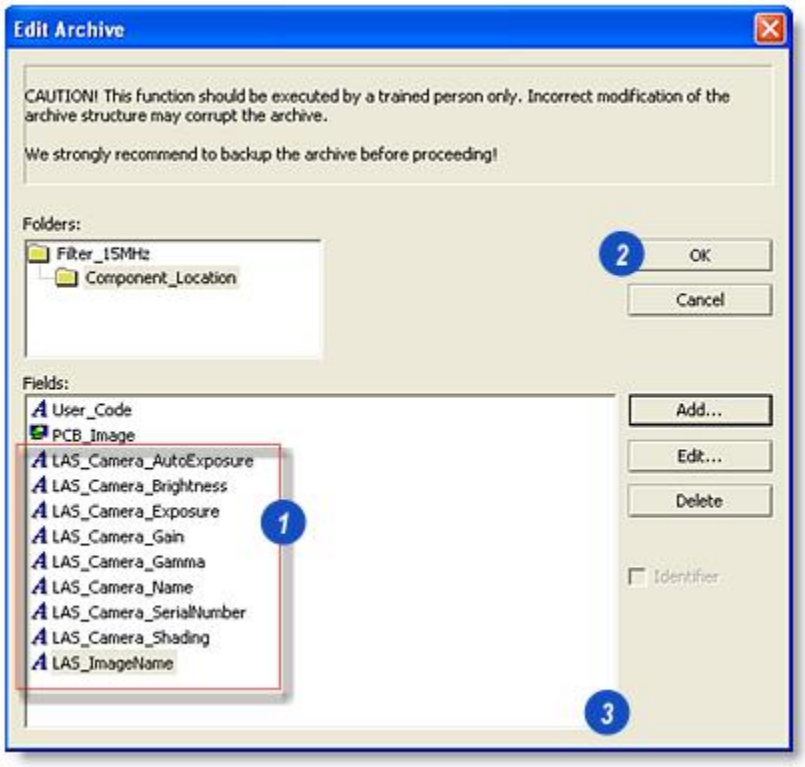
Select the LAS Fields: Finish

Returning to the *Edit Archive* dialog:

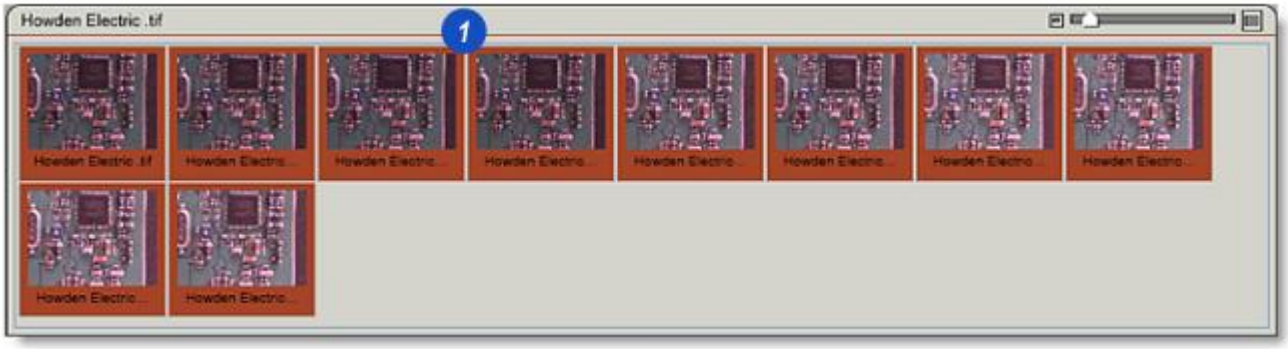
1: The selected LAS fields have been added to the list.

2: Click *OK* to complete the Leica Image Manager setup.

3: Double-click the desk-top icon to start *Leica Application Suite.*



Select the Images to Export



In Leica Application Suite, select the images to be exported to Leica Image Manager – it can be a single image or an entire range.

1: Click to select a thumbnail in the *Gallery* or if a range of images is required click on the first thumbnail to be included and holding down the keyboard *Shift* key, click on the last thumbnail in the range. All of the images encompassed by the two selections are highlighted.

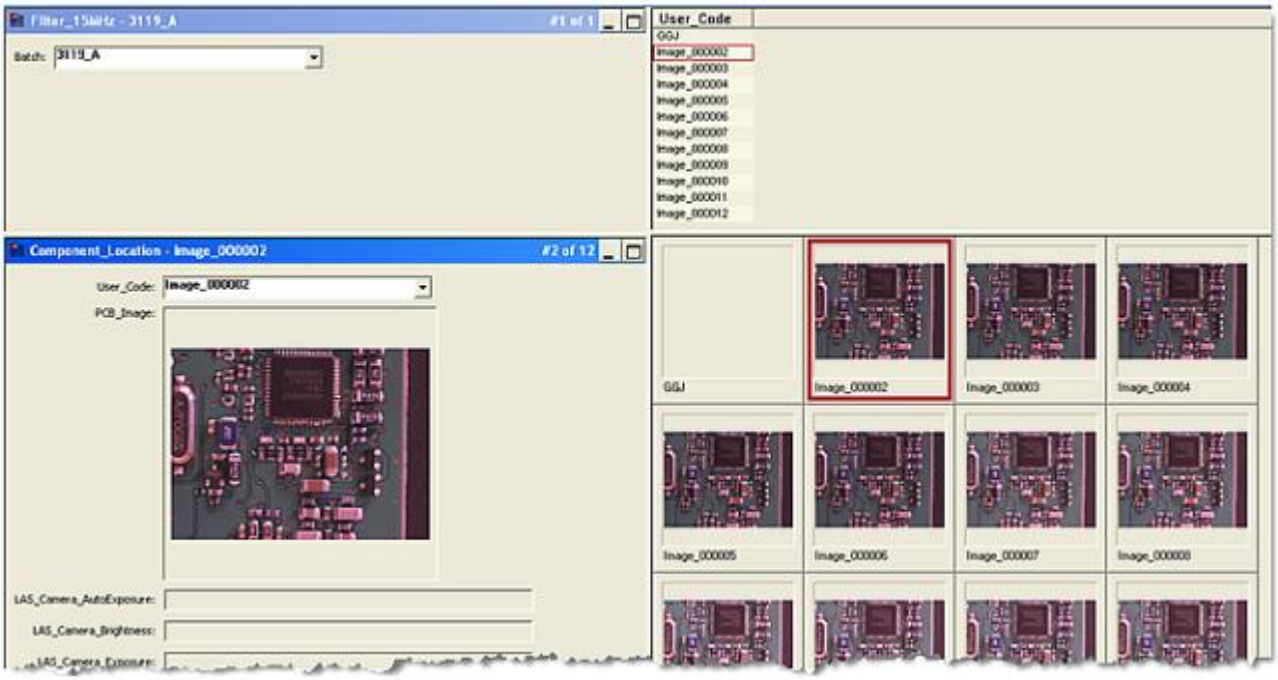
2: Click on the *Export* button.

3: On the *Export Images* dialog, click to select *Image Manager* and if required…

4: …click on the *Don't ask again* check box to skip the *Export Images* dialog in the future.

5: Click *OK* and the export begins.

Images exported from Leica Application Suite in Leica Image Manager.



Existing images, sequences or movie clips stored in *Leica Image Manager 5 (IM V5)* prior to and including *Leica Application Suite 3.3*, can be easily imported into an LAS Archive or Folder created after Version 3.3. The process is slightly different for archives or folders.

Start LAS and then...

Start Image Manager.

Import into an LAS Archive:
Importing is carried out on a field-by-field basis so the target (receiving) archive must have the same structure and field names as the source otherwise data will be lost.

1: Click on the *Setup Workflow.*

2: Select the *Archive* tab.

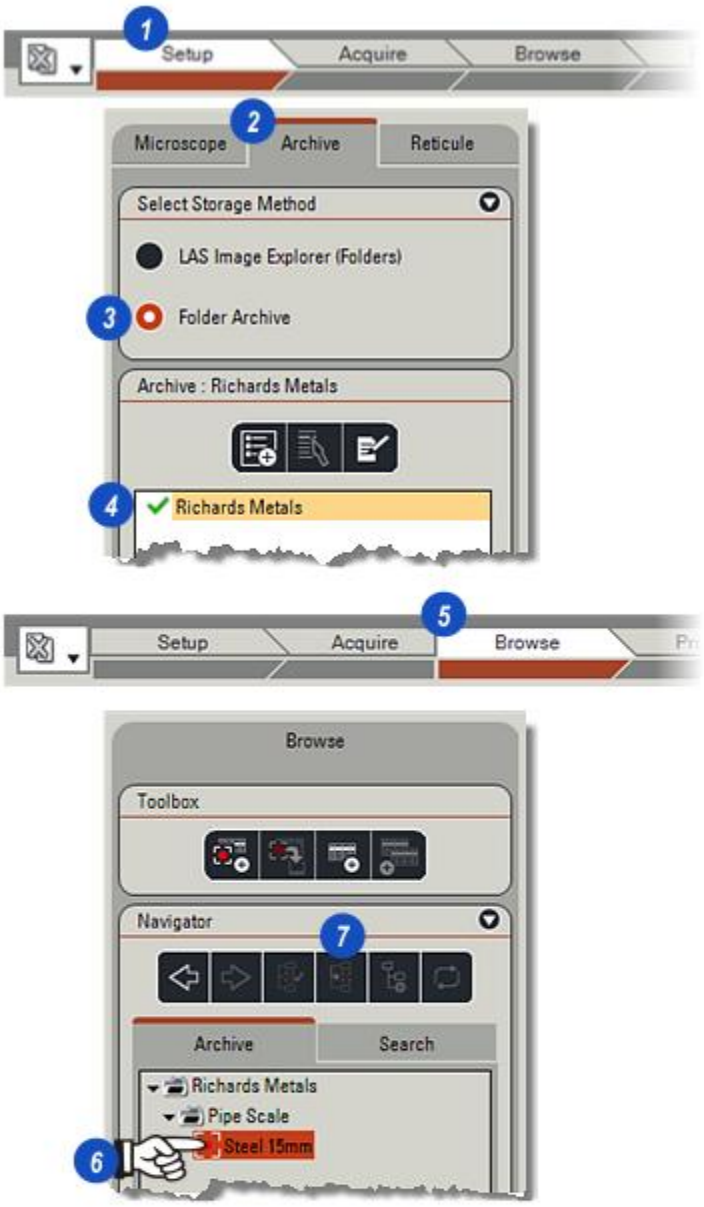
3: Click on the *Folder Archive* button.

4: On the list of available archives click to select the target archive into which the images will be imported.

5: Return to the *Browse Workflow.*

6: Click the target archive and...

7: ...select it as the *Capture Archive*.



Select the Image in Leica IM V5

Go to Image Manager and navigate to the location of the image(s) to be sent to LAS Archive:

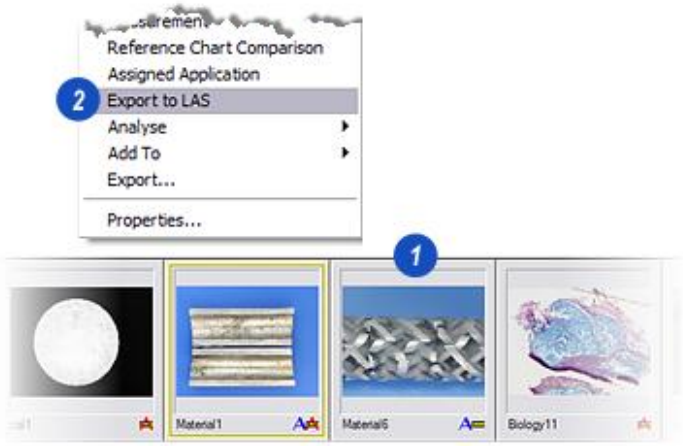
1: Right click on the thumbnail of the image to be sent to LAS in the *Leica IM5* gallery.

2: From the *context menu*, click to select *Export to LAS*.
If this option is not available on the menu see the instructions in the Appendix: Go there... 548

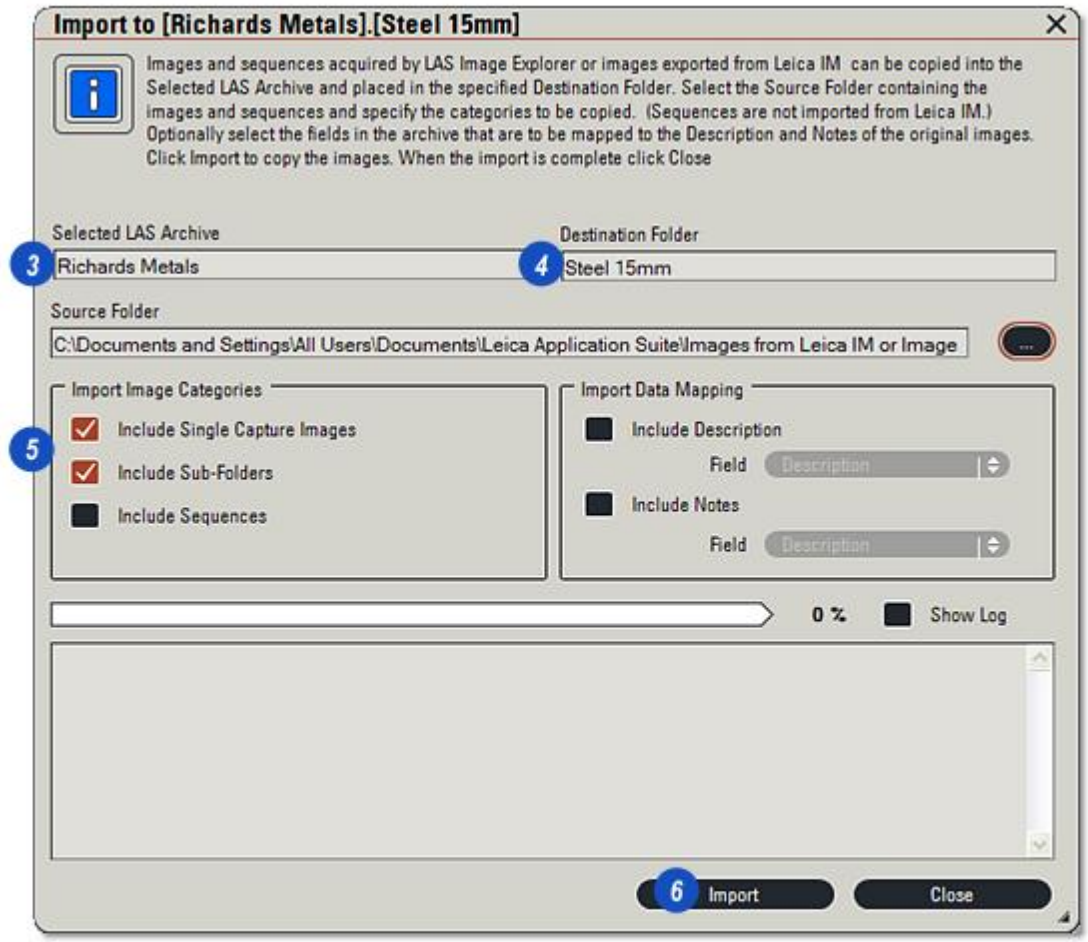
3: The *Import to* dialog appears with the target archive in LAS and...

4: ...the destination folder in the appropriate window.

5: Click to enable both *Include Single Capture Images* and *Include Sub-Folders.*

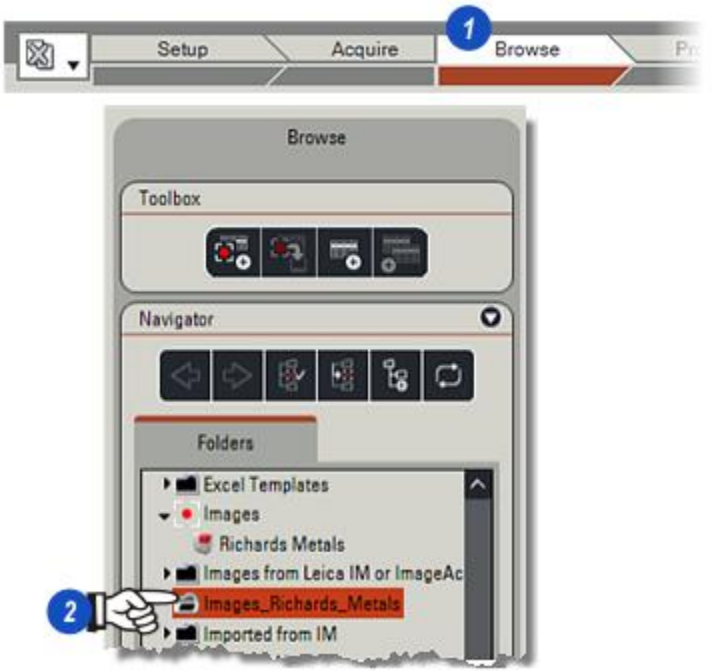


6: Click *OK* and the image will be sent to LAS.



1: Click on the *Browse Workflow*.

2: On the *Folders* tab, navigate to the target folder in LAS into which the images from *Leica IM5* will be imported.

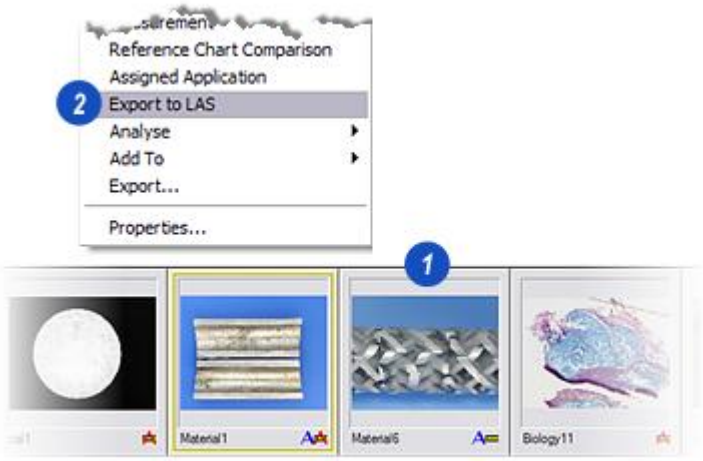


Go to Leica Image Manager and navigate to the location of the image(s) to be sent to LAS Archive:

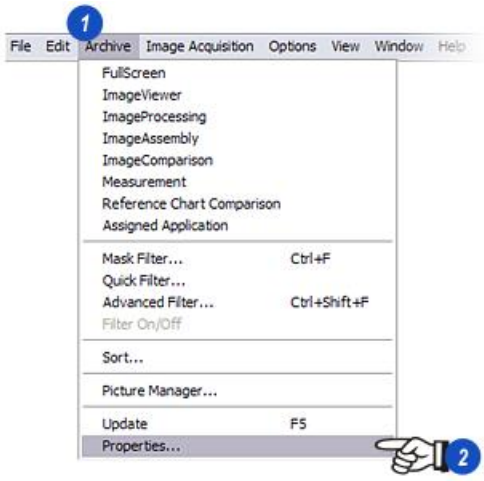
1: Right click on the thumbnail of the image to be sent to LAS in the *Leica IM5* gallery.

2: From the *context menu*, click to select *Export to LAS*.
If this option is not available on the menu see the instructions in the Appendix: Go there... 548

3: The *LAS Import* dialog appears with the target archive in LAS listed. Click *Yes* to confirm the import and the image will be imported.



Right clicking on a thumbnail in the *Image Manager* gallery, reveals a context menu. To be able to export images from *Leica IM5* into *LAS* archives or folders, a small program must be enabled. The program resides in *Leica Application Suite* but is launched from *Leica Image Manager 5* by clicking on an entry in the context menu called *Export to LAS*.



To put the option on the context menu:

1: In *Leica IM5*, click on *Archive* on the main menu and...

2: ..from the drop down menu click the *Properties* option.

3: On the *Archives* dialog, click to enable *Assigned application*.

4: Click in the *Name* text box and type *Export to LAS*.

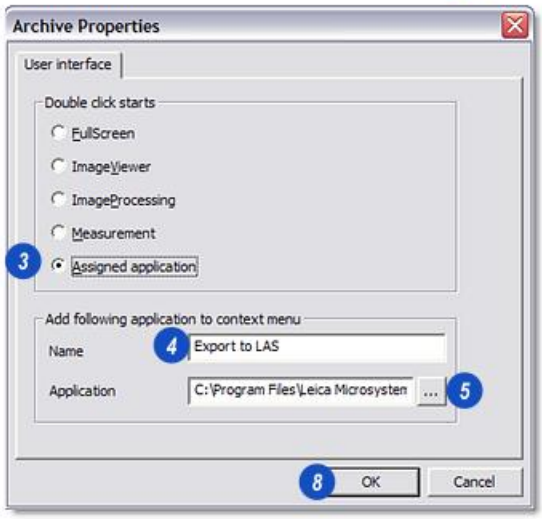
5: Click on the browse button to the right of the *Application* text box and...

6: ...on the *Open* dialog navigate to:
C:\Program Files\Leica Microsystems\Leica Application Suite V3...

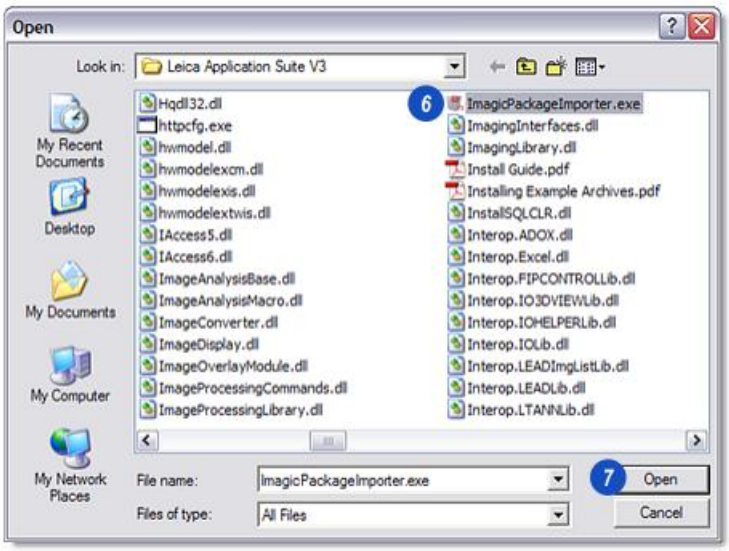
...and click to select *ImagicPackageImporter.exe*

7: Click *Open.*

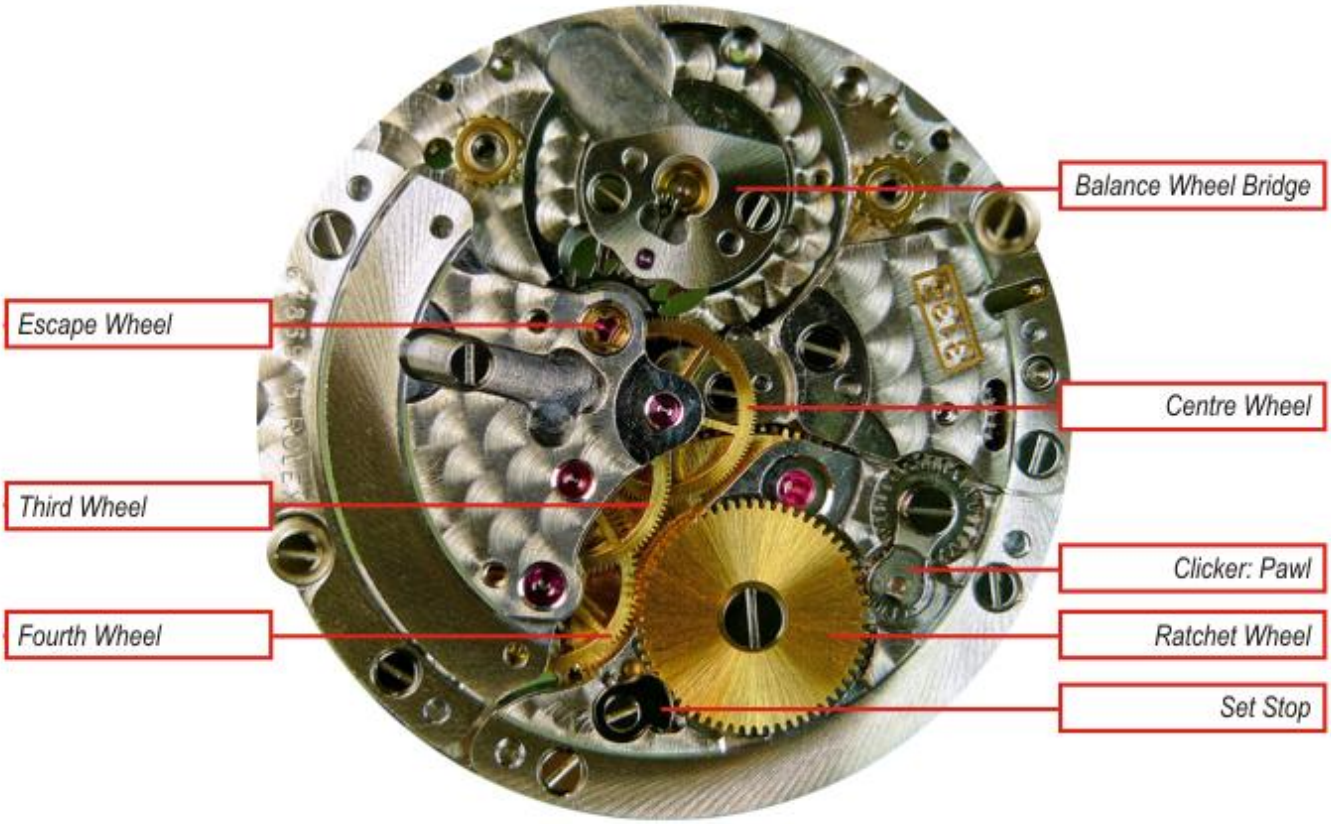
8: Click *OK*



The *Export to LAS* option will now be available on the *Leica IM5* context menu.



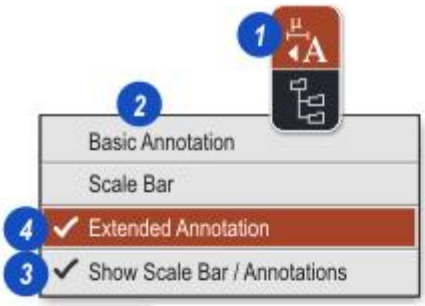
Extended Annotations

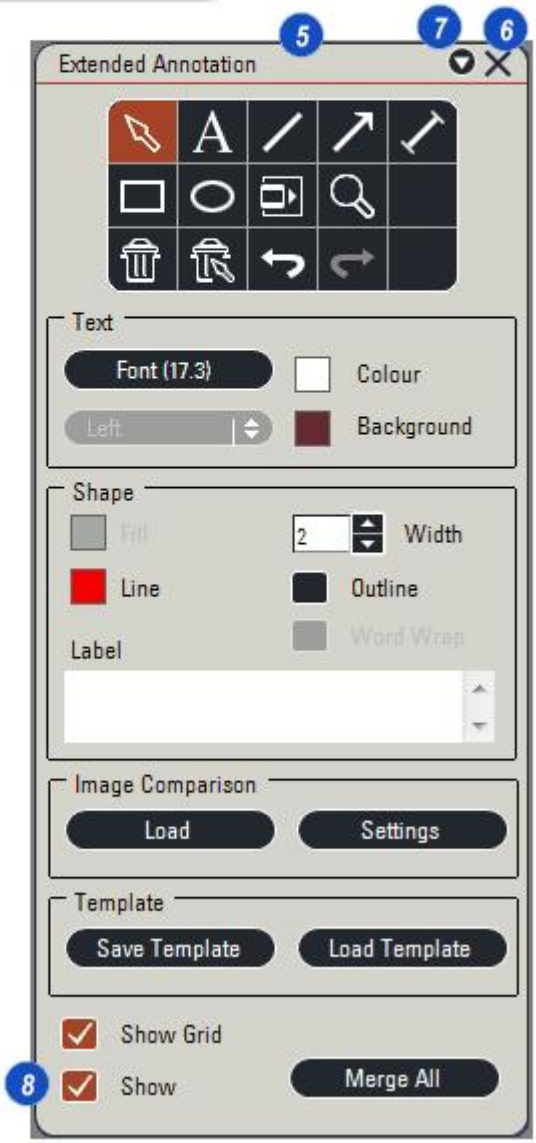


Leica Application Suite *Extended Annotation* gives users an easy-to-use yet powerful collection of tools to add comprehensive detail and information to images.

- Choose[560] from two leader lines - plain and arrow
- Add labels with selectable[556] colours and text to leaders.
- Use the distance line[560] to measure between features and automatically display the distance on a label.
- Draw[564] rectangles, squares, ellipses and circles all with selectable outline widths and colour.
- Shapes fills[563] can be set to almost any colour with transparency set between clear and totally opaque, to allow the underlying image to show through.
- Apply text[554] either directly to the image or automatically as lines and shapes are drawn.
- Use templates[575] as a flexible save-to-disc 'clipboard' with the advantage of applying copies of annotations to images at any time in the future.
- Image comparison[568] allows an image to be laid over either a live or captured image. Change the transparency to compare the images and detect where there are differences.
- Merge[582] the live or captured image with drawn annotations to make a permanent, shareable image.

The optional module *Extended Annotation* is available on *Acquire* - for live images -, *Browse, Process* and *Analysis* workflows for captured images. The control panel normally resides on the *Process Workflow*.





1: Clicking the *Show Annotation Options* button on the *Side Tool Bar...*

2: ...displays the *Annotations and Scale Bar Quick Launch* menu.

3: Click to select the *Show Scale Bar/ Annotations* option and...

4: ...the *Extended Annotation* option. The *Extended Annotation* control panel appears to the right of the image.

5: The *Extended Annotation* control panel can be dragged by the header and 'parked' on any part of the*Viewer*.

6: Close the dialog and return it to the *Process Workflow* by clicking the 'X' on the dialog caption.

7: If the dialog is obscuring the image or controls, collapse it by clicking on the small arrow to the right of the header. Expand it by clicking the arrow again.

8: Activate the *Extended Annotation* tools and features by clicking to enable (Tick mark visible) the *Show* check box

For a detailed explanation of the *Extended Annotation* features click the links below:

1: Drawing and Control Tools[552]

2: Annotation *Label*[559] text size, colour and alignment

3: *Shape*[562] - rectangles and ellipses - outline thickness, colour and fill. The *Label* feature attaches numbers or text to an annotation

4: The *Image*[568] controls allow users to adjust the overlaid image for transparency, scaling and effect

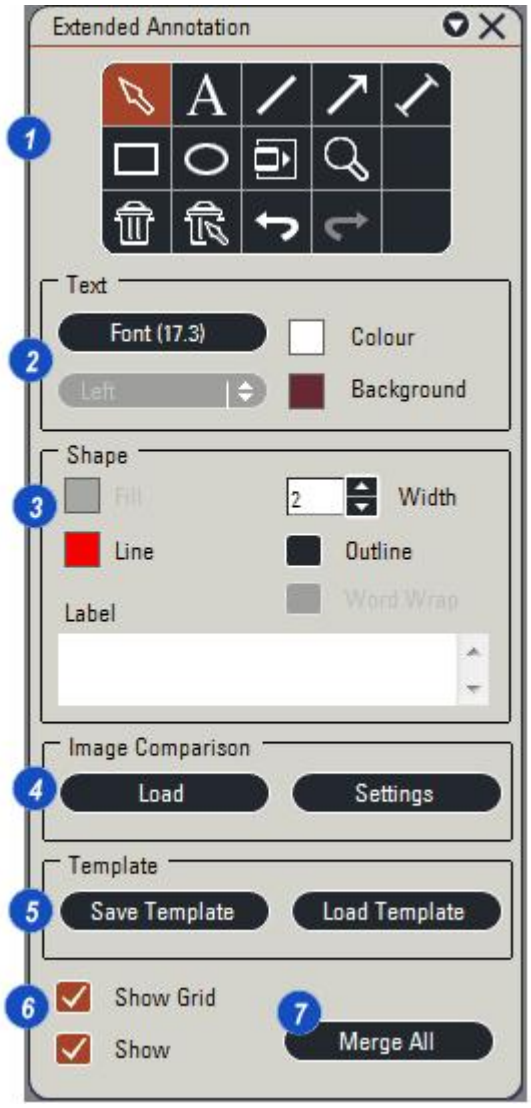
5: *Templates*[575] allow users to save annotations as a separate image that can be laid over another to check the differences between the two. Can be used on both live and captured images

6: When enabled, the *Show Grid*[581] control displays a list of all the annotations. Clicking on an entry immediately selects the annotation on the image

7: Clicking the *Merge All*[582] button incorporates all of the annotations into the image. Once merged, annotations are a part of the image and cannot be changed

Creating *Key Numbers: Template*[578] example

Working with *Image Comparison*[568]



Magnification pane

If you use the Zoom Region[574] tool, the Extended Annotation panel has a new Magnification pane.

Click on the tool button to link to detailed information:

The *Selection* tool:
Does not actually draw but is used to select annotations already made on the image ready to edit or move them.

The *Text* tool:
Type *Label* text including blocks of text.

The *Line* tool:
Draws a simple line between two points.

The *Arrow* tool:
Draws a line between two points with an arrow head at the starting point.

The *Distance Line*:
Draws a line between two points with strokes at both ends and a caption that displays the distance between the strokes in the current measurement units.

The *Rectangle* tool:
Draws rectangle including squares with selected fill and outline.

The *Ellipse* tool:
Draws an ellipse or circle with selected fill and outline.

The *Image* tool:
Enables the Image Comparison feature.

The *Zoom Region* tool:
Draw one or more zoom regions on the image.

Delete All Annotations:
Deletes all current annotations with a user confirm dialog.

Delete Selected Annotation:
Deletes only the selected annotation.

Undo and Redo:
Undo the last action and *Redo* the last action after an *Undo*.

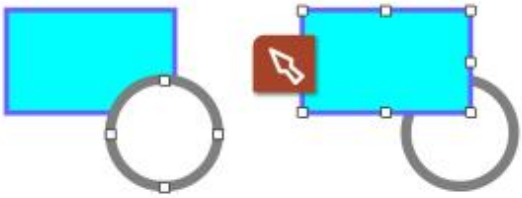
The *Selection* tool does not actually draw but is used to select annotations already made on the image ready to edit or move them.

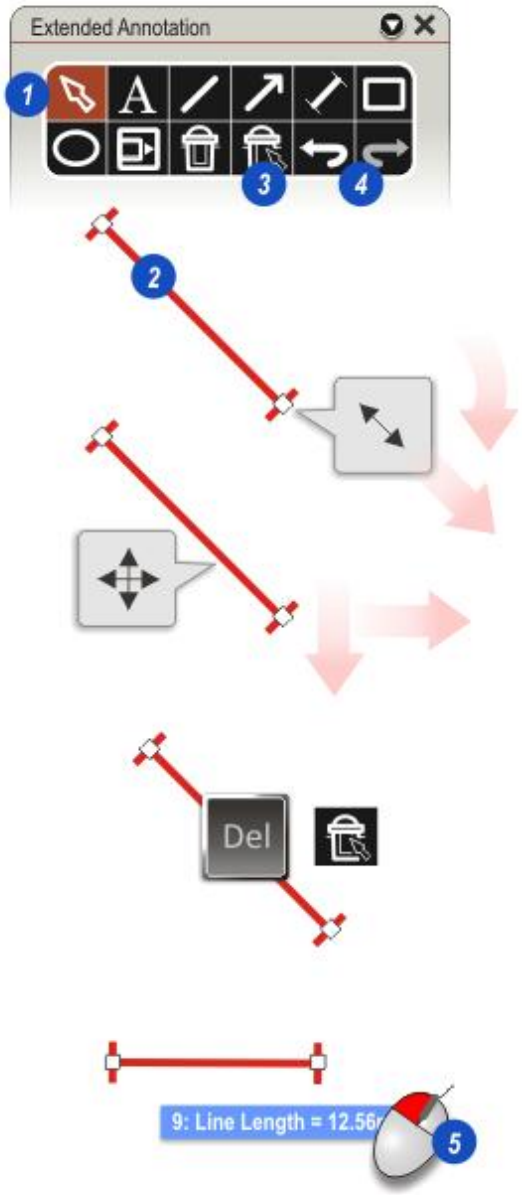
1: Click on the *Selection* tool button...

2: ... and then on the annotation, the end-points of which will appear as small 'boxes' or handles to indicate it is selected. The cursor changes to a double-ended arrow.

- *Adjust an Annotation:* Click and drag on a handle to extend or reduce length or swing the annotation around the opposite handle..
- *Re-position:* Click on the annotation - not on a handle. The cursor changes to a cross. Holding down the mouse key, drag to re-position it.
- *Delete:* Press the keyboard *Delete* key or click on the *Trash Can* (3) to remove a selected annotation.
- *Undo/Redo Actions:* Use the Undo button to restore the last deletion (4).
- *Re-position Label:* Click and drag on a label to re-position it independently of the annotation (5).

As text, lines and shapes are drawn on the image each occupies a separate 'layer' stacked on upon another. The last annotation occupies the topmost layer. Clicking on an annotation moves it to the top layer so if required it can be overlaid on another annotation.





There are two methods of applying text to an image:

- Drawing a background panel directly on the image and typing text into it or...
- Typing into the *Label* [555] text box on the *Extended Annotation* panel and then drawing a background on the image with the text automatically inserted

Drawing directly on the image:

1: Click on the *Text* button. If necessary:

- Change the font settings [556].
- Choose the text position [557].
- Select a font colour [557].
- Set the background colour/transparency [558].

2: Click on the image and drag a rectangle to create the *Label* background. Release the mouse button. The size and position are not critical and can be adjusted later.

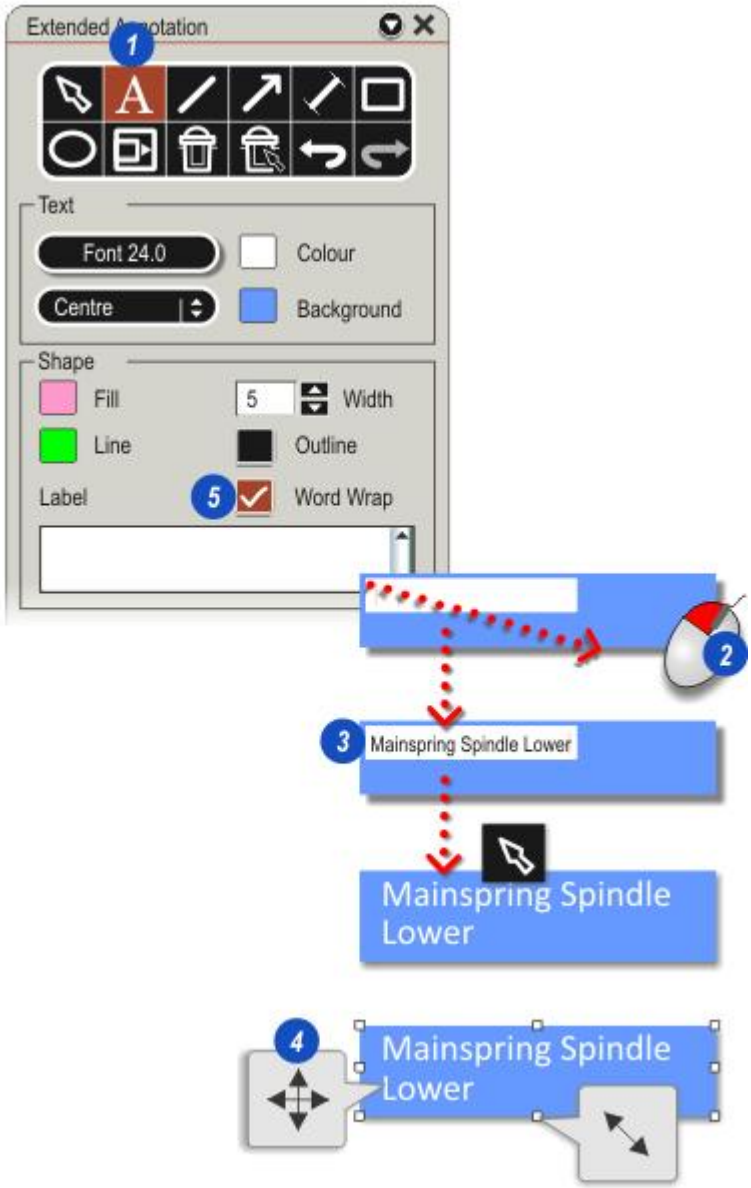
3: As the background is drawn a white text box appears top left. Click inside the text box and type the caption text.

When finished click on the *Selection* tool - do not use a carriage return *(Enter)* to end. The text is displayed in the selected colour, font and size.

4: Adjust the size of the *Label* by clicking it with the *Selection* tool and then clicking and dragging the appropriate 'handle'.

Move the label by clicking inside it with the *Selection* tool - the cursor changes to 4-arrow cross - and dragging to the required position.

5: Click to enable (Tick mark visible) the *Word Wrap* check box to ensure that the text fits the *Label* otherwise it will be displayed as a continuous line.



Type text directly into the *Label* text box so that when a label is drawn the text is automatically placed and displayed.

Typing into the *Label* text box::

1: Click on the *Text* button. If necessary:

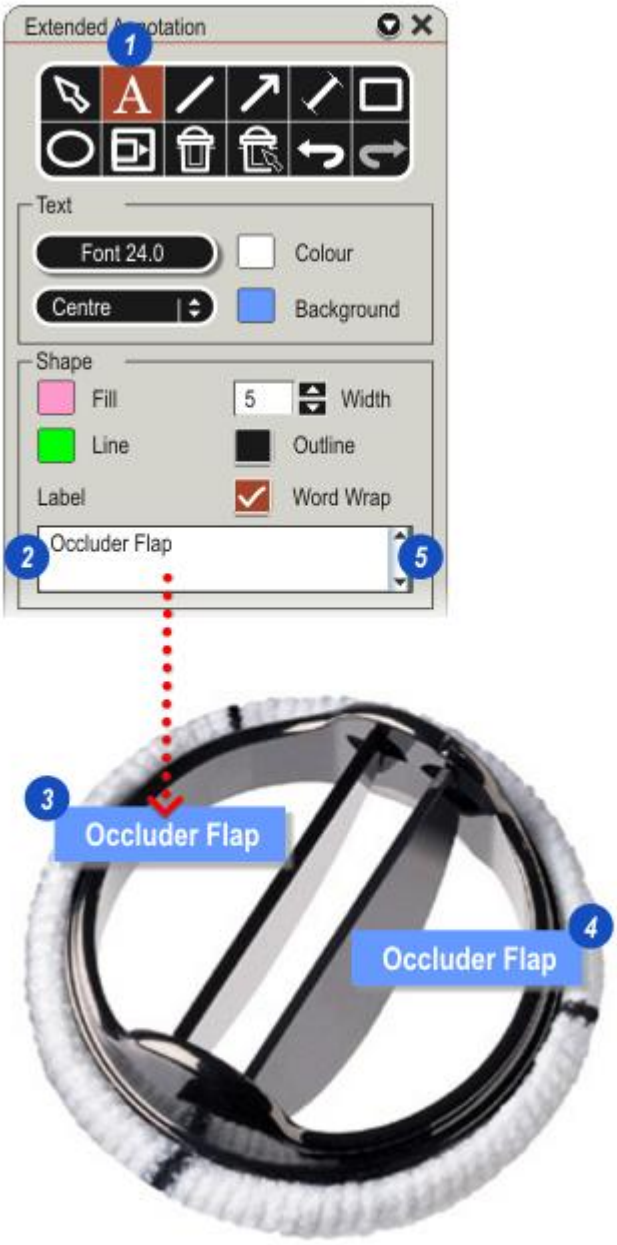
- Change the font settings.
- Choose the text position.
- Select a font colour.
- Set the background colour/transparency.

2: Click inside the *Label* text box and type the text.

3: Click on the image and, holding down the mouse button drag down and to the right to create label. As it is drawn the text appears over it.

4: If settings are not changed the text remains intact and further labels can be drawn displaying the same text.

5: Blocks of text can be typed into the Label text box and scrolled using the scroll bar to the right. Check to enable the *Word Wrap* check box to keep the text within the perimeter of the label.



1: Change the font properties by clicking on the *Text* button. This will enable the *Text* property controls.

2: Click the *Font* button and...

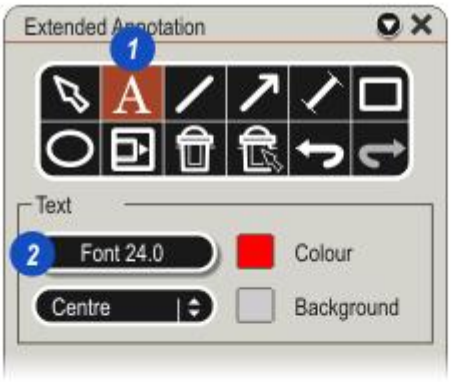
3: ...on the *Font* dialog, use the side scroll bars to locate the required font and click to select it.

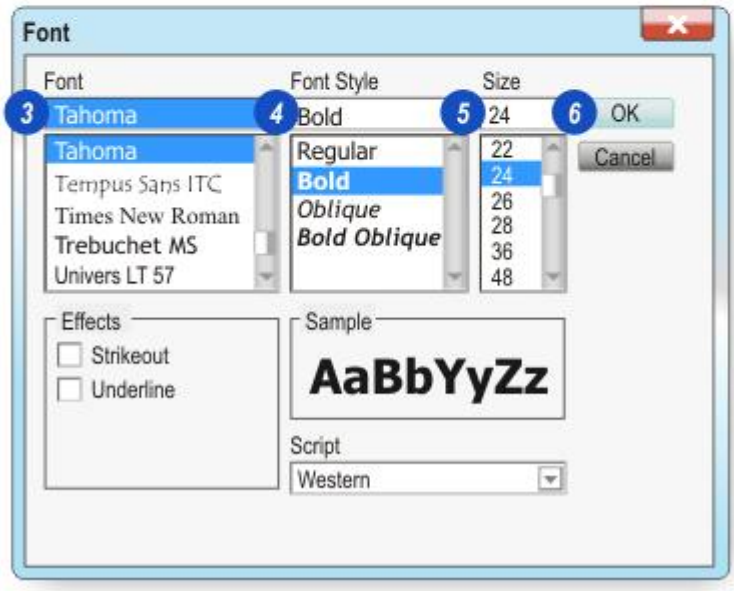
4: Click to select the *Font Style* – bold, italic etc and also...

5: …the *Size* in points.

6: Click *OK*.

The *Font Effects* - *Strikeout* and *Underline* have no effect.





Text can be positioned relative to a background panel either as *Left, Centred* or *Right*:

1: Click on the *Text* tool.

2: Click on the small arrows to the right of the text position button and...

3: ...from the drop-down list click to select the required position type.

Both the *Font* and *Background* colours are selected in the same way:

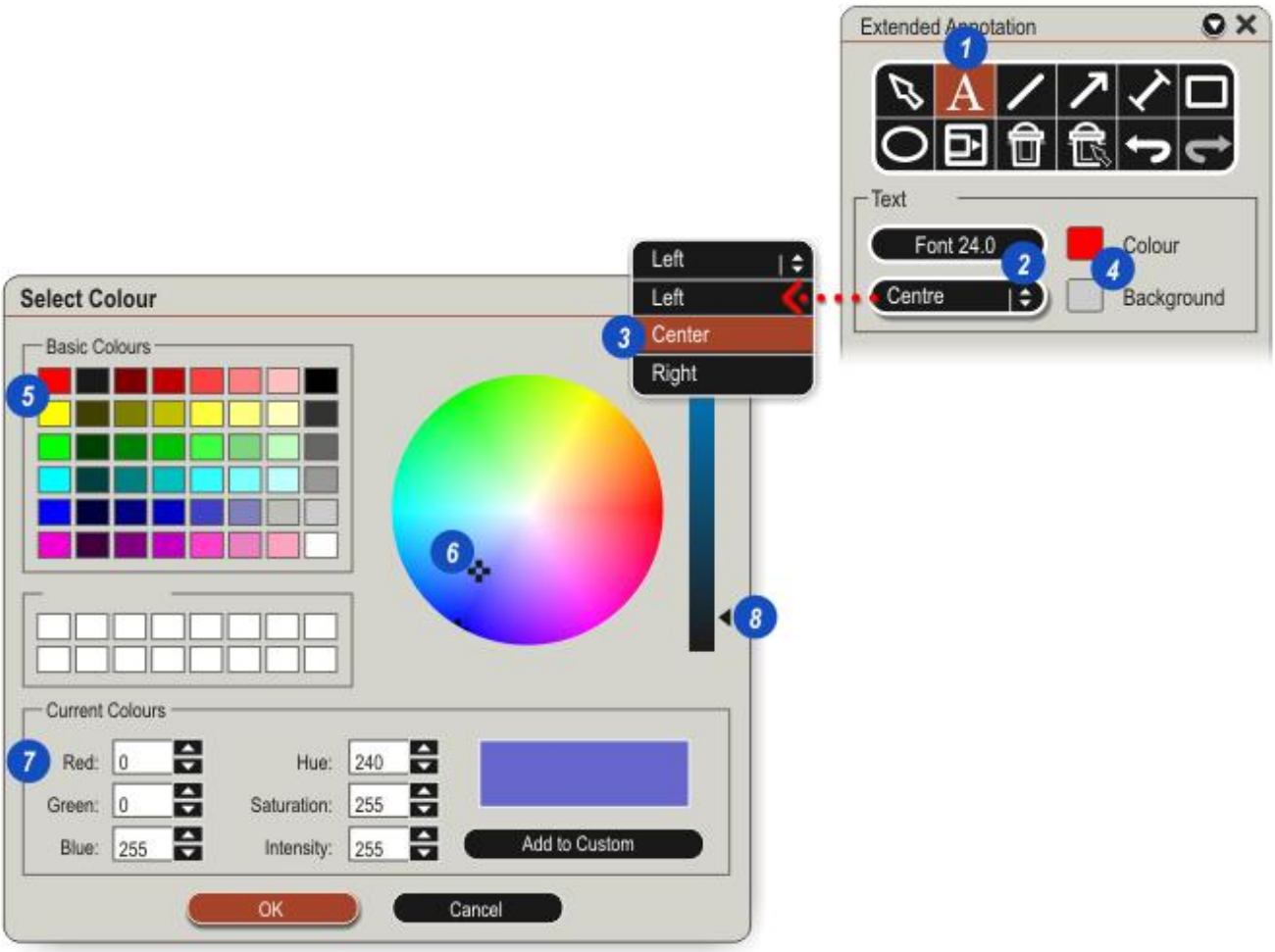
4: Change the *Font* colour by clicking the *Colour* button or the background colour by clicking the *Background* button.

5: On the *Select Colour* dialog select a new colour by clicking on a swatch, …

6: …clicking and dragging the small 'target' on the *Colour Wheel*, or …

7: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range *0* to *255.*

8: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

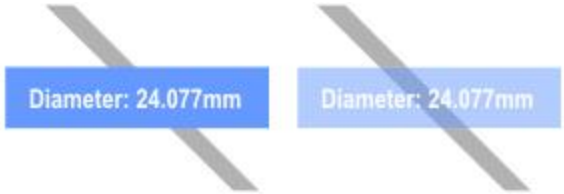


1: Colour transparency for both the font and background can be set by...

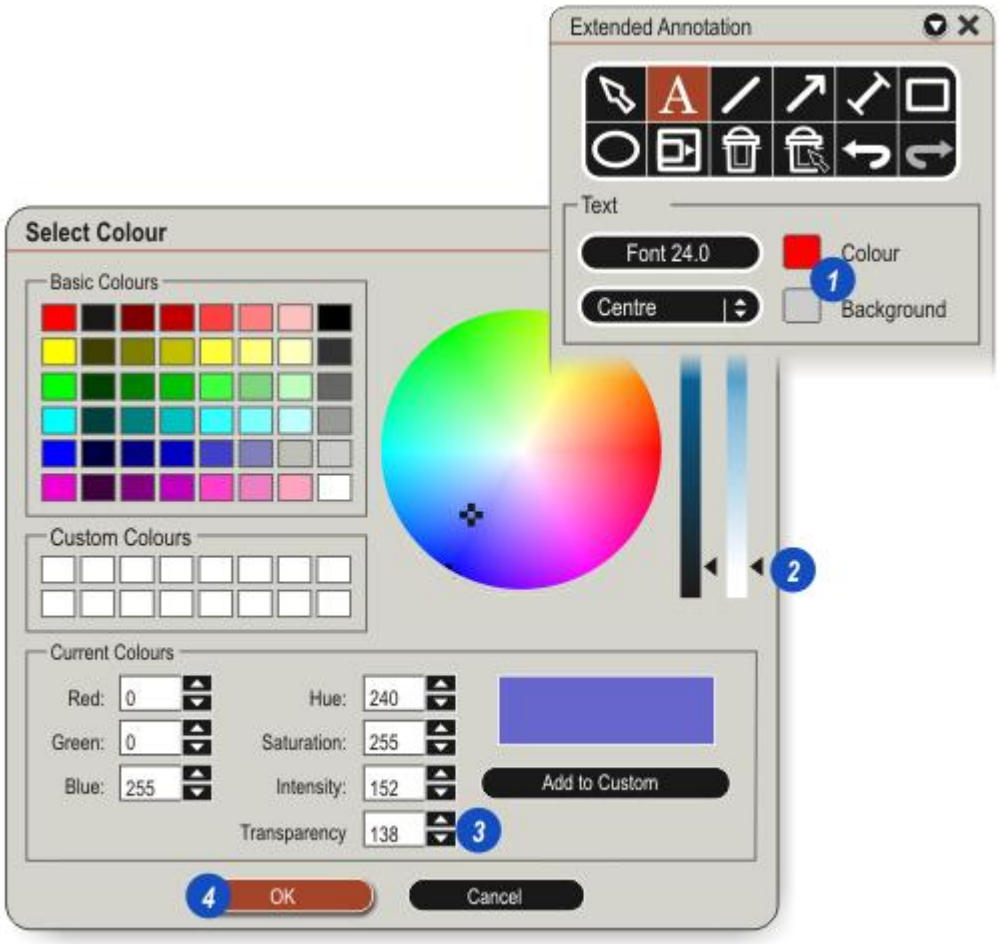
2: ... clicking and dragging the slider on the *Transparency Bar* or…

3: …clicking in the *Transparency* text box and typing a value in the range 255 for a solid colour to 0 for complete transparency.

4: Click *OK.*



The label on the right in the diagram above has a transparency setting of 125 but the font transparency has been set at 255 for a solid colour.



There are three *Line* variants in *Extended Annotation:*

- *Plain Line*.
- *Arrow Line* and
- *Distance Line*.

The *Thickness* and *Colour* of all three type are set in the same way:

1: Click to select the *Line* type.

2: Change the *Line Thickness* by clicking on the Up/ Down (Increase/Decrease) arrows to the right of the *Width* text box or...

...click inside the *Width* text box and type a new value in pixels.

The maximum thickness allowed is 20 pixels.

3: Change the *Line Colour* by clicking on the *Colour* button and on the *Select Colour* dialog choosing a new colour by...

4: …clicking on a standard colour swatch, …

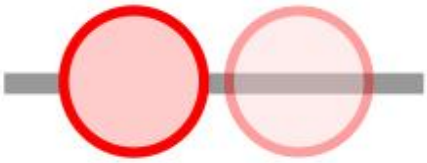
5: …clicking and dragging the small 'target' on the *Colour Wheel* or …

6: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range 0 to 255.

7: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

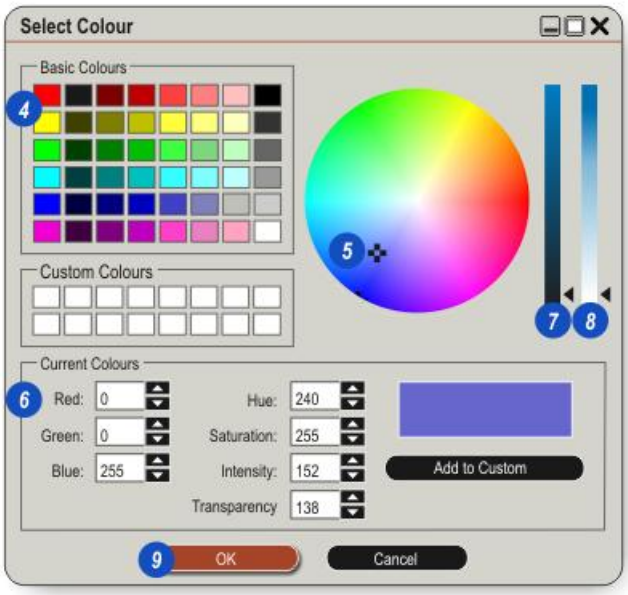
8: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

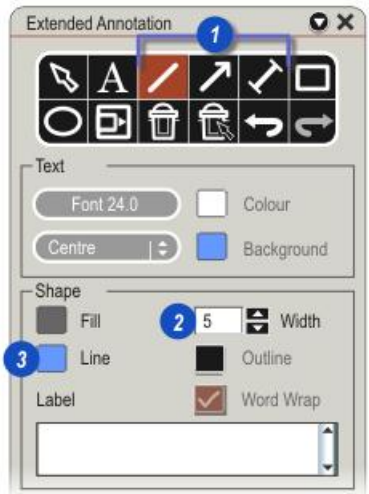
…clicking in the*Transparency* text box and typing a value in the range *255* for a solid colour to *0* for complete transparency.



The circle on the right in the diagram has an outline transparency setting of *100*.

9: Click *OK*.





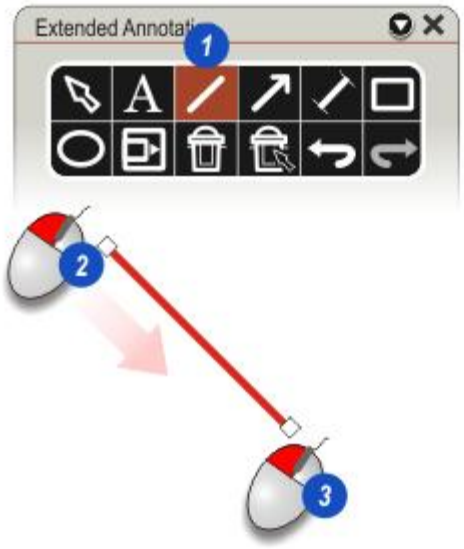
Plain Line:

1: Click on the *Line* button.

2: Click on the image at the starting point and holding down the mouse button...

3: ...drag to the end point and release the button.

Adjust the length, rotation and position of the line using the Selection tool [553]



Arrow Line:

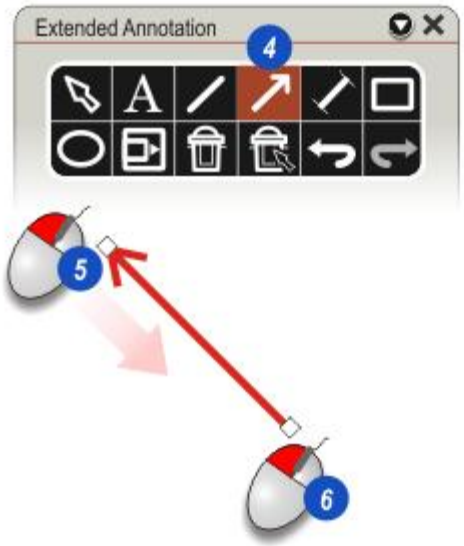
The *Arrow Line* has a single arrow head which is drawn at the starting point:

4: Click on the *Arrow* button.

5: Click on the image at the starting point and holding down the mouse button...

6: ...drag to the end point and release the button.

Adjust the length, rotation and position of the line using the Selection tool [553]



Distance Line:

This is a measuring tool which displays the distance between two points:

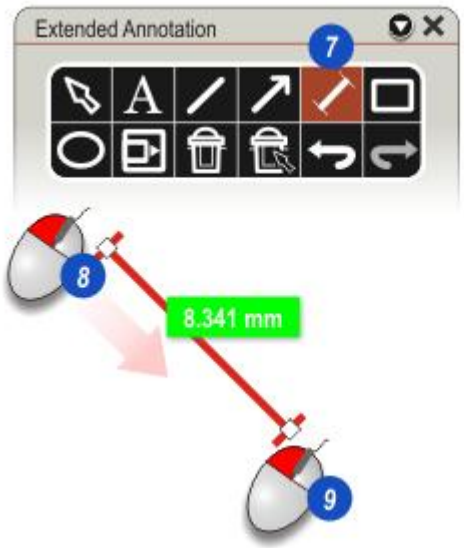
7: Click on the *Distance* button.

8: Click on the image at the starting point and holding down the mouse button...

9: ...drag to the end point and release the button. A label displaying the distance between the two pints is displayed automatically.

The measurement units - millimetres, microns etc - are those of the current calibration for live images or for captured image, those active when the image was captured. To change the measurement units see *Calibration* [318] for live images or *Update Calibration* [85] for captured images.

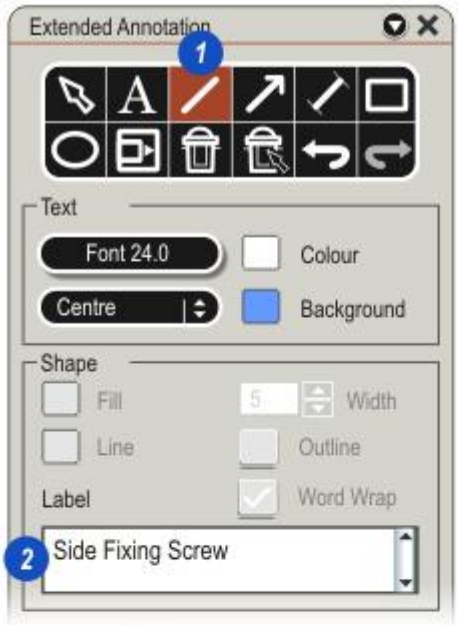
Adjust the length, rotation and position of the line using the Selection tool [553]



A *Line* or *Arrow* can have a label attached and drawn simultaneously. All that is required is some text in the *Label* text box. The *Distance Line* already has a label.

To display a label automatically:

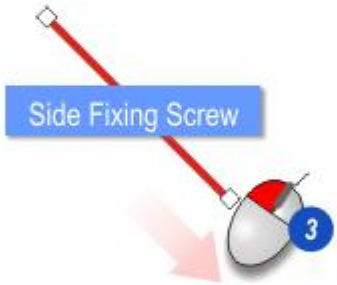
- Select the font, style and size.
- Choose the alignment
- Set the font Colour and Background Colour



1: Click to select either the *Line* or *Arrow* tool.

2: Click inside the *Label* text box - it will accept text even though the *Text* tool is not selected - and type the label text.

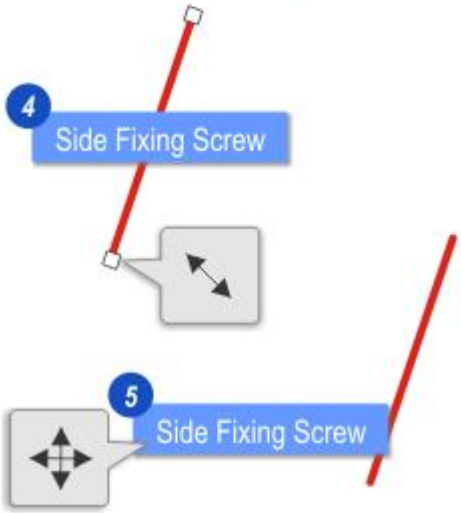
3: Draw the line and the label is automatically drawn with it.



Providing the drawing has been selected with the *Selection* tool:

- Any of the line attributes can be altered.
- The font and background settings can changed.
- The label text can be changed simply by changing the text in the *Label* text box.

If the label has been drawn automatically it is attached to the line so that when the line is re-positioned the label moves also (4). However, the label can be moved independently by clicking on it (not the line) and, holding down the mouse button, dragging it to a new location (5).



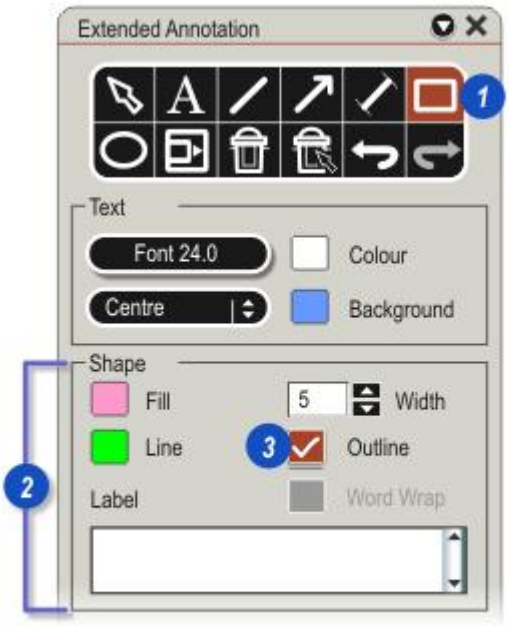
Extended Annotation has two shape tools:

- *Rectangle* and.
- *Ellipse*.

Squares can be drawn with the *Rectangle* tool and circles with the *Ellipse* tool.

Settings include:

- Show/hide shape *Outline*.
- Outline *Thickness* and *Colour*.
- Fill *Colour* and *Transparency*.

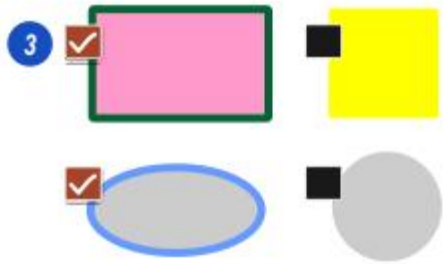


Enable the *Shape* settings by:

1: Click on the *Rectangle* or *Ellipse* tool.

2: This enables the *Shape* settings panel.

3: Show or hide the shape outline by clicking to enable (Tick mark showing)/disable the *Outline* check box.



The *Outline Thickness* and *Fill Colour* of both shapes are set in the same way:

1: Click to select the *Shape* type.

2: Change the *Line Thickness* by clicking on the Up/ Down (Increase/Decrease) arrows to the right of the *Width* text box or...

...click inside the *Width* text box and type a new value in pixels.

The maximum thickness allowed is 20 pixels.

3: Click either the Fill or Line colour button and on the *Select Colour* dialog choosing a new colour by...

4: …clicking on a standard colour swatch, …

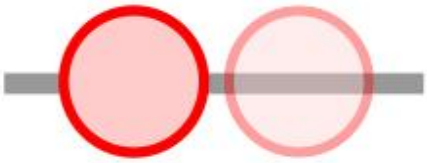
5: …clicking and dragging the small 'target' on the *Colour Wheel* or …

6: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range 0 to 255.

7: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

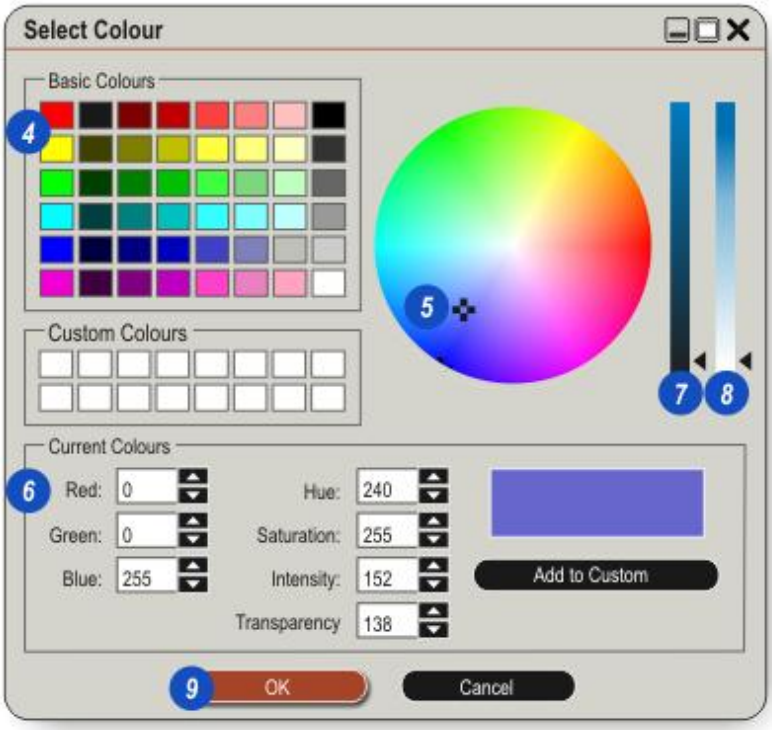
8: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

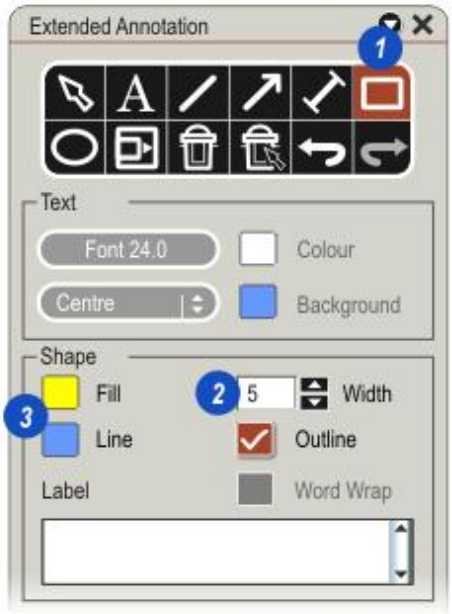
…clicking in the*Transparency* text box and typing a value in the range *255* for a solid colour to *0* for complete transparency.



The circle on the right in the diagram has an outline transparency setting of *100*.

9: Click *OK*.





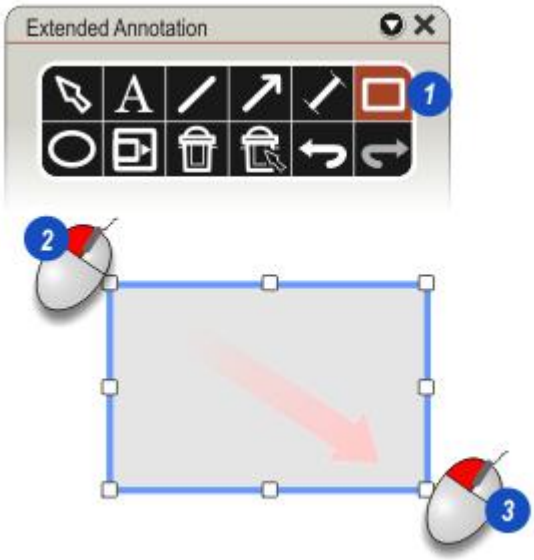
Rectangle:

1: Click on the *Rectangle* button.

2: Click on the image at the starting point and holding down the mouse button...

3: ...drag to the end point and release the button.

Adjust the shape dimensions and position using the Selection tool[553]



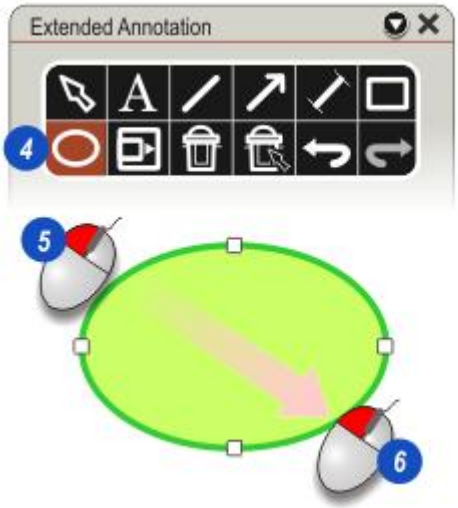
Ellipse:

1: Click on the *Ellipse* button.

2: Click on the image at the starting point and holding down the mouse button...

3: ...drag to the end point and release the button.

Adjust the shape dimensions and position using the *Selection* tool

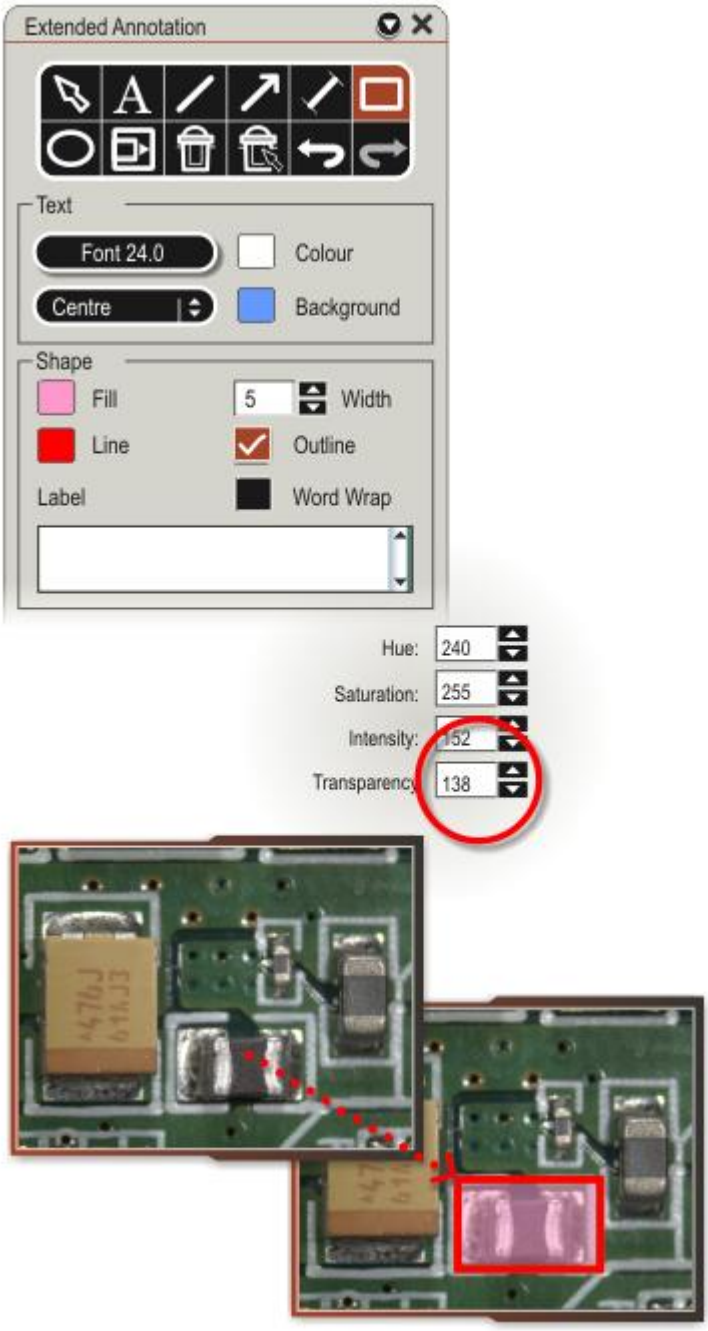


Transparency can be effective when it is used to highlight part of an image.

Add impact by keeping the shape outline solid - transparency = 255 - possible because the colour settings for line and fill are independent in Extended Annotation.

The process is fast and simple:

- Select the shape required - *Rectangle* or *Ellipse*.
- Choose the *Line* colour and set the *Transparency* to 255 (Solid).
  Enable the *Outline* check box.
- Select the *Fill* colour.
- Adjust the *Fill* transparency to allow the underlying detail to show though. It can be adjusted for best effect after the shape is drawn.
- Draw the shape.
- Position it over the detail to be high-lighted.



A *Label* can be drawn simultaneously with a *Shape* by:

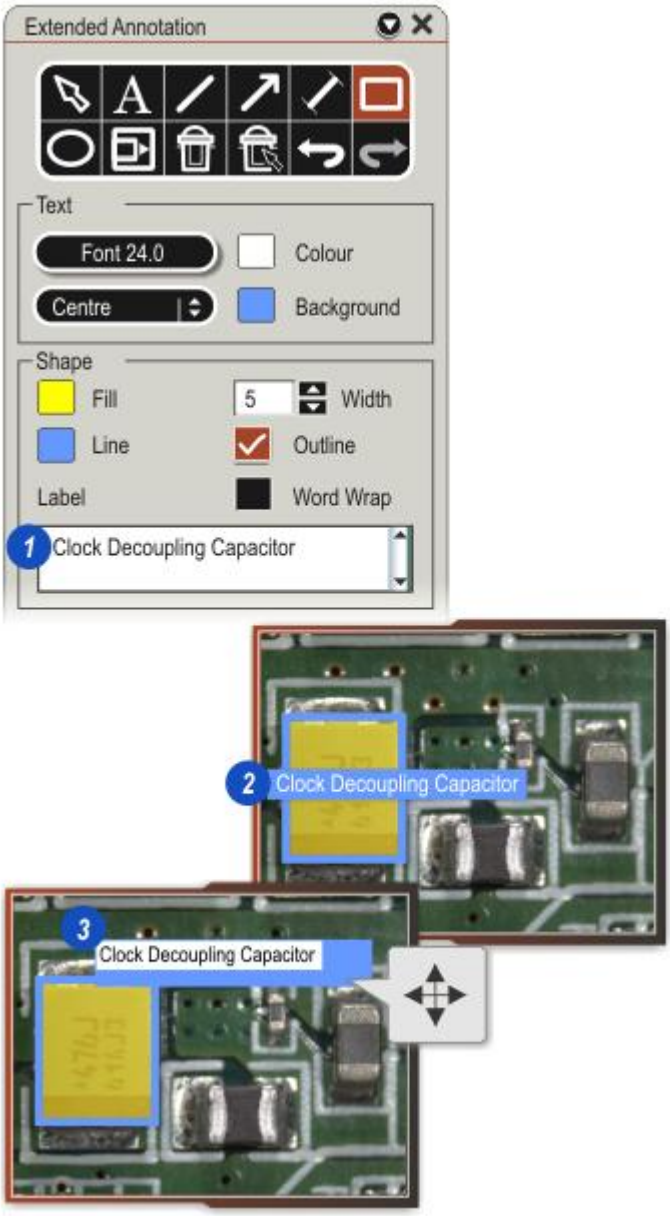
- Select the *Font*, font position on the label, *Colour* and *Background*.
- Select the *Shape* and choose the *Fill* and *Line* colours.

1: Click inside the *Label* text box and type the label text.

2: Draw the shape. The label with its text is drawn at the same time centrally on the shape.

Shape and label are linked so that as the shape is re-positioned the label moves as well.

3: If the label is to be moved independently of the shape, leave the label text box empty and, using the *Text* [554] tool draw directly on the image. The label can then be positioned precisely without affecting the shape.

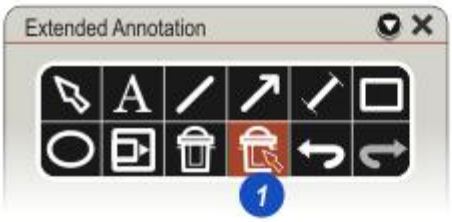


Delete, Undo and Redo

To delete the current selected object - annotation, line or shape:

1: Click the *Delete (Trash Can)* button.

Delete cannot be undone.

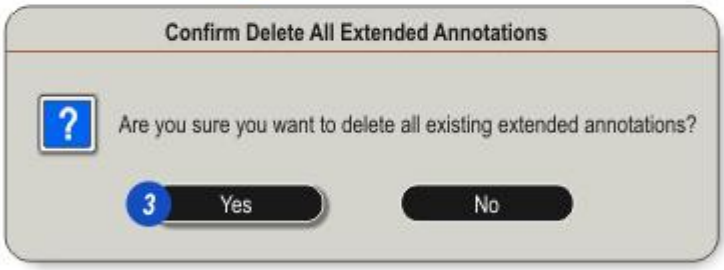


To delete all of the annotations - selected or not:

2: Click the *Delete All*... button and...

3: ...confirm or abort the deletion.

Delete All cannot be undone: Use with care.



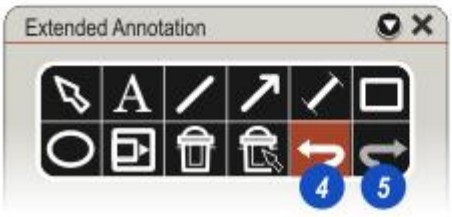
Undo and Redo:

4: *Undo* the last action and...

5: ... *Redo* the last action after an *Undo.*

Individual annotations can be deleted or restored using the *Undo* and *Redo* buttons.

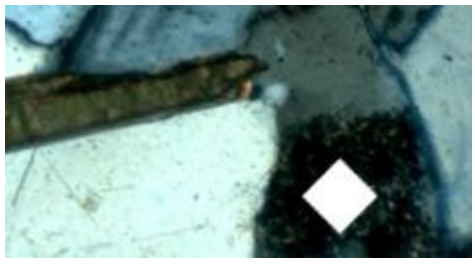
Hover the cursor over the *Undo* or *Redo* button to determine the action that will occur when the button is clicked.



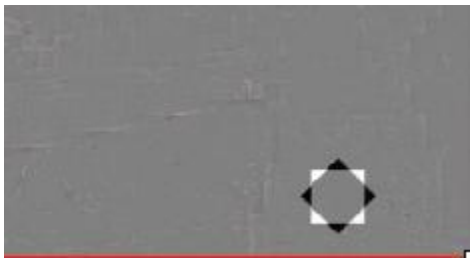
The *Image Comparison* feature in *Extended Annotations* allows you to superimpose one image (the *Layer*) over another (the *Base*).

This allows you to do the following:

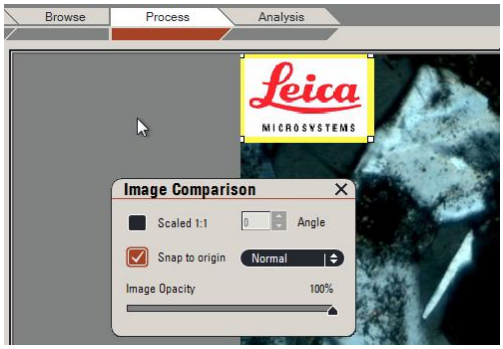
- Compare common features and detect differences, by adjusting the Layer image's position, transparency and effects.
  For example, here are two similar images (with a very obvious difference, for the purposes of illustration):

  Here is the result of superimposing one on the other using the Difference[572] effect:

- Combine two or more images.
  For example, you could add a logo to the Base image:

Note: The Layer image must be previously captured, but the Base can be live or captured.

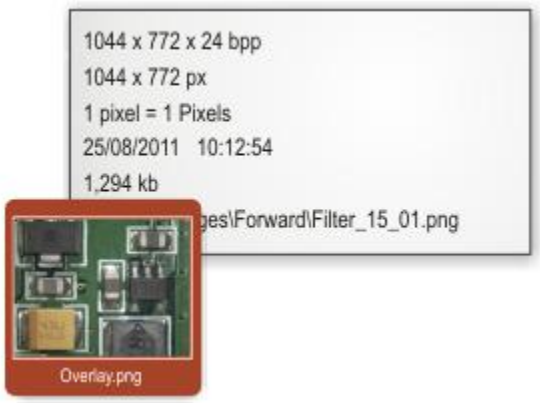
Comparing features and detecting differences

1. Load[570] the Layer image.
2. Scale and position[571] the Layer image so that it aligns exactly with the Base image.
3. Apply Layer Effects[572] and opacity to the Layer image.

See also Combining Images[573].

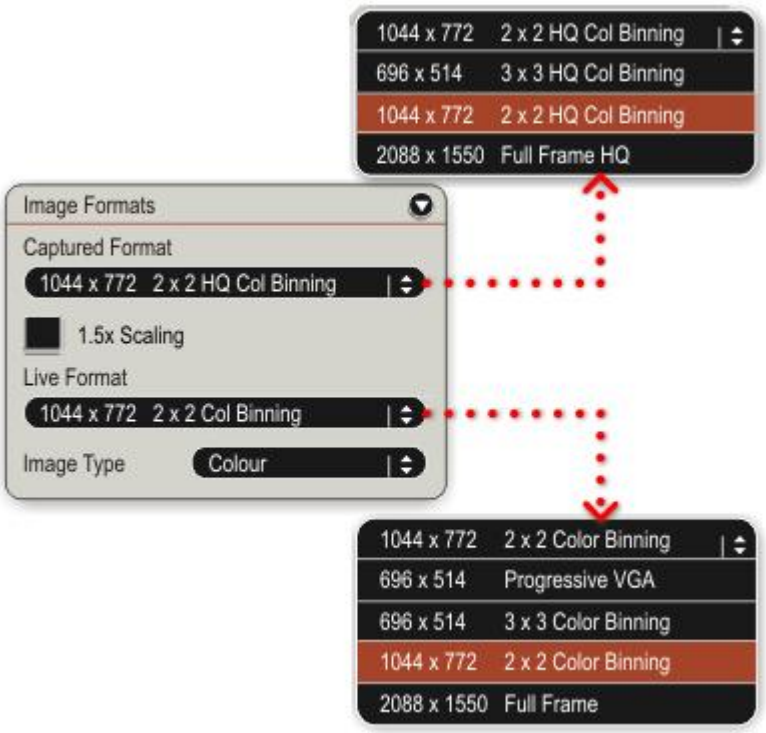
For 1:1 comparison, both Base and Layer images must have the same format (otherwise the image sizes will not match and the Image Annotation Error warning will be displayed).

Check the Layer image format by hovering the mouse over the *Gallery* thumbnail. The image properties are displayed with the captured format.



To change image format:

1. Click on the *Acquire* Workflow.
2. Select the *Camera* tab.
3. Expand the *Image Formats* panel.
4. Display the *Live* or *Captured Format* drop-down menu.
5. Select the format that matches the overlay image.



To select and place a Layer image:

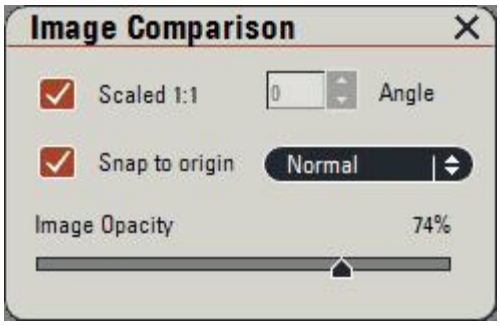
1. Click the *Image* tool.

2. Click the *Settings* button in the *Image Comparison* pane of the *Extended Annotations* panel.

   This displays the *Image Comparison* settings dialog.

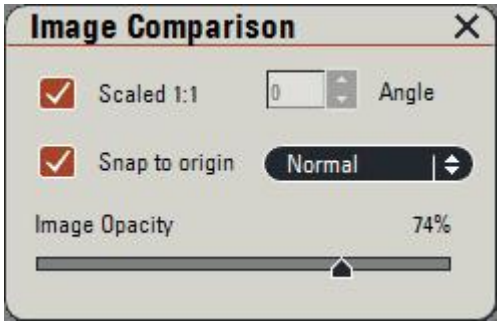
3. Specify how you want the Layer image to appear.
   See Scale and Position the Layer[571] and Layer Effects[572].
   You can also apply a border[563] (but not a fill) to the Layer image.
4. Do one of the following:
   - Click the *Load* button; the Layer image will be centred in the *Image Viewer*, unless *Snap to origin* is enabled
   - Click in the *Image Viewer* where you want the top left-hand corner of the Layer image to be positioned (this is ignored if *Snap to origin* is enabled)
   - Drag to draw a frame to hold the Layer image; the Layer image will be scaled to fit (while retaining its aspect ratio), unless *Scaled 1:1* is enabled
5. In the resulting dialog, navigate to your chosen Layer image and click *Open* to place the image.

Notes

- If *Snap to origin* is enabled, the top left-hand corners of the Layer and Base images will coincide (regardless of how or where you place the image)
- If *Scaled 1:1* is enabled, the Layer image will always be placed at actual size.

For a 1:1 comparison:

1. With the Layer selected, click the *Settings* button in the *Image Comparison* pane to display the *Image Comparison* dialog.

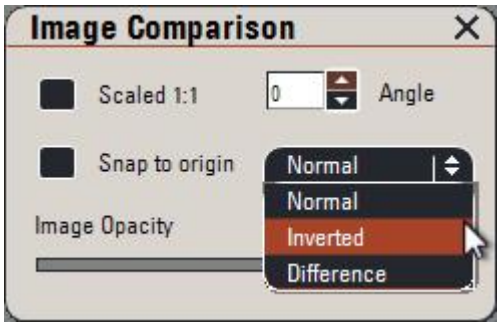


2. Enable the *Scaled 1:1* check box.
   The Layer image expands to its actual size.
3. Disable the *Snap to origin* check box; this will allow you to align the images manually.
4. With *Snap to origin* disabled, you can also adjust the *Angle* of the Layer so that it aligns exactly with the Base.
5. To assist positioning, select *Normal* from the drop-down effects menu and adjust the *Image Opacity* slider to reveal the Base image through the Layer overlay.

Notes:

- If the Base and Layer images are different sizes, disable the *Scaled 1:1* and *Snap to origin* check boxes.
- If *Snap to origin* is disabled, you can also adjust the *Angle* of the Layer in the *Image Comparison* dialog so that it aligns exactly with the Base.

Use the drop-down menu in the *Image Comparison* dialog to apply Layer effects.



- *Normal*: The Layer image is superimposed on the Base image, with no colour changes. Use the *Image Opacity* slider to reveal the Base image, to help with positioning.
- *Invert*: Layer image colours are inverted. Used in conjunction with *Image Opacity*, alignment is easier, and differences between the two images become more obvious.
- *Difference*: Differences in the Base and Layer images are shown as hard, dark edges or white highlights.

Note: You cannot change the *Image Opacity* in *Difference* mode. The slider is greyed out, and opacity is set automatically (regardless of the value displayed on the slider).

You can combine images, for example to add logos, QA stamps or employee images to an original (Base) image. The Layer image can have a smaller (but not larger) format than the Base image.

1. Click the *Image* button.
2. If the Layer image is too large, disable the *Scaled 1:1* check box so that the Layer will fill only the drawn frame.
3. Select the *Normal* effect from the drop-down menu.
4. Draw the frame, select and place the Layer image.

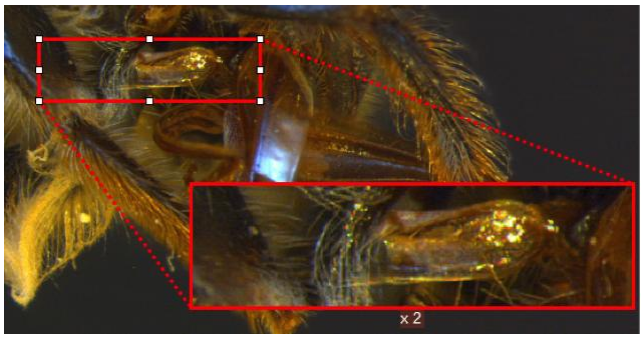
If a Layer is superimposed on a live image, you can combine them permanently using the Merge All[582] feature in either the *Browse*, *Process* or *Analysis* Workflow.

The Zoom Region tool allows you to draw one or more regions of Interest (ROI) on an image, and displays a Zoom Box for each ROI.

1. Click on the *Zoom Region* tool.
   The *Extended Annotation* panel changes to display a *Magnification* pane.

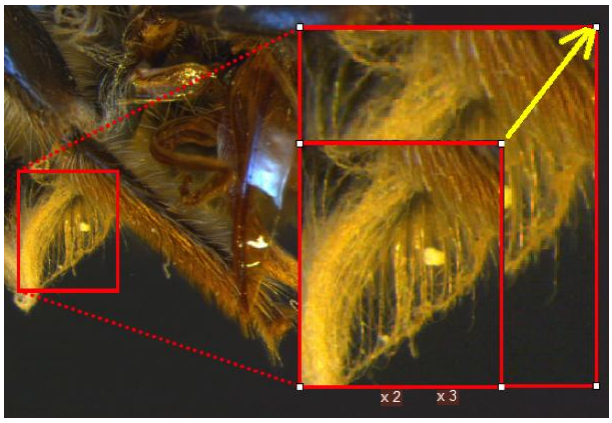
2. Set the required zoom level.
3. Click to enable the *Show Label* box. This will display a magnification label below each *Zoom Box* on the image.
4. Drag to draw an *ROI* on the image.
   When you release the mouse button, the *ROI* and its associated *Zoom Box* will be displayed on the image.
5. If necessary, drag the *Zoom Box* so that is is not directly over the *ROI*.

Moving and resizing the ROI



- Drag the *ROI* to zoom in on a different part of the image. The *Zoom Box* stays in the same location, but always displays the contents of the *ROI*.
- Dragging the handles on the *ROI* change the size and shape of the *ROI* but the zoom factor will remain the same.

Moving and resizing the Zoom Box



- You can move the *Zoom Box* freely to any location on the image. It will still display the contents of its associated *ROI*.
- Drag the handles on the *Zoom Box* to change its size (and hence the zoom factor). For example, the diagram on the right shows the zoom factor changing from 2 to 3.
- With either the *ROI* or the *Zoom Box* selected, you can change the *Magnification* setting in the *Extended Annotation* panel.

Notes

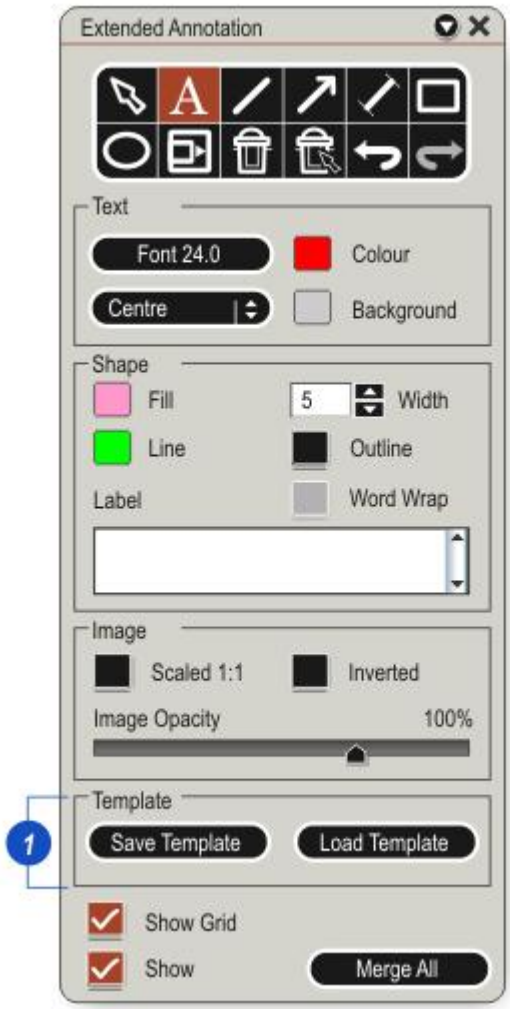
- Enable *Merge All* on the *Extended Annotation* panel to merge all currently displayed ROIs and Zoom Boxes into the image.
- When printing a report, only one *Zoom Box* is included (either the first drawn, or the currently selected).

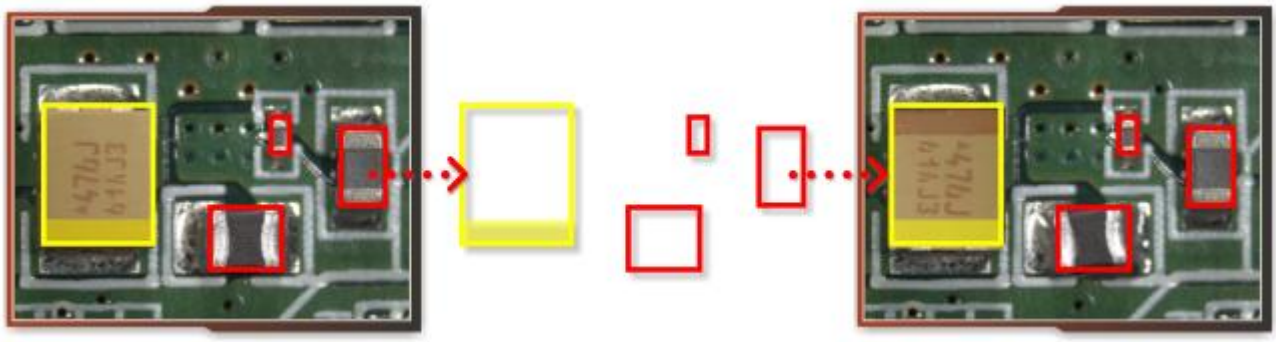
A *Template* is a collection of annotations - lines, shapes, labels and measurements in any combination - that is saved on the computer hard drive. It can be recalled and applied to live or captured images often to check the positioning and presence of components.

1: Two controls are used with *Templates*:

- *Save Template:* Stores the annotations on the computer drive under a user-defined file name and...
- *Load Template:* Allows the user to select a template and apply it to the image.

The illustration below shows a template - four rectangles drawn and positioned on a 'perfect' product - being applied to the live image of a printed circuit. The template clearly shows that the resistor (bottom centre) is mis-aligned and the capacitor (left) which is polarised, is upside down - the polarity marker bar should be at the bottom.





To save a *Template*:

1: Draw the annotations. All lines, shapes, measurements and labels are allowed in any combination.

2: Click the *Save Template* button. All of the annotations will be saved to the template automatically.

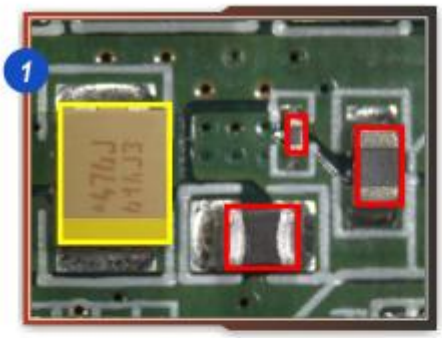
3: Although users can select a folder of their choice, LAS will default to the installed folder at:

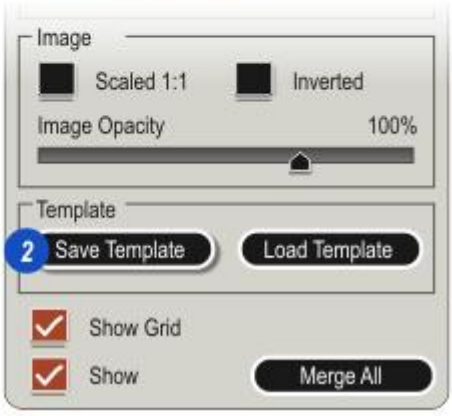
Users/Public/Public Documents/Leica Application Suite/Annotation Templates.

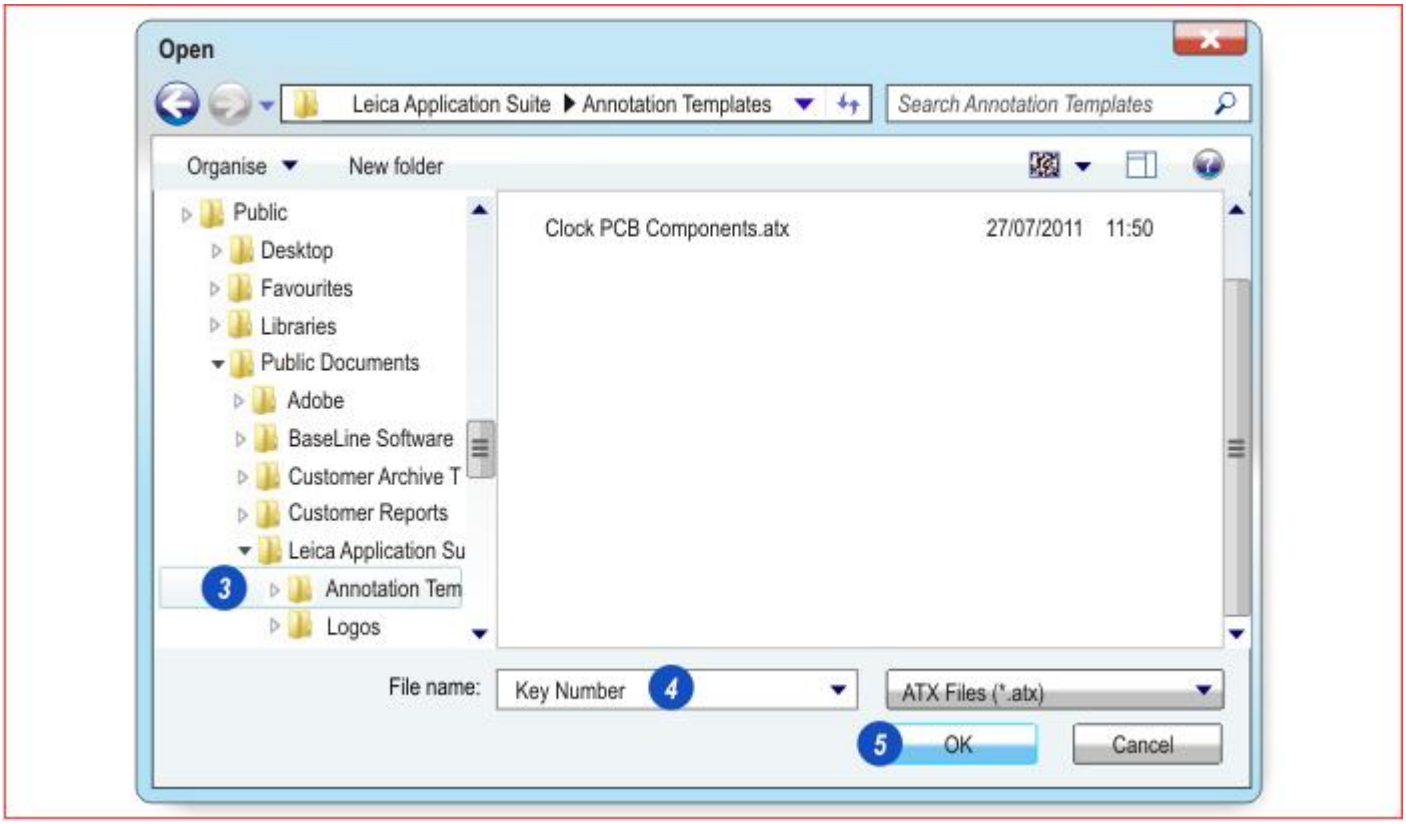
4: Click inside the *File name* text box and type a name for the template. If the name is already in use, users will be prompted to overwrite or cancel.

5: Click *OK*.

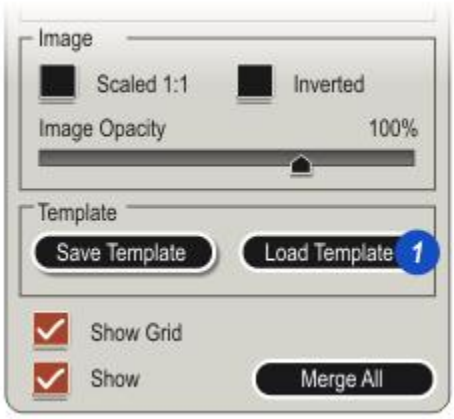
Continued 577







Loading a template retrieves the template from the computer hard drive and places it over the image like and overlay. Templates can be added to both live and captured images:

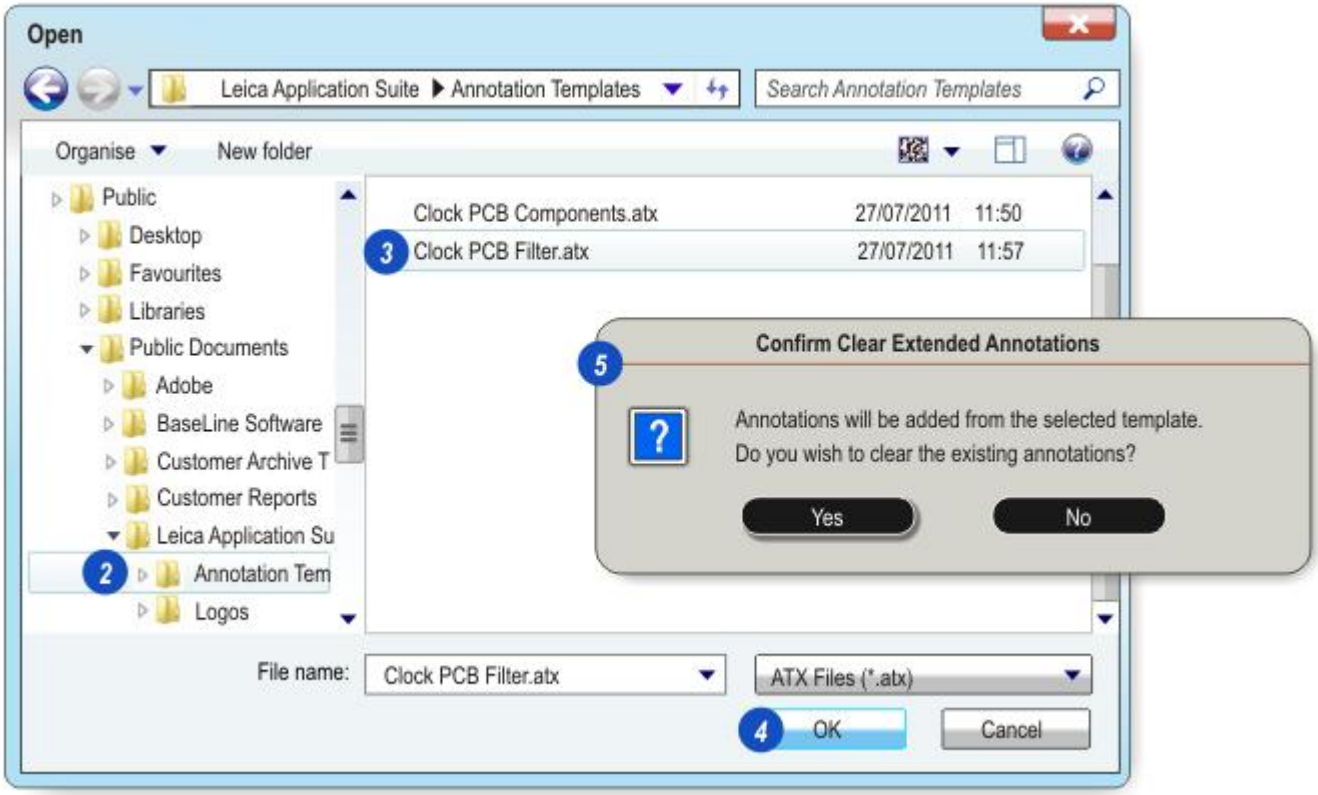


1: Click on the *Load Template* button.

2: Navigate to the template folder and...

3: ...click to select the required template. These are files that have the *atx* extension.

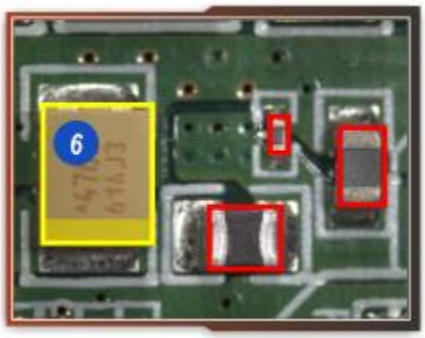
4: Click *OK*.



5: Users now have a choice. On the *Confirm Clear Annotation* dialog:

- *No*: Add the template annotations to those that may already exist on the image, or...
- *Yes:* Delete any existing annotations and add only those on the template.

6: The template 'overlaid' on the image.



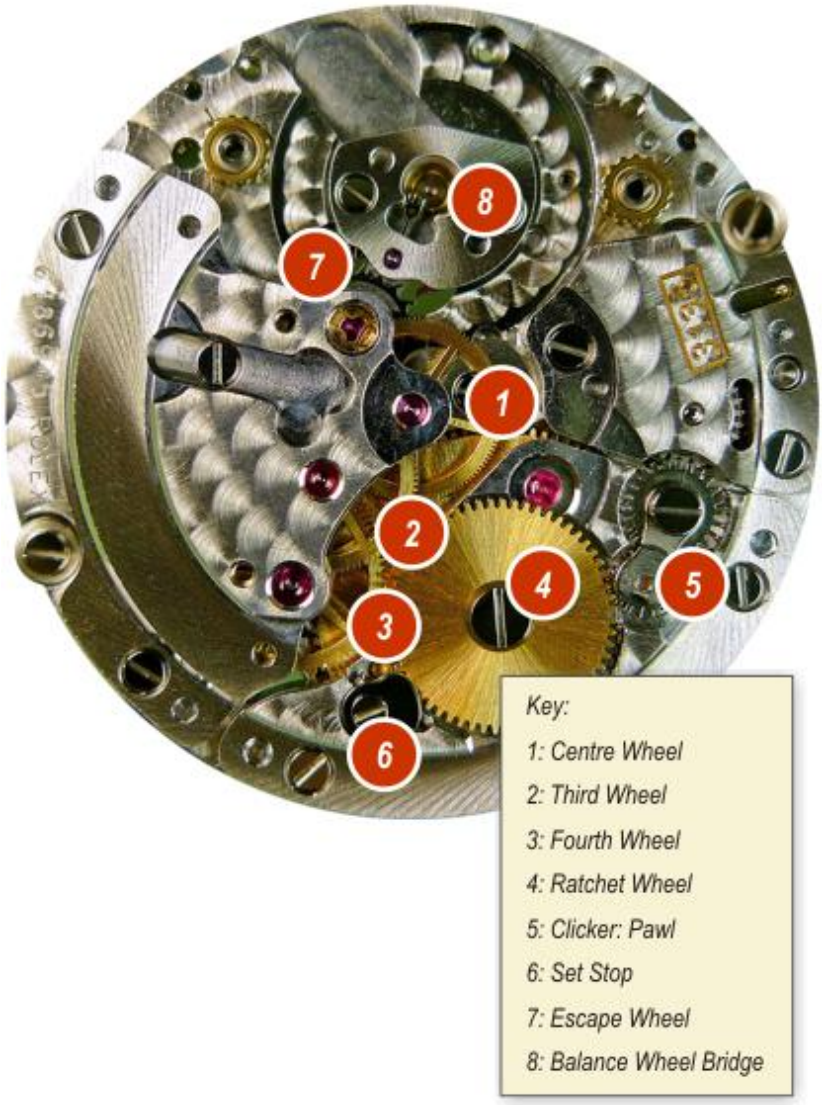
Key Numbers: A Template Example

As well as being a valuable aid to checking the integrity of items on an image, templates can also be used as a scratchpad or clipboard. This is because once saved they can be applied to an image any number of times. They are quick to save and fast to load.

Individual elements of a template can be moved about the image and colours, outlines, size and text changed as needed. This is an example of a template used as a clipboard.

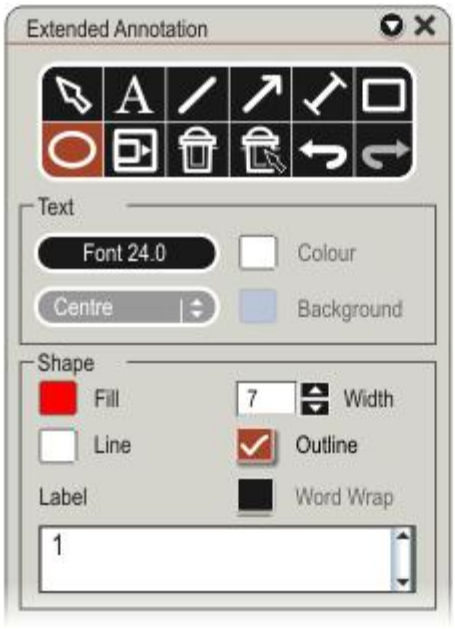
Key Numbers - small squares or circles displaying a number or letter - are invaluable where labels with text would be difficult to place and obscure the image. The wrist watch mechanism opposite is a good example.

The numbers or letters are listed in a *Key* that references each item and explains what it is. Both *Key Numbers* and *Key* are easily achieved in *Extended Annotations* and because the *Key Number* is drawn only once, saved to a template and then continually loaded, the size and style remain completely consistent: Only the numbers are changed.



To create the *Key Number* template:

- Click the *Text* button:
  Select the font, style and size
  Set the text position as *Centre*
  Select the font *Colour*
  Make the *Background Transparency* = 0
- Click the *Rectangle* or *Ellipse* button:
  Set the *Outline Width*
  Enable the *Outline* check box
  Select the *Outline Colour*
  Select the shape *Fill* colour
- Type the first number or letter in the *Label* text box:



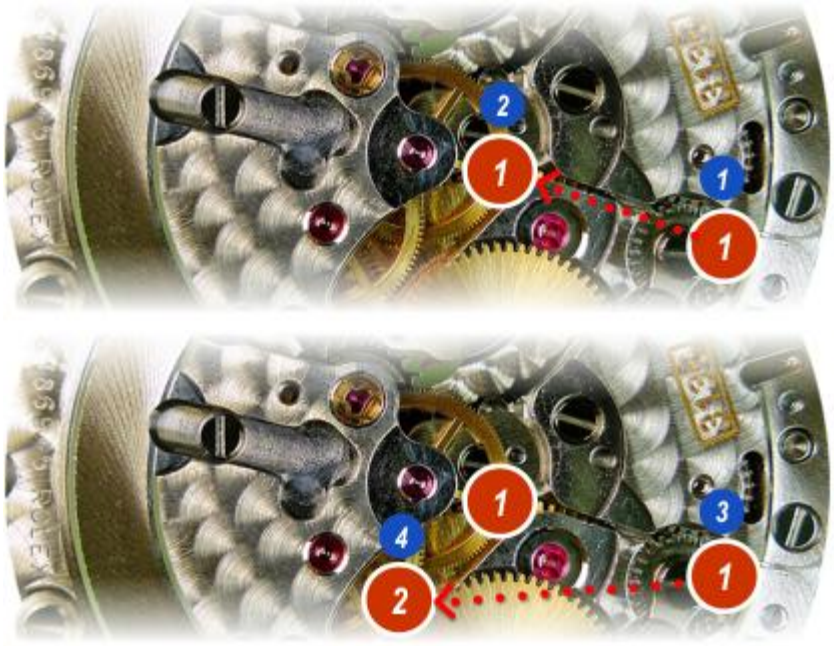
1: Click on the image - position is not important - and draw the *Key Number*. Save the *Key Number* as a template

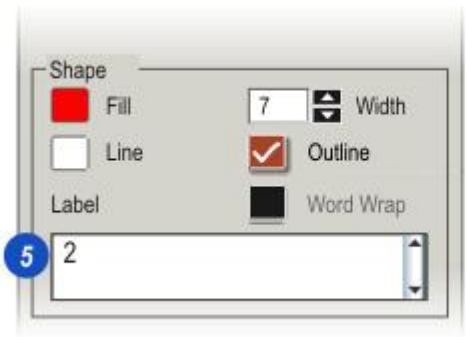
2: Click on the *Selection* tool and then on the *Key Number* and drag it to its proper position.

3: Load the template and ...

4: ...drag the *Key Number* to the next position.

5: Change the number or letter by clicking inside the *Label* text box and typing the new number.





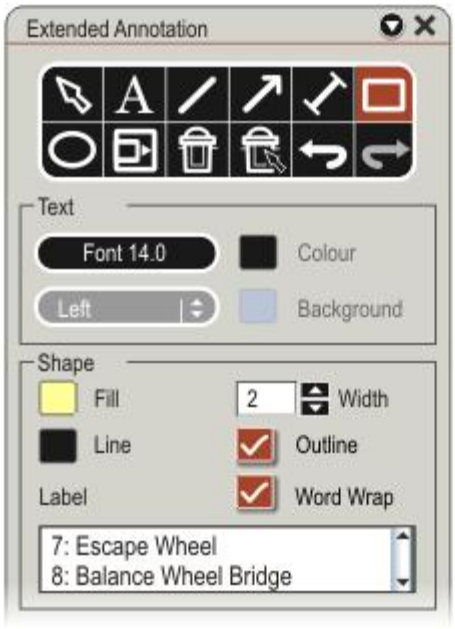
To create the *Key* list - *a* references to each *Key Number* and a description of what it represents:

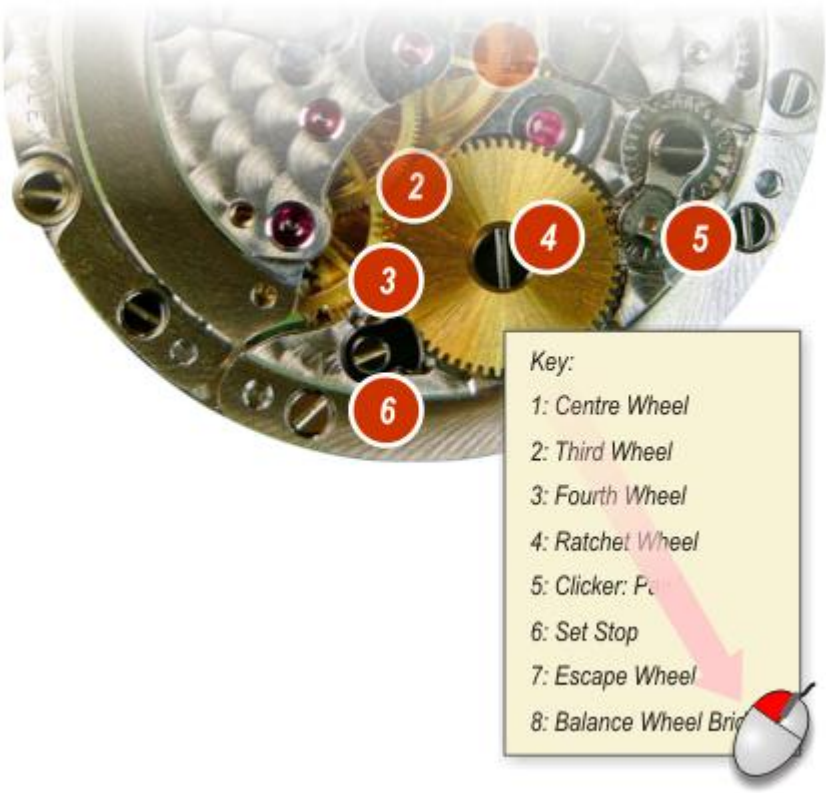
- Click the *Text* button:
  Select the font, style and size
  Set the text position as *Left*
  Select the font *Colour*
  Make the *Background Transparency* = 0

- Click to enable the *Word Wrap* check box to that the text will remain inside the outline.
  Click inside the *Label* text box and type the lines of *Key* text.
  Use the *Return (Enter)* key to start a new line.
  Use the *Scroll Bar* on the right to move through the text.

- Click the *Rectangle* button:
  Set the *Outline Width*
  Enable the *Outline* check box
  Select the *Outline Colour*
  Select the shape *Fill* colour

- Click on the image and drag down and to the right to draw the *Key*. The text is drawn at the same time.
  Use the *Selection* tool to re-size or re-position the *Key*



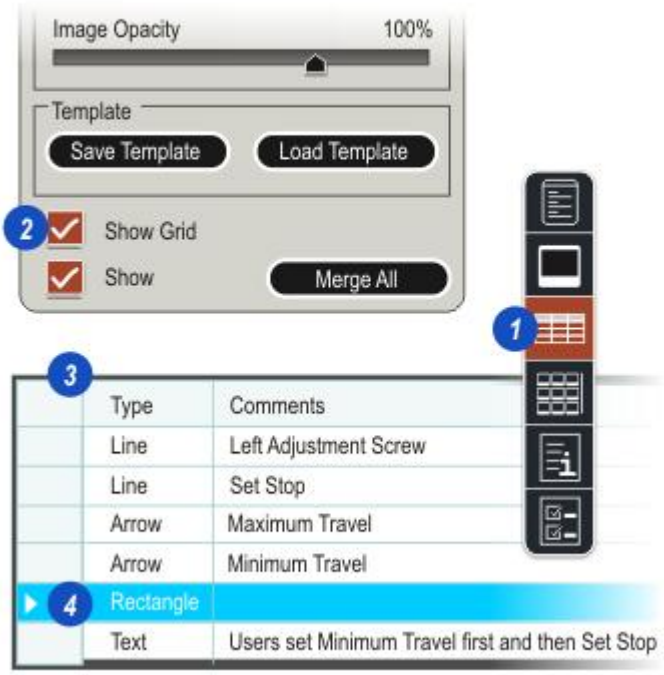


The *Extended Annotations Grid* is a list of all of the annotations on the current image. The line or shape description is listed under *Type*, and any label text under *Comments*.

1: The *Grid* button on the *Side Tool Bar* must be enabled - red background.

2: The *Show Grid* check box when enabled will show the *Extended Annotation Grid* (3); When disabled the normal captured images *Grid* is displayed.

4: Clicking an entry on the *Extended Annotation Grid* will highlight the annotation on the image - especially useful when there are many annotations on an image.



Merging is the process of combining annotation drawings including other images, into a single image. It is especially useful if image and annotations are being distributed and may not be viewed in LAS. A merged image can be opened in most office software with the annotations intact.

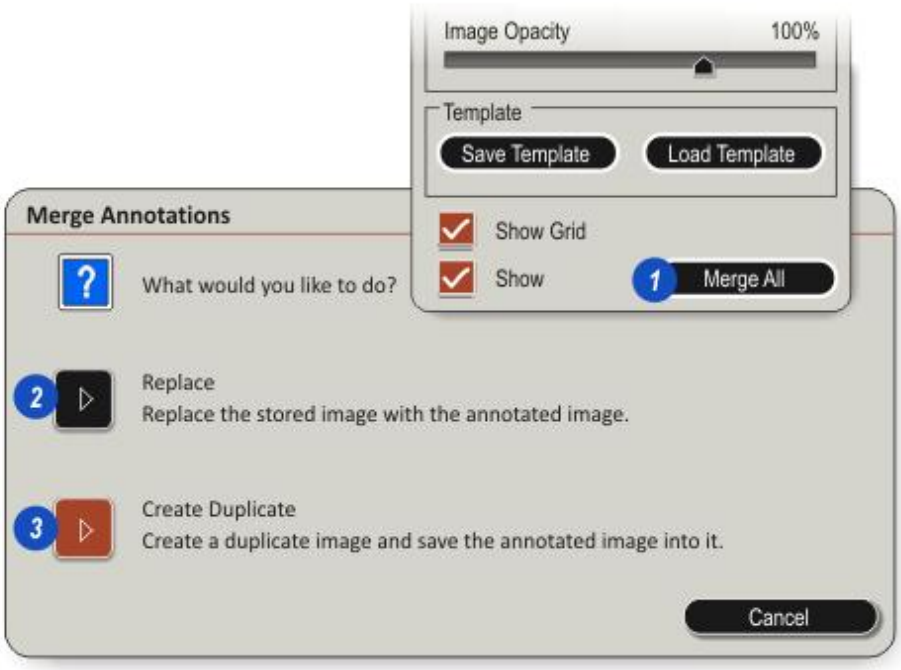
Once merged, the image will always appear with the annotations and cannot be altered. *Merging* is available on for captured images in the *Browse, Process* or *Analysis Workflows.*

1: Click the *Merge All* button.

2: Two options are available: *Replace* the captured image and the annotations with it, or …

3: …*Create Duplicate* which makes a copy of the existing captured image and merges the annotations with the copy.

Click the required option button.



Although Extended Annotation was designed as a cost effective, easy to use drawing program, it has some remarkably versatile features to add style and effectiveness to users images.

For example, the annotations around the watch image on the opening page, were created simply by:

- Drawing a plain line leading to the object.
- Draw a rectangle that will enclose the longest text line. The fill must be solid - transparency = 255.
- Set the text position to the left for left-hand side annotations.
- Copy the rectangle as a template.
- Drag the rectangle over the leader line.
- Load the template and change the text for the next annotation.

Creating a drop shadow is easy and adds presence to text and shapes:

- Create the text. In this example two words are butted together because they are different fonts. The fill is a pale grey.
- Copy the text as a template and then load it. This places a copy over the original.
- Change the text fill to the desired colour.
- Drag the copy up and to the left to expose the original grey text.

Image transparency together with templates can create some interesting effect. The 'doughnut' below has 'roundness' simply by:

- Draw the shape - in this case a circle with an outline width of 8. Set its transparency to 80%. It can have a solid fill.
- Copy the circle as a template and load it.
- Change the outline width of the copy to 4. Leave the transparency setting at 80%.
- Load the template again and change the outline width to 2.

Highlights and cast shadows can also be created with transparency and templates:

The purple circle is saved as a template and loaded.

The size of the copy is adjusted, the fill changed to white and transparency to 75%.

Repeat the process changing the diameter and position each time.

The top highlight is effective against dark backgrounds:

The white circle is saved as a template, loaded and the size, position and fill changed.

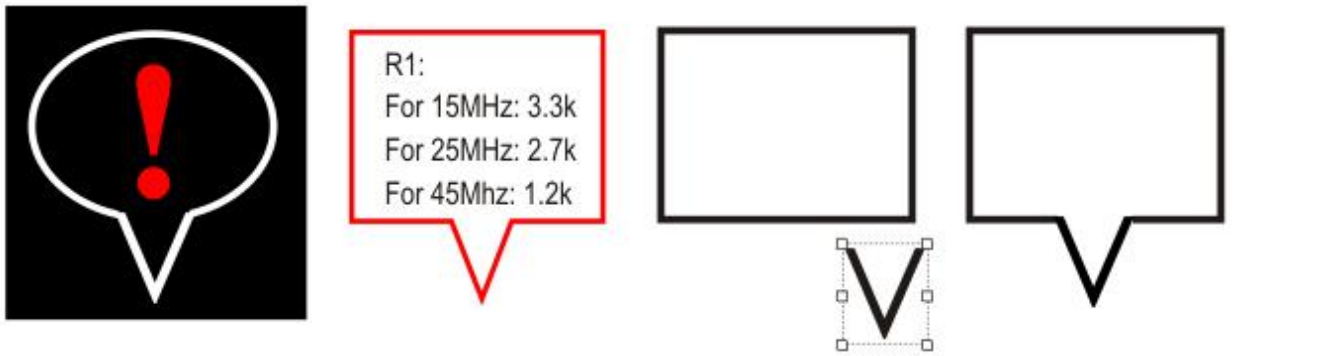
Cast shadows are created in the same way:

The shadow is a grey-filled ellipse with a transparency of 80%.

It is saved as a template, loaded and re-sized.

A callout is a text box with an attached pointer that indicates a detail on the image. The callouts described here are suitable for black or white backgrounds.

- Draw a shape - rectangle or ellipse with a solid white or black fill.
- Using the text tool with a sans-serif font - Arial or Helvetica for example - type a capital 'V' with fill and outline colour to match the shape. Set the font size to suit the callout.
- Drag the text background handles to touch the edges of the 'V'.
- Drag the 'V' so that its background overlaps and obscures the shape outline.
- Type the text and drag it over the shape.



There are many symbol 'fonts' available - some of them free to download - that are collections of symbols, each of which is related to a normal font character. For example, the tick mark opposite is drawn by typing the number 4 in the *Zapf Dingbats* font. They can be treated like any other text character for size and fill colour.

The Leica Application Suite *MultiTime* optional module is a highly effective solution for the automatic acquisition of images over time. The time span and acquisition may range from many images per second or just a few, delayed by minutes.

After acquisition they may be viewed, enhanced and documented. Other LAS modules can perform analysis and image measurement.

There are 2 separate components in *MultiTime* that are enabled and operated independently:

- *Time-lapse*[586]*:* Images are acquired with a delay starting at 1 sec .
- *Movie*[606]*:* Images are acquired in a compressed image stream directly to the hard drive as fast as possible, equivalent to making a video recording.

If images are to be collected over a long time, the user should ensure that the specimen and microscope are stable, unaffected by changes in temperature, focus position and the electrical supply.

TimeLapse is an imaging technique that captures images with a user-selected delay between each. This delay time is the distinguishing feature between normal imaging and *Time Lapse* imaging.

The images are stored on the hard drive at defined intervals and can be recalled individually, in a continuous loop or as an AVI movie file.

TimeLapse is best suited for continuous image capture over long periods, or where there is no need for image data at full frame rates during the operation.

TimeLapse imaging has a significant time delay between each capture and the camera acquires at its full frame rate. But only one image is processed because it is inefficient to process every single image if only certain images are of interest.

The *MultiTime* module must be installed and enabled.

1: Click on the *Acquisition Mode* selector.

2: From the menu, click on the *MultiTime* icon.

Select the Fixed Capture Folder:

The *Time Lapse* images are captured into a folder of the users choosing.

3: Click on the *Browse Workflow* and...

4: ...if necessary click on the *Browse* tab.

5: Navigate to and click to select the capture folder and then...

6: ...click on the *Set Fixed Location* button. To indicate the fixed capture location archive, a red dot appears to the left of it.

! Images will only be captured to the *Fixed Location* folder if *'Capture to fixed'* is enabled in *Preferences*[588].

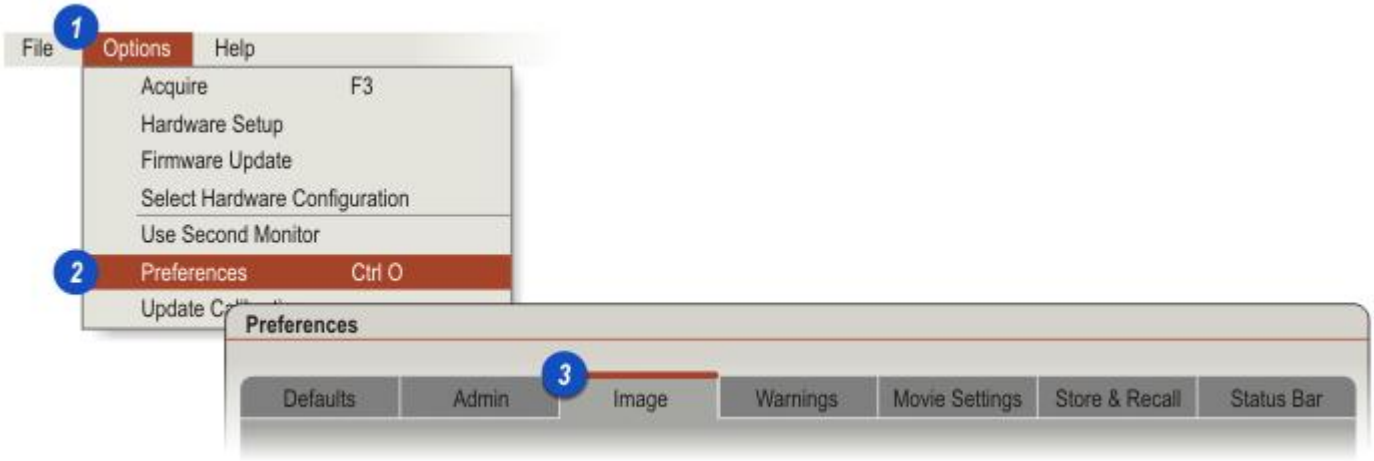
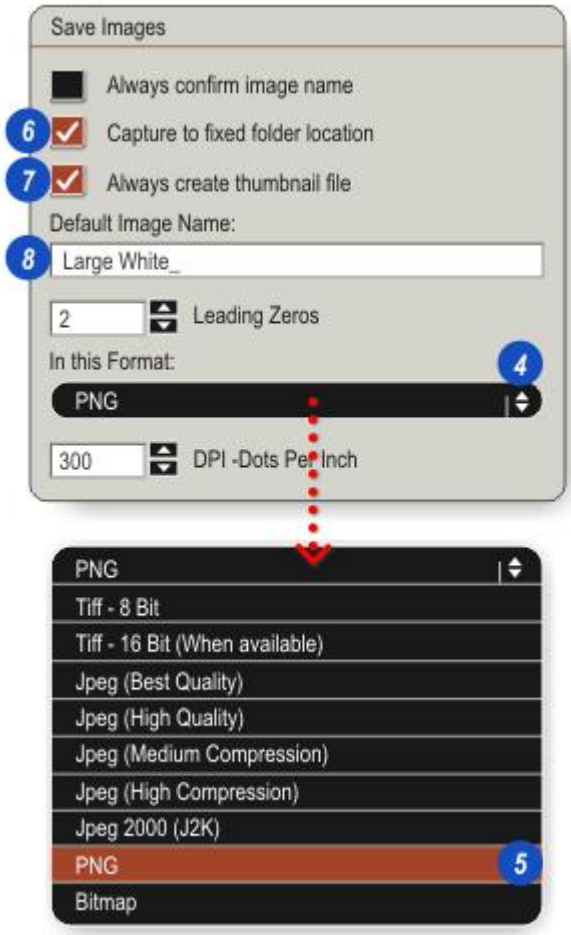


Image compression affects the data size and quality of the captured image. For long *TimeLapse* sessions involving many images it can also have an important bearing on disk space. High quality, little or no compression images are large and disk-hungry.

The *Compression* setting is made in *Preferences* > *Save Images* panel and at the same time users should enable *Capture to a fixed folder*.



Select the Image Compression:

1: Click on *Options* on the *Main Header* and...

2: ...click to select *Preferences*.

3: On the *Preferences* dialog, click the *Image* tab.

4: Click on the arrows to the right of the *In this format* header and...

5: ...click to select the required *Compression* type.

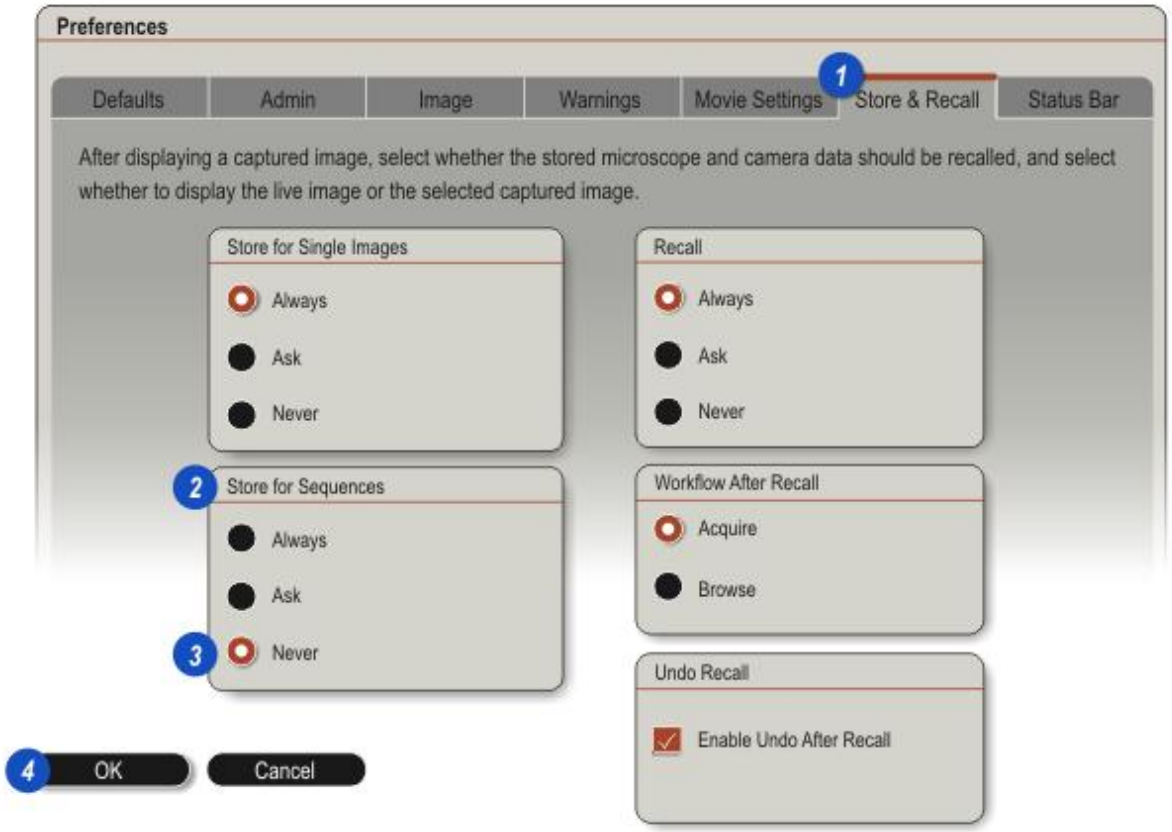
Fixed Capture Folder:

6: Click to enable (tick mark visible) the *Capture to fixed folder location.* The folder is selected in *Browse.*

7: *TimeLapse* always creates a thumbnail for each capture regardless of the check box setting..

8: The *Default Image Name* is not used for the captured images. Instead, the default *t_nn* is used where *nn* is an incremental number.

TimeLapse creates a new folder inside the *Fixed Capture Folder* giving it a *Sequence Name*[602] entered by the user on the *Options* panel.



The *Store and Recall* feature allows the microscope and camera setting to be stored with an image so that precisely the same conditions may be repeated at a later date. It can also provide consistency across a range of different specimens.

For *TimeLapse* sequences this may not be necessary and because it also adds a small amount of time to the image capture processing, for short time lapse durations *Store & Recall* should be switched off:

1: Click on the *Store & Recall* tab.

2: On the *Store for Sequences* panel...

3: ...click the *Never* radio button to select it.

4: Click *OK* to save the changes and exit *Preferences*.

Both exposure time and capture format depend on the time lapse duration between individual image captures.

For example, a short time lapse of say 1 second, will demand a short exposure time to allow:

- Image capture and processing.
- Data written to disk.
- Control files (if selected) written and saved with image.
- Camera elements to be cleared ready for the next image.

If all this take longer than the time lapse duration, images containing the desired information may not be captured.

TimeLapse does however, have a *Test* [597] tool which will check if adequate time has been allowed between captures.

> ! *TimeLapse* captures all of the images required by the user, but expected activity may not be present on the images if the duration is too short.

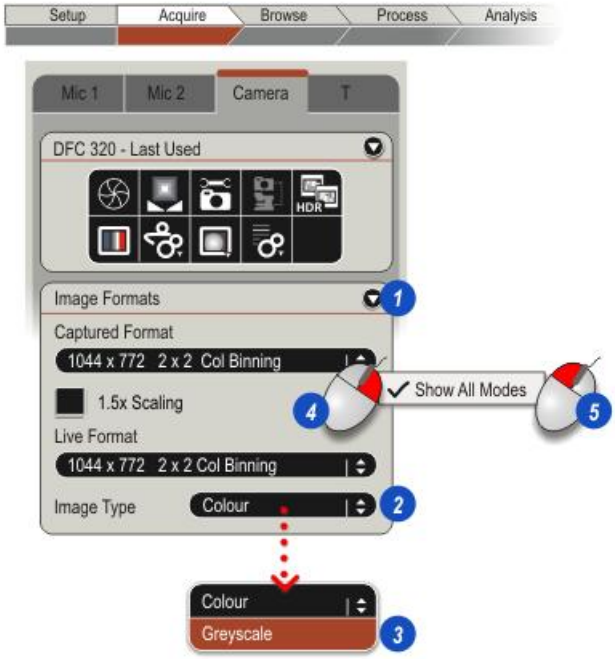
Select the Capture Format:

High capture resolutions take longer to process so for a short time lapse, *High Quality (HQ)* formats should be avoided and medium to lower resolutions chosen - *1044 x 772* or *696 x 514* depending upon the camera type.

For details of Image Formats [290]

Click on the *Acquire Workflow* and select the *Camera* tab:

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.



2: Click on the arrows to the right of the *Image Type* header bar...

3: ...and from the drop down menu select *Colour* or *Greyscale (Monochrome).*

4: Right click on the *Captured Format* text box and...

5: ...left click on the *Show All Modes* label to check it.

Selecting the Captured Format:

1: Click on the arrows to the right of the *Captured Format* header bar.

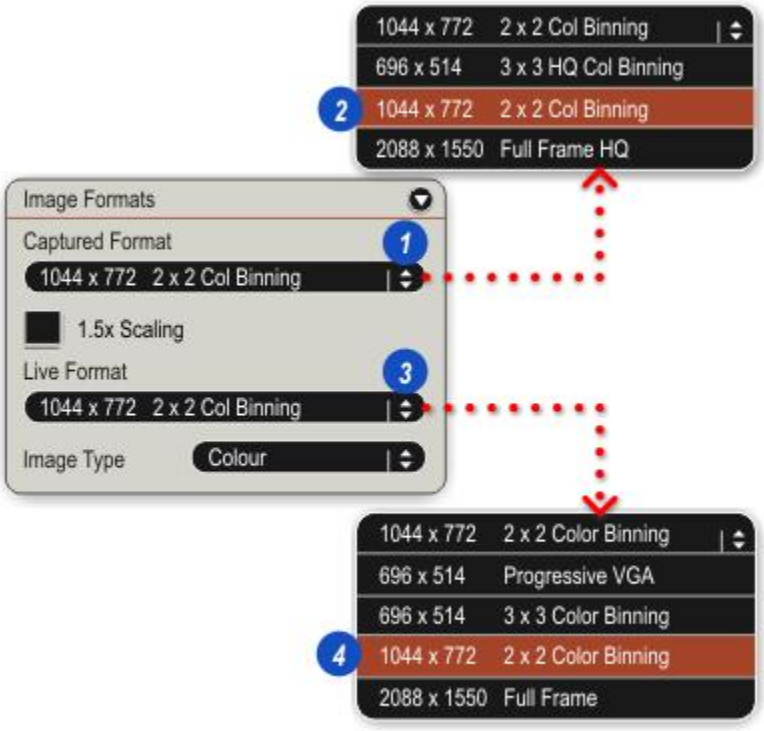
2: From the drop down menu click on the format required.

Selecting the Live Format:

This is the format displayed in the *Viewer*. In most cases it can be the same as the captured format.

3: Click on the arrows to the right of the *Live Format* header bar and...

4: ... from the menu click to select a format. If the camera supports a wide range of formats, small *Scrolling Arrows* will appear top and bottom of the drop down list. Click to scroll up and down.



There are two options for adjusting the exposure:

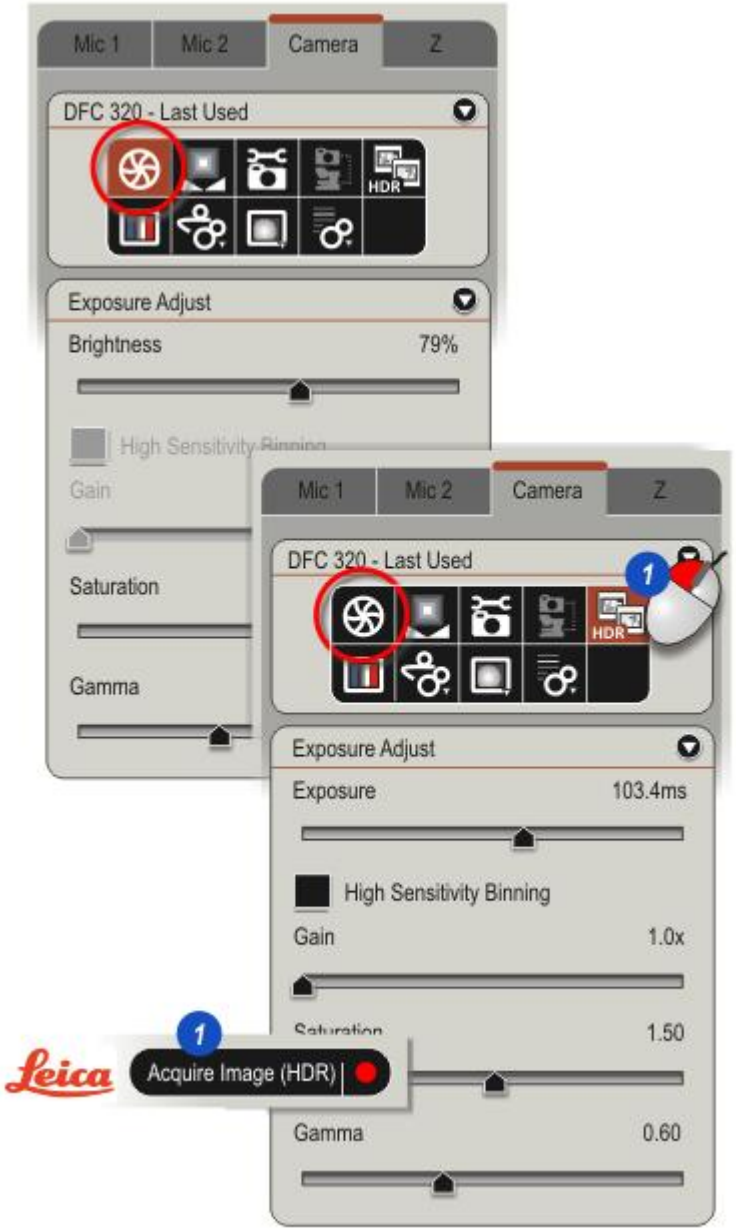
- *Automatic* with some fine-tuning, and...
- *Manual* with a range of precision controls.

As a start, using *Automatic Exposure* is a good option because combined with *Automatic White Balance* it could produce a perfectly acceptable image very quickly.

Disable HDR and AVG:

HDR and *AVG* features are two methods of capturing multiple images in memory and combining them to create a clearer image. The process takes a substantial amount of time for each capture so, for most *TimeLapse* projects is not suitable.

1: If either *HDR* or *AVG* is enabled, left click the *Camera Toolbox HDR* button to disable it. The button is not highlighted.



The *TimeLapse* controls are grouped into 4 panels:

1: *Start*[594]: Sets whether there will be a delay before the capture sequence starts or if it should begin immediately the *Acquire TimeLapse* button is clicked

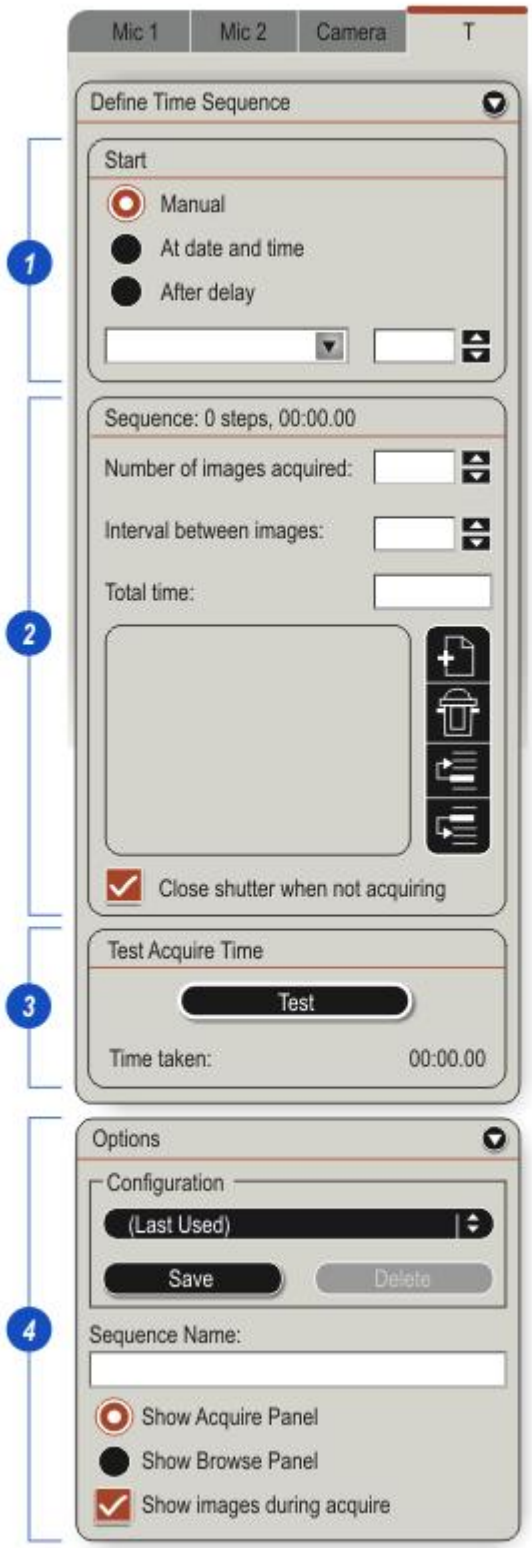
2: *Sequence*[596]*:* Sets the number of images the user requires and the interval between them. Sequences with differing image counts and intervals can be mixed in a *Sequence List* displayed on this panel

3: *Test*[597]*:* The *Test* facility compares the user set interval with the actual time required to capture the image and write it to disk. If the interval is too short some images may not contain the data expected

4: *Options*[600]*:* The *TimeLapse* setup can be saved as a *Configuration* that can be recalled on this panel. The *Sequence Name* is also entered here and the user can select whether to display live or acquired images during the sequence capture

There are also controls for:

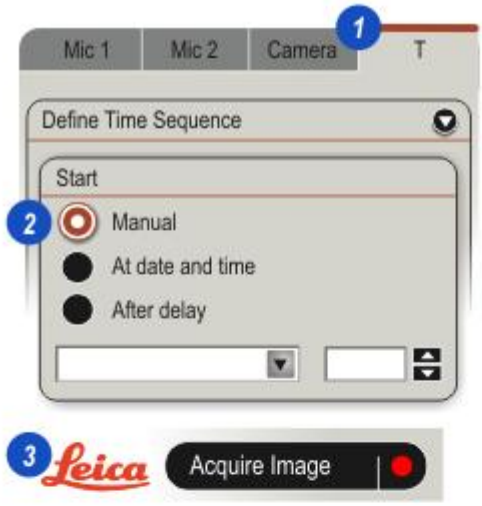
- Showing capture progress[603] with pause and stop buttons.
- Scrolling though the captured images as a loop or individually, and also converting the sequence to a movie. See Capture Complete[604].



The *Start* panel provides three start up options for the *TimeLapse* sequence:

- *Manual:* The sequence starts as soon as the *Acquire Image* button is clicked.
- *At date and time:* Starts the capture on a specific date and at a specified time.
- *After delay*: Waits for a specified time before starting the capture.

1: Click on the *TimeLapse (T)* tab to reveal the control panels.



Manual start:

2: Click on the *Manual* button to start the sequence as soon as the *Acquire Image* button (3) clicked. Go directly to the *Sequence*[596] panel to set up the time lapse.

At date and time:

4: Click the *At date and time* button.

5: Click on the arrow to the right of the date window and the calendar appears.

6: Use the left/right arrows to scroll through the months and years.

7: To select a month quickly, click on the calendar header and the month list appears. Click to select a month.

Click on the day date on which the sequence will start.

With the At date and time option selected and the date chosen, set the start time.

The time is divided into three fields – *hours: minutes* and *seconds.* The hours field (24 hour clock) rolls over to zero at a count of *24*, and the minutes and seconds at *60*.

1: In the *Time* text box...

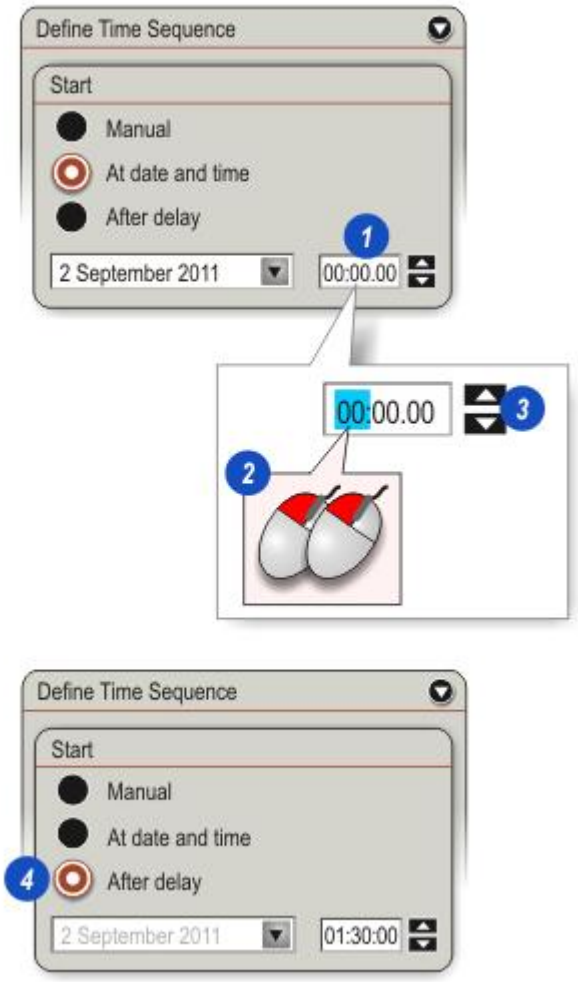
2: ...double-click on a field to select it...

3: ...and then use the up/down arrows to the right of the window to set the required value. Click and hold on an arrow for a fast scroll.

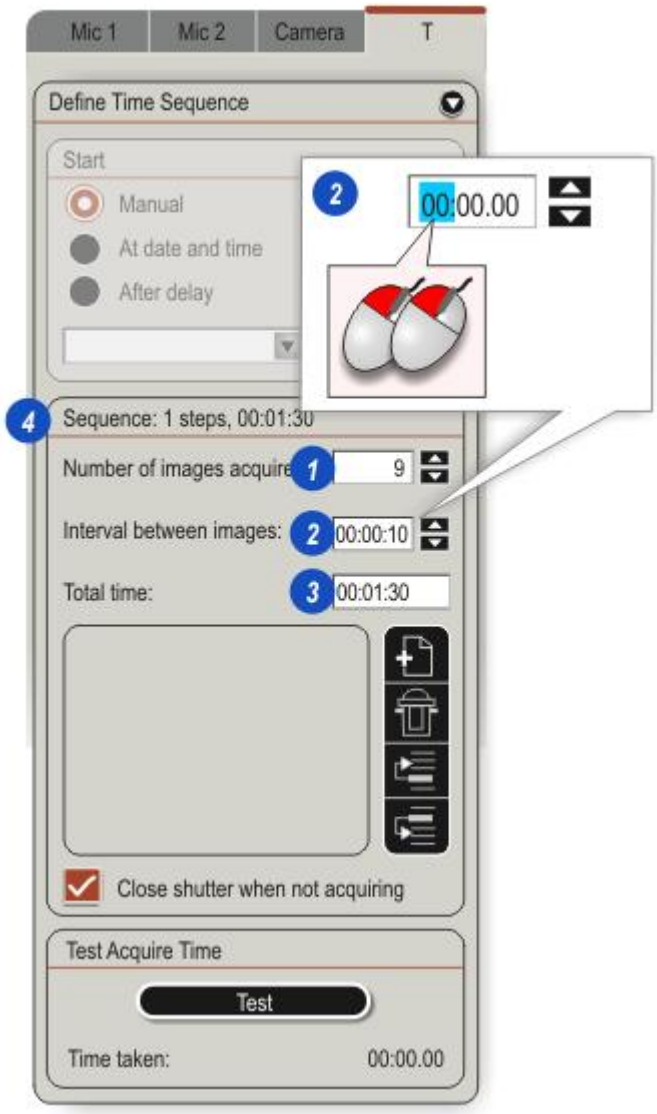
The After delay option:

4: Click to select the *After delay* option which will start the sequence when the set time has expired.

The time is set using the steps above.



The *Sequence Panel* contains the controls for setting up the structure of the *TimeLapse* sequence.



Number of Images acquired:

The user sets the number of images that will be required in the sequence by:

1: Clicking the up/down arrows to the right of *Number of images acquired* window to set the number of images in the sequence.
Alternatively, click inside the text box and, holding down the mouse button swipe the existing value to highlight it. Then type a new value on the keyboard.

Interval between images:

Sets the delay - time lapse - between each of the image captures.

2: The text box is divided into three fields – hours: minutes: seconds. Double-click on a field to select it and either type a value or use the up/down arrows to the right of the window to set the required value.
Click and hold on an arrow for a fast scroll.
The hours field (*24* hour clock) rolls over to zero at a count of *24*, and the minutes and seconds at counts of *60*.
Intervals may extend from *1* second to *23* hours: *59* minutes: *59* seconds.

Total time:

The number of images is multiplied by the interval and the overall sequence duration is displayed.

3: In the illustration, 9 images are required with an interval of 10 seconds. Including an interval for capture and data writing for the 6th. image (in case further images are added to the sequence), this equates to 60 seconds or 1 minute 30 seconds *(00:01:00)*. However, if no further images are added, the final interval is ignored.

4: The sequence details are displayed on the header - the number of sequences (steps) and the total estimated time to complete.

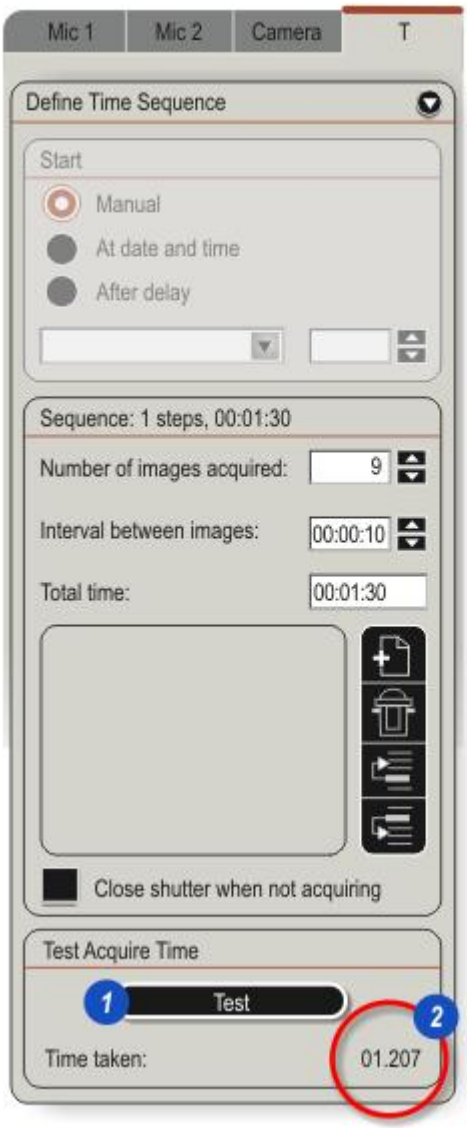
The Total time display is simply the number of images required multiplied by the time lapse interval. It does not take into account the camera capture, processing and writing to disc. The *Test Acquire Time* tool makes the complete calculation, including the effects of exposure and image format, for a single capture.

The result is displayed in seconds to decimal three places:

1: Click the *Test* button. This is a software emulation - images are not being captured - and...

2: ...the result is shown bottom right of the panel.
In the illustration the test reports that each image will take *1.207* seconds to capture and process - well within the 10 seconds interval.

The selected interval must always be larger than the test result otherwise images may not contain the expected information.



1: If Fluorescence techniques are being used, click to enable the *Close shutter when not acquiring* check box to avoid damaging the specimen.

Before a sequence can be used it has to be added to the Sequence List.

2: Click on the *Add Sequence* button and...

3: ... the image count and interval appear in the *Sequence List*.

4: Click on the *Acquire TimeLapse* button to start the sequence capture.

If, on the *Start* panel *At date and time* or *After delay* have been selected and set, this period will start and run, triggering the sequence capture after it has expired.

With the *Manual* option selected the capture will start immediately.

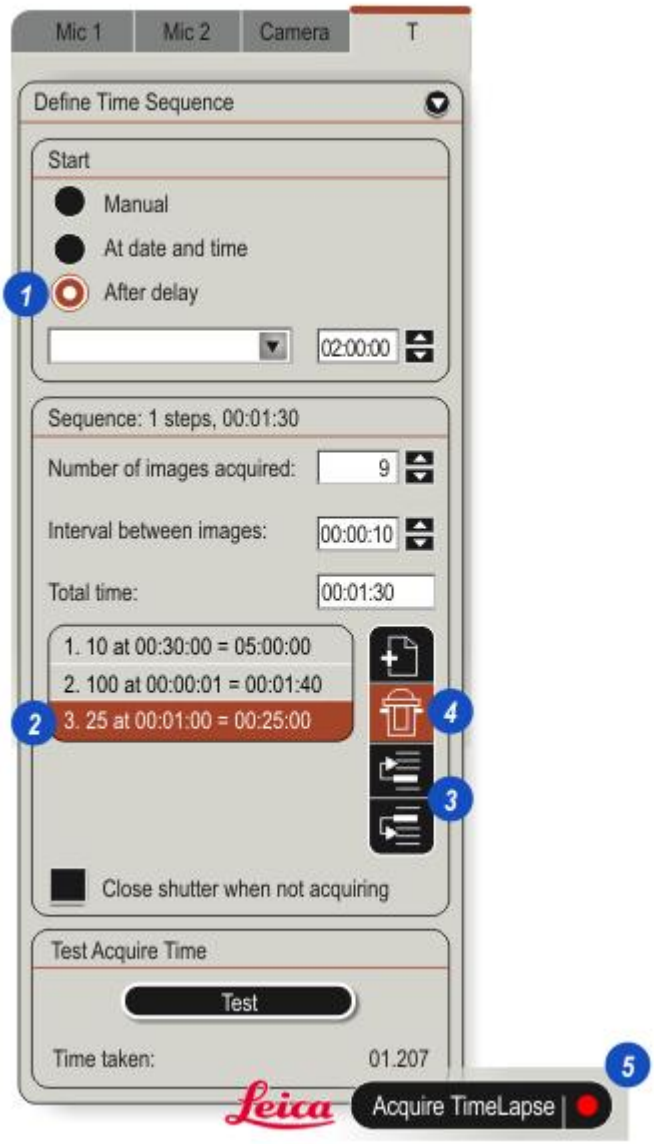
To achieve complete flexibility, almost any number of capture sequences can be added to the *Sequence List* and they will run in turn.

1: The *Start* option is executed first – in the example *After delay* of 2 hours has been selected. When the delay has elapsed each of the sequences in the list will be executed.

The example sequences on the illustration are:

- 10 images with a 30 minute delay between each.
- 100 images with a short 1 second delay between each image, and…
- ...25 images with 1 minute between.

Each sequence was added to the overall program by clicking on the *Add Sequence* icon and was given a sequence number. A scroll bar is added automatically to long lists.



Changing the Sequence order:

To move a sequence up or down the execution order:

2: Click on the sequence to be moved.

3: Click on the *Up/Down* order buttons. As a sequence moves up or down the list its sequence number changes. The list always executes top to bottom.

Deleting a sequence:

2: Click on the sequence to be deleted.

4: Click on the Trash Can icon.

Start the Sequences:

5: To start the capture sequences click on the *Acquire TimeLapse* button.

The Options panel provides controls for:

- Saving the sequence or *Sequence List* with all the timings as a *Configuration* that can be recalled and used at any time.
- Determining which *Workflow - Acquire* or *Browse* to show during capture. Both display the live image but....
- ...only if *Show images during capture* is enabled.
- Giving the sequence a *Name* that will be used in for the capture folder(s).

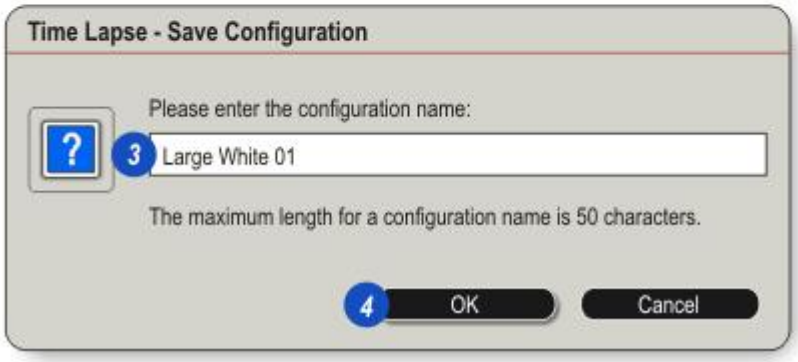
1: Reveal the *Options* panel by clicking the arrow to the right of the header.

To Save the Sequence(s) as a Configuration:

2: Click the *Save* button.

3: On the *Save Configuration* dialog, click inside the *Name* text box and type a unique name for the configuration.

4: Click *OK*.



Recall a Configuration:

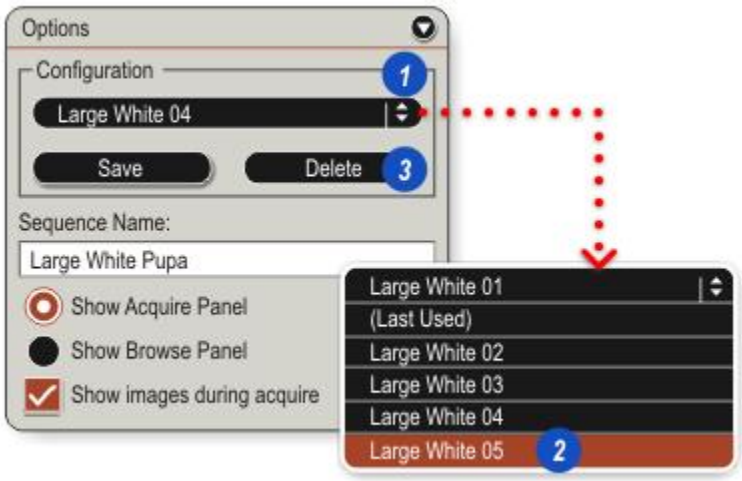
1: Click on the arrows to the right of the *Configuration* text box.

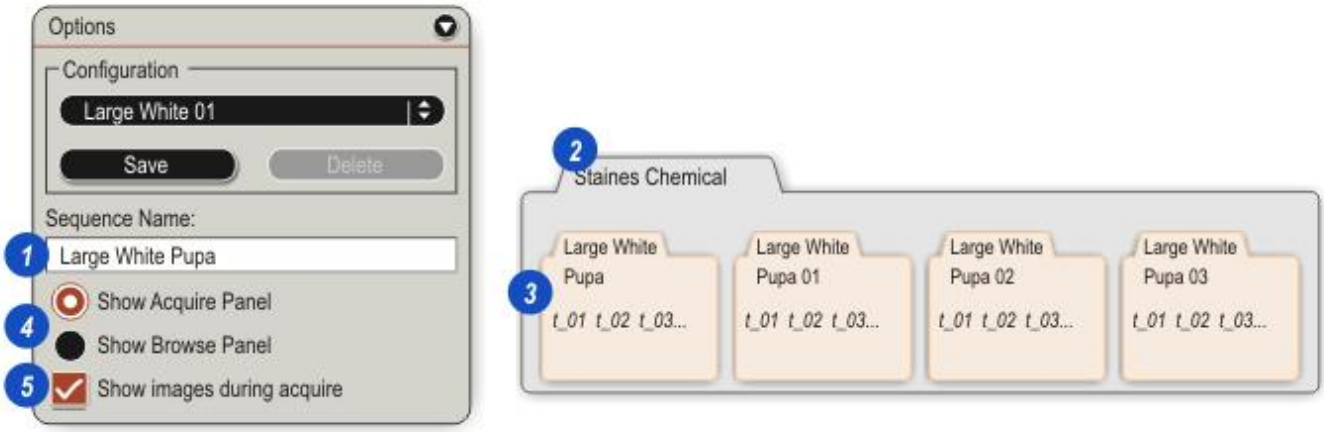
2: From the drop down list, click to select a configuration.

To Delete a configuration:

2: Select a configuration from the list.

3: Click on the *Delete* button and the configuration is removed from the list. It cannot be retrieved.





When a *TimeLapse* sequence starts, a new *Sequence Folder* is created inside the *Fixed Capture Folder* and it is into this that all of the images and their data files will be saved.

The Sequence Folder must be given a name:

1: Click inside the *Sequence Name* text box and type a new name. In this example, the *Sequence Folder* is named Large White Pupa and it will be created inside...

2: ...the *Fixed Capture Folder* called Staines Chemical in this example. The captured images are automatically named *t_nn* where *nn* is a numeric increment.

3: If another sequence is captured, another new folder is created with the *Sequence Name* plus a numeric suffix. Again, the captured images are named *t_nn*.

4: Click to select if either the *Acquire* or *Browse Workflow* is displayed during capture.

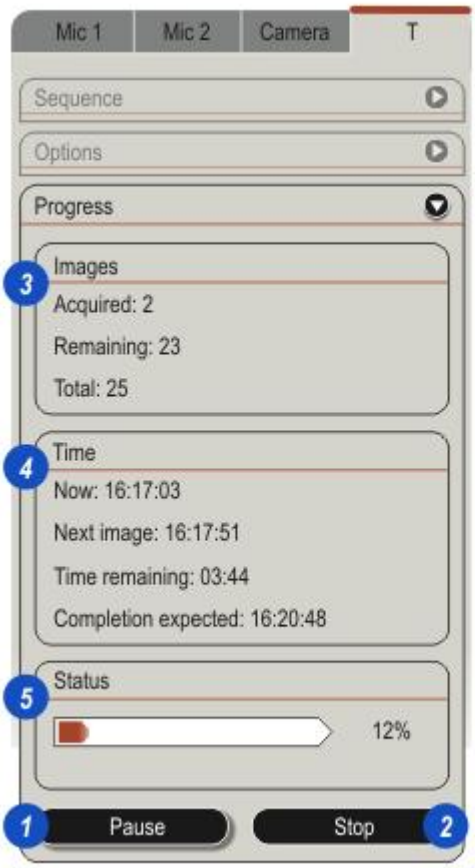
In the *Browse Workflow* the captured image is displayed in the *Viewer*.

With *Acquire* selected the live image is displayed but only if...

5: ... the *Show images during acquire* check box is enabled.

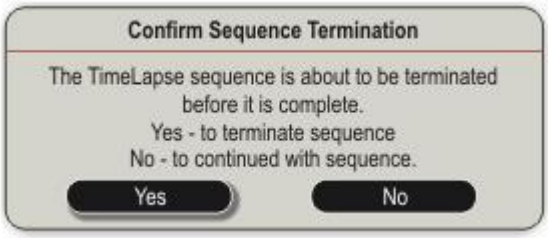
When the sequence is complete, the *Browse Workflow* is automatically displayed.

When the capture sequence starts the *Progress* panel opens.



1: Users can *Pause* the sequence by clicking the *Pause* button and resume the sequence by clicking it again. Some images may be lost if the sequence is paused.

2: Clicking the *Stop* button halts the capture sequence and gives the user the option to abort the sequence or to continue.

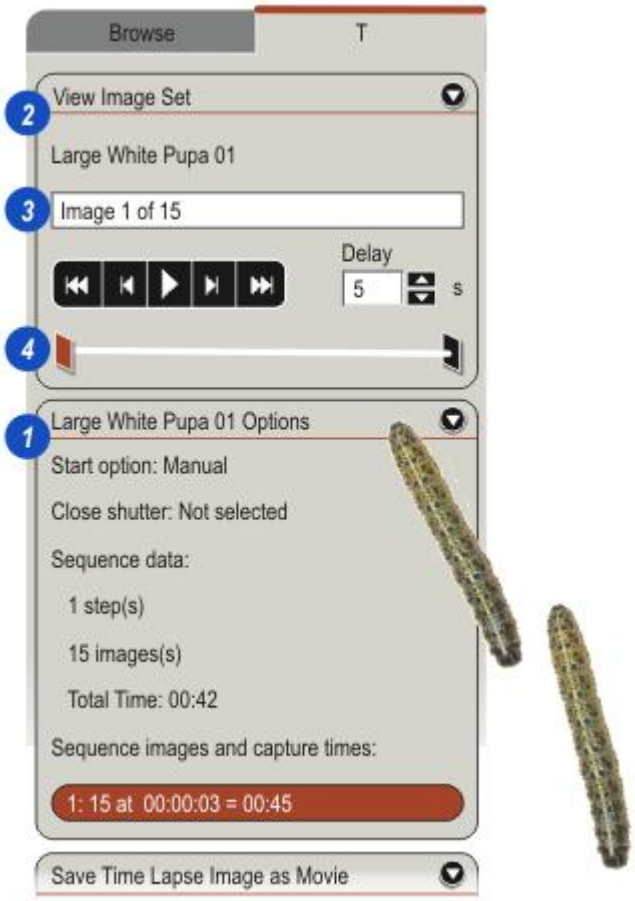


3: The *Images* panel displays the captured image count so far, how many remain and the total.

4: Real time is displayed on the *Time* panel with the current time (as set in the computer clock), when the next image is due for capture, total time remaining for the entire sequence at expected completion.

5: The *Status* bar shows progress graphically.

When the capture sequence finishes the *Browse Workflow* opens automatically. If the *Gallery* with thumbnails is not displayed click the *Show Gallery* button on the *Side Tool Bar.*



1: The *Options* panel shows a synopsis of the sequence.

2: The *Sequence Name* is displayed on the *View Image Set* panel with...

3: ...the number of captured images shown. The first image is selected and displayed in the *Viewer*.

4: Scroll through the images by clicking on the slider and holding down the mouse button drag right or left. The images are displayed in order in the *Viewer*.

The *TimeLapse* sequence can be displayed as a loop by:

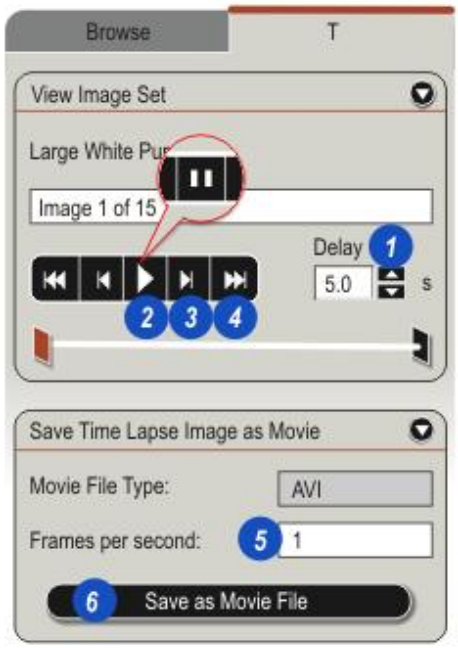
1: Use the up/down arrows to set a delay between the images being displayed. This gives users time to examine the images. Increments are *0.1* seconds.

2: To start the loop, click the *Play* button. When the loop finishes it will roll over to the first image and start playing again.

Click the *Play* button again to stop the loop.

3: Click the single arrow to move forward/backward one image.

4: Click the double arrow to go to the last/first image.



Convert TimeLapse Sequence to a Movie.

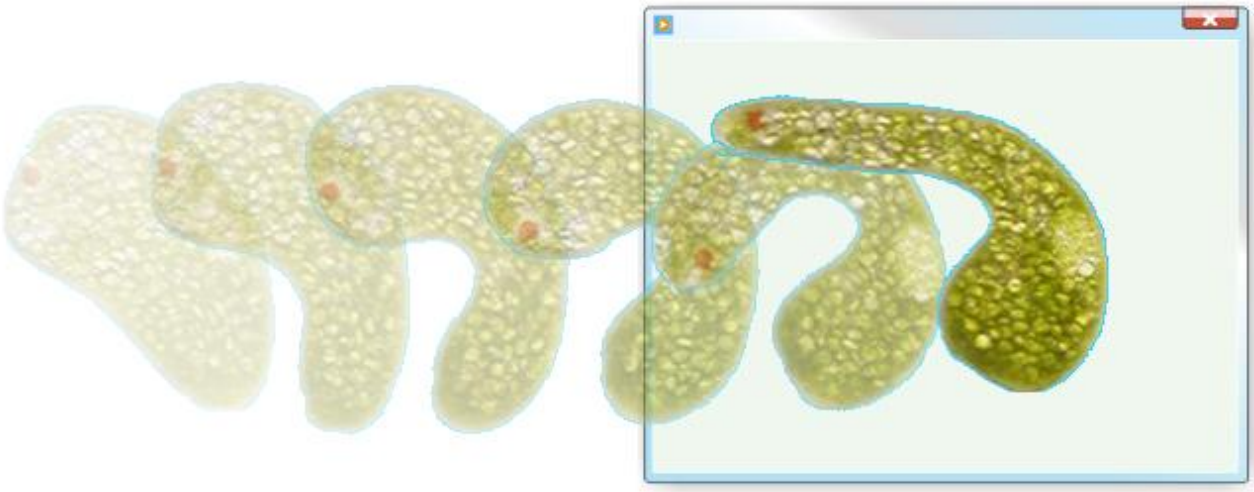
For portability, the captured sequence can be converted to movie; The *AVI* format is the default.

5: Set the movie speed - the number of images that will be displayed in one second - by clicking inside the *Frames per second* text box and typing a value. The higher the number the faster the action.

6: Click the *Save as Movie File* button.

The movie is saved in the same folder as the TimeLapse sequence with the name *MovieAt_* followed by the number of frames per second - *10.00fps.avi* for example.

Double click the file in *Windows* to launch the system movie player software.



Leica Application Suite *Movie Module* is an easy to use intuitive program that can capture sequential images and play them back either in LAS or in a selected media player.

All of the LAS controls are used conventionally to set up the images and the two *Movie* modes can accommodate slowly or erratic specimen changes.

A movie can comprise any number of individual, self-contained clips that can be played 'standalone' or as a continuous sequence.

Intervals can be set to run without capturing images between clips so that files are not unnecessarily 'bloated' with periods of specimen inactivity

Click on a heading for more information.

Features Check List

Continuous Mode 618
Movie duration limited only by the disk space allocation. Ideal for continuously changing specimens.

Define Clip Sequence Mode 619
Set up Clip and Interval sequences for specimens with irregular changes.

Precision Clock for Clip and Interval duration 619

Wide Compression Range 621
Reduces file size and increases playing time.

Timestamp Option 622
Merges an displays the real time with the movie. Font and colour set by user.

Save Settings as a Use-again Configuration 623

Single Frame Capture 629
Copy the current frame as a single image.

Play and Revue single Clips 629
Move between clips without having to stop and load. Variable playback speed shows specimen activity in slow motion.

Three Playback Options 628
Play in LAS Viewer or any appropriate media player.

Movie Information 631
Analysis of the movie and clips.

Setup Check List

Choose Capture Folder 610

Check Preference settings 611

Turn On Shading 613

Adjust the Exposure 615

Select the Live Image Format 616

Choose the Movie Settings 621

Check the Estimated Length 623

Start Capturing 624

The *Movie* optional module must be installed and enabled.

1: Click on the *Acquisition Mode* selector.

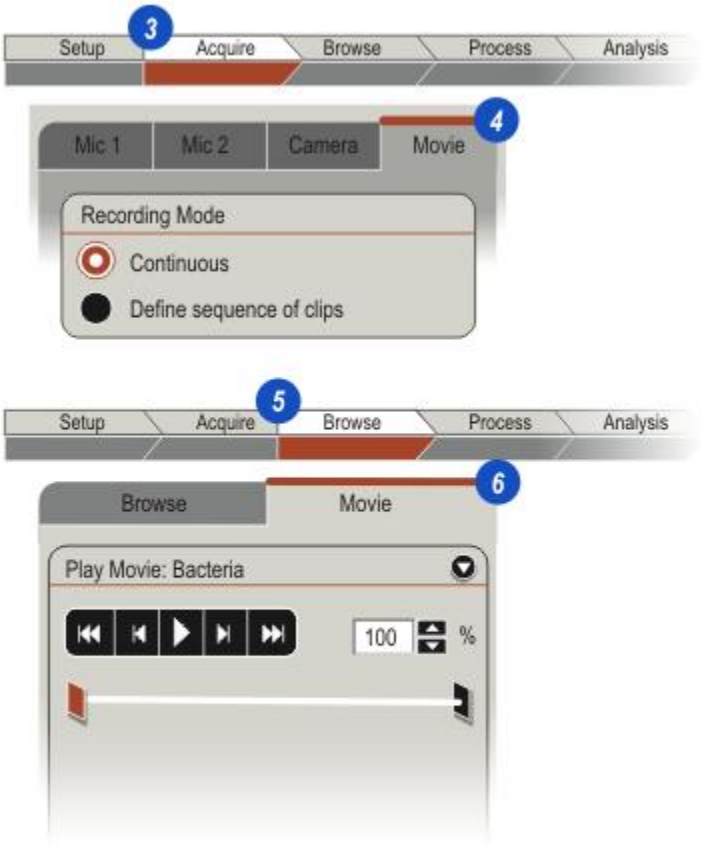
2: From the menu, click on the *Movie* icon.

3: With the *Acquire* Workflow selected...

4: ...click to reveal the *Movie* setup controls.

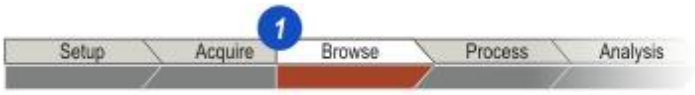
5: In *Browse*...

6: ...click the *Movie* tab to play the movie and check its properties.



Microscope Setup: Choosing the Capture Folder

The *Movie* image sequence can be saved into a folder of the users choosing.

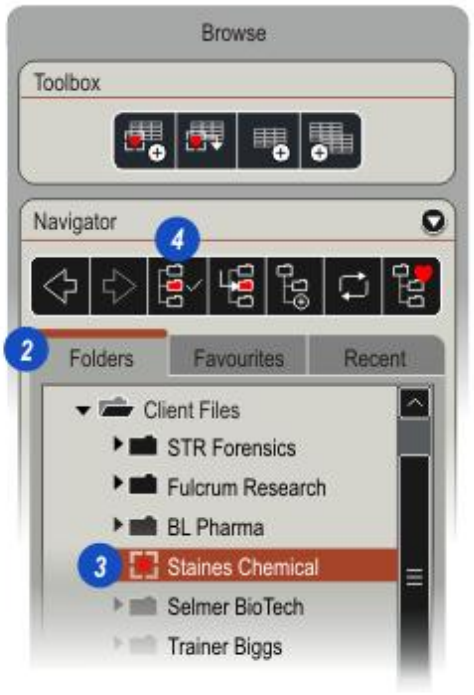


1: Click on the *Browse Workflow* and...

2: ...if necessary, click on the *Folders* tab.

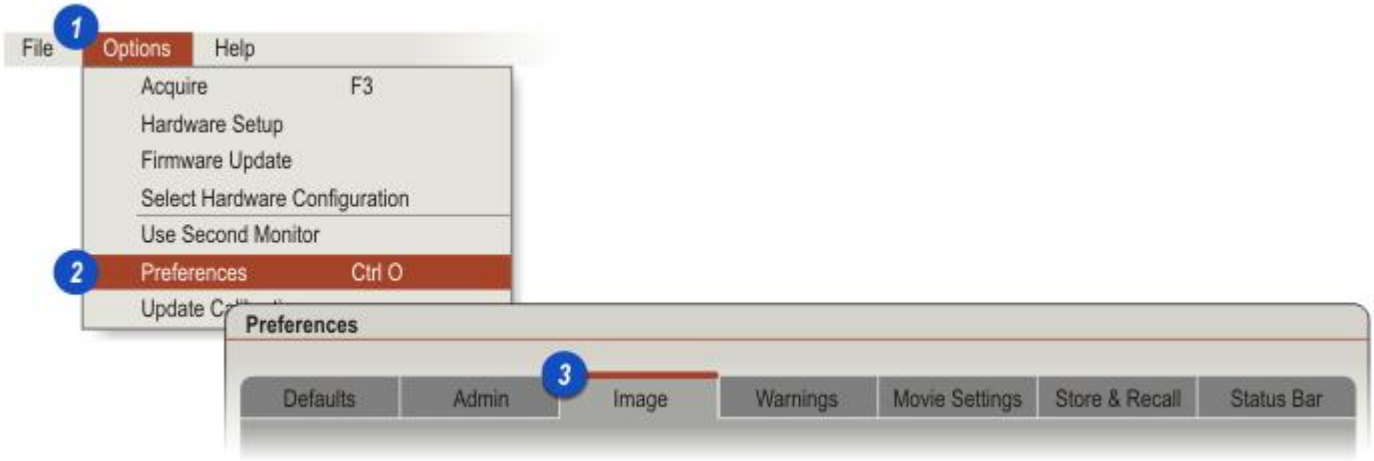
3: Navigate to and click to select the capture folder and then...

4: ...click on the *Set Fixed Location* button. To indicate the fixed capture location archive, a red dot appears to the left of it.



! Images will only be captured to the *Fixed Location* folder if *'Capture to fixed'* is enabled in *Preferences* 63

Preferences: Fixed Folder Location

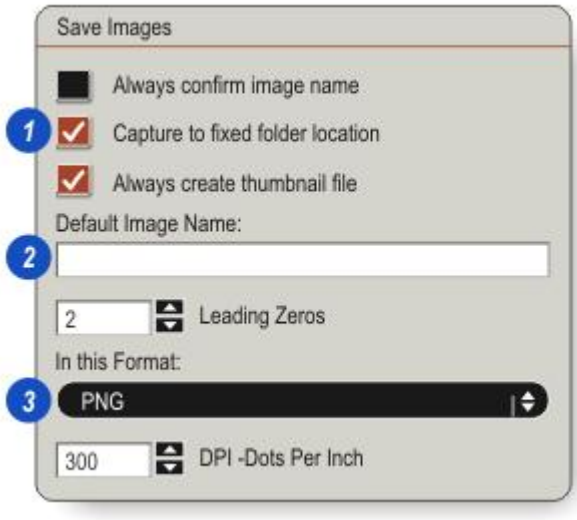


1: Click to enable (tick mark visible) the *Capture to fixed folder location.* The folder is selected in *Browse*

2: The *Default image Name* and...

3: ...compression *(In this Format*) have no effect.

The compression level *(Quality)* and name are set on the *Acquire > Movie* [621] controls

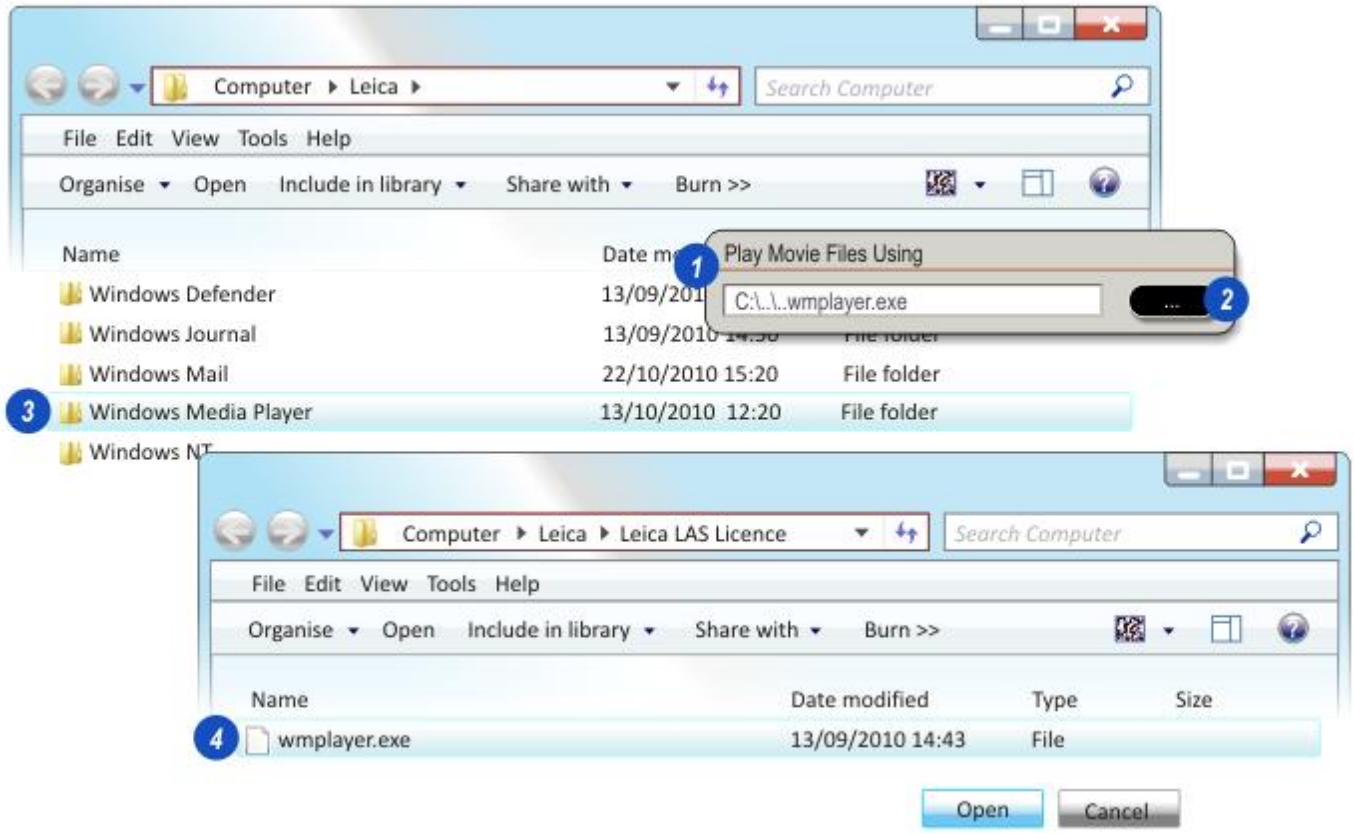


1: After a movie has been created, it may be played using a nominated application.

2: Still on the *Image* tab, click on the browse button to the right of the *Play Movie Files* text box and...

3: ... from the *Windows Navigator* navigate to...

4: .. and select the application required. Click on the *Open* button and the application name will appear in the *Play Movie Files Using* text box.

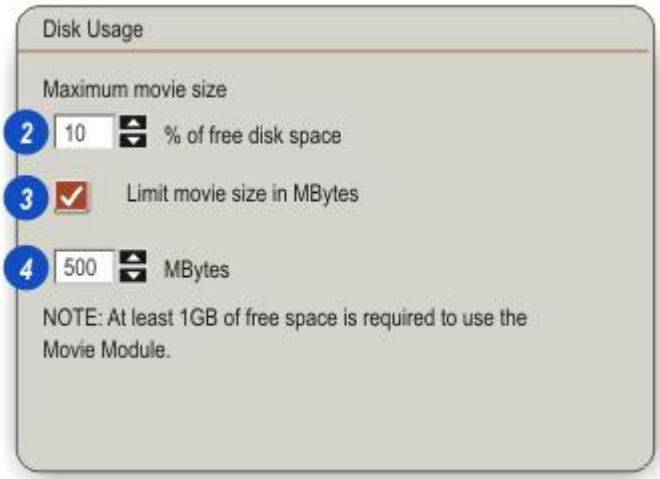


Because movies can be disk-space 'hungry', the *Movie Settings* tab provides two ways of limiting movie size:

- *Maximum Movie Size* limits file size in terms of free disk space whereas:
- *Limit Movie Size* prevents files exceeding a physical size measured in Megabytes (MBytes).

If *Limit Movie Size* is enabled, both features will run together to control movie size.

1: Click on the *Movie Settings* tab to reveal the *Disk Usage* panel.



Limit the size of movies as disk space:

2: Click on the up/down arrows to the right of the *Maximum Movie Size* window to increase/decrease the percentage of disk space that can be allocated to movies.

Note that at least 1 Gigabyte (GByte) of free disk space is required simply to run the movie application.

Limit movie files to a specific size:

3: Click on the *Limit Movie Size* checkbox to enable size limiting. The checkbox will become red with a white tick.

4: Click on the up/down arrows to the right of the *Limit Movie Size* window to increase/decrease the maximum file size. Each click is a 1MByte step.

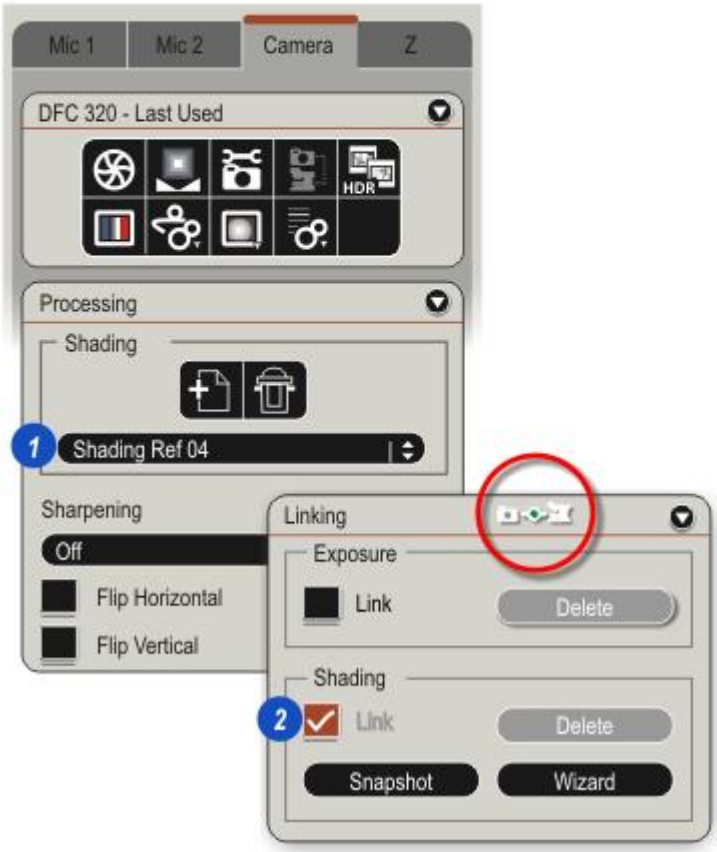
Shading refers to variations in the background light level across an image.

The image on the left shows how the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even 'illumination' on live images can be achieved in software by applying a 'blank area' value to the entire image area. The effect is shown in the right image.

Alternatively, a *Shading Link* can be created which corrects the shading effect on individual objective and illumination setups.

Expand either the *Processing* or the *Linking* panel depending upon the shading type to be used:



1: On the *Processing* panel select a user configured *Shading Reference*. *More information* [307].

2: On the *Linking* panel click to enable (tick mark displayed) the *Shading* check box. *More information* [341]

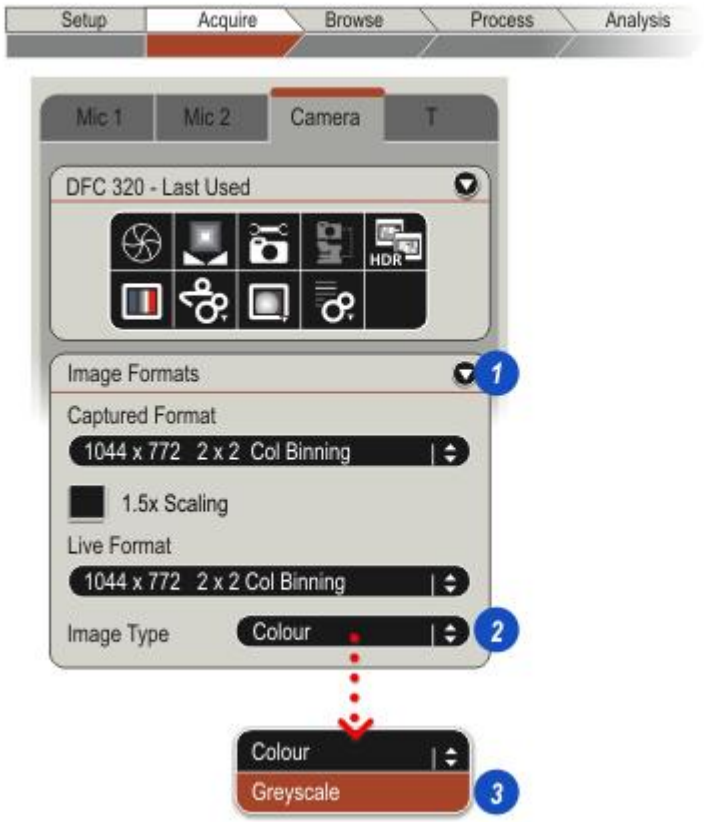
Both colour and monochrome (Greyscale) images are suitable with *Movie*.

On the *Acquire Workflow* select the *Camera* tab and check that the *Image Type* is set to *Colour*;

1: Click the arrow to the right of the *Image Formats* header and check the *Image Type.*

2: Change to *Colour* by clicking on the arrows to the right of the *Image Type* header and...

3: ...clicking to select the *Colour* or *Greyscale* option.

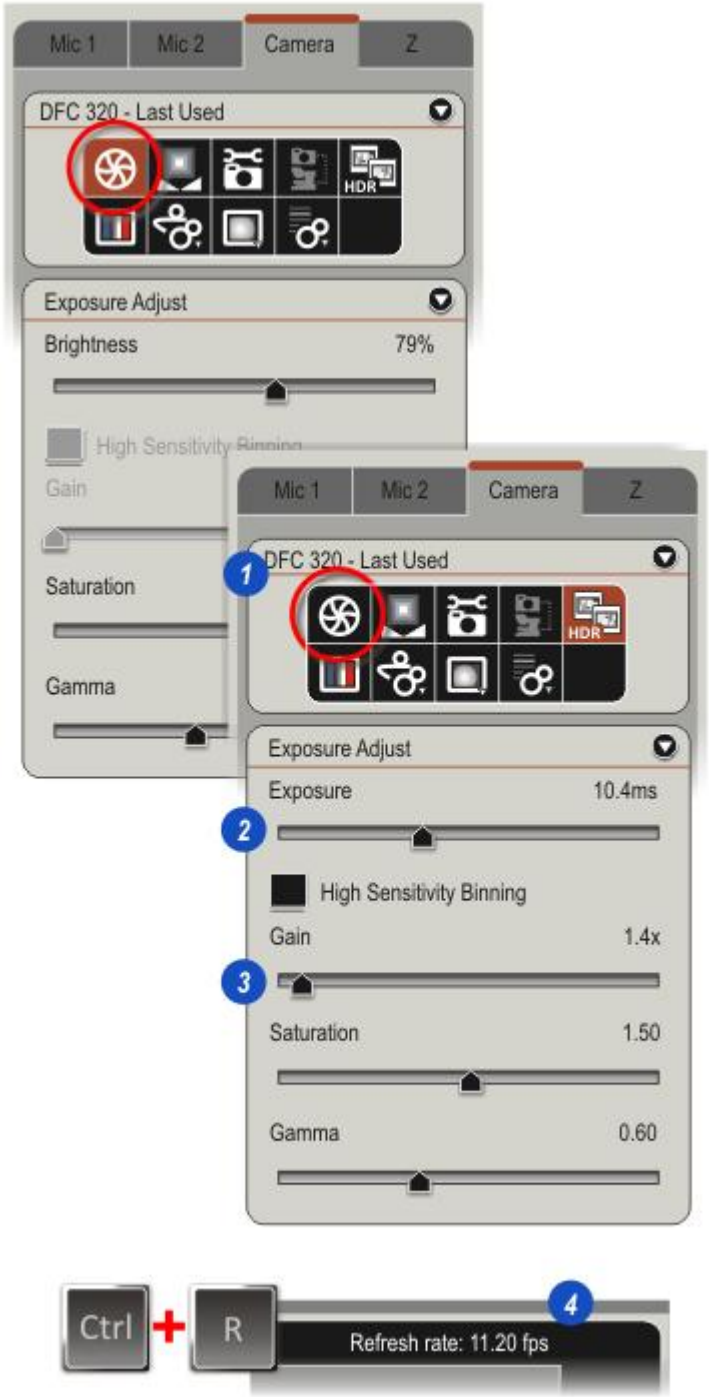


There are two options for adjusting the exposure:

- *Automatic* with some fine-tuning, and...
- *Manual* with a range of precision controls.

As a start, using *Automatic Exposure* is a good option because combined with *Automatic White Balance* it could produce a perfectly acceptable image very quickly.

Information about *Exposure* 282



1: Use the *Manual Exposure Adjust* to make fine adjustments to:

2: The *Exposure* time balanced with...

3: ...small increases in *Gain* to achieve scans of acceptable quality in reasonable times.

Users will ultimately aim for a camera frame rate (the number of images the camera can capture in 1 second) of between 18 and 24 frames per second (fps).

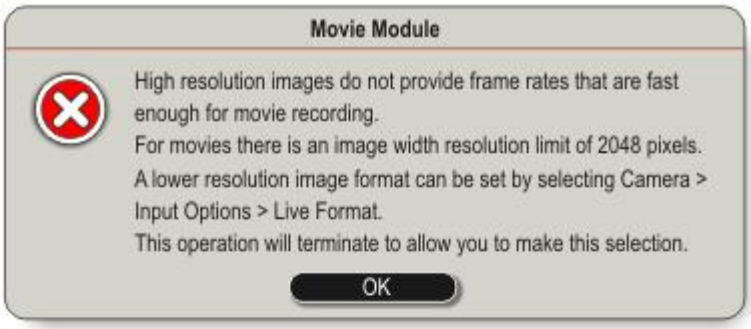
However, *Exposure* only plays a part in achieving a high frame rate - the next step, selecting the *Live Image Format* has a major effect.

The actual frame rate is shown on the *Viewer* border and changes as the *Live Image Format* changes so shows how close the 18 - 24 frames per second target is:

4: With the *Camera* tab selected, press and hold down the keyboard *Ctrl* key and then press the *R* key to reveal the camera *Refresh rate* as fps - *Frames per Second* - top right on the *Viewer*.

- *HDR* and *AVG* are disabled with *Movie* selected.

The *Live Format* determines the quality of the image displayed in the *Viewer* affects the speed at which the camera can capture fields - the camera frame rate (fps = frames per second). A high resolution format will slow capture and processing and, if very high will stop the module and display the *Movie Module* warning.

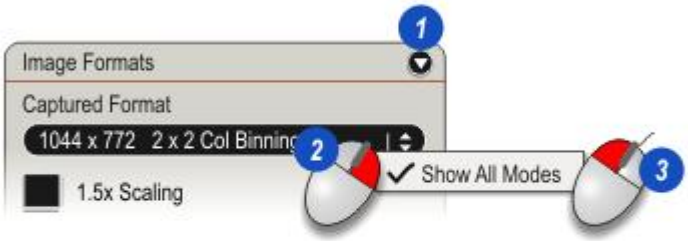


On the Acquire Workflow and Camera tab:

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.

2: Right-click on either the *Live* or *Captured Format* headers and...

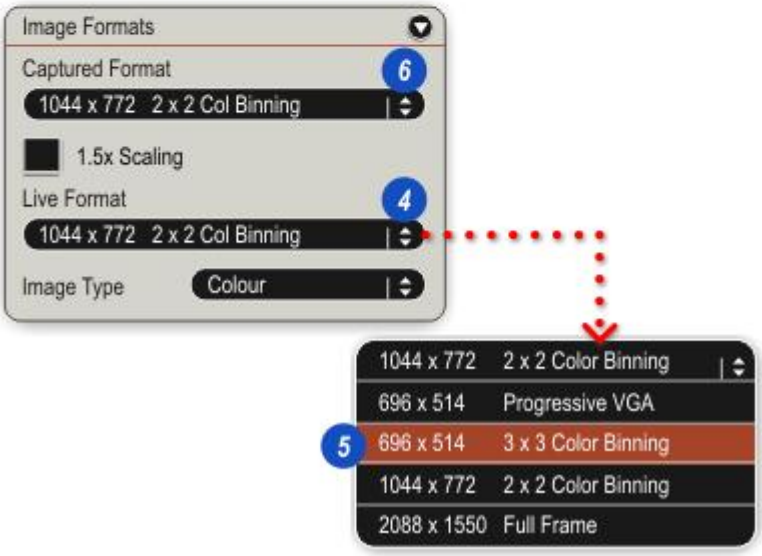
3: ...left-click to turn on *Show All* modes which will display all of the formats that the camera is capable of displaying.



4: Click on the arrows to the right of the *Live Format* header bar and...

5: ... from the menu click to select a format. If the camera supports a wide range of formats, small *Scrolling Arrows* will appear top and bottom of the drop down list. Click to scroll up and down.

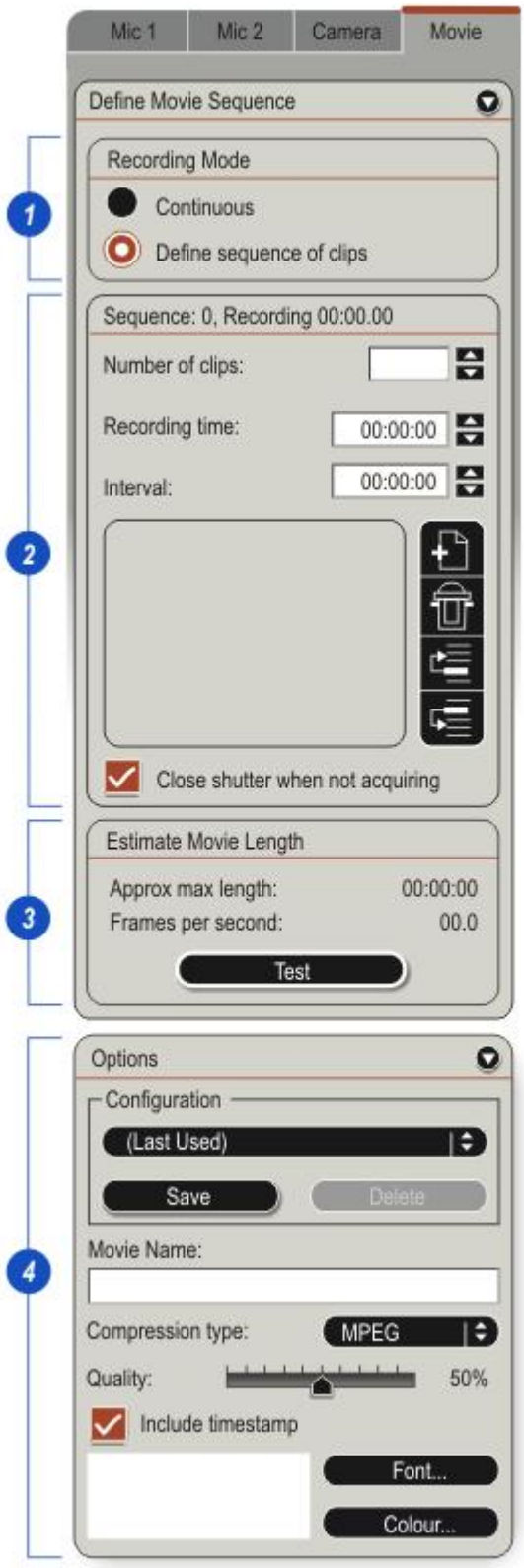
- A *696 x 514 2 x 2 Colour Binning* (depending upon the camera) format is a good starting point.
- Check the camera frame rate (*Viewer* border top right) with a target of 18 to 24 fps.
- Choose a lower format if the camera frame rate is too low.



6: The *Captured Format* does not affect *Movie*.

The *Acquire* > *Movie* interface comprises two main panels:

- *Define Movie Sequence* and...
- *Options*.



On the Define Movie Sequence panel:

1: Users select the *Recording Mode*:
Continuous will grab images at the highest possible frame rate, saving them to the hard drive until the space allocated to *Movie* is used up. The amount of disk space reserved is set in *Preferences*[612].

Define Sequence of clips provides for almost any number of differing length movies - clips - to be captured with an interval between. Clips can be assigned to *Sequences* with varying recording duration and intervals.

2: *Sequence Setup:*
Only available when *Define sequence*... is selected, these controls set up the recording times and intervals.

3: *Estimate Movie Length:*
Can be used in either mode to provide a calculated estimate of the movie duration. Intervals are not included in the calculations, only the recording times.

On the Options panel:

4: *Options:*
Allows the current settings to be saved as a *Configuration* that can be retrieved from disk to automatically set up an identical movie session.

The *Movie* is given a *Name* here...

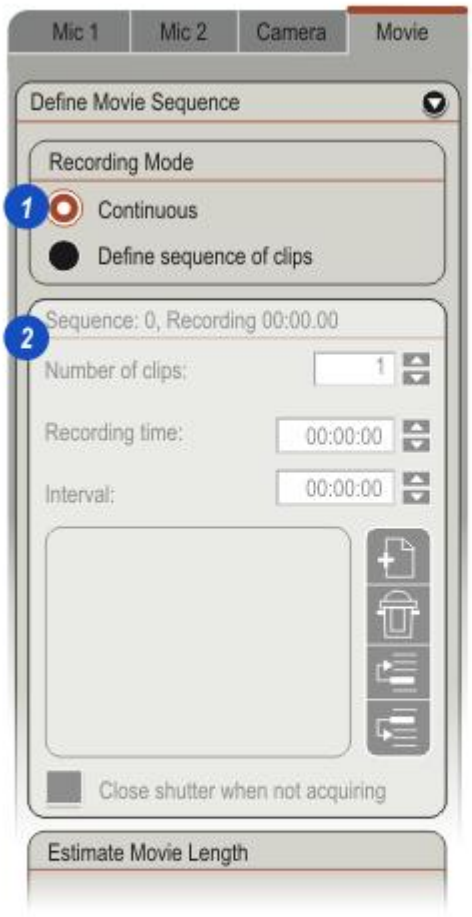
...the *Compression* level is set...

...and, if needed a *Timestamp* merged and displayed with the Movie.

To create a Movie the duration of which is limited only by the amount of hard drive space allocated:

1: Click to select the *Continuous* option on the *Recording Mode* panel.

2: The *Sequence* panel is disabled in this mode.



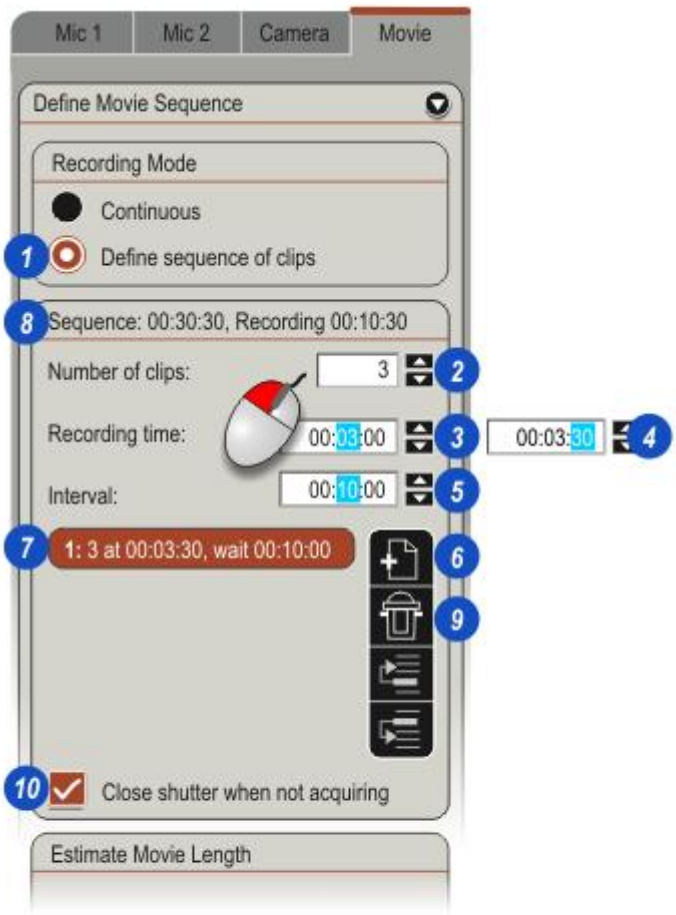
Before estimating the movie duration, users should:

- *Name* the *Movie*.
- Set the *Compression Level* (Quality).
- Determine if a *Timestamp* is required and if it is, set up the *Font* and *Colour*.
- Save the settings as a *Configuration* if required again in the future...

...all in *Options* 621

Clips are generally short movies and in the *Movie* module they can be grouped together as a *Sequence*.

Additionally, an *Interval* can be set between each clip when capture is suspended for a given amount of time. Intervals are not part of the movie, only a means of avoiding periods of inactivity in the specimen and saving disk space and processing time.



On the Recording Mode panel:

1: Click to select *Define sequence...*

On the Sequence panel:

2: Use the up/down arrow buttons to set the number of clips required.

3: Set the *Recording* duration for the clips - they will all be the same length - by clicking on either *hours:minutes:seconds (00:00:00)* and setting a value with the up/down arrows.

4: In the example the Recording time has been set to 3minutes and 30 seconds.

5: Set the *Interval* between clips in the same way. During an *Interval,* image capture is suspended and only re-starts when the interval has elapsed. The interval delay is not part of the movie - the end of one clip butts the start of the next - but the specimen action has moved on.

6: Click the *Add Sequence* button and...

7: ...the *Sequence* is displayed together with...

8: ... a simple analysis across the panel header.

In this example there are 3 clips, each lasting 3 minutes and 30 seconds. Between clips *1 & 2* and *2 & 3* an Interval of 10 minutes occurs. An *Interval* is not added to the end of the movie.

9: Delete a *Sequence* by clicking to select and clicking the *Delete* (Trash Can) button.

10: To protect delicate specimens from heat and light, check to enable (Tick mark visible) the *Close Shutter...* check box. This will close the light source shutter whilst images are being written to disk.

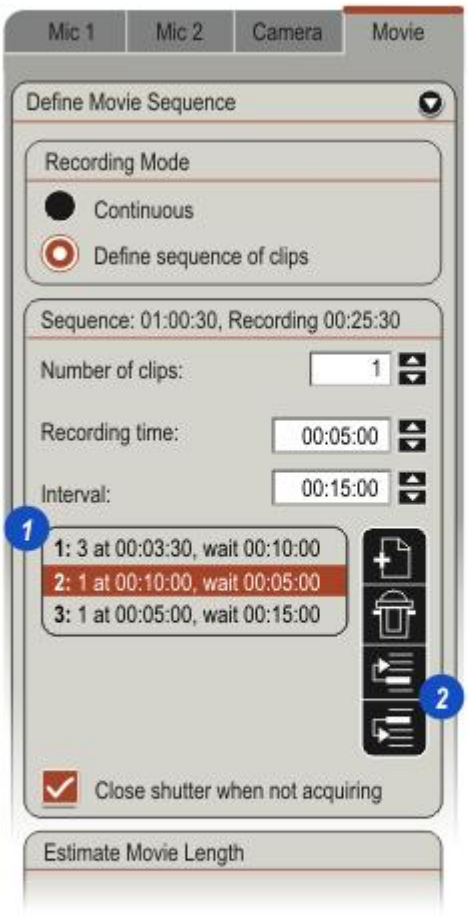
In the same way that *Clips* are grouped into *Sequences*, so sequences can also be grouped into a *Sequence List* with each containing different recording times and intervals.

Images are captured in *Clip* sequence and then *Sequence by Sequence* until all are complete.

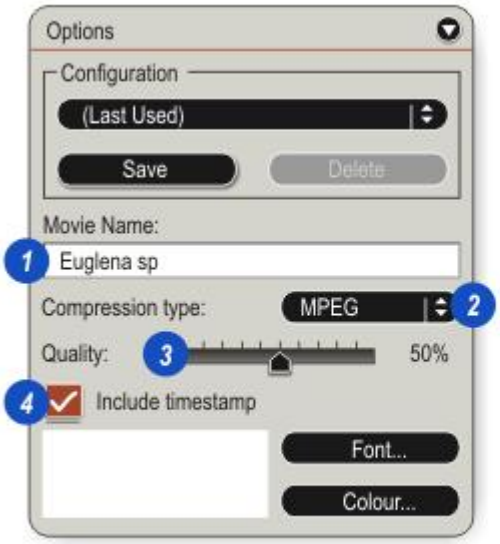
Each *Sequence* is created using the process described on the previous page and each is added to the *Sequence List* (1) by clicking the *Add Sequence* button.

Intervals as specified are added between sequences. So, the first sequence will have a 10 minute interval between each clip, plus 10 minutes at the end before the second sequence starts.

Individual sequences can be clicked to select and deleted with the *Delete* (Trash Can) button, or moved up and down the *Sequence List* (2) - changing the time at which it is implemented - with the *Move up* and *Move down* buttons.



On the Options panel:



1: Click inside the *Movie* name text box and type a unique name for the movie. It is automatically prefixed with *'M-'* to indicate it is a movie.

If the user does not provide a name the default *'M-Movie'* is used.

If there are multiple *Clips* within a *Sequence* then each is saved as an .avi movie with the name 'm-' plus an automatic numerical suffix. When the entire movie is played the individual clips are displayed seamlessly in sequence, but because the clips are saved and shown separately in the *Gallery* they can be clicked and played individually.

2: Click on the small arrows to the right of the *Compression Type* header to reveal the compression options. Click to select the required compression type. The default is *MPEG.*

3: The *Quality* slider set the *Compression Level* where:

- 0%: No compression, large file, good quality and...
- 100%: Maximum compression, smaller file, acceptable quality.

4: A *Timestamp* - the time in *hh:mm:ss* at which the movie was created - can be included to display top right corner of the movie. Click to enable the *Include timestamp* check box.

1: With the *Include Timestamp* check box enabled:

Change the Timestamp Font:

2: Change the font properties by clicking on the *Font* button.

3: On the *Font* dialog, use the side scroll bars to locate the required font and click to select it.

4: Click to select the *Font Style* – bold, italic etc, and…

5: …the *Size* in points.

6: Click *OK*.

Change the Font Colour:

7: Click on the *Colour* button and on the *Select Colour* dialog choosing a new colour by...

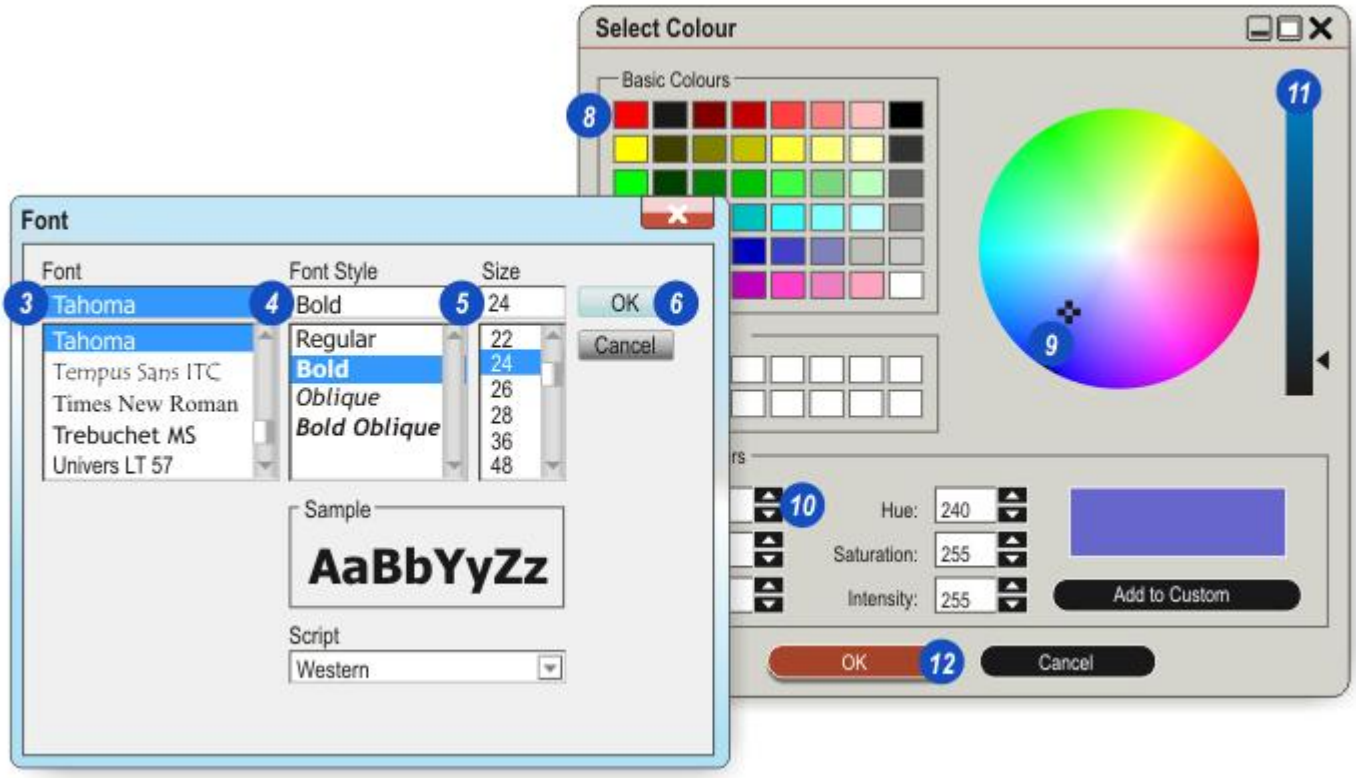
8: …clicking on a standard colour swatch, …

9: …clicking and dragging the small 'target' on the *Colour Wheel* or …

10: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range *0* to *255.*

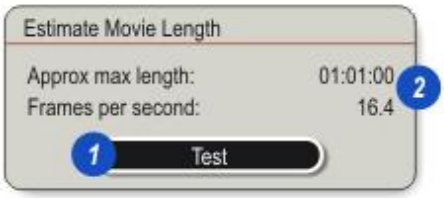
11: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

12: Click *OK.*



To calculate the movie length:

1: On the *Estimate Movie Length* panel click the *Test* button and...



2: ...the *Frame Rate* and duration in *hh:mm:ss* are displayed.

Save, Recall and Delete a Configuration:

3: Save the movie settings as a *Configuration* that can be recalled and applied at any time to re-create the movie setup, by clicking the *Save* button...

4: ...and on the *Save Configuration* dialog, click inside the text box and type a unique name for the configuration.

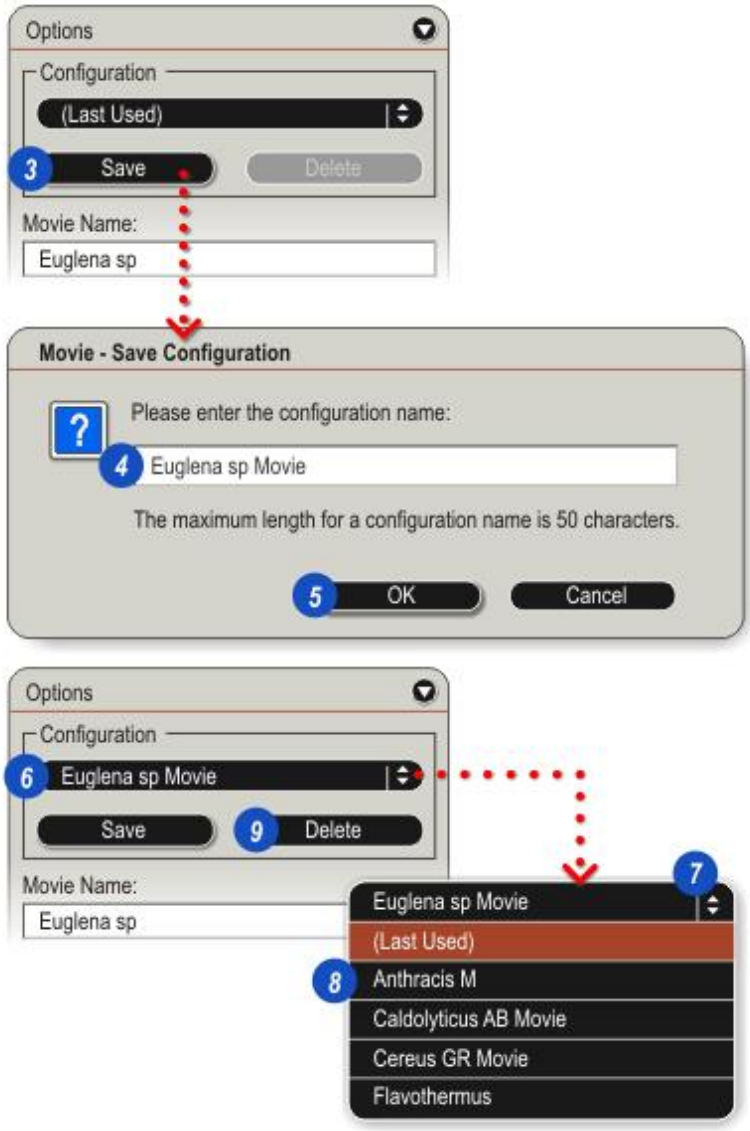
5: Click *OK.*

6: The new name is displayed on the *Configuration* header.

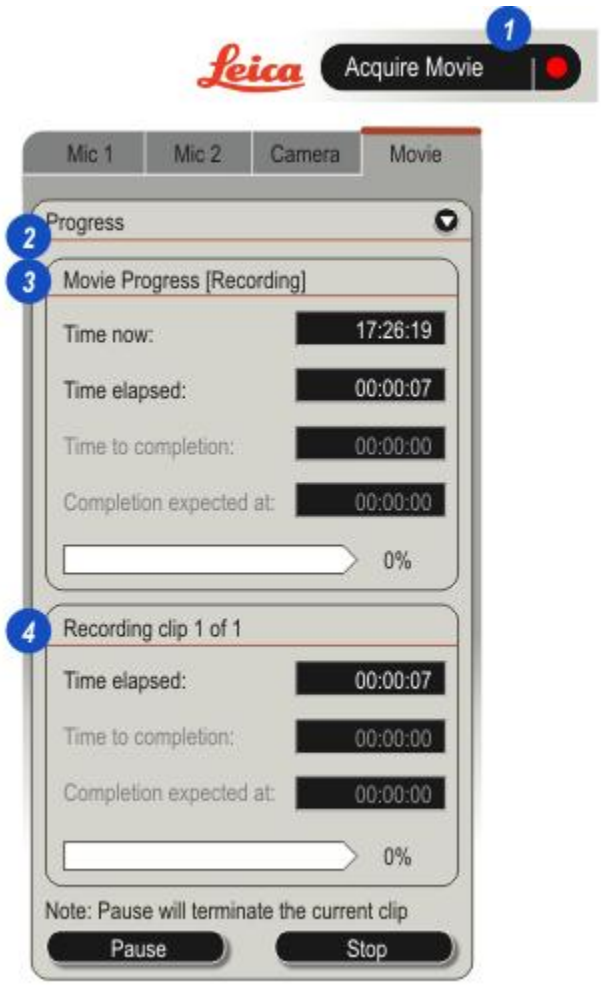
7: Almost any number of configurations can be saved and accessed by clicking on the arrows to the right of the header...

8: ...and clicking to select a configuration from the list.

9: The selected configuration can be removed by clicking the *Delete* button.



With the Continuous Recording setup complete:



1: Click on the *Acquire Movie* button.

2: The *Progress* panel opens with two sub-panels:

3: The overall *Movie Progress* the header of which changes to indicate either a *Recording* phase or an *Interval* phase. Times are shown in *hh:mm:ss* for:

- *Time now:* The current time.
- *Time elapsed:* Time into the movie.

4: Progress for individual clips as they are captured is shown on the *Recording Clip* panel:

- *Time elapsed:* Time into the clip.

  In *Continuous* mode the movie duration is limited only by the amount of disk space allocated to it (see *Preferences* 612), *Time to completion* and *Completion expected at* cannot be calculated precisely and are not displayed.

Stop and Pause

There are two controls on the *Progress* panel:

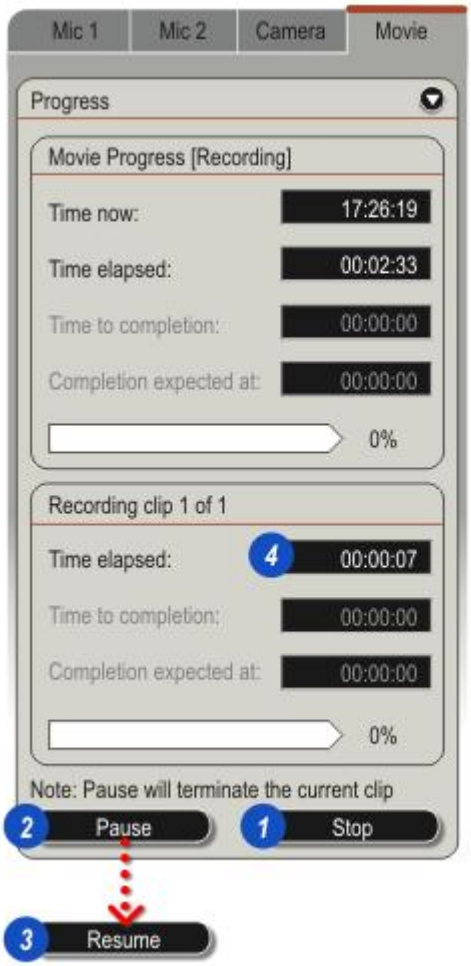
- *Stop:* Ends the movie immediately with the shortened movie saved and processed.
- *Pause:* Can be used as an 'on the fly' editor to skip periods of specimen inactivity.

1: Click the *Stop* button to end the movie and start processing.

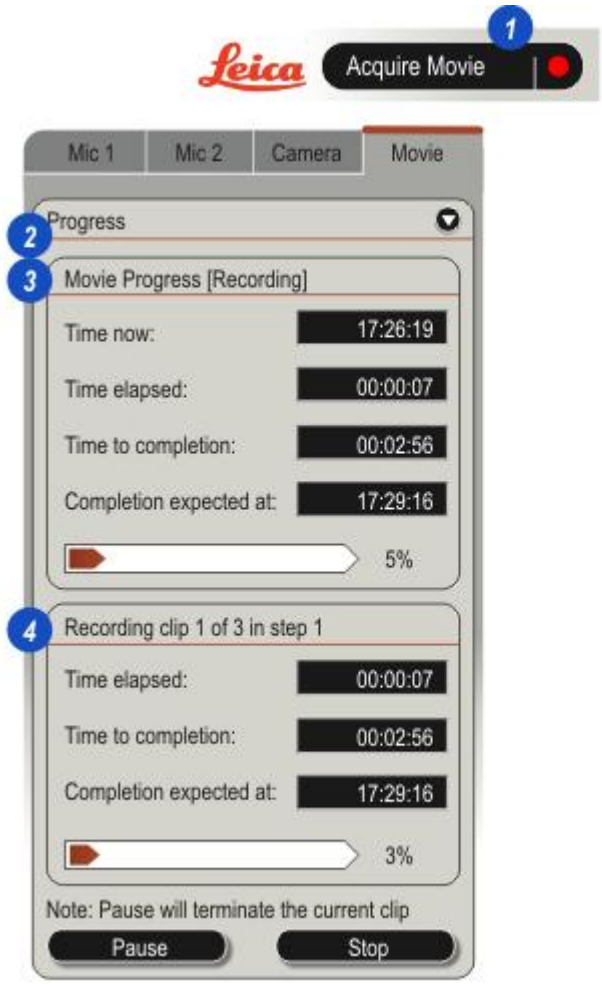
2: Click the *Pause* button to suspend image capture. Frames already captured are retained and processed being saved as a distinct clip with the name *'m-'* and an automatically incremented suffix.

3: The *Pause* button works as a toggle - the caption changes to *Resume* and clicking again will continue recording.

4: Time elapsed displays both the duration of the *Pause* and a 'resumed' capture.



With the Define Sequence setup complete:



1: Click on the *Acquire Movie* button.

2: The *Progress* panel opens with two sub-panels:

3: The overall *Movie Progress* the header of which changes to indicate either a *Recording* phase or an *Interval* phase. Times are shown in *hh:mm:ss* for:

- *Time now:* The current time.
- *Time elapsed:* Time into the movie.
- *Time to completion:*
- *Completion expected at:* Real time for completion.

  Visual progress is shown on the progress bar.

4: Progress for individual clips as they are captured is shown on the *Recording Clip* panel:

- *Time elapsed:* Time into the clip.
- *Time to completion:* For this clip or interval.
- *Completion expected at:* Actual time for clip or interval completion.

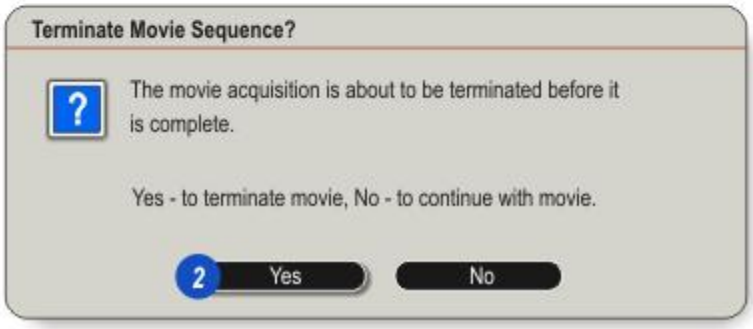
  Visual progress is shown on the progress bar.

There are two controls on the *Progress* panel:

- *Stop:* Ends the movie immediately with user confirmation, with the shortened movie saved and processed.
- *Pause:* Can be used as an 'on the fly' editor to shorten clips. It is only available when a sequence contains more than one clip.

1: Click the *Stop* button.

2: Click the *Yes* button on the *Terminate Movie Sequence* to end the movie.

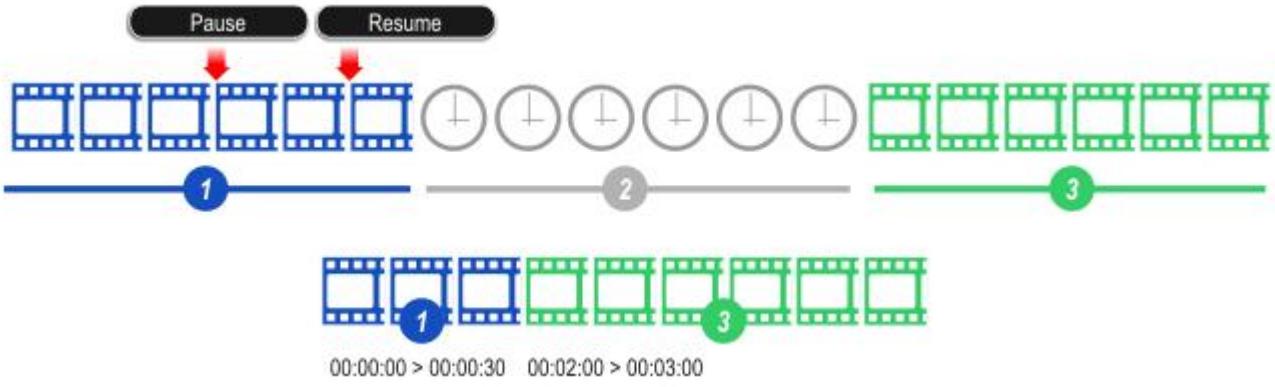


3: Click the *Pause* button to suspend image capture. Frames already captured are retained but no more are captured for the remainder of the remaining time allocated to the clip.

In the diagram below the sequence comprises a clip of 1 minute (1), an interval of 1 minute (2) and another clip of 1 minute (3).

The *Pause* button is clicked after 30 seconds and *Resumed* at 50 seconds but only the first 30 seconds of capture is kept.

Pause and intervals both occur in real time so the *Pause* button has no effect on intervals because they are equivalent. Capture of the second clip starts as scheduled at 2 minutes.



Movie creates a folder with either the user-specified name in *Options* or the default *M-Movie-* with an automatically incremented suffix.

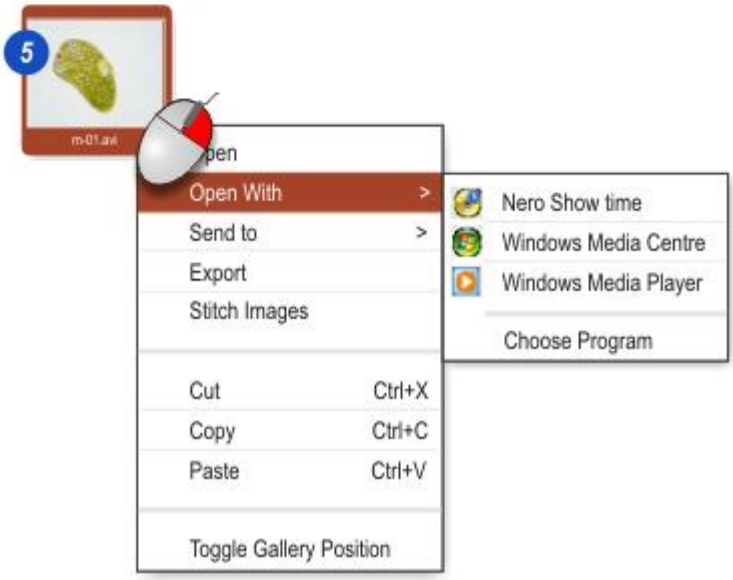
1: Within the folder are one or more clips each given the name *'m-'* plus an incremented suffix. The clips are represented by thumbnail in the *Gallery*. Any clip can be played individually by clicking to select it and then choosing any one of three possible options:

2: *Play in the LAS Viewer*[629]*:* Using the *Play Movie* controls

3: *Play in the default media player*[630]*:* Double-clicking the clip in the *Gallery* launches the computer media player

4: *Play with nominated media player*[611]*:* This is the media player selected in *Preferences* - if there is one

5: Alternatively, right-click on the clip thumbnail, from the drop-down menu click to select *Open With* and choose an application from the context menu.



When capture is complete the *Browse Workflow* is automatically displayed.

1: Click on the *Movie* tab to show...

2: ...the *Play Movie* panel and controls appear.

For playback in the LAS *Viewer* using the *Play Movie* controls:

3: If the *Gallery* is not displayed click the *Show Gallery* button on the *Side Tool Bar* (*****). Click on the thumbnail to select the clip to be played...

4: ...and the clip name appears in the *Current movie* window.

5: The *Clip Navigation* controls can be used to move back and forth between clips. They can also be used while clips are playing.

6: The play speed can be adjusted by clicking on the up/ down arrows. The value range is *1%* = Very slow to *100%* = Normal captured frame rate.

7: Click the *Play* button to start the movie. The button icon changes to *Stop* - click to halt playback.

8: Skip forward one frame...

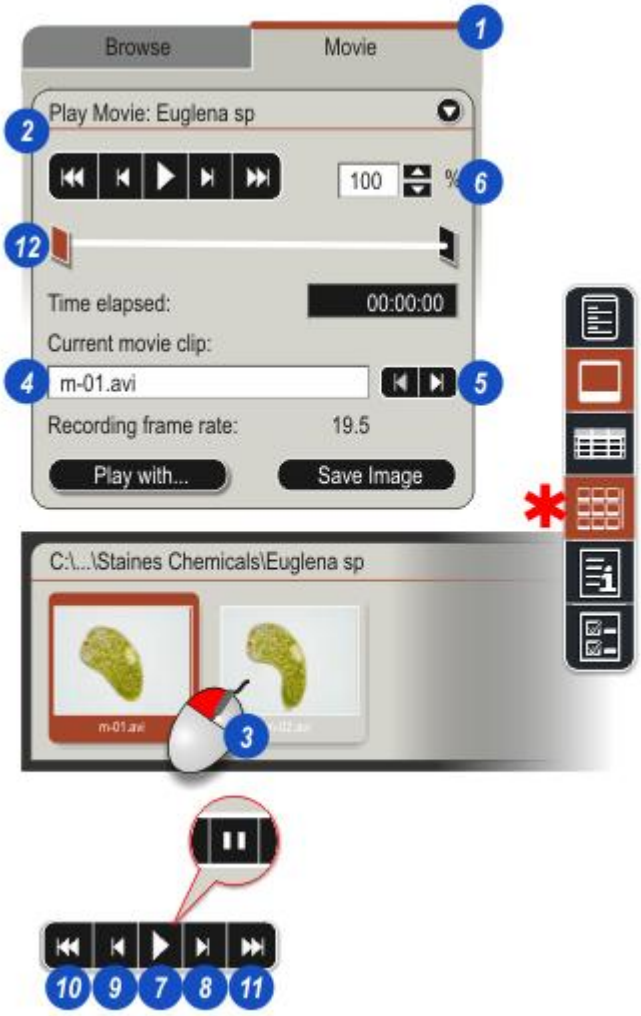
9: ...skip backward one frame.

10: Go to the start of the movie...

11: ...go to the end.

12: Move playback to any part of the movie by clicking *Position* slider and dragging it along the track.

If the clips are part of a sequence, play them all in order by selecting the first clip and then starting playback. Sequential clips are automatically detected and played.



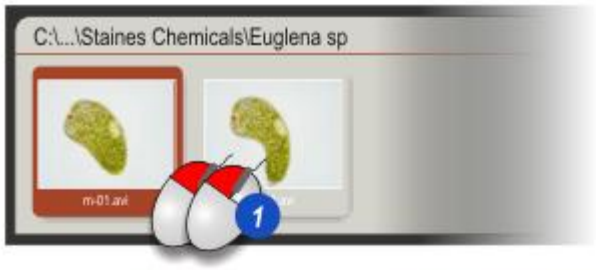
Play in Selected Media Player

The computer will have a *default* media player either chosen by the user or selected when the operating system was installed.

Users should check that the *.avi* format can be played on the default.

To play the movie in the default player:

1: Double-click on the clip thumbnail.



2: The default media player is launched and the movie starts to play. Movie control is achieved with the media player and not within LAS.

To play a movie with the nominated player:

3: In *Preferences*[611], users can nominate a media player to be used with *Movie*.

If a player has been selected, the *Play with...* button is available on the *Play Movie* panel.

Click to launch the nominated player and play the movie. Movie control remains with the player and not with LAS.

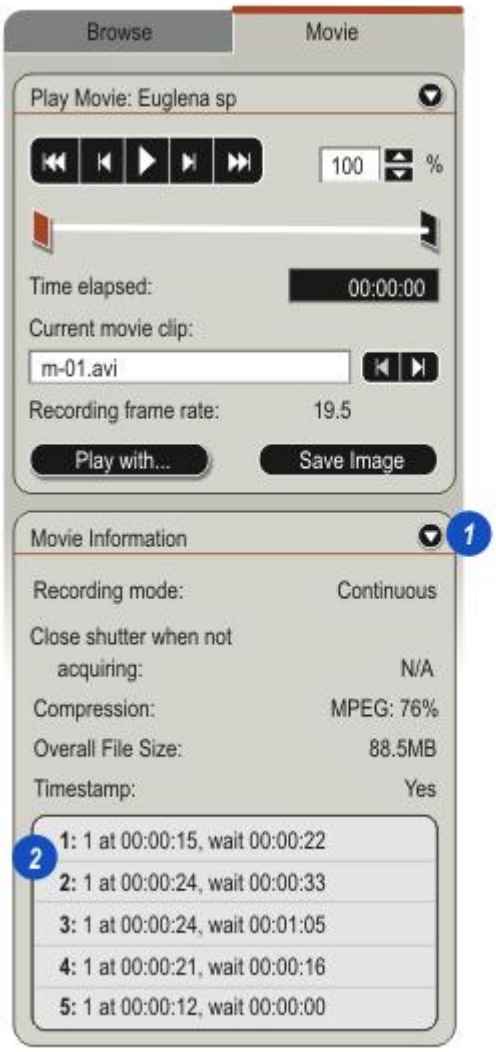
4: Clicking the *Save image* button copies the current frame to the *Fixed Folder* location, not to the movie folder.

Use *Save image* when the movie is not playing - before it starts or during a pause.

To display an analysis of the movie:

1: Click on the arrow to the right of the *Movie Information* header. The information is revealed...

2: ...with a list of all of the clips that make the complete movie, their duration and intervals.



Images obtained from microscopes have a known and limited depth-of -focus. For specimens with varying surface height, when the Z-position of the specimen is adjusted, different regions of the specimen appear in focus. By collecting digital images at these Z-positions, they can be combined by an image processing algorithm into one single sharp composite image that the effectively extends the depth-of-focus of the image.

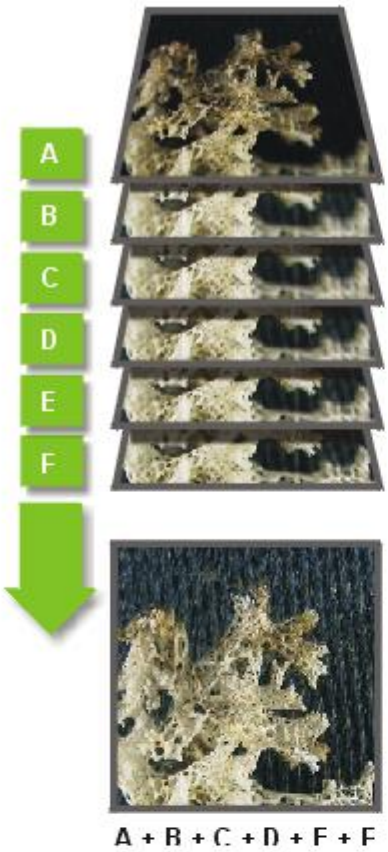
Specimens that can benefit from *LAS MultiFocus* are predominately those that are imaged by light reflecting from the surface such as geological and fossil specimens, plant and marine biology, histology and materials such as paper, electronic components, metallurgy, surface coatings and fractures

LAS MultiFocus provides fully integrated software controlling microscopes with motorised focus, cameras with high resolution and colour fidelity that are combined with a computer that comfortably handles digital images. The performance and relative economy of modern computing makes the use of sophisticated imaging algorithms practicable for acquiring and processing Z-Stacks of multiple images.

The principle is simple: An image that varies in focus, is taken at steps across the thickness of the specimen. These ‘slices’ are then mathematically combined to form a *MultiFocus* Image – all of the slices blended into a single, uniformly sharp image.

Almost any number of slices (together called a Z-Stack) can be captured – the higher the number, generally the better the *MultiFocus* Image – and each may be saved and examined.

The MultiFocus application is suitable for both manual and automatic microscopes.



Enable MultiFocus

MultiFocus is an optional module which must be installed and operational before use

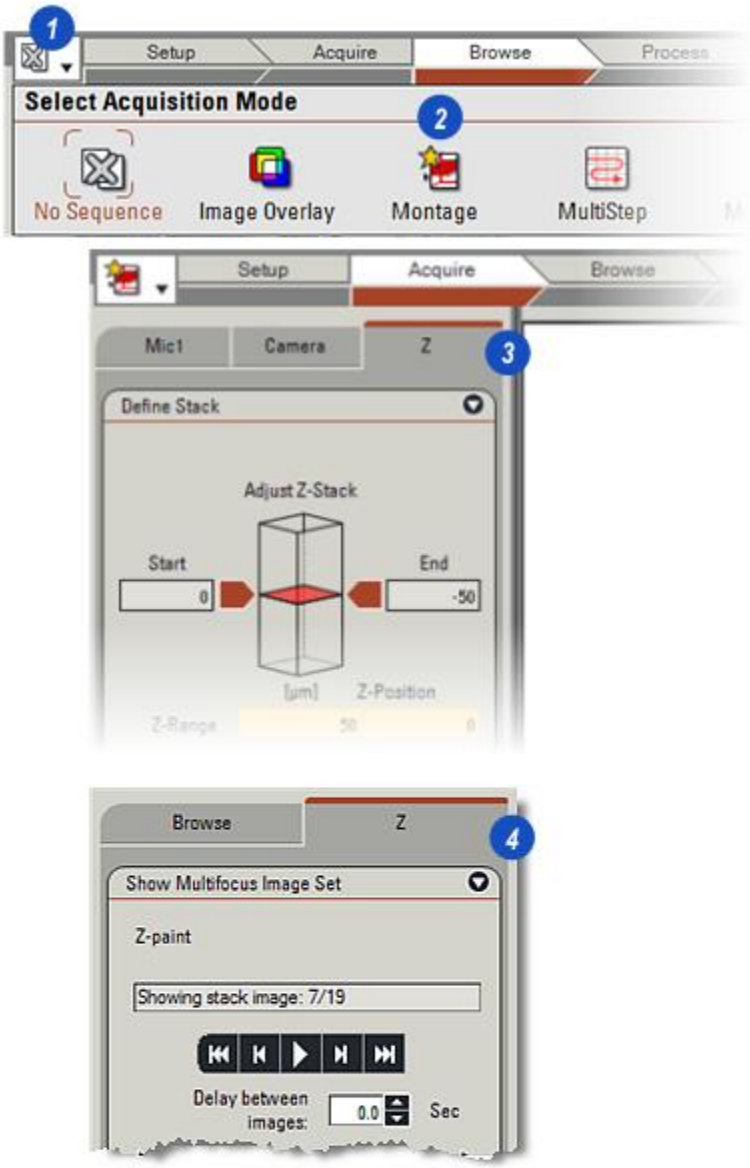
To enable MultiFocus:

1: Click on the *Module S*elect icon.

2: From the drop down menu click on the *MultiFocus (Montage)* icon to enable it. The icon will only be available if *MultiFocus* is installed.

3: The 'Z' logo appears on a new tab in the *Acquire Workflow* and…

4: Also in the *Browse Workflow.*

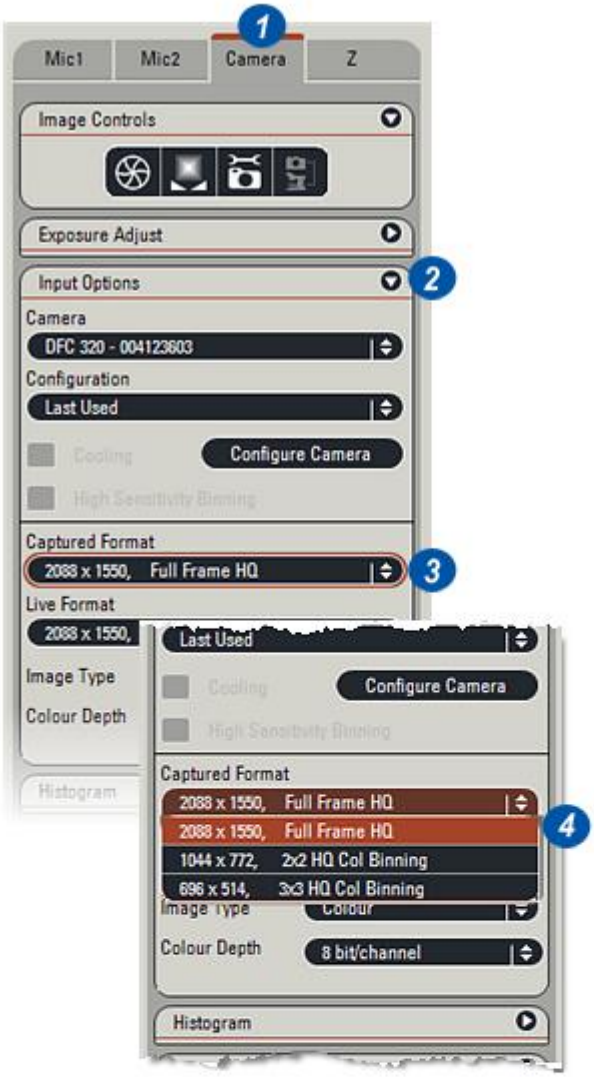


1: On the *Acquire Workflow* click on the *Camera* tab.

2: Click on the arrow to the right of the *Options* panel to reveal it.

3: Click on the arrow to the right of the *Captured Format* header bar and from the list of options…

4: Click to select the appropriate format. Thick specimens may require a large number of steps so consider a more 'compact' format that does not compromise quality but speeds the capture time, reduces disk space and makes processing quicker.



Select or Create Capture Folder

Check the *Preferences* 63 settings to ensure images have the correct settings:

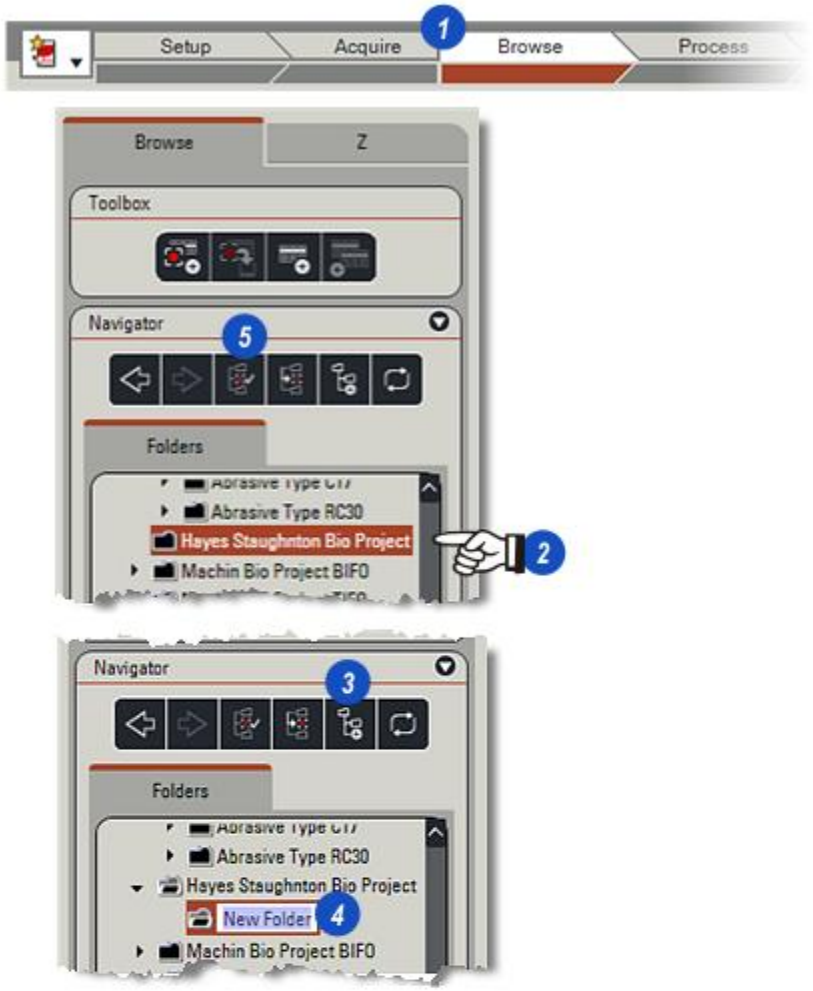
1: Click on the *Browse* Workflow and...

2: ...navigate to the folder in which to capture the images.

3: If a new folder is required click on the *New Folder* button and...

4: ...rename the folder appropriately.

5: Click on the *Set Capture Location* button to automatically save the images to the selected folder. Make sure *Capture to Fixed Location* checkbox in *Preferences* is enabled.



1: Click on the *Acquire Workflow* to select it.

2: Click on the *Z* tab to display the *MultiFocus*control panels.

3: If necessary, click on the arrow to the right of the *Options* header bar to reveal the Options panel.

Create MultiFocus after Stack Acquire:

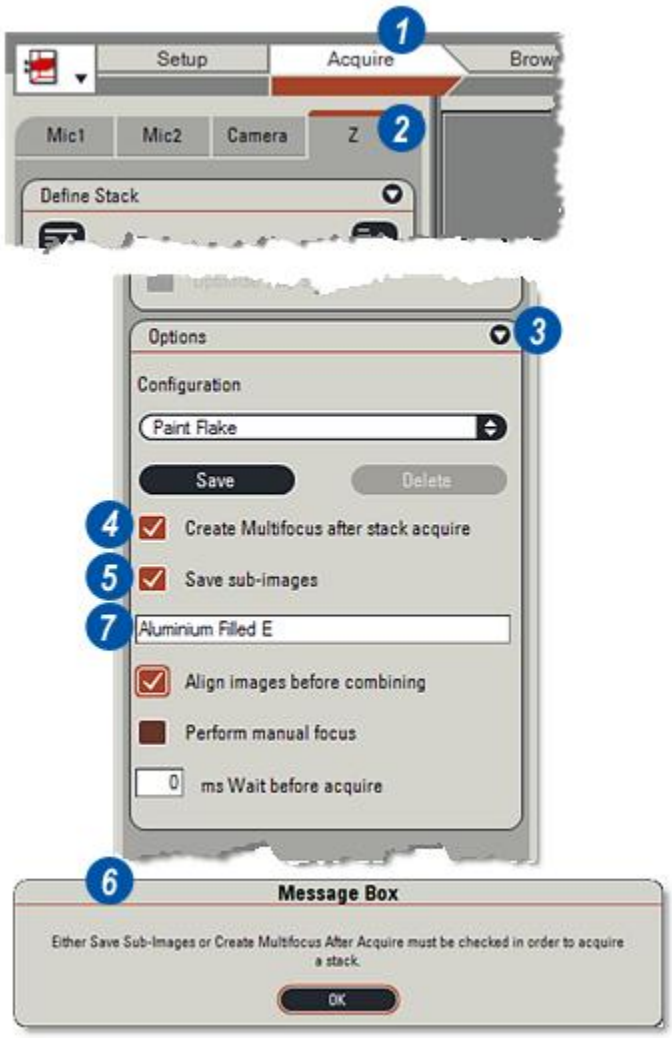
4: Selecting this option – clicking the checkbox – will automatically create a MultiFocus Image when the last Z-slice is acquired. Left un-checked, the *MultiFocus* Image can be created manually on the Viewer.

Save Sub-images:

5: If the *MultiFocus* Image is created automatically (See **4** above), it may not be necessary to save all of the Z-slices or sub-images. Check this option to discard the layers. *Save sub-images* must be selected if *Create MultiFocus* is unchecked otherwise the prompt **(6)** appears. If *Save sub-images* is checked, the *MultiFocus* Image cannot be re-created again; the entire process will have to be repeated.

Enter the Stack Name:

7: Click in the *Stack Name* text box and type an appropriate name for the Z-Stack. The letter Z is automatically prefixed to denote a *MultiFocus* group, and a sequential number is appended every time a new stack is acquired.



Align Images before combining:

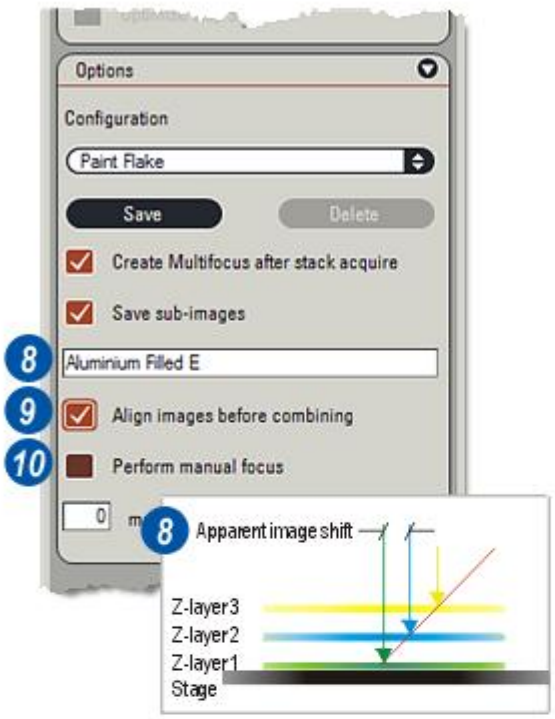
8: Optical variations - predominantly in stereo microscopes - can cause an apparent shift in the point of focus from layer to layer. The shift will be corrected in software if the *Align Images* option is checked so that all X-Y points of focus are in exact alignment.

Perform Manual Focus:

9: Check this option if the microscope does not have a motorised focus and each image is to be focussed manually.

Wait Before Acquire:

10: Measured in milli-seconds, this option inserts a delay between the *Acquire* button being clicked and the acquisition of the first layer. Click in the text box and type a value.



The *Configuration* facility allows *MultiFocus* Z-step settings and options to be saved, retrieved and used again.

Create a New Configuration:

1: Click in the *Configuration* text box and type an appropriate file name for the MultiFocus session.

2: Click on the *Save* button. The settings are saved to disk and the file name added to existing *Configurations*. After the *Z-Stack* has been set up, the Configuration will be saved again to include the new stack settings.

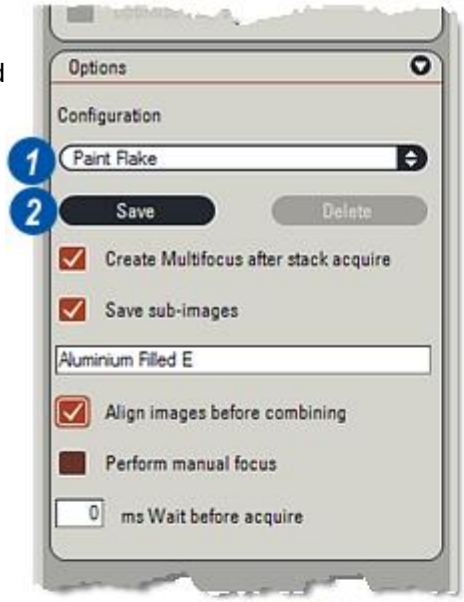
Retrieve an Existing Configuration:

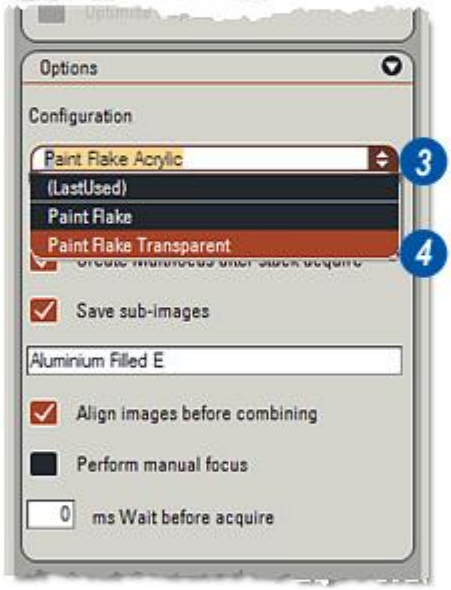
3: Click on the arrows to the right of the *Configuration* text box.

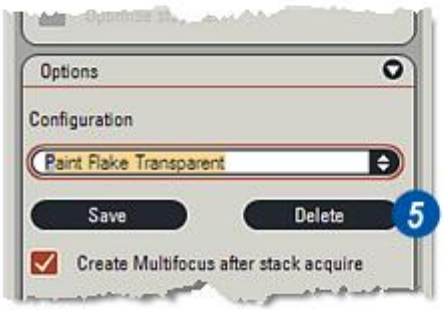
4: From the drop down list click to select the Configuration required. The *Last Used* option will automatically load the settings used during the last *MultiFocus* session.

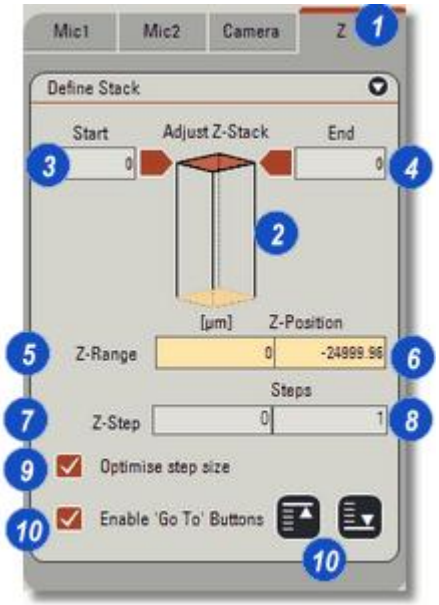
Delete a Configuration:

5: Click the *Delete* button to remove the Configuration displayed in the text box. The settings cannot be retrieved. The *Last Used* option may not be deleted.









1: If necessary, click on the *Acquire: Z* tab to reveal the *MultiFocus* control panels.
Defining the stack consists of setting the first Z position on the specimen, called the *Start*, setting the last Z position called the *End*, and then deciding the number of focussed 'slices' or steps to capture between *Start* and *End*.

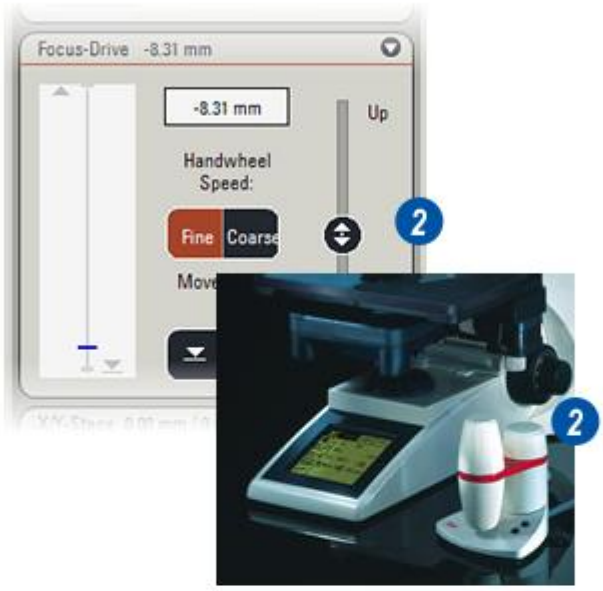
Start and *End* may encompass either the whole specimen from bottom to top or any part of the specimen. The positions chosen are displayed on a graphical 'stack' **(2)** with the *Start* position (**3)** shown as an arrow on the left hand side, and the *End* position as an arrow on the right hand side.
Windows attached to the arrows display the microscope focus values.

The *Z-Range* window **(5)** will show the numerical difference between the *Start* and the *End* positions.
Z-Position **(6)** is the current microscope focus position.
Z-Step **(7)** represents the distance between each of the Z-steps and *Steps* **(8)** is the number of images that will be captured.
The *Optimise step size* button **(9)** will determine the best step size based upon the microscope and the optics.
Go To buttons **(10)** will automatically move the objective to either the *Start* or *End* position.

Note: The *Go To* buttons **(10)** are disabled by default, so that an operator cannot move the stage unintentionally. To enable them, check the *Enable 'Go To' Buttons* box then click *OK* when you have read the warning.

Generally, the *Start* position is the part of the specimen closest to the stage. It provides the opportunity to place the specimen on a textured background which, when in sharp focus is a precise reference for the software. In subsequent 'slices' the background will be out of focus and so ignored.

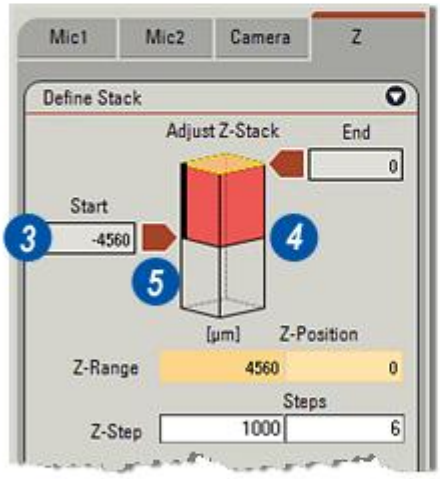
1: Click on the *Set Start* arrow. It will turn black indicating it is active and will automatically track the microscope movements.



2: Adjust the microscope either with its manual controls or by using the *Application Suite* interface, until the specimen at the Start position is in focus.

3: The microscope settings are reflected in the *Start* window and the Start arrow moves down the virtual ‘stack’ **(4).**

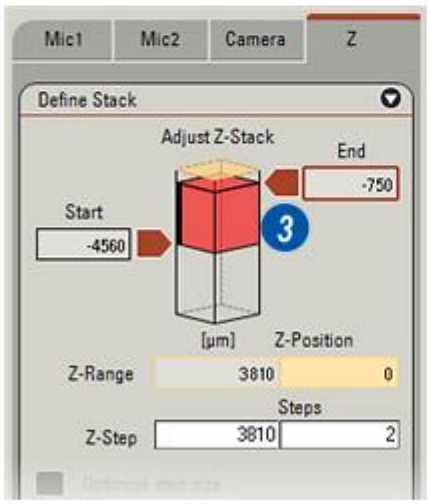
5: Click on the *Start* arrow which will become red and locked at the focussed position.



1: Click on the *Set End* arrow. It will turn black to indicate it is active and tracking the microscope movements.

2: Focus on the part of the specimen that represents the last Z-step using either the microscopes manual controls or the *Application Suite* interface. The *End* arrow will move along the virtual 'stack' in response to the microscope.

3: Click on the *End* arrow which will become red again and locked in the *End* Z-step position.



The illustrations show two images:

1: The *Start Z-layer* with part of the specimen in focus together with a sharp, textured background, and…

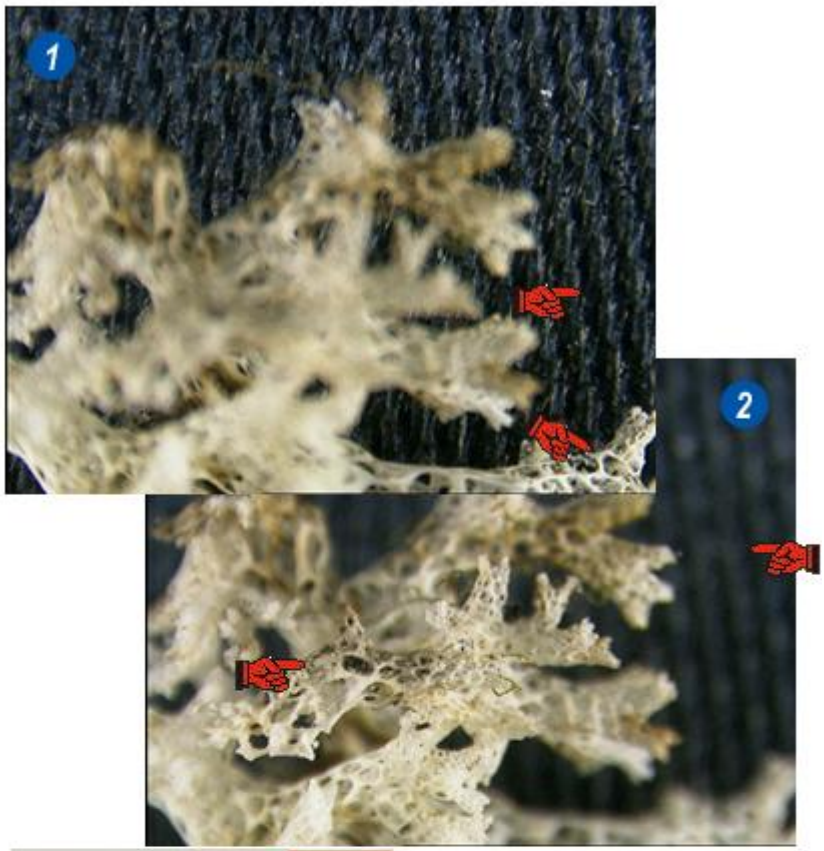
2: The *End Z-layer* with a different part of the specimen 'sharp' but the background out of focus.

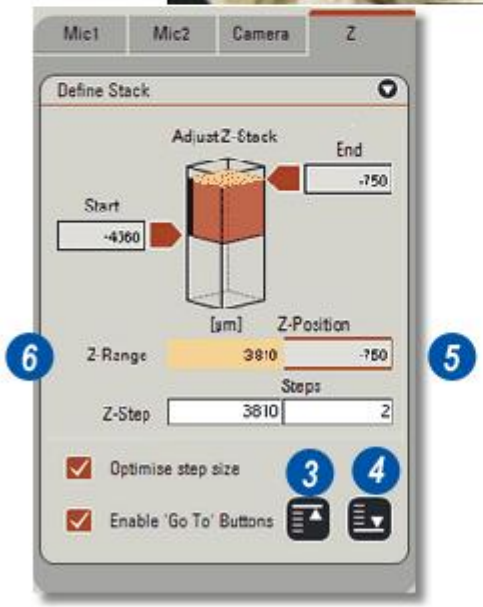
Using the 'Go To' buttons:

3: The *Go To Start* button when clicked will drive the microscope to the Start position with the value displayed in the *Z-Position* window **(5)** for a focussing check.

4: The *Go To End* button drives the microscope to the End position for a focussing check. Again, the microscope value is displayed in the *Z-Position* window **(5).**

6: The *Z-Range* window shows the distance between the *Start* and *End* positions.





Set Z-Stack Steps

With the *Start* and *End* points set, the distance between the two is calculated and displayed in the *Z-Range* and *Z-Step* windows.

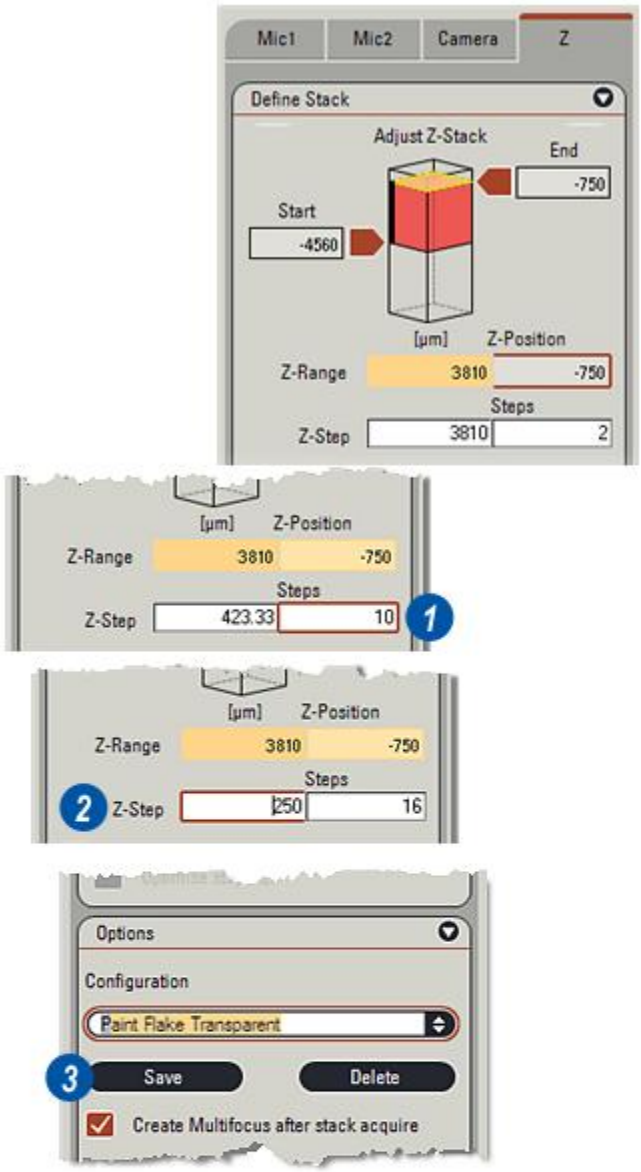
There are two methods of setting the number of layers or steps:

1: Click in the *Steps* text box and type the number of steps required. The distance between each is calculated and shown in the *Z-Step* text box.

2: Click in the *Z-Step* text box and type the distance required between each step. The range value is divided by the entered figure and the resulting number of steps displayed in the *Steps* text box.

3: With the Stack Definition complete, click the *Options: Save* button to save the settings.

See *Acquire Z-Stack Images.* 644

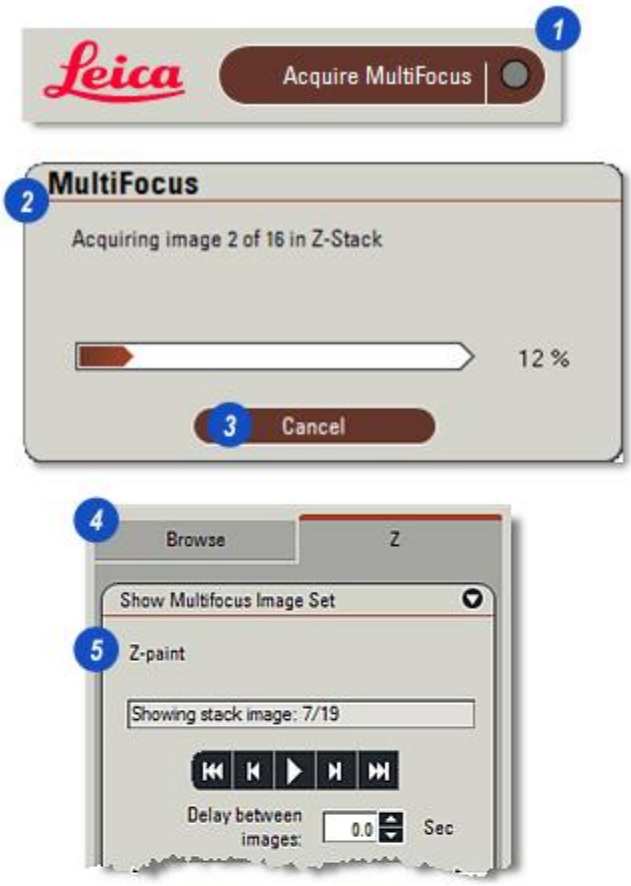


1: Click on the *Acquire MultiFocus* button to start the acquisition.

2: At each step position, the microscope will automatically move to the next step and focus. A progress message is displayed.

3: To cancel the sequence, click on the *Cancel* button.

4: When all of the images have been captured, the *Browse Workflow* opens with the *Show MultiFocus Image Set* control panel displayed **(5).**



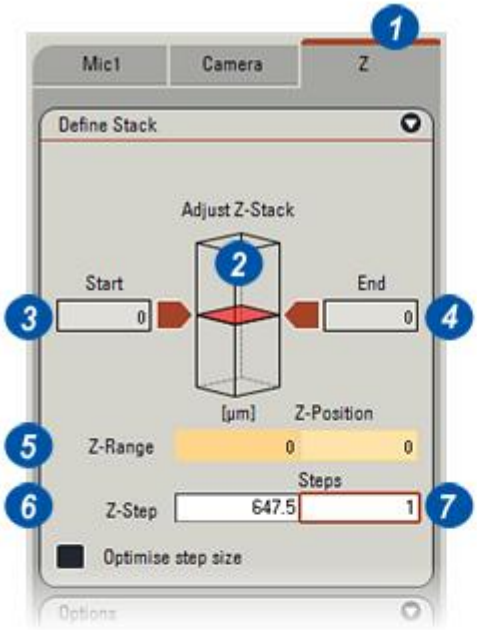
1: If necessary, click on the *Acquire: Z* tab to reveal the *MultiFocus* control panels.

Defining the stack consists of setting the first Z position on the specimen, called the *Start*, setting the last Z position called the *End*, and then deciding the number of 'slices' or steps to capture between *Start* and *End*.

Start and *End* may encompass either the whole specimen from bottom to top or any part of the specimen. The positions chosen are displayed on a graphical 'stack' **(2)** with the *Start* position **(3)** shown as an arrow on the left hand side, and the *End* position as an arrow on the right hand side.
Windows attached to the arrows display the microscope focus positions.

The *Z-Range* window **(5)** will show the numerical difference between the *Start* and the *End* positions.

Z-Step **(6)** represents the distance between each of the Z-steps and *Steps* **(7)** is the number of images that will be captured.



Generally, the *Start* position is closest to the stage because the specimen can be set against a textured background which will also be in focus. This gives the software a benchmark for the background so that the out-of-focus background of subsequent steps will be ignored.

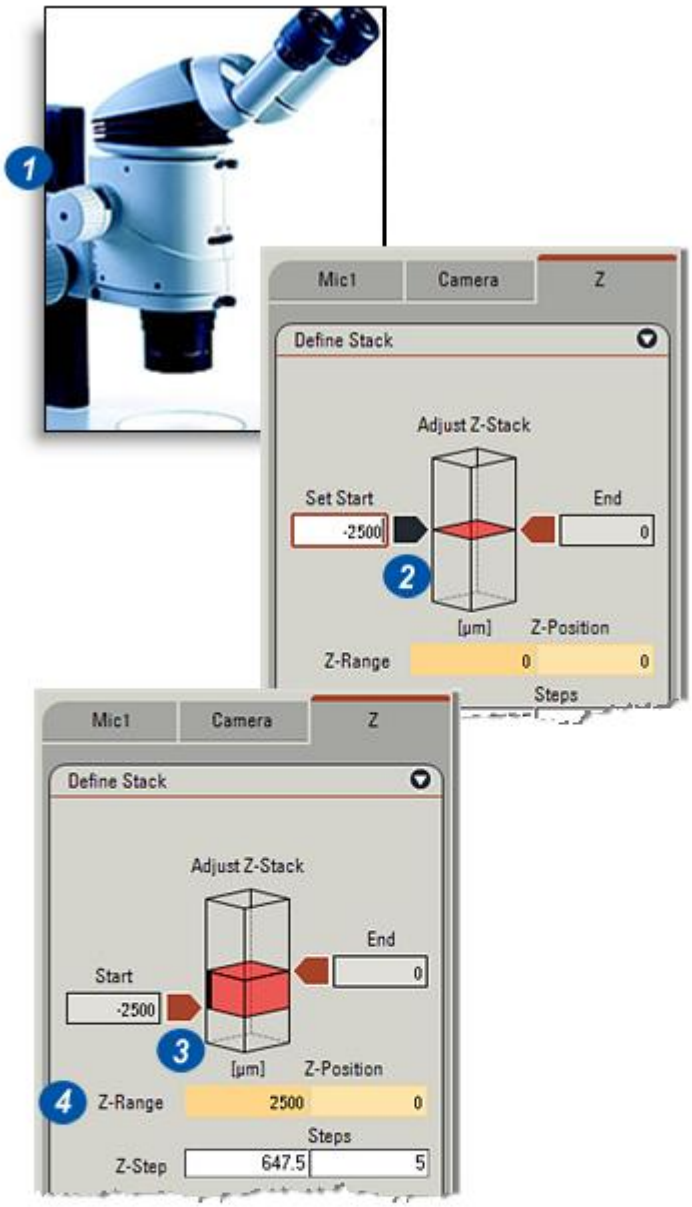
1: Focus on the start region of the specimen making a note of the microscope scale reading.

2: Click on the *Set Start* arrow which will turn black, and type in the scale reading.

3: Click on the *Set Start* arrow which will become red again. The virtual 'stack' displays a graphical representation of 'depth'.

4: The *Z-Range* value reflects the entered *Start* position.

See: Set End position: Manual Microscopes. 647



End Position

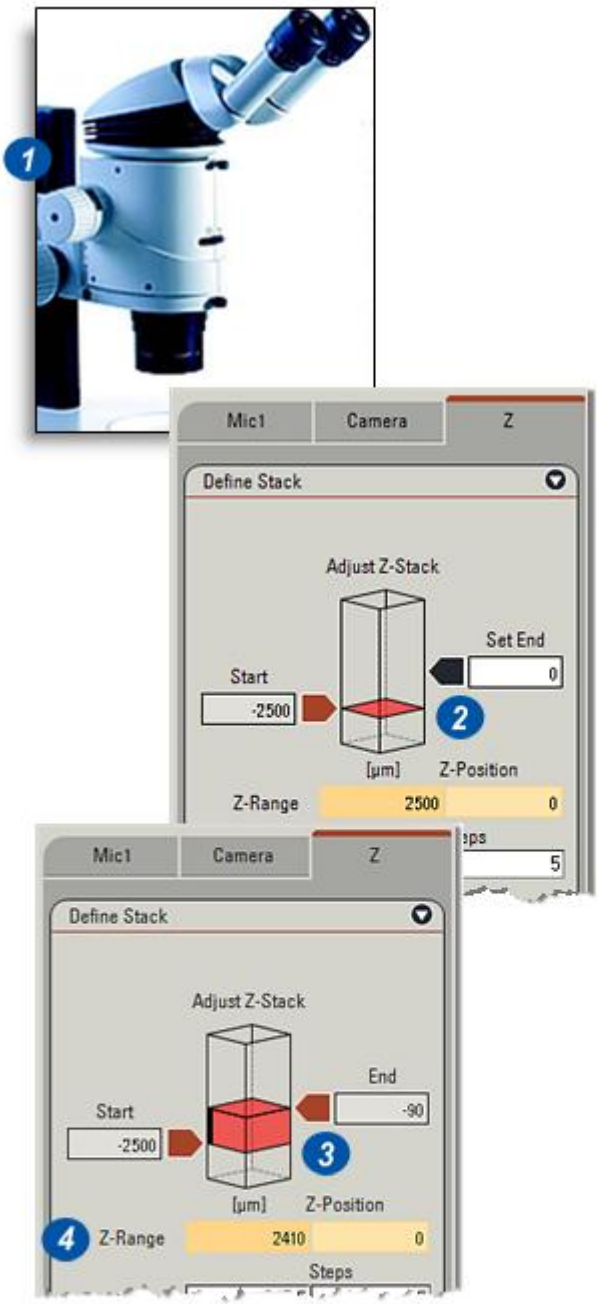
1: Focus on the region of the specimen that represents the *End* position. Make a note of the microscope scale reading.

2: Click on the *Set End* arrow which will turn black, and type the scale reading in the window.

3: Click on the *Set End* arrow which will become red. The virtual 'stack' will display a graphical representation of the distance between the *Start* and *End* positions.

4: The *Z-Range* window displays the difference between the *Start* and *End* microscope readings.

See: Set Start position: Manual Microscopes. 646



With the *Start* and *End* points set, the distance between the two is calculated and displayed in the *Z-Range* and *Z-Step* windows.

There are two manual methods of setting the number of layers or steps:

1: Click in the *Steps* text box and type the number of steps required. The distance between each is calculated and shown in the *Z-Step* text box, or...

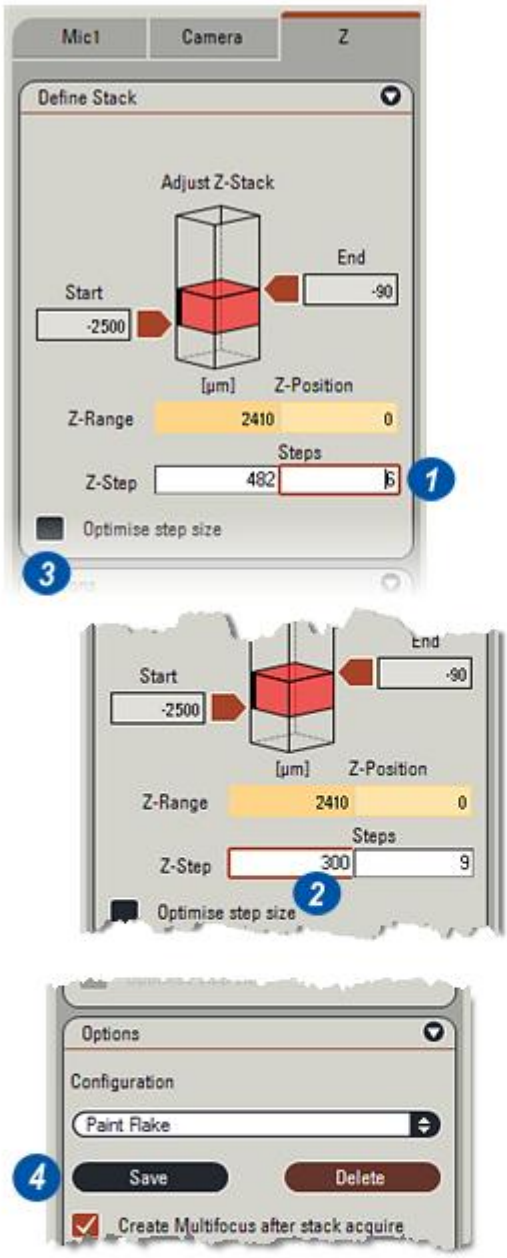
2: Click on the *Z-Step* text box and type the distance required between each step. The range value is divided by the entered figure and the resulting number of steps displayed in the *Steps* text box.

Optimise step size:

3: Enable the *Optimise step size* button to have the steps calculated automatically using the microscope aperture. On stereo microscopes check that the actual and displayed iris settings correspond.

4: With the Stack Definition complete, click the *Options: Save* button to save the settings.

See: Acquire Z-Stack images: Manual Microscopes. 650



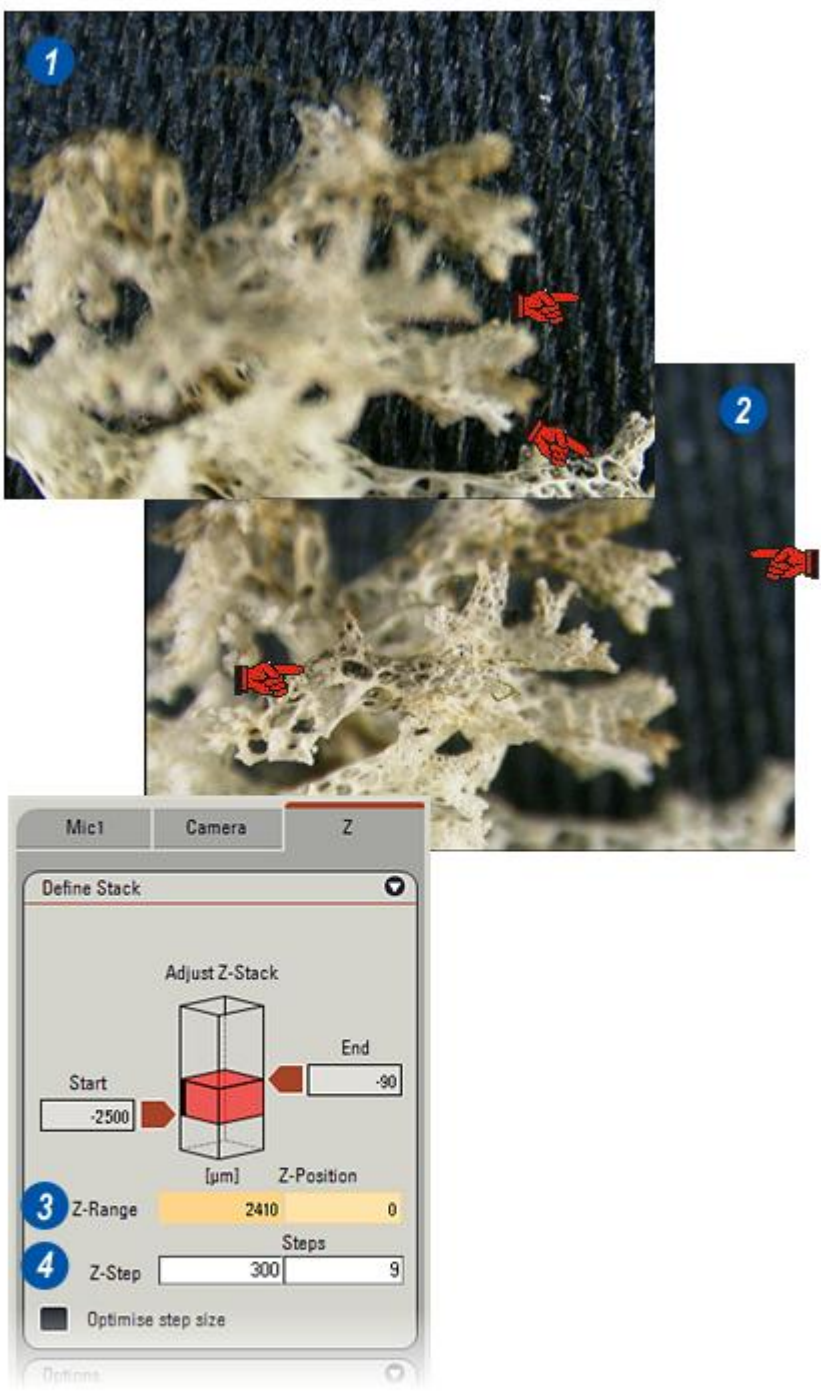
The illustrations show two images:

1: The *Start Z-layer* with part of the specimen in focus together with a sharp, textured background, and…

2: The *End Z-layer* with a different part of the specimen 'sharp' but the background out of focus.

3: The *Z-Range* window shows the distance between the *Start* and *End* Z-layers, and…

4: The number of steps and the 'size' of each step in the *Z-Step* window.

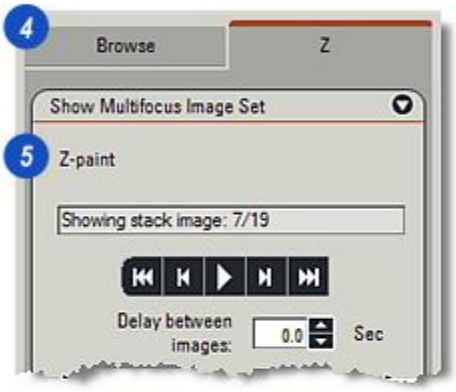


1: Click on the *Acquire MultiFocus* button to start the acquisition.

2: At each step position, the prompt appears. Re-focus the microscope using the *Z-Step* value, always focussing in the same direction.

3: Click *OK* to capture the image. To cancel the sequence if it is believed sufficient slices have been captured, click on the *Cancel* button. The number of captured images does not have to correspond with the calculated or entered number of steps.

4: When all of the images have been captured, the *Browse Workflow* opens with the *Show MultiFocus Image Set* control panel displayed **(5).**



When all of the *Multifocus* images are captured, the *Browse Workflow* opens with the *Show Multifocus Image Set* panel displayed.

The panel provides the controls necessary to view the entire set as a 'slide show', a single layer or the *Multifocus* Image.

1: Set the delay between images in seconds by:
Clicking on the *Delay Between Images* text box and typing a number or…

Using the up/down arrows to the right of the text box.

2: Click on the *Stack Images* button to select the images.

3: Click the *Play* button. It will change to red with the pause symbol.

4: The current image and total images are displayed in the *Progress Window* and…

5: ...an indicator moves across the *Progress Bar*as each image is displayed in turn in the *Viewer*.

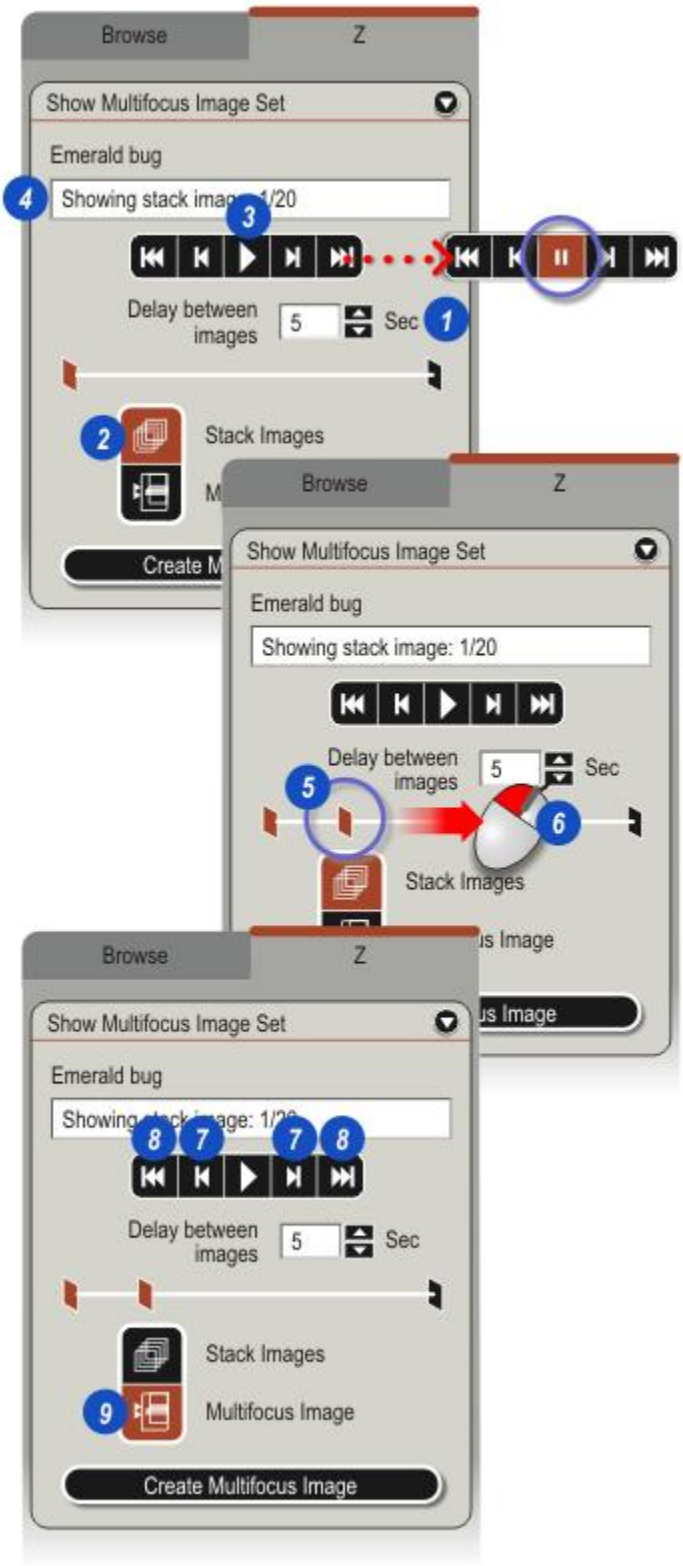
Click the *Play* button again to 'freeze' the current image.

6: Move to individual images in the set by clicking and holding the *Progress Bar* indicator and dragging it left or right.

7: Skip an image backward or forward by clicking the *Skip* buttons.

8: Choose the first or last images by clicking the *Beginning* or *End* buttons.

9: Select and display the *Multifocus* image by clicking the *Multifocus Image* button.



Montage Multifocus

Leica Application Suite *Montage Multifocus* provides advanced, versatile features for producing excellent extended depth-of-focus images using the renowned technology of *Auto-Montage* from *Syncroscopy*.

Digital images from a Z-Stack, spread over the focus range of the specimen, are acquired using the same features provided by *LAS Multifocus* but because *LAS Montage* provides tunable algorithms is able to create excellent extended depth-of-focus images covering a wider range of microscopy contrast methods.

LAS Multifocus provides only one montage method and is effective only on narrow range of specimen types. There are no facilities for adapting to different situations and no means of enhancing result image or alternative means of visualising the result image.

LAS Montage has many additional capabilities to extend the imaging conditions that can be used and improve the quality of the resulting image.

LAS *Montage* extends *Multifocus* by adding functions to provide:

- *Depth Map:* An image which contains depth information for all points on the image,
- *Confidence Map:* An image which contains an estimate of the accuracy of the depth map at all points on the image..

Additionally, the image can be viewed in several different ways to examine the surface in greater detail:

- *Anaglyph*.
- *Stereo Pair*
- *Colour Relief*
- *3D Model* (Optional extra module) which provides a perspective visualisation of the extended focus image for which the user can change the viewpoint so that the image give an impression of depth.

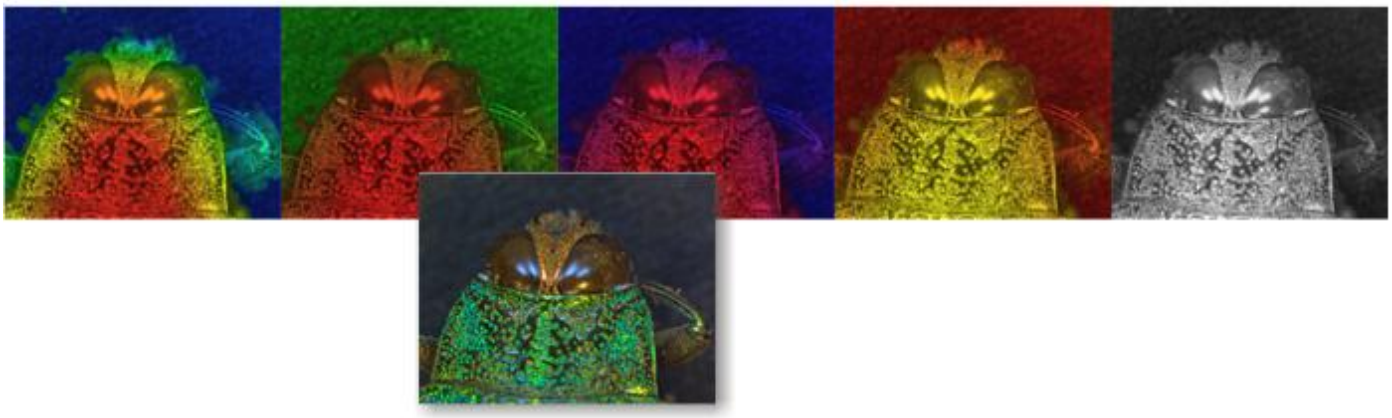
  Note: LAS *Montage* is a pre-requisite for the use of LAS *3D Viewer.*

The extensive *Measure Tools* include a fast

- *Profile display*...

...that maps and measures the contours of a user-drawn path.

Z-Stack acquisition is the same for both *Multifocus* and *Montage*. To find out more 632 .



Launch *Montage Multifocus* by:

1: Clicking on the *Acquisition Mode* button...

2: ...and then on the the *Montage* icon.

Montage Multifocus OperatingSteps:

- *Acquire the Z-Stack* 654
- *Select the Z-Stack* 654
- *Select the Z-Stack images* 655 *to use*
- *Create the Montage Multifocus image* 657
- *Make Enhancements* 659
- *View the Results* 663
- *Edit the Results* 672
- *Create additional views if needed* 664
- *Use the Measure Tools* 675

Select the Z Stack

1: Click to select the *Browse Workflow.*

2: Click the *Browse* tab and...

3: ... select the required Z-Stack on the *Navigator*.

4: Click on the ***Z*** tab to open the *Montage* control panel.

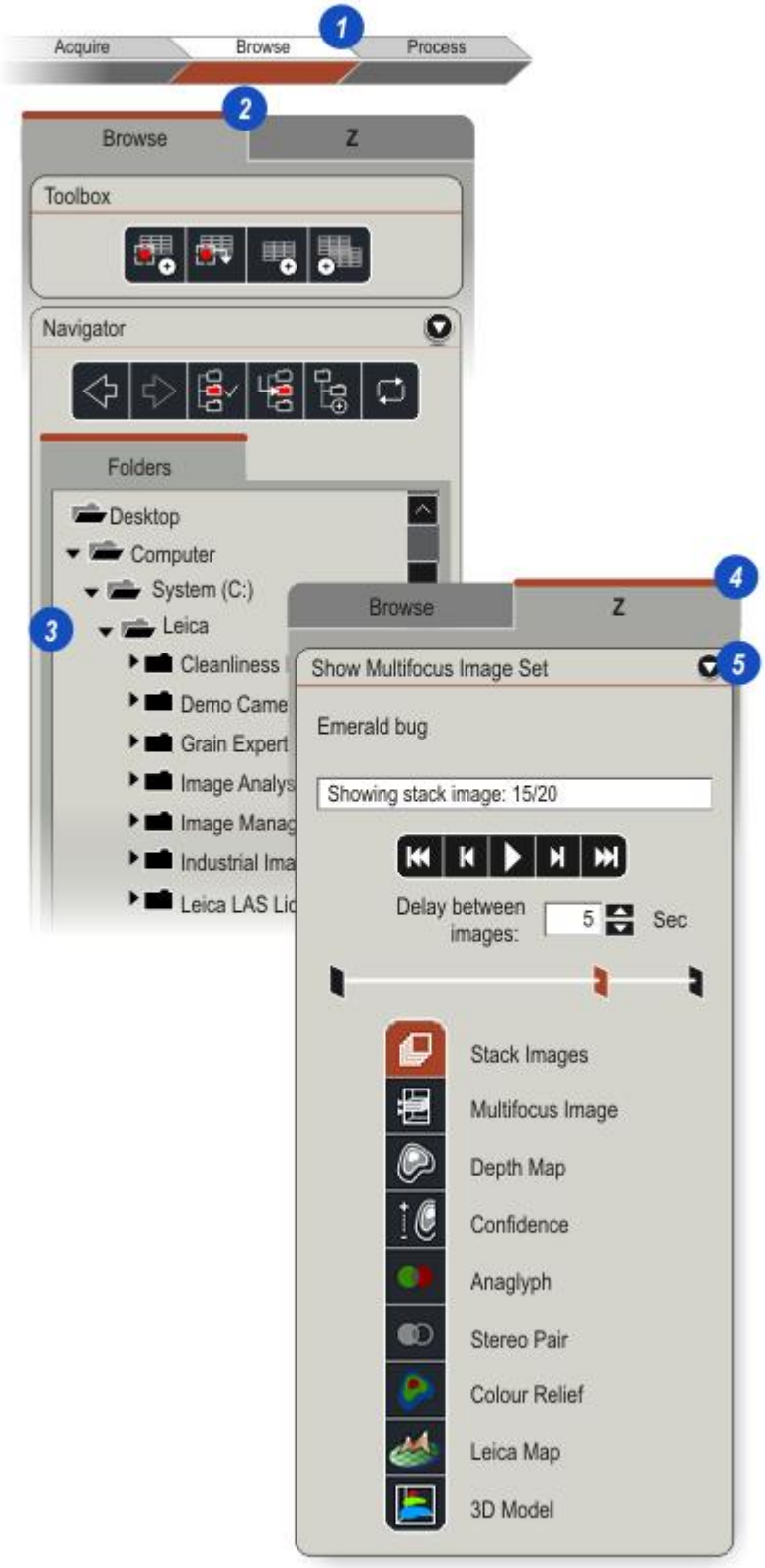
5: If necessary, click to expand the *Show Multifocus Image Set* panel.

The Z-Stack images will be loaded into the gallery together with any associated *Multifocus* image and maps.

Clicking on a thumbnail in the *Gallery* will display the image.

From this panel click to review the following images:

- *Stack Images* 655
- *Multifocus Image* 657
- *Depth Map* 662
- *Confidence Map* 665
- *Anaglyph* 666
- *Stereo Pair* 667
- *Colour Relief* 668
- *3D Model* 670
- *Leica Map* 683*, if it is installed, has its own help*



1: To view the Z-Stack sequence click on the *Stack Images* button. This will display the first image in the stack and enable the controls to view the stack.

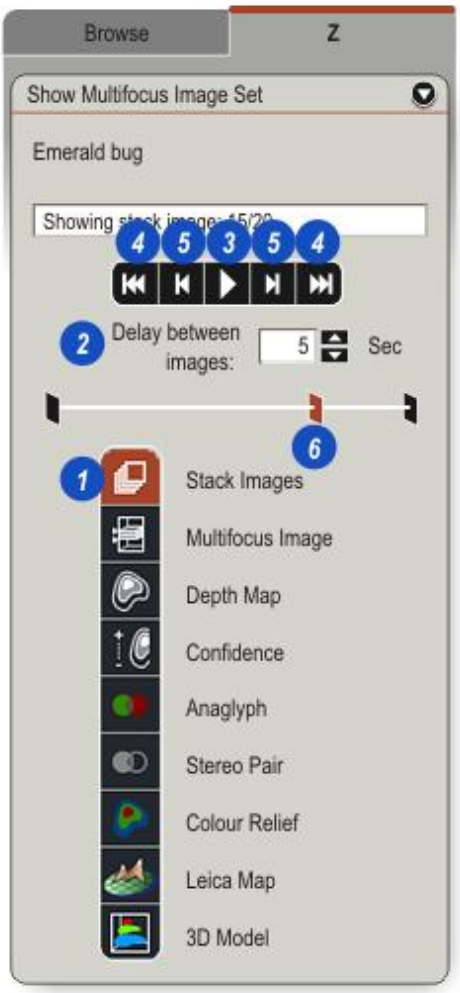
2: Enter a delay time between images and then use the control buttons to scan the images.

3: Click to play the image sequence continuously with the specified delay time between each image. Click again to stop.

4: Moves to first and last image in the sequence.

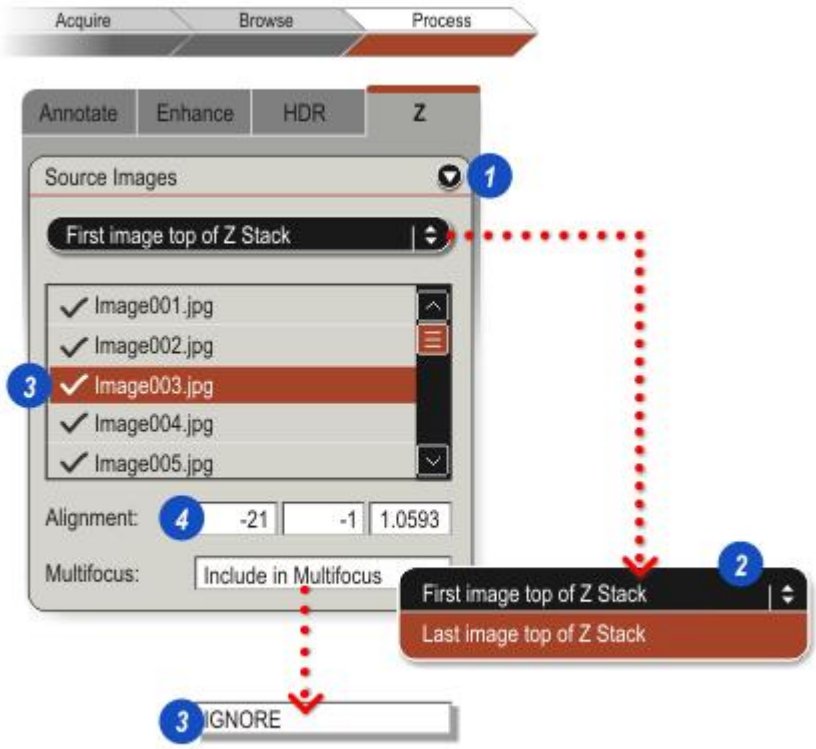
5: Steps forward or back in the sequence.

6: Click and drag on the red slider to move forward or back through the sequence. The display also shows the position in the sequence while the sequence is played.



The stack images are listed in the *Source Images* panel from where they can be selected for inclusion in the *Montage.*

Click to select the *Process Workflow* and then the ***Z*** tab.



1: Click on the arrow to the right of the *Source Images* header to reveal the panel.

2: The image sequence can be swapped between starting with the top of stack or starting with the bottom of the stack by clicking the arrows and selecting the option from the drop-down list.

3: Clicking on an image in the list will select it and display it in the *Image Viewer.*

Clicking again toggles between *IGNORE* and *Include in Multifocus*. Any image that is ignored will not be used in the generation of the *Multifocus* image.

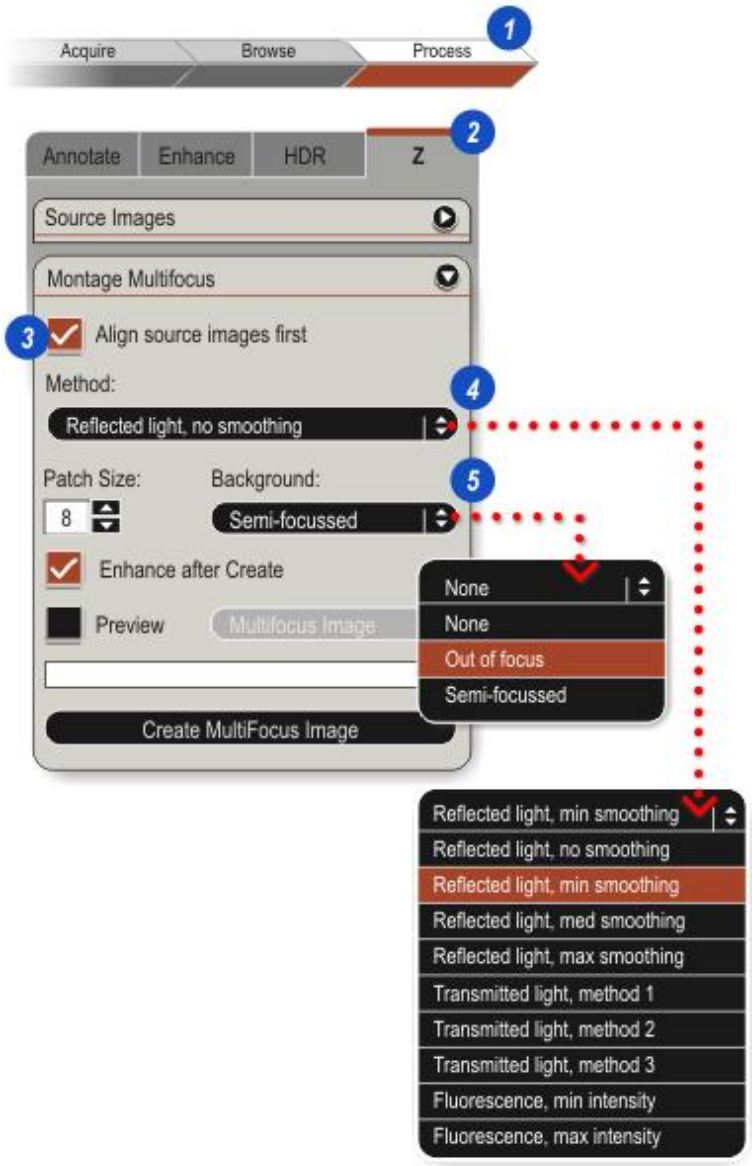
4: If the *Align Images* before combining box is checked on the *Montage Multifocus* [657] panel then relative alignment data will be shown here.

If the source images are not exactly registered with each other, having been captured with a stereo microscope or from scanned photographs, this should be corrected with the *Align Source Images* function before proceeding with the *Montage* operation.

1: Click on the *Process Workflow.*

2: Click on the **Z** tab.

3: On the *Montage Multifocus* panel, click to enable (tick mark visible) *Align source images first.*



4: Choose the *Montage Method* that is closest to the contrast method used when the Z-Stack was created by clicking on the arrows to the right of the *Method* header and selecting from the drop-down list:

- *Reflected light:* Four degrees of smoothing - none, minimum, medium or maximum ...
- *Transmitted light:* Methods 1, 2 and 3.
- *Fluorescence:* Minimum and maximum intensity.

5: Select the *Background* to reduce the effect the background has on the *Multifocus* image.

1: The *Patch Size* refers to the spread of pixels used to calculate the montage. A recommended range is 8 to 20: Too small and and the detail will be good but noise could be a problem. Too large and whilst the depth maps will be smoother there could be less detail.

Adjust the *Patch Size* by clicking the up/down arrows to increase/decrease it.

2: Click to enable the *Enhance after Create* check box to automatically apply any settings made on the *Enhance* [659] dialogs

This is a convenient way of creating a final *Multifocus* image but enhancements can always be made after the image is created.

3: To preview a result, click to enable the *Preview* check box and...

4: ...click on the arrows to the right of the header and choose the image to be previewed from the drop-down list.

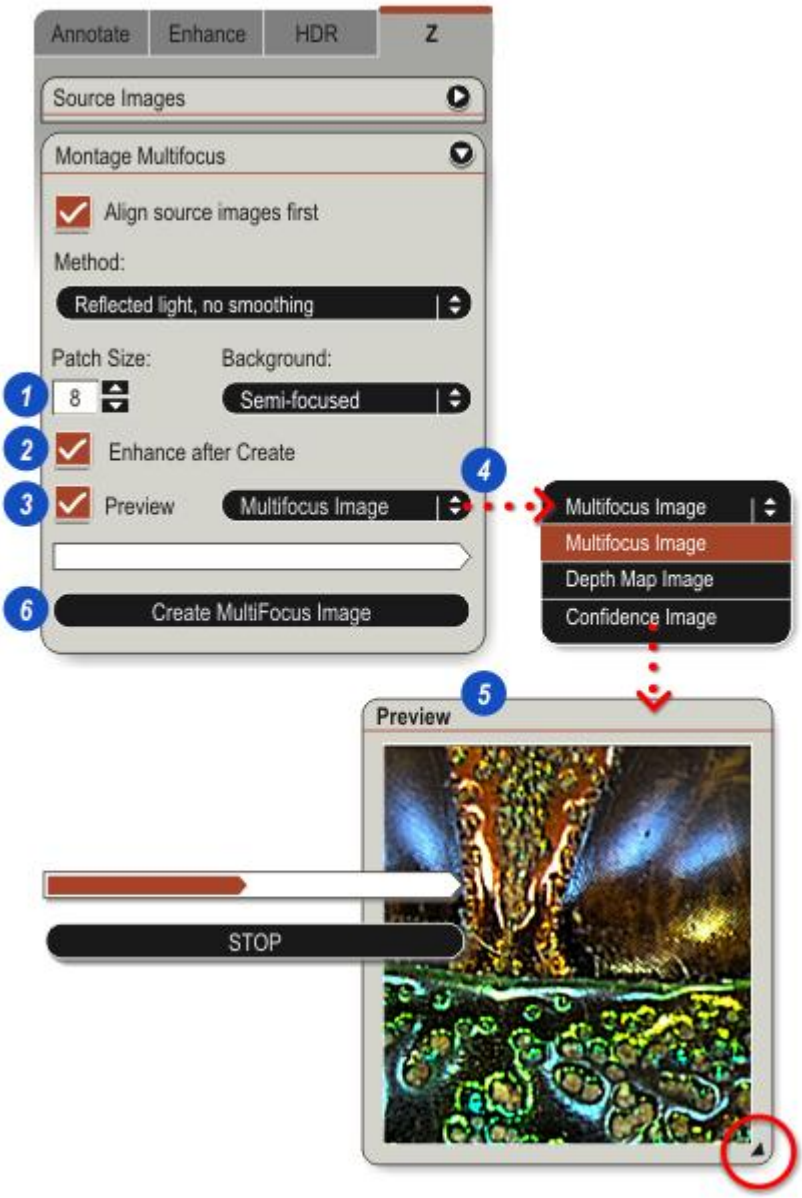
5: The *Preview* is automatically generated and the area can be enlarged or reduced by clicking and dragging the corner arrow.

Click the *STOP* button to halt the preview generation.

Click and drag inside the *Preview* window to move the area of interest.

To close the *Preview* window, click to disable the *Preview* check box (2).

6: Click the *Create Multifocus Image* button.

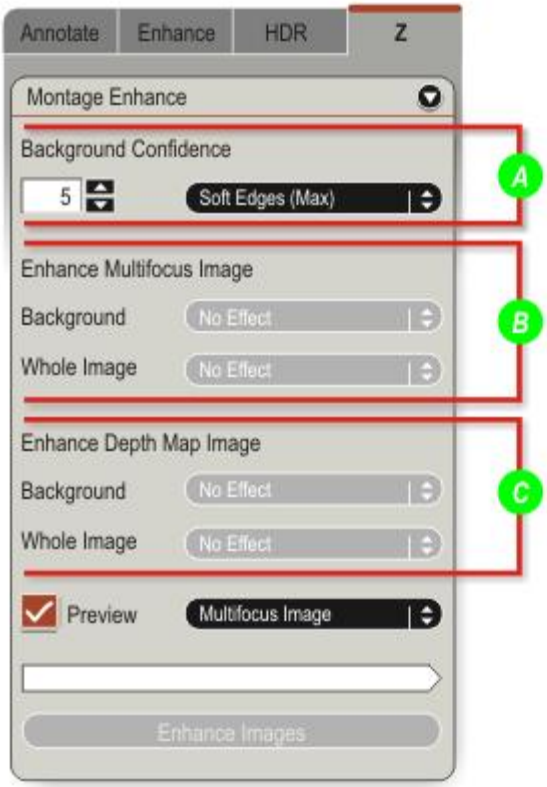


The *Montage* and *Depth Map* images may be processed prior to publication. Smoothing the *Depth Map* can be used to remove artefacts that may appear in regions that lack in focus detail. Sharpening the *Montage* image may improve the images appearance.

The *Montage Enhance* panel is divided into three main control areas:

- *Background Confidence (A)..*
- *Enhance Multifocus Image (B) and*
- *Enhance Depth Map Image (C).*

Additionally, there is a *Preview* facility and the *Enhance Images* button that applies the enhancements.

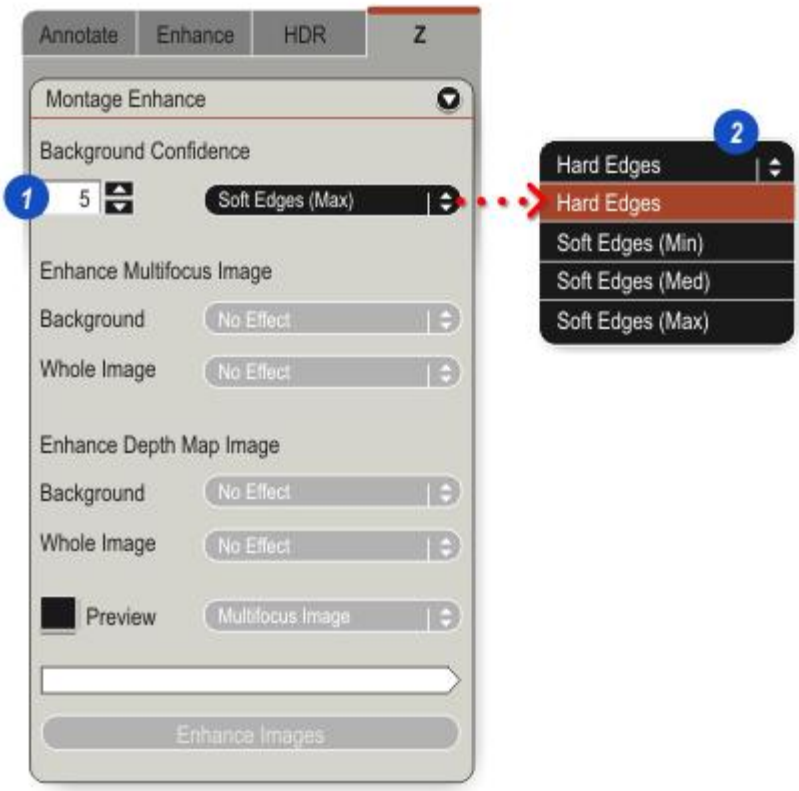


Note - Background is now removed using the *Background* selection from the *Montage MultiFocus* panel. This information is retained only for reference.

Please set control (**1)** to 0.

Adjusting the *Background Confidence* value will define a new *Background Mask* layer which can be applied to both the *Multifocus* and *Depth Map* images.

The *Background Mask* can be derived from any or all of the layers and is simply the parts of the images that remains after all of the desirable image information has been extracted.



1: The threshold for the *Background Confidence* can be set to a value between 0 and 100% by clicking inside the text box and typing a value or using the up/down arrows.

Any pixel with a confidence value in the range *0* to *Background Confidence%* will be regarded as a background pixel.

2: The boundary between the *Background Mask* layer and the rest of the image may be smoothed depending on the value of the smoothing factor. Click on the arrows to the right of the header and from the drop-down list select from:

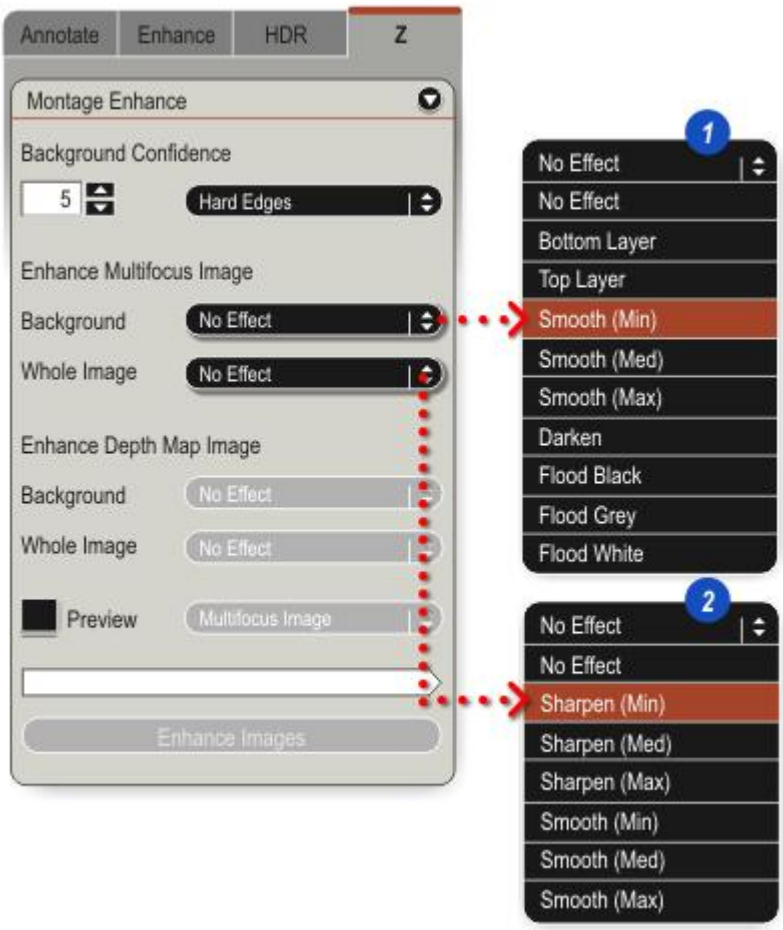
- *Hard edges*.
- *Soft Edges(Min)*
- *Soft Edges(Med)*
- *Soft Edges(Max)*

Background:

Apply various effects to the *Montage* image, but only within the region where a pixel has a confidence value in the range *0 - Background Confidence%* - the *Background Mask.*

1: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Montage* image..
- *Background*: Over the *Background Mask* region the background layer is copied to the *Montage* image
- *Smooth: (3 levels):* Over the *Background Mask* region a smoothing filter is applied to the *Montage* image
- *Flood Black, Grey or White:* Over the *Background Mask* region, the *Montage* image is set to a solid colour
- *Darken*: Over the *Background* mask region, the intensity of the *Montage* image is reduced



Whole Image:

Sharpen or smooth the parts of the *Multifocus* image where the confidence is higher than the *Background Confidence%*.

2: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Montage* image..
- *Sharpen (3 levels):* or...
- *Smooth (3 levels).*

Background:

Apply various effects to the *Depth Map* image, but only within the filter region where a pixel has a confidence value in the range *0* - *Background Confidence%* - the *Background mask.*

1: Click on the arrows to the right of the header and from the drop-down list click to select:

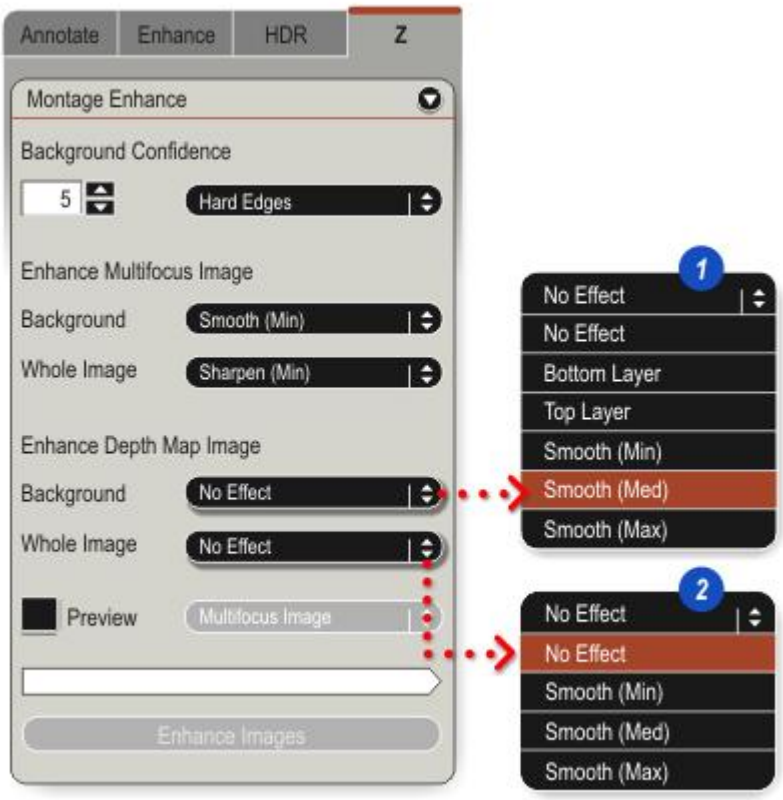
- *No Effect:* No effect on *Depth Map* image..
- *Smooth: (3 levels):* Over the *Background* mask region a smoothing filter is applied to the *Depth Map* image

Whole Image:

Sharpen or smooth the parts of the *Depth Map* image where the confidence is higher than the *Background Confidence%*.

2: Click on the arrows to the right of the header and from the drop-down list click to select:

- *No Effect:* No effect on *Depth Map* image or..
- *Smooth (3 levels)*.

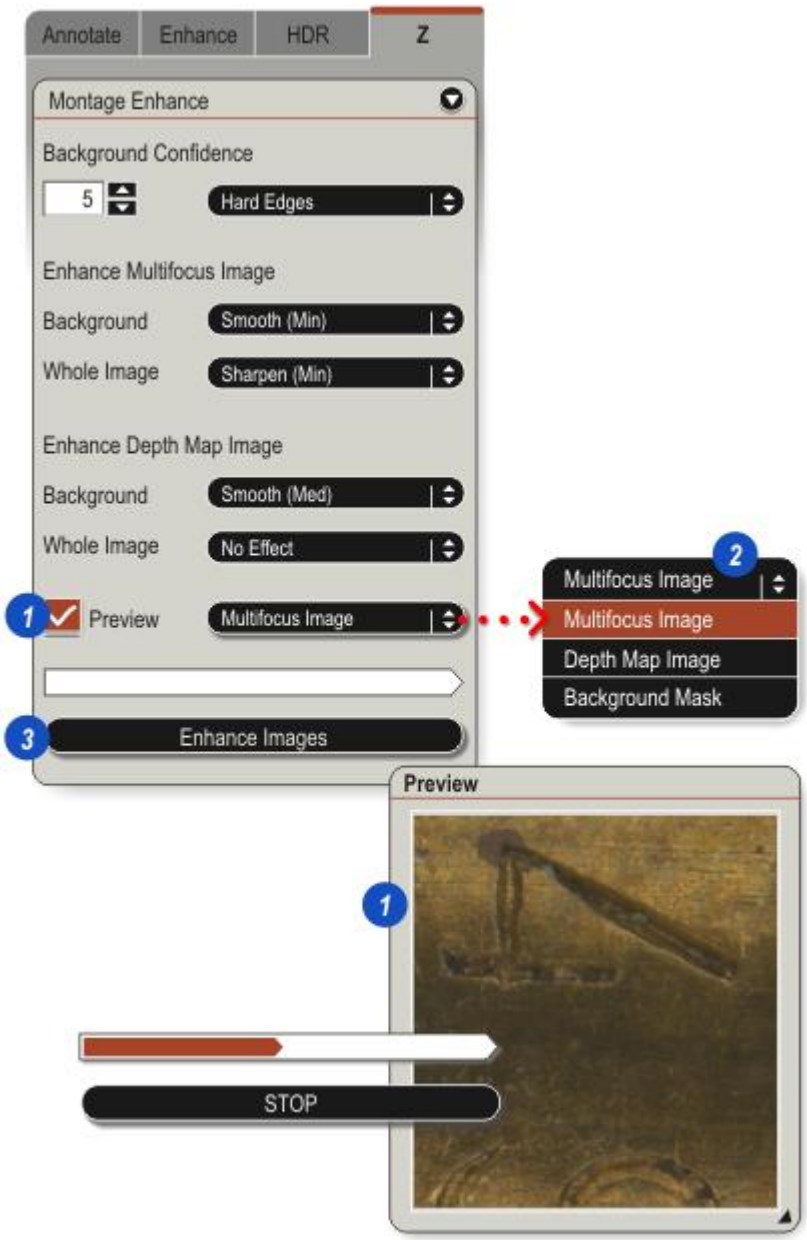


To preview the *Montage Enhance* settings:

1: Click to enable the *Preview* check box.

2: Select the image type to preview by clicking on the arrows to the right of the header and choosing from the drop-down list. The *Preview Viewer* opens.
To move the area of interest within the *Viewer*, click and drag the image.

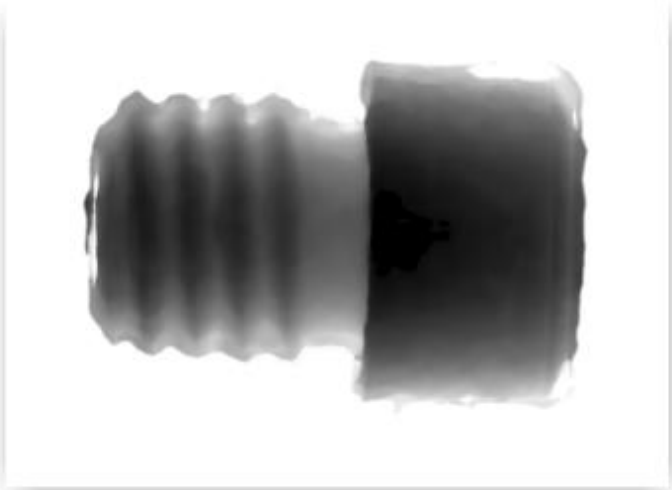
3: When the *Enhance* settings are acceptable, click on the *Enhance Images* button to apply them to the images.



A *Depth Map* image is generated as part of the Montage process.

It is a record of which source image provided the in-focus region for the montage image at each pixel location, expressed as a grey level.

The *Depth Map* image is the same size as the source images used to generate it, but is always monochrome. The grey levels depend on the particular *Montage Method* used to generate the depth map.

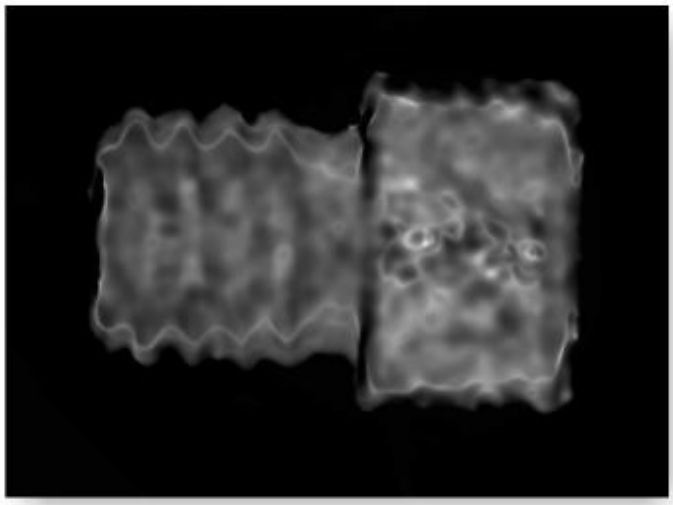


The *Depth Map* image is displayed in the depth map image window and is saved in the dataset file itself, but can also be exported to an image file if required.

A *Confidence Map* is also generated for each montage operation. It is an estimate of accuracy of the depth map image at each pixel location, expressed as a grey level.

White represents high confidence and therefore an accurate depth value. Black represents low confidence and an uncertain depth value.

Low confidence will often represent areas of low contrast where several planes will appear to be in focus.

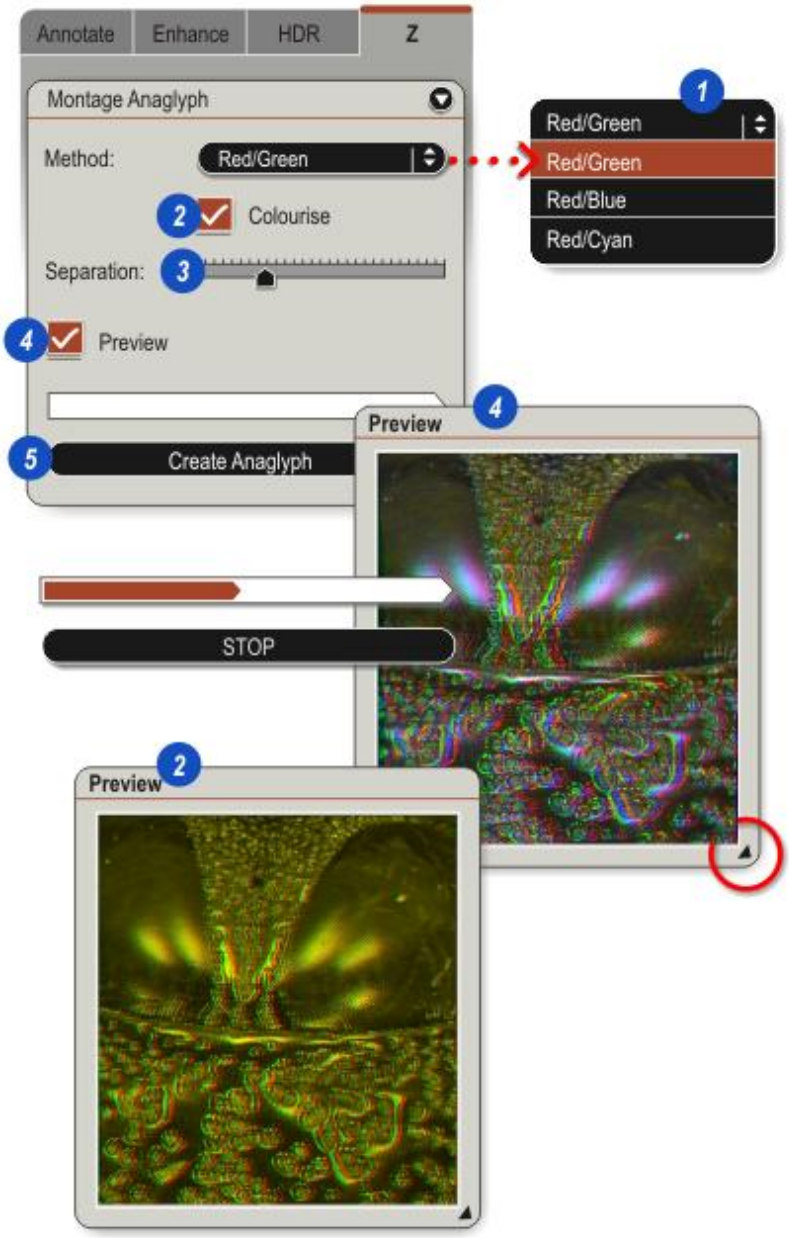


The *Confidence* image is the same size as the source images used to generate it, but is always monochrome. Values are expressed as percentages, ranging from 0% (no confidence that depth map is correct for that pixel) to 100% (complete confidence).

The *Montage Anaglyph* operation generates the anaglyph image, which when viewed through suitable red and green/blue lenses offers a 3-dimensional monochrome view of the montage image, with depth information synthesized from the depth map.

It is the same size as the *Montage* image used to generate it, but always comprises 3 RGB planes.

The anaglyph image will be saved as one of the result image files.



1: Select *Method* from drop-down list. Choose *Red/Green* or *Red/Blue* to match the glasses to be used. Choose *Red/Cyan* for better printed results - the effect depends on the *Montage* image.

2: Check C*olourise* to add a hint of the original *Montage* image colour back to the *Anaglyph* image - the final effect depends on the *Montage* image.

Colourise is not available with *Red/Cyan* option because all 3 colours are effectively already used.

3: Set the amount of *Separation* - the maximum amount, in pixels, by which left and right-eye images are displaced to provide the stereo effect. Lower values are recommended in most cases.
Click and drag the slider or if the mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.

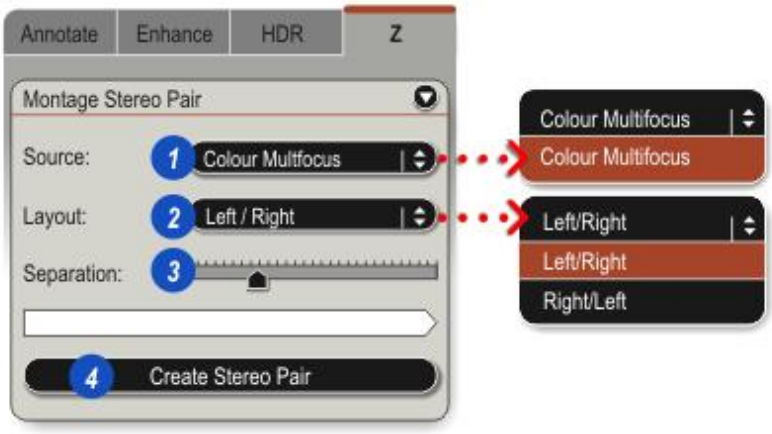
4: Check *Preview* to see the effect of the settings in the Preview window

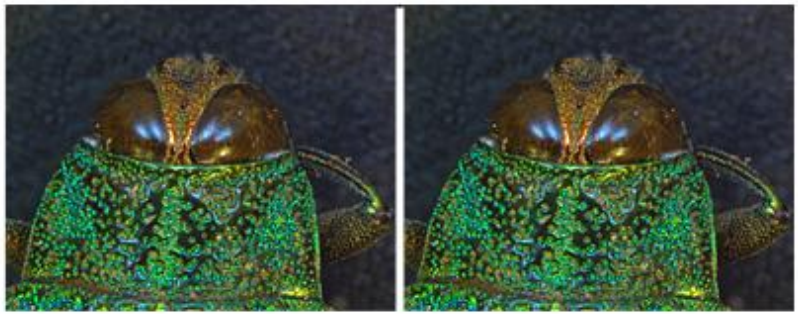
5: Click *Create Anaglyph* to create see the finished *Anaglyph* image in the Viewer.

The *Montage Stereo Pair* operation generates the *Stereo Pair* image, which when viewed correctly offers a 3-dimensional colour view of the montage image - with depth information synthesized from the depth map.

The stereo pair image comprises two similar images, in colour or greyscale depending on the source image, each the same size and pixel depth as the *Montage* image used to generate it.

The *Stereo Pair* image will be saved as one of the result image files.





1: Select *Source* from drop down box. The stereo pair may be generated from:

- A monochrome representation of the *Montage* image,.
- From the original colour *Montage* image - if the source images is colour, or
- From the colour relief image if that has been generated.

2: Select *Layout*. For normal use, select *Left/ Right.* However, some people find it easier to view a stereo pair with the images flipped *Right/Left*

3: Set the amount of *Separation* - the maximum amount, in pixels, by which left and right-eye images are displaced to provide the stereo effect. Lower values are recommended in most cases.
Hint**:** *If your mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.*

4: Click *Create Stereo Pair.*

Montage Colour Relief generates the colour relief image that offers a view of the *Montage* image colour-coded with depth information from the *Depth Map.*

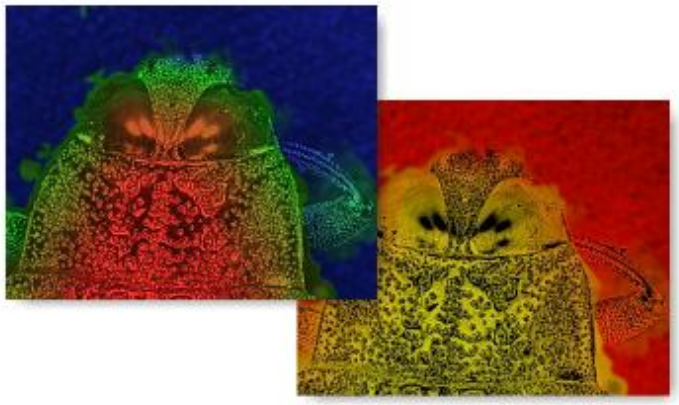
The *Colour Relief* image is the same size as the *Montage* image used to generate it, but always comprises 3 RGB planes.

Experiment with the controls to find a representation which works well with your particular images:

Images of surfaces consisting mainly of highlight detail tend to look good with mode set to *Light Features* and *Saturation* slightly left of centre.
Images of biological samples with dark fibres tend to look good with mode set to *Invert*.

A pair of *red/gr*een or *red/cy*an spectacles as used for anaglyphs, may help to interpret colour as depth.

The *Colour Relief* image will be saved as one of the result image files.



1: Select *Method* from drop down box. Experiment with this control to find which mode works best with the images:

- *Light Features* applies the colour effect mostly to the lighter parts of the montage image.
- *Dark Features* applies the colour effect mostly to the darker parts of the montage image.
- *Invert* applies the colour effect to the darker parts of the montage image and darkens the background.

2: Click on *Apply Confidence* to use the confidence image to suppress unfocused parts of the montage image.

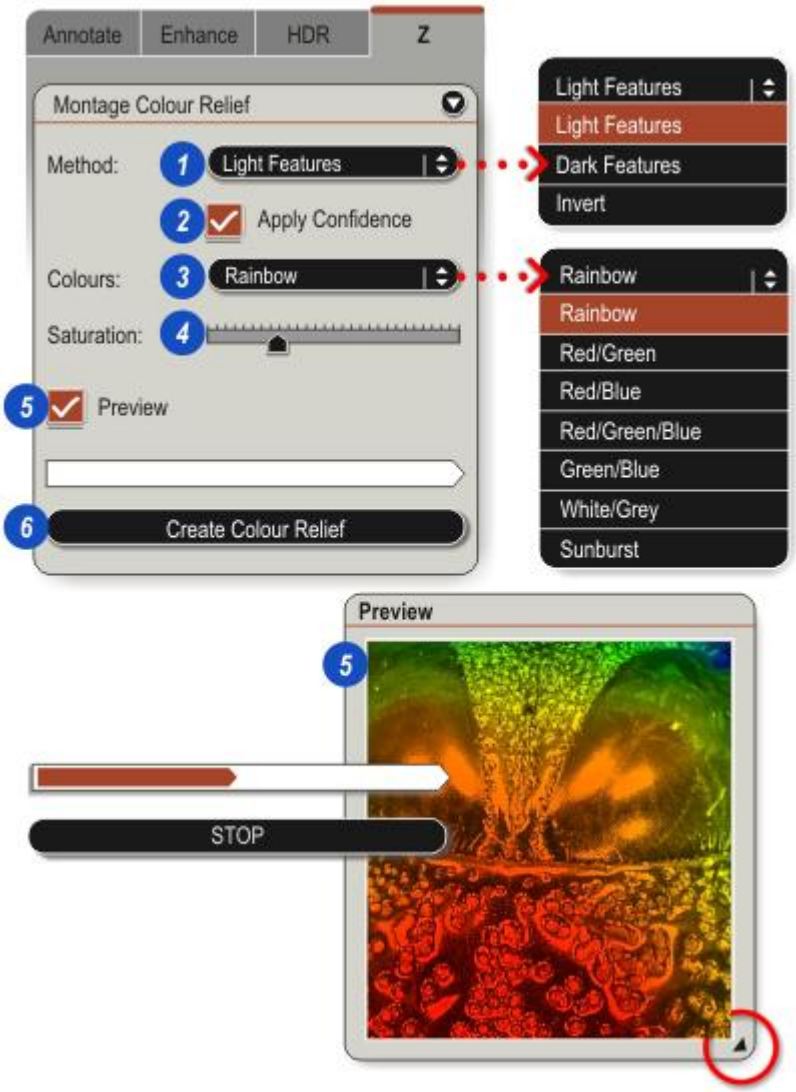
3: Select the *Colours* scheme in which to plot the colour relief image.

4: Adjust the *Saturation*. Saturation varies from purely monochrome intensity (left-most position) to full-coloured depth map with no intensity information (right-most position).

Click and drag the slider or if the mouse has a wheel for scrolling, click on the slider and use the wheel to adjust the parameter value.

5: Check *Preview* to see the effect of your settings in the *Preview* window

6: Click *Create Colour Relief* to see the finished image in the *Viewer*.



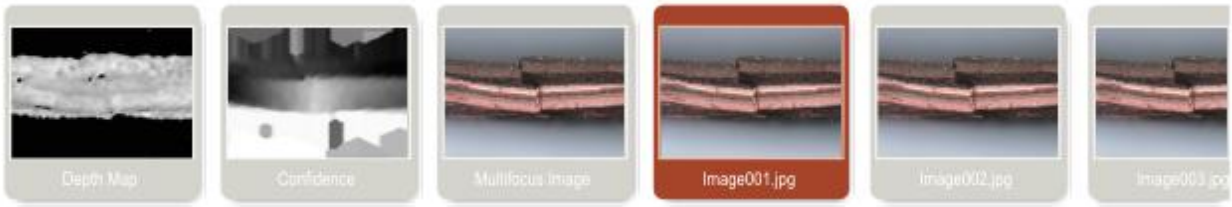
The *3D Model* is an Optional Module that must be licensed and installed. If it is not, the button will not appear in the tool list.

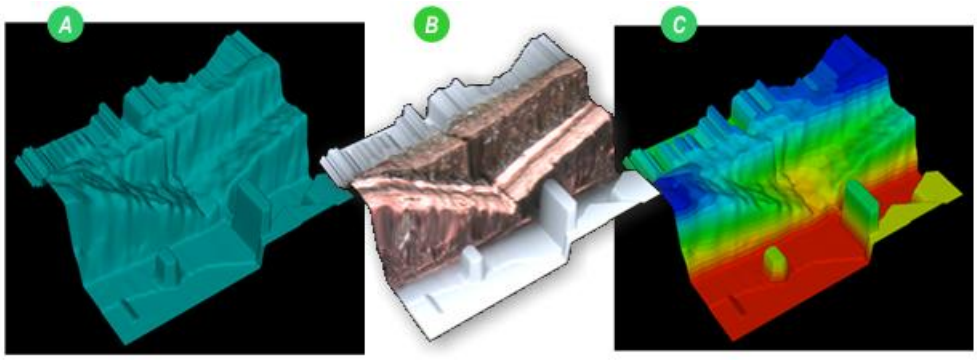
The module is launched from the *Browse Workflow:*

1: Click on the *Browse Workflow*.

2: Click to select the ***Z*** Tab.

3: On the tool list, click the *3D Model* button.

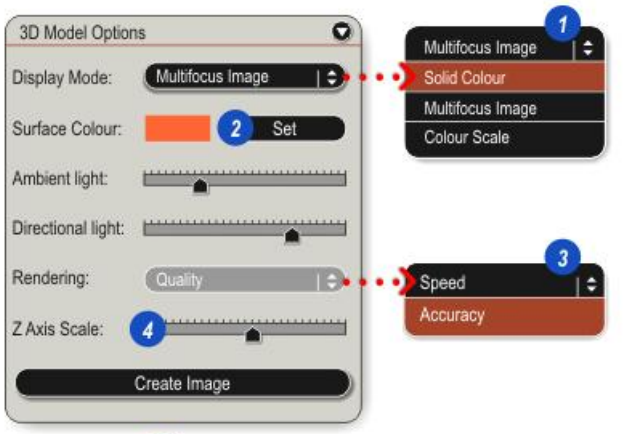


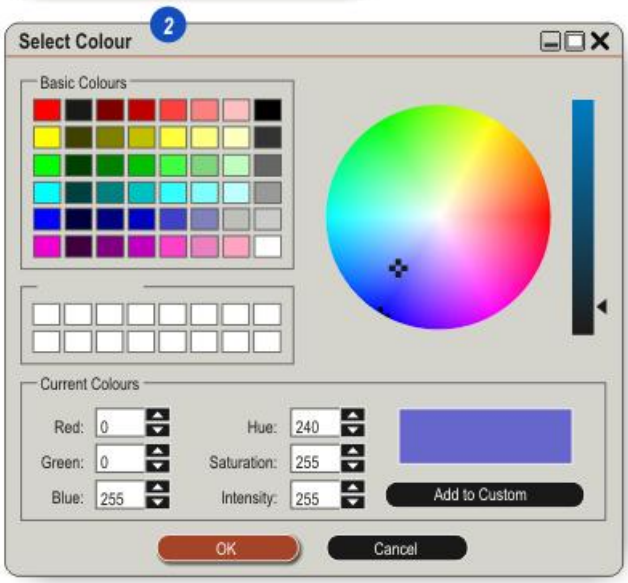


The *3D Model* is a 3D surface view of the image based on the *Depth Map* information and can be viewed from different angles by clicking on the model and dragging the mouse.

The model can be created in three different *Display Modes*:

- ***A:** Solid Colour:* The model is displayed as a surface relief with a single colour. The colour can be set by clicking on the *Set* button..
- ***B:** Multifocus Image:* The model is displayed as a surface relief overlayed with the *Multifocus* image.
- ***C:** Colour Scale:* The model is displayed as a surface relief overlayed with the colour relief image showing the depth map contours.





1: Select the display mode from the drop down box.

The brightness of the image can be adjusted using the *Ambient light* slider and the illumination direction with the *Directional light* slider.

2: With the *Solid Colour* mode selected, the colour can be changed by clicking the *Set* button and choosing a colour from the dialog.

3: Set *Rendering* to *Speed* or *Accuracy* to change the response time of the model display in *Colour Scale* mode.

4: The image can be 'stretched' along the Z axis by clicking and dragging the *Z Axis Scale* slider.

5: Click the *Create Image* button.

The *Montage Edit* tool is used to copy and blend pixels from one image - the *source*, - to another - the *target*. It is best described using a hypothetical example.

Image **(A)** is the resulting *Montage* from the *Z Stack* of a bolt. Most of the image is sharp with the exception of the serial number (circled) which is important to the job but out of focus and indecipherable. This will be the target of some edited pixels.

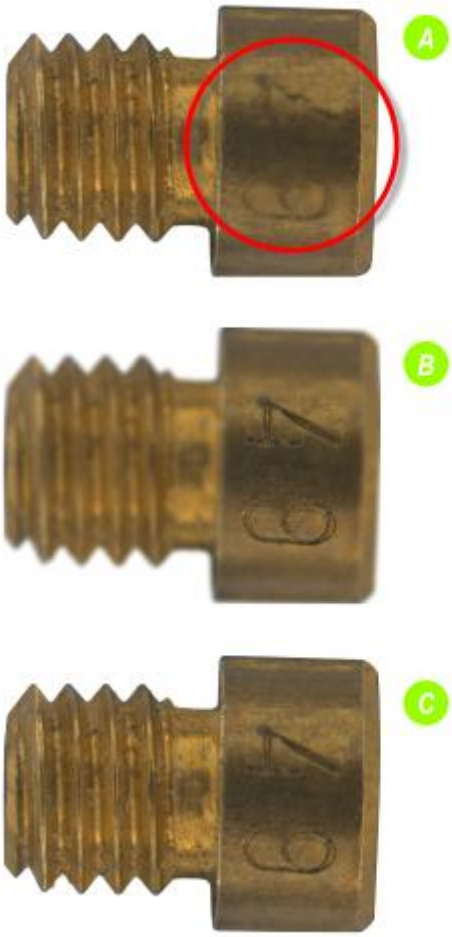
Image **(B)** is *Z Stack* image number (10) and whilst it is mainly out of focus, the serial number is sharp and readable. This will be the source of the edited pixels.

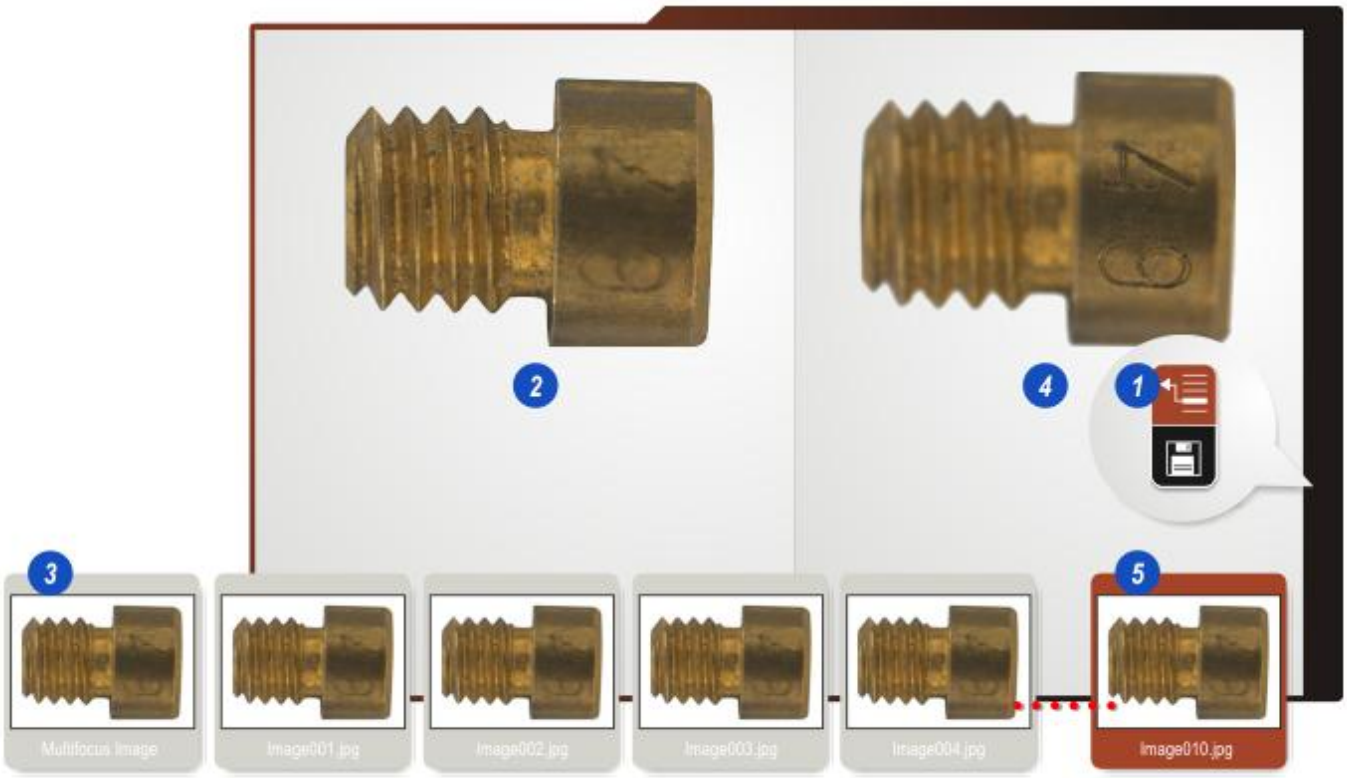
(C) *Montage Edit* allows the user to extract a section of a selected stack image and 'blend' it into the final montage.

Now the *Montage* is sharp and the serial number from Z Stack image (10) has been included and maintains its sharpness.

The *Dual Viewer* facility allows the user to view both the final montage and the edit source side-by-side so that the result can be seen immediately and editing is an easy and fast operation.

The *Depth Map* can also be the target of the edit.





Both the source and target of the edited pixels can be viewed side-by-side by using the *Dual Viewer* facility:

1: Click on *Viewer Options* icon on the *Side Tool Bar*. From the drop-down list click to select *Dual Viewer*. The *Viewer* separates into two panes.

2: Click in the left-hand pane.

3: In the *Gallery*, click on the target thumbnail - in this case the *Montage Multifocus* image but it could be the *Depth Map*. The image appears in the left-hand pane.

4: Click in the right-hand pane.

5: Click on the source of the pixels - in this case *Image010.jpg.* The image appears in the right-hand pane.

1: Click the *Edit Mode* check box to enable editing.

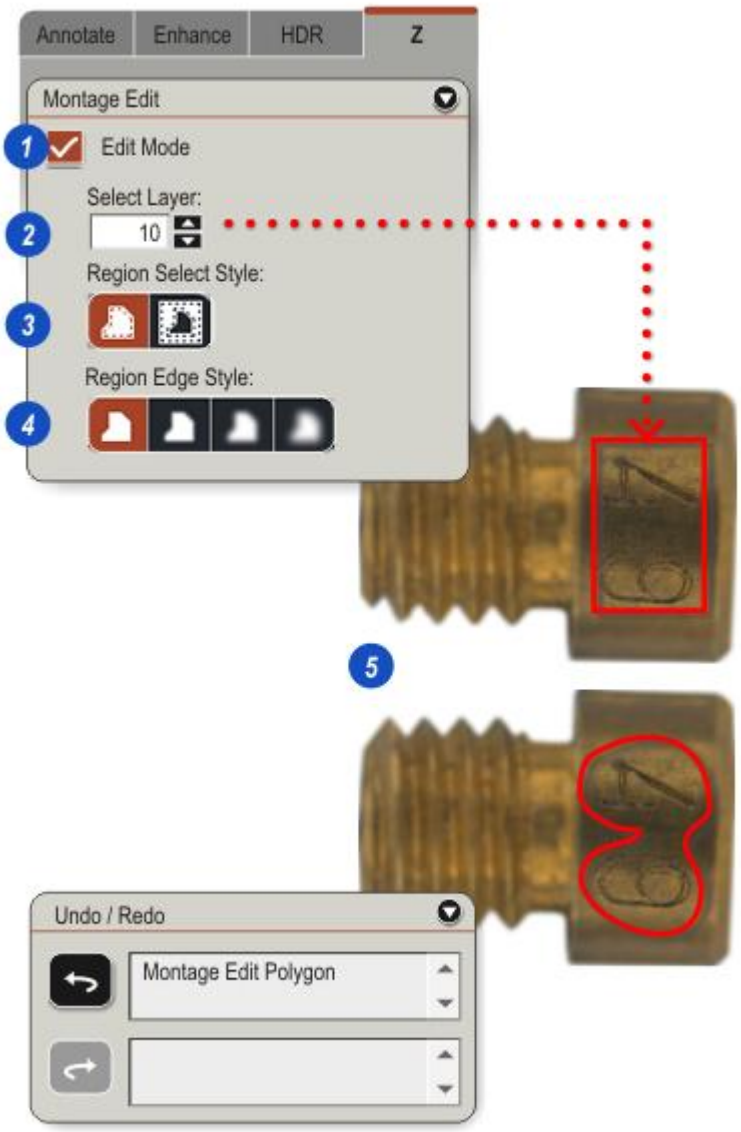
2: Click inside the *Select Layer* text box and type the source image number - in the example image 10.
Alternatively, click inside the text box and then click on the image thumbnail which will automatically transfer the image number.

3: *Region Select Style* allows the easy selection of an area using the mouse. The selected area is outlined with a red line.

4: There are 4 possibilities for the *Region Edge Style*:
The left-most selection uses a hard edge with no blending of the edited area and the background image.
Each of the other 3 edge styles add progressively heavier blurring of the compound image and the selected layer, while still remaining within the selected outline. Click to select.

5: Define the source edit area:

- Create a *rectangular* area by clicking at each corner..
- Create a *freehand* area by clicking and dragging to draw the edit area.
- Double-click to close the shape and define the edit area.



If a mistake is made while defining an outline, a right mouse click can be used to delete the last point. This can be repeated until the line is entirely removed.
To quickly remove an entire line, uncheck the *Edit Mode* checkbox.
The *Undo / Redo* panel can be used if the results of an edit operation are not as desired.

The edited target is automatically saved. To revert to the original *Montage Multifocus* [657] image create a new image that is not influenced by the edit.

Measure Tools allows the user to read depth information from a point or line on a *Montage Multifocus* Image and display a profile of the surface as a graph on a chart. Measurements may be absolute or relative to a reference point set by the user.

This mode is controlled by several checkboxes on the panel. The panel is inactive if no depth map is available for the *Multifocus* image. (The depth map is created when *Montage Multifocus* makes a new *Multifocus* image.)

1: Check to enable the *Position Readout* check box to give a continuous readout of the current mouse position as the mouse is moved over the image.

2: The ***X*** and ***Y*** positions are given in calibrated units relative to the top left corner of the image.

The ***Z*** position is taken from interpreting the depth map and is displayed in microns.

Distance is measured from the top left corner of the image or from a *Reference Point* placed on the image. *Distance* follows a path constrained to the surface and prescribed by the *Depth Map.* The *Distance* along the surface will be longer than the straight-line distance between the start and end point.

3: Set a *Reference Point* on the image by clicking the *Set* button and...

4: ...clicking on the image where the *Reference* is to be placed.

5: Click the *Clear* button to remove a *Reference Point.*

- It is best practice to use calibrated images for *Distance* measurement. A nominal one micron per pixel is used as a default for un-calibrated images.

Two check boxes control the *Profile* display:

1: Enable *Profile* (with *Edit* disabled) to draw...

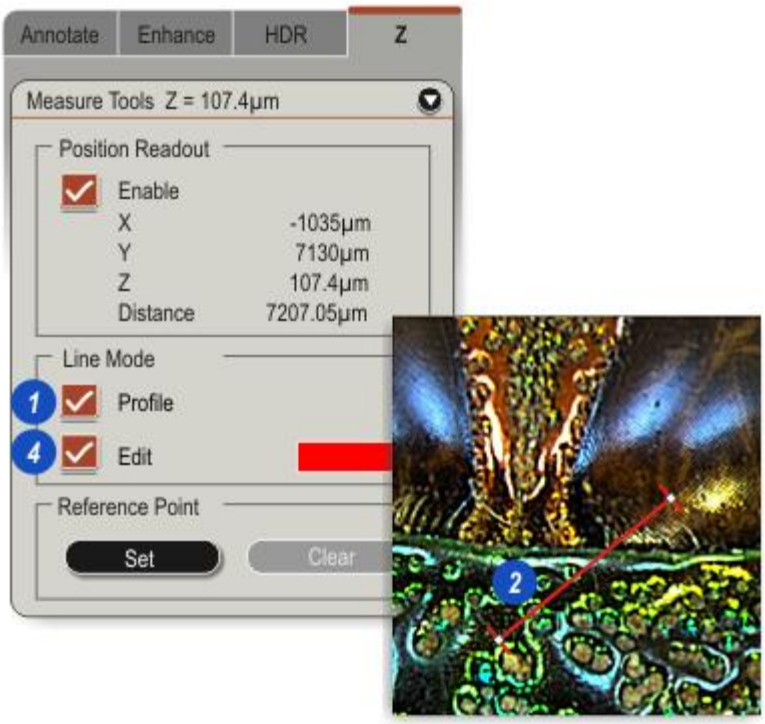
2: ... a straight line on the image with the mouse. Each new line will replace any previous one.

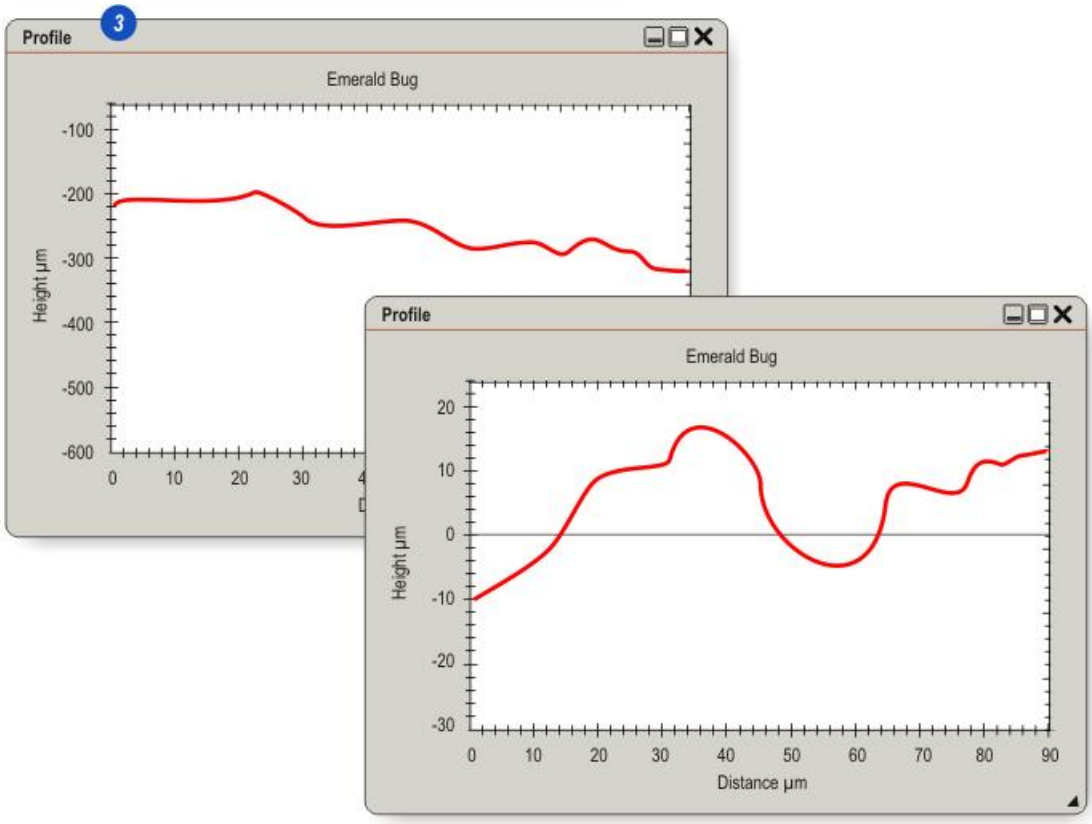
3: The *Profile* display will show the graph of the surface section. Use the controls top-right to display the *Profile* full screen or to close it.

4: Click to enable *Edit* to interact with an existing line or move a reference point. The line can be moved by clicking and dragging it or rotated by clicking a dragging an end point.

A *Reference Point* can also be repositioned. The *Profile* graph changes accordingly.

Placing a *Reference Point* on the image sets a zero level for ***Z*** values and the display will change to include a Zero line with heights both above and below it.



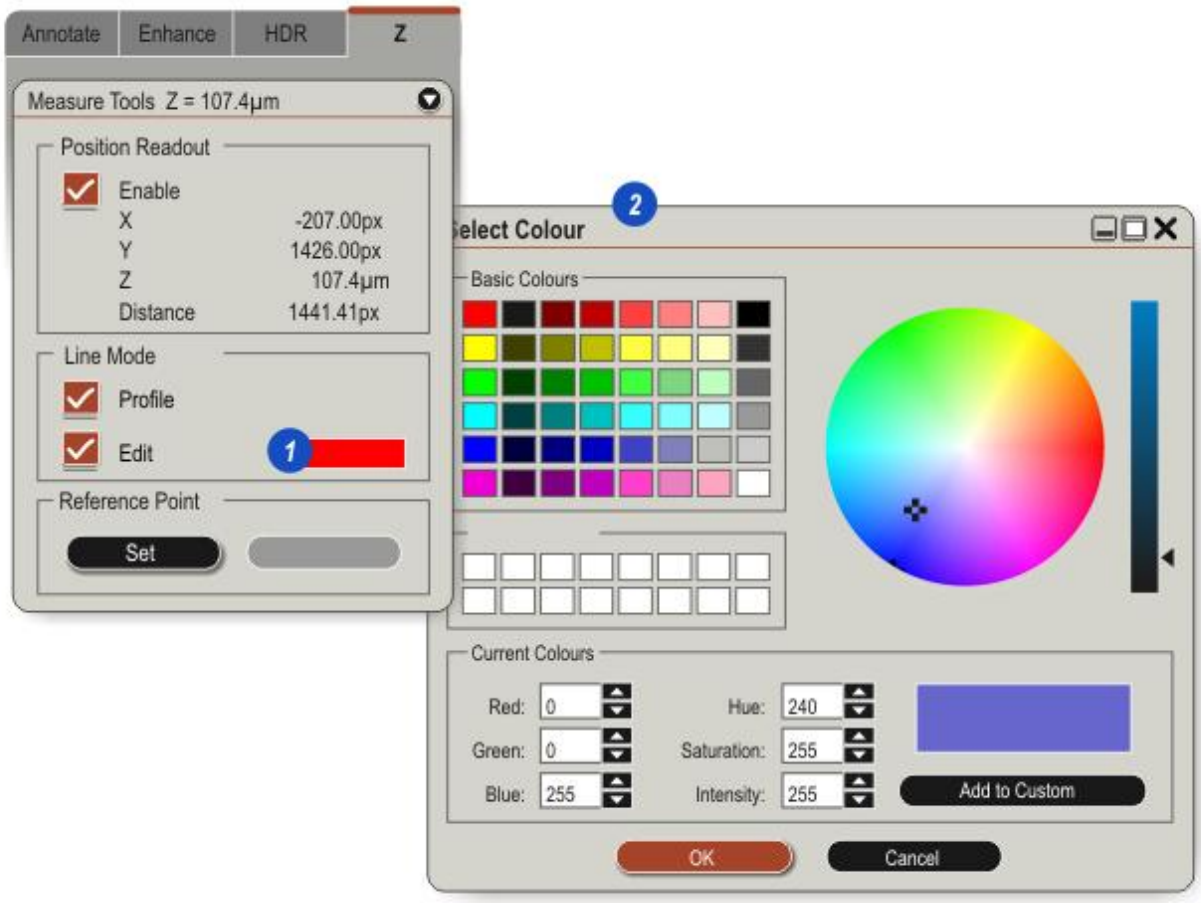


Change Line Colour

Change the *Line* and *Reference Point* colour by:

1: Click on the *Line Mode* colour button.

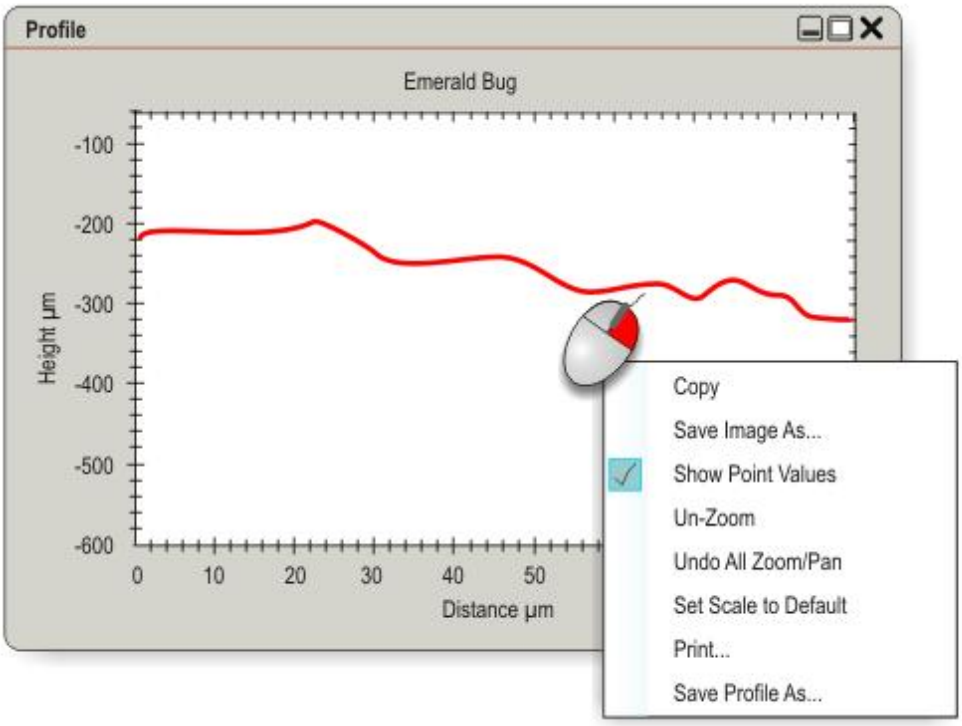
2: From the *Select Colour* dialog click to select a basic colour, drag the target to the required colour on the circle or enter values in the *Current Colours* text boxes.



Profile Panel Context Menu

The *Profile Panel* has a context menu selected with a right-click.

- *The Profile* can be copied to the clipboard,.
- *Saved* to a named file and...
- *Printed.*
- *Selecting Show Point Values* provides a read-out of the graphs co-ordinates when the cursor is placed near the graph.
- *To zoom* a portion of the graph, left-click and drag a rectangle around the area of interest.
- *Select Un-Zoom* to remove the zoomed area.
- *To Pan* a zoomed chart hold down the left *Shift* key, click and drag the mouse.
- *Save Profile As* exports the profile to a text file that can be imported into *Microsoft Excel.*



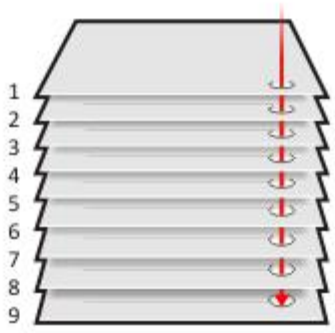
The purpose of the *Montage Methods* is to create a single in-focus image by combining parts of multiple images taken at different Z positions. Each individual image may contributes some focussed areas to the final image.

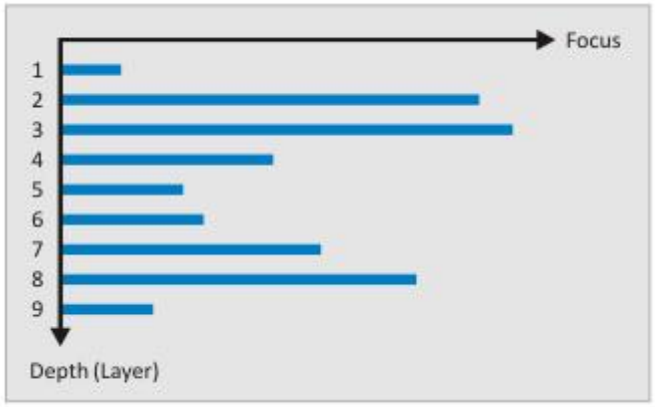
The multiple images are acquired in order of Z position ideally at steps in the Z position that are separated by less than the depth of focus of the objective being used. This set of images is called an image- or Z- 'stack' and the individual images are referred to as slices.

Consider a single pixel from each source image (slice) at the same *X/Y* location. Nine source images comprise the Z-Stack in the illustration with the single pixel location represented bottom right of each.

On comparison with neighbouring pixels in the same image, a numerical measure of 'sharpness of focus' is calculated for that pixel within each source image.
A graph of focus against depth (*Source Image Index*) is then plotted for that pixel location in all source images.

The focus comparison and the algorithms described in the following illustrations are repeated for every pixel *X/Y* location within the series of source images.





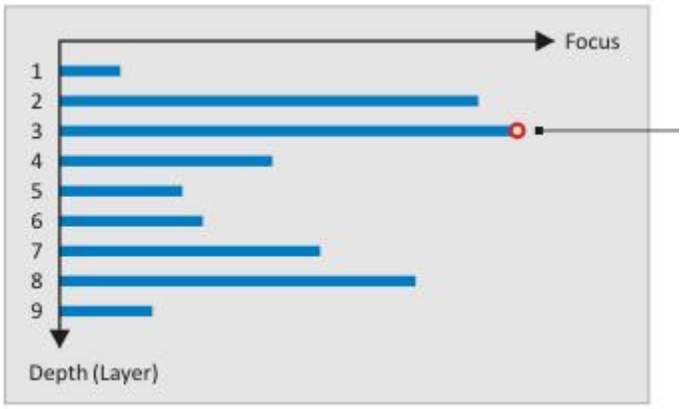
Reflected Light: No Smoothing: Fixed:

Selects the single source image plane which is in best focus at each pixel location.

The resulting *Depth Map* - a list of each in-focus pixel with its source slice and X/Y co-ordinates - is generated with whole integer values referring to a single source image index for each location and the depth map image will be seen to have a 'layered' appearance.

The resulting *Montage* image is generated by pasting in the single pixel value from the selected source image at that location.

Reflected Light No Smoothing is the quickest algorithm, so is useful for determining optimal parameter values before performing a *Reflected Light with Smoothing* or *Transmitted Light* montage operation. The *Depth Maps* produced are not suitable for 3D viewing or measurement.



Note - This method is used by the *MultiFocus* module.

Depth Map image value (3).
Montage Image value copied from single source image (3).

Reflected Light: Smoothing: Blended:

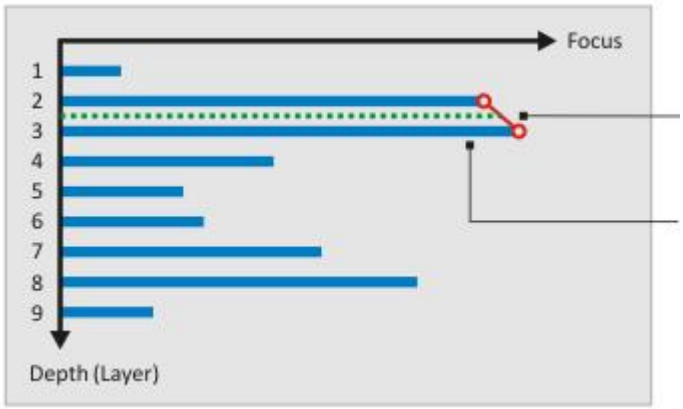
Takes into account the effects of adjacent in-focus planes at any one pixel location, and attempts to predict the point of maximum focus more accurately than the no smoothing method.

The *Depth Map* is generated with fractional floating-point values which shows slopes within the sample more accurately.

The *Depth Map* image will be seen to have a 'smooth' appearance.

The *Montage* image is generated by interpolating between adjacent source images according to the fractional part of the depth map value.

Reflected Light with Smoothing gives good results with many types of specimen.



Depth Map image value 2.7.
Montage Image value calculated from relative proportions of source images (2) and (3).

Blended Variations Min, Med & Max:

Blended (Min): The best focus for each pixel is compared with its focus in the two immediately adjacent source images and an interpolation is performed between those three values. This is expected to give the best results for most cases.

Blended (Med): The best focus for each pixel is biased towards whichever of the immediately adjacent source images has stronger focus in a ratio calculated using all three values. This tends to squeeze the *Depth Map* a bit more enthusiastically and should cope better when the sample has a continuous gradient but focus has been moved too far between successive source images.

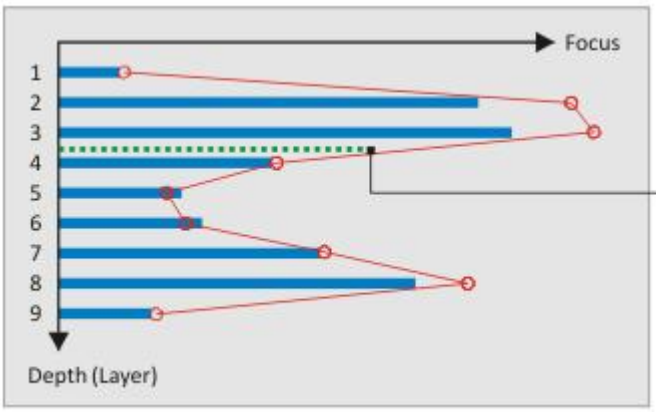
Blended (Max): This is like *Med* above but then applies a gentle smoothing to the *Depth Map* only. This should probably be viewed as a last resort to pull out acceptable-looking results when the other two have failed.

Transmitted Light: Method 1: Weighted:

Takes into account multiple in-focus planes at any one pixel location.

The *Depth Map* is generated with fractional floating-point values but may contain inaccurate depth values which do not correspond to an optimally-focussed source image.

The *Montage* image is generated by calculating relative proportions of all source images.



Transmitted Light: Method 1 depth is particularly effective on transparent biological samples, where more than one plane may contain focussed detail; The result is a combination of all focused planes, rather than a choice between them.

The result is expected to be a 'soft' focus not sharp.

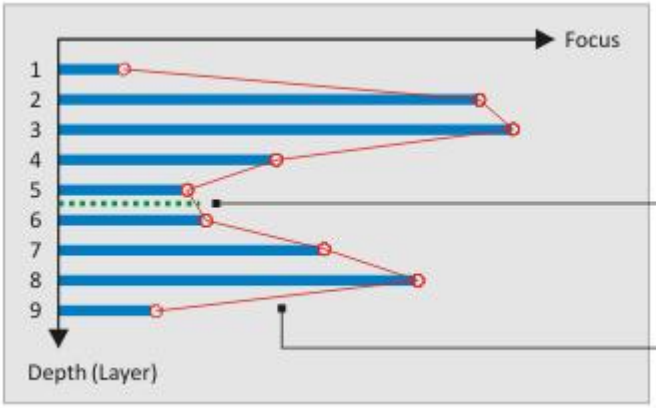
Depth Map image value of 3.6 is not accurate.

Montage Image calculated from relative proportions of all source images.

Transmitted Light: Method 2: Weighted Exponentially:

Takes into account multiple in-focus planes at any one pixel location, but biased more strongly towards the best focus.

The *Depth Map* is similar to that generated by the weighted depth algorithm, although it will usually be more accurate.



The resulting *Montage* image is generated by calculating relative proportions of all source images.

Particularly effective on biological samples with intrusive background.

Depth Map image value of 5.4 is not accurate.

Montage Image calculated from relative proportions of all source images.

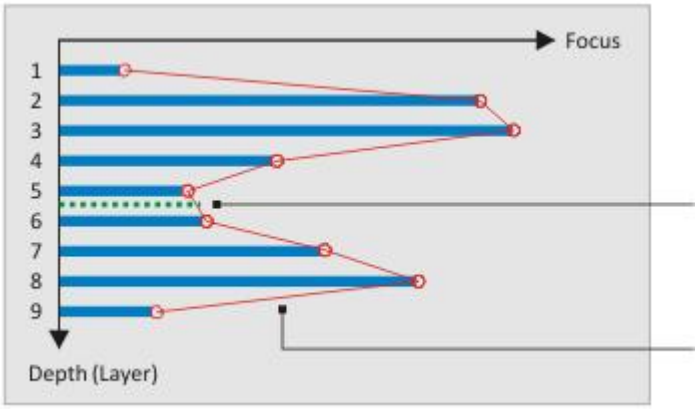
Transmitted Light: Method 3: Compound Weighted:

Takes into account multiple in-focus planes at any one pixel location, but biased more strongly towards the best focus.

The *Depth Map* is similar to that generated by the weighted depth algorithm, although it will usually be more accurate.

The resulting *Montage* image is generated by calculating relative proportions of all source images.

Particularly effective on biological samples with intrusive background.



Depth Map image value of 5.4 is not accurate.

Montage Image calculated from relative proportions of all source images.

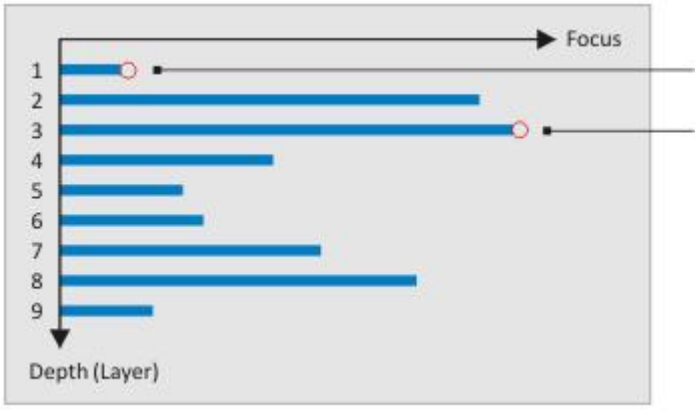
Fluorescence Min and Max Intensity: Projection:

A single pixel from each source image at the same X/Y locations is used as in all other cases.

Determine the maximum or minimum value that is found.

Place this value in the result image and repeat for all X/Y locations.

Often used on fluorescent images where light is being generated from various Z positions in the specimen.



Minimum

Maximum

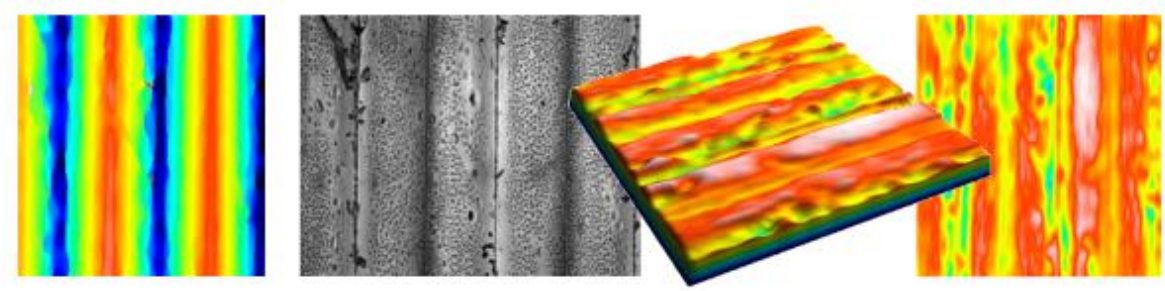
Leica Map is a powerful and sophisticated metrological application for analysing complex surface profiles and structures.

It works in conjunction with the *LAS Montage* module to produce comprehensive reports using a wide range of tools for both surface data manipulation and display. The reports can be customised to suite corporate or project styles and guidelines.

The working data is sourced from a *Montage* image sequence that has a processed montage image and depth map. This is the only data 'route' so *Montage* must be installed and licensed for *Leica Map* to work properly. The *Montage* stack must be captured using a well-maintained motorised microscope stand because the Z-positions must be accurate. *Refer to the Montage help: Go there...* 652

The following pages show how to export data to and launch *Leica Map,* and also provides a brief illustration of how it can be used. It has its own extensive help that can be launched from the *Main Menu* or used contextually by pressing key *F1.*

Leica Map is a very flexible and adaptable program but outcomes are always dependent upon the suitability of the original specimen and the users interpretation of the results.



Leica Map is a Windows application *not* an optional module that runs independently from LAS.and is installed from the LAS DVD. The dongle provided must be fitted to a USB slot on the computer whilst the program is running.

Leica Map can be used initially in demo mode with full functionality for a limited period. It is recommended that it is not installed until you are ready to start an evaluation.

However after the demo period expires, a license must be purchased for continued operation. A Leica Map dongle (separate from the LAS dongle) will be .supplied to enable the license. It is recommended that the Leica Map dongle is attached to a USB port on the computer before it is booted to make sure of validation before *Leica Map* is launched.

Leica Map can be launched directly from the desktop icon once installed for use with images directly from the computer hard drive.

Select the File Format

Leica Map must be configured to accept the Montage file formats and layer structure.

To select the file format:

1: Click on *Options* on the main menu and...

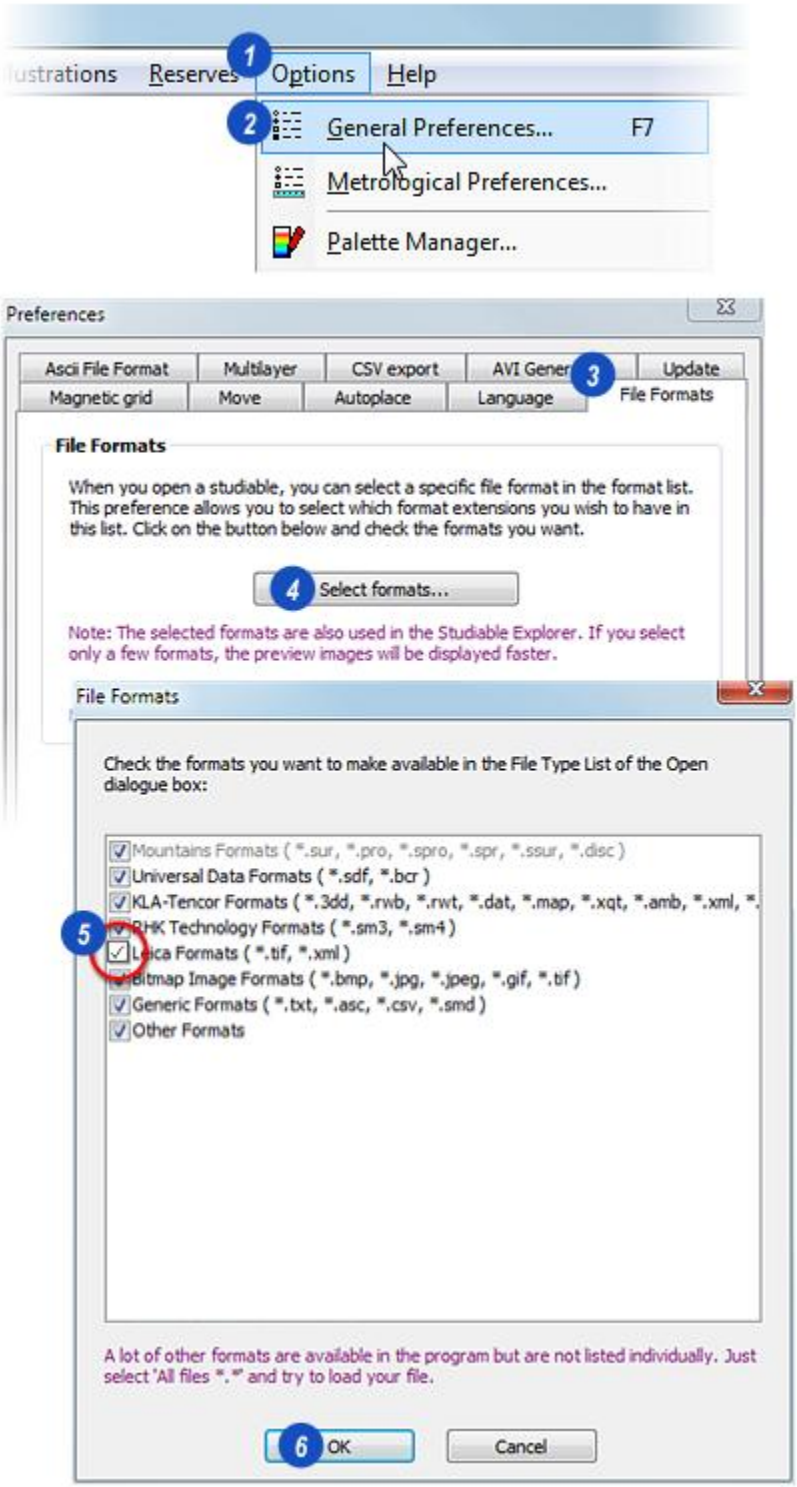
2: ...from the drop-down menu click to select *General Preferences*. Alternatively, press *F7* on the keyboard.

3: On the *Preferences* panel, click the *File Formats* tab and...

4: ...the *Select Formats* button.

5: The list of file formats appears. Scroll down the list until the Leica Formats entry appears and click to enable the check box.

6: Click *OK*.



Select the Layer type as follows:

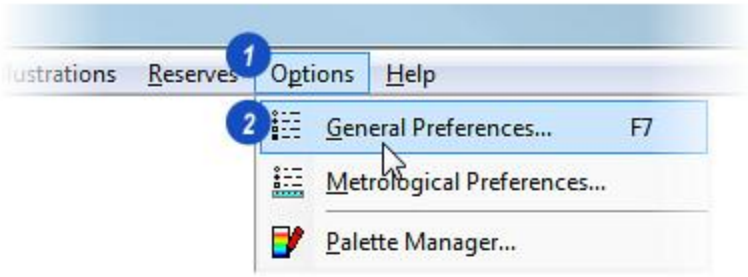
1: Click on *Options* on the main menu and...

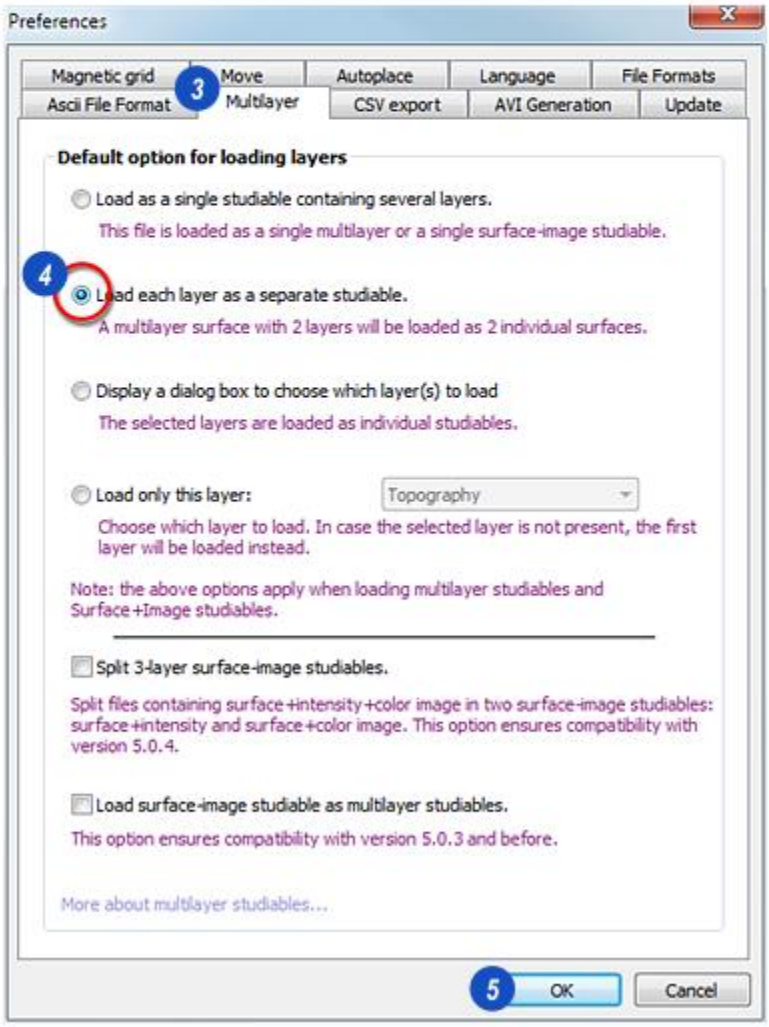
2: ...from the drop-down menu click to select *General Preferences*. Alternatively, press *F7* on the keyboard.

3: On the *Preferences* panel click the *Multilayer* tab.

4: Click to select the *Load each layer as a separate studiable* radio button.

5: Click *OK*.





Users that have just captured a Z-Stack sequence in *Acquire* and have automatically come to the *Browse Workflow* because of the *'After Capture'* settings in *Preferences*, can skip this page.

For a previously captured Z-Stack sequence:

1: The *Montage* module must be active. If it is not click on the *Selector* button and...

2: ...from the *Acquisition Mode* list click to select *Montage*.

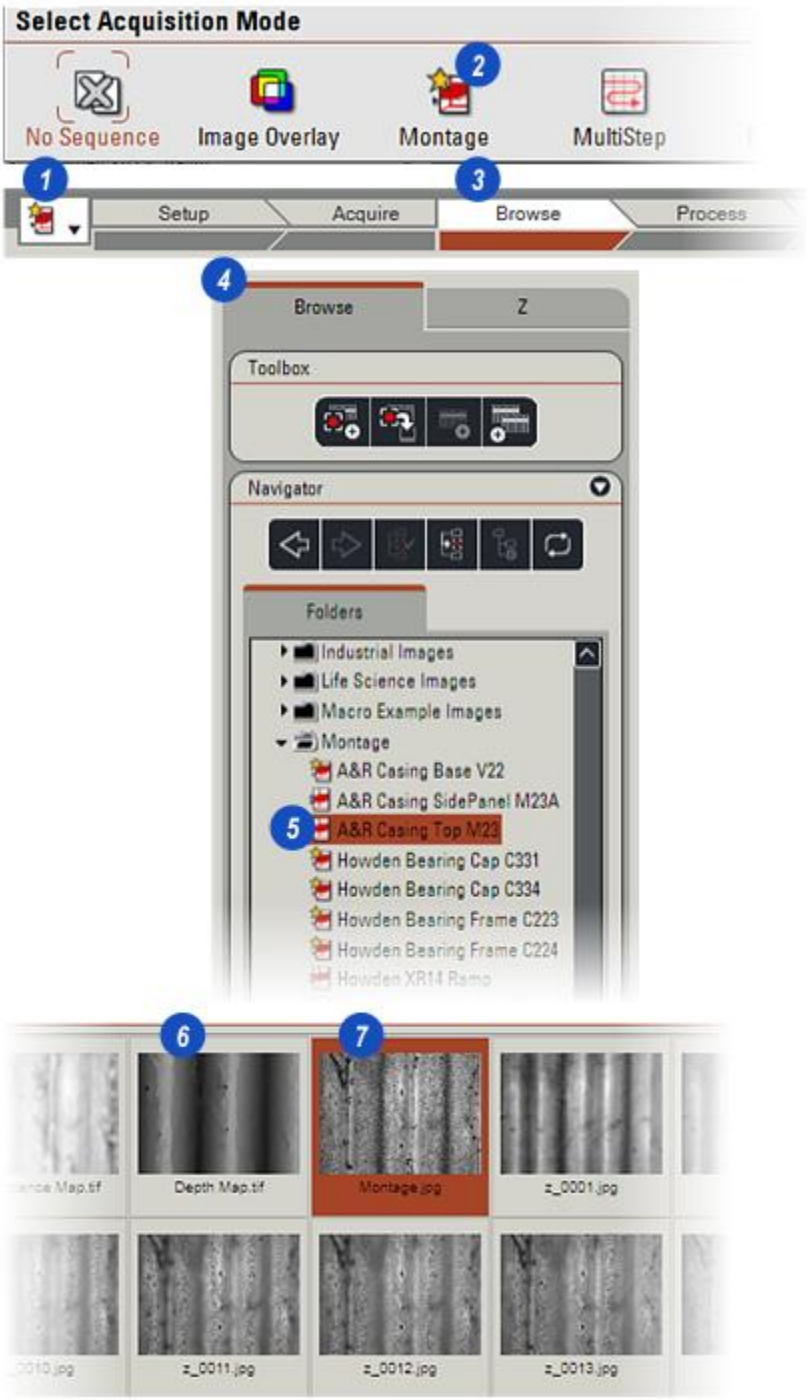
3: Click on the *Browse Workflow* and...

4: ...on the *Browse* tab.

5: In the *Navigator* locate the folder containing the *Montage Z-Stack* sequence and open it.

6: In the Z-Stack sequence there must be a *Depth Map* and...

7: ...a *Montage* extended focus image.



To launch the *Leica Map* application from *Leica Application Suite* with *Montage* active:

1: Still in the *Browse Workflow*, click on the *Z* tab.

2: Click on the *Leica Map* button. This will only be available if Leica Map is installed and the dongle is present and validated.

3: *Leica* Map is launched and the flash screen appears.

If *Leica Map* is already active it is detected and the *Montage* data transferred to it automatically.

Before image analysis begins, the user has the template option:

- Use an existing template - either the default provided with Leica or one created by the user, or...
- A basic report which can have specific analyses added to suit the project.

Using a Template:

1: To use a template for the report, click to enable the *Template* check box - a tick mark appears.

2: Select a template by clicking the *browse* button and...

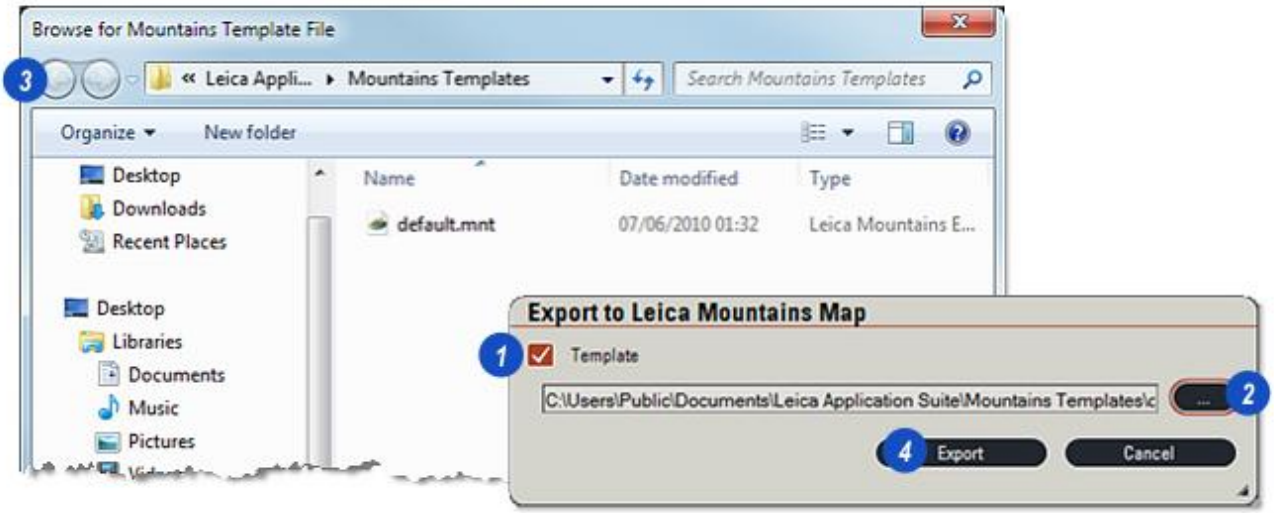
3: ...navigating to the folder in which the templates are stored. The location for the default template is:

C:\Users\Public\Documents\Leica Application Suite \Leica Map Templates\default.mnt.

4: Click the *Export* button.

Any previously saved *Leica Map* report with the *.mnt* extension stored in any location can be used as a template. However, but some *Montage* images may not be appropriate to the *Operators* and *Studies* used in the template and in this case *Leica Map* will automatically close.

Template examples are shown on the following pages.



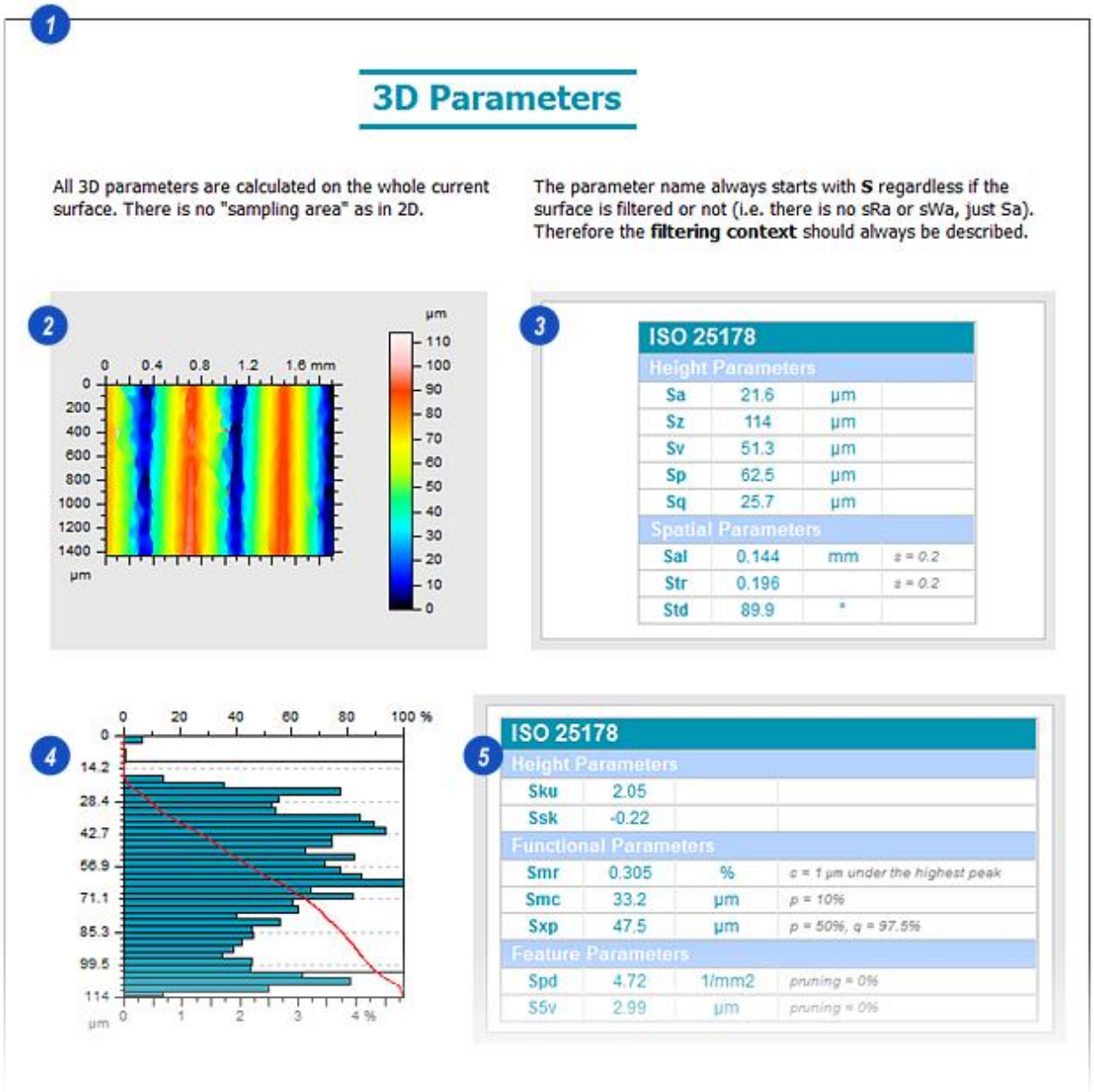
The default *3D Parameters* template **(1)** creates a report that contains much of the data prescribed in the *ISO 25178 Surface Texture Standards.*

A *Topography* pseudo colour image **(2)** is accompanied by a *Height Parameters* table **(3)** containing data such as *Mean Height of the Surface, Maximum Height of the Peaks, Valleys and the entire Surface,* and the *Root Mean Square Height. Spatial Parameters* include *Texture Aspect Ratio* and *Texture Direction.*

The *Abbot-Firestone Curve* **(4)** is represented as well as a table **(5)** showing *Height Parameters (*such as *Kurtosis Height distribution* and *Skewness), Functional Parameters* (*Surface Bearing* and *Extreme Height*) and *Features* (*Peak Density* etc) derived from segmentation.

Also included but not shown on the illustration, are *Waviness* and *Roughness* pseudo colour images.

Pages can be added to the report together with a wide range of specific data and images. *Operators and Studies: Go there...* 692



1: If the *Template* option is cleared the browse button is no longer available and a basic report is created.

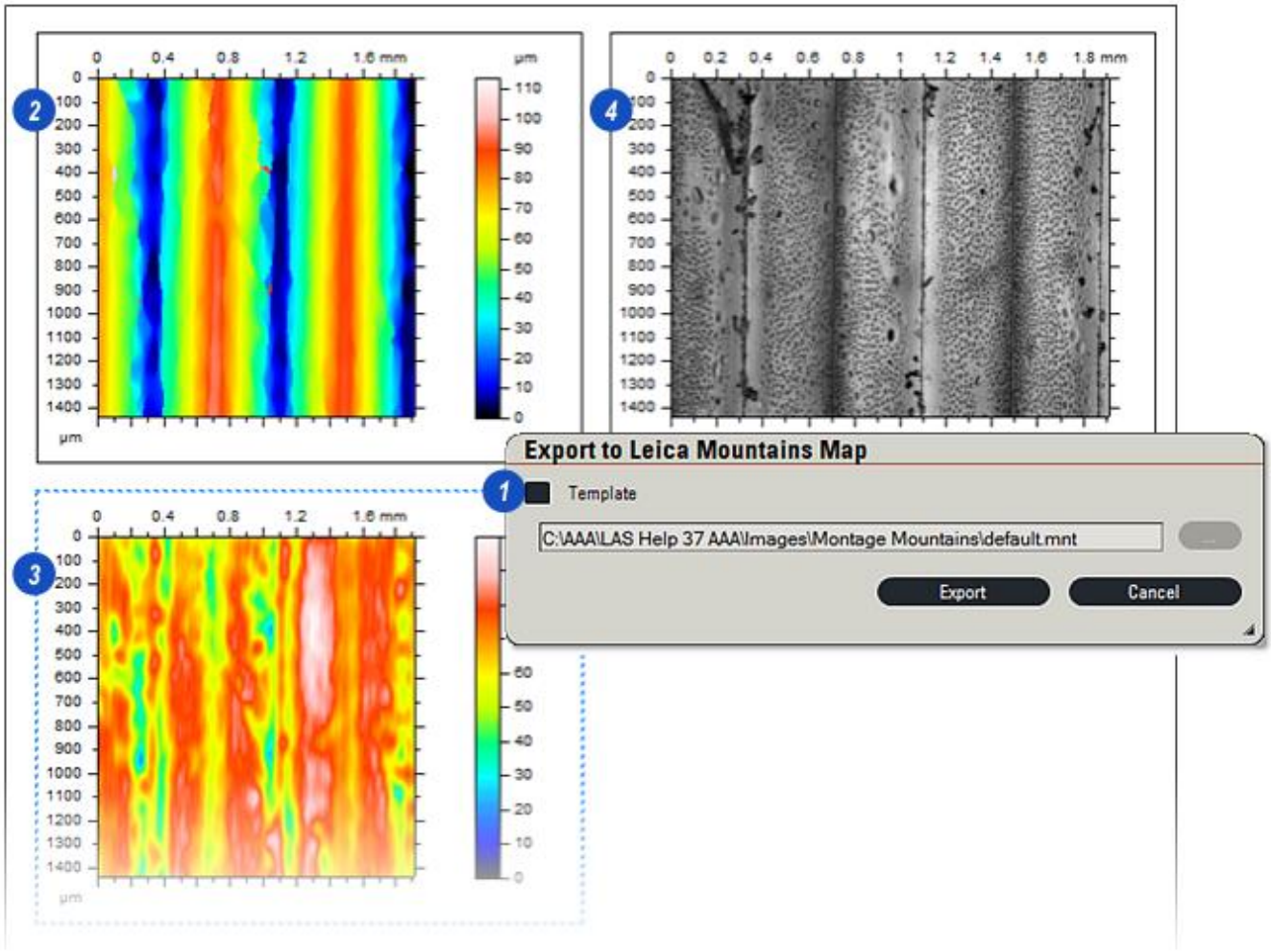
2: Click *Export* and only essential images such as the *Topography*...

3: ...and *Intensity* layers are included on an otherwise blank report sheet.

4: The report example shown here also includes the original *Montage* image.

The user can then add tables, analyses and images - called *Studiables* - appropriate to the project using *Operators and Studies:* 692 *Go there...* 692

More pages can be added and the various components moved around with borders, text, captions, company logos, names and report headers added as required. The resulting document can then be used as a template for other, similar projects.

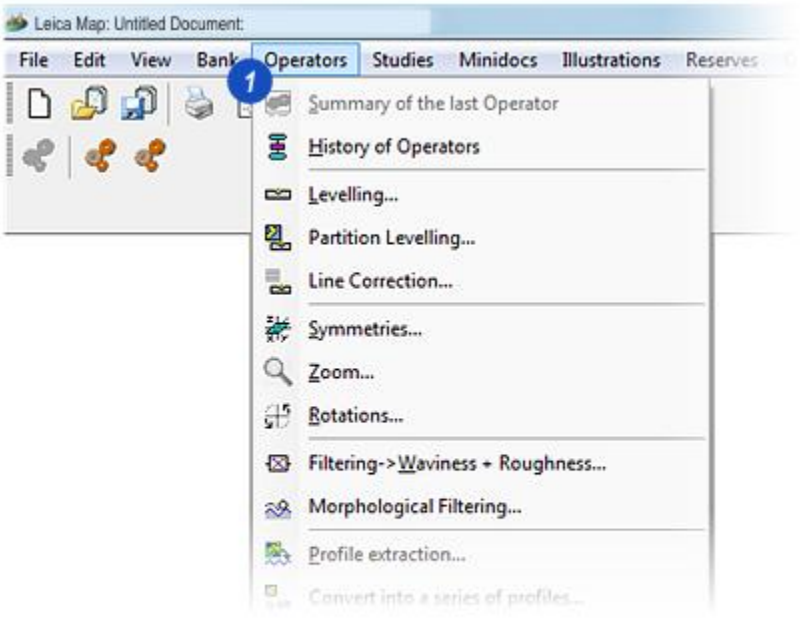


Leica Map performs surface analysis by applying *Studies* and *Operators* to the data being analysed (known as a Studiable), in this case *Depth Map* and *Montage Images*.

Studies are graphical representations of a studiable such as a 3D view or an analysis performed on a studiable such as a step height measurement.

Operators are mathematical transformations used to modify a studiable by adjusting settings in an associated dialog box. Examples would be to level a surface or to extract the waviness by filtering.

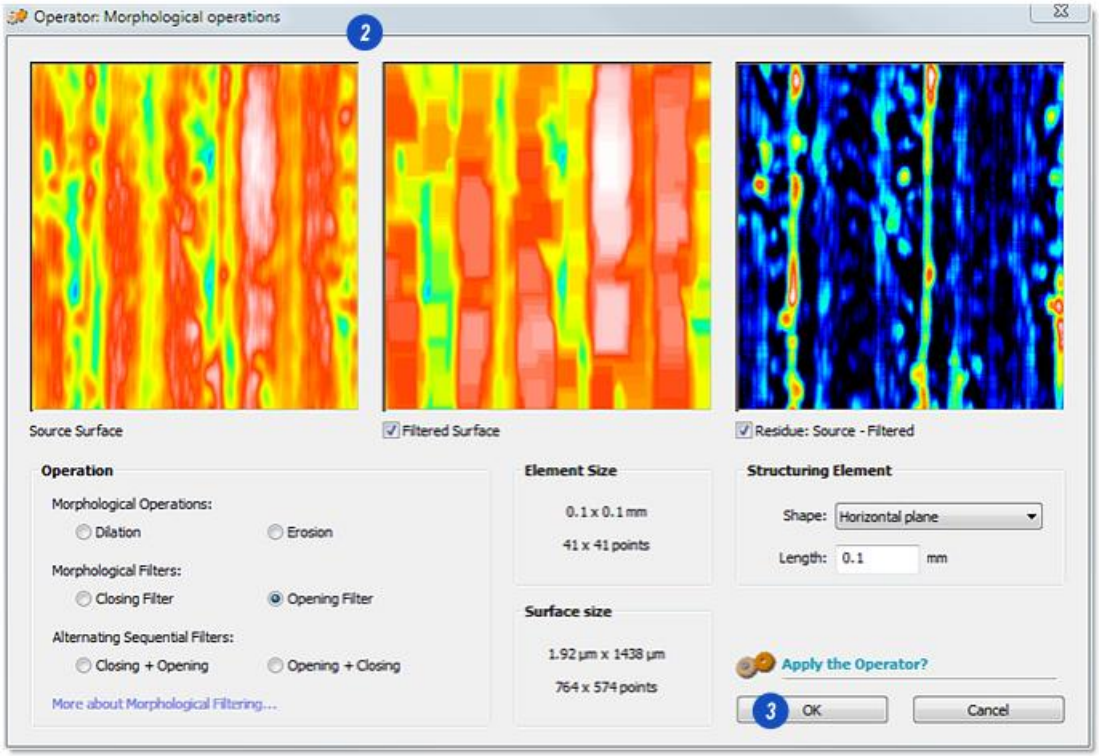
Many *Operators* are interactive providing controls to adjust for example, filter structures, thresholds and edge detection.



1: With an image on the report selected, on the *Main Menu* click the *Operators* option.

2: The *Operator* dialog appears. The controls and output displays differ with the *Operator* chosen. Change the parameters as needed and...

3: ...to include the results in the report click the *OK* button. Clicking the *Cancel* button will close the dialog and not include the results in the report.



Studies provide different methods for representing the surface data.

1: On th *Main Menu* click the *Studies* option.

2: From the drop-down, click to select the required study type.

3: If necessary, a new page is added automatically to the report and the study graphic displayed. *3D View* and *Distance Measurement* are illustrated.

Some studies have built-in interactivity. For example, on a corner of the *3D View* click and hold down the mouse button whilst moving the mouse will rotate and tilt the image. Similarly, the dotted lines on the *Distance Measurement* can be dragged to measure a specific distance or angle.

4: Right-click a study to reveal and change its parameters.

5: Display features such as axis colours and graduation, image tinting and slicing for both *Operators* and *Studies* can be changed using the mini-toolbars available when an image is selected.

An additional set of tools can change border colour and thickness and alter the image sequence if they are overlapping.

Illustrations and *Reserves*, both available from the *Main Menu* can be used to add captions, images and and a range of graphical features to enhance and customise a report.

Leica Map has a dedicated help file accessed through the help option on the *Main Menu* or contextually by pressing the *F1* key when the program is running.

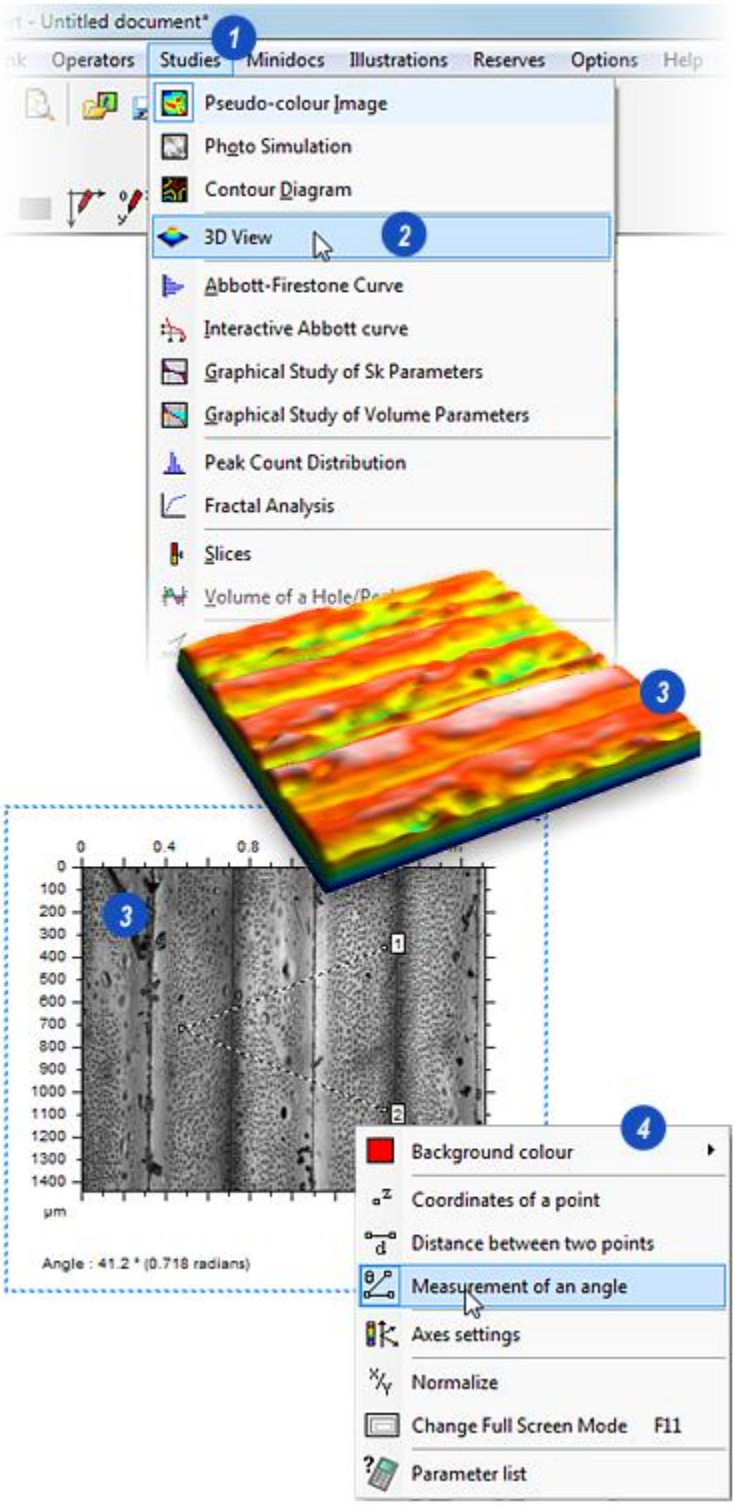
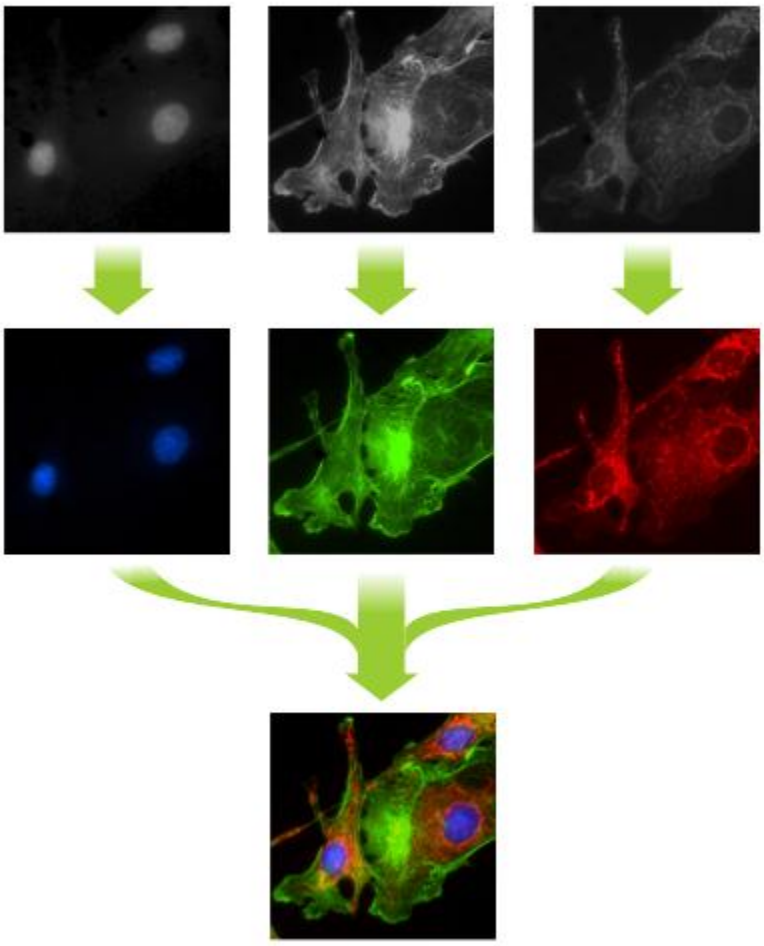


Image Overlay

Image Overlay is an LAS module that enables the acquisition of Channel images with a LAS compatible microscope and the creation of a composite image from a sequence of these images. The Channels are typically Fluorescent channels but can also be any other available contrast method.



Sequences of channels may be defined and for each channel the microscope and camera settings set and saved. Once a sequence is acquired a coloured overlay image may be made using a variety of methods.

If using a colour camera the *Image Type* should be set to greyscale.

See *Acquire > Camera > Image Formats* 290

Fully motorised microscopes will automatically select filters, but users of manual machines may still use Image Overlay simply by physically turning the filter turret when prompted.

Between 2 and 8 images are captured during an Overlay sequence. The subject and magnification are the same for all, but individual images use a different filter and exposure to optimise contrast for a specific part of the image.

Additional enhancement and ‘separation’ is achieved with pseudo colour – a digital staining technique – all controlled within Leica Application Suite.

The final step is to bring all of the images together in a single combined overlay in which individual parts can still be easily identified to illustrate their place in the ‘whole’.

Converting a folder of images into an Image Overlay

You can combine a folder of existing images into an Image Overlay. For example, you might have obtained images from elsewhere (i.e. **not** using the Image Overlay *Acquire* tab).

See Converting a Folder of Images 715.

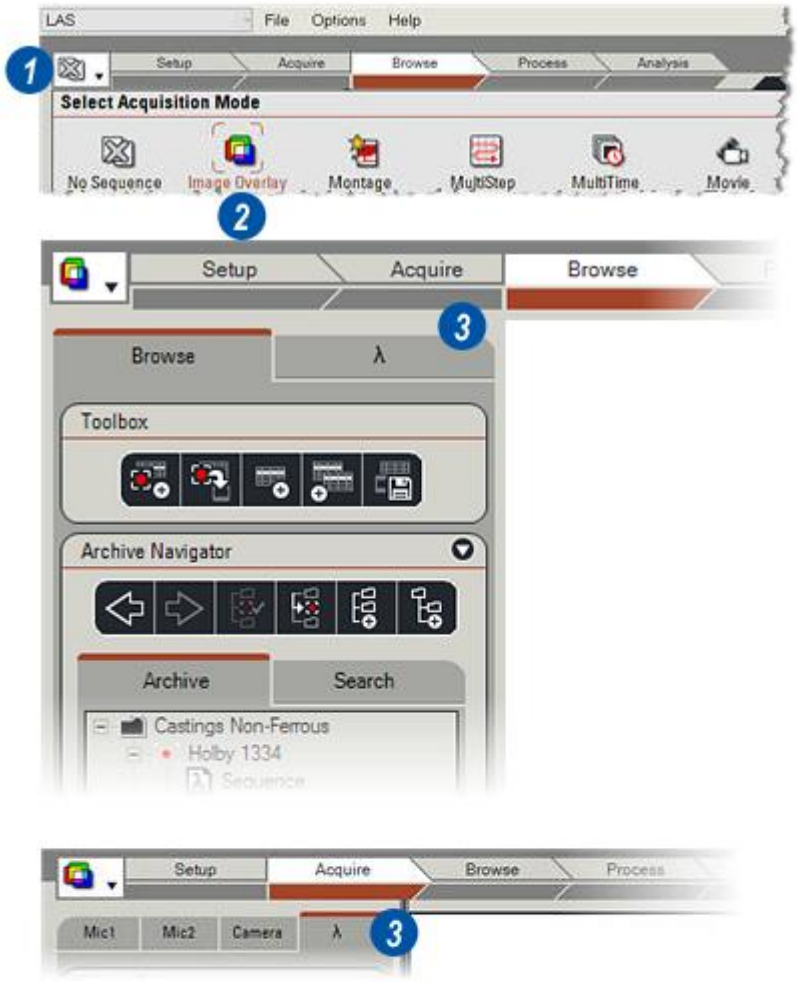
Enable Image Overlay

The *Image Overlay* module must be installed and enabled; If it is not the icon will not appear on the *Acquisition* menu **(2).**

To start Image Overlay:

1: Click on the *Select Acquisition* icon and from the menu…

2: Click to select *Image Overlay*. After it is selected an additional tab marked with the Lambda (**λ**) symbol **(3)** appears in both the *Browse* and *Acquire Workflows*.



1: Select the *Acquire W*orkflow by clicking on its tab.

2: Click on the *Image Overlay* tab marked with the Lambda (λ) symbol to reveal the Channel Setup panel comprising…

3: The *Setup Tools* and…

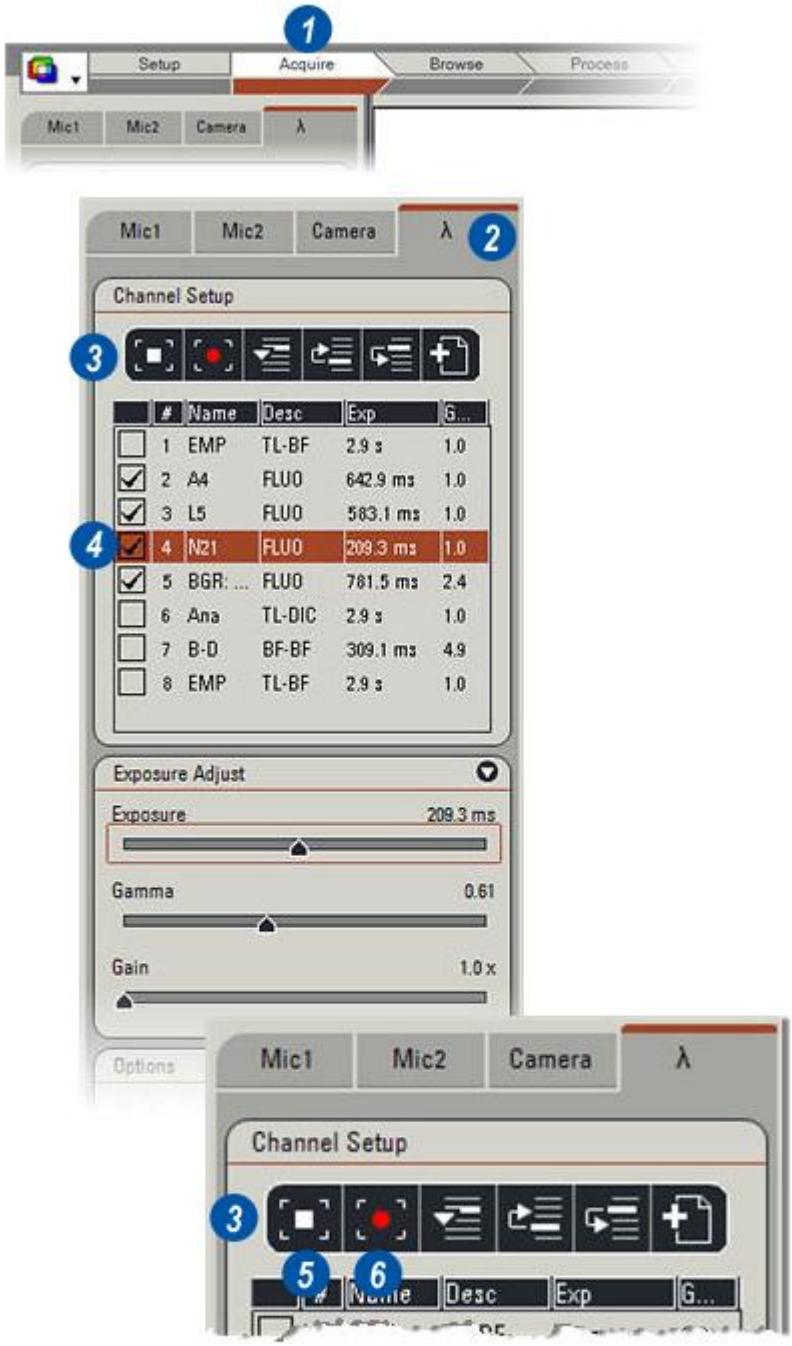
4: The *Channel Dialog.*

Using the Setup Tools:

5: *Camera Live* 'freeze' halts the camera activity leaving the latest image on the Viewer. Click again to resume taking live images. Use the *freeze* button in combination with…

6: *Capture Single Image* which will save the current image to the capture folder. The filename is created automatically and a thumbnail of the image is displayed in the Working Gallery.

See: Channel Dialog 699

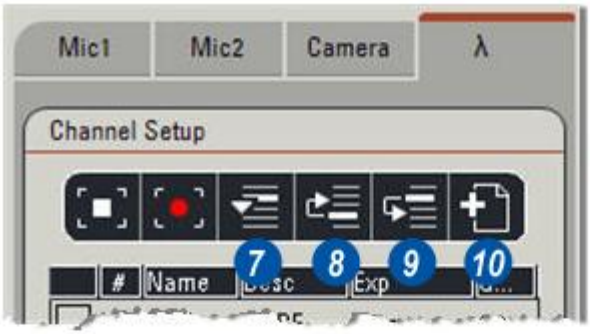


7: The *Next Filter* button moves the focus of interest to the next available filter on the Channel dialog. The focus 'rolls over' from bottom to top automatically.

8: Click the *Move Up* button to move the selected channel up in the filter sequence.

9: Click the *Move Down* button to move the selected channel down in the filter sequence.

10: To save the channel dialog sequence, click the *Create New Sequence* button.



See: Channel Dialog 699

The Dialog is a list of the filters available on the microscope with details of:

1: The filter position on the turret. Initially, this will also represent the image capture sequence in automatic mode but may be changed.

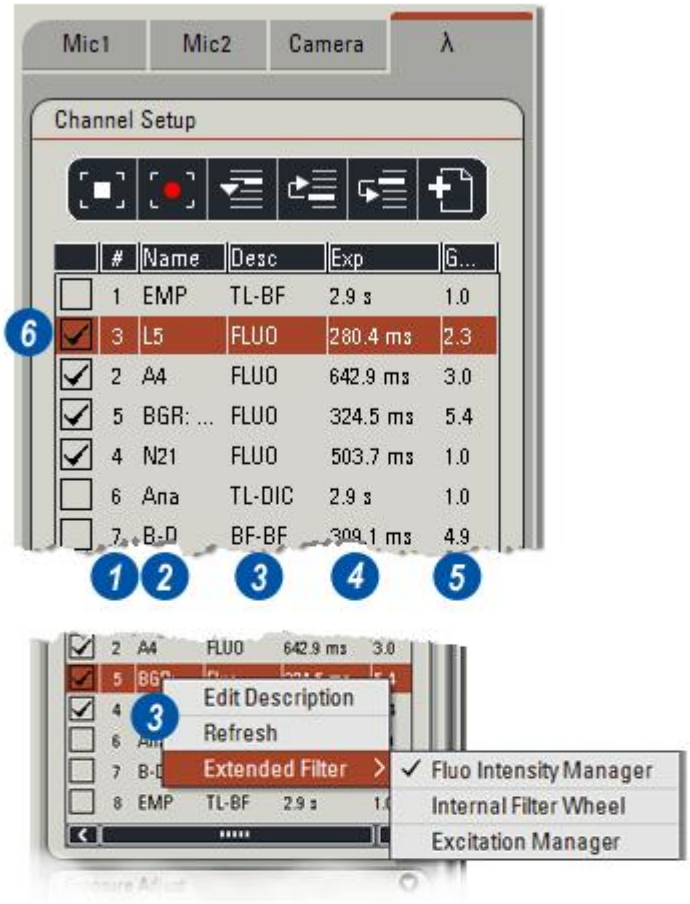
2: The filter name.

3: The contrast method. Right click on the entry to reveal a menu with options based upon the filter type:
Edit Description to change the description:
Refresh to update the filter information:
Reset Pseudo Colour reverts to the filter's default value. This is only available on monochrome cameras.
Extended Filter reveals an additional menu to determine how the filter works with the internal filter wheel.

4: Image exposure time. Initially, this value will be the same for all filters but may be changed individually to achieve the best results.

5: The Gain value. Again, these will be set to the same common value but may be altered for each filter.

6: To the left of each filter entry is a check box. When this is enabled (ticked) the filter will be actively used in the sequence and, with its associated image becomes a Channel.



Select a Channel by:

7: Clicking anywhere on a filter entry.

8: Clicking to enable the checkbox. At least two and up to the total filters available may be chosen for a complete Channel sequence.

Changing sequence order:

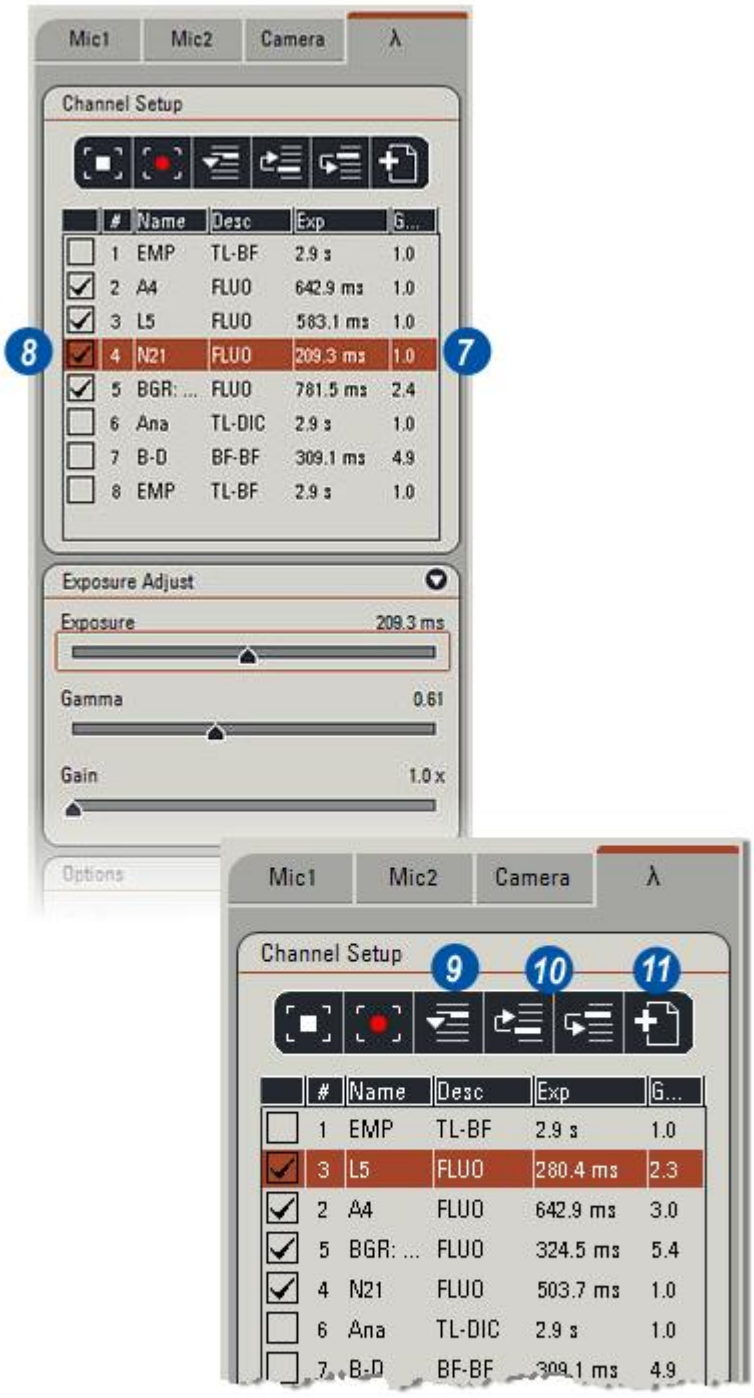
9: Select an enabled Channel by clicking the *Next Filter* button.

10: Click either the *Move Up* or *Move Down* buttons to change the order in which images will be captured.

Saving a Channel sequence:

When all of the required filters have been selected:

11: Click on the *Create new sequence* button to save the Channel sequence.

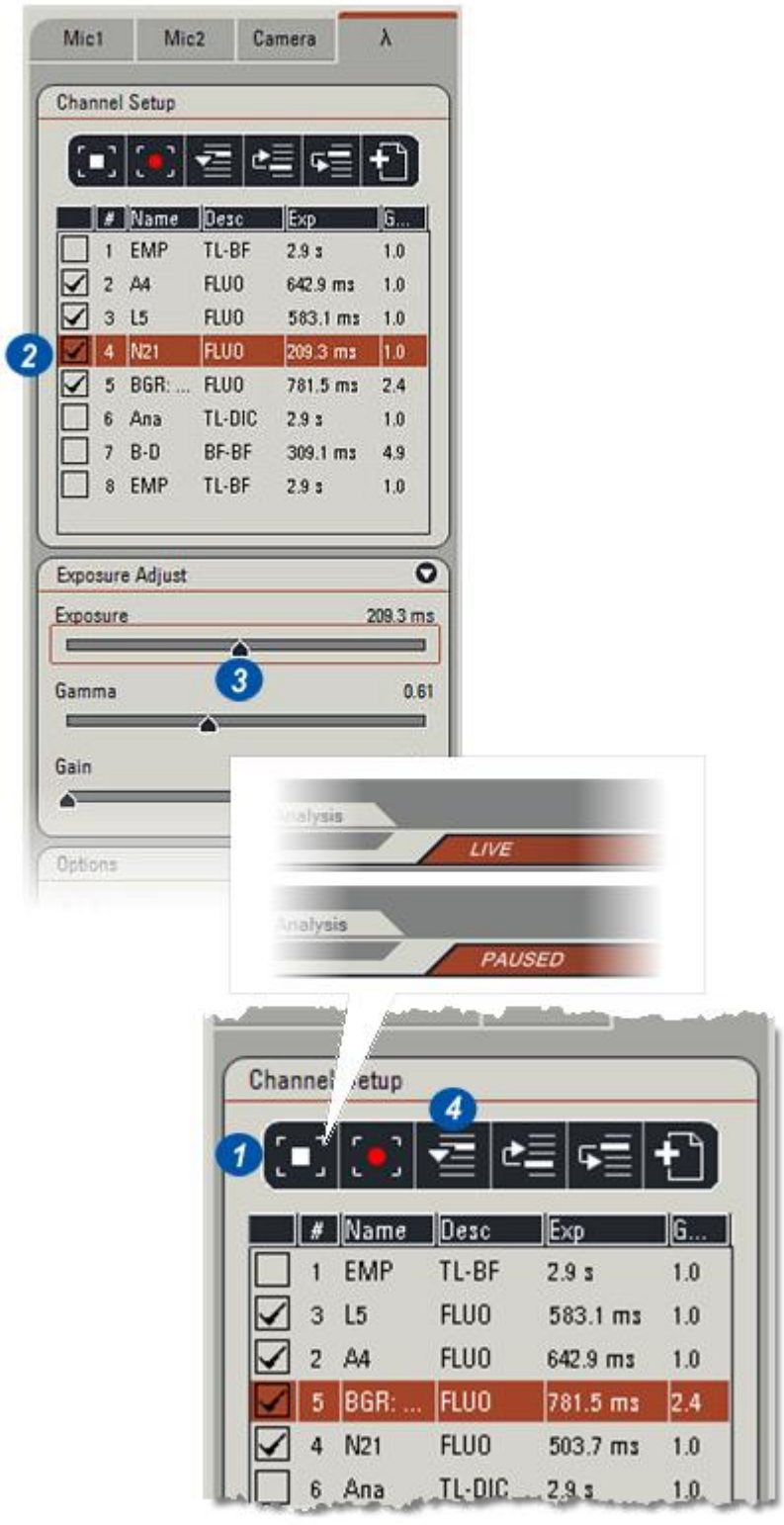


Each Channel may have *Exposure, Gamma* and *Gain* values set individually to achieve the best image.

1: Check that the *Camera* is in live mode. The message on the top bar of the *Viewer* indicates the camera state. If it reads *Paused* then click the *freeze* button to return to live. The button icon should have a small square in the centre, not an arrow.

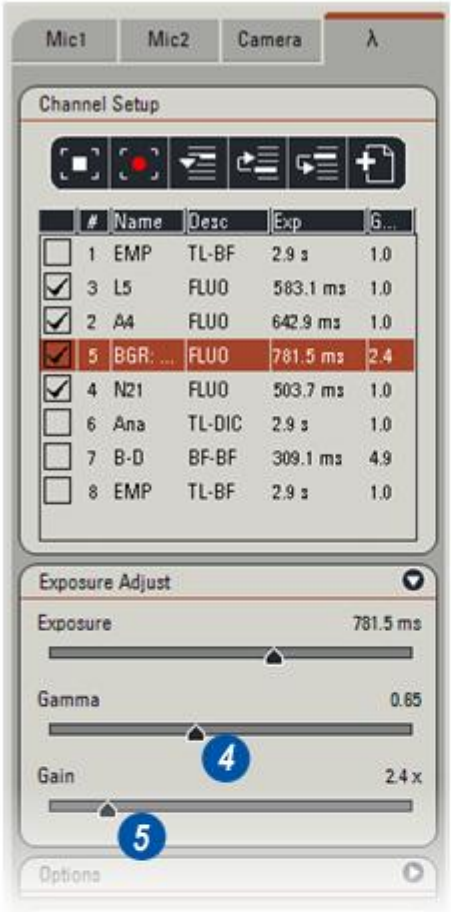
2: Click on the *Next Filter* button to move to the Channel required. Motorised microscopes will automatically turn the filter turret to the correct position, but non-motorised machines will have to be set manually. The filtered image will appear in the *Viewer*.

3: If necessary, alter the *Exposure* time by clicking and holding the *Exposure* slider and drag it left, to decrease exposure, or right to increase it.



4: The *Gamma* value is normally set to best suit the viewing medium – in this case a colour monitor. Small changes can have a dramatic effect on colour density but in some cases can help to improve contrast. Click and hold the *Gamma* slider and drag left to reduce the *Gamma* value or right to increase it.

5: *Gain* will brighten or darken the image without affecting the *Exposure* time. Try to keep *Gain* to low values to avoid introducing 'noise' into the images. Click and hold the *Gain* slider and drag it left to darken the image or right to lighten it.



The *Capture Single Image* function provides a simple ‘snapshot’ method of checking images from individual Channels after making changes to the Exposure settings.

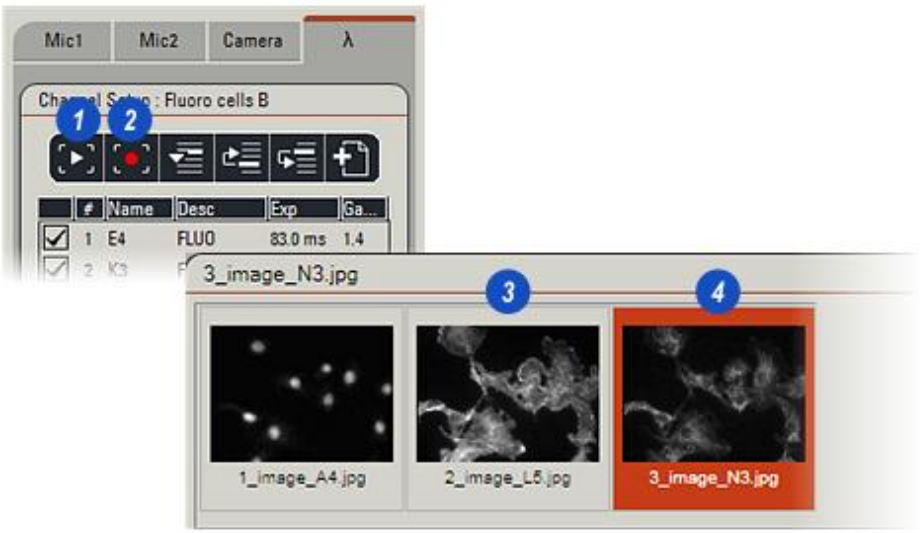
If necessary, click on the *Channel* to snap. Wait for the *Viewer* to settle and then:

1: Click on the *Camera Live* button to halt the *Camera*. Check the *Viewer* top bar: The message should read *Paused.*

2: Click on the *Capture Single Image* button.

3: The 'snapped' image appears in the *Gallery*.

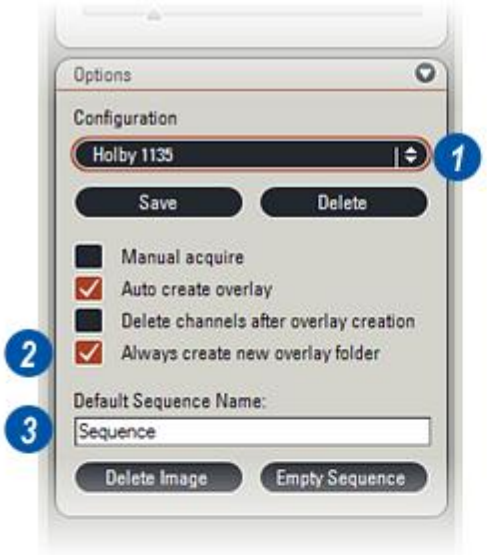
4: Remove snapshots from the *Gallery* by clicking on the thumbnail to select it and then pressing the keyboard *Delete* key.



Changes made to any of the Channel settings are immediately reflected on the live image in the Viewer and also appear in the appropriate columns in the Channel dialog.

Always check that the correct filters are in the specified positions on the filter turret – some microscopes can do this automatically, but most require a manual check.

Final settings are made on the Options panel:



1: Click on the arrows to the right of the *Options* bar to reveal the panel.

2: If the *Always create new overlay folder* check box is enabled, every time an Overlay sequence is acquired a new folder within the current capture archive will be created.
Situations, in which a substantial throughput is expected, will benefit from this time-saving option.

3: If *Auto create overlay* is selected, the text in the *Default Sequence Name* is used as part of the folder name.

Continued 704 *...*

4: The folder name comprises the *Default Sequence Name* and a sequential number. Creation and naming are fully automatic.

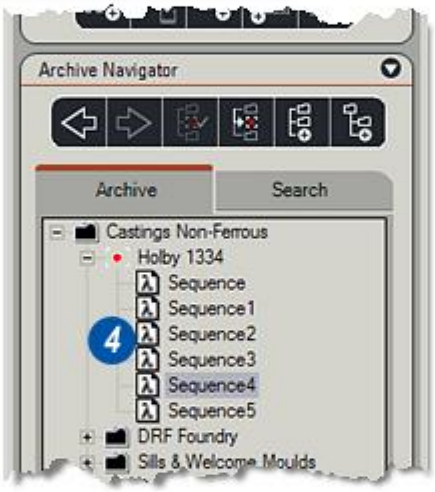
5: Save the configuration, including the Channel sequence by clicking on the *Save* button and…

6: Entering an appropriate name for the configuration and…

7: Clicking the *OK* button.

8: The new name appears in the *Configuration* window and the settings may be used again for future overlays.

See: Selecting an existing Configuration. 708



For speed and efficiency, select:

1: *Auto create overlay* by clicking the check box and…

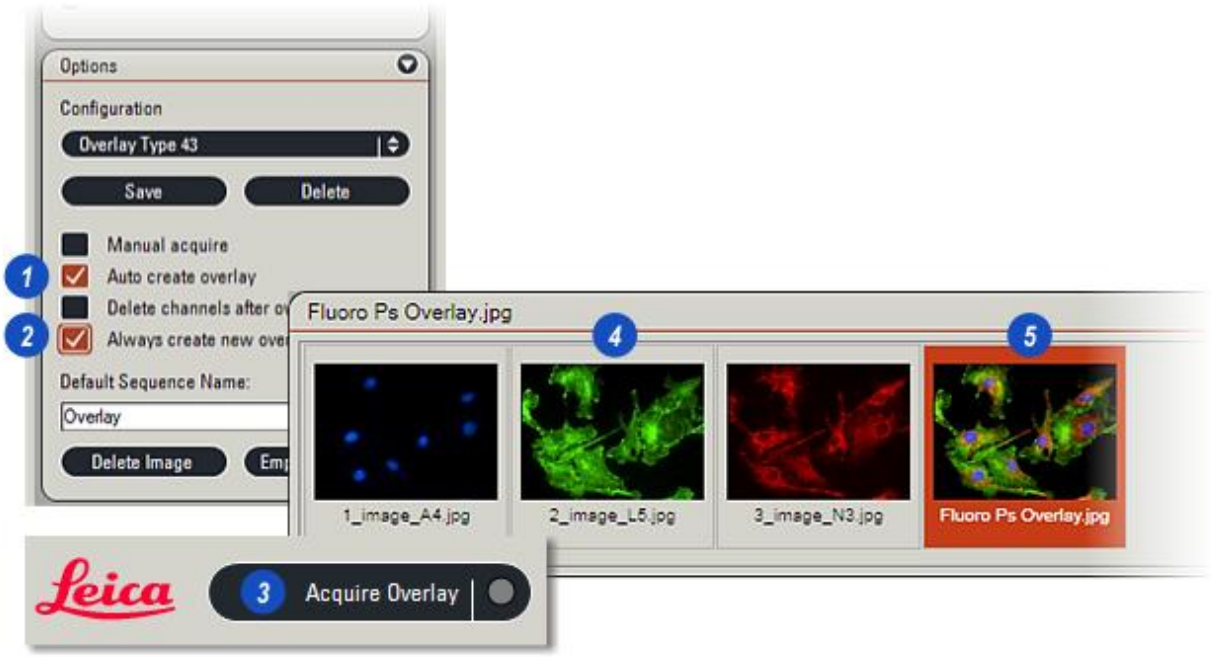
2: Click on the *Always create new overlay folder* check box.

3: Click on the *Acquire Overlay* button.

These options will create a new folder within the capture folder with the *Default Sequence Name* as part of its name. The images for each of the filter channels **(4)** will be saved inside the folder and they will be automatically combined to produce the Overlay **(5).**

Adjusting Channels before creating:

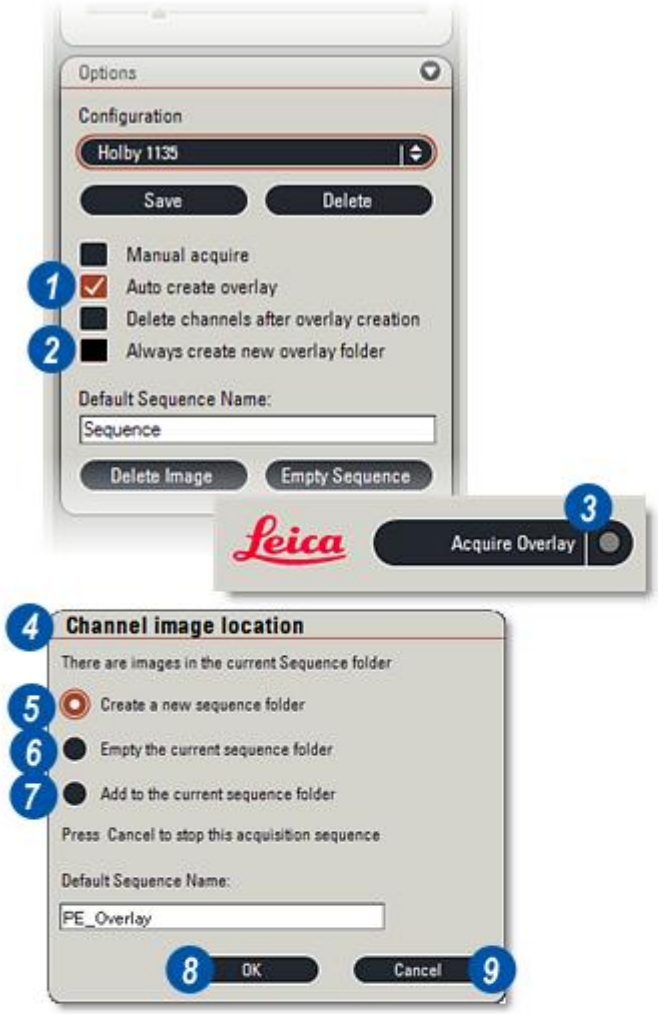
1: Uncheck the *Auto* create overlay check box for a preview before committing. Each of the images may then be examined and adjusted for colour balance and brightness before creating the overlay.



1: Click on the *Auto create overlay* check box to enable it.

2: Disable *Always create new overlay folder* by clicking the check box until the tick symbol disappears.

3: Click on the *Acquire Overlay* button. The Channel image location dialog appears (**4).**



There are 3 options on the dialog:

Create a new sequence folder **(5)** will create a new folder within the capture folder and save the images to it – the equivalent of checking the *Always create new overlay folder* check box.

Empty the current sequence folder **(6)** will delete all of the images inside the current folder and replace them with the new acquisition.

Add to the current sequence folder **(7)** is a powerful option that allows any number of channel images and subsequent overlays to be stored in the same folder. Each acquisition is given its own sequential number to differentiate between the sets**.**

Select the required option by clicking the button next to it and clicking on the *OK* button **(8).**

9: The entire acquisition sequence may be stopped by clicking the *Cancel*

Auto Overlay With Delete Channels

Enabling the *Delete channels after overlay creation* option, removes the individual Channel images after the overlay has been created automatically. This is an excellent 'housekeeping' arrangement for very high throughput where there is no requirement to add pseudo colour to the images.

1: Check the *Auto create overlay* check box and…

2: ...the *Delete channels after Overlay creation* check box.

3: Click on the *Acquire Overlay* button. The Channel images are deleted after the overlay is created and saved in the current *Capture Folder* – not in a newly created folder or the last newly created folder.

Previously saved configurations stored in the current capture folder can be re-called by clicking on the arrows to the right of the *Configuration* window **(1)** and then choosing from the list **(2)**. Changes may then be made to the exposures and to the Options with the altered settings saved as a new configuration **(3).**

Manual Acquire:
Primarily designed for non-motorised microscopes, the *Manual Acquire* facility prompts for the filter turret to be moved manually to the correct position before the image is captured.

4: Click to enable the *Manual acquire* option.

5: Any or all of the other options may be selected in *Manual Acquire* mode.

6: Click on the *Acquire Overlay* button.

7: When the *Manual Sequence Prompt* appears, turn the turret to the filter position specified on the prompt and…

8: Click *Continue*. The image will be acquired and appear as a thumbnail in the working *Gallery*. The prompting process repeats for all of the selected filters.

9: To end a capture sequence prematurely, click on the *Finish* button.

When the Channel images have been captured…

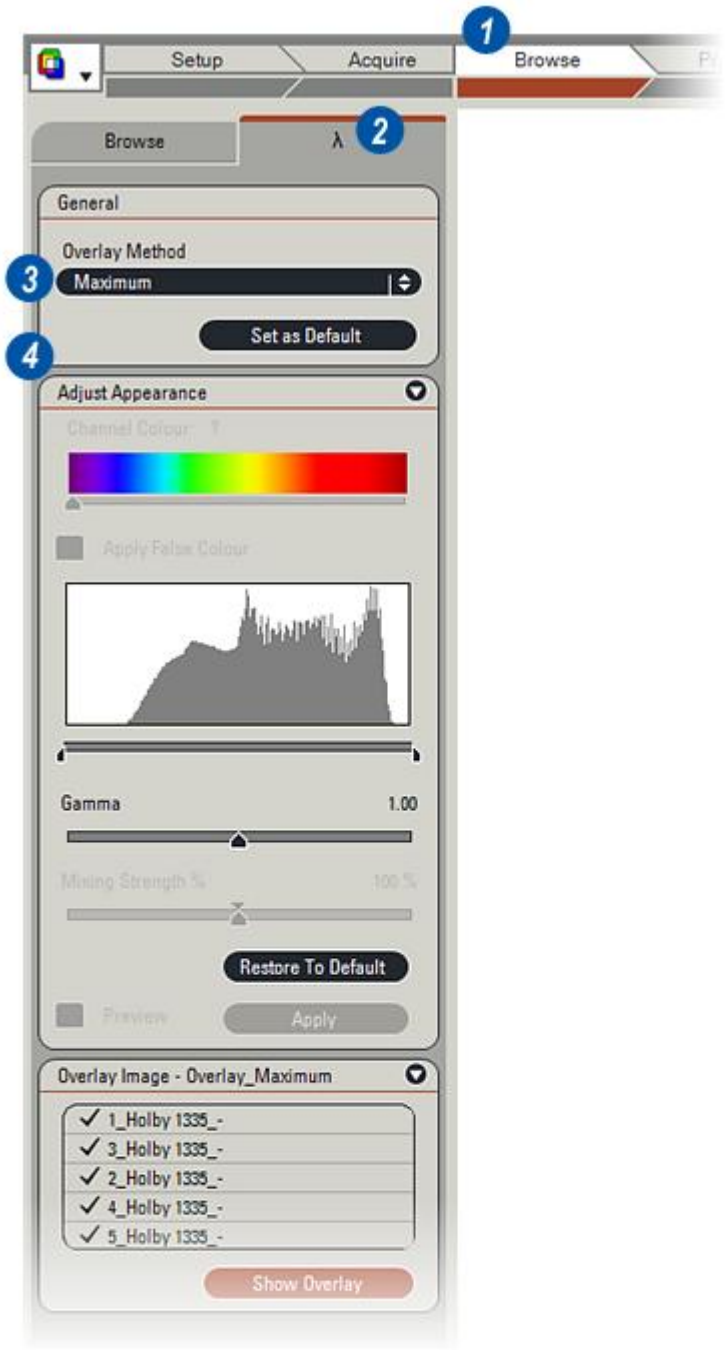
1: The *Browse Workflow* opens with…

2: ...the *Image Overlay* or a preview selected. Thumbnails of the captured channel images together with the composite overlay if auto-create were selected, will be displayed in the *Gallery*. The *Viewer* shows the last image captured or the overlay.

There are three panels on the Image *Overlay* tab:

3: The *General* panel on which the *Overlay Method* determines the manner in which the channel images are combined to create the overlay.

4: The *Image Groups* and *Overlay Method* settings can be saved as the ongoing default.



The *Adjust Appearance* panel provides the controls for:

6: Adding false (pseudo) colour to monochrome images.

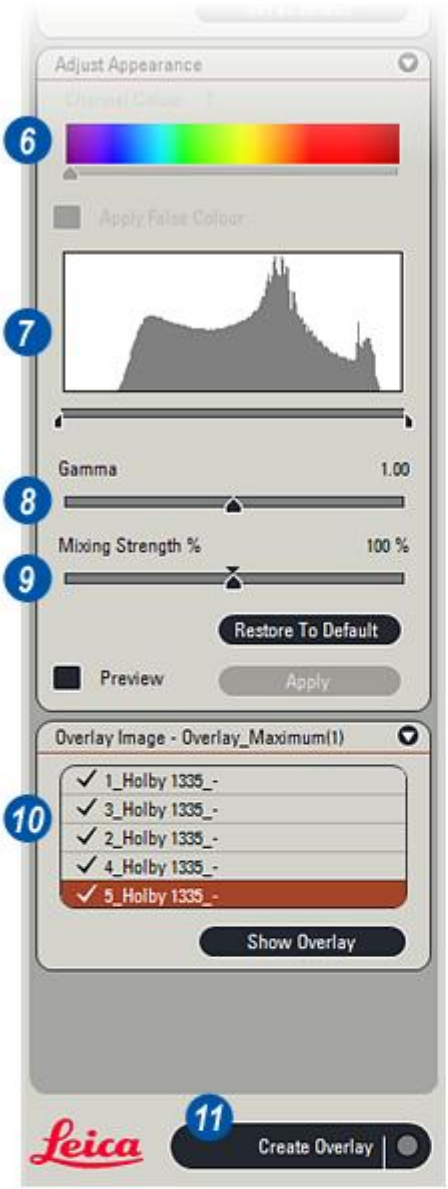
7: Adjusting colour balance with *Histogram* controls.

8: Changing the *Gamma* value of the channel images and the overlay, and

9: ...varying the 'mix' strength or dominance of a channel image. Images captured in colour will not have all of the colour controls available.

The *Overlay Image* panel **(10)** determines which of the channel images are to be included in the overlay. At least 2 channels must be selected before an overlay can be created.

11: The *Create Overlay* button creates a new Overlay based upon the selected settings. Once the channel images have been captured they may be altered at will and almost any number of different overlays created.



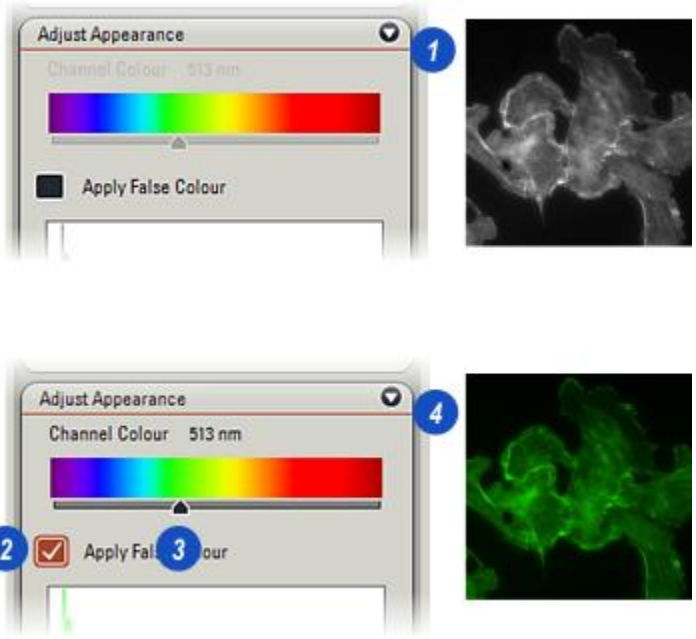
Monochrome Channel images can have false or pseudo colour applied to them before they are combined into a single overlay to improve contrast and clarity of the final image.

1: The *Monochrome Channel* image with the *Adjust Appearance* panel as it looks when capture is complete. Had this been a colour image the *Apply False Colour* check box would not be available.
Select another image by clicking on its thumbnail in the *Gallery*. It will be displayed in the *Viewer*.

2: To apply colour, click on the *Apply False Colour* check box to enable it. A basic colour may be applied to the image based upon the type of filter used in its capture.

3: Move the *Channel Colour* slider to the desired colour.

4: The applied colour is immediately displayed on the *Viewer*.



5: Refine the image by adjusting the sliders below the *Histogram* and also...

6: ...the *Gamma* value.

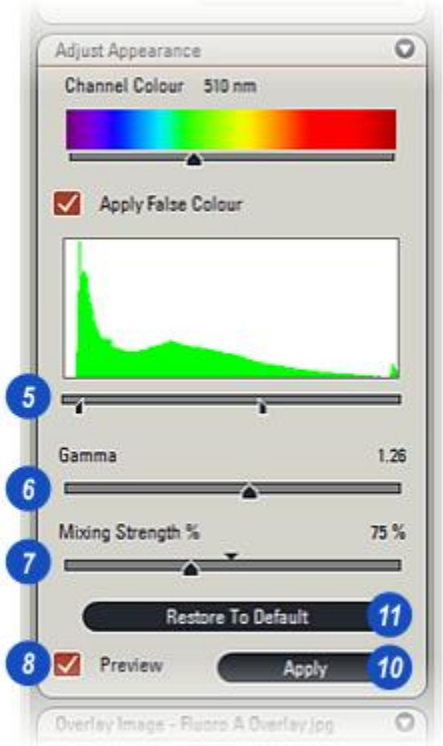
7: The *Mixing Strength* slider controls the predominance the Channel image will have in the overall result. The range is from 0 to 200%. The higher the value, then the greater the 'presence' of the image in the overlay.

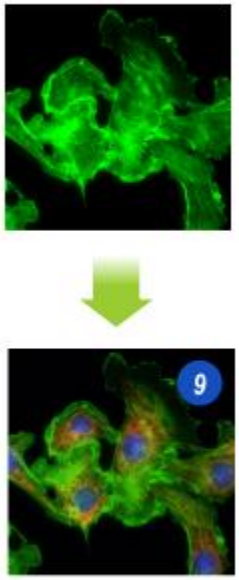
8: Test the result by clicking the *Preview* check box.

9: All of the channels are combined in a temporary overlay.

10: If the result is acceptable click on the *Apply* button to create an overlay. A new thumbnail will appear in the *Gallery*. In this way any number of overlays can be created to illustrate various aspects of the image.

11: To reset the channel images to their original filter colour values, click on a thumbnail and then on the *Restore To Default* button.





1: To select the method of combining the Channel images to create the overlay, click on the arrows to the right of the *Overlay Method* text box and from the drop down list…

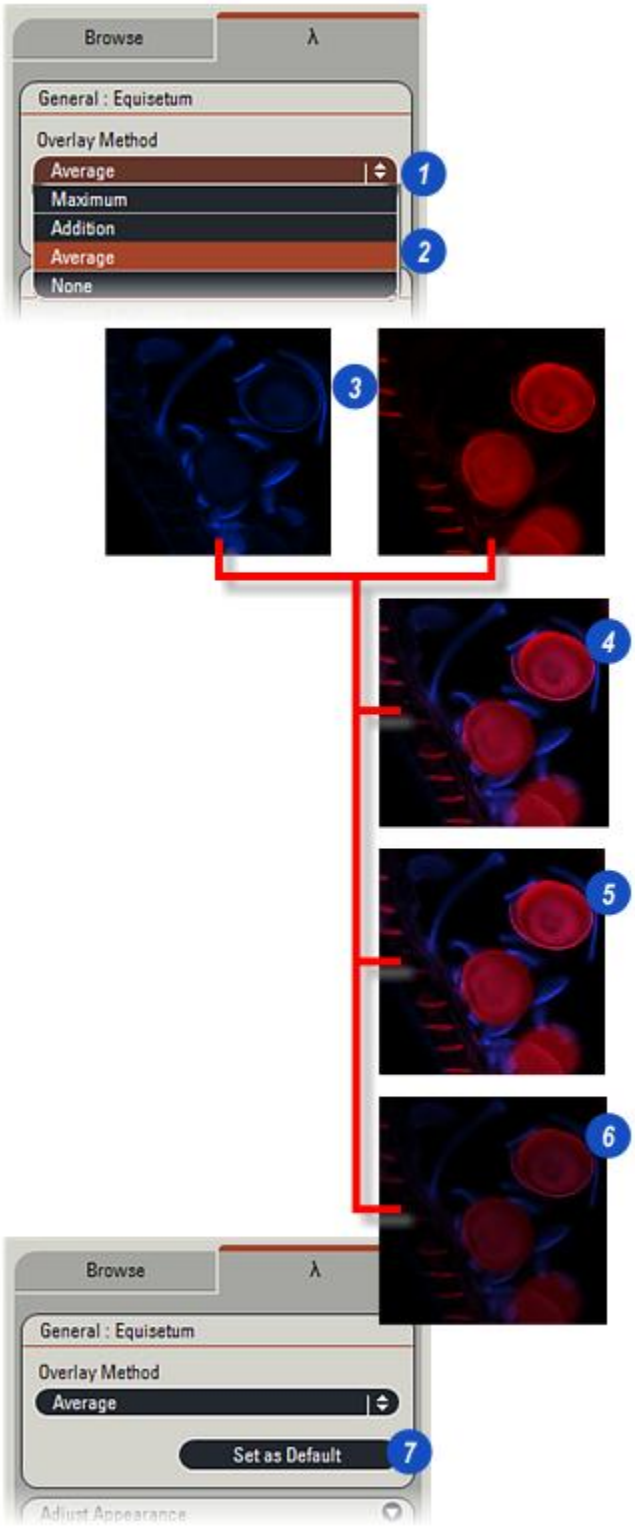
2: ...click to select the required method. In the illustrations, images **(3)** are the original, common channel images that have had false (pseudo) colour applied.

4: Represents the overlay combining the three common channel images using the *Maximum* option. The highest pixel value from the same location in the three common images is used to create the overlay.

5: The same process using the *Addition* option. The pixel value from the same location in all three channel images are added together.

6: Shows the result with the *Average* option. The pixel values in the same location in all three channel images are added together and then averaged.

7: To save the settings as default, click the *Set as Default* button.



False colour is applied to a channel image electronically by association only – the original remains intact and unchanged. This means that colour may be ‘removed’ completely or ‘fine tuned’ at any time after capture and a new overlay created.

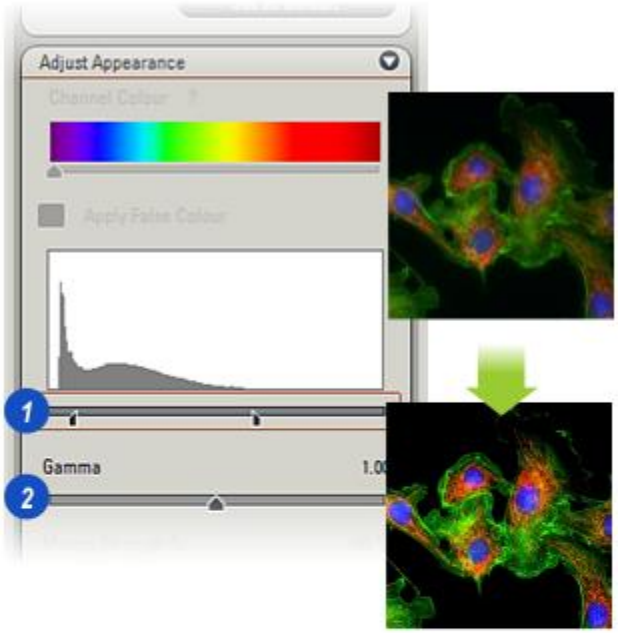
Individual colours in overlays cannot be altered, but the *Histogram* **(1)** and the *Gamma* **(2)** controls are available to lighten or intensify overall effect and in the process enhance a particular feature.

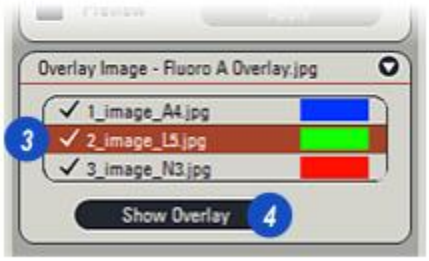
It is not necessary, nor always desirable to include all of the channel images in an overlay. The *Overlay Image* panel provides the means to include or exclude individual channels.

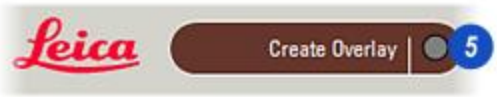
3: A channel is included when the check box to its left is checked. Click to un-check and exclude.
At least two channels must be selected in order to create an overlay. If only two are still selected the program will prevent any more exclusions.
The colour bar to the right of a channel name indicates the filter colour used in its capture.

4: Click the *Show Overlay* button to select and display the last overlay created or…

5: Click on the *Create Overlay* button to produce a new overlay using the revised channel images.

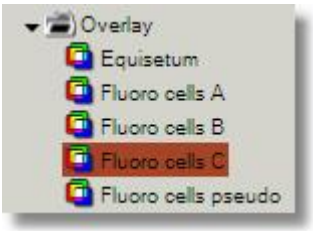






You can combine a folder of existing images into an Image Overlay. For example, you might have obtained images from elsewhere (i.e. **not** using the Image Overlay *Acquire* tab).

You cannot simply copy images into an existing Overlay folder – they are special folders marked with an Overlay icon:



Instead, the method is as follows:

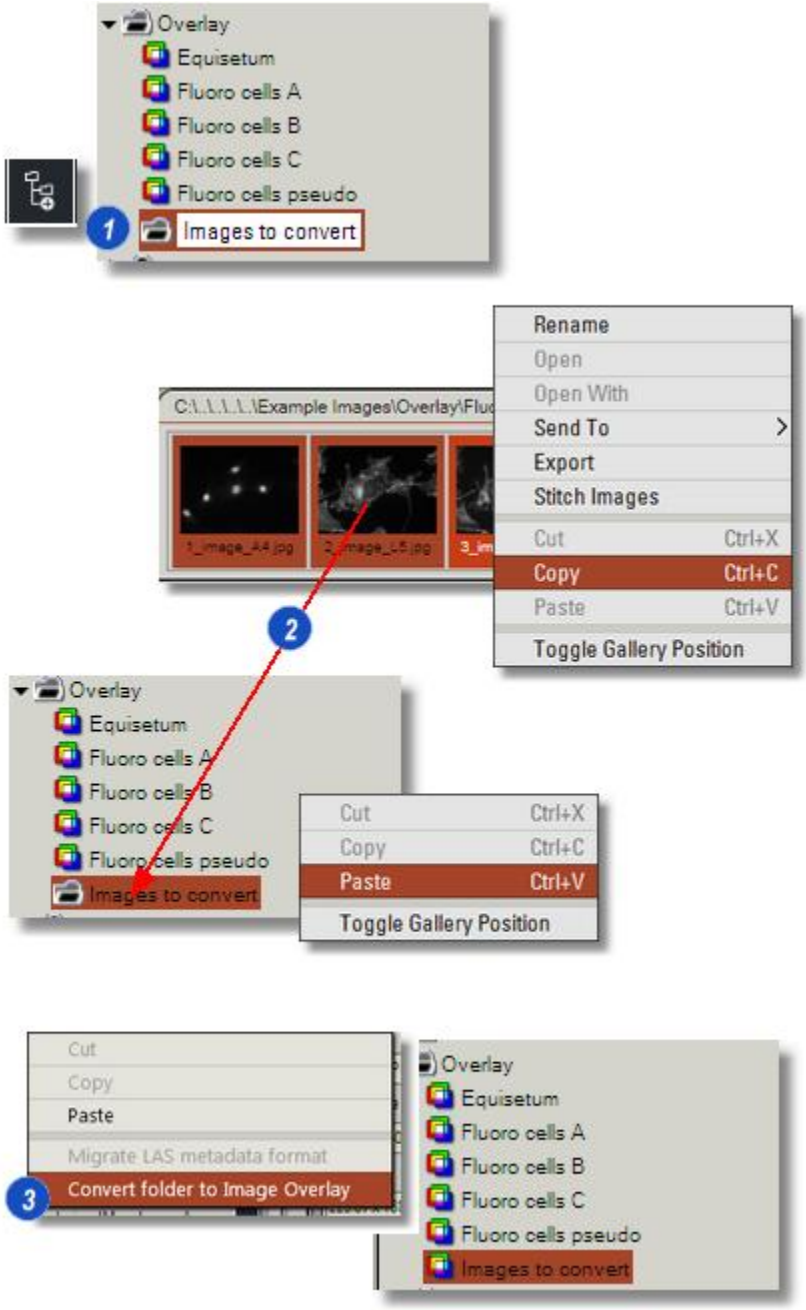
1: Use the *Navigator* to create a suitable folder to hold the images you want to combine.

2: Place all the images that you want to be part of the Overlay into the new folder. You can copy and paste *Gallery* images from other folders in the *Navigator*.

Note: The images must all be the same resolution and bit-depth or the conversion will not be performed.

3: Right-click on the folder in the *Navigator* and select *Convert folder to Image Overlay*.

4: Now you can create [709] an overlay image in the normal way. **Note**: You will need to adjust the false colour [711] of the component images (something that is usually done as part of the Overlay > Acquire process).

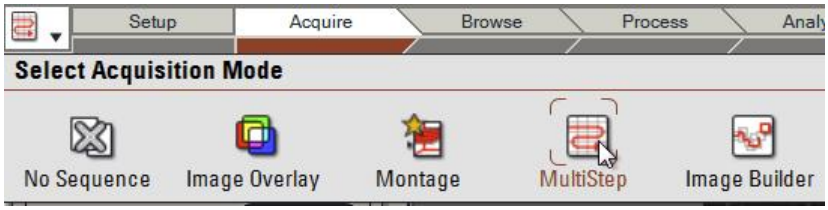


Hints and tips

Bear the following in mind when converting to an Image Overlay:

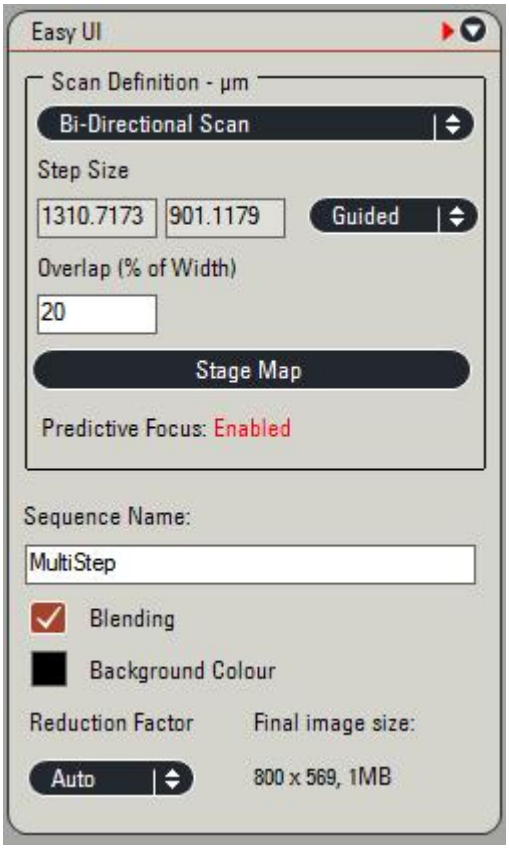
- Try not to exceed 10 images in a folder
- Once the folder has been converted, the process cannot be reversed; be sure that you want to do this
- The Image Overlay module must be licensed and selected

Introduction



This module allows you to create *Scan Patterns* using Motorised X/Y Stage control through the Leica Application Suite interface. Once you have acquired the images, they can be joined together to create one large image or mosaic.

Note: Before using *MultiStep,* please ensure that the system is accurately calibrated and the stage is carefully aligned with the X and Y axes of the camera. Also ensure that camera Shading Correction[725] is correctly set.



To load the *MultiStep* module:

1: Click the *Select Acquisition Mode* button to the left of the *Workflow* bar, then click *MultiStep*.

2: A new tab labelled *S* will appear in the *Acquire* and *Browse Workflow* modes.

You can choose one of two modes in which to work in the *Acquire Workflow.* By default, *MultiStep* opens in *EasyUI* mode. See MultiStep Operating Modes[717].

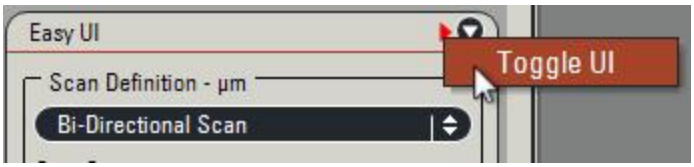
MultiStep has two operating modes:

- **Easy UI**[722]: This is the default startup mode, and contains the essential features to enable you to quickly perform a *MultiStep* Acquisition.
- **Define MultiStep Sequence**[723]: This contains the full feature set for operators needing complete control.

To switch between modes

1: In the *Acquire Workflow*, right-click the red triangle on the right of the top panel in the *S* tab.

2: Select *Toggle UI* from the drop-down menu.



There is a preference setting that applies specifically to *MultiStep*, if Predictive Focus[730] is enabled. It triggers a warning message, asking you to confirm that you want to continue before performing a scan. This acts as a reminder to check that your Predictive Focus settings are appropriate for your sample.

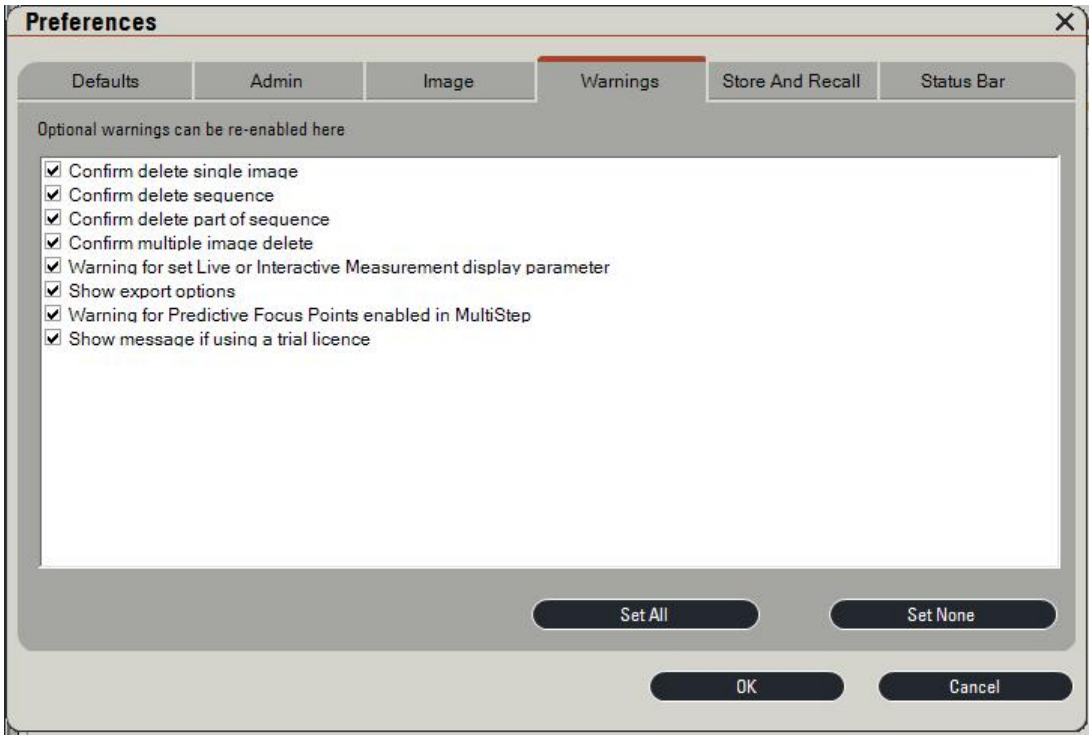
To enable/disable Z focus warnings

1: Select *Options* > *Preferences* from the main menu bar.

2: Display the *Warnings* tab.

3: Check or uncheck the Predictive Focus Points item to enable or disable the warning message.

4: Click *OK*.



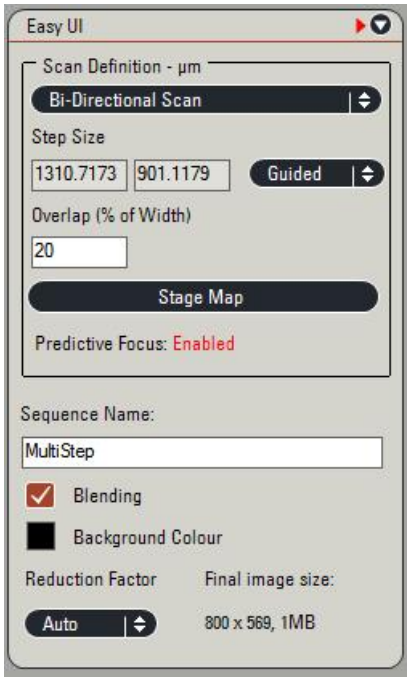
On the *Acquire Workflow* panel an *S* tab will be visible if the *MultiStep* option is active. The panels displayed in the *S* tab depend which operating mode is active.

A further control panel, *Stage Map*, is also used with MultiStep. This gives access to stage movement and predictive focus controls.

See Stage Map 720.

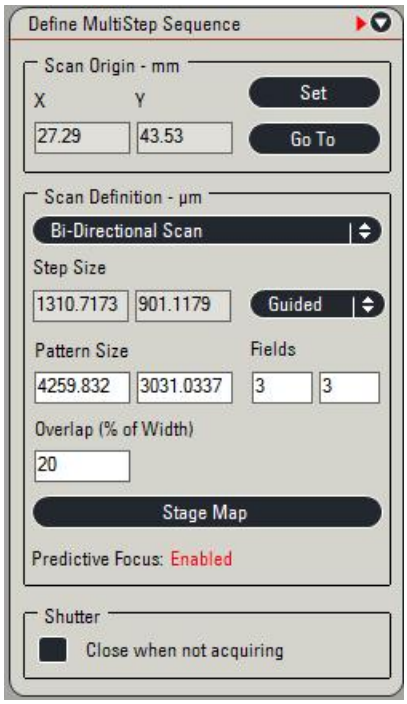
Easy UI mode

Easy UI panel 722

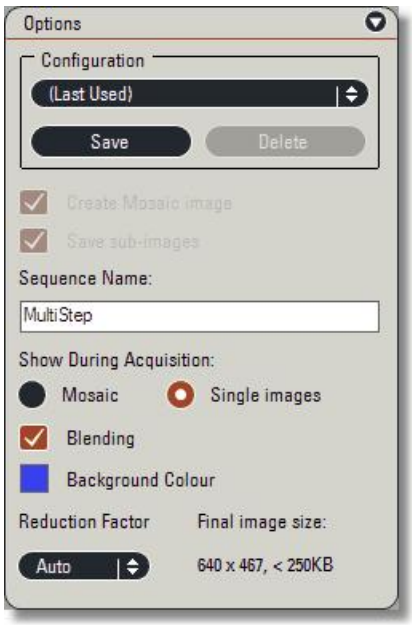


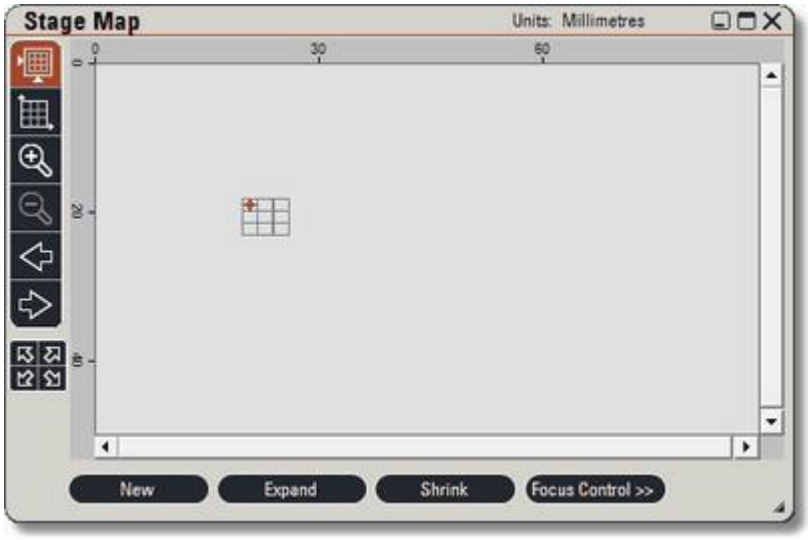
Define MultiStep Sequence mode

Define MultiStep Sequence panel 723



Options panel 731





The *Stage Map* shows the area over which the stage can travel. The position of the current field of view within this area is indicated by a red cross: +. A scan pattern is shown as a diagram on the *Stage Map*. The live window shows the image at the current position of the stage.

See also Stage and Predictive Focus Controls 721.

In Original Size mode, the Stage Map shows the entire stage area as defined by the stage limits. The scales on the axes indicate the size of the area, for example in millimetres. This mode is used to place in context the current stage and scan pattern position within the stage area.

In Fit to Display mode, the Stage Map shows the detail of the current scan pattern.

For both modes, you can zoom in and out. When more detail is shown, the area displayed will pan to keep the scan pattern in view.
Move (pan) the Map around by clicking and dragging (or by using the scroll bars) to show different regions of the stage.

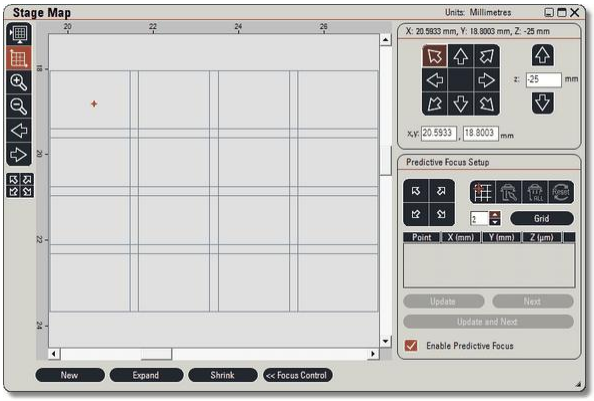
When you have defined a scan pattern, use the arrow buttons to step from one field to the next.

Double-click on the stage area to move the stage to that position.

Right-click and drag with the right mouse button to move the scan pattern.

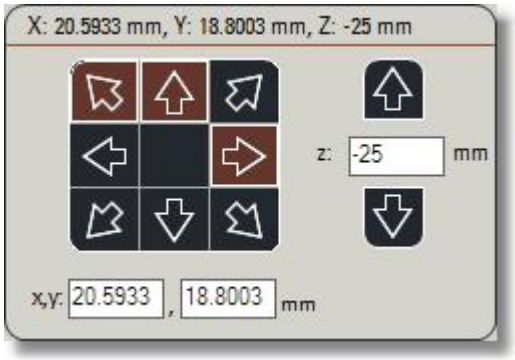
Use the smaller arrow buttons to move the stage position to the corners of the scan pattern.

At the bottom of the *Stage Map*, click *Focus Control* to display a fly-out panel containing stage and predictive focus controls.



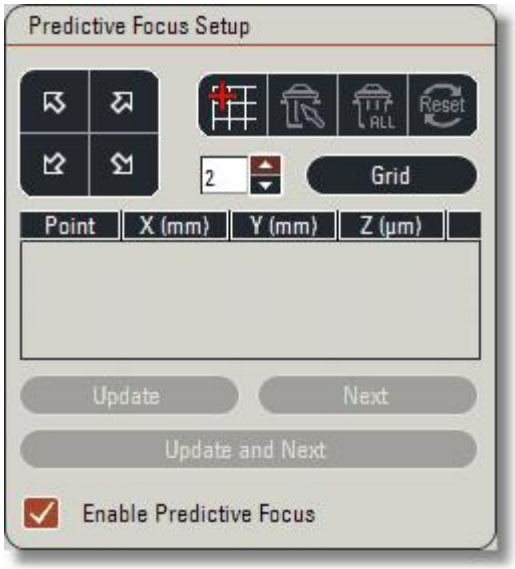
Soft Joystick controls

- Use the *Soft Joystick* controls to navigate to new positions. Click and hold a button to move the stage in the direction selected.
- The red cross will move to the new position on the *Stage Map*.
- Move the focus using the *Z* up and down arrows. A warning will be given if you exceed the Z range limits.



Predictive Focus Setup

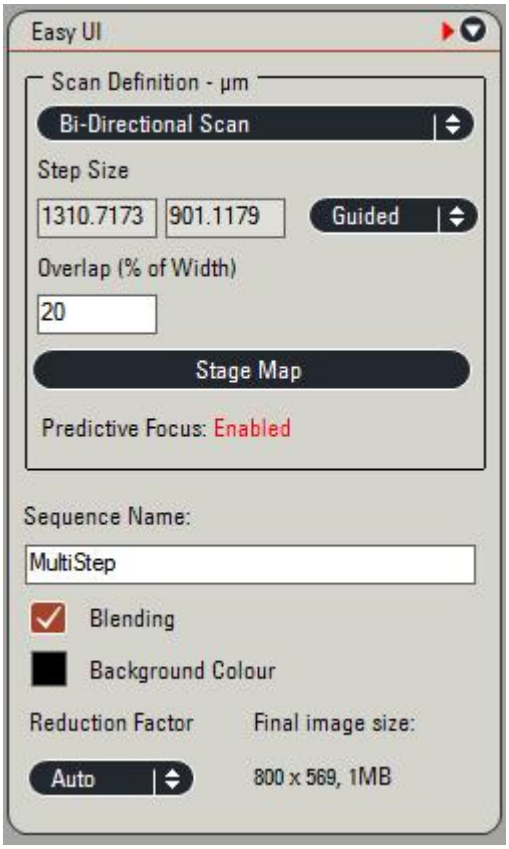
- See Predictive Focus[730]. The controls are exactly the same for Easy UI and Define MultiStep Sequence modes.
- **Note**: Predictive Focus is **disabled** by default every time you start LAS.



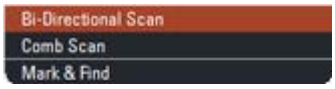
Easy UI mode has been designed to make MultiStep image acquisition as simple as possible. If you need more control over the image acquisition settings, use Define MultiStep Sequence mode 723.

Note: Easy UI mode uses some of the Options 731 settings from Define MultiStep Sequence mode, so you may need to check those settings periodically to confirm that they are suitable for your scans.

You define a scan pattern on the *Stage Map* and fine tune it by entering data on the *Easy UI* panel.



1: Select a Scan Definition 726 from the drop-down menu.



2: Select a Scan Method 727 (e.g. *Guard*) from the drop-down menu.

3: Enter a *Step Size* if appropriate to the scan method.

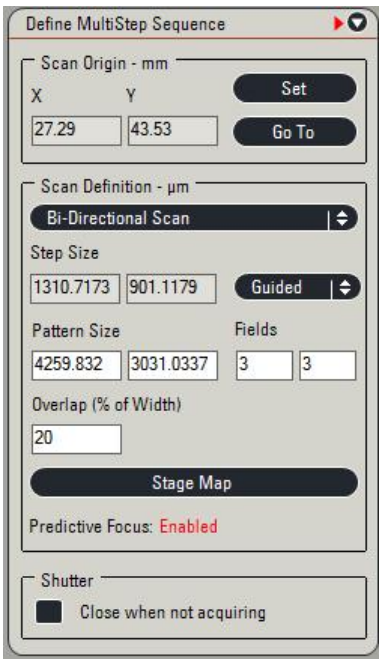
4: Enter a Particle Size Overlap (Guard mode) or Overlap (Guided mode).

5: Use the *Stage Map* 720 button to set up a scan pattern, and define your predictive focus points.

6: Acquire 734 the image sequence.

See also Tips for Best Results 724.

You define a scan pattern on the *Stage Map* and fine tune it by entering data on the *Define MultiStep Sequence* panel.



1: Click the *Stage Map* button to show the *Stage Map*.

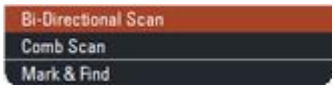
2: Move the stage to the first field and click *New* to create a *Scan Pattern* of one field.

3: Move the stage to the field at the opposite corner of the scan pattern and click *Expand.* A grid representing the scan area is shown.

4: If necessary change the number of fields by typing into the *Fields* boxes.

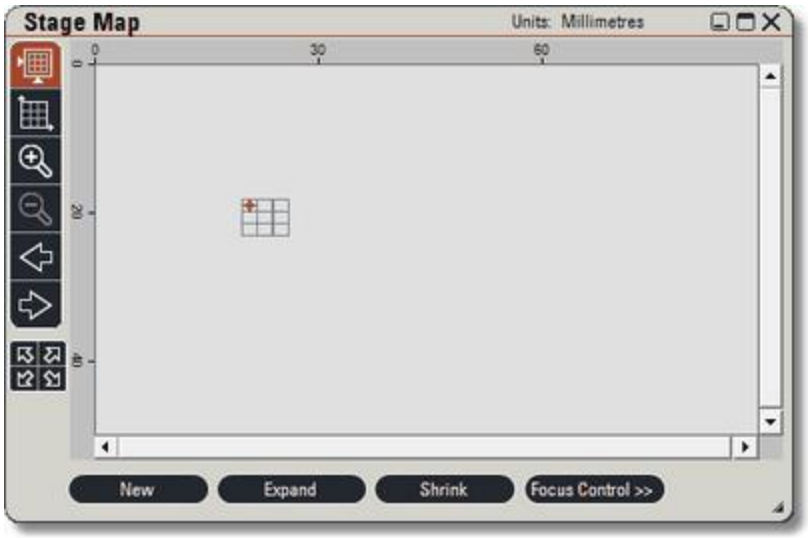
5: To reduce the size of the scan area, double-click in an existing field then click *Shrink*.

6: Select a *Scan Pattern*[726] (e.g. *Bi-Directional Scan*) from the drop-down menu.



7: Select a *Scan Method*[727] (e.g. *Auto*) from the drop-down menu.

8: If you chose *Off* as the *Scan Method,* enter the *Step Size* (the distance between fields, both horizontally and vertically).



See also Tips for Best Results[724].

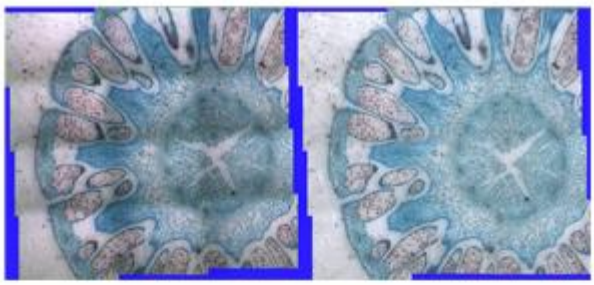
To ensure that you obtain the best possible results when creating MultiStep Sequences:

- Ensure the stage is accurately calibrated
- Ensure camera is accurately aligned with the x and y axes of the stage
- Shading correction 725 should always be used when using any form of sequence operation:
  - Adjust the camera and lighting to ensure the sample image is correctly exposed. There should be no large areas of saturation or under-exposure
  - Move the stage to view an empty field on the specimen, or remove the specimen slide and replace it with a slide (and cover slip if necessary) of the same type and quality.
  - A very small amount of microscope de-focus may be helpful to prevent contaminants affecting the Shading reference.
  - Click *Snapshot* in the *Linking* panel and a shading image will be acquired and stored.
  - The matching is carried out in the area of overlap, so it is a good idea to have an overlap of at least 10% of the image.

Shading refers to variations in the background light level across an image.

The left-hand image shows how poor shading correction can ruin a mosaic image.

Even ‘illumination’ on live images can be achieved in software by applying a ‘blank area’ value to the entire image area. The effect is shown in the right-hand image.



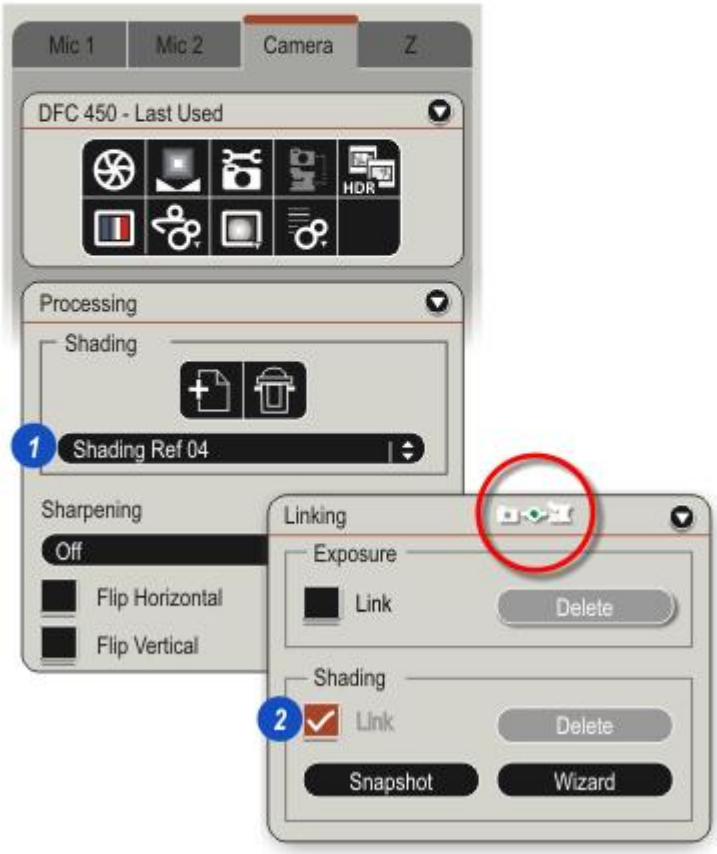
Alternatively, you can create a *Shading Link* which corrects the shading effect on individual objective and illumination setups.

- Shading should be turned **on** if the lighting is causing obvious 'seams' on the *Builder Image*.

Expand either the *Processing* or the *Linking* panel depending upon the shading type to be used:

1: On the *Processing* panel select a user-defined *Shading Reference.* *More information* 307.

2: On the *Linking* panel click to enable the *Shading* check box. *More information* 341.



Bi-Directional

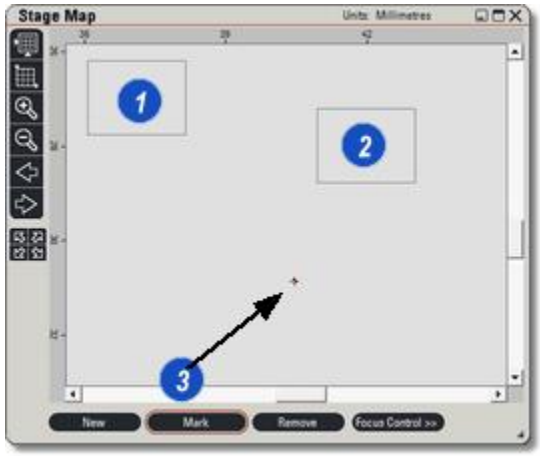
Scans left-to-right and right-to-left. This is slightly faster than *Comb Scan*, as the stage does not need to return to the beginning of the next row before scanning.

Comb Scan

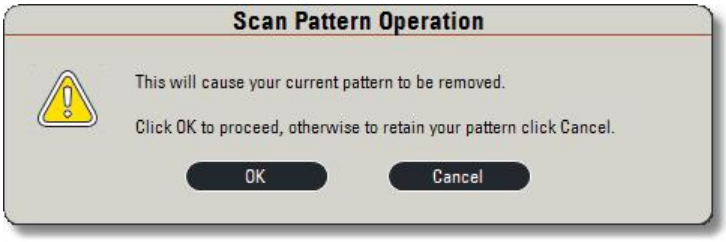
Scans one row of images, returns to the beginning of the next row, then scans that row.

Mark and Find

Scans a user-defined set of points on the stage. Double-click on the *Stage Map* to move the stage to a designated point, then click *Mark (3)*. Repeat until you have defined all points of interest.

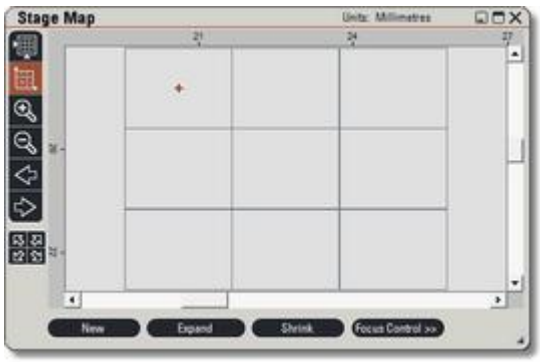


Note: If you change the Scan Definition at any time, you will be prompted to confirm the operation (since it will cause your current scan pattern to be removed.



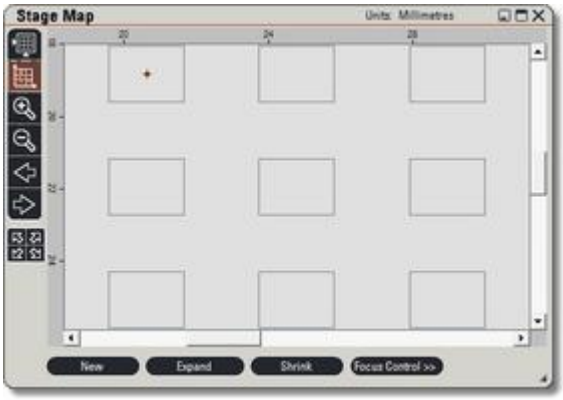
Auto

A MultiStep sequence will be acquired with no overlaps. A mosaic image will be created with each tile butting up to the next. In other words, the Step Size is equal to the size of the image.



Off

You can capture a MultiStep sequence and define the step size between images. For example, if you have a sample with a repetitive structure that has a pitch greater than one frame, you can set the *Step Size* to capture an image of each structure but not the areas in between.



More 728

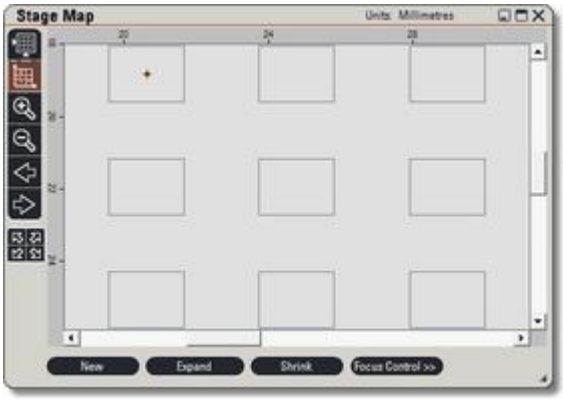
Guard

Guard method is used primarily when the sequence of images are to be analysed using Image Analysis. The MultiStep sequence will be acquired with an overlap between each image that is greater than the maximum particle size (µm) that you specify in the Particle Size Overlap box. This ensures that, when a sequence is acquired, any particle that is equal to or smaller than the maximum particle size specified and lies close to the edge of an image is included in only one image. When the sequence is analysed the guard region used during capture can be taken into account to ensure that these particles will only be measured once.

Guided

In Guided Mode images are captured with a small overlap. At least 10% is recommended, and this is the default. Features in the overlap region between adjacent images are matched and the images placed in the canvas based on these matched coordinates. The result is improved matching between adjacent images, improving the quality of the final, combined image. If there is limited detail in your image, you can increase the overlap to improve matching accuracy.

This technique can provide some compensation for mechanical inaccuracies in the Stage and camera alignment. However, please note that in order to obtain the best results you should always start by setting up and calibrating your system as accurately as possible.



Data Container

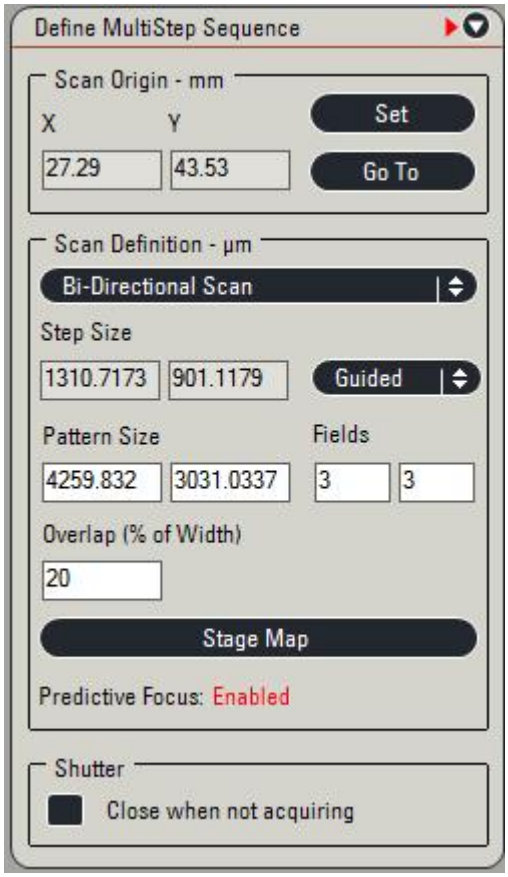
The Data Container is an area on the hard disk that LAS uses as a temporary image store. The advantage of the Data Container is that it allows MultiStep to capture many more images, in a fast and efficient way, than there is RAM available. Currently this is only used by the MultiStep Module in Guided mode.

The maximum image size that can be acquired in MultiStep with Guided mode is now 4GB (2GB for BMP images). If the final image is likely to be larger than this then set a larger reduction value in the Multistep Options[731] panel.

- *Set* takes the current stage position and sets it as the Scan Origin; the scan pattern is shifted appropriately.
- *Go To* drives the stage to the Scan Origin.
- The *Pattern Size* gives the x and y dimensions of the rectangular pattern. If you type in these values, the number of steps will be adjusted accordingly.
- The *Fields* boxes show the number of whole fields of view in the pattern.
- *Particle Size Overlap* is used in conjunction with the *Guard* 727 method to acquire images for use with *LAS Image Analysis*. The overlap should be set to the size of the largest particle to be measured, in microns.

  If the method is set to *Guided* 727, this field is named *Overlap* and the value set as a percentage of the image width.

Changing the *Step Size* changes the *Pattern Size,* as does changing the number of *Fields* of view.



Note: Predictive Focus is disabled by default; you must enable it each time you start a new LAS session.

1: Click *Focus Control* on the Stage Map to display the *Predictive Focus Setup* panel.

2: The *Predictive Focus Setup* panel displays the points on the scan pattern at which the focus has been set. The focus at other positions is interpolated from the known positions.

3: Enter the *Grid* size for the number of predictive focus points - the number of points on the first row. A number of predictive focus points will now be generated with an equal spacing over the scan pattern.

Alternatively you can enter the points individually.

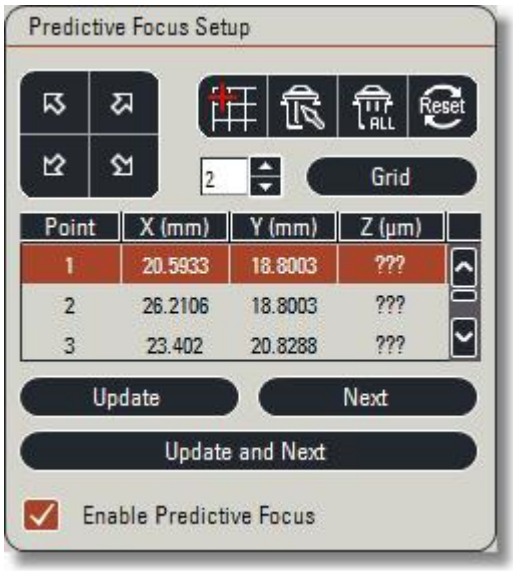
4: The predictive focus points are shown on the *Stage Map*. An open circle means that the predictive focus has not been set. A green dot means the focus is set.

Note: Predictive Focus points are saved between sessions. You can also save them as part of a Configuration 731. This is useful if you often perform similar scans, such as Mark and Find on a microtitre plate (MTP), where you set the focus point for each of the wells.

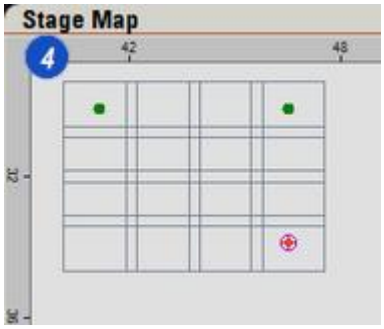
5: Use *Update* and *Next* to visit each predictive focus point and to set the focus.

6: When the points are all set, activate *Enable Predictive Focus* to so that it is used during the scan pattern.

7: To close the *Shutter* during stage moves (so that it is only opened prior to an acquisition) enable *Close when not acquiring* on the *Define MultiStep Sequence* panel. This is particularly useful when working with fluorescence specimens.



The predictive focus points above have been placed but not yet updated, as shown by the question marks in the Z column



This panel allows you to set advanced options that are used in Define MultiStep Sequence mode.

The *Configuration* section allows you to store and recall previously saved configurations. (The *Options* dialog has slightly different settings available, depending which Scan Method[727] you are using.)

- To use a previously saved configuration, select it from the drop-down menu.
- To save a new configuration based on the current settings, click *Save*, enter a name in the resulting dialog and click *OK*. This entry defines the mosaic name and the sub-folder name if sub-images are saved .
- To remove a previously saved configuration, select it from the drop-down list and click *Delete*.
- If *Create Mosaic image* is checked, a mosaic image will be created after the last image in the Scan Pattern has been acquired. This will be displayed and stored in the Gallery.
- If *Save sub-images* is checked, all acquired images will be saved as an image set. If unchecked, these images will not be saved.
- Enter a *Sequence Name* for the acquired image sequence. This will be visible in the *Navigator*, in the *Browse Workflow*.

Show During Acquisition

- *Mosaic* shows the dynamic build up of the mosaic on the screen as each image is acquired
- *Single Images* shows each acquired image on the main screen as it is captured in turn.

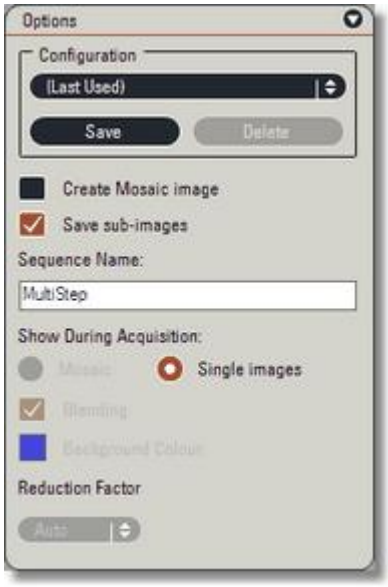
Reduction Factor

- See this topic[733]

Options also used in Easy UI mode

Be aware that some of these Options panel settings (Create Mosaic Image, Save sub-images, and the selected Configuration) are also used as the default settings in Easy UI mode[722], even though you cannot access them from that mode.

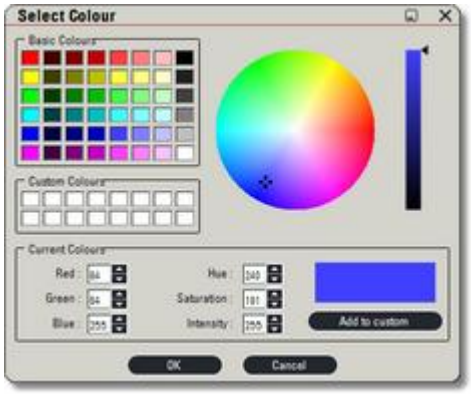
Options window (all Scan Methods except Guided)



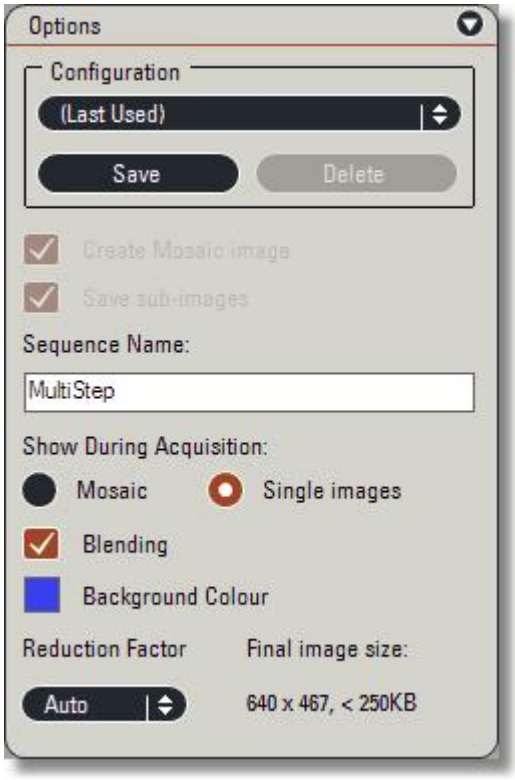
See Extra Options for Guided Scan[732]

Extra options for Guided Scan Method

- *Blending:* When enabled, if two images overlap, they will be blended to show a smooth transition across the overlap region.
- *Background Colour* allows you to pick a different canvas colour. In general, it is better to choose a colour that contrasts with the sample.



Options window (Guided Scan Method)

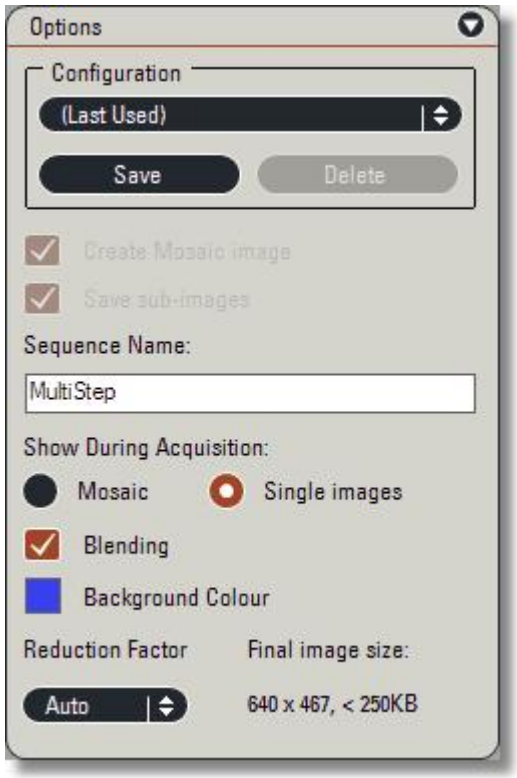


- *Auto* provides a final image at a size comparable with an individual field image size.
- *None* provides an image at max size; the size of the final image is the sum of sizes of individual images. The final size will take account of the overlap, if set.

  Note that there are practical limits to the size of images that can be generated due to the memory available to Windows programs. The number of bytes in an image will not be the same as the number of pixels, since a pixel may be 3 bytes (e.g. an RGB colour image).

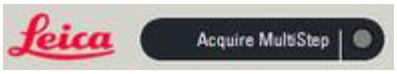
- Values (e.g. x2) indicate an image of size Max Size / (value* value). Highest value is capped to give an image of approx 640 x 480. It displays the image size and amount of storage that will be used.

Note: Memory requirements can only be shown accurately if the image is to be saved in BMP or TIF formats. JPGs will show the maximum size for the image. Disk space requirements are additional because field images need disk space too. Even if system is set up to acquire 16-bit images, mosaic images will be acquired in 8 bits

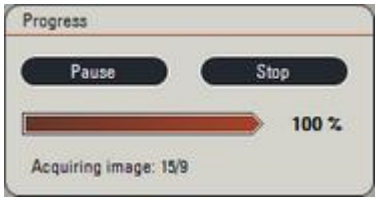


Once you have defined the parameters of the sequence, you can acquire the images.

1: Click *Acquire MultiStep* to start the Scan Pattern acquisition process as defined above.



A *Progress* bar will be displayed:

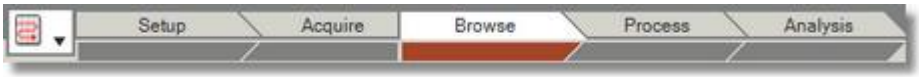


- *Pause/Resume*: Pause or resume the acquisition.
- *Stop*: Stop the acquisition process completely, resulting in either a partial mosaic being created and/or a partial set of images (depending upon your settings).

2: Once complete, the *Browse Workflow* will be entered automatically.

Under the *Browse* > *MultiStep* tab *S* there are two control panels:

- *View Image Set*[736].
- *MultiStep*[737]



View Image Set Panel

This allows you to select a previously saved set of *MultiStep* acquisition images.

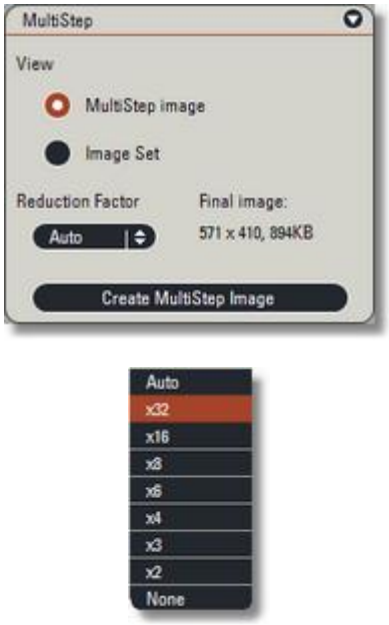
- The images may then be stepped through (like a slide show), by using the video play controls which allow the user to go to the beginning or end of the sequence or step through images one at a time forward or back or play the sequence at the specified frame rate.
- The rate at which images can be displayed is altered by entering a value into the *Delay* box.
- The red slider in the sequence visualization can also be grabbed using the mouse (like using a scroll bar) and the sequence navigated by moving this back or forth through the image sequence.
- The image being displayed is listed in the text box.

After selecting the sequence in *Browse*, the *MultiStep* panel allows you to view the images and create a Mosaic image.

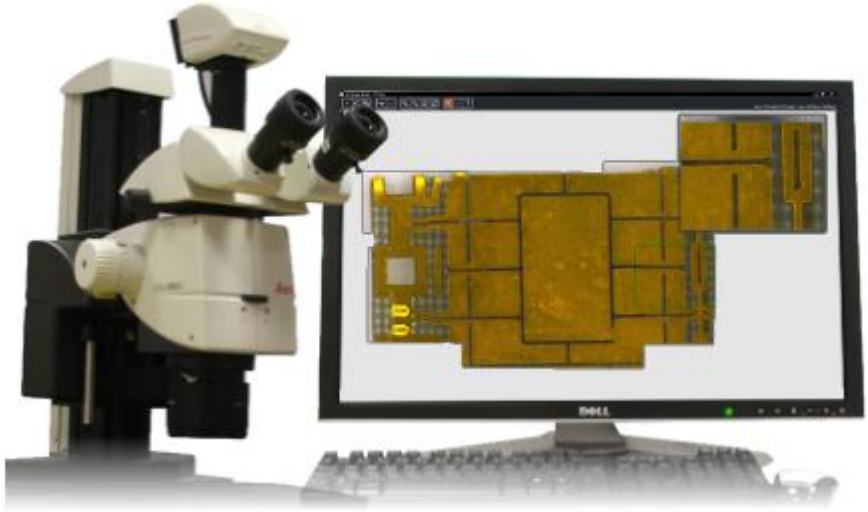
- *MultiStep Image*: Selecting this automatically loads the *MultiStep* image from the currently selected image set in the gallery. The *View Image Set* controls will be disabled as the mosaic image is being displayed.
- *Image Set*: Selecting this option loads the first image from the image set.
- The *Create MultiStep Image* button allows you to re-create the *MultiStep* Image from the current image set.

- *Auto* provides a final image at a size comparable with an individual field image size.
- *None* provides an image at max size. Size of final image is sum of sizes of individual images
- *Values* (e.g. x2) indicate an image of size Max Size / (value* value). Highest value is capped to give an image of approx 640 x 480. It displays the image size (in pixels) and file size.

Note*:* File size can only be shown accurately if the image is to be saved as in BMP or TIF formats. JPGs will show the maximum size for the image. Even if LAS is set up to acquire 16-bit images, mosaic images will be acquired as 8-bit files.



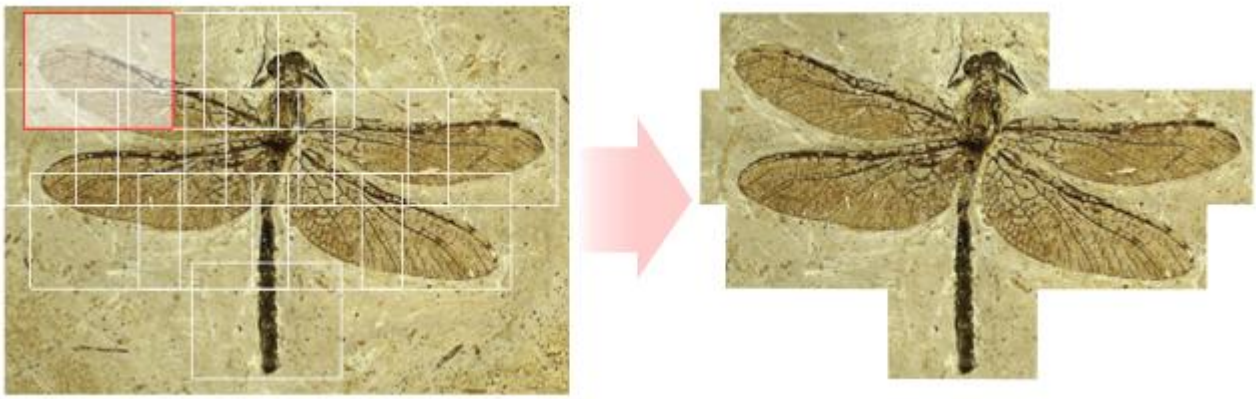
Live XY Builder



Designed for users with manually operated stages, *Live XY Builder* creates a complete image of specimens that extend beyond the field of view of the microscope.

Live XY Builder is smart software:

- It detects when the stage has been moved to a new position and extends the image automatically. No need to click buttons to capture.
- The image is built dynamically in real time as you work.
- You can concentrate on capturing the salient parts of the specimen, ignoring areas that are not important.
- Each tile is matched and its pixels precisely stitched to the growing image .
- The *Blending* option minimises edges and seams by smoothing the pixel-to-pixel transition .
- A *Live Image Window* allows focussing for each tile if needed, without leaving the *Live XY Builder* interface.
- *Backtracking* allows you to replace parts of the image simply by re-tracing steps and re-capturing tiles along the way.
- *Leica Application Suite* calibration, image formats, exposure and shading all work in the normal way.
- A wide range of *Zoom* options is available.
- You can save the *Builder Image* with all the usual LAS attributes; it is available immediately for measurements, manipulation and annotation.



Terminology in this help:

- *Field:* The microscope field of view.
- *Tile:* The *Field* captured to computer memory.
- *Builder Image:* Tile pixels are added dynamically to the growing *Builder Image* and, as soon the pixels are matched and integrated, the *Tile* is discarded. The *Builder Image* is not made up of individual, discernable tiles. See *The Builder Concept* 741.
- *The Canvas:* A part of computer memory reserved for the *Builder Image.*
- *Stitching*: Combining any or all of a *Tile's* edges with the existing *Builder Image* on a pixel-by-pixel basis.
- *Overlap:* The area of a *Tile* that duplicates and therefore matches a part of the existing *Builder Image*. Matching and stitching occur in the *Overlap* area.
- *Scanning:* The sequence moving the stage to different parts of the specimen and adding *Tile* pixels to the *Builder Image*.

The Stage:

Live XY Builder has been designed for use with manual XYstages (i.e. those not moved automatically by software).

The stage controls may be mechanical (attached to the stage) or a remote joystick or *SmartMove* that drives axis motors.

Regardless of the control type, it is essential that all stage movements are made slowly and at a consistent speed.

Specimens:

- Specimens with random, non-repeating detail are ideal for *Live XY Builder*. Regular patterns and a narrow contrast range will make matching difficult and unpredictable. Large areas of uniform colour without detail can often be avoided during scanning.
- The specimen should be as flat as possible. *Live XY Builder* will allow focussing during the scan to accommodate small thickness variations, but a focus change will also affect neighbouring tiles in the overlap area.
- Make sure the specimen is properly and evenly lit and secure.
- Avoid optics that introduce barrel distortion, which can compromise stitching.

Live XY Builder detects changes in the microscope *Field* - when you move the stage to a new part of the specimen.

When the movement stops and the stage has settled, the software captures the new *Field* in computer memory; It becomes the *Tile*.

Then it tries to match the *Tile* with detail on the *Builder Image*. If a match is successful, pixel information from the *Tile* is merged (*Stitched)* to the *Builder Image* to become an integral part of it, and the *Tile* is discarded.

Live XY Builder does not collect and save a series of tiles to be merged later. Matching and *Stitching* are dynamic and happen in real time as you work.

In the illustration:

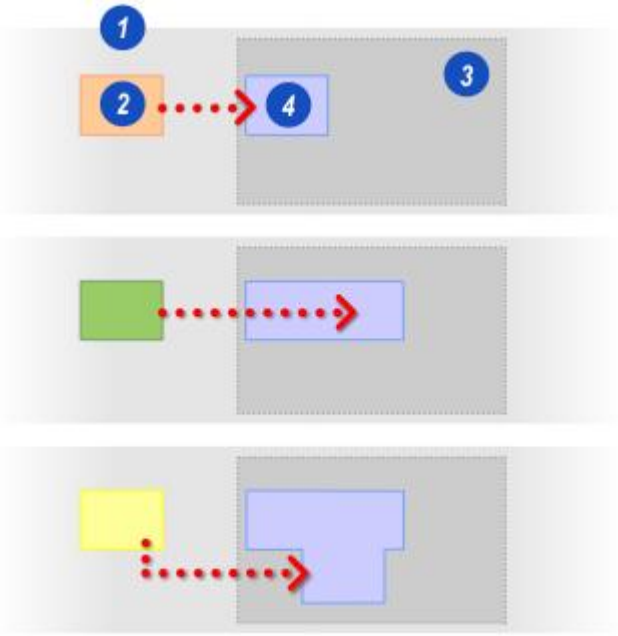
1: Represents the *Live XY Builder* working area.

2: Represents a *Tile* which is the current microscope *Field.*

3: Represents a region dedicated to the *Canvas*.

4: Represents the *Canvas*, where the *Builder Image* is constructed.

As successive *Tiles* are captured, they are matched; the pixel information is *Stitched* to the *Builder Image,* which grows accordingly.



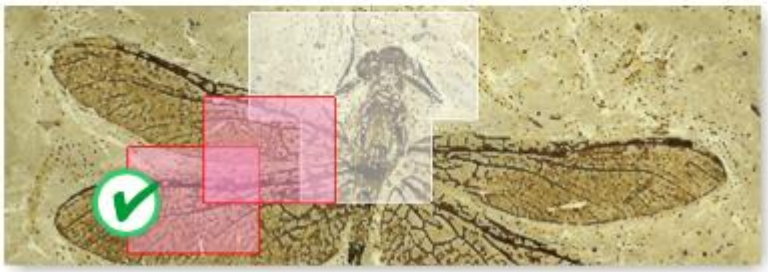
Successive *Tile* edges must not butt up against each other.

There must always be an overlap with the *Builder Image* of at least 30% of the *Tile* width or height on all adjacent edges.

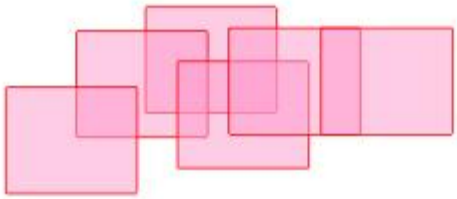
The *Overlap* is the region in which the pixel matching and *Stitching* takes places. If the *Overlap* is too small, *Stitching* may not be accurate, seams could appear, or matching may fail completely.

Every tile must overlap on at least one edge with the *Builder Image*. A *Tile* cannot stand alone…

…there must be a connection to the *Builder Image*.



Overlaps do not have to be precise or regular on the edges as long as they are adequate - at least 30% of the *Tile* width or height.



If you are new to *Live XY Builder,* use these check lists to familiarise yourself with the program features and set up before building.

User Interface Overview

The Live XY Builder Interface 753
What the user sees. Where the controls are and the live image and scan areas.

The Tool Bar 754
Details of the tools and what they do.

The Live Image Window and Blending 756
Focus during a seam-free scan.

The Canvas 758

Zooming the Scan and Capture Status 759

Workflow

Choose a Capture Folder 744
Determine where the final scan image will be saved.

Check the Preference Settings 745

Has Calibration been carried out recently? 746
For accurate measurements on the final image, calibration is essential.

Check the Image Type 747
Live XY Builder works only with colour images.

Set the Live Image Format 748
Image Format is important in determining the speed of Live XY Builder.

Turn on Shading 749
Helps to eliminate tile seams on the final scan.

Adjust the Exposure 750
Important in setting a camera frame rate for fast scans.

Planning the Scan 751

Launch Live XY Builder 752

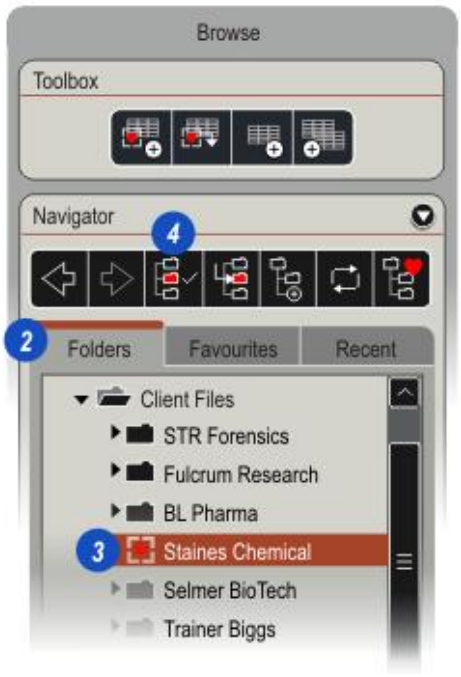
Start Building 756

Re-focussing and Backtracking 760
Live XY Builder allows tiles to be re-focussed and captured again.

Save the scan image 761

Choosing the Capture Folder

You can save the *Live XY Builder* scan into a folder of your choosing.



1: Click on the *Browse Workflow.*

2: If necessary, display the *Folders* tab.

3: Navigate to and click to select the capture folder.

4: Click *Set Fixed Location*.
A red dot indicates the fixed capture location archive.

! Images will only be captured to the *Fixed Location* folder if *'Capture to fixed'* is enabled in *Preferences*[745].

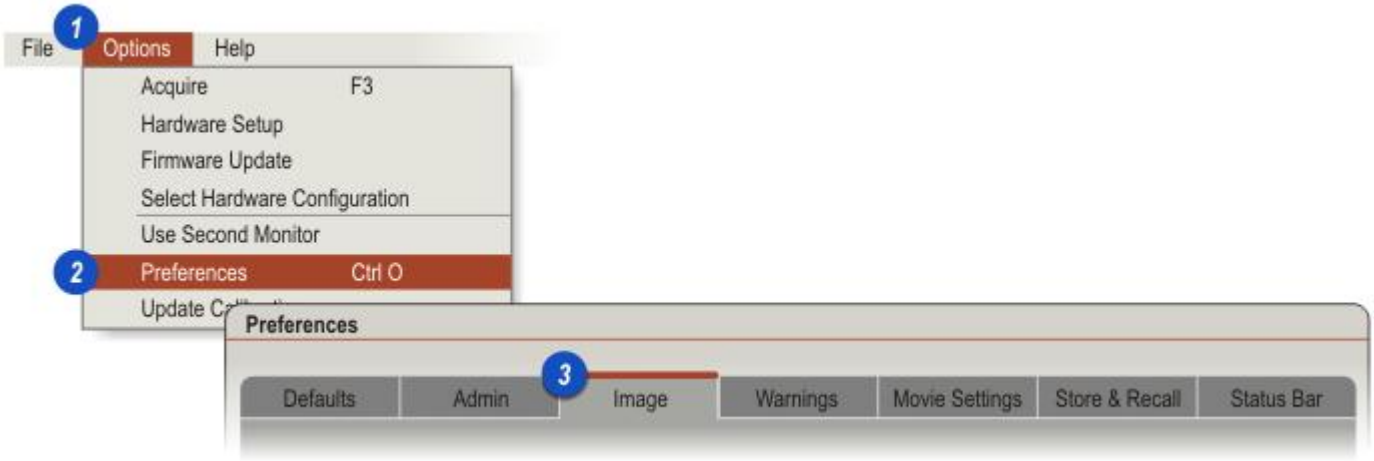
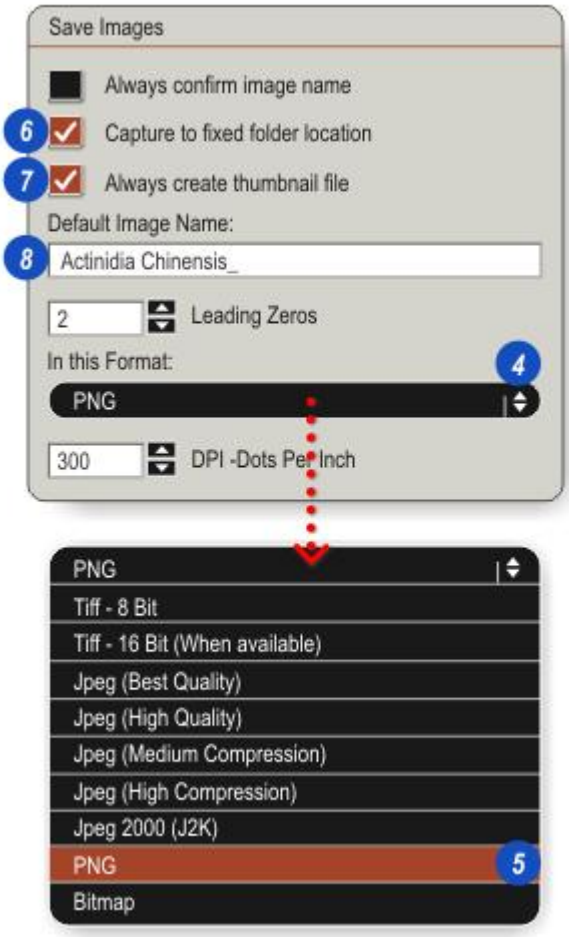


Image compression affects the file size and quality of the captured image. For complex *Live XY Builder* scans that may be shared, it can have an important bearing on the size of e-mail messages for example. High quality images with little or no compression can be very large.

Set the file type and quality level in the *Preferences* > *Save Images* panel; you should also enable *Capture to a fixed folder* and give the saved scan a *Name*.



1: Click *Options* on the *Main Header.*

2: Select *Preferences* from the menu.

3: On the *Preferences* dialog, display the *Image* tab.

4: Click to display the *In this Format* drop-down menu.

5: Select the required *Compression* type.

6: Click to enable *Capture to fixed folder location. (*The folder location is set here[744] *.)*

7: *Live XY Builder* always creates a thumbnail for each capture, regardless of the check box setting.

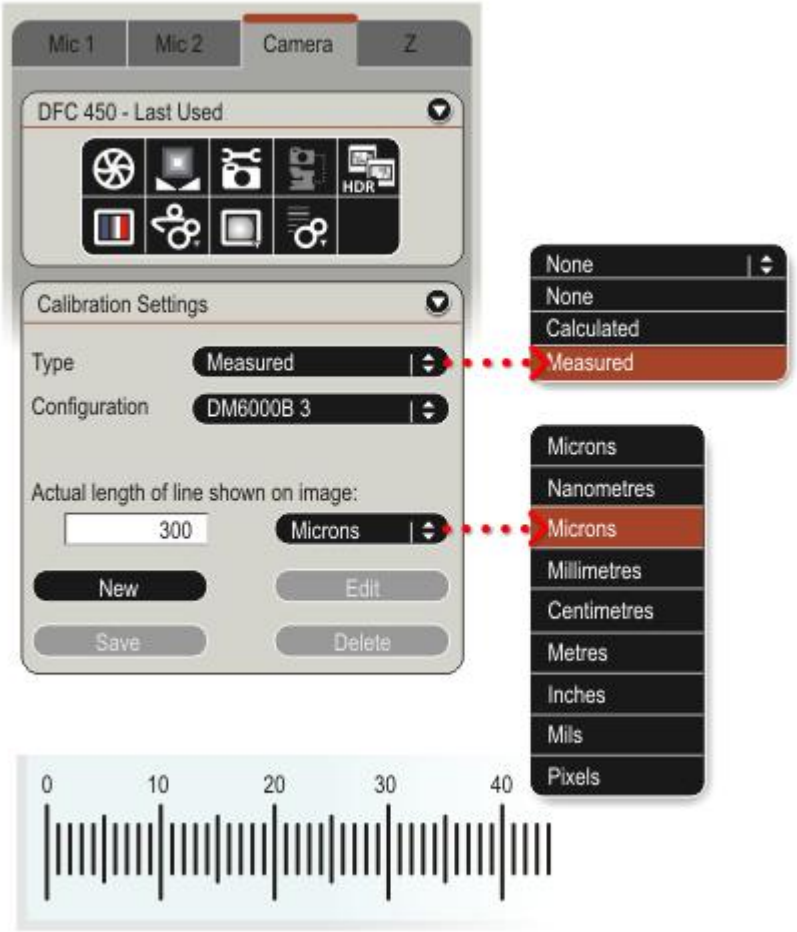
8: Enter a *Default Image Name* for saved scans. All subsequent scans are given the same name with an incremental numeric suffix.

9: If required, enter a number of leading zeros that will appear at the start of the file name.

10: Set the DPI for saved images. Larger values will create larger file sizes.

Calibration ensures that measurements shown by the software are in real world units - microns, millimetres etc. - taking into account the microscope optical magnification and the camera pixel size.

You should check that the microscope has been recently calibrated; If not, calibrate the equipment before running *Live XY Builder*.



Check the Image Type

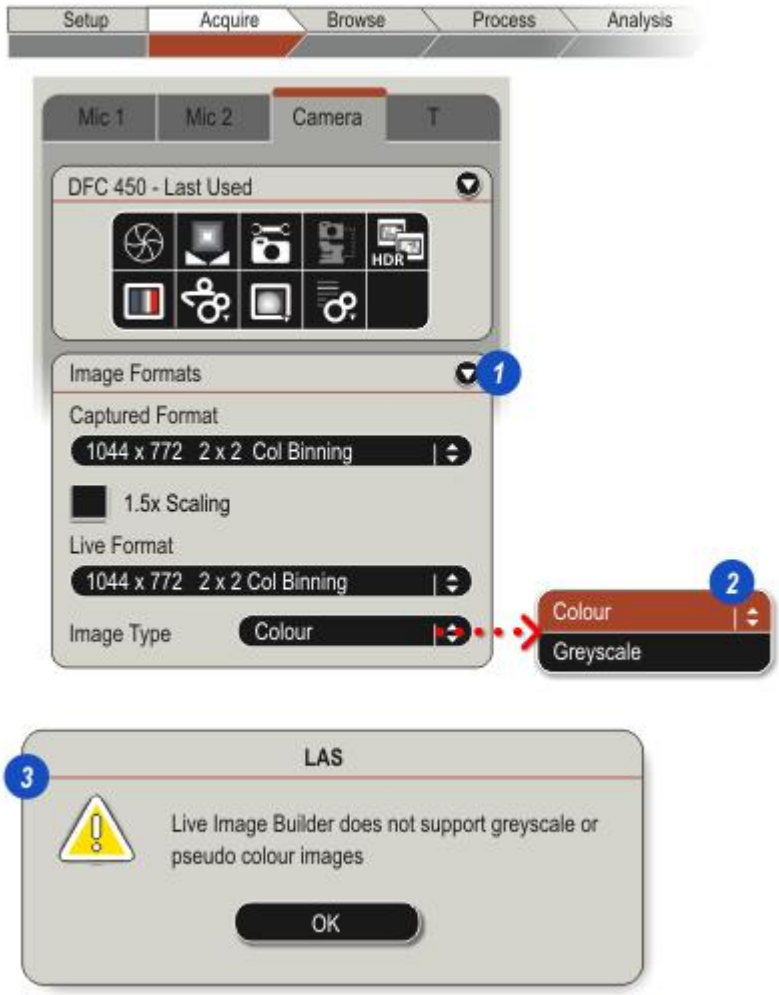
Live XY Builder works with *DFC* cameras and colour images.

On the *Acquire Workflow,* select the *Camera* tab and check that the *Image Type* is set to *Colour*;

1: Click the arrow to the right of the *Image Formats* header and check the *Image Type.*

2: Set the *Image Type* to *Colour*.

3: Attempting to use any other image type will display a warning when *Live XY Builder* is launched.



The *Live Format* determines the quality of the image displayed in the *Viewer* and will affect the speed at which the camera can capture fields - the camera frame rate (fps = frames per second). Higher resolution formats will cause slower capture and processing.

On the *Acquire Workflow* and *Camera* tab:

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.

2: Right-click on the *Live* and *Captured Format* headers..

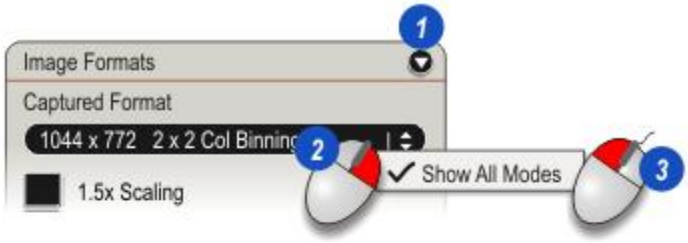
3: Enable *Show All* modes, which will list all the formats that the camera is capable of displaying.

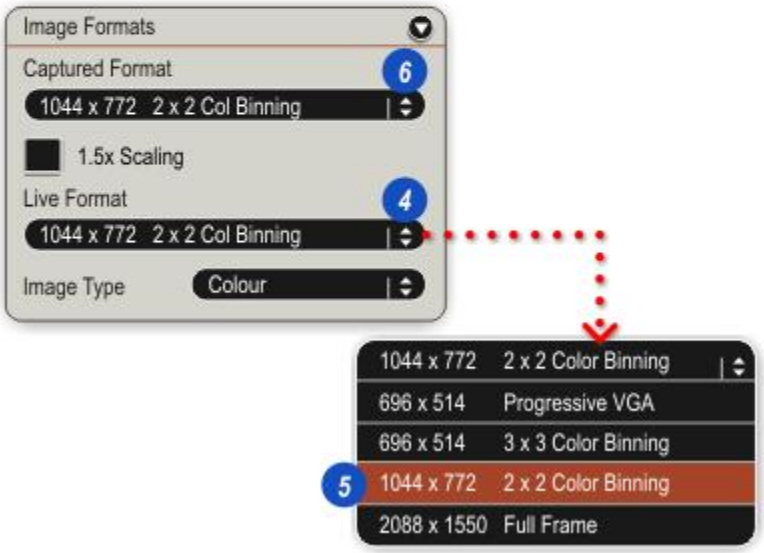
4: Display the *Live Format* drop-down menu.

5: Select a format. If the camera supports a wide range of formats, use the scroll arrows to navigate to your chosen format.

- If the *Live Format* is the default setup when LAS is installed, continue to use that. Otherwise, *1044 x 772 2 x 2 Colour Binning* (depending upon the camera) is a good starting point. Choose a lower-resolution format if the camera frame rate is too low and cannot be increased sufficiently using the *Exposure* controls.

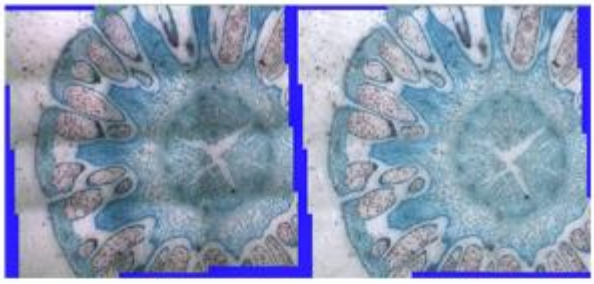
6: The *Captured Format* does not affect *Live XY Builder.*





Shading refers to variations in the background light level across an image.

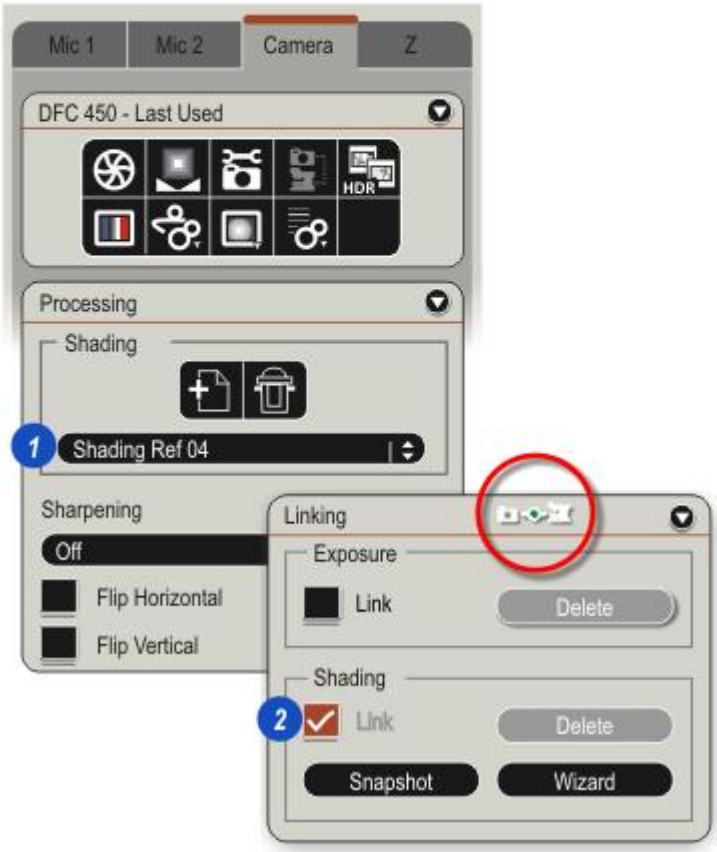
The image on the left shows how the light source and the optics conspire to create a shading gradient across an image. This shows up as a grid of darker regions on a composite image.



You can achieve even 'illumination' on live images in software, by applying a 'blank area' value to the entire image area. The effect is shown in the image on the right.

Alternatively, you can create a *Shading Link,* which corrects the shading effect on individual objective and illumination setups.

- Shading should be turned on if the lighting is causing obvious 'seams' on the *Builder Image* .



Expand either the *Processing* or the *Linking* panel, depending upon the shading type to be used:

1: On the *Processing* panel select a user-defined *Shading Reference.*
More information 307 .

2: On the *Linking* panel click to enable the *Shading* check box.
More information 341

There are two options for adjusting the exposure:

- *Automatic* with some fine-tuning
- *Manual* with a range of precision controls.

Automatic Exposure is a good starting point because, when combined with *Automatic White Balance,* it can produce a perfectly acceptable image very quickly.

Information about *Exposure*[282].

1: Use the *Manual Exposure Adjust* to make fine adjustments to:

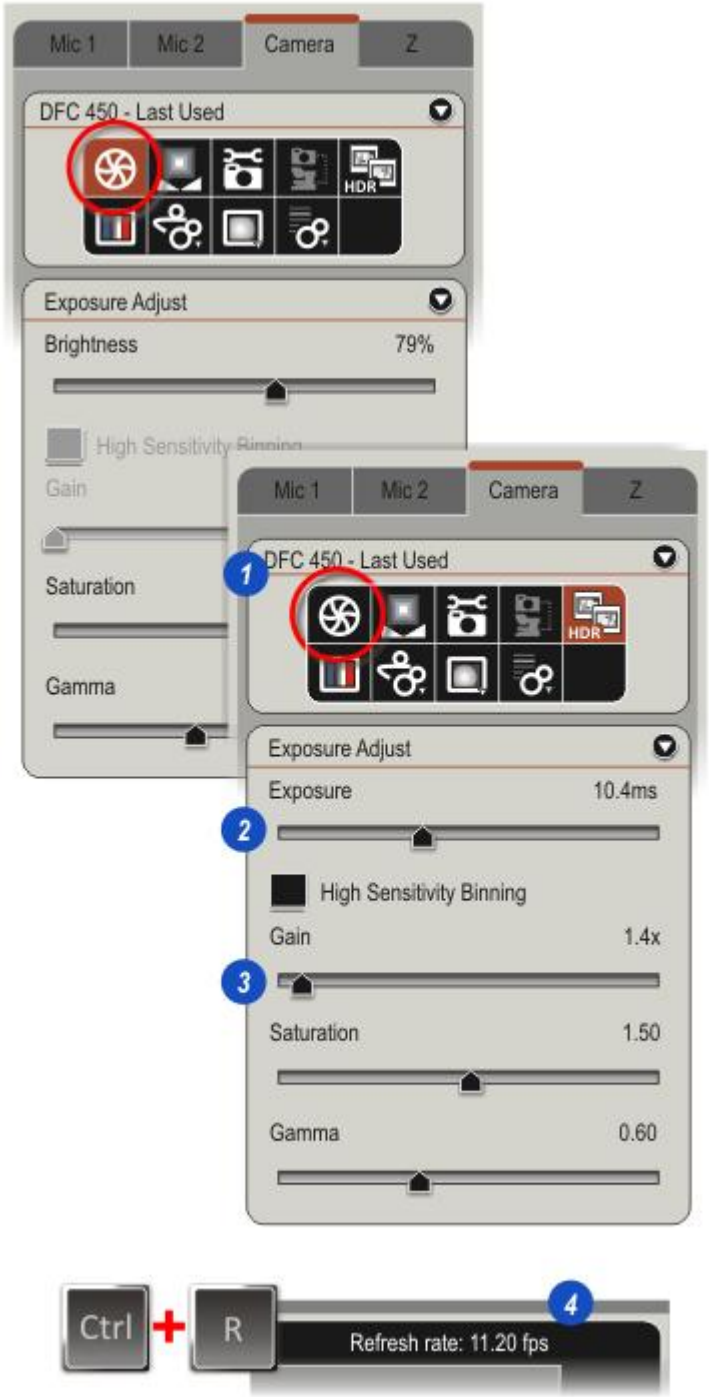
2: The *Exposure* time balanced with...

3: ...small increases in *Gain* to achieve scans of acceptable quality in reasonable times.

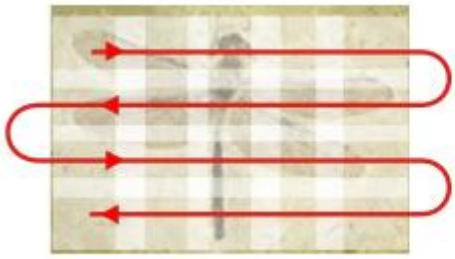
Aim for a camera frame rate of between *9* and *12* fps. The actual frame rate is shown on the *Live XY Builder* live image window.

4: With the *Camera* tab selected, press *Ctrl+R* to reveal the camera *Refresh rate* as frames per second (fps) in the top right of the *Viewer*.

Note: *HDR, AVG and automatic exposure* are disabled in *Live XY Builder* so that the exposure settings remain consistent throughout the scan.



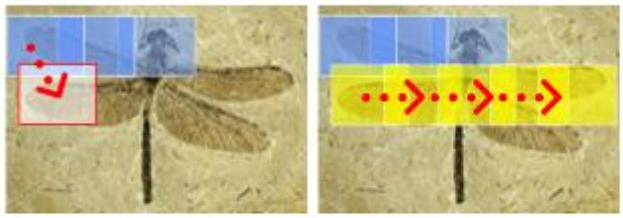
For many specimens it may be appropriate to start at one corner and capture tiles in a bi-directional (zig-zag, row-by-row) pattern until the scan is complete.



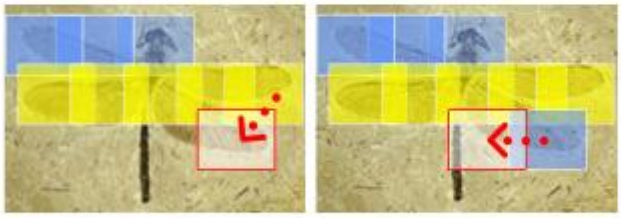
Other, perhaps more irregular specimens can be scanned more quickly and efficiently given some planning. The dragonfly fossil at the top used 20 physical stage steps to complete; With a little planning and the sequence shown, the number of steps was reduced to just 11.

Starting with the dragonfly head in sharp focus, the stage was moved left toward the tip of the wing in 3 tiles.

The stage was moved diagonally down and right, to pick up the lower left wing then continued to scan towards the right wing.

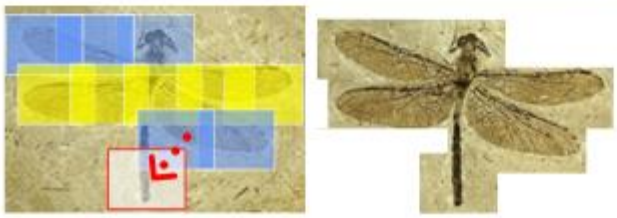


The stage was moved diagonally down and left to complete the tiles of the right lower right wing in two steps.



Finally, a single tile captured the tail.

A saving of 9 steps and a lot of time.

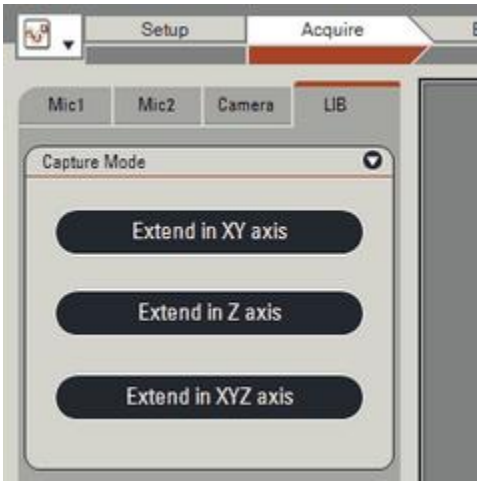


Before you start, the *Live XY Builder* module must be installed and enabled.

There are two optional modules in *Live Image Builder*, which need to be installed and activated separately:

- *Live XY Builder*
- *Live Z Builder*.

After the 60 day evaluation period they must be licensed individually.



To launch the *Live XY Builder* module:

1: Click on the *Acquisition Mode* selector.

2: From the menu, click on the *Image Builder* icon.

3: Click the *Acquire Workflow.*

4: Display the *LIB* (Live Image Builder) tab. The *LIB* tab is only present if at least one of the modules is installed and activated.

5: Click the required button to launch the module.

Live XY Builder Interface 753

The user interface opens in a new window. The main areas are:

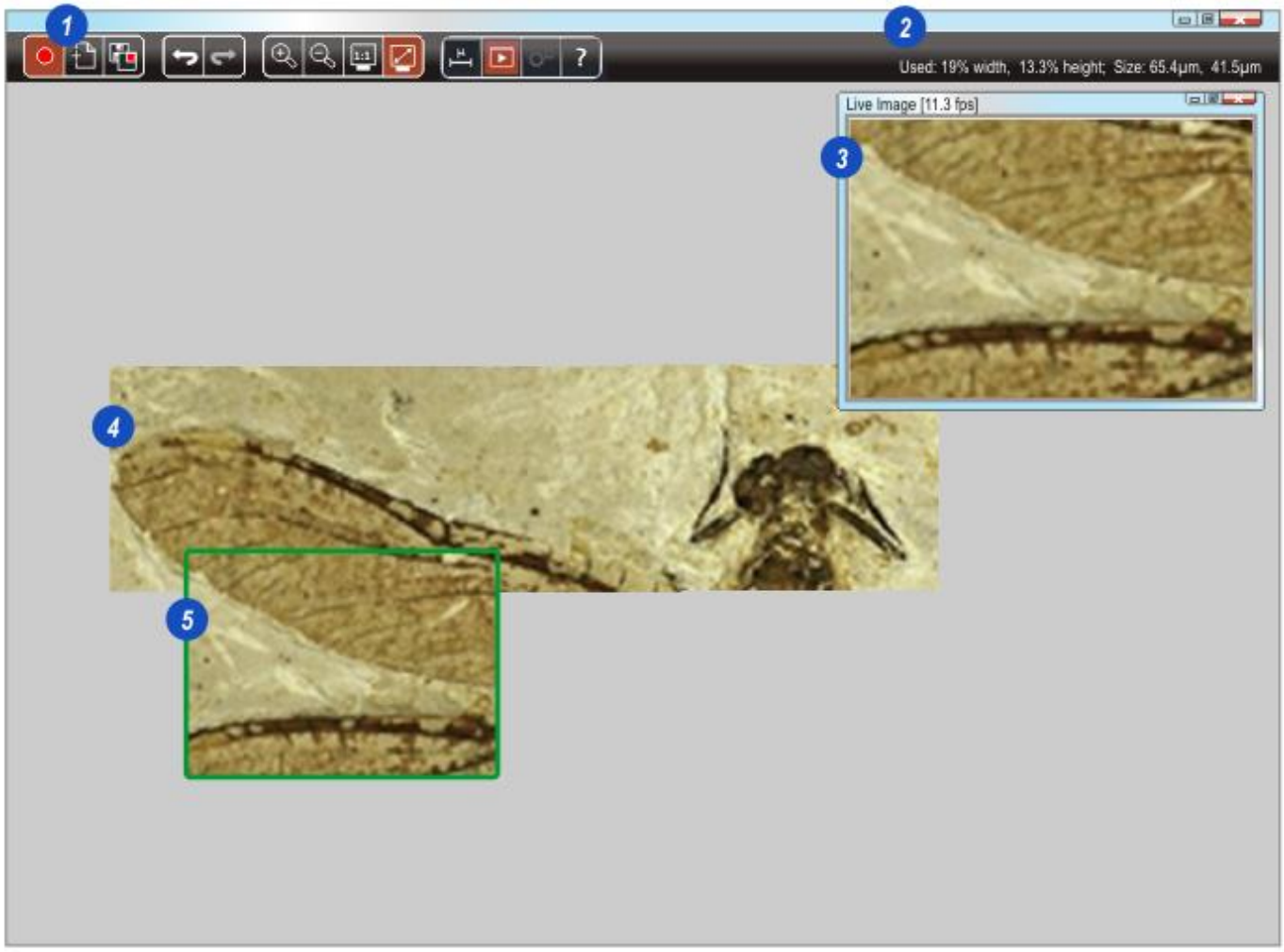
1: The *Tool Bar.*

2: The *Status Bar*: Showing how the scan is progressing in terms of physical size - not file size.

3: The *Live Image Window*: This is the current camera field showing position on the specimen. Positioning co-ordinates are not recorded or 'remembered'.

4: The *Builder Image* so far.

5: The current *Tile* (same as the *Live Image Window*) that is being processed.




[Start Building/Pause](#) 758 :
Starts and pauses the live build process.

[New Canvas](#) 758 :
Clears the existing *Builder Image* and starts building a new one.

[Return to LAS](#) 761 :
Returns to the LAS interface with the option to save the *Builder Image*.


Undo/Redo:
Step backwards and forwards through recent actions.


[Zoom In/Out](#) 759
Increase or decrease the display size of the *Tile* and *Builder Image*. If available, you can also use the mouse wheel to zoom.

[Display at Original Size](#) 759 :
Displays the *Builder Image* at its actual size - the same size the specimen appears in the *Acquire Viewer.*

[Fit image to Viewer](#) 759 :
Enlarges the *Builder Image* to fit the available viewing area.

[Show/ hide Scalebar](#) 755 :
Click to hide or reveal the *Scalebar* in the top right corner of the *Builder Image*.

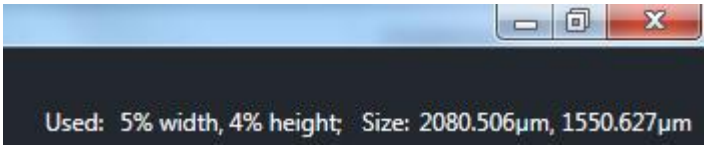
[Live Window Hide](#) 756 :
Click to hide or reveal the *Live Image Window* to see more of the *Builder Image*.

[Preferences](#) 757 :
Displays the *User Preferences* dialog.

Help:
Displays this help file in a new window.

[Capture Status](#) 759 :
The border around the *Tile* shows whether it was successfully matched and stitched.

[Status Bar](#) 758 :
At the top right of the *Live Image Builder* window, this displays the ongoing scan area used and the scan size.

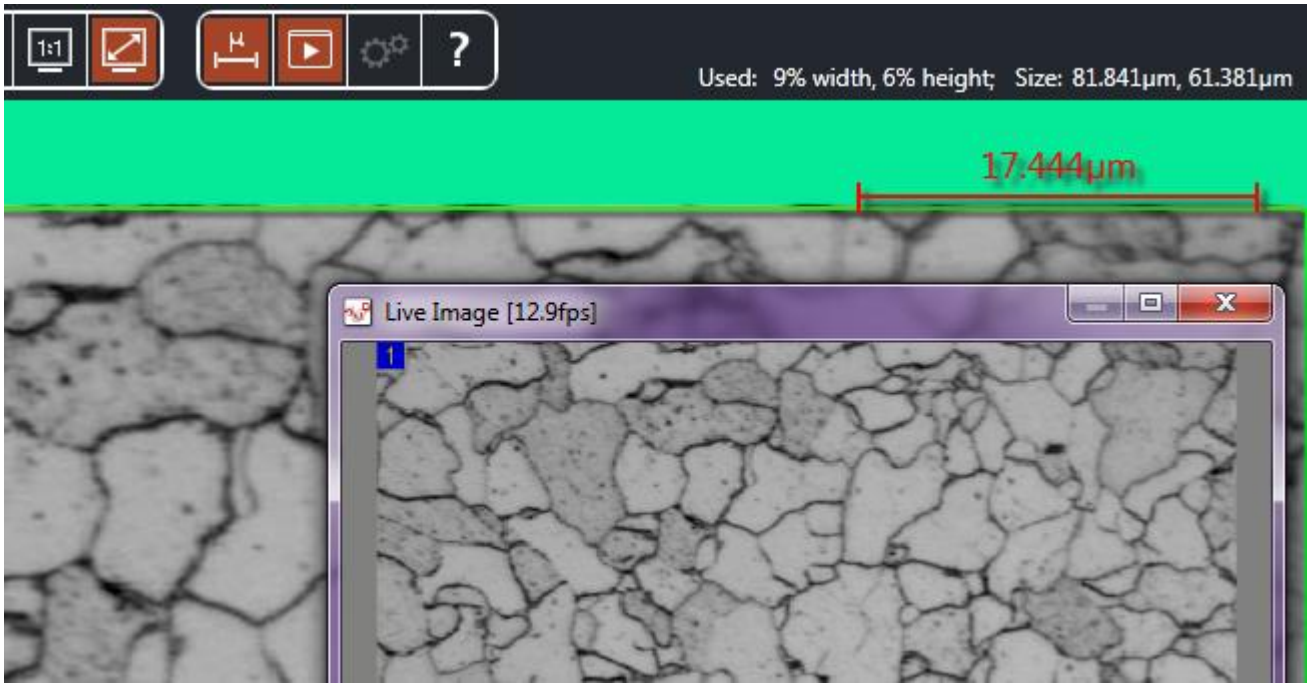


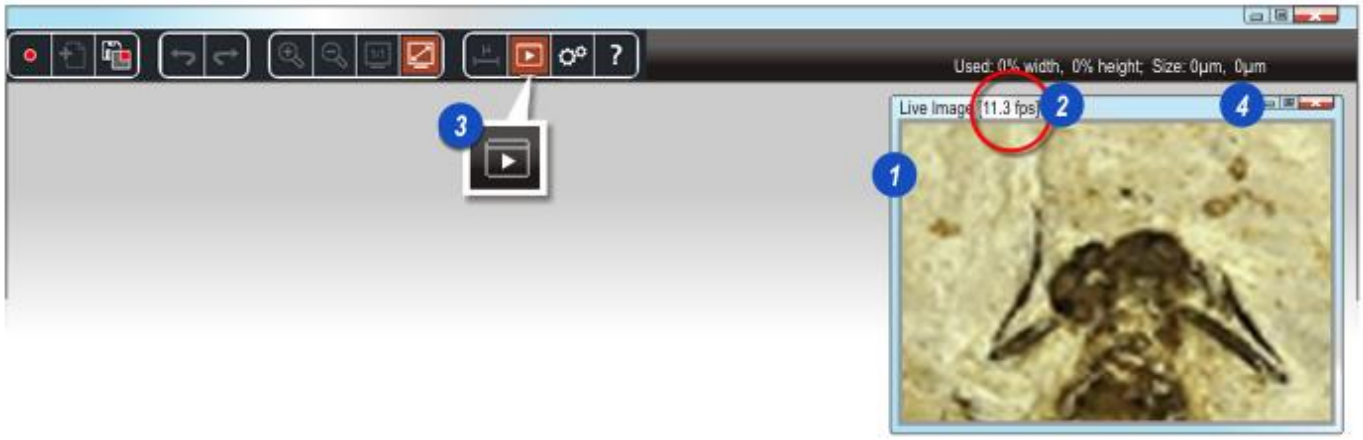
You can display a *Scalebar* in the top right corner of the LIB canvas. The colour of the scale bar can be set in the User Preferences 757 window. It is a good idea to choose a contrasting colour to that of your sample. The scale bar is only shown in LIB XY and LIB XYZ. It is not displayed in LIB Z.

The *Scalebar* units displayed are taken from the calibration of LAS. It is important that when using a manual microscope the correct objective / magnification is set in LAS. If no calibration is set then the *Scalebar* units will be shown in pixels. The *Scalebar* in LIB is rounded to the nearest whole number as it is intended to provide an indication only, not to be a measurement tool. For accurate measurements, please save the image and use the measurement tools within LAS.

Note: If you are viewing in *1:1* mode, the measurement displayed by the *Scale Bar* will not change. If you are in *Fit to Window* mode, the size shown will change according to the area viewed.

The *Scalebar* is a fixed length and location; you can toggle it on and off using the button on the tool bar.





The *Live XY Builder* interface opens with the following setup:

1: The current *Field* is displayed on the right, in the *Live Image Window.*

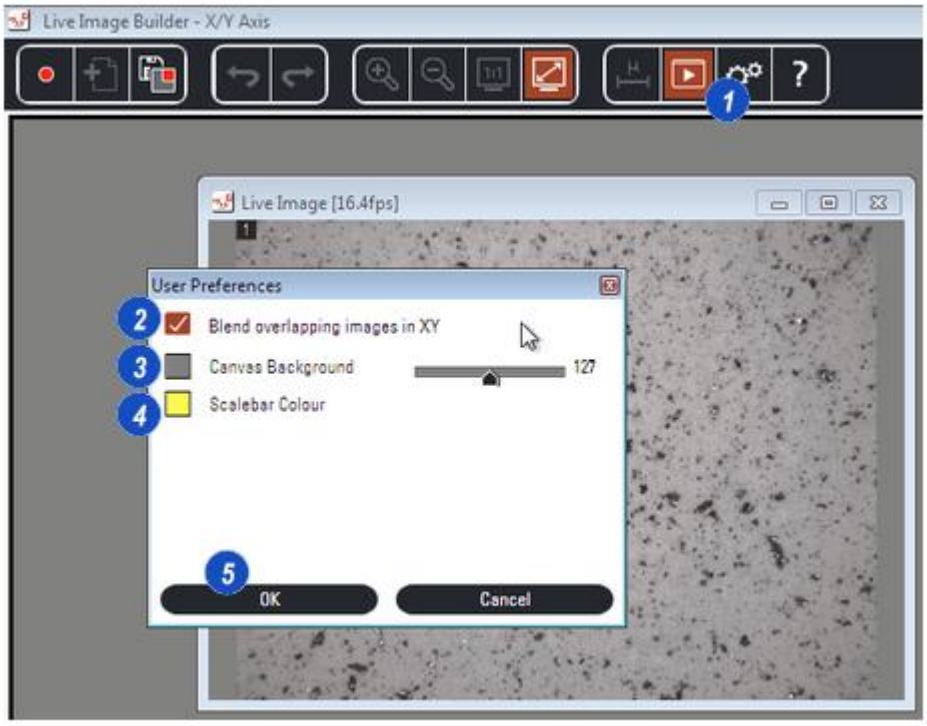
2: The camera frame rate in frames per second (fps) is shown in the header. You should aim for a frame rate of greater than *9* frames per second.

The *Live Image Window* allows you to re-focus while the scan is in progress.

3: You can hide the *Live Image Window* to allow more of Click *Builder Image* to be seen by clicking the *Hide/ Reveal Live Image* button. Click again to reveal it.

4: You can close or minimise the *Live Image Window* with the usual Windows controls. Use the *Hide/ Reveal* button to open it again.

Decide upon the XY start position. Do not start in the middle of the sample. Find a corner of the sample and plan to work in a zig-zag pattern across and down the sample.



1: Click the *User Preferences* button to open the *User Preferences* widow.

2: Enable or disable the *Blend Overlapping Images* check box:

- *Enabled*: The pixels in the *Overlap* are 'smoothed' to eliminate visible seams caused by shading. The blending process may cause very fine detail to loose sharpness.

  If the next tile focus is changed, the focus in the overlap area will change also.

- *Disabled*: Each tile can be focussed separately without affecting its neighbours. Fine detail is retained. Changes in focus may be obvious as seams.

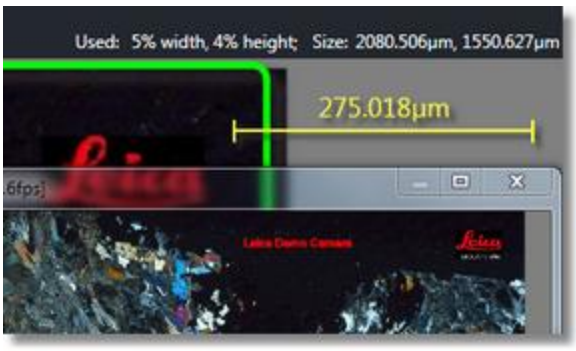
3: Click the *Canvas Background* tile if you want to pick a different canvas colour. In general, it is better to choose a colour that contrasts with the sample. (**Note**: You cannot change the canvas colour if you are using a greyscale camera. Use the slider to specify the greyscale value from 0 to 255.)

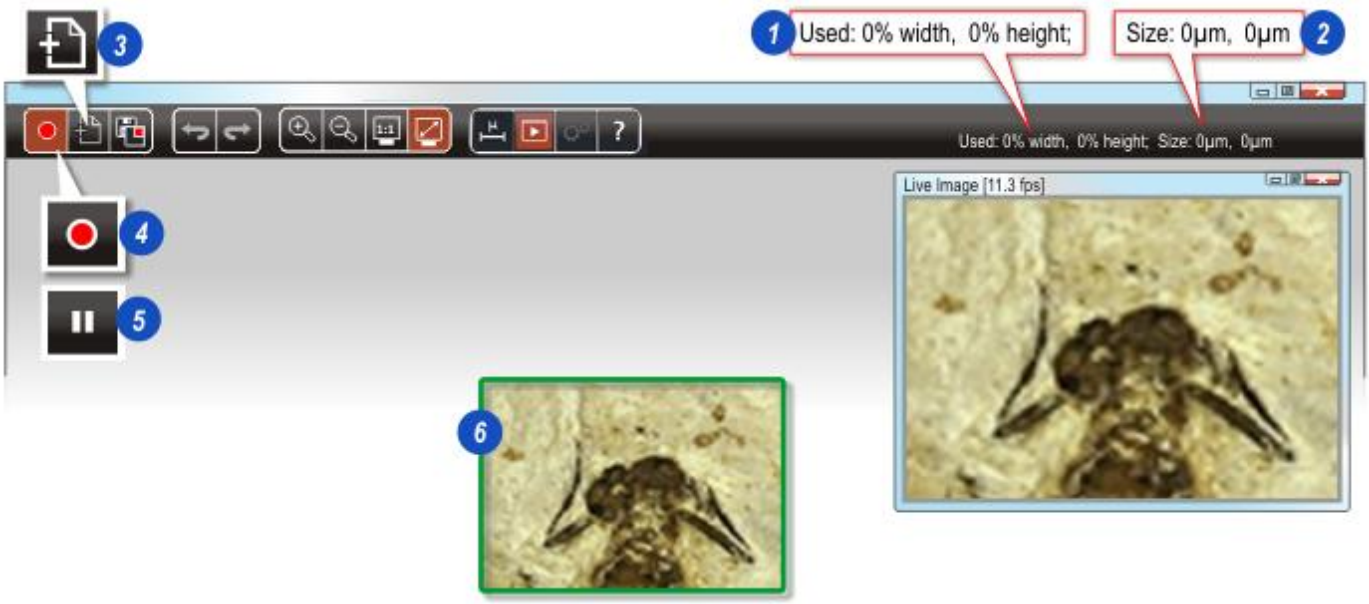
4: Click the *Scalebar Colour* tile if you want to pick a different colour for the Scalebar. In general, it is better to choose a colour that contrasts with the canvas.

5: Click *OK*.

Colour Picker dialog

Scalebar colour changed to yellow





Live XY Builder creates the *Builder Image* on *The Canvas.*

The *Status Bar* provides information about *The Canvas*:

1: The amount of the *The Canvas* used so far.

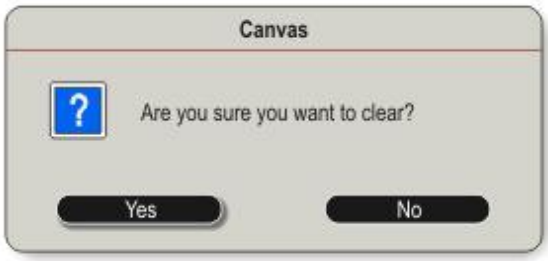
2: The size (in *µm*) of the scan.

When the *Builder Image* extends so that it touches the boundary of *The Canvas*, the display is shifted to allow more *Tiles* to be added - providing there is free space within *The Canvas*.

If the *Builder Image* exceeds the limits of *The Canvas,* stitching *w*ill stop but the *Builder Image* is still available to save.

When *Live XY Builder* starts, *The Canvas* is blank; as work progresses you can decide to discard the current *Builder Image* and start afresh.

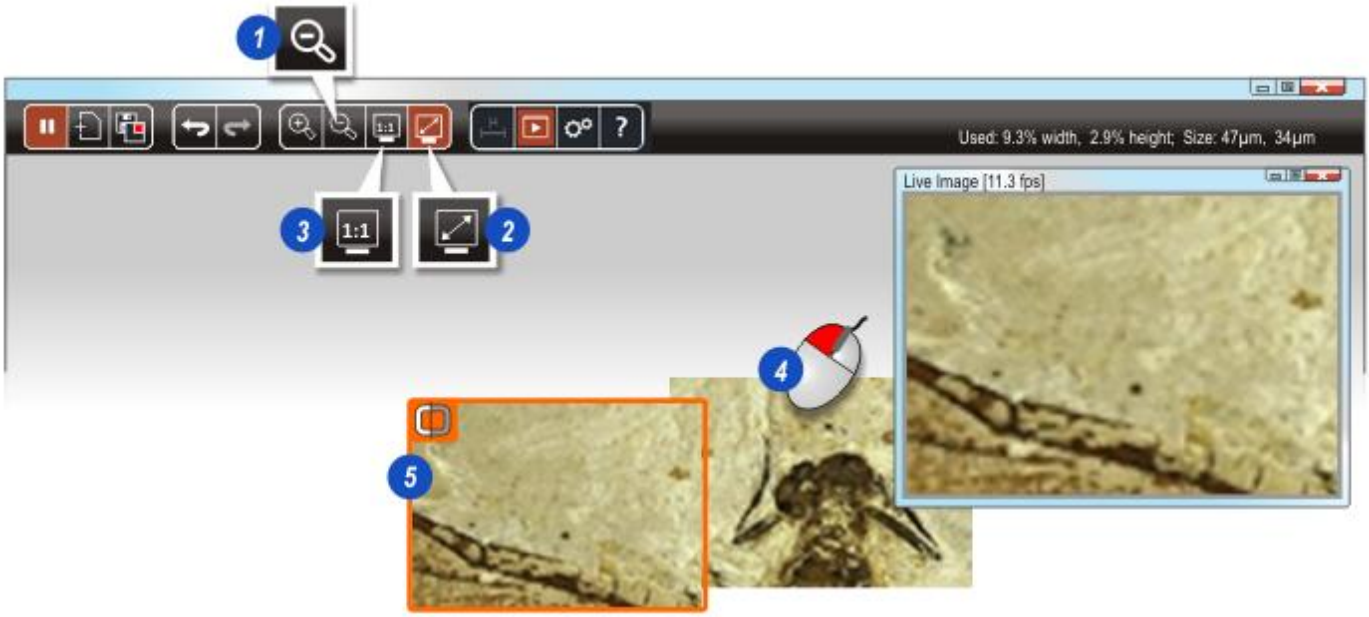
3: Click *New Canvas* to clear all existing work and reset *The Canvas* to its full size. Click *Yes* to confirm, and the existing scan will be cleared. It cannot be retrieved.



4: Start the scan by clicking the *Start Building* button.

5: The button icon changes to *Pause*. Click to suspend the scan and click again to resume.

6: The first tile - a copy of the live image - is displayed and the *Status Bar* is updated.



1: Resize the first tile as required using the *Zoom* tools. The *Builder Image* will be displayed at the selected zoom assuming that neither the *Fit to Window* or *Scale 1:1* tools are used.

Zoom is centred around the current mouse position.

2: *Fit to Window:* When clicked will fill the screen with the *Builder Image* so far. As tiles are added the *Image* is scaled down so that all of it fits inside the window.

This has the advantage that all of the *Builder Image* can been seen, which helps with stage positioning .

However, for larger images, the detail can become too small to be seen easily.

3: *Scale 1:1:* Tiles are always added at the current magnification and the display is shifted within the window to maintain this.

The advantage is that detail will always be visible and focus can be easily adjusted if required.

However, if *1:1* proves to be too large, reduce the *Builder Image* to about 25% of the screen area; as the image expands, the *Canvas* is relocated so it always sits in the centre of the screen.

As the *Builder Image* gets larger, tracking can become more difficult. If position is lost, click on the *Pause* button (this will stop captures) and backtrack[760] on the stage to re-orientate.

4: The first tile becomes the first part of the *Builder Image* and is positioned centrally in the widow; you can drag it to a more convenient position.

Tile capture and stitching are dynamic and completely automatic. A new tile is captured when:

- The *Field* has moved and at least 1 second has elapsed after movement has stopped.

  The software will then capture the *Tile* and try to match and stitch.

5: Each new *Tile* has a border that indicates its status:

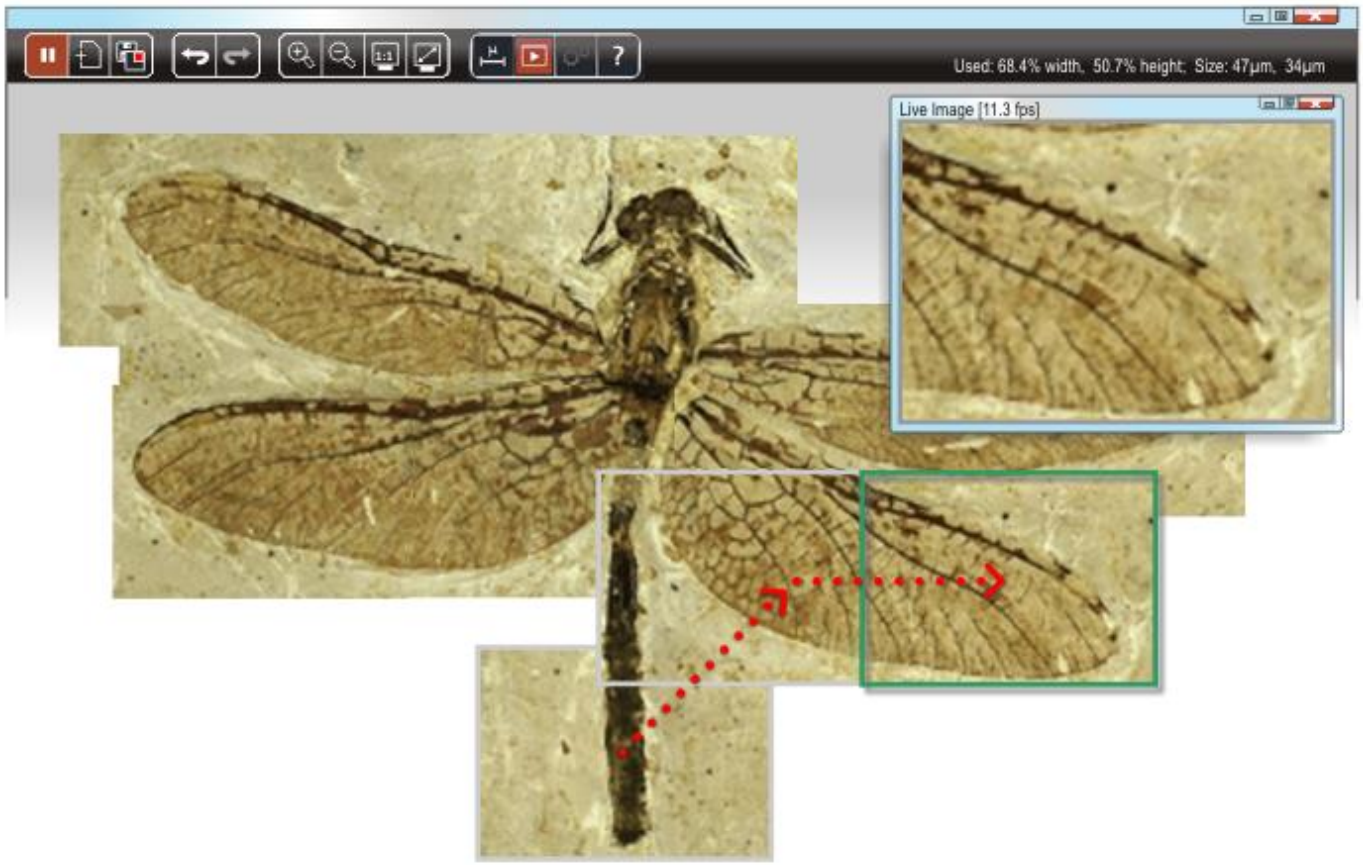
Capture and stitching successful. Pixel data added to the *Builder Image*. Ready for the next.

Capture cannot be matched or stitched: No tile created. Backtrack and try again using smaller stage movements and increased overlap.

Partially matched but perhaps the pattern was not sufficiently different to guarantee proper stitching. No tile created. Consider backtracking and offsetting the tile slightly.

Probably insufficient overlap. No tile created. Move the stage slightly to increase overlap.

Error status can often be resolve by small, slow stage movements. Wait for the border to change to green.



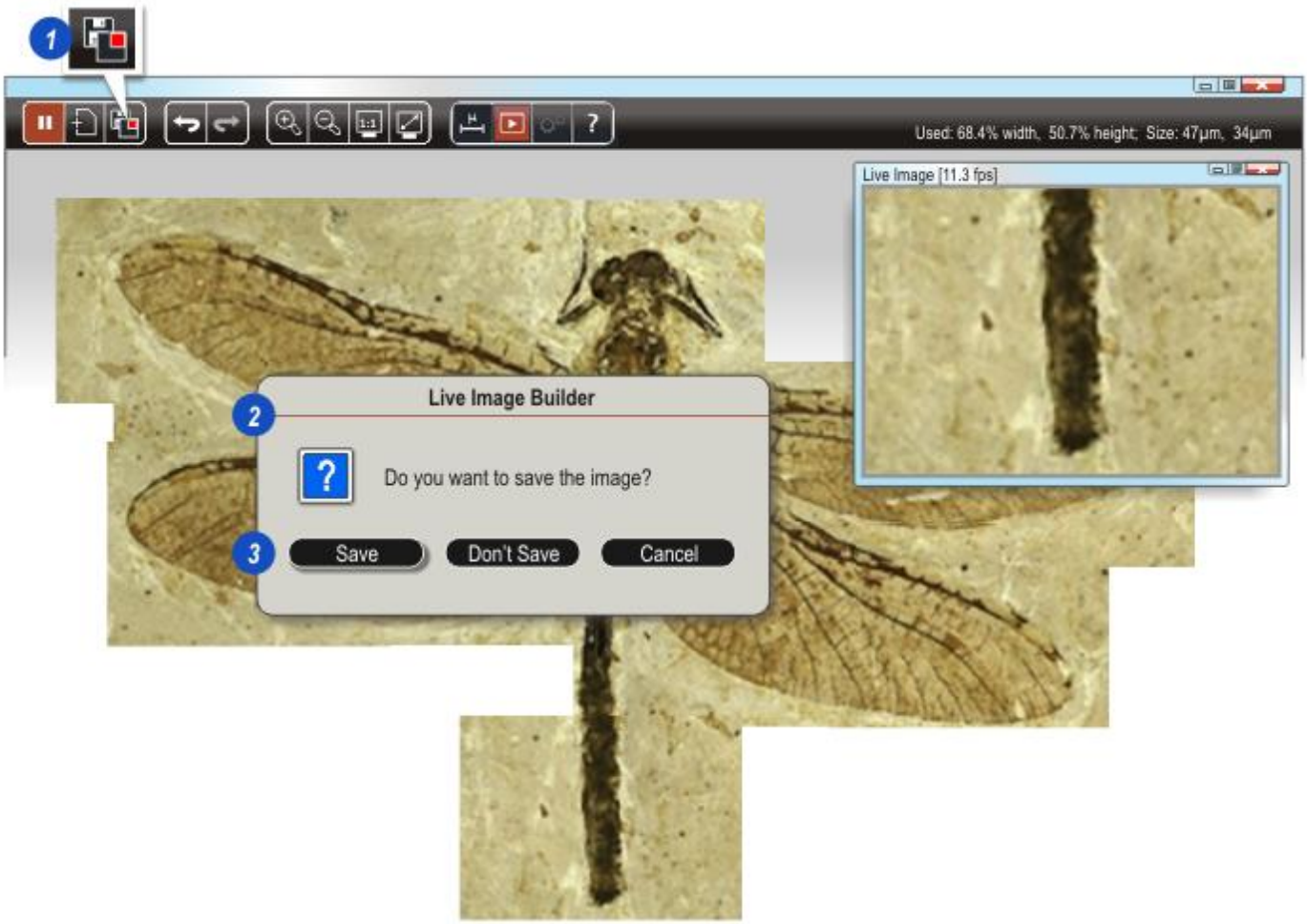
Live XY Builder does not record *Tile* co-ordinates relative to the specimen. This means that you cannot drive the stage to a known position, re-capture a *Tile* and replace it on the *Builder Image.*

There will be occasions however when, because some detail was missing or out of focus, you need to capture a tile again and update the image.

Backtracking simply means going over parts of the *Builder Image* already created and replacing the pixels already there with new ones from new tiles. Maintain the overlap rules, and carry out re-focussing or re-positioning on each tile as necessary.

If the original track is lost, it is helpful to *Pause* building and make small adjustments to the stage position until an acceptable tile is found.

On the diagram, the tiles outlined in grey have been re-captured and replaced on the image to get to the one with the green border which needed attention.



With the scan complete, the *Builder Image* can be saved to the selected capture folder.

1: Click the *Return to LAS* button.

2: The *Save Image* dialog appears.

3: Click to *Save*, return to LAS without saving *(Don't Save)* or *Cancel* and continue building.

Live Z Builder

Designed for microscope users with manually controlled focus, *Live Z Builder* creates a single image of the field of view with sharp focus through the depth of the specimen.

In operation, the specimen focus is gradually changed over its thickness so that all parts of it are, at some time in sharp focus. A digital image - a *Slice* - is continually captured as the focussing progresses and tested pixel-by-pixel with those captured previously. Pixels that are sharp replace those on the image that are not - then the *Slice* is discarded. Slice-by-slice the sharp pixels are blended to produce an image that is uniformly sharp.

Live Z Builder is smart, fast software:

- It *continually* captures and checks the field pixel-by-pixel. These rapid, tiny *Z-Steps* yield images with excellent sharpness over the *entire* specimen thickness.
- Capture is automatic - no need to click buttons. All the user has to do is to smoothly drive the focus control.
- The *Z Builder Image* is built dynamically in real time as the user works.
- Individual fields or *'Slices'* are not saved to hard disk, saving space, increasing speed and making the *Z Builder Image* available as soon as focussing is complete. No post-processing is required.
- The *Live Image Window* allows initial focussing to be checked before processing begins.
- Images can be captured top-to-bottom or bottom-to-top of the specimen.
- *Leica Application Suite* calibration, image formats, exposure and shading all work in the normal way.
- A wide range of *Zoom* options to suit the user's working methods.
- The final *Z Builder Image* can be saved with all the usual LAS attributes so is available immediately for measurements, manipulation and annotation.

In this help the cutter blades from an electric razor are going to be processed to result in a sharp image from blade edge to assembly base.

Terminology in this help:

Field: The microscope field of view.

Slice: The *Field* captured during focus movements. Because *Live Z Builder* is capturing fields continuously the *Slices* are very close together and almost contiguous. The *Slice* is captured temporarily to computer memory. It is not saved to the hard disk.

Z Builder Image. As focus changes across the specimen thickness, each *Layer* pixel is compared with the *Z Builder Image* equivalent for sharpness. If the *Layer* pixel is sharper it replaces the *Builder* pixel. When comparison and replacement is complete, the *Layer* is discarded.

The Canvas: A part of computer memory reserved for the *Z Builder Image.*

Live Z Builder has been designed for use with manually controlled focus. The controller can be any one of the range produced by Leica, but regardless of the control type, it is essential that focussing is carried out slowly and at an even speed.

Because *Live Z Builder* is fast, users can quickly practice their focussing technique, saving only the *Z Builder Image* they are completely satisfied with.

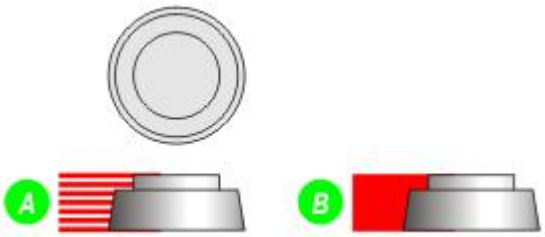
Specimens:

Almost any specimen imaged by light reflecting from the surface such as geological and fossil specimens, plant and marine biology, histology and materials such as paper, electronic components, metallurgy, surface coatings and fractures is suitable for use with *Live Z Builder* providing:

- It is secure.
- Not so tall that it could collide with the objective during focus travel.
- Well and evenly lit. This is particularly important for specimens with deep recesses the bottom of which could be in shadow making sharpness difficult to determine.
- Specimens must have a surface texture that allows the focus region to be identified.
- Stereomicroscopes preferably should have the *AX Carrier* fitted to minimise parallax effects, but the software will allow for size and shift changes during the creation of the extended focus image.

Many depth of field software packages capture fields at distinct steps - *Slices* - across the focus range (A). If the steps are not sufficiently close some of the image will not be captured and resolved.

Live Z Builder (B) captures and analyzes continuously - there are no pre-determined steps so, providing the user changes focus at a moderate and even speed, all of the specimen is captured.



Capturing, pixel comparison and replacement is carried out in real time whilst the user is working.

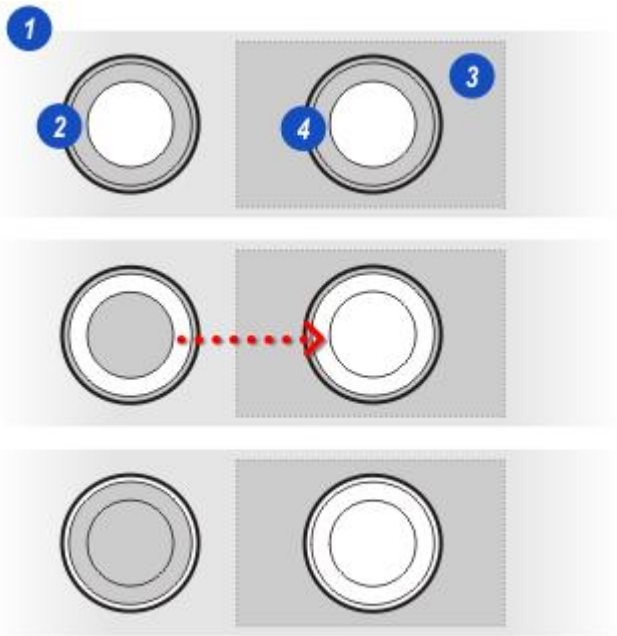
In the diagrams:

1: Represents the *Live Z Builder* working area within which are...

2: ...the *Slice* which is the current microscope *Field,* and...

3: ...a region dedicated to *The Canvas* - which is where...

4: ...the *Z Builder Image* is constructed.



The illustration shows three steps to represent a continuous process. The left-hand images are the specimen and the right-hand are the *Z Builder Image* on the *Canvas:*

The first diagram represents the starting *Slice* - the top face coloured white is in sharp focus; The rest, coloured grey is out of focus. It is captured in its entirety and copied to the *Canvas* as the starting *Z Builder Image*.

In the next *Slice* focus has changed and now the shoulder of the specimen (white) is in focus. The sharp pixels replace those out of focus on the *Canvas.*

Finally, the bottom edge of the specimen is in focus (white) and again the sharp pixels replace those on the *Z Builder Image* that are out of focus to end with a single image all of which is sharp.

If you are new to *Live Z Builder,* use these check lists to familiarise yourself with the program features and set up before building.

User Interface Overview

The Live Z Builder Interface 774
What the user sees. Where the controls are and the live image and scan areas.

The Tool Bar 775
Details of the tools and what they do.

The Canvas 777

Zooming 778

Workflow

Choose a Capture Folder 766
Determine where the final Z Builder Image will be saved.

Check the Preference Settings 767

Has Calibration been carried out recently 768
For accurate measurements on the final image, calibration is essential.

Check the Image Type 769
Live Z Builder works only with colour images.

Set the Live Image Format 770
Image Format is important in determining the speed of Live Z Builder.

Turn on Shading 771
Help to maintain even lighting across the Builder image.

Adjust the Exposure 772
Important in setting a camera frame rate for fast sampling.

Launch Live Z Builder 773

Start Building 776

Scan in either direction 776

Save the image 779

The *Live Z Builder* image can be saved into a folder of the users choosing.

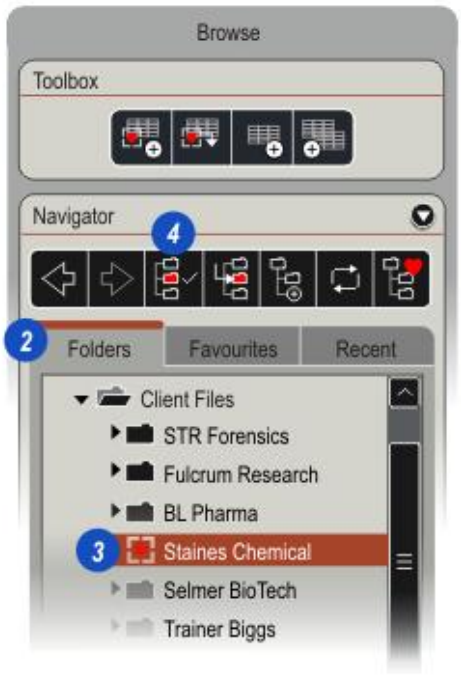
1: Click on the *Browse Workflow* and...

2: ...if necessary, click on the *Folders* tab.

3: Navigate to and click to select the capture folder and then...

4: ...click on the *Set Fixed Location* button. To indicate the fixed capture location archive, a red dot appears to the left of it.

! Images will only be captured to the *Fixed Location* folder if *'Capture to fixed'* is enabled in *Preferences* 63



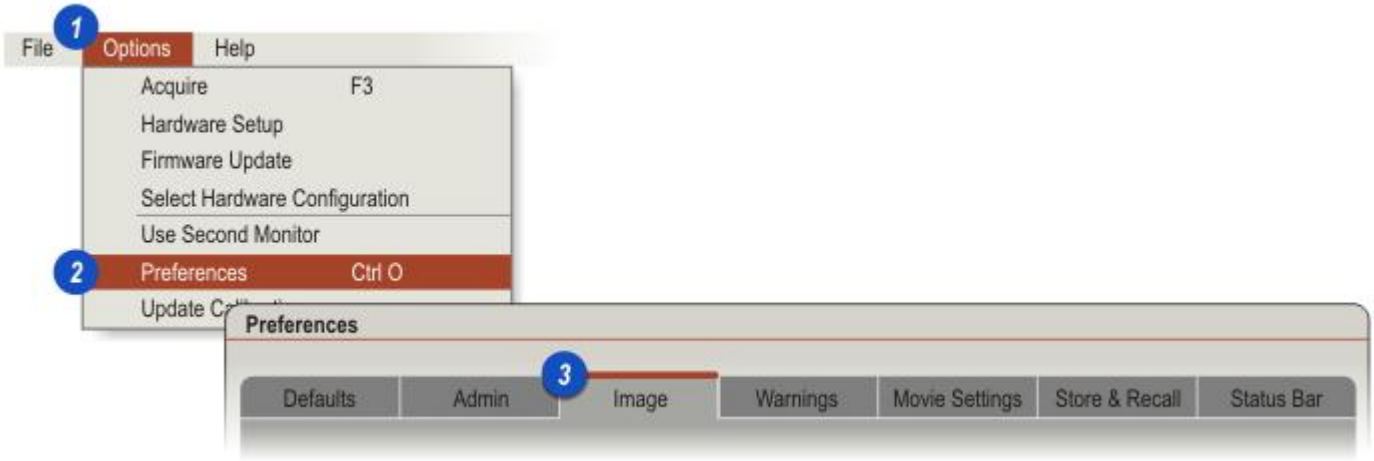
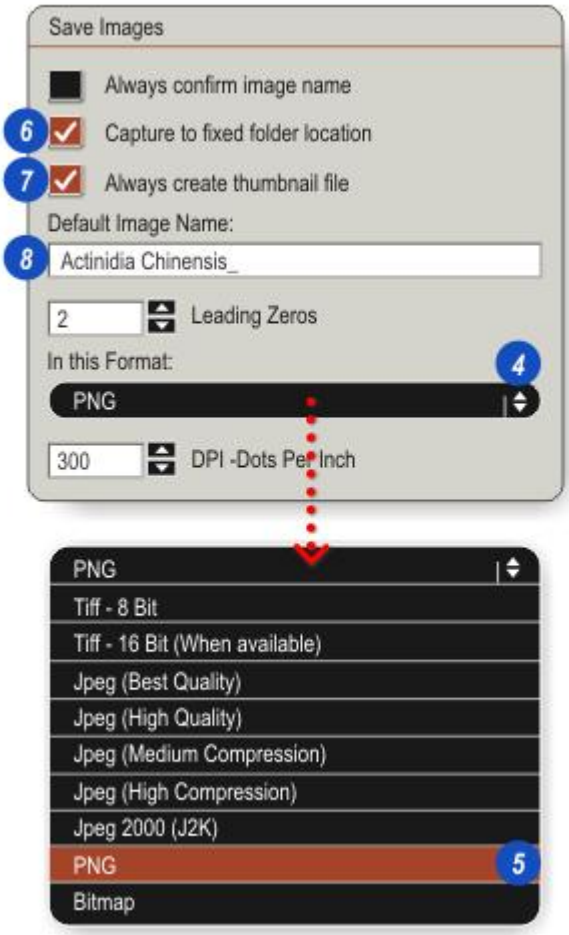


Image compression affects the data size and quality of the captured image. For complex *Live Z Builder* scans that may be shared, it can have an important bearing on the size of e-mail messages for example. High quality, little or no compression images can be very large.

The *Compression* setting is made in *Preferences* > *Save Images* panel and at the same time users should enable *Capture to a fixed folder* and give the saved scan a *Name*.



Select the Image Compression:

1: Click on *Options* on the *Main Header* and...

2: ...click to select *Preferences*.

3: On the *Preferences* dialog, click the *Image* tab.

4: Click on the arrows to the right of the *In this format* header and...

5: ...click to select the required *Compression* type.

Fixed Capture Folder and Image Name:

6: Click to enable (tick mark visible) the *Capture to fixed folder location.* The folder is selected in *Browse* 766

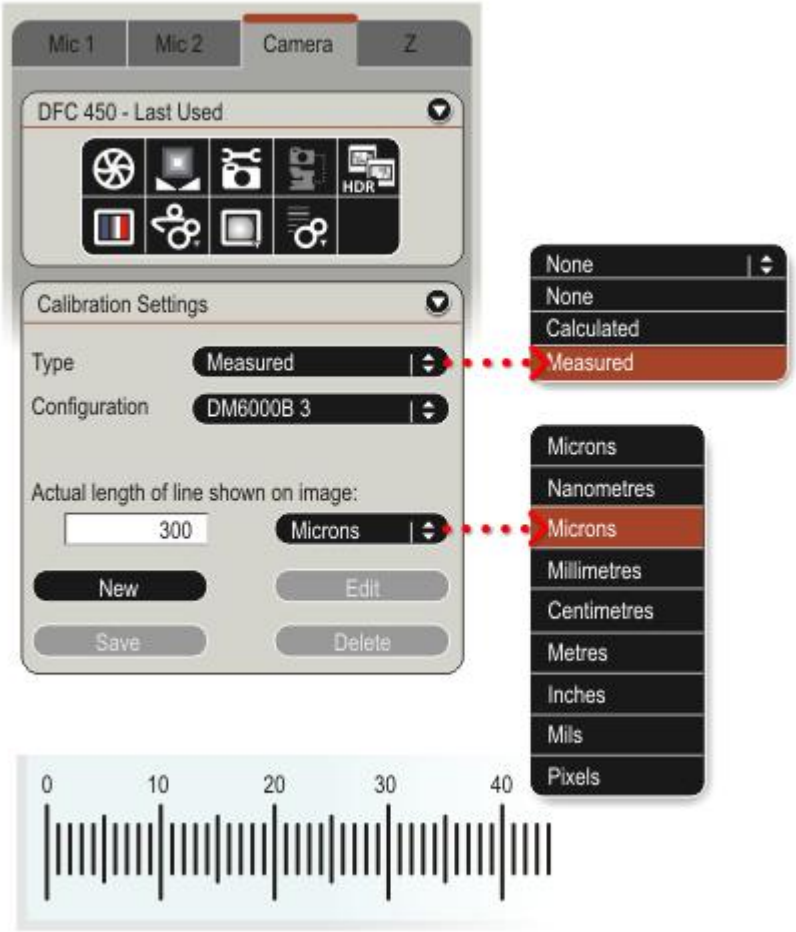
7: *Live Z Builder* always creates a thumbnail for each capture regardless of the check box setting.

8: The *Default Image Name* is given to a saved scan. Subsequent scans are given the same name (unless it is changed) with an incremental numeric suffix.

Calibration ensures that measurements shown by the software are in real world units - microns, millimetres etc - taking into account the microscope optical magnification and the camera pixel size.

Users should check that the microscope has been recently calibrated; If not, calibration should be carried out before running *Live Z Builder*.

More information 318



Check the Image Type

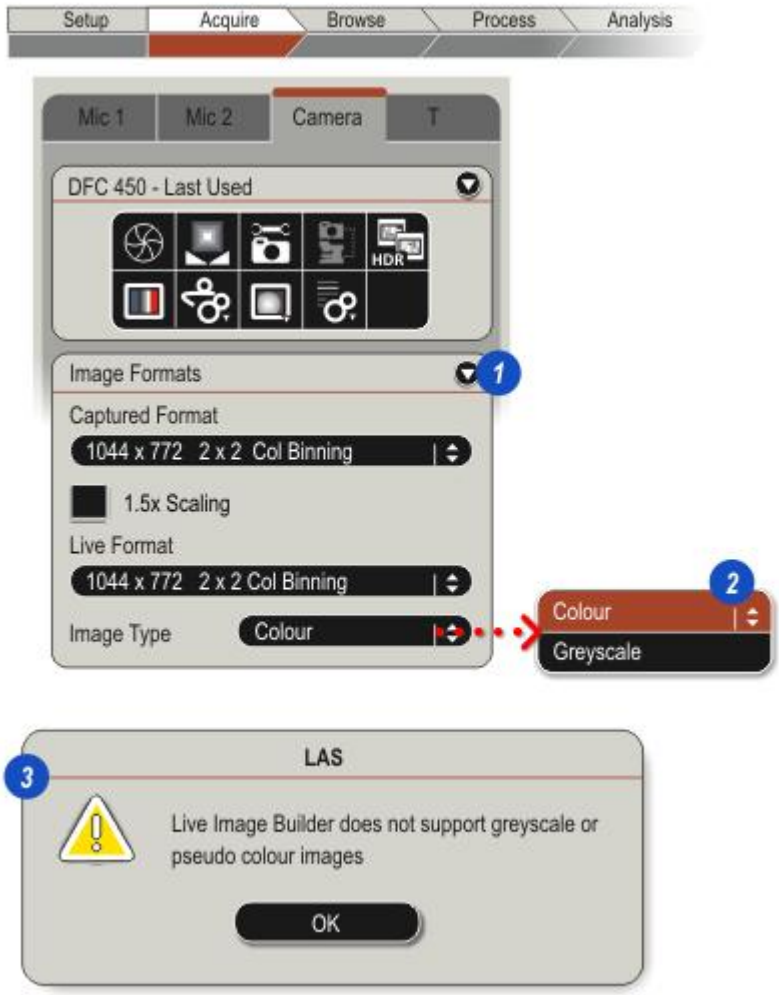
Live Z Builder works with *DFC* cameras and colour images.

On the *Acquire Workflow* select the *Camera* tab and check that the *Image Type* is set to *Colour*;

1: Click the arrow to the right of the *Image Formats* header and check the *Image Type.*

2: Change to *Colour* by clicking on the arrows to the right of the *Image Type* header and selecting the *Colour* option.

3: Attempting to use any other image type will display the warning when *Live Z Builder* is launched.



The *Live Format* determines the quality of the image displayed in the *Viewer* and will affect the speed at which the camera can capture fields - the camera frame rate (fps = frames per second). A high resolution format will slow capture and processing.

On the Acquire Workflow and Camera tab:

1: Click on the arrow to the right of the *Image Formats* header to reveal the panel.

2: Right-click on the *Live* and *Captured Format* headers and...

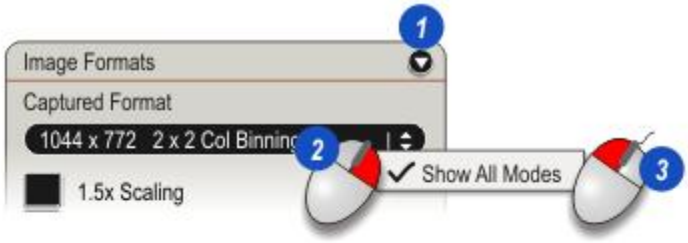
3: ...left-click to turn on *Show All* modes which will display all of the formats that the camera is capable of displaying.

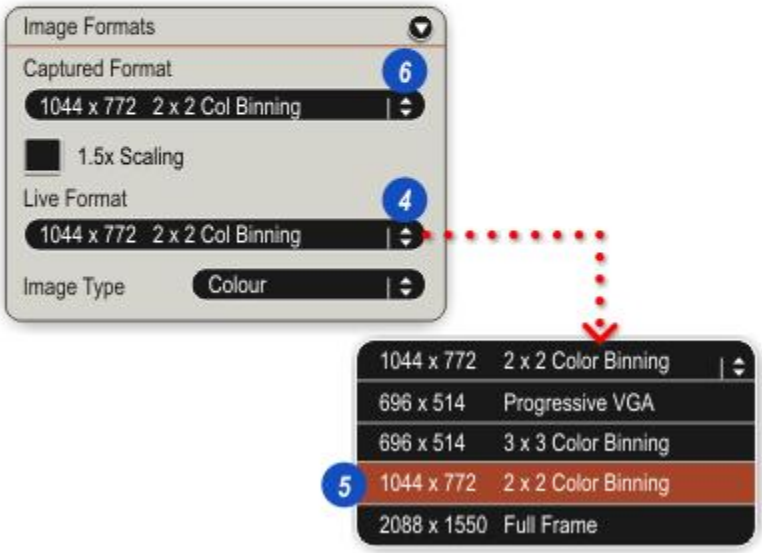
4: Click on the arrows to the right of the *Live Format* header bar and...

5: ... from the menu click to select a format. If the camera supports a wide range of formats, small *Scrolling Arrows* will appear top and bottom of the drop down list. Click to scroll up and down.

- If the *Live Format* is the default setup when LAS is installed, continue to use that. Otherwise...
- A *1044 x 772 2 x 2 Colour Binning* (depending upon the camera) format is a good starting point. Choose a lower format if the camera frame rate is too low and cannot be increased sufficiently using the *Exposure* controls.

6: The *Captured Format* does not affect *Live Z Builder.*





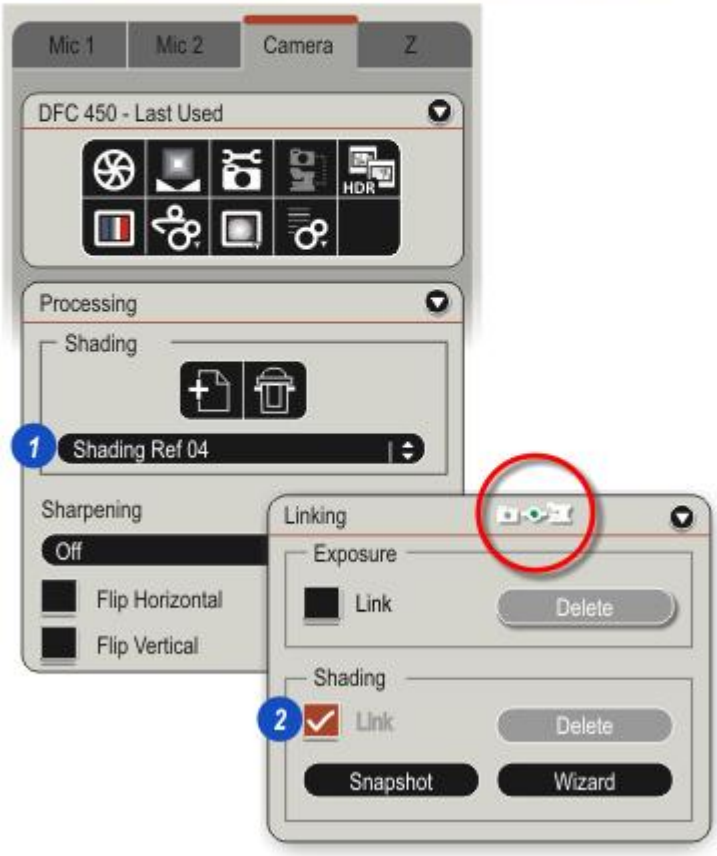
Shading refers to variations in the background light level across an image.

The image on the left shows how the light source and the optics conspire to create a bright spot in the centre of the image which gradually becomes less and less bright toward the edges.

Even ‘illumination’ on live images can be achieved in software by applying a ‘blank area’ value to the entire image area. The effect is shown in the right image.

Alternatively, a *Shading Link* can be created which corrects the shading effect on individual objective and illumination setups.

- *Shading* should be turned on if the lighting is causing obvious bright spots.



Expand either the *Processing* or the *Linking* panel depending upon the shading type to be used:

1: On the *Processing* panel select a user configured *Shading Reference*. *More information*[307].

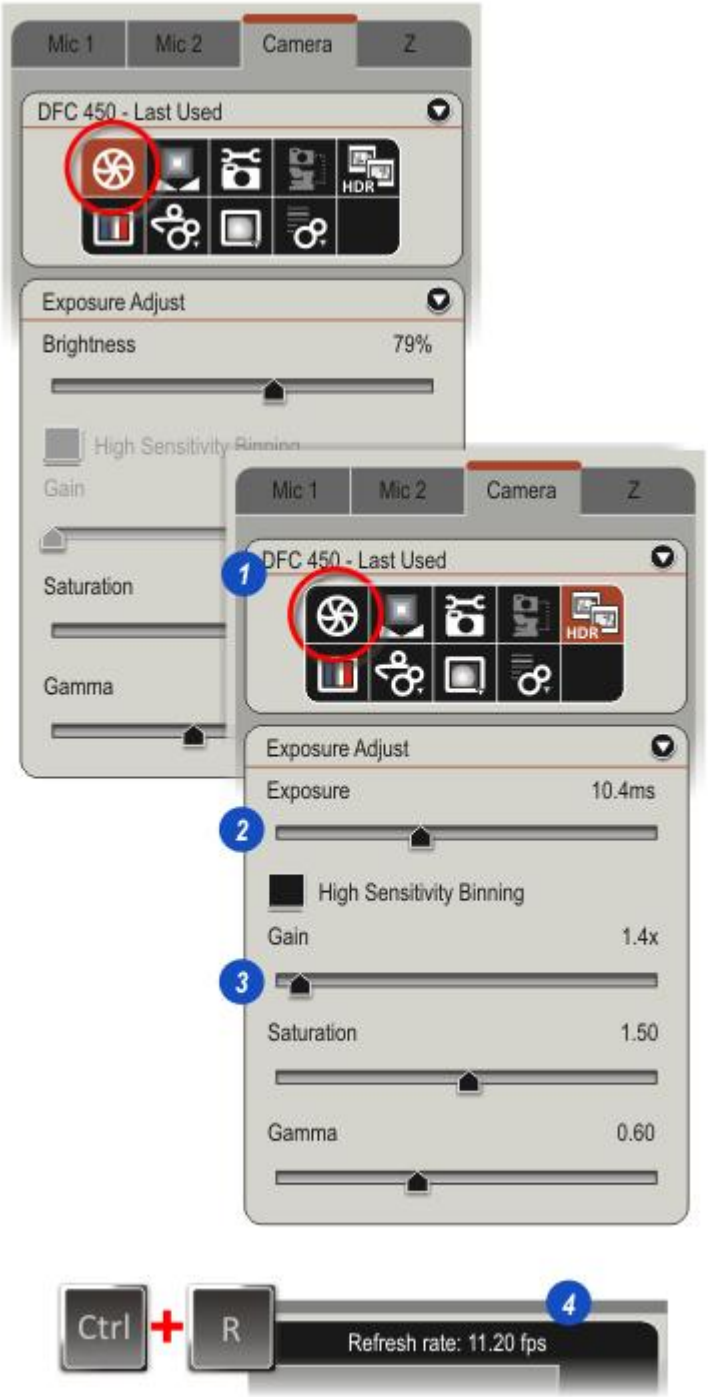
2: On the *Linking* panel click to enable (tick mark displayed) the *Shading* check box. *More information*[341].

There are two options for adjusting the exposure:

- *Automatic* with some fine-tuning, and...
- *Manual* with a range of precision controls.

As a start, using *Automatic Exposure* is a good option because combined with *Automatic White Balance* it could produce a perfectly acceptable image very quickly.

Information about *Exposure* 282



1: Use the *Manual Exposure Adjust* to make fine adjustments to:

2: The *Exposure* time balanced with...

3: ...small increases in *Gain* to achieve scans of acceptable quality in reasonable times.

- Aim for a camera frame rate (the number of images - *Slices* - the camera can capture in 1 second) of between *9* and *12* fps. The actual frame rate is shown on the *Live Z Builder* live image window or...

4: ...with the *Camera* tab selected, press and hold down the keyboard *Ctrl* key and then press the *R* key to reveal the camera *Refresh rate* as fps - *Frames per Second* - top right on the *Viewer*.

- *HDR, AVG and automatic exposure* are disabled in *Live Z Builder* so that the exposure settings remain consistent.

Launch Live Z Builder

The *Live Z Builder* module must be installed and enabled.

1: Click on the *Acquisition Mode* selector.

2: From the menu, click on the *Image Builder* icon.

There are two optional modules in *Live Image Builder*:

- *Live XY Builder* and...
- *Live Z Builder*.

They have to be installed and activated separately: After the 60 day evaluation period they must be licensed individually.

3: Click the *Acquire Workflow.*

4: Click to select the *LIB* (Live Image Builder) tab. The *LIB* tab is only present if at least one of the modules is installed and activated.

5: Click the required button to launch the module. If a module is not installed the button will be greyed and not accessible.

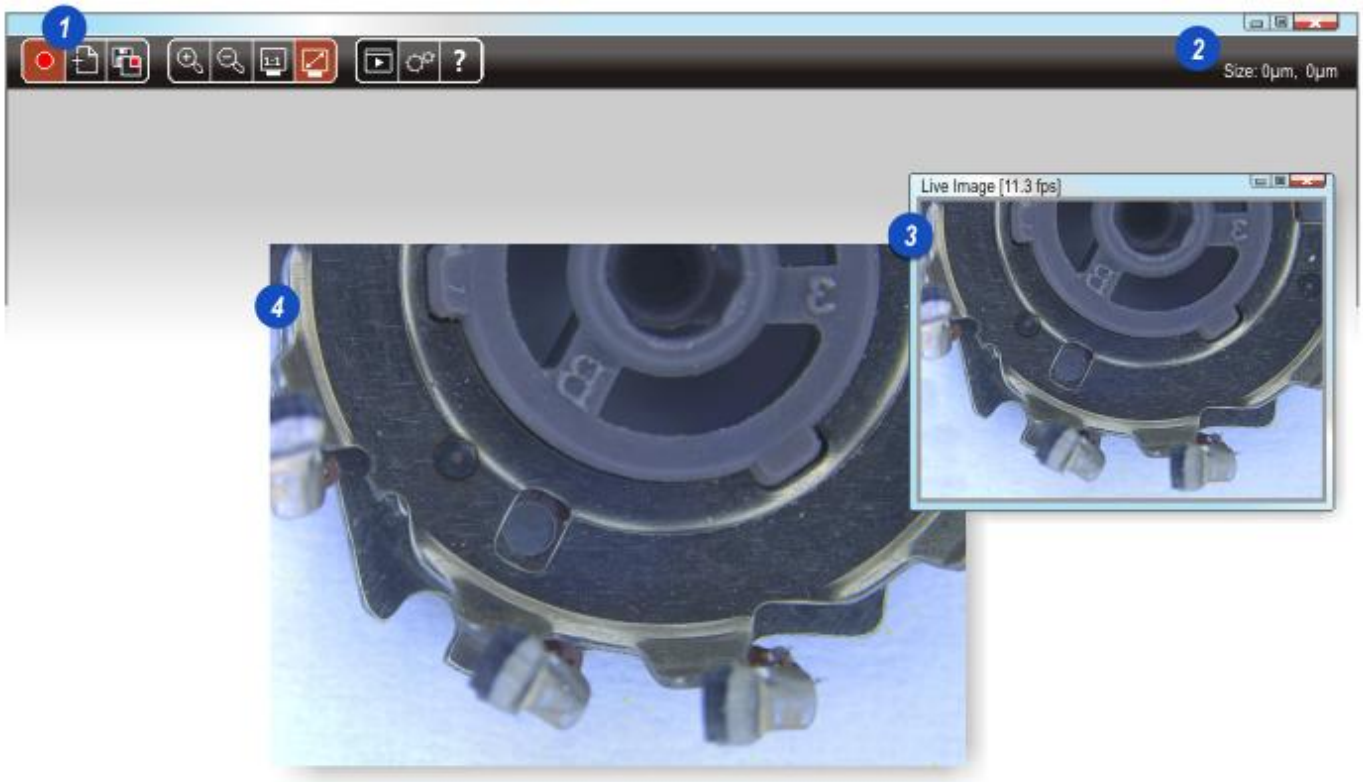
The user interface opens in a new window. The main areas are:

1: The *Tool Bar.*

2: The *Status Bar*: Showing the *Z Builder Image* size - not file size.

3: The *Live Image Window*: This is the camera live field.

4: The *Z Builder Image* so far.



Click on a button below for more information:

Start Building and...
Pause:
Starts the live build process: The button changes to *Pause* which when clicked halts the build. Clicking again will resume the build.

New Canvas:
Clears the existing *Z Builder Image* and starts building a new one.

Return to LAS:
Returns to the LAS interface with the user option to save the *Z Builder Image*.

Zoom in and...
Zoom Out.
Increases or decreases the display size of the *Tile* and *Z Builder Image*. If users have a wheel on the mouse this can also be used to zoom.
When the mouse left button is held down, movement of the mouse will pan the canvas.

Display at Actual Size:
Displays the *Z Builder Image* at its actual size - the same size the specimen appears in the *Acquire Viewer.*

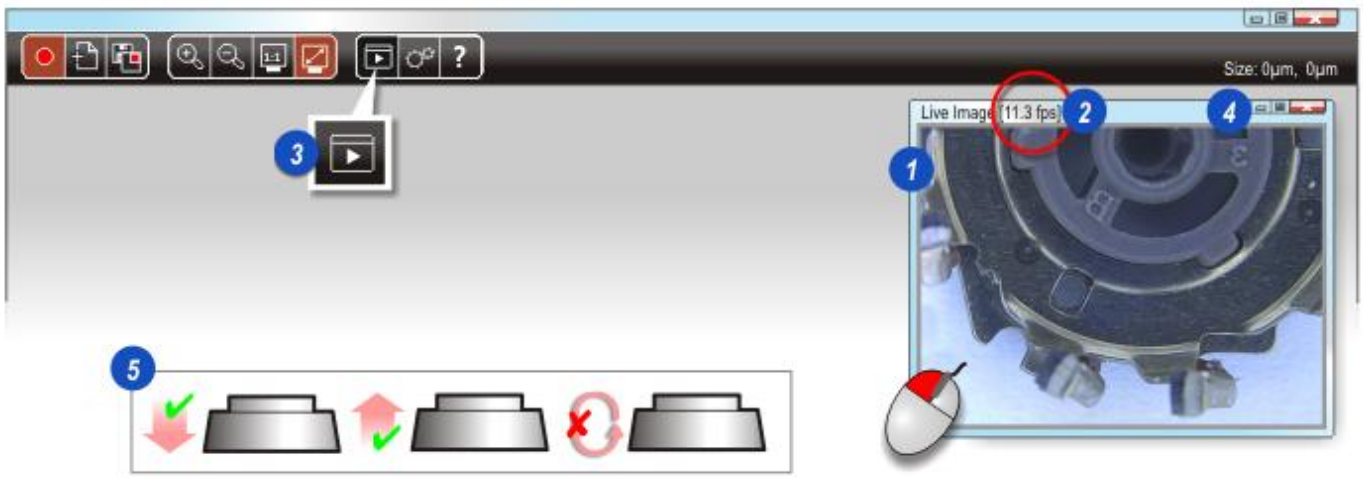
Fit image to Viewer:
Enlarges the *Z Builder Image* to fit the available viewing area.

Live Window Hide:
Click to hide or reveal the *Live Image Window* to see more of the *Z Builder Image*.

Preferences:
Not available in Live Z Builder..

Help:
Displays this help file in a new window.

1: *The Status Bar:*
Displays the ongoing scan area used and the scan size



The *Live Z Builder* interface opens with:

1: ...the camera image displayed on the right in the *Live Image Window.*

2: The camera frame rate in frames per second (fps) is shown on the header. User should aim for a frame rate of over *9* frames per second.

The *Live Image Window* allows users to initially focus on either the top or bottom of the focus range and see the changes in focus as the build proceeds.

3: Hide the *Live Image Widow* to allow more of the *Z Builder Image* to be seen by clicking the *Hide/Reveal Live Image* button. Click again to reveal it.

4: The *Live Image Window* can be closed or minimised with the usual Windows controls. Use the *Hide/ Reveal* button to open it again.

Click and drag on a corner to change the window size.

5: Decide upon the focus direction - start at the top and work down, or at the bottom and work up. Do not start in the middle of the specimen and work in a 'loop'.

Focus on the starting point in the *Live Image Window*.

1: The *Status Bar* shows the user the image size (in µm).

Although the *Z Builder Image* is a fixed field, the size reported will change slightly as the focus changes. This is an inherent optical effect and because of that some *Builder Images* may display a narrow, fuzzy band along one edge

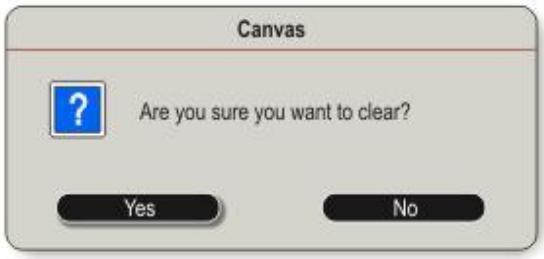
2: Start building by clicking the *Start Building* button.

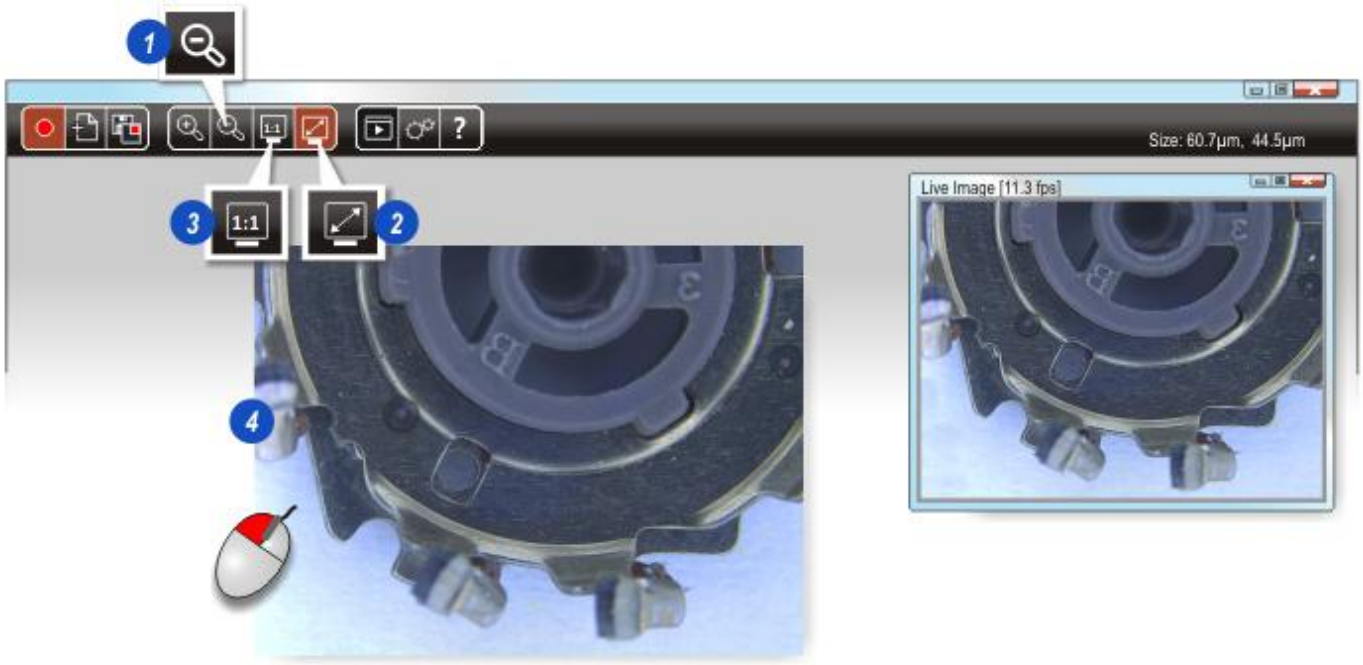
3: The button icon changes to *Pause*. Click to suspend the build and click again to resume.

Live Z Builder creates the *Z Builder Image* in computer memory on a 'drawing area' called conceptually *The Canvas.*

4: The first *Slice* - a copy of the live image - starts the *Z Builder Image* on the *Canvas* and the *Status Bar* is updated.

5: Clicking the *New Canvas* button will clear all of the existing work. Confirm clearing *The Canvas* and the existing *Z Builder Image* will be cleared. It cannot be retrieved.





1: Resize the *Z Builder Image* as required using the *Zoom* tools. It will be displayed at the selected zoom assuming that neither the *Fit to Window* or *Scale 1:1* tools are used.

Zoom is centred on the current mouse position.

2: *Fit to Window:* When clicked will fill the screen with the *Z Builder Image*.

3: *Scale 1:1:* The Z Builder Image is displayed at the same magnification and size as the Camera viewer.

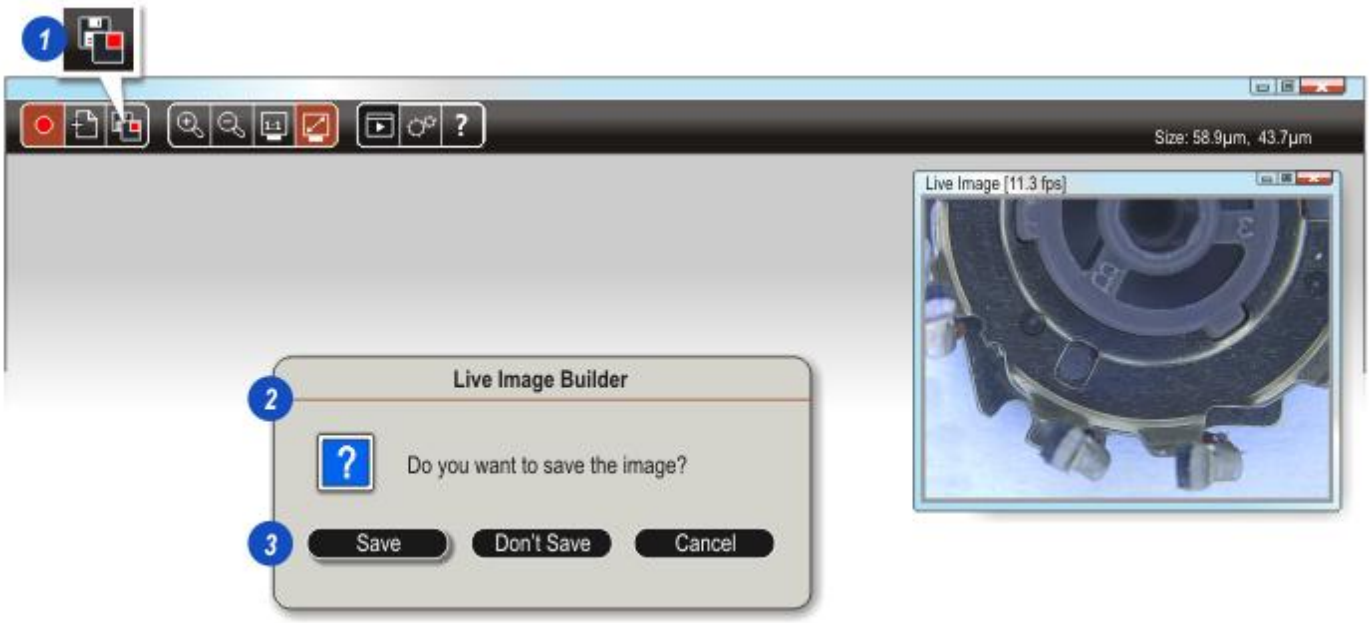
4: The first *Slice* becomes the first part of the *Z Builder Image* and is positioned centrally in the widow but it can be dragged to a user-preferred position.

Live Z Builder samples the live image continually at the camera frame rate and not just when the user changes focus.

If the software detects no change in focus then the *Z Builder Image* remains unchanged, but as soon as focus changes, pixels becoming sharper replace those in the *Z Builder Image* that are less sharp.

Users should move smoothly and evenly between the focus extremes. Stopping part way is acceptable but reversing is not. See the focus change in the live image window and watch sharpness maintained across the entire *Z Builder Image.*

Because *Live Z Builder* is very fast, if users believe they have driven the focus control too quickly and perhaps compromised the overall image sharpness, it is better to clear the *Canvas*, refocus at the starting point and re-build.



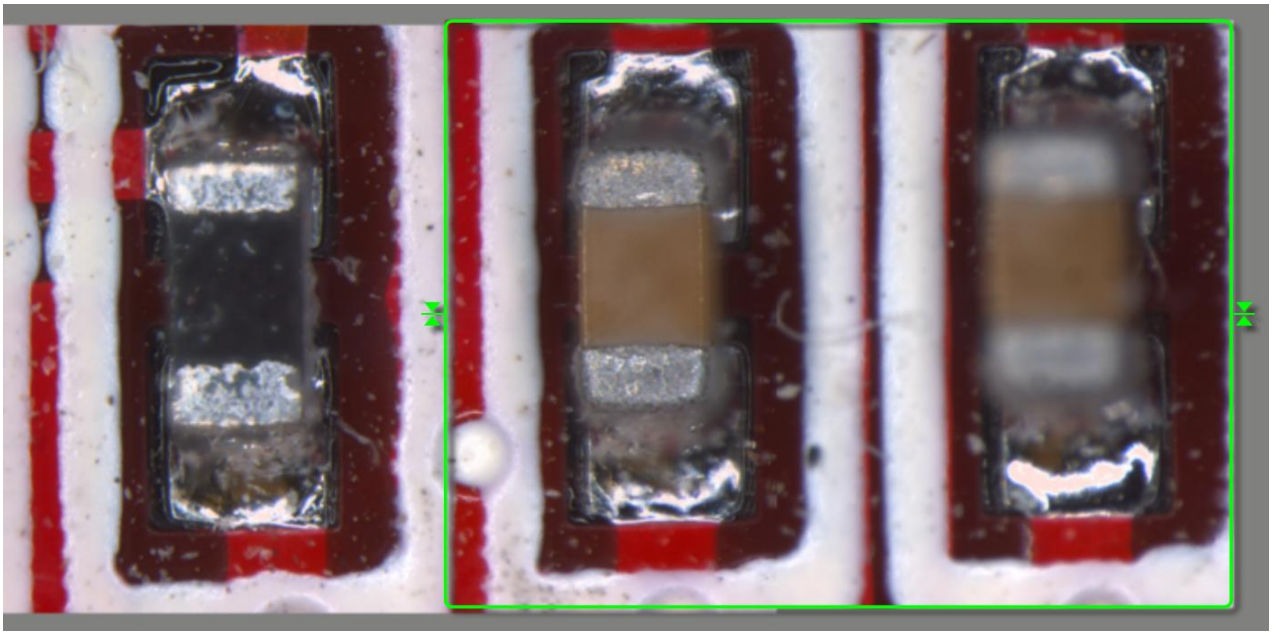
With focussing is complete, the *Z Builder Image* can be saved to the selected capture folder.

1: Click on the *Return to LAS* button.

2: The *Save Image* dialog appears.

3: Choose to *Save* the image or scrap it *(Don't Save),* or *Cancel* and continue building.

Live XYZ Builder



Designed for microscope users with manually controlled XY stages and focus (Z), *Live XYZ Builder* creates a complete image of specimens that extend beyond the field of view of the microscope and additionally with sharp focus through the depth of the specimen.

In operation, you move the specimen gradually in XY while the image is extended. When you notice that parts of the field are not in focus, swap the XY mode to focus mode and gradually change the Z position over the sample depth so that all parts of it are, at some time, in sharp focus. At this point you can swap back to XY mode to carry on extending. Proceed in this way until you have acquired the extended in-focus image.

Live XYZ Builder is smart, fast software:

- It continually matches and tracks the XY motion, adding XY images to the canvas when the movement is paused.
- At this point it switches to Z mode to continually capture and merge the most in-focus parts of the image. When XY movement is again detected, the mode switches back.
- Capture is automatic - no need to click buttons. All the user has to do is to smoothly move the sample.
- The XYZ Builder Image is built dynamically in real time as you work.
- A manual switching mode is provided for samples where detailed control over the Z acquisition is required.
- Individual fields or Z 'Slices' are not saved to hard disk, saving space, increasing speed and making the XYZ Builder Image available as soon as the movement is complete. No post-processing is required.
- The *Live Image Window* allows initial position and focussing to be checked before processing begins.
- Leica Application Suite calibration, image formats, exposure and shading all work in the normal way.
- A wide range of *Zoom* options is available.
- You can save the final XYZ Builder Image with all the usual LAS attributes; it is available immediately for measurements, manipulation and annotation.

Terminology in this help:

Please refer to the help for the Live XY 739 and Live Z 762 Builders.

Live XYZ Builder has been designed for use with manually controlled XY stages and focus. The controller can be any one of the range produced by Leica. However, regardless of the control type, it is essential that sample XY movement and focussing is carried out steadily and at an even speed.

Stereomicroscopes should have the ***AX Carrier*** fitted to minimise parallax effects.

The software will allow for small size and shift changes during the creation of the extended focus component of the XYZ image. However, sample movement and parallax movement can be confused if there is significant change.

Concepts and Fast Track

Please refer to the help for the Live XY 739 and Live Z 762 Builders.

The *Live XYZ Builder* module must be installed and enabled.

1: Click on the *Acquisition Mode* selector.

2: From the menu, click on the *Image Builder* icon.

There are three optional modules in *Live Image Builder*:

- *Live XY Builder*
- *Live Z Builder*
- Live XYZ Builder

They have to be installed and activated separately: After the 60 day evaluation period they must be licensed individually. Licensing XY and Z will enable the combined XYZ.

3: Click the *Acquire Workflow.*

4: Click to select the *LIB* (Live Image Builder) tab. The *LIB* tab is only present if at least one of the modules is installed and activated.

5: Click the required button to launch the appropriate module. If a module is not installed the button will be greyed and not accessible.

The user interface opens in a new window. The main areas are:

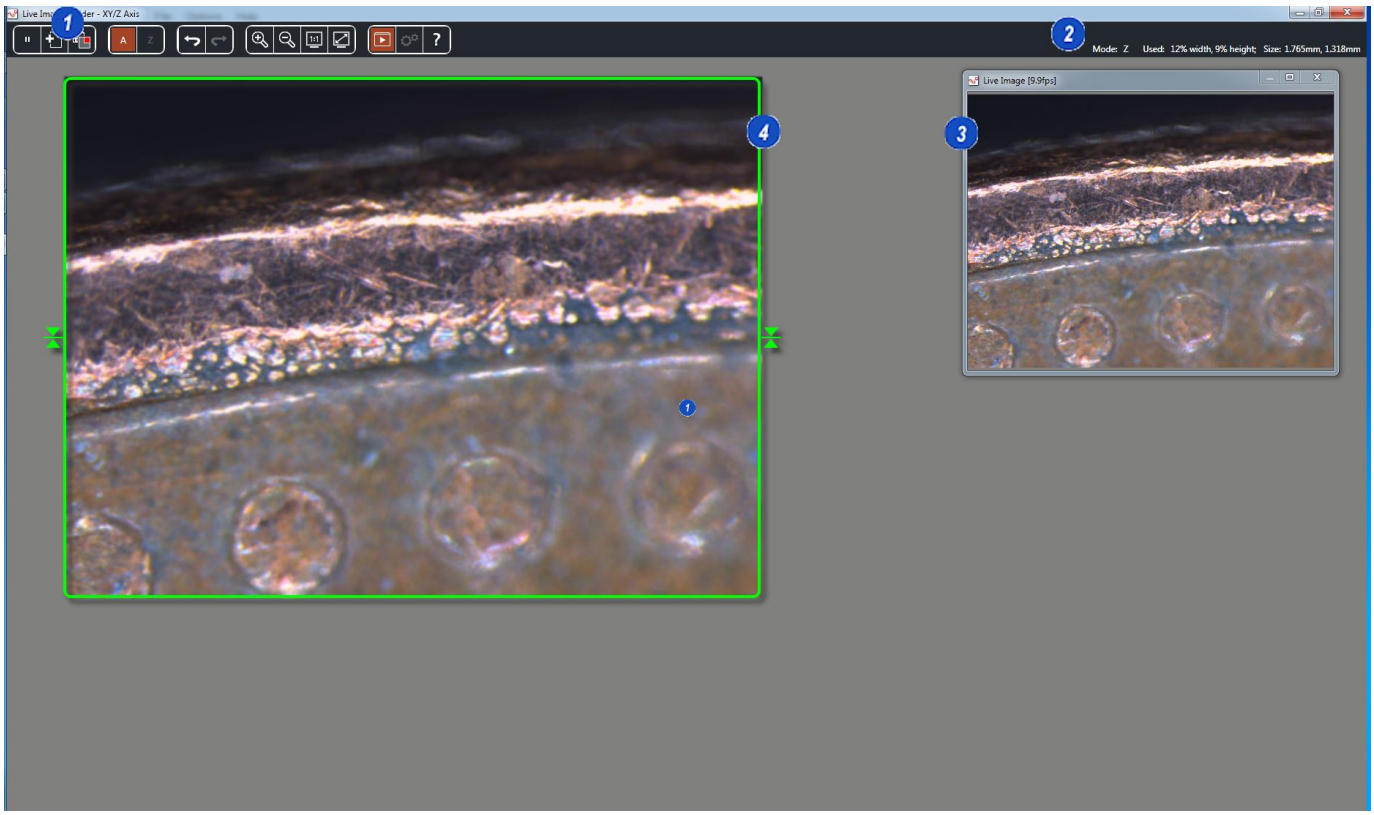
1: The *Tool Bar.*

2: The *Status Bar*: Showing the XY*Z Builder Image* size - not file size.

3: The *Live Image Window*: This is the camera live image.

4: The XY*Z Builder Image* so far - in Z mode.

Continued 784



Click on a button below for more information:

Start Building and...
Pause:
Starts the live build process: The button changes to *Pause* which when clicked halts the build. Clicking again will resume the build.

New Canvas:
Clears the existing XY*Z Builder Image* and starts building a new one.

Return to LAS:
Returns to the LAS interface with the user option to save the XY*Z Builder Image*.

Auto Switch:
The software will automatically switch to Z mode when movement stops. And back to XY mode when movement starts.

Manual Z or XY:
The user chooses the mode

Zoom in and...
Zoom Out.
Increases or decreases the display size of the XY*Z Builder Image*. If users have a wheel on the mouse this can also be used to zoom.
When the mouse left button is held down, movement of the mouse will pan the canvas.

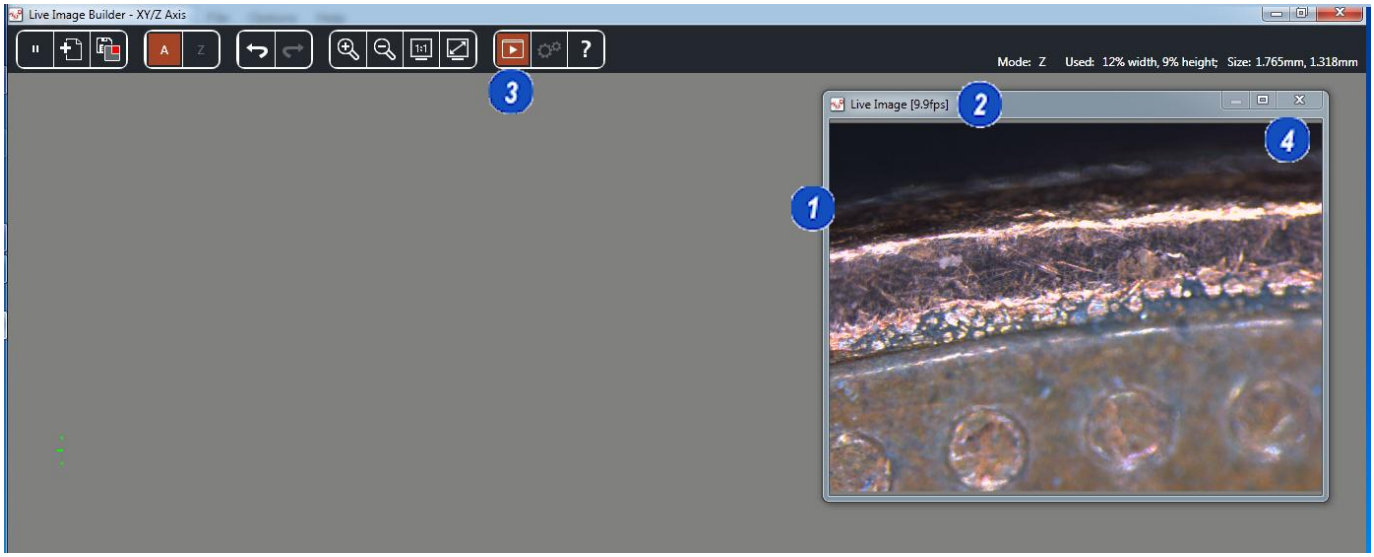
Display at Actual Size:
Displays the *Z Builder Image* at its actual size - the same size the specimen appears in the *Acquire Viewer.*

Fit image to Viewer:
Enlarges the *Z Builder Image* to fit the available viewing area.

Live Window Hide:
Click to hide or reveal the *Live Image Window* to see more of the *Z Builder Image*.

Preferences:
Displays the *User Preferences* dialog. See the description here 757 .

Help:
Displays this help file in a new window.



The *Live XYZ Builder* interface opens with:

1: ...the camera image displayed on the right in the *Live Image Window.*

2: The camera frame rate in frames per second (fps) is shown on the header. User should aim for a frame rate of over *9* frames per second.

The *Live Image Window* allows users to initially focus on the first image and either the top or bottom of the focus range and see the changes in position and focus as the build proceeds.

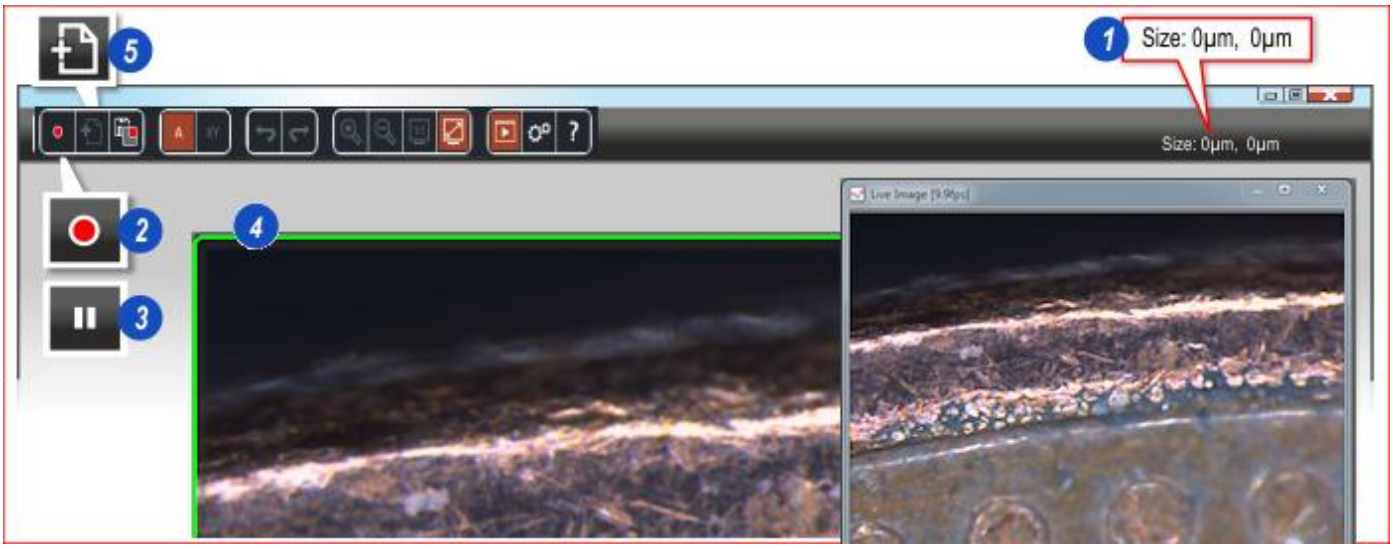
3: Hide the *Live Image Widow* to allow more of the XY*Z Builder Image* to be seen by clicking the *Hide/Reveal Live Image* button. Click again to reveal it.

4: The *Live Image Window* can be closed or minimised with the usual Windows controls. Use the *Hide/ Reveal* button to open it again.

Click and drag on a corner to change the window size.

Decide upon the XY start position. Do not start in the middle of the sample. Find a corner of the sample and plan to work in a ZigZag pattern across and down the sample.

Focus on the starting point in the *Live Image Window*.



1: The *Status Bar* shows the user the image size (in µm).

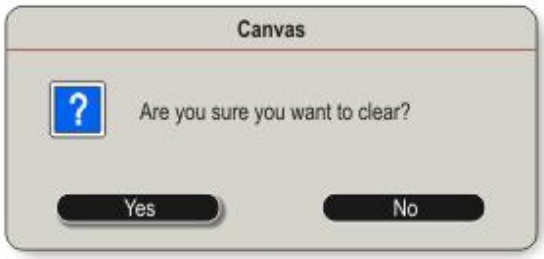
2: Start building by clicking the *Start Building* button.

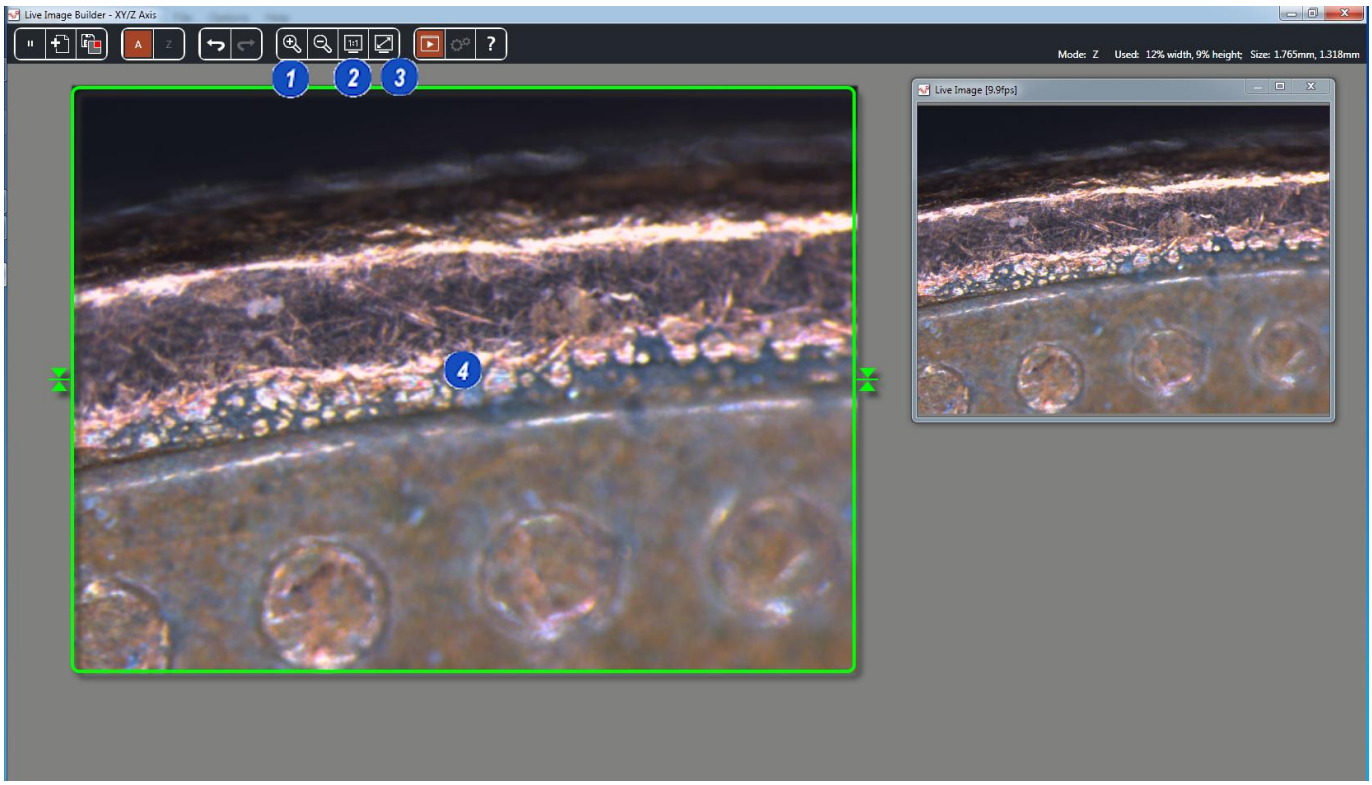
3: The button icon changes to *Pause*. Click to suspend the build and click again to resume.

Live XYZ Builder creates the XY *Builder Image* in computer memory on a 'drawing area' called conceptually *The Canvas.*

4: The first image - a copy of the live image - starts the XY*Z Builder Image* on the *Canvas* and the *Status Bar* is updated.

5: Clicking the *New Canvas* button will clear all of the existing work. Confirm clearing *The Canvas* and the existing XY*Z Builder Image* will be cleared. It cannot be retrieved.





1: Resize the XY*Z Builder Image* as required using the *Zoom* tools. It will be displayed at the selected zoom assuming that neither the *Fit to Window* or *Scale 1:1* tools are used.

Zoom is centred on the current mouse position.

2: *Scale 1:1:* The XYZ Builder Image is displayed at the same magnification and size as the Camera viewer.

3: *Fit to Window:* When clicked will fill the screen with the *Z Builder Image*.

4: The first image becomes the first part of the XY*Z Builder Image* and is positioned centrally in the widow but it can be dragged to a user-preferred position.

Live XYZ Builder samples the live image continually at the camera frame rate and not just when the user changes move the sample.

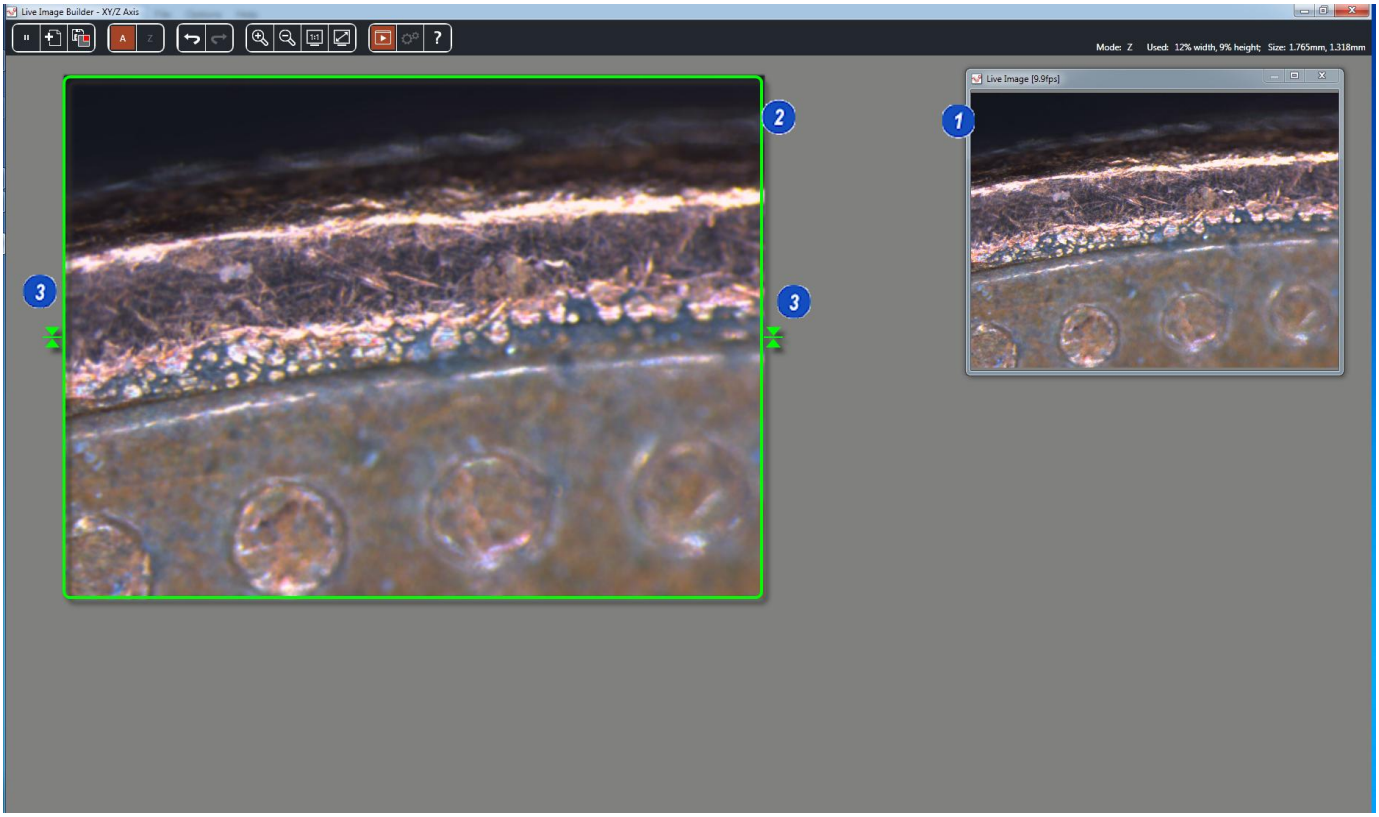
Start moving the sample in XY. If the software detects that movement has stopped, then the image is added to the canvas.

Z mode is selected and as soon as focus changes, pixels becoming sharper replace those in the XY*Z Builder Image* that are less sharp.

Once the focus is satisfactory, it is suggested that you move back to the start focus plane as this will allow the previous base level of focus position to be extended.

Move in XY again and continue to switch between XY and Z until the entire image is built.

Users should move smoothly and evenly between the focus extremes. Stopping part way is acceptable but reversing may cause artefacts to appear. You will need to judge what works on your samples. See the focus change in the live image window and watch sharpness maintained across the entire *Z Builder Image.*



While the Live XYZ image is being created, you can observe several coloured frames that appear drawn over the canvas. These are to assist in the creation of the image by indicating the current status of the matching process.

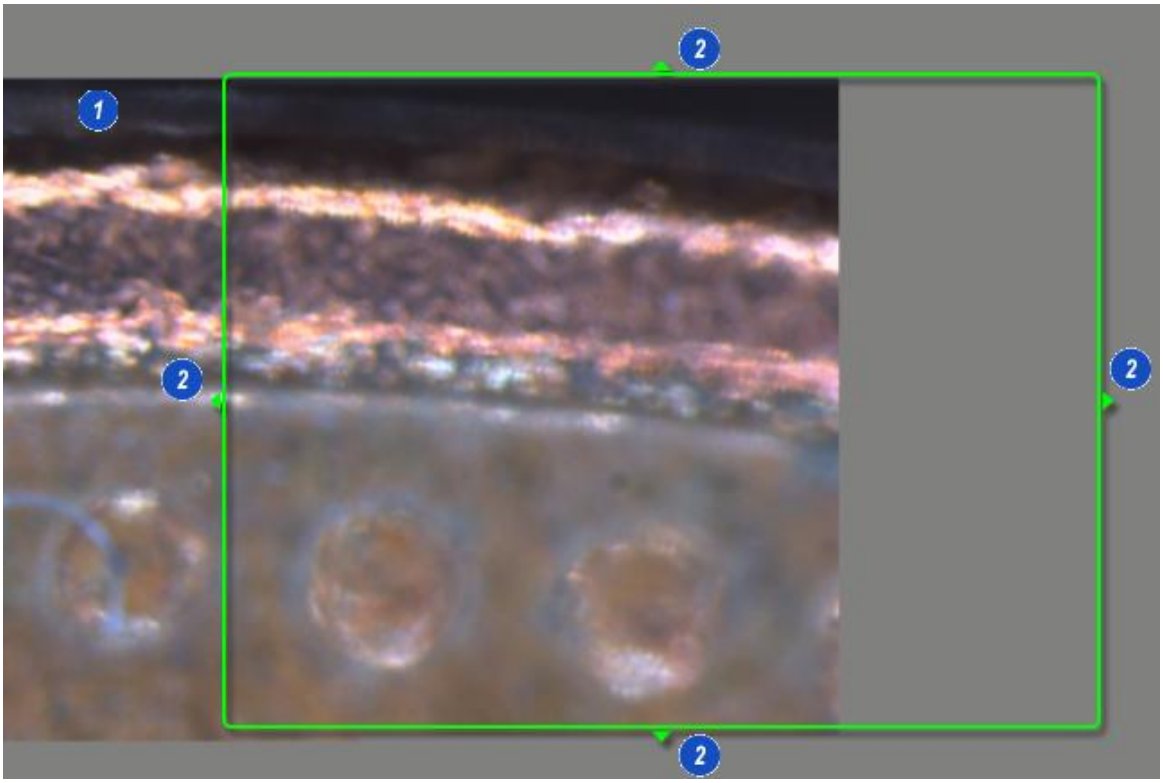
Immediately after starting building, the screen will typically appear as shown.

1: Live image window

2: The XYZ builder window showing the first image has been placed on the canvas.

3: A green frame is show. Green indicates that the software estimates that the live image from the camera is matching with the canvas. The arrow icons on the side of the green frame tell you that the image building is currently in Z mode.

Any changes you make to the focus, will now result in sharper regions being added to the canvas.



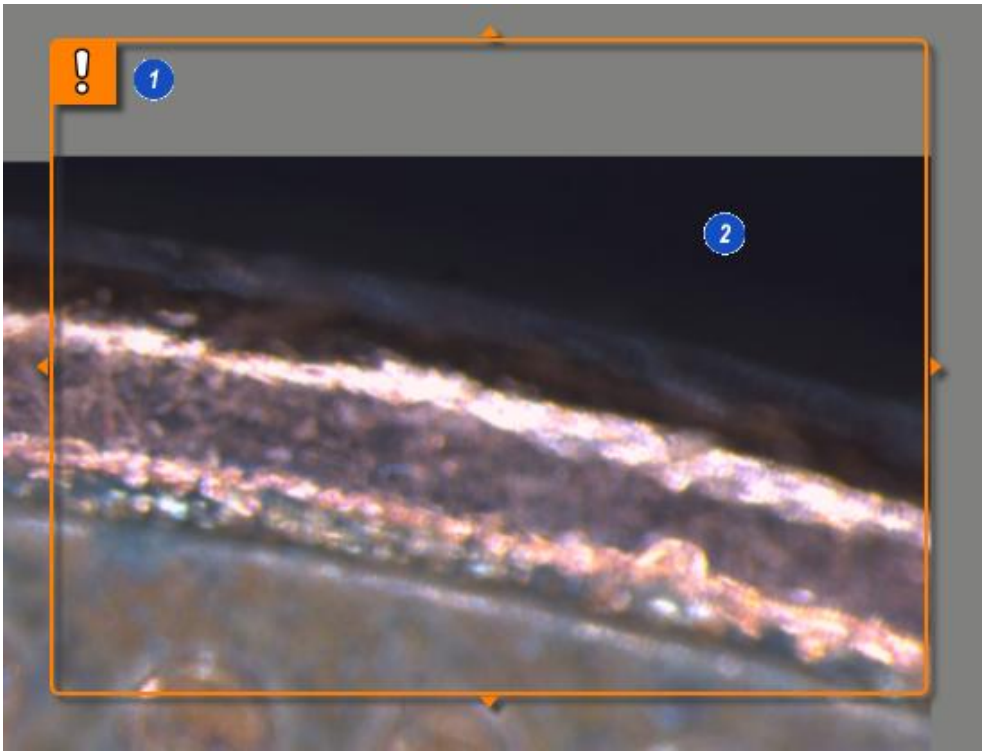
While the sample is being moved, the XY frame is displayed. As this is green, it appears that the software is matching the live image to the canvas. Continue to move the sample steadily while the green frame is shown.

1: Image in the canvas.

2: The XY frame showing that the live image is matched with the canvas. The arrows show that this is the XY frame.

Movement of the sample is being detected, so the frame will move a corresponding distance.

When you have moved about halfway along the sample, it is time to pause and allow the live image to be added to the canvas.



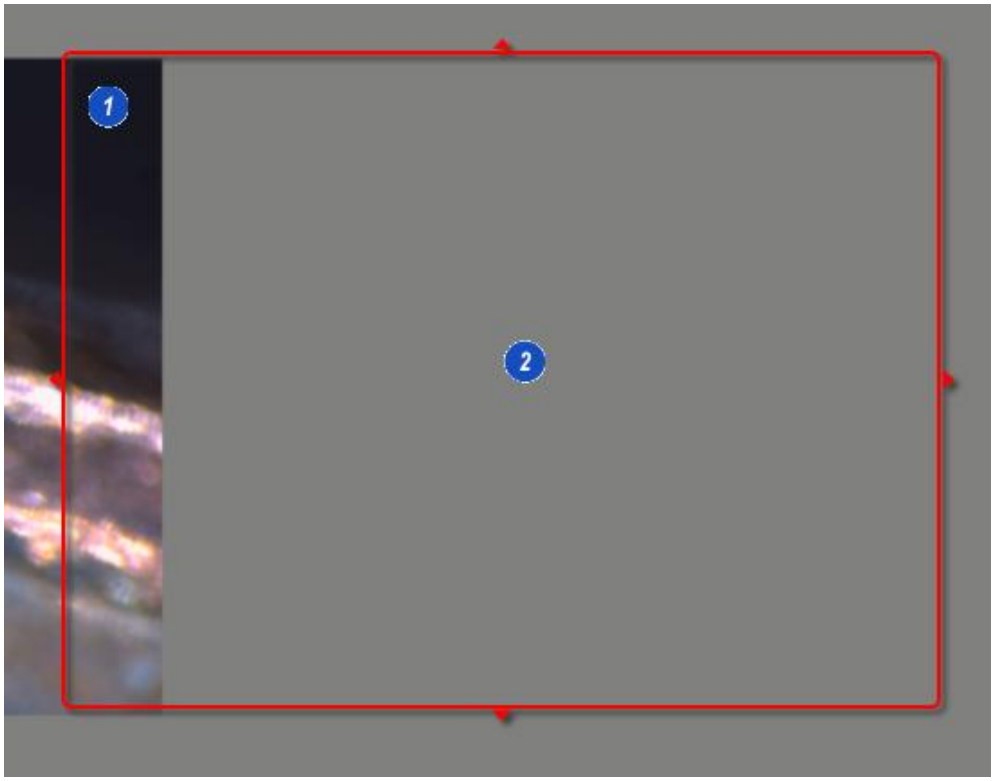
While the sample is being moved, the XY frame is displayed. If it changes from green to orange, this is saying that the software is loosing confidence in the matching. You should stop moving the sample and move back a little until the green frame is shown again.

Some reasons for the matching confidence being low are:

No enough detail in the image
The detail has repetitive structures
Out of focus
Moving too fast
Sample not secure
Camera frame rate too slow

1: Warning frame showing that you have moved to a region where the matching is poor.

2: This region is featureless. So although the sample has not moved far, the region available for good matching is rather small. hence the warning is given before matching is lost completely. At this point is it usually easy to recover matching.



If the sample is moved too far before you pause to allow the canvas to be built, the live image to canvas matching may be lost completely. If this happens you must try to move the sample back to find the region where you last had a good match. The software will attempt to regain the match and if the frame turns green you can continue, moving the sample at a slightly slower speed.

If you see that images are placed on the canvas that appear to be in the wrong position. Use the Undo button to remove them. When you are satisfied that you have corrected the canvas, click the Red start button to continue building.

1: Red error frame showing that you have moved to a region where the matching is lost.

2: This position of the red frame will jump around because there is no matching detail.

With focussing is complete, the XY*Z Builder Image* can be saved to the selected capture folder.

1: Click on the *Return to LAS* button.

2: The *Save Image* dialog appears.

3: Choose to *Save* the image or scrap it *(Don't Save),* or *Cancel* and continue building.

Power Mosaic and Power Mosaic Plus

LAS Power Mosaic software integrates high-performance specimen scanning into the Leica Application Suite to provide an easy-to-use application for creating, viewing, and saving ultra-high resolution mosaic images. LAS Power Mosaic is using in conjunction with a Leica microscope, Leica DFC digital camera and a stepping stage.

With LAS Power Mosaic you can scan a selected area or an entire slide quickly and accurately, and then effortlessly relocate to areas of interest with a simple click. Additionally, with the LAS Power Mosaic Plus software, it is possible to acquire images at multiple focus positions and view the complete mosaic while scrolling through focus.

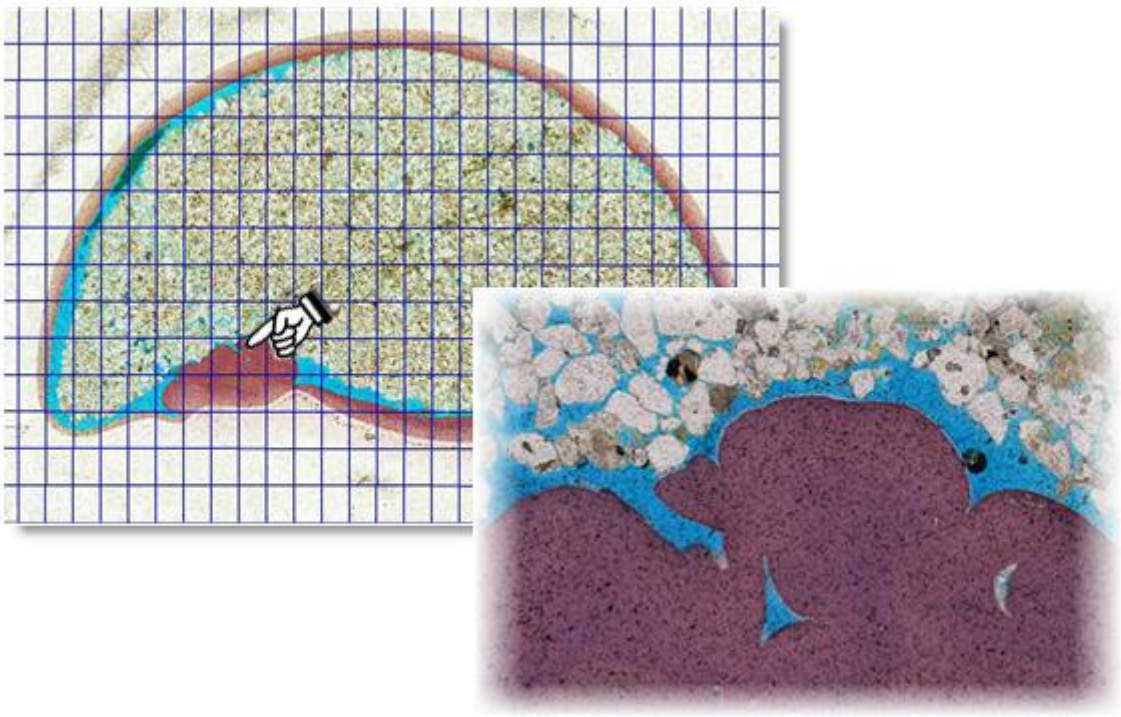
The ability to generate high-resolution mosaic images provides a powerful and novel method of visualising a specimen. The specimen overview is an aid to understanding the relationships between microscopic features and overall structure.

Once you have created a scanned mosaic, you can save your specimen's details as a workspace, or export the full-resolution mosaic for use with image publishing and analysis packages.

The software is designed to offer a comprehensive range of facilities with a strong emphasis on versatility and ease of use. With minimal experience it is possible to produce excellent mosaic images. In particular, the accurate calibration of the camera and motorised stage is essential and is made convenient by being performed automatically.

Good results depend, however, on the system being well configured and set-up and on the user becoming reasonably familiar with the available facilities and functionality.

The principal purpose of this manual is to provide practical guidance on configuration, set-up and use of LAS Power Mosaic.



Navigate specimens from LAS

- Power Mosaic integrates seamlessly within LAS.
- Power Mosaic images can be acquired and reviewed.
- Images from LAS Power Mosaic can be saved and measured in LAS.
- Specimen movement is performed by a stepper stage.
- A Leica DFC camera acquires images.

Power Mosaic Acquisition

- Uses triggered image capture for very fast continuous scan and acquire on suitable cameras.
- Standard scan using step and acquire for low light applications.
- Image streaming for mosaic sizes limited only by free disk space.
- Additional scans can be added to the initial scan to include all parts of a specimen.

Scan Patterns

- Rectangle, Circular, Annular, Cross (+ and x), Random.
- Overlap of tiles allows smooth merging of joins.
- Correction for camera rotation is performed
- Save / load scan patterns

Export/Import

- Save and load mosaic scan pattern and Power Mosaic environment as a ‘workspace’.
- Select workspace for recall from LAS.
- Load last used workspace on start up.
- Images exported to tif, bmp or jpg.
- Export mosaic as full resolution bitmap.
- Export mosaic as reduced resolution bitmap.
- Export user-selected region of interest.
- Export image to current LAS capture folder.

Specimen Map

- Switch between display of stage overview and pattern view.
- Drag to create pattern scan or enter exact details.
- Point and click relocation of stage to indicated position.
- Real-time graphic display of current stage position on specimen map.
- Zoom and pan over entire mosaic.

Calibration

- Automatically measures calibration and updates scan patterns according to selected magnification on microscope using a correlation-based calibration procedure.
- Camera rotation and sage skew is measured to allow convenient adjustment.
- Small rotation of camera is compensated for in the mosaic creation.
- Automatic stage orthogonality correction.

Microscope Automation

- Oasis XY stage and Z focus control drive board is used.
- Software joystick for stage and focus movement.
- Compatible with Leica Microsystems LAS configured microscopes controlling focus, turret, condenser, and lamp control as available
- Stage and focus speed defined per objective linked to LAS.

Automatic Focus

- Multi-point predictive focus for continuous focus tracking.
- Predictive focus setup by combined auto scan with user review.
- Autofocus can be used for specimens that are not flat.
- Predictive and Autofocus can be combined.

LAS Power Mosaic Plus

This is an extended version of Power Mosaic and includes all the features of Power Mosaic and in addition:

- Z-Scan optional at each field.
- Definition of Z-Scan positions, step number and step width.
- Extended focus imager can be created.
- Mosaic image can be reviewed while sweeping through all focus positions.
- Navigator for multiple scan patterns

The following procedure lists the steps required to obtain high-quality mosaic images. Once the system is correctly aligned and adjusted, some of the steps will not be necessary again.
It is assumed that the system is already installed according to the installation procedure detailed in the release notes.

1: Prepare the specimen:
Check the following:

- The specimen is as flat as possible to minimise the need for focus changes.
- The slide surface is clean.
- The specimen is firmly fixed to the stage - loose slides are a common problem.

2: Setup the microscope:

- To gain experience with Power Mosaic, start with an x5 objective as this magnification probably does not require focus compensation during the scan. This makes it easier to check that the scanning is working correctly.
- Ensure the condenser is in focus and apertures are set for Koehler illumination. With an automatic microscope this should be checked for all of the objectives expected to be used.

3: Initialise the Stage and Focussing:

- This checks the stage travel limits, finishing at the centre point. The small green 'target' in the stage area indicates current position. The 'hatched' border around the periphery indicates the 'soft' limit switch area.
- Lower the condenser and check that stage will not collide with objectives.
- Check/adjust the stage speed: *See: Stage Initialisation.* 812
- Initialise/adjust the focus limits: *See Focus Initialisation.* 813
- *See Autofocus Setup:* 814

4: Check the camera and adjust the exposure:
On a clear field of view, debris in the optical path will be seen as fixed dark regions on the live image when the specimen is moved. If cleaning is necessary, a qualified technician should perform it.

- Check that there is no visible debris in the optical path.
- For a colour camera Saturation = 1.75: Gamma = 0.6: Gain = 1: Make fine tuning adjustments around these values to achieve the required image.
- For a mono camera: Saturation = 1.75: Gamma = 1.0: Gain = 1.
- Ensure that the black and white levels on the histogram are reset.

See: Input Options: 809

Turboscan and Standard scan require different adjustments:

Adjusting the camera for Turboscan:
Turboscan, the fastest scanning method, can be used if:

- The camera has progressive scan mode.
- There is sufficient light to give an exposure of less than 200 µs and
- The specimen is flat enough to use no focus change or predictive focus.
- Select an exposure mode with Progressive scan.
- Set Exposure to approximately 100 µs.
- On the Histogram display, click to enable *'Show Under/Over Exposure'.*
- Adjust the lamp voltage until red flecks appear on the image white highlights. *Note:* On a DM microscope, fine lamp voltage control is achieved by pressing both stand lamp buttons together. Expect a high lamp voltage and very bright image.
- If there is insufficient light, increase the camera gain by small amounts to give the correct exposure. Check that this does not increase significantly image noise.

WARNING: Switch 100% light to the camera. DO NOT look at the specimen through the eyepieces.

Adjusting the camera for Standard scan
Standard scan is used when the conditions for Turboscan are not met.

- Select the camera image format to suit the specimen detail required. Choose the lowest resolution without compromising image quality to save disk space.
- Set the lamp voltage to give a comfortable image in the eyepiece.
- Adjust lamp voltage and camera exposure to until red flecks appear on a white region of the image.

5: Set shading
Shading correction has to be set in Power Mosaic. LAS settings are not used.

- Shading correction must be repeated every time the objective is changed.
- Move the specimen so a clear region without artefacts is visible over the whole image.
- Set the shading correction: *See: Shading:* 818

6: Calibration for each objective
LAS Power Mosaic derives its calibration from the stage movement to accurately align the tile edges. Calibration must be carried out for every objective on first use.
A warning message appears if an objective has not been calibrated.
Calibration tests that the values returned are reasonable: If they are not a warning is displayed. Camera rotation must be less than 0.1°. Conditions can alter over time and systems are susceptible to dirt, heat and vibration. Periodically check the calibration to ensure that it remains accurate.

- Check microscopes with a manual turret, that the selected objective matches that selected on the Acquire:Mic1 tab. The same applies to the Mag Changer if it is fitted.
- Set calibration: *See: Calibration:* 816
- If necessary adjust the camera rotation: *See: Camera rotation:* 817

7: Create and perform a test scan

- Select the Split Screen view. The 'crosshair' shows the current stage position.
- Move the stage so that the specimen can be seen in the live image window.
- Select the New Pattern tool, click close to the stage 'crosshair' and drag a small scan region.
- Click Acquire Power Mosaic.
- Use Zoom and Pan to check that the mosaic is formed correctly. *See: Create Pattern Grid:* 835

8: Extend the scan pattern to the required region
To include parts of the specimen not included in the test scan:

- Click on the Create/Expand tool.
- Click on the stage in an area outside the boundary of the test scan and drag to include the required parts of the specimen. *See: Create Pattern Grid:* 835

9: Select the Focus Method:

During scanning, two focussing methods are available:

- *Predictive Focus* is best suited to uniform specimens and low magnification - up to x10. Focussing is performed either manually or automatically on a number of points across the specimen to create a table of values. Points which have not been pre-focussed use the table to predict a focus position without going through the time-consuming process of an Autofocus on every field.
- *Autofocus* should be used for irregular specimens at any magnification. Focussing is carried out at regular intervals across the specimen with options to set the repetitiveness.
- Both *Predictive* and *Autofocus* may be used in combination to benefit from the speed of Predictive and the precision of Autofocus.

Using Predictive focus:

- Select the focus points manually or use the automatic Grid.
- Select and focus each point manually or choose *'Autofocus on all points'*
- Run Predictive focus: *See: Predictive Focus:* 830

Using Autofocus:

- Set the repeat pattern for fields to be focussed.
- Enable/disable field skipping on focus failure. *See: Focus Methods:* 829

Power Mosaic: Loading the module:

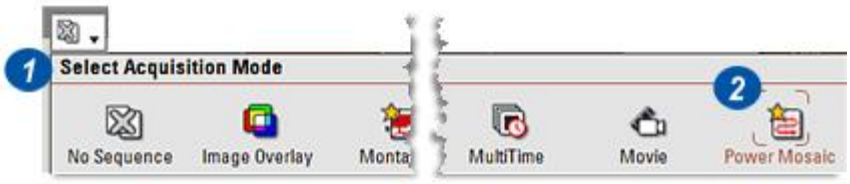
1: Click on the *Select Acquisition* button to reveal the available modules. If a module is already loaded and running the button icon will differ from the illustration.

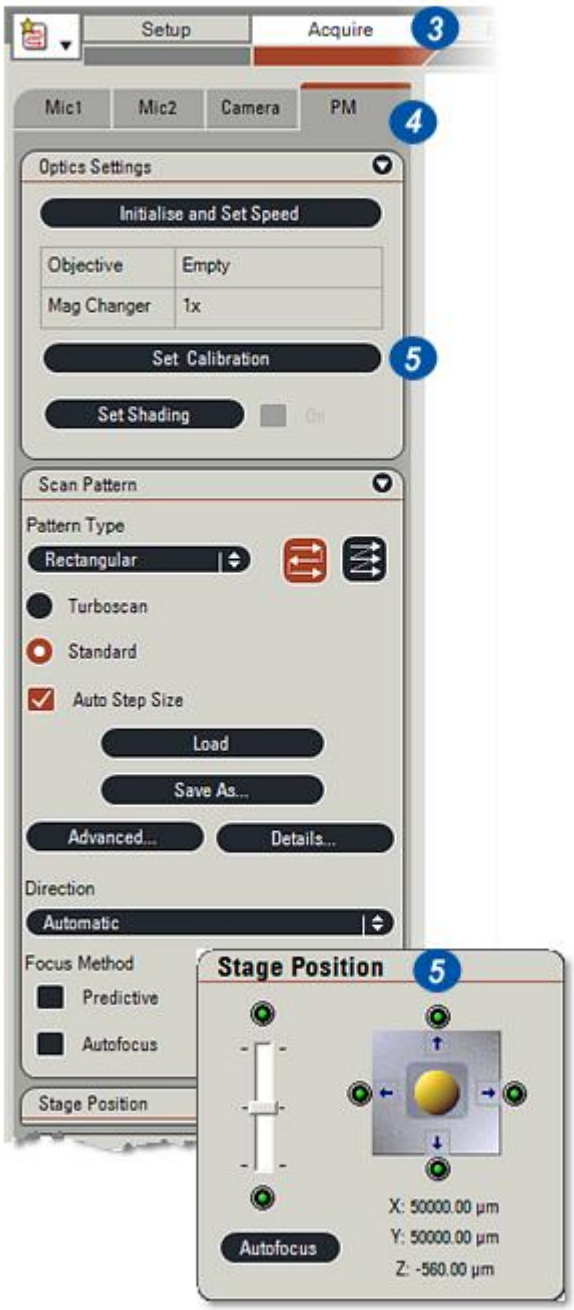
2: The *Power Mosaic* icon will be present only if it is installed. However, it will also need to be enabled:
See: Installing, Configuration and Licensing: Registration Information.
Click on the icon.

3: Click on the *Acquire Workflow* tab.

4: When Power Mosaic is loaded and running the PM tab will be present with the main panels displayed.

5: An additional panel – the On-screen Joystick – is available by clicking the *Show Joystick Tools* button.
Click and hold on the *Joystick* header to drag and dock it on any part of the screen.





The User Interface is shown in the illustration with the Acquire Workflow selected and the Power Mosaic tab active**.**

1: *Workflow* tabs.

2: *Control Panels* and function tabs.

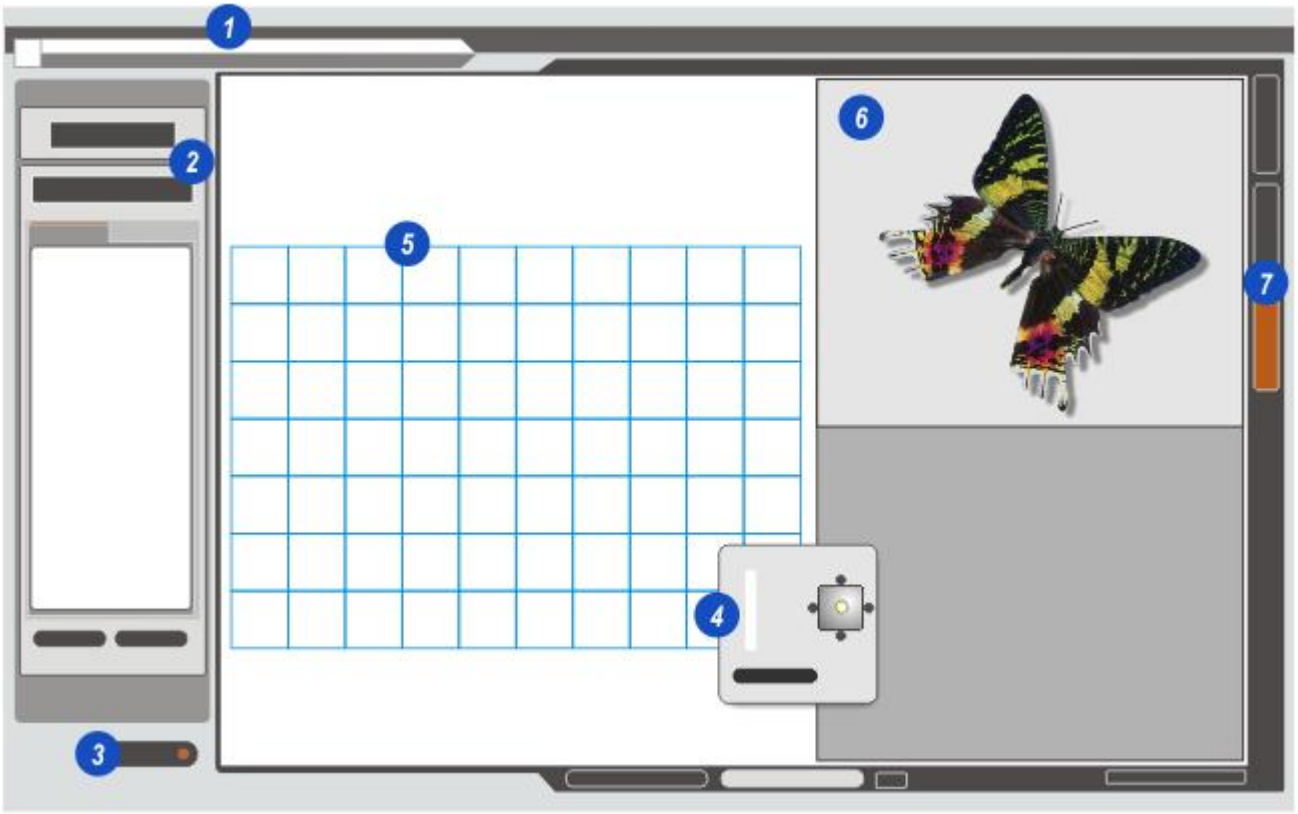
3: *Acquire* scan button.

4: *Scan* viewing area with on-screen Joystick shown.

5: *Scan Grid Pattern.*

6: *Live Image Field:* Split screen view is selected.

7: *Tool Bar.* The tools are explained on the following page.



The Toolbar is located on the right-hand side of the screen. The tools descriptions are:

1: *Live Screen:* The entire screen is devoted to the live image.

2: *Split Screen*: Divided between viewer part of live screen.

3: *Grid Pattern View:* Entire screen is devoted to the scan grid.

4: *No Tool* selected.

5: *Stage Area:* displayed on Viewer.

6: *Display Grid Pattern.*

7: *Hide/Display Grid Pattern.*

8: *Zoom In/Zoom Out.*

9: *Pan.*

10: *Create a New Scan Grid.*

11: *Extend/Contract* existing scan grid.

12: *Go to Stage point.*

13: *Move Scan Pattern.*

14: *Clear scanned Tiles.*

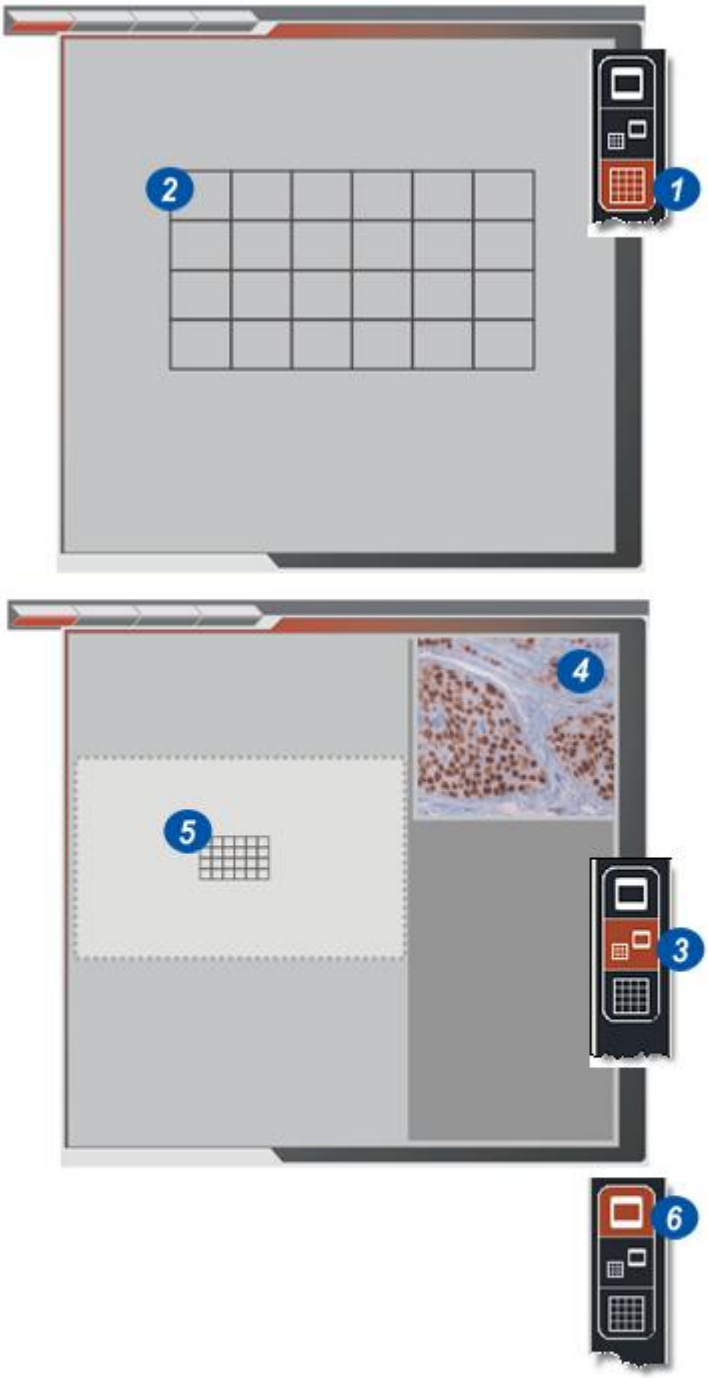
There are three viewing options available, each selected by clicking the appropriate button.

1: *View Stage Map:* fills the viewing area with…

2: …the scan pattern grid and scanned tiles scaled to fit.

3: *Split View:* displays the pattern grid, and scanned tiles on the left **(5)** and a live image on the right **(4)**.

6: *Live Image:* fills the viewer with a live image.



With the On-Screen Joystick visible, it can be customised to suit individual preferences.

1: Right click on the *Joystick* to reveal the *Speed* and *Properties* menu.

2: Click to select the speed at which the stage will be driven during when the joystick is moved.
Three options are available: *Normal, Fast or Slow.*
Actual speed will depend upon the stage type so 'select and test' to find the most appropriate.

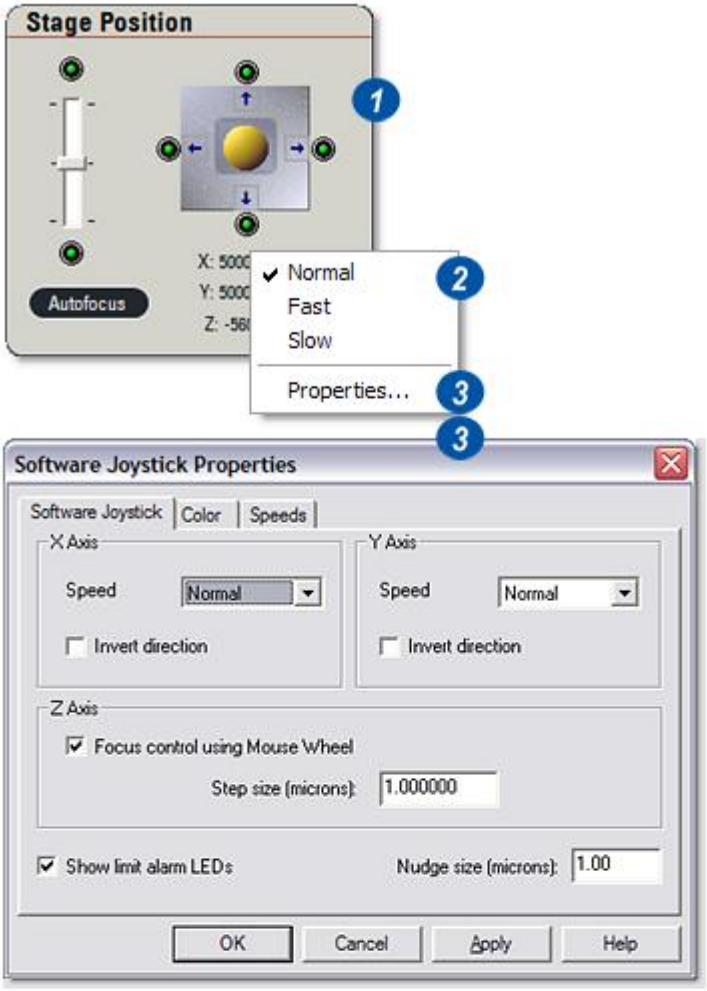
3: Click on *Properties* to reveal the Properties Dialog. Three tabs provide:

Speed: Similar to **(2)** above but on this tab X and Y speeds may be selected individually and the travel direction can be inverted.

Z-Axis: Allows the Mouse Wheel (if fitted) to act as a focussing control and sets the focus step size. To change it, click on the Step size window, press the Delete key to clear the existing value and type a new value in µms.

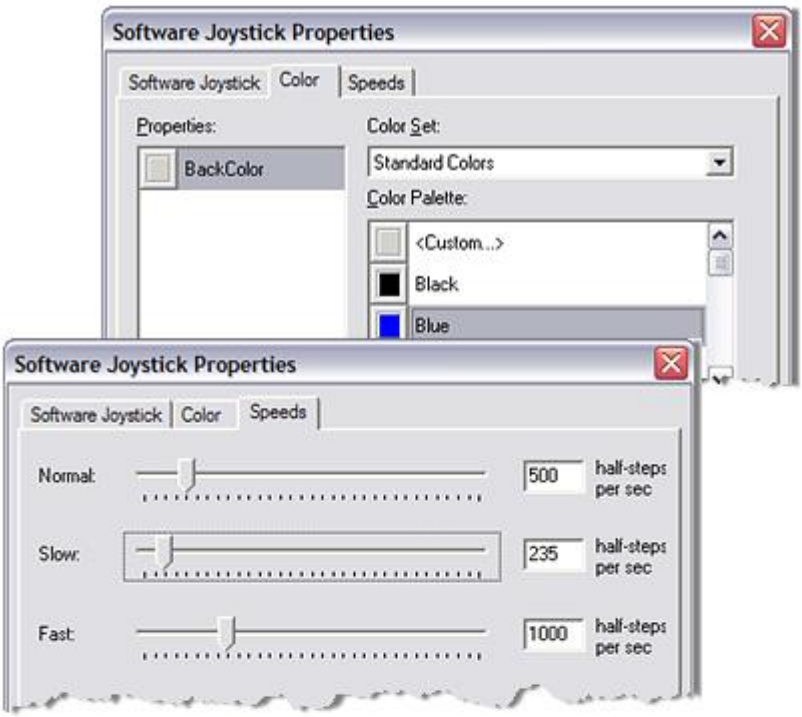
Limit Alarm: There is a small indicator at each of the joystick quadrants and also top and bottom of the focus slider. Normally they are green when the stage and the focus are within limits. If the Show Limit Alarm LEDs box is checked, the indicators will turn red as the travel limits are approached.

Nudge size: Each joystick quadrant also has a small arrow displayed. Clicking an arrow 'nudges' the stage in that direction. It is a very useful facility for precise positioning. Set the nudge value in µms by clicking the window, deleting the existing value and typing a new one.



Color: The area around the joystick defaults to pale grey (silver) but may be changed by clicking to select a required color. Various color sets are available.

Speeds (Calibration): The three speed options may be calibrated individually by clicking and dragging the appropriate slider. Again, actual speeds will vary with the stage type: Change and test to achieve the best settings



If Power Mosaic Turboscan is going to be used, the exposure times must be very short – typically less than 200µs (microseconds). This requires high light levels making some contrast methods unsuitable. If the required exposure times cannot be achieved, choose Standard scan instead for which light levels and exposure times are not critical.

1: Click on the appropriate Mic(roscope) tab and…

2: …select the required Objective, Magnification and…

3: …Contrast method.

4: Set the Aperture and...

5: …Intensity to suit specimen and scan type.

Often, a good image can be achieved quickly by setting basic exposure parameters, using Auto Exposure and then fine-tuning the result with white balance. This is especially suitable for Standard scan - Turboscan will probably require closer attention to exposure:

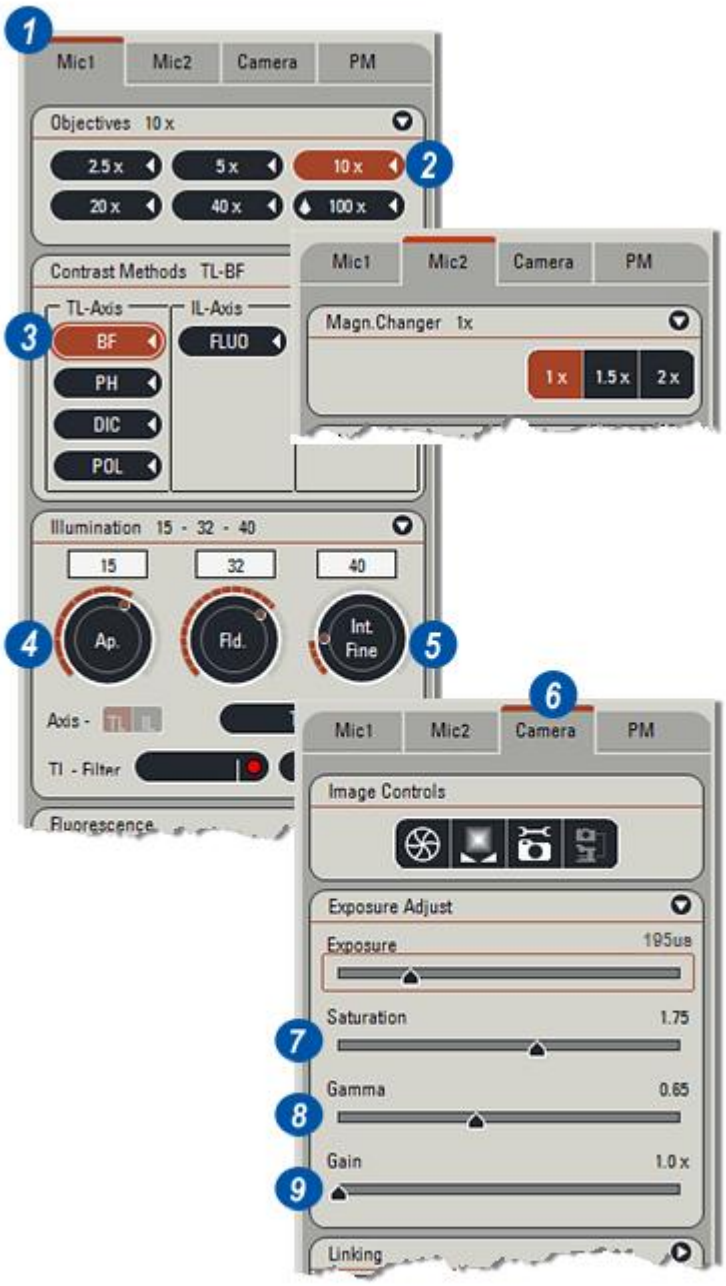
6: Click on the Camera tab to reveal the exposure controls.

7: Set the Saturation to 1.75 by clicking on the slider and dragging it – to the left to reduce the value and to the right to increase it.

8: Set the Gamma to 0.6 (1.0 for greyscale).

9: Gain should be as low as possible – start with a value of 1.

See: Acquire: Camera for more detailed information.



The image must be properly focussed.

On the Histogram:

1: Set the *Black level* to 0 and…

2: …the *White level* to 255 by clicking and dragging the sliders.

3: Enable *Under/Over Exposure* by clicking the check box.

Run Auto Exposure:

4: Click on the *Auto Exposure* icon and then click again to turn Auto Exposure off.

Manual Exposure setting for Turboscan

5: Adjust the Exposure slider to give a reading of about 100µs. Gradually increase the light intensity (see previous page) until intermittent red flecking indicating slight over- exposure, appears.

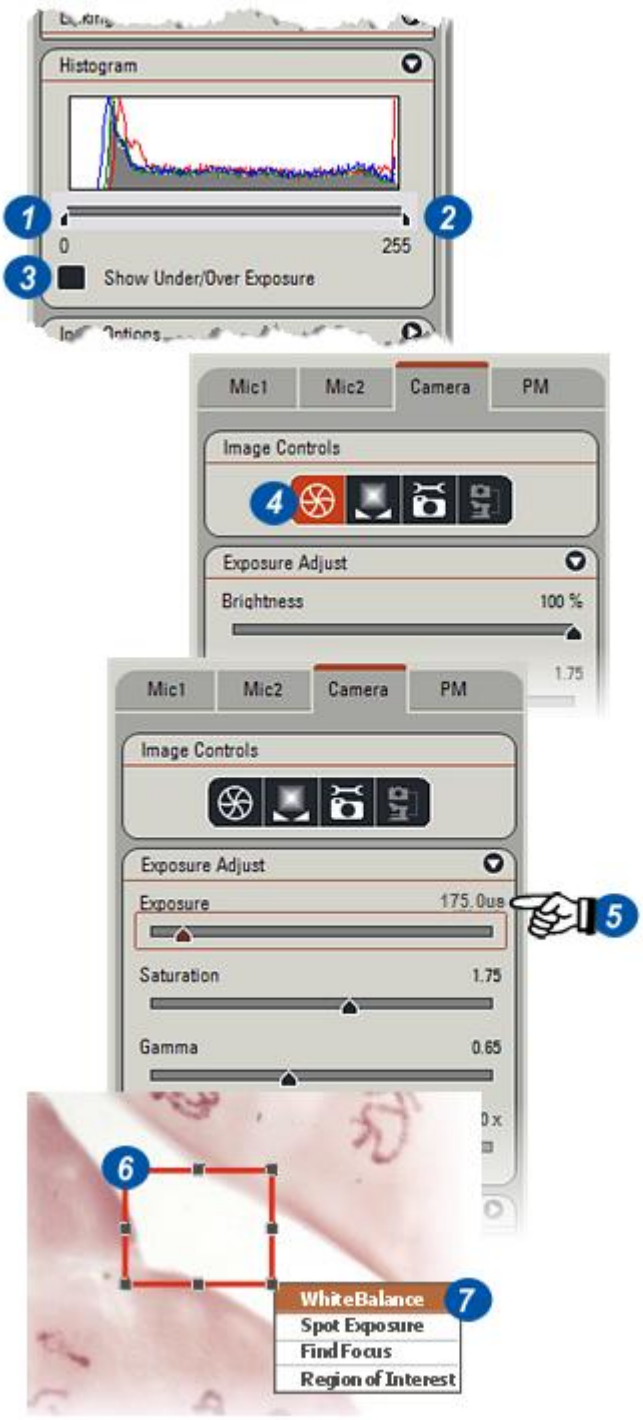
Set the white balance:

6: Click and drag a Region of Interest around a white area.

7: Select White Balance from the menu.

Fine tuning:

If necessary, fine tune the image with the Saturation and Gain controls.



Selecting the Input Options:

1: Click on the *Camera* tab.

2: If the *Input Options* panel is concealed click on the arrow to the right of the header to reveal it.

The panel provides options for:

3: Using a pre-saved Input Options Configuration or creating a new one.

4: Selecting the *Image Type* – colour or greyscale.

5: Setting the *Colour Depth*.

6: Determining the *Captured Format* – how the image will be saved and stored on disc and…

7: Selecting the *Live Format* – how the images will be displayed on the Viewer.

Selecting the Camera:
If Turboscan is going to be used, only designated high speed, progressive scan cameras with a trigger facility can be selected.

Choosing a Configuration:
If an Input Options configuration has been previously saved, it can be recalled from the Configuration menu.

8: Click on the arrows to the right of the *Configuration* header.

9: From the drop down menu, click to select the saved configuration. Each will have a unique name. All of the saved settings will be loaded and the remainder of the Input Options may be skipped.
A newly created configuration can also be saved using the *Configuration* menu and selecting the '*Save Current*' option.

See: Acquire:Camera:Input Options for detailed procedures.

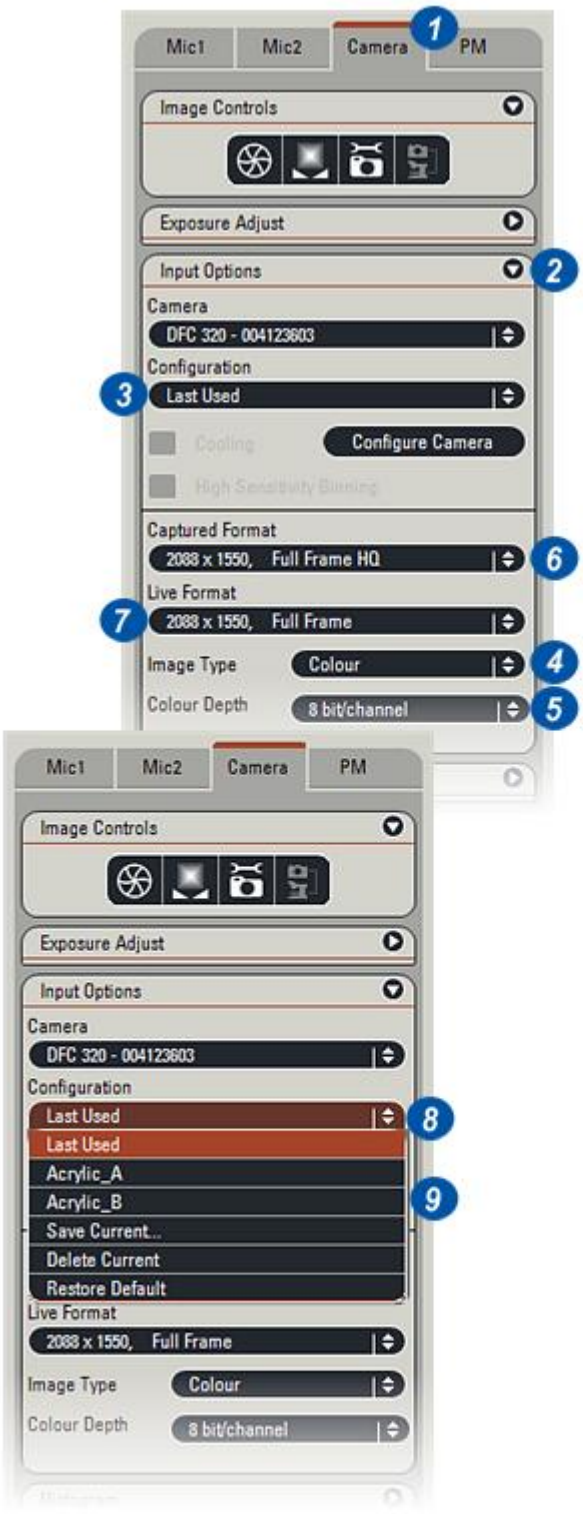
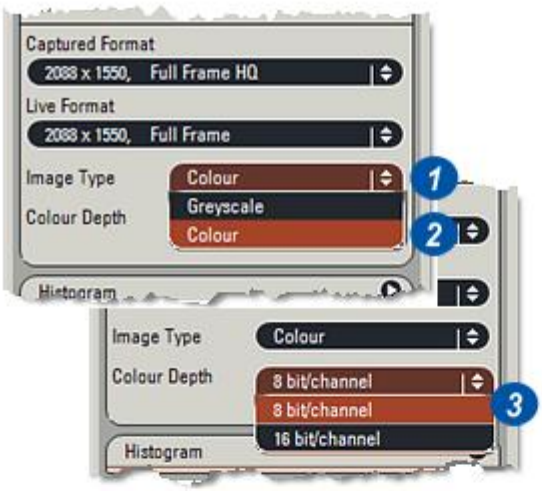


Image Type:
Colour cameras may be used in either colour or monochrome (Greyscale) mode.

1: Click on the arrows to the right of the *Image Type* header and from the drop down menu…

2: …select either *Colour* or *Greyscale*. If Turboscan is being used with a Progressive Capture Format, the Image Type will automatically revert to Greyscale.



Captured Colour Depth:

3: Click on the arrows to the right of the *Captured Colour Depth* header and select 8 bit. Avoid the 16 bit option if it is available. It will greatly increase stored image size, slow the scan and may not be compatible with other image processing software.

Captured Format:
Power Mosaic has been designed to capture high quality images so avoid using the low resolution options. For Turboscan, select one of the Progressive options (but not VGA):

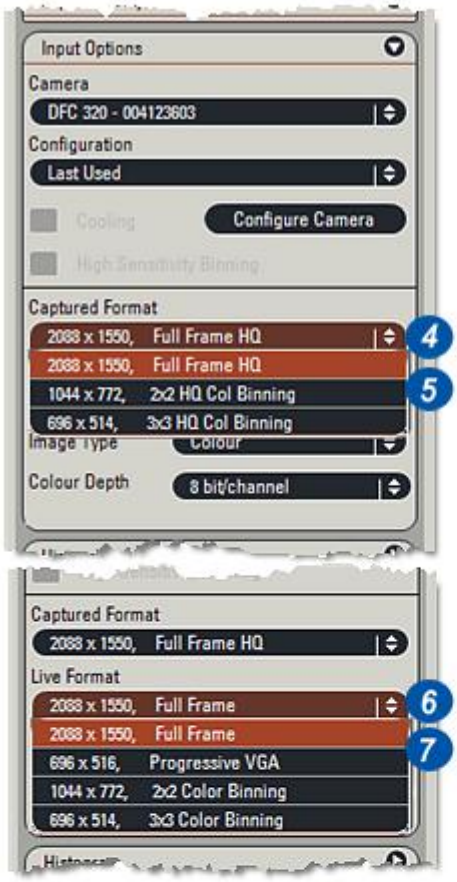
4: Click on the arrows to the right of the *Captured Format* header and from the drop down menu…

5: …choose the highest possible resolution. The 'binning' options will save disk space but are unlikely to improve scan speed. Use the navigation arrows to reveal other options.

Live Format:
Live format is not used during Power Mosaic, but for the sake of completeness keep the Live Format the same as the Captured Format.

6: Click on the arrows to the right of the *Live Format* header and from the drop down menu…

7: Select the same format as the *Captured Format*.



See: Acquire: Camera for more detailed information: 290

The Stage must be initialised the first time an objective is selected and again if the stage stalls for any reason.

Initialising determines the limits of travel in both the X and Y directions and 'matches' the stage speed to the objective.

Warning: Ensure that there are no obstructions before initialising – turn to an empty turret position and lower the sub-stage condenser.

To compensate for different stage types, ages, slackness and stiffness in the mechanisms as well as various imaging setups, the stage speed can be de-rated from its maximum. Lowering stage speed also helps prevent 'stalling' which results in a loss of initialisation values.

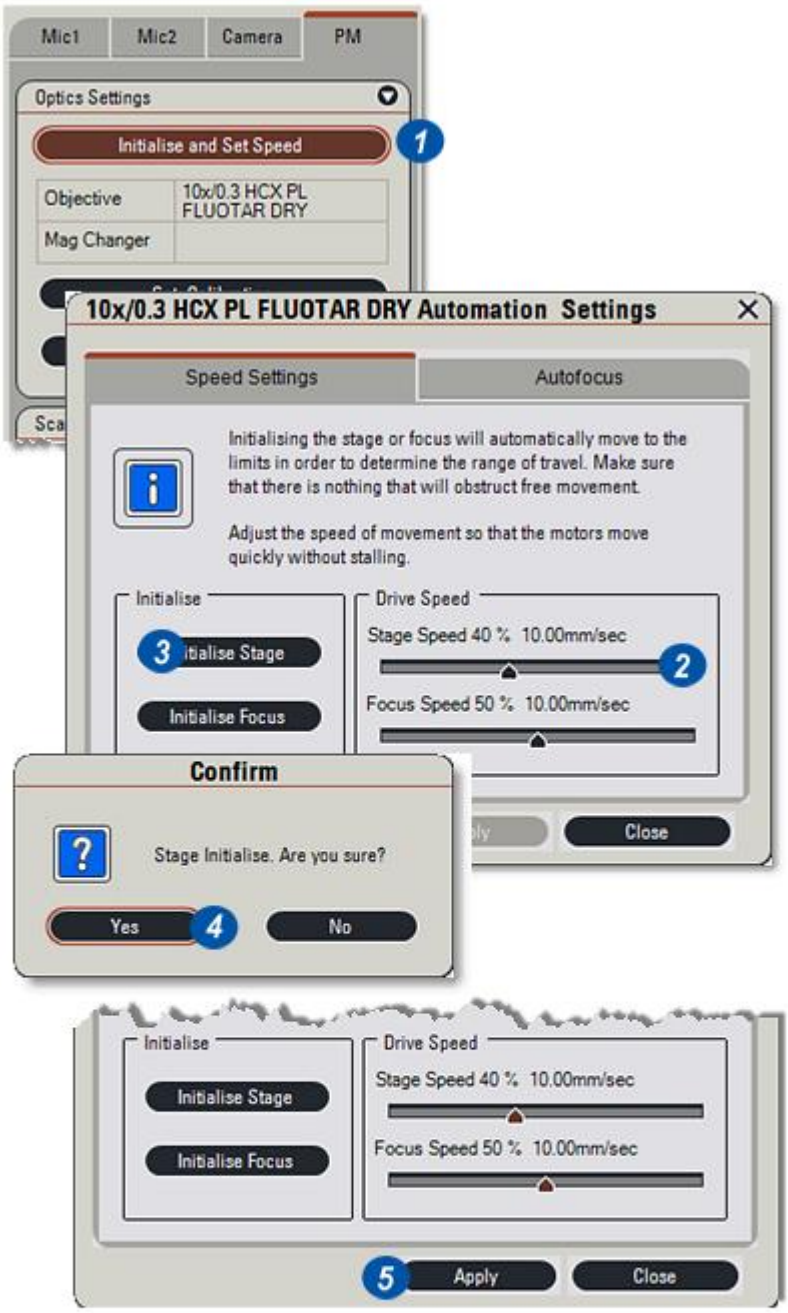
1: Click on the *Initialise and Set Speed* button.

2: On the *Automation Settings* dialog, set the Drive Speed slider to a high value - start at 90%.

3: Click the *Initialise Stage* button.

4: Click *Yes* on the *Confirm* message.
If the stage stalls, reduce the speed and re-initialise.

5: After initialisation completes successfully, reduce the speed by 10% and click on the *Apply* button.



Focus Initialisation

The speed of the focus drive mechanism can be adjusted below its maximum to allow for possible overrun and minimise shaking.

Additionally, the limits between which the focus mechanism can operate are set to prevent the specimen colliding with the objective (Upper) and allow sufficient clearance for the specimen to be accessed (Lower).

Select Live Image and focus.

1: Click on the *Initialise and Set Speed* button.

2: On the *Automation Settings* dialog, adjust the *Focus Speed* by moving the slider. Determining the actual value will depend upon the microscope and the imaging setup and may require some trials.

3: Click on the *Initialise Focus* button.

4: Set the *Upper Focus Limit* (relative to the current position) by clicking on the text box and entering a new value. For example, to limit the focus position to 100um above the current position, type in '100'.

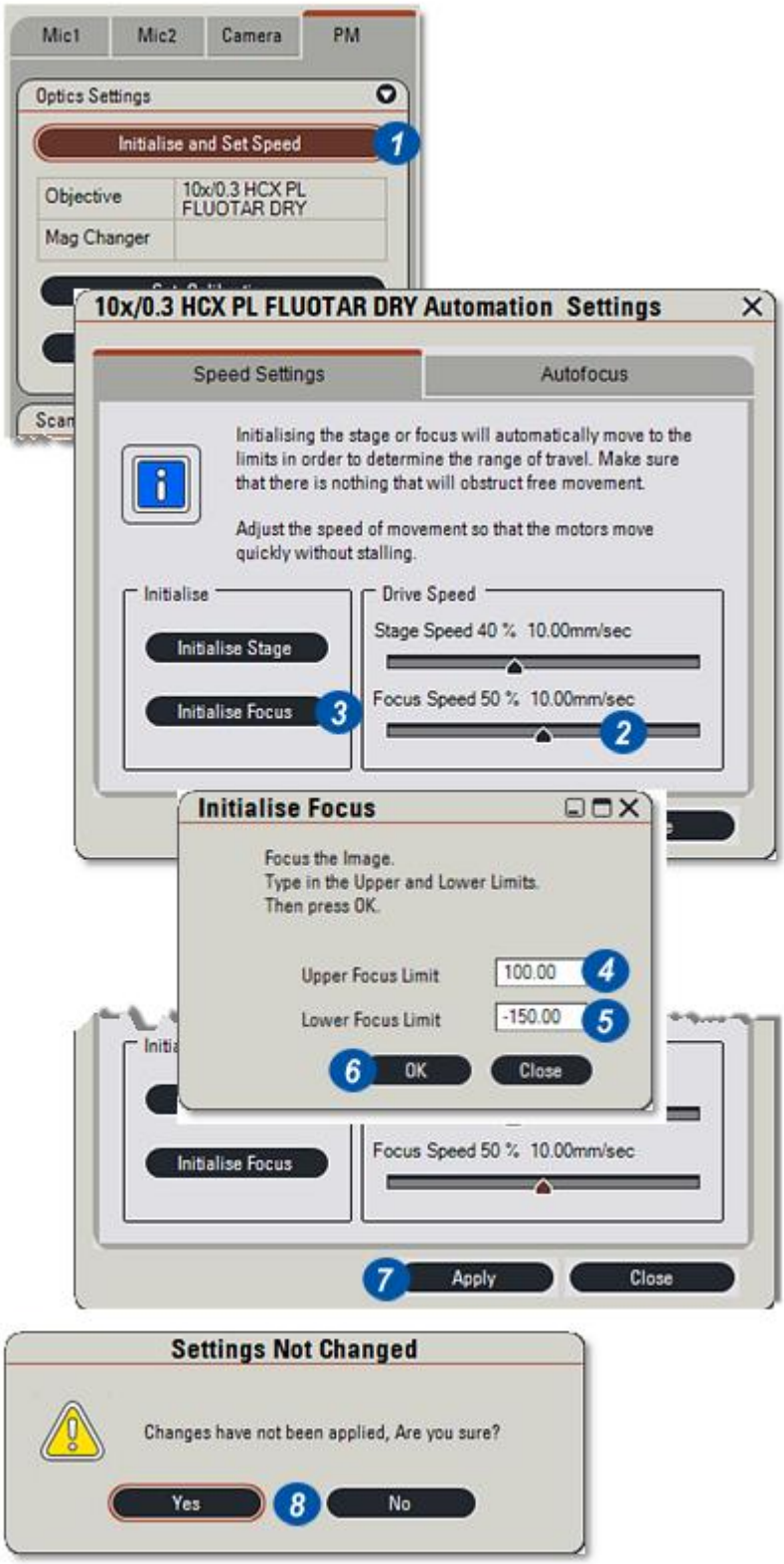
5: Set the *Lower Focus Limit* in the same way. Positive numbers will be automatically converted to a negative value so no need to type the leading sign.

6: Click *OK*.

7: Click *Apply* to apply the settings and Close to save them.

8: If Apply is not clicked, the *Settings Not Changed* message appears. Click *No* to apply the new settings

Go to Autofocus Setup: 814



Auto Focus initialisation sets the travel range of the focussing mechanism and also the threshold value for focussing ‘success’.

Select the split screen display option.

1: Click on the *Auto Focus* tab.

2: The current stage position on the Z Axis is shown by a vertical green line.

3: A blue horizontal line represents the *Focus Curve*.

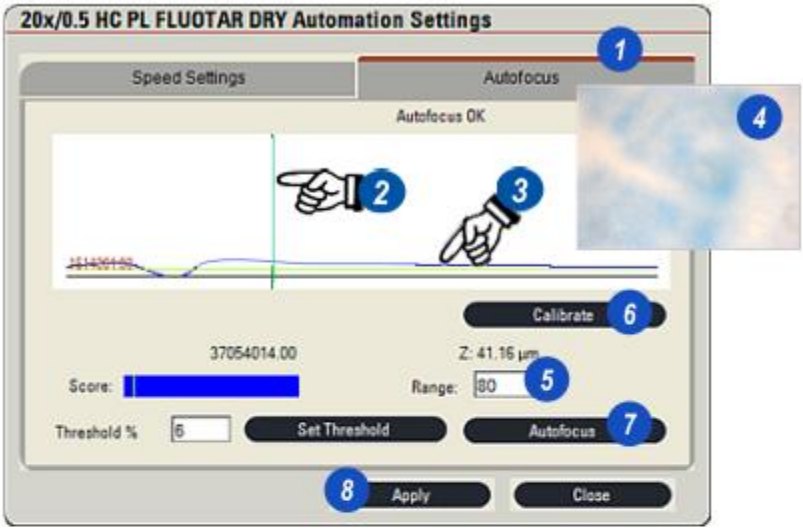
4: Using the on-screen Joystick, SmartMove or stage controls, navigate in the live image pane to a part of the specimen that contains some detail, avoiding repetitive or symmetrical patterns. It may be helpful in ‘proving’ the Auto Focus effectiveness to de-focus the specimen.

5: The *Range* value (in microns) determines the travel limits of the focussing mechanism. It reflects the thickness of the specimen. Click in the Range text box and enter a value – the mechanism will drive upward from the current stage Z position by 50% of the value, and downward by an identical distance. Between these limits, Auto Focus will achieve the best focus possible.

6: Find a field on the image that can be sharply focussed and click the *Calibrate* button. This will measure any drive backlash and store the value to be used with the current objective.

7: Click the *Apply* button. *ALWAYS click Apply when changing values.*

8: Click on the *Auto Focus* button.



1: If there is sufficient detail and contrast in the specimen area chosen, the curve will look similar to this centred about the new stage Z Axis position, and…

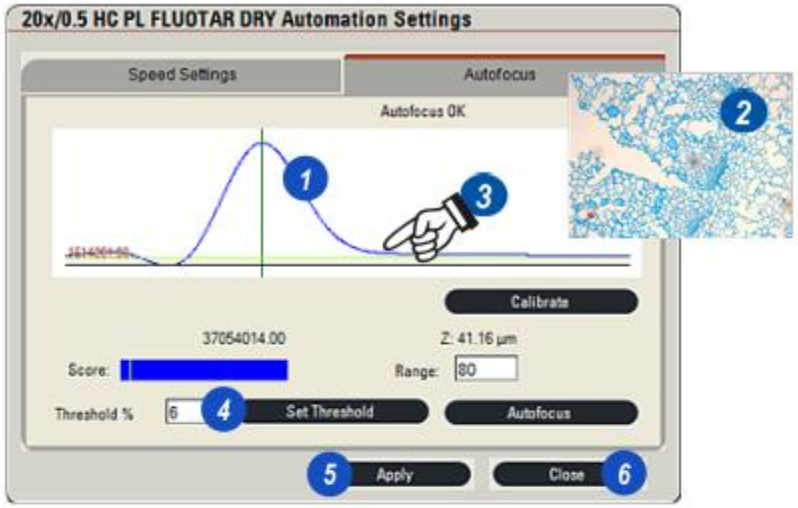
2: …the image should be sharp. The *Score* bar will extend across to the right.

3: The *Threshold* value represents a difference (%) of the lightest to darkest pixels in the image. For Auto Focus to 'succeed' the calculated value must be at or above the Threshold. Below the Threshold and there probably was not sufficient detail to guarantee accuracy.

4: Set the *Threshold* by clicking in the text box, typing a new value and clicking the *Set Threshold* button.

5: Click *Apply*. The Threshold line will be drawn to reflect the new value.
After changing Threshold repeat Auto Focus to ensure that the value is not so high it prevents focussing.

6: Click *Close.*



Note: In the unlikely event that the Z focus shows at the peak but the Score indicates something less than peak, gearing backlash could have caused the discrepancy. In this situation change the Z scan direction to 'Up' (bottom to top of specimen). Please refer to the Oasis manual.

Input options and lighting levels must be set and the specimen in place.

The Calibration process is automatic: It will determine the 'pixel/micron' value for the objective being used. As objective magnification increases so the actual field of view on the specimen decreases. Calibration correlates the field dimensions (microns) to the number of camera elements (pixels) required to capture it. It must be carried out at the start of a session, and whenever the optics are changed.

Calibration also checks for camera rotation – the angle of rotation from the Stage X and Y axes.

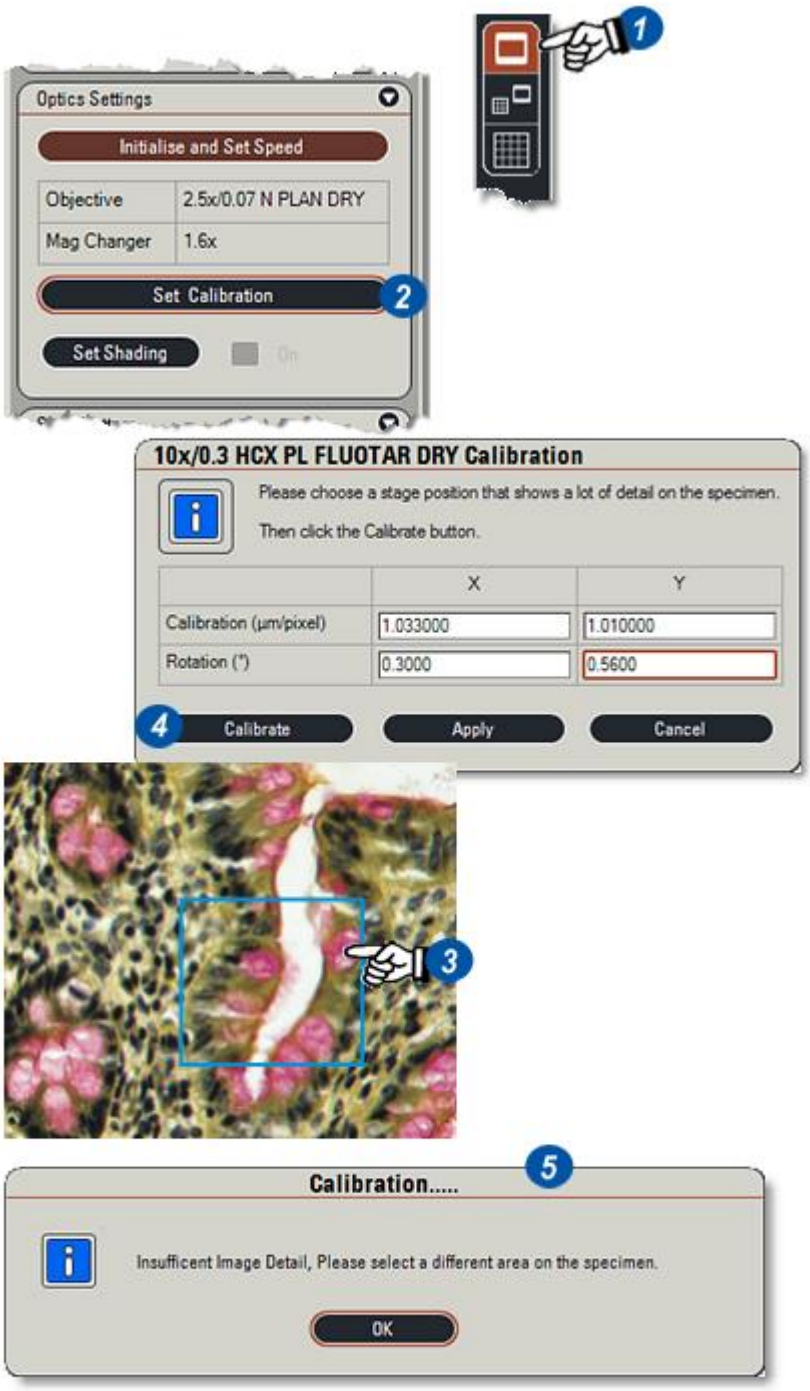
1: Select the *Live Screen* view.

2: Click on the *Set Calibration* button. When the Calibration Dialog appears…

3: …a small rectangle is displayed on the live image. Use the on-screen joystick or stage controls to navigate to a well defined and detailed region of the image lying within the rectangle. Avoid uniform patterns.

4: Click on the *Calibrate* button.

5: If there is insufficient detail in the selected region a warning will appear. Repeat the process from Step **(3)**.



The values for camera rotation returned by the Calibration, relate to the stage X and Y axes. If the rotation is excessive a warning will be displayed **(2).**

Rotation for both axes should not exceed 0.10 degrees **(1).**

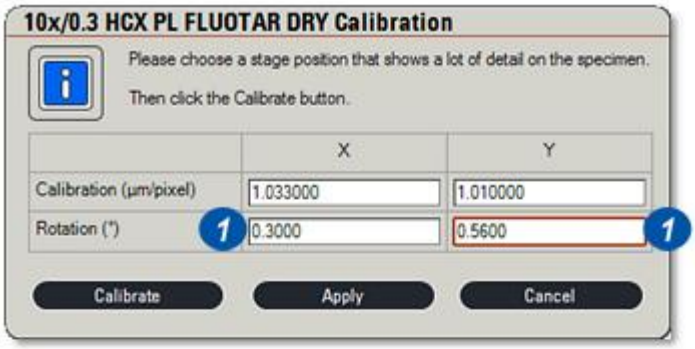
Check that the camera mount is secure.

Loosen slightly the camera clamp **(3)** so that the camera may be rotated.

Rotate it by very small amounts and repeat the calibration until the rotation angle is within limits.

Tighten the clamp.

Once set correctly and providing the camera and stage do not change, rotation should seldom require adjustment.



The Shading Reference electronically corrects any light level fall-off toward the edges of the image that is often inherent in optical systems.

The exposure, light intensity, required objective and mag level must all be properly set with the specimen in place. If there is a change in objective, the Shading Reference must be carried out again.

Select Live Screen view:

1: Navigate to a portion of the image that is free of detail with a clear background.

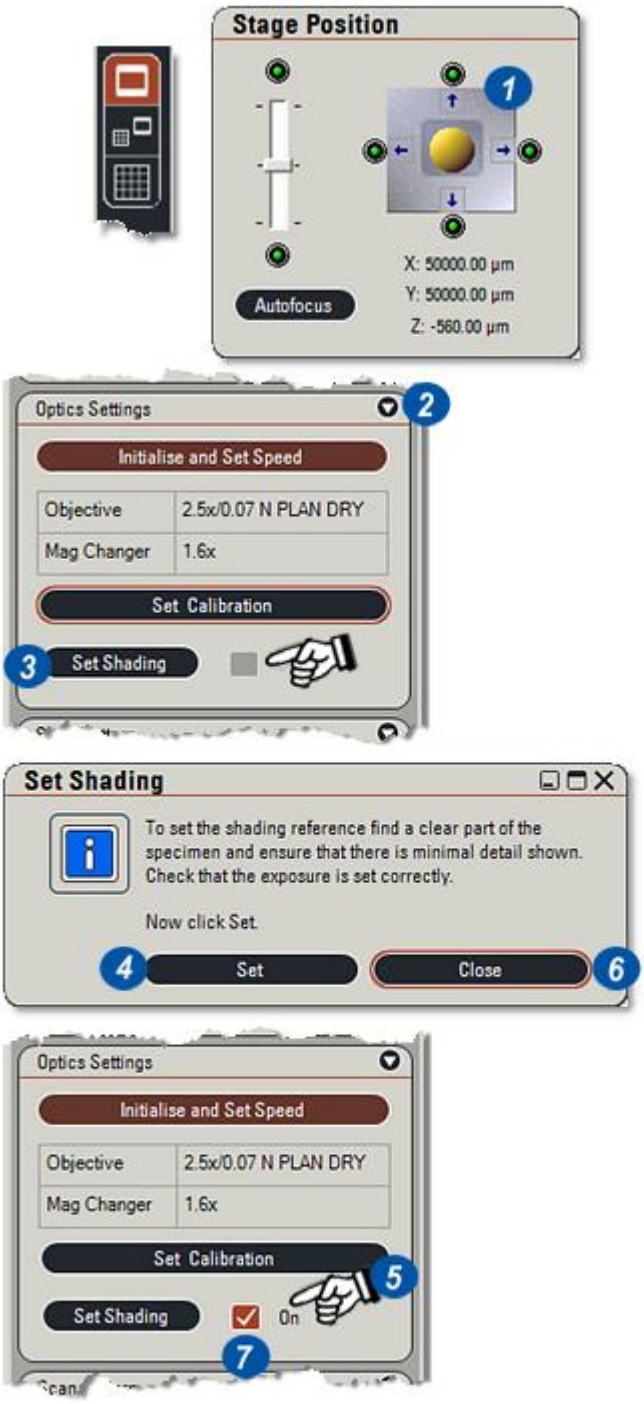
2: If necessary, click on the *Optics Setting* header to reveal the panel. If a Shading Reference has not been carried out, the check box will appear greyed out.

3: Click the *Set Shading* button and…

4: …on the dialog click the *Set* button.

5: When complete, the *Set Shading* box **(7)** will be checked and shading will be enabled.
If required, it can be disabled by clicking the check box.

6: Click the *Close* button on the dialog.



Load Configuration

A Scan Pattern configuration may be saved for future recall as a fast and accurate way of loading settings.

To retrieve and automatically load a saved configuration:

1: If necessary, click on the arrow to the right of the *Scan Pattern* panel to reveal it.

2: Click on the *Load* button.

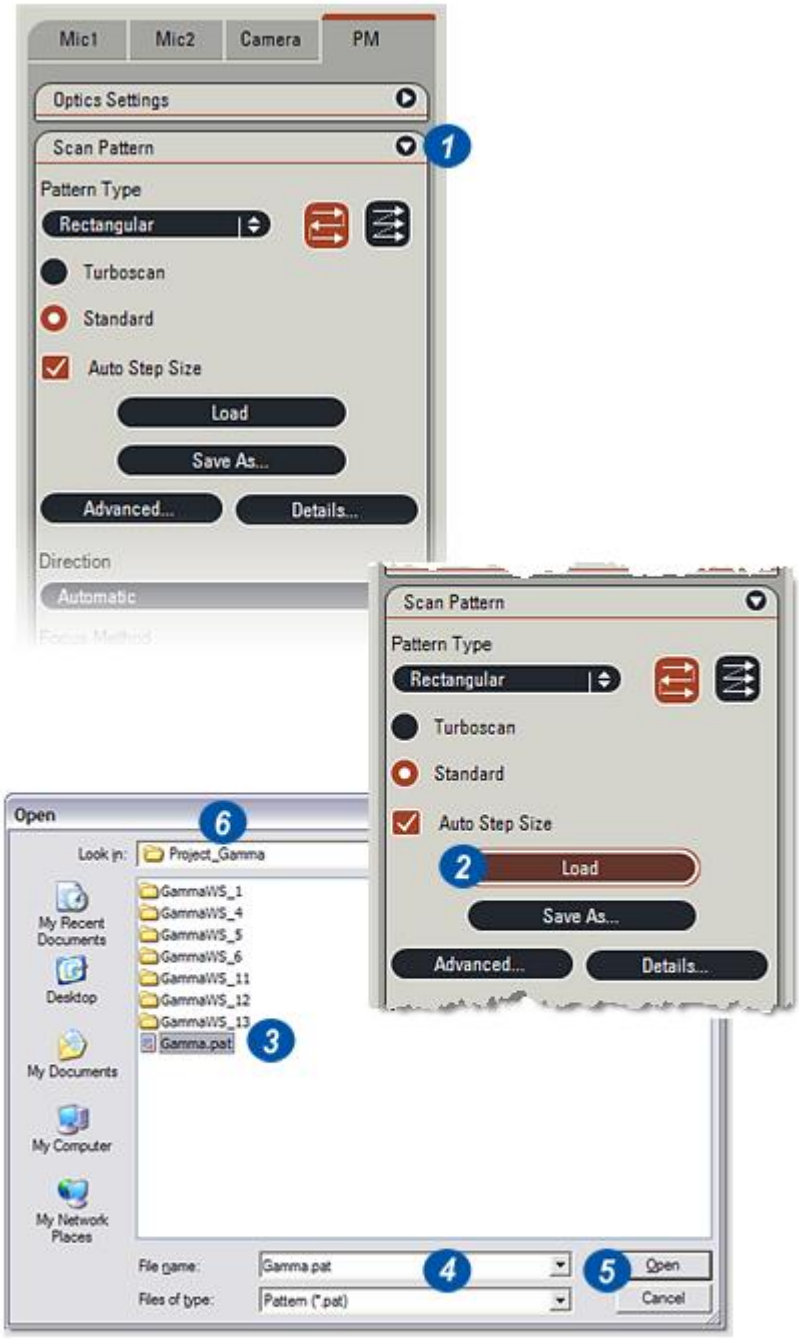
3: On the *Open* dialog, click to select the configuration required – the selected file name appears in the window **(4)**. Scan Pattern configuration files have the file extension *.pat*.

5: Click on the *Open* button.
Only the Scan Pattern settings are loaded; Optics and Z-Stack settings must be adjusted for each session.

6: The Folder defaults to the selection made either in *Preferences* or *Browse*. Use Windows navigation to open an alternative folder.

See: Preferences:Save in Directory:

See: Browse:Select Default Directory.



There are two scan pattern options which govern the stage tracking as the image tiles are collected – bi-directional and uni-directional.

The bi-directional pattern scans left to right, moves down to the next row, reverses and scans right to left.

The uni-directional pattern scans left to right, returns to the left, moves to the next row and scans from left to right again. (Depending upon the specimen shape the scan direction may be from top to bottom).

1: Choose bi-directional for speed.

2: Choose uni-directional for greater accuracy.

The selected check box is coloured red.

Select Scan Speed:
Turboscan is the fastest way to capture a image, but it does require a very fast exposure time - 200µs (micro seconds) or less and a progressive scan camera with trigger facility. During Turboscan, the stage moves continuously – it does not stop to make an exposure – which is why a short exposure is needed.

During Standard scan the stage halts at every tile position so the exposure time is immaterial. Standard scan can also operate with non-progressive cameras and does not require a trigger facility.

3: Choose *Turboscan* for speed but only if the exposure time is less than 200µs. Trying to scan with a higher exposure time will display the warning message **(4).**

5: Click to select *Standard* scan speed for exposure times greater than 200µs and non-progressive cameras.

The Step Size represents the distance between adjacent tiles in µm (micro-meters). Normally, Auto Step Size is enabled to allow mosaic creation, and the program calculates the number of tiles required to cover the image on the basis that they will all abut adjacent tiles **(2).** Turning off Auto Step Size allows the step to be adjusted so that tiles may be overlapped or spaced apart **(8).**

1: Enable *Auto Step Size*. The check box will be coloured red with a tick. Auto Step Size must be enabled for a mosaic to be created.

2: The tiles are butting and arranged to cover the image. In these examples the Cross Scan Pattern has been used for clarity.

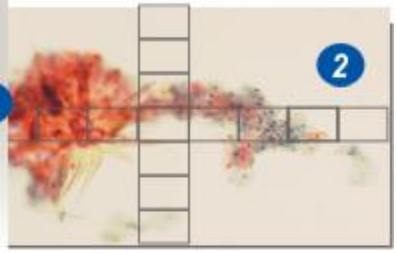
3: Click to disable *Auto Step Size*. The check box becomes grey.

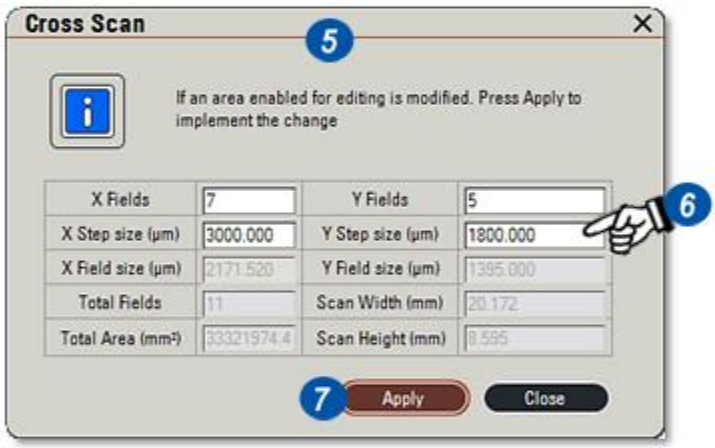
4: Click the *Details* button.

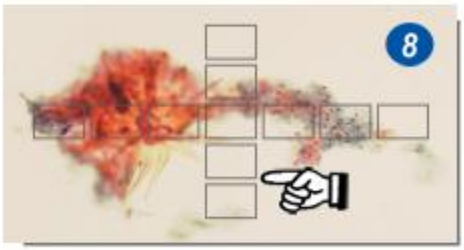
5: The *Details Dialog* appears with…

6:…the tile X and Y co-ordinates enabled for editing. To change the step size, click on either the X or Y text box and type a new value.

7: Click *Apply*. If necessary, re-enter the step values to get the required spacing or overlap **(8).**





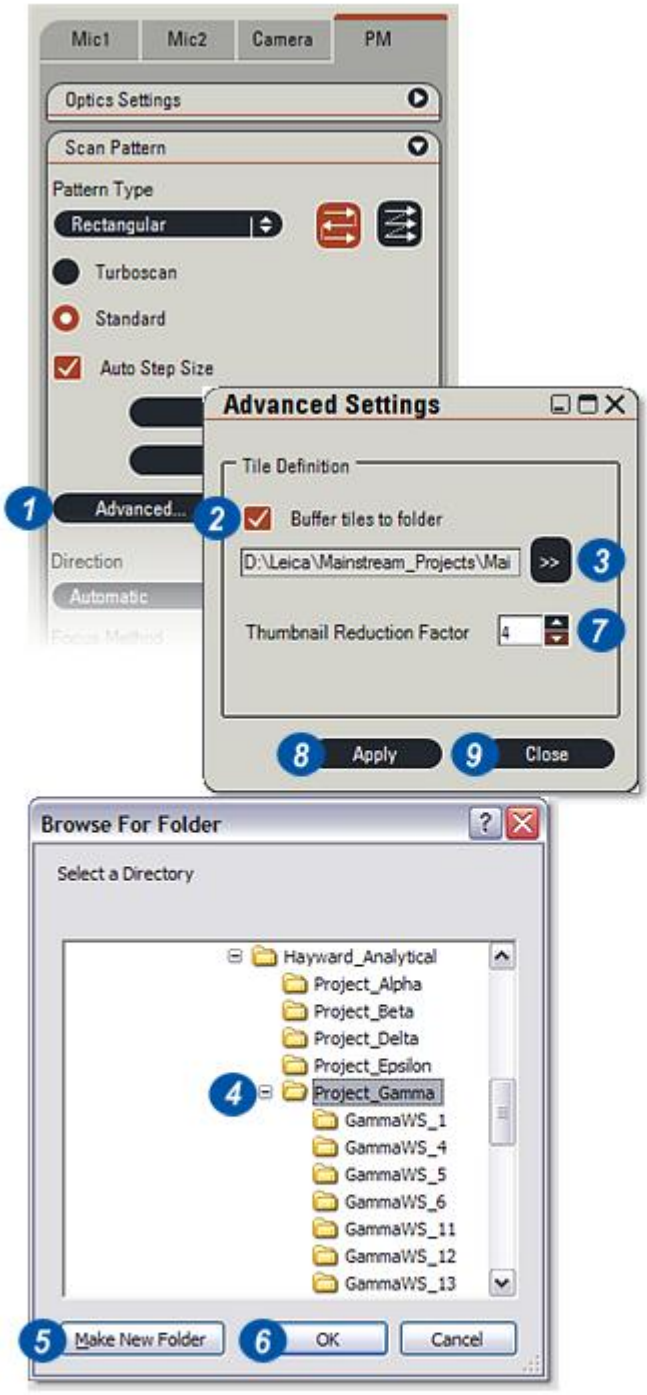


The Advanced options allow individual image tiles to be saved temporarily (Buffered) to a nominated folder and, if they are saved, set the size of the thumbnails in that folder.

Saving tiles has the advantage of identifying and keeping relevant parts of the image, and discarding less important parts so saving valuable disc space.

It is important that there is sufficient disk space to accommodate all of the images otherwise the scan will stop. The recommended approach is to have a partitioned section of the disk – D:PMTemp for example – in which to buffer the images. Make sure that computer privileges extend to the partition.

Thumbnail size is also important in that they are initially stored in RAM for fast access. If the thumbnails are too large, volatile RAM becomes clogged and the speed advantage is lost.



1: Click on the *Advanced* button.

2: On the dialog click to enable or disable tile buffering.

3: If tile buffering is required, click on the *Browse* button to reveal the *Browse for Folder* dialog.

4: Click to select a folder or…

5: …create a new folder. Use Windows navigation to reach other levels or directories.

6: Click *OK.*

7: If necessary, use the arrows to the right of the *Thumbnail Reduction Factor* text box. The larger the number the greater the thumbnail detail – and also the amount of disc space occupied.

8: Click *Apply* and…

9:…click *Close.*

The stage Scan Direction may be either side-to-side or front-to-back. The options are:

Automatic: Allow the software to determine the best direction,

Horizontal: Select side-to-side, or

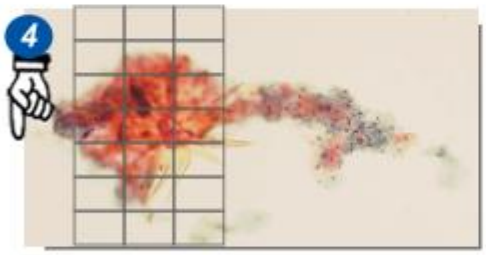
***Vertical*:** Select front-to-back.

Generally, Automatic is the best option to ensure the most efficient scan, especially when Turboscan is being used.

1: Click on the arrows to the right of the *Direction* header to reveal the options drop down menu.

2: Click to select the required direction.

Although both specimens **3** and **4** illustrated are the same, a vertical scan on **Figure (4)** would be best because there are fewer direction changes.



Select Pattern Type:
There is a wide range of scan patterns available designed to provide total flexibility and efficiency in the capture and storage of images.

Each pattern type can be configured to best suit the task in hand. On the following illustrations, each small rectangle represents a separate tile.

1: Click on the arrows to the right of the *Pattern Type* header.

2: From the drop down menu select the pattern required.

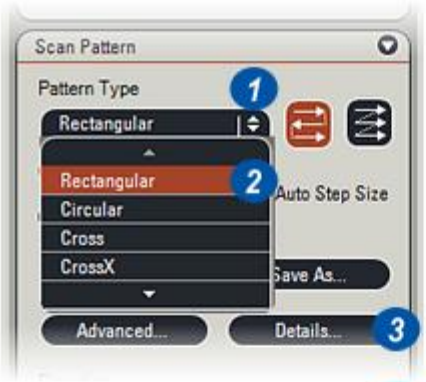
3: Click on the *Details* button. This opens the appropriate pattern dialog **(4).**

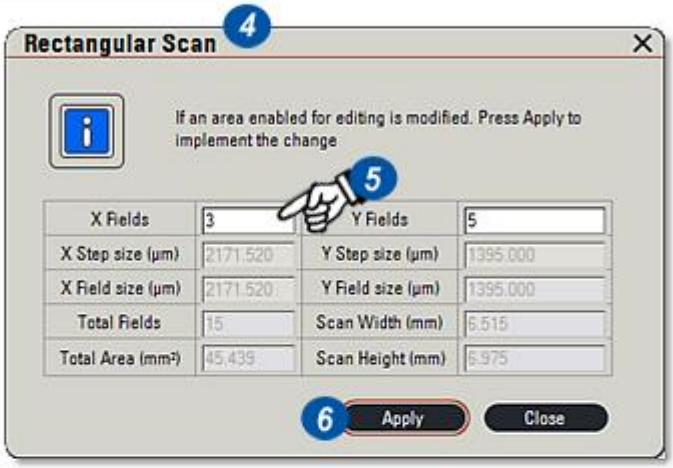
5: For the *Rectangle Pattern* **(7)**, the X and Y tile counts are available for editing. Click in the X or Y text box and enter a new value.

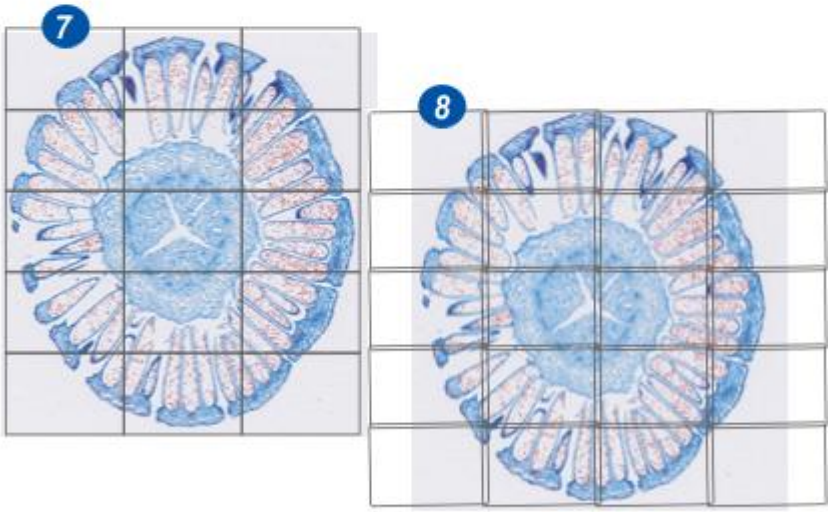
6: Click on the *Apply* button and the new configuration is applied to the image. Repeat steps **(5 and 6)** until the pattern is suitable.

For clarity, the tile layouts on the following pages are shown neatly abutting each other. However, if the camera rotation is not perfect the tiles may appear at an angle and possibly overlapping **(8)**. The software has been designed to accommodate these variations.

See: Create the Pattern Grid: 835





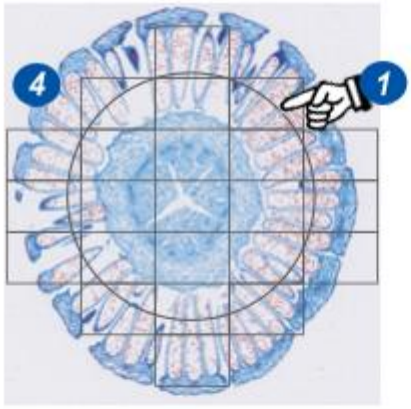


The following pages illustrate the Pattern Types available and the options for each.

Circular Pattern Type:
The options are:

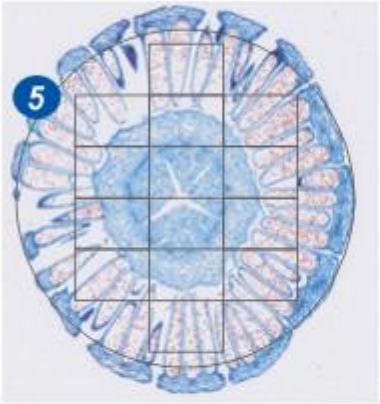
Full Coverage and

Inside only.



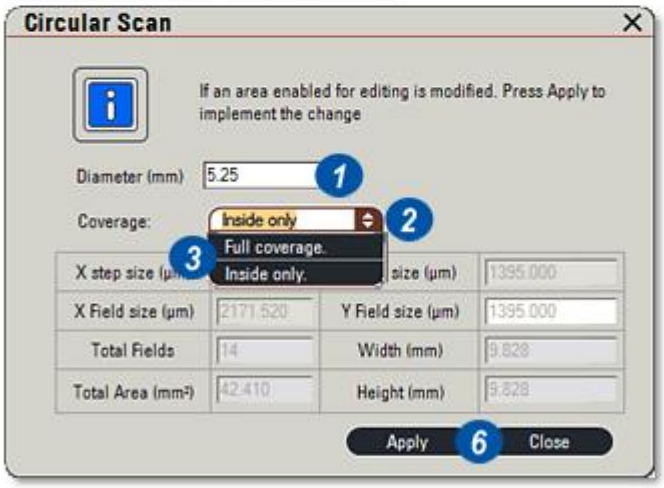
1: Click on the *Diameter* text box and enter a value for the circle diameter in millimetres. The circle does not have to cover the entire image.

2: Click on the arrows to the right of the *Coverage* text box and…



3:…select either *Full Coverage* **(4)** or *Inside only* **(5)** from the drop down menu.
Full Coverage covers the entire circle with overlap where necessary.
Inside only places tiles within the circle.

6: Click *Apply* to apply the values to the image. Repeat from Step **(1)** if necessary to adjust the pattern.
Click *Close* to save the pattern and exit.



Cross and CrossX Pattern Types:
The options are:

X and Y tiles for the Cross pattern:

Tile count only for the CrossX pattern*:*

1: For the *Cross* pattern:

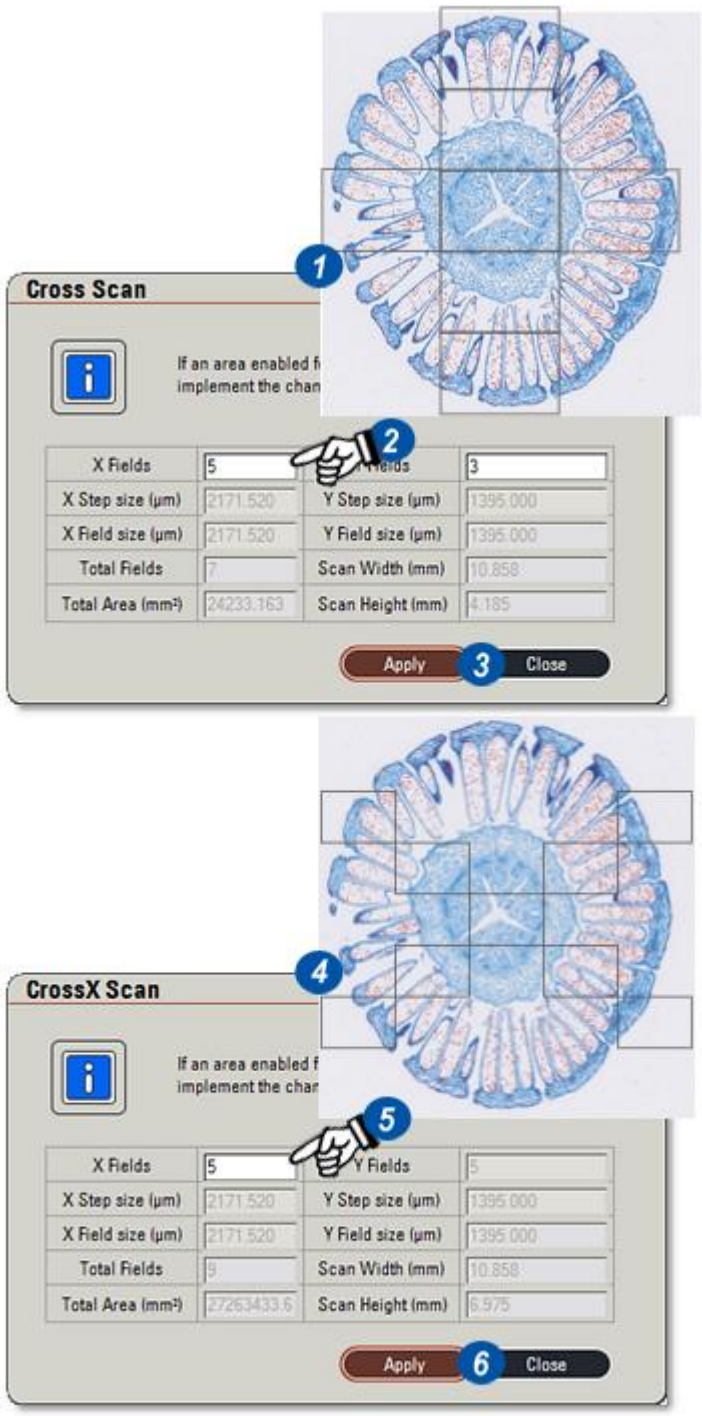
2 Click on the *X or Y text boxes* and enter a value. The X value represents the horizontal tile count and the Y value the vertical count.

3: Click *Apply* to apply the settings. Repeat from Step **(2)** to change the pattern. Click *Close* to save and exit.

4: For the *CrossX* pattern which is a regular X:

5: Click on the *X text box* to enter a new total tiles values.

6: Click *Apply* to apply the setting. Repeat from Step **(5)** to change the pattern. Click *Close* to save and exit.



Random and Random without overlap patterns:
The options are:

Random the number of tiles some of which may overlap:

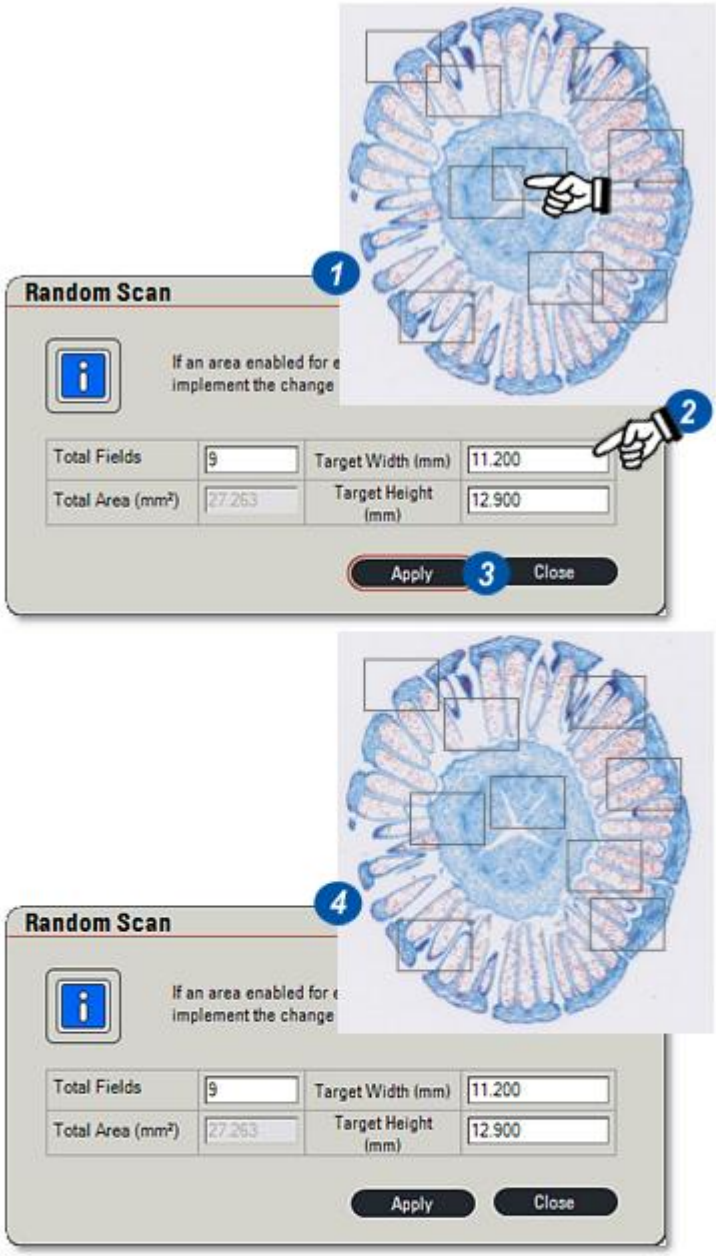
Random without Overlap the number of tiles none of which will overlap:

1: *Random* pattern creates a specified number of tiles randomly inside a specified boundary. Some of the tiles may overlap.

2: Click on the *Target Width and Target Height* text boxes and enter values for the scan area boundary. The scan area does not have to cover the entire image.
Click on the *Total Fields* (tiles) text box and enter a value for the number of tiles.

3: Click *Apply* to apply the settings. Repeat from Step **(2)** the change the values.
Click on the *Close* button to save and exit.

4: *Random Pattern without Overlap*:
This option is the same as Random except that none of the tiles will overlap.



Annular:
Options are:

Full Coverage and

Inside only.

This option automatically populates an area defined between two concentric circles with a calculated number of tiles.

1: Click on the *Diameter* text box and enter a value for the outer (larger) circle in millimetres. The circle does not have to cover the entire image.

2: Click on the *Inner Diameter* circle text box and type a value for the smaller circle (shown coloured on the illustrations for clarity). It must be smaller than the outer diameter otherwise the settings will be ignored.

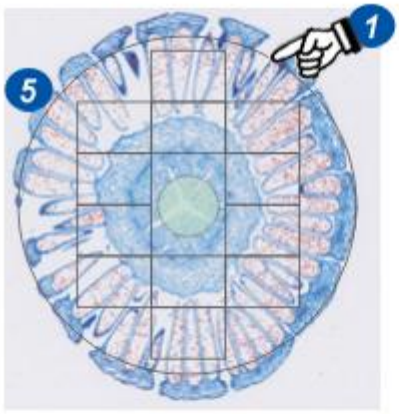
3: Click on the arrows to the right of *Coverage* text box and from the drop down menu…

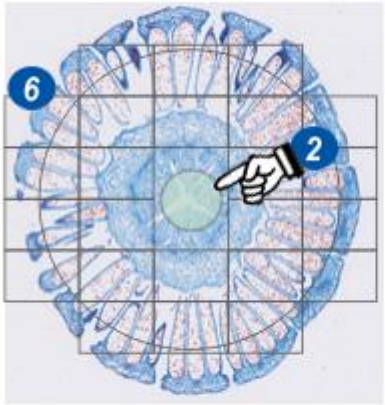
4: …click to select either *Full Coverage* or *Inner only*.

5: *Inner only* places the tile within the outer circle avoiding inner circle.

6: *Full Coverage* ensures the entire outer circle is covered without including the area of the smaller circle.

7: Click *Apply* to apply the settings. Repeat from Step **(1)** to change the values.
Click on the *Close* button to save and exit.





Three automatic focus methods are available:

Predictive Focus: which uses a set of known focus points to interpolate (predict) any unknown points. Predictive Focus is fast with good results but should be used only when the specimen is either flat or has a uniform, slope across focus points. Use objectives up to 10x or for very flat specimens up to 20x.

Autofocus: uses contrast differences in groups of adjacent pixels to establish sharpness. Because it is a continuous process of focussing and checking, Autofocus is slower than Predictive but yields extremely good results. especially over irregular specimens.

Predictive and *Autofocus:* in combination uses the speed of Predictive and the quality of Autofocus to achieve very good results, quickly. Use for specimens that are predominantly uniform but also have local irregularities. Predictive will come close to focus and the Auto will fine tune it.

Both *Predictive* and *Autofocus* methods and the combination are selected by enabling the check boxes **(1).**

When a method is enabled its setup button becomes active **(2)**. Setup is explained on the following pages.

For perfectly flat specimens both methods can be turned off.

The Predictive Focus function, focuses on a number of predetermined points on the image and creates a ‘table’ of their focus values. The focus position for any other point can be predicted by interpolating the table values.

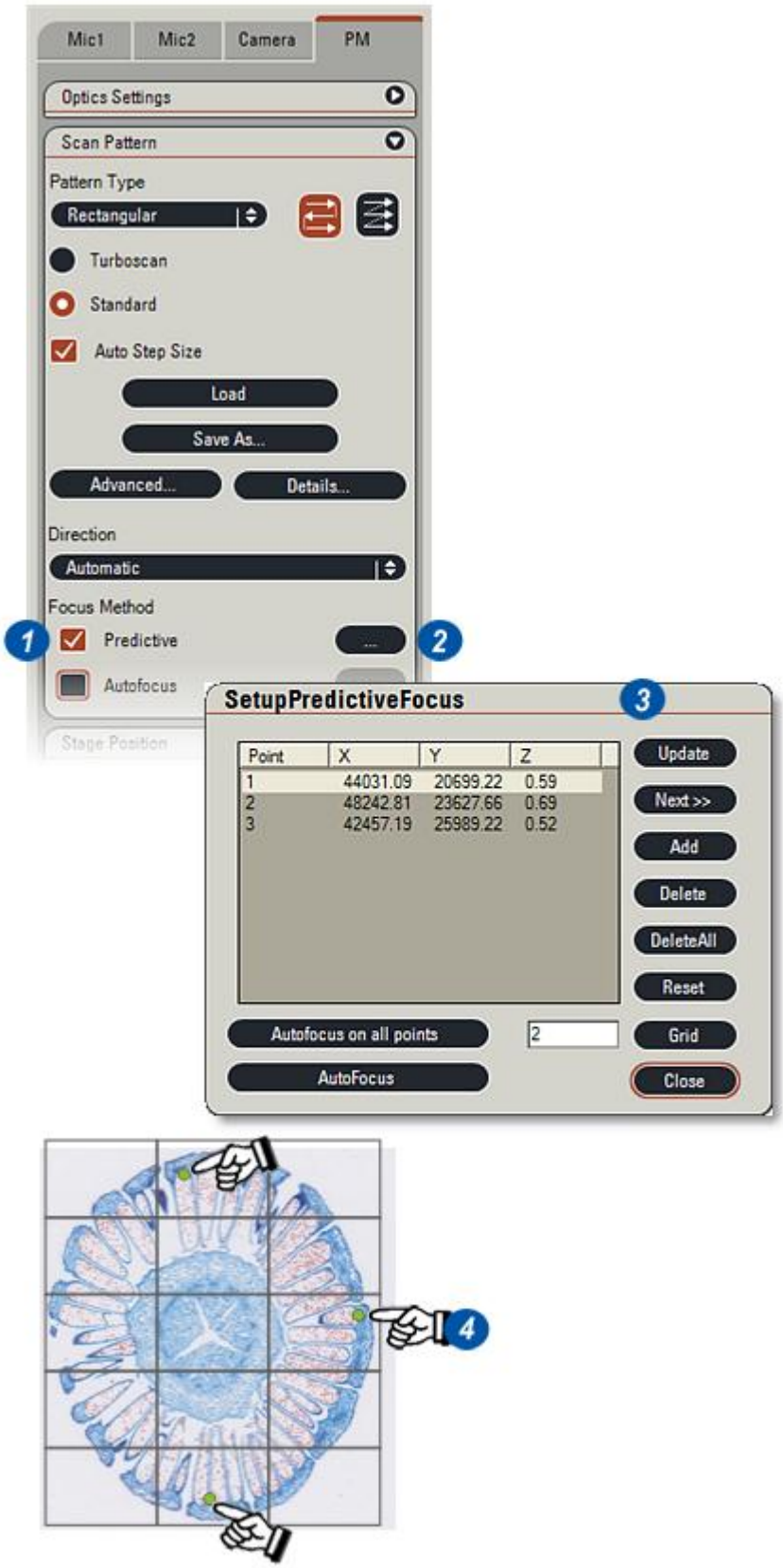
The greater the number of pre-determined points then the greater the accuracy of the prediction and better the overall focus. This is especially true for very specimens that have irregular focus.

1: Click on the *Focus Method: Predictive* checkbox to enable it.

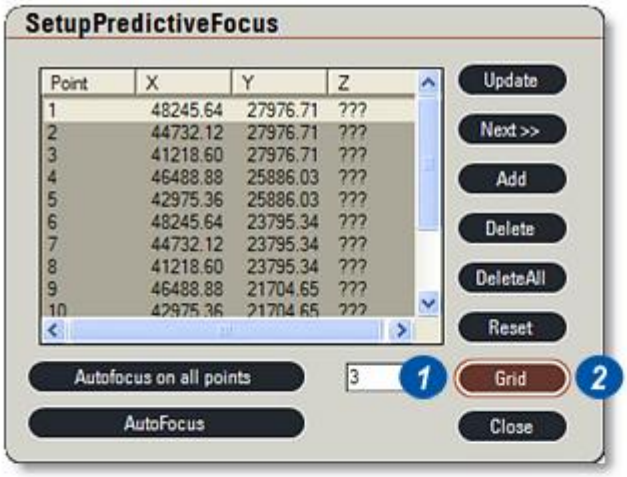
2: Click on the *Setup* button to display…

3: …the *Predictive Focus* dialog.

4: Three pre-determined points are automatically placed on the image. Their positions are displayed in the X/Y columns on the dialog.
Normally, the set points will be coloured red to indicate that they require focussing. If they are green and have a value in the Z column denoting that they are already focussed, possibly as the result of a previous scan, the points should be focussed again.

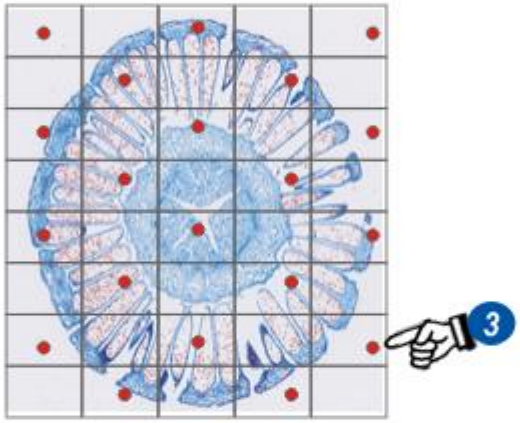


The Grid feature automatically creates a regular pattern of points across the image. The number of points can be established by typing a value.



1: Click on the *Grid* text box to high light the existing value. Press the keyboard delete key to remove the value. Type a new value.
The value represents the number of points that will be created along the top row of the grid. On the second row the number of points is reduced by one. The next row is increased by 1 and so on across the entire grid. In the illustration, a value of 3 has been typed.

2: Click on the *Grid* button and the new points will be created and listed on the dialog.
Points sitting on completely clear areas of the image **(3)** will result in a failed Autofocus because there is no detail to focus on. To avoid a failure, either delete the points *(See: Delete a focus set point)* 832 or adjust the Focus Threshold Setting upward to include values closer to white (clear). However, setting the threshold to a very high value can affect the precision of Autofocus.*See: Initialisation: Focus* 813



Reset Focus Point values:

1: To clear all focus point values, click on the *Reset* button. The values will clear and '???' will be displayed in the Z column of the dialog.

Delete a Focus Point:

2: To delete a focus point click on the point on the dialog list.

3: Click the *Delete* button. The list entry and the point disappear.

To create a New Point:

4: Click on the *Move Stage To Point* button.

5: Select a new point on the image and click. The point appears on the image coloured red to indicate that it requires focussing.

6: Click on the *Add* button.

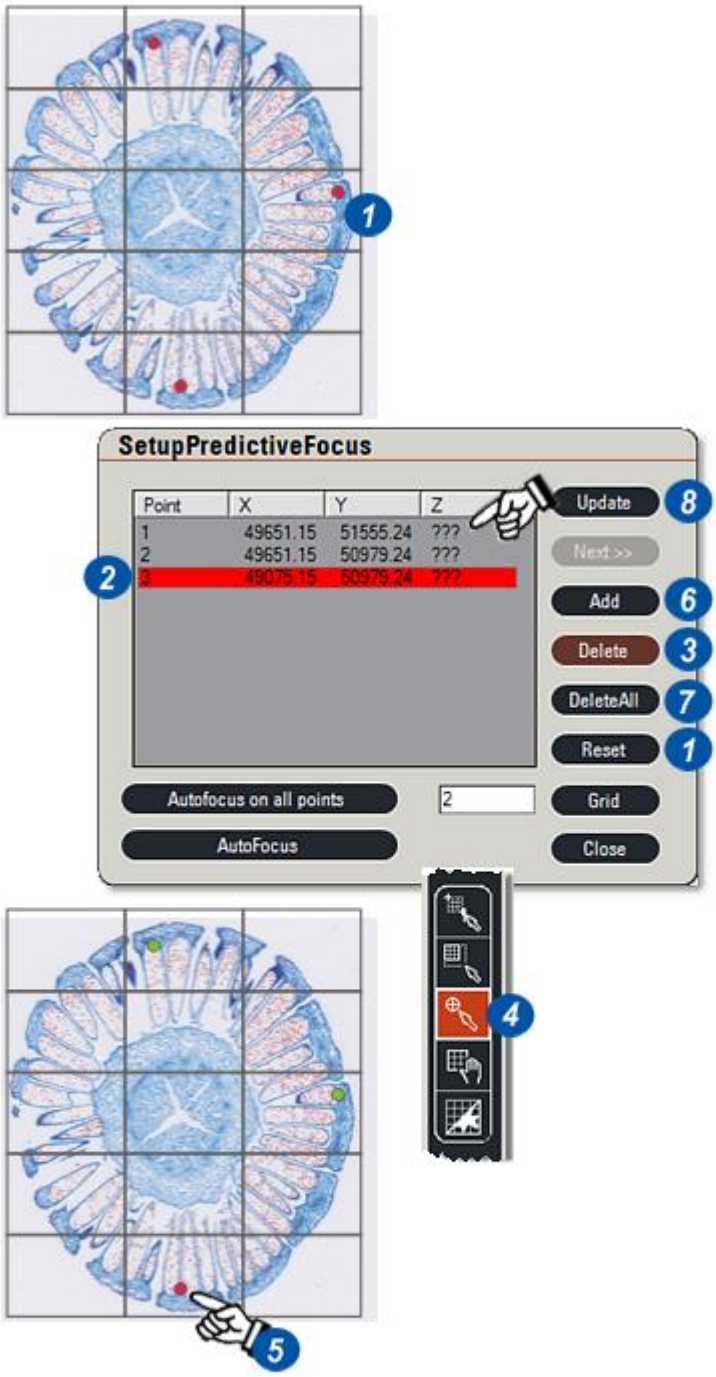
Delete all Points:

7: All of the points may be deleted byclicking on the *Delete All* button. The dialog list will clear and the points will disappear from the image.

Update:

Having added or deleted points, update the list and displayed points by…

8: …clicking on the *Update* button.



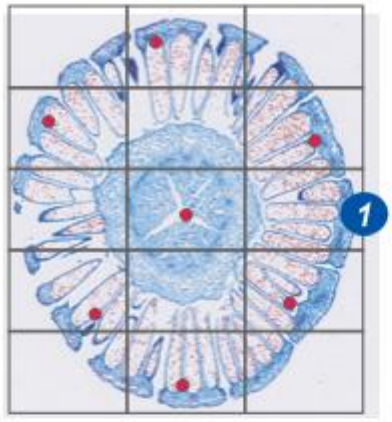
For multiple focus points:

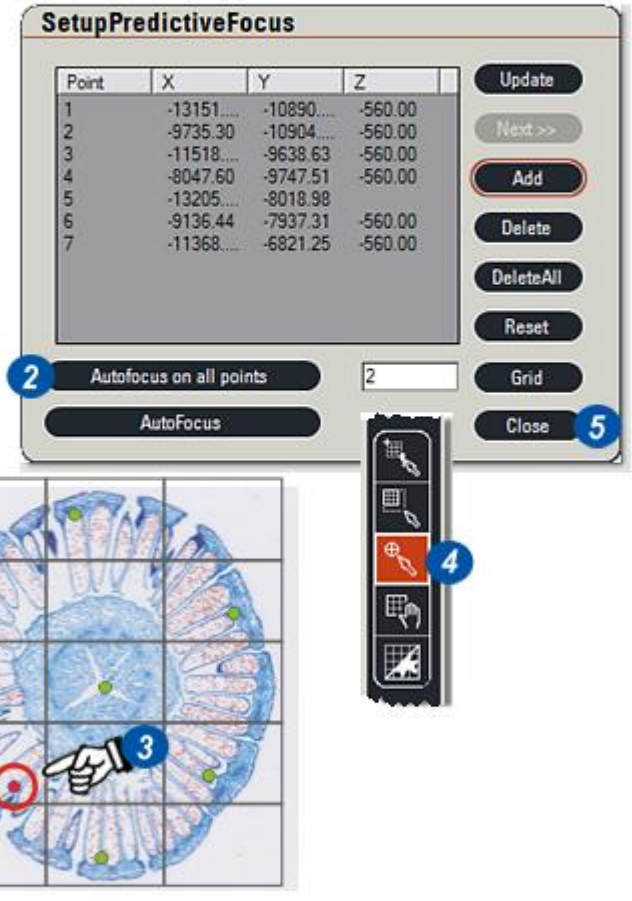
1: The illustration shows a range of pre-determined points that have been added manually to comprehensively cover the specimen.

2: Click on the *Autofocus on all Points* button, or for a single point, click on the *Autofocus* button.
The program will cycle through each of the set points automatically focussing. As each is completed the point turns green and the value appears in the Z column of the dialog.

3: Check 'failed' points (circled for clarity) by…

4: …selecting the *Move Stage To* button and then on the focus point. Manually check the point for detail. Either delete the point or adjust the Focus Threshold Setting, reset the points and repeat the process.





Setup Autofocus Skipping:
To speed up the scan especially on specimens that are more-or-less uniform, automatic focussing can be skipped on some tiles. The number of tiles to skip is set up with Autofocus.

It is also possible to resume focussing on a tile immediately following a focus 'failure' rather than skipping tiles, to maintain focus integrity.

Autofocus failure generally occurs when there is insufficient detail in the specimen.

1: Click to enable the *Autofocus* check box.

2: Click on the *Setup* button and the Setup Autofocus dialog appears.

3: To enable tile skipping click on the *Autofocus every…* check box.

4: Set the number of tiles to skip by either clicking on the text box, deleting the existing value and typing another, or clicking on the increase/decrease arrows to the right of the text box.

5: To *Retry on Failure*, click the check box to enable.

6: Click on the *Apply* button and…

7: Click on the *Close* button.

Go to Create the Pattern Grid... 835

Create Pattern Grid

This step draws a pattern grid representing the scan tiles over the image. Power Mosaic will use this grid as a scanning 'map'. Because the specimen has yet to be scanned, its precise position on the stage is unknown so the first grid drawing locates it.

1: Click on the *Split View* button and navigate to an appropriate part of the specimen using the on-screen joystick.

2: Click on the *Pattern View* button.

3: Click on the arrows to the right of the *Pattern Type* header and from the menu...

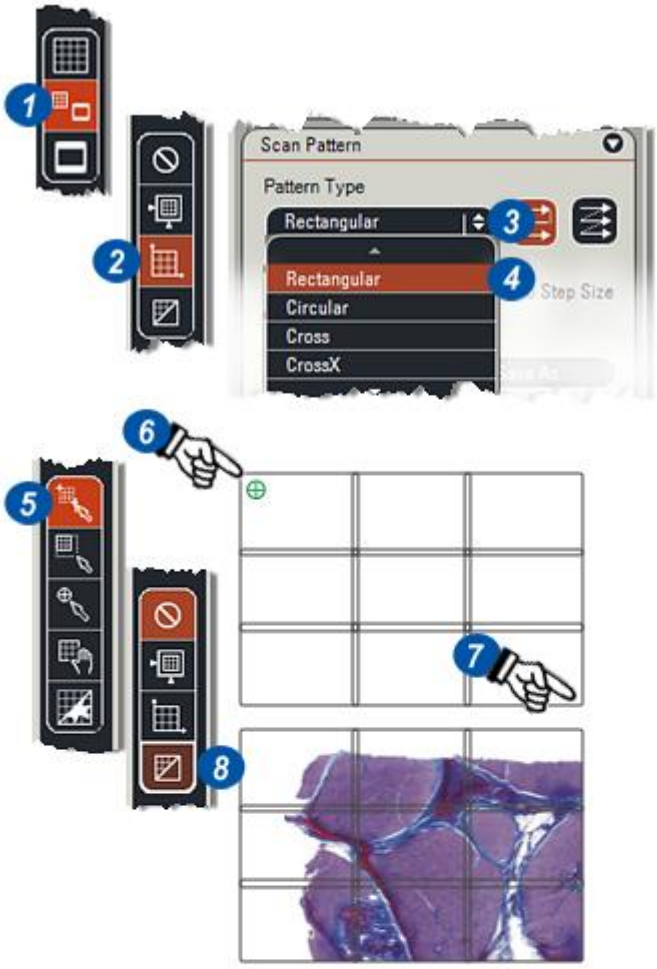
4: …select by clicking the *Rectangular* option. A different pattern may be selected later.

5: Click on the *Draw Pattern* tool and…

6: …positioning the cursor close to the stage marker (small, green 'crosshair'), click and hold...

7: …and drag diagonally to the right. An arbitrary grid pattern should appear. If it does not, …

8: …click on the *Show Grid Lines* button to reveal it.

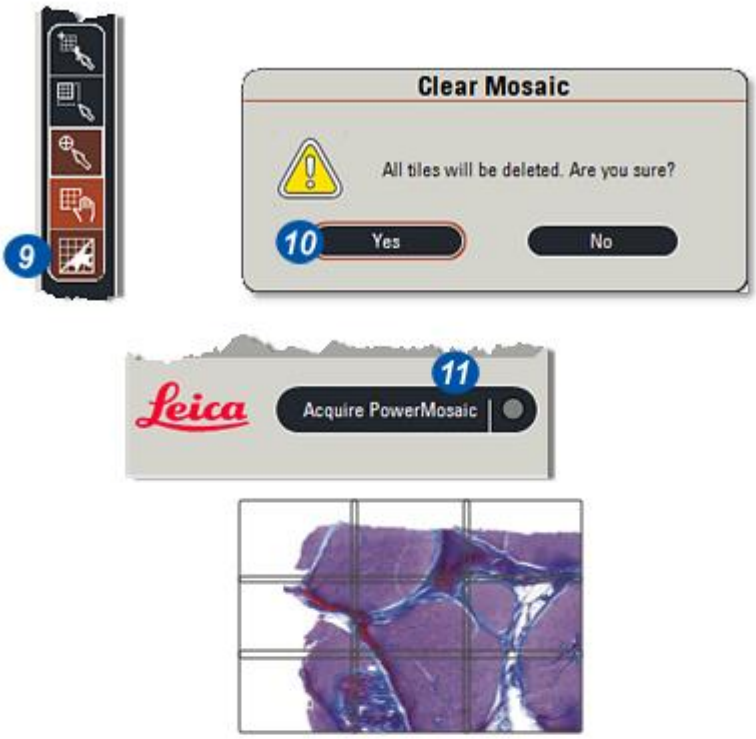


9: Click on the *Clear Tiles* button to clear any previous images.

10: Confirm clearing the images on the warning message by clicking *Yes*.

11: Click on the *Acquire Power Mosaic* button. The specimen will be scanned with the tiles filled in sequence.
The first drawing usually encloses only part of the image as shown in the illustration. If this is the case go to the next page.

9: If none of the specimen is found click on the *Clear Tiles* button and repeat the process starting the drawing at a different location.



With the pattern grid enclosing only part of the specimen, it can be extended so that the specimen is fully covered.

1: Click on the *Create/Expand* button. This feature allows the existing grid to be expanded.
The Create/Expand button may also be used to reduce the pattern grid if it is too large.

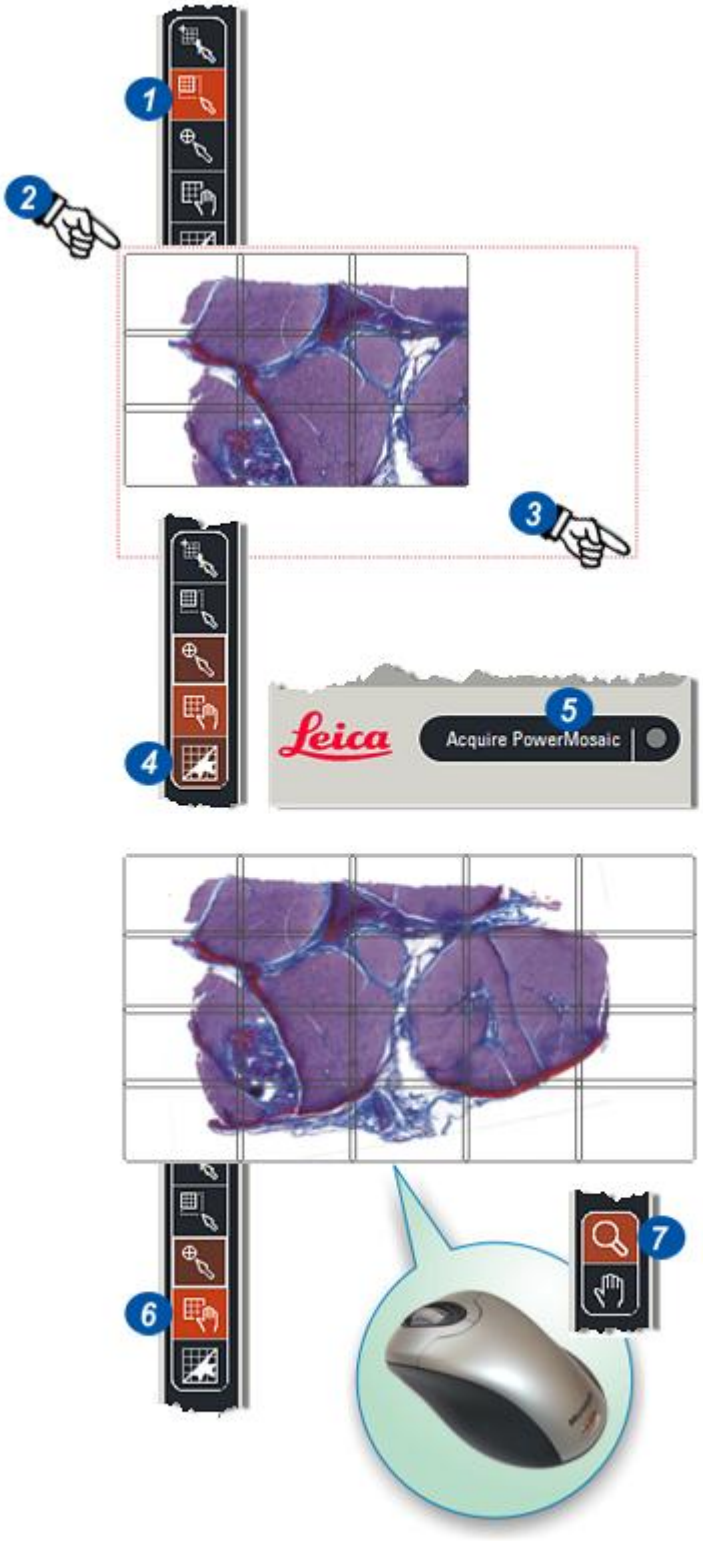
2: Click on the top left corner of the existing pattern grid, hold and drag diagonally to the right. A dotted rectangle follows the cursor to indicate the extent of the grid. Release the mouse button when the specimen is considered to be completely enclosed **(3).**

4: Click on the *Clear Tiles* button and confirm the clear on the warning message.

5: Re-scan by clicking the *Acquire Power Mosaic* button.

6: To re-position the scanned image within the grid, click on the *Move Scan Pattern* button, click and hold on the scanned image and drag it to reposition.

7: The *Magnifier* may help in the repositioning - click on the tool and use the mouse buttons to enlarge (left button) or reduce (right button) the view.



Having re-positioned the image within the pattern grid, it is possible that there are too many tiles. Excess tiles may be removed as columns or rows only by:

1: Click on the *Details* button and on the dialog, change the fields (tiles) to remove any excess.

2: Click the *Apply* button and…

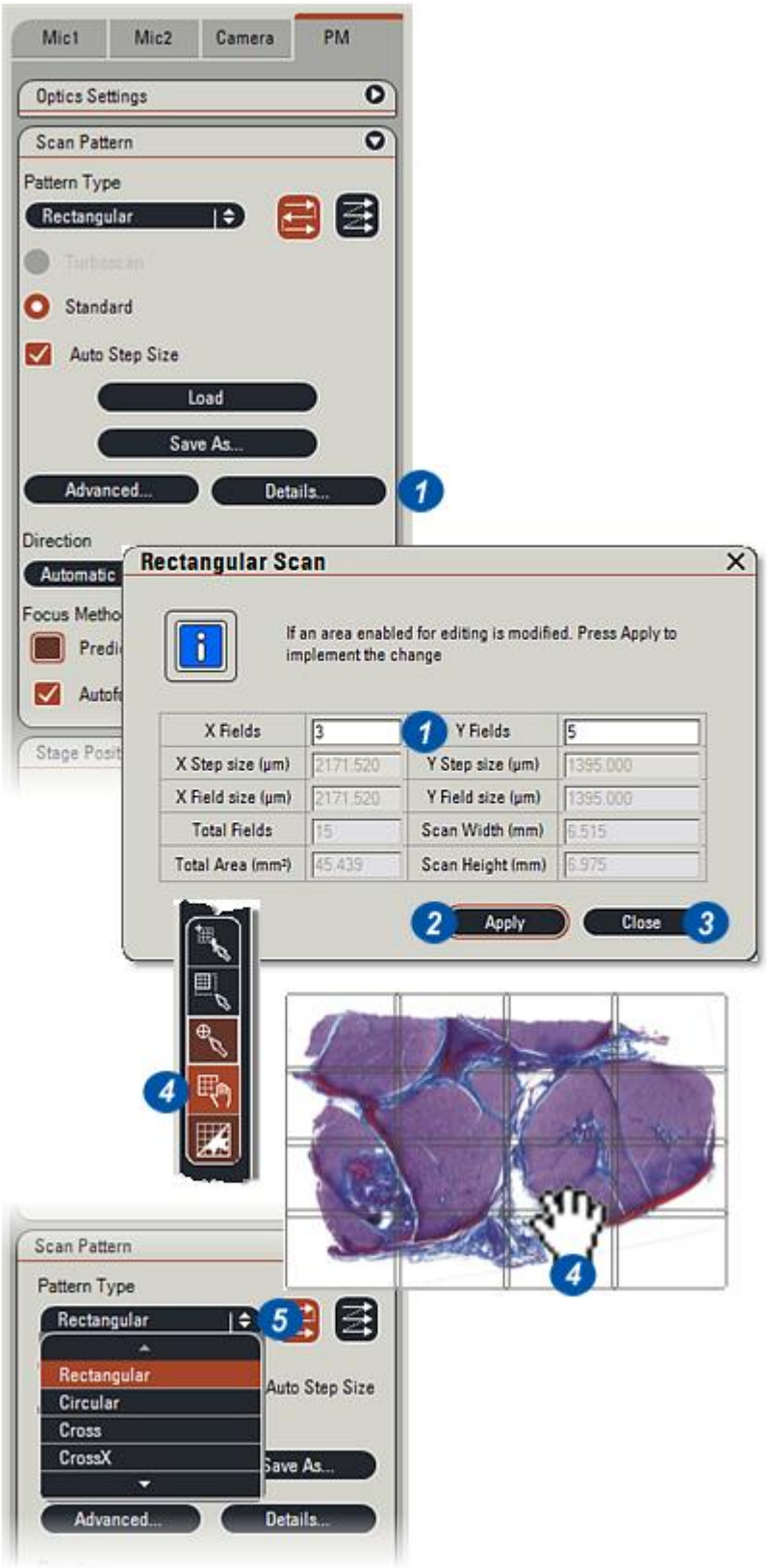
3: …click the *Close* button.

4: Select the *Move Scan Pattern* button and re-position the grid to make sure the fit over the specimen is acceptable.

5: If an alternative *Pattern Type* is required, click on the arrows to the right of the Pattern Type header and from the drop down menu click to select the required pattern.

It may be necessary to re-adjust the pattern grid-to-specimen fit using the *Move Scan Pattern* button.

Go to Save Configuration... 839



Save Configuration

With the Power Mosaic configuration complete, it is possible to save the settings for instant use in the future.

To save the current settings:

1: On the *Scan Pattern* panel click the *Save As* button.

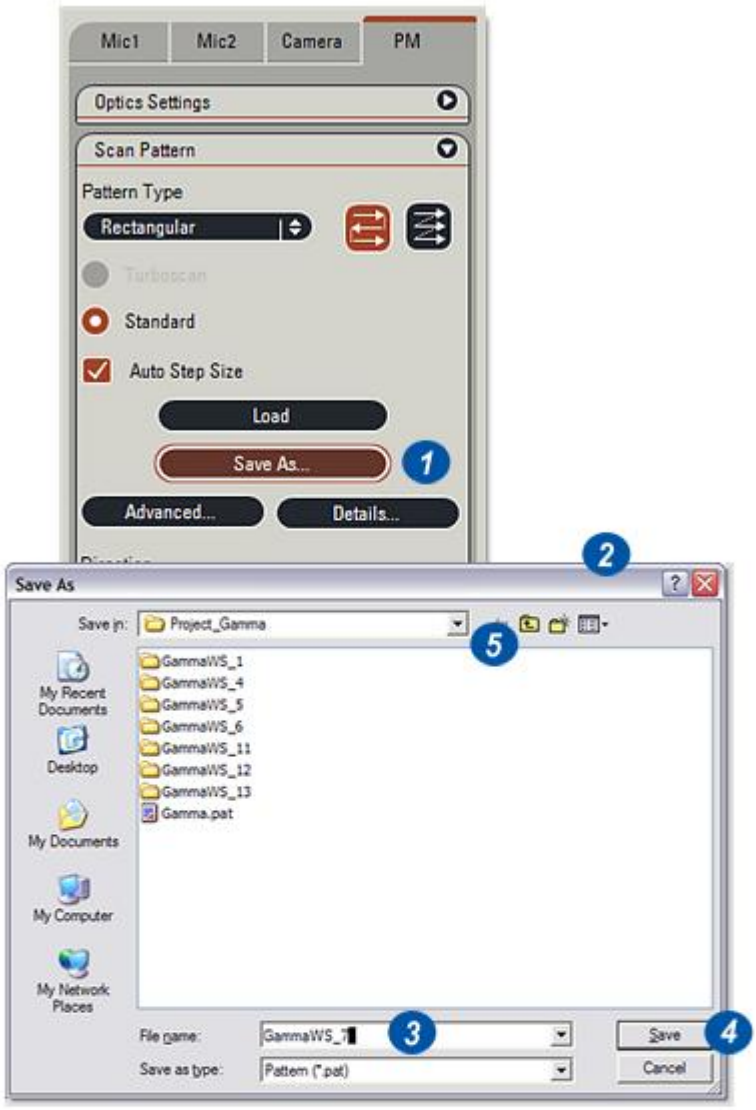
2: On the *Save As* dialog…

3: …type a file name and…

4: …click the *Save* button. Files are automatically saved with the *.pat* extension.
The *Save As* dialog defaults to the folder selected in *Preferences* or *Browse.*

See: Preferences:Save in Folder:
See: Browse:Select Default Folder:

5: The *Save As* folder may be changed on the Save As dialog using Windows navigation.



Before starting a Power Mosaic scan, the thumbnails and tiles from previous scans, if not required, should be deleted. This is not mandatory – keeping previous information can be a vital part of an ongoing session in which the specimen is in several parts for instance.

Thumbnails of each tile are stored in RAM to make access fast and immediate. They are used to 'paint' the mosaic on the Viewer. Keeping thumbnail size as small as possible *(See: Advanced Options: Thumbnail Reduction Factor 822)* will help prevent filling the RAM too quickly.

The tiles are stored initially in a temporary file on the hard drive. The individual images are much larger and used to paint the mosaic at greater resolution to preserve detail.

Clearing previous scans removes the thumbnails from RAM and the tiles from the temporary file. If the previous scan is left intact, new thumbnails and tiles will be added to the existing with sequential image numbers.

1: Click on the *Clear Tiles* button to clear the previous scan. This is only the previous scan and does not remove any prior to that.

2: The *Clear Mosaic* message appears. Click *Yes* to continue.

3: Click on the *Acquire Power Mosaic* button.

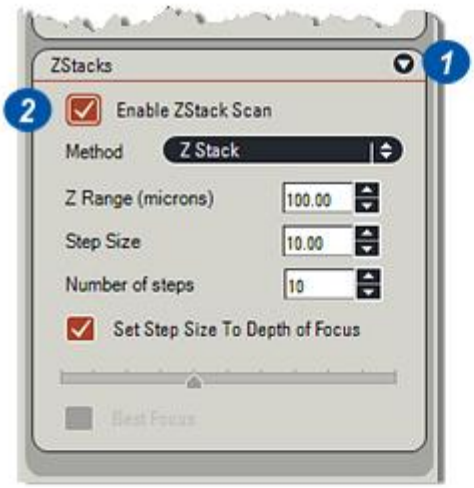
4: If Turboscan is selected and the exposure speed is too high, a dialog appears giving the option to use Standard scan instead or to cancel.

If Power Mosaic Plus is installed and enabled, the Z-Stack option is available. For every tile position on the XY plane, Power Mosaic Plus creates a stack of tiles in the third dimension or Z plane.

The number of tiles in the stack and the distance between them can be set to reflect the specimen.

Once captured the individual stack tiles can be processed in several ways and eventually into a single composite image that represents the best focus across the entire specimen regardless of thickness.

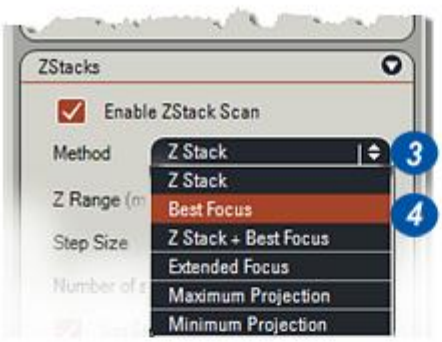
Z-Stacks can occupy very large amounts of disc space especially if all of the tiles are saved, so ensure that a liberal amount of space is available. The best arrangement for image storage is to have a partitioned area on the hard drive *(Drive D:* for example) separate from other programs and data, or a completely independent drive.



1: Click on the arrows to the right of the *Z-Stacks* panel to reveal it.

2: Click the *Enable Z-Stack* check box to enable scanning.

3: To select the required processing method, click on the arrows to the right of the *Method* header and from the drop down menu…



4: Select the method:

Z-Stack will save all of the captured stack tiles for further, individual viewing.

Best Focus chooses the tile with the sharpest image from each stack and discards the rest.

Z-Stack + Best Focus combines both options, retaining all of the stack tiles but also selecting the best from each stack which it places at the lowest level.

Extended Focus examines all of the tiles from a stack, choosing the 'best' pixel from each at a given location. These are then combined into a single tile and the rest discarded.

Maximum and *Minimum Projections* emulate Extended Focus to create composite images based upon the darkest and lightest pixels in each pixel column in each stack tile.

The Z-Range – the focussing distance based upon objective depth of focus - in microns that will be travelled – Step Size and Number of Steps, are inter-related; Change one value and the others will be calculated and changed automatically. Actual settings will depend upon the specimen.

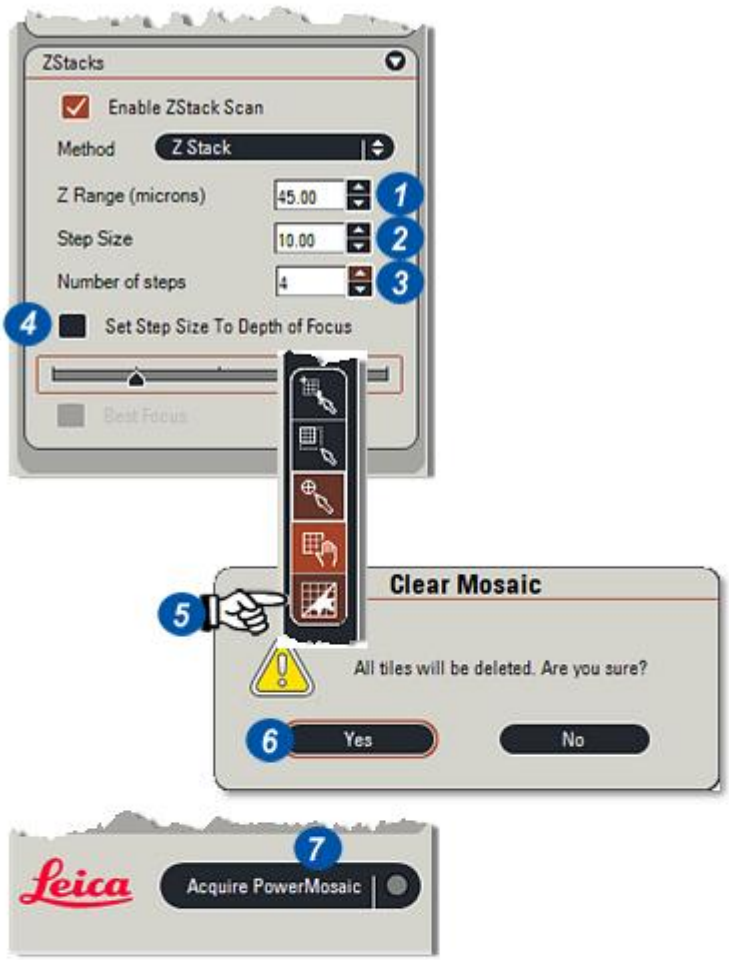
1, 2 and **3**: Use the up/down arrows to the right of each text box to enter a value. For large values, click on the appropriate text box and type a value.

4: The *Set Step Size* check box if enabled will automatically load a step distance based upon the depth of focus and adjust the Z-Range and Number of Steps accordingly. Changing the Step Size manually will turn off Set Step Size.

5: Click on the *Clear Tiles* button to discard any previous scans. The *Clear Mosaic* warning will appear…

6:…click *Yes* to clear.

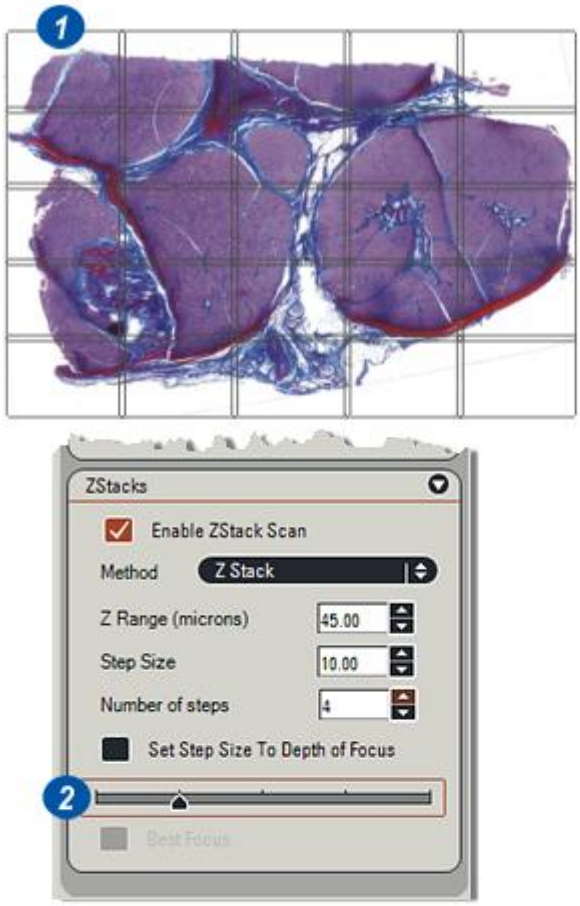
7: Click the *Acquire Power Mosaic* button. Z-Stacks are only available in Standard Scan mode. If Turboscan has been selected it will be ignored



When the scan is complete the entire image is displayed with the tile grid overlaid **(1).**

Two methods – *Z-Stack* and *Z-Stack + Best Focus* – have a slider **(2)** associated with them which allows each stack step to be viewed individually, a powerful image analysis tool.

The slider scale represents the number of steps or multiples of steps. Click, hold and drag the slider to display the step results in turn.



Pattern Navigator is a module that is part of and licensed by *Power Mosaic Plus.* It allows users to create *Scan Patterns* randomly in any position across the stage and then scan them either all together or at selected sites. The great advantage of this facility is that only areas of interest are captured substantially reducing both processing time and required disk space.

Since patterns can be saved and restored, tasks that routinely use the same specimen layout on a slide (say), benefit from the speed that having a pre-defined scanning template can offer. For example, illustration **(A)** shows a standard 75 x 25 microscope slide (enlarged for clarity) divided into 12 specimen 'wells'. The wells are not spread evenly across the slide – there is a larger area at the top than there is at the bottom - nor are they of uniform size. To complicate matters even further, only 6 of the specimens are usually scanned – they are marked with a red border in the illustration. It is a simple task for *Pattern Navigator* to locate the required wells, create a scan pattern from them and to store that as a template for future processing.

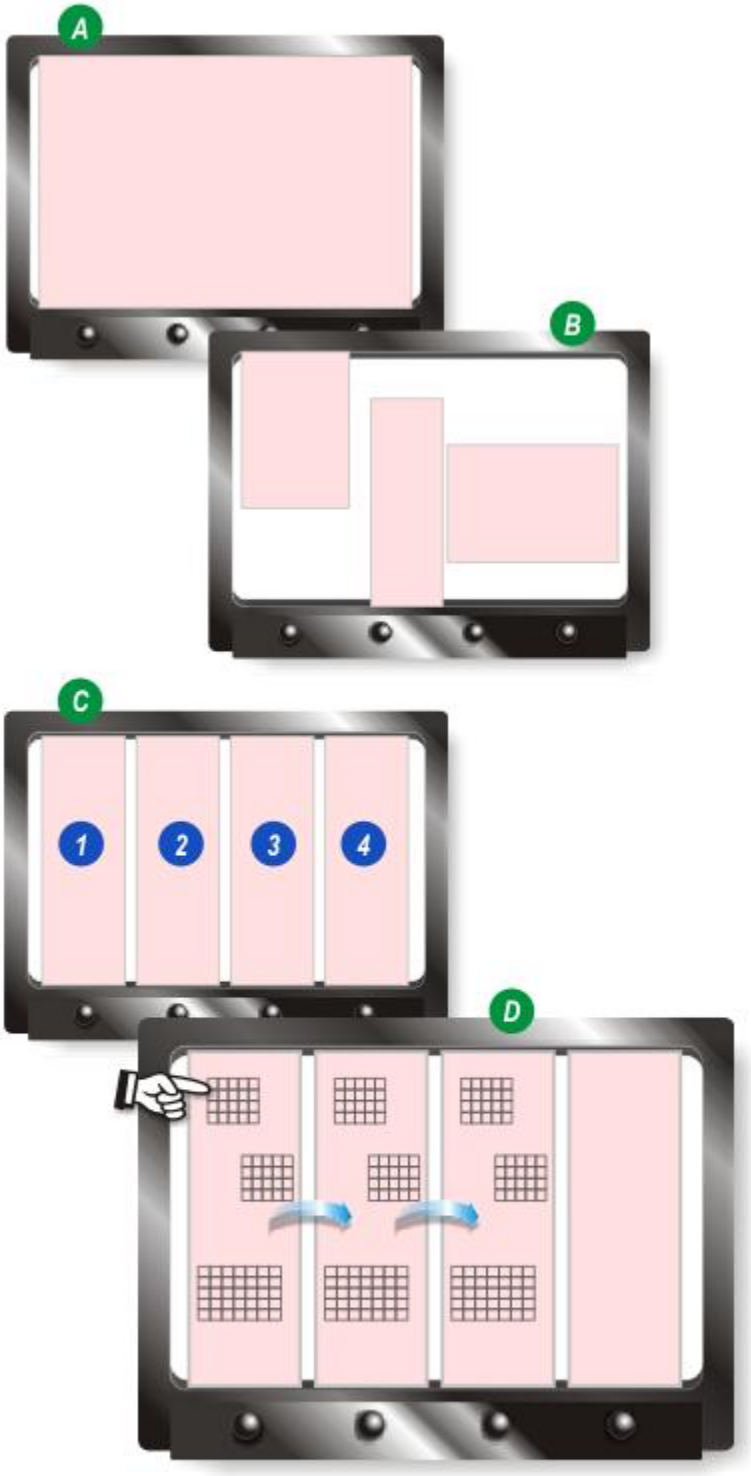
Pattern Navigator allows the user to divide the viewable stage into distinct areas called *Workspaces*. The number and position of the *Workspaces* is determined by the users for ease of working. The entire stage could be classed as a single *Workspace* **(A)**; It could be divided into several *Workspaces* of differing areas **(B)** or a group of uniform *Workspaces* like 4 slides in a row for example **(C)**. The only restriction is that *Workspaces* must not overlap.

Each *Workspace* encloses its own group of *Scan Patterns* **(D)**. An individual pattern can be re-sized and moved within its *Workspace* providing it does not overlap with another. *Scan Pattern* shapes – rectangular, square, circular, cross etc – can be mixed within a *Workspace* to achieve the most efficient and economical image capturing.

Because a *Workspace* acts as a container for its *Scan Patterns*, it can be moved to any location on the stage and its patterns will move precisely with it.

Workspaces can also be copied, so having set up a group of patterns the entire *Workspace* can be duplicated and 'pasted' to other parts of the stage. This is especially useful for repetitive specimens – four identical slides in a row for example.

By default the Workspace outline and fill is turned off. To turn it on and change its properties: *Go there...* 874



Capturing specimen mosaics using *Pattern Navigator* comprises three simple steps:

1: Creating and mapping the *Workspace*.

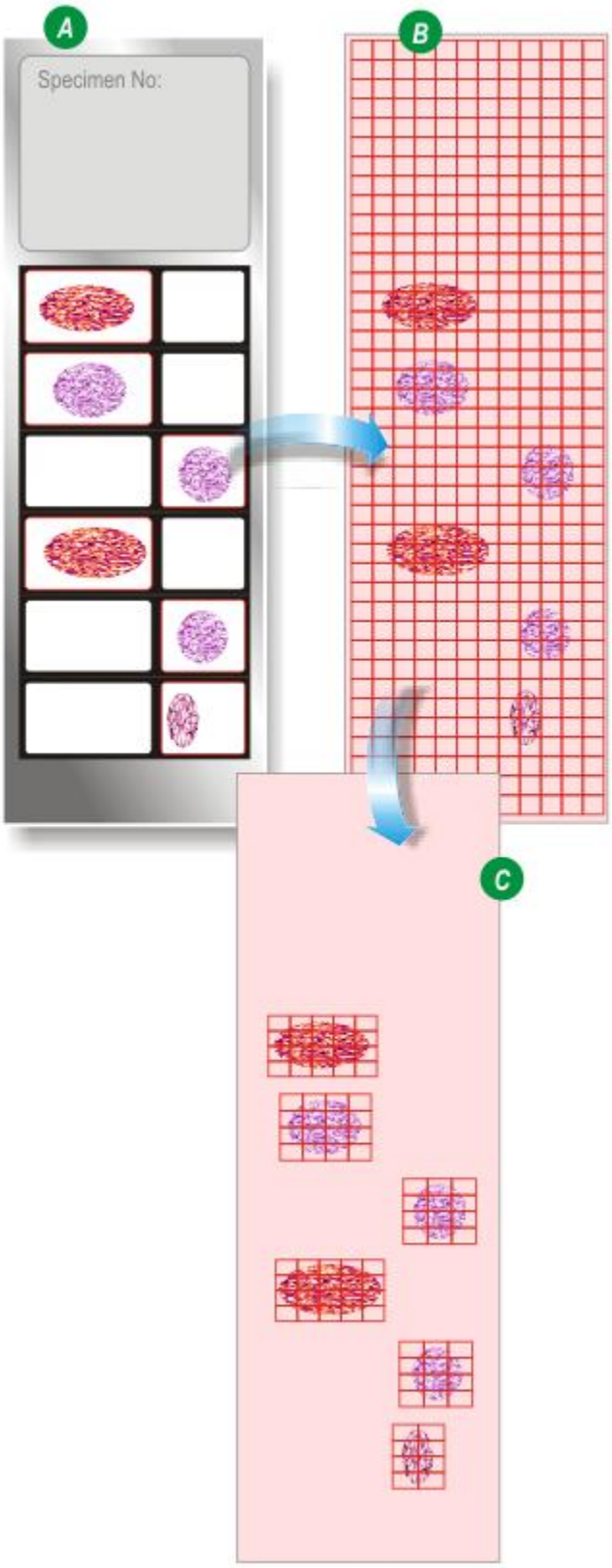
2: Plotting the individual *Scan Patterns* within the *Workspace*.

3: Scanning using the *Scan Patterns.*

Creating and Mapping the Workspace: Illustration **(A)** shows the sample microscope slide described earlier.

To map the locations of the required specimens, a rapid scan is made using a scan pattern that covers the entire *Workspace* – in this case the slide area **(B)**.

Using this as a guide, individual *Scan Patterns* are created and placed (just by dragging and dropping) over the specimens. The overall scan pattern is then removed **(C).**



To map the *Workspace* only low resolution images are required because they are to be used only as a guide to the specimen location. Low resolution capture formats also reduce the scanning time.

1: In the *Acquire Workflow* click on the *Camera* tab.

2: Click on the arrow to the right of the *Image Formats* panel.

3: Click on the arrows to the right of the image type header and…

4: …select *Colour* or *Greyscale* to suit the image.

5: Click on the *Mic* tab and...

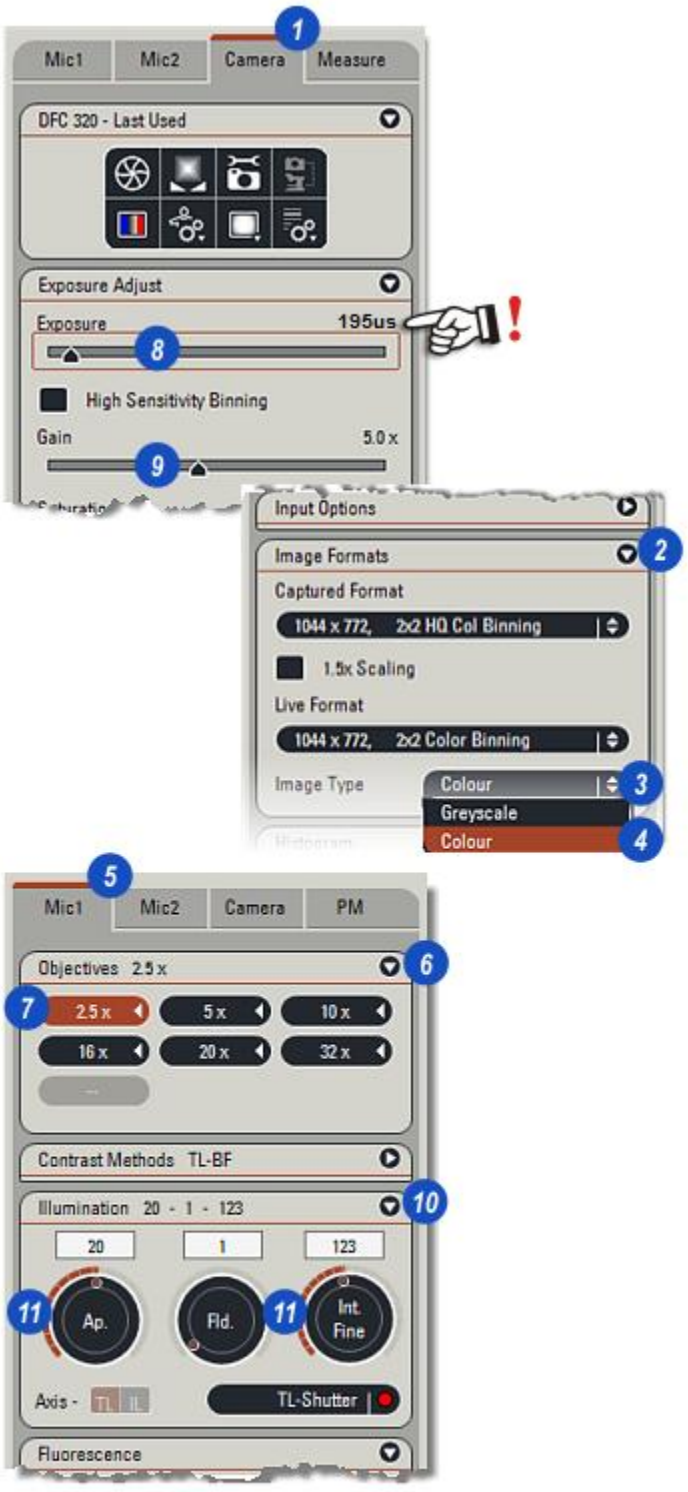
6: ..on the arrow to the right of the *Objectives* header.

7: Select the lowest magnification objective available.

8: Adjust the *Exposure* and…

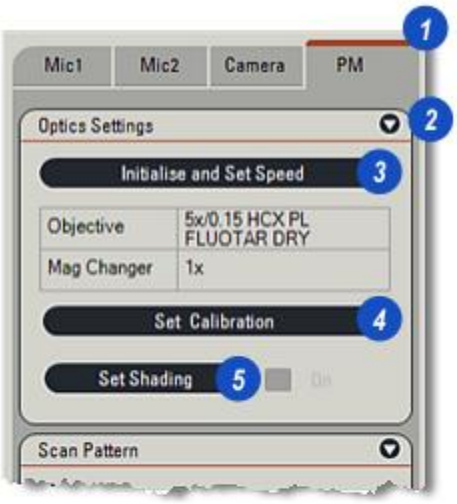
9: …the *Gain* to achieve an acceptable image with an exposure time no greater than *200µs*, necessary to run *Power Mosaic* in *TurboScan* mode.

10: It may be necessary to adjust the *Aperture* and lamp *Intensity* **(11)** on the *Microscope* tab.



1: On the *Acquire Workflow*, click on the *PM* (Power Mosaic) tab.

2: Click on the small arrow to the right of the *Optics Settings* header to reveal the panel.



Stage Initialisation:

3: Click on the *Initialise and Set Speed* button: Follow the Initialisation procedure: *Go there…*

Objective Calibration:

4: Click on the *Set Calibration* button: Follow the Calibration procedure: *Go there…*

Shading:

5: Click on the *Set Shading* button and follow the *Shading* procedures: *Go there…*

On the *PM* tab:

1: Click on the small arrow to the right of the *Scan Pattern* header to reveal the panel.

2: Click on the arrows to the right of the *Pattern Type* menu and from the drop down list click to select *Rectangular* pattern.

3: Click to enable the *TurboScan* button.

4: Click to enable the *Auto Step Size* check box.

5: Click to enable bi-directional scanning, the fastest option.

6: Disable both *Focus Method* options.

On the *Side Tool Bar* **(1)** click to select:

2: …the *Stage and Live Image* option,

3: …the *Stage View,*

4: …and *Show Scan Pattern.*

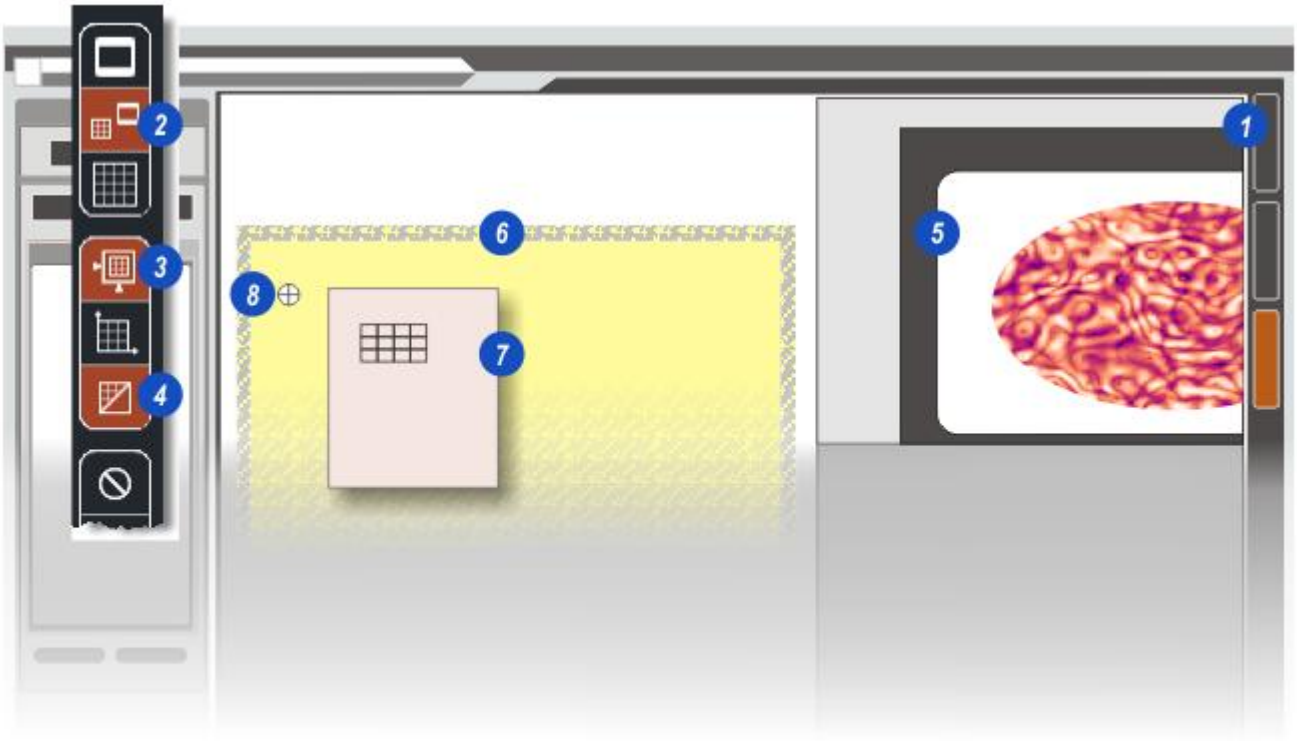
With this arrangement:

5: The Live Image appears top right of the *Viewer*.

6: The *Stage Viewable* area shown as a hatched outline.

7: A *Workspace* with *Scan Pattern* which at first use will be arbitrary sizes and positions. Subsequently, the last used *Workspace* will be displayed.

8: The *Stage Marker* which is the current stage position.



On the *PM* (Power Mosaic) tab:

1: click the small arrow to the right of the *Power Mosaic Plus* header to reveal the panel.

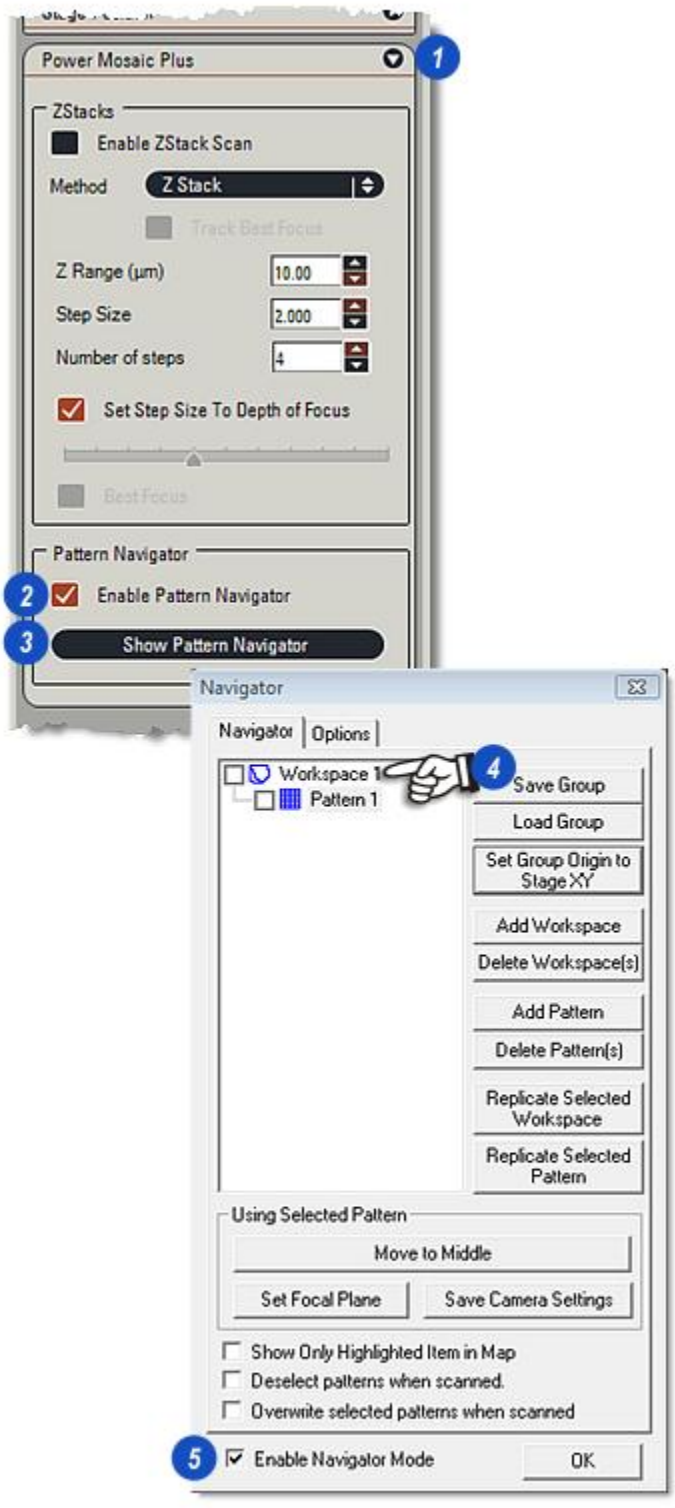
2: On the *Pattern Navigator* panel, click to enable *Pattern Navigator* and…

3: …click the *Show Pattern Navigator* button. The *Navigator* dialog appears.

4: On the dialog, the current displayed *Workspace* is listed together with the *Scan Pattern*(s) associated with it. Both *Workspace* and *Pattern* names can be changed to suit the user, so the words may differ but the layout will be the same.

5: The *Enable Navigator Mode* check box should be enabled.

Change Workspace and Scan Pattern Names: *Go there...* 867



In this example, the specimens are mounted on a standard 25 x 75mm microscope slide so the *Workspace* will be re-sized to the same dimensions.

1: *Right* click on the *Workspace* name.

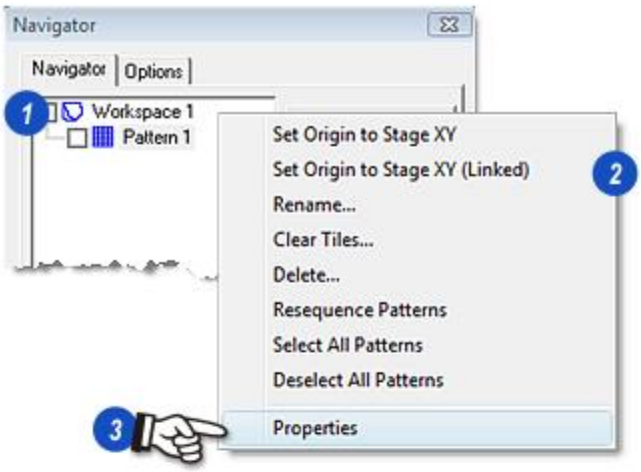
2: The *Workspace* dialog appears.

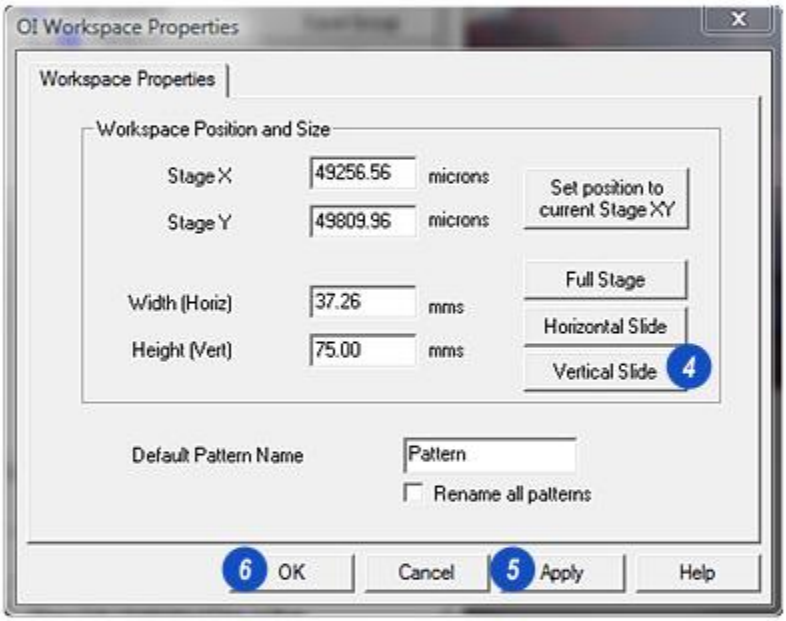
3: Click on the *Properties* option.

4: On the *Workspace Properties* dialog, click on the *Vertical Slide* button to automatically re-size the *Workspace* to 25 x 75mm orientated vertically.

5: Click *Apply* and…

6: …click *OK*.





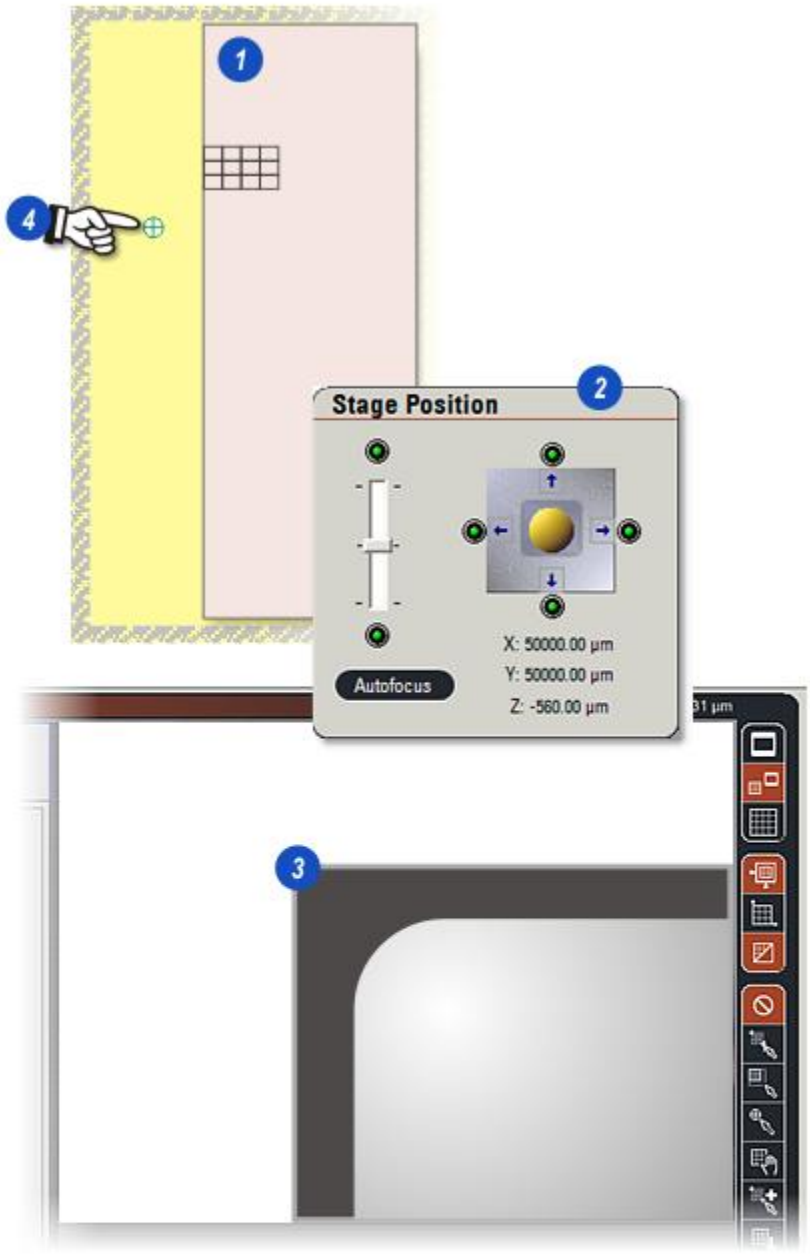
1: The *Workspace* has been re-sized to represent a standard 25 x 75 microscope slide.

The next step positions the *Workspace* over the specimen slide with their top –left-hand corners aligned:

2: Using either the on-screen *Joystick* or *Leica SmartMove* (if fitted),

3: …drive the *Stage* to the top left-hand corner of the specimen slide.

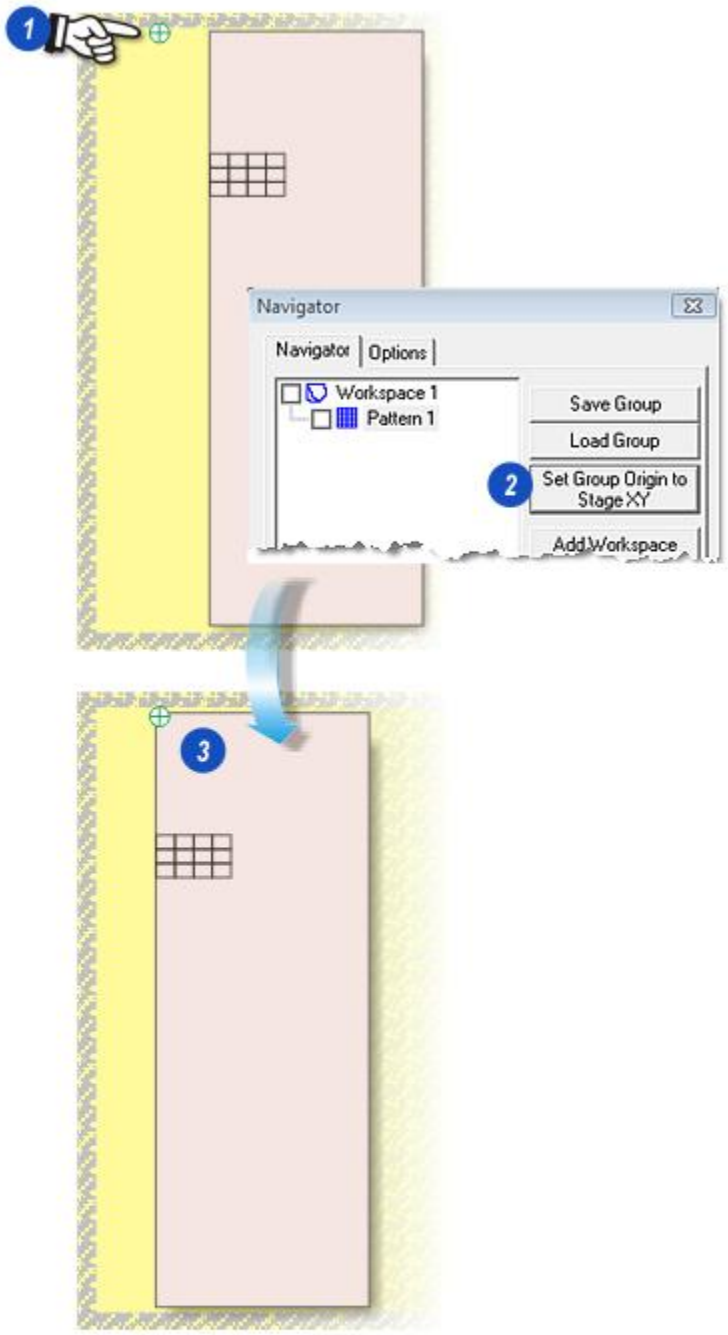
4: The *Stage Marker* moves to reflect the current stage position.



1: The *Stage Marker* has moved to the current *Stage* position which is now the top left-hand corner of the microscope specimen slide.

2: On the *Navigator* dialog, click the *Set Group to Stage XY* button.

3: The *Workspace* and *Scan Pattern* immediately move to align with the *Stage Marker* and therefore, the specimen slide.



Create a *Scan Pattern* covering the entire *Workspace* – in this example the size and shape of a microscope slide.

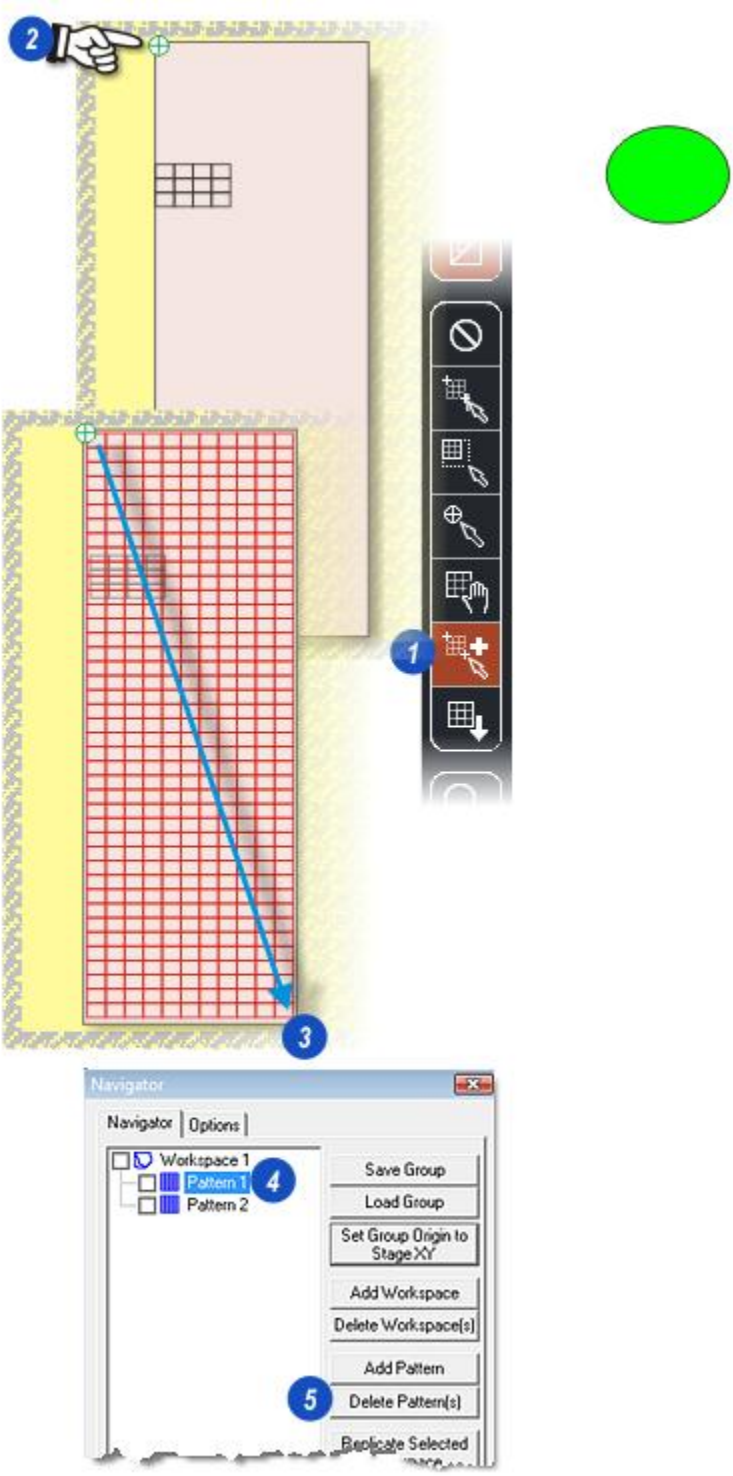
1: Click on the *Create New Pattern* button.

2: Click on the top left-hand corner of the *Workspace* and…

3: …holding the mouse button down, drag to the bottom right-hand corner and release the button.

4: Delete the original (small) *Scan Pattern* by, on the *Navigator* dialog, clicking the pattern name in the list.

5: Click on the *Delete Pattern(s)* button. Only the full-sized *Scan Pattern* now remains.

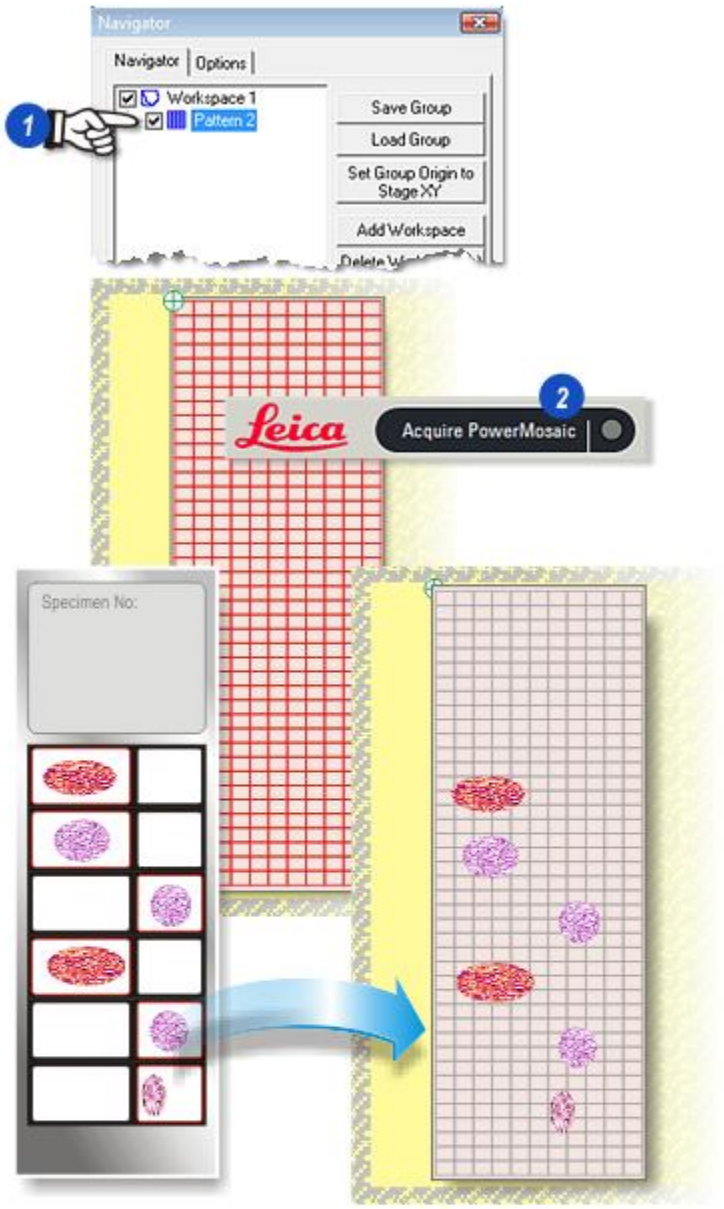


Only the *Workspaces* and *Scan Patterns* that are selected are scanned – the check box alongside has to be enabled:

1: Click to enable the check boxes to the left of the *Workspace* and *Scan Pattern.*

2: Start the scan of the entire specimen slide by clicking on the *Acquire Power Mosaic* button.

All of the specimens appear positioned against the *Scan Pattern* that will be used to precisely locate individual patterns ready for high quality image captures.



Each specimen is now given its own individual *Scan Pattern* and it is these that will be used to capture the final, high quality images.

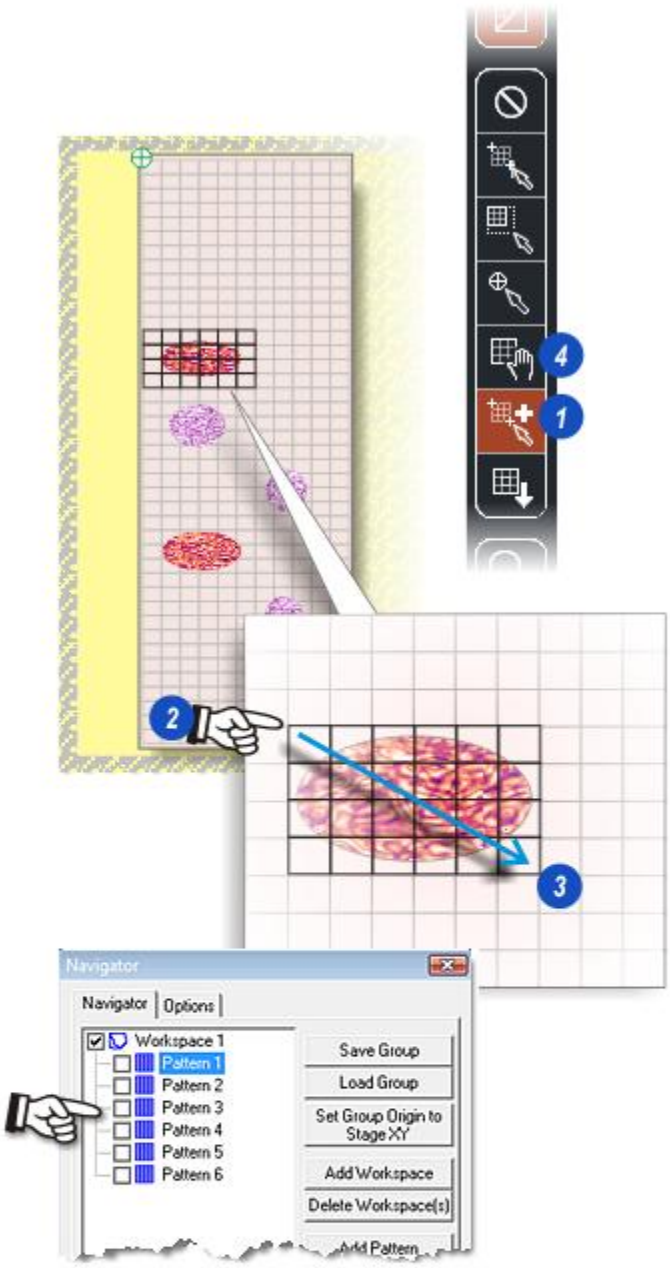
1: Click on the *Create New Pattern* button.

2: Click on the large *Scan Pattern* above and to the left of the specimen. Holding down the mouse button drag down and to the right to create a pattern that fully encloses the specimen.

3: Release the mouse button.

4: If necessary, click on the *Move Pattern* button and then on the new pattern. With the mouse button held down drag the *Scan Pattern* to the correct position.

Repeat the process for all of the other specimen areas. As the patterns are created they are added to the *Workspace* list.



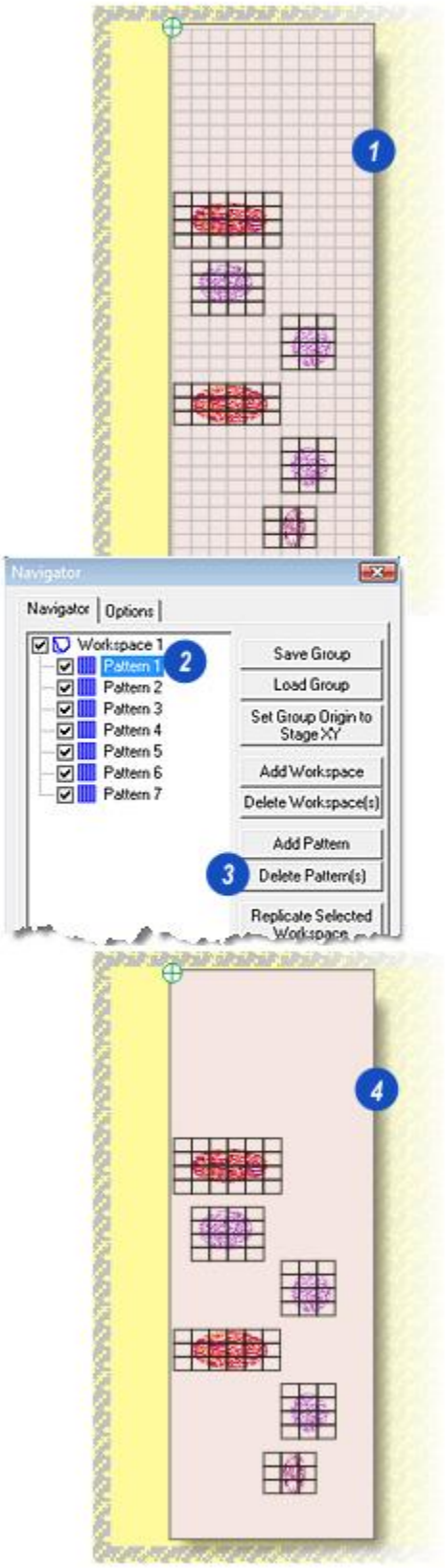
Remove Guide Pattern

1: All of the required individual *Scan Patterns* are in place. The next task is to remove the large guide *Scan Pattern.*

2: Click on the large *Scan Pattern* on the *Navigator* dialog to select it. Selected *Scan Patterns* are highlighted on the *Workspace*.

3: Click on the *Delete Pattern(s)* button.

4: The large guide pattern is removed leaving just the individual patterns.



At this point it is advisable to save the *Workspace* and its *Scan Patterns.*

1: On the *Navigator* dialog, click on the *Save Group* button.

2: The *Windows Save As* dialog appears.

3: The default folder for saved *Workspaces* is *OIGroups* but this can be changed to suit the user simply by navigating to an existing folder or creating a new one.

4: Type a unique name for the *Workspace* group.

5: Click *Save*.

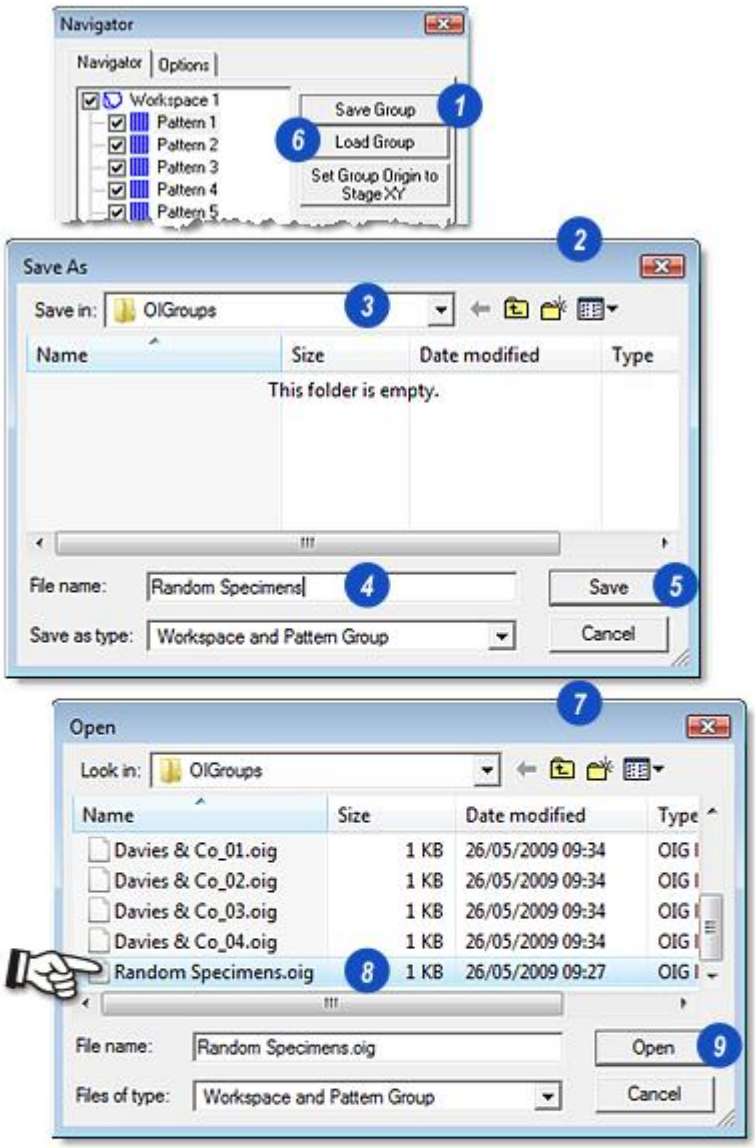
Loading a Saved Workspace Group:
To re-load a *Workspace* and its *Scan Patterns:*

6: Click on the *Navigator Load* button.

7: On the *Open* dialog…

8: …click on the required file with the ***.oig*** extension – ***not*** on the folder of the same name.

9: Click *Open* and the *Workspace* will be loaded and displayed.



Before scanning with the individual Scan Patterns:

1: On the *Camera* tab, select a higher quality *Captured Format.*

2: On the *PM* tab, select the *Scan Pattern* panel and decide upon *TurboScan* (high speed but lower precision) or *Standard Scan* (slower but higher precision). The higher quality image formats will often preclude using *TurboScan* due to the high capture time.

3: Decide upon bi-directional (faster but lower precision) or uni-directional scanning (slower but better precision).

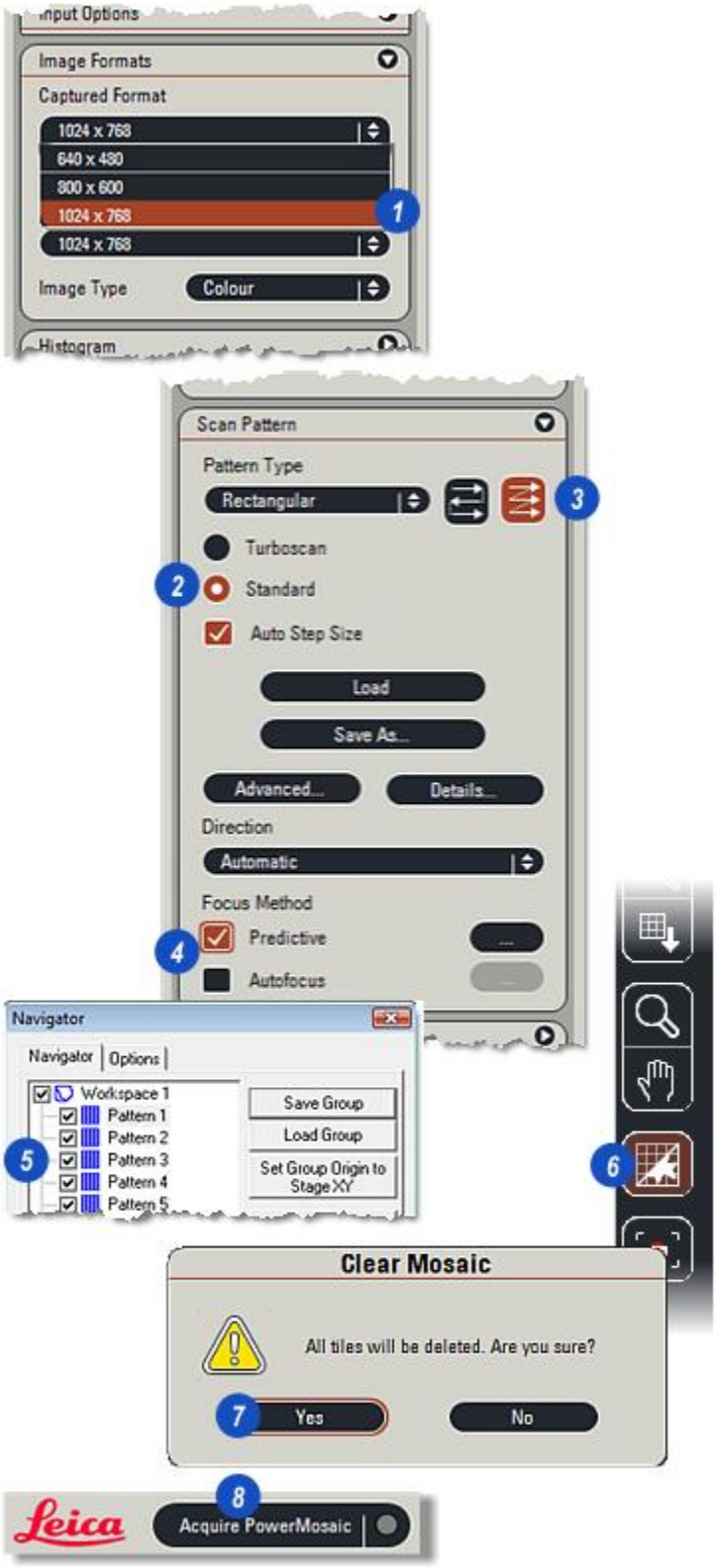
4: Select the *Focus Method*. This may require re-calibrating the focus.

5: Select the specimens to scan by enabling the check box to the left of each pattern.

6: Click on the *Clear Tiles* button to remove the previous scans and...

7: ...confirm the deletion.

8: Then start scanning…



This section describes the wide range of features available in *Pattern Navigator.*

On the *Navigator* dialog with a *Scan Pattern* selected:

1: Clicking *Move to Middle*, drives the stage to the centre of the selected *Scan Pattern.*

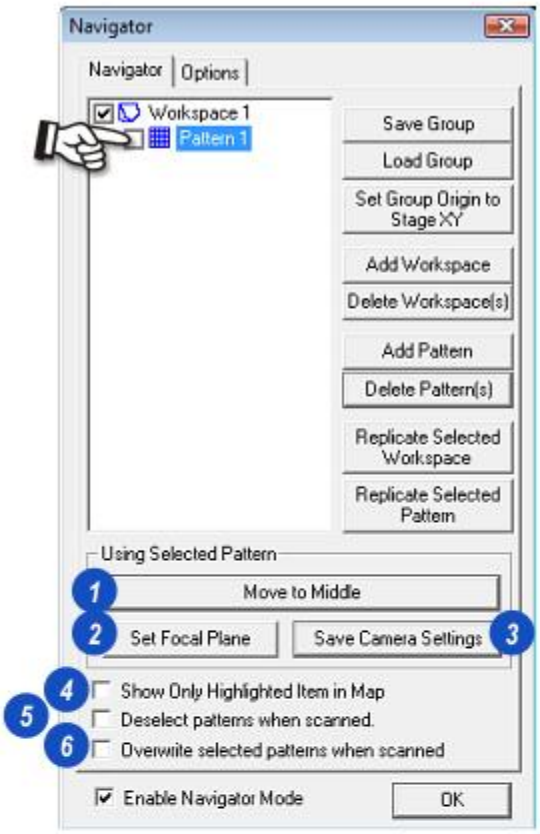
2: *Set Focal Plane* sets the Z axis to the centre of the current *Scan Pattern*.

3: *Save Camera Settings:* Not required.

4: Enable the check box to hide all of the *Scan Patterns* except the one selected.

5: Enable the check box to have *Scan Patterns* de-selected (check box to the left of the pattern name is cleared) after they have been scanned. The check box will have to be enabled before the pattern can be scanned again.

6: When *Overwrite* is enabled, any scanned images already present will be overwritten with a new image from the current scan.



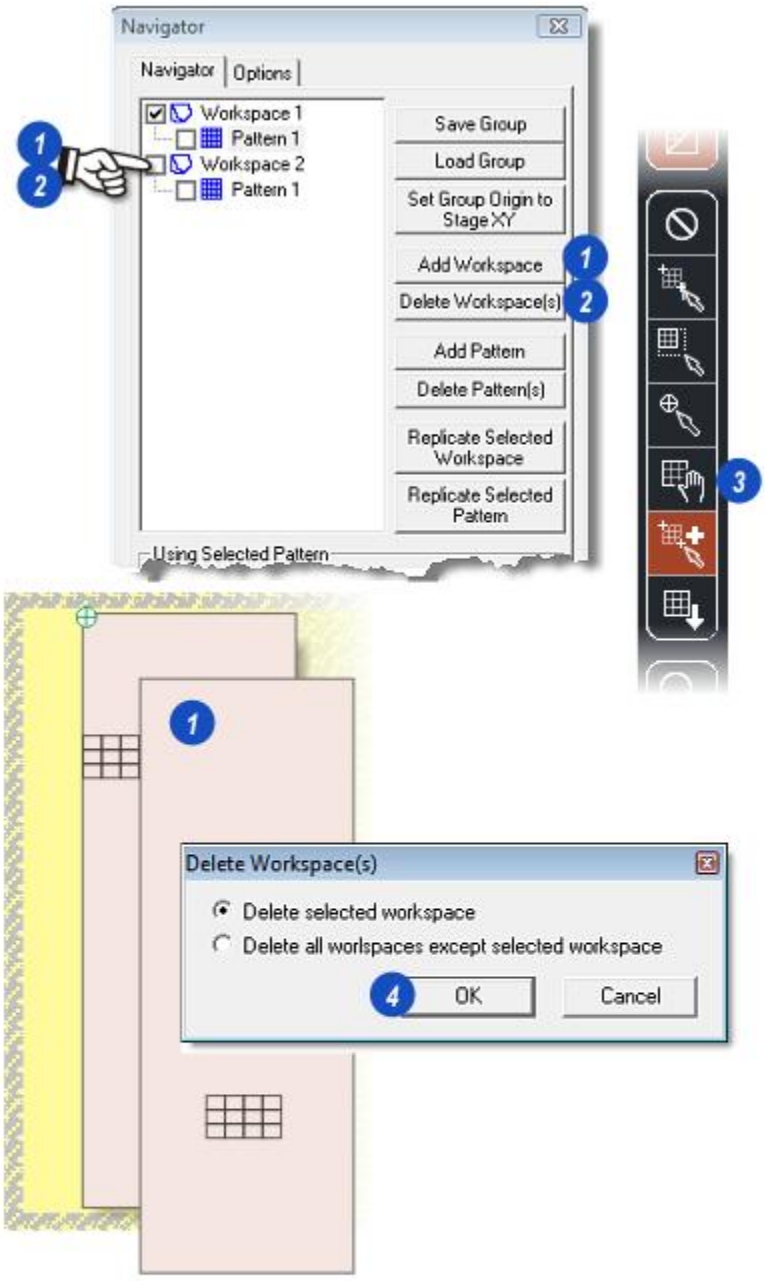
To add a Workspace:

1: Click on a *Workspace* name on the *Navigator* list. This is the *Workspace* that will be copied and added. Click on the *Add Workspace* button. A duplicate of the selected *Workspace* appears with a new entry in the list. At least one *Scan Pattern* is created with a new *Workspace*.

Reposition the copy by selecting the *Drag and Drop* button **(3)** on the *Side Toolbar*, clicking on the *Workspace* copy and dragging it to a new location. A *Workspace* can be positioned precisely by using the *Workspace Properties* dialog*: Go there…*

Delete a Workspace:

2: Click on the *Workspace* name to be deleted on the *Navigator* list. Click on the *Delete Workspace(s)* button. On the *Delete Confirm* dialog **(4)** select either *Delete the Selected Workspace* or *Delete All but the Selected Workspace*. Click *OK* and the *Workspace(s)* will be removed permanently.



To Add a Scan Pattern:

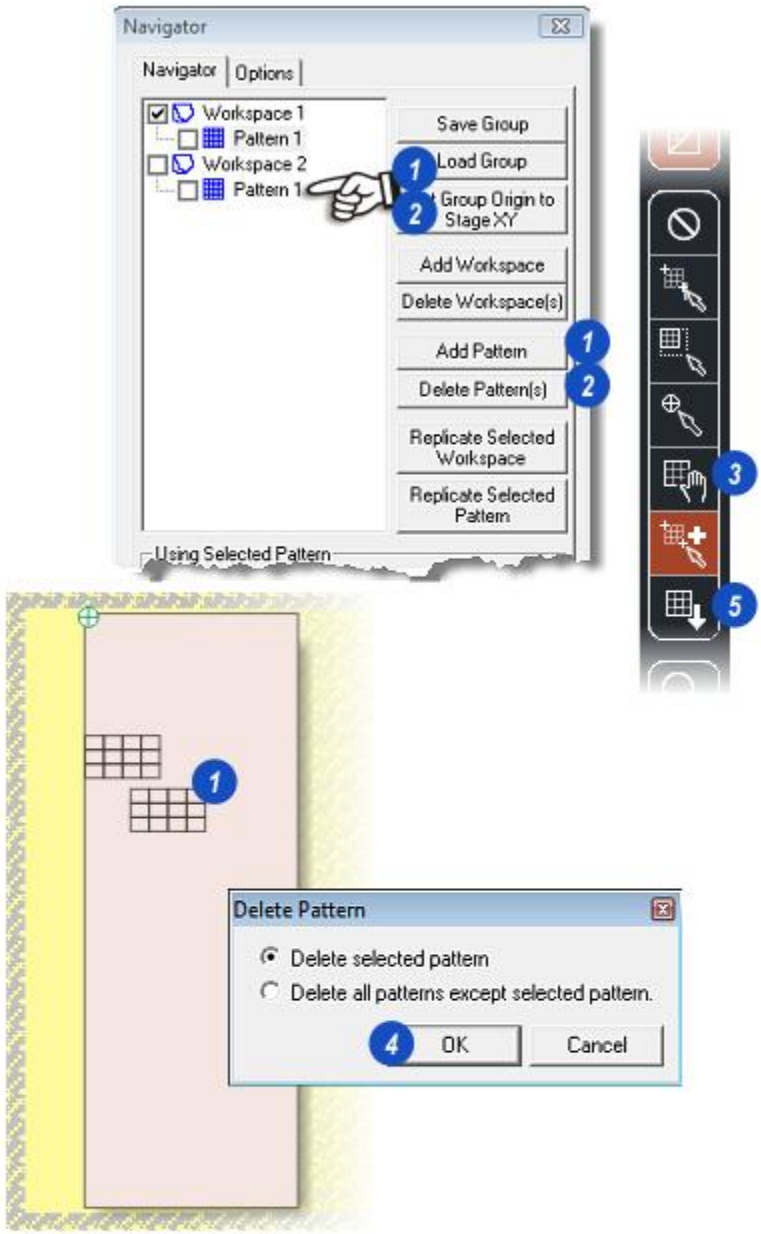
1: Click on the pattern name on the *Navigator* list to be duplicated and added. Click the *Add Pattern* button and a copy of the pattern will appear. Use the *Drag and Drop* tool **(3)** to re-position it.

Delete a Scan Pattern:

2: Click on the pattern name to be deleted on the *Navigator* list. Click on the *Delete Pattern(s)* button. On the *Delete Confirm* dialog **(4)** select either *Delete the Selected Pattern* or *Delete All but the Selected Pattern.* Click *OK* and the patterns(s) will be removed permanently.

Copy a Scan Pattern:

5: Click on the *Copy Pattern* button to copy the currently selected *Scan Pattern* to the 'cursor' and click inside the Workspace to place a copy. Repeat for as many patterns required.



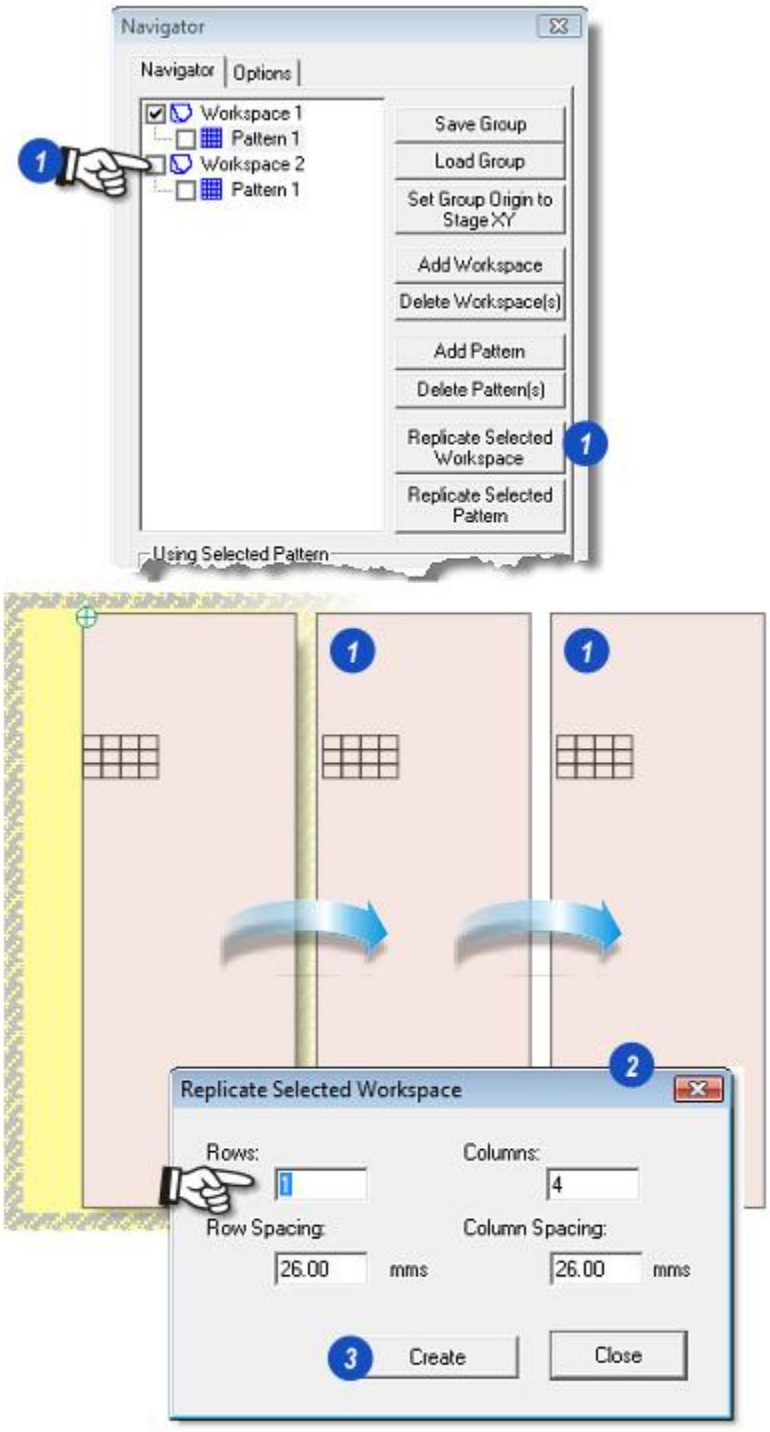
Workspace replication makes multiple copies of the selected *Workspace*, including any attached *Scan Patterns*, and places them in rows and columns determined by the user. The original *Workspace* is removed.

1: Click on the *Workspace* name on the *Navigator* list that is to be replicated. And click on the *Replicated Selected Workspace* button.

2: The *Replicate* dialog appears. Set the number of *Rows* and *Columns* required and their spacing by clicking in the appropriate window and typing a value.

3: Click **Create**. A warning appears advising that the original will be deleted and replaced by a new group each *Workspace* of which will be a faithful copy of the original.

The *Row* and *Column* settings can be adjusted and the *Create* button clicked again without closing the dialog.



Scan Pattern replication, makes multiple copies of the selected pattern and arranges them precisely in a matrix either created by the user or based upon the range of templates supplied with the Navigator.

1: Click on the *Pattern* name on the *Navigator* list and click the *Replicate Selected Pattern* button.

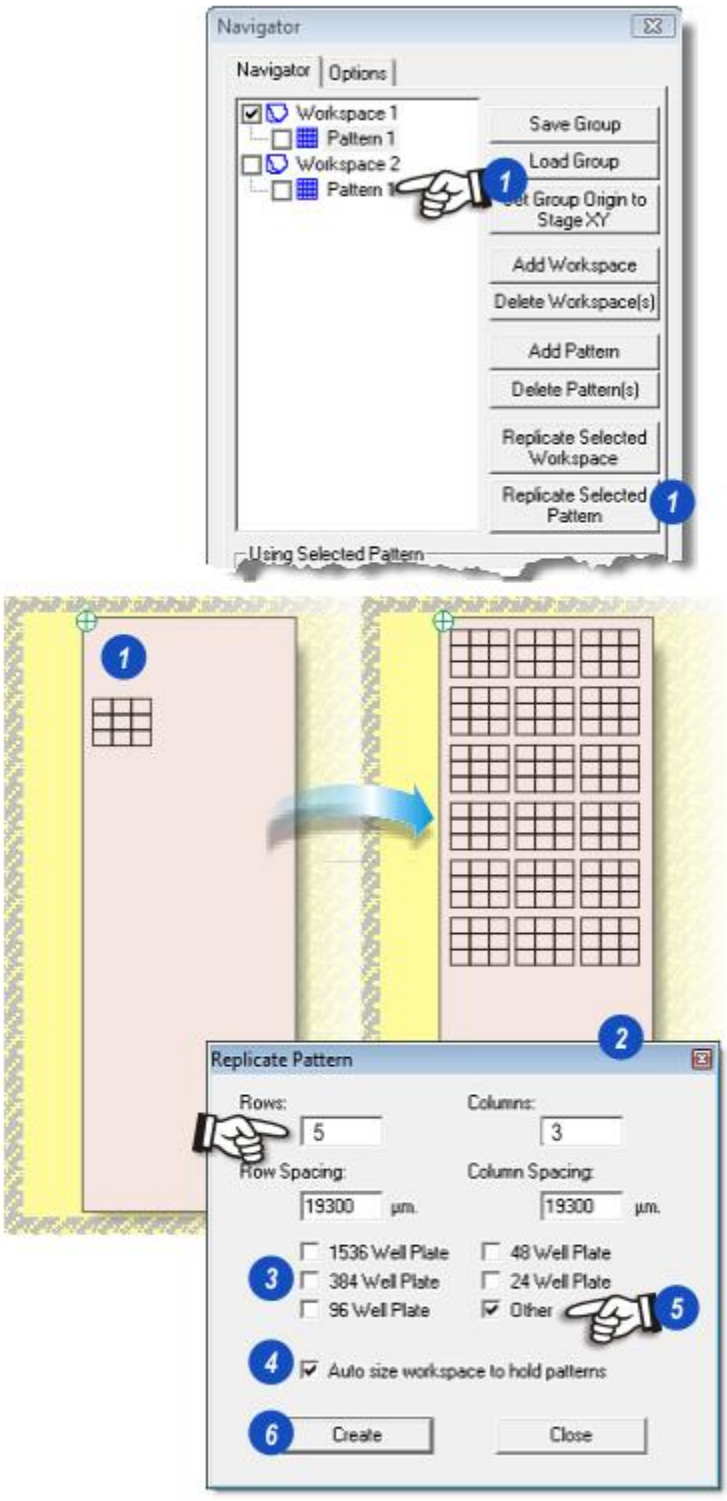
2: On the *Replicate Pattern* dialog, enter the number of *Rows* and *Columns* as well as the spacing between, by clicking inside the appropriate text box and typing a new value.

3: A range of templates for standard *Well Plate* configurations are provided and a template can be selected by clicking to enable the check box to its left.

4: If the current *Workspace* is too small to accommodate the selected template, it is automatically re-sized.

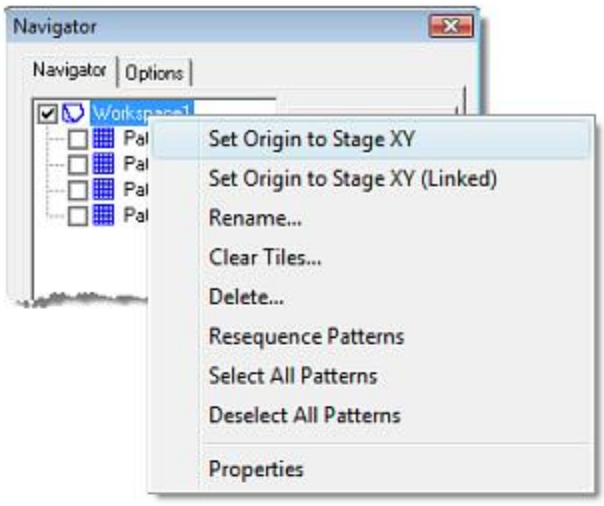
5: For user-defined column and row layouts click to enable the *Other* option and, if necessary, enable the automatic re-sizing facility **(4).**

6: Click the *Create* button. The selected pattern is replicated on its *Workspace*. The *Row*, *Column* and spacing values can be adjusted and the Create button clicked again without closing the dialog.



Workspace Alternate Menu

Right-clicking on a *Workspace* name on the *Navigator*, reveals an alternate menu. Click to select the high-lighted option:



Set Origin to Stage X/Y: Moves the entire selected *Workspace* and patterns to the current stage position. The *Stage Marker* is centred on the 'first' *Scan Pattern.*

Rename: Type a new name for the *Workspace*. It must be unique.

Clear Tiles: Clears all of the mosaic filed images form all of the patterns on the *Workspace*.

Delete: Deletes the selected *Workspace* and its patterns.

Re-sequence Patterns: Re-numbers the pattern sequence starting at '1'.

Select All Patterns: Selects and high lights all of the patterns on the *Workspace*.

Deselect All Patterns: Deselects all selected *Scan Patterns.*

Right click on a *Workspace* name on the *Navigator* list and from the drop down menu:

1: Click on the *Properties* option.

2: The *Workspace* (top left corner) can be positioned precisely by setting the *Stage-X* and *Y* co-ordinates and clicking the *Apply* button **(7).**

3: *Set Position to Current Stage XY* when clicked will move the *Workspace* to the current stage position.

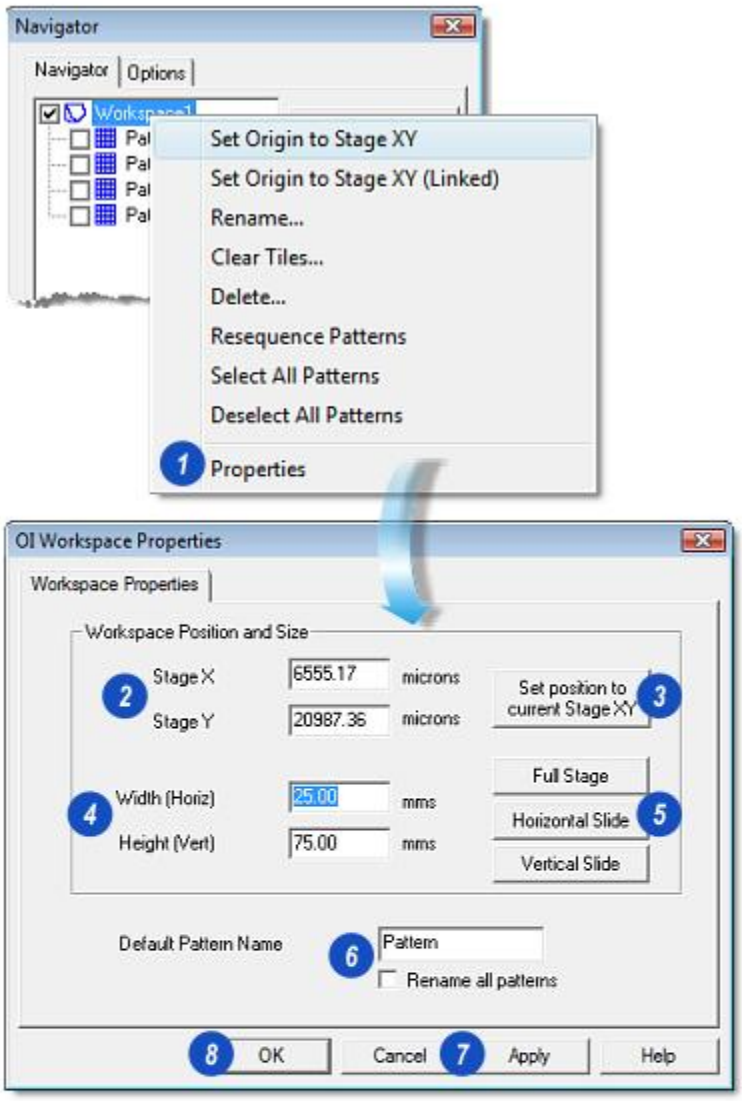
4: The *Workspace* can be re-sized by clicking in the *Height* and *Width* text boxes and typing a new value. Click the *Apply* button **(7).**

5: Three pre-set templates – *Full Stage, Horizontal* and *Vertical Slides* – are provided. Click on an option to automatically size the *Workspace*.

6: The default name for *Scan Patterns* is 'Pattern' followed by a sequential number as they are created. To change the default name, click in the *Default Pattern Name* text box and type a new name. To retrospectively rename all existing names, click to enable the *Rename All* check box.

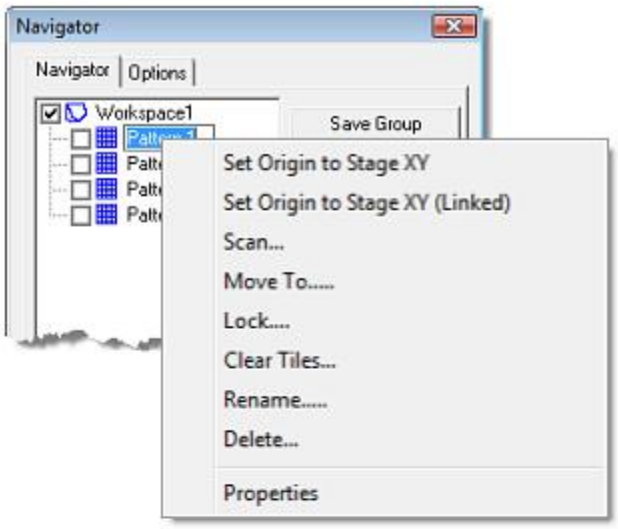
7: Click *Apply* and…

8: …click *OK*.



Scan Pattern Alternate Menu

Right clicking on a *Scan Pattern* name on the *Navigator*, reveals an extended menu of options. Click to select a menu item:



Set Origin to Stage XY: Moves the *Scan Pattern* and its *Workspace* to the current stage position. The *Stage Marker* is located at the centre of the *Scan Pattern.*

Scan: Starts a scan for the selected pattern only.

Move To..: Selects the *Drag and Drop* tool. Click on the *Scan Pattern* and drag it to the required location.

Lock: Locks the *Scan Pattern* at its current position and a small padlock icon appears to the left of its name on the *Navigator*. Click *Lock* again to unlock it.

Clear Tiles: Removes any mosaic fields in the selected pattern only.

Rename: Type a unique name for the pattern.

Delete: Deletes the selected *Scan Pattern* only.

To display the *Scan Pattern* properties:

1: Click on the arrow to the right of the *Scan Pattern* header to reveal the panel.

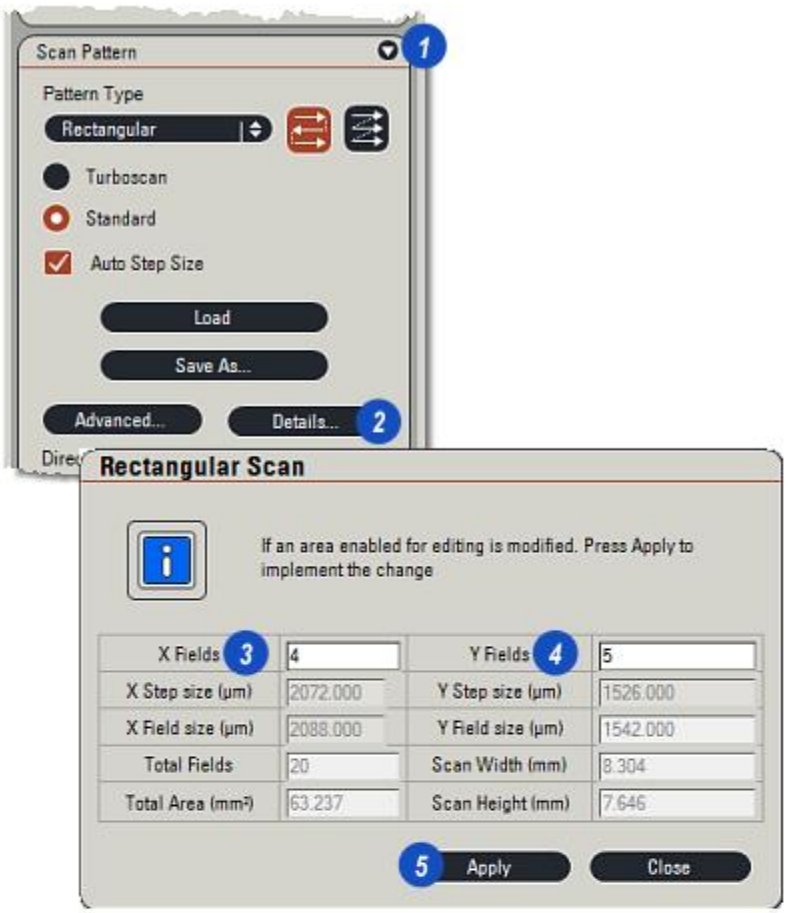
2: click on the *Details* button. The *Scan* dialog appears – in this case *Rectangular* layout.

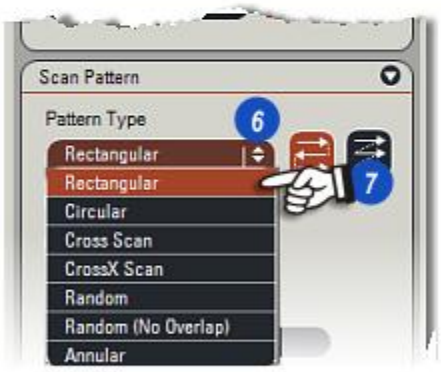
3 & 4: Change the number of *Columns* (X) and *Rows* (Y) by clicking inside the appropriate text box and typing a value.

5: Click *Apply* and the selected pattern will change to reflect the new values.

6: A *Scan Pattern Type*– in this example the *Rectangular* layout has been used – can be changed to better suit the specimen shape and optimise the number of images required. Click on the arrows to the right of the *Pattern Type* header and from the drop down list…

7: …click to select the required type.





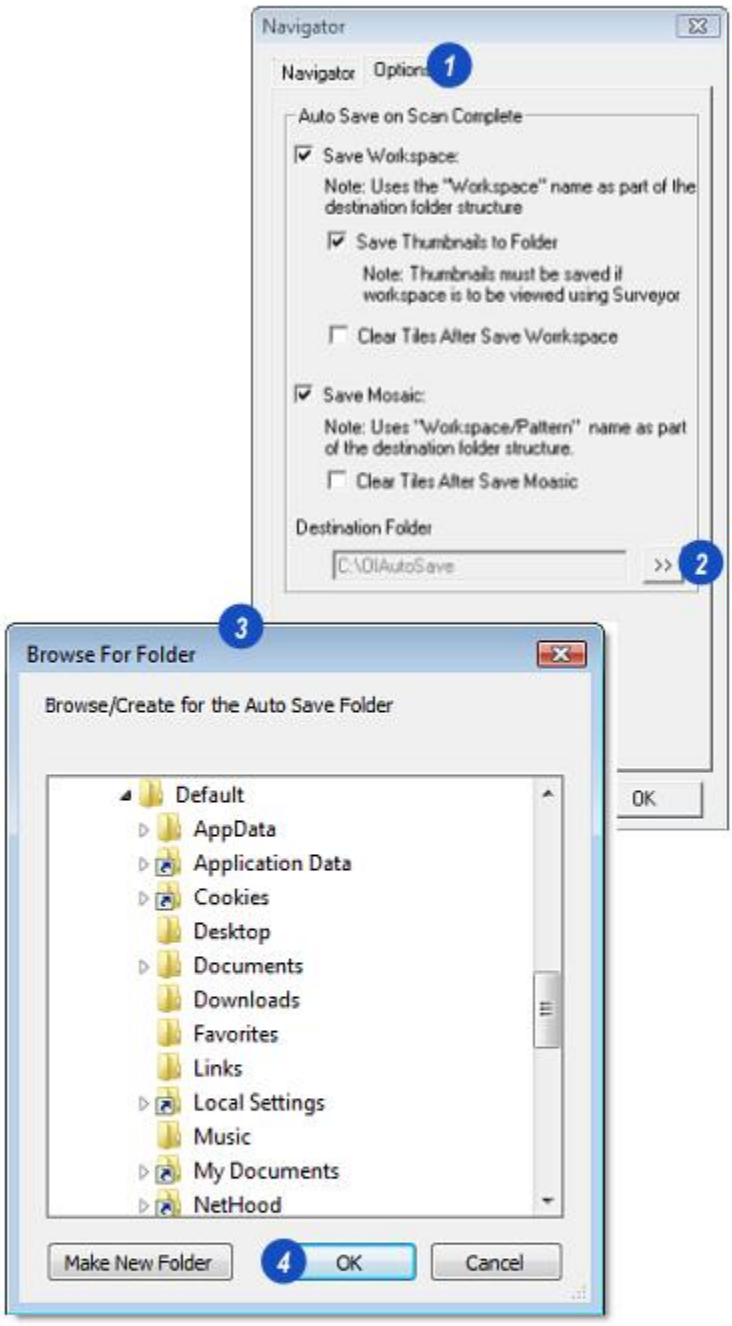
When the scan is complete, there are several options that can be invoked automatically – such as saving the mosaic.

1: Click on the *Navigator Options* tab to reveal the options. Check to enable the required options:

Save Workspace: Automatically saves the current *Workspace* with the word '*Workspace*' as the file name followed by a sequential number – *Workspace-05* for example.

Save Thumbnails to Folder: Saves a low resolution thumbnail of each scan tile within the *Workspace* folder to be used in the *Gallery*.

Clear Tiles After Save Workspace/ Mosaic: Clears the scan tiles from all *Scan Patterns*. Especially important where machine memory is at a premium. Use a greater Display Thumbnail Reduction Factor to reduce memory usage. *Go there...* 872



There are a large number of options that can be used to tailor *Pattern Navigator* exactly to a user's needs. The major options are explained here. The *Map Properties* dialog is reached by:

1: Click on the *Interactive Mouse* button on the *Side Tool Bar* and then right-click anywhere on the stage display area. The *Map Properties* dialog appears.

2: If necessary, click on the *Map* tab to reveal the main functions.

3: The *View* panel determines the current *Navigator* view:

Stage: The viewable *Stage* area with the *Workspaces* and *Scan Pattern(s).*

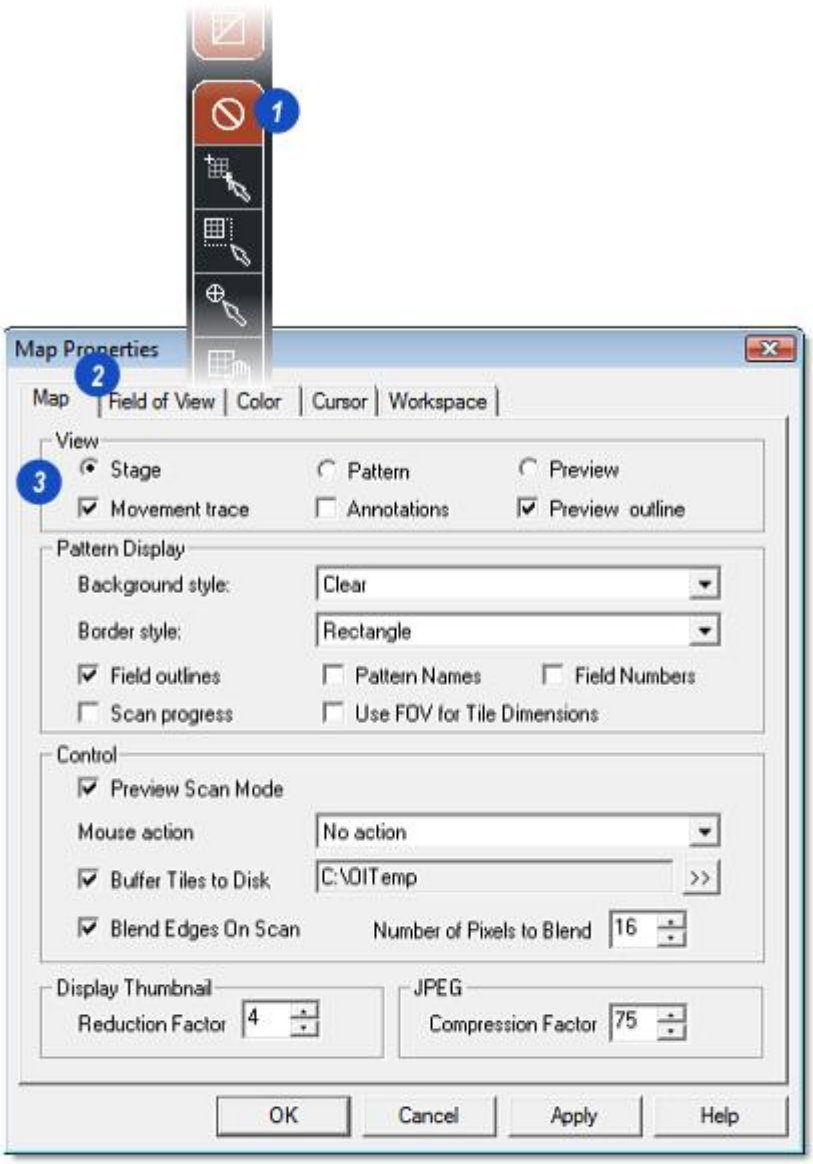
Pattern: The selected pattern is displayed enlarged.

Preview: Not required.

Movement Trace: Displays the trace path over the *Scan Pattern* **(4).**

Annotations: Turns On and Off *Pattern Name* and *Field Numbers* if they are enabled.

Preview Outline: Not required.



In the *Pattern Display Panel*:

1: Click on the arrow to the right of *Background Style* menu to reveal how the *Scan Pattern Fields* should be highlighted and filled. Click a menu item to select it.

2: The *Scan Pattern* border style can be changed by clicking on the arrow to the right of the *Border Style* menu and clicking to select an option. *Rectangle* **(3)** is the 'normal' rectangle configuration and…

4: …*Circle* configures the pattern as a series of overlapping, circular fields.

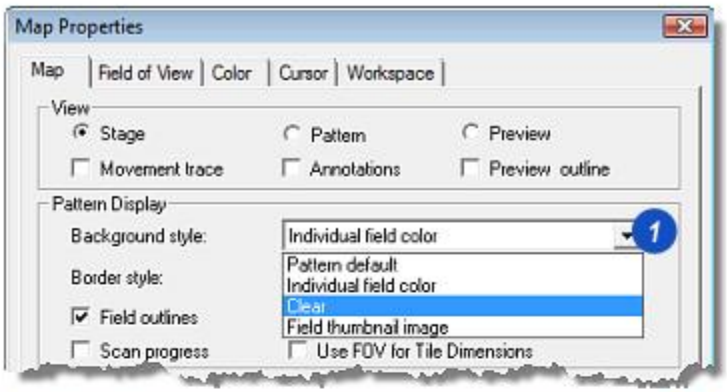
Field Outlines: Displays an outline around each field.

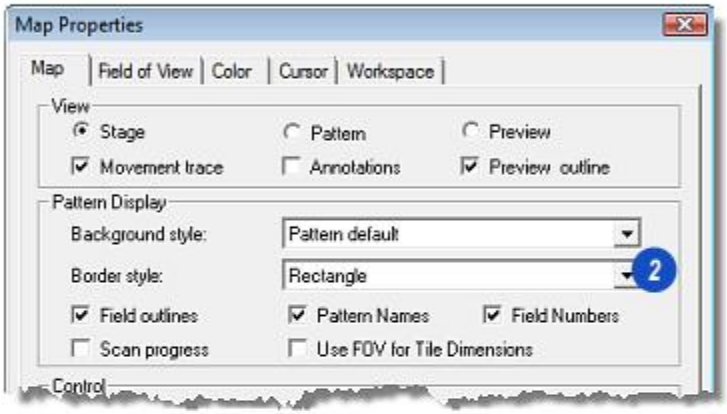
Pattern Names: Displays the *Pattern Name* beneath the pattern **(4).**

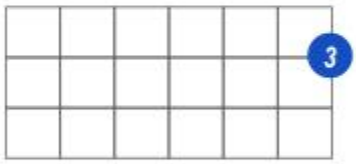
Field Numbers: Displays a sequential number against each field **(4)**.

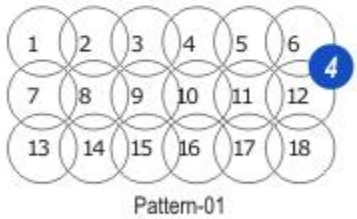
Scan Progress: Displays a progress bar during scanning.

Use FOV for Tiles: The *Field of View* determines the field dimensions.









Pattern-01

1: Click on the *Colour* tab to reveal the colour options.

2: On the left-hand panel, click to select the item to have a colour change. Use the slider on the right to display all of the items.

3: Choose a colour from the right-hand display. The first colour entry has the option to select a user colour via the *Windows Colour* dialog.

4: Click on the *Cursor* tab to modify the *Cursor* – stage marker.

5: Adjust the values to construct the cursor of choice – even an elliptical outline **(6)**.

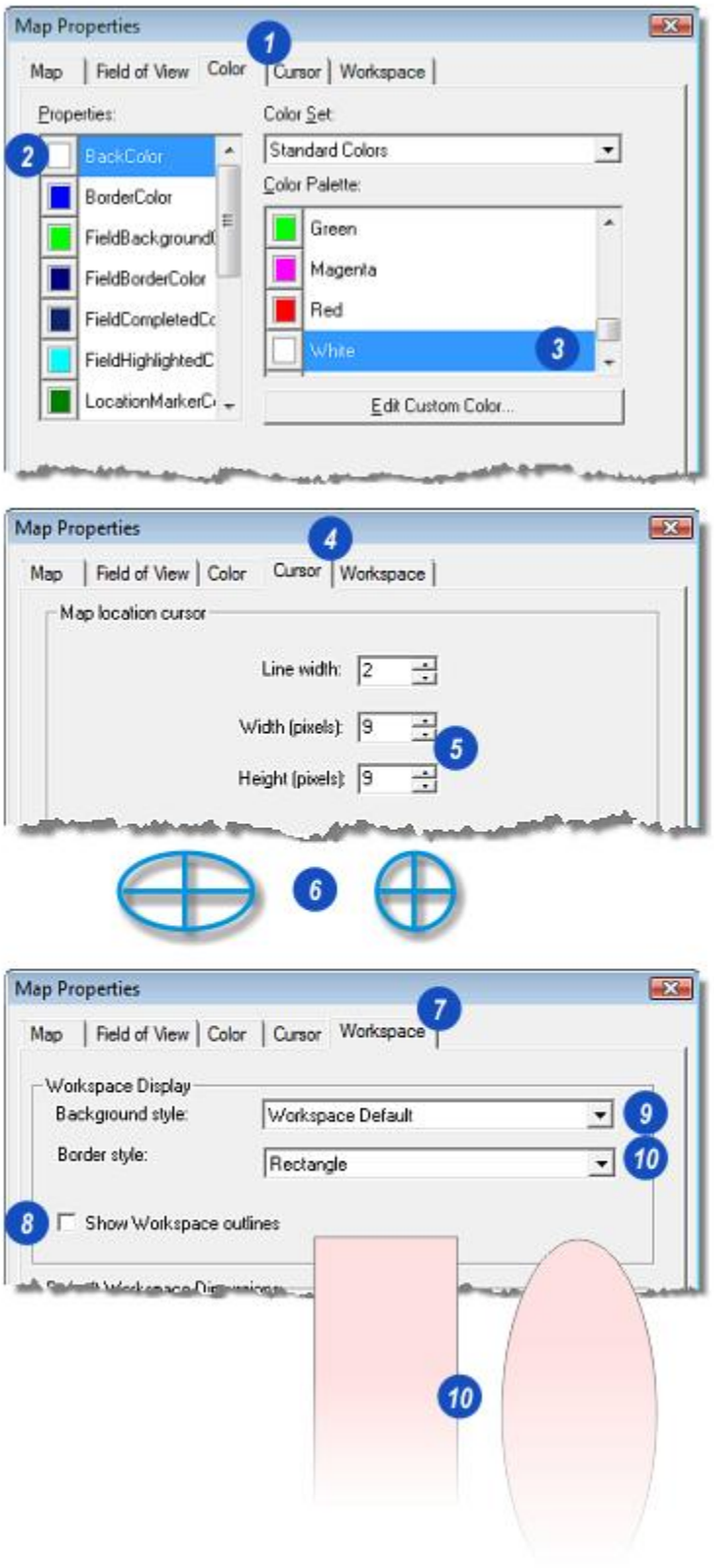
The *Workspace* can be displayed or hidden and its shape and colour changed by:

7: Click on the *Workspace* tab.

8: Display or hide the *Workspace* by checking or clearing the check box.

9: Click on the small arrow to the right of the *Background Style* menu to list the options - display in selected colour (See Step 2 above) or display the outline only.

10: The shape of the *Workspace* is set by clicking the small arrow to the right of the *Border Style* menu and selecting from *Rectangular* or *Circular*.



Some of the main *Pattern Navigator* properties can be accessed through the *LAS Power Mosaic* panels as follows:

1: Click on the small arrow to the right of the *Scan Pattern* header.

2: Click on the *Advanced* button.

On the *Advanced Settings* dialog:

3: Set the scan tiles capture folder by enabling the *Buffer Tiles to Folder* check box,…

4: …click on the browse button and, on the Windows dialog, navigate to and select the destination folder.

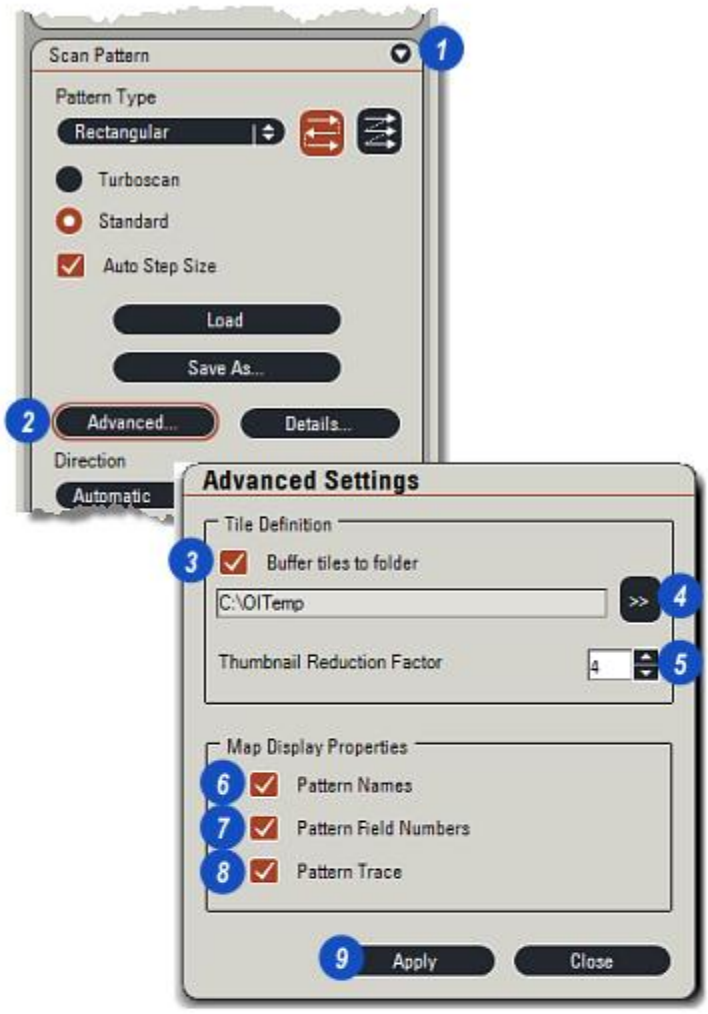
5: Set the *Thumbnail Reduction Factor* – a larger number results in more memory space-saving – by clicking on the Up/Down arrows to the right of the text box.

6: Display/Hide the *Scan Pattern Names* by checking or clearing the check box.

7: Display/Hide the *Scan Pattern Field Numbers* by checking or clearing the check box.

8: Enable/Disable the stage travel *Trace* by checking or clearing the check box.

9: Click *Apply* to load the settings and close the dialog.



After a scan (or scans), the tiles are saved in a temporary file on the hard drive and the thumbnails in RAM. Both may be saved to a nominated folder - the Capture Folder - on the hard drive.

Select the Capture Folder:

1: Click on the *Browse Workflow* tab.

2: Click on the archive folder in which to save the scans.

3: Click on the *Set Fixed Capture Location* button.
A red dot appears to the left of the selected folder.

Save Tiles

There are two parts to saving the scanned images – saving the collection of individual tiles which at this point are still being stored in a temporary file, and thumbnails of the tiles that are in volatile RAM, - and then saving the tiles as a composite image or mosaic.

1: Click on the *PM* (Power Mosaic) tab.

2: Click on the *Save Workspace Location* button. A Workspace is a detailed description of the display and image settings saved as a file with the extension *.sws* within the Workspace Location. Additionally, another folder to contain the individual tiles is created within the Workspace Location together with Thumbnail images of each tile.

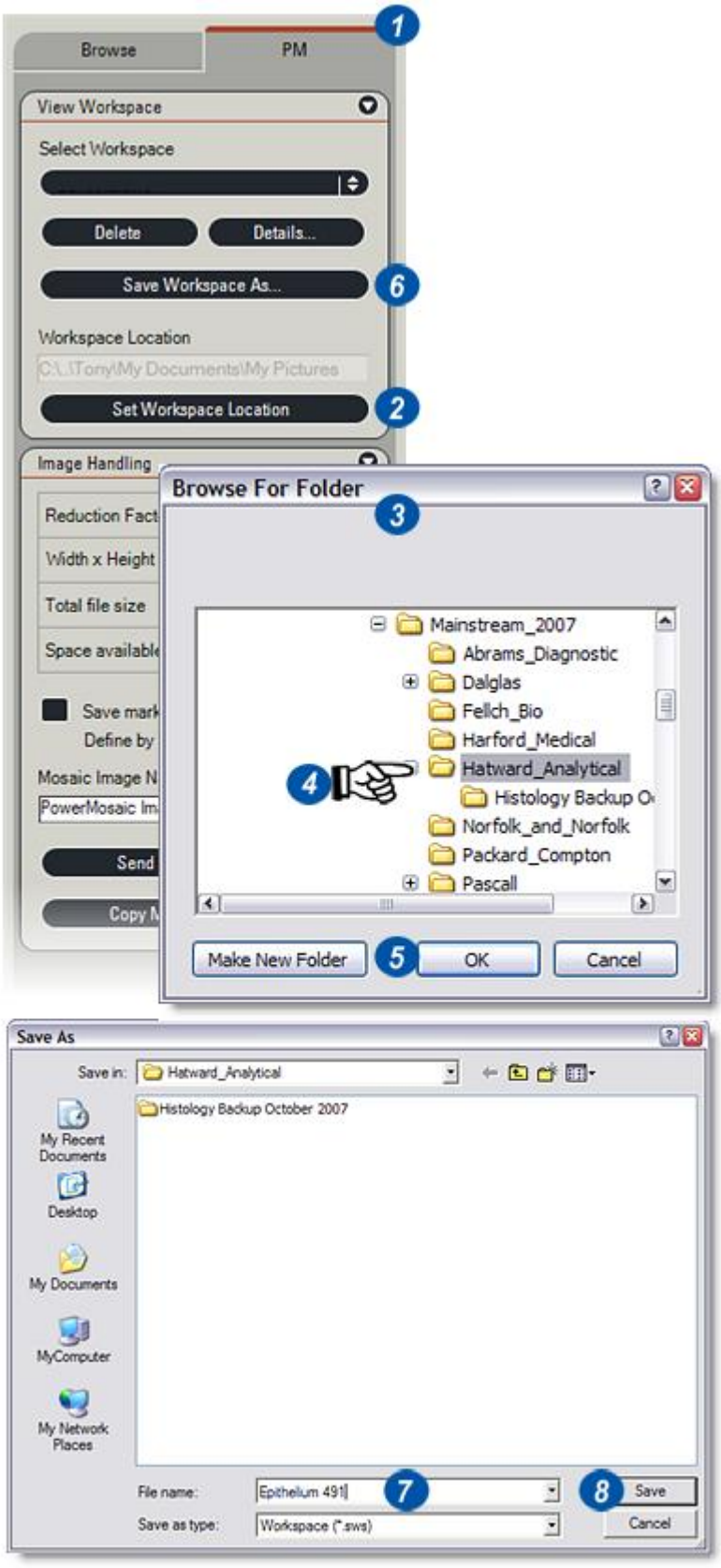
3: On the Browse For Folder dialog...

4: ...navigate to the folder in which to save the Workspace and...

5: ...click *OK*.

6: Click on the *Save Workspace As* button and on the *Save As* dialog...

7: ...type a unique name for the Workspace and click *Save* **(8)**.
A *Saving mosaic images progress* message appears and the tiles are saved at the format and resolution selected on Camera:Input Options.

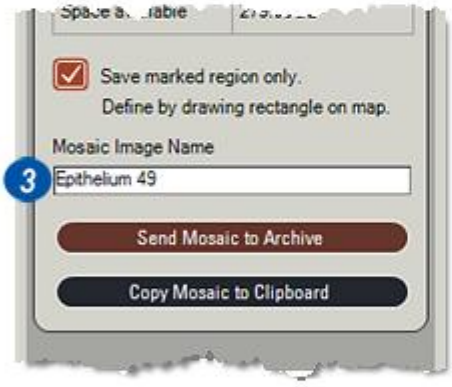


The composite image or mosaic file is saved directly in the Capture Folder with several associated control files. Because mosaic images can be very large in terms of disk space occupied – several gigabytes is not unusual – there is a facility to reduce the size of the saved mosaic. This is called the reduction factor.

1: Click on the arrow to the right of the *Image Handling* header to reveal the panel.

2: Using the arrows to the right of the *Reduction Factor* text box, increase or decrease the Reduction Factor. The illustrations show the considerable difference in image size with factors of 1 and 4.

3: Click in the *Mosaic Image Name* text box and type a name for the mosaic. The image will be stored with this name in the Capture Folder.



It is not necessary to save the entire mosaic – very often only one part is of real interest and the remainder can be discarded. It is possible to 'crop' the image to a selected region and save that alone. The individual tiles are not affected – they remain intact.

To save a selected part of the mosaic:

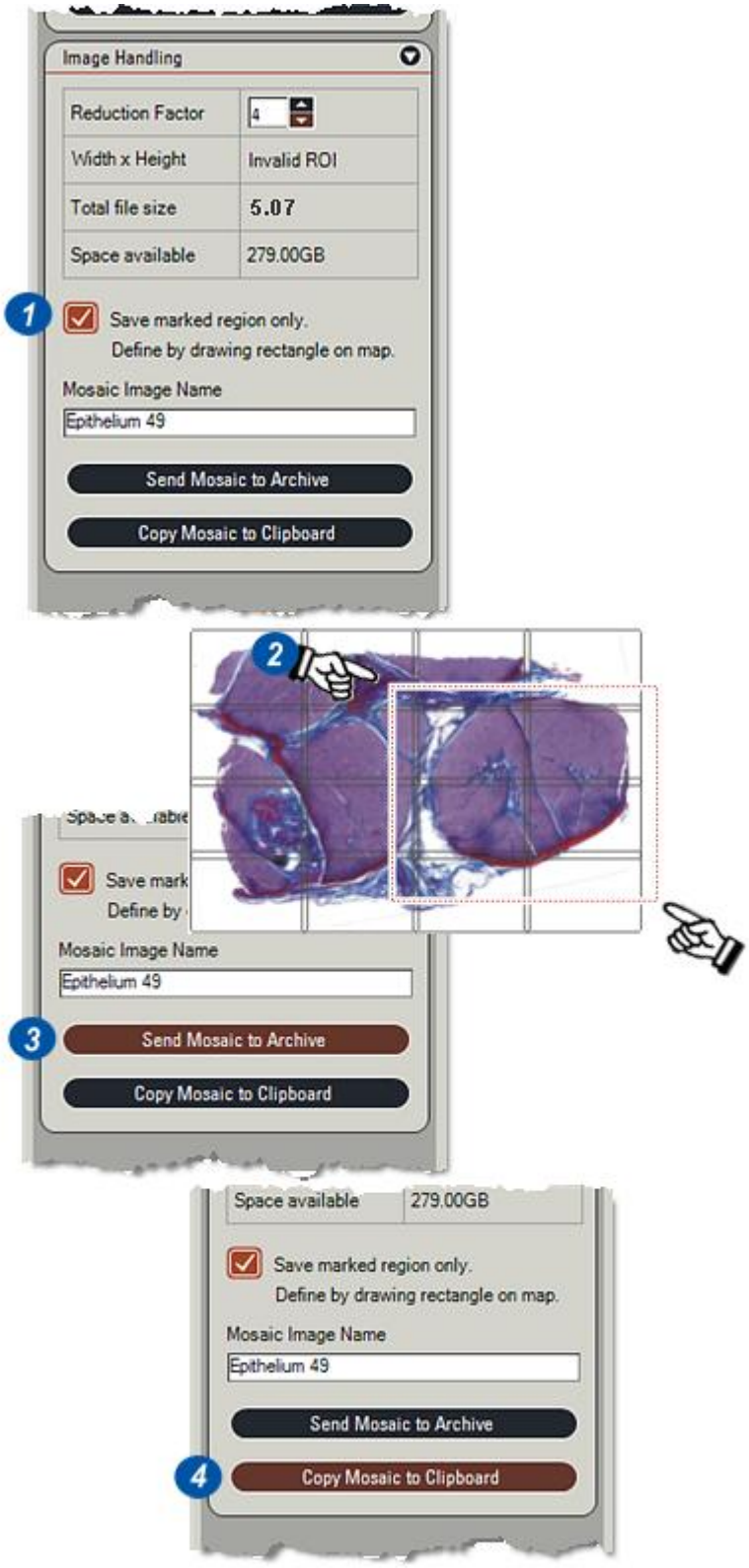
1: Click on the *Saved Marked Region* check box to enable it.

2: Click on the edge of the region to be saved, hold and drag diagonally to the right to encompass the region. Release the mouse button. For clarity, the illustration shows a red dotted line – on screen it is black.

3: Save the mosaic by clicking *Send Mosaic to Capture Folder.*

4: The mosaic can also be saved to the Windows Clipboard for loading into another application. Click on the Copy *Mosaic to Clipboard* button.

Go to Retrieving the Mosaic... 880



When a mosaic is retrieved and re-displayed, it is the Workspace and therefore the tiles that are targeted with a *new* full size mosaic created on the viewer. This could be saved again as a mosaic either as a selected region or at a different size.

To retrieve a mosaic:

1: Click on the arrows to the right of the *Select Workspace* header.

2: From the drop down list, click to select a *Workspace*.

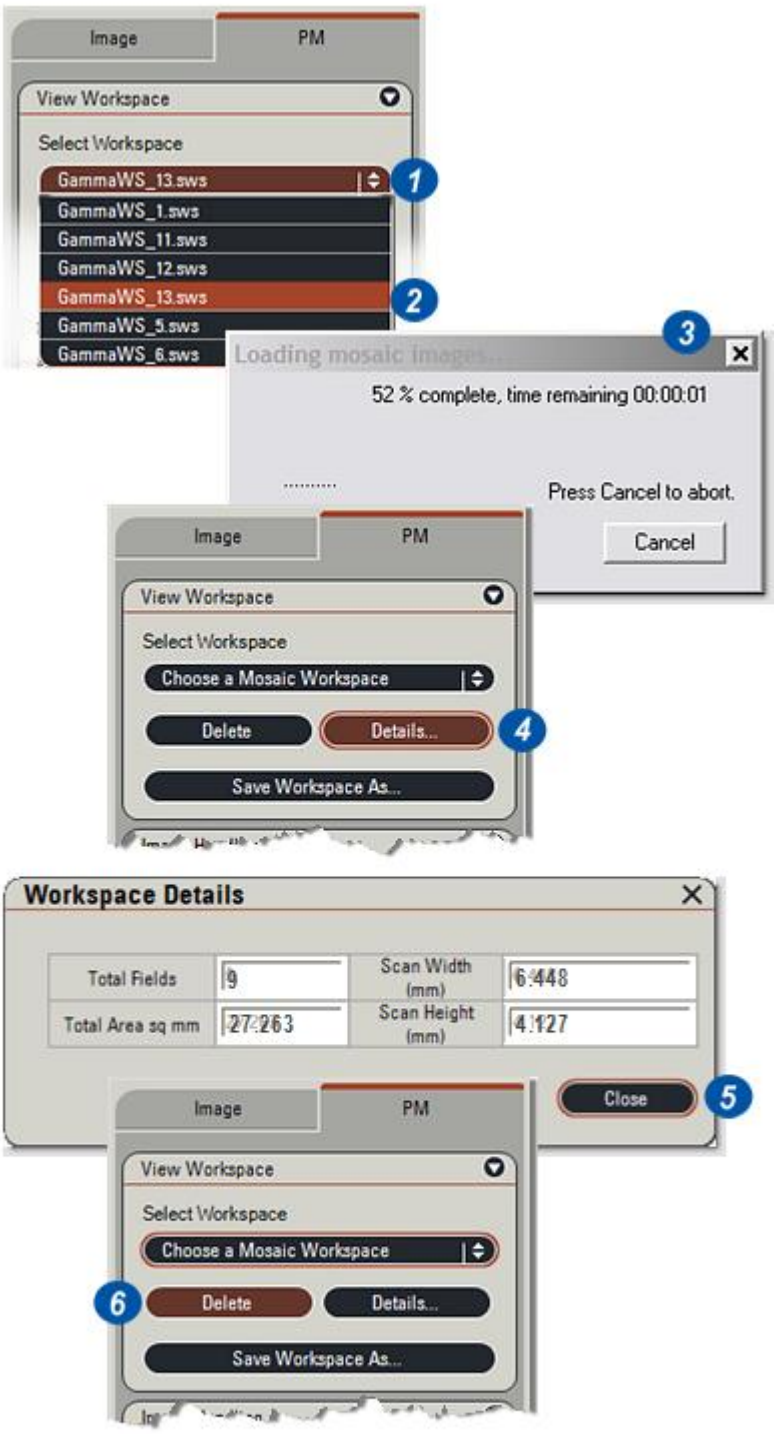
3: A progress panel (Loading Mosaic mages) appears and the tiles are displayed as a mosaic on the viewer.

4: Detail of the mosaic can be displayed by clicking the *Details* button and…

5: …the *Workspace Details* panel appears specifying the number of tiles and image area.

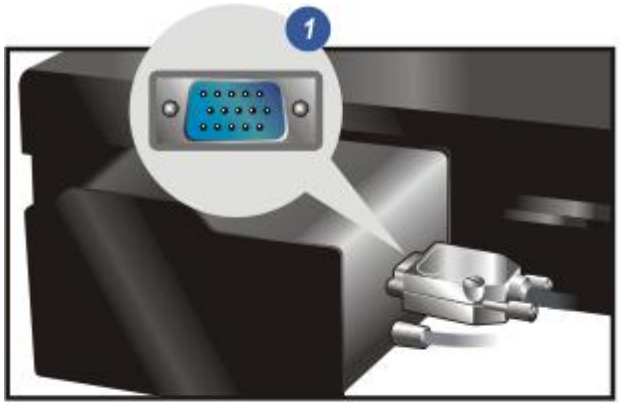
To delete a Workspace:

6: Click on the *Delete* button to delete the selected Workspace (Steps 1 and 2 above). Deleted Workspaces cannot be retrieved.



Although the Leica ISO Pro stage is driven directly by Leica Application Suite, it still requires an Oasis Controller Board to report the X and Y co-ordinates.

Connection between the stage and the computer is by a 15 pin (not the usual 9 pin) 'D' style DIN plug **(1).**

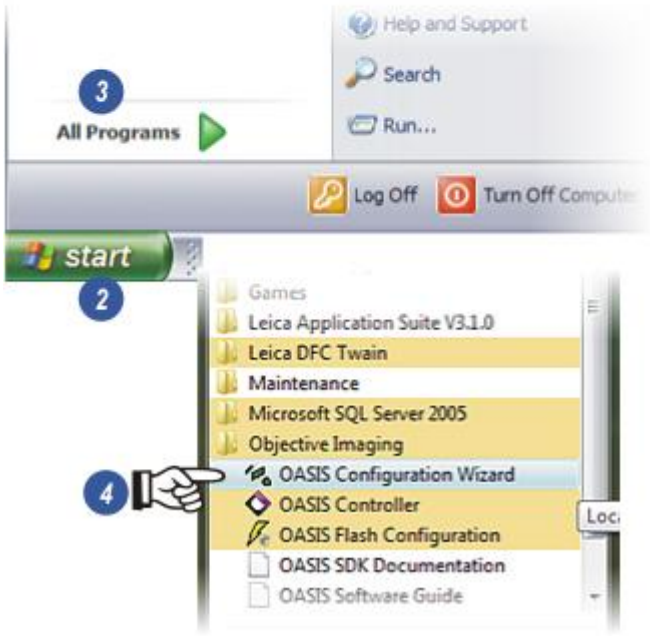


Selecting the Stage:
LAS needs to 'know' that an ISO Pro stage is to be used and this is setup using the Oasis Configuration Wizard. The illustrations are from a typical Windows XP operating system - Windows Vista is very similar.

2: Click on the *Start* button and...

3: ...on *All Programs.*

4: From the pop up menu, click to select the *Oasis Configuration Wizard.*

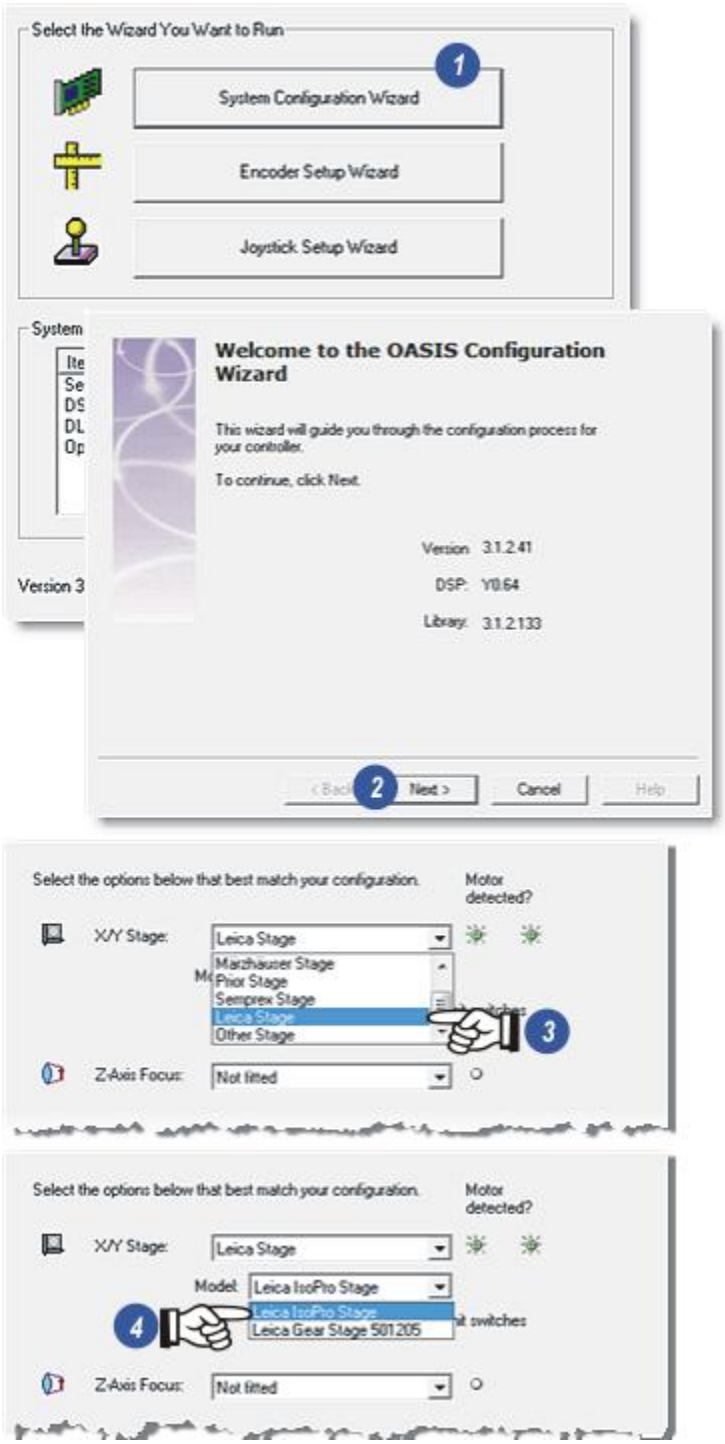


1: When the Oasis Configuration Wizard opens, click on the *System Configuration Wizards* button.

2: Click *Next* on the introduction dialog.

3: In the *X/Y* list box, use the scroll bar on the right to find the *Leica Stage* entry. Click to select it.

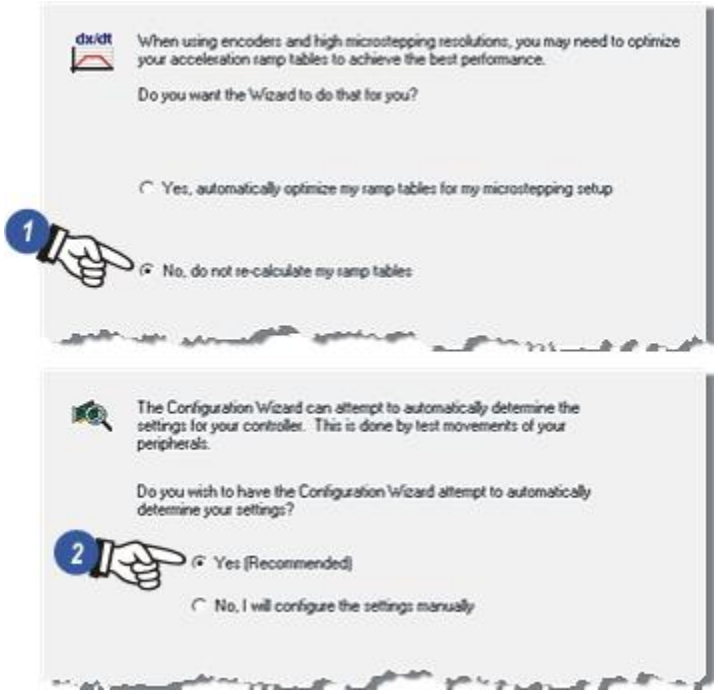
4: In the *Model* list box, click to select the *Leica ISO Pro* stage. Click *Next.*



1: For the Acceleration Ramp tables click the *No, do not re-calculate* radio button. LAS automatically handles the motor power.

2: Most of the details of the data exchange between Oasis and LAS can be setup automatically so click to select *Yes (Recommended)*.

Make sure the stage is clear of any obstructions and click the **Finish** button to exit the wizard.



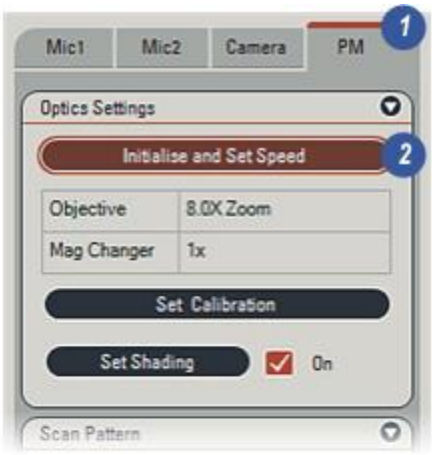
On the Acquire Workflow:

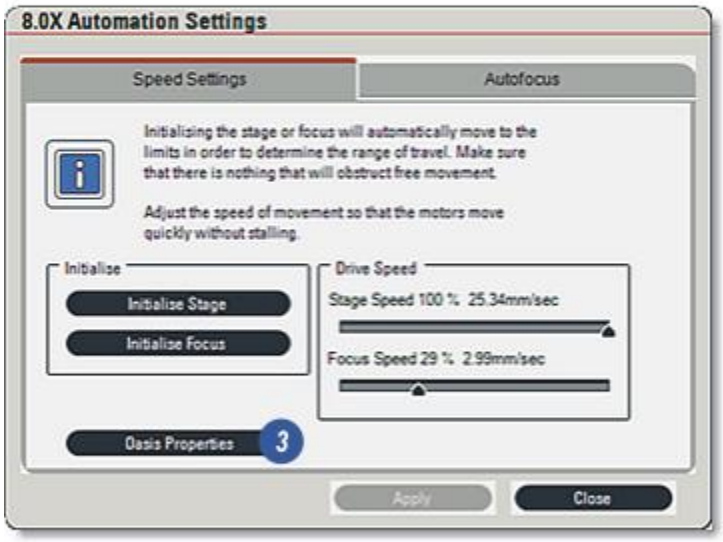
1: ...if necessary click the *PM* (Power Mosaic) tab to reveal the Optics Settings panel. If the PM tab is not visible the Power Mosaic module has not been loaded: *See Starting: Go there...* 801

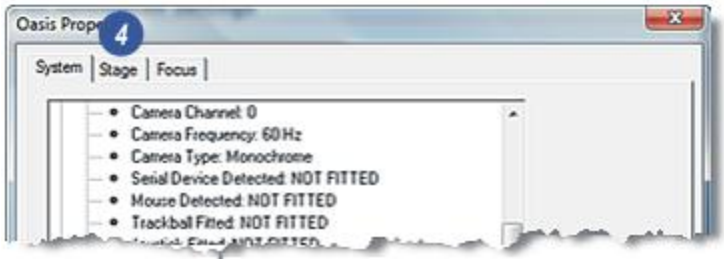
2: Click on the *Initialise and Set Speed* button.

3: On the Automation Settings dialog, click on the *Oasis Properties* button.

4: When the Oasis Properties dialog opens, click on the *Stage* tab.







On the Oasis Properties > Stage dialog:

1: Check that *Encoders Fitted* is set to *Yes*. If it is not the cable connection may be loose or not fitted, or the wrong stage has been selected. Repeat the Oasis Configuration process: *Go there...* 881

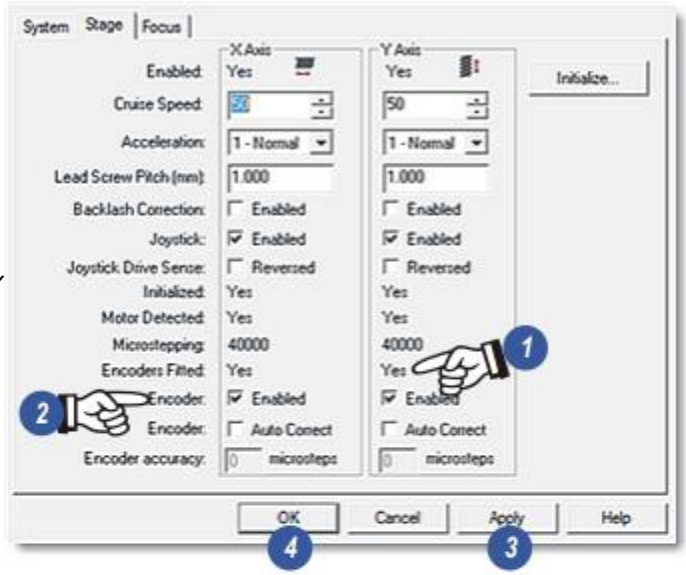
2: Click to enable both of the *Encoder X* and *Y Axes* check boxes.

3: Click *Apply*.

4: Click *OK*.

5: The ISO Pro stage will perform well in both bi- and uni-direction modes, but for scanning precision the uni-direction mode is recommended.

6: Again, for accuracy and precision opt for the *Standard* scan speed in most situations.

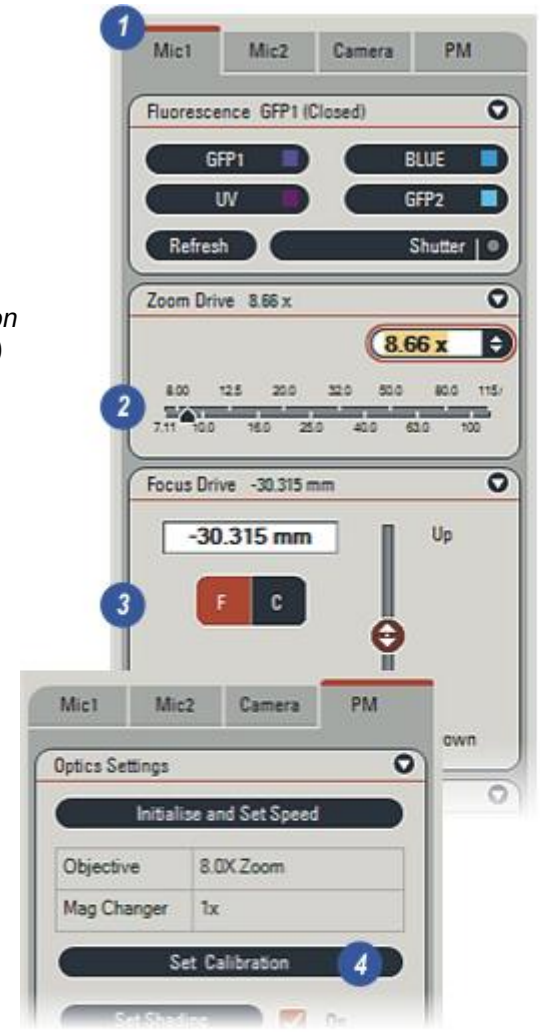


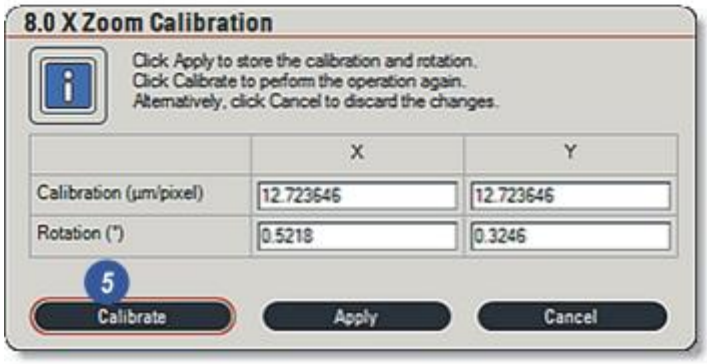
There are three essential steps to successful imaging using the ISO Pro stage with both motorised *(Select the Mic1 tab: **1**)* and manual stands:

Zoom: **(2)**

Focus: **(3)** and

Calibrate: **(4)** by clicking on the *Set Calibration* button and then on the *Calibrate* button **(5)** on the Zoom Calibration dialog. *For more details:* *Go there...* 816





Live and Interactive Measurements

Optional modules *Live Measurements* and *Interactive Measurements* bring all the flexibility and precision of Leica Application Suite to image measurement tasks.

Both modules provide the user with a wide range of precision tools such as distance, angle, parallel distance and counting. They also generate parameters like dimensions, area, perimeter and display co-ordinates.

The modules are licensed separately. However, they do share many common features, so there is only one Help file; any differences are identified in the Help text.

Live Measurements:

- Optimised for working on the live image
- Measurements made using microscopes with coded zoom are automatically updated as magnification is changed.

Interactive Measurements:

- Optimised for working on captured images
- Measurements are calibrated using the calibration factor stored with the image.

Users can choose how to label the measurements on screen using:

- *Parameters:* For example a circle can be labeled with diameter, radius, area or circumference whichever is more appropriate.
- *Properties:* For line thickness, colour, label colour - including a transparency control - position and type font.
- *Display or Hide:* Conceal or reveal parameter label display to suit the image.
- *Comments:* User-entered text to display alongside and enhance the parameters.

Capabilities include:

- *Classes*: Objects can be grouped together to make reporting and analysis quick and convenient.
- *Configurations:* To save *Classes*, *Properties* and *Settings* together in a file that can be retrieved and applied to images at any time.
- *Templates:* Used as moveable 'overlays' to benchmark important positions and locations on live images.
- *Reports:* Display and analysis of selected measurements in the correct corporate style with a *Microsoft Excel* report.

Microsoft Excel does not have to be installed on the computer to view reports, but a complete installation is required if templates are to be changed.

The main areas of the user interface:

1: *Menu Bar*: For Options and Help.

2: *Workflows*: Live Measurements is on the Acquire Workflow; Interactive Measurements is on the Analysis Workflow.

3: *Measurements Tab*: Click to reveal all the tools and features.

4: *Measurement Toolbox*: Click to select measurement type.

5: *Control Tools*: Commands for actions such as New Measurements, Redo and Undo.

6: *Function Panels*: Reveal Properties, Classes, Configuration, Report and Template controls.

7: *Acquire button*: (**Live Measurements only**) Click to capture an image and data.

8: *Side Tool Bar*: Hide and reveal the Grid (for Results) and Thumbnail Gallery.

9: *Gallery* Zoom: Move the slider to resize the thumbnails.

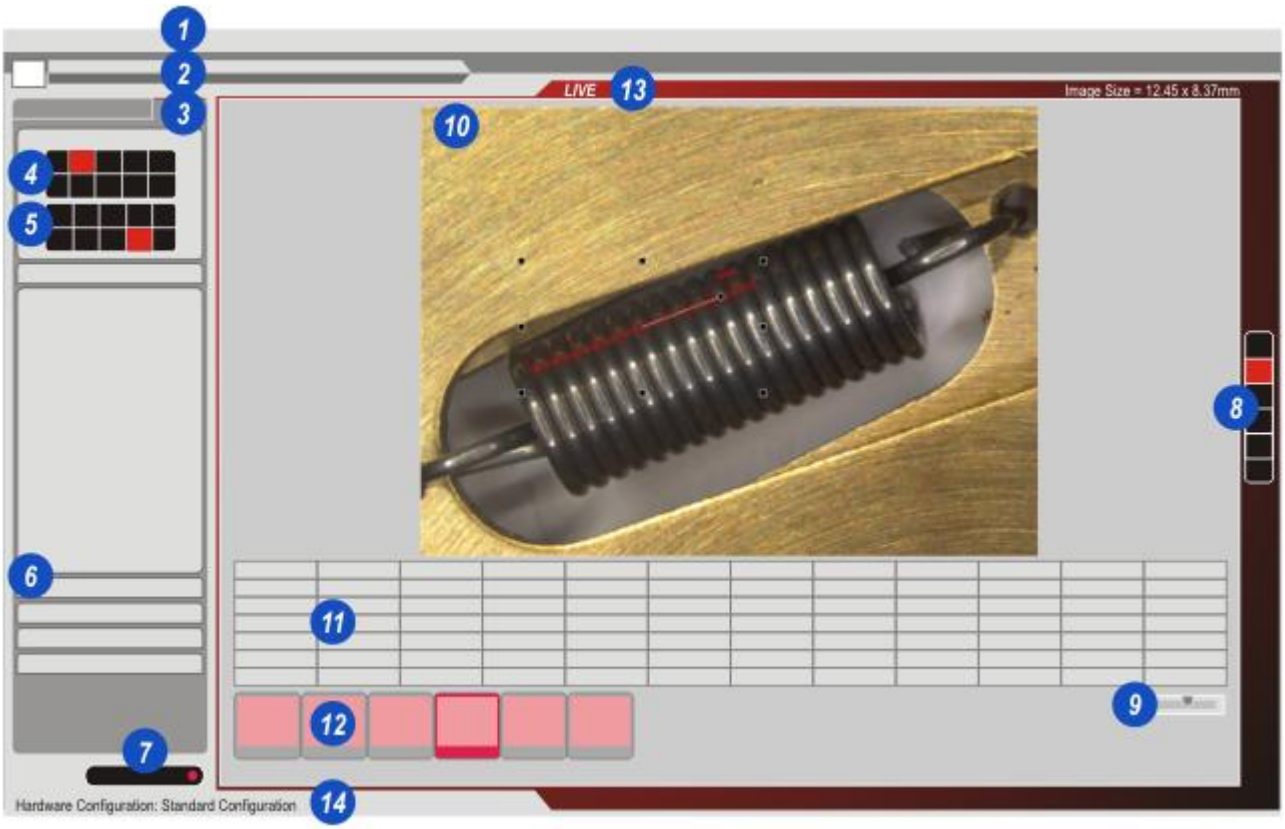
10: *Viewer*: Displays the live or captured image.

11: *Results Grid*: Configure for Details or Summary of measurements. Hide to increase the Viewer area.

12: *Thumbnail Gallery*. Hide to increase the Viewer area.

13: *Image Status Display*.

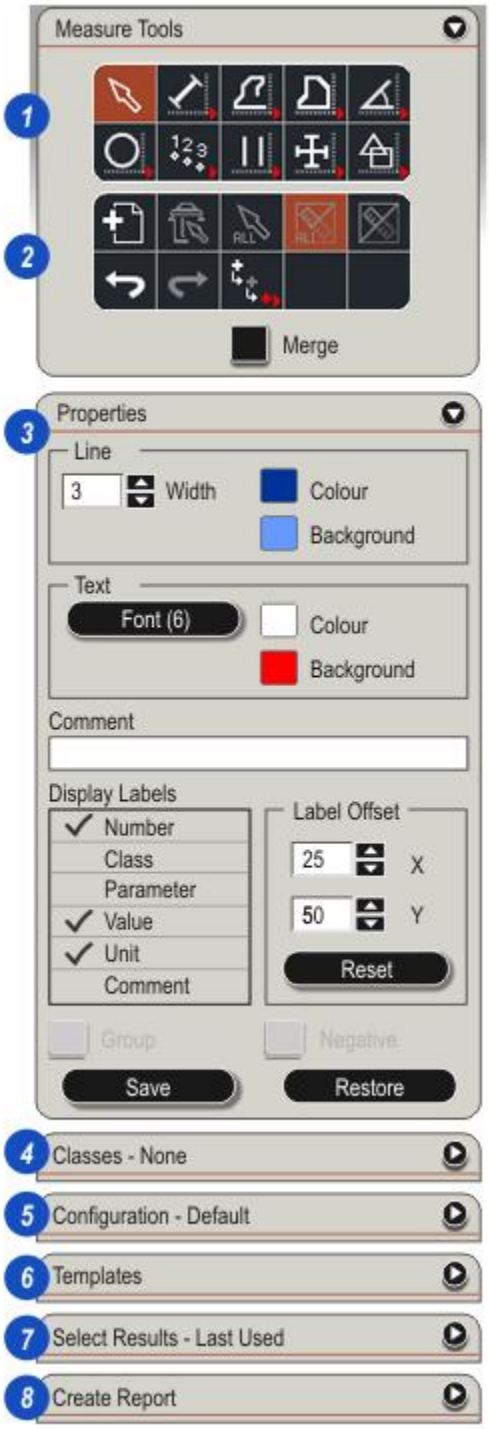
14: *Status Bar*.



Direct links to the *Live Measurement* features:

- *Calibration and Preferences*[891]*:* Check the calibration to ensure measurement precision.
- *Fast Track Measurements*[890]*:* Step-by-Step sequence to start making measurements quickly
- *Launching the Module*[895]*:* Starting Live Measurements
- *Drawing Tools*[903] *(1):* Selecting and using measuring tools
- *Controls Tools*[896] *(2):* Start a new measurement, clear existing, hide and navigate actions
- *Properties*[904] *(3):* Set up line thicknesses, colours, fills, fonts and labels
- *Classes*[954] *(4):* Set up measurement properties to identify feature groups
- *Configurations*[963] *(5):* Save and re-load properties
- *Templates*[967]*:* Create 'overlays' from measurements that can be applied to other images
- *Select Results*[999] *(7):* Choose the results to display on the *Grid* and use in reports
- *Create Report*[1006] *(8):* Comprehensive reporting using measurement details or summary in either *Excel* or *CSV* formats
- *Acquire and Merging*[1019]*:* Merge measurements and image into a single entity

When the *Results Grid* is selected, a range of shortcut key combinations are available. For a complete list of all the *LAS Keyboard Shortcuts*[35]



Fast Track is a simple checklist of steps to take to get into precise measuring quickly.

Live Measurements

- **Acquire Workflow:** Measurements are made on a live camera image in the *Acquire Workflow* - no need to capture the image first.
- **Calibrate** [318]**:** Carry out a calibration before making measurements
- **Large Image:** Make the image as large as possible - close the *Gallery* and *Grid* if they are open
- **Select an Image Format** [290]**:** Choose an image *Format* that provides good resolution with a fast refresh rate
- **Consider Classes** [954]**:** Determine if image measurements can be grouped together to make reporting and analysis quick and convenient. Set up *Classes* on the *Measure* tab to reflect the groups
- **Set up Properties** [903]**:** If *Classes* are not being used, set up the *Line, Background* and *Font* properties for initial measurements. They can be altered later if necessary
- **Comments** [909]**:** Add a comment if needed
- **Select the Display Label Options** [909]**:** Select the items to display on the *Display Labels*
- **Save a Configuration** [963]**:** If required, save the settings as a *Configuration*
- **Start drawing measurements...**

Points to Note:

- *Live Measurements* cannot be used with sequence images created with modules such as *MultiTime* or *Movie*. If a sequence module is selected the *Measure* tab is not available.
- If a stereo microscope zoom is being used, the measurement drawings will change size but their positions will be incorrect because of an image shift due to the focus change. Use the *AX-Carrier* option to correct.

Interactive Measurements

- **Analysis Workflow:** Measurements are made on a captured image in the *Analysis Workflow* with the *Interactive* tab selected.
- **Calibrate** [318]**:** Carry out a calibration before making measurements
- **Consider Classes** [954]**:** Determine if image measurements can be grouped together to make reporting and analysis quick and convenient. Set up *Classes* on the *Measure* tab to reflect the groups
- **Templates** [967]**:** Existing measurements stored as templates can speed repetitive measurement tasks
- **Set up Properties** [903]**:** If Classes are not being used, set up the Line, Background and Font properties for initial measurements. They can be altered later if necessary
- **Comments** [909]**:** Add a comment if needed
- **Select the Display Label Options** [909]**:** Select the items to display on the *Display Labels*
- **Save a Configuration** [963]**:** If required, save the settings as a *Configuration*
- **Start drawing measurements...**

Live Measurements

To maintain ongoing measurement precision, calibration should be carried out at regular intervals.

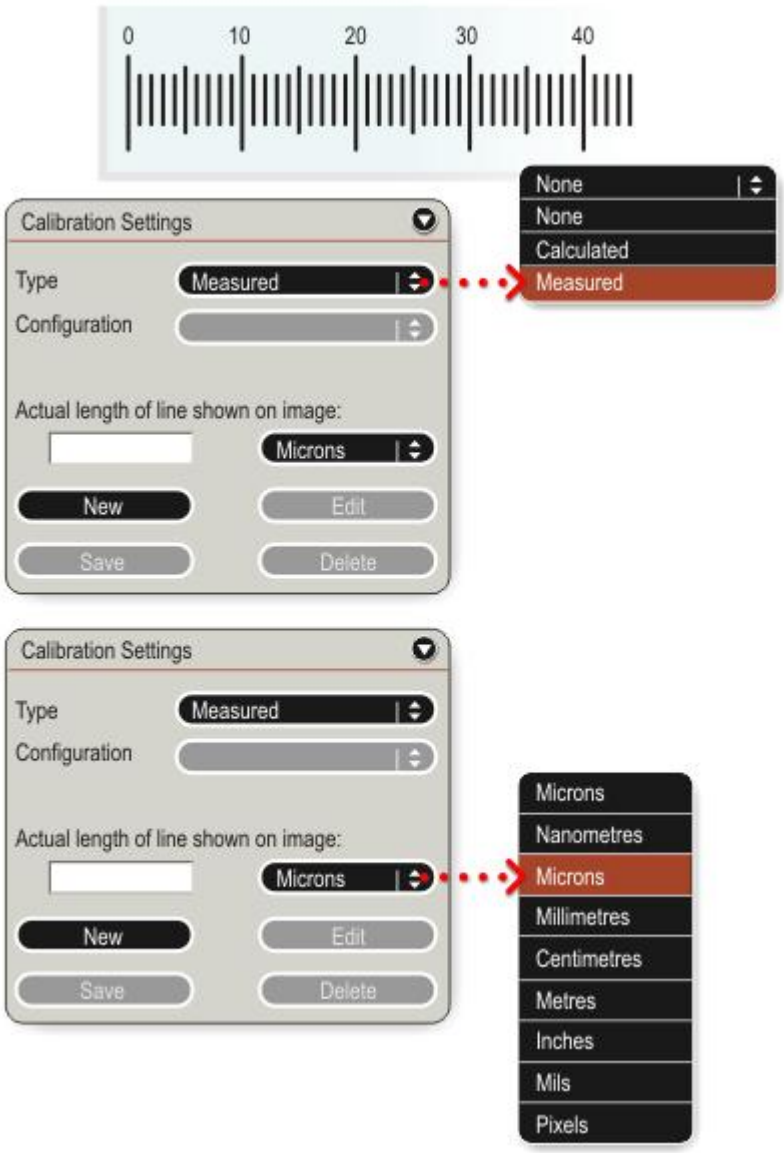
More 892

Interactive Measurements

Images captured in LAS with a properly calibrated objective can be used directly with Interactive Measurements, but if the system calibration has changed or images are being used that do not reflect the current calibration values, *Update Calibration* provides a simple and quick way to bring images up to date.

More 893

For the Live Measurements module, the calibration functions can be found in the *Acquire Workflow*[318] in the *Camera* section

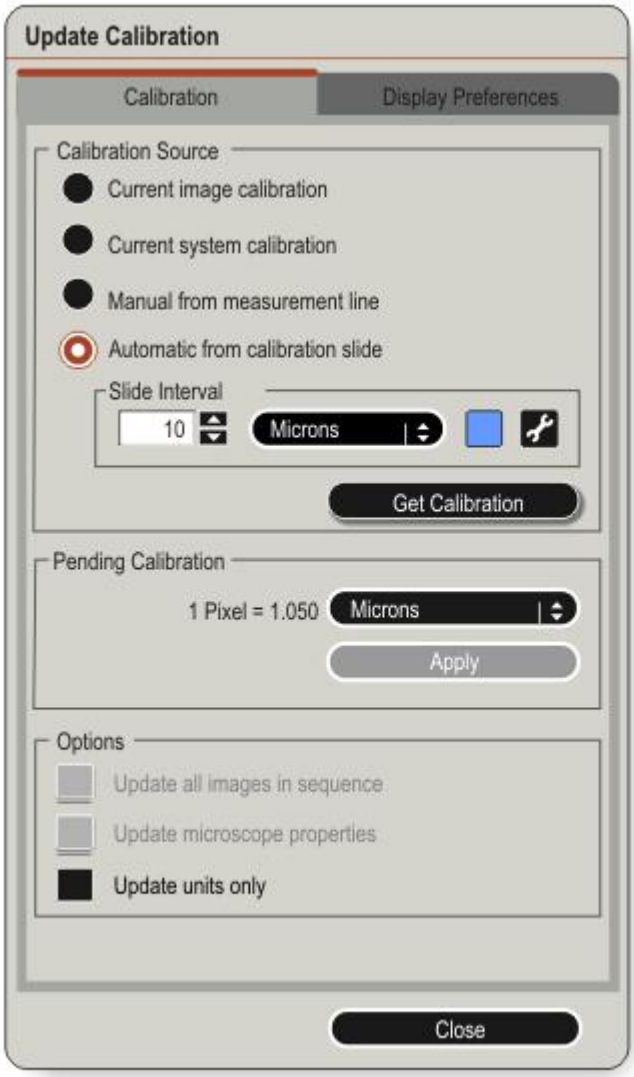


Update Calibration is used on captured images including those that have a *Scale Bar* 'burnt in' (merged), but in those cases the merged displayed value will not change.

Four options for the calibration source are available:

- *Current image calibration:* Uses the calibration values of the selected image.
- *Current system calibration:* Uses the prevailing calibration settings.
- *Manual from measurement line:* Allows new calibration values to set up directly from a known distance on the displayed image.
- *Automatic from calibration slide:* The calibration is calculated automatically from an image of a calibration slide captured at the same time as those to be updated.

Link to *Update Calibration* 85



LAS measurements are stored with with many digits because floating point calculations are used. To display all of these digits could hinder measurement interpretation, so the number of digits displayed after the decimal point can be set by the user to aid clarity.

Reducing the display places does not affect the stored calculations.

To set the number of display places after the decimal point:

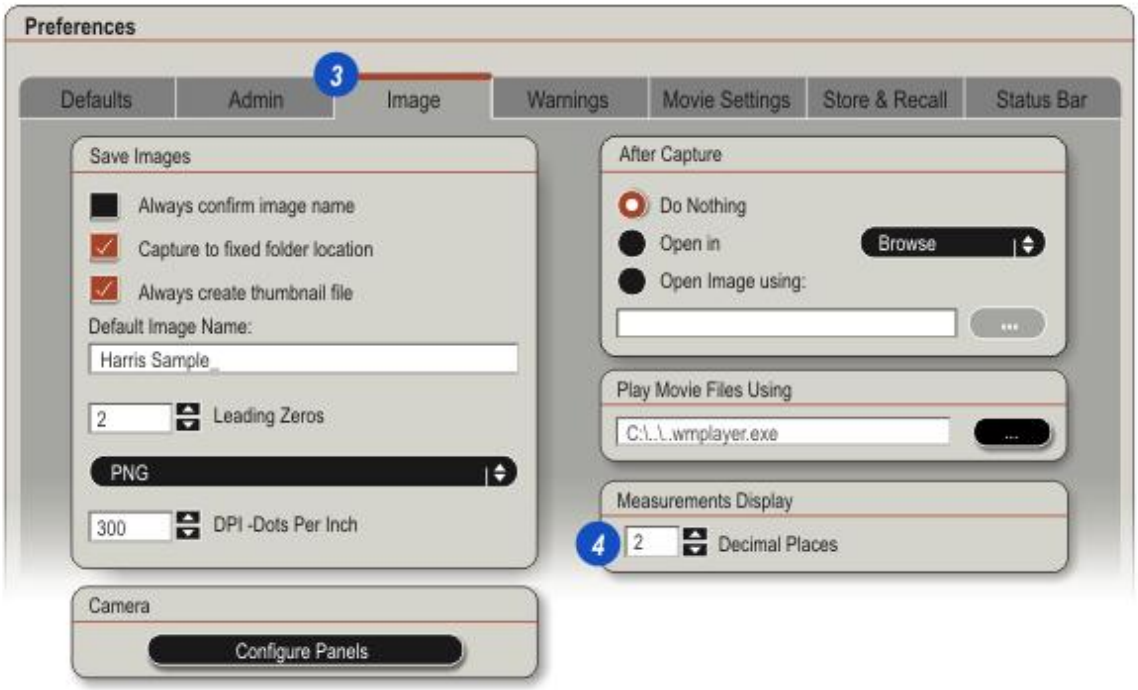
1: On the main header click on *Options*...

2: ...and from the drop down click to select *Preferences*.

3: On the *Preferences* dialog, if necessary, click on the *Image* tab.

4: On the *Measurements Display* panel, in the *Decimal Places* text box, either click in the box and type a value or use the up/down arrows to the right of the text box to increase/decrease the value.

5: Click *OK* to save the value. Any measurements being displayed will be updated immediately.



Live Measurements is selected from the *Acquire Workflow:*

1: If the *Measure* tab (3) is not displayed, check that the *Acquisition Mode* icon is showing *No Sequence* selected. If a sequence has been previously selected, click on the small arrow bottom right of the icon and click *No Sequence* from the options.

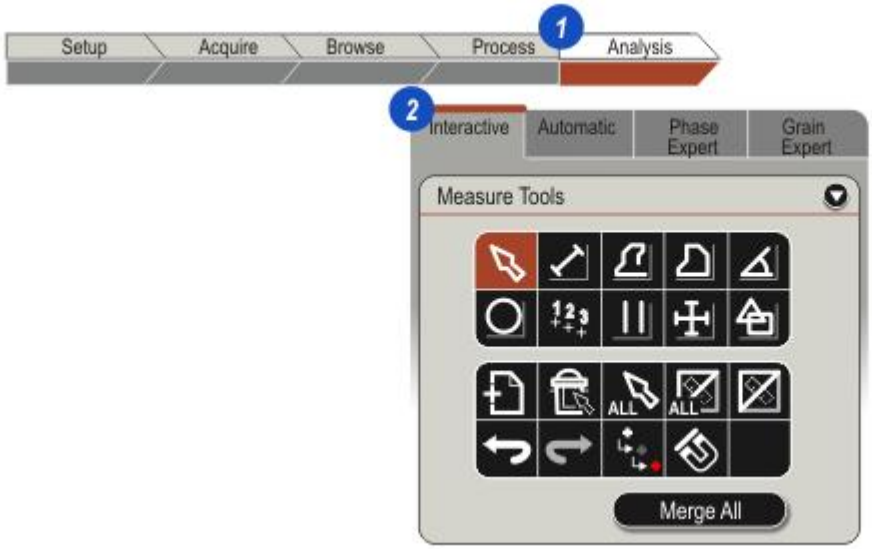
2: Click on the *Acquire Workflow*.

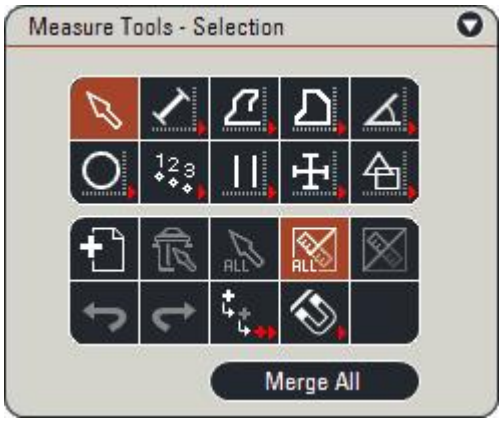
3: Click on the *Measure* tab to reveal the *Toolbox* and other panels.

Interactive Measurements is selected from the *Analysis Workflow:*

1: Click on the *Analysis Workflow*.

2: Click on the *Interactive* tab to reveal the *Toolbox* and other panels.





The lower set of tools are utilities used to start new measurements, make multiple deletes and move between actions.

For a detailed explanation of individual tool modes, parameters and the drawing methods, click on the tool button.

Start New Measurements:
Deletes all existing measurements ready to start a new set of measurements.

Delete (Trash Can):
Deletes selected measurements.

Select All:
A single click selects all measurements.

Hide All:
Selects and hides all measurements. Click again to reveal the measurements.

Hide Selected:
Hides selected measurements. Click again to reveal the measurements.

Undo and Redo:
Undo the last action and *Redo* the last action after an *Undo*.

Toggle Cursor Colour:
Changes the cursor colour including a user-selected colour.

Snap: (Interactive Measurements only)
Toggles the 'Snap to edge' feature.

Start a new set of measurements:

Deletes all existing measurements ready to start a new set. Because this action also clears the *Undo/Redo* history, it cannot be reversed.

1: Click on the *Start New Measurements* button.

2: Click *OK* to confirm the *Start New Measurements*.

Deleting Measurements

Select a measurement for deletion:

1: Click to choose the *Selection* tool and click on the measurement to be deleted or...

Click on the measurement entry on the *Grid* if it is being displayed.

Select several measurements for deletion:

1: Click to choose the *Selection* tool. Hold down the keyboard *Ctrl* key and click on the measurements to be deleted or...

Hold down the keyboard *Ctrl* key and click on the measurements on the *Grid* if it is being displayed.

Select all measurements for deletion:

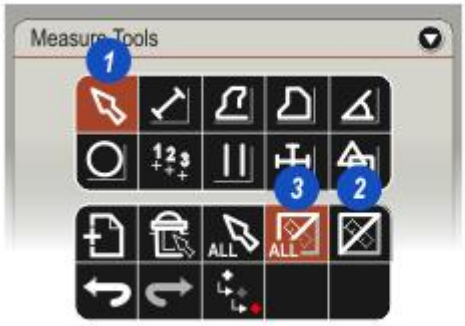
2: Click on the *Select All* button and all measurements are selected.

Delete the selected measurements:

3: Click on the *Delete (Trash Can)* button or...

Press the keyboard *Del* key to remove all of the selected measurements.

Hide Measurements



The *Hide* tools conceal selected measurement(s):

Measurements are not deleted and can be revealed by clicking the Hide button again.

Select a measurement to hide:

1: Click to choose the *Selection* tool and click on the measurement to be hidden or...

Click on the measurement entry on the *Grid* if it is being displayed.

Select several measurements for hiding:

1: Click to choose the *Selection* tool. Hold down the keyboard *Ctrl* key and click on the measurements to be hidden or...

Hold down the keyboard *Ctrl* key and click on the measurements on the *Grid* if it is being displayed.

Hide the selected measurements:

2: Click on the *Hide Selected* button.

Reveal the measurements by clicking the *Hide Selected* button again.

To hide **all** measurements, even if they are not selected, simply hold down the *Ctrl* key at the same time as clicking the Hide button. This action takes precedence over changing the state of the *Hide* button.

Hide all measurements:

3: Click the *Hide All* button.

Reveal all the measurements by clicking the *Hide All* button again.

Undo and Redo

1: *Undo* the last action and...

2: ... *Redo* the last action after an *Undo.*

Individual measurements or points can be deleted or restored using the *Undo* and *Redo* button.

Hover the cursor over the *Undo* or *Redo* button to determine the action that will occur when the button is clicked.

Cursor Colour

Users can change the cursor colour to suit the live image.

3: Each click on the *Toggle Cursor Colour* button will switch the cursor between black, white or a user-defined colour.

Changing the Cursor colour

4: Right-click on the *Toggle Cursor Colour* button and click on *Set Custom Colour for Cursor*.

5: On the *Select Colour* dialog, select a new colour by:

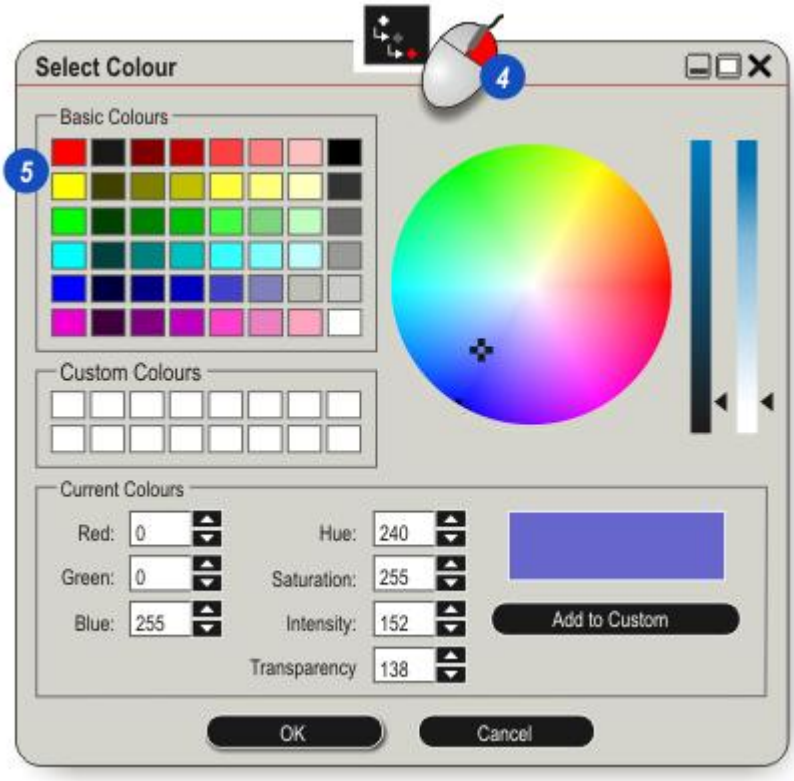
Clicking a colour swatch

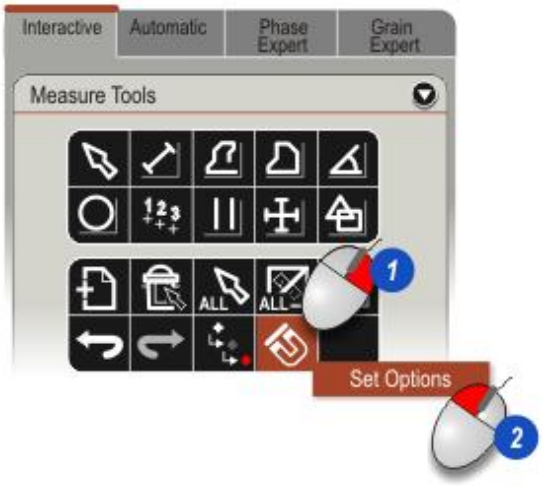
Clicking and dragging the target on the colour wheel.

Clicking inside the Current Colours text boxes and typing appropriate values.

6: Click *Add to Custom* if you want this colour to be available as a custom colour.

7: Click *OK.*





The *Snap* tool (*Interactive Measurements* only) allows the measuring tools - *Distance, Circle, Parallel, Angle and Cross* - to precisely 'snap' to adjacent sharp edges on the image.

When the *Snap* tool is enabled, the drawing tool cursor changes to a 'box' - a *Patch Box* - with a cross marking the tool position.

Optionally, a line indicating the angle of the closest sharp edge on the image can be displayed inside the box.

Snap Options:

To set up the Snap tool options:

1: Right-click on the *Snap* tool and...

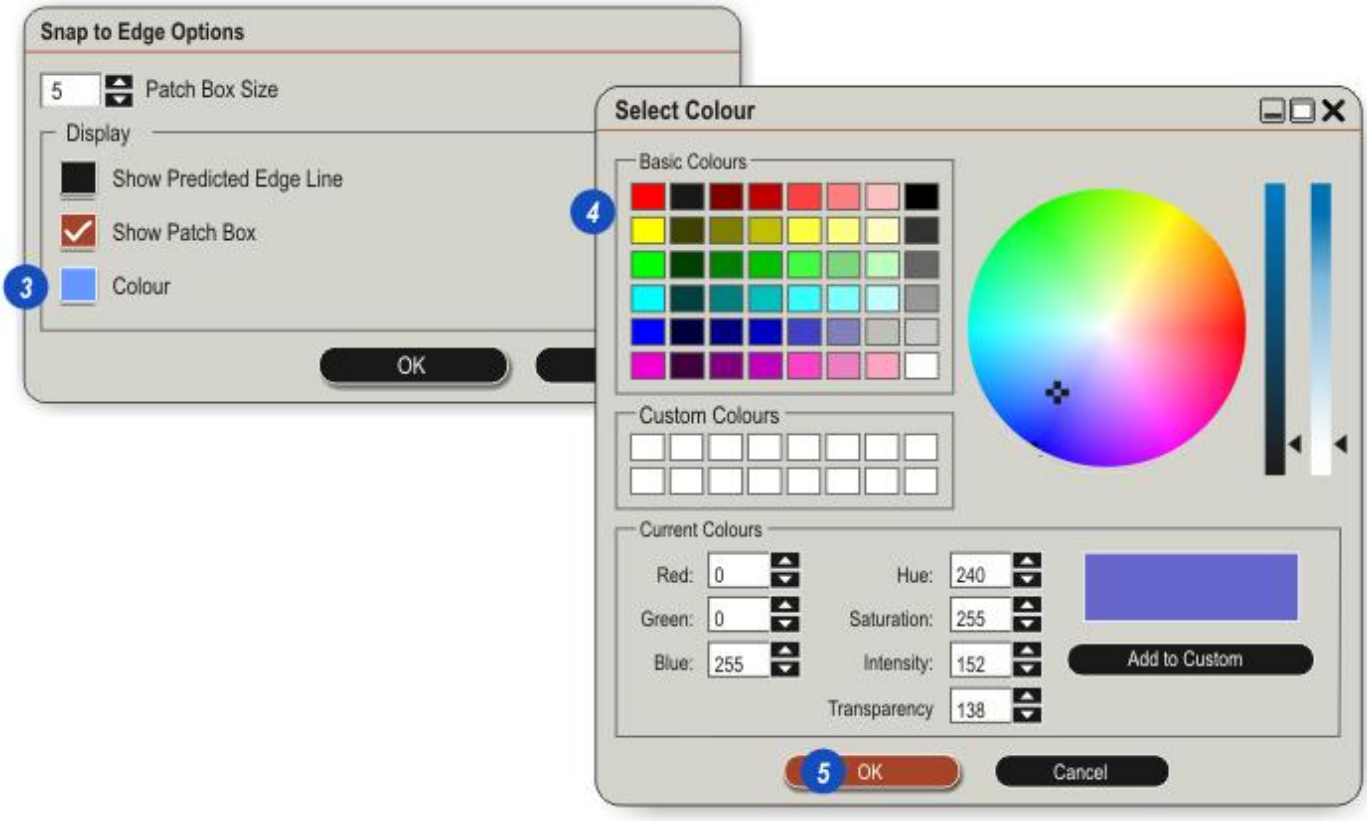
2: ...left-click on the *Set Options* context to display the *Snap Options* dialog.

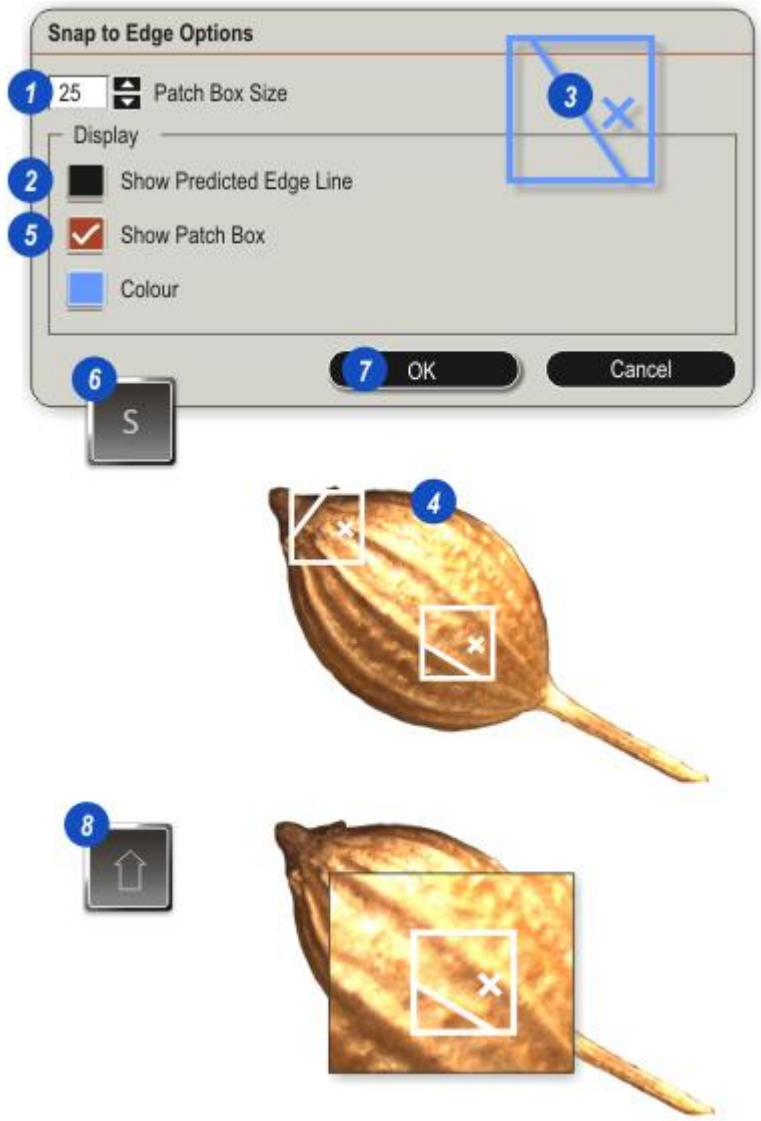
Change the *Patch Box* colour to suit the image:

3: Click on the *Colour* button.

4: Choose a new colour by clicking on the swatches, clicking and dragging the target mark on the wheel or typing values in the *Current Colours* text boxes.

5: Click *OK.*





1: Increase or decrease the size (10 to 50) of the *Patch Box* by clicking the up/down arrows.

The box size determines the area that is 'searched' for a sharp edge. If the box is too small edges may be difficult to detect; Too large and several edges may prove to be snap targets. Adjust the *Patch Box* size to suit the image complexity.

2: Enable the *Predicted Edge Line* by clicking the check box.

3: A line appears within the *Patch Box* and...

4: ...rotates to indicate the *direction* of the nearest edge. Bring the *Patch Box* close to a circle and the *Edge Line* appears as a tangent, faithfully following the curve of the circle.

5: Click the *Show Patch Box* check box to display the *Patch Box*.

6: Hold down the keyboard *'S'* button to disable snapping. Release the key to enable it again.

7: Click *OK* to save the settings.

8: Enlarge the *Patch Box* and the area of the image around it by holding down the keyboard *Shift* key.

Using the Snap Tool:

Click to enable the *Snap* tool; Then select a drawing tool and use it in the normal way.

The *Live Measurements* and *Interactive Measurements* modules use common drawing tools:

Some tool buttons have several drawing modes and parameters: right-click on any tool button with a red triangle to reveal the context menu. *More information* 916

Note: Some tools are not available in both modules. For example, Area - Auto Trace 928 is only available in *Interactive Measurements* as part of the *Analysis Workflow.*

Note: The accuracy of any measurements is dependent on the accuracy of your motorised stage.

Selection 915 tool:

Line - Draw 918
Line - Two-Point 919
Line - Short Line 920
Line - Long Distance Line 921
Line - Vector Line 925

Segment Tool 926

Area - Freehand 927
Area - Auto Trace 928

Baseline Angle 931
Apex Angle 932
Four Point Angle 933

Circle - Three Point 935
Circle - Diameter 941
Circle - Radius 937
Ellipse 938
Dual Circle - Three Point 939
Dual Circle - Diameter 941
Dual Circle - Radius 943
Circle Sector - Three Point 945
Circle Sector - Radius 946

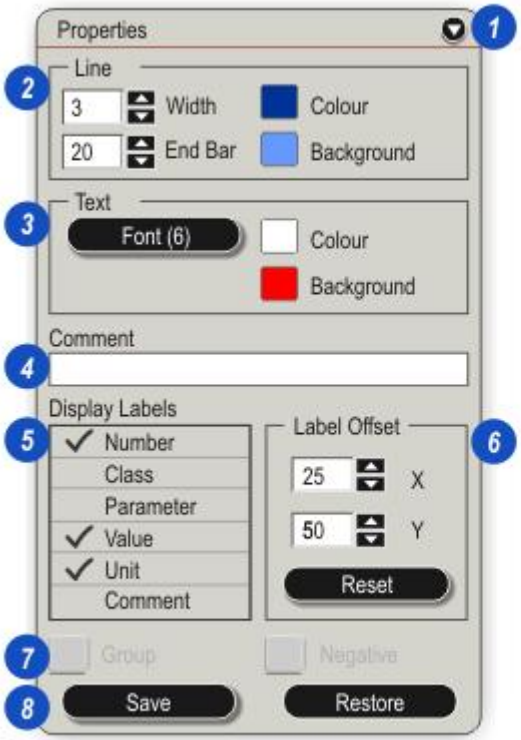
Count 947
Point 948

Multiple Distance Line 949
Parallel Distance Line 950

The Cross 951

Rectangle 952
Triangle 953

The *Properties Panel* provides all the facilities for tailoring the appearance of measurements to suit the user.



1: Click on the arrow to the right of the header to expand the panel.

2: *Line:* Change the line thickness, colour[905] and, for enclosed shapes, the fill (*Background*[906])

3: *Text*[907]*:* Change the font face, weight, size and colour as well as the label background

4: *Comment*[909]*:* A user comment can be added to the display label

5: *Display Labels*[909]*:* Users can select the parameters to be displayed on the measurement label

6: *Label Offset*[910]*:* Labels are usually displayed at a default position relative to the measurement but this can be changed by the user to avoid underlying detail

7: *Group and Negative*[911]

8: *Save and Restore*[914]*:* Saves changes to the current configuration or restores the previous settings

Line Thickness:

1: Change the *Line Thickness* by clicking on the *Up/ Down* (Increase/Decrease) arrows to the right of the *Width* text box or...

...click inside the *Width* text box and type a new value in pixels.

The maximum thickness allowed is 20 pixels.

Line End Bar:

2: Change the *Line End Bar Width* by clicking on the *Up/ Down* (Increase/Decrease) arrows to the right of the *End Bar* text box or...

...click inside the *End Bar* text box and type a new value in pixels.

Line Colour:

3: Change the *Line Colour* by clicking on the *Colour* button and on the *Select Colour* dialog choosing a new colour by...

4: …clicking on a standard colour swatch, …

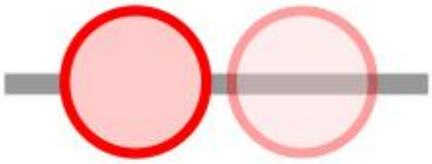
5: …clicking and dragging the small 'target' on the *Colour Wheel* or …

6: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range 0 to 255.

7: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

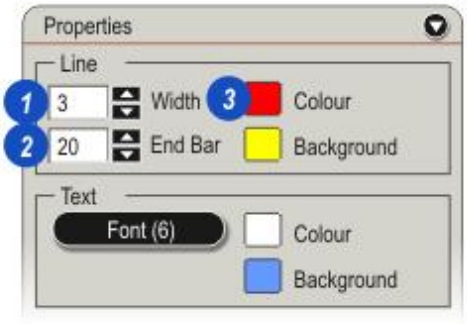
8: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

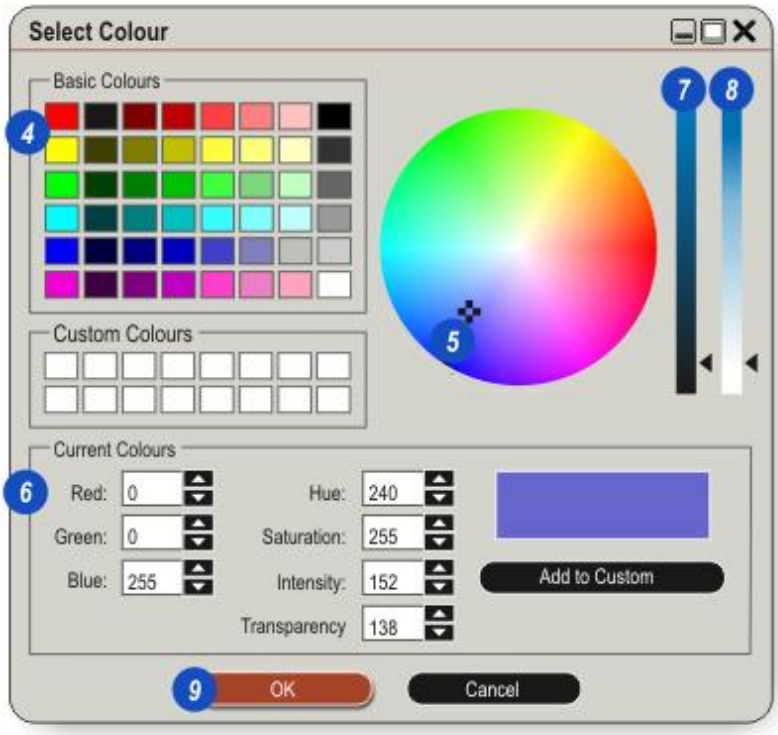
…clicking in the *Transparency* text box and typing a value in the range *255* for a solid colour to *0* for complete transparency.



The circle on the right in the diagram has an outline transparency setting of 100.

9: Click *OK.*





1: Change the *Background (Fill)* colour of a closed shape by clicking the *Background* button.

2: On the *Select Colour* dialog select a new colour by clicking on a swatch, …

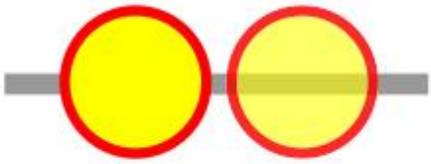
3: …clicking and dragging the small 'target' on the *Colour Wheel*, or …

4: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range *0* to *255.*

5: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

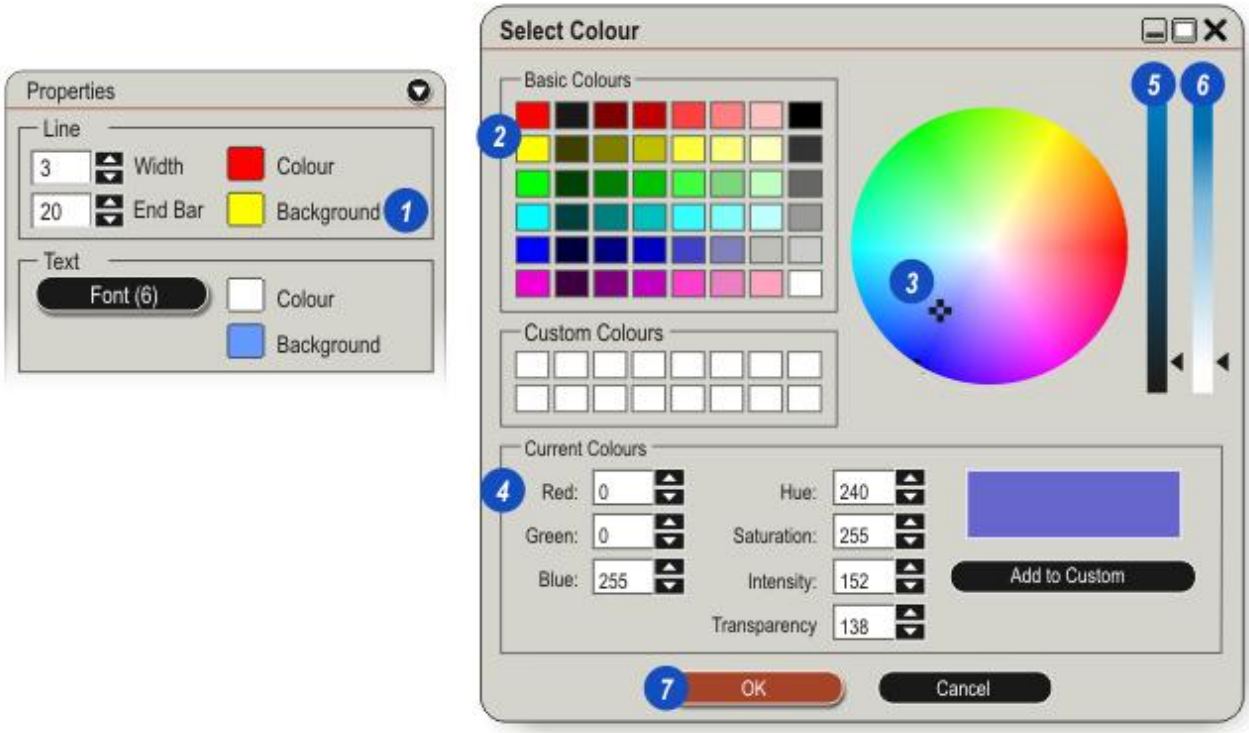
6: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

…clicking in the *Transparency* text box and typing a value in the range *255* for a solid colour to *0* for complete transparency which, when used with an outline will display an unfilled shape.



The circle on the right in the diagram has a transparency setting of *100* but the line transparency has been set at *255* for a solid outline.

7: Click *OK.*



1: Change the font properties by clicking on the *Font* button.

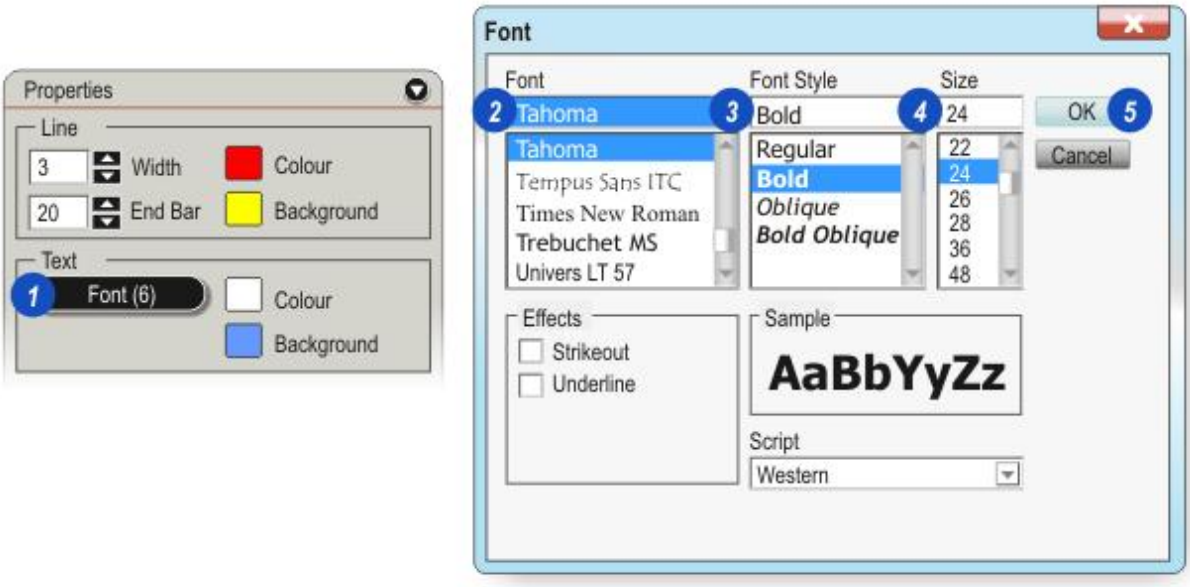
2: On the *Font* dialog, use the side scroll bars to locate the required font and click to select it.

3: Click to select the *Font Style* – bold, italic etc, and…

4: …the *Size* in points.

5: Click *OK*.

The *Font Effects* - *Strikeout* and *Underline* have no effect.



The *Label* is the background 'panel' for the *Font*. Both the *Font* and *Label* colours are selected in the same way:

1: Change the *Font* colour by clicking the *Colour* button or...

2: ...change the *Label* colour by clicking the *Background* button.

3: On the *Select Colour* dialog select a new colour by clicking on a swatch, …

4: …clicking and dragging the small 'target' on the *Colour Wheel*, or …

5: ….by clicking in the *red, green* and *blue* text boxes and typing a value in the range *0* to *255.*

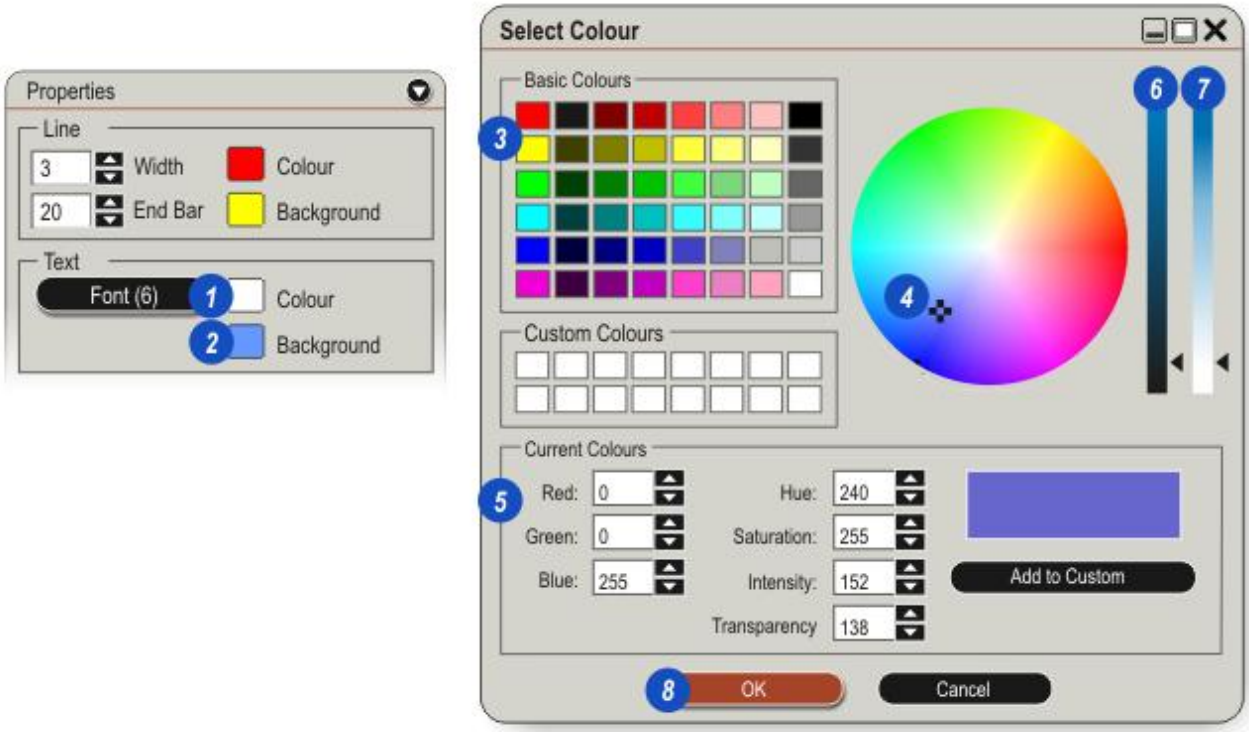
6: Adjust the shade by clicking and dragging the slider on the *Shade Bar*.

7: Colour transparency can be set by clicking and dragging the slider on the *Transparency Bar* or…

…clicking in the *Transparency* text box and typing a value in the range *255* for a solid colour to *0* for complete transparency.

The label on the right in the diagram has a transparency setting of *125* but the font transparency has been set at *255* for a solid colour.

8: Click *OK.*



Comments are often a useful addition to a measurement and can be added by:

1: Clicking in the *Comments* text box and typing a comment or note.

Display Labels:

2: This is a list of the parameters that can be displayed on a label.

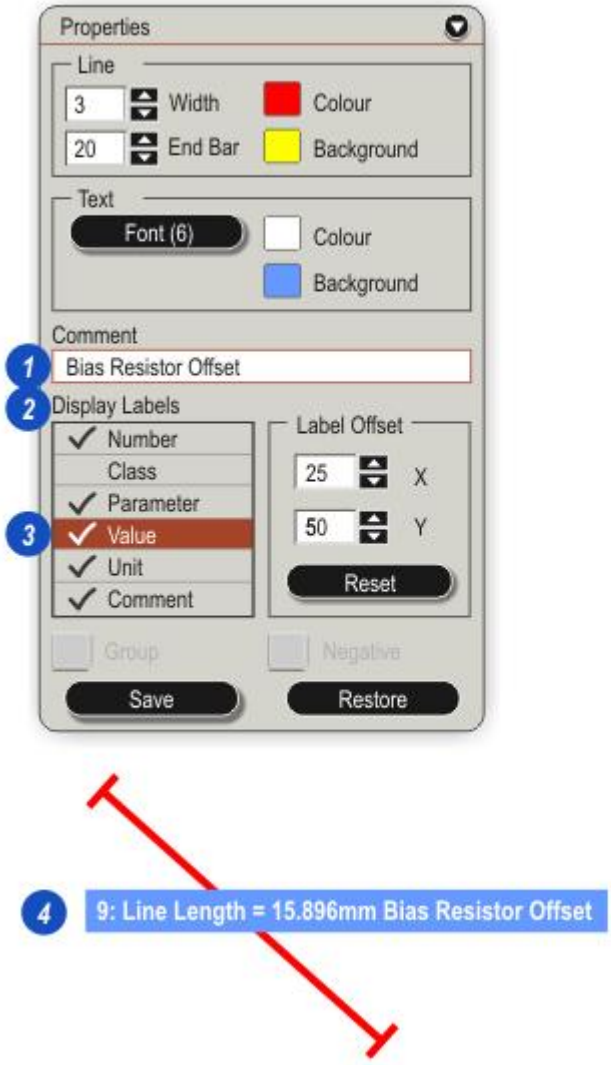
3: To select a parameter for display, click to select the parameter (it is highlighted) and click again so that a tick mark appears to the left.

A single click will remove the tick mark and the parameter will not be displayed.

Parameters are:

- *Number*: Is a sequential measurement number.
- *Class*: The *Class Name* if one has been selected.
- *Parameter*: Shows the tool value – *Length, Area, Angle* etc.
- *Value*: The actual measurement.
- *Unit*: The measurement units chosen in *Camera* > *Calibration* – *mm* for example.
- *Comment*: A comment or note entered in the *Comment* text box.

4: An example of a label displaying the chosen parameters.



The default position for a label is approximately the centre of the line or shape. The *Label Offset* tool allows the user to precisely change the position of the label with respect to the measurement line.

On the *Label Offset* panel...

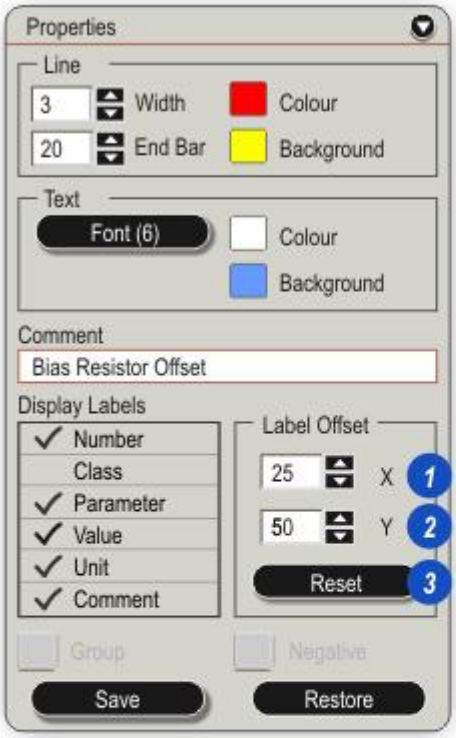
1: ...either click inside the *X or*

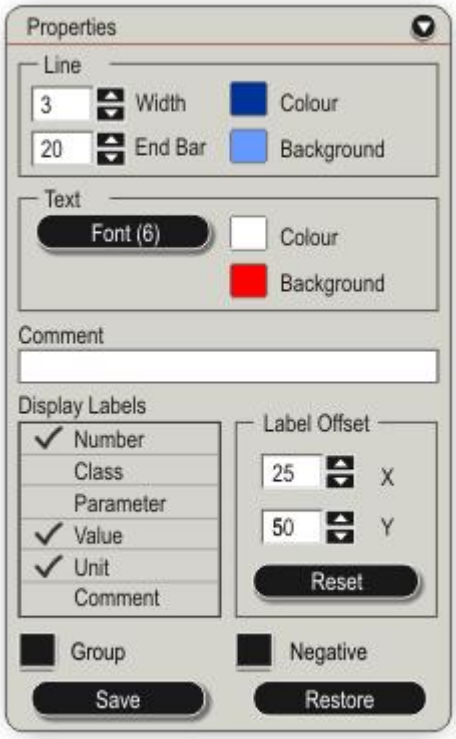
2: ...*Y* text boxes and type a new value, or use the small up/down arrows to set the label position.

The *X* value moves the label horizontally and the *Y* value moves it vertically.

The new *X/Y* co-ordinates are used to position any subsequent labels until the values are changed or...

3: ...the *Reset* button is clicked which will revert the label positions to the default.





Group and Negative:

Two complementary controls on the *Properties* panel for adding or subtracting the parameters of several objects.

Two or more measurements can be grouped together and their values added. For example, three rectangles can be grouped and their total area calculated and displayed.

The following is a useful example of the *Group* and *Negative* controls - determining the effective area of an object, such as a washer or toroid, with a hole in it.

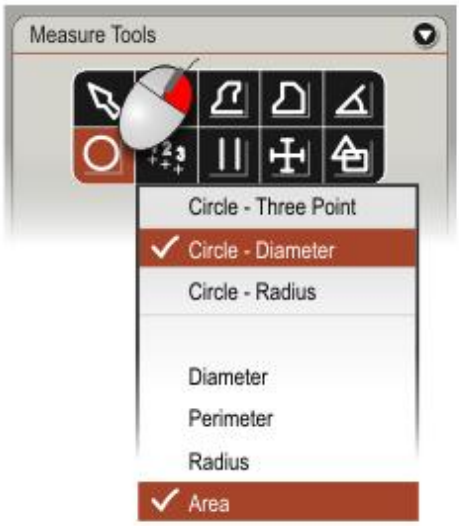
For a circular object the steps are:

- Select the *Circle* drawing mode.
- Select the *Circle* parameter - area in this example.
- Draw two circles around both the larger and smaller features.
- Using the *Ctrl* key and *Selection* tool, select both circles.
- Click to check (tick mark visible) the *Group* check box.
- Click to check (tick mark visible) the *Negative* check box.
- The difference in area between the two circles is displayed.

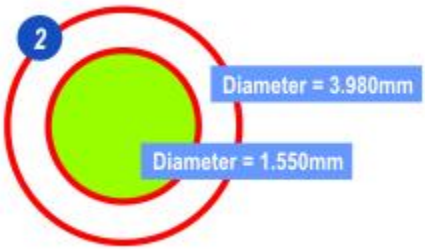
Note: If grouped drawings completely overlap, to select the drawing 'underneath' the larger one, click twice on the smaller one.

Example: Determining the effective area of an object, such as a washer or toroid, with a hole in it.

1: Select the appropriate tool and parameter - in this example *Circle* and *Area*.



2: Draw a circle around both the larger and smaller features.



To help distinguish between the two circles the outline and fill can be changed before drawing the second circle by de-selecting the first circle and then changing the properties.

3: Click to enable the *Display Grid* button in the *Side Tool Bar.*

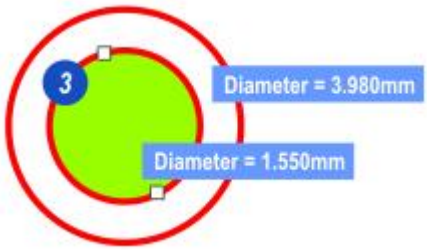
The values for both circles are displayed on the *Grid*.

Measurement	Image Name	Tool	Width(mm)	Diameter(mm)	Area(mm²)	
1	Live Image	Diameter Circle	3.983	3.983	12.463	-
2	Live Image	Diameter Circle	1.558	1.558	1.907	-

1: Click to enable the *Properties* > *Group* check box.

2: The values of both circles are added together and displayed as totals. The *Group* entry on the *Grid* shows the number of circles grouped.

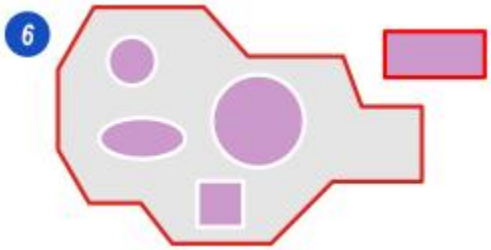
3: On the image, click to select the smaller circle.



4: Click to enable the *Properties* > *Negative* check box.

5: The value of the smaller circle is subtracted from the larger. The result is the effective area of the large circle.

6: The same principle can be applied to multiple shapes that can be both enclosed or separated.



Measurement	Image Name	Group	Tool	Width(mm)	Diameter(mm)	Area(mm²)
3	Live Image	Count = 2, Negative = 0	Diameter Circle	5.542	5.542	14.370

2

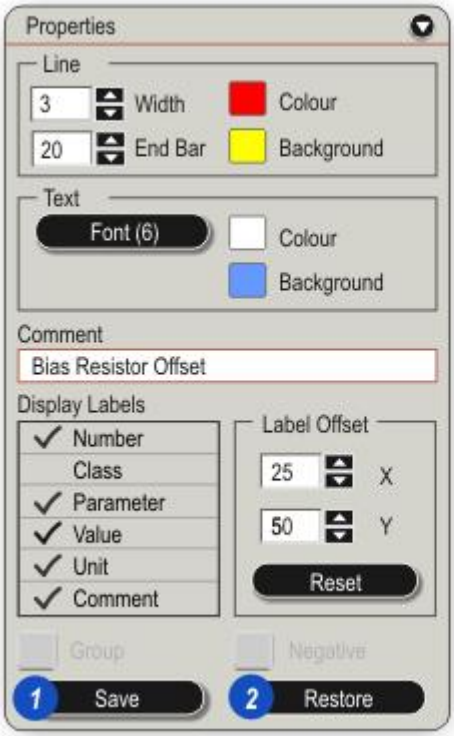
Measurement	Image Name	Group	Tool	Width(mm)	Diameter(mm)	Area(mm²)
3	Live Image	Count = 2, Negative = 1	Diameter Circle	2.425	2.425	10.556

5

1: *Save*: If a user changes the properties, the current *Configuration* can be updated without leaving the *Properties* panel by clicking the *Save* button: More about Configurations[963]

However, if the *Default* configuration is being used the properties are factory set, cannot be changed and so the *Save* feature is not available.

2: *Restore*: Re-load the current configurations by clicking the *Restore* button.



The *Selection* tool does not actually draw but is used to select measurements already made on the image ready to edit or move them.

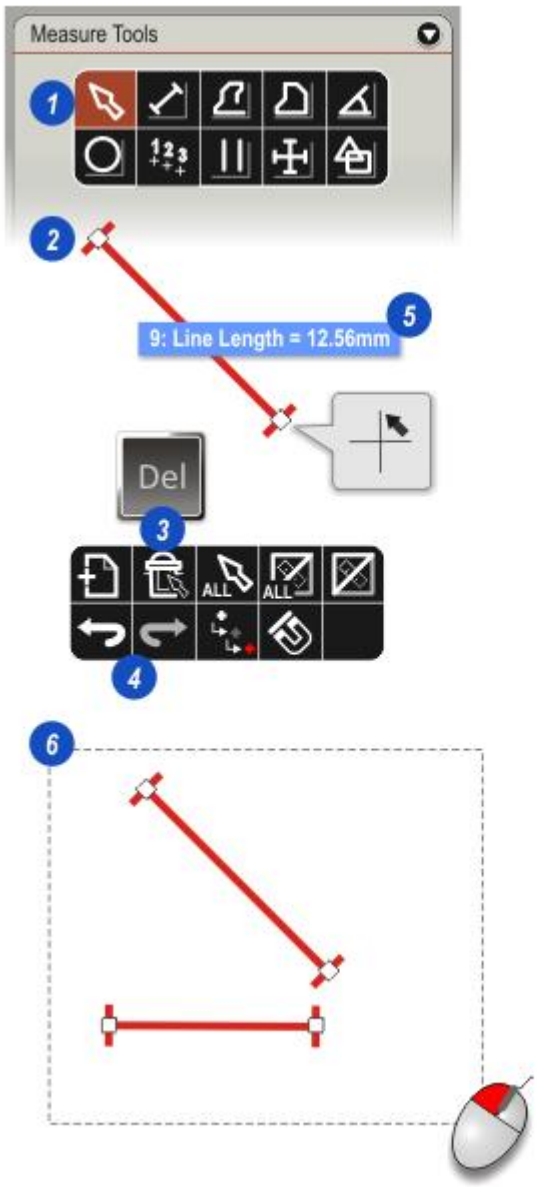
1: Click on the *Selection* tool button...

2: ... and then on the measurement, the end-points of which will appear as small 'boxes' or handles to indicate it is selected. The cursor changes to crosshairs and arrow.

- *Adjust a Measurement:* Click and drag on a handle.
- *Delete:* Press the keyboard *Delete* key or click on the *Trash Can* **(3)** to remove a selected measurement.
- *Undo/Redo Actions:* Use the Undo button to restore the last deletion **(4)**.
- *Re-position:* Click and drag on a measurement line (not a handle) to re-position it. The label follows.
- *Re-position Label:* Click and drag on a Label to re-position it independently of the measurement **(5)**.

Labels can be positioned precisely by typing values for the *X* and *Y* positions in the *Label Offset* text boxes.

- *Multiple Selection:* Click and drag on the image (not a measurement) to draw an enclosing box or 'marquee' around any number of measurements to simultaneously select them **(6)**.



From LAS 4.2 onwards, small red triangles on some panels indicate that a *Context Menu* with further options is available.

A good example is the *Measure Tools - Selection* panel in the *Acquire* module, where you can right-click to select a tool type and its parameter:

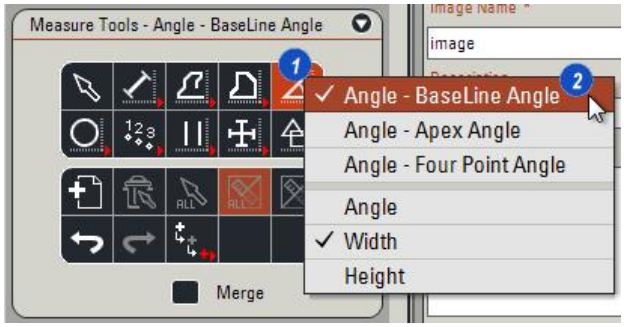
1: Right-click on a tool button, such as the *Angle Tool*.

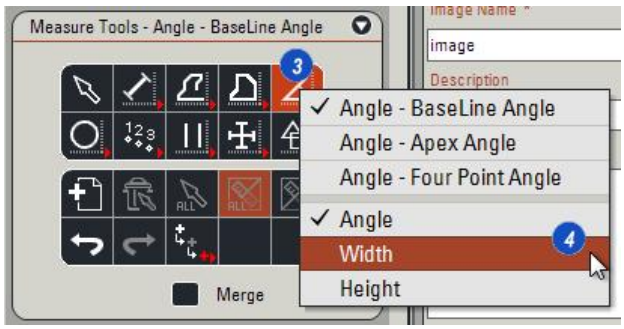
2: Left-click on the *Context Menu* to select the tool type (e.g. *Angle - Baseline Angle*).

3: Right-click on the tool button again.

4: Left-click on the *Context Menu* to select the parameter (e.g. *Width*).

5: Left-click on the tool to activate it.

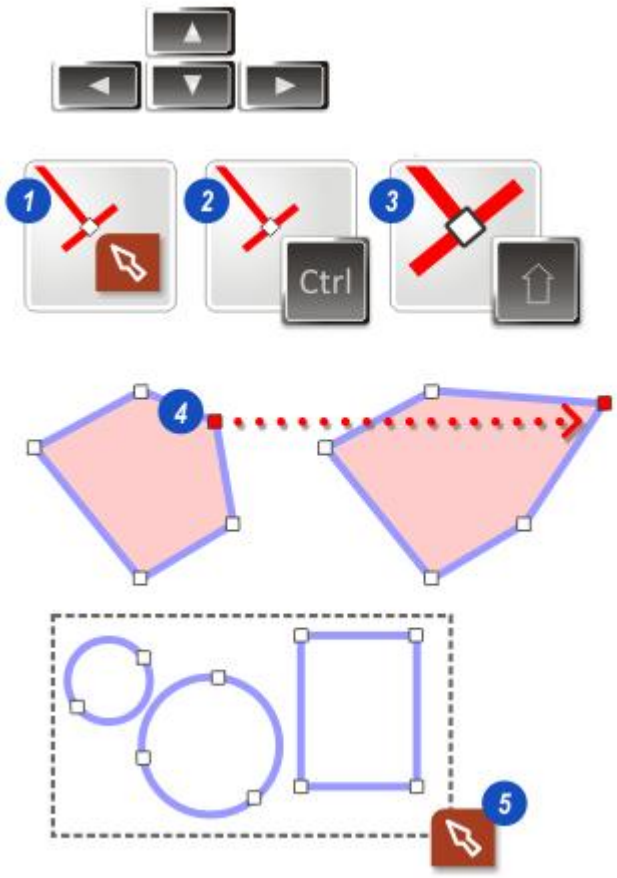




Nudge and Super-Nudge

The keyboard arrow keys can be used to 'nudge' - move in small increments - groups of measurement drawings, individual measurements or even single nodes on a drawing.

All four keys can be used - up, down, left and right.



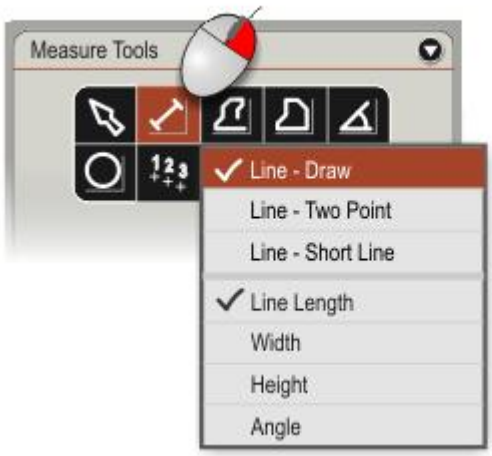
1: To move a single node on a drawing, use the *Selection* tool to highlight the node and then press the required arrow key. Movement is a single pixel for each key-press or...

2: ...hold down the *Ctrl* key to move the node 10 pixels - super-nudge - for each arrow key press.

3: Use the *Shift* key to magnify the node whilst pressing the arrow key to achieve very precise positioning.

4: Shapes as well as lines can be modified by selecting and nudging a single node.

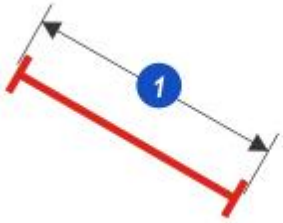
5: Individual measurements or groups of measurements can be nudged by choosing the *Selection* tool and then clicking and dragging a marquee around the required measurements. Release the *Selection* tool and use the arrow keys to precisely move the group.



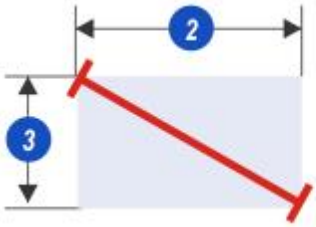
Mode: *Line - Draw*
Draws a distance line by dragging from a point.

Parameters:

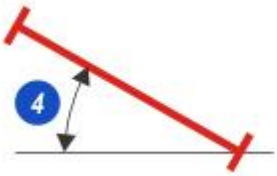
1: *Length:* Overall length of the drawn line measured to the centre of the line ending strokes.



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the line.



4: *Angle:* The angle swept between the line and the horizontal.

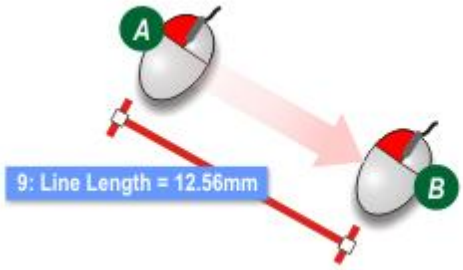


Method:

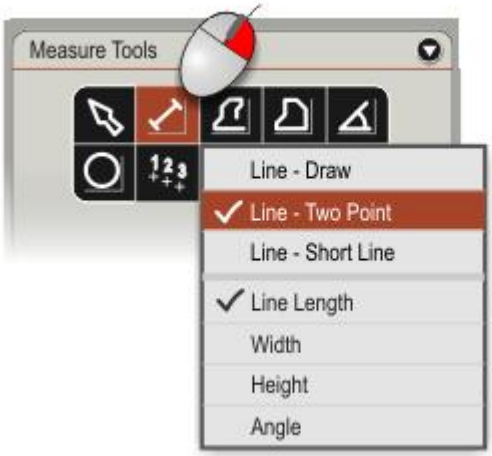
A: Left-click on the starting point and holding down the mouse button...

B: ...drag the line to the end point. Release the mouse button.

The *Label* is drawn at the same time.



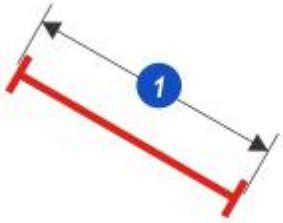
Distance Line: 2 Point



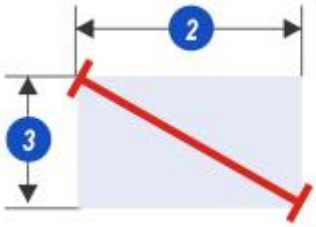
Mode: *Line - Two-Point:*
Draws a distance line between two clicked points.

Parameters:

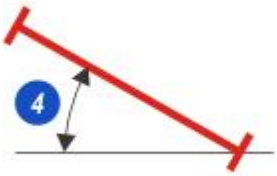
1: *Length:* Overall length of the drawn line measured to the centre of the line ending strokes.



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the line.



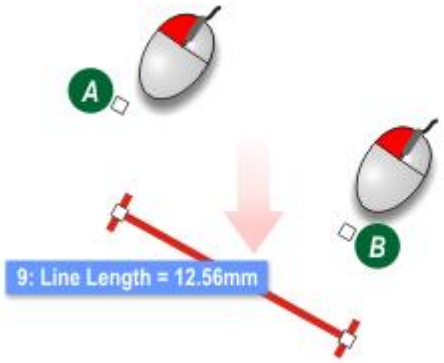
4: *Angle:* The angle swept between the line and the horizontal.

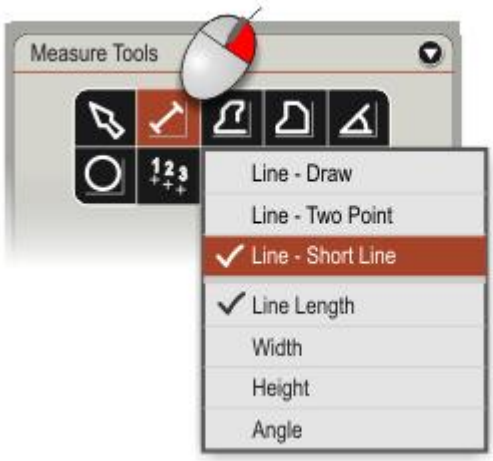


Method:

A: Left-click on the starting point. Release the mouse button.

B: Left-click on the end point and as the mouse button is released the line is drawn between the two points and the label displayed.



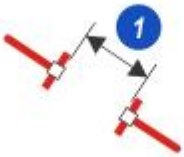


Mode: *Line - Short Line*

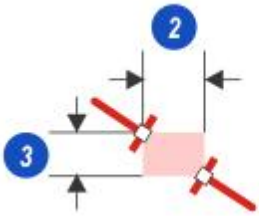
The *Short Line* mode is used to dimension narrow objects in which a single-ended line is placed either side of the object.

Parameters:

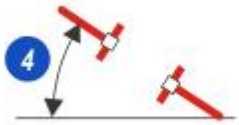
1: *Length:* Overall length of the drawn line measured to the centre of the line ending strokes.



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the line.



4: *Angle:* The angle swept between the line and the horizontal.

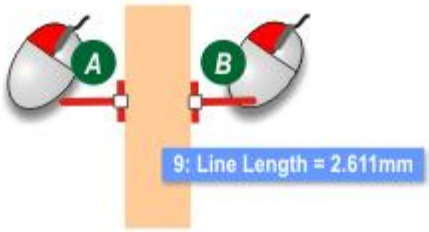


Method:

A: Left-click on one side of the object. The single-ended line is drawn.

B: Click on the opposite side of the object and the opposite facing line is drawn.

The *Label* is drawn at the same time.



A motorised stage controlled by LAS is an essential requirement for this feature. Similar in operation to Distance Line: 2 Point[919], it draws a distance line between two clicked points. However, it has the following advantages:

- It allows you to move the stage after clicking the start point, to measure line lengths that extend beyond the field of view
- You can refocus after clicking the start point, and the resulting measurement will also include Z-plane information

Notes:

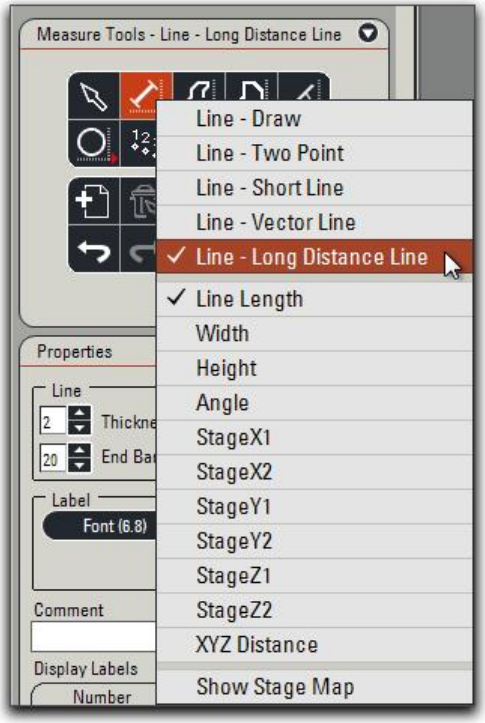
- This tool is only available as follows:
  - o In *Live Measurements* as part of the *Acquire Workflow*
  - o For microscopes with motorized or coded stages
- The microscope stage needs to be set up[924] correctly for multistep mosaic images

Mode: *Line - Long Distance Line*:
To measure automatically the area of an enclosed shape.

Parameters:

- *Line Length*: The 2D (X-Y) line length.
- *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.
- *Angle*: The angle swept between the line and the horizontal (as viewed on the X-Y plane).
- *StageX1, etc.*: The 3D stage co-ordinates of the start and end points.
- *XYZ Distance*: The 3D line length.

See Method[922]



To draw a Long Distance Line:

1: Left-click to place the start point.

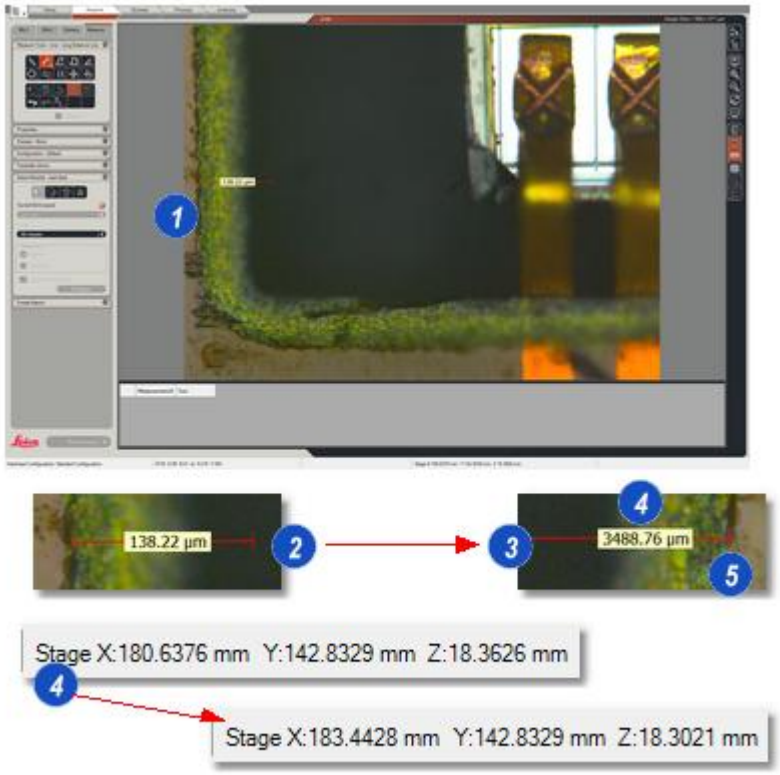
2: If your chosen end point lies outside the field of view, move (pan) the microscope stage manually using the joystick.

3: If required, you can also move the microscope stage in the Z plane to refocus on your chosen end point.

4: As you move the stage, the parameter value (e.g. *Line Length*) updates in the *Label*, and the *Stage Co-ordinates* update in the *Status Bar* 73 .

(If the Stage Co-ordinates are not displayed, choose *Options* > *Preferences* and enable the *Stage Position* option on the *Status Bar* tab.)

5: Left-click to mark the end point of the Long Distance Line.



The measurement will be displayed in the Grid.

Note: The accuracy of any measurements depends on:

- The mechanical condition of the Stage
- The accuracy of movement of the Stage; LAS cannot calibrate for any inaccuracies.

	Measurement #	Image Name	Tool	Line Length (µm)	Width (µm)	Height (µm)	Angle (°)
▶	6	Live Image	LongDistanceL...	3,487.25	3,487.25	3.78	0.06

XYZ Distance (µm)	StageX1 (µm)	StageY1 (µm)	StageZ1 (µm)	StageX2 (µm)	StageY2 (µm)	StageZ2 (µm)
3,487.25	180,209.65	142,793.62	18,302.05	183,696.90	142,789.84	18,302.05

Viewing Long Lines with the Stage Map

If you have drawn multiple Long Lines, or a mixture of line types, you can use the Stage Map to navigate your way around them in the Image Viewer:

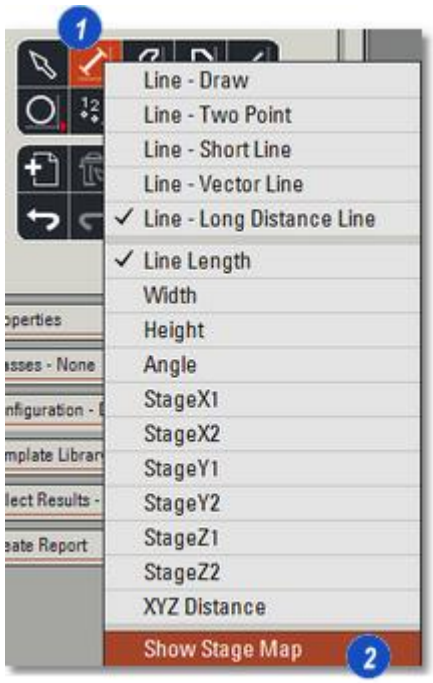
1: Right-click on the Line tool button in the *Measure Tools - Selection* panel.

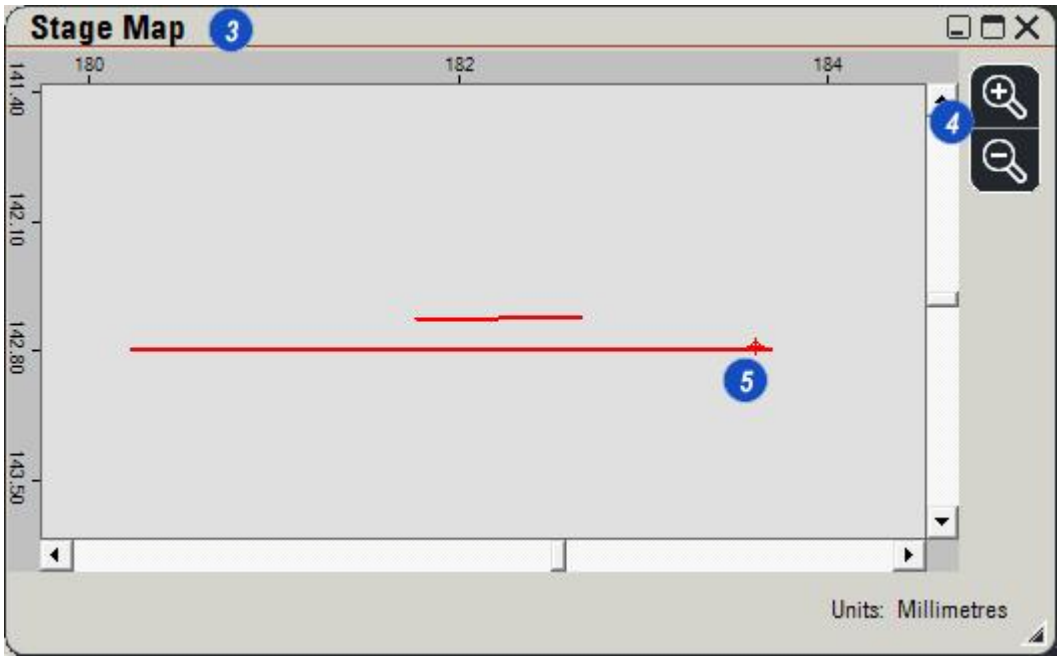
2: Choose *Show Stage Map* from the context menu.

3: The *Stage Map* will appear as a floating window. Red lines show the positions of any measurements; a red cross shows the current position on the microscope stage.

4: Zoom in or out using the zoom buttons, or...

5: ...Double-click on the Stage Map to move the Stage and centre the Image Viewer on a particular point.

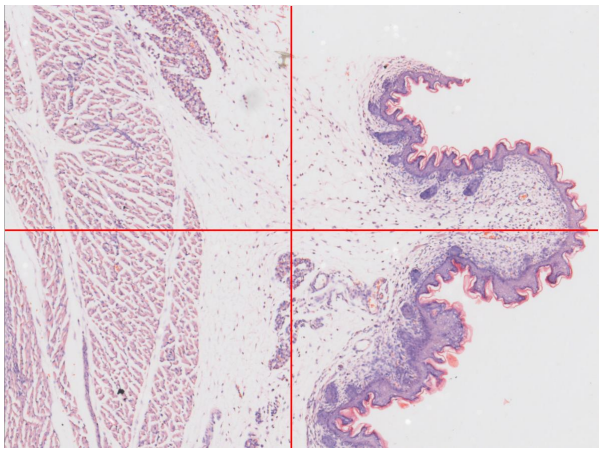




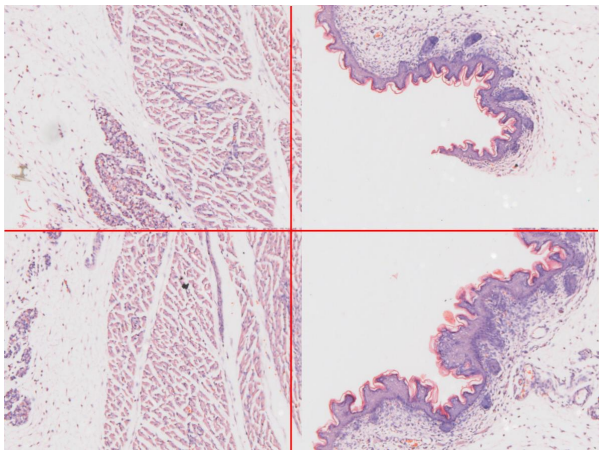
LAS can create Mosaic images combining several fields of view - built up using Multistep Sequences[716]. You can still make Distance Line measurements on such Mosaic images. However, bear the following important points in mind:

- Check the orientation of the stage is correct by making trial mosaics of 2x2 and 3x3 images.
- Before using MultiStep please ensure that the system is accurately calibrated and the stage is carefully aligned with the X and Y axes of the camera. The camera shading correction should also be set.
- Ensure that image flipping[311] is set up so that the mosaic images are correctly and accurately stitched. It is obvious when this is incorrect, because the mosaic will be wrong.

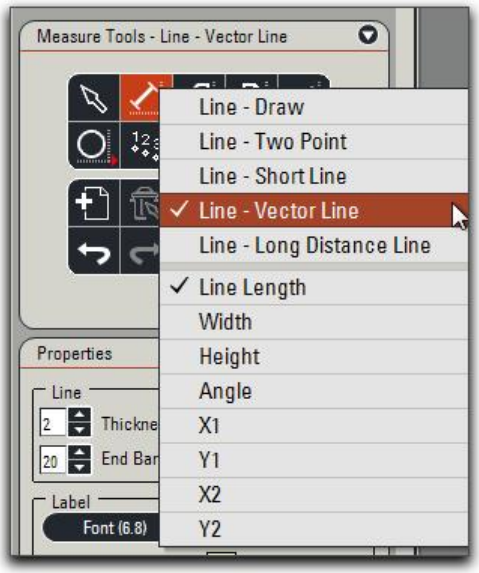
Correct mosaic stitching



Incorrect: images flipped vertically and horizontally



Similar to a Distance Line[918], this tool draws a distance line between two points, but also displays the X-Y co-ordinates of the start and end points.



Note: This tool is available in:

- *Live Measurements* as part of the *Acquire Workflow*
- *Interactive Measurements* as part of the *Analysis Workflow*

Mode: *Line - Vector Line*:
Draws a distance line between two clicked points.

Parameters:

- *Line Length*: The 2D (X-Y) line length.
- *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the line.
- *Angle*: The angle swept between the line and the horizontal.
- *X1, etc.*: The X and Y co-ordinates of the start (1) and end (2) points.

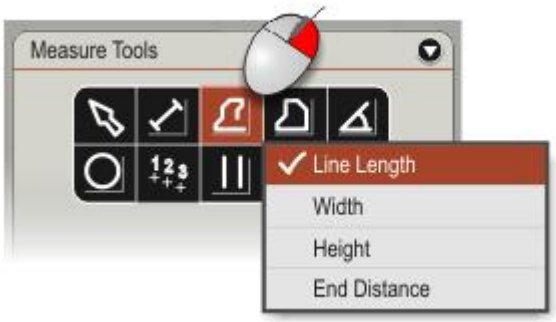
Method:

1: Left-click on the starting point.

2: Drag the pointer and release on the end point. The line is drawn between the two points and the label displayed.

3: The X-Y co-ordinates are displayed in the Grid.

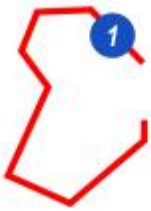
Line Length (µm)	Width (µm)	Height (µm)	Angle (°)	X1	Y1	X2	Y2
421.385	419.088	43.930	-5.984	162.000	215.000	291.000	228.000



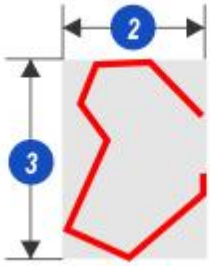
Mode: *Segment Tool*: Single option:
To measure the distance around the periphery of an irregular shape.

Parameters:

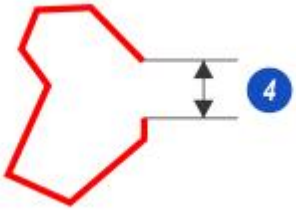
1: *Line Length:* The total length of all the segments added together.



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.



4: *End Distance:* The distance between the two end points of the figure.

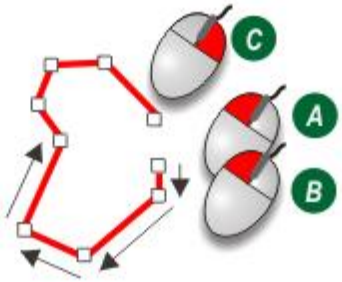


Method:

A: Click and release to start.

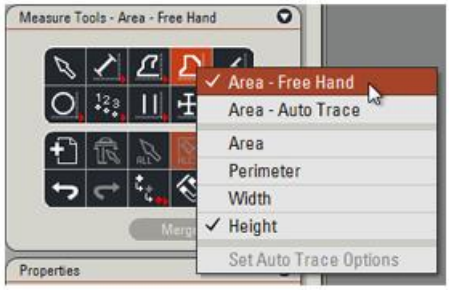
B: Click to end the segment and start another. A line is drawn between the two points.
Repeat around the periphery.

C: Right click to end. The *Label* is displayed at the same time.



Alternatively, click, hold and drag for a continuous line that tracks the mouse path.

Area Tool: Free Hand



Mode: *Area Tool*: *Free Hand*:
To measure the area of an enclosed irregular shape.

Parameters:

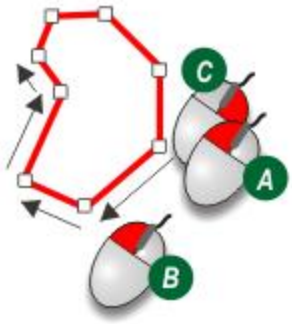
- *Area:* The enclosed space returned in the selected measurement squared (for example mm²).
- *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.
- *Perimeter:* The distance around the figure.

Method:

A: Click and release to place the first vertex.

B: Move the cursor to the next vertex and click.
Repeat for all sides and back to the start point.

C: Right click to end. The *Label* is displayed at the same time.



Alternatively, click, hold and drag for a continuous line that tracks the mouse path.

Note: If the drawn shape pulsates when complete, some points on the outline cross. These must be removed to give a correct area measurement.

Note: This tool is only available in *Interactive Measurements* as part of the *Analysis Workflow.*

Mode: *Area Tool*: *Auto-Trace*:
To measure automatically the area of an enclosed shape.

Parameters:

- *Area:* The enclosed space returned in the selected measurement squared (for example mm²).
- *Perimeter:* The distance around the shape.
- *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.

Auto Trace Options

- *Intensity Spread:* The range of greyscale or RGB levels used to define the area automatically.

For example, if you set this value to 40, LAS takes the greyscale or RGB value of the pixel you click on as the reference value, and measures ± 40 levels.

- *Edge Smoothing*: Choose from *None*, *Low*, *Medium* or *High*.
- *Maximum Points Per Trace After Smoothing:* You can adjust this value to make the traced shape more or less accurate. Higher values are more accurate, but take more processing power. Values range from 0 to 50000.

See Method 929 and Possible Error Messages 930

1: Right-click and select the *Area - Auto Trace* tool from the context menu.

2: Right-click and select which parameter should be displayed (e.g. *Area*) from the context menu.

3: Right-click on the *Area Tool* and select *Set Auto Trace Options* from the context menu.

4: Set the *Intensity Spread*.

5: Select the amount of *Edge Smoothing*.

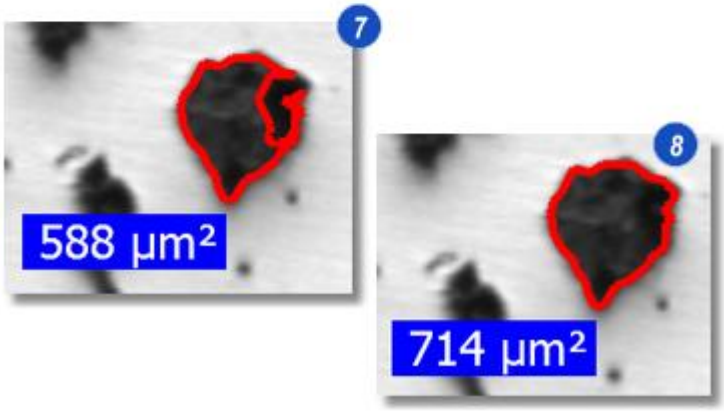
6: Set the *Maximum Points Per Trace After Smoothing* and click *OK*.

7: In the *Image Viewer*, click on a point inside an enclosed shape that you want to trace, or click and drag a small rectangle for a more representative selection of pixels.

- The area drawn is based on the current Auto Trace Options (in particular, the *Intensity Spread*).
- Any holes enclosed by the region are filled in.

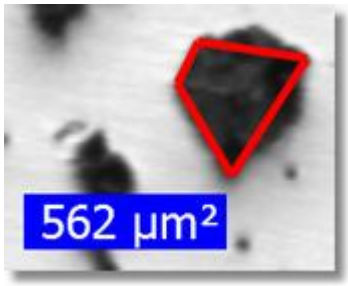
8: Ctrl-click to extend the current region.

Note: The point that you Ctrl-click should be close enough to the selected region so as not to create a disconnected region (which is not allowed).



9: The measurement appears in the Grid.

Effect of higher smoothing and fewer maximum points:

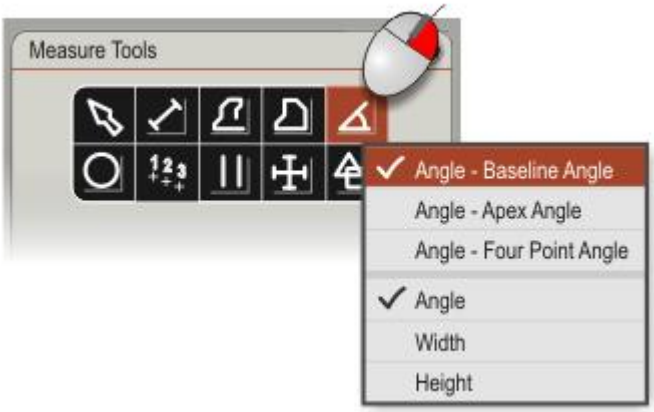


Measurement #	Image Name	Tool	Width (µm)	Height (µm)	Area (µm²)	User Value
5	gr01.tif	AreaAutoTrace	31	35	714	2147483647

See Possible Error Messages 930

Message	Description	Suggestion
No object traced, the tracing appears to be too complex.	You have clicked on a point for which LAS cannot easily detect an enclosing region. Trying to trace such a region will probably cause LAS to run out of memory.	Try clicking on a different point, that has a more obvious enclosing region.
The image is too big to be traced.	The image is approaching the maximum size that LAS can handle.	If possible, reacquire the image using a lower resolution.
No object was created as the whole image would have been covered.	With the current Auto Trace Options, the traced region would extend right out to the borders of the image.	Try decreasing the *Intensity Spread* setting.
The selected object cannot be extended because the new region is not joined to it.	With the current Auto Trace Options, the new point would cause a separate region to be created, rather than an extension of the existing region.	Click on a point that is closer in location and intensity to the original point, or try increasing the *Intensity Spread* setting.
No object was created as this would have been a single pixel	With the current Auto Trace Options, the traced region would only contain a single pixel.	Try increasing the *Intensity Spread* setting, or click on a different point that is surrounded by pixels of similar intensity.

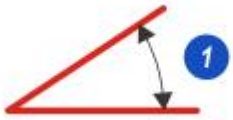
Angle: Baseline



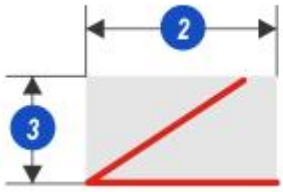
Mode: *Baseline Angle:*
Represents the angle between a horizontal or 'base' line and an extension.

Parameters:

1: *Angle*: The angle described with reference to the horizontal..



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.

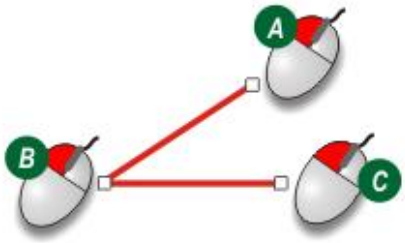


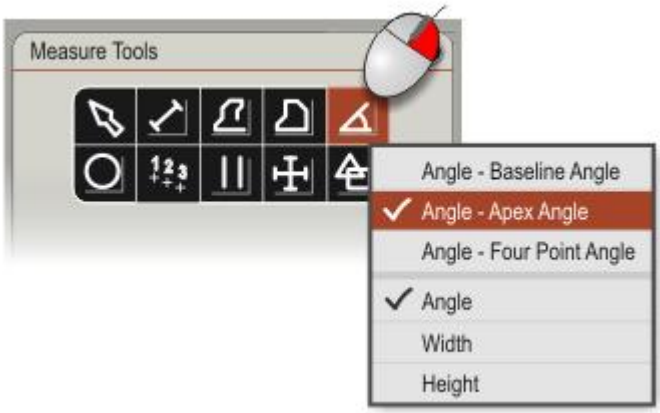
Method:

A: Click on the starting point.

B: Move the cursor to the junction between the two legs and click once.

C: Move the cursor to the end of the horizontal leg and click to finish.

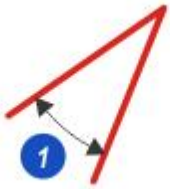




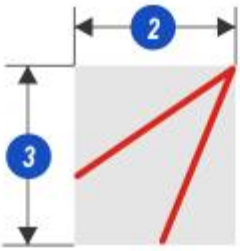
Mode: *Apex Angle:*
The angle between the two legs irrespective of the inclination.

Parameters:

1: Angle: The uppermost angle described between the two drawn 'legs'.



2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.

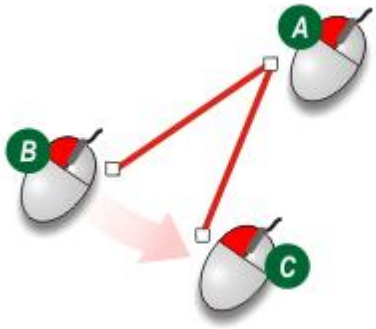


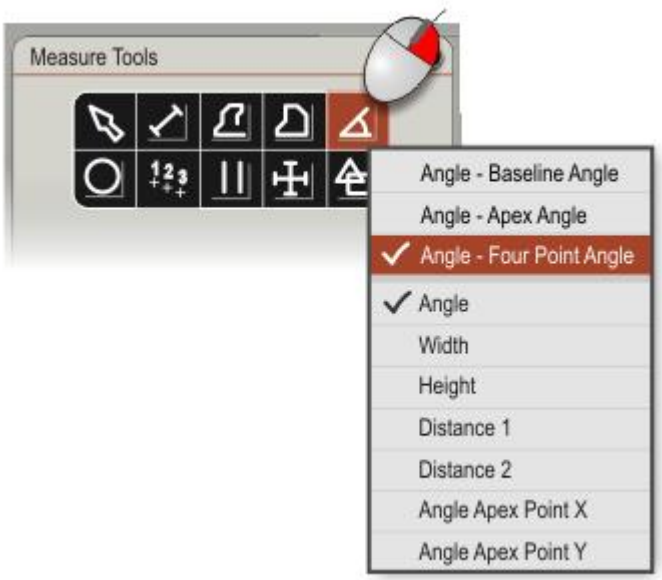
Method:

A: Click on the apex of the angle.

B: Move to the end point of the first leg and click.

C: Describe an arc to the end point of the second leg and click to finish.



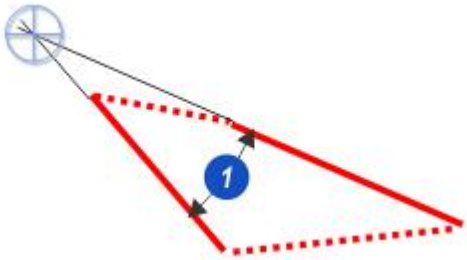


Mode: *Four Point Angle*

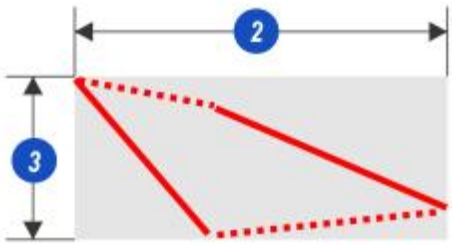
The angle between two lines that are not connected at a point. Furthermore, the lines projected intersection could be beyond the limits of the image or the *Viewer*.

Parameters:

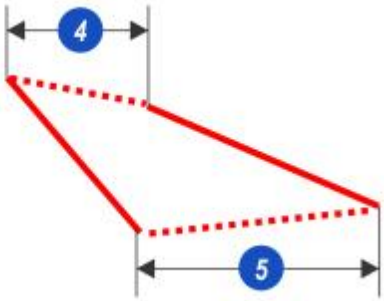
1: *Angle*: The angle described at the intersection of two extended lines.



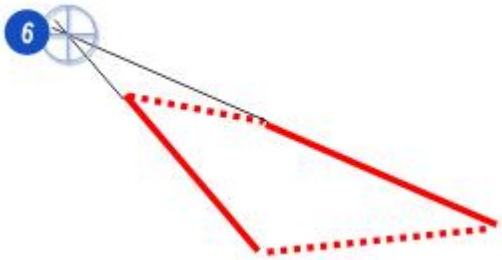
2 & 3: *Width and Height:* The horizontal and vertical measurements of a bounding box enclosing the outline.



4 & 5: *Distance 1 and Distance 2:* The shortest and longest distance between the ends of the two lines respectively.



6: *Angle Apex Point X and Point Y:* Displays the point at which the two sides would meet if extended to an apex either as the *X* co-ordinate or the *Y* co-ordinate.



Method:

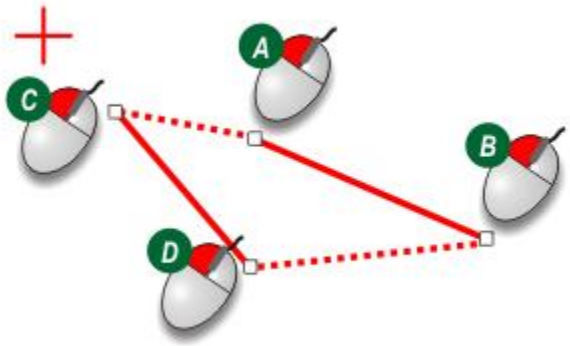
A: Click on the starting point of the first line.

B: Move the cursor to the end of the first line and click again. The line is drawn between the two points.

C: Click on the starting point of the second line.

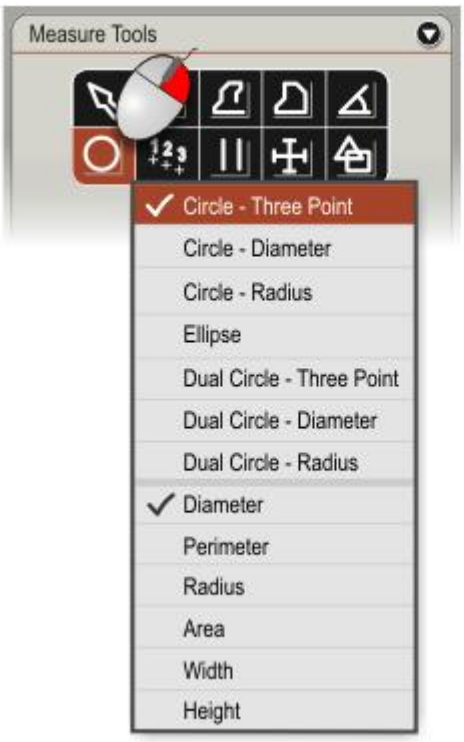
D: Move the cursor to the end of the second line and click again. The line is drawn between the two points.

Dotted lines are drawn between the ends of the lines and also the point of imaginary intersection.



- If the lines are nearly parallel an imaginary point of intersection will be beyond the limits of the image or even the *Viewer*, but the software can accommodate this situation and return an accurate angle.
- If the lines are parallel then the angle will be 0°.

Circle: Three Point



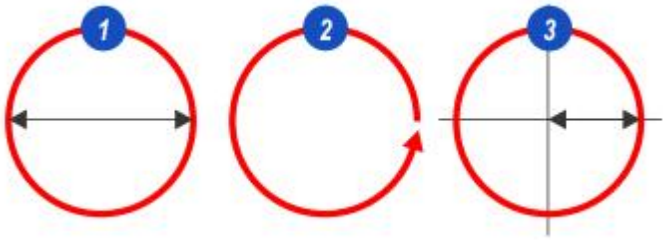
Mode: *Three Point*:
Draws a circle intersecting 3 points created by the user.

Parameters:

1: *Diameter*: Distance across the circle in any direction.

2: *Perimeter*: The circle circumference.

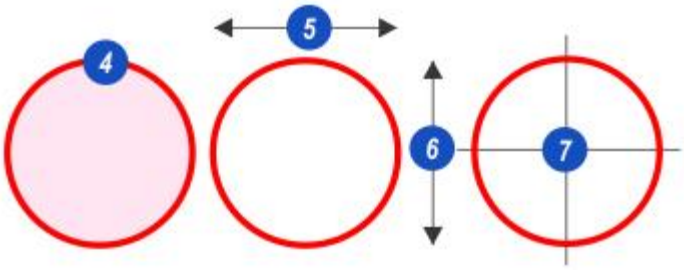
3: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



4: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

5: *Width*: Distance across the circle horizontally.

6: *Height*: Distance across the circle vertically.



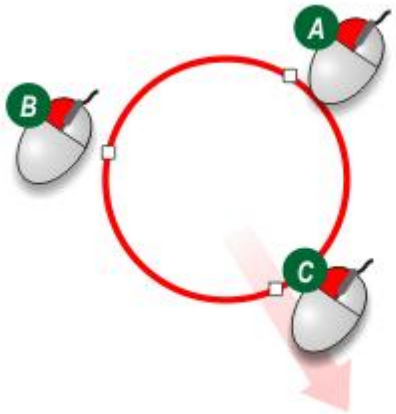
7: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.

Method:

A: Click on a point on the image.

B: Click on a second point on the image.

C: Click on a third point and, holding down the mouse button drag the point 'handle' to describe the diameter required.



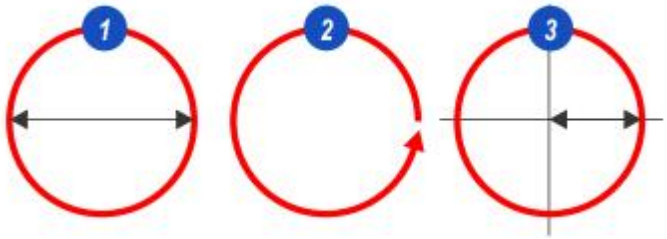
Mode: *Diameter:*
Draws a circle by dragging on the circle edge. To aid precise drawing the *Diameter* value is displayed continuously during drawing.

Parameters:

1: *Diameter*: Distance across the circle in any direction.

2: *Perimeter*: The circle circumference.

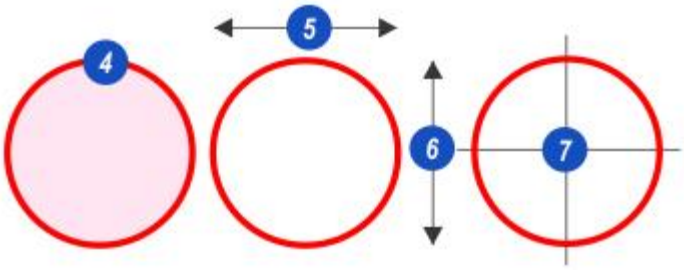
3: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



4: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

5: *Width*: Distance across the circle horizontally.

6: *Height*: Distance across the circle vertically.

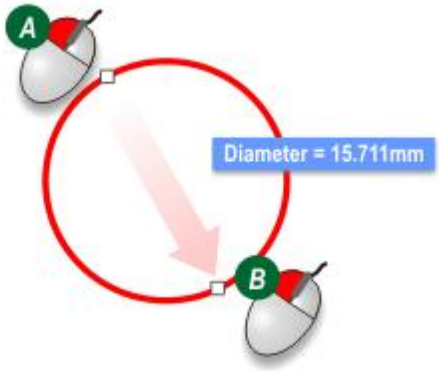


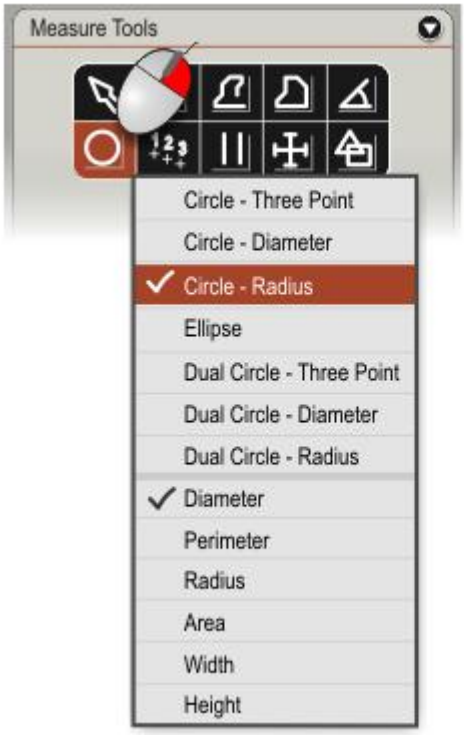
7: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.

Method:

A: Click on a point on the image and, holding down the mouse button...

B: ...drag to the diameter required - the diameter is displayed continuously as the circle is drawn. Release the mouse button.





Mode: *Radius:*

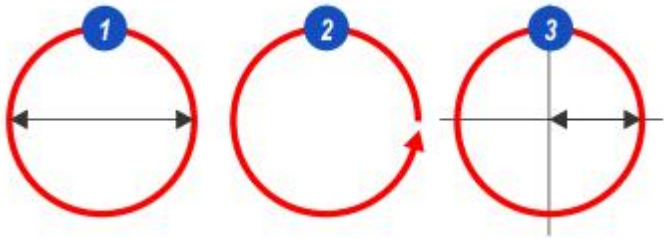
Draws a circle by dragging on the circle edge. To aid precise drawing the *Radius* value is displayed continuously during drawing.

Parameters:

1: *Diameter*: Distance across the circle in any direction.

2: *Perimeter*: The circle circumference.

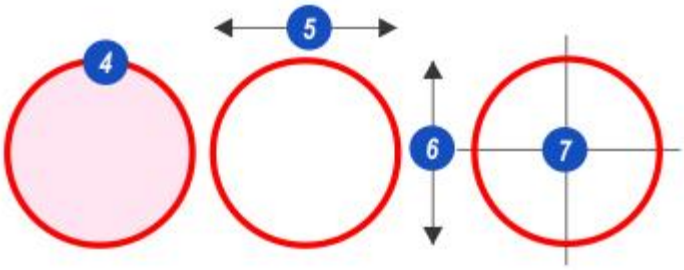
3: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



4: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

5: *Width*: Distance across the circle horizontally.

6: *Height*: Distance across the circle vertically.

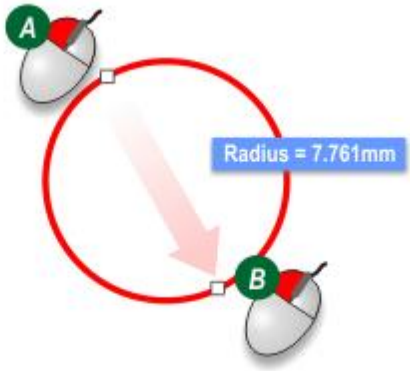


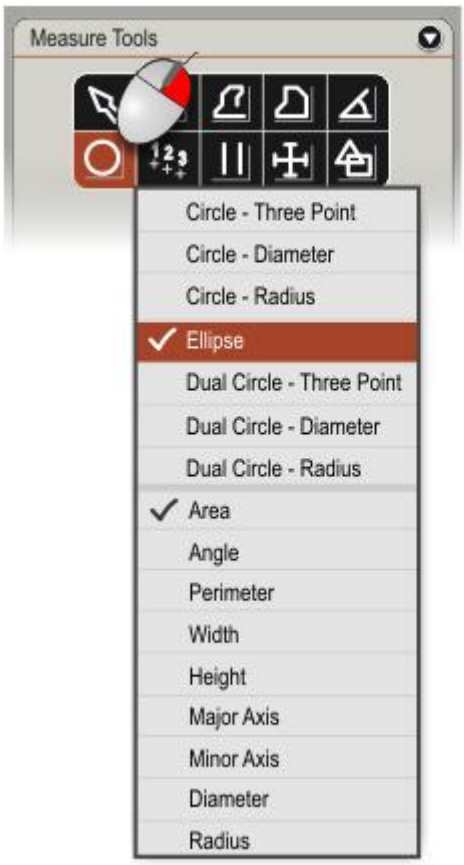
7: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.

Method:

A: Click on a point on the image and, holding down the mouse button...

B: ...drag to the radius required - the radius is displayed continuously as the circle is drawn. Release the mouse button.





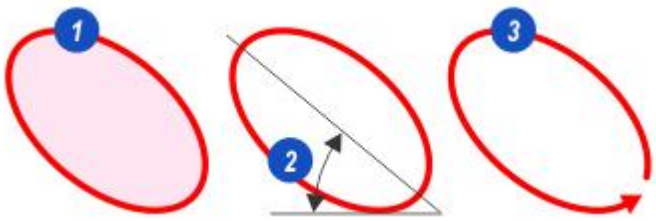
Mode: *Ellipse*: Single option:
Part of the *Circle* class but having unequal axes.

Parameters:

1: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

2: *Angle*: Angle between the horizontal and the Major Axis.

3: *Perimeter*: The distance around the ellipse.

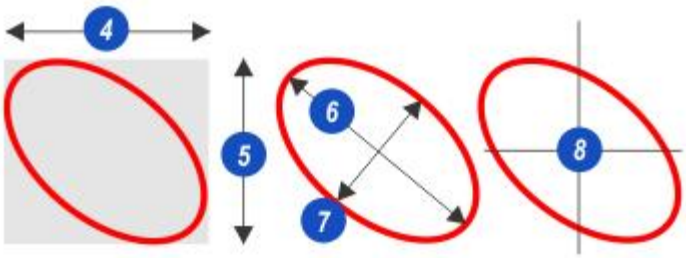


4: *Width*: Distance across the ellipse horizontally.

5: *Height*: Distance across the ellipse vertically.

6: *Major Axis:* The greatest dimension.

7: *Minor Axis:* The smallest dimension.



Diameter and *Radius* are retained as part of the *Circle* class but are principally the *Major Axis* and the *Major Axis / 2.*

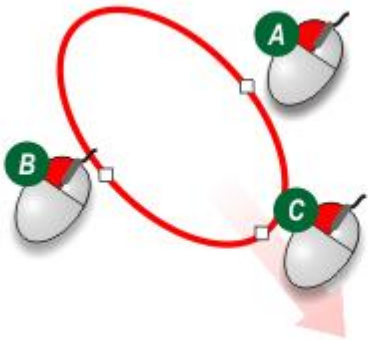
8: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.

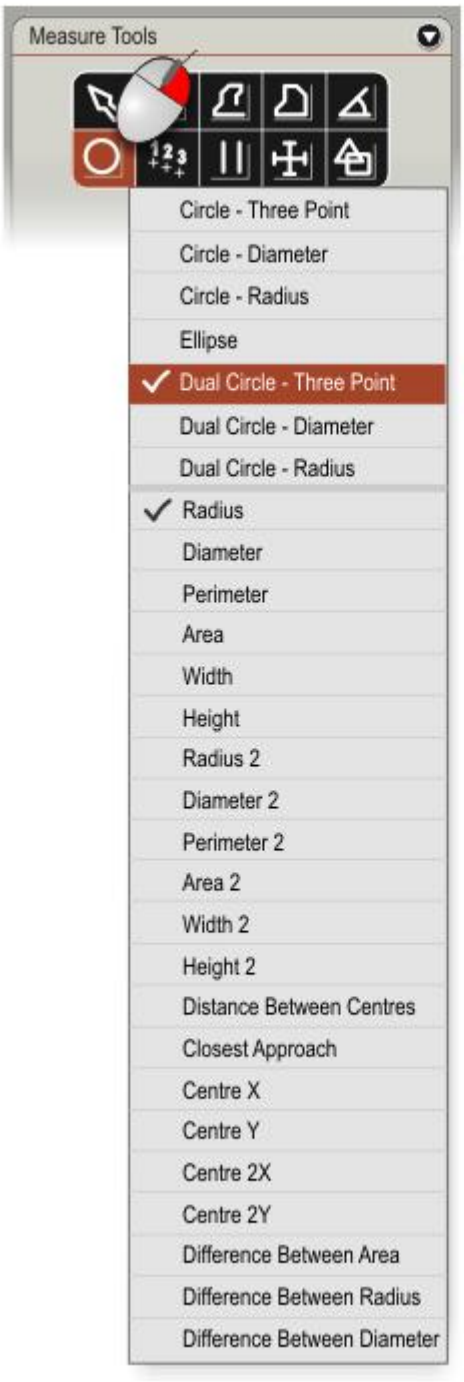
Method:

A: Click on a point on the image.

B: Click on a second point representing either the major or minor axis of the *Ellipse* and...

C: ... still holding down the mouse button drag to describe the other axis. Click to complete.





Mode: *Three Point:*
Draws two circles and provides a range of comparison measurement.

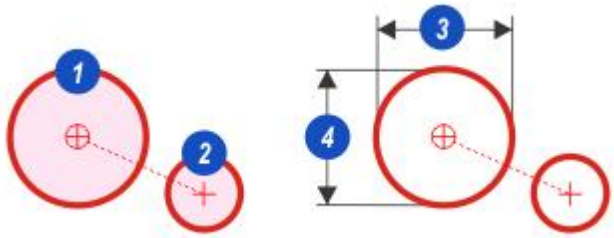
Parameters:

The parameters with the *'2'* suffix (for example *Radius 2*) refer to the second circle:

1 & 2: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

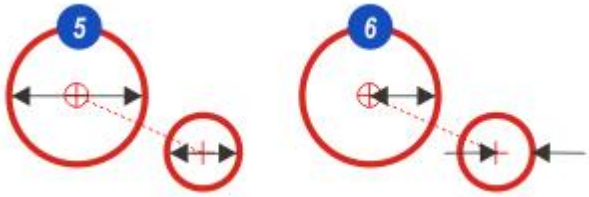
3: *Width*: Distance across the circle horizontally.

4: *Height*: Distance across the circle vertically.



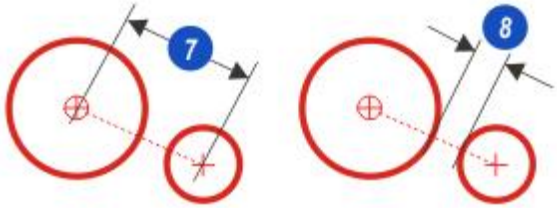
5: *Diameter*: Distance across the circle in any direction.

6: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



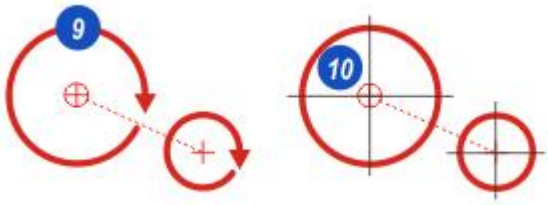
7: *Distance Between Centres* is the distance between the centres of the first and second circles.

8: *Closest Approach* is the smallest distance between the two perimeters.



9: *Perimeter*: The circle circumference.

10: The *Centre X* and *Centre Y* dimensions are the *X* (Horizontal) and *Y* (Vertical) distances from the top/left corner of the image.



Difference Between displays the result of subtracting the smaller circle value - *Area, Radius* or *Diameter* - from the larger.

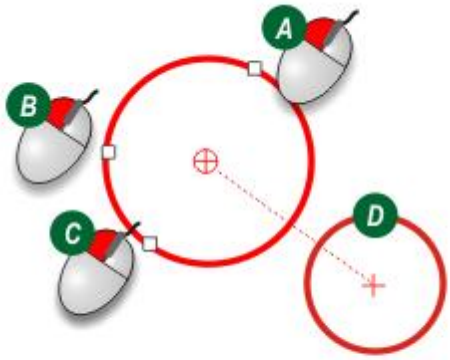
Method:

A: Click on a point on the image.

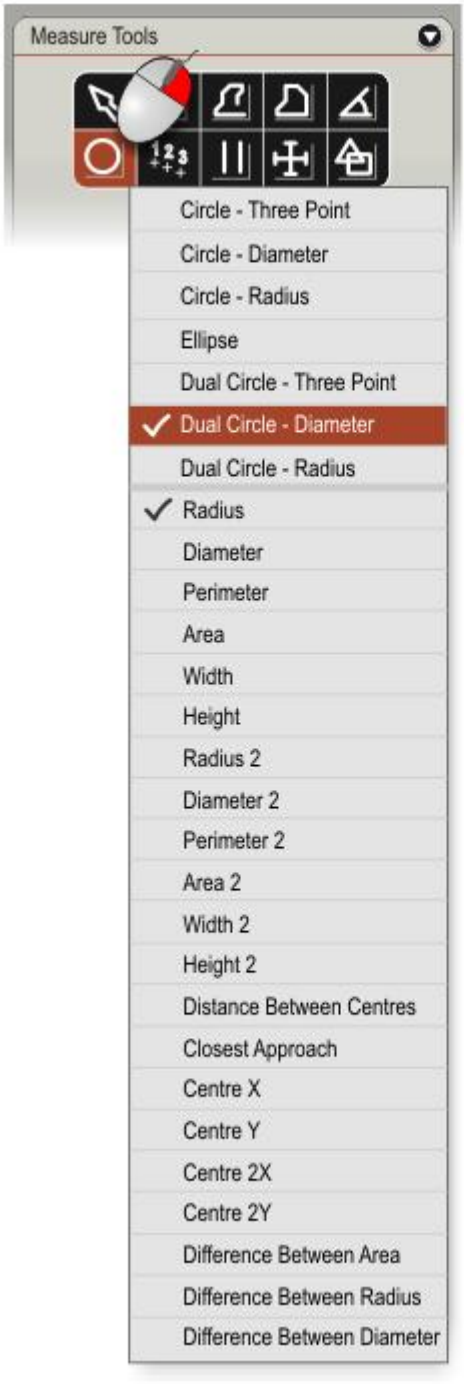
B: Click on a second point on the image.

C: Click on a third point and, holding down the mouse button drag the point 'handle' to describe the diameter required.

D: Repeat the process for the second circle. A dotted line is drawn between the two centres.



Dual Circle: Diameter



Mode: *Diameter:*

Draws two circles based upon diameter and provides a range of comparison measurement.

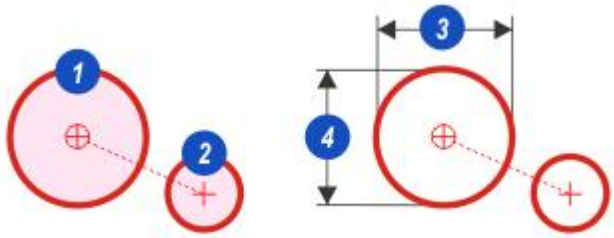
Parameters:

The parameters with the *'2'* suffix (for example *Radius 2*) refer to the second circle:

1 & 2: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

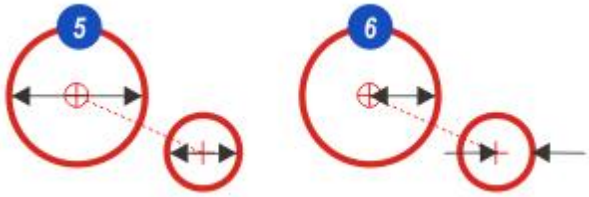
3: *Width*: Distance across the circle horizontally.

4: *Height*: Distance across the circle vertically.



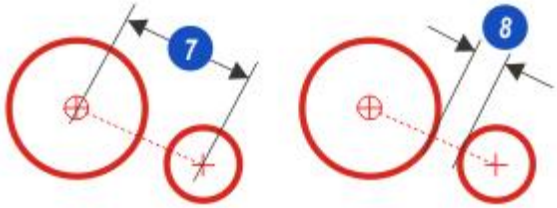
5: *Diameter*: Distance across the circle in any direction.

6: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



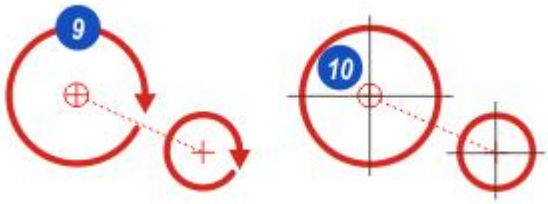
7: *Distance Between Centres* is the distance between the centres of the first and second circles.

8: *Closest Approach* is the smallest distance between the two perimeters.



9: *Perimeter*: The circle circumference.

10: The *Centre X* and *Centre Y* dimensions are the *X* (Horizontal) and *Y* (Verttical) distances from the top/left corner of the image.



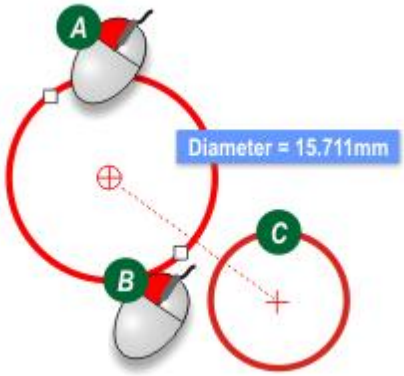
Difference Between displays the result of subtracting the smaller circle value - *Area, Radius* or *Diameter* - from the larger.

Method:

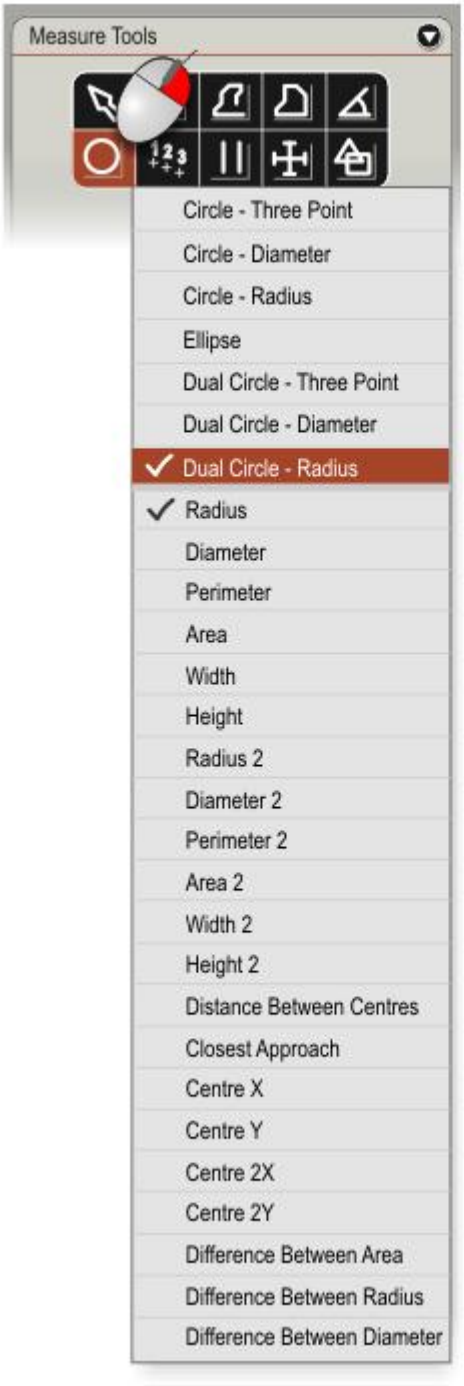
A: Click on a point on the image and, holding down the mouse button...

B: ...drag to the diameter required - the diameter is displayed continuously as the circle is drawn. Release the mouse button.

C: Repeat the process for the second circle. A dotted line is drawn between the two centres.



Dual Circle: Radius



Mode: *Radius:*

Draws two circles based upon radius and provides a range of comparison measurement.

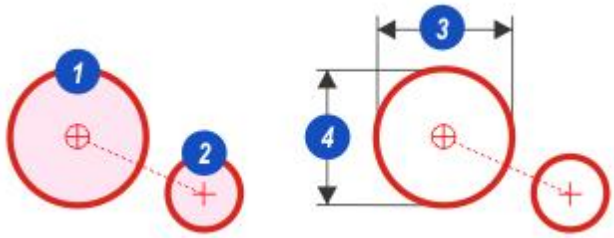
Parameters:

The parameters with the *'2'* suffix (for example *Radius 2*) refer to the second circle:

1 & 2: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

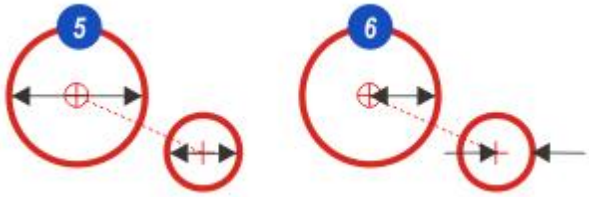
3: *Width*: Distance across the circle horizontally.

4: *Height*: Distance across the circle vertically.



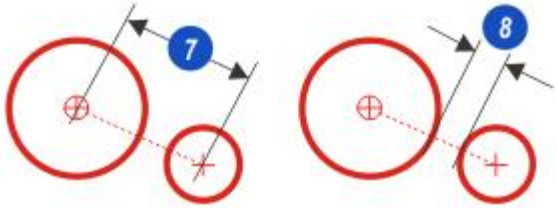
5: *Diameter*: Distance across the circle in any direction.

6: *Radius*: The distance from the centre of the circle to the centre of the enclosing line.



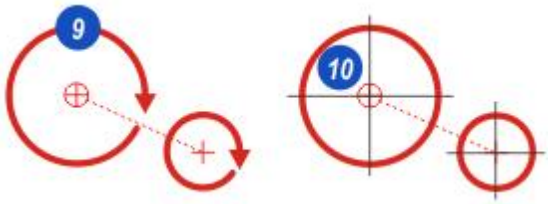
7: *Distance Between Centres* is the distance between the centres of the first and second circles.

8: *Closest Approach* is the smallest distance between the two perimeters.



9: *Perimeter*: The circle circumference.

10: The *Centre X* and *Centre Y* dimensions are the *X* (Horizontal) and *Y* (Verttical) distances from the top/left corner of the image.



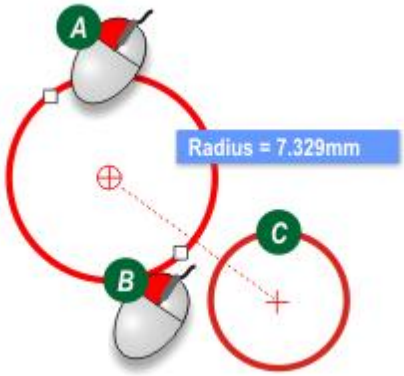
Difference Between displays the result of subtracting the smaller circle value - *Area, Radius* or *Diameter* - from the larger.

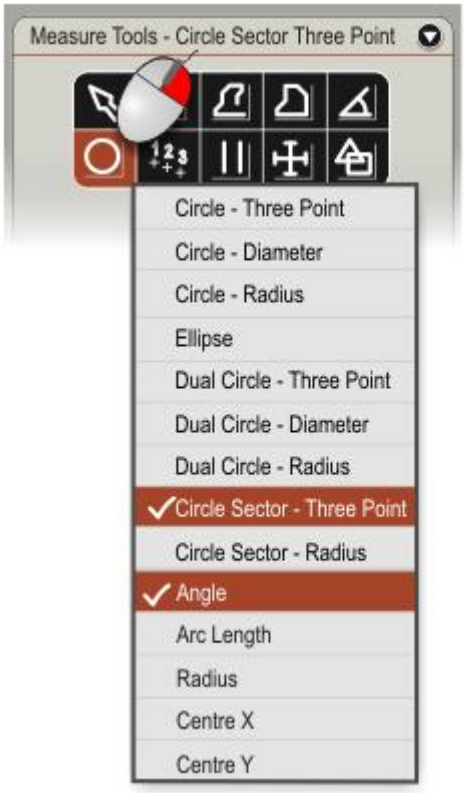
Method:

A: Click on a point on the image and, holding down the mouse button...

B: ...drag to the radius required - the radius is displayed continuously as the circle is drawn. Release the mouse button.

C: Repeat the process for the second circle. A dotted line is drawn between the two centres.





Mode: *Three Point*:

Draws a circle sector arc intersecting 3 points created by the user.

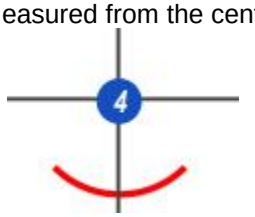
Parameters:

1: *Angle:* The sector angle.

2: *Arc Length:* Distance along the arc.

3: *Radius*: The distance from the centre of the sector to the perimeter.

4: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.



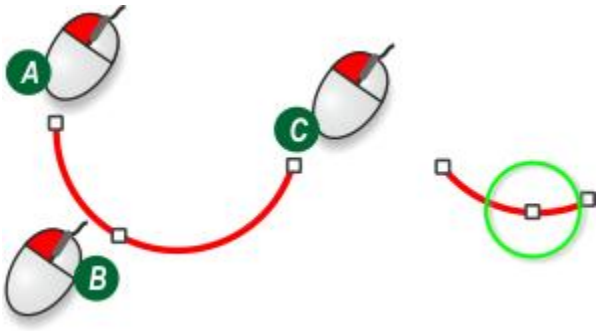
Method:

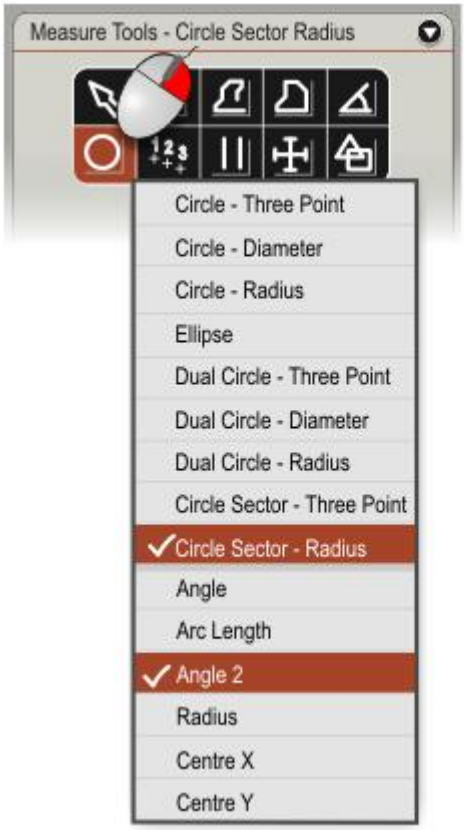
A: Click on a point on the image at the start of the sector arc.

B: Click on a second point further along the arc. The initial arc is drawn.

C: Move the cursor. The arc will be extended to and follow the movement of the cursor. When the position is at the end of the arc, click the mouse button to set the third point.

The shape and dimensions can be changed by clicking and dragging on a node with the *Selection* tool.





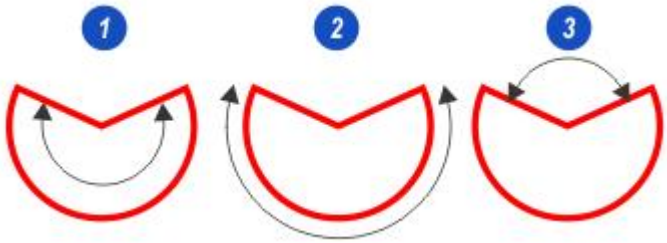
Mode: *Radius*:
Draws a circle sector by using 2 points to represent the radius and dragging a third point to describe the sector arc.

Parameters:

1: *Angle:* The sector angle.

2: *Arc Length:* Distance around the perimeter of the sector.

3: *Angle 2:* The sector external angle.

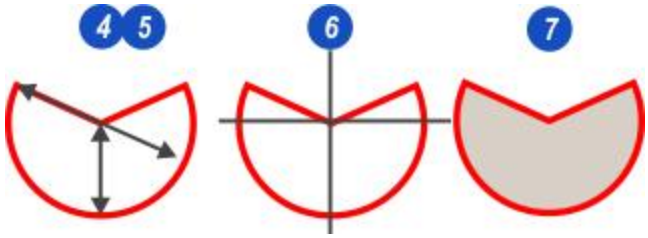


4: *Radius*: The distance from the centre of the segment to the perimeter.

5: Diameter: Of the circle of which the arc is part.

6: *Centre X and Centre Y:* Horizontal and vertical position co-ordinates measured from the centre of the image.

7: *Area*: Enclosed by sector



Method:

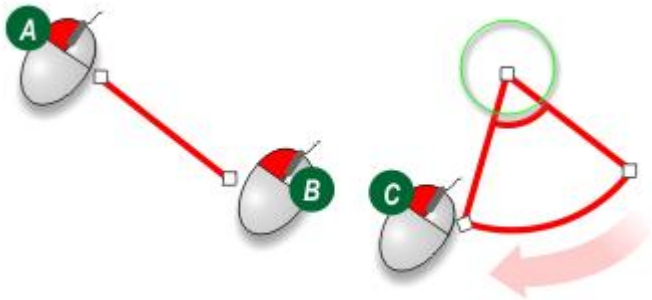
A: Click on a point on the image representing the centre of the sector circle and, holding down the mouse button...

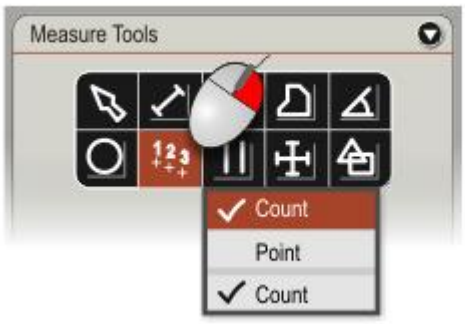
B: ...drag to a second point representing the radius. Click and release to set the point.

C: Move the mouse to describe the segment arc and finish by left-clicking.

The shape and dimensions can be changed by clicking and dragging on a node with the *Selection* tool.

Alter and invert the sector - acute to obtuse for example - by clicking and dragging the centre node.





Mode: *Count*:
Counts each item clicked and displays the count sequence:

Parameter:

Count: Incremental value of number of items clicked.

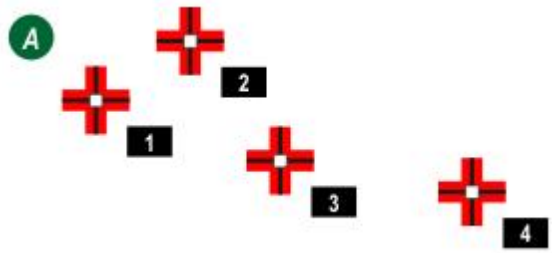
Method:

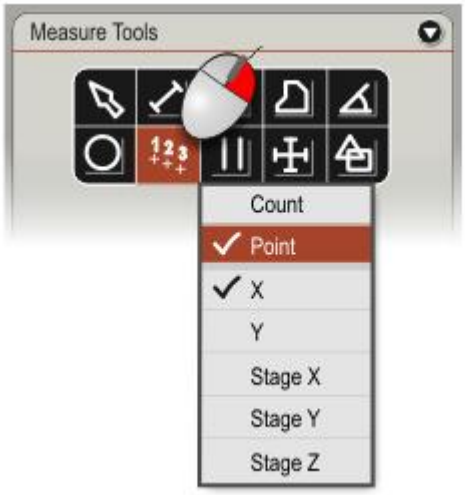
A: Click on each of the items to be counted.

The count value automatically increments and a small ‘target’ is drawn over the clicked point.

Individual ‘targets’ and labels can be moved for clarity.

Use line thickness to change the 'target' size.





Mode: *Point*:
Displays a co-ordinate and cross-hairs at a point on the image that is clicked.

Parameters:

X and *Y:* The selected co-ordinate relative to the centre of the image.

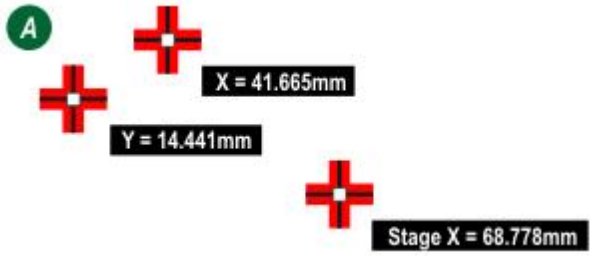
Stage X and *Stage Y:* The selected co-ordinate relative to the centre of the stage.

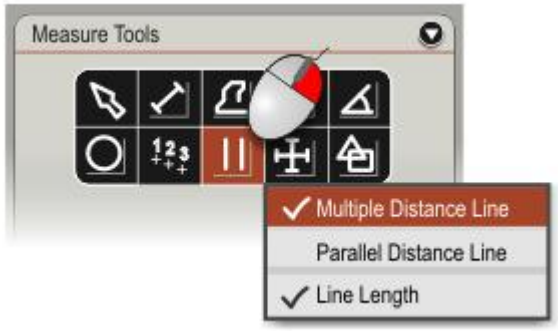
Stage Z: Focus position.

Method:

A: Click on a point to display the selected co-ordinate.

Use line thickness to change the 'target' size.

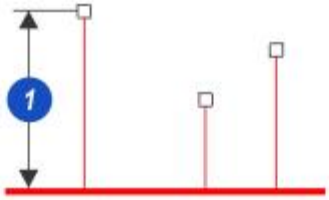




Mode: *Multiple Distance Line:*
Uses a single datum line and then measures the distance of objects from that line by individual offset lines.

Parameters:

1: *Line Length (Default):* The distance from a datum line to an object indicated by a drawn line.



Method:

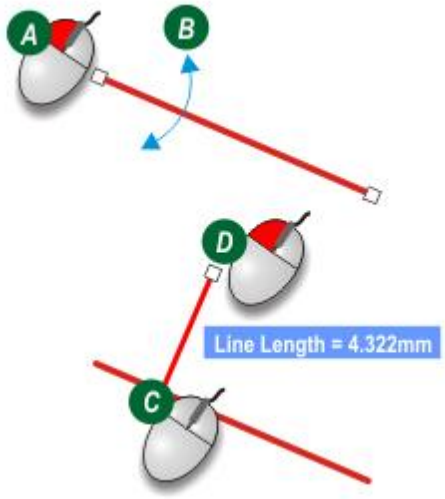
A: Click on the image to establish the datum line starting position.

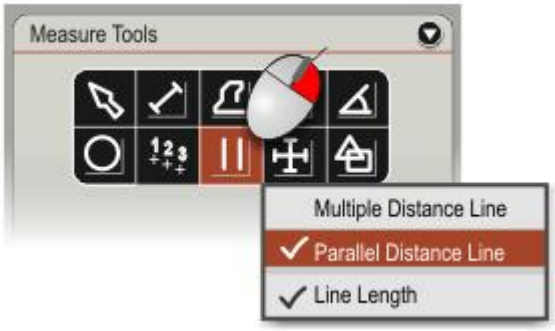
B: Move the cursor to rotate the datum line if necessary. Click again to anchor it.

C: Move the cursor to the distance to be measured left or right, top or bottom of the datum.

D: Click on the object to be measured.

Repeat the process for subsequent lines ending with a right-click.

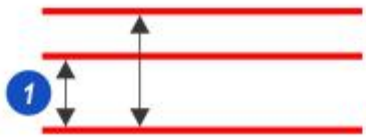




Mode: *Parallel Distance Line:*
Uses a single datum line and then measures the distance of objects from that line by individual offset parallel lines.

Parameters:

1: *Line Length (Default):* The distance from a datum line to a parallel drawn line.



Method:

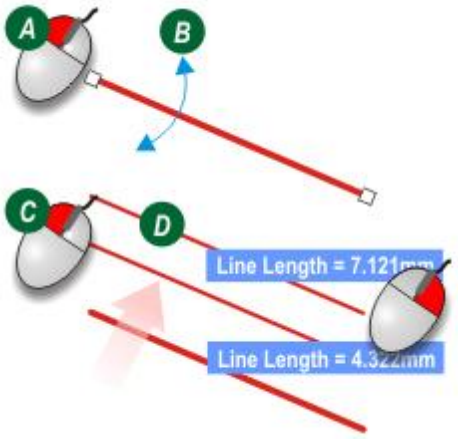
A: Click on the image to establish the datum line starting position.

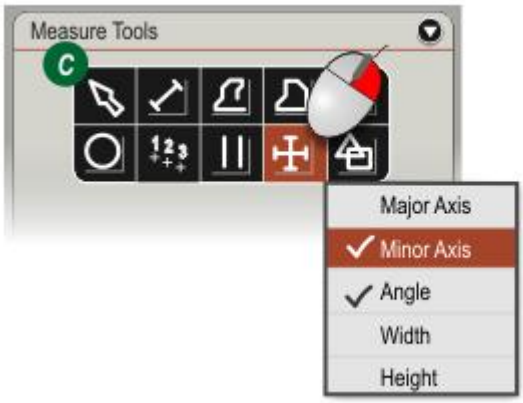
B: Move the cursor to rotate the datum line if necessary.

C: Click and move the cursor to the distance to be measured left or right, top or bottom of the datum.

D: Click to anchor the line.

Repeat the process for subsequent lines ending with a right-click.





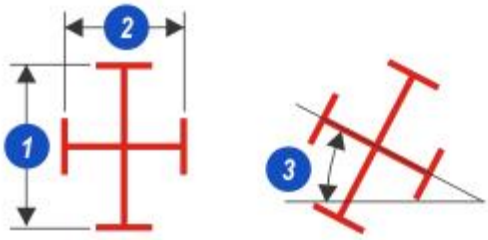
Mode: *The Cross:* Single option:
Measures the major and minor axes of an object by drawing a cross over it. The *Cross* may be drawn and rotated to any angle.

Parameters:

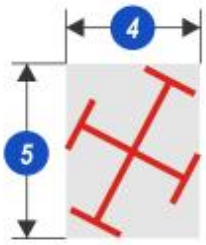
1: *Major Axis:* The length of the major (longest) axis.

2: *Minor Axis:* The length of the minor (shortest) axis.

3: *Angle*: Displays the angle from the horizontal: Positive values above the horizontal and negative values below the horizontal.



4 & 5: *Width and Height:* The dimensions of a bounding box enclosing the *Cross*.



Method:

A: Click on the edge of the area to be measured.

B: Move the mouse cursor (do not drag) to the opposite edge.

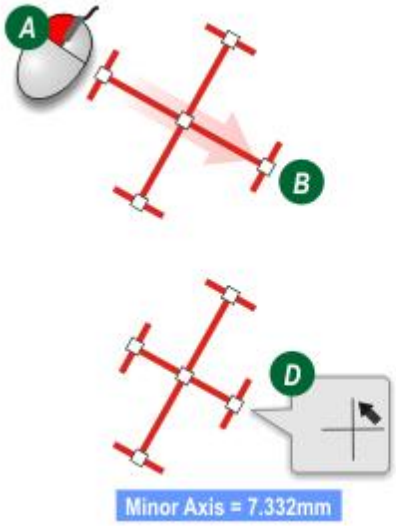
The *Cross* can swing around the first point.

Click to end.

The *Cross* is drawn initially as an equilateral figure - both axes are the same length. To adjust axis lengths:

C: Click on the *Selection* tool and then on the *Cross* to reveal handles for re-sizing and rotating.

D: Click on a handle to drag and change the axis length.



Re-position the *Cross* by clicking on the *Selection* tool and then on the *Cross*. Click on the centre handle and drag the *Cross* to a new position.

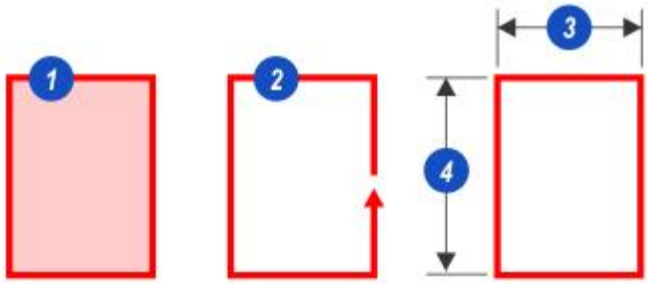
Mode: *Rectangle:*
Draws an enclosed 4-sided rectangle.

Parameters:

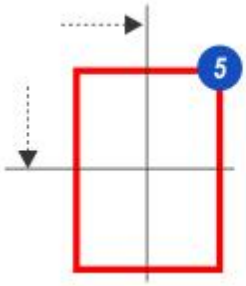
1: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

2: *Perimeter*: The distance around the *Rectangle*.

3 & 4: *Width and Height:* Horizontal and vertical dimensions of the *Rectangle*.



5: *CentreX and CentreY:* The distances horizontally *(X)* and vertically *(Y)* from the edge of the image to the centre of the *Rectangle*.

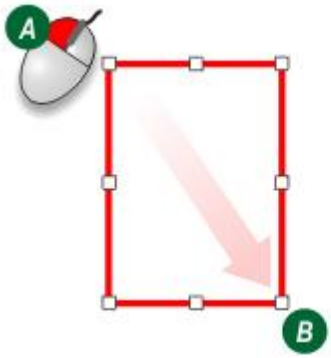


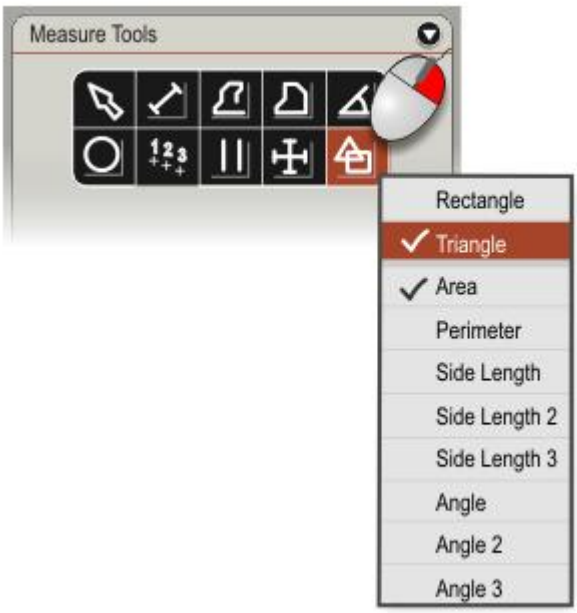
Method:

A: Click on the starting point of the *Rectangle*.

B: Move the mouse cursor (do not drag) to the opposite point of the *Rectangle*.

Left-click to end.



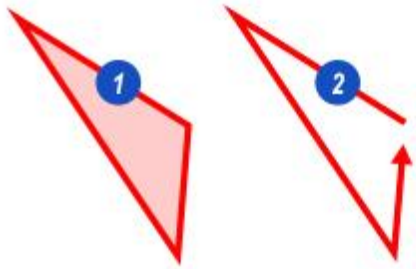


Mode: *Triangle*:
Draws a Triangle by clicking 3 points.

Parameters:

1: *Area*: The enclosed space returned in the selected measurement squared (for example mm²).

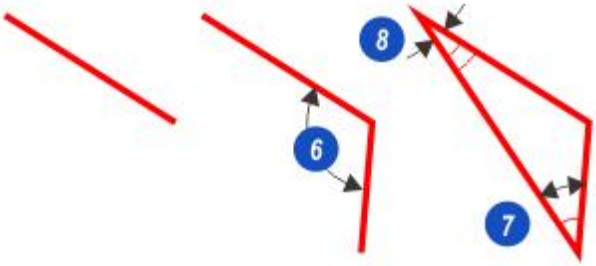
2: *Perimeter*: The distance resulting in the sum of the sides.



3: *Side Length:* The length of the side drawn first.

4: *Side Length 2:* The length of the side drawn second.

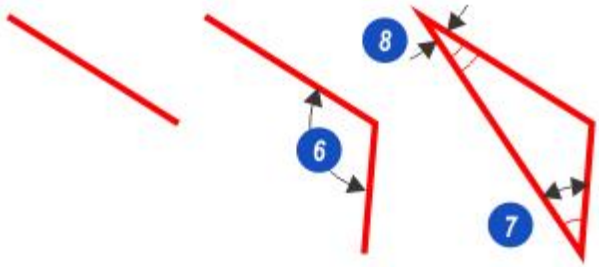
5: *Side Length 3:* The length of the side drawn last.



6: *Angle*: The angle between the first line drawn and the second line drawn. No arc.

7: *Angle 2:* The angle between the second line drawn and the last line drawn. Denoted by a single arc.

8: *Angle 3:* The angle between the last line drawn and the first line drawn. Denoted by two arcs.



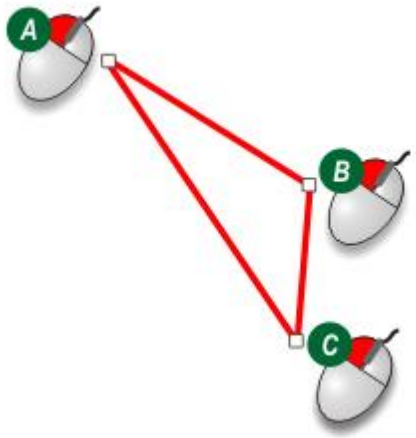
Method:

A: Click on the starting point of the *Triangle*.

B: Move the mouse cursor (do not drag) to the second point. Left click.

C: Move the mouse cursor (do not drag) to the third point.

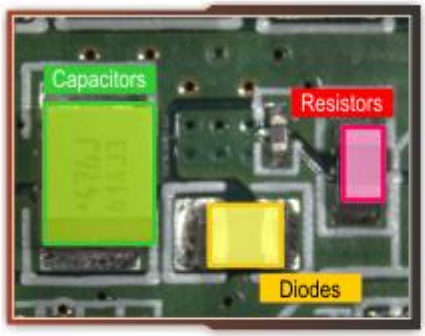
Left click to finish.

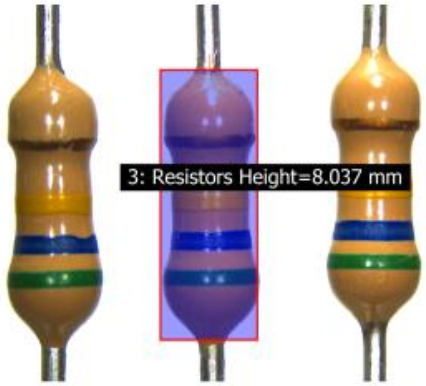


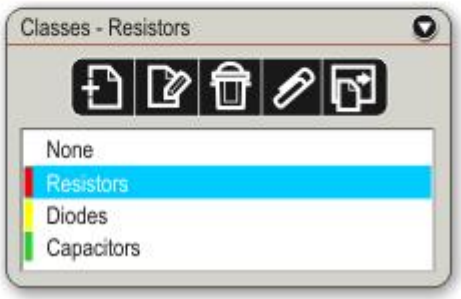
Classes represent a quick and simple method of 'grouping' the measurements of related objects under a common name - the *Class* name.

On a report classes can be used as a filter to help summarise related data and to aid identification on the image, each class can be given individual tool, label and font properties.

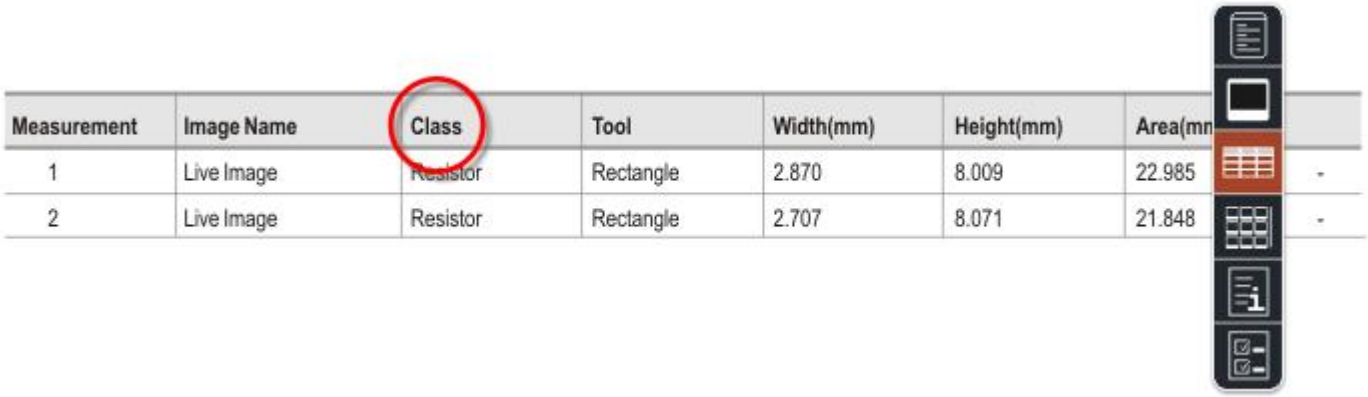
Any tool can be used with a class and the class name can be included on the measurement label and on the *Grid* 999



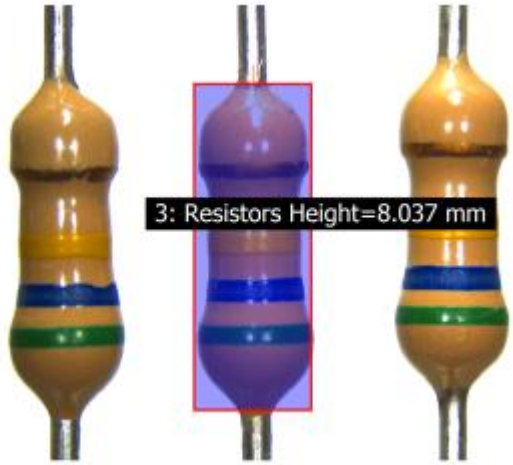


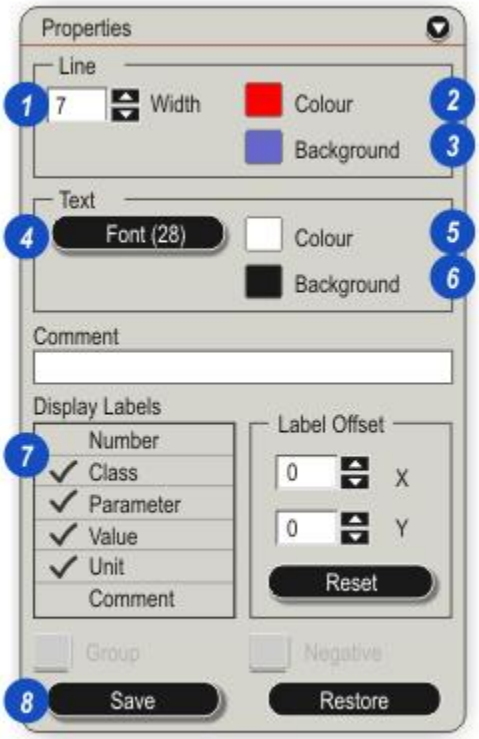


The following pages describe how this and any other *Class* can be set up.



Measurement	Image Name	Class	Tool	Width(mm)	Height(mm)	Area(mm
1	Live Image	Resistor	Rectangle	2.870	8.009	22.985
2	Live Image	Resistor	Rectangle	2.707	8.071	21.848





To create a *Class* and its settings which include the graphical properties:

1: Set the *Line Thickness*[905]

2: Select the *Line Colour*[905]

3: Set *Fill*[906] *(Background)* for closed shapes

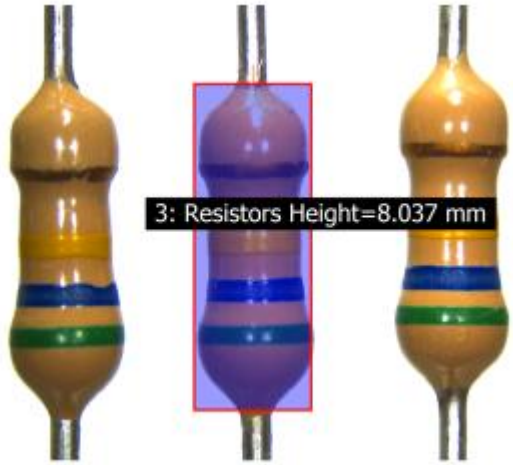
4: Set the *Text Font*[907], *Style* and *Size*

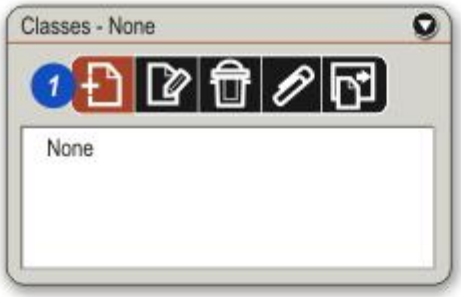
5: Select the *Font Colour*[908]

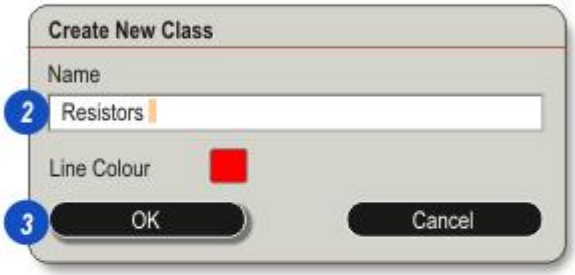
6: Select the *Label Background*[908] colour

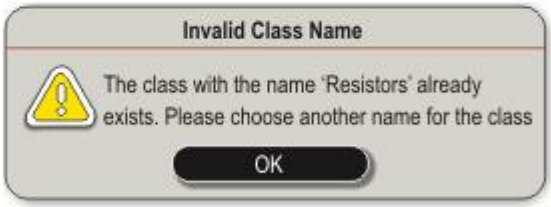
7: Choose the *Parameters*[909] to display

8: ...and click the Save button to temporarily store the *Properties*.









Give the new *Class* a name by:

1: Click on the *Create Class* button.

2: Click inside the *Name* text box and type a unique name for the new *Class*.

The new class name must be unique. If it is not a warning appears and the name is not accepted unless it is removed from the *Class* List first.

3: Click *OK*.

4: The new *Class* name appears on the *Classes* panel with...

5: ...the *Class* line colour displayed as a tag to the left of the name.

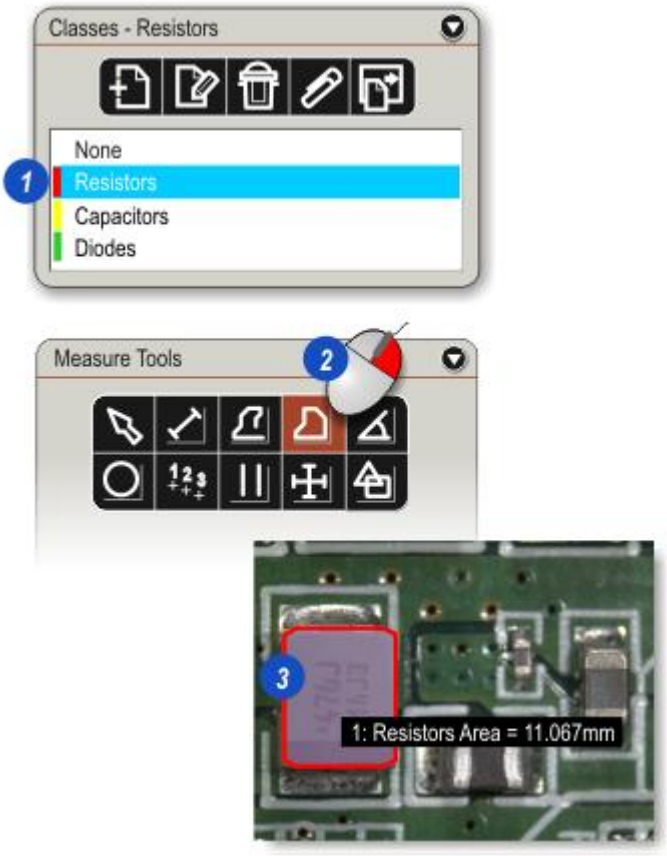
Using a Class

To use *Class* properties with a drawing tool:

1: Click to select the required *Class* on the *Classes* panel.

2: Select the drawing tool and parameter[916]

3: Create the measurement on the image.



All class descriptions are stored in a single file called the *Master Class* file and can be retrieved and shared between any number of projects.

Individual classes may be retrieved from the *Master Class* and attached to new projects or and they can be moved back into the file - detached - if they are no longer required. (Detach).

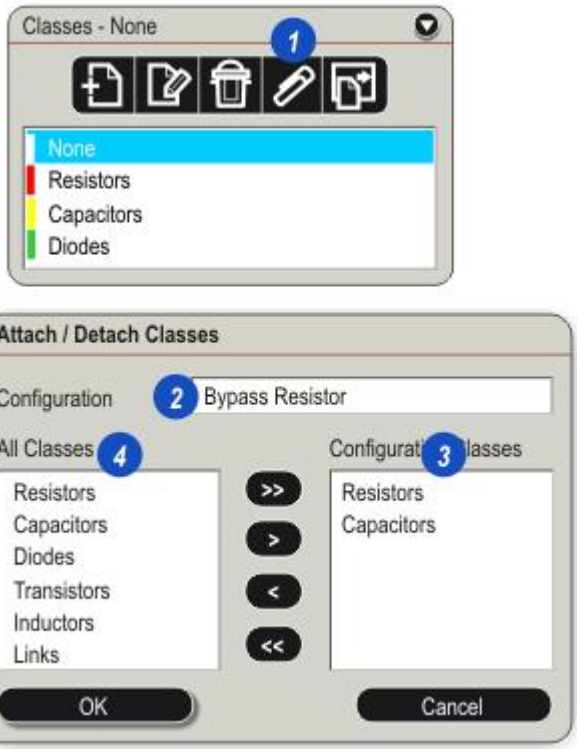
To attach or detach a class or classes:

1: Click on the 'paper clip' *Attach/Detach* button.

2: On the *Attach/Detach* dialog the current Configuration is displayed at the top.

3: The Classes attached to the current Configuration are displayed in the right-hand pane.

4: All of the classes in the *Master Class* file are listed in the left-hand pane and those in the current project in the right-hand pane.



The buttons between the panes move either a single class or all classes between the two panes:

Attach all classes in the *Master Class* file to the current project.

Select a single class from the *Master Class* file and click this button to attach it to the current project.

Select a single class in the current project and click the button to remove it.

Remove (detach) all classes from the current project.

Detaching a Class example

In this example the current project has three classes attached to it. The *Diode* class is no longer needed and will be removed:

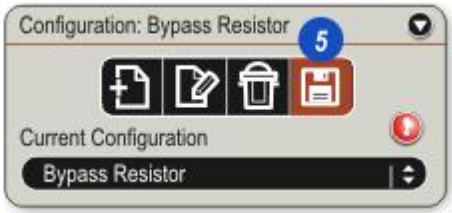
1: Click on the *'Paper Clip' (Attach / Detach)* button.

2: The classes attached to the current project are listed in the right-hand pane on the *Attach / Detach Classes* dialog. Click to select the *Diode* class.

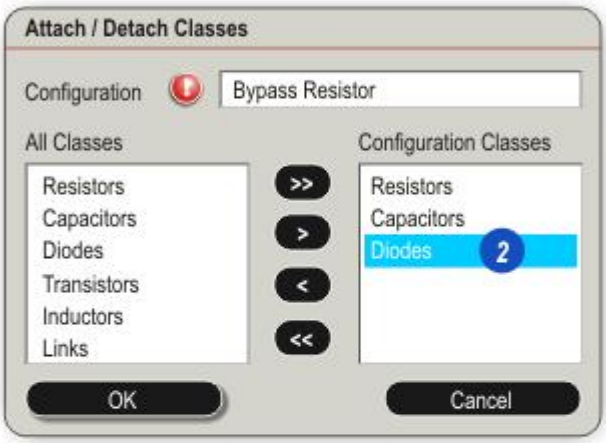
3: Click the *Detach Single Class* button and...

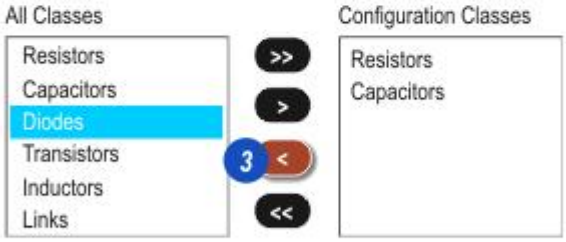
4: ...the *Diode* class is removed from the list and no longer appears on the *Classes* panel.

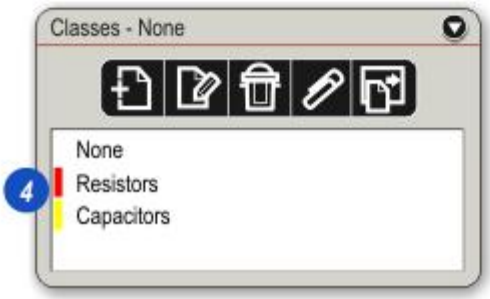
When a class is attached or detached the *Configuration* - even if it is only the *Default* - has changed and the *Properties Changed* warning flashes on the dialog.



5: The *Configuration* will need to be saved again or a new one created.







Deleting a Class

There are two options for deleting a *Class*:

- Detach it from the current project or *Configuration* or...
- Delete it completely from the *Master Class* file.

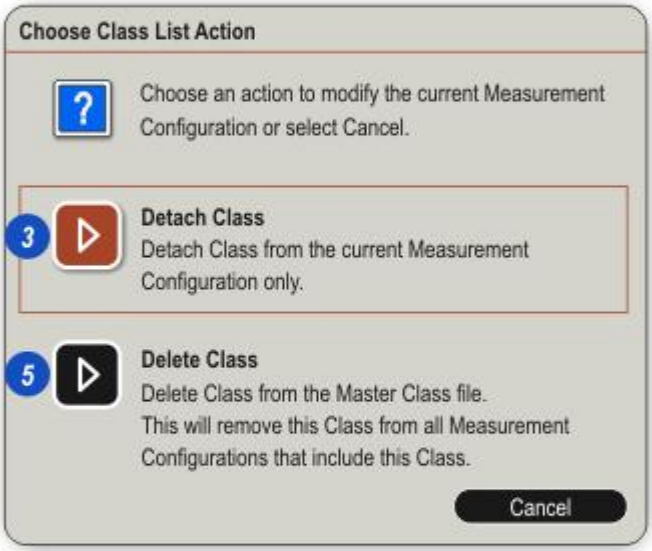
1: Click to select the class to be deleted on the *Classes* panel.

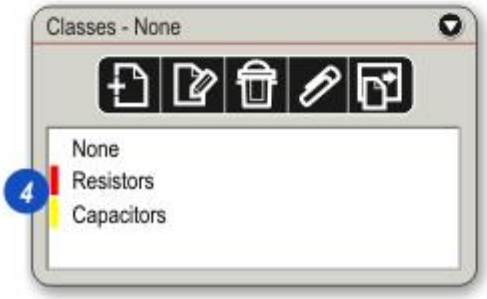
2: Click on the *Delete (Trash Can)* button.

3: The default option on the *Choose Class List Action* dialog is to remove (detach) the class from the current project or *Configuration* only.

Click the *Detach Class* button and...

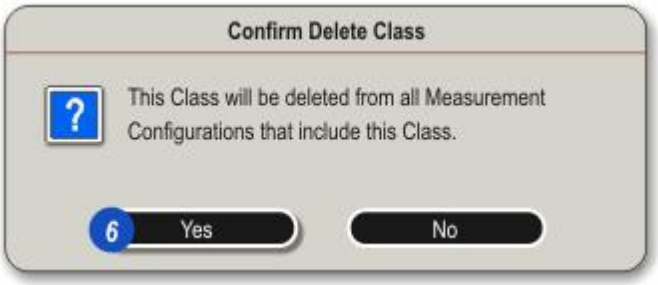
4: ...the *Class* is removed from the *Classes* panel.





Delete Class:

5: Click on the *Delete Class* button to remove the *Class* from the *Master Class* file and from all the *Configurations* to which it is attached.



6: Click *Yes* to confirm the deletion.

Edit a Class

Users can change the name and outline colour of a class by:

1: click to select the class to be edited in the *Classes* list.

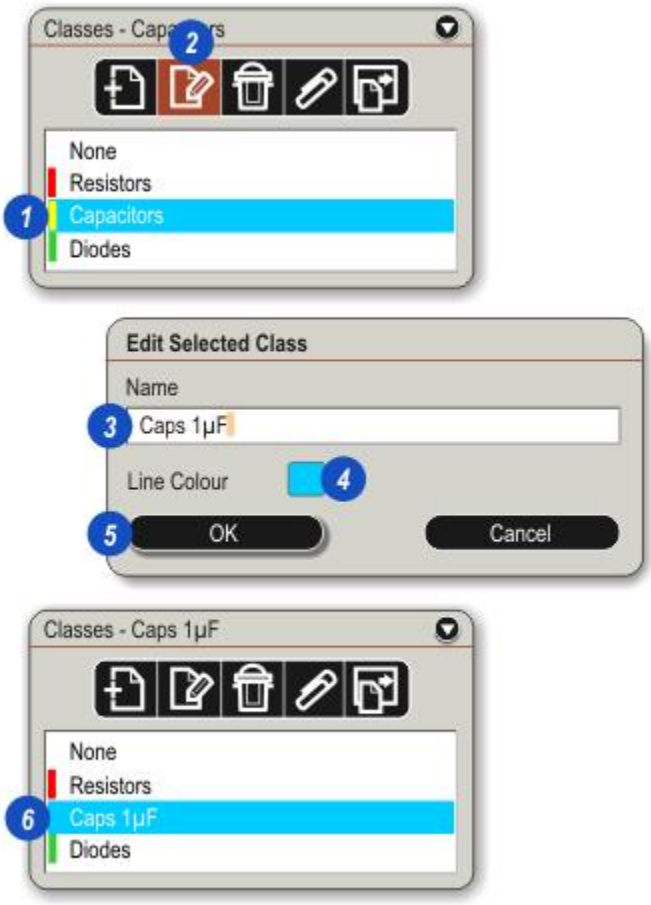
2: Click the *Edit Class* button.

3: On the *Edit* dialog, to change the class name click inside the *Name* text box and type a new name.

4: Change the outline colour by clicking on the *Line Colour* button and selecting a new colour from the *Select Colour* dialog.

5: Click *OK*.

6: The new class name and line colour appear in the *Classes* list.



Changing a Measurements Class

The class associated with a measurement can be changed by:

1: Using the *Selection* tool...

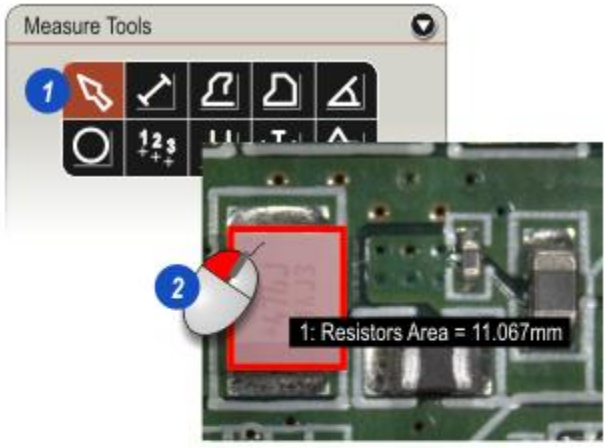
2: ...click to select the measurement to be changed.

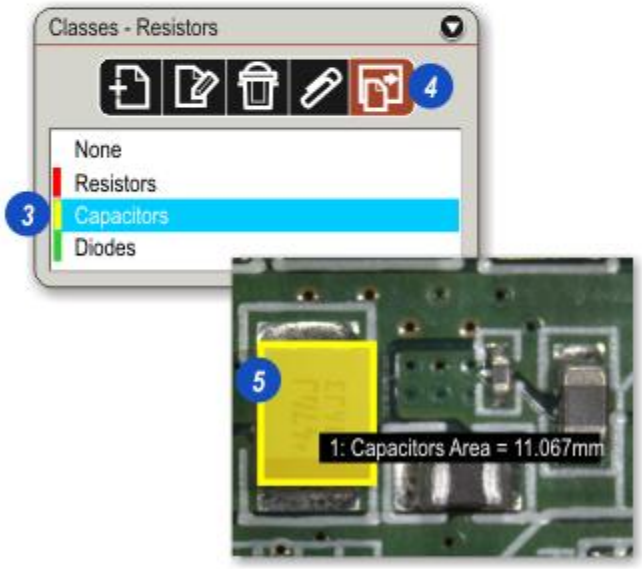
3: Click to select the new class to be applied.

4: Click the *Apply Class* button and...

5: ...the measurement line colour and fill together with the caption are changed to the new class.

The class name will only be displayed in the caption if it is selected in *Properties > Display Labels*[909].





The current *Classes* and properties can be saved as a *Configuration* file associated with the image, and retrieved to be used at a later date.

1: Each *Configuration* has a unique name and can be accessed by clicking on the arrow to the right of the *Current Configuration* window and from the drop down list clicking to select the required configuration.

See also *Properties > Save and Restore* 914

2: Two configurations are provided with Leica Application Suite:

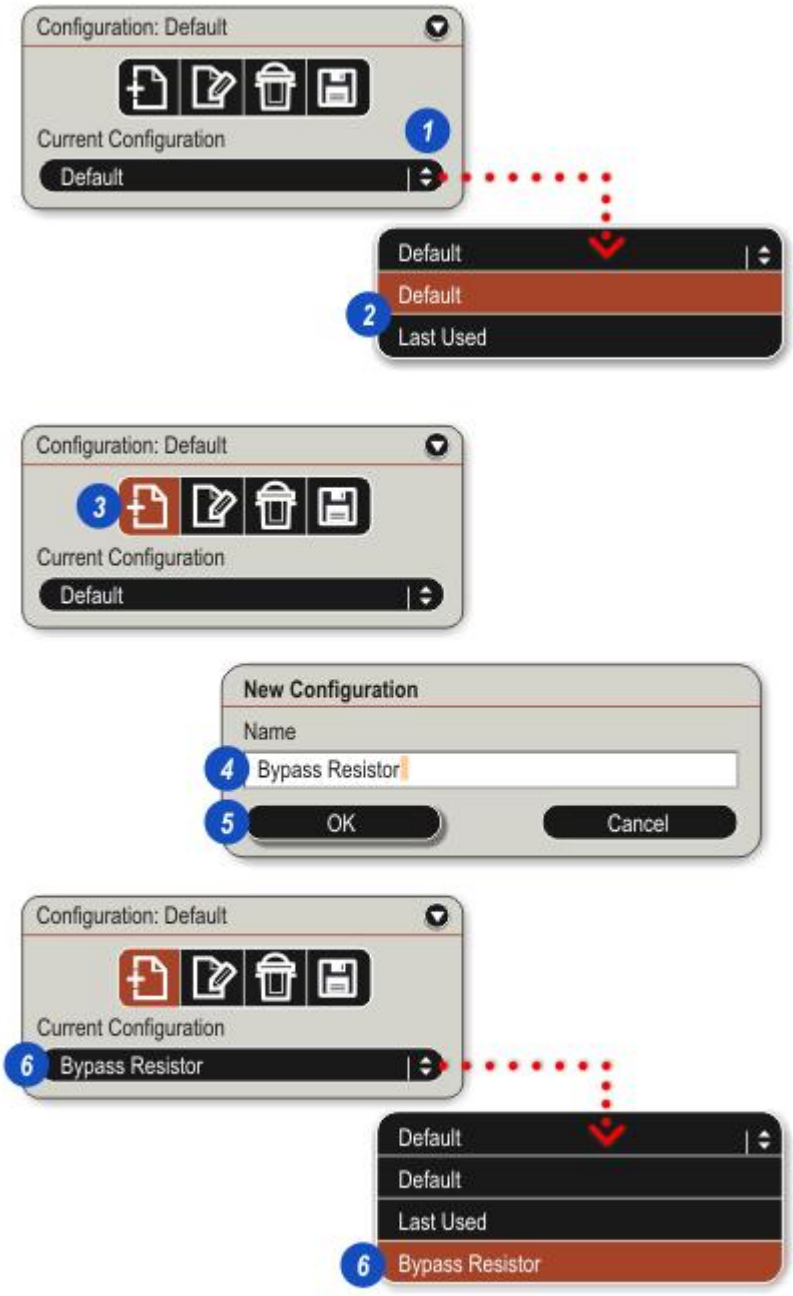
- *Default*, which are 'factory' settings that can be subsequently changed in *Preferences*, and...
- *Last Used* which, as its name implies, re-loads the configuration that was last being used and had been saved - that could be the *Default*.

3: To save the current settings as a new configuration, click on the *New* button and…

4: …type a unique name for the new configuration.

5: Click *OK* to save the setting.

6: The new configuration appears in the drop down list.



To Edit a Configuration Name:

1: Select the configuration to be changed from the drop down list.

2: Click on the *Edit Configuration* button.

3: On the *Edit Configuration* dialog, change the name by clicking in the text box and typing a new name and…

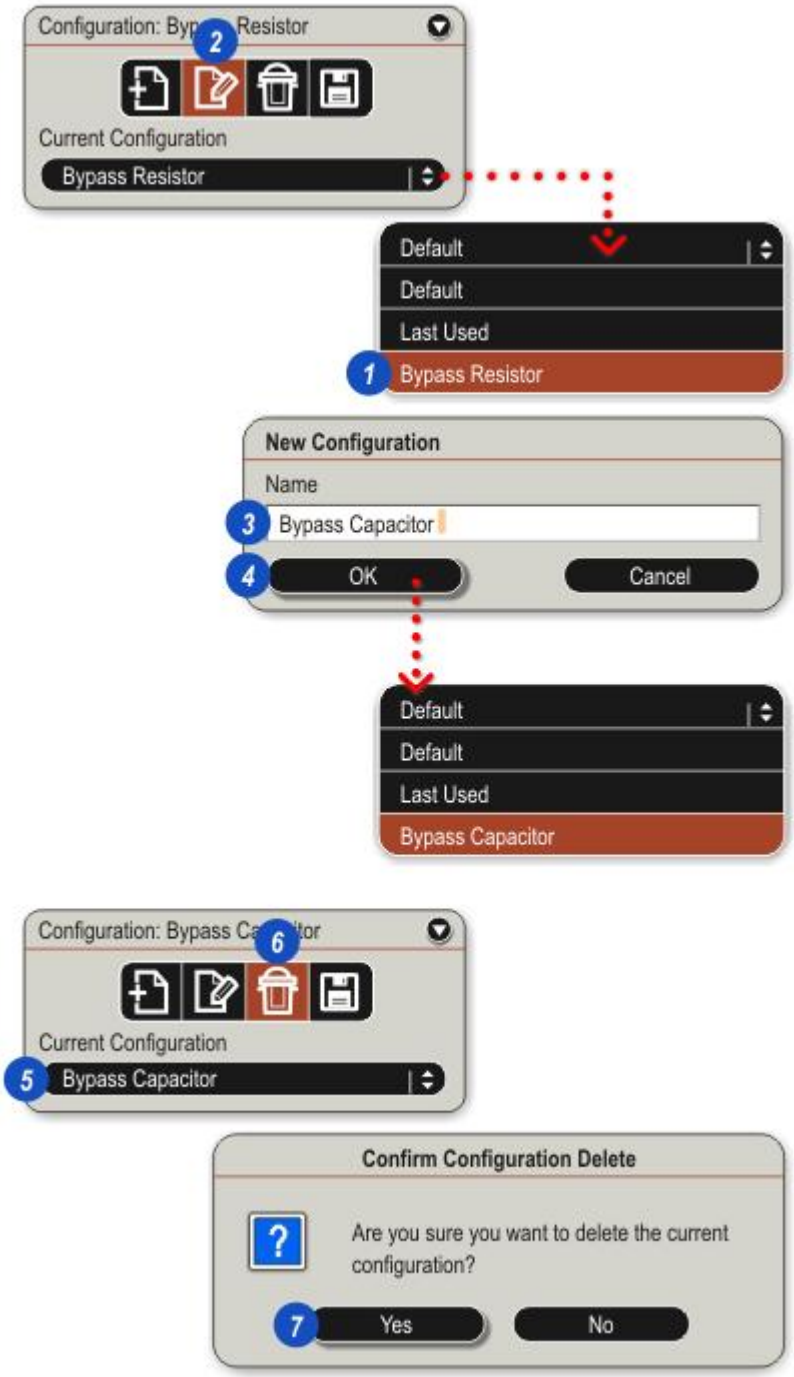
4: …clicking *OK*.

To Delete a Configuration:

5: Select the configuration to be deleted from the drop down list.

6: Click the *Delete* (Trash Can) button.

7: Confirm the deletion and the *Configuration* will be removed permanently. The operation cannot be reversed.



Properties have changed

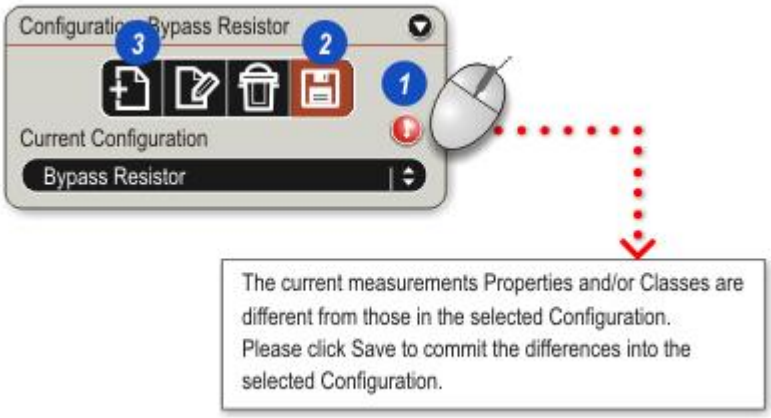
If, after a configuration has been loaded, properties or classes are changed, the user is warned that the configuration needs to be updated or a new configuration created.

1: The *Properties Changed* warning appears next to the current configuration.

Hover the mouse over the warning to see details.

2: Either click the *Save* button to update the current configuration or...

3: ...create a new configuration which will include both old settings and the changes.



If the current configuration is *Default* and changes are made to the *Properties* or *Parameters*, the *Properties Changed* warning appears.

But the *Default* configuration cannot be changed - they are factory settings - so if the user needs to save the changes it has to be to a new configuration:

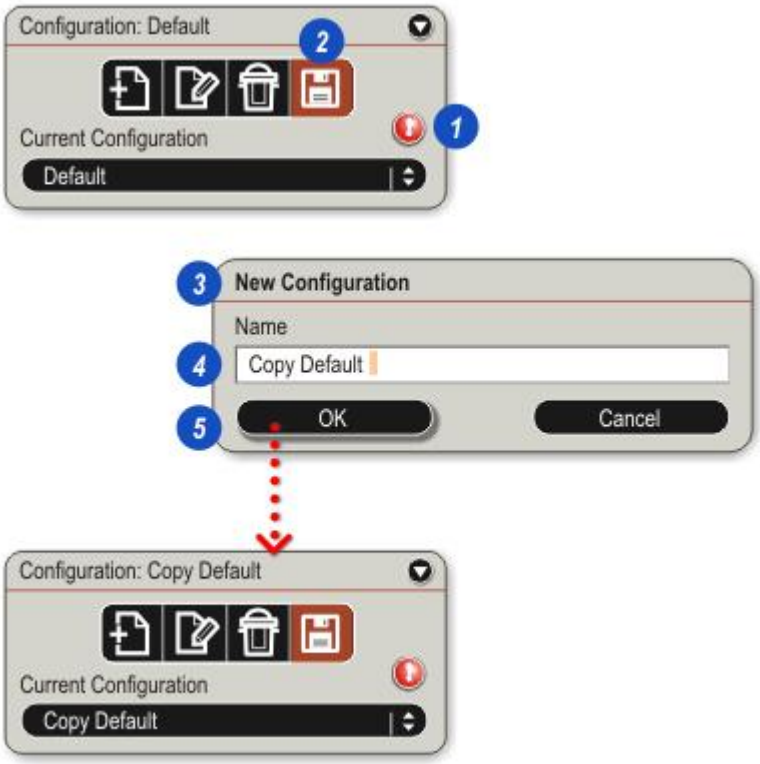
1: The *Properties Changed* warning appears.

2: Click on the *Save* button.

3: The *New Configuration* dialog automatically appears - the programs 'knows' that *Default* cannot be changed.

4: Click inside the *Name* text box and type a new name.

5: Click *OK*.



A *Template* is a measurement or a collection of measurements that can be saved, re-loaded and then:

- Overlaid on a live image being displayed in *Live Measurements*[968] or...
- Overlaid on a captured image, in *Interactive Measurements*[980].

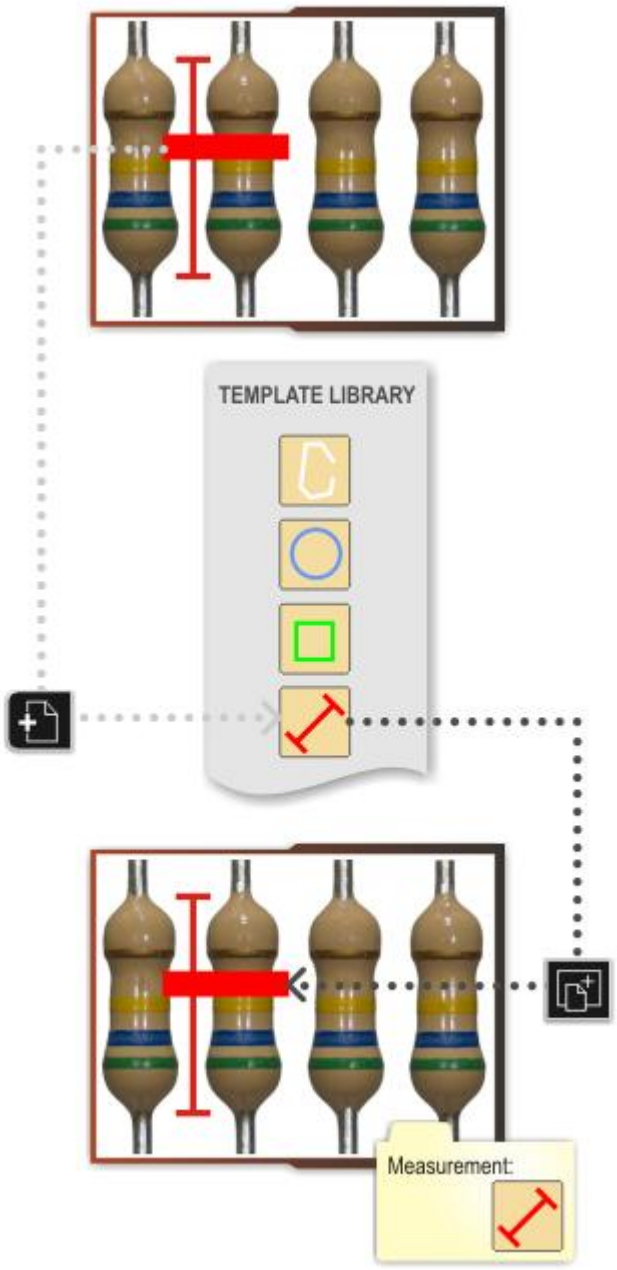
Templates as overlays allow users to check for conformity across images.

When LAS is installed a *Template Library* is created. Users cannot access it directly, only through *Live Measurements* or *Interactive Measurements.*

A measurement drawn by the user can be saved as a *Template* with a unique name in the *Template Library* and from there retrieved and loaded to other images.

When a *Template* from the *Library* is applied to a live image it is copied, converted to a measurement and attached to the image. It can then be edited in *Interactive Measurements*.

Measurements made directly on the live image can be mixed with measurements applied by using *Templates.*

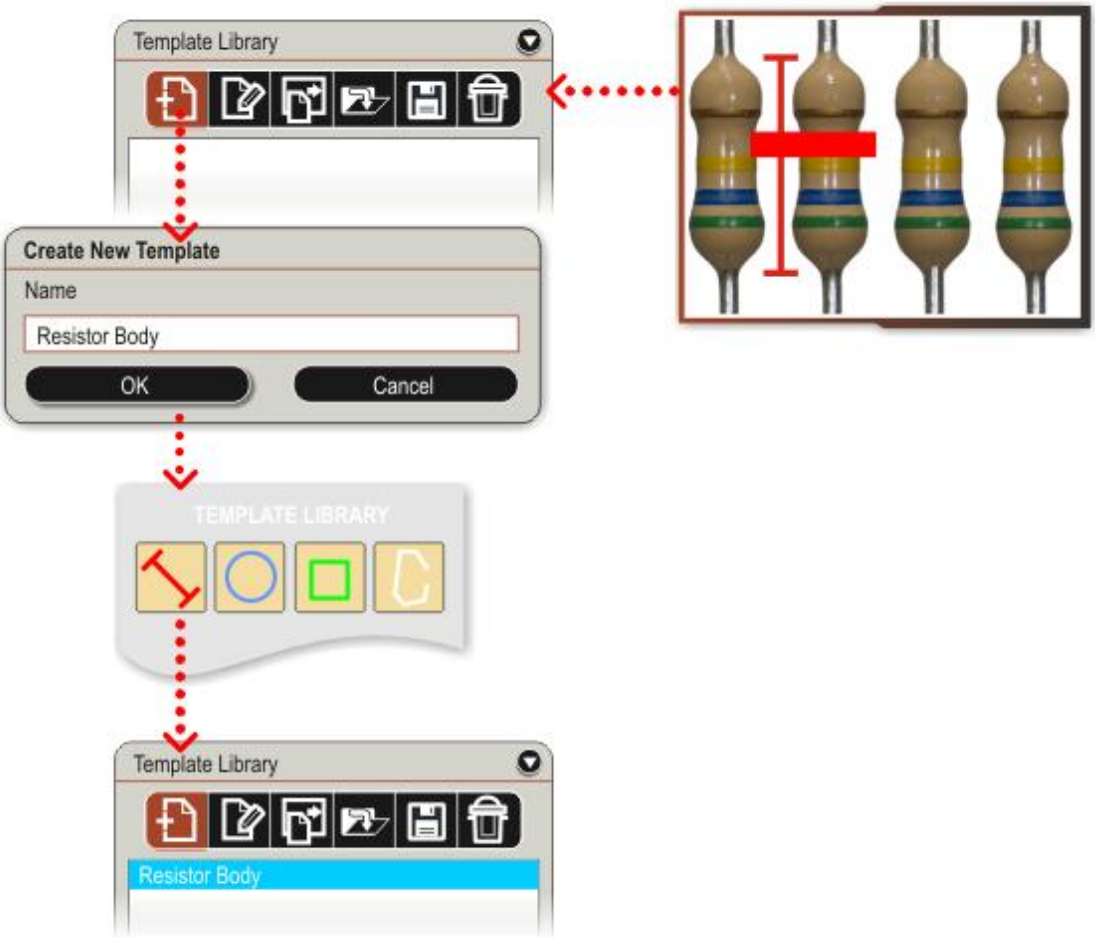


Creating a Template

The diagram shows how *Templates* are created from a measurement drawn on a live image, given a unique name, stored in the *Template Library* and become available ti use on other images.

The steps are:

- Draw a measurement on the live image.
- Click the *Create Template* button.
- Give the measurement a unique name.
- The measurement is converted to a *Template* and stored in the *Library*.
- It becomes available to use on other live images.



To create a *Template*:

1: Select the required tool and...

2: ...draw measurements on the live image. Measurement tools, line colours and thickness, fonts and backgrounds can be mixed and there can be any number of them.

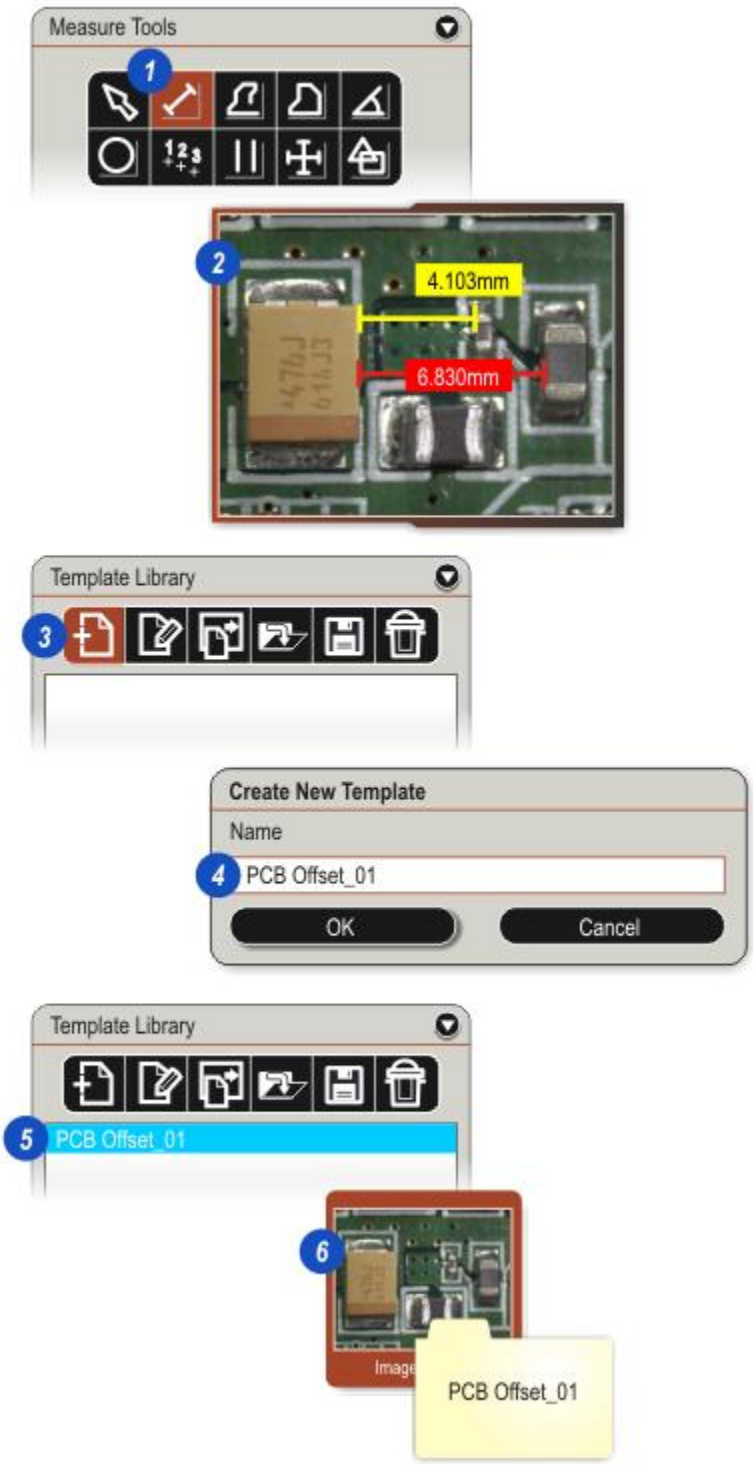
3: Click on the Create Template button.

4: On the *Create New Template* dialog, click inside the text box and type a unique name for the template.

Click *OK.*

5: The measurements are converted to a *Template* and saved in the *Library* but remain on the image.

6: When the image is captured, the measurements are saved with it. This is not the *Template* but a discreet file that is attached to the image.

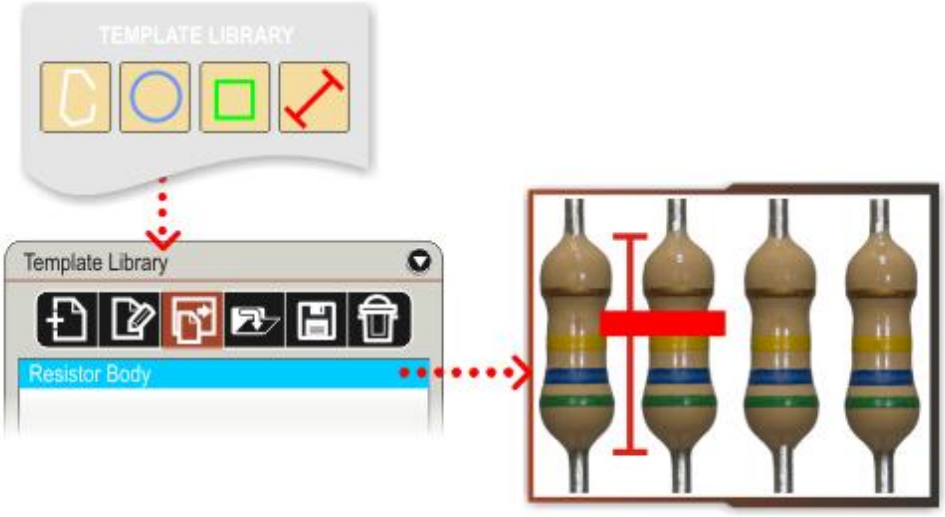


Applying a Template as a Measurement

The *Templates* panel displays a list of templates that are in the *Template Library.*

When a template is applied to a live image, it is automatically converted to a measurement. The process is:

- Select the *Template* to be applied in the *Template* panel.
- Click the *Apply Template* button.
- Any existing measurements on the image are removed before the selected template is applied.
- To add[973] a template to existing measurements



A template is converted to a measurement before it can be applied to a live image. This is an automatic process that takes place when the *Apply Template* button is clicked.

In this example the template comprises two measurements:

1: A new live image without measurements.

2: Click to select the template to be applied from the *Template* panel.

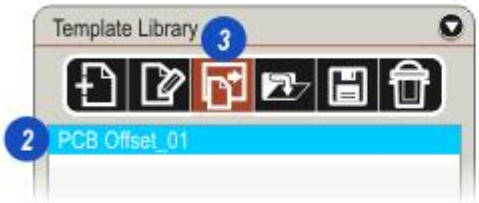
3: Click on the *Apply Template* button and...

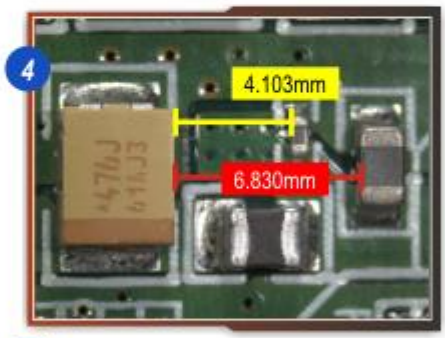
4: ...the template is converted to a measurement and drawn on the image.

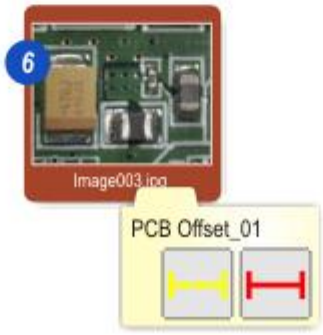
5: If the image is captured...

6: ...the measurements are captured with it.

This is not the template or a link to it, but simply the measurements.





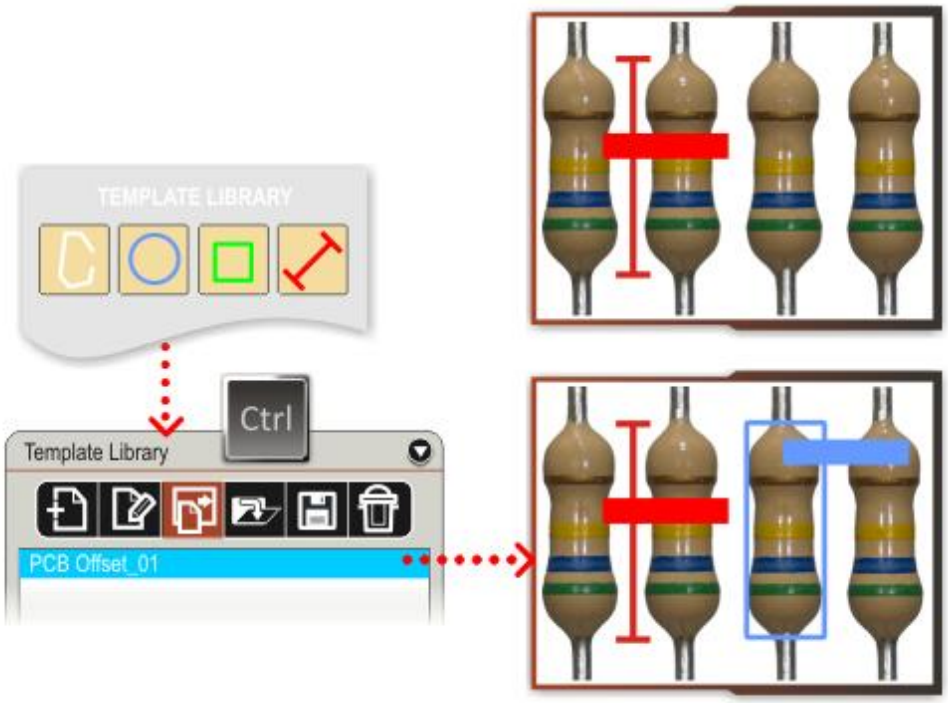


Adding a Template to a Measurement

A template (or several templates) can be applied to a live image that already has measurements on it so that both drawn and template measurements appear together.

The process is:

- Draw measurement(s) on the live image.
- Select the template to be added from the *Template* panel.
- Hold down the *Ctrl* key.
- Click the *Apply Template* button.



A *Template* is converted to a measurement and added to an existing measurement:

1: A new live image with a measurement drawn on it.

2: Click to select the template to be applied from the *Template* panel.

3: Press and hold down the keyboard *Ctrl* key. Click on the *Apply Template* button and...

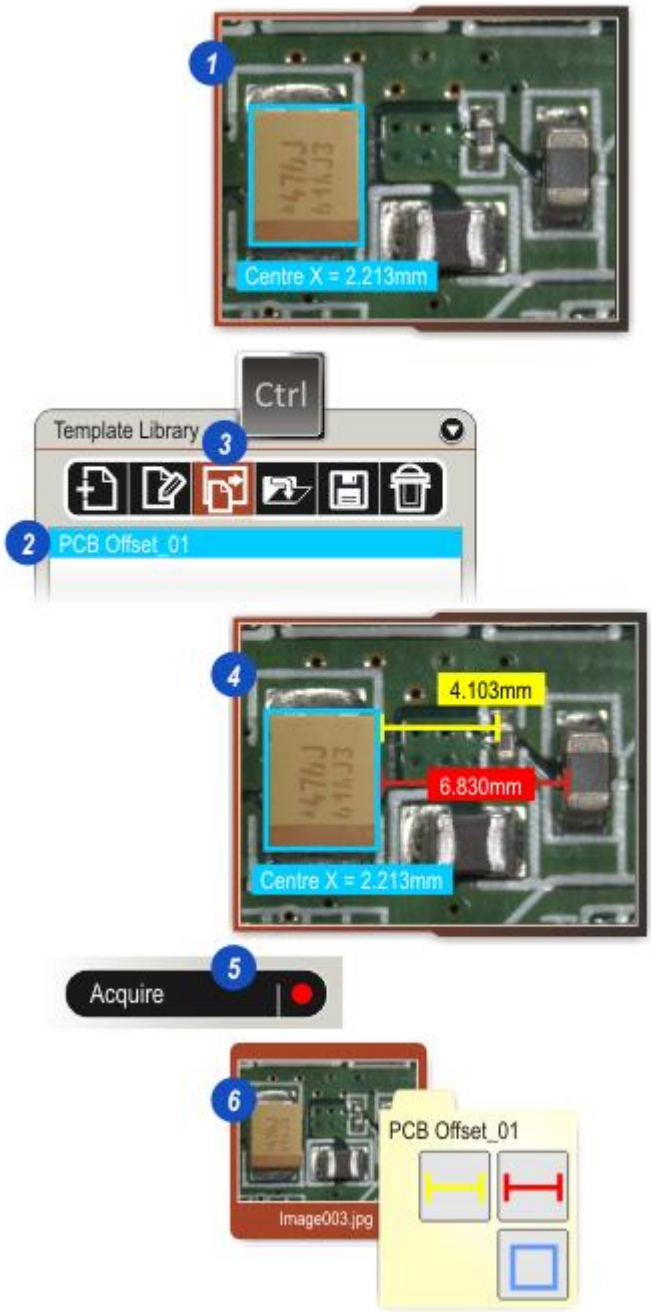
4: ...the template is converted to a measurement and drawn on the image together with the original measurement.

5: If the image is captured...

6: ...the measurements are captured with it.

This is not the template or a link to it, but just a set of instructions for drawing all of the measurements.

- This process can be used to add multiple templates.
- Multiple measurements can be saved to a new template as a group but the individual measurements can be edited in optional module *Interactive Measurements* if it is installed.
- Drawn measurements can be added after the template is applied.



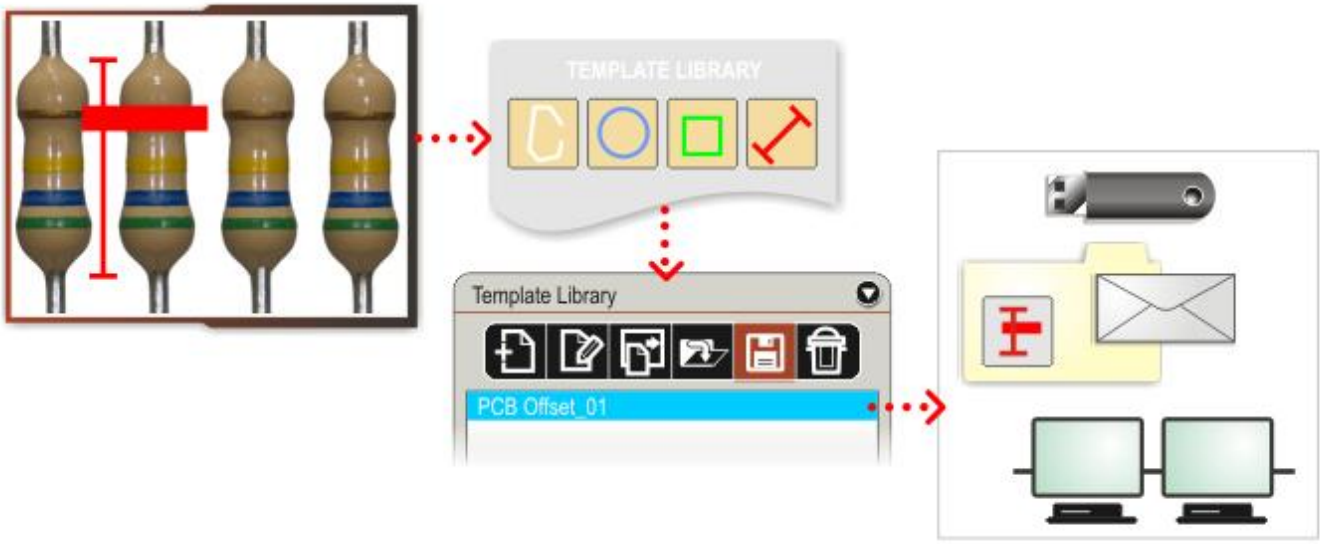
Saving Templates

Measurements that have been named and stored as *Templates* can be saved to most storage media using the *Save To* feature.

Targets for *Save To* include pen drives, removable backup hard drives and folders on this or on networked computers.

Templates can be attached to an e-mail but only through an intermediate folder.

Save To makes a copy of the template (*.tem* file extension).



The *Save To* feature copies a *Template* from the *Library* to:

- A Pen Drive (Stick).
- A removable Hard Drive (Backup),
- A shared folder on a Network,
- A folder on this computer.

Because the *Library* is inaccessible to users outside *Live Measurements*, to attach a template to an e-mail or write to a CD or DVD, save the template to a folder on this computer and use that as source for the mail or writing.

1: On the *Template* panel, click to select the template to be saved.

2: Click the *Save To* button and...

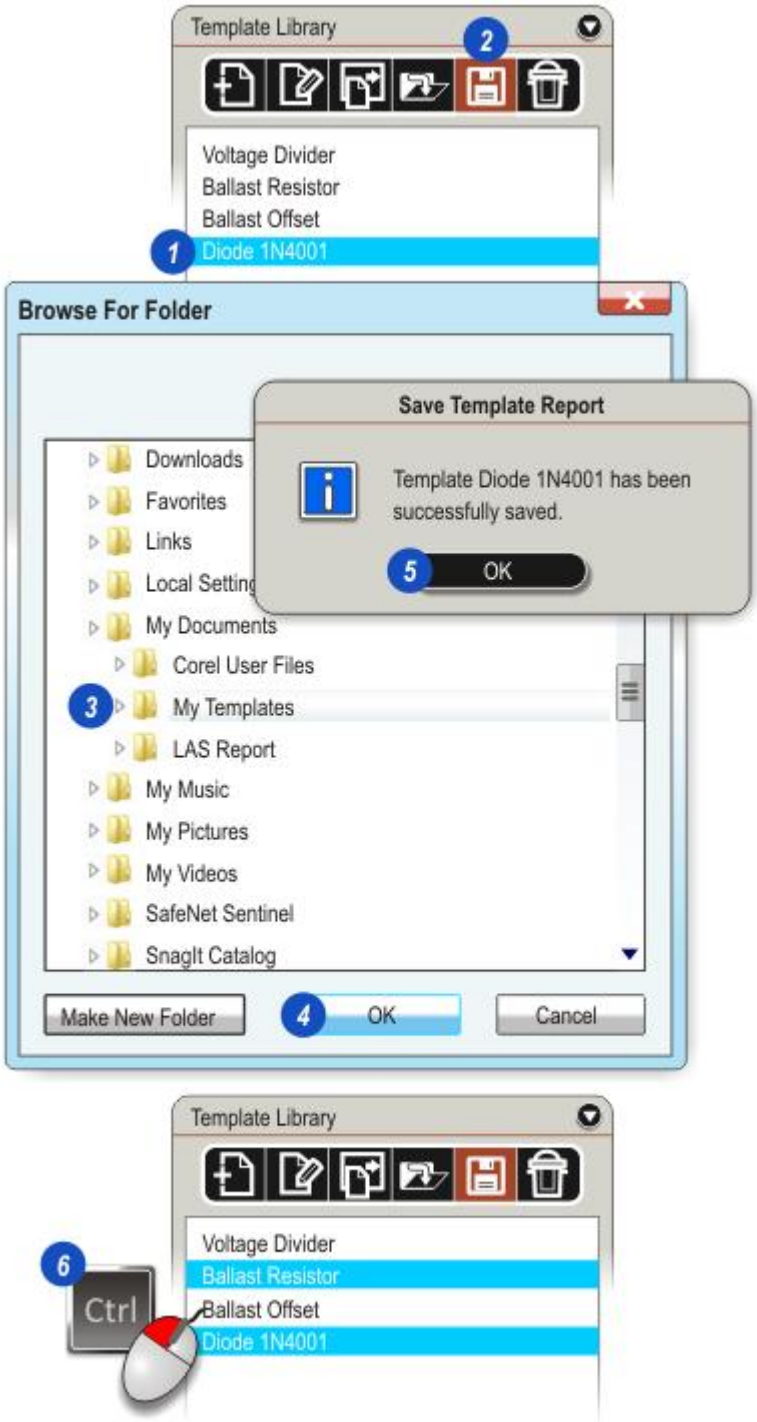
3: ...on the *Browse for Folder* dialog navigate to the target folder or media.

4: Click *OK.*

5: The *Save Template Report* will display success (or failure).

Several templates can be saved simultaneously:

6: Hold down the keyboard *Ctrl* key and click the individual templates to save and follow the sequence above.

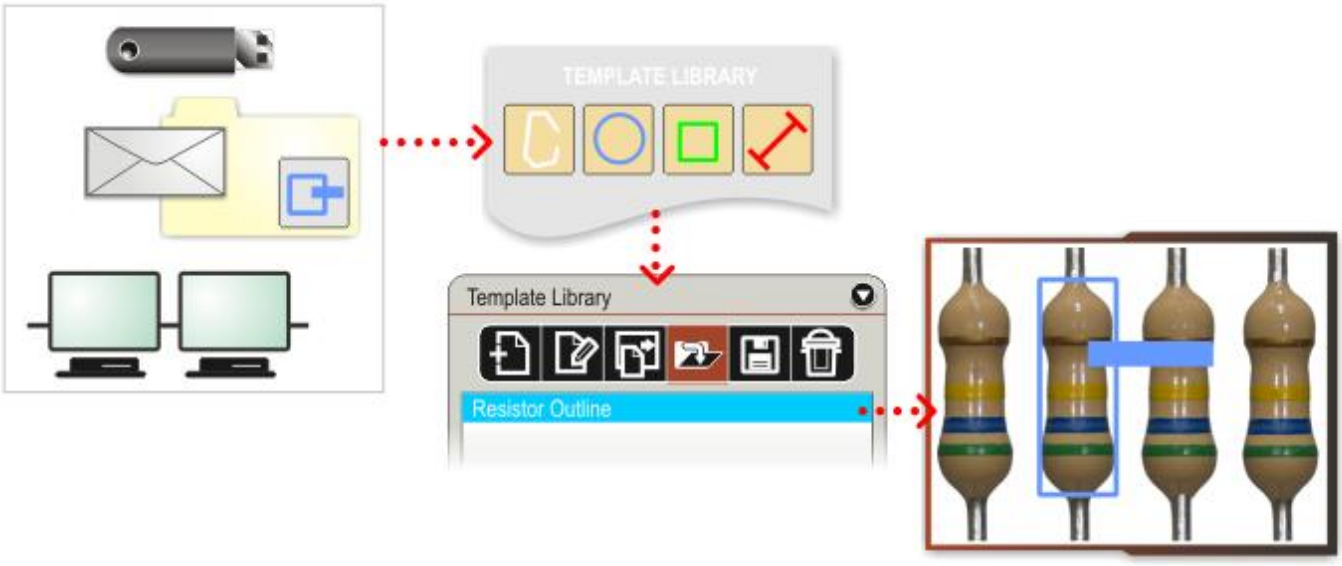


Templates can be loaded from most storage media using the *Load From* feature.

Sources for *Load From* include pen drives, removable backup hard drives and folders on this or on networked computers.

Load From copies a *.tem* file from the source media to the *Library and displays it on the Templates panel.* From there it can be converted to a measurement and used on live images.

If there is already a template of the same name in the *Library* the loaded file is given an incremental suffix.



The *Load From* feature copies a template from:

- A Pen Drive (Stick).
- A CD or DVD,
- A removable Hard Drive (Backup),
- A shared folder on a Network,
- A folder on this computer.

...and places it in the *Library*. From there it can be applied to a live image.

1: Click on the *Load From* button.

2: On the *Open* dialog navigate to the folder or media containing the template file and...

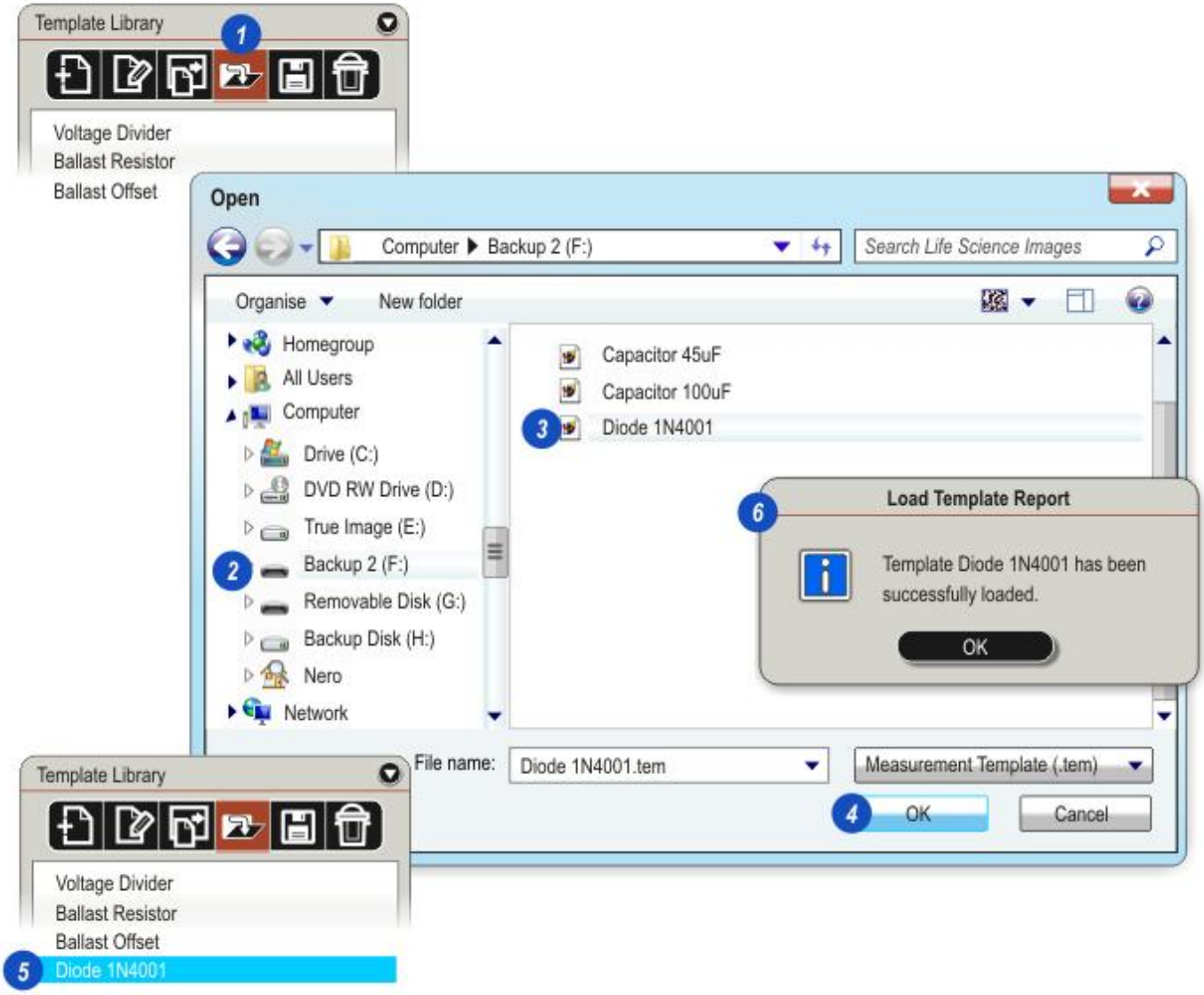
3: ...click to select it.

4: Click the *OK* button. The template is copied to the *Library...*

5: ...its name appears in the *Template* panel list and is available to add to the image.

If a template of the same name already exists in the *Library* the new file will be given an incremental suffix.

6: The *Load Template Report* displays success (or failure).



Delete a Template

Delete a *Template* from the *Library* by:

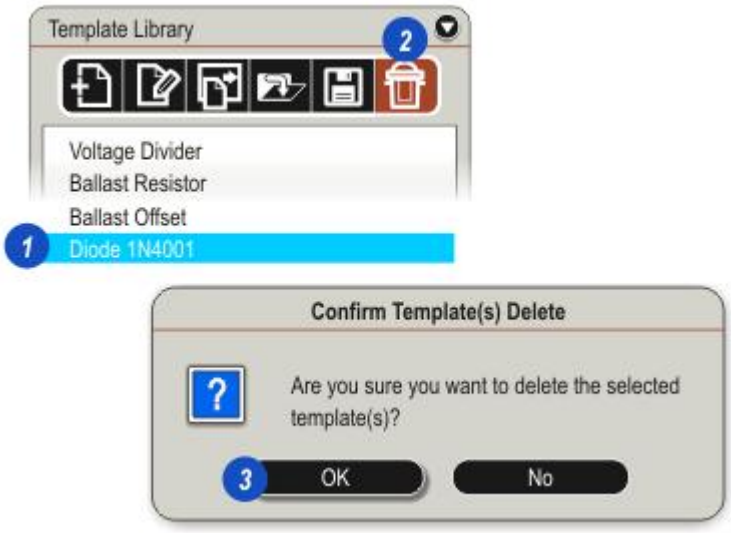
1: Click on the template to be deleted in the *Templates* panel.

2: Click on the *Delete (Trash Can)* button.

3: Confirm the deletion by clicking *OK* and the template is removed.

Images that have used the template as a source of measurements are not affected - they have the measurement attached which remains intact.

Templates cannot be recovered once deleted, but an image that has had the template applied as a measurement can be used to create a duplicate.



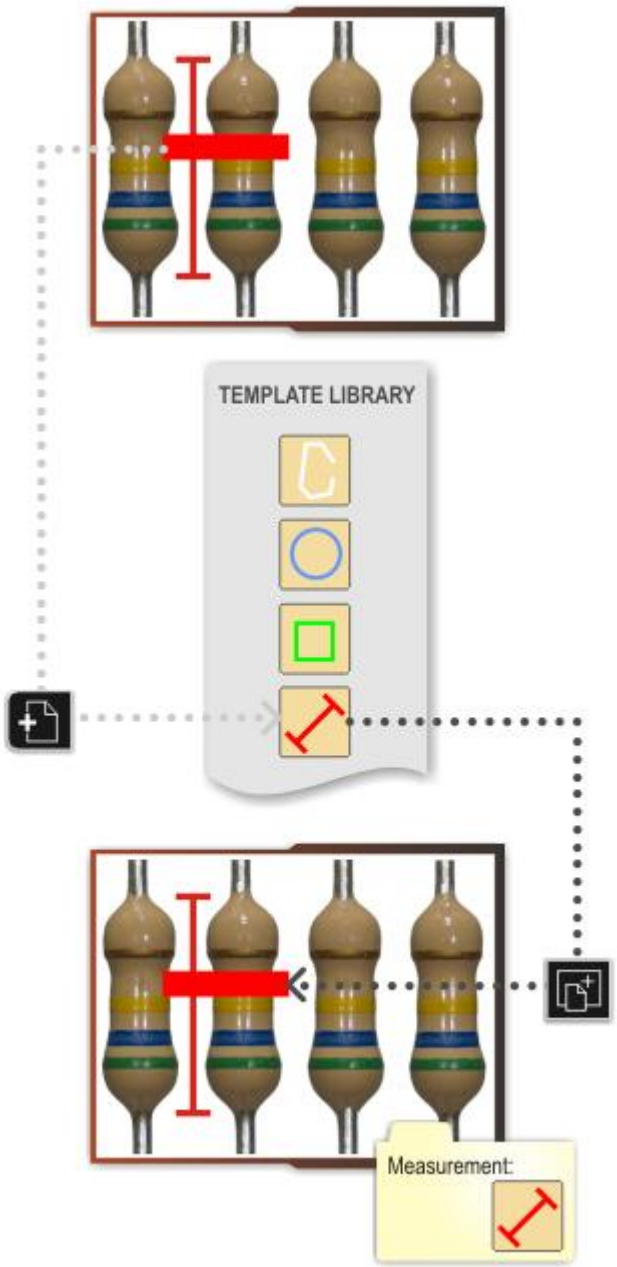
Templates created in optional module *Live Measurements* are saved in the same *Template Library* and are also available in *Interactive Measurements.*

Templates can be shared between other users using:

- *Save As* to copy a template *to* a distributable medium - memory stick, e-mail or network, or....
- *Load From* to copy *from* distributable media.

When a *Template* from the *Template Library* is applied to a captured image it is copied, converted to a measurement and attached to the image. Then it is displayed over the image in the *Viewer.* There it can be edited if necessary or simply used to check object location.

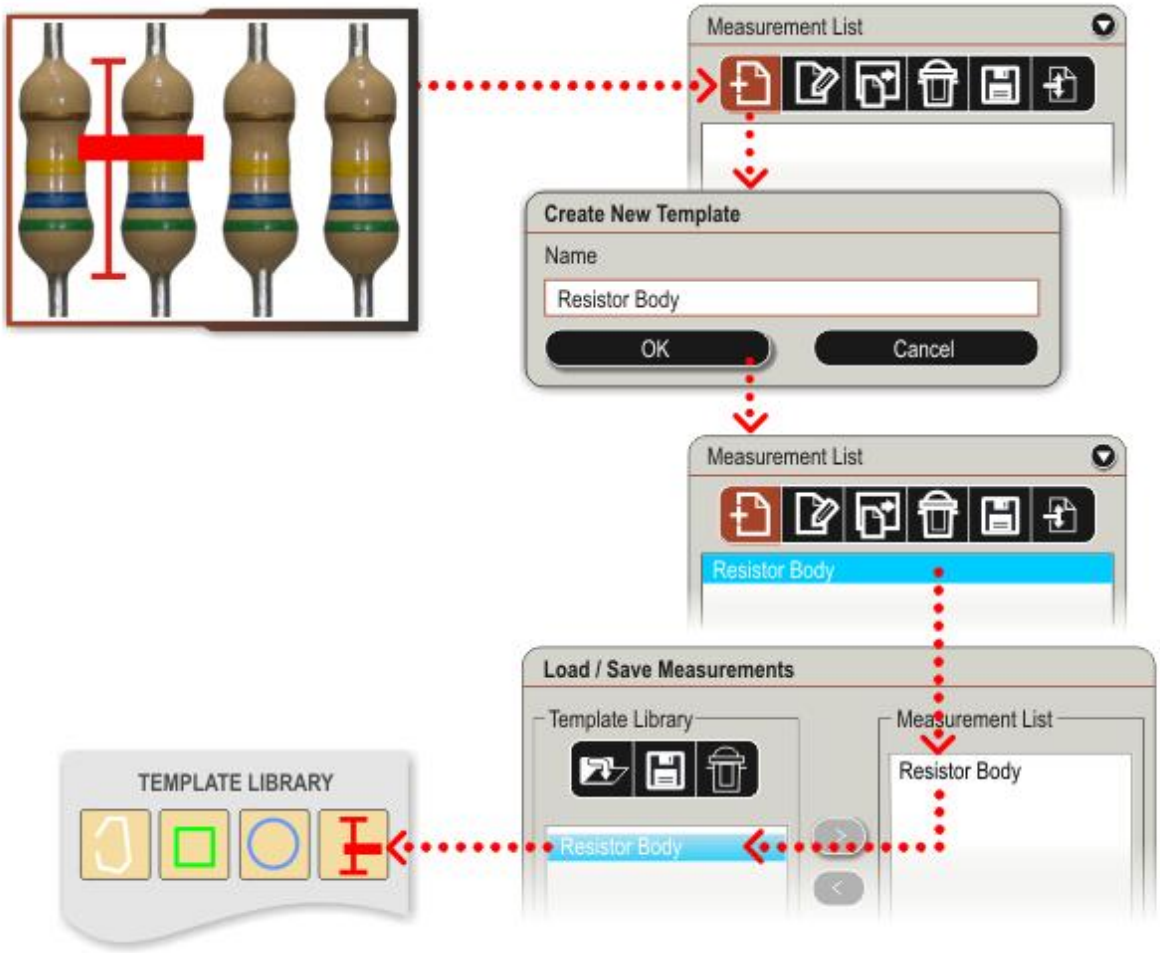
Additional measurements can be added to a template and that then either updated or saved as another template.



The diagram shows how *Templates* are created from a measurement drawn on a captured image and given a unique name, are stored in the *Template Library* and become available to use on other images.

The steps are:

- Draw a measurement on the image.
- Click the *Create New Measurement* button.
- Give the measurement a unique name.
- The measurement is added to the *Measurement List*.
- From the *Measurement List* the measurement is added to the *Template Library*.



Start by making a measurement and adding it to the *Measurement List*:

1: Select the required tool and...

2: ...draw measurements on the captured image.

Measurement tools, line colours and thickness, fonts and backgrounds can be mixed and there can be any number of them.

3: Click on the *Create New Measurement* button.

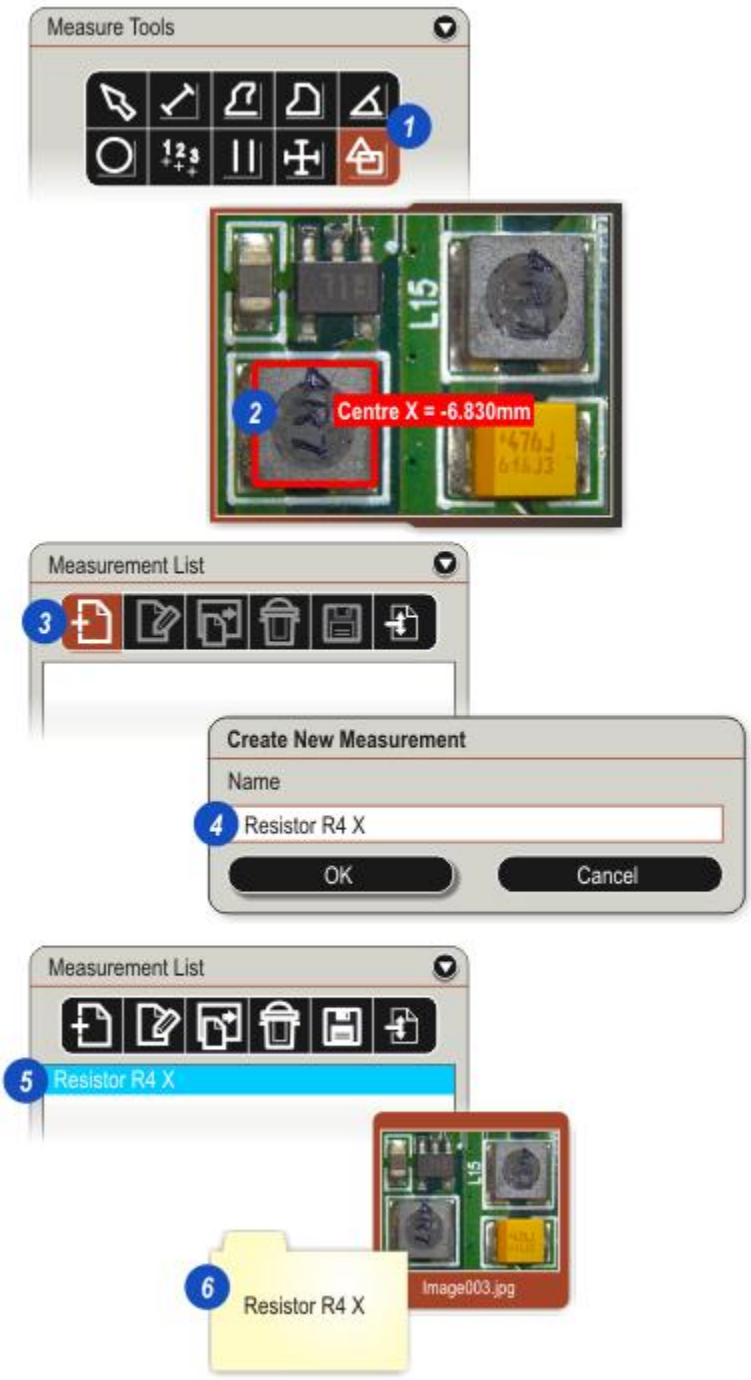
4: On the *Create New Measurement* dialog, click inside the text box and type a unique name for the template.

Click *OK.*

5: The measurement(s) is added to the *Measurement List.*

6: The measurement(s) is attached to the image.

Continued 983



Add a Measurement to the Template Library

To add the measurement to the *Template Library* so that it becomes available to other images:

1: On the *Measurements List* panel, click on the *Load / Save* button.

2: On the *Load / Save Measurements* dialog, the measurement is shown in the *Measurements List*, the pane to the right.

3: Add the measurement to the *Template Library* by clicking the *Save* button - the left-facing arrow.

4: The measurement is converted to a template and copied to the *Template Library*, appearing in the left-hand pane.

The measurement is still available to the current image.

5: Click *OK.*

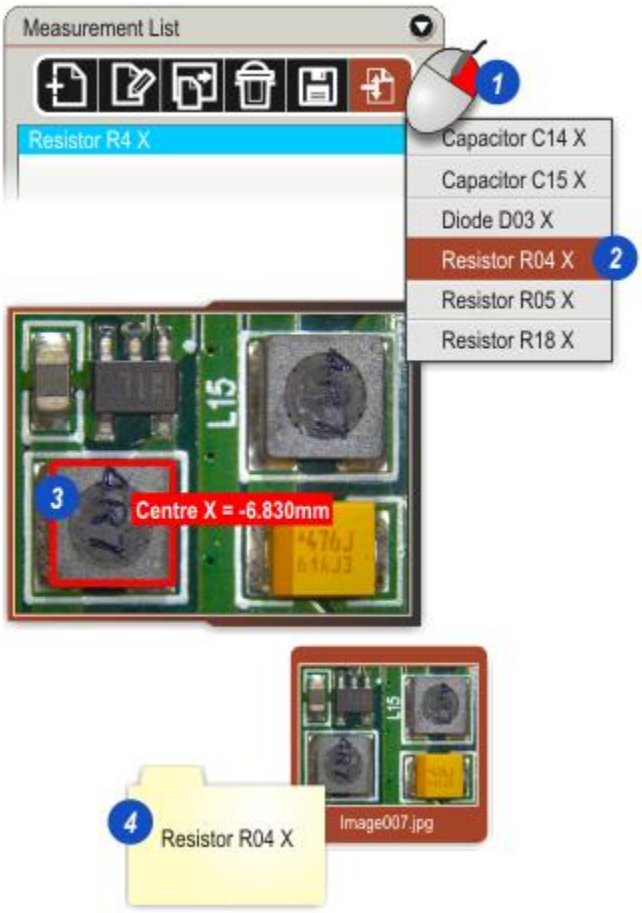
Templates are converted to measurements before being applied to an image. The process is automatic:

1: Right-click the *Load / Save Measurements* button. The drop-down shows all of the templates stored in the *Template Library.*

2: Click to select the template required.

3: The measurement is applied to the image...

4: ...and stored with it.



Applying another Template

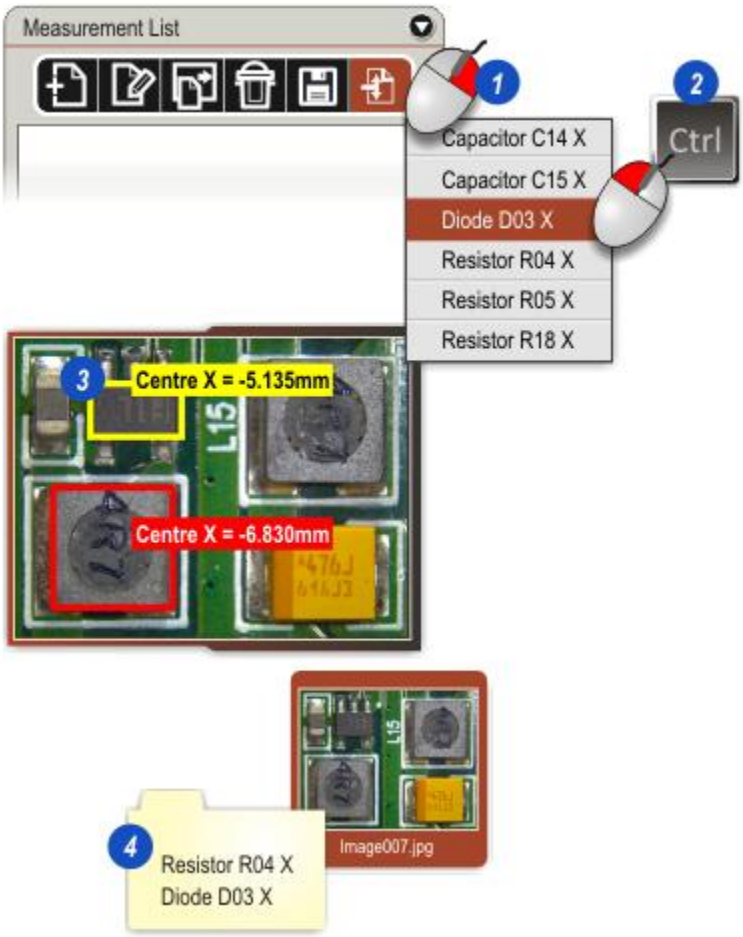
Add further templates to those existing on the image by:

1: Right-click the *Load / Save Measurements* button. The drop-down shows all of the templates stored in the *Template Library.*

2: Press and hold down the keyboard *Ctrl* key and left-click to select the template required.

3: The measurement is applied to the image...

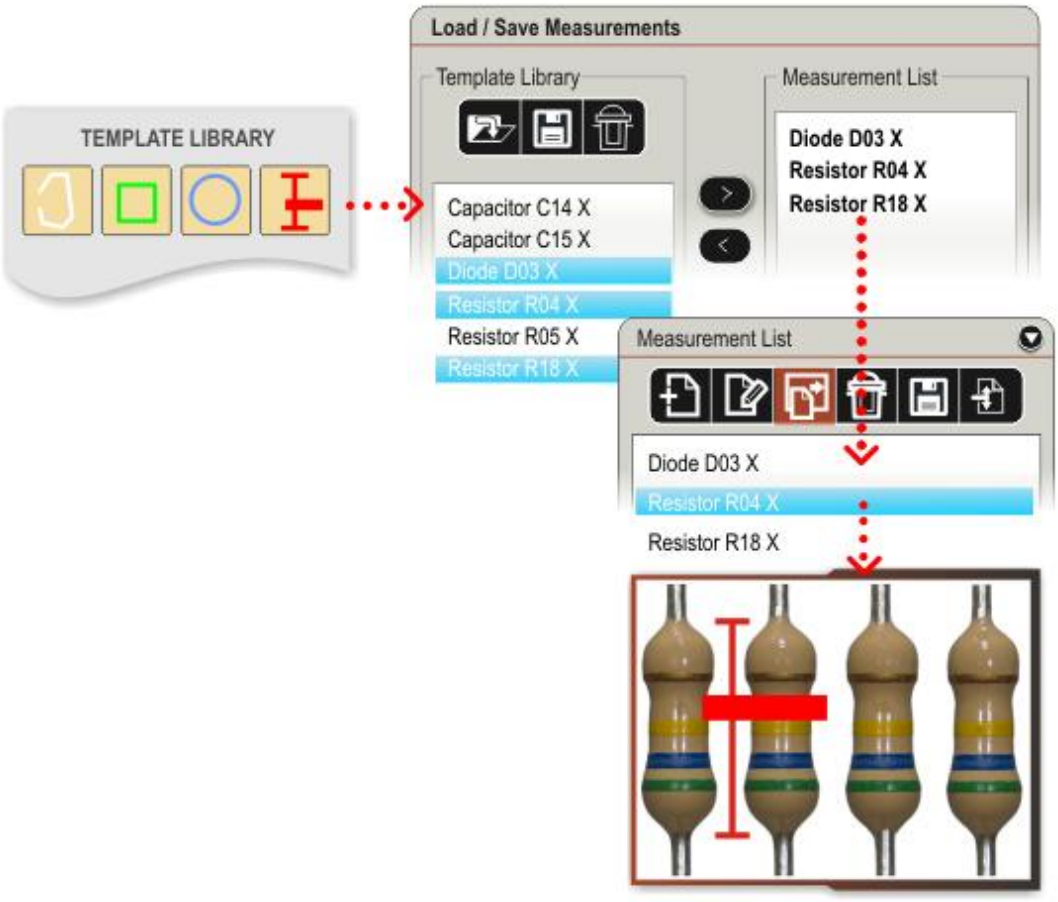
4: ...and stored with it together with existing measurements.



Some users may need to apply templates in sequence to an image - for example to check the positioning of individual components. The *Measurement List* is a quick and convenient way of achieving this - as each template is applied it replaces the previous one.

The first step is to create a *Measurement List* from the *Template Library* of the required templates. The diagram shows how *Templates* are retrieved from the *Template Library* and converted to measurements. The measurements are then added to the *Measurement List* and can be applied to a new image.

- The templates to be used are selected from the *Template Library*..
- The templates are copied to the *Measurement List* and automatically converted to measurements..
- The measurements appears on the *Measurement List* panel ready to be applied to the image.



Creating the Measurement List

Create the Measurement List from selected templates by:

1: On the *Measurements List* panel click on the *Load / Save button.*

2: On the *Load / Save Measurements* dialog, the available templates in the *Library* are listed in the left-hand pane.

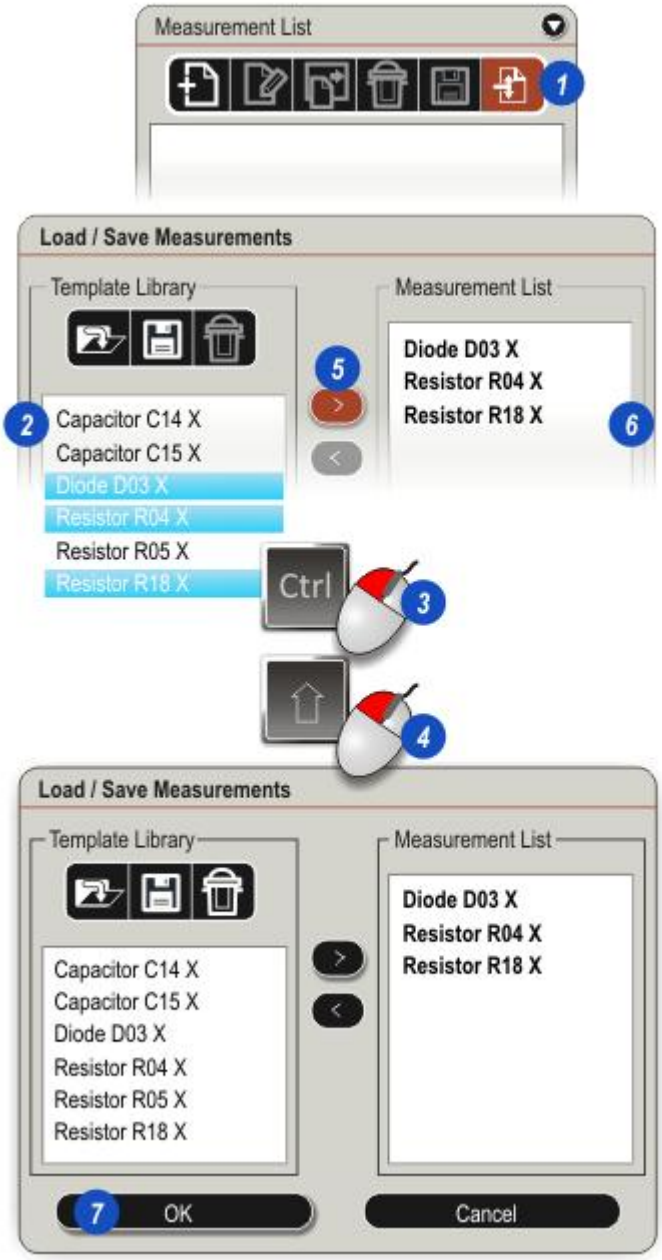
3: Select individual templates by pressing and holding down the keyboard *Ctrl* key and left-clicking each required template or...

4: ...select a sequence of templates by clicking on the first, pressing and holding down the keyboard *Shift* key and left-clicking on the last template. All of the templates between will be selected.

5: Click on the *Load Template* button and the selected templates are converted to measurements and...

6: ...shown on the *Measurement List* - the right-hand pane.

7: Click *OK.*



Templates converted to measurements are also displayed on the *Measurements List* panel. From here they can be applied to the image by:

1: Click on the measurement to be applied.

2: Click the *Load Measurement* button.

3: The measurement is applied to the image.

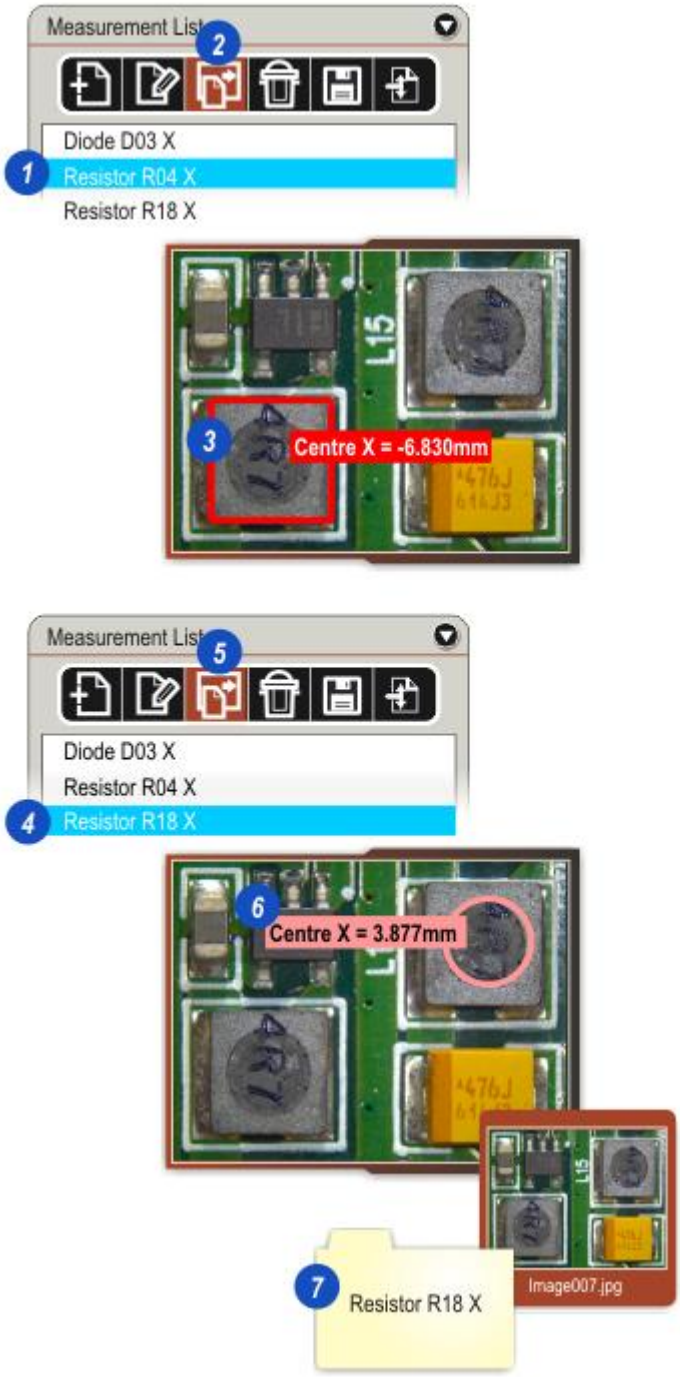
To apply the next measurement repeat the process:

4: Select the measurement.

5: Click the *Load Measurement* button and...

6: ...the existing measurement is removed and the new one applied.

7: Only the last measurement is saved with the image.



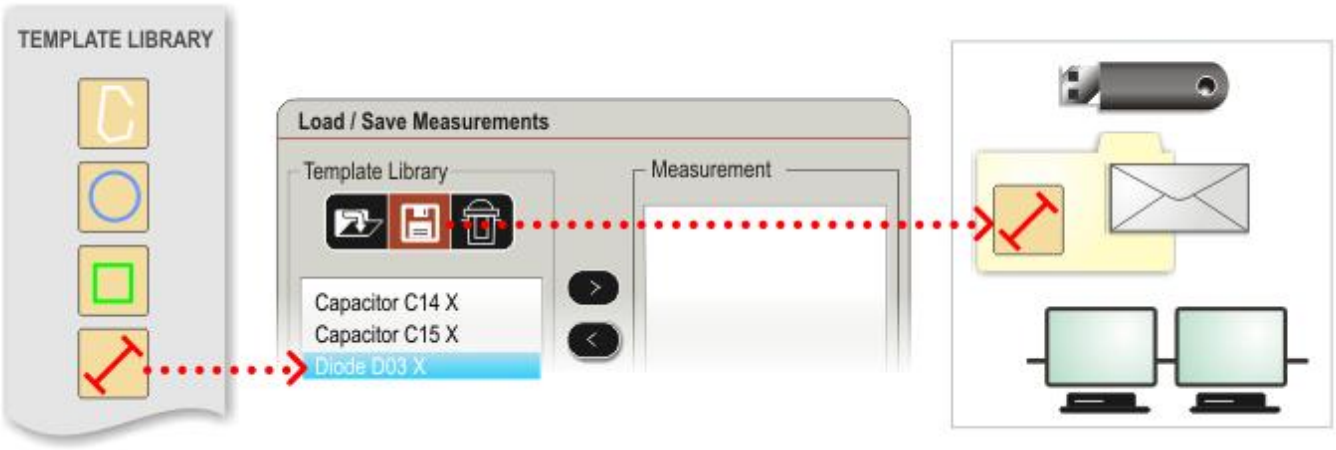
Saving Templates

Measurements that have been named and stored as templates in the *Template Library* can be saved to most storage media using the *Save Template To* feature.

Targets for *Save Template To* include pen drives, removable backup hard drives and folders on this or on networked computers.

Templates can be attached to an e-mail but only through an intermediate folder.

Save Template To makes a copy of the template (*.tem* file extension).



The *Save Template To* feature copies a template from the *Library* to:

- A Pen Drive (Stick).
- A removable Hard Drive (Backup),
- A shared folder on a Network,
- A folder on this computer.

Because the *Template Library* is inaccessible to users outside *Interactive Measurements*, to attach a template to an e-mail or write to a CD or DVD, save the template to a folder on this computer and use that as source for the mail or writing.

1: On the *Measurements* panel, click the *Load / Save* button.

2: On the *Load / Save Measurements* dialog, *Template Library*, click to select the template to be saved.

3: Click the *Save Template To* button...

4: ...and on the *Browse for Folder* dialog navigate to the target folder or media.

5: Click *OK.*

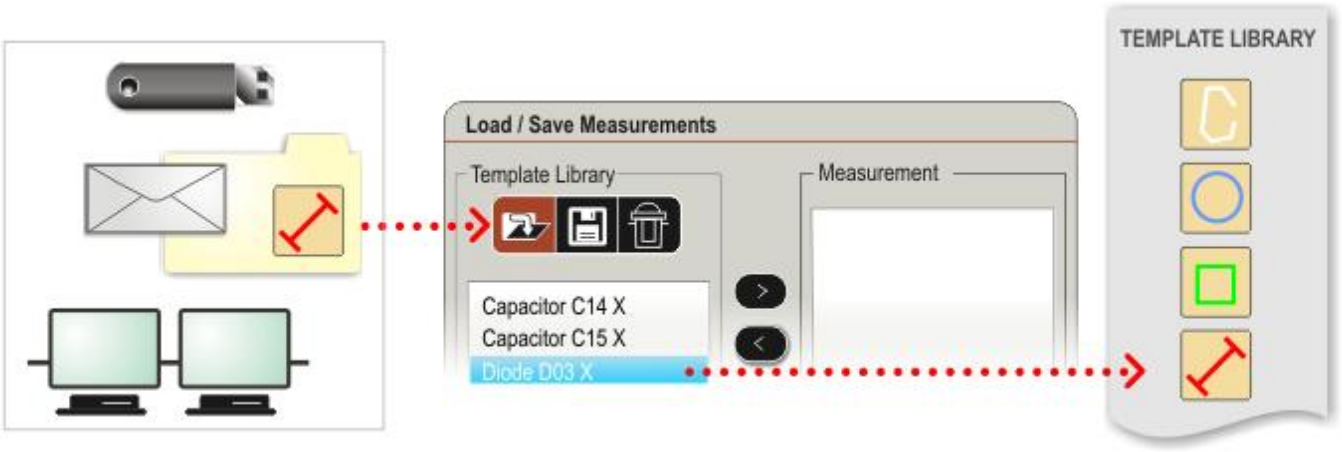
6: The *Save Template Report* will display success (or failure).

Templates can be loaded from most storage media using the *Load Template From* feature.

Sources for *Load Template From* include pen drives, removable backup hard drives and folders on this or on networked computers.

Load Template From copies a *.tem* file from the source media to the *Library and displays it on the Template Library pane.* From there it can be converted to a measurement and used on images.

If there is already a template of the same name in the *Library* the loaded file is given an incremental suffix.



Load a Template

The *Load From* feature copies a template from:

- A Pen Drive (Stick).
- A CD or DVD,
- A removable Hard Drive (Backup),
- A shared folder on a Network,
- A folder on this computer.

...and places it in the *Library*. From there it can be applied to a live image.

1: On the Measurements panel click the *Load / Save* button.

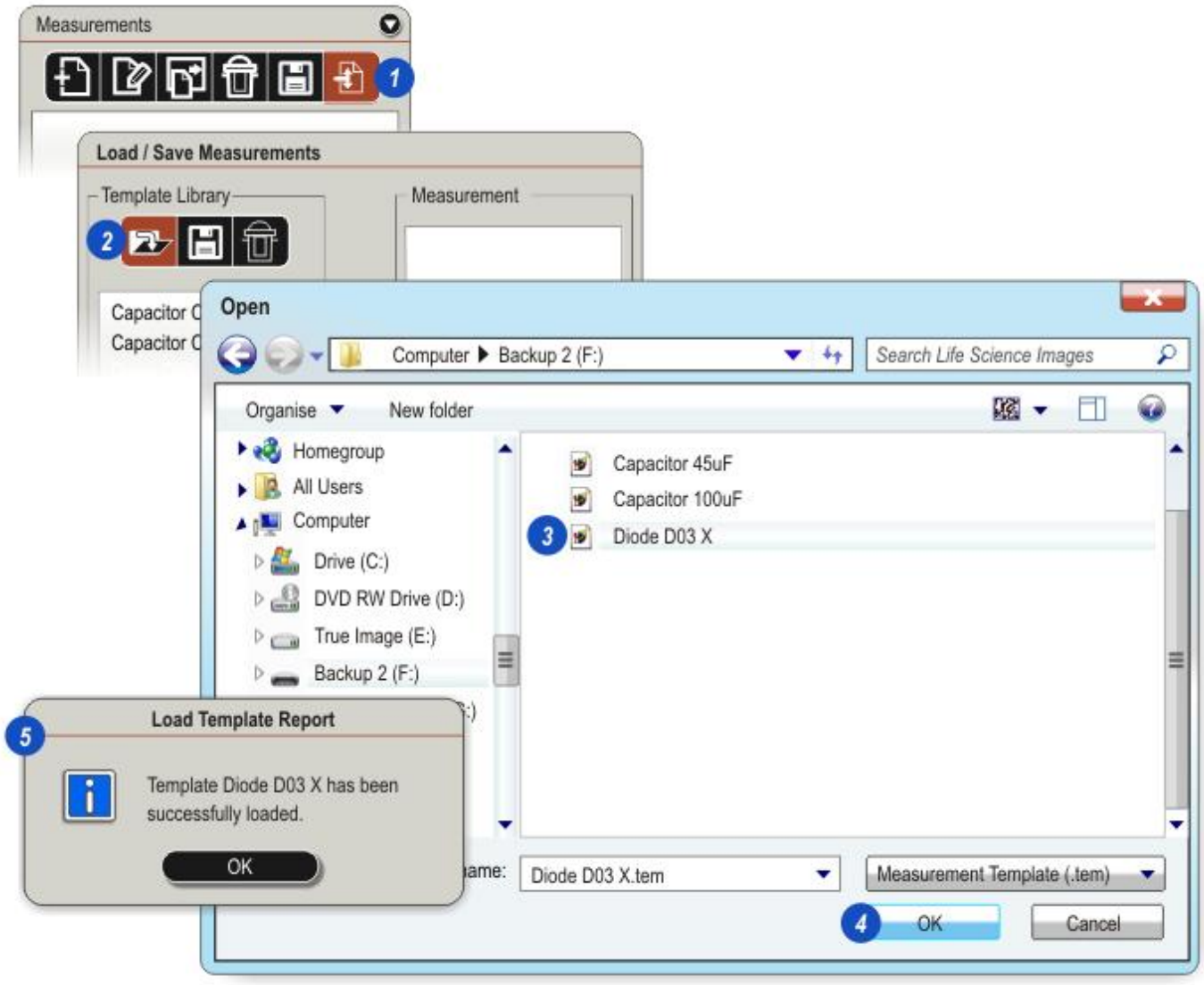
2: Click the *Load Template From* button on the *Load / Save Measurements* dialog.

3: On the *Open* dialog navigate to the folder or media containing the template file and click to select it.

4: Click the *OK* button. The template is copied to the *Template Library*

If a template of the same name already exists in the *Library* the new file will be given an incremental suffix.

5: The *Load Template Report* displays success (or failure).



Delete a Measurement

Delete a measurement from the Measurement List by:

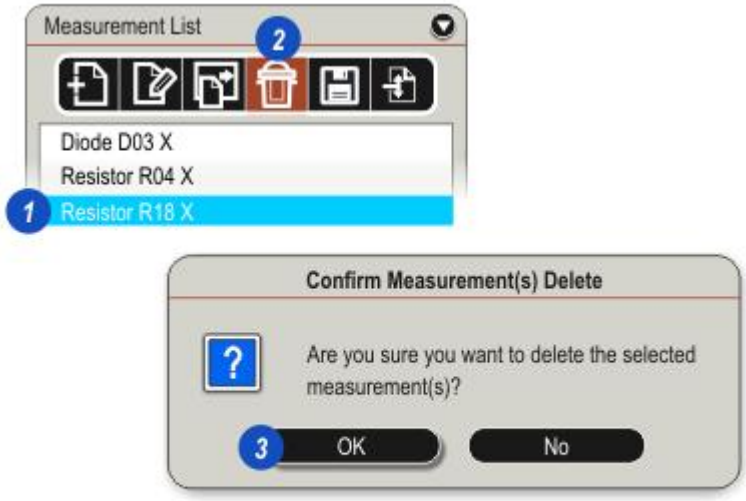
1: Click on the measurement to be deleted in the *Measurements List* panel.

2: Click on the *Delete (Trash Can)* button.

3: Confirm the deletion by clicking *OK* and the template is removed.

Images that have used the measurement are not affected - they have the measurement attached which remains intact.

Measurements are only removed from the *Measurement List*[987]. To reinstate and use the measurement again, copy and convert the template.



Edit a Measurement

The *Edit* feature allows a user to change a measurement name:

1: Click on the measurement to be edited on the *Measurement List*.

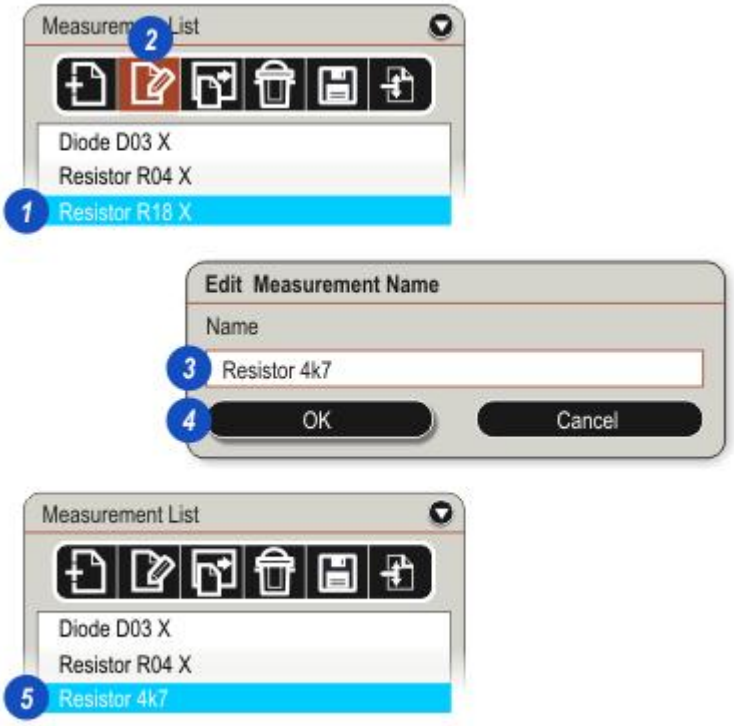
2: Click the *Edit* button.

3: On the *Edit Measurement Name* dialog, click in the text box and type a new name.

4: Click *OK* and....

5: ...the new name appears on the *Measurement List.*

Changing a measurement name does not change the associated template name. The measurement will have to be saved as a new[983] template if required.



Measurements made on a live image using optional module *Live Measurements* (if it is installed and licensed) are saved with the image when it is captured.

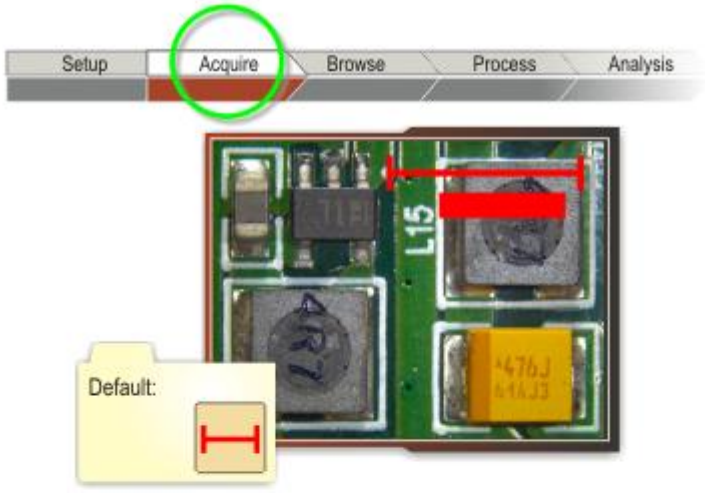
Although a single measurement is shown in the diagrams it could be a collection of measurements.

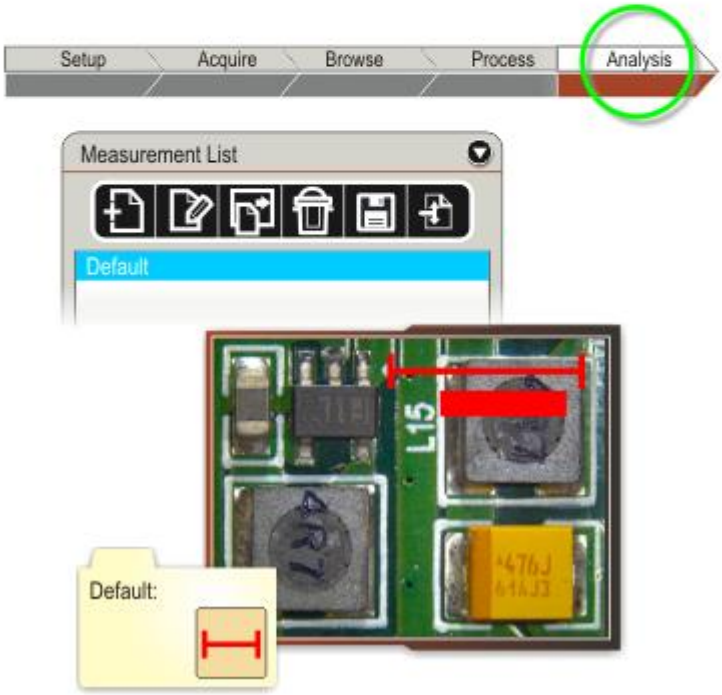
The measurement itself does not have a name so when the image is opened in *Interactive Measurements* it is given the default name of *'Default'*.

The name can be changed to something more meaningful using the *Edit*[994] feature

The options for the user are:

- Make changes[996] to the *Default* measurement and re-save it with the image
- Add[997] further measurements to the *Default* file and re-save it with the image - still as a single measurements file
- Keep[998] the original *Default* measurement but make a copy of it with a new name and add further measurements as required. In this way both the *Default* and a new file that includes the original *Default* measurement are attached to the image as two separate files
- Save[994] the measurement(s) as a template to be used on other images





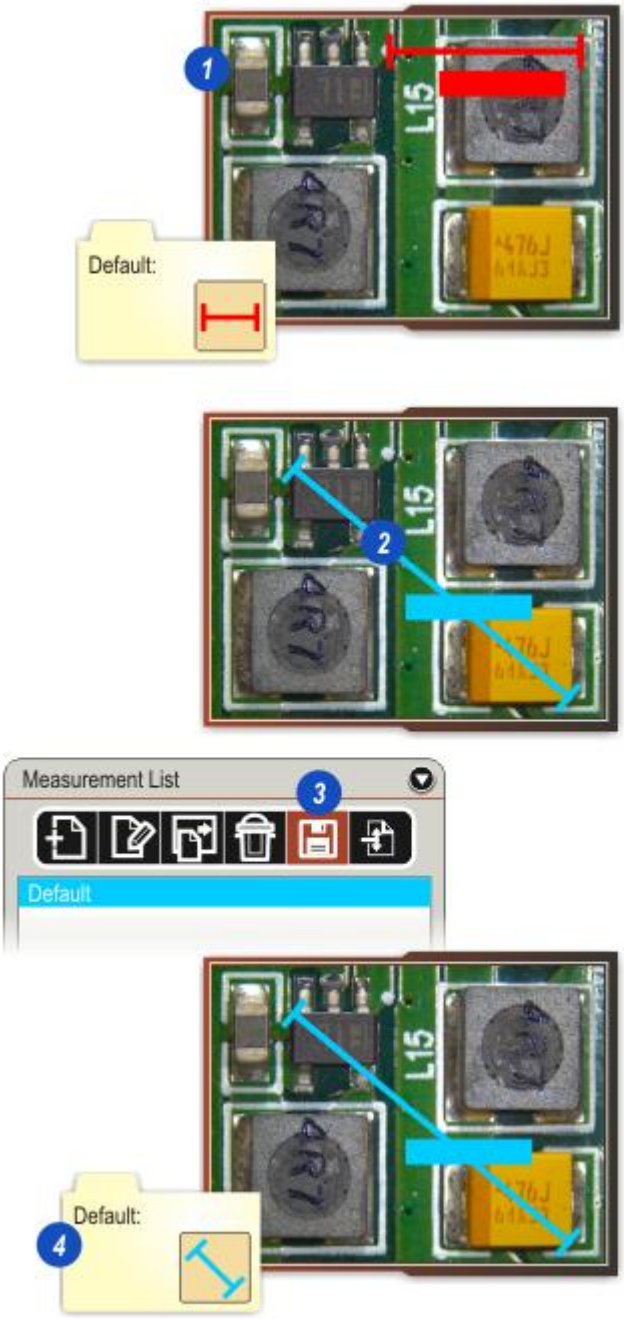
In these steps, changes are made to the original *Default* measurement and the changes saved as a modified *Default* file:

1: The original captured image and *Default* measurement.

2: The measurement is altered.

3: Click on the *Save Measurement* button.

4: The *Default* file is saved reflecting the changes.



Adding Measurements to Default

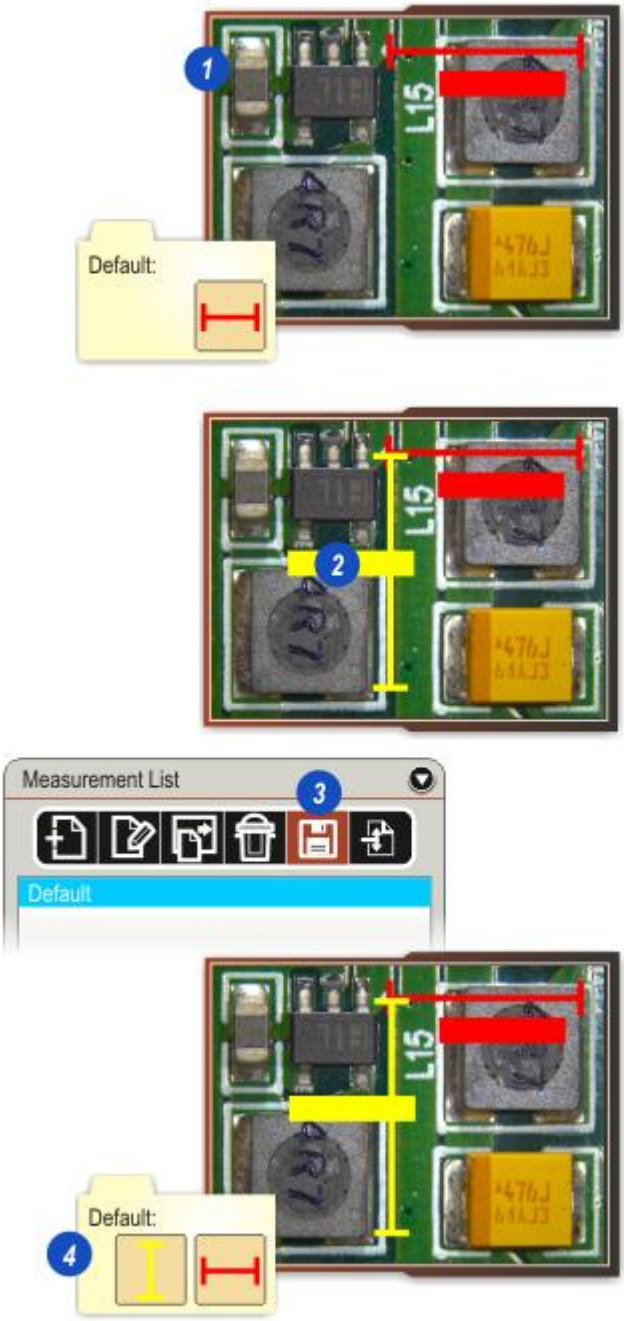
This procedure adds another measurement to the *Default* and saves the result as a single file - still called *Default*:

1: The original image and *Default* measurement made in *Live Measurements* (if installed and licensed) opened in *Interactive Measurements.*

2: Another measurement is made on the image.

3: Click the *Save Measurement* button.

4: The new measurement is added to the *Default* file.



Default as a Basic Measurement

This procedure uses *Default* as the basis for a new measurement whilst keeping *Default* intact.

Start by copying *Default* under a new name:

1: The image with *Default* measurement.

2: Click to select *Default* in the *Measurement List.*

3: Click the *Create New Measurement* button.

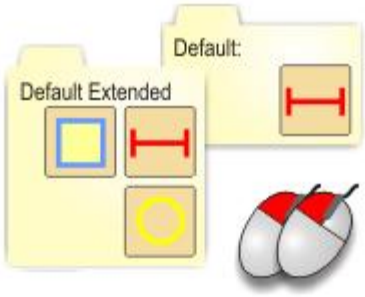
4: On the dialog, click inside the text box and type a unique name for the *Default* copy.

5: The copy appears in the *Measurement List* under its new name.

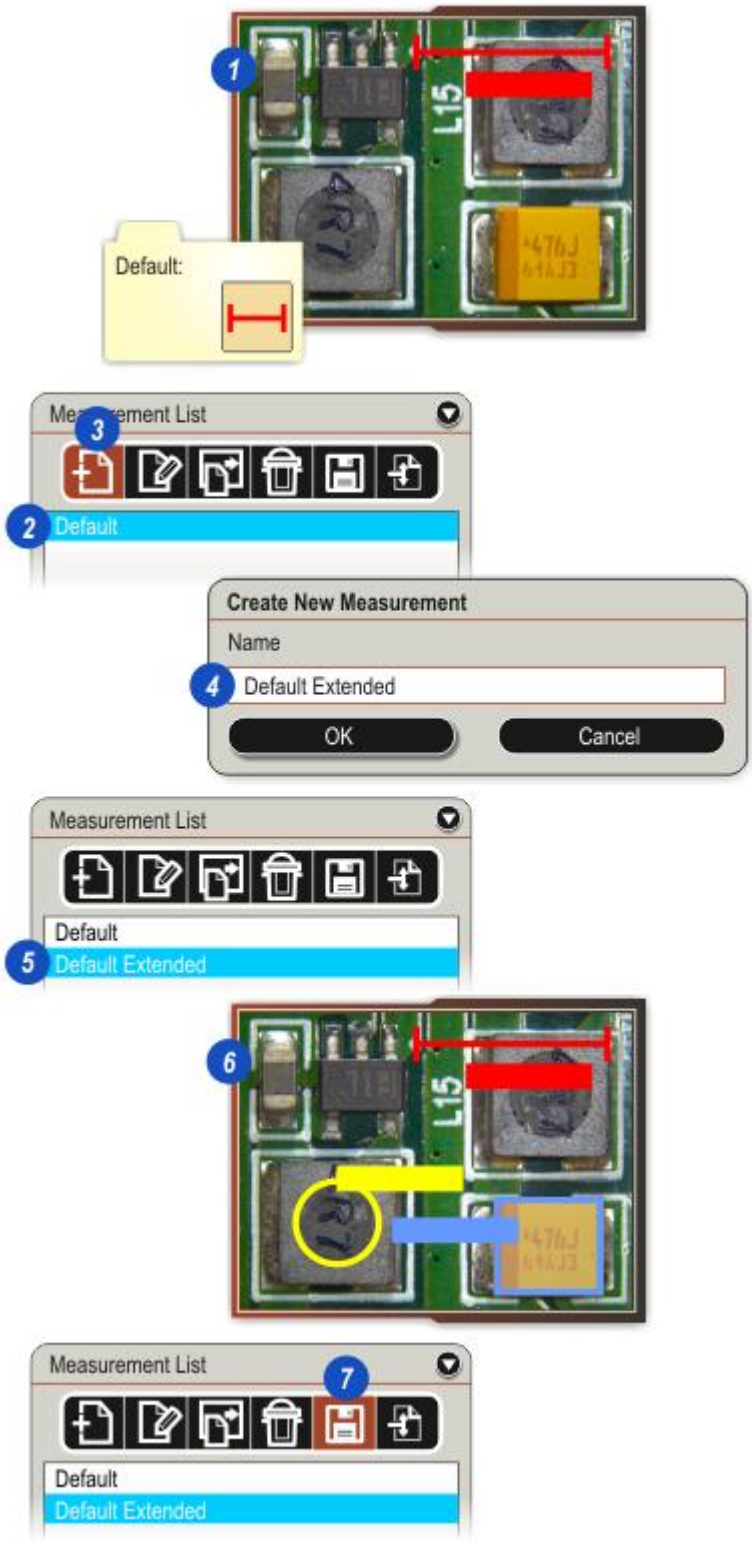
6: Add new measurements leaving the original intact.

7: Click the *Save Measurement* button.

The image now has two attached files - *Default* and the new copy with the original *Default* measurement in both.



Either measurement file can be applied to the image by double-clicking its entry in the *Measurements List.*



Select Results

The *Select Results* function displays an analysis of all the measurements taken on an image and presents them either as:

- *Details (1)*: Each measurement is shown with all its parameters (or those that are selected in the *Configuration*), or ...
- *Summary (2)*: The measurements are treated as a complete set of data tabulated statistically.

Both options present the information as a grid arranged below the image. The information can be later edited, deleted or attached to the image.

1

Measurement	Tool	Line Length (µm)	Width (µm)	Width 2 (µm)	Height (µm)	Height 2 (µm)
2	Distance Line	3 826.009	2 614.416	-	2 794.380	-
3	Three Point Circle	-	2 692.014	-	2.692.014	-
4	Three Point Circle	-	2 272.638	-	2 272.638	-
5	Rectangle	-	2 380.548	-	1 389.800	-

2

Statistic Type	Line Length (µm)	Width (µm)	Width 2 (µm)	Height (µm)
Total	-	18 462.729	-	8 548.058
Mean	-	3 692.546	-	1 709.612
Mode	-	-	-	-
Median	1 579.484	3 302.890	-	1 754.516
Maximum	1 571.739	7 365.657	-	3 302.890
Minimum	1 579.484	1 241.403	-	314.488

Set up the Grid Headings for Details:

1: If necessary, click on the small arrow to the right of the Select Results panel.

2: Click to enable the *Details* button.

3: Click the *Configure* button.

4: On the *Configure Details Grid* dialog...

5: ...click the *Hide All* button to disable all of the parameters.

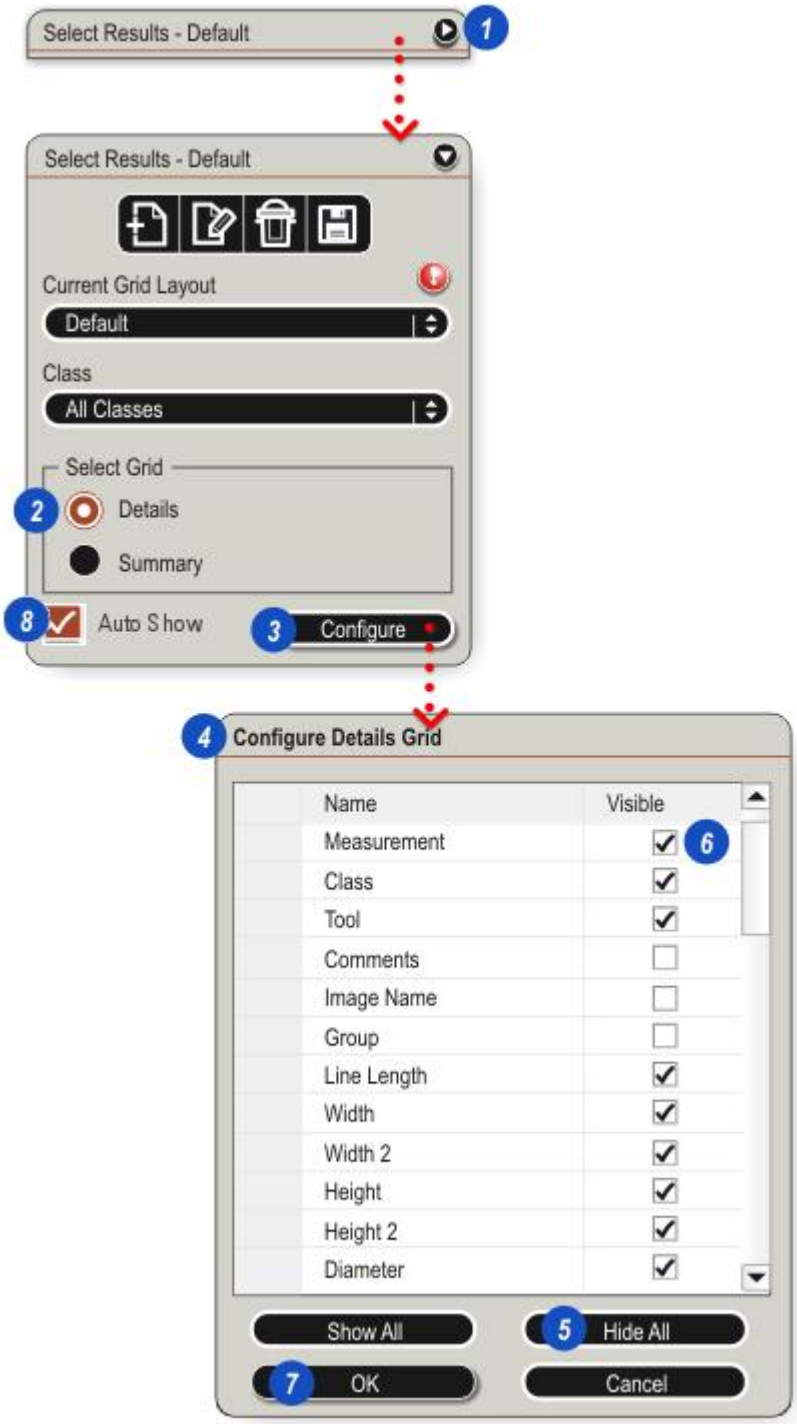
6: Individual parameters can then be enabled by clicking to enable (Tick mark displayed) the check box to the right of the parameter.

7: Click *OK*.

Those parameters that have been enabled will appear as a heading on the *Grid* with the appropriate results beneath. If a chosen parameter is not applicable to a measurement - for instance, *Line Length* is not a parameter of a *3-Point Circle* - then the value is reported as a dash (-).

Click the *Show All* button to enable all of the parameters.

8: *Auto Show Columns* when checked will show only the parameters for the selected Drawing Tool. This reduces the number of empty columns that are displayed. If you select more than one Drawing Tool, then you see the tools from all selected Tools. When this option is checked off, the parameters selection is Configured as described earlier.



To display the results as tabulated data using the *Summary* information, set up the *Grid Headings:*

1: Click to enable the *Summary* button.

2: Click the *Configure* button.

3: On the *Configure Summary Grid* dialog...

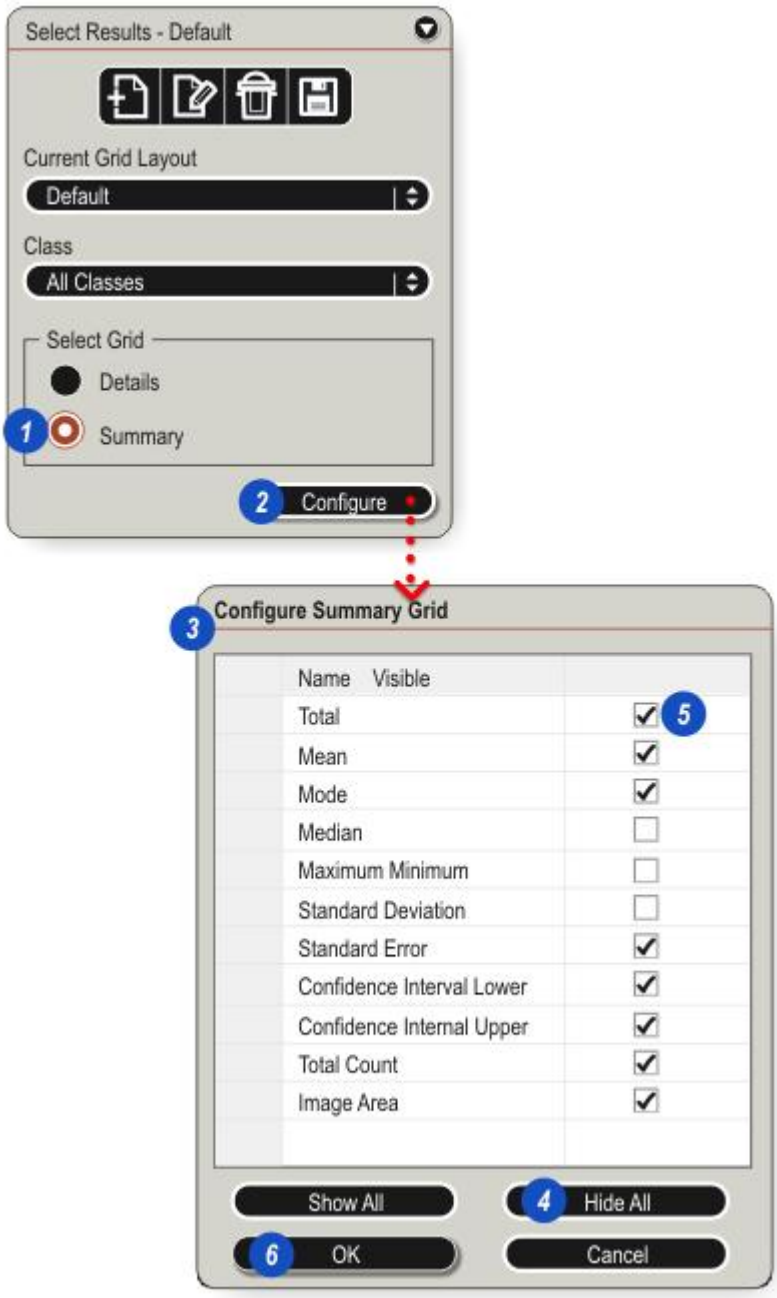
4: ...click the *Hide All* button to disable all of the parameters.

5: Individual parameters can then be enabled or disabled.

6: Click *OK*.

Those parameters that have been enabled will appear as a heading on the *Grid* with the appropriate results beneath. If a chosen parameter is not applicable to a measurement - for instance, Line Length is not a parameter of a *3-Point Circle* - then the value is reported as a dash (-).

Click the *Show All* button to enable all of the parameters.



1: When changes are made to the *Grid* either in *Details* or *Summary*, the *Data Changed Warning* ! flashes.

2: The current *Grid Layout* configuration (but not *Default*) can be updated by clicking on the *Save* button.

3: Save the new *Grid Layout* as a new configuration by clicking the *Create New Grid* button and...

4: ...on the *Create New Grid* Layout dialog, click inside the *Name* text box and type a new unique name for the configuration.

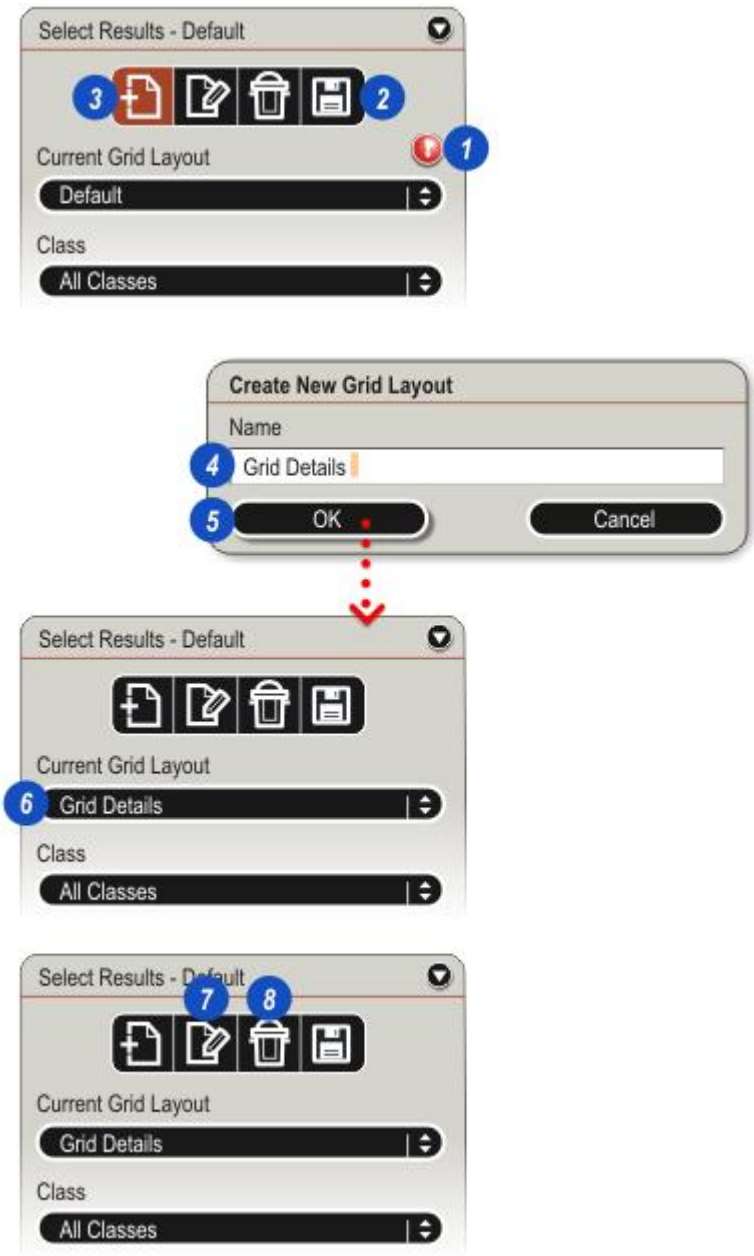
5: Click *OK.*

6: The new configuration name appears in the *Current Grid Layout* header.

When the *Results Grid* is selected, a range of shortcut key combinations are available. For a complete list of all the *LAS Keyboard Shortcuts* 35

7: To change the name of a configuration click on the *Edit* button and change the name on the dialog.

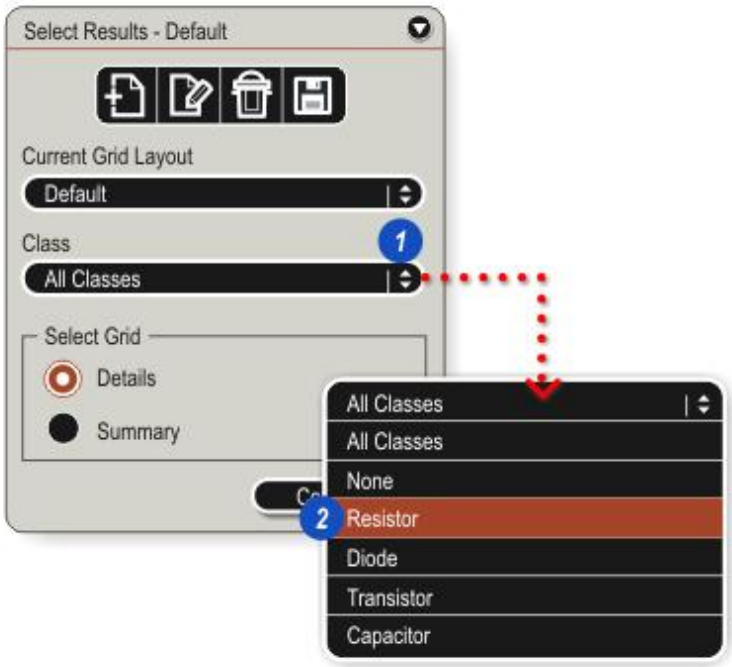
8: Delete a configuration by selecting it from the drop-down menu and clicking the *Delete (Trash Can)* button.



Selecting Classes

Results can be 'filtered' using the *Class* option. If classes are being used results can be displayed on the *Grid* by selecting:

- *All Classes:* Displays all the results.
- *Selected Class:* Only measurements associated to the selected class are displayed.
- *None:* Only measurements not associated with a class are displayed. Default if classes are not being used.



To select a *Class* option:

1: Click on the arrow to the right of the *Class* header.

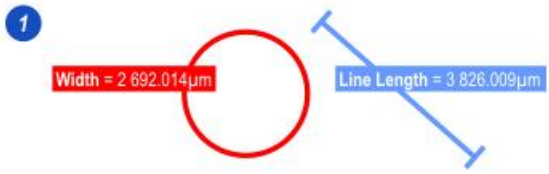
2: Click to select a *Class* option from the drop-down.

If a specific class is going to be used as a filter, users will find it helpful to include the *Class* heading in the *Details Grid* 1000

Measurement	Class	Tool	Line Length (µm)	Width (µm)	Width 2 (µm)	Height (µm)
2	Resistor	Distance Line	3 826.009	2 614.416	-	2 794.380
3	Resistor	Three Point Circle	-	2 692.014	-	2.692.014
4	Resistor	Three Point Circle	-	2 272.638	-	2 272.638
5	Resistor	Rectangle	-	2 380.548	-	1 389.800

Displayed measurement parameters and values can be changed by simply double-clicking a header on the grid.

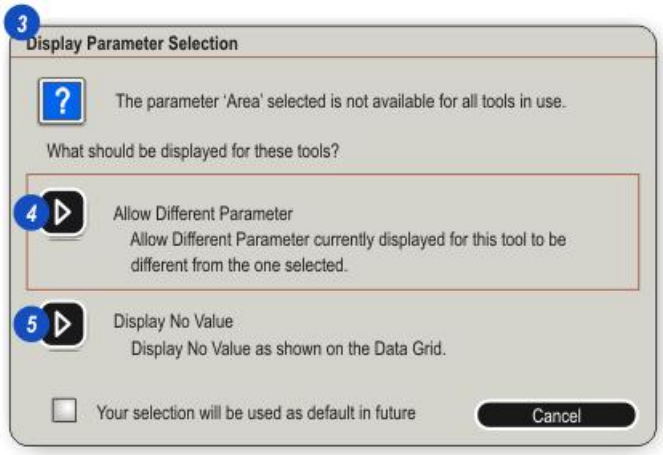
The program will try to change all of the measurements to the selected parameter but there will be occasions when the chosen parameter is not appropriate to some measurements. For example:



1: The illustration shows two measurements - a *Distance Line* and a *Circle* - with *Line Length* and *Width* labelled respectively.

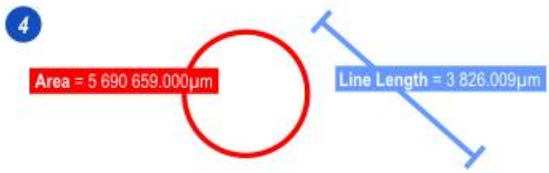
Measurement	Tool	Line Length (µm)	Width (µm)	Height (µm)	Area(µm)
2	Distance Line	3 826.009	2 614.416	2 794.380	-
3	Three Point Circle	-	2 692.014	2.692.014	5 690 659.000
4	Three Point Circle	-	2 272.638	2 272.638	4 055 724.500
5	Rectangle	-	2 380.548	1 389.800	3 308 485.600

2: The user decides to change the parameter to *Area* and display the *Area* value by double-clicking on the *Area* header. The *Display Parameter Selection* dialog appears.

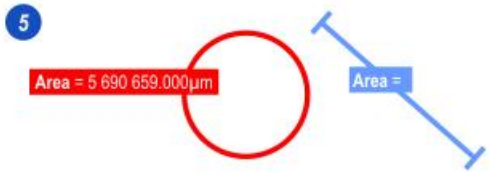


3: *Area* is appropriate to the *Circle* measurement but not to the *Distance Line* so, on the dialog there are two choices:

4: *Allow Different Parameter*: Clicking the button will change those parameters that are appropriate - the *Circle* parameter and value has changed - but the *Distance Line* is unaffected because *Area* is not appropriate and it still displays *Line Length*.



5: *Display No Value:* Changes all measurement parameters whether appropriate or not, but because in the case of the *Distance Line* the value for the *Area* parameter would have no meaning, it is left blank.

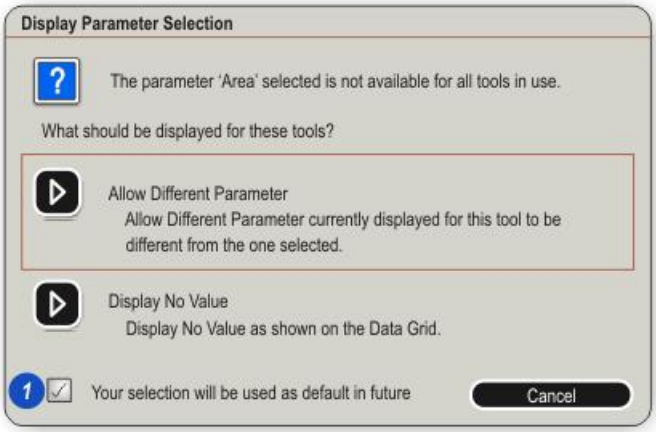


Users can revert to the original parameters and values by double-clicking another header.

Because the *Change Parameters* function is designed for fast checking and comparison of measurements, some users will want to skip the dialog in the interests of speed and have either:

- *Allow Different Parameter* or .
- *Display No Value...*

...applied automatically every time,



1: Enabling (tick mark visible) the *Your selection...* check box will will apply the last choice automatically.

Restore the dialog by enabling (tick mark visible) the *Warning to set Measurements display parameter* on the *Preferences > Warning* 68 tab

The sequence of results in the *Grid* can be displayed high value to low value or the reverse by:

2: Click to the right of a header. The sequence is reversed and a small arrow appears. Click on the arrow to change the sequence again.

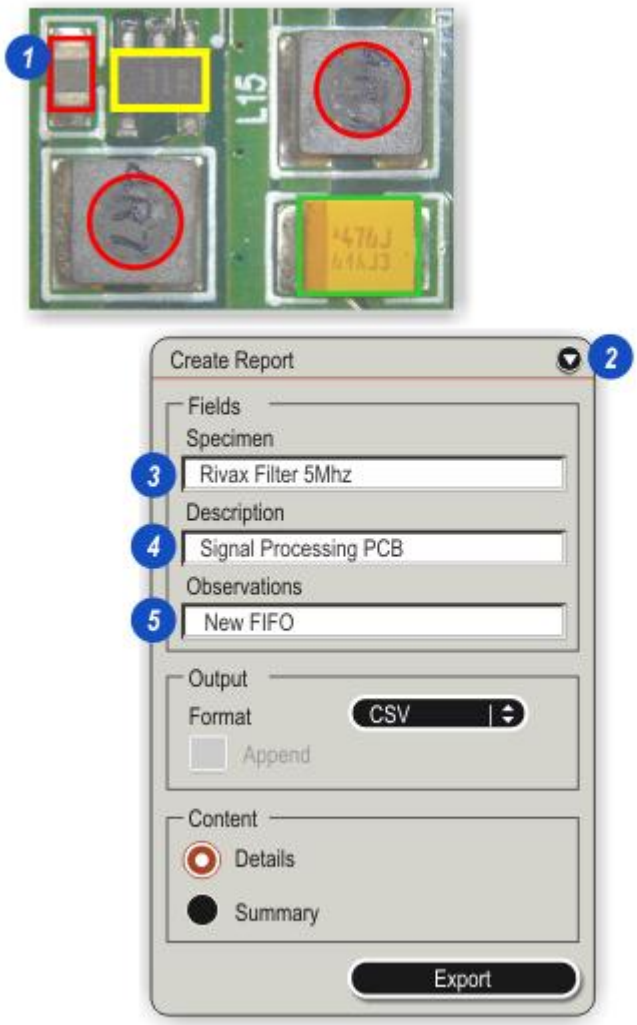
Measurement	Tool	Line Length (µm)	Width (µm)	Height (µm)	Area(µm)
2	Distance Line	3 826.009	2 614.416	2 794.380	-
3	Three Point Circle	-	2 692.014	2.692.014	5 690 659.000
4	Three Point Circle	-	2 272.638	2 272.638	4 055 724.500
5	Rectangle	-	2 380.548	1 389.800	3 308 485.600

A report collects the measurement information and stores it in one of the following readable formats:

- *Microsoft Excel*
- *CSV:* Comma Separated Value file.

CSV files are stored as plain text and are very compact, making them ideal as e-mail attachments. They can also be used in a wide range of text processing applications.

The parameters that are exported reflect those chosen to display in *Select Results* 1000



1: A typical live image with several measurement tools and classes being used.

2: Click on the arrows to the right of the *Create Report* header to reveal the panel.

3, 4 and 5: Three *Field* text boxes – *Specimen*, *Description* and *Observations* – are provided as optional headers for the measurement file. They do not have to contain information but to add text to them click inside a text box and type.

Create Excel Report

The method used for creating Excel Reports depends on which optional module you are using:

- Live Measurements 1008
- Interactive Measurements 1009

1: Click on the arrows to the right of the *Format* header to reveal the output options.

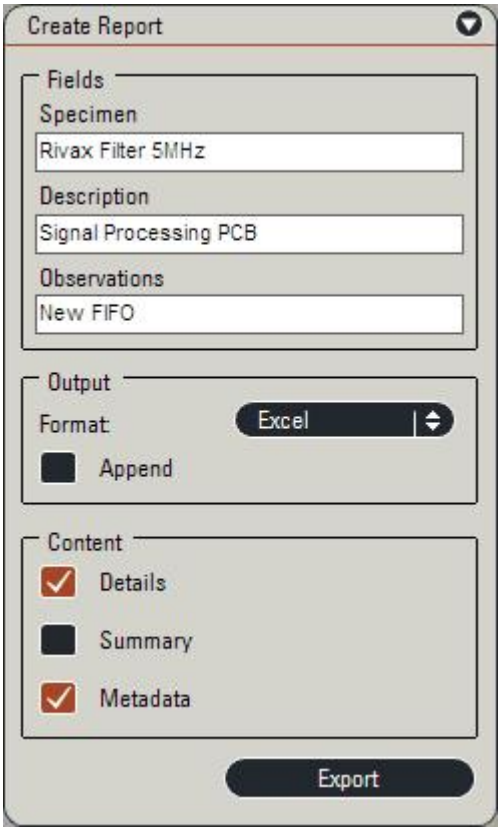
2: Select *Excel* from the drop down menu.

3: The report content can contain any mixture of the following:

- *Details:* The actual measurements, classes and results
- *Summary:* The detailed analysis of the measurements
- *Metadata*: File metadata, including information on the camera and microscope (Note: if LAS Archive is enabled, the report will also contain all archive metadata)

4: To add the report data to an existing Excel spreadsheet, click the *Append* button. This arrangement allows results to be analysed individually or as a group.

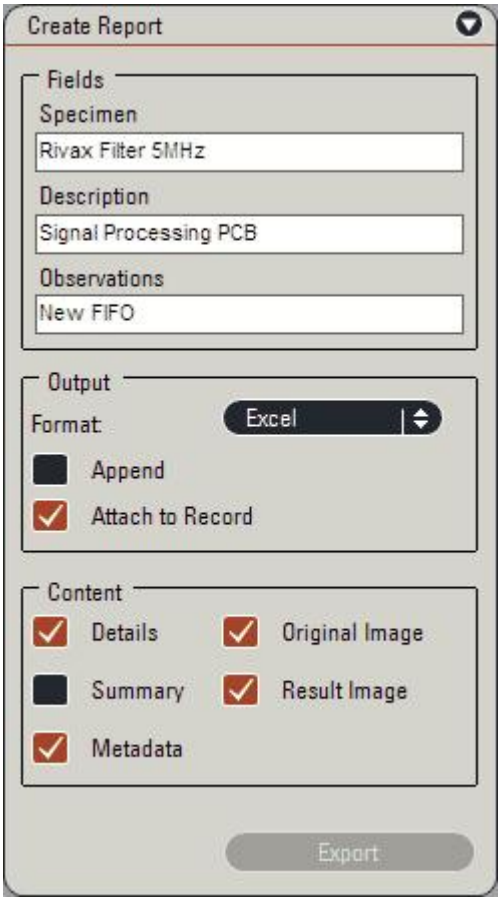
5: Click the *Export* button.



With the *Attach to Record* check box enabled (tick mark showing), the report data is attached to the image as a separate file.

The report can contain:

- *Details*: The actual measurements, classes and results
- *Summary*: The detailed analysis of the measurements
- *Metadata*: File metadata (Note: if LAS Archive is enabled, the report will also contain all archive metadata)
- *Original Image*
- *Result Image*



Browse for or Name Report

When LAS is installed, a default location for reports is created at:

- *Users > UserName > Documents > LAS Reports >* .

On first use this will be the displayed path. Users can use this and simply change the report name, or create their own folders elsewhere on the computer or network.

1: On the *Export* dialog the *Report* text box displays the last report path and name. This is convenient for overwriting or appending the report.

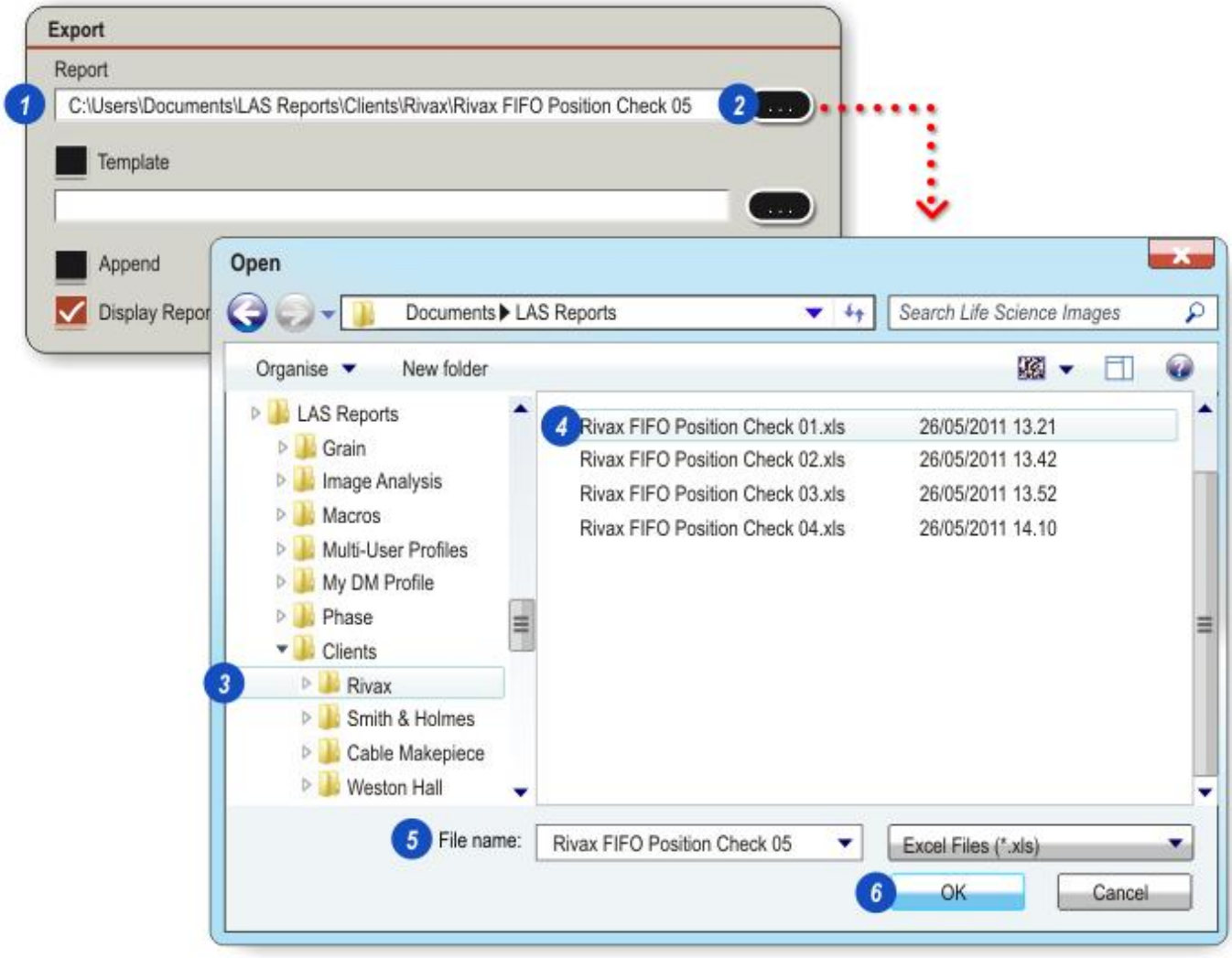
2: To create a new report, click the browse button and...

3: ...on the *Open* dialog navigate to the required folder,...

4: ...click to select and existing file to overwrite or append to.

5: Alternatively, click inside the *File name* text box and type a name for a new report.

6: Click *OK*.



Select a Template

When the *Live Measurements* module is installed several Excel templates are included. These will determine the layout of the data and analysis. *Live Measurement* reports cannot include images so result tabs are available for the *Cover* that can also show selected data (in this case *Circle* measurements), *Summary* and *Details*. Users can opt to include *Summary* data, *Details* or both.

By default, the templates are stored in the following location:

- *Users > Public Documents > Leica Application Suite > Excel Templates > Measurements..*.

Alternatively, users can create their own templates, store them in a location of choice and use them to configure the report data.

1: If the *Append* check box is enabled the option to choose a template is not available - the report data will be configured with the original report template. The last template used is displayed in the *Template* text box. To select another template...

2: ...click on the browse button and...

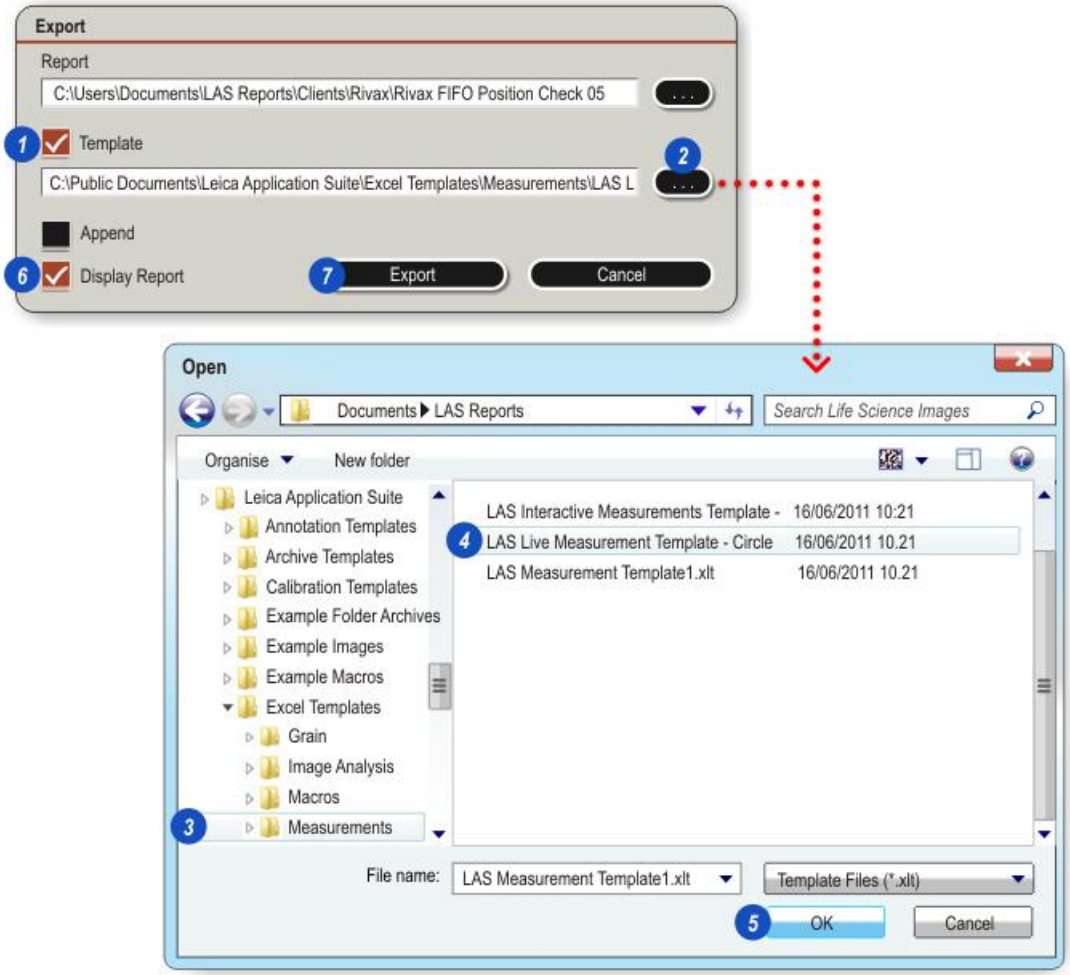
3: ...on the *Open* dialog navigate to the required template folder.

4: Click to select a template - in this case the *Live Measurements* template which does not include an image.

5: Click *OK*. The selected template path and name appears in the *Template* text box.

6: To display the Excel report as soon as it has been created, click to enable (tick mark visible) the *Display Report* check box.

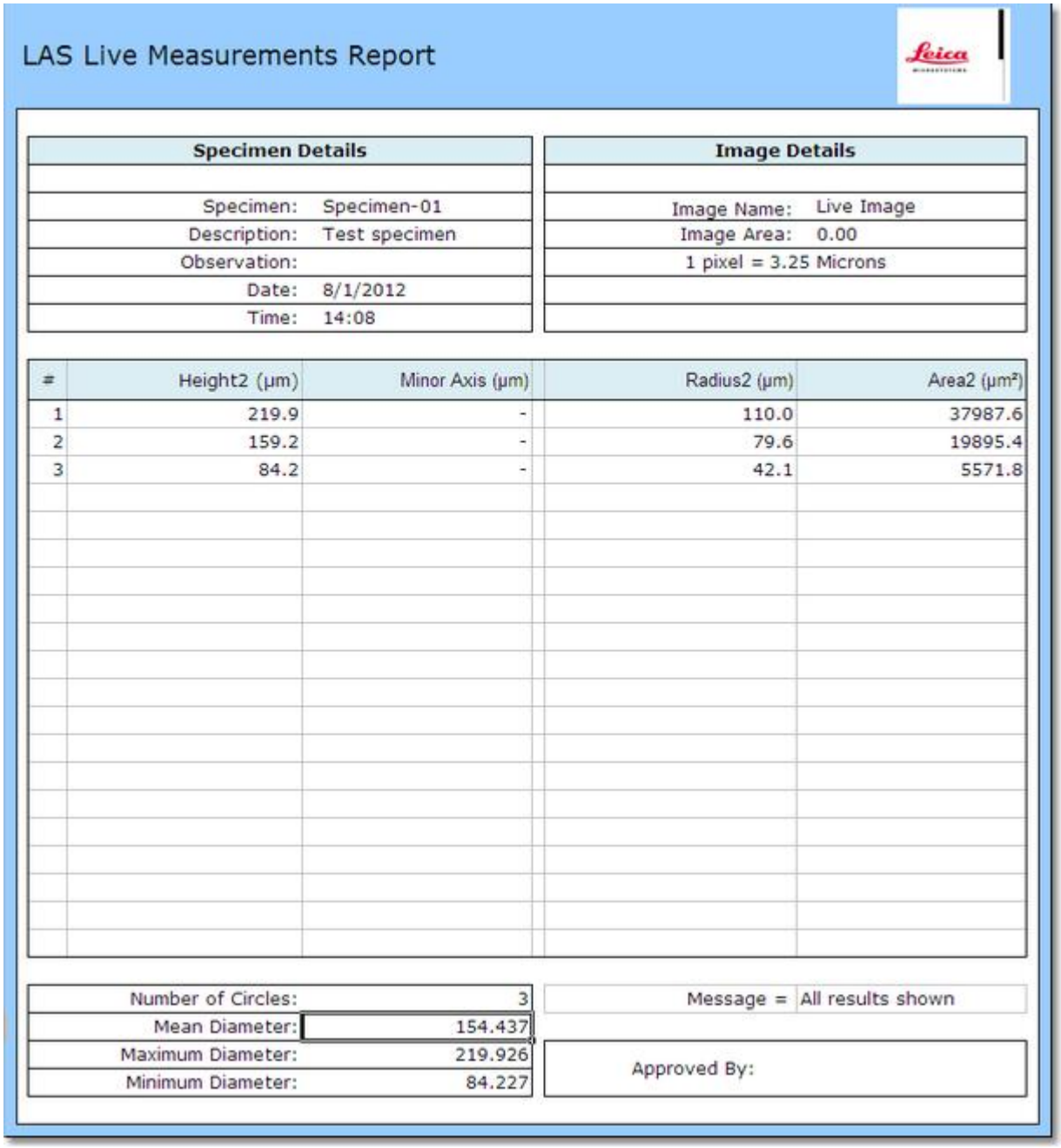
7: Click *Export* to create the report.



Two sample templates are installed with *Live Measurements:*

- *LAS Live Measurements Template - Circles.xlt:* designed to summarise the circle measurements but include any other measurements as details. Users should select both *Details* and *Summary* as *Content* options.
- *LAS Measurement Template.xlt:* A general purpose template for reporting all measurements as both *Summary* and *Detailed* data.

Both can be used as a ready-made templates but also as the basis for users to create their own specialised, company-styled templates.



LAS Live Measurements Report

Specimen Details	
Specimen:	Specimen-01
Description:	Test specimen
Observation:	
Date:	8/1/2012
Time:	14:08

Image Details	
Image Name:	Live Image
Image Area:	0.00
1 pixel = 3.25 Microns	

#	Height2 (µm)	Minor Axis (µm)	Radius2 (µm)	Area2 (µm²)
1	219.9	-	110.0	37987.6
2	159.2	-	79.6	19895.4
3	84.2	-	42.1	5571.8

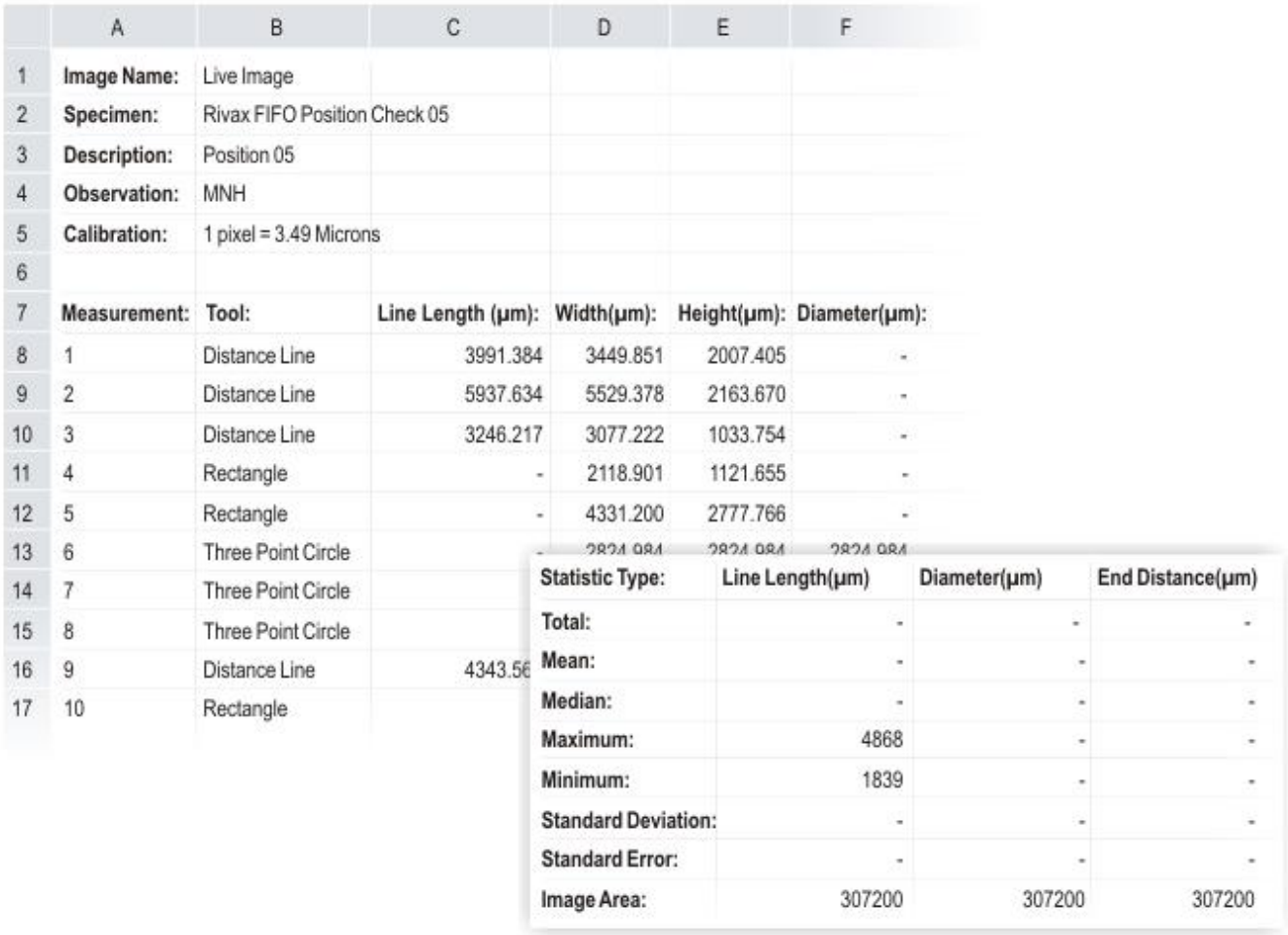
Number of Circles:	3
Mean Diameter:	154.437
Maximum Diameter:	219.926
Minimum Diameter:	84.227

Message = All results shown

Approved By:

A typical Excel report with *Details* and *Summary*. The report style depends upon the template chosen.

See the *Appendix*[1022] for the explanations of *Summary* parameters



	A	B	C	D	E	F
1	**Image Name:**	Live Image				
2	**Specimen:**	Rivax FIFO Position Check 05				
3	**Description:**	Position 05				
4	**Observation:**	MNH				
5	**Calibration:**	1 pixel = 3.49 Microns				
6						
7	**Measurement:**	**Tool:**	**Line Length (µm):**	**Width(µm):**	**Height(µm):**	**Diameter(µm):**
8	1	Distance Line	3991.384	3449.851	2007.405	-
9	2	Distance Line	5937.634	5529.378	2163.670	-
10	3	Distance Line	3246.217	3077.222	1033.754	-
11	4	Rectangle	-	2118.901	1121.655	-
12	5	Rectangle	-	4331.200	2777.766	-
13	6	Three Point Circle	-	2824.984	2824.984	2824.984
14	7	Three Point Circle				
15	8	Three Point Circle				
16	9	Distance Line	4343.56			
17	10	Rectangle				

Statistic Type:	**Line Length(µm)**	**Diameter(µm)**	**End Distance(µm)**
Total:	-	-	-
Mean:	-	-	-
Median:	-	-	-
Maximum:	4868	-	-
Minimum:	1839	-	-
Standard Deviation:	-	-	-
Standard Error:	-	-	-
Image Area:	307200	307200	307200

Excel Tags:

Excel reports can be styled to suit the user by using *Tags* that can determine the results and images being displayed. Tags are simple text strings that can be typed directly into an Excel cell or copied using the *Excel Macro* feature.

More information about *Excel Tags*[425].

The tags for *Live Measurements* have a specific format and are listed below. Click on the required tag for help in using it.

<LAS LM User Data>[435] Reference Data

<LAS LM Results>[436] Feature Results

<LAS LM Summary>[436] Feature Summary

Create a CSV Report

The method used for creating CSV Reports depends on which optional module you are using:

- Live Measurements[1015]
- Interactive Measurements[1016]

Live Measurements:

1: Click on the arrows to the right of the *Format* header to reveal the output options.

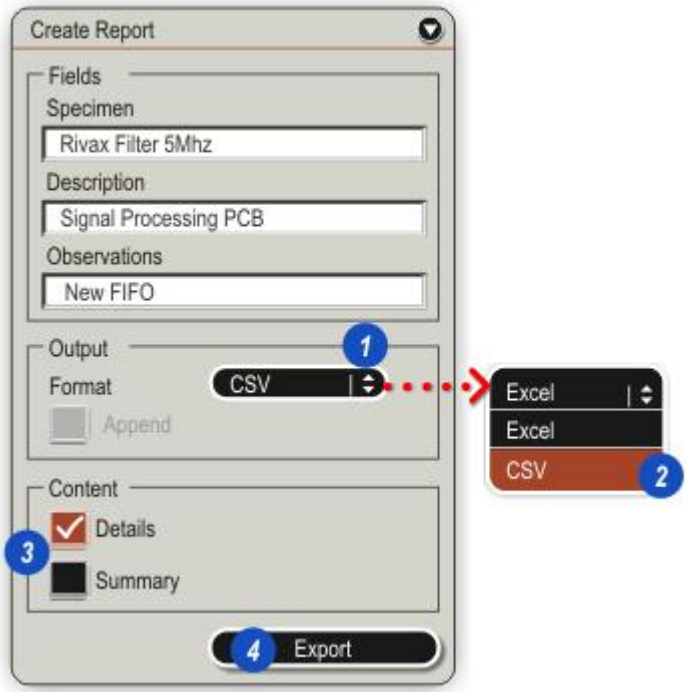
2: Select *CSV* from the drop down menu.

3: The report content can be:

- *Details:* The actual measurements, classes and results, or…
- *Summary:* The detailed analysis of the measurements

...but not both together.

4: Click the *Export* button.



1: With the *Attach to Record* check box enabled (tick mark showing), the report data is attached to the image as a separate file.

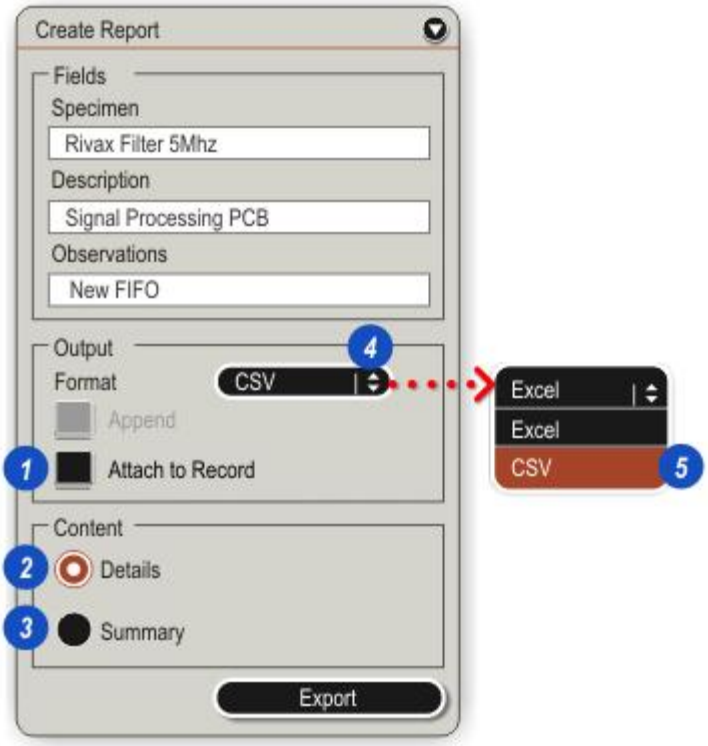
2 and 3: The report content can be:

- *Details:* The actual measurements, classes and results, or…
- *Summary:* The detailed analysis of the measurements

...but not both together.

4: Click on the arrows to the right of the *Format* header to reveal the output options.

5: Select *CSV* from the drop down menu.



Browse for or Name Report

When LAS is installed, a default location for reports is created at:

- *Users > UserName > Documents > LAS Reports >* .

On first use this will be the displayed path. Users can use this and simply change the report name or create their own folders elsewhere on the computer or network.

1: On the *Export* dialog the *Report* text box displays the last report path and name. This is convenient for overwriting or appending the report.

The *Template* and *Append* options are not available with CSV files.

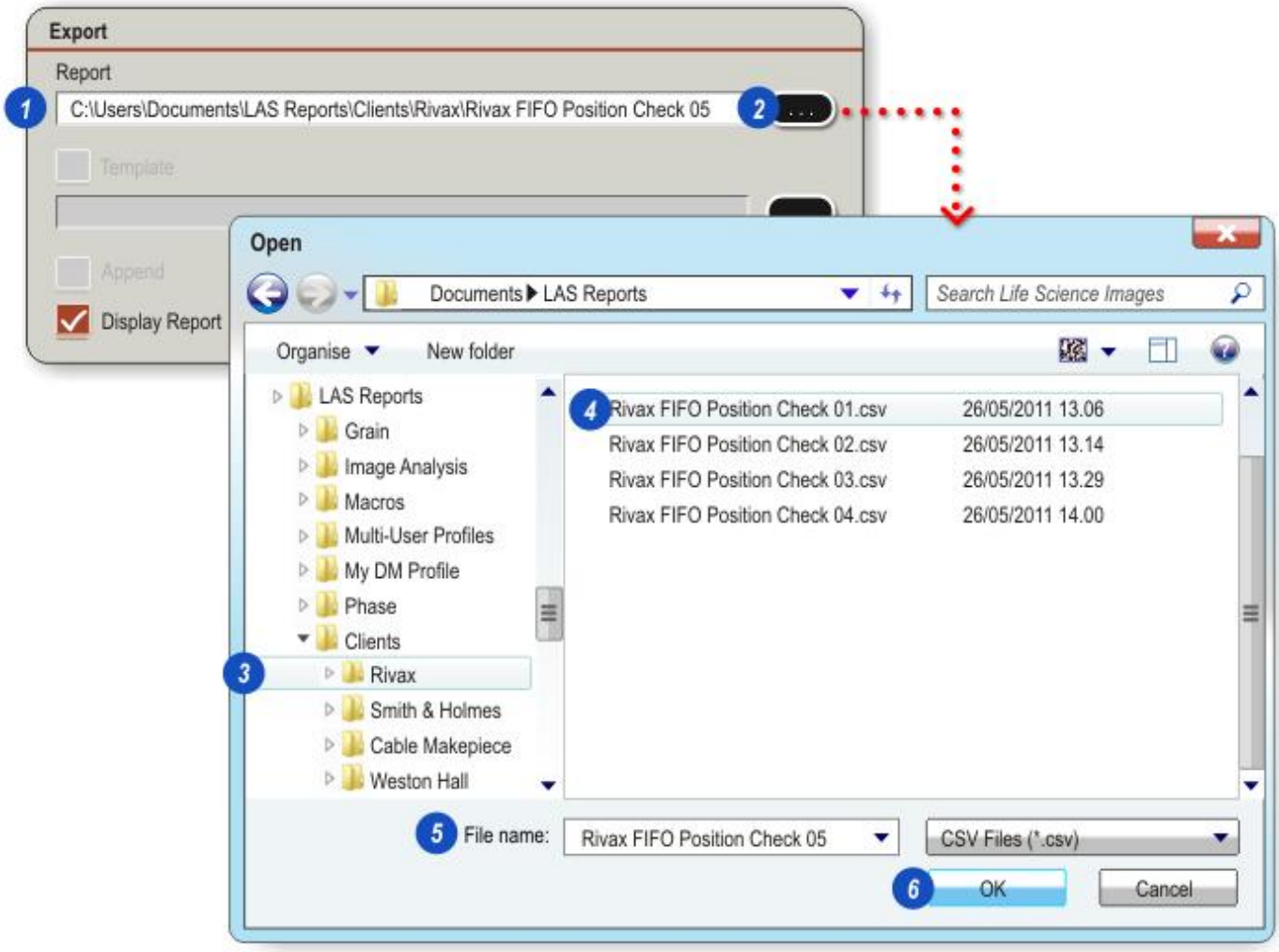
2: To create a new report, click the browse button and...

3: ...on the *Open* dialog navigate to the required folder,...

4: ...click to select and existing file to overwrite.

5: Alternatively, click inside the *File name* text box and type a name for a new report.

6: Click *OK*.

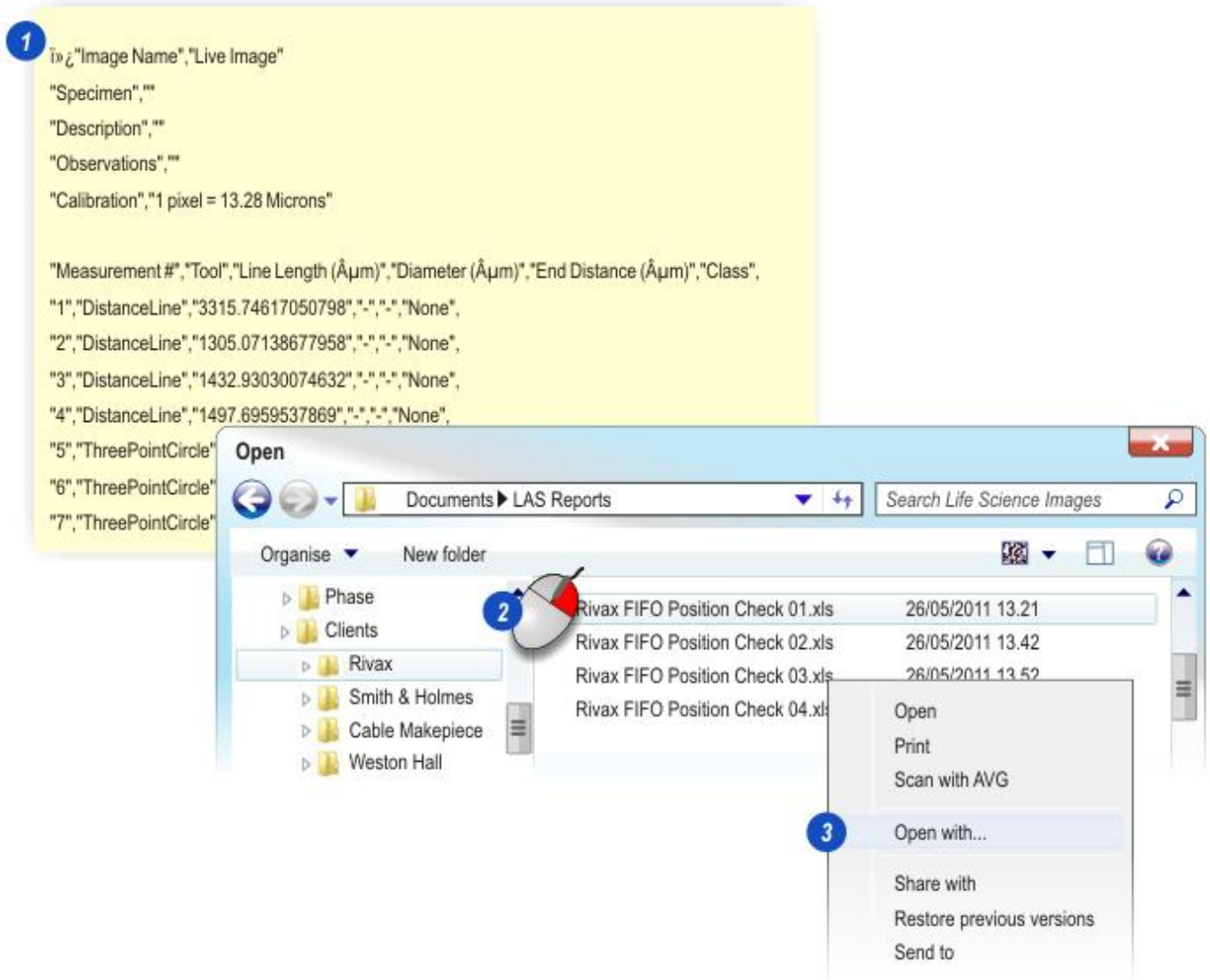


1: An example of a CSV files opened in *Microsoft Word* or *Wordpad*. Formatting is very limited but the values can be clearly seen. Some computer configurations will default to *Excel* to display CSV files but the column widths may need adjusting.

2: To select an alternative application to display CSV files, right-click on the file name and...

3: ...from the drop-down menu, click *Open with* and choose a program from the dialog.

See the *Appendix* [1022] for the explanations of *Summary* parameters.



Merging is the process of combining drawn measurements with an image so that they become a single entity.

- Process for Live Measurements[1020]
- Process for Interactive Measurements[1021]

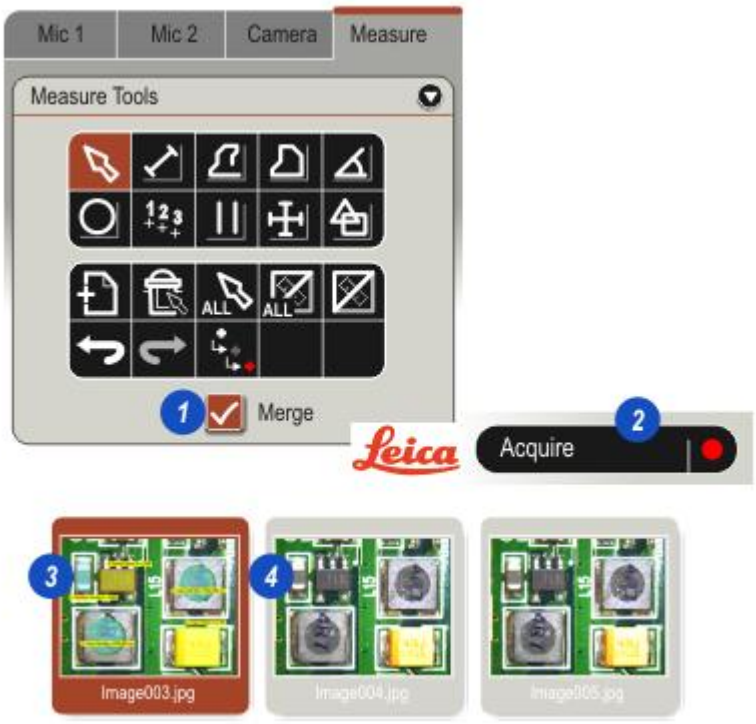
Measurements merged with an image cannot be edited and altered later.

1: Click to enable the *Merge* check box - tick mark visible.

2: Click on the *Acquire* button.

3: In *Browse Gallery* a thumbnail with the measurements merged and visible, and...

4: ...no measurements with *Merge* disabled.



Measurements merged with an image cannot be edited and altered later.

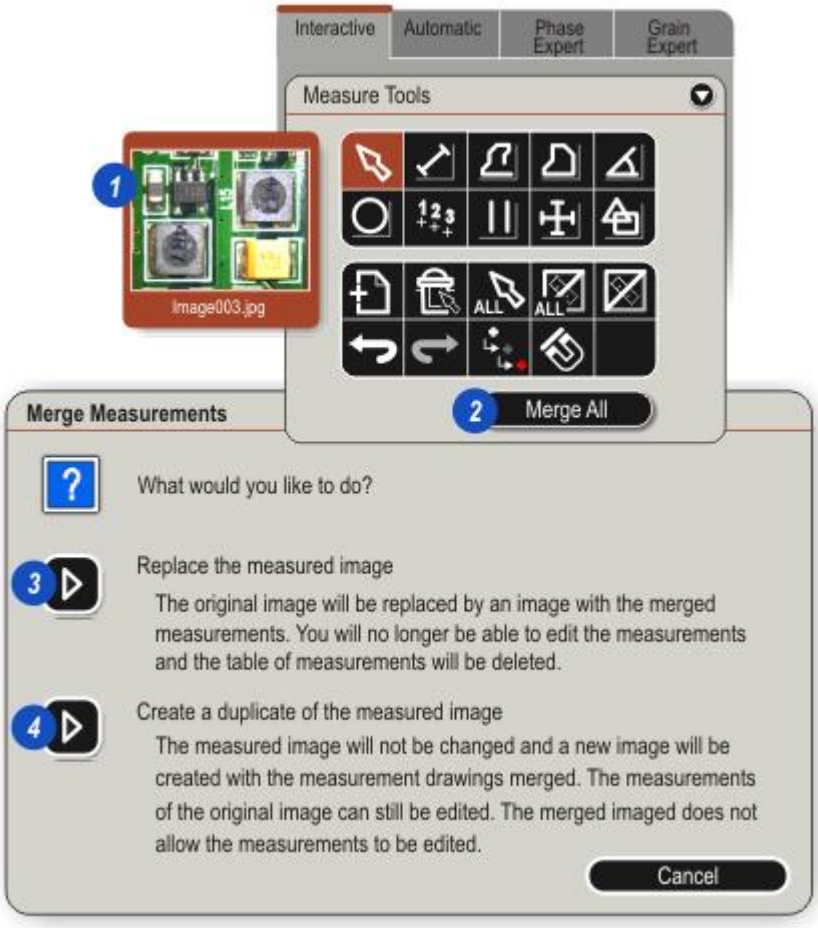
1: Select the image and measurements to be merged.

2: Click on the *Merge All* button.

On the *Merge Measurements* dialog the user has two options:

3: *Replace*: Overwrites the original image with the merged image.

4: *Create Duplicate:* Copies the original image and measurements and merges them. The original image remains intact.



Total: Is the total for all tools beneath that heading. For example, Total Line Length is the total measurement for all the tools - Distance and Line - that have length as a parameter.

Mean: Represents the Total divided by the number of measurements made (Total/Total Count).

Mode: The most frequently occurring parameter value from measurements using this parameter. If multiple modes exist, that with the lowest value is displayed.

Median: The actual mid-value in a list of values. For example 676 is the Median of 214, 676 and 1031. For an even numbered list of values, the values either side of the mid-point are averaged.

Maximum and Minimum: The largest and smallest measurements made regardless of the tool used.

Standard Deviation: A measure of the spread of a parameter values from the measurements using that parameter. It is based upon a random sample taken from the values.

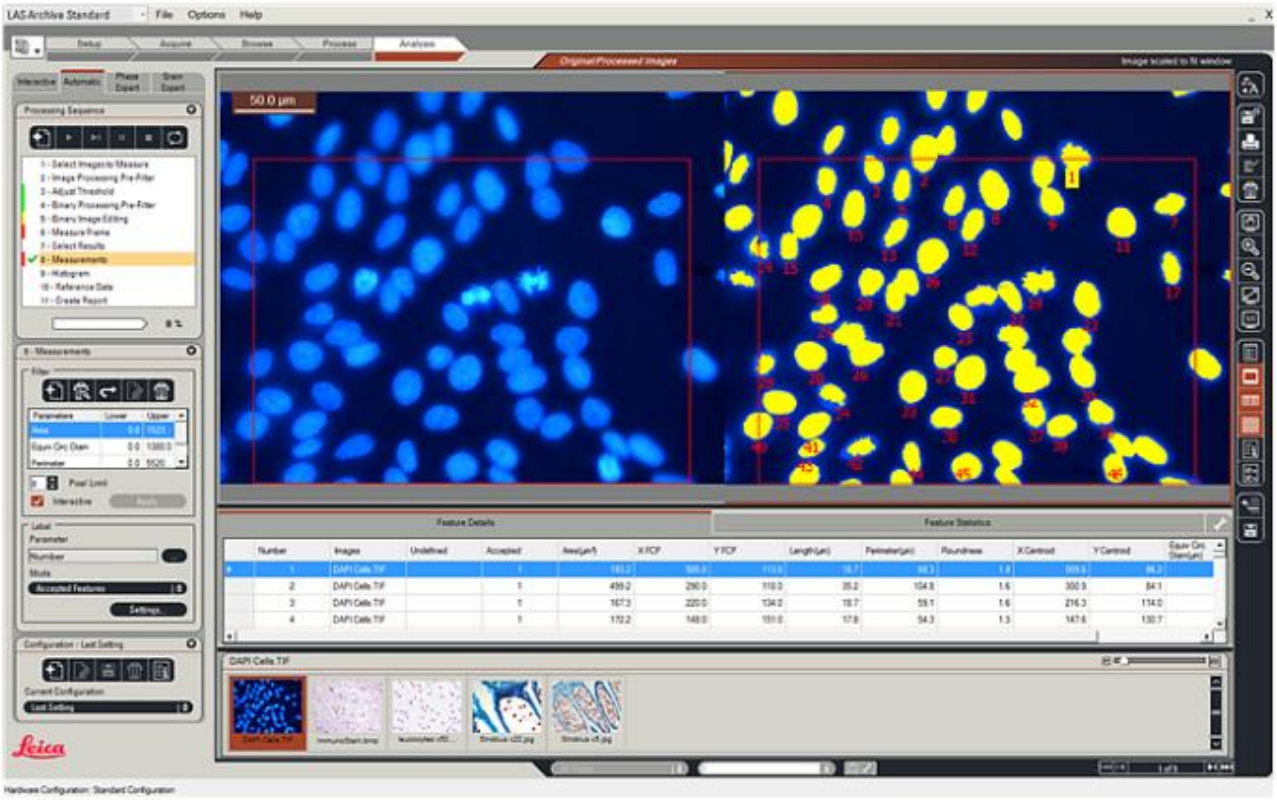
Standard Error: Uses the convention Standard Deviation/Square root(n) where 'n' is the number of measurements made or Total Count.

Confidence Interval Lower: The lower range of parameter values within which 95% of parameter values are likely to fall. It is based upon the assumption that the values are normally distributed with a mean of the same value as Mean.

Confidence Interval Upper: The upper range of parameter values within which 95% of parameter values are likely to fall. It is based upon the assumption that the values are normally distributed with a mean of the same value as Mean.

Total Count: The number of measurements made with this parameter.

Total Image Area: Is the area (selected units^2) of the entire image.



Leica Application Suite (LAS) *Image Analysis* is an optional software module for image processing and analysis in quantitative microscopy. LAS *Image Analysis* allows you to:

- Measure parameters (e.g. size, shape, position, orientation, intensity) for individual features (e.g. cells, fibres, nodules, particulates, pores)
- Measure the area percent, total area, perimeter and other parameters that are summed for the entire Field of View (e.g. bone sections, reflective minerals, tissue sections)
- Analyse multiple images and accumulate their data
- Show a list of selected parameters for all features measured
- Calculate a range of statistics
- Create histograms to display the distribution of sizes and shapes
- Store and display images in the *Gallery*
- Export data to Excel to create user-defined reports
- Use LAS Macro Editor functionality.

The combination of digital camera and microscope control makes *LAS Image Analysis* the best application for automatic measurement in a diverse range of imaging tasks, including:

- Analysis of the size distribution of porosity
- Characterisation of the shape of a population of features
- Measurement of particulate dimensions
- Counting powders from pharmaceutical preparations
- Analysis of fibre cross-section

Getting Started

This section explains how to start LAS Image Analysis, set up your workspace, and make some initial settings.

Starting LAS Image Analysis 1025

Setting up the Workspace 1026

Process Settings (Options) 1027

Starting LAS Image Analysis

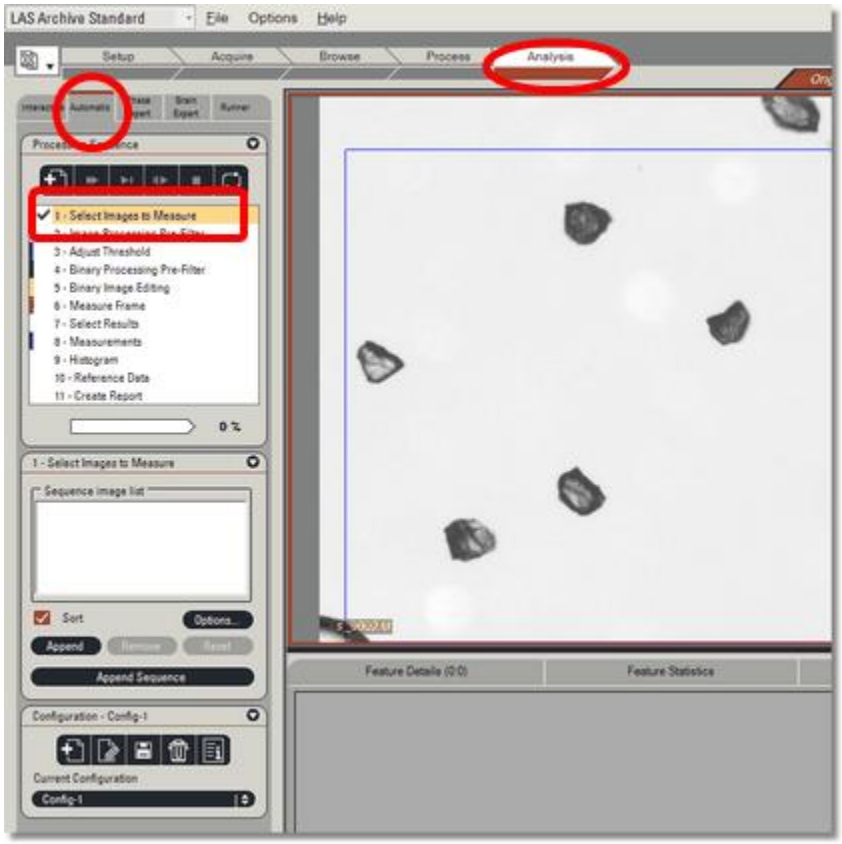
LAS Image Analysis is part of the *Analysis* Workflow.

To start using LAS Image Analysis:

1. Click on the *Analysis* Workflow.
2. Click on the *Automatic* tab.
3. Set up the workspace 1026 for optimum viewing. (You only need to do this once, the first time you start LAS Image Analysis.)
4. Click on the *Select Images to Measure* entry in the main *Processing Sequence* control panel.

You are now ready to add images to your processing sequence, and set up the measurement parameters. Take a look at the following topics:

- The User Interface 1029
- The Processing Sequence 1035
- Main Steps 1040



There are some application settings that will make your Image Analysis tasks easier:

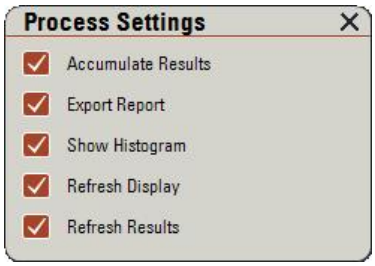
1. Display the *Navigator, Grid* and *Gallery*, by clicking on their icons in the *Side Tool Bar*[1031].

2. Click on the *Show Viewer Options* button on the *Side Tool Bar* and from the context menu, click to enable *Dual Viewer* – this displays the original *Input Image* on the left and the adjusted *Output Image* on the right of the *Viewer*. The other options are explained here[1031].

Process Settings (Options)

You can make some global application settings for *LAS Image Analysis*:

1. Click on the *Select Images to Measure* entry in the *Processing Sequence* panel.
2. Click on the *Options* button in the *Select Images to Measure* panel.
3. Set up the options on the *Process Settings* dialog.



The options are as follows:

- *Accumulate Results*: Produce a continuous list of results for all images.
  Disable this option to speed up processing if there is a large number of images in the sequence.
- *Export Report*: The results of the Processing Sequence will be loaded automatically to an Excel spreadsheet 1146 when processing is complete.
- *Show Histogram*: Display the Histogram 1127 during the Processing Sequence for each image (the *Show Histogram* option on the Histogram 1127 panel must be enabled).
- *Refresh Display*: Refresh the *Image Viewer* display between each image analysis.
- *Refresh Results*: Update the *Grid* results display for every image.

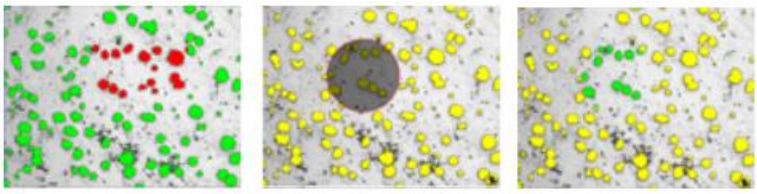
Image Analysis works on images that have already been captured or imported (in *Browse* or *Acquire)*; Image Analysis itself cannot capture original images. You can use individual images, or part of a sequence captured with another module (such as *MultiStep)*.

The image detail to be processed must be different in either contrast or colour from the background, and should be evenly illuminated (use shading correction).

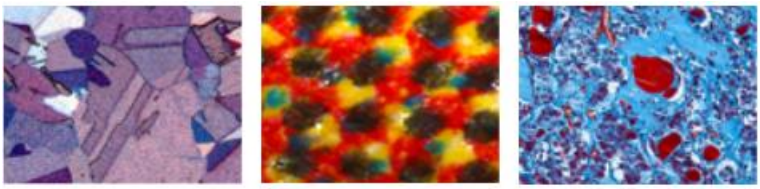
The way you use *LAS Image Analysis* depends on the type of image being processed and the features to be measured. Here are three possible usage scenarios:

- *Fast Track Automatic*: For clear, evenly illuminated images with defined features. No image processing is needed, just detect features, measure, and display results.

- *Fast Track with User Interaction*: Images need some work to define and isolate features of interest. Minimal image processing is required, but some artefacts need to be removed. Well defined features may be touching or overlapping.



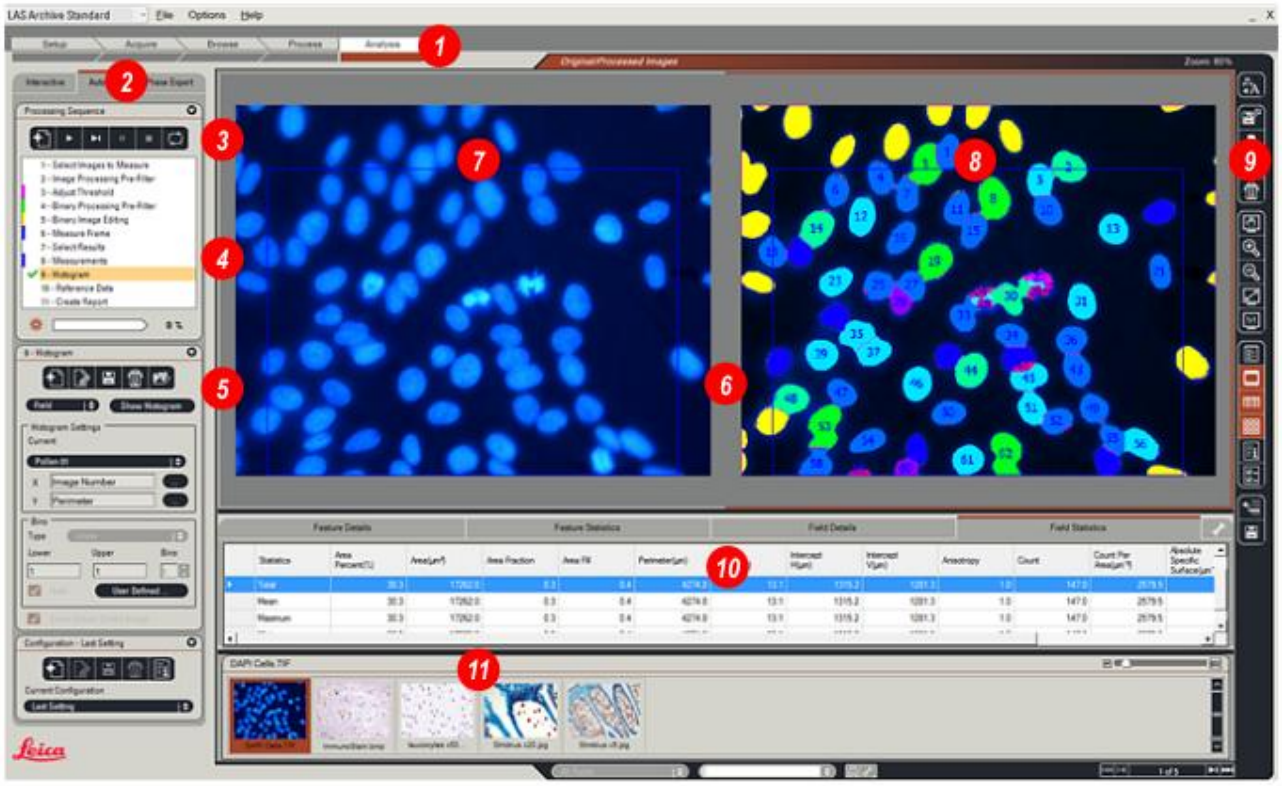
- *Advanced Measurements*: Images are highly detailed, but may exhibit problems such as lack of contrast or incorrect focus. Use the powerful Image Processing Pre-Filter[1048] and Binary Processing Pre-Filter[1081] tools to process detailed images with many features, noisy backgrounds and unwanted artefacts.



Use the Processing Sequence[1035] control panel to decide how to handle your different images.

The *LAS Image Analysis* user interface has the following main features:

1. *Analysis* Workflow: Launch area for LAS Image Analysis optional module.
2. *Automatic* tab: Contains the Image Analysis controls.
3. Automatic *Processing Sequence* tool bar[1037].
4. *Processing Sequence*[1035] menu: Click to select a step in the sequence.
5. Control panels[1030] (each Processing Sequence step has its own control panels).
6. *Viewer*: Can be split to show original and processed images.
7. Viewer (in split mode) can be locked so that both displays respond together to navigation and setup.
8. In this example a Measure Frame created on the original is duplicated automatically on the processed image.
9. *Side Tool Bar*[1031].
10. *Grid*[1033]: Displays measurement results.
11. *Gallery*[1034]: Displays image thumbnails.



Each step in the Processing Sequence[1035] has its own control panel.

- Collapse and expand control panels by clicking on the arrow to the right of the header.
- To float (undock) a control panel, drag its header to a new position on the screen.
- To restore a control panel to its original docked position, click on the 'X' to the right of the panel header.
- Many panels feature drop-down menus.
- Some panels have dedicated Tool Bars; Hover the cursor above a tool button to display a tool tip. For example, here is the *Configuration*[1041] control panel, which is displayed during every Image Analysis step:

The *Side Tool Bar* is situated on the right-hand side of the *Viewer*. Buttons are grouped according to function:

Viewer primary controls

 Show/Hide Pan window

 Zoom in and zoom out

 Fit the image to Viewer

 Display the Image at actual size

Image source and data controls

 Display Data Form

 Hide/Show Image

 Hide/Show Results Grid: Only available if an LAS Archive is installed

 Hide/Show the thumbnail *Gallery*

 View all details: Displays *Record Details* for the current image.

 Select details to display: Displays the *Select Visible Fields* dialog, which allows you to specify which fields are populated in the Record Details window when you click *View all details*.

Viewer options

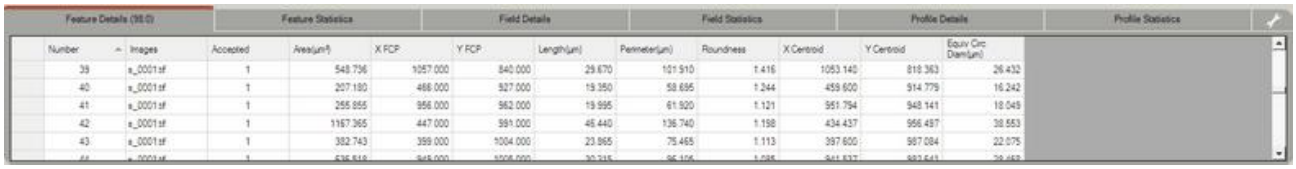
Click the *Show Viewer Options* tool on the *Side Tool Bar* to display a drop-down menu with the following options:

- *Dual Viewer*: When enabled, the Viewer is split into two panes with the original image on the left and the processed image on the right. When this option is disabled, use the Original/ Processed option to select either the original image or the processed image.
- *Original/Result from previous step*: Displays the processed image from the previous operation on the left. Click again to return to the work. Use this option to quickly compare processing steps.
- *Original/Processed*: Toggles between the original image and the processed image when the Dual Viewer is disabled.
- *Show Background Image*: Hides (masks) or reveals the underlying processed image to show only those areas that have been modified. Hiding the background image displays only the masked pixels.
- *Show Binary Masks*: Highlights the processed areas (those falling within the threshold values) in your chosen colour (the default is red). Use this option to quickly show the effects of binary editing. You can select different highlight colours for the following steps: Adjust Threshold, Binary Processing Pre-Filter, Binary Image Editing and Measure Frame.
- *Lock View*: If you *Zoom* in sufficiently on the Viewer, scroll bars are automatically displayed. Enable *Lock View* to automatically synchronise both displays in Dual Viewer mode: scrolling in one view is reflected in the other view.

(Continued on next page)

Saving the output image

Save output image in current location: Saves the results image, including all masks currently displayed. A thumbnail appears in the *Gallery* and the image can be used at a later date for documentation.



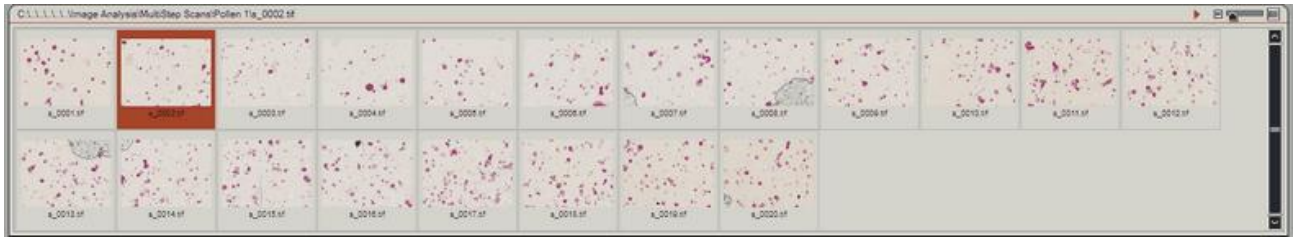
Click the Show grid tool on the Side Tool Bar[1031] to display results on the screen.

- *Grid* column headings are determined by the range of results chosen in Select Results[1108] – either a *Predefined* range or *All Measurements*.
- Tab visibility (e.g. Feature Details and Field Statistics) is defined in Select Results[1108].
- Click on an entry in the *Grid* to highlight the feature on the image; the label will be shown in a contrasting colour.
- Click on a feature on the *Binary Output Image* to highlight the results for that feature in the *Grid*. If an image sequence is being measured, the appropriate image will be automatically displayed.
- Ctrl-click on rows to make multiple selections. Shift-click to make a contiguous selection.
- Press the Delete key to remove the currently selected features from the Grid and measurement results; they will be coloured as not included. Reinstate features in Binary Editing using the Keep[1100] tool.
- Click on the *Configure* tool (the white spanner icon at the right-hand end of the the *Grid* header) to filter the results displayed on the current Grid tab. For example, if the *Feature Details* tab is visible, the *Select Feature Details* dialog will be displayed.

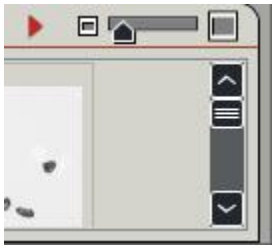
  Click to enable the *Visible* check box for each item you want to display on the *Results Grid*. You can also click on *Show All* or *Hide All*.

- To change the sort order of a column in the *Grid*, hover the pointer over a column header and click the small arrow that appears.
- Drag the vertical line between two column headers to change column width.
- Use standard Windows control keys to copy data from the *Grid*:
  - Ctrl + C: Copy selected items to the clipboard.
  - Ctrl + A: Select all the grid data.

-  Click the *Show gallery* tool on the *Side Tool Bar* 1031 to display the *Gallery*.



- Drag the slider (top right of the *Gallery*) to change the thumbnail size.



- Drag the horizontal bar at the top of the *Gallery* to change the number of thumbnails displayed.
- Use the scroll bar and arrows to scroll through the thumbnails.

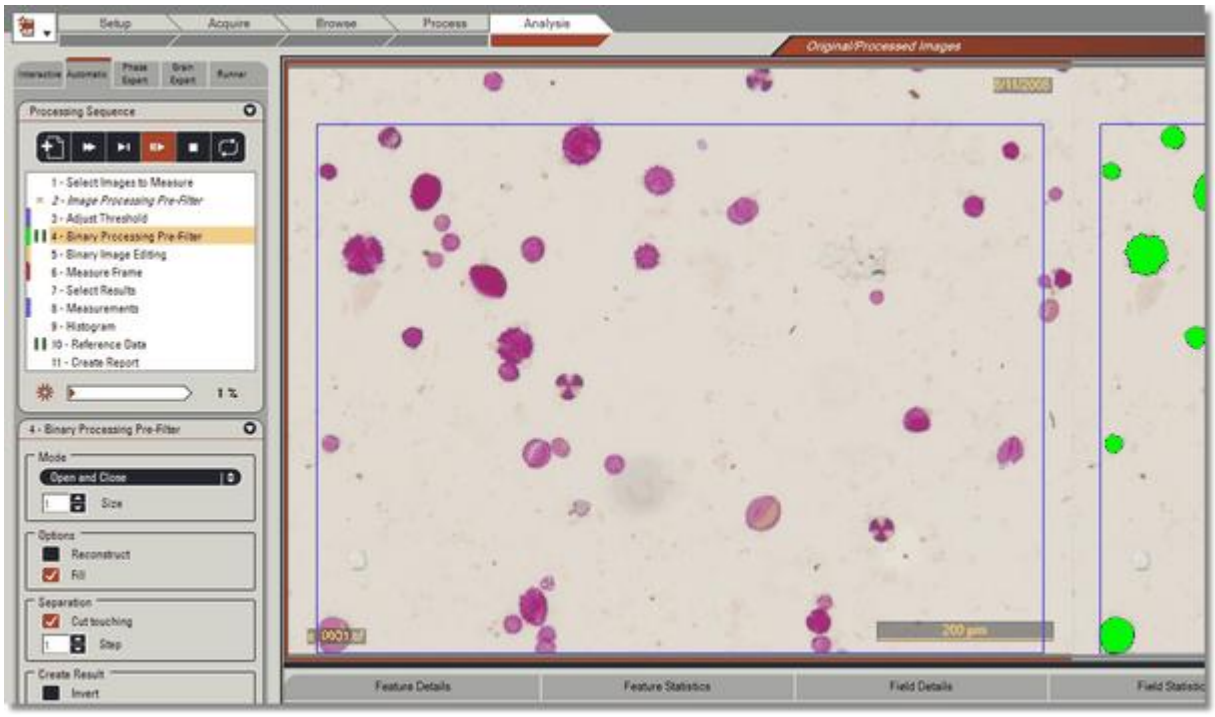
The *Processing Sequence* applies your chosen tools and settings automatically to groups or sequences of images.

- The coloured bars next to each item indicate the colour used to highlight different features in the Viewer (colour is specified on the appropriate control panel).
- Processing progress is indicated by the rotating 'star' and the Progress Bar.

LAS *Image Analysis* is designed to make each step as simple as possible. The *Processing Sequence* control panel guides you through the acquisition, detection and measurement steps; once defined, you can use these steps again and again.

1. Work through the analysis steps, in order, on a single image (you can click on a step in the *Processing Sequence* control panel, or click the *Next Measurement Step* button in the Toolbar[1037]).

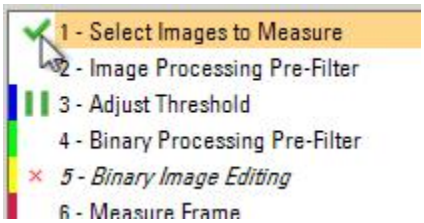
   The settings for each step are remembered by LAS Image Analysis when you subsequently run the sequence (note that some images in a sequence may require different settings[1039]).
2. If necessary, you can program *LAS Image Analysis* to pause at any step in the processing sequence, so that you can refine an image – to delete or separate features for example – before continuing.
3. Optionally, choose to generate and display comprehensive report when processing is complete.
4. Save the Processing Sequence as a *Configuration*[1041], so that you can run it again at a later date.
5. Automatically process all the images using the saved settings.



To start a new Processing Sequence:

1. Click the *Start a New Measurement* button. Note that this will delete all existing measurements.

2. Click *Yes* to confirm.
3. Click *Select Images to Measure* (step 1 in the *Processing Sequence* panel).
4. Append all the images that you want to process so they appear in the Sequence image list. See Select Images to Measure 1043.
5. Select a typical image from the *Sequence Image List*. (You can select from the *Gallery*, but remember that the Gallery might contain images that you have not added to the sequence.)
6. Apply the processing tools to the image (steps 2 to 5 in the *Processing Sequence* panel). As you choose settings, *LAS Image Analysis* keeps track of them displaying a green tick mark to the left of the name to indicate normal processing with the current settings.



7. Configure the other steps in the sequence (steps 6 to 11 in the *Processing Sequence* panel).
8. If required, Pause or Skip 1038 one or more steps in the sequence.
9. Save the sequence settings as a Configuration 1041.

 *Start a New Measurement*: Clear all existing sequence information.

 *Start Running Measurement Steps*: Start the current sequence.

 *Next Measurement Step*: Stop the automatic sequence so that you can proceed one step at a time. Click the *Run* button to resume automatic processing.

 *Continue from Present Measurement Step*: Resume the automated sequence (process all images using the current settings).

 *Stop running Measurement Step*: Interrupt the current sequence (without processing all the files). Click *Yes* to confirm.

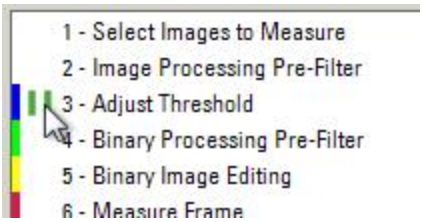
 *Update*: Refresh the processing sequence if you make any changes to individual images.

Pausing the sequence

Some images may require specific additional processing – for instance, you may need to remove artefacts on an image-by-image basis using the *Binary Edit* tool.

To pause an automatic image processing sequence at the same step for every image:

1. Click to the left of the step name in the *Processing Sequence* control panel to display the green pause symbol (each time you click, the status toggles between Pause, Skip and Normal).



2. Run the measurement sequence (click the *Start Running Measurement Steps* button in the toolbar, or click the *Run Measurement* button at the bottom of the *Automatic* tab).

3. The sequence will pause at this step for the first image, allowing you to make changes.
4. Resume the sequence (click the *Continue from Current Measurement Step* button in the toolbar, or click the *Continue* button at the bottom of the *Automatic* tab).

5. Repeat steps 3 and 4 until the measurement sequence is complete.

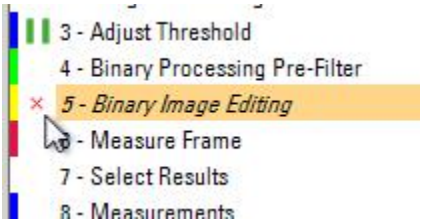
Skipping a step

You can skip any of the following steps:

- Adjust Threshold
- Binary Processing Pre-Filter
- Binary Image Editing
- Reference Data

To skip a step:

1. Click to the left of the step name in the Processing Sequence control panel to display the red Stop (cross) symbol; each time you click, the status toggles between Pause, Skip and Normal. The step text will be shown in italic.



2. Run the measurement sequence (click the *Start Running Measurement Steps* button in the toolbar, or click the *Run Measurement* button at the bottom of the *Automatic* tab).

You might need to apply different filters or settings to images in a sequence or group.

For example, consider a sequence containing images A, B, C and D.

1. Select image A and set up the filters and parameters.
2. Run the sequence. At this stage, the settings you made to image A will apply to *all* images in the sequence.
3. Now select image B and modify a setting (e.g. the Threshold).
4. Click the *Update* button to apply the changes to the Processing Sequence.
5. Run the sequence again.
   In this example, only the changed Threshold will apply to image B; all other settings (for all images in the sequence) are as defined for image A.

Note: If you need to change a setting for *every* image in a sequence, it is better to Pause[1038] the processing sequence at this step.

Working with other images

Sometimes you might want to work on an image in the *Gallery* that is *not* part of the *Sequence List*. LAS Image Analysis allows you to do this, without affecting the Processing Sequence settings.

Main Steps

This section describes the major components and tools of the LAS *Image Analysis* module. Generally, you follow the order of steps in the Processing Sequence panel, but you can jump between steps without losing settings.

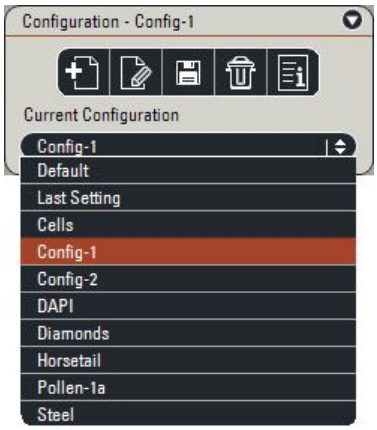
- *Select Images to Measure* 1043*:* Choose the image folders, groups and sequences
- *Image Processing Pre-Filter* 1048*:* Improve and clarify features to measure
- *Adjust Threshold* 1073*:* Isolate features and create binary output image prior to measurement
- *Binary Processing Pre-Filter* 1081*:* Fill holes and fissures in features
- *Binary Image Editing* 1095*:* Add, remove, select and deselect features, draw and fill shapes, and group objects
- *Measure Frame* 1103*:* Create a frame to contain the features to be measured
- *Select Results* 1108*:* Select and configure the results to display in the *Grid* and report
- *Measurements* 1109*:* Count and measure the selected features
- *Histogram* 1127*:* Display the measurement results graphically
- *Reference Data* 1140*:* Information to be displayed on the *Report*
- *Create Report* 1146*:* Configure, display and print the *Report* data and images.

The *Configurations* panel is available at the bottom of the *Automatic* tab, during every step of the LAS Image Analysis process. It allows you to save the current settings for most tools and panels as a *Configuration*. You can then use the same measurement settings for different image sequences.

Selecting an existing Configuration

To select an existing (saved) Configuration:

1. Display the *Current Configuration* drop-down menu.

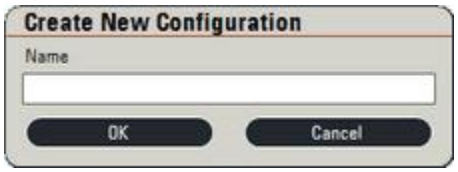


2. Click to select a named Configuration.

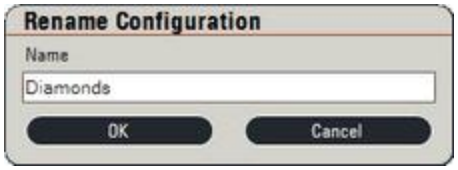
(Continued on next page)

Managing configurations with the toolbar

- *Create New Configuration*
  Saves all the current settings to a Configuration with your chosen name. Click to display the *Create* dialog, enter a name, and click *OK*.



- *Rename Selected Configuration*
  Overwrites the existing Configuration name. Click to display the *Rename* dialog, enter a new name, and click *OK*.



- *Save Selected Configuration*
  Saves any changes you have made to the current Configuration (using the same name).
- *Delete Selected Configuration*
  Removes the current Configuration from the drop-down list. Click to delete, and click *Yes* to confirm the action.
- *Display Selected Configuration*
  Saves the current Configuration settings into an Excel file. If Excel is installed on your machine, the file will be displayed on screen. **Note**: This is for information only; If you make changes to the Excel file and save it, these changes are not reflected in LAS Image Analysis.

Select Images to Measure

Before you can start making measurements, you need to select the images to be used in your Processing Sequence (with *Image Explorer,* or *LAS Archives,* if installed).

- LAS Archive or Image Explorer? 1044
- Browse for images 1045
- Loading Sequences and Random Images 1046
- Sorting and Removing Images 1047

LAS Archive or Image Explorer?

To specify whether to use LAS Image Explorer or an LAS Archive:

1. Click on the *Setup* Workflow tab.
2. Display the *Archive* tab to reveal the control panels.
3. Click on the appropriate button (*LAS Image Explorer* or *Folder Archive*).
4. If you selected *Folder Archive*, double-click the required archive entry to make it active.

Browse for images 1045

Browse for Images

Once you have decided which type of image archive to use, you can browse for the images that you want to measure. The simplest way is to use the *Navigator*; this saves you time, since you can use it directly from the *Analysis* Workflow, rather than having to switch between the *Browse* and *Analysis* Workflows:

1. Select the *Analysis* Workflow and display the *Automatic* tab.
   (Note: At this point, the *Viewer* will display the last image that was last selected in the *Browse* Workflow.)
2. Click on the *Select Images to Measure* step in the *Processing Sequence* panel.
3. If they are not already visible, display the *Navigator* and the *Gallery*, by clicking on their icons in the *Side Tool Bar*.

4. In the *Navigator*, click to select the folder containing the images you want to measure.
5. If there is more than one image in the folder, the first image will be displayed in the *Viewer* and highlighted in the *Gallery*.
6. Click in the *Gallery* to select the image you want to measure.
7. Click *Append* in the *Select Images to Measure* panel.

Loading Sequences and Random Images

Before you can automatically process an image sequence or a collection of individual images, you need to add them to the Sequence image list.

Loading a complete image sequence

If the images to be processed form part of a sequence:

1. Click on an image thumbnail in the *Gallery*.
2. In the *Select Images* panel, click the *Append* button. LAS *Image Analysis* will recognise that the image is part of a sequence.
3. Click the *Append Sequence* button.

All the sequence images will be automatically added to the *Sequence image list*.

Loading a selection of images

To load a selection of images from the *Gallery*:

1. Use the standard Windows methods for selecting items from a list: Ctrl-click and Shift-click on image thumbnails in the *Gallery*.
2. Click the *Append* button; all the selected images will appear in the *Sequence image list*.

See also:

Sorting and Removing Images 1047

Process Settings (Options) 1027

Creating a Process Sequence 1036

To remove a single image from the Sequence image list

1. Click to select the image.
2. Click the *Remove* button.

To remove all images from the Sequence image list

1. Click the *Reset* button.

To sort the images in numerical order

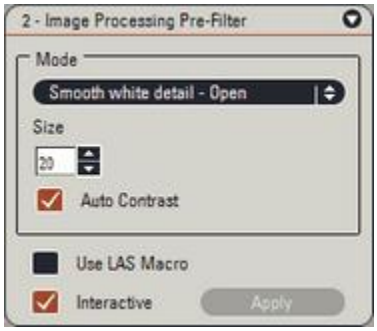
1. Enable the *Sort* check box.

Image Processing Pre-Filter

The Image Processing Pre-Filter allows you to enhance feature recognition in colour and monochrome images.

When Greyscale Pre-Filters are applied to an image, the original image is not affected at all – it remains intact on the hard drive. Applying a filter creates a Processed Greyscale Output Image in memory which is then passed on to other functions (such as Adjust Threshold[1073]) for additional processing.

Although the processed Greyscale Output Image is created in memory, you can save it to disk[1031] and export it to another application.



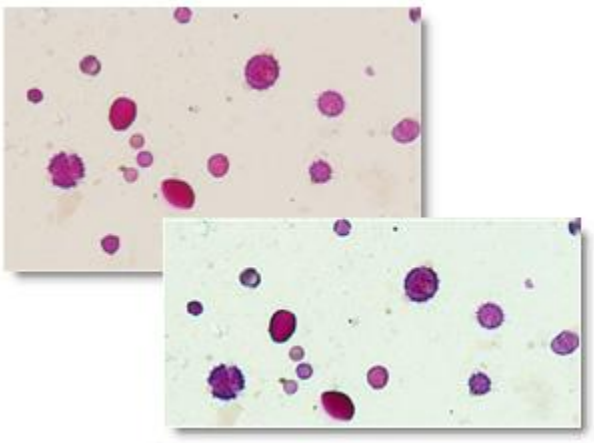
To use the *Image Processing Pre-Filter*:

1. Click on the *Image Processing Pre-Filter* option in the *Processing Sequence* panel.
2. Select a pre-filter Mode[1050] from the drop-down menu.
3. Set the structuring element matrix using the *Size* text box. Small increments are preferable, because there will be a point at which the image cannot be improved by making another 'pass'.
4. Decide whether to use Auto Contrast[1049].
5. Decide whether to use *LAS Macro Runner. See here for details.*
6. Decide whether pre-filtering results should be displayed interactively:
   - *Interactive* enabled: Each change in the settings is automatically reflected in the Output Image. This is the best setting for simple images.
   - *Interactive* disabled: Changes are only updated when you click *Apply*. This is better for more complex images that may take a little longer to process.

Experimentation is an inevitable part of improving image definition. These examples[1061] demonstrate the power of the Image Processing toolkit – use them to decide which tools to use.

Auto Contrast

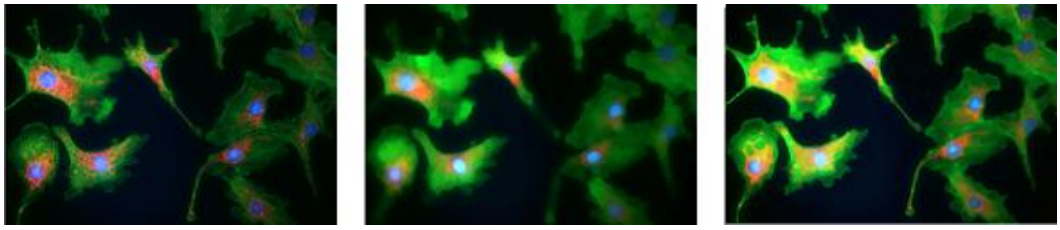
Enable *Auto Contrast* to improve the contrast between features and background. The examples below show an image before and after Auto Contrast is applied:



The Pre-Filters available using the *Mode* drop-down menu are as follows:

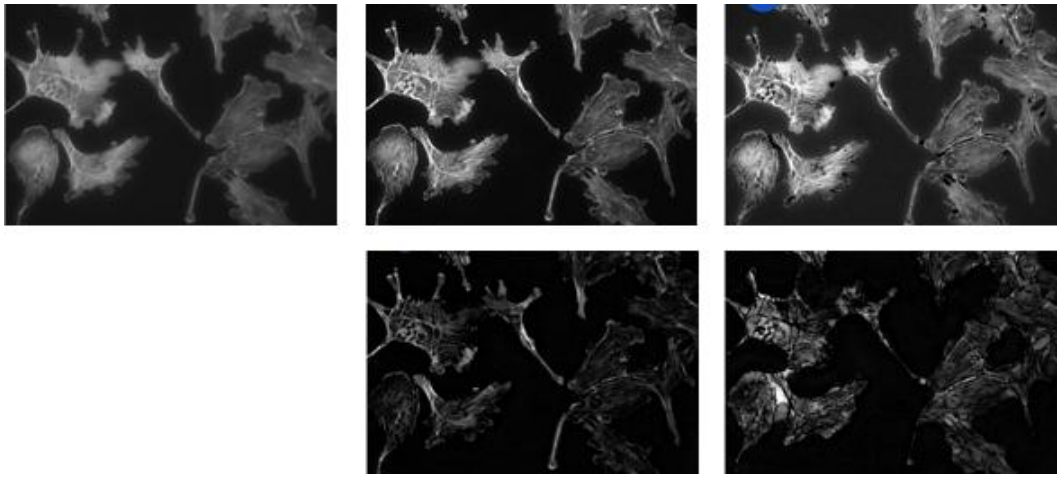
- *Smooth White Detail* brightens the Output Image by accentuating the lighter tones. This is an open filter – Erosion followed by Dilation.
- *Smooth Black Detail* darkens the Output Image by strengthening the darker tones. This is a close filter – Dilation followed by Erosion.

  The illustration below shows original, *Smooth White* and *Smooth Black* images:



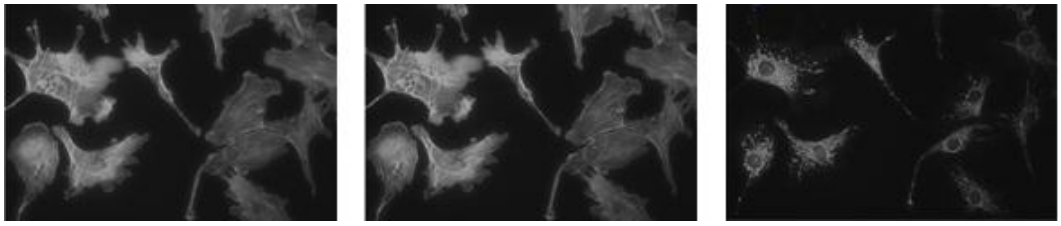
- *Enhance White Detail* accentuates the white – values approaching 255 – areas of the image.
- *Enhance Black Detail* improves the contrast between the black (values toward 0) and the lighter areas of the image.
- *Find White Detail Top Hat* detects and removes lighter detail from the image depending upon the size of the Structuring Element.
- *Find Black Detail Top Hat* detects and removes darker detail from the image depending upon the size of the Structuring Element.

  The illustration below shows original, *Enhance White* and *Enhance Black* images, then *Find White* and *Find Black* top hat images:



- *Sharpen Grey Transitions (Delineate)* removes intermediate grey tones to provide greater contrast between areas of the feature.
- *Noise Removal (Median)* filter removes artefacts down to pixel size from the image.

  The illustration below shows original, *Sharpen Grey* and *Noise Removal* images:

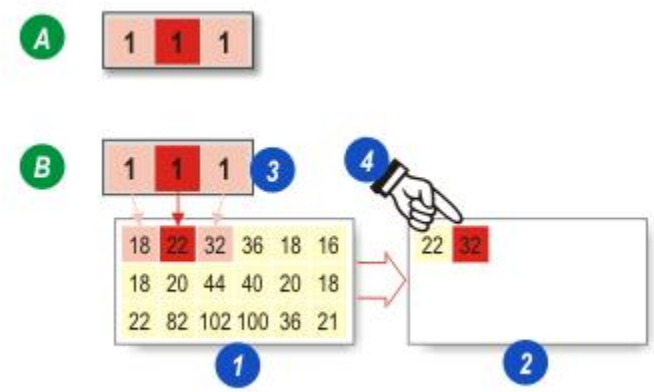


At the heart of most image processing is a concept called a Structuring Element, shown in its simplest form in Illustration (**A**). It comprises a matrix of cells, each of which will be a binary value – either 1 or 0. The centre (red) cell is called the Origin.

In Illustration (**B**):

1. The original *Greyscale Input Image,* with each pixel represented as a value in the range 0 to 255 - black to white.
2. **T**he *Output Image* which will be created using values determined by the Input Image and the *Structuring Element.*
3. The Structuring Element is used as an 'overlay' with the *Origin (red) Cell* positioned over a pixel on the Input Image. This is also coloured red and called the *Input Pixel.*

   Those pixels either side of the *Input Pixel* are called *Neighbours*, shaded pink.
4. The value of the *Neighbour* pixels are used to create a new *Output Pixel* in the Output Image.



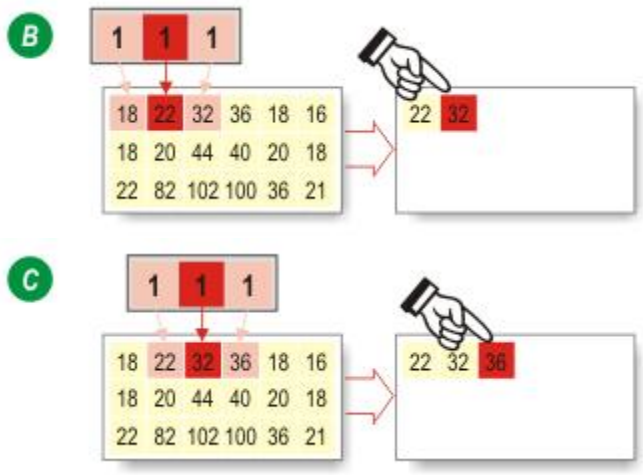
A *Neighbour* pixel is only tested if the corresponding cell in the *Structuring Element* is set (=1). In the example, both cells in the *Structuring Element* are set so the *Neighbour* pixels either side of the *Input Pixel* will be evaluated.

Brightening detail (Dilation):

In this process, the *Neighbour* pixel with the *greatest* value determines the value of the *Output* pixel. Since, in a greyscale image, the lighter pixels have the greatest values (closer to white at 255), the effect is to increase the area of lighter detail and reduce that of darker detail.

In the Illustration **(B)** the *Structuring Element Origin* (red) is positioned above an *Input Pixel* with the value 22. The *Neighbour* pixels values are 18 and 32. The intention is to brighten the image and so the *Neighbour* pixel with the *greatest* value is used for the new *Output Pixel* – in this case 32.

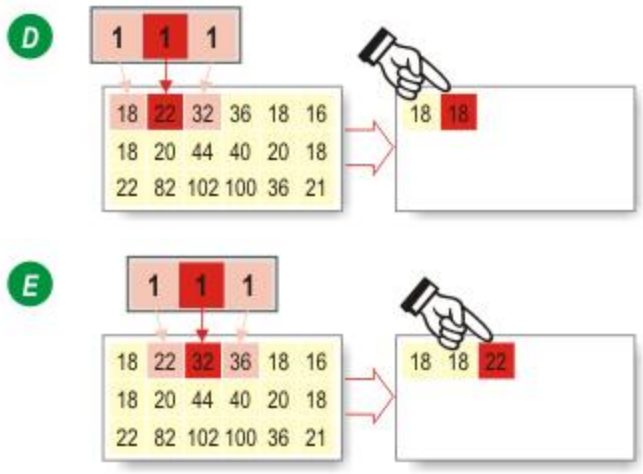
In Illustration **(C)** the *Structuring Element* has moved right to the next *Input Pixel*. Now the values in the *Neighbour* pixels are 22 and 36 and again the *greatest* is used for the *Output Pixel*.



Darkening detail (Erosion):

To darken the image the same evaluation process is used but in this process the *Neighbour* pixel with the *lowest* value (closer to black at 0) is used as the *Output Pixel*. Illustrations **(D)** and **(E)** show the process.

All of the greyscale *Input Image* pixels are tested until the *Output Image* is complete.



A simple example

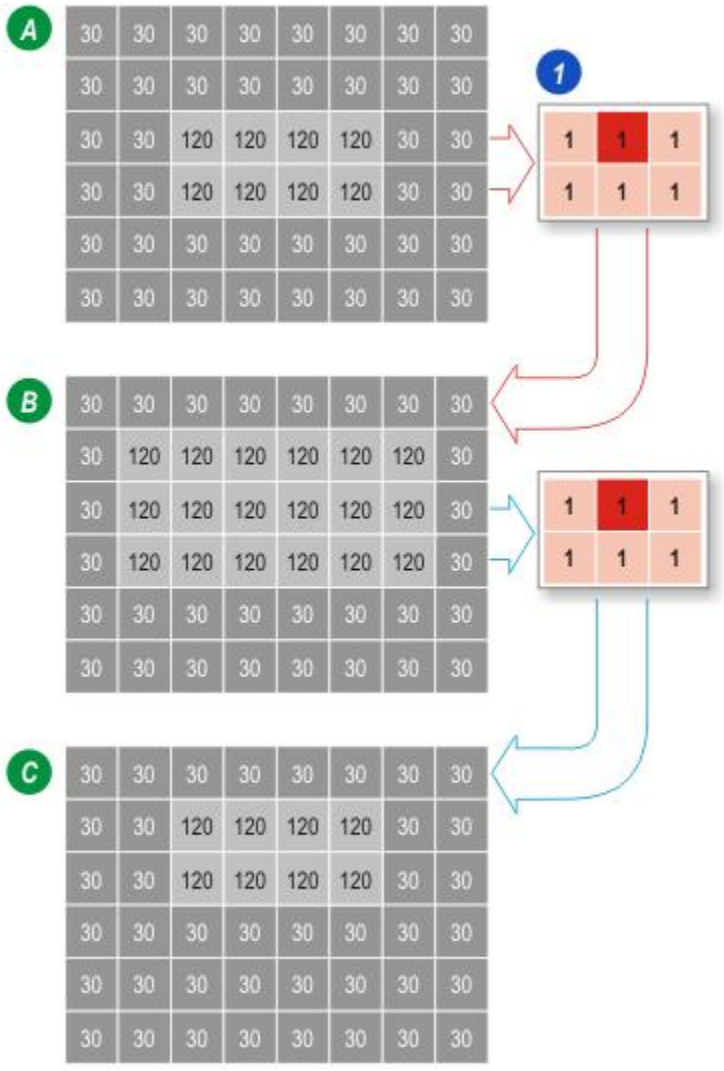
Illustration **(A)** is a greyscale image – a small block of pale grey pixels each with a value of 120, surrounded by dark grey pixels with a value of 30.

A 2 x 3 *Structuring Element* **(1)** is to be applied to the image, first as a *Dilation* and then as an *Erosion*.

Dilation uses the *Neighbour* pixels with the *greatest* values so in Illustration **(B)** the pale grey block has 'grown' adding pixels to the top and sides.

The process is reversed by *Erosion* in Illustration **(C).** *Erosion* uses the *smallest* values found in the *Neighbour* pixels so the pale grey pixels (with a value of 120 closer to white) have been replaced by dark grey pixels (with a value of 30 closer to black).

The image shift – the pale grey block has moved upward by a row of pixels – is due to the design of the *Structuring Element*, and can be prevented by using a more appropriate matrix.



A practical example

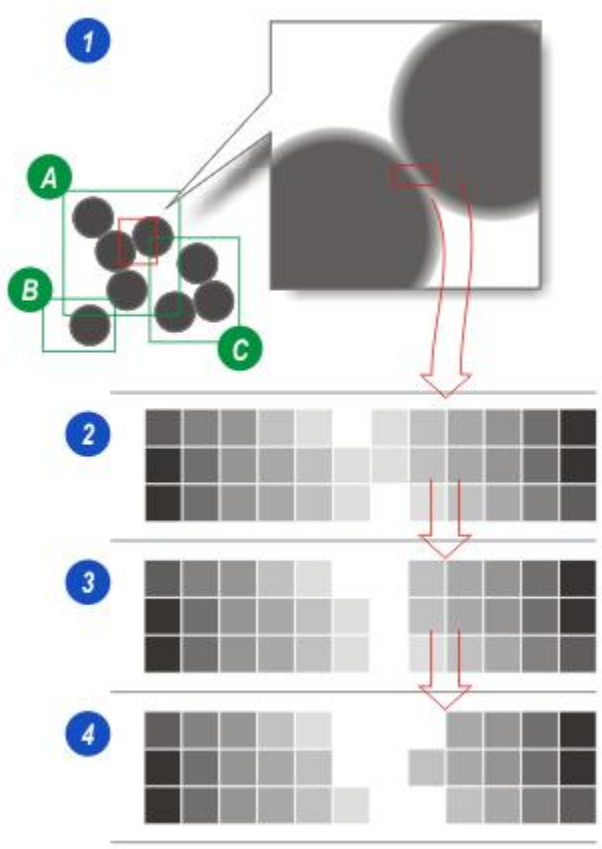
Greyscale image **(1)** shows a group of 8 black discs on a white background. The transition between black (0) and white (255) on an image is seldom that distinct – very often there will be several grey pixels around the boundary of a dark feature giving it a 'blurred' appearance. The blurring may be due to incorrect focussing, poor lighting or perhaps the feature itself is fuzzy.

The blurred boundary can make adjacent features appear to be touching each other and, if the intention was to count the features in an image, the discs in Figure **(1)** would be treated as 3 features – groups **(A), (B)** and **(C) -** instead of 8.

The boundaries between 2 adjacent discs may look like the pixels in Illustration **(2),** the black pixels of the discs blurring through shades of grey with white background pixels appearing at the extreme edges.

A *Dilation* (Illustration **3**) uses the greatest *Neighbour* pixel values to create an *Output Image* so at the edges of the blurring, white (value of 255) would replace pale grey pixels (value say of 210). The 'new' white background pixels can be seen in the centre.

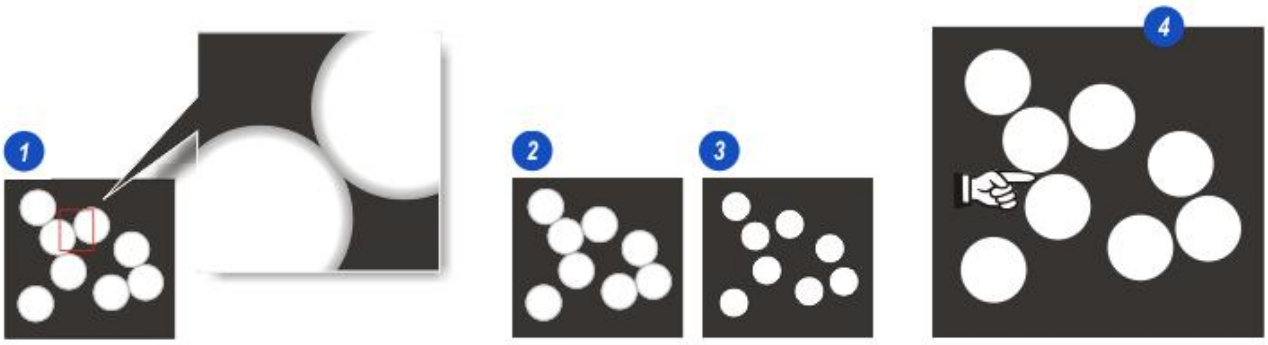
The amount of *Dilation* depends upon the *Structuring Element* matrix. Illustration **(4)** shows the effect of applying a *Structuring Element* with a greater number of cells testing a wider group of *Neighbours*. Here several grey pixels have been replaced with white pixels to create a larger gap between adjacent discs.



Erosion then Dilation

An Erosion followed by a Dilation is called an Opening Filter.

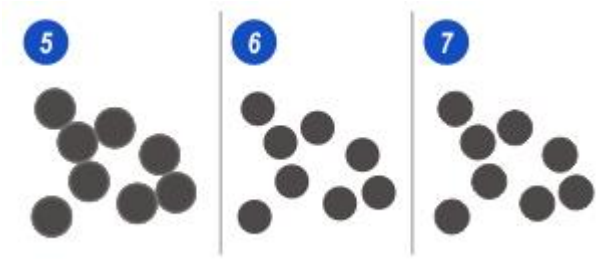
1. The same greyscale image but this time 8 white discs on a black background and apparently ‘grouped’ because of edge blurring. The task is the same – separate the discs without losing important dimensional data.
   Light features against a dark background are often better separated by using *Erosion* followed by *Dilation*.
2. The original ‘blurred’ image.
3. *After Erosion* – *Neighbour* pixels with the smallest value (nearer to black at 0) replace those with *greater* value (closer to white at 255) – the grey pixels around the boundaries of the discs have been replaced with black.
4. *After Dilation* – *Neighbour* pixels with the greatest value (closer to white at 255) replace those with closer to black (at 0). The effect is to ‘grow’ the white discs and, with the appropriate *Structuring Element*, to closely re-establish the discs’ diameter without causing them to touch again.



Dilation followed by *Erosion* is called a *Closing Filter.*

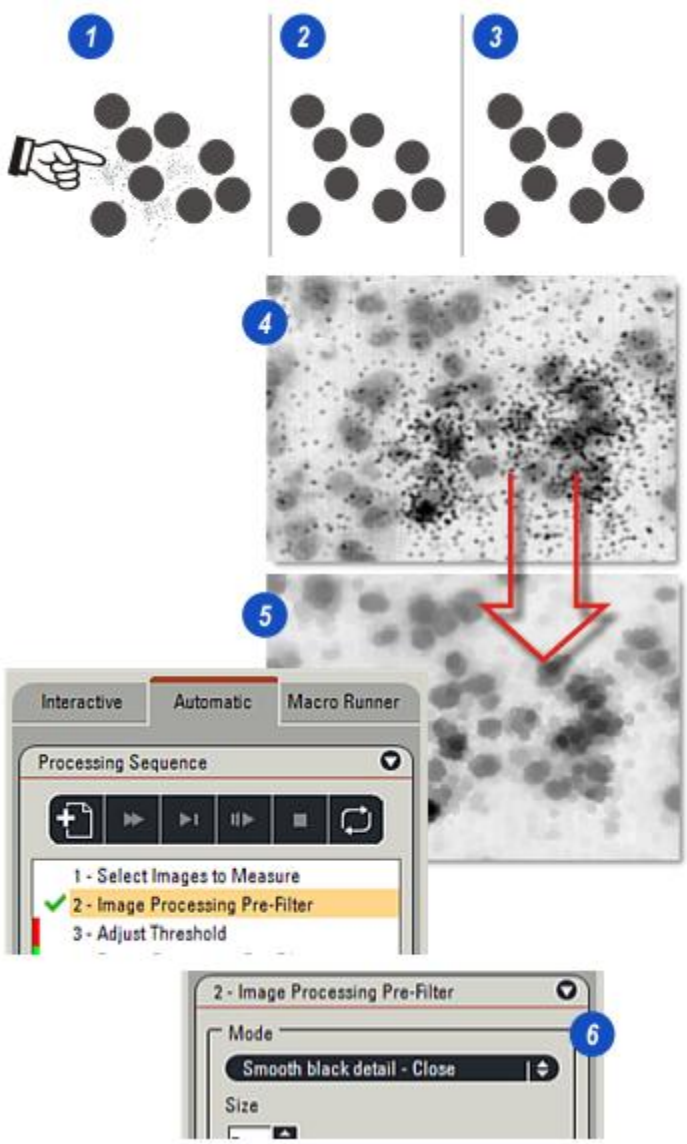
Illustration **(5)** is the original blurred image and **(6)** is the same image with successive *Dilations* until all of the grey pixels have been replaced with white – only distinct, separate black discs and white background remain – 8 features now instead of 3.

However, removing the grey pixels may have also reduced the actual size of the discs, which could be detrimental if areas or dimensions are to be measured. An *Erosion* will make the black discs grow – *smallest* value *Neighbour* pixels (those nearest black at 0) replace higher value pixels (those closest to white 255). *Erosions* continue until the black discs are close but not touching to complete the transition **(7)**.



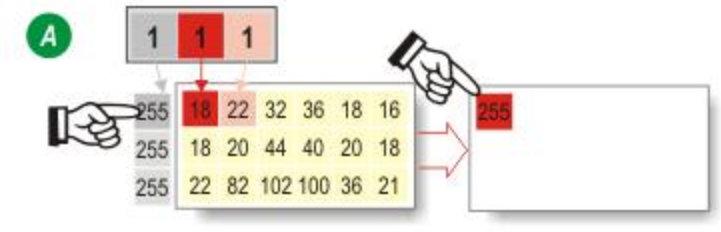
Open and *Closed Filters* have a wide range of applications in image processing, especially in noise removal.

1. A greyscale image with clumps of small features in the background which could be ‘noise’ or just insignificant parts of the image (unwanted artefacts that need to be removed before accurate measurements can be mad) e.
2. Successive Dilations have not only separated the black discs but also removed the ‘noise’ artefacts completely – simply because they are small.
3. Erosion enlarges the discs to their original size but because the ‘noise’ no longer exists there is nothing to enlarge.
4. Removing small artefacts in Image Analysis; The original Input Image.
5. The Output Image with the artefacts removed and the grey features restored to their original size.
6. The Image Analysis panel with *Image Processing Pre-Filter* tools in *Smooth Black Detail* Mode.

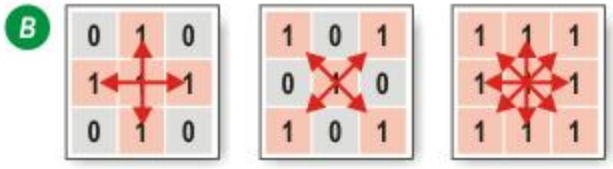


[Smooth Black Detail Mode](#) 1050

On the edge of the Input Image, the first *Neighbour Cell* of the Structuring Element will point to a non-existent pixel beyond the edge of the image. For greyscale images **(**Illustration **A)** and depending upon the process – *Dilation* or *Erosion* – these pixels are assumed to have values of 0 for *Dilation* and 255 for *Erosion*. This arrangement prevents border effects – fine, dark lines appearing around the output image.



Almost any layout can be applied to a *Structuring Element*; **(**Illustration **B)** shows a common 3 x 3 cell configuration in which a set cell (=1) is an active *Neighbour* used to evaluate the Input Image pixel values, whereas a cell cleared (=0) will be ignored.



The *Structuring Element* matrix – how many cells it contains – generally reflects the size and shape of the features being detected within the Input image. Image Analysis uses a circular configuration which expands or contracts depending upon the size selected by the user **(**Illustration **C).**

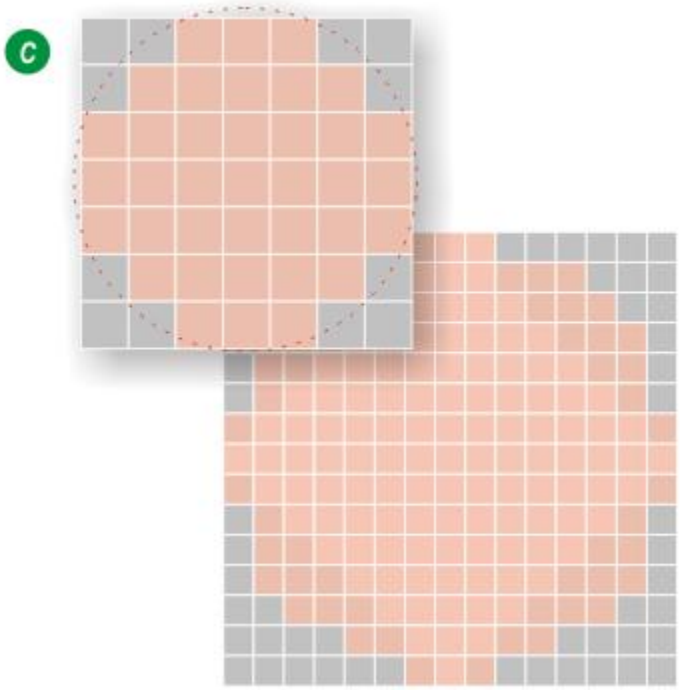


Illustration (**A**) depicts a Kernel with a 3 x 3 matrix – the Origin has 8 Neighbour Cells. The filter extracts the 9 pixels from the Input Image and calculates a Median value, that is a value at the mid-point when the pixels are arranged in order of value, not an average.

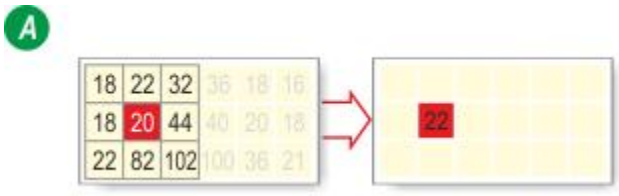
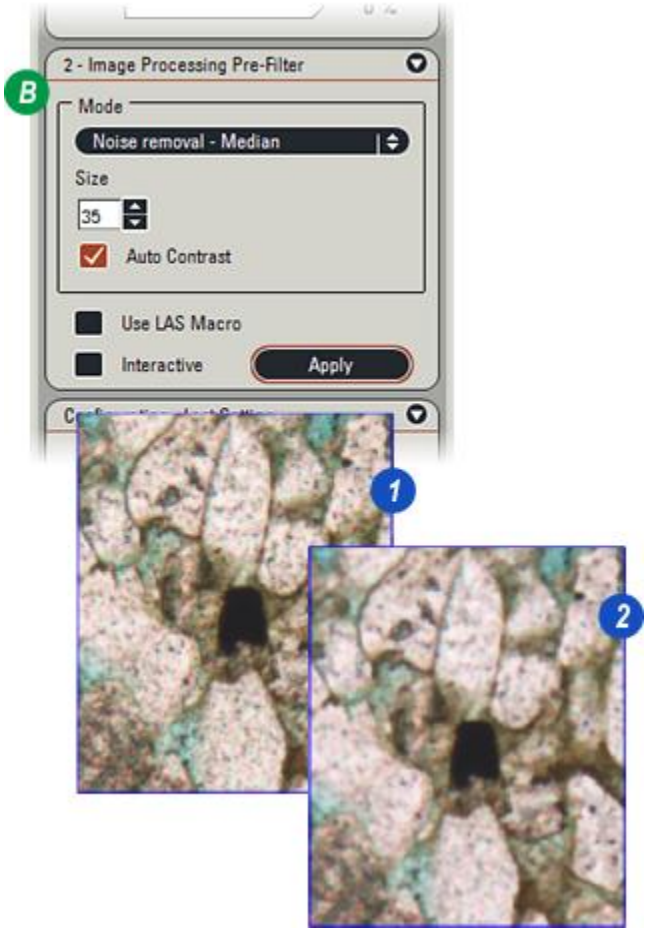


Illustration **(B)** shows Image Analysis in action with the *Median Noise Removal* tool selected. **(1)** is the Input Image and **(2)** the Output Image.



Some of the most useful morphological filters, *Tophats,* belong to a sub-class of operations called residues (since they produce something which is 'left over' from the original image).

Tophats can be defined as:

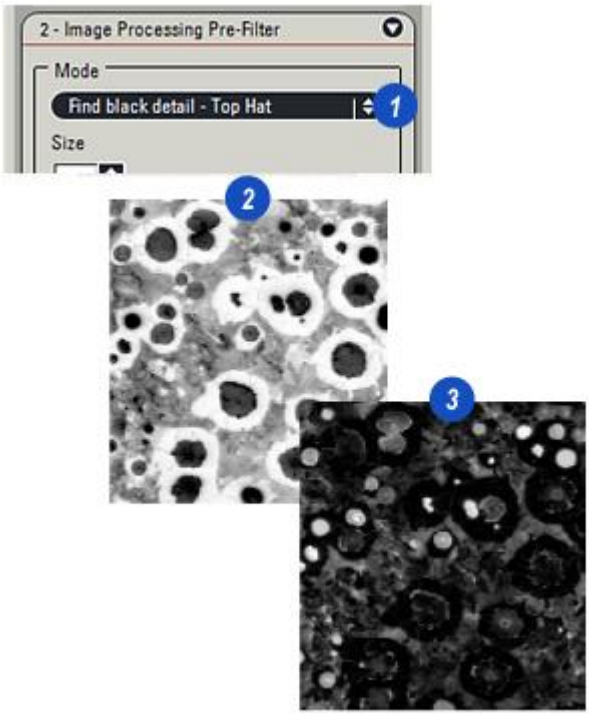
Smoothing > Difference > Tophat

The net effect is to enhance whatever was removed during the smoothing process, which will usually be the detail of the image.

The most common *Tophats* are constructed by using grey openings and closings as the smoothing operations. The Find black detail -*Tophat* is defined as:

Tophat = Closed Image - Original Image

1. The effect of a black *Tophat* is to pick out the small dark features in the image. The closing will remove the dark features up to a size defined by the cycles used, replacing them with the grey level of the lighter regions which surround them, and the subtraction of the original image will thus show the grey level difference between these 'filled in' pixels and their grey levels in the original image.
2. The original image.
3. After *Find black detail Tophat*.



Examples of Pre-Filter usage

The following topics give some examples of pre-filter usage.

Example 1 1062

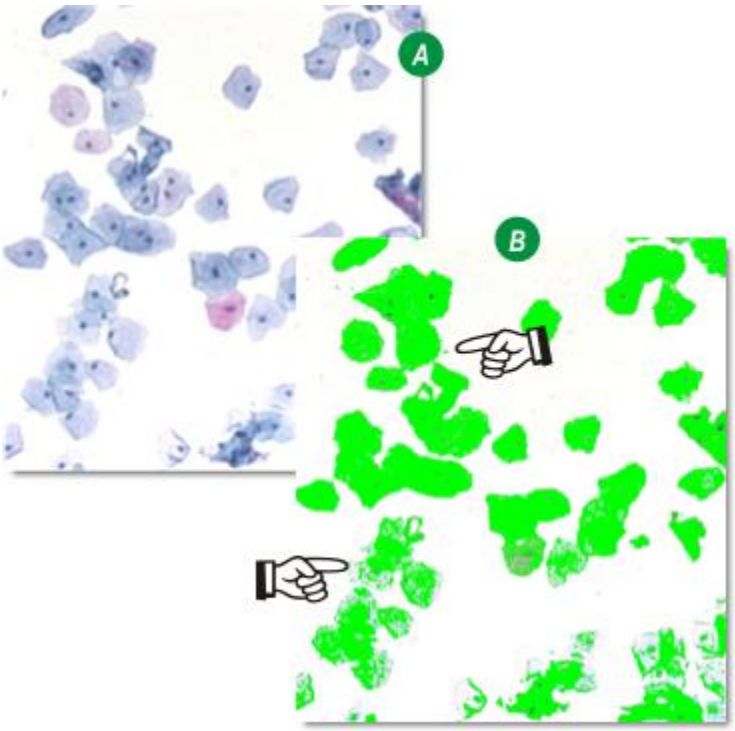
Example 2 1066

Example 3 1069

The aim of this example is to measure the number of biological cells on an image. The problem with the original image (**A**) is that the cells overlap, have a range of differing colours, have irregular outlines with varying contrast and some have coalesced into indeterminate 'blobs'.

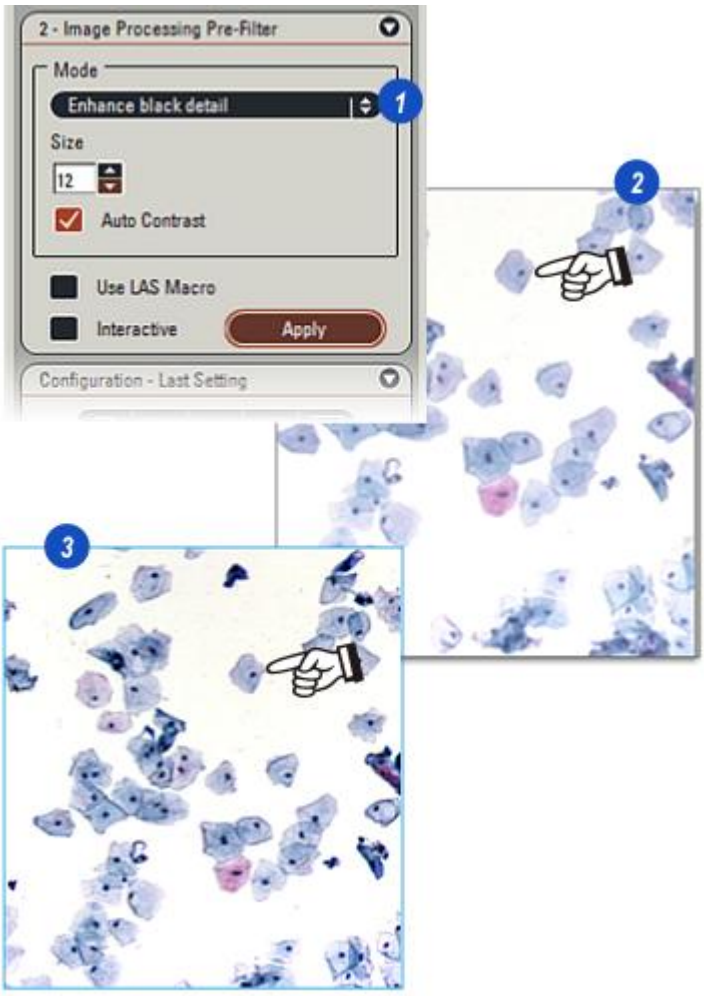
Skipping a Greyscale Pre-Filter and going directly to *Adjust Threshold* (Illustration **B**) results in a fuzzy Binary Output Image with scattered additional artefacts all of which could be included in the measured count. Adjustments to intensity might have improved the image slightly, but it would be a time-consuming hit-and-miss process not necessarily resulting in a better count. Better to use a Pre-Filter to isolate individual cells allowing each to be counted with acceptable accuracy.

Because the cell outlines and contrast are so variable, an alternative solution would be to count the 'nuclei' – they have a more-or-less consistent colour and size – and where cells overlap the nucleus tends to show through and can be counted.



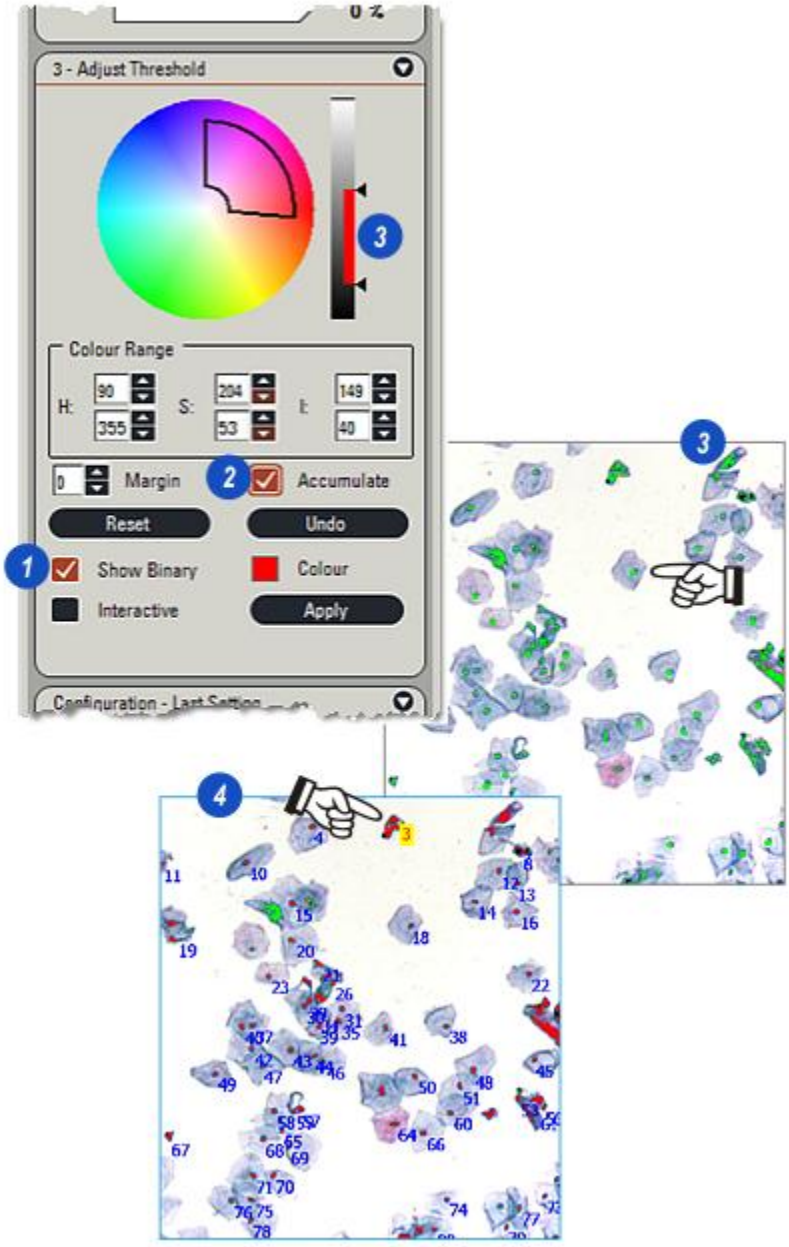
1. The *Enhance Black Detail* filter was chosen to improve the contrast between the nuclei and the surrounding cell tissue. Applying a structuring element of size 12, many of the intermediate greys have disappeared, the cell edges have become more clearly defined and the nuclei contrast is especially enhanced.

   There are still areas of the image in which the cells have clumped together but these may be removed or more clearly defined during *Adjust Threshold* or with *Binary Image Edit*.
2. The original image.
3. The Greyscale Output Image.



The next step is to apply the Thresholds.

1. Check that *Show Binary* is enabled.
2. Check that *Accumulate* is enabled.
3. With adjustments to the upper and lower Intensity Thresholds, the resulting Binary Output Image highlighted the nuclei and some clumps of artefacts all shown in green the default colour.
4. A first measurement for cell count yielded 86 but that included the clumps of artefacts as well. The feature highlighted in yellow is currently selected on the Grid view.



Adjust Threshold[1073]

Measurements[1109]

Grid View[1108]

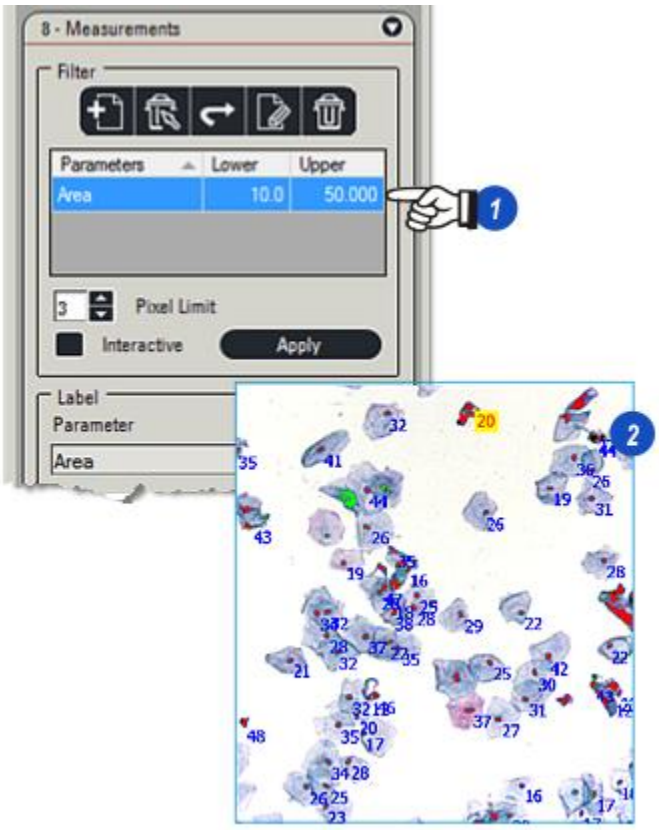
There are several methods for removing unwanted features – *Reject* and *Delete* Modes in Binary Edit for example, but in this example they were excluded by refining the upper and lower limits of the *Area Measurement* tool.

A first pass with a wide difference between the upper and lower limits produced an area value for all of the features, nuclei as well as unwanted artefacts. From this it could be seen that the nuclei areas fell between 10 and 50µm^2 whereas the unwanted artefacts were considerably larger.

The *Area Parameter* limits where then set to include the nuclei **(1)** with the result that all of the unwanted artefacts were ignored and the count reduced to 80 **(2)**.

In this example a *Measure Frame* was used to select just a small part of the image since the features were evenly distributed across the entire image and the smaller *Measure Frame* would yield a good mean result. It also speeds some of the processing time.

Once the correct filtering and thresholds have been established, the *Measure Frame* can be set to *Entire Image* and a further measurement made – in this case it resulted in a total count of 294 cells.



Measurement Tools 1109

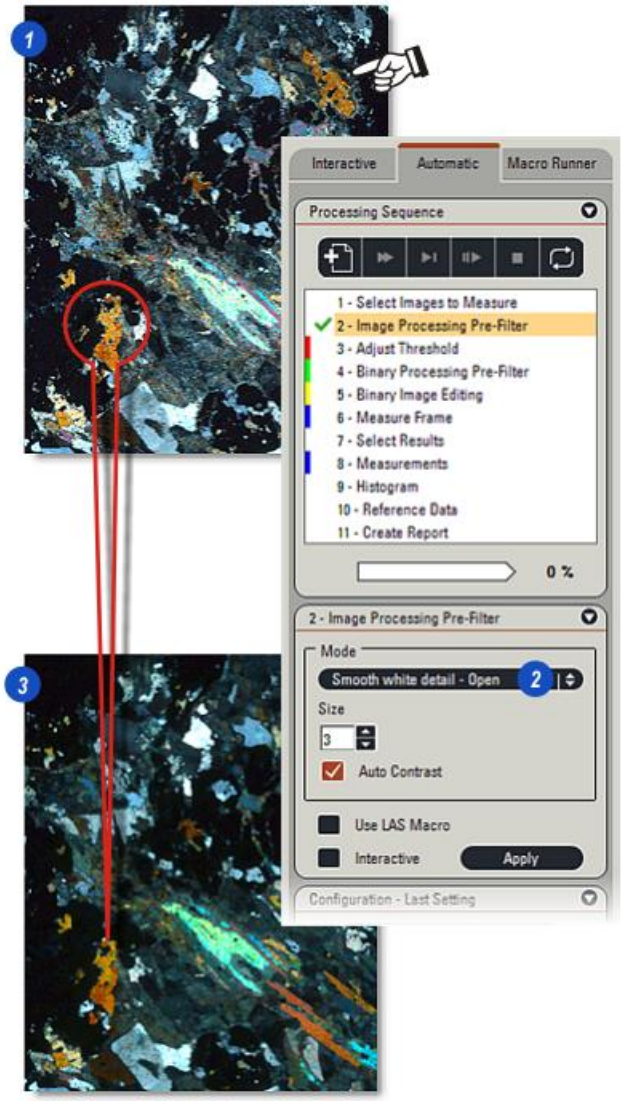
Area Parameter 1114

In this example a piece of schist, a crystalline rock, is the original image **(1)**. The specimen contains some iron ore which shows up as red-orange areas scattered randomly across the image. The test is to determine what percentage of the sample comprises iron ore.

In such a complex image there are likely to be many single, unconnected pixels that are close to the iron ore colour range for which we will be searching. Leaving these pixels in place will slow the measurement process considerably without beneficially affecting the end result, so an overall ‘tidy-up’ to remove ‘stray’ pixels and consolidate larger groupings is a good starting point.

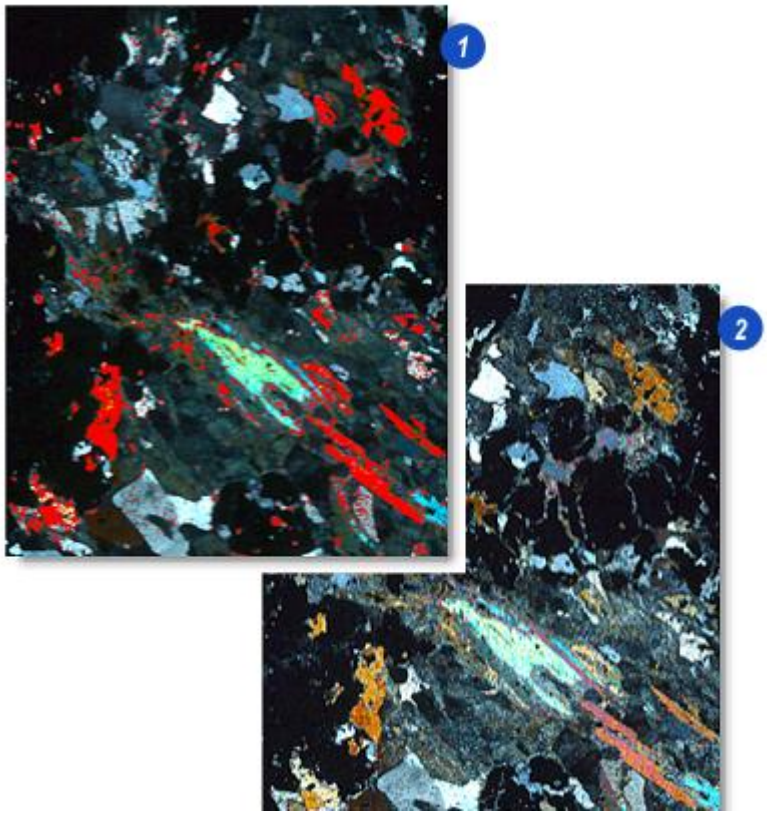
The Pre-Filter chosen was *Smooth White Detail* **(2)** with a structuring element of Size 3. Because Smooth White Detail is an open filter – erosion first and then dilation – some features disappear completely which is good for removing the stray pixels, but it can also start to reduce the area of the wanted features. Since this example will measure area, the filter has to be used sparingly.

The result of the Smooth White Detail Pre-Filter **(3)**.



It took only minor alterations to the *Adjust Threshold* [1073] intensity and saturation to produce a clean, well-delineated Binary Output Image **(1)** which, when compared with the original **(2)** proved to have faithfully pin-pointed the traces of iron ore and ignored everything else.

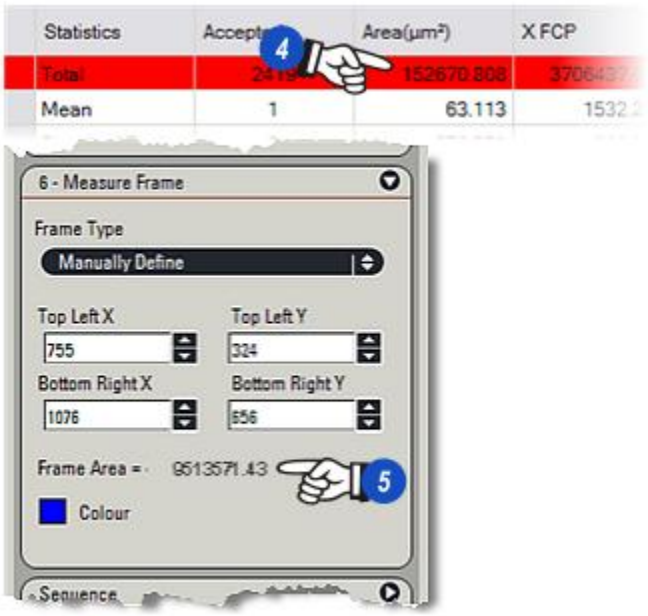
However, there were some holes and fissures in the main accretions which looked as though they should be included, but trying to fill them with the Threshold controls also tended to draw unwanted artefacts into the Binary Image.



There is an easier way to fill holes: Adjust Threshold [1073]

1. The *Combine Detail* filter, which is part of the *Binary Processing Pre-Filter collection*[1081], can quickly, simply and precisely fill holes and fissures in a Binary Output Image.
2. The *Reconstruct* and *Fill* options were enabled.
3. The structuring element *Size* control was incremented in single steps until the holes in the major features were filled without gathering together some of the more separated features.
4. A first measurement yielded a total feature (iron ore) area of 152670µm^2 which was displayed on the *Grid View.*
5. This was within a manually defined *Measure Frame* of 2587631µm^2 giving a value of 5.9% iron ore.

To check that the measure frame represented an average part of the image, the frame was reset to Entire image and the area measurement was taken again. This yielded a feature area of 577912µm^2 within an image area of 9513571µm^2 - 6.0%.



Binary Processing Pre-Filters[1081]

Grid View[1033]

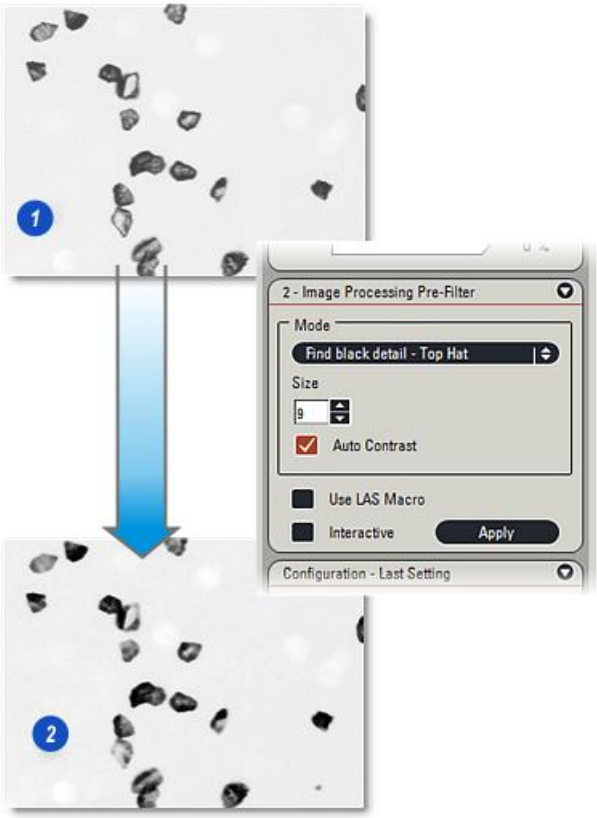
Example 3

The diamond chips in the original image **(1)** produce an acceptable Binary Output image if passed directly through the Adjust Threshold, but some fast processing with a Greyscale Pre-Filter reduces the amount of fine adjustments made to the Threshold and can improve measurement precision considerably.

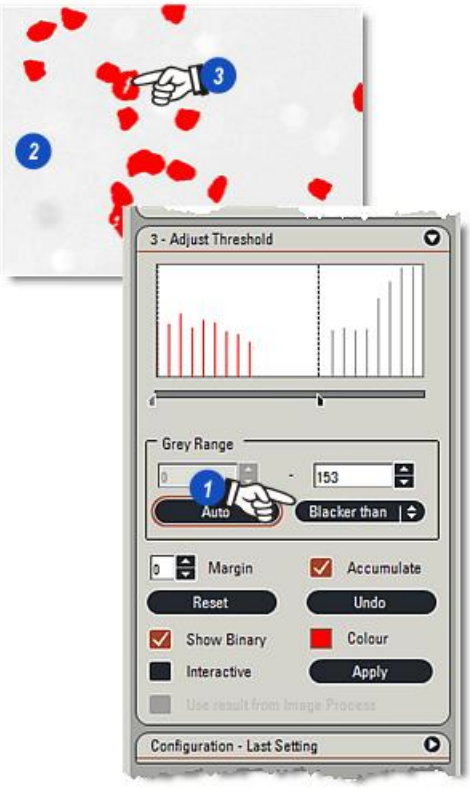
LAS Image Analysis is so fast that it is always beneficial to run an image through several greyscale filters to check for improvements.

In this example the image was processed with the Smooth Black Detail Pre-Filter that removed some of the intermediate grey values and increased the chip edge contrast **(2).**

However, it had to be used carefully since this was to be a feature count – how many chips in the image – and because several features overlap, the Smooth Black filter could have blended them together resulting in a single feature rather than two.



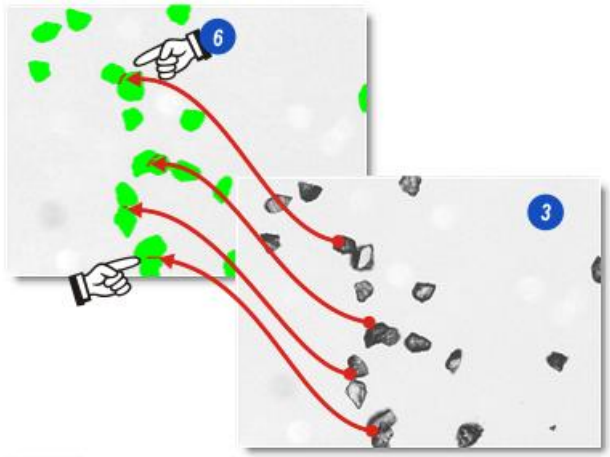
1. Diamond chips is a monochrome image with predominant greys closer to the black end of the scale, so the *Grey Range Selector* was set to *Blacker than* and minor adjustments made to the *Histogram* sliders.
2. The result is a very good *Binary Output Image* with small holes **(3)** in just two of the chips.

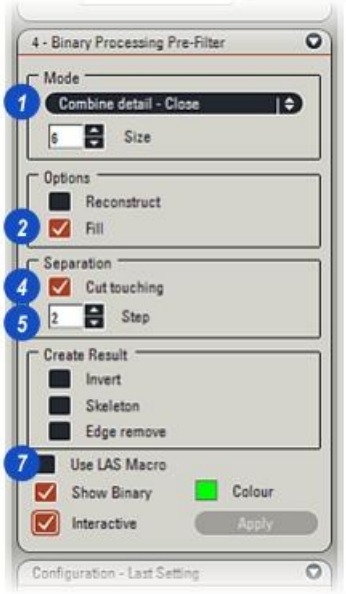


In this example the holes in several of the diamond chips would not have made a difference to the final feature count, but since it was necessary to pass the image though a *Binary Pre-Filter* to make sure that there was good separation where the chips overlapped, the *Combine Detail* with the *Fill* option enabled was used to fill the holes with the *Size* setting at 6. Again, this was used sparingly because some of the chips are close together and could have been combined.

There were 4 chip pairs that were either overlapping or touching; They can be clearly seen on the original **(3)**.

With *Separation* > *Cut Touching* enabled, two steps were sufficient to separate the overlaps, indicated by the red lines on the *Output Image* **(6).**

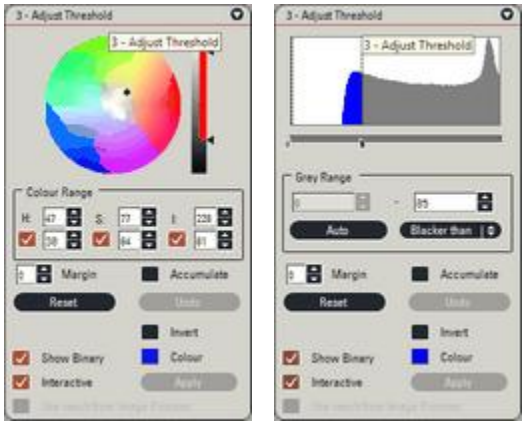




The final step before counting the diamond chips was to remove the fragment (bottom right) to prevent it being counted. This was achieved with *Binary Edit Delete Mode*. The fragment was not actually removed from the Binary Output Image but only highlighted and ignored in the count.

The count **(3)** shows 18 chips in the image and shows that the *Cut Touching* tool is working well.

Adjust Threshold allows you to select the features that you want to measure. *LAS Image Analysis* recognises colour and monochrome images, and displays appropriate tools during the *Adjust Threshold* step.



Both versions of the control panel have common[1078] tools and buttons.

You can use the following methods to select features to be included in measurements:

- Drawing a Region of Interest[1074] (colour and monochrome images)
- Using the Grey Range Selector[1075] (monochrome images only)
- Using the Colour Range Selector[1077] (colour images only).

Note: You cannot add images that use different colour modes to the same processing sequence.

Setting up the workspace

To get the best experience when setting an image threshold, we recommend that you make the following Workspace settings:

1. Click on the *Show Viewer Options* button on the *Side Tool Bar*.
2. From the context menu, click to enable all the options:

Drawing a Region of Interest

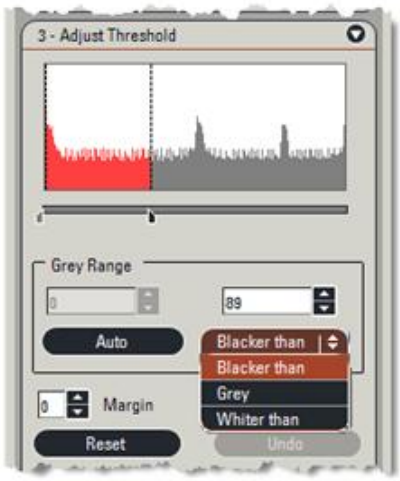
LAS Image Analysis recognises colour and monochrome images, and displays the appropriate control panel during the *Adjust Threshold* step.

Note: You cannot add images that use different colour modes to the same processing sequence.

To draw a Region of Interest (RoI):

1. Drag the pointer over a feature on the *Viewer*. Any pixels within the drawn rectangle will be used to define the threshold.
2. To fine-tune your selection, use the appropriate greyscale or colour controls:
   - Using the Grey Range Selector 1075
   - Using the Colour Range Selector 1077

LAS Image Analysis automatically detects monochrome images and displays the *Histogram* and *Grey Range* selector in the *Adjust Threshold* panel.



Using the Auto button

If the image has good contrast and well-defined features:

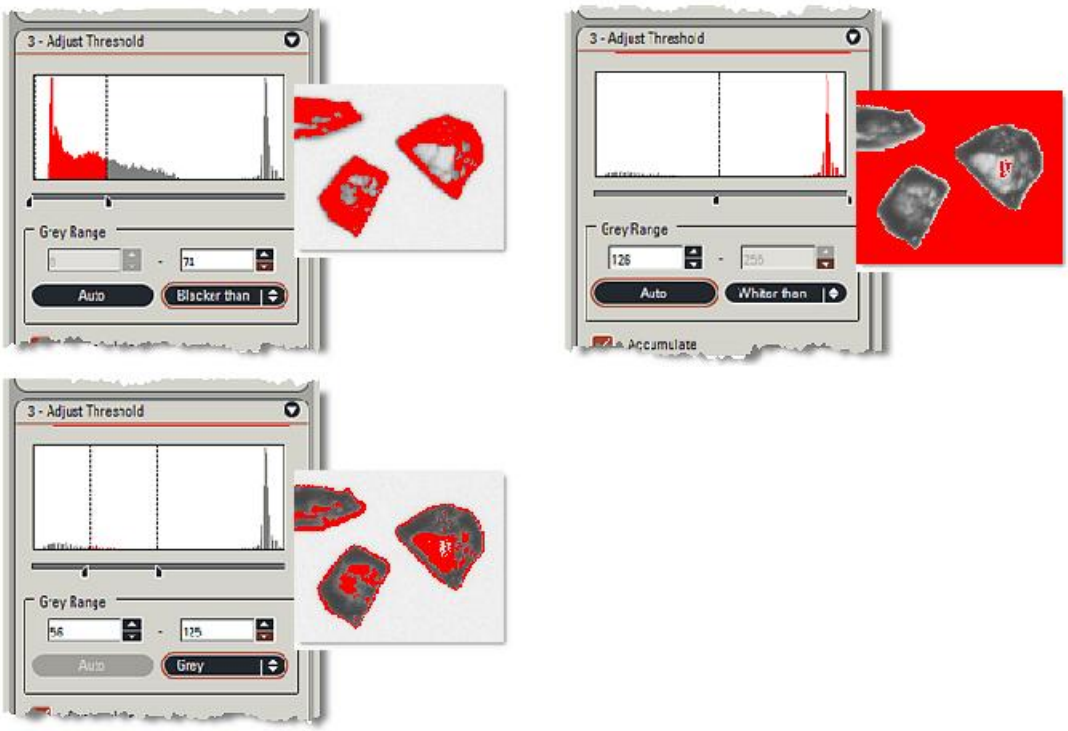
1. Click the *Auto* button.

(Continued on next page)

Using the Grey Range Selector and the Histogram

If you need greater control when setting the threshold:

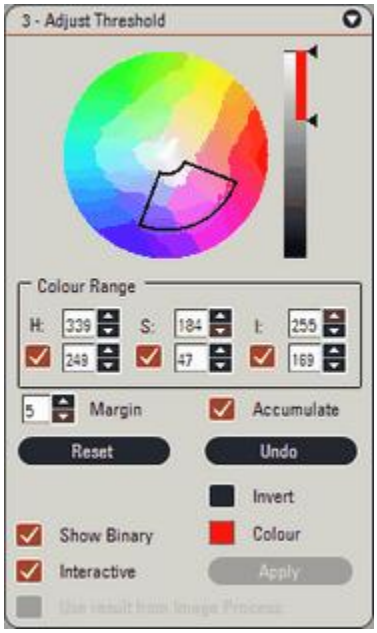
1. Select a thresholding method using the drop-down menu in the *Grey Range* pane.
   - *Blacker than* selects all features at or below the entered grey value
   - *Whiter than* selects all features at or above the entered value
   - *Grey* automatically sets two values (Blacker than + 1 and Whiter than – 1) as a centre band.



2. Fine tune the selection using any of the following methods:
   - Drag the sliders at the bottom of the *Histogram*
   - Enter values in the text boxes
3. Use any of the common tools[1078] to optimise your feature selection and display.

Changes made to the grey values are automatically reflected in the Blacker than and Whiter than settings.

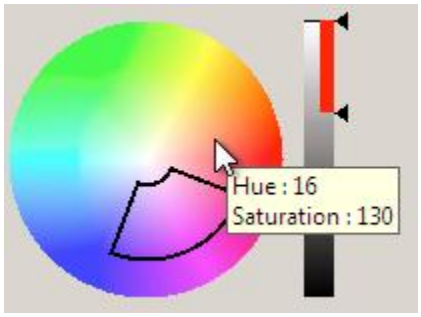
LAS Image Analysis automatically detects colour images and displays the *Colour Wheel* and *Colour Range* selector in the *Adjust Threshold* panel.

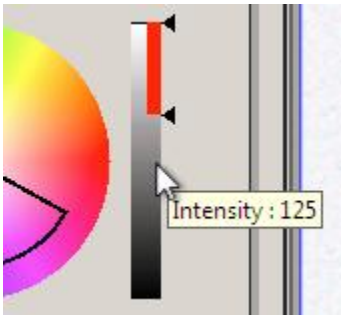


Use the *Colour Wheel* to adjust and experiment with the Hue and Saturation values. *H=Hue, S=Saturation* and *I=Intensity.*

- *Hue* is measured along the Wheel's circumference; *Saturation* is measured along the *Wheel*'s radius.
- The section of the Wheel outlined in black shows the current range of the Hue and Saturation settings used to select features for measurement.

- As you move the cursor over the Colour Wheel, the *Hue* and *Saturation* values are displayed in a *Tooltip;* As you move the cursor over the *Intensity Bar*, the *Intensity* value is displayed in a *Tooltip*





- Drag the black outline of the *Wheel* segment to alter the *Hue* and *Saturation;* you can drag the radii (to alter the *Hue* boundaries), or the inner and outer arcs (to alter the *Saturation* boundaries); the actual values are displayed in the *Colour Range* windows
- Drag the *Intensity Bar Threshold* pointers to alter the *Intensity* boundaries
- Fine-tune all three parameters using the Up/Down arrows to the right of the *Colour Range* windows. Each pair of windows represents the span of values for that parameter
- You can disable one or two (but not all three) HSI check boxes while adjusting the other parameters. For example, it is often simpler to ignore the colour saturation, since you are interested in the predominant colour.

The following Threshold tools are common to both greyscale and colour images.

Margin

Use the Margin up/down arrows to adjust the spread of selected threshold values.

For example, with Margin set to 5, a threshold value of 12 on the image would actually include all values in the range 7 to 17 (i.e. 12 ± 5).

Accumulate

The *Accumulate* tool adds the values of successive drawn regions to extend the Threshold range, including more features.

Reset and Undo

- *Reset*: Clears all Threshold values
- *Undo*: Removes the last pass.

Invert

Providing the background is reasonably uniform and has colour content distinct from the 'target' features, the *Invert* [1079] tool can make selection much simpler.

Show Binary

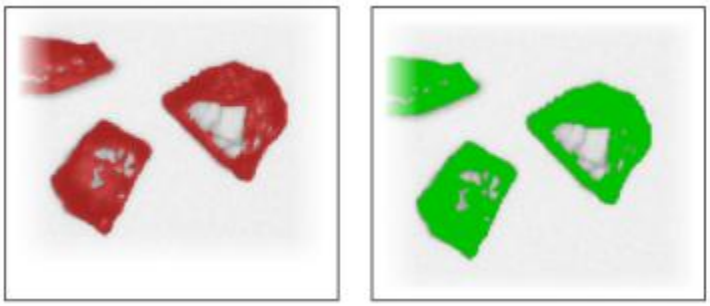
When the *Show Binary* checkbox is enabled, features selected for measurement are displayed in the right-hand side of the *Viewer*. You can change the default highlight colour (see below).

Colour

You can change the colour used to display the selected pixels in the *Binary Output Image*. For example, if the features you are trying to measure are red, you might want to change the highlight Colour to a bright blue so the features are easy to distinguish.

1. Click on the *Colour* button.
2. Use the standard Windows *Select Colour* dialog to choose a new highlight colour.
3. Click *OK*.

The illustration below shows the effect of changing the binary *Colour* from red to green.



Don't forget, you can right-click in the *Viewer* to temporarily disable the highlighted features, to help you see whether you have set your threshold correctly.

Interactive

- *Interactive* enabled: Each change in the settings is automatically reflected in the Output Image. This is the best setting for simple images.
- *Interactive* disabled: Changes are only updated when you click *Apply*. This is better for more complex images that may take a little longer to process.

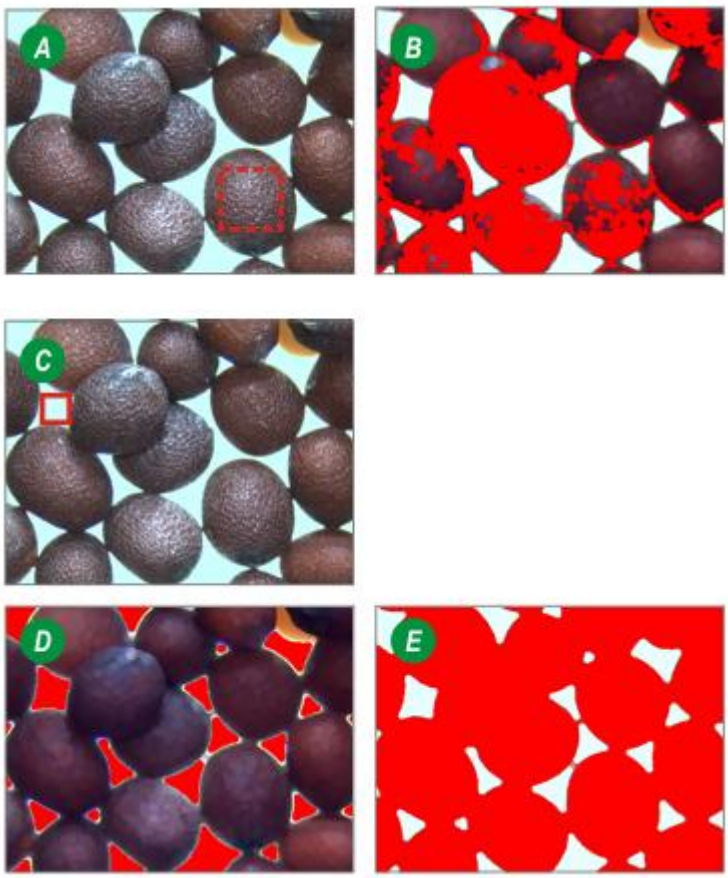
Using the Invert Tool

Specimens that have complex detail can be time consuming to select. The seeds in *Image (A)* display a wide colour range, detailed textures and highlights. Drawing several *Regions of Interest* may still fail to pick up all of the variations - *Image (B).*

Providing the background is reasonably uniform and has colour content distinct from the 'target' features, the *Invert* tool can make it much easier to select features. The principle is to select the background (not the features) and then to invert the selection so that the features are selected and the background is ignored.

1. Click the *Reset* button the clear any previous selections.
   Image C: Click and drag to draw a *Region of Interest* over an area of the background. If Interactive is disabled, click the *Apply* button.
   Image D: The background selected - red in this example.
2. Click to enable the *Invert* check box.
   Image E: The features are selected and the background deselected.

Note: If the background is very uniform, enable the *Invert* check box *before* drawing the *Region of Interest* on the background and the features will be selected directly.

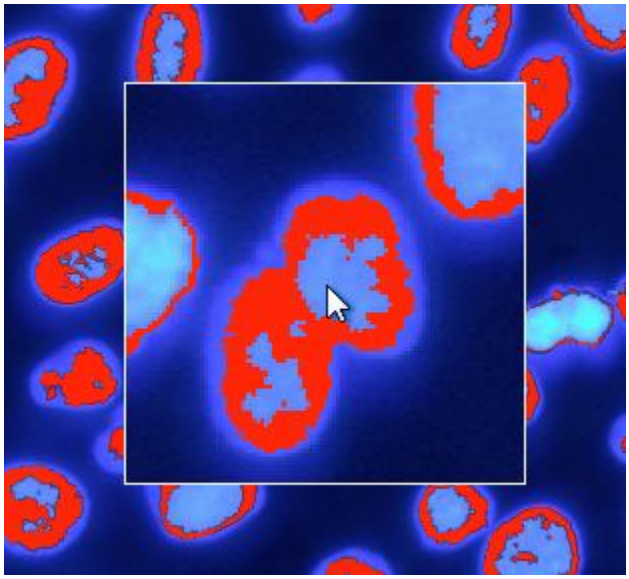


To switch between the *Binary Output Image* and the *Input Image:*

- Right-click on the *Binary Output Image* to hide the binary image and reveal the original input.

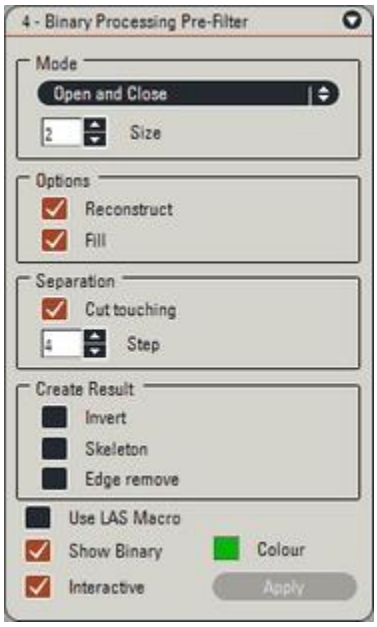
To enlarge parts of either the *Input Image* or the *Binary Output Image:*

- Hold down the Shift key (but do **not** click). The magnified area tracks the mouse movements. Release the Shift key to close the magnifier.



Binary Processing Pre-Filter

The *Binary Processing Pre-Filter* uses the same techniques as the Image Processing Pre-Filter[1048] for improving and modifying images. However, instead of working with values ranging from 0 to 255, only binary pixel values of 1 and 0 are used.



Usually, the image presented to the *Binary Processing Pre-Filter* has passed through the Image Processing Pre-Filter[1048] and Adjust Threshold[1073] stages.

If you need to learn about the binary processing principles in detail, see this topic[1092].

To use the *Binary Processing Pre-Filter*:

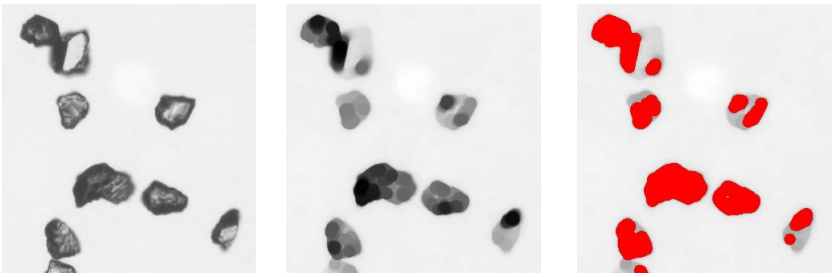
1. Click on the *Binary Processing Pre-Filter* option in the *Processing Sequence* panel.
2. Select an active filter from the *Mode* drop-down menu.
   - No Filter
   - Discard detail - Open[1082]
   - Combine detail - Close[1083]
   - Open and Close[1084]
3. Enable or disable tools in the *Options* panel:
   - Reconstruct[1085]
   - Fill[1086]
4. Set up the Cut Touching[1083] tool, which helps to separate overlapping or touching features.
5. Decide which of the *Create Result* tools to use; these allow you to modify the Binary Input Image in very specific ways:
   - Invert[1088]
   - Skeleton[1089]
   - Edge Remove[1090]

Note: If you want to use *LAS Macro Runner*, instead of the Binary Processing Pre-Filter, click to enable the check box. You can find detailed help for LAS Macro *here*.

The *Combine Detail - Open* filter performs a sequence of erosion followed by the same amount of dilation. This cleans the image by removing small objects.

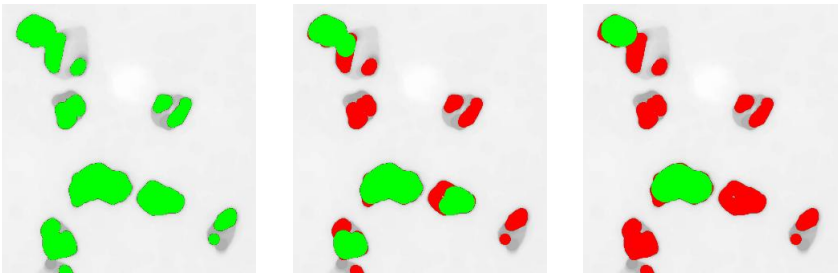
We will use an image of diamond chips as an example.

- The darker areas have been enhanced with the Image Processing Pre-Filter[1050] > *Smooth Black Detail*.
- Applying *Adjust Threshold* has selected most of the darker areas but also included some mid-grey tones that are not required.

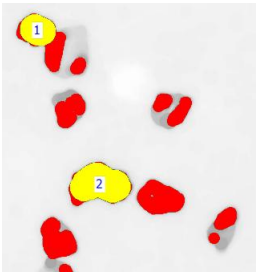


1. Using the *Binary Pre-Filter* in *Discard Detail - Open* mode, we gradually increase the *Size* of the Structuring Element[1093] to shrink the selected areas, starting with the lighter tones, until only those required for use in measurements are highlighted.

   The selected feature areas are highlighted in green, shown below with *Size* settings of 10, 20, then 30 (this retains only the very dark areas, which was the aim of using the *Discard Detail* filter):



2. A quick *Measurements > Number (Count)* check reveals that only the required features are included.

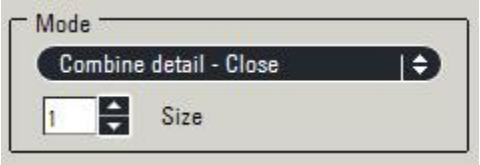


However, a part of the image has been rejected – possibly because two diamond chips at the top left were overlapping; this needs to be included without drawing in any other lighter features. See Reconstruct[1085].

Mode: Combine Detail - Close

The *Combine Detail - Close* filter performs a sequence of dilation followed by the same amount of erosion. This removes fissures in features, leaving them well defined and ready for measurement.

1. Select the *Combine Detail - Close* filter from the *Mode* drop-down menu.
2. Gradually increase the Structuring Element[1093] *Size*.



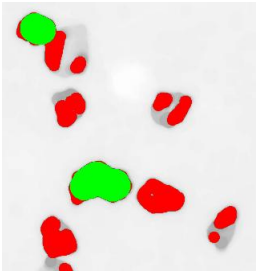
Mode: Open and Close

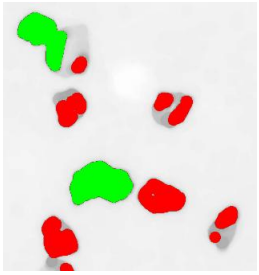
The *Open and Close* filter performs a sequence of dilation followed by the same amount of erosion. This removes unwanted small objects, along with fissures in features, leaving them well defined and ready for measurement.

1. Select the *Open and Close* filter from the *Mode* drop-down menu.
2. Gradually increase the Structuring Element[1093] *Size*.

The Reconstruct tool targets small, closely-related areas to be included in measurements.

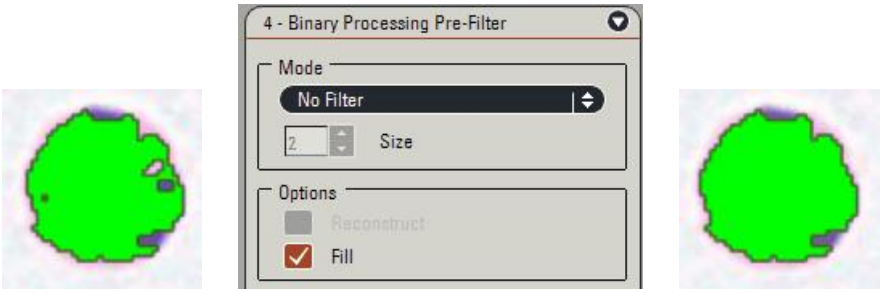
We will continue the example started in Mode: Discard Detail - Open 1082. By enabling the Reconstruct tool, we can include the part of the image that was originally discarded.





The previously excluded area at the top left of the image is now included; while this would not affect a Number (Count) measurement, it would be significant if Area was being measured.

The Fill tool can improve the Binary Input Mask for features that have been left with holes and fissures after passing through the Image Processing Pre-Filter[1048] and Adjust Threshold[1073] stages. This fills the holes well but leaves some fissures open.

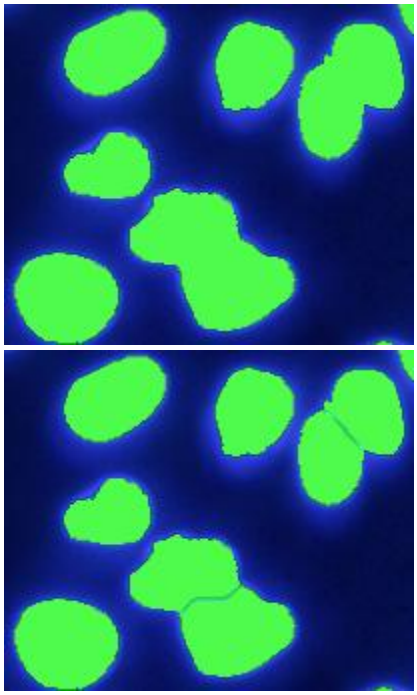


It is usually better use the Fill tool, rather than the *Threshold*[1077] *Hue* and *Intensity* (which creates noise on the background).

Cut Touching

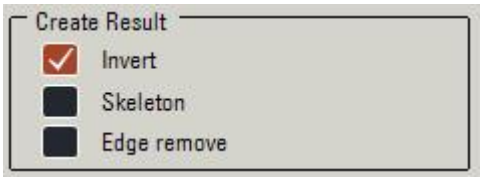
The *Cut Touching* tool allows you to separate two or more discrete features that appear coalesced (either because the Threshold has been set incorrectly, or because they are actually overlapping):

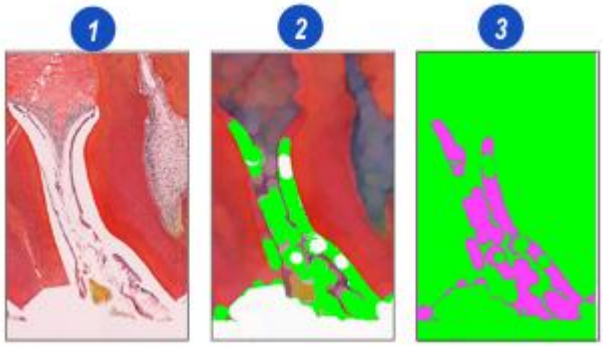
1. Enable the *Cut Touching* tool in the *Separation* panel.
2. Gradually increase the *Step* value until a line appears, indicating that the features have been detected and separated.



The illustration below shows part of a section through soft tissue.

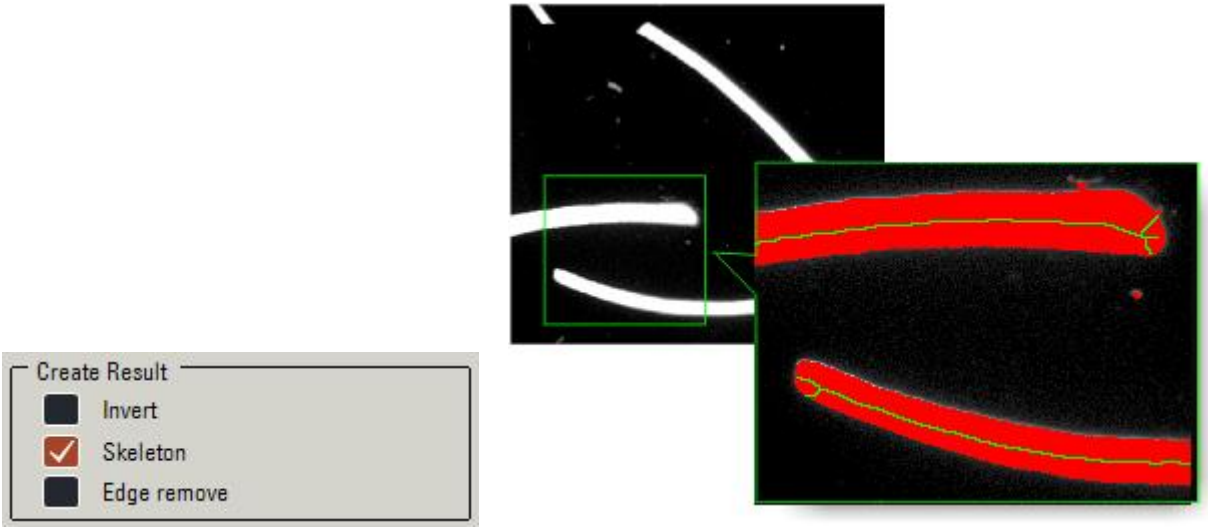
1. The original image.
2. The image has been passed through the *Smooth White* greyscale filter and the *Threshold* filter to select the predominantly white area of the image. The selected areas are shown coloured green.
3. Enabling the *Invert* check box deselects the previously selected areas – now displayed in a different colour – and selects everything else. It is essentially a 'swap' tool.



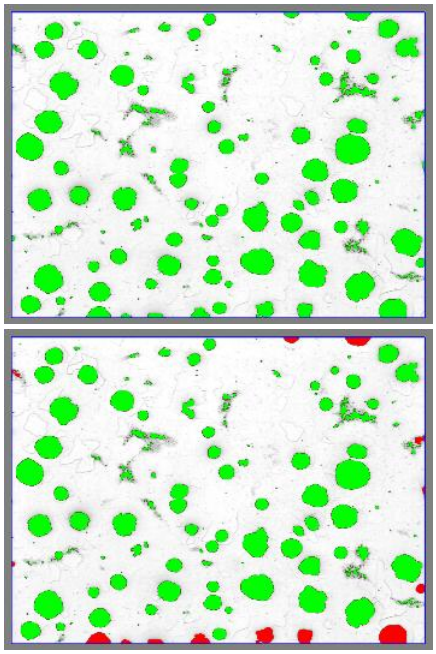


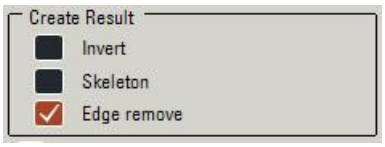
Enabling the Skeleton tool erodes selected features to the point where only a ‘backbone’, just 1 pixel wide remains.

The illustration below shows wool fibres 'traced' using the Skeleton tool.



The Edge Remove tool excludes from measurement any objects that are not completely within the image boundary. Such objects are coloured red in the Binary Output Image.



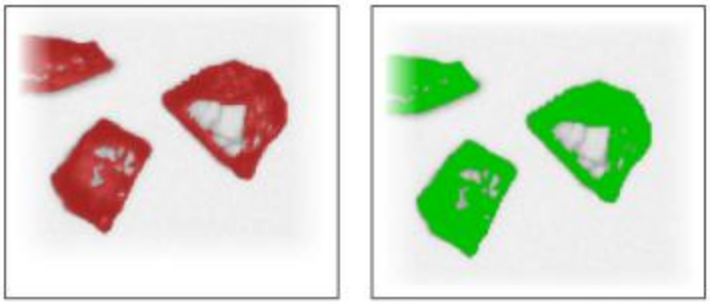


Colour

You can change the colour used to display the selected pixels in the *Binary Output Image*. For example, if the features you are trying to measure are red, you might want to change the highlight Colour to a bright blue so the features are easy to distinguish.

1. Click on the *Colour* button.
2. Use the standard Windows *Select Colour* dialog to choose a new highlight colour.
3. Click *OK*.

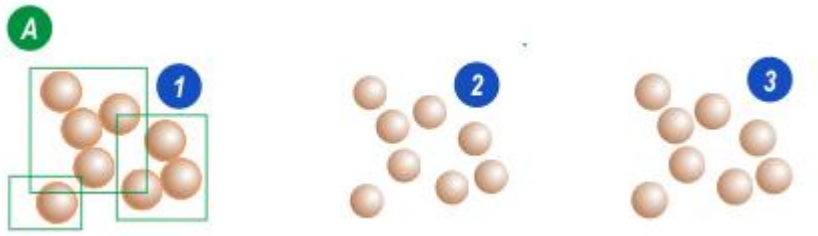
The illustration below shows the effect of changing the binary *Colour* from red to green.



Don't forget, you can right-click in the *Viewer* to temporarily disable the highlighted features, to help you see whether you have set your threshold correctly.

Illustration **(A)** shows a typical Binary Processing sequence:

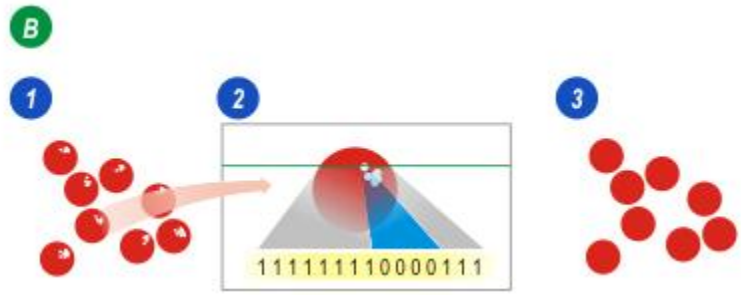
1. The Greyscale Input Image comprising 8 touching disks; the task is to count the disks and measure their areas. Performing a *Number* (Count) and *Area* calculation on this image would yield just 3 features with diverse areas because some of the disks are touching.
2. The Output Image after a Greyscale Processing *Erosion* – the disks are no longer touching so a *Number* (Count) would yield 8 features. But some diameter has been lost so the *Area* calculation would be understated.
3. The Output Image after a Greyscale *Dilation*. The disks are still separate, and much of their original diameter has been restored. A count measurement would now be much more accurate.



In Illustration **(B)**:

1. The image has passed though *Adjust Threshold* and those grey values falling within the threshold limits are coloured red. The highlights on the disks have not fallen within the threshold limits and so remain white.

   The *Output Image* from the *Adjust Threshold* has converted a Greyscale Input with grey value ranging from 0 (black) to 255 (white), to a *Binary Output Image* in which those pixels selected are set to a binary value of '1' and those not selected cleared to a binary value of '0'.
2. An enlarged view of a disk. The string of binary digits below the illustration represents a single row of pixels (shown by the green line in the illustration). The selected (red) areas of the disk are set to '1' and the unselected highlight (white) areas cleared to '0'.
   There are 'holes' in the disks!
3. For a proper *Area* measurement those 'holes' need to be selected and filled. *Binary Processing* can do that quickly and efficiently.



Binary Dilation 1093

Binary Erosion 1094

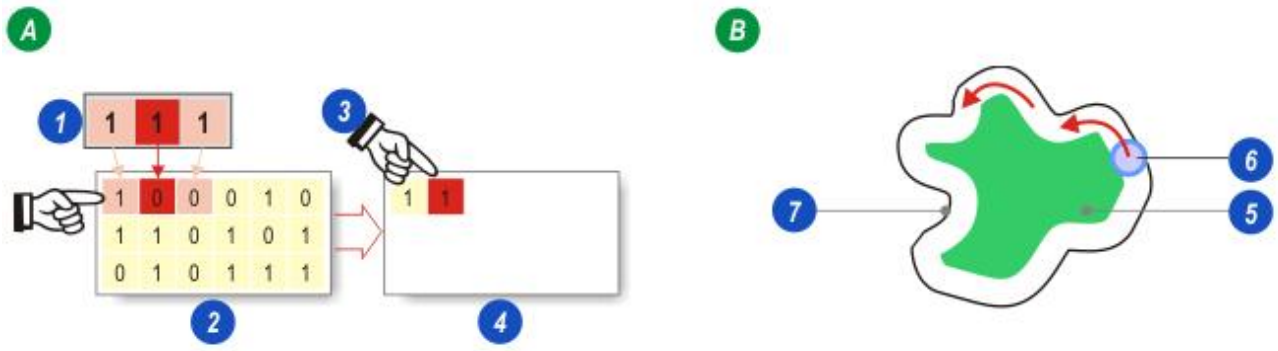
The *Binary Pre-Filters* provide the tools for modifying the *Binary Image* in the same manner as Greyscale Pre-Filters.

In Illustration **(A)**:

- The Structuring Element **(1)** is an 'electronic overlay' with cells that are also set or cleared to binary values. There are three cells, all are set to have a binary value of 1.
- The middle cell **(2)** is called the Origin (coloured red) and it 'looks' at a single pixel (also coloured red) called the Input Pixel in the Binary Input Image.
- The process examines the pixels neighbouring the Input Pixel to determine whether the corresponding pixel **(3)** in the Binary Output Image **(4)** should be set or cleared. The Structuring Element settings determine whether a neighbour is tested (1) or ignored (0).
- The process described in this illustration is called a Dilation – if any of the neighbours are set (=1) then the Output Pixel is also set – which is the case in Illustration (A). A Dilation has the effect of increasing the number of selected pixels.

Illustration **(B)** shows a simplified form of dilation:

- The original object **(5)**.
- An imaginary 'roller' **(6)** tracing around the periphery of the object.
- The new outline of the object **(7)**.



In Illustration **(C)** the *Structuring Element* has moved one pixel to the right. Now the *Input Pixel* (red) and its neighbours are all cleared (=0) and so the pixel in the *Binary Output Image* is also cleared **(1)**. The process continues for every pixel until a complete and new *Binary Output Image* has been created.

Binary *Dilation* would be a most suitable pre-filter for filling the highlight 'holes' in the example image **(D).**

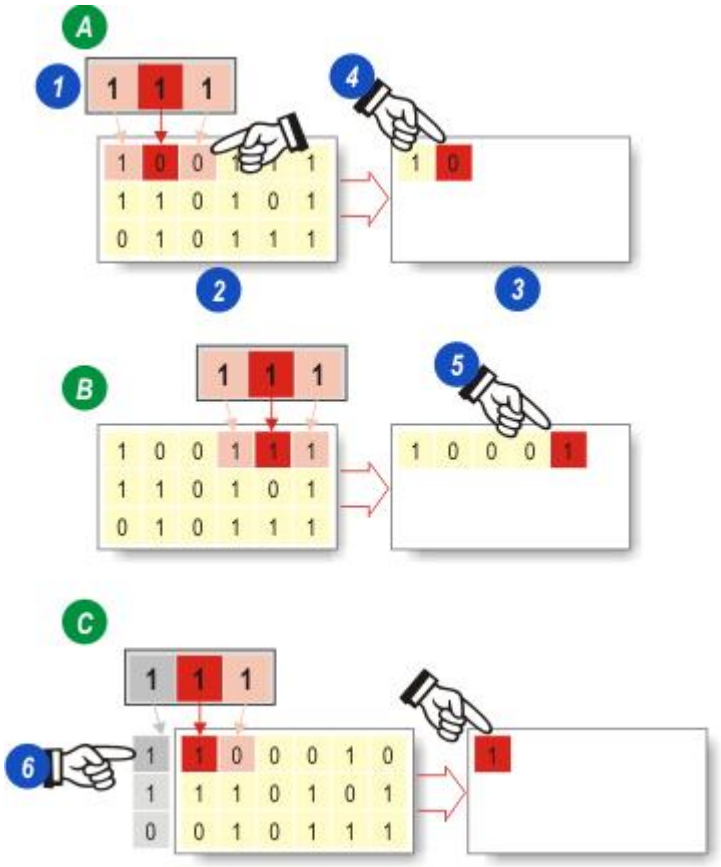
1. The *Structuring Element*.
2. The *Binary Input Im*age with pixels either set (=1) or cleared (=0).
3. The *Binary Output Image*. Set pixels are included in a measurement but cleared pixels are not.

The *Erosion* process examines the *Input Pixel's* neighbours and if any one is cleared (=0) then the Output Pixel is also cleared. This has the effect of removing selected pixels from the image.

In Illustration **(A)** a neighbouring pixel is cleared (=0) and so the *Output Pixel* **(4)** is also cleared.

The *Structuring Element* has moved four pixels to the right in Illustration **(B)** and now both of the neighbours are set (=1) so the *Output Pixel* **(5)** is also set.

To determine the value of pixels on the extreme edges of the *Output Image*, Illustration **(C)**, the Input Pixel (coloured red) is 'ghosted' **(6)** to become a neighbour. The illustration represents a *Dilation* so the *Output Pixel* is set, but if this were an *Erosion* the *Output Pixel* would be cleared because the right-hand neighbour is cleared.



Erosion and *Dilation* can be used in combination to achieve specific results.

- Dilation followed by an Erosion is called a closing filter, and is used for filling 'holes' in an image.
- Erosion followed by Dilation is called an opening filter and is often used for removing small details such as noise and dust.

Binary Image Editing tools allow you to specify which features from the *Binary Processing Pre-Filter* and *Adjust Threshold* steps are included in your measurements.

Binary Editing uses three separate images:

- The original *Greyscale* or *Colour* image, which remains unchanged.
- The *Binary Input Image,* which is the result of the thresholding and binary processing steps, showing the features of interest.
- The *Binary Output Image,* which represents the edited image still in binary format.

Basic procedure

The basic procedure for Binary Image Editing is as follows:

1. In the *Processing Sequence* control panel, click on *Binary Image Editing*. The *Binary Edit* panel appears.
2. Use the *Select Method* pane to control the way in which the editing is performed.
3. Use the *Select Editing Tool* to add or erase features from your measurements. The tools available depend on which *Method* you choose.
4. Use the *Common* controls to undo and redo actions, delete all drawn regions, and control the cursor colour.
5. Use the *Show Binary* check box to see the effect of your drawn shapes and selections before applying your changes.
6. Click *Apply* to merge all the drawings and selections with the Binary Mask.

(Continued on next page)

Setting up the workspace

To get the best experience when setting an image threshold, we recommend that you make the following Workspace settings:

1. Click on the *Show Viewer Options* button on the *Side Tool Bar*.
2. From the context menu, click to enable all the options:

Toggling the cursor colour

You can toggle the cursor colour between red, black or white to suit the image by clicking on the Cursor Colour button in the *Common* pane.

You can draw straight lines on a Binary Image Mask to join or separate features that were not perfectly detected in previous processing steps. This method works in one of two modes:

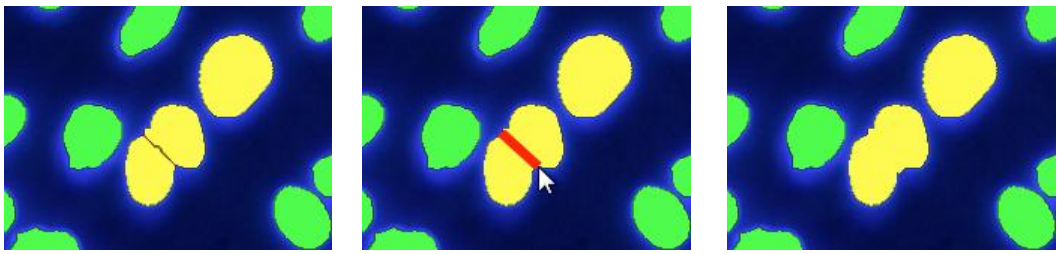
- *Add* mode: Use this to join features that may have been cut as part of the Binary Processing Pre-Filter[1081] step, or to include fibres that were not detected
- *Erase* mode: Use this to cut features that may have been joined as part of the Binary Processing Pre-Filter[1081] step, or to ignore fibres that were detected

To draw a line:

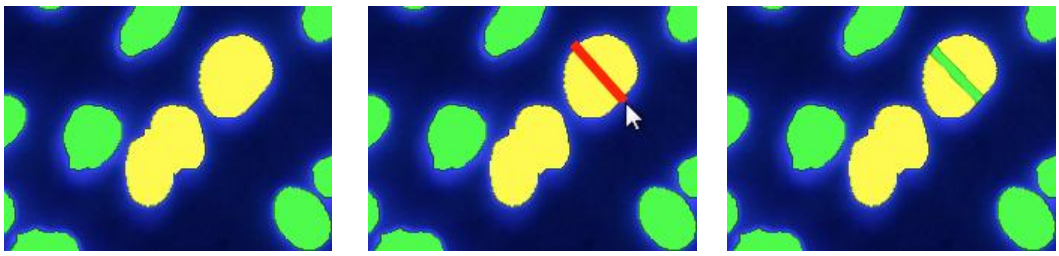
1. Enable *Show Binary* to display the selected features in the current binary mask colour.
2. Choose the *Draw Straight Lines* method from the drop-down menu.

   The *Line* tool is selected automatically.
3. Click to enable the *Add* or *Erase* radio button.
4. Set the *Line Width*.
5. Drag the cursor to draw a line.
   - *Add* mode: The line is shown in red until you click *Apply*, then it becomes part of the Binary Output Image
   - *Erase* mode: As the line affects the mask, it is shown in the mask colour set in the Binary Processing Pre-Filter[1081] step
6. If necessary, use the *Undo* and Redo buttons to review any lines you have drawn.
7. When you have finished drawing lines, click on the *Apply* button.

Example: Add mode

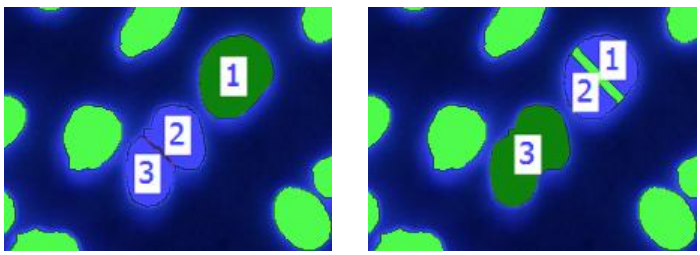


Example: Erase mode



Processed image, before and after drawn lines

The illustrations below show the results of processing an image before and after the Add and Erase lines used in the examples above.



Select Exisiting Regions

You can draw regions of interest on an image mask to include or exclude features that were missed or included incorrectly in previous processing steps. This method works in one of two modes:

- *Accept* mode: Enclosed features in selected regions will be included in the measurements
- *Reject* mode: Enclosed features in selected regions will be excluded from the measurements

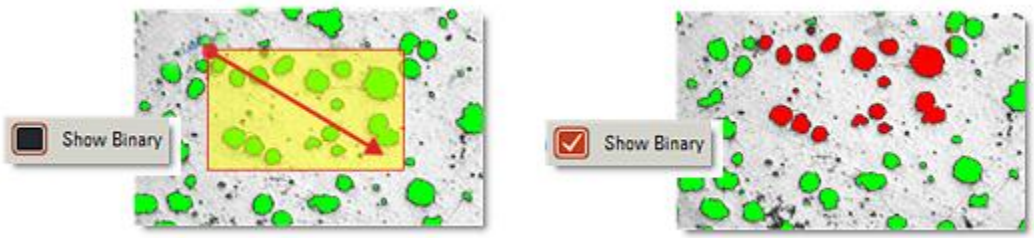
To select an existing region:

1. Enable *Show Binary* to display the selected features in the current binary mask colour.
2. Choose the *Select Existing Regions* method from the drop-down menu.
3. Click to enable the *Accept* or *Reject* radio button.
4. Click to activate a drawing tool to create the RoI:
   - *Fill Area*
   - *Rectangle*
   - *Circle*
   - *Ellipse*
5. Drag the cursor to draw the RoI.

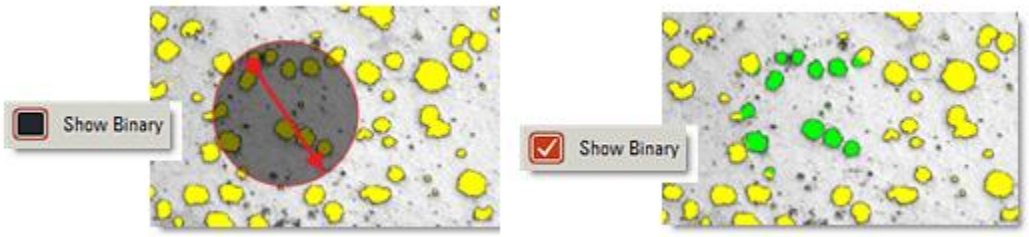
   Fill Area tool: Left-click at each point on the shape, and right-click on the final point to complete the filled area.

   The drawn region is filled with a semi-transparent colour to make it easier to identify the selected features.
6. If necessary, use the *Undo* and Redo buttons to review any regions you have drawn.
7. When you have finished drawing regions, click on the *Apply* button.

Example: Accept mode



Example: Reject mode



Draw New Shapes

You can draw lines and shapes on an Binary Image Mask to add or erase regions that were not perfectly detected in previous processing steps. This method works in one of two modes:

- *Add* mode: Use this to edit features that may have been missed as part of the Binary Processing Pre-Filter[1081] step
- *Erase* mode: Use this to exclude features that may have been included as part of the Binary Processing Pre-Filter[1081] step

To draw a shape:

1. Enable *Show Binary* to display the selected features in the current binary mask colour.
2. Choose the *Draw New Shapes* method from the drop-down menu.
3. Click to activate a drawing tool:
    - *Line:* Use this in the same way as Draw Straight Lines[1097], for example to separate features that may have been joined incorrectly
    - *Freehand Line:* Use this to trace and join features that are not closed shapes, such as fibres; left-click at each point on the shape, and right-click on the final point to complete it
    - *Filled Area*: Left-click at each point on the shape, and right-click on the final point to complete it
    - *Rectangle*
    - *Circle*
    - *Ellipse*
4. Click to enable the *Add* or *Erase* radio button.
5. Set the *Line Width*.
6. Draw a shape:
    - *Add* mode: The shape is shown in red until you have finished drawing, then it becomes part of the Binary Output Image
    - *Erase* mode: As the line affects the mask, it is shown in the mask colour set in the Binary Processing Pre-Filter[1081] step
7. If necessary, use the *Undo* and Redo buttons to review any shapes you have drawn.
8. When you have finished drawing shapes, click on the *Apply* button.

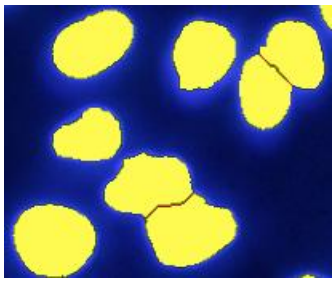
Click to Select Regions

The *Click to Select Regions* method lets you quickly include or exclude features that have already been detected.

- *Keep* mode: Use this when your image has many detected features, but you only want to include a few; All features are deselected to begin with; simply click on those you wish to keep
- *Delete* mode: Use this when your image has many detected features, but you only want to delete (reject) a few; All features are included to begin with; simply click on those you wish to delete

To select an existing region:

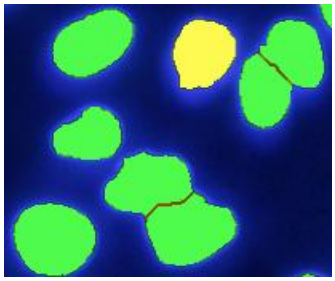
1. Enable *Show Binary* to display the selected features in the current binary mask colour.



2. Choose the *Click to Select Regions* method from the drop-down menu. The *Selection* tool is enabled (you cannot select any other tools).
3. Click to enable the *Keep* or *Delete* radio button.
4. Click on features to keep or delete them, depending on the setting in the previous step.
5. If necessary, use the *Undo* and Redo buttons to review your changes.

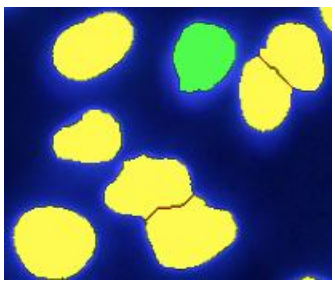
Example: Keep mode

When you click on a feature, it is marked as 'keep'; all others are marked as 'deleted'. You can click to select multiple regions.



Example: Delete mode

When you click on a feature, it is marked as 'deleted'; all others are marked as 'keep'. You can click to select multiple regions.



Show Binary

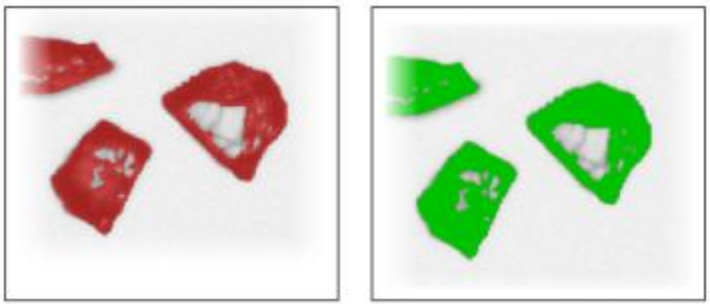
When the *Show Binary* checkbox is enabled, features selected for measurement are displayed in the right-hand side of the *Viewer*. You can change the default highlight colour (see below).

Colour

You can change the colour used to display the selected pixels in the *Binary Output Image*. For example, if the features you are trying to measure are red, you might want to change the highlight Colour to a bright blue so the features are easy to distinguish.

1. Click on the *Colour* button.
2. Use the standard Windows *Select Colour* dialog to choose a new highlight colour.
3. Click *OK*.

The illustration below shows the effect of changing the binary *Colour* from red to green.



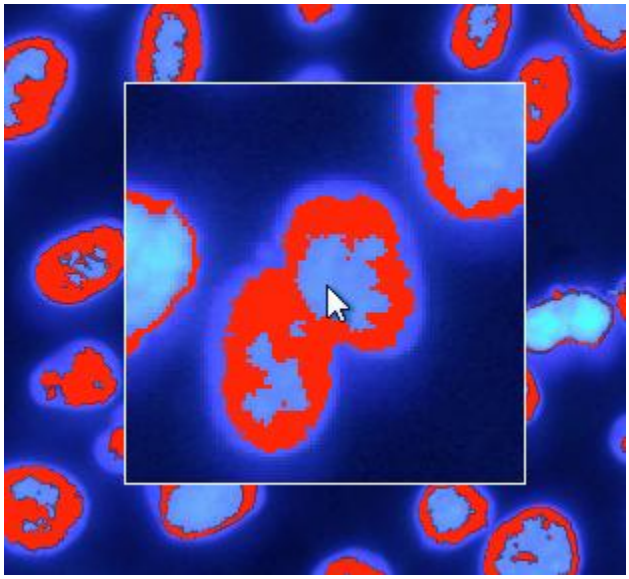
Don't forget, you can right-click in the *Viewer* to temporarily disable the highlighted features, to help you see whether you have set your threshold correctly.

To switch between the *Binary Output Image* and the *Input Image:*

- Right-click on the *Binary Output Image* to hide the binary image and reveal the original input.

To enlarge parts of either the *Input Image* or the *Binary Output Image:*

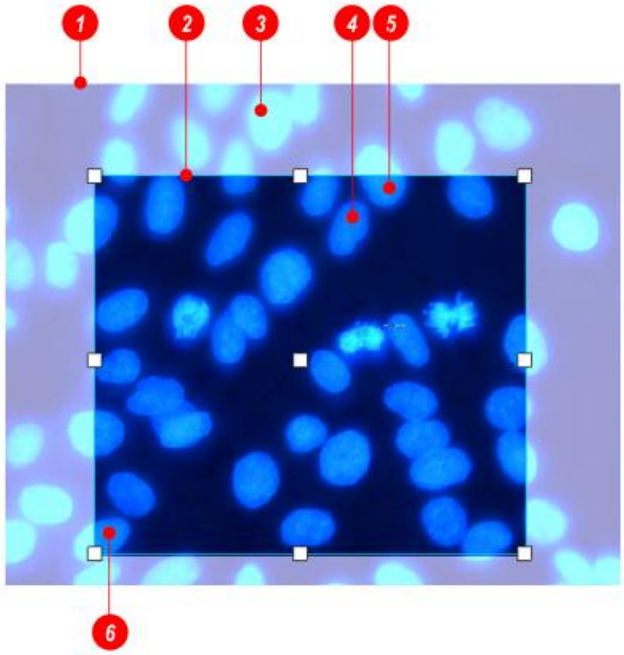
- Hold down the Shift key (but do **not** click). The magnified area tracks the mouse movements. Release the Shift key to close the magnifier.



A *Measure Frame* determines the area of an image that will be used for analysis and measurement. It can be the entire image or more usually a selected, representative area.

- A *Field Measurement* processes all binary pixels inside the Measure Frame and none outside it.
- For a *Feature Measurement*, the Measure Frame determines which features to include or exclude, as described below. A valid feature will have all visible pixels processed, even if they fall outside the Measure Frame, while a feature rejected by the Measure Frame will not be considered at all. (**Important**: see Clear Detail Outside Frame 1107.)

The main function of the Measure Frame is to avoid measurement errors, especially with Feature Measurements and objects cut by the edge of the field of view. When acquiring an image set by automatically stepping a motorised stage, the images will be overlapped so that every feature appears in full in at least one image. The Measure Frame is set to correspond to the overlap and ensures that incomplete features are rejected, and that every feature is measured only once.



The illustration shows the following features:

1. The edge of the image.
2. A typical *Measure Frame* outlined in blue.
3. The Guard Region.
4. An object that is completely within the Measure Frame, and is included in the results.
5. An object that lies partly outside the Measure Frame but, since its bottom right-hand pixel is inside the Measure Frame, it *is* included.
6. An object that is mostly within the Measure Frame but, since its bottom right-hand pixel is either touching or outside the frame boundary, it is *not* included.

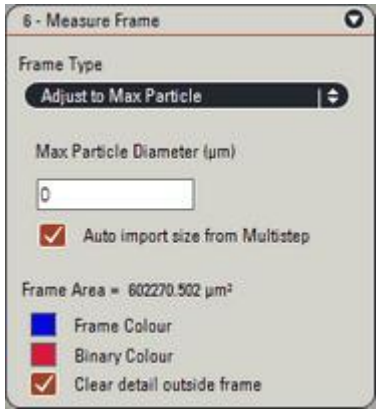
Rules for including/excluding objects

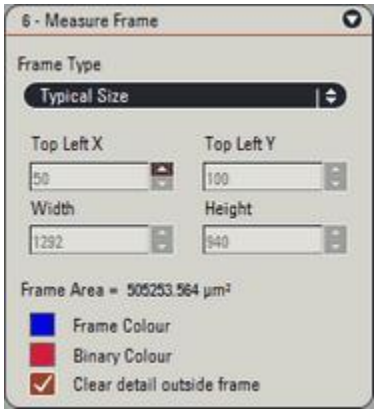
- Any object that has its bottom right-hand pixel lying within (not on) the Measure Frame boundary is included in the analysis, even if any of its other pixels lie in the Guard Region – outside the Measure Frame boundary.
- Field measurements are performed within the Measure Frame; any pixels outside the Measure Frame are not included.

Create a Measure Frame

To create a Measure Frame:

1. Click on the *Measure Frame* option in the *Processing Sequence* panel.
2. Display the *Frame Type*[1105] drop-down menu and select the required option from the list.





Entire Image

The frame boundary coincides with the edges of the image.

Adjust to Max Particle

To create a frame based on a user-defined particle size:

1. Enter a value (µm) in the *Max Particle Diameter* field.

The *Guard Region* is based on:

- 100% of the particle diameter at the top
- 50% of the particle diameter on the sides
- 0% of the particle diameter at the bottom.

To create a *Measure Frame* based on the particle size in a sequence, such as a series of *Multistep* images:

1. Click to enable the *Auto Import Size from MultiStep* check box.

The check box is only available if the sequence is selected for measurement and appended.

Typical Size

This option will suit a wide variety of microscope images. Based on an 'average' particle size, it creates a *Guard Region* with:

- 100% of average particle diameter at the top
- 50% of average particle diameter on the sides
- 0% at the bottom.

Manually Define

This option allows you to create a frame of specific dimensions and position it anywhere on the image.

1. Select *Manually Define* from the *Frame Type* drop down menu.
2. Enter the *Top Left X/Y* coordinates.
3. Enter the *Bottom Right X/Y* coordinates.
4. (Alternatively, drag the *Measure Frame* handles to the required size.)
5. Reposition the *Measure Frame* by dragging its centre handle.

Measure Frame colour

1. Click on the Frame *Colour* button.
2. On the *Select Colour* dialog, choose a new colour and click *OK*.

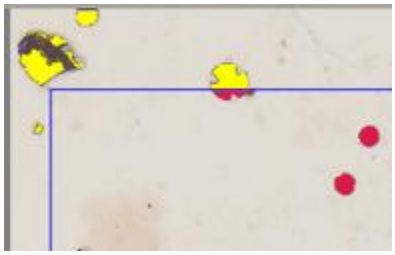
Binary colour

1. Click on the Binary *Colour* button.
2. On the *Select Colour* dialog, choose a new colour and click *OK*.

This function should be used for visualisation only, and should normally be *deselected* before proceeding. In particular, it is NOT recommended with Feature Measurements, since it actually modifies the binary. (In the illustration below, the red part of the feature at the top of the frame would be *incorrectly* measured).

To highlight the difference between features inside and outside the *Measure Frame*:

1. Click to enable the *Clear detail outside frame* check box.



Select Results

Use the *Select Results* panel to control the range of results presented on the *Grid*.

Note: You can fine-tune the results displayed on the *Grid* using the Configure tool[1033].

Feature Measurement

Measurements relate to individual features, and include parameters such as size and length.

1. Enable the check box to make the *Feature Details* and *Feature Statistics* tabs visible on the *Grid*.
2. Use the drop-down menu to specify which parameters are measured for each feature:
   - *All Parameters*: All possible measurements are made but the range can be modified.
   - *Predefined Parameters*: Reduces processing time; only commonly-used parameters are measured.

Field Measurement

Field measurements relate to a complete *Field of View;* the data produced is called *Field Data,* and includes parameters such as *Field Area* and *Field Perimeter*.

Field Data represents the summed value for all objects within the *Measure Frame*[1103], regardless of whether they are touching or separate (whereas *Feature Data* produces a separate value for each isolated feature that ends inside the *Measure Frame)*.

1. Enable the check box to make the *Field Details* and *Field Statistics* tabs visible on the *Grid*.
2. Use the drop-down menu to specify which parameters are measured for each feature:
   - *Field Parameters:* All 2D field measurements are made.
   - *Field with Grey Parameters:* In addition to the 2D measurements, the RGB or Intensity values are also measured within the binary mask.

Profile Measurement

Shows the variation in grey level intensity for a monochrome image or the RGB values for a colour image along a specified line. See also Profile Measurements[1125].

1. Enable the check box to make the *Profile Details* and *Profile Statistics* tabs visible on the *Grid*.

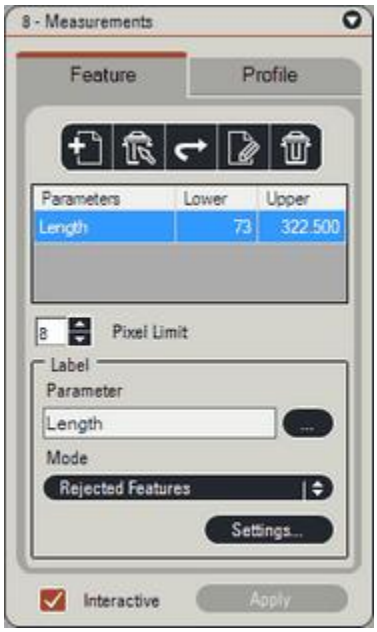
See also:

Configure the Grid[1033]

After deciding which measurement results you want to work with (using the Select Results[1108] step), use the *Measurements* panel to control which features are included in the results, and labelled on the binary output image.

Most Parameters can accept lower and upper limits so that only features falling within the scope of the limits are found and labelled.

These range pair values can be saved together with all of the other settings as a *Configuration*[1041] to be restored and used at any time.



- Use the *Filter*[1111] toolbar to control which features are included in the results. Active filter items appear in the parameter filter list, below the toolbar (you can fine-tune the filter limits here)
- Use the *Pixel Limit* field to apply a tolerance (in pixels) to the lower and upper limits of a Filter; Thee purpose of this function is to eliminate small spurious particles due to noise in the image.
- In the *Label* pane, use the *Parameter*[1113] browse button to determine which parameter is labelled on the output image.

  See here[1115] for detailed descriptions of the parameters.
- Use the *Mode*[1122] drop-down menu to specify which features are labelled (you can choose to label only accepted or rejected features, for example).
- Click on the *Settings*[1122] button to configure the appearance and position of feature labels.
- Use the *Interactive* check box to specify whether changes you make to the output image are updated in real time as you work, or only when you click the *Apply* button.

See also:

Profile Measurements[1125]

Filter Tool Bar

The *Filter* tool bar on the *Measurements* control panel contains the following tools:

 *New Filter*: See Creating a New Filter 1111

 *Delete Selected Filter*: Deletes the currently selected filter from the list

 *Reset to Default Limits*:

 *Reset to Measured Limits*: Resets filter limits to values based on the current image. The lower and upper limits actually measured on the image are used in the filter.
For example, if the Area Filter was originally set to 500 (Lower) and 2500 (Upper), and a measurement yielded a feature low of 549 and a feature high of 2317, these two values would be used in the filter

 *Clear All Filters*:

Creating a New Filter

When creating a filter, the list of available parameters will depend upon the *All* or *Predefined* option in the Select Results[1108] step.

1. Click on the *New Filter* button in the tool bar.

2. On the *Select Measurement Parameters* dialog, expand a parameter group if necessary and enable one or more *Parameters.*
3. Click *OK*.

The chosen *Parameter* names appear in the *Filter List* with the default lower and upper values. These values are based upon the image in the *Viewer*.

See Setting Filter Limits Manually[1112]

Setting Filter Limits Manually

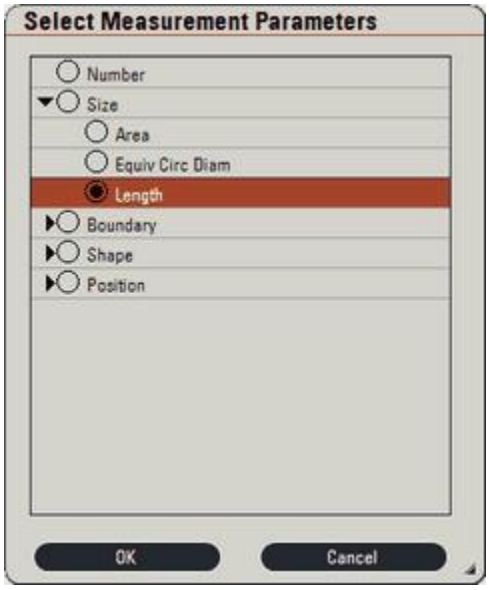
To edit filter limits manually:

1. Click in a *Lower* or *Upper* field in the filter list.
2. Enter the required limit for this parameter.
3. Repeat until you have set all the upper and lower limits.

Choosing which Parameter is Labelled

You can choose which parameter[1114] is labelled on the binary output image.

1. In the *Label* pane of the *Measurement* panel, click on the browse button to the right of the *Parameter* field. This will display the *Select Measurement Parameters* dialog.



   Remember, the list of parameters from which you can choose is set in the Select Results[1108] step:

   - If you selected *Predefined Parameters*, the *Select Measurement Parameters* dialog will display only the most commonly used parameters.
   - If you selected *All Parameters*, all possible measurement parameters will appear.

2. If necessary, expand a parameter sub list (e.g. *Size*) by clicking the arrow.
3. Select the parameter to display (e.g. *Length*) by enabling the appropriate radio button.
4. Click *OK.*

You can resize the dialog by dragging the small arrow in the bottom right corner.

See also:

Parameter Descriptions[1114]

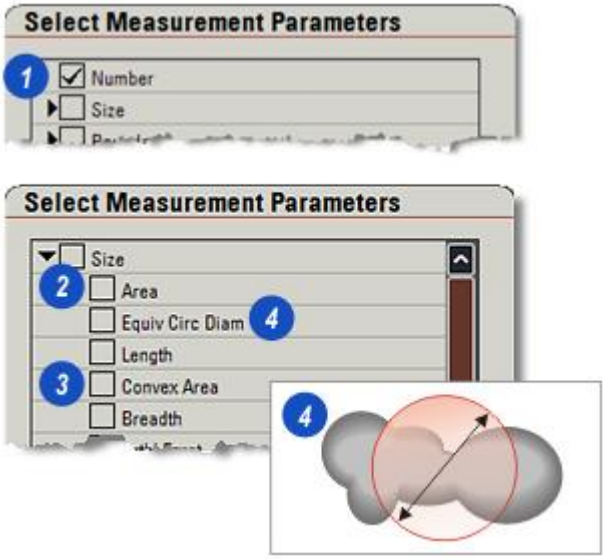
Labelling Features[1122]

Parameter Descriptions

This section describes the different measurement parameters.

- Number and Size 1115
- Shape 1117
- Boundary 1118
- Position 1119
- Topology/Intensity 1121

All measurements are quoted in calibrated units.



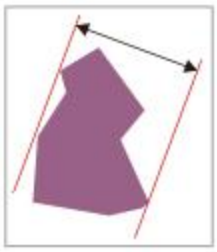
Number

- *Number*: Carries out a count of all the selected objects on the Binary Output Image.

Size

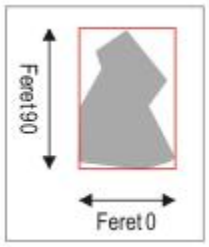
- *Area*: Measures the Area of every selected object.
- *Convex Area*: Derived from the mean Feret diameter approximating to the enclosing polygon.
- *Equivalent Circle Diameter*: The diameter of a circle that has the same area as the feature.
- *Length*: The greatest distance between parallel lines drawn through 2 points on a feature's boundary regardless of orientation. Also called Max Feret Diameter.

- *Breadth*: The shortest distance between parallel lines drawn through 2 points on a feature's boundary regardless of orientation.



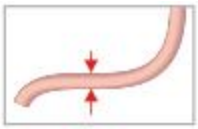
(Continued on next page)

- *Orthogonal Feret*: The Feret diameter perpendicular to the Max Feret diameter or length.
- *Feret 0*: The greatest horizontal distance (width) measured in the horizontal direction.
- *Feret 90*: The greatest vertical distance (height) measured between parallel lines in the vertical direction. Feret 0 and Feret 90 are the equivalent of the width and height of a Bounding Box around the feature.

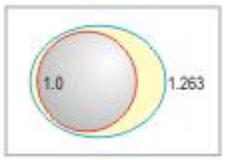


- *Curve Length*: The Curve Length can measure the actual length of an irregular feature – a piece of string for example - the curves of which may overlap.

- *Curve Width*: Measures the mean or average width of a feature (the feature will probably not have a uniform thickness like it does in the illustration below).

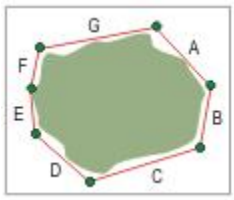


- *Roundness*: The perfect circle has a notional value of 1.0. Any variations from the circular are reflected by an increase in the notional value.

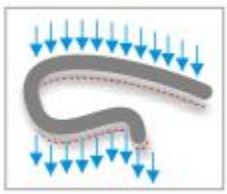


- *Aspect Ratio*: Object length divided by object breadth or Feret Length/Feret Breadth.
- *Fullness Ratio*: The square root of the object area divided by the object's Convex Area. v(A/CA).

- *Perimeter*: Distance around the boundary of the feature, given in the current caibration units. It includes all inlets and projections and compensates for the edge orientation.
- *Convex Perimeter*: Derived from the Feret diameter and approximates to the perimeter of the enclosing polygon.



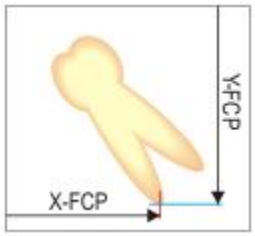
- *Vertical Projection*: The distance representing the shadow cast by a feature if light were impinging upon it from a vertical direction. Presented in the current calibration units.



- *Horizontal Projection*: The distance representing the shadow cast by a feature if light were impinging upon it from a horizontal direction. Presented in the current calibration units.

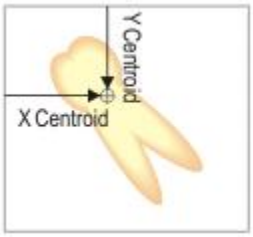
Note: All the parameters on this page are in pixels.

FCP = Feature Count Point; represents the bottom rightmost pixel in the feature.



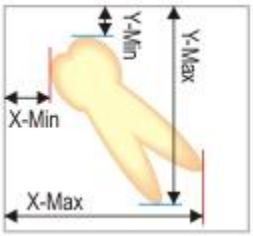
- *X-FCP*: measured from the left-hand edge of the image to the Feature Count Point
- *Y-FCP*: measured from the top edge of the image to the Feature Count Point

Centroid is the Centre of Mass of a feature. Its position is measured from the edges of the image.



- *X Centroid*: The distance from the left hand edge
- *Y Centroid*: The distance from the feature's top edge.

The feature's position co-ordinates on the image:

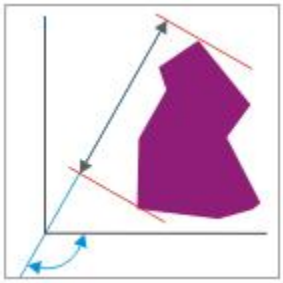


- *X-Min* and *X-Max*: The boundary pixel closest to and furthest from, the left hand edge of the image.
- *Y-Min* and *Y-Max*: The boundary pixel closest to and furthest from, the top edge of the image.

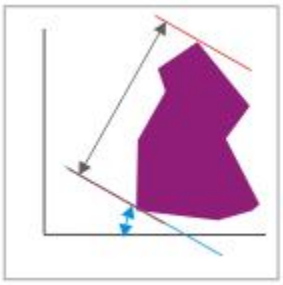
Note: All the parameters on this page are in degrees, counting clockwise from vertical, which is 0°.

Derived Orientation is recommended over orientation for measurement of direction. It gives more accurate results than the traditional Orientation.

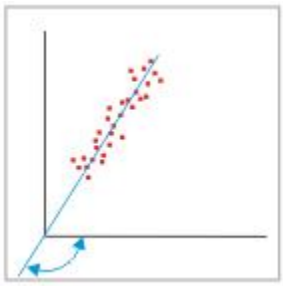
- *Orientation*: The angle of the Feret Length to the horizontal.



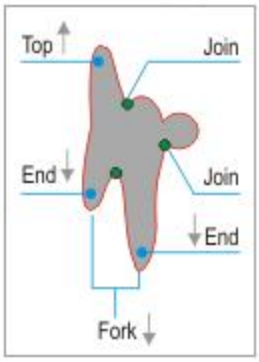
- *Orthogonal Orientation*: The angle perpendicular to the Orientation (Feret Length) - essentially the Orientation minus 90°).



- *Derived Orientation*: The angle representing a line plotted through a range of pixel co-ordinates.



Topology



- Forks: The number of downward projecting fork-shaped limbs.
- Joins: The number of points at which a projection (limb) joins the feature.
- Tops: The count of upward-extending projections (limbs).
- Ends: The number of downward-extending projections (limbs).

Intensity

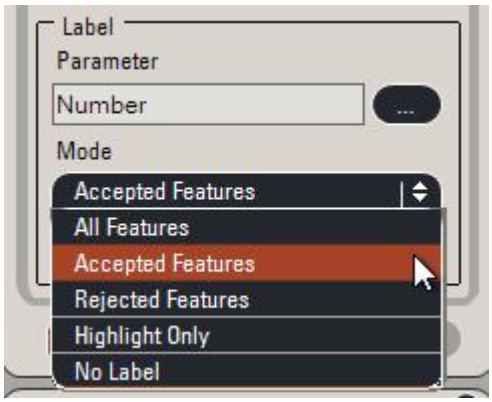
All intensity parameters return values from 0-255 where zero represents black and 255 is white.

- *Integrated Grey*: Sum of the grey value of all of the pixels within the feature.
- *Grey Mean*: Average brightness of the pixels in the feature; calculated from the sum of the value of all of the pixels in the feature (Integrated Grey) divided by the number of pixels in the feature (Equivalent to Area).
- *Grey Variance*: Variance (Standard Deviation^2) of the values of the pixels in the feature. Grey Variance can give an indication of surface texture; smooth objects have a lower GV.

Labelling Mode

To specify which features on the image are labelled:

1. Display the *Mode* drop-down menu in the *Label* pane.



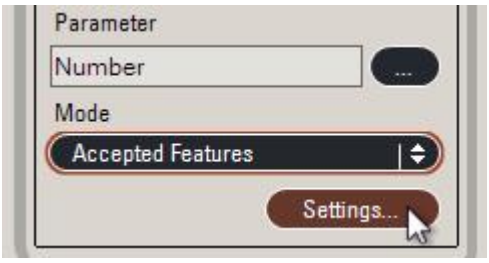
2. Click to select an option.
   - *All Features*: All the features highlighted on the *Binary Output Image* are labelled regardless of whether they fall inside limits or not.
   - *Accepted Features*: Features are labelled that fall within specified limits. For example, if the *Area* parameters are set to find image features no smaller than 50px2 and no greater than 150px2, then only those features that fall within the parameters are labelled. All others are ignored.
   - *Rejected Features:* Use this option to label features that fall outside the set parameters. Using the example above, features smaller than 50px2 and greater than 150px2 would be labelled.
   - *None*: Turns off labelling.

(Continued on next page)

Label Control

To change the way *Labels* and results are displayed on the *Measurements Binary Output Image:*

1. On the *Label* panel, click on the *Settings* button to display the *Label Control* dialog.

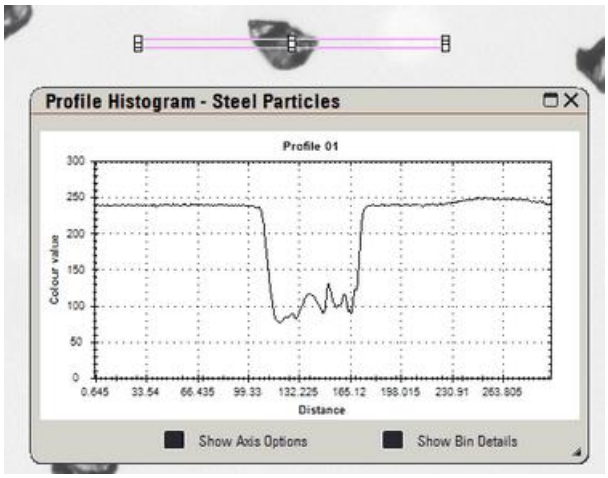


2. Use the *Position* drop-down menu to set the location of the label with respect to a feature.
   - *Centroid* displays the label in the centre of the object.
   - *Below-Centre* displays the label below and on the centre line of the object.
   - *Below-Left* displays the label below and to the left of the feature.
3. Specify whether the parameter *Name* and *Units* should be displayed:
   - Enable the *Name* check box to display the *Parameter* – Length, Number, Diameter etc – alongside the feature and result.
   - Enable the *Unit* check box to display the measurement units – pixels (px), millimetres (mm) – next to the feature.
   - **Note**: To set the number of digits following the decimal place on the display, change the *Measurement Display* value on the main *Preferences* > *Image* dialog.
4. Click on the *Colour* swatch to display a standard colour selector, and specify the label colour.
5. Click on the *Font* button to display a standard font selector dialog, and specify the label colour.
6. Click on the *Background* swatch to display a standard colour selector dialog, and specify a background colour for the label text boxes.
7. Use the *Outline* box to display an outline around the selected features. A value of '0' turns off outlining.
   Note: The *Outline* colour is the same as the *Label Font* colour.

See Results Grid[1033] and Select Results[1108] for details of displaying and managing results.

The *Profile* panel allows you to measure the distribution of grey or colour levels along a line drawn across an image. For example, you might want to measure the distance between the bands on a chromosome. You can view a line graph of the profile using the Histogram[1127] panel.

- For greyscale images, you just get one set of measurements (the greyscale levels).
- For colour images, you can measure the levels of the separate RGB channels across the image.



To set up a Profile measurement:

1. Display the *Profile* tab on the *Measurements* panel.
2. Set the *Profile Type* using the drop-down menu:
   - *Horizontal*: The profile measurement runs along a horizontal line through the centre line of your measurement rectangle.
   - *Vertical*: The profile measurement runs along a vertical line through the centre line of your measurement rectangle.
   - *Line*: The profile measurements are taken along a freehand path that you draw on the image.
3. Choose whether to enable the *Average* option; This can provide smoothing of the profile for regular Vertical or Horizontal structures, or for a set number of pixels around a Line profile.
   - In Horizontal mode, this averages each column in the measurement rectangle; In Vertical mode, it averages each row.
   - In *Line* mode, this averages the profile for a set number of pixels around the line you draw (defined by *Thickness* in the next step).
4. If you selected a *Line* profile and enabled the *Average* option, set the line *Thickness*.
5. Draw the measurement rectangle or line on the image, using the mouse.
   - If you chose *Horizontal* or *Vertical* profile type, drag and re-size the measurement rectangle using the mouse. You can also change the size and position of the measurement rectangle using the *Top Left X/Y* and *Width/Height* fields.
   - If you chose the *Line* profile type, draw a line on the image by left-clicking to place anchor points, then right-click to place the final point.

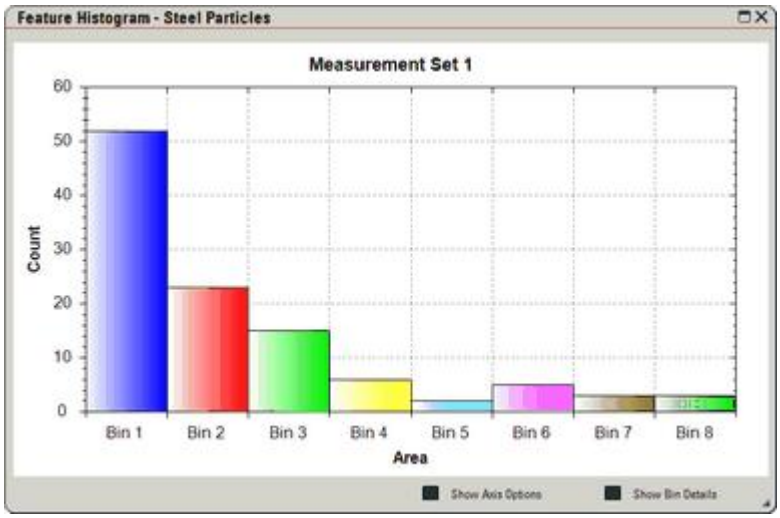
(Continued on next page)

6. If you are working on a colour image, choose which of the *R G B* channels to include in the measurements (you must include at least one).
7. Click on the *Profile Colour* swatch to display a standard colour picker dialog, and select the colour used to display the measurement region or line.

You will see the results of your measurements in the *Profile Details* and *Profile Statistics* tabs in the Results Grid [1033].

Use the Histogram [1130] panel to view a graph of the results.

Use the *Histogram* control panel to display results graphically[1139] as a vertical or horizontal bar chart, or a pie chart.



You can also display a Profile[1125] histogram, which shows how the colour or greyscale value of an image varies with distance from a specified origin.

Results are grouped into Bins. You can control[1134] how Bins are displayed.

To access the *Histogram* control panel:

1. Click on the *Histogram* entry in the main *Processing Sequence* control panel.

The *Histogram* control panel has the following features:

- *Tool Bar*[1128]
- Feature, Field, Profile[1130] menu
- *Show Histogram* button
- *Settings*[1138] group
- Bins[1134] configuration. You can specify the number of Bins, and the upper and lower bounds.
- *Show Colour Coded Image:* Enable this to preview how features will be allocated to Bins on the Histogram (colours are defined here[1135]).

Histogram Tool Bar

The Histogram control panel has the following tools:

- *Create new histogram*: Click this button, enter a name for a new Histogram and click *OK*. You can now set up the new Histogram then click the *Save* button.
- *Edit current histogram*: allows you to change the name of the currently selected Histogram. Click in the *Name* text box on the dialog and type a new unique name. Click *OK*.
- *Save selected Histogram Setting*: Save the settings so far. Any subsequent alterations to the Histogram settings can be saved and loaded (retrieved) at a later date to replicate the Histogram display.
- *Delete current histogram*: Delete all the Histogram settings. Click *OK* to confirm the deletion. This action cannot be reversed.
- *Load selected Histogram Settings*: Click to revert to the saved settings for the current Histogram.

Create a New Histogram

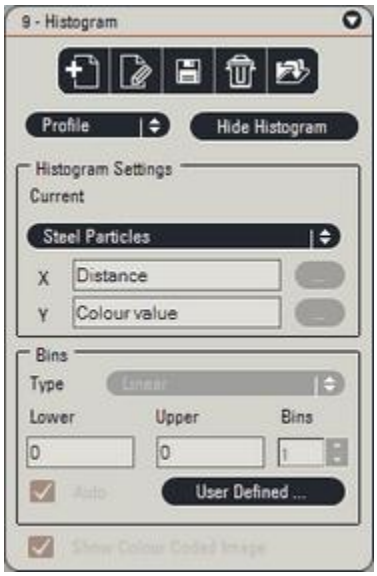
1. Click on the *Create new histogram* button on the *Tool Bar*. The resulting file will contain the current settings, along with the Field and Feature definitions. These are used in the report.

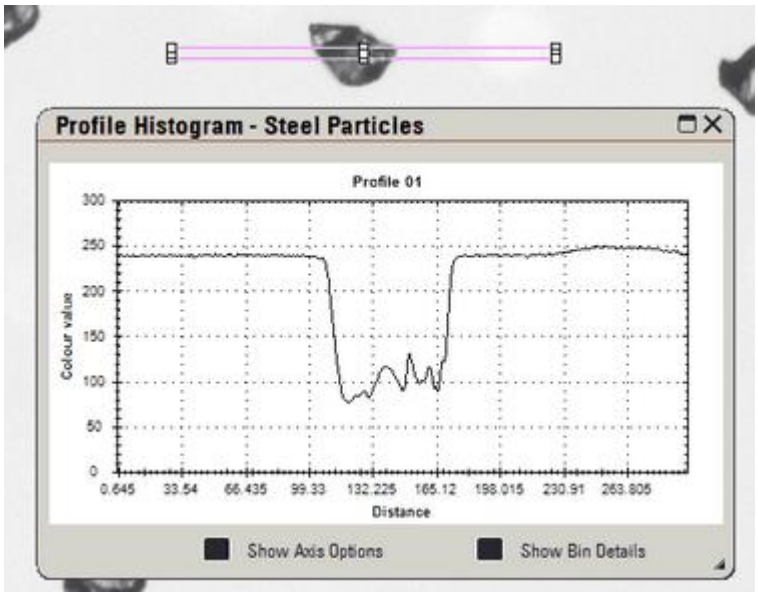
2. On the *Create New Histogram Settings* dialog, click inside the *Name* text box and type a new name for the Histogram.

3. Click *OK*.
4. The name of the new Histogram is now available in the *Current* drop-down menu.

Switch between different Histogram types using the drop-down menu:

- *Feature*: Includes parameters such as size, shape, position, orientation, intensity
- *Field*: Includes parameters that are summed for the entire Field of View, such as area percent, total area and perimeter
- *Profile*: A line graph, plotting colour or greyscale value against distance from a specified origin (defined in the *Profile* [1125] tab of the *Measurements* panel)





Note: The options available in the drop-down menu are defined by the settings in Select Results [1108].

Showing and Hiding the Histogram

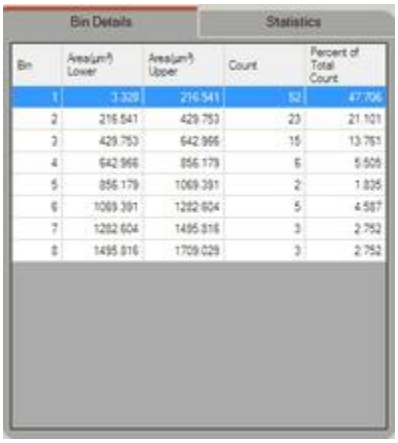
The button near the top of the *Histogram* control panel changes, depending whether the Histogram is currently displayed:

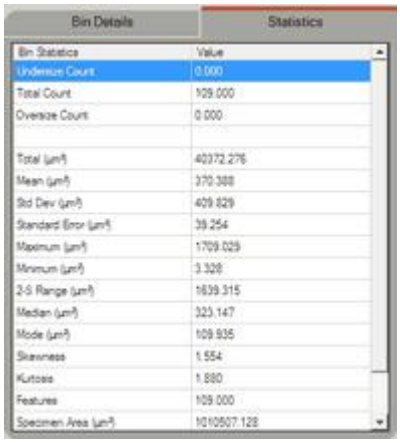
- If the Histogram is not displayed, click *Show Histogram*

- If the Histogram is displayed, click *Hide Histogram, or* click the cross icon in the top of the Histogram window

Enable the *Show Bin Details* check box at the bottom of the *Histogram* window.

- *Bin Details* tab: displays a comprehensive breakdown of the results in each bin
- *Statistics* tab: Gives statistical information about the results (e.g. Standard Deviation and 2-S range).





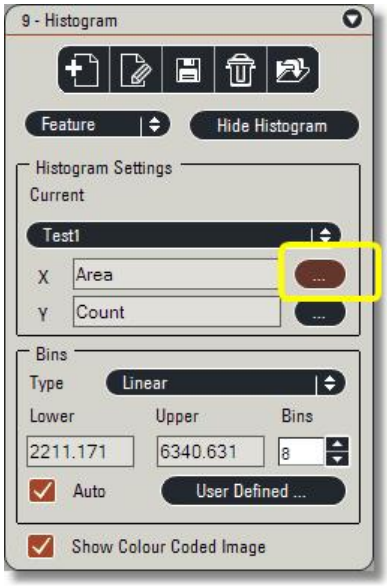
Setting X and Y axis parameters

You can specify which measurement parameter is used for each axis of the Histogram.

Note: For Profile[1125] histograms, the axis parameters are not editable; you are measuring levels against distance from an origin.

For example, to set the X axis parameter:

1. Click on the Browse button to the right of the X field.



2. On the *Select Measurement Parameters* dialog, expand the required parameter group.
3. Select the parameter that will be used on the X axis and click *OK*.

Selecting a configuration

If you have previously created[1129] or saved a Histogram configuration, you can select it using the *Current* drop-down menu in the *Histogram Settings* panel.

To configure the bounds for Histogram Bins:

1. Choose an option from the *Type* drop-down menu in the *Bins* pane:
   - *Linear* applies limits to each bin in a linear progression from the lower limit to the upper limit.
   - *Log* uses the same value range and bin count but applies logarithmic increments.
   - *User Defined* allows you to customise the bin limits, and the names and colours used on the histogram. See Bin Setup: User Defined Settings 1135.

Auto mode

In Auto mode, the upper and lower limits are automatically set to the largest and smallest values measured in the current feature results (which could possibly come from more than one image).

This is the default setting, so that you are sure to see something in the histogram (if, for example, you don't know what values to expect).

Setting the Upper and Lower limits manually

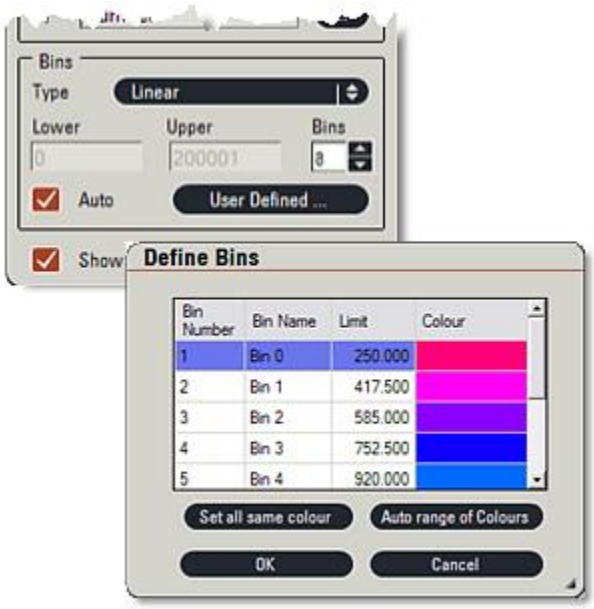
Turn off *Auto* mode if you know the size range you want, and don't want the limits to change with every measurement:

1. Disable the *Auto* check box.
2. Enter the highest and lowest results to be displayed on the Histogram in the *Upper* and *Lower Limit* fields.
3. Set the number of Bins.

See also Bin Setup: User Defined Settings 1135.

In the Type drop-down menu, *User Defined* allows you to manually define the limits for your classes, rather than having them imposed by the software. This is so you can have different sized classes corresponding to special requirements. Clicking the *User Defined* button opens the Define Bins dialog where you can define the size range, name and colour for each bin.

You have to select the *User Defined* mode for these settings to be applied. In Linear or Logarithmic modes the class limits are automatically calculated.



Setting user-defined limits

Note: While *Auto* mode is enabled, you can change all bin limits *except* the Upper and Lower limits.

1. On the *Histogram* control panel, disable *Auto* mode.
2. Select *User Defined* from the *Type* drop-down menu.
3. Click the *User Defined* button.
4. Click in a *Limit* field and enter a new limit for the selected Bin.
5. If *Auto* mode is off, you can change the upper and lower limits, and these will be reflected on the main Histogram control panel.
6. Click *OK*.

Setting user-defined colours and bin names

You can always override the default bin names and colours (even when *Linear* or *Logarithmic* is selected in the *Type* drop-down menu):

1. Click the *User Defined* button.
2. In the *Define Bins* dialog, click in a *Bin Name* field and enter a new name.
3. Do one of the following to change bin colours:
   - Click *Auto Range* to set the Bin colours to preset shades
   - Double-click an individual *Colour* swatch and select a colour in the resulting dialog
   - Click *Set All Same Colour* and select a colour for all the bins in the resulting dialog
4. Click *OK*.

There are two check boxes at the bottom right of the Histogram display:

- *Show Axis Options* 1137: Reveals the *Y Axis Options* panel, where you can set the display mode, style and scales; you can also see the configuration panels for the results and the Histogram labels.
- *Show Bin Details* 1132: Displays the results alongside the Histogram.

Y Axis Options

- *Range*: If Auto is disabled, you can specify the Y axis range
- *Auto*: If enabled, optimum Y axis range will be calculated and displayed automatically
- *%*: Results displayed as a percentage of all measurements
- *Log*: Results displayed on a logarithmic scale

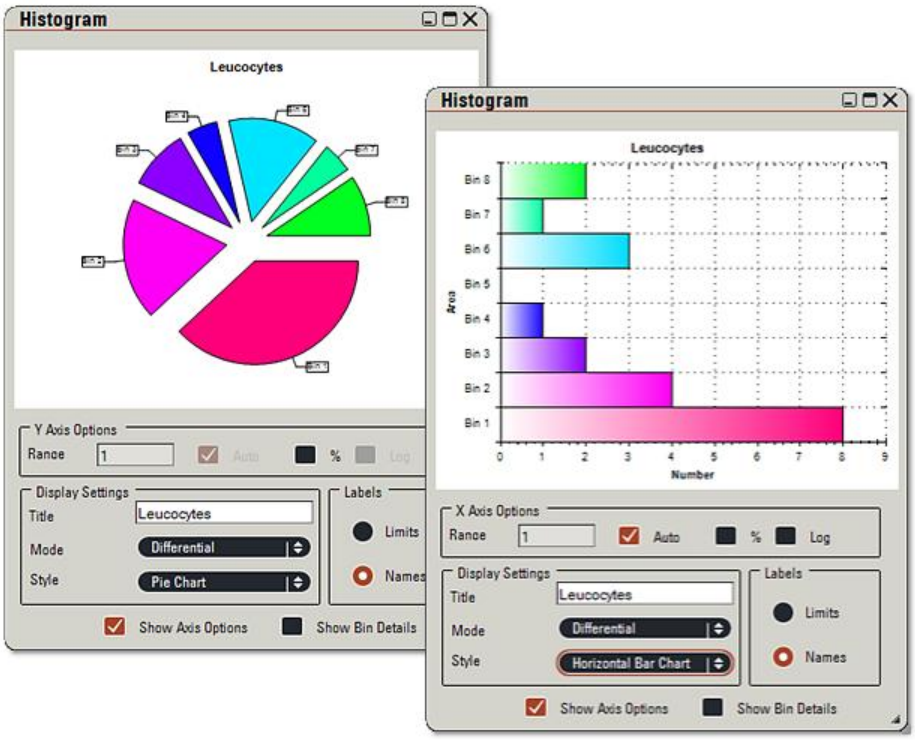
Labels

- *Limits*: Display the range of results allocated to each bin along the X axis.
- *Names*: Display the Bin Name along the X axis.

1. Enable *Show Axis Options* at the bottom of the Histogram window.
2. Choose a display mode from the *Mode* drop-down menu in the *Display Settings* pane:
   - *Differential*: displays the results in each bin as a direct value against the Y-Axis scale.
   - *Cumulative+*: adds the results progressively across the Histogram to display an ascending ramp
   - *Cumulative-*: subtracts the results across the bins resulting in a declining ramp

To change the way your results are displayed, choose an option from the *Style* drop-down menu in the *Display Settings* pane:

- *Bar Chart*: Standard bar chart with measurements along the Y axis and Bin Names or Limits along the X axis
- *Pie Chart*: Standard pie chart
- *Horizontal Bar Chart*: Bar chart, but with measurements along the X axis and Bin Names or Limits along the Y axis



Any *Reference Data* you enter as part of the Processing Sequence is included when you create a Report[1146].

To edit a Reference Data field quickly:

1. Click on the *Reference Data* entry in the main menu.

2. In the *Reference Data* control panel, enter relevant text in the *Data* text boxes.

Use the following buttons if you need greater control over *Reference Data* entries:

- *Edit*[1141]: Edit the keywords in the currently selected field (this is like clicking in the field, but displays a dialog box)
- *User Define*[1142]: Edit existing field names, add and delete fields, and control field visibility.

To edit the keywords in a *Reference Data* field:

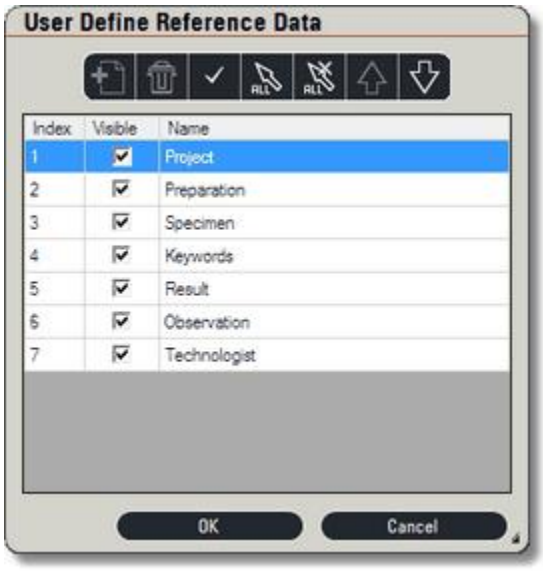
1. In the *Reference Data* control panel, do one of the following:
   - Select a *Data* field and click on the *Edit* button
   - Double-click on the *Data* header
2. Make your changes in the resulting dialog (the dialog header displays the name of the field being edited); you can use line returns.

3. If necessary, click *Next* or *Previous* to navigate between fields; the dialog title changes as you do this.
4. Click *OK* when you have finished editing.

To manage Reference Data topics:

1. In the *Reference Data* control panel, click the *User Define* button.

   The *User Define Reference Data* dialog is displayed.



2. Use the *Tool Bar* buttons to make changes.
3. Click *OK*.

See:

- Adding and Deleting Reference fields 1143
- Changing Field Visibility 1144
- Changing Field Order 1145

Note: The *Create* and *Delete* buttons are only available if you are logged in as an Administrator.

To add a new reference field

1. Click the *Create a new reference field* button.

2. Enter a Name for the field.

To delete a reference field

1. Select the fields you want to delete.

   You can Shift-click and Ctrl-click to select multiple fields.

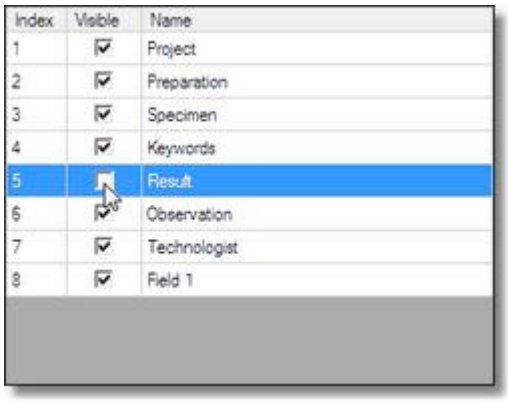
2. Click the *Delete selected reference fields* button.

Changing Field Visibility

You can specify which Reference Data fields are included in a report. Using this method, the fields remain in the configuration (so you can make them visible again if you wish). You can also permanently delete fields from a configuration (see this topic 1143).

Changing the visibility of a single field

1. Click to enable or disable the *Visible* check box for the field.



Changing the visibility of multiple fields

1. Select the fields whose visibility you want to change by Shift-clicking and Ctrl-clicking on their entries.
2. Click *Toggle visibility of selected reference fields*.

Making all fields visible or invisible

1. Click *Show all reference fields* or *Hide all reference fields*.

Changing Field Order

To change the order in which Reference Data fields appear:

1. Select a field in the User Define Reference Data dialog.
2. Click *Move selected reference field up/down*.

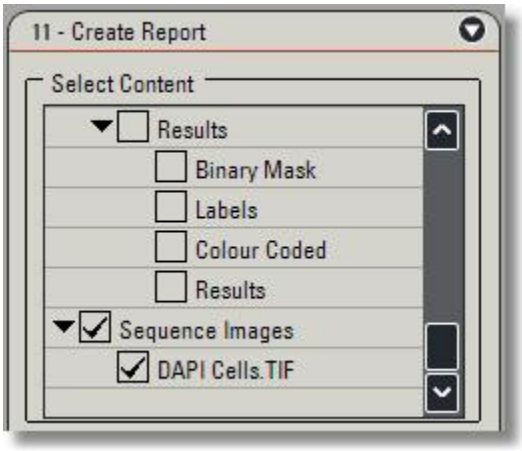
A Report saves data generated by a Processing Sequence in a Microsoft Excel spreadsheet.

A standard Excel-compatible template is supplied with *Image Analysis*. If you want to develop your own template, open the standard template, save it with a different name, and work with that copy.

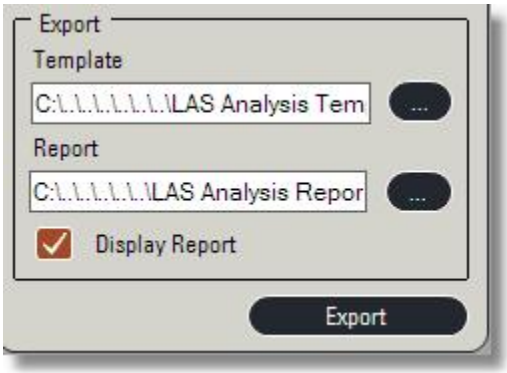
To set up and create a Report:

1. Click on the *Create Report* entry in the *Processing Sequence* panel.

2. In the *Create Report* control panel, enable check boxes in the *Select Content* panel to include images and other data items in the Report.



3. If necessary, use the browse buttons in the *Export* panel to select a Report Template and specify the output file name.



4. If you want to view the report automatically after it has been generated, enable the *Display Report* check box.
5. Click *Export*.

By default, Report templates are stored in the following folder:

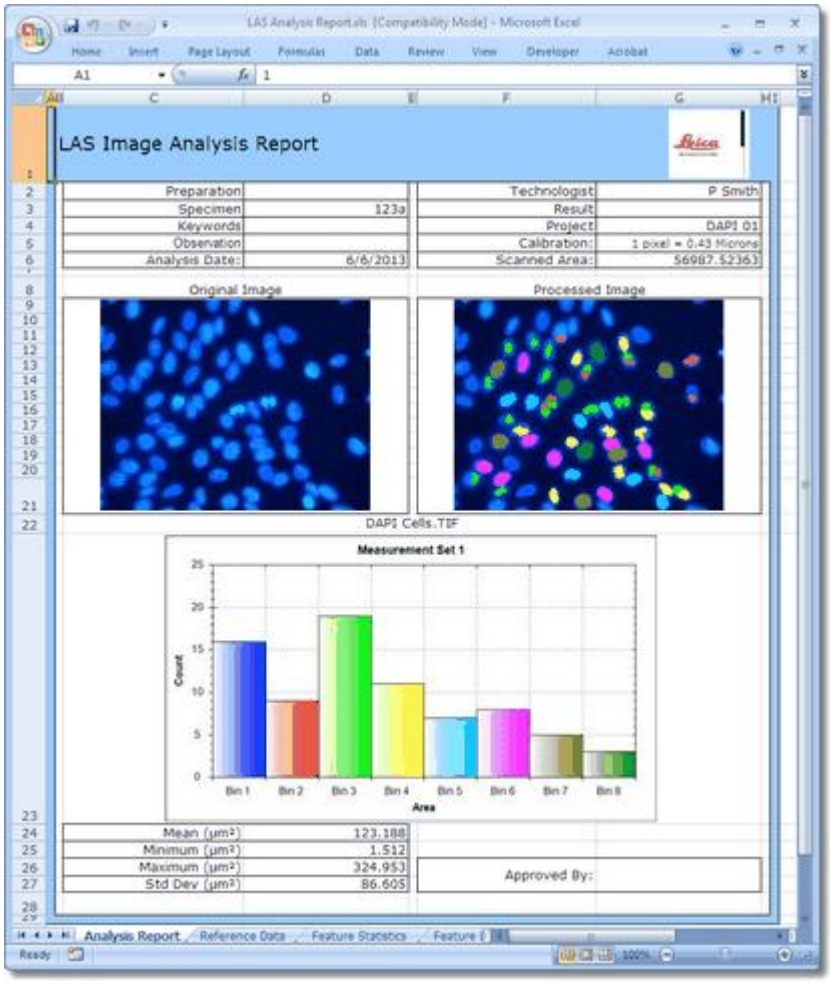
- *My Computer > Shared Documents > Leica Application Suite > Analysis > Templates*

By default, Report files are stored with the following path and file name:

- *My Computer > Shared Documents > Leica Application Suite > Analysis > LAS Analysis Report.xls*

A *LAS Image Analysis Report* has a number of tabbed Excel sheets. Depending on which options you enabled on the *Select Results* [1108] panel, some sheets may not contain any data.

- *Analysis Report*: A summary of the results; some cells refer to data contained on other sheets (such as the Feature Histogram).
- *Reference Data*: Reflects the information you entered on the *Reference Data* [1140] panel.
- *Feature Statistics*: Summary and full measurement statistics.
- *Feature Details*: Automatic measurement results (summary and full details).
- *Feature Histogram*: Histogram statistics, bin data and histogram chart.
- *Field Measurements*: Tables containing field measurements (summary and full details); Histogram statistics, bin data and histogram chart.
- *Profile Measurement*: Profile measurement results, bin data and histogram chart.
- *Images*: For each image specified in the *Select Content* pane of the *Create Report* panel, this sheet contains: Image data (name, pixel size, etc), source image, binary mask image, label image and colour coding image.



Report Excel Tags

You can format Excel reports by using Tags. Tags are simple text strings that can be typed directly into an Excel cell or copied using the Excel Macro feature.

More information about Excel Tags[425].

The tags for Image Analysis have a specific format and are listed below. Click on a tag for help.

- <LAS AM User Data>[458] Reference Data
- <LAS AM Results>[459] Feature Results
- <LAS AM Summary>[461] Feature Summary
- <LAS AM Histogram Statistics>[464] Feature Statistics
- <LAS AM Histogram Bin Data>[465] Feature Bin Data
- <LAS AM Histogram Chart>[465] Feature Chart Graphic
- <LAS AM Field Results>[462] Field Results
- <LAS AM Field Summary Full>[463] Field Summary
- <LAS AM Field Histogram Bin Data>[464] Field Bin Data
- <LAS AM Field Histogram Statistics>[465] Field Statistics
- <LAS AM Field Histogram Chart>[465] Field Chart Graphic
- <LAS AM Profile Results>[466] Profile Results
- <LAS AM Profile Bin Data>[466] Profile Bin Data
- <LAS AM Profile Histogram Chart>[429] Profile Chart Graphic
- <LAS AM Images>[467] All Images
- <LAS AM Image>[468] Selected Image
- <LAS AM Function>[469] Functions

Runner with Applications

LAS Applications use Runner to select and start solutions that automate image processing, analysis and measurements for quantitative microscopy. The richness of image processing functions in LAS is adapted to dedicated imaging tasks.

Leica Expert optional applications offer the user comprehensive solutions for the microscope analysis for Industrial Materials. The user can be confident that the analysis process conforms to the individual particular laboratory requirements.

The following Leica Expert applications use Runner:

Leica Cast Iron Expert is used for ductile irons and incorporates industry standards including ASTM E247, ISO 945-2 and JIS5502.

Leica Layer Thickness Expert is used for layers and coatings of many different materials such as paint, chrome plating, plastic coatings and incorporates industry standards including ASTM B487 and ISO 1463 for Metallic and Oxide coatings.

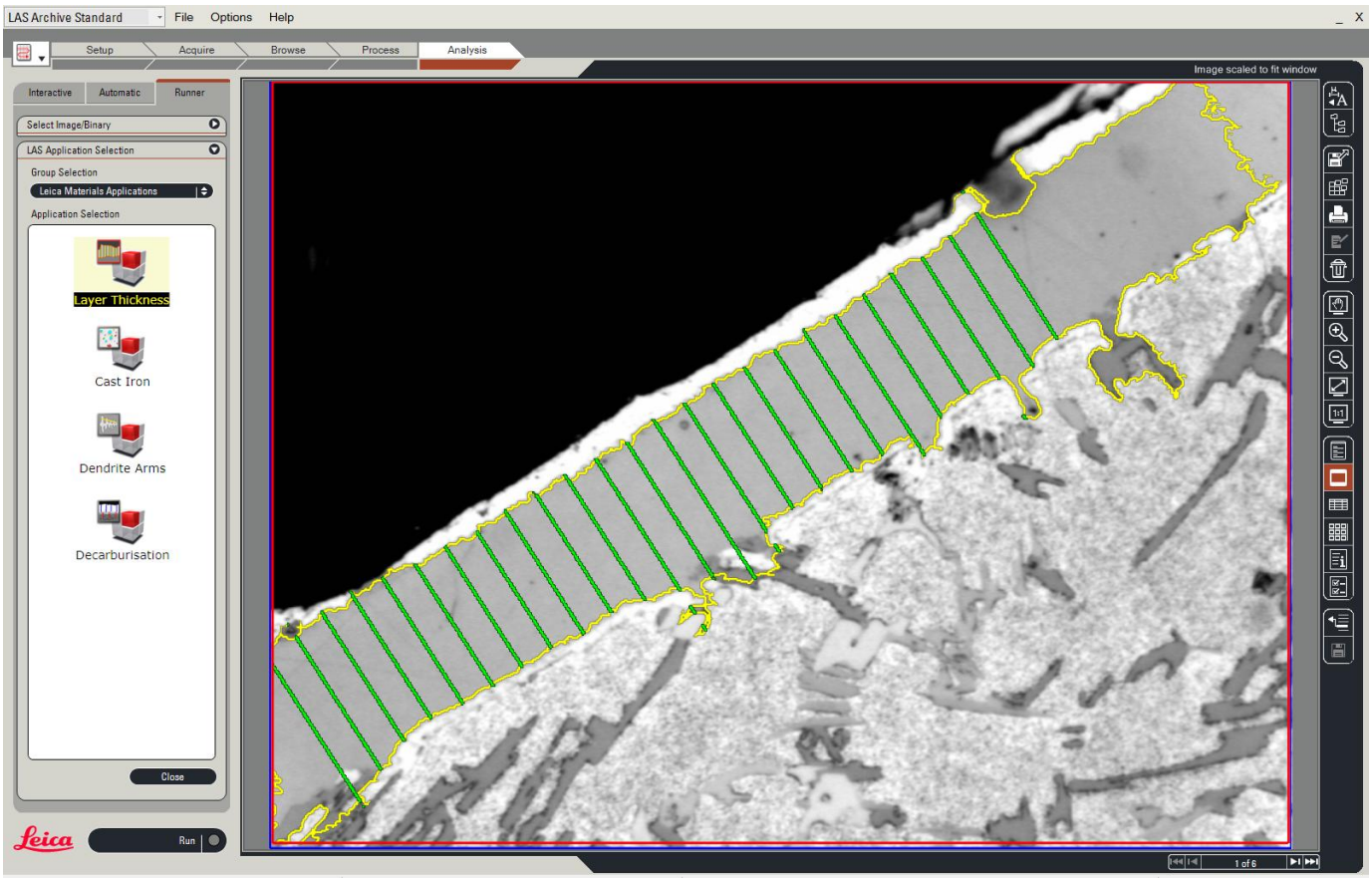
Leica Dendrite Arms Expert is used to help assess the mechanical properties of wrought materials, aluminium, copper and alloys.

Leica Decarburisation Expert is used in estimating the depth of decarburisation of steel specimens. It incorporates industry standards ASTM E1077, ISO 3887, JIS G0558 and DIN 50192

See:

Starting Expert Applications 1150

Using Runner with LAS Macros 1151



1. Start LAS and select the *Analysis* workflow.

2. Click on the *Runner* tab and wait a few seconds while the software loads. Under the Runner tab, the *LAS Application Selection* panel will appear.

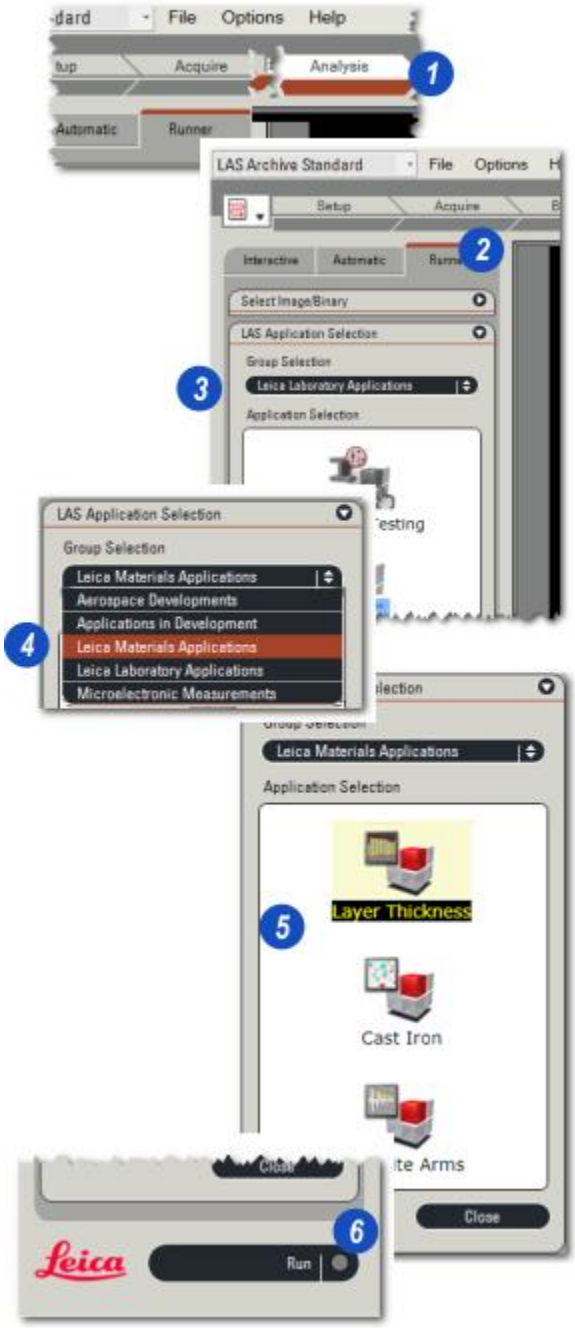
3. If necessary, choose *Leica Materials Applications* from the *Group Selection* drop-down menu (generally this will be the only option available, and will be selected automatically).

4. (If other application groups have been defined using *LAS Macro Runner*, the menu might look like this.)

5. Double-click on the application icon that you want to use or...

6. ...Select the icon and click *Run*.

On exiting the application, the *LAS Application Selection* panel will reappear so that you can select another one, or use other LAS facilities.



Using Runner with LAS Macros

LAS Applications using *Runner* automate image processing, analysis and measurements for quantitative microscopy. The richness of image processing functions in LAS can be adapted to a diverse range of imaging tasks.

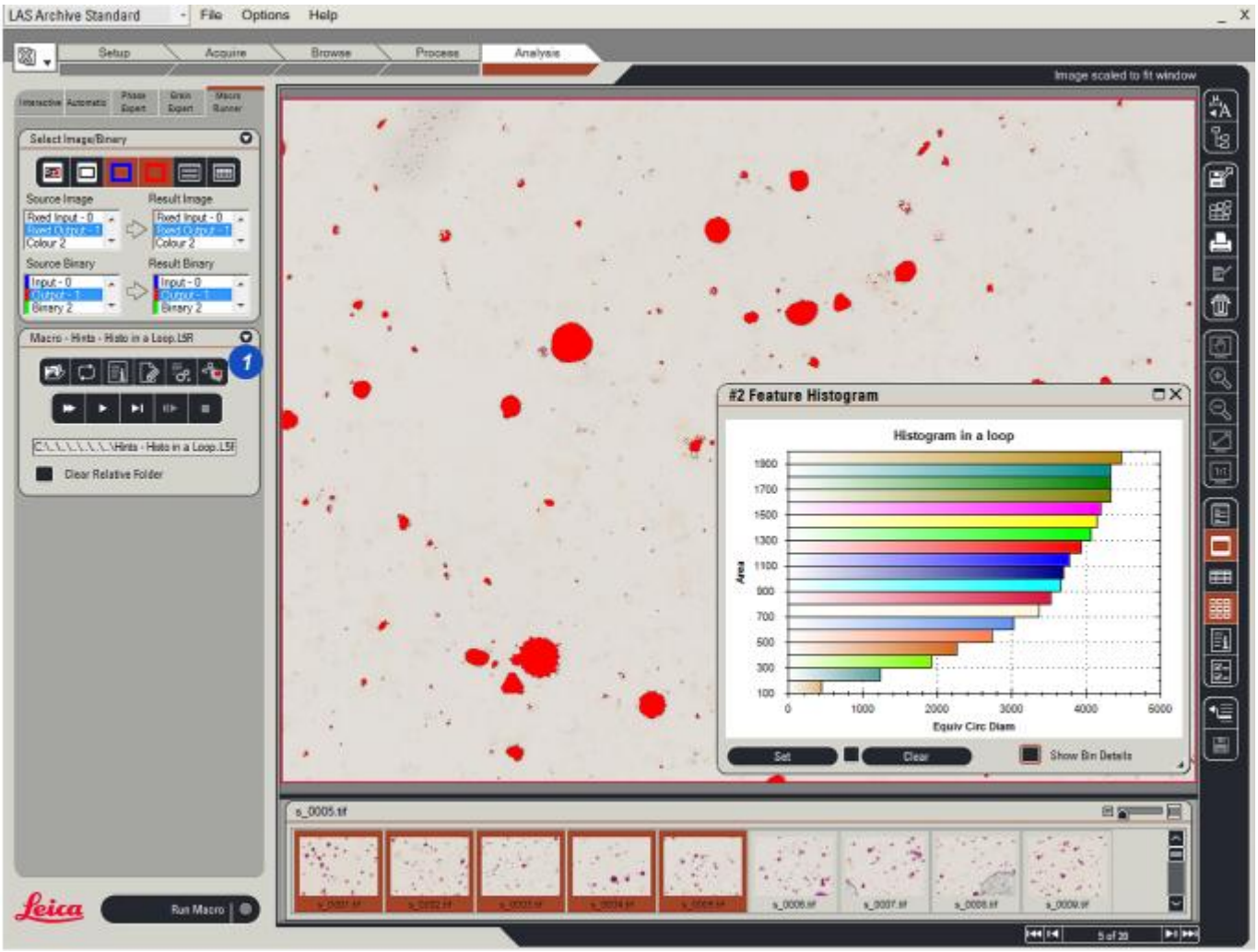
LAS Macro allows repetitive tasks to be customised to the needs of particular applications, optimising imaging solutions in a wide range of fields. This versatile software processes images obtained by Leica digital cameras and digital microscopes using the Leica Application Suite.

The *LAS Macro Editor* defines the instructions for image processing, binary processing and measurement. An LAS Macro routine is like a conventional computer program. The difference is that you create it interactively using the *LAS Macro Editor*. An instruction is automatically created and inserted into the *LAS Macro* when you press the *Insert* button. There is no need to write any software code!

Macro programs are run within the LAS environment either using the *LAS Macro Runner* or in combination with *LAS Image Analysis*. *LAS Macros* are included in the analysis sequence at the step for Image *Processing* or *Binary Processing.* This combination makes a complete application solution that can be repeatedly used by operators with no specialist knowledge of LAS Macros.

Adding the versatility of *LAS Macros* to the automation of the *LAS Image Analysis* sequence or to the simplicity of the *LAS Macro Runner* provides an efficient solution to demanding and unconventional tasks in analytical microscopy.

For detailed information about the optional *LAS Macro Runner*, please refer to the *LAS Macro Editor* help file. *Go there...*



Phase Expert

Optional module *Phase Expert* has been designed to measure precisely up to ten different phases – regions of the image that can be identified by their homogenous colour or grey level.

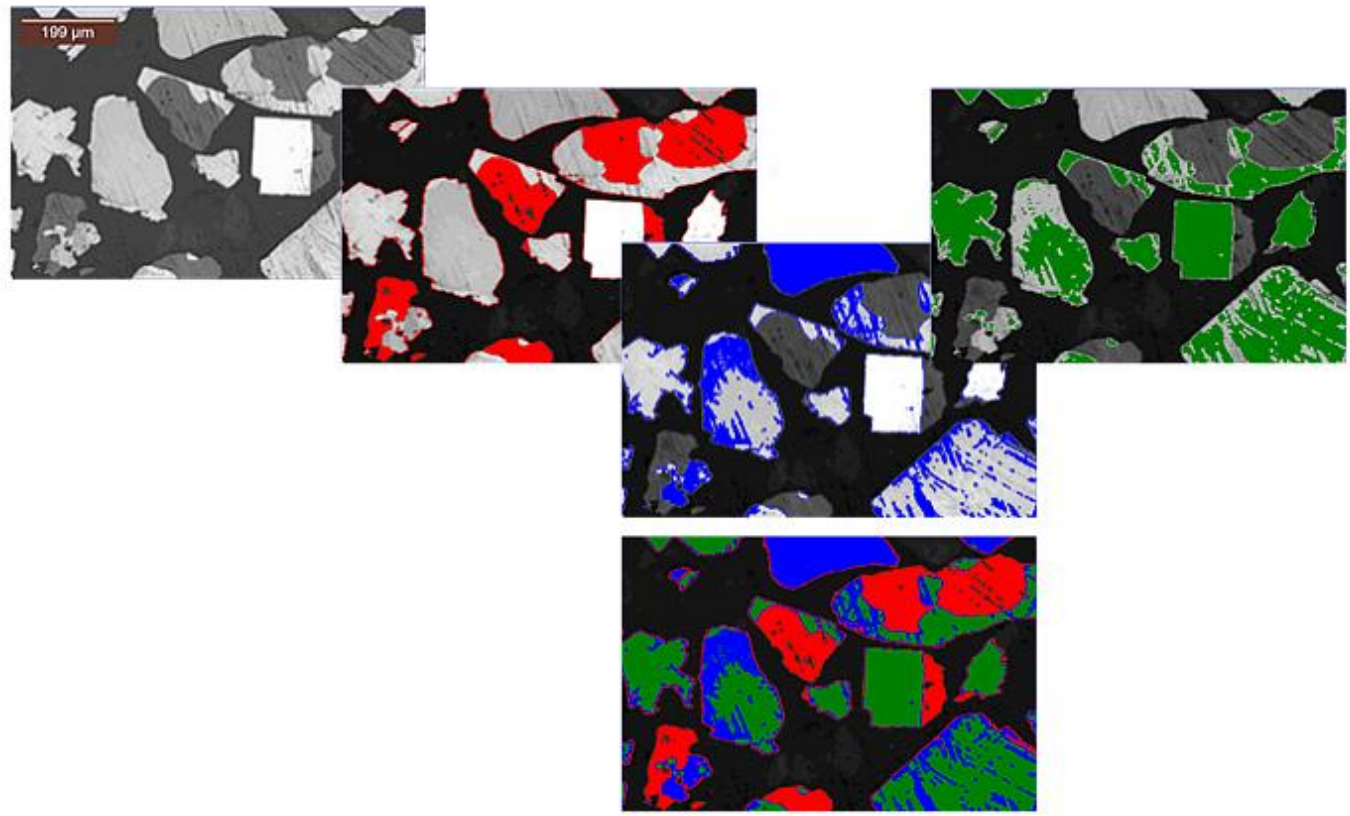
For example, regions of differing reflectivity in oil-shale; Colours due to the polarised light of different constituents in a rock section or stain variations in tissue or bone sections. Phase Expert can determine the occurrence of these phases both in terms of the overall image or with reference to just one of the selected phases.

Samples are typically embedded in a resin billet with the image face ground and polished to good reflective flatness.

Single or multiple images can be processed automatically once any required grey processing filters specified and thresholds set.

Results can be displayed on screen in a variety of formats both as graphics and as tabular in the *Grid*. And they can be saved as a report to *Microsoft Excel Spreadsheet* for easy distribution. Displayed results and reports provide wide scope for tailoring to the user's needs.

Phase Expert works in conjunction with *Image Analysis* which must be installed, licensed and enabled.



These steps represent the sequence users should follow to successfully carry out *Phase* measurements.

Because Phase Expert works in conjunction with Image Analysis, in most steps links are provided to both modules:
Red, will take the reader to the appropriate place in the Image *Analysis (Automatic)* help file for more detailed information, and
Blue, connects to help files that are specific to *Phase Expert*.
Sometimes, it will be beneficial to follow both links if they are provided.

Set up the Phases:
Each of the *Phases* to be measured is colour-coded and that colour is used on the processed image to distinguish it from other *Phases*.
- Names and colours of the *Phases* can be selected by the user.
- Up to 10 different *Phases* can be chosen for measurement.

Phase Expert help >>> 1155

Select the Images to Process:
One or more images can be processed in sequence simply by selecting the *Thumbnail(s)* in the *Gallery* and then clicking the *Append* button on the *Select Images to Measure* panel.
Image Analysis help >>> 1043 *Phase Expert help >>>* 1159

Choose the Reference:
Phase Expert provides two paradigms for determining the measurements:
- Measure each *Phase* against the *Field* – the entire image, or:
- Measure each *Phase* against a selected *Phase* called the *Reference*.

If the entire Field is to be used check that none of the phases have the *(Ref)* marker set against and go to the next step.
Phase Expert help >>> 1158

Set the Measure Frame:
The *Measure Frame* determines the part of the *Field* that is measured and analysed.
- *Phases* lying outside the *Measure Frame* will be ignored.
- For *Phase Expert* the usual option is *Entire Image* (Field).

Image Analysis help >>> 1103 *Phase Expert help >>>* 1161

Image Processing Pre-Filter:
A wide and powerful range of filters to improve *Phase* boundaries and recognition making measurement and analysis faster are more precise.
Image Analysis help >>> 1081

Continued... 1154

Adjust Thresholds:

Adjust Thresholds allows the upper and lower pixel values for each *Phase* to be adjusted so that they accurately represent the desired boundaries. The *Phase* detection process uses the pixel range values set during *Threshold* adjustment.

- Clear, easy to use *Histogram* shows each *Phase* in its own colour with a simple *Bar* display beneath.
- Range adjustment can be made by dragging on the *Histogram* or by entering values.
- Two modes – *Continuous* and *Overlapping* – are available.

Image Analysis help >>> 1073 *Phase Expert help >>>* 1162

Binary Processing Pre-Filters:

The *Binary Pre-Filters* provide the tools for modifying the Binary Processed Image in the same manner as *Greyscale Pre-Filters* work on the original image but with finer precision – small, individual details can be targeted and modified.

- Discard and Combine details.
- Fill Holes and Separate Touching details.

Image Analysis help >>> 1081 *Phase Expert help >>>* 1168

Binary Image Editing:

Binary Image Editing is a tool collection that provides the methods for working directly on processed *Binary Images* to add, remove, select and de-select details.

- Facilities also include drawing and filling shapes as well as grouping features.

Image Analysis help >>> 1095 *Phase Expert help >>>* 1169

Results and Histogram:

There is a wide range of options for displaying *Phase Expert* results both as graphics – *Bar* and *Pie* charts – and tabular in *Detail* and *Summary*.

- Create personalised layouts and save as re-usable Configurations.
- Display tabular results for multiple fields as aggregates or individual fields and phases.

Image Analysis help >>> *Phase Expert help >>>* 1170

Reference Data:

A comprehensive range of *Data Items* can be appended to the *Phase Expert* results that will identify important details such as the *Project Name,* the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

- Administrators can add to the supplied list of data headings to comply with corporate demands.

Image Analysis help >>> 1140 *Phase Expert help >>>* 1177

Create Report:

Phase Expert Reports are created and saved in *Microsoft Excel* spreadsheet format. *Excel* must be installed on the computer to create the report but only the *Excel Viewer* is needed to display it. Images can be included and each report is structured to show the results comprehensively on individual sheets.

- A flexible Template is supplied with Phase Expert.
- The default Template can be modified to suit the user's needs.
- The report can be displayed as it is created.

Image Analysis help >>> 1146 *Phase Expert help >>>* 1178

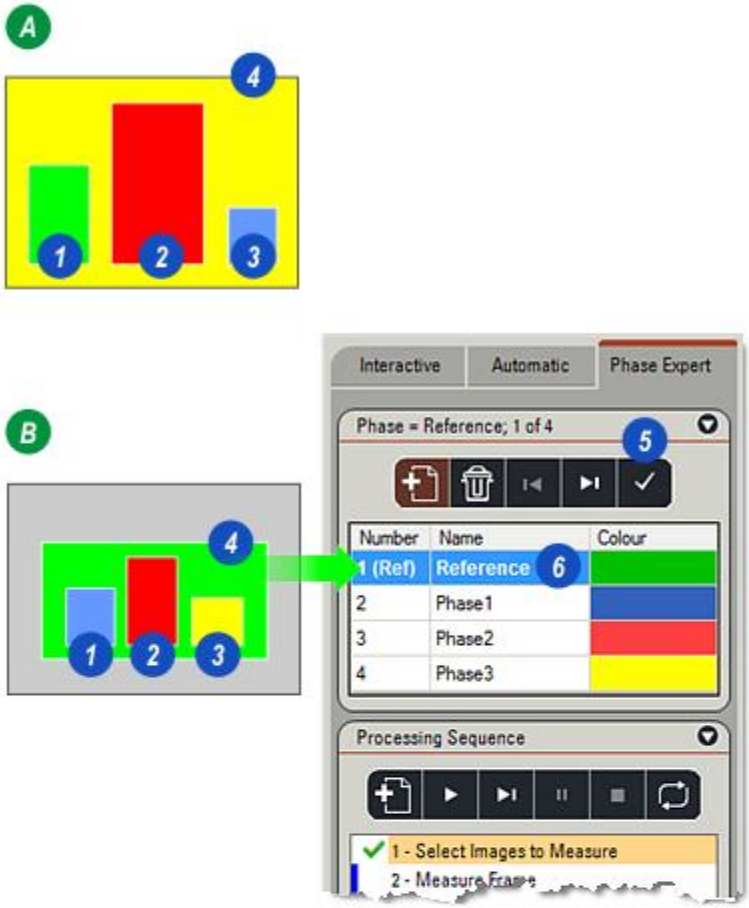
Phase Expert measurements can function in either of two modes:

A: *Field Mode*: In which each of the phases *Green* **(1),** *Red* **(2)** and *Blue* **(3)** in the diagram are measured with respect to the entire *Visible Field* **(4)**.

B: *Reference Mode:* The phases *Blue*, *Red* and *Yellow* are measured with respect to another phase coloured *Green* **(4)** in the illustration, that generally encompasses the others. The *Field* parameters are ignored in the measurement comparisons but are reported.

5: *Reference Mode* is selected by clicking the *Tick* button on the tool bar. Clicking again turns off *Reference Mode.*

6: When *Reference Mode* is active the tag ***'Ref'*** appears next to the chosen phase. In the illustration the word *Reference* has been typed in by the user.



Each phase to be measured is colour-coded on the binary output image. Set up the phases by:

1: Click on the arrow to the right of the *Phase* header to reveal the panel. On opening a single phase with the default name *Phase 1* is automatically displayed.

For some jobs a single Phase will be sufficient, but many images will contain several Phases all of which need to be identified. In these cases add further Phases as described on the following pages.

2: Change the *Phase Name* by clicking in the name text box and typing a new name.

3: To change the *Phase Colour*, double-click on the colour and…

4: …the *Colour* dialog appears. Choose a colour from the swatches, wheel or hue slider or type the required values.

5: Click *OK*.

6: An example of a *Phase Name* and *Colour* change.

1: Add further *Phases* by clicking on the *Create New Phase* button. Change the *Phase Name* and *Colour* using the procedure described on the previous page.

As additional *Phases* are added they are given a sequential number shown in the left-hand column. The lowest number represents pixel values close to 0, the black end of the greyscale. Each additional *Phase* represents a higher span of pixel values so the three *Phases* in the illustration might represent the greyscale pixel values:

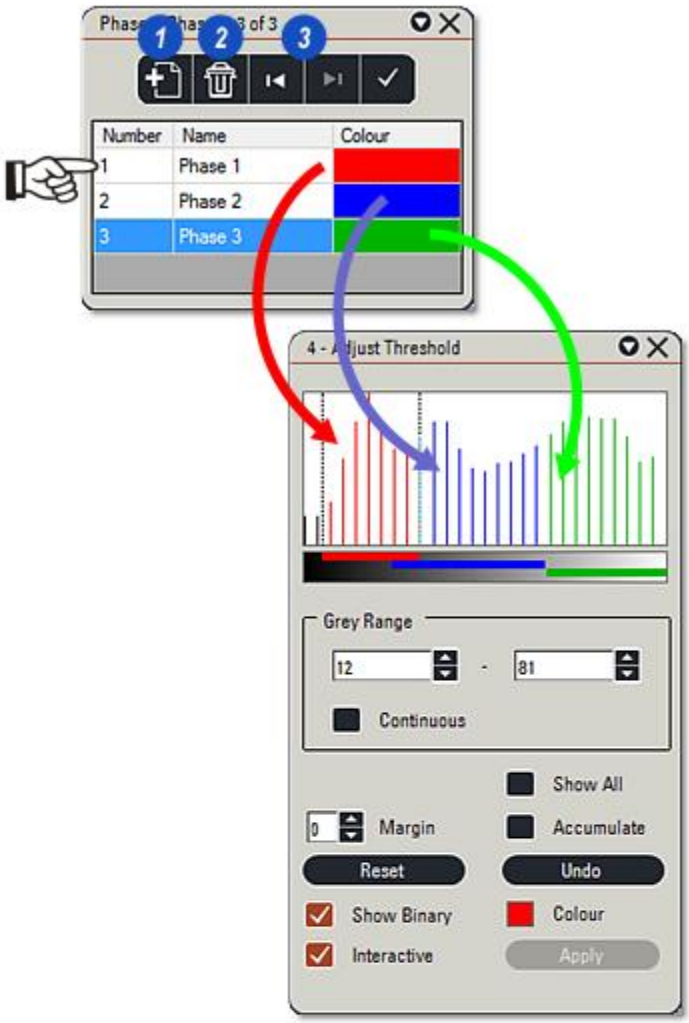
Phase 1: Red: 0 (Black) to 72.
Phase 2: Blue: 73 to 154.
Phase 3: Green: 155 to 255 (White).

...for example.

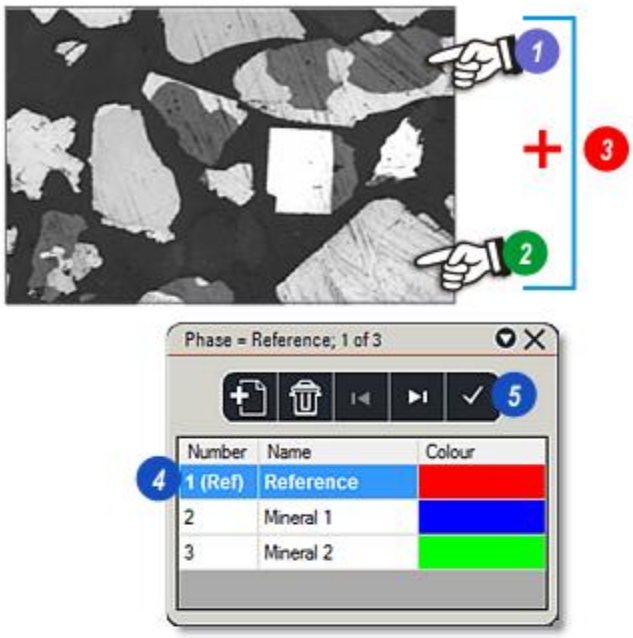
The initial range values are established by software based on a broad analysis of the image. The user will adjust them during a later step.

2: Delete a *Phase* by clicking to highlight it and then clicking on the *Trash Can* (Delete) button. If a *Phase* is deleted by mistake, immediately click the *Create New Phase* button and it will be restored.

3: Navigate through the *Phases* using the *Previous* (Left) and *Next* (Right) buttons.



In this example, flakes of two minerals - dark grey **(1)** and light grey **(2)** on the image, exist blended together. The task is to determine what percentage of each is represented by the blend. The area of both minerals together **(3)** is represented by a combined phase called the *Reference*.



- The first step is to measure the area of *both* minerals to determine the combined *Reference*.
- The next step is to measure *Mineral 1* separately and finally...
- ...measure *Mineral 2* separately.

4: The combined measurement - the *Reference* - is colour-coded red. *Mineral 1* is coloured Blue and *Mineral 2,* green. The colours have been set up as phases with appropriate names.

5: Set the *Reference* phase - the combined measurement - by clicking on the phase and then on the *Set Reference* button. *(Ref)* appears against the phase to indicate it is the *Reference*.

To see the outcome of this example: *Go there…*
1165

Select Images to Measure

1: On the *Processing Sequence* click on *Select Images to Measure.*

2: Click on a *Thumbnail* in the *Gallery*. *If a single image is to be processed it does not have to be added to the Sequence Image List*. Continue only if multiple images are going to be processed.

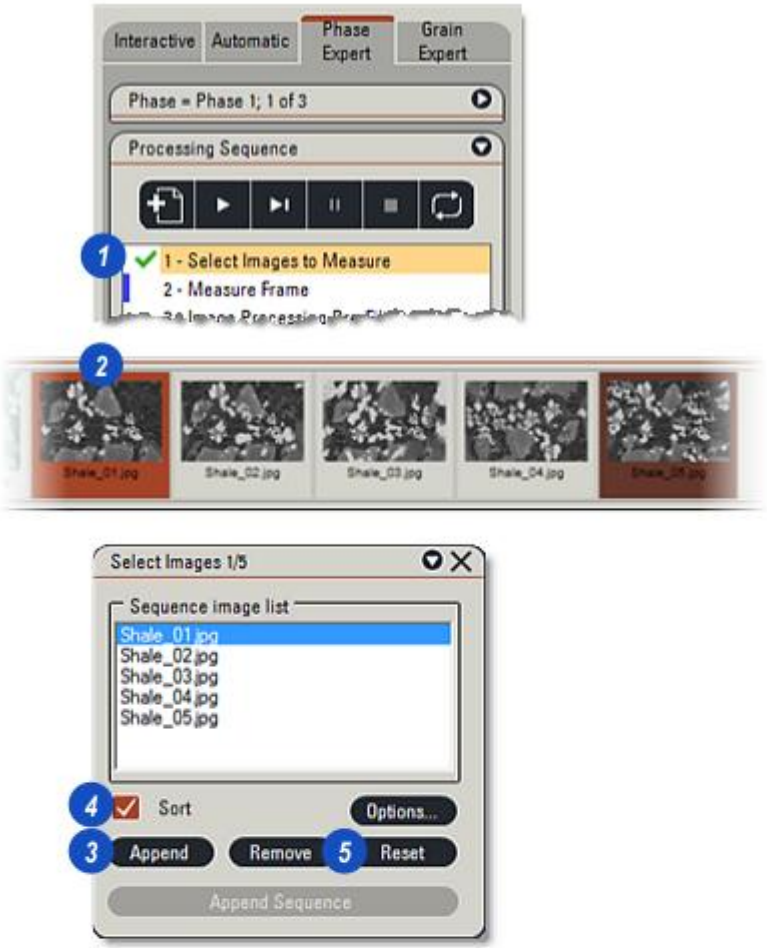
3: Click to *Append* button on the *Select images to measure* dialog.

Repeat the steps to add more images to be measured as a batch.

All selected images must have the same type - for example *.bmp, .png, .jpg* - although it is recommended that images of type *tif* or *jpg* are used because these are not compressed. Sometimes artefacts of the compression process are seen in formats using compression.

4: Images can be selected in any order from the *Gallery*. To sort them into numerical sequence click to enable the *Sort* check box.

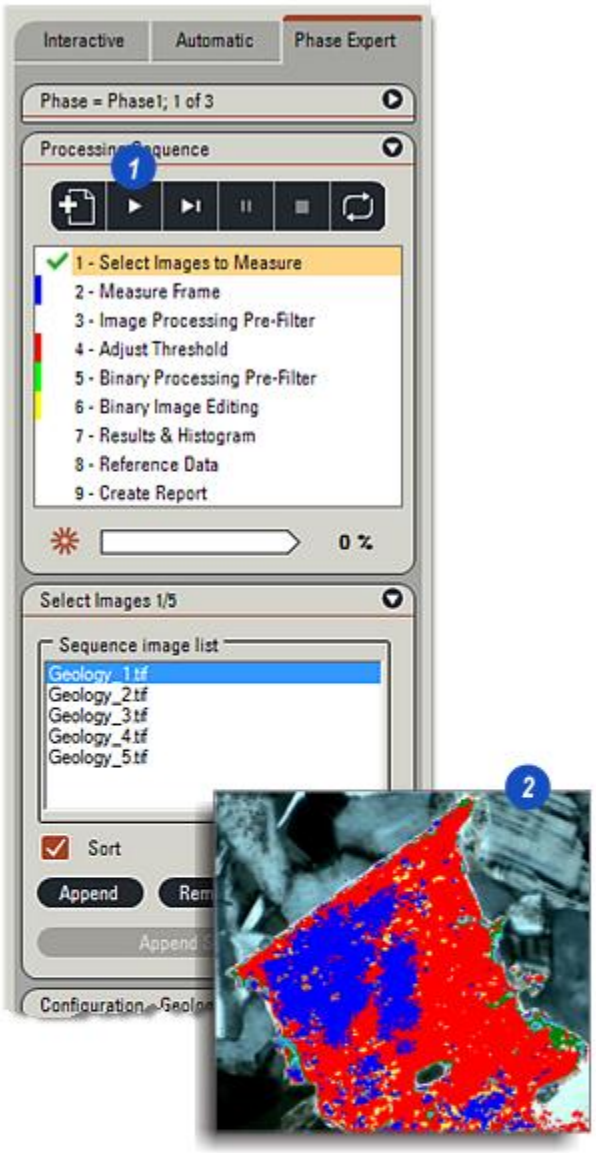
5: Use the *Remove* button to remove a single selected image from the list, or click the *Reset* button to remove all images.



When multiple images have been added to the *Image List*, the process so far has been carried out on just one of them. But the program 'remembers' the settings so by clicking on the *Run Sequence* button **(1)** all of the images are processed one after the other and measured using the same settings. This is sometimes called Batch Mode.

The image **(2)** shows all of the phases displayed together. Use the *Show All* check box on the *Adjust Threshold* panel to see this result.

For further information about the Processing Sequence see *Image Analysis* 1035 help.

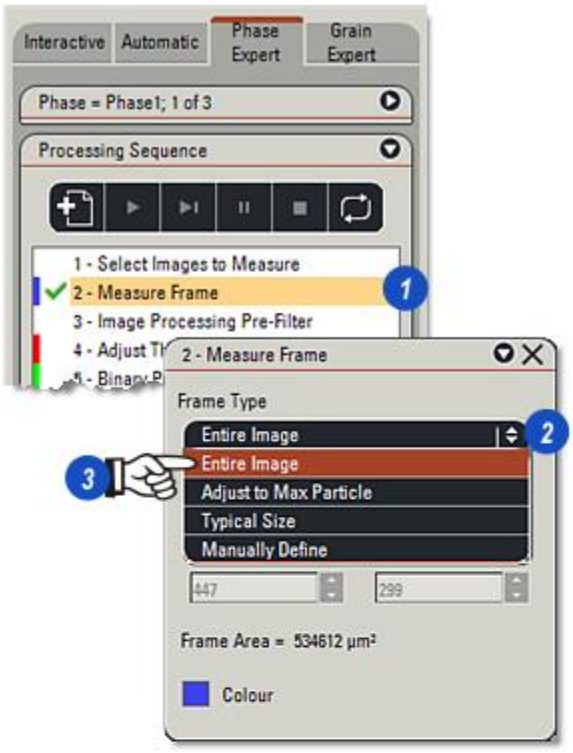


Set the Measure Frame

1: In the *Processing Sequence* click to select *Measure Frame*.

2: On the *Measure Frame* panel click on the arrows to the right of the header.

3: Select the *Entire Image* option from the drop down menu. Although other options are available and can be used, *Entire Image* is the usual selection for *Phase Expert.*



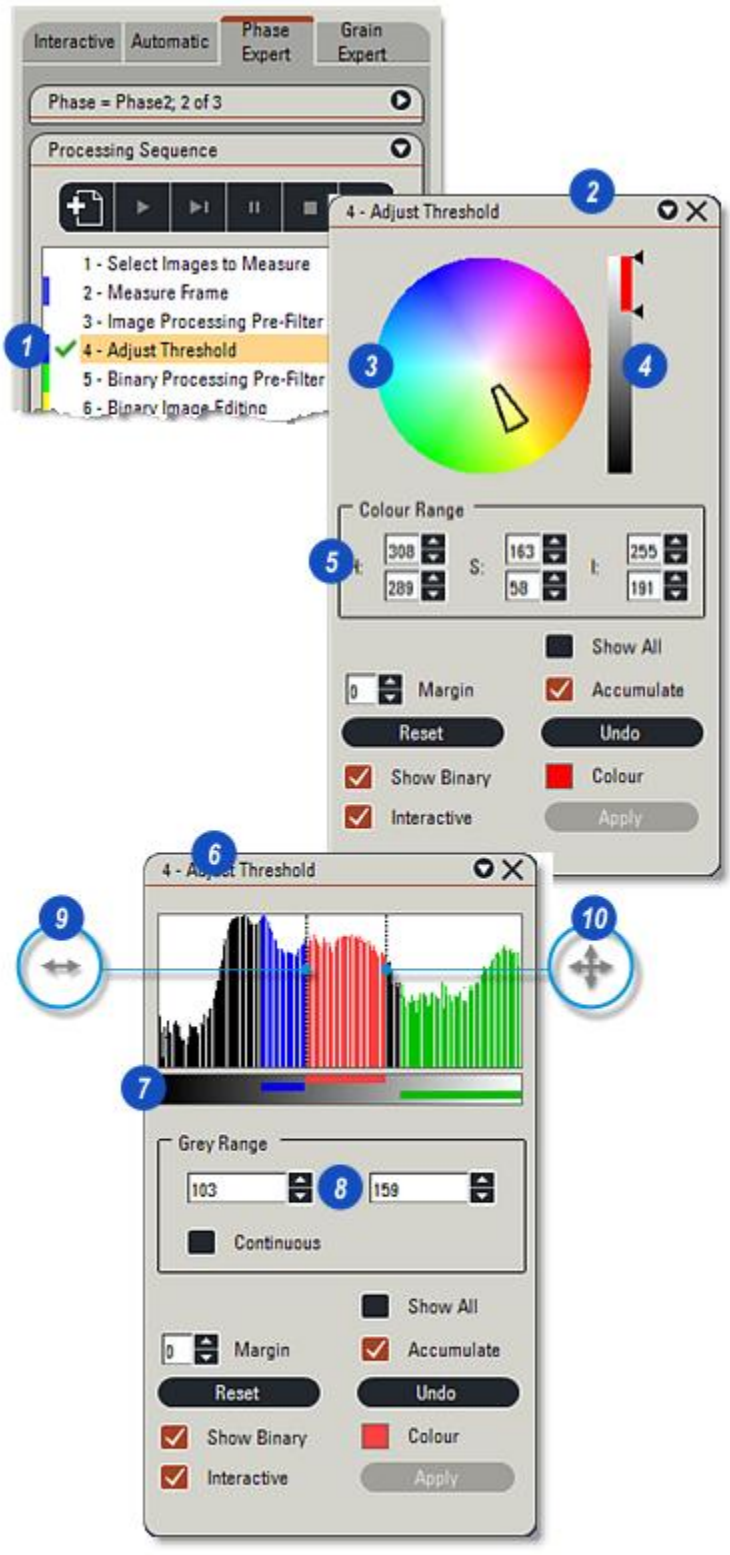
Adjust Threshold allows the pixel range values of each phase to be adjusted so that they accurately represent the phases boundaries. The phase detection process uses the upper and lower pixel values set during the *Threshold* adjustment.

1: Click to select *Adjust Threshold* on the *Processing Sequence* menu.

Colour images use the colour wheel dialog **(2)** that allows *Hue* and *Saturation* to be set on the *Colour Wheel* **(3)** and Intensity controlled with the *Slider* **(4)**. Actual values can be typed into the *Colour Range* text boxes **(5)**.

Monochrome or greyscale images use the *Histogram* panel **(6)** that also has *Colour Bars* that, in some instances show how pixel values overlap each other. Values can be typed into the text boxes **(8)**. The value range on the *Histogram* can be adjusted by clicking and dragging on the vertical dashed bars when the cursor is represented by a double-ended arrow *(9)*, or the entire range can be shifted up or down when the four-ended arrow **(10)** is displayed.

The following pages illustrate *Threshold* adjustment for both colour and mono images.



In this example three phases are going to be detected and measured with respect to the entire visible field, hence *Field Mode.*

On original image **(1),** the three areas of interest are the yellow flecks, the orange speckles and the pale blue blobs that surround the yellow area. Each area will be detected in turn starting with the yellow flecks – *Phase 1* detected in *Red*.

2: On the *Phase* panel, click to select the *Phase 1 (Red).*

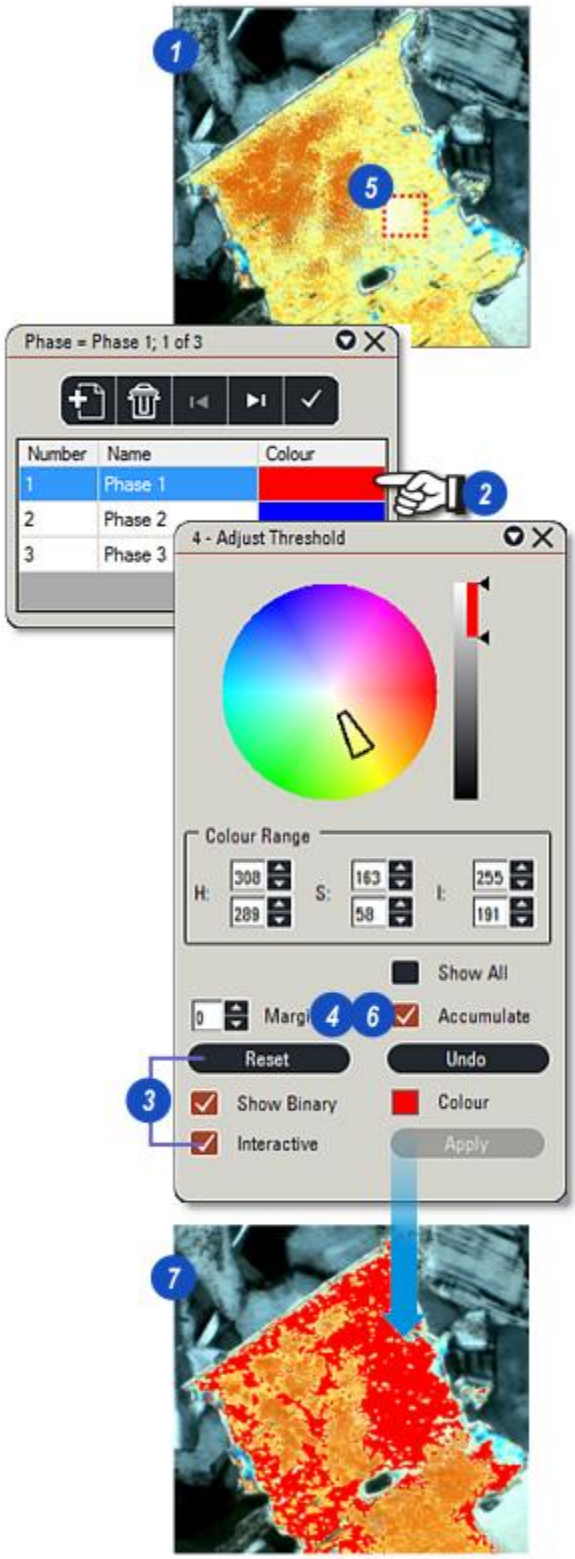
3: Enable *Interactive* and *Show Binary* and click the *Reset* button to remove any existing detection.

4: Disable *Accumulate*.

5: Click on part of the area of interest – in this case the yellow flecks – and drag a small rectangle. The pixel values contained within the rectangle are immediately displayed on the *Colour Wheel* and in the text boxes and the binary image **(7)** will be shaded with the phase colour – in this case *Red..*

6: Click to enable *Accumulate*. This will add any further selections to the existing. Keep repeating step **(5)** to include any (yellow flecked) area not yet detected. Make fine adjustments on the *Colour Wheel, Intensity Slider* and in the *Colour Range* text boxes.

7: The final image with *Phase 1 (Red)* detecting all of the yellow flecks.



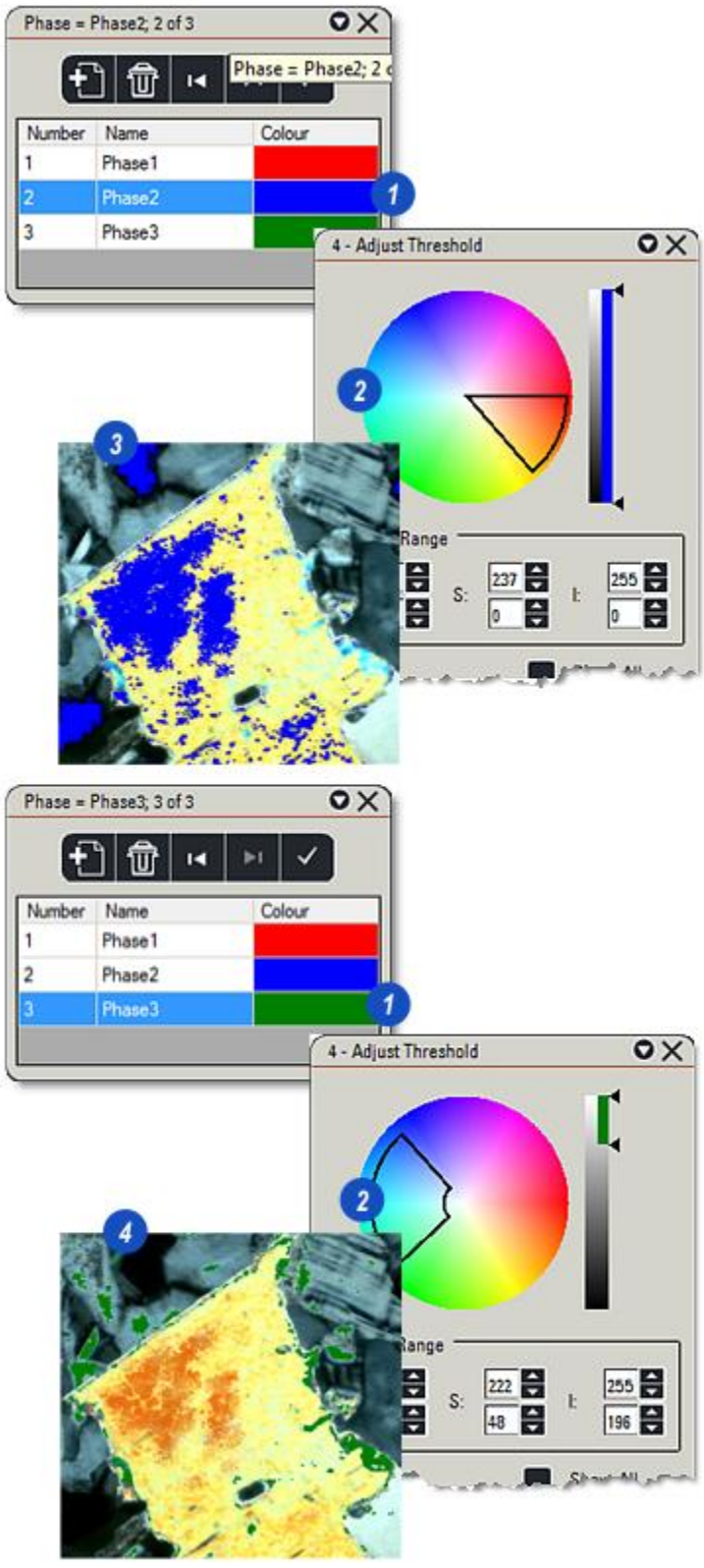
The process for detecting the two remaining phases – orange speckles and pale blue blobs, *Phase 2* and *3* – is the same as threshold detection for *Phase 1* but selecting relevant areas on the image.

1: Click to select the phase to be detected.

2: Select and detect an appropriate area of the image and adjust the threshold on the *Colour Wheel* controls.

3: The result of *Adjust Threshold* for the orange speckles – *Phase 2 Blue*, and…

4: …for *Phase 3 Green*, the pale blue blobs.



1: Click on the *Reference* phase on the phase list. This indicates the phase that is going to be selected. In this example the *Reference* is going to include both *Mineral 1* and *Mineral 2.*

2: Click to enable *Show Binary*, *Interactive* and also on *Reset* to clear any automatic detection.

3: Click to disable (OFF) *Accumulate*.

4: Disable the *Continuous* function on the *Histogram*.

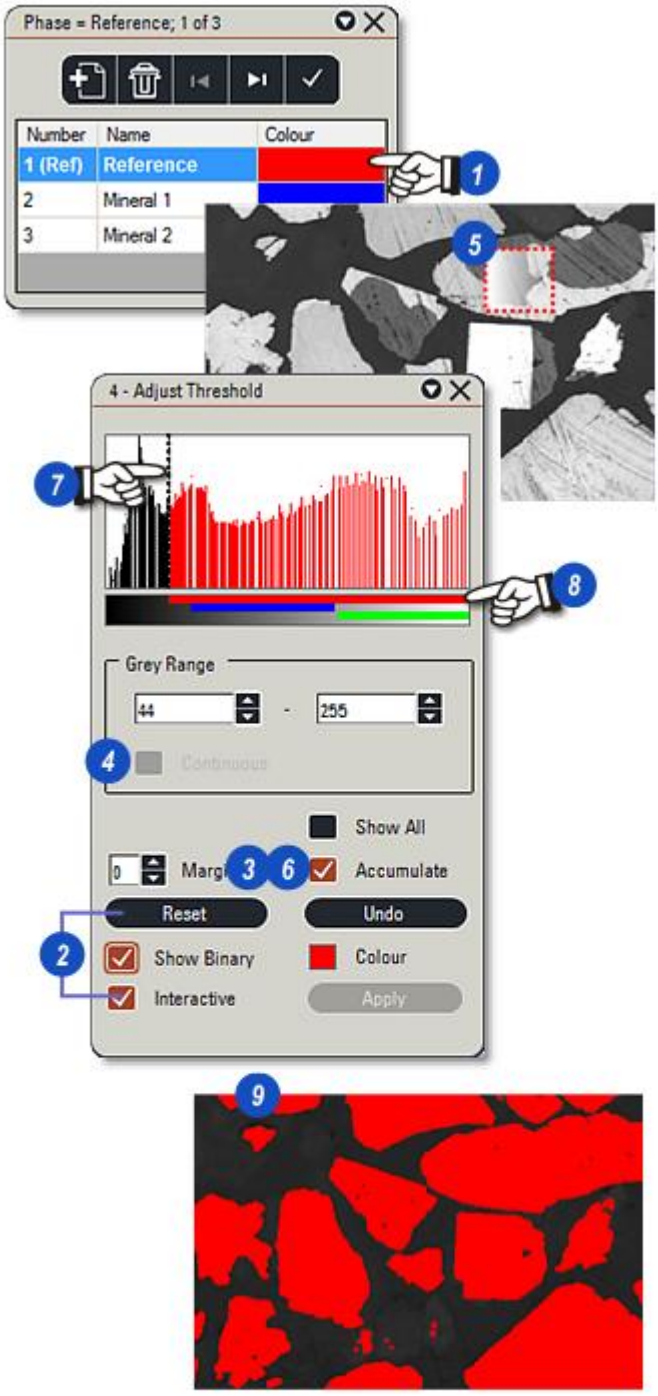
5: Select an area that represents either of the minerals, click and drag to create a region. All of the pixels values within the region will be displayed on the image and on the *Histogram*.

6: Click to enable (ON) *Accumulate* and repeat procedure **(5)** on another part of the minerals that is not yet detected. The *Accumulate* feature will add the selection to the previous values. Continue like this until all areas of the two minerals are detected and combined.

7: Adjust the detected phases by dragging the vertical dotted lines on the *Histogram* until only the areas covered by *Mineral 1* and *Mineral 2* are represented. Everything else is ignored.

8: On the *Histogram* the different phases are allowed to overlap because *Continuous* is disabled.

9: The *Reference* phase binary image.



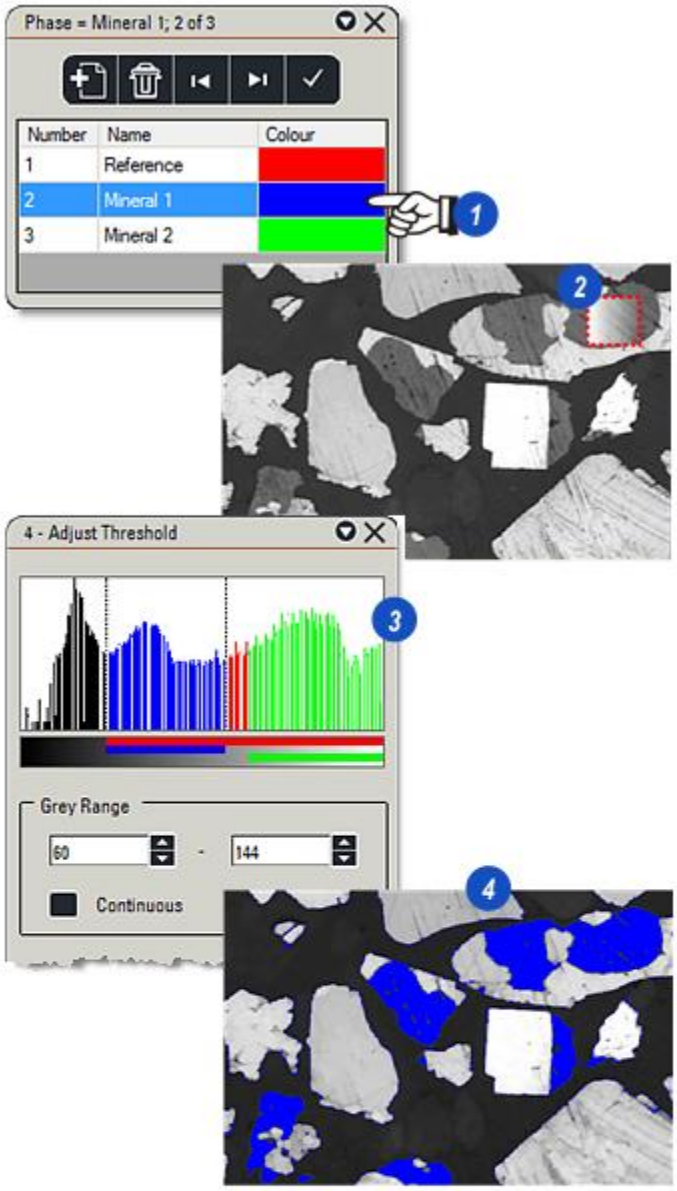
Detect *Mineral 1* that is colour-coded Blue on the phase list.

1: Click to select the *Blue (Mineral 1)* phase.

2: Follow the steps on the previous page to start the detection but this time select a region that represents only *Mineral 1*, repeating the selection until all of the phase has been detected.

3: Fine tune the detection with the *Histogram* controls – notice how the *Mineral 1 phase (Blue)* overlaps the *Reference (Red)* phase.

4: The *Mineral 1* phase binary image after detection.



Detect *Mineral 2*, colour-coded Green on the phase list.

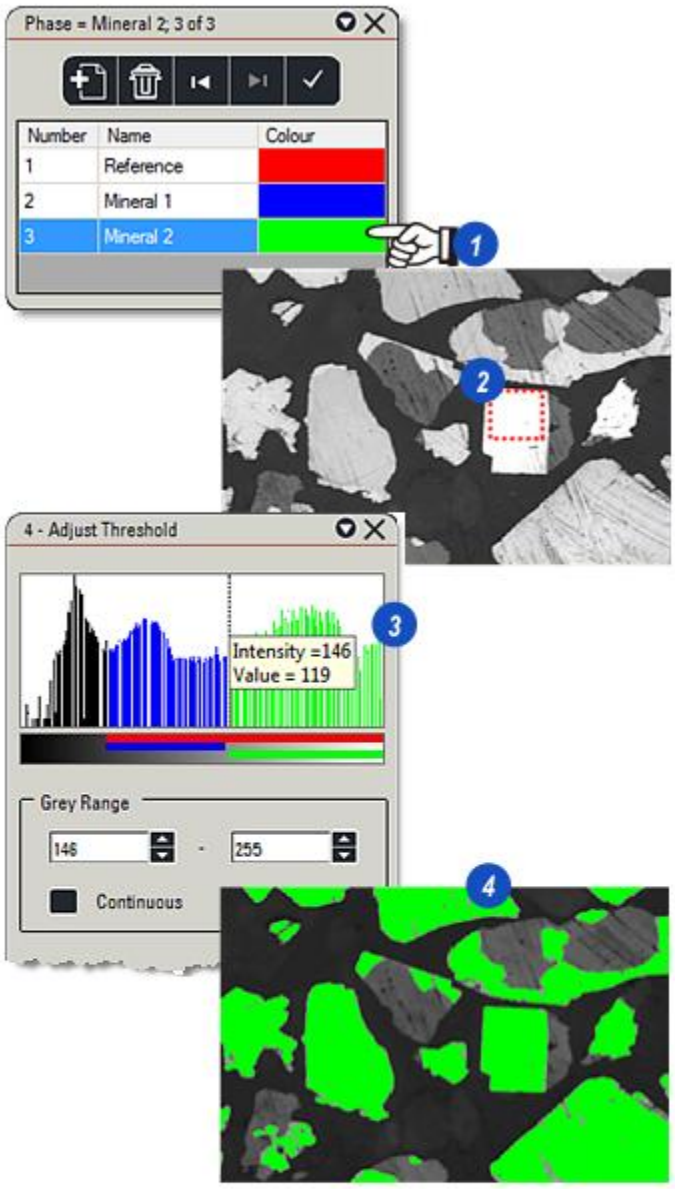
1: Click to select the *Green (Mineral 2)* phase.

2: Follow the steps on the previous pages to start the detection but this time select a region that represents only *Mineral 2*, repeating the selection until all of the phase has been detected.

3: Fine tune the detection with the *Histogram* controls – notice how the *Mineral 2* phase (*Green*) overlaps the *Reference (Red)* phase but butts against the highest value for the *Mineral 1* phase (*Blue*).

4: The *Mineral 2* phase binary image after detection.

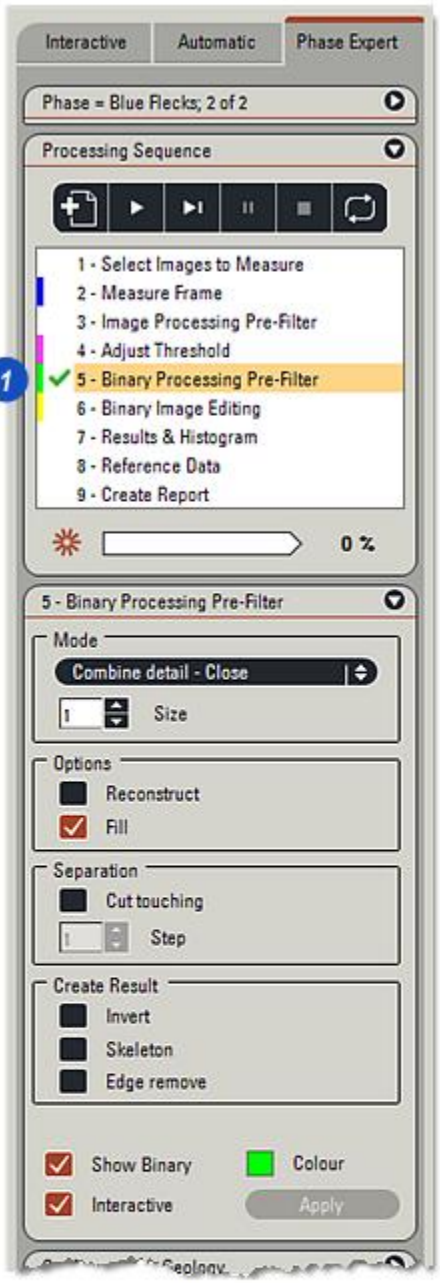
That completes the *Threshold* phase detection for this example.



The *Binary Processing Pre-Filters* provides several filters and a range of tools that can quickly and easily improve a binary image - combining or separating detail, filling holes, separating phases that are touching and reconstructing 'fractured' phases.

1: Click on the *Binary Processing Pre-Filter* option on the *Processing Sequence* menu to open the *Pre-Filter* panel.

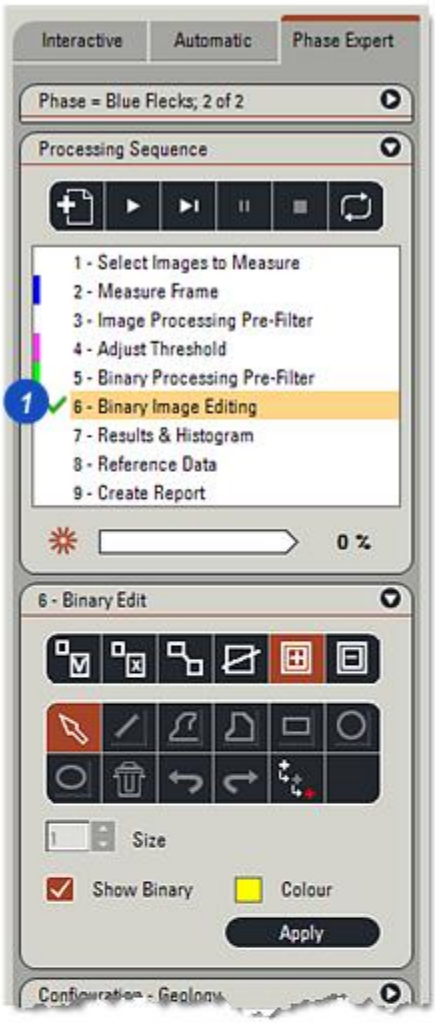
For detailed information and techniques about *Binary Processing Pre-Filters*, see *Image Analysis* [1081] help.



Binary Image Editing is a tool collection that provides the methods for working directly on *Binary Images* to add, remove, select and de-select features. Facilities also include drawing and filling shapes as well as grouping features.

1: To use the *Binary Editing* tools, click on the *Binary Image Editing* option on the *Processing Sequence* menu.

For detailed information about *Binary Editing*, see the *Image Analysis* [1095] help:



1: Click to select *Results & Histogram* on the *Processing Sequence* menu.

2: Click on the arrows to the right of the *Phase/Field* header and…

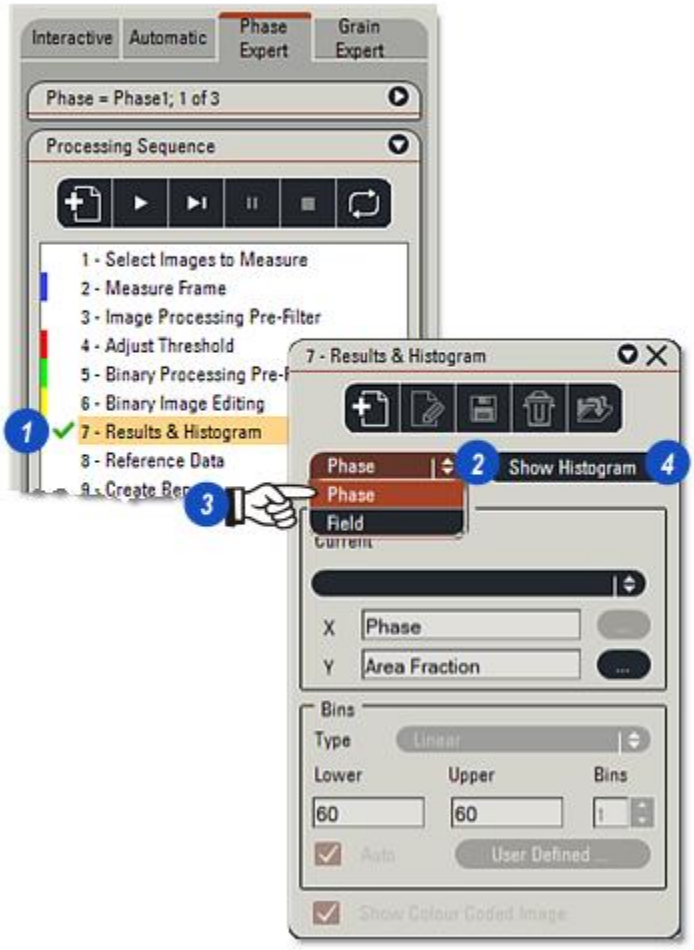
3: …from the drop-down list click on the *Phase* option.

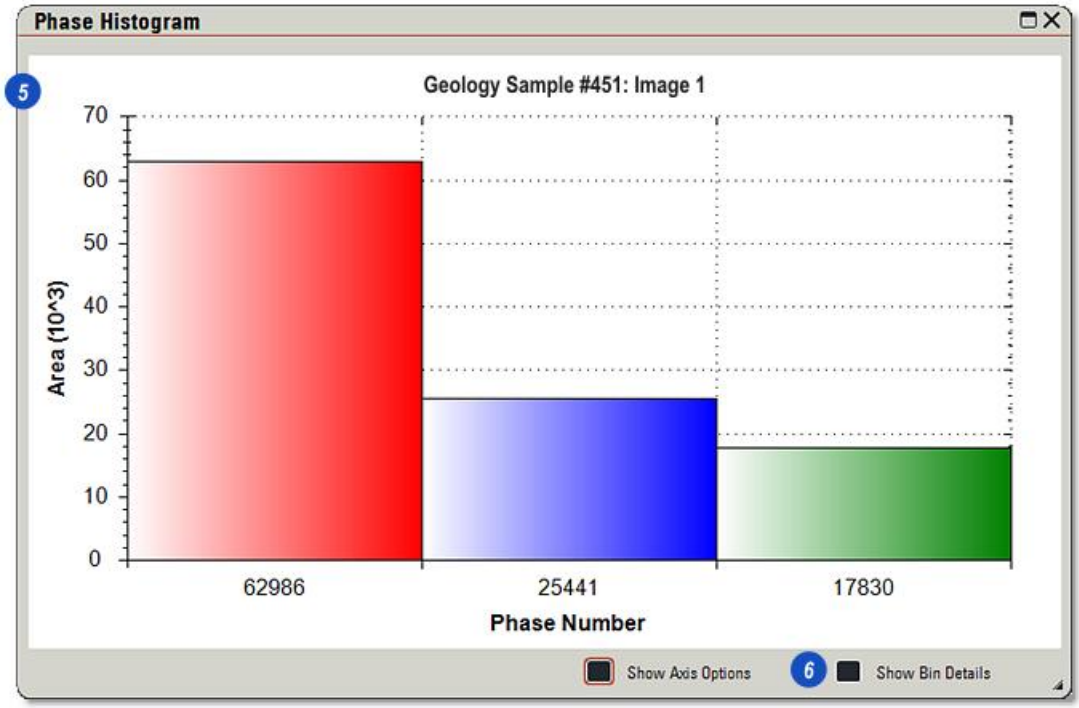
4: Click on the *Show Histogram* button.

5: The *Phase Histogram* shows the phases and their results plotted against the parameter chosen for the *Y* axis.

With *Phase Histogram* selected, the *X* axis always shows the *Phases* and cannot be changed. The *Y* axis which represents the measured parameter, can be user selected. *Go there…* 1172

6: For details of other *Axis* and *Bin* options, see *Image Analysis* 1134 help.





1: Click on the arrows to the right of the *Phase/Field* header.

2: From the drop-down menu click to select the *Field* option.

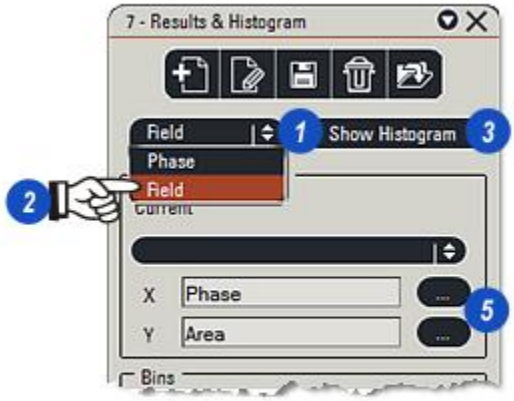
3: Click the *Show Histogram* button.

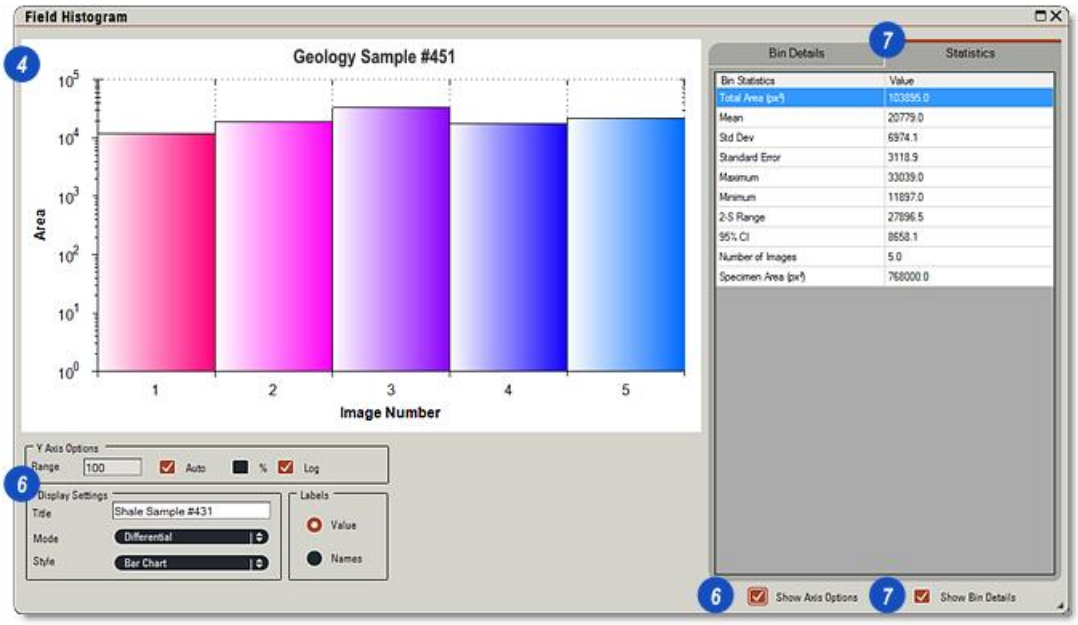
4: The *Field Histogram* is displayed. This is the result of the entire image – *Field* – plotted against the parameters chosen by the user for both the *X* and *Y* axes.

5: Selecting the parameters for the measurement axes: *Go there…* 1172

6: The *Y* axis and *Display Settings* dialog revealed by enabling the *Axis Options* check box, and...

7: ...the *Bin Details* panel when the *Show Bin Details* check box is enabled.



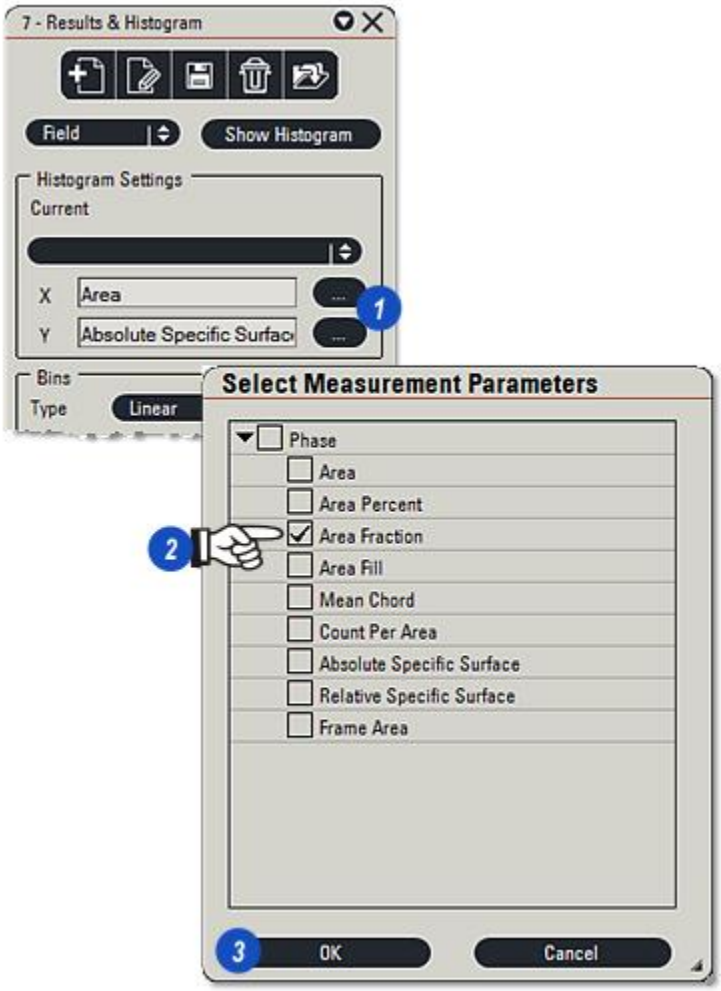


With the *Phase* option selected for the *Histogram* display, only the *Y* axis parameter can be changed. For the *Field* option both *X* and *Y* parameters can be selected.

1: Click on the *Browse* button against the axis to be changed. If the button is greyed out the option is not available.

2: The *Select Measurement Parameters* list will depend upon the display option chosen and the axis to be altered. Click to select the parameter required.

3: Click *OK*.



If multiple fields are measured, results can be aggregated or shown for individual fields by:

1: Clicking on the *Options* button on the *Select Images* dialog and ...

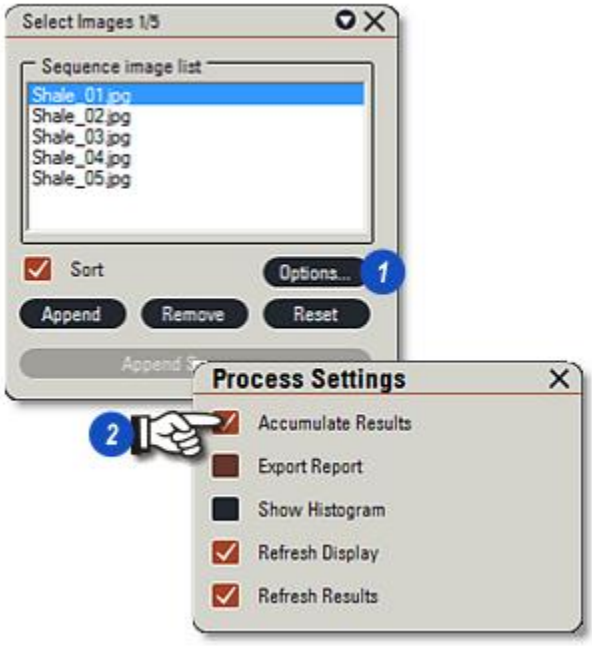
2: ...clearing/enabling the *Accumulate Results* check box on the *Select Images to Measure* panel.

The check box affects the display only so there is no need to re-process the images.

3: Click on the *Phase Details* tab to reveal the results as each *Phase* compared to the *Field* or compared to the *Reference Phase* is one has been set up.

4: Click on the *Phase Summary* tab to show each *Phase* as a set of user-defined statistics.

To change the oder of the results - low-to-high or high-to-low, double-click a column header.



3

Phase Details

Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
1	Shale_01.jpg	Phase1	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
		Phase2	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
		Phase3	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

4

Phase Summary

Phase	Statistics	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
Phase1	Total	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Mean	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
	Minimum	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
Phase2	Total	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Mean	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
	Minimum	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
Phase3	Total	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Mean	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Std Dev	-	-	-	-	-	-	-
	Maximum	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000
	Minimum	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

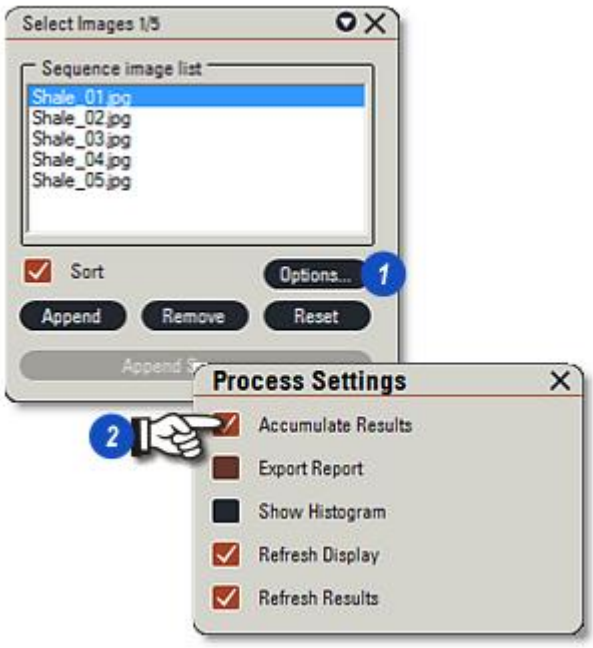
If multiple fields are measured, results can be aggregated or shown for individual fields by:

1: Clicking on the *Options* button on the *Select Images* dialog and ...

2: ...clearing/enabling the *Accumulate Results* check box on the *Select Images to Measure* panel.

The check box affects the display only so there is no need to re-process the images.

3: Click on the *Phase Details* tab to reveal the results as each *Phase* compared to the *Field* or compared to the *Reference Phase* is one has been set up.
Click on the *Phase Summary* tab to show each phase as a set of user-defined statistics.



To change the order of the results - low-to-high or high-to-low, double-click a column header.

3

Phase Details

Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Mean Chord(px)	Count Per Area(px⁻²)	Frame Area(px²)
4	Shale_04.jpg	Phase1	36.247	55675.000	0.362	0.569	10.996	7760.417	153600.000
2	Shale_02.jpg	Phase1	30.521	46881.000	0.305	0.439	9.710	7584.635	153600.000
1	Shale_01.jpg	Phase1	27.318	41960.000	0.273	0.376	11.307	5963.542	153600.000
3	Shale_03.jpg	Phase1	26.269	40349.000	0.263	0.356	8.122	9479.167	153600.000
5	Shale_05.jpg	Phase1	25.982	39908.000	0.260	0.351	7.390	11217.448	153600.000
3	Shale_03.jpg	Phase3	24.098	37014.000	0.241	0.317	15.257	598.958	153600.000
1	Shale_01.jpg	Phase2	23.775	36518.000	0.238	0.312	3.682	15585.938	153600.000
5	Shale_05.jpg	Phase2	20.327	31223.000	0.203	0.255	3.296	17207.031	153600.000
2	Shale_02.jpg	Phase2	20.243	31094.000	0.202	0.254	3.526	15071.615	153600.000
4	Shale_04.jpg	Phase2	17.050	26189.000	0.171	0.206	3.374	13170.573	153600.000
5	Shale_05.jpg	Phase3	16.867	25908.000	0.169	0.203	11.196	774.740	153600.000
3	Shale_03.jpg	Phase2	16.762	25747.000	0.168	0.201	3.305	13190.104	153600.000
2	Shale_02.jpg	Phase3	14.092	21645.000	0.141	0.164	12.938	501.302	153600.000
4	Shale_04.jpg	Phase3	13.482	20708.000	0.135	0.156	11.139	579.427	153600.000
1	Shale_01.jpg	Phase3	8.968	13775.000	0.090	0.099	11.624	390.625	153600.000

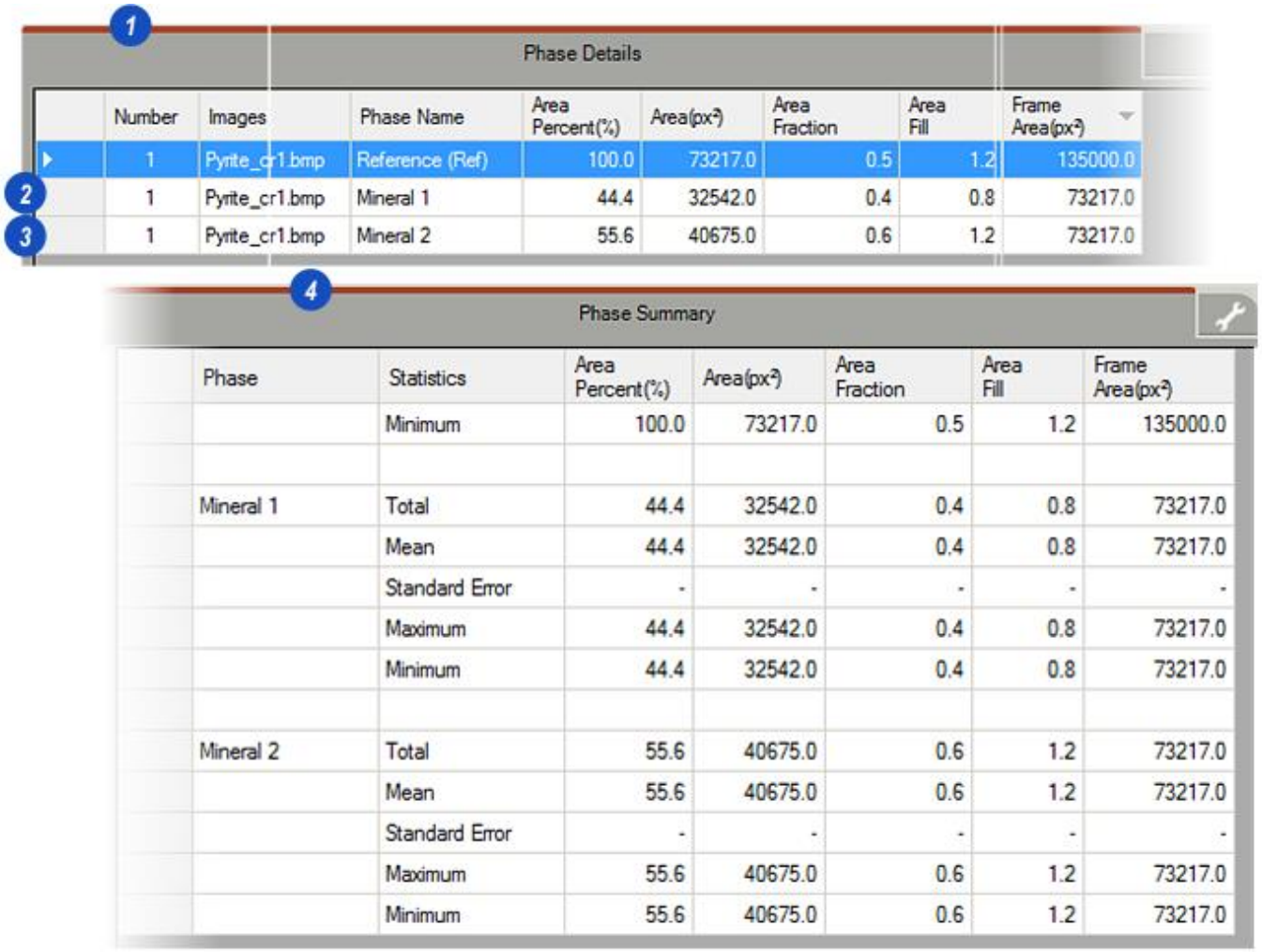
Tabular Results: The Reference example

Shown below are the tabular results for the *Reference* example.

1: Click on the *Details* tab to show the overall relationship between the *Reference* phase (Highlighted) - always shown as representing 100% of the area - and...

2 & 3: ...*Minerals 1* (44.4%) and *2* (55.6%) that together add up to 100%.

4: Click on the *Summary* tab to show the basic statistics.



Phase Details

Number	Images	Phase Name	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Frame Area(px²)
1	Pyrite_cr1.bmp	Reference (Ref)	100.0	73217.0	0.5	1.2	135000.0
1	Pyrite_cr1.bmp	Mineral 1	44.4	32542.0	0.4	0.8	73217.0
1	Pyrite_cr1.bmp	Mineral 2	55.6	40675.0	0.6	1.2	73217.0

Phase Summary

Phase	Statistics	Area Percent(%)	Area(px²)	Area Fraction	Area Fill	Frame Area(px²)
	Minimum	100.0	73217.0	0.5	1.2	135000.0
Mineral 1	Total	44.4	32542.0	0.4	0.8	73217.0
	Mean	44.4	32542.0	0.4	0.8	73217.0
	Standard Error	-	-	-	-	-
	Maximum	44.4	32542.0	0.4	0.8	73217.0
	Minimum	44.4	32542.0	0.4	0.8	73217.0
Mineral 2	Total	55.6	40675.0	0.6	1.2	73217.0
	Mean	55.6	40675.0	0.6	1.2	73217.0
	Standard Error	-	-	-	-	-
	Maximum	55.6	40675.0	0.6	1.2	73217.0
	Minimum	55.6	40675.0	0.6	1.2	73217.0

Select the Tabular Display Parameters

1: To change the *Phase Details* column header parameters or the *Phase Summary* statistic parameters, click on the appropriate results tab.

2: Click on the *Tool Tab* and, depending upon the results tab selected the *Select Phase Details* or *Select Phase Summary* dialog will appear.

3: Enable the required check boxes to include the parameter required.

4: Click OK.

The *Show All* option when enabled will select all of the options.

Click on the *Hide All* button to clear all of the options before starting a new range of selections.

Note: The meaning of most of the parameters is clear, but the following may need a little more explanation:

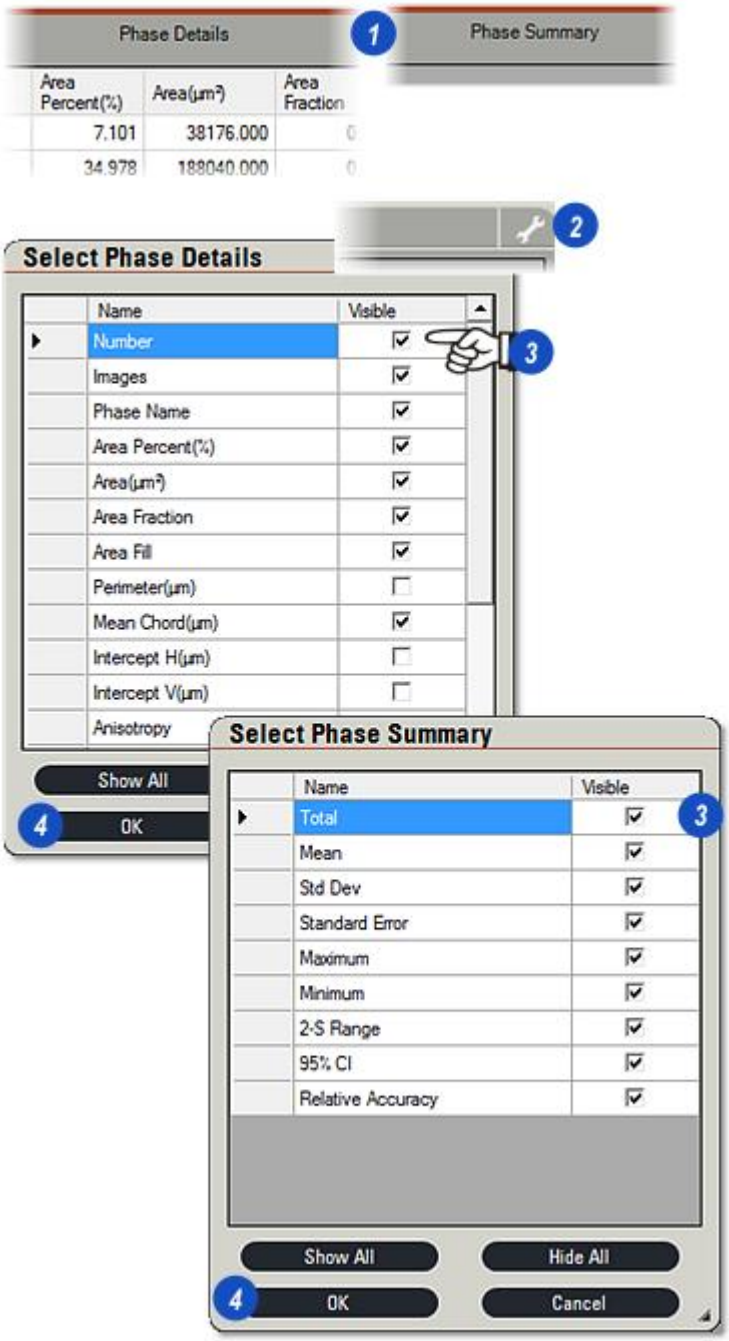
Relative Specific Surface = (perimeter / area) * 4 / pi

Absolute Specific Surface = (perimeter / frame area) * 4 / pi

Specific surface is a property of solids that measures the total surface area per unit of volume or cross-sectional area.

It is defined as: Surface area divided by the volume (units of $\mu m^2/\mu m^3$ or μm^1)

Stereology is used to determine this parameter from planar cross sections; here it is the perimeter/area ($\mu m/\mu m^2$) .

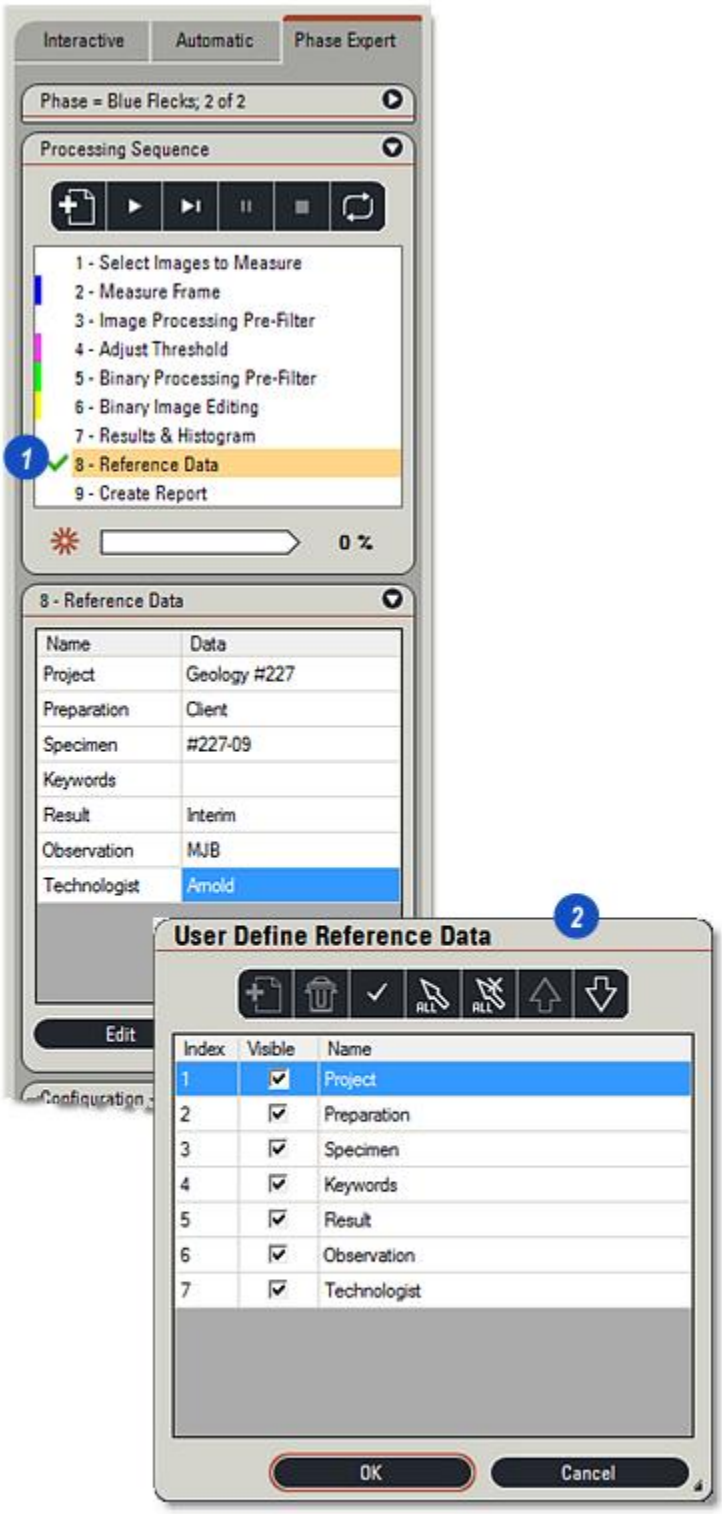


A comprehensive range of *Data* Items can be appended to the *Phase Expert* results that will identify important details such as the *Project Name*, the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

1: Click on the *Reference Data* option on the *Processing Sequence* menu to reveal the *Reference Data* dialog.

Administrators can add to the supplied list of data headings to comply with corporate needs **(2)**.

For details refer to *Image Analysis*[1140] help:



1: Click to select *Create Report* on the *Processing Sequence* menu.

2: On the *Create Report* panel, click to select/ de-select the images to be included in the report.

3: Nominate a *Report Layout Template* by clicking on the browse button and…

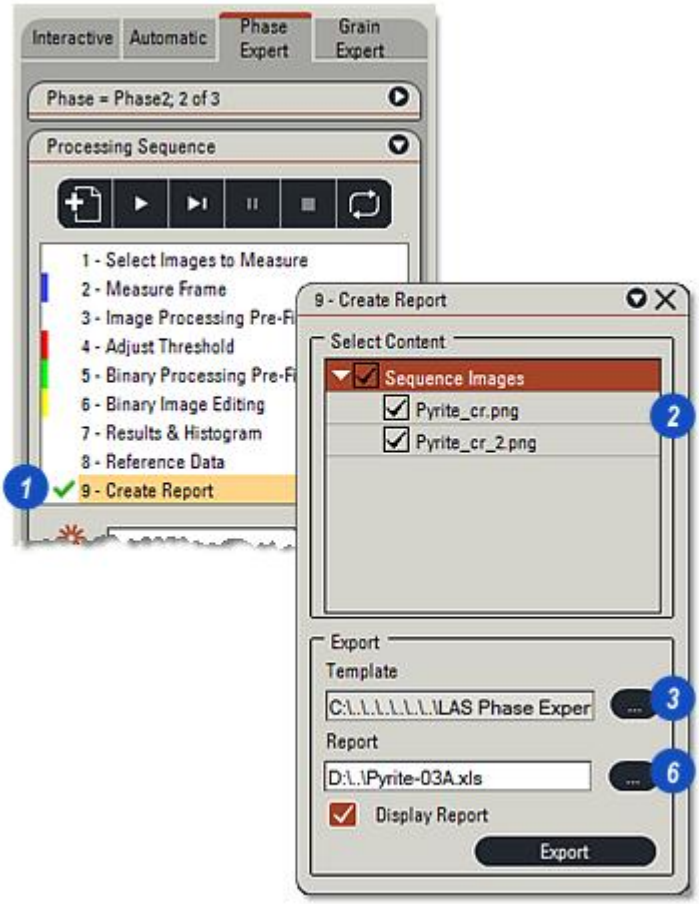
4: …navigating to and clicking to select a template. A default template is supplied with *Phase Expert* and is located in the *Excel Templates\Phase\LAS Phase Expert Templates* folder.
The supplied template can be modified to suit the user's requirements and saved under a separate file name.

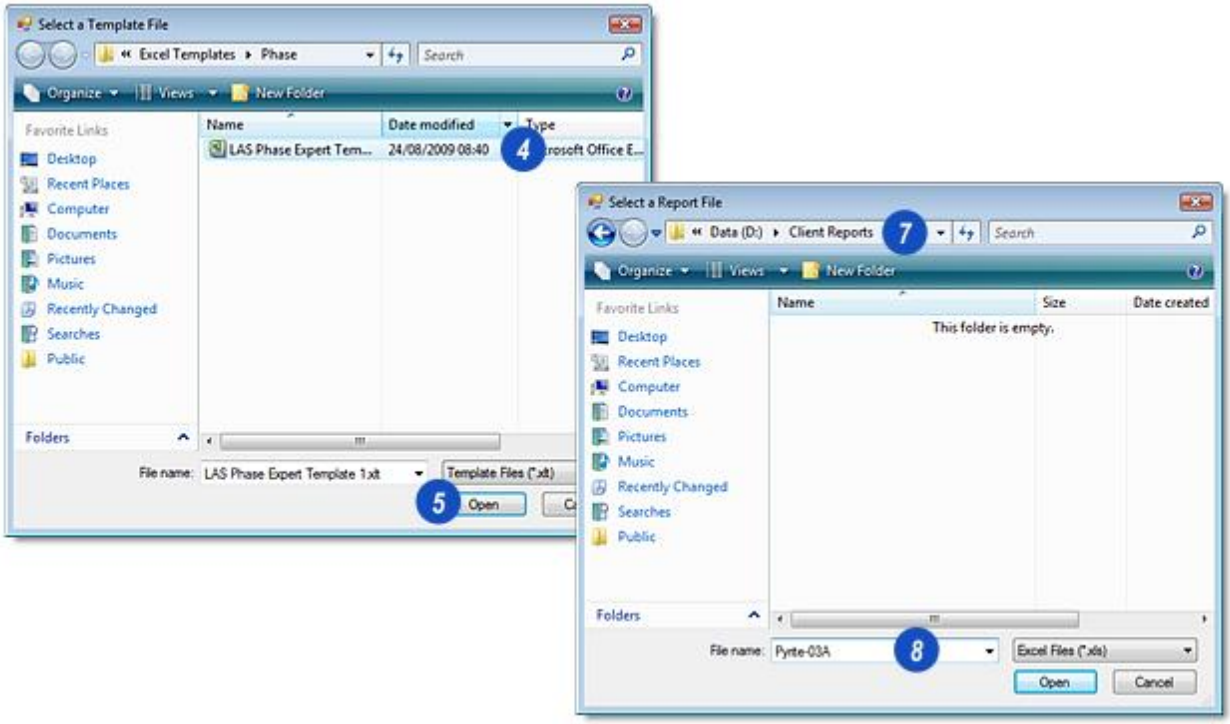
5: Click *Open*.

6: Specify where the report is to be saved by clicking the browse button to the right of the *Report* text box and…

7: …navigating to the destination folder, ...

8: …typing a file name for the report and clicking *Open*.

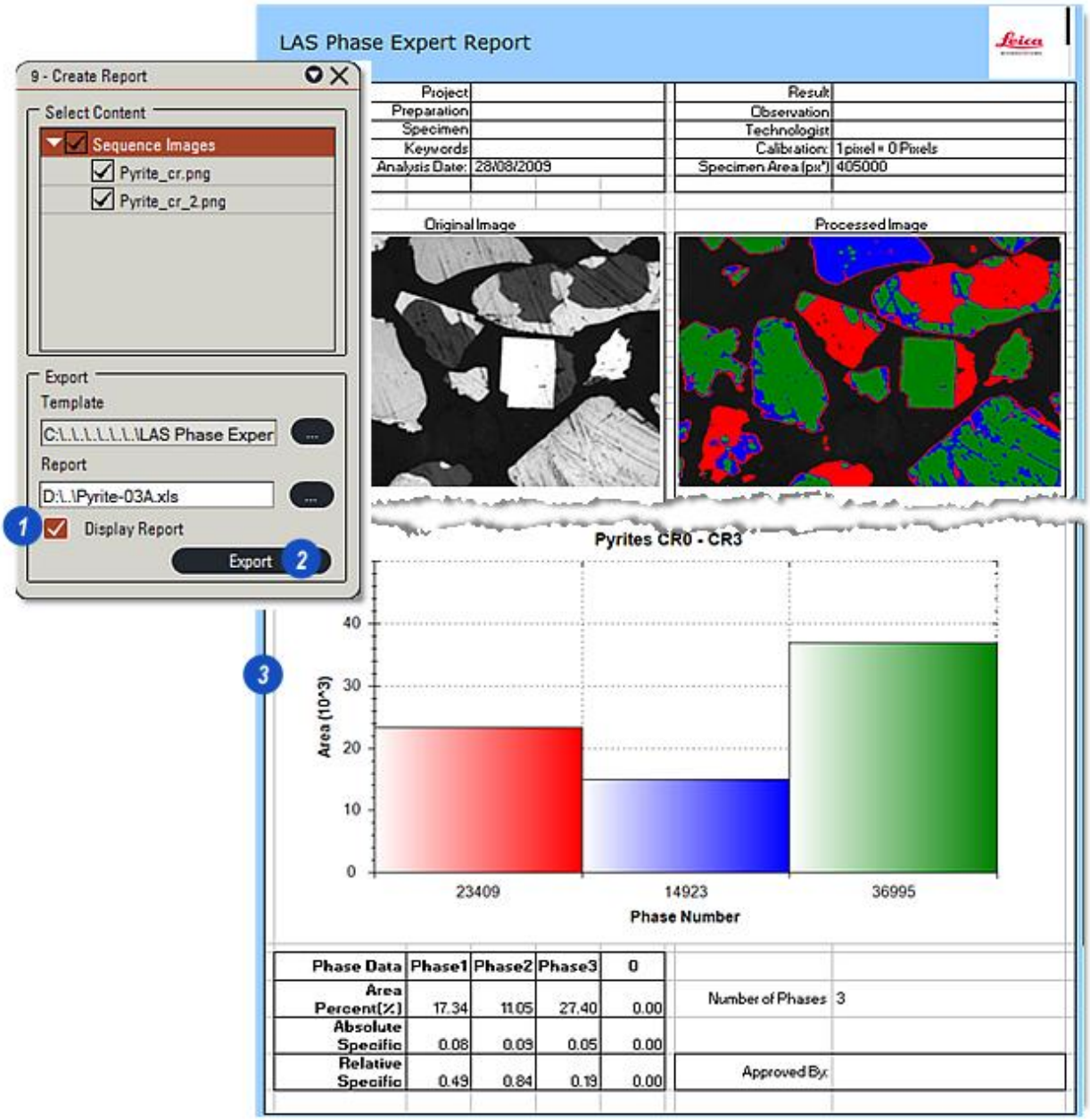




1: To display the report automatically as it is created, click to enable the *Display Report* button.

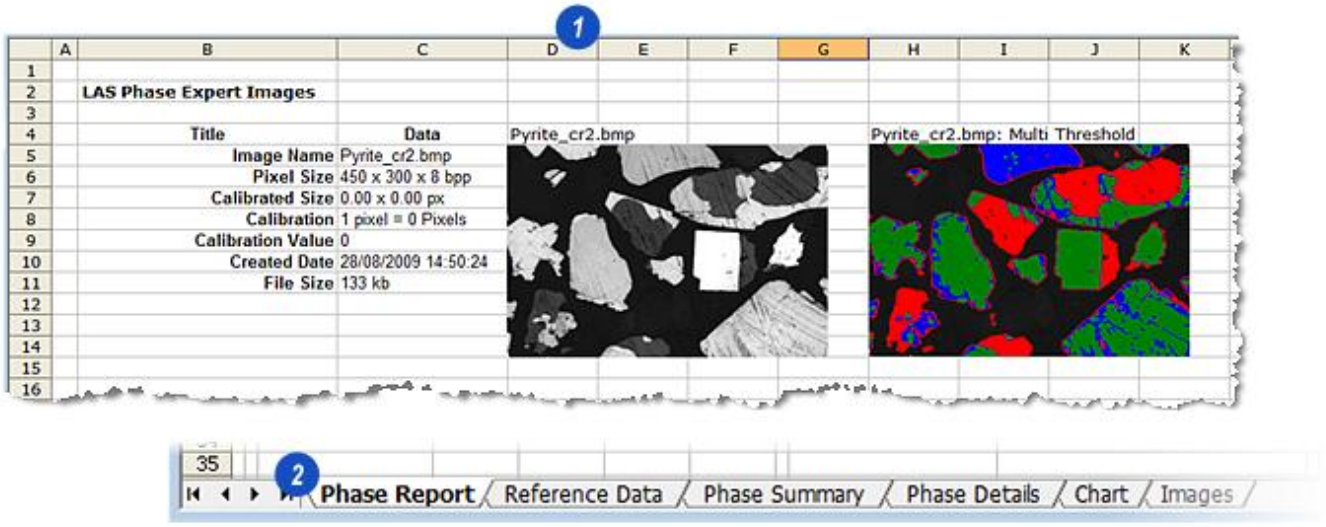
2: To create the report click the *Export* button.

3: The front page of the report displayed in *Microsoft Excel*.



1: Sample page from the *Microsoft Excel* report.

2: The *Excel Spreadsheet* tab structure.



Excel Tags:

Excel reports can be styled to suit the user by using *Tags* that can determine the results and images being displayed. Tags are simple text strings that can be typed directly into an Excel cell or copied using the *Excel Macro* feature.

More information about *Excel Tags*[425]

The Tags for *Phase Expert* have a specific format and are listed opposite. Click on the required tag for help in using it.

<LAS AM User Data>[450] Reference Data

<LAS AM Phase Results>[451] Phase Results

<LAS AM Phase Summary>[452] Phase Summary

<LAS AM Phase Histogram Statistics>[453] Phase Statistics

<LAS AM Phase Histogram Bin Data>[454] Phase Bin Data

<LAS AM Phase Histogram Chart>[453] Phase Chart Graphic

<LAS AM Images>[455] All Images

<LAS AM Image>[456] Selected Image

<LAS AM Function>[457] Functions

The current settings for most editing panels can be saved with the archive as a *Configuration* to be retrieved at a later date. Retrieved *Configuration* settings are applied to automatically to the tools

1: Each configuration has a unique name and can be accessed by clicking on the arrow to the right of the *Current Configuration* window and from the drop down list…

2: …clicking to select the required configuration.

3: Click the *Display Configuration Settings* button to list the configuration details.

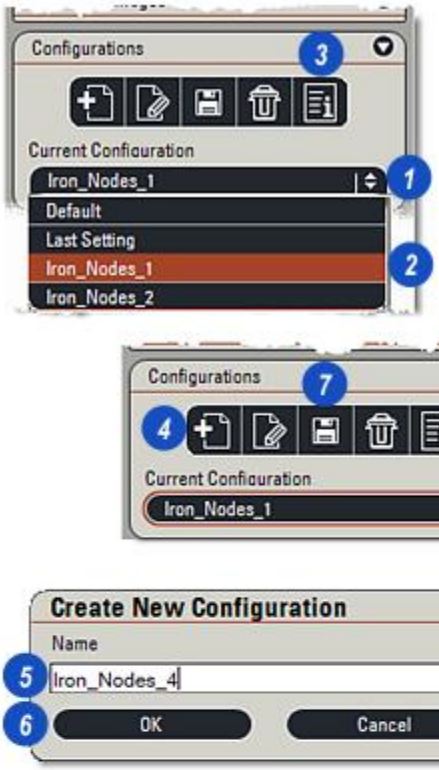
Save Configuration:
Saves all of the current settings and process sequences in a unique file.

4: To save the current settings as a new configuration, click on the *New* button and…

5: …type a unique name for the new configuration.

6: Click *OK* to save the setting.

7: Click on the *Save Configuration* button The new configuration appears in the drop down list.



Edit Configuration:
A *Configuration* name can be changed by:

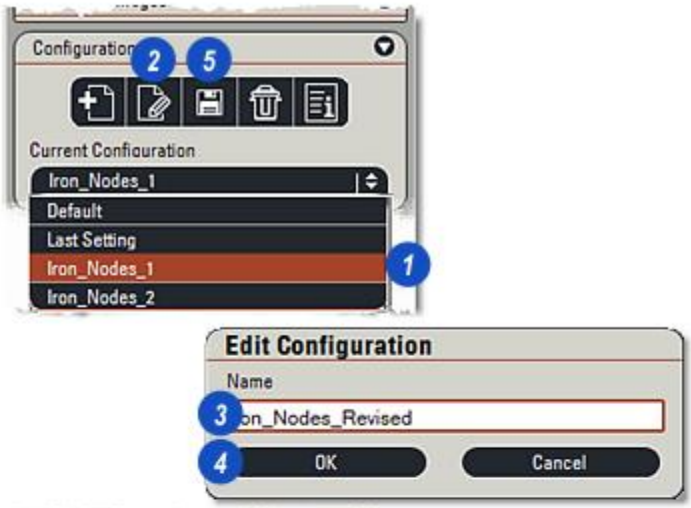
1: Select the configuration to be changed from the drop down list.

2: Click on the *Edit Configuration* button.

3: On the *Edit Configuration* dialog, change the name by clicking in the text box and typing a new name and…

4: …clicking *OK*.

5: Click the *Save Configuration* button.

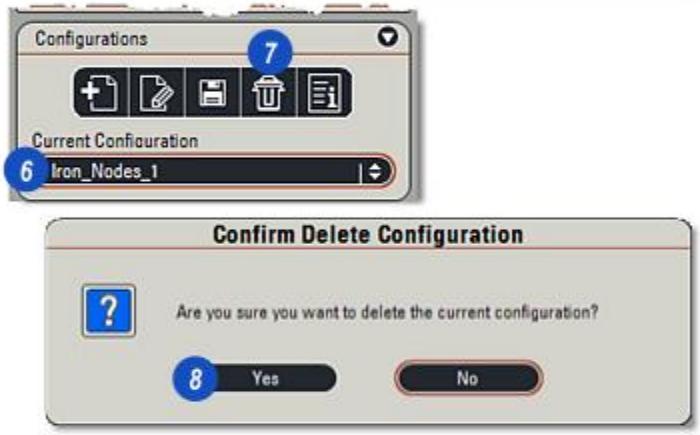


Remove a Configuration:
A *Configuration* can be removed from the list by:

6: Selecting the configuration to be deleted from the drop down list.

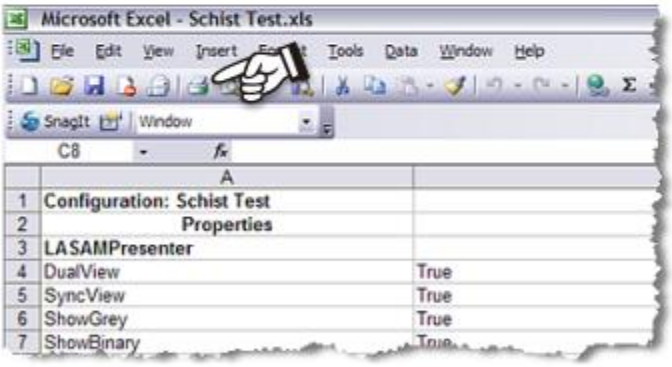
7: Clicking the *Delete* (Trash Can) button.

8: Confirm the deletion and the *Configuration* will be removed permanently. The operation cannot be reversed.



Print a Configuration:
Check that the printer is on and connected to the computer. The configuration is captured to an Excel spreadsheet and printed from there.

1: Select the *Configuration* to be printed from the drop down list. When the Configuration settings appears on an *Excel* spreadsheet, use the print function to print the settings list.



Grain Expert

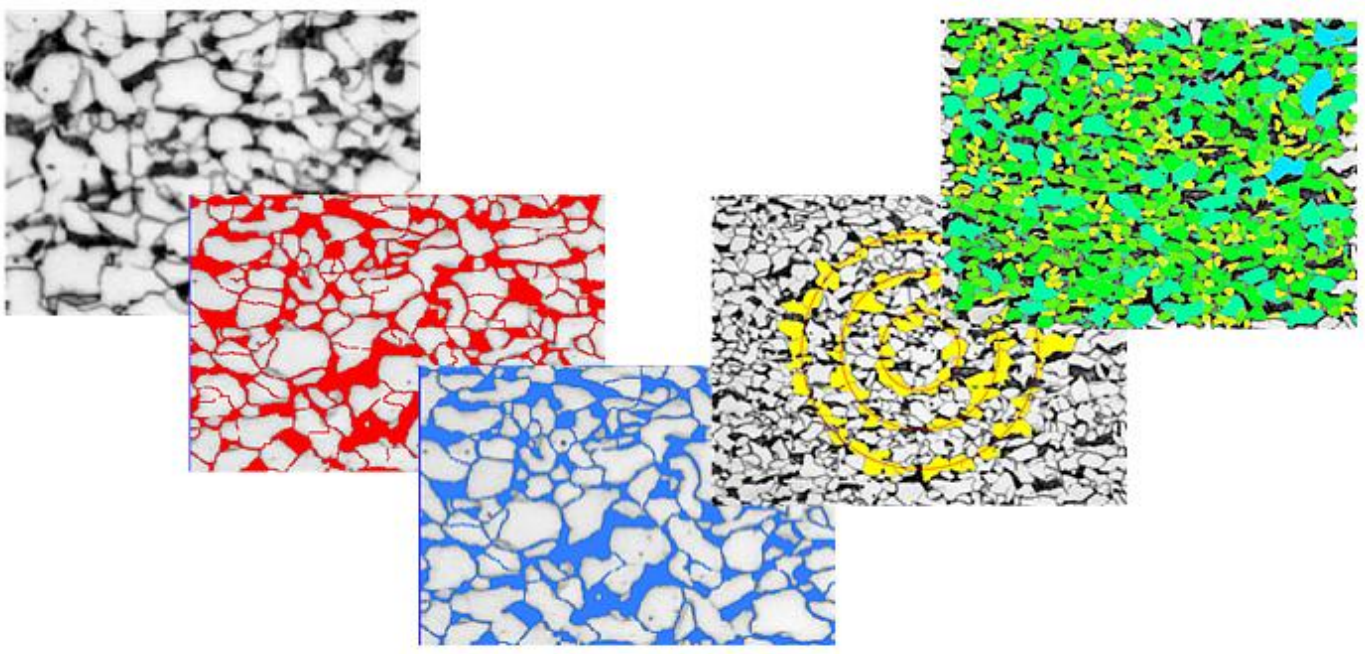
The grain sizes in a metal, plastic, composite or material is an important factor in determining its strength or machinability. *LAS Grain Expert* allows you to measure the average grain size of visible grains on the surface of a specimen. Grain boundaries are identified on the basis of contrast in the image. Grains can be measured using a number of different standards and techniques using automatic methods to identify boundaries. For example, on appropriate images:

- Automatically identify grains and measure their size.
- Count the intersections of grain boundaries with a test line
- Mark the diameter of selected grains on an image
- Edit the image to trace the shape of selected grains on an image
- Compare a sample image with a selection of typical example images to select the most appropriate boundary identification method.

LAS Grain Expert calculates:

- Average grain size, expressed in terms of a grain size number.
- Mean grain area.
- Maximum and minimum grain size.
- Confidence level.
- Relative accuracy.

The results obtained depend on the standard and technique employed.



Grain Expert must be installed, licensed and enabled. Click on the *Analysis Workflow* **(1)** and then on the *Grain Expert* tab **(2)**.

If the tab is not present, *Grain Expert* has either not been installed or is not enabled: Refer to the *LAS Installation Guide*.

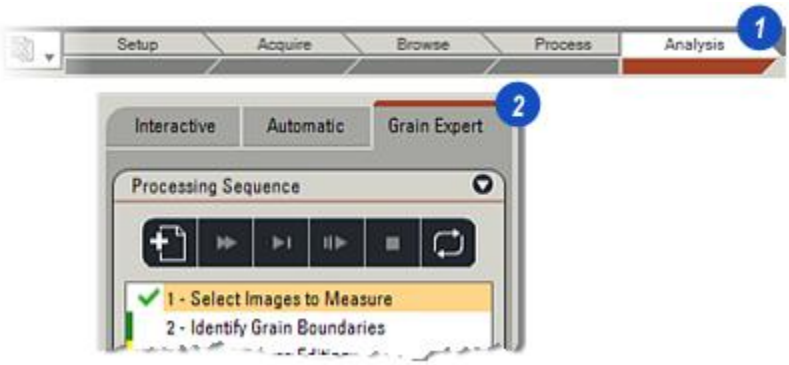
These steps represent the sequence users should follow to successfully carry out *Grain* measurements.

Because *Grain Expert* works in conjunction with *Image Analysis*, in most steps links are provided to both modules:
Red, will take the reader to the appropriate place in the *Image Analysis (Automatic)* help file for more detailed information, and *Blue,* connects to help files that are specific to *Grain Expert*. Sometimes, it will be beneficial to follow both links if they are provided.

Select the Images to Process
One or more images can be processed in sequence simply by selecting the *Thumbnail (s)* in the *Gallery* and then clicking the *Append* button on the *Select Images to Measure* panel.
Image Analysis help 1043
Grain Expert help 1185

Identify the Grain Boundaries
A range of *Reconstruction Images* are supplied with *Grain Expert* to help to simplify and speed the task of finding the grain boundaries.
Additional close refinement can then be achieved with the *Threshold* and *Sensitivity* controls.
Grain Expert help 1187

Binary Image Editing
Repair boundaries, remove features and generally fine-tune the *Binary Image* prior to analysis.
Image Analysis help 1095
Grain Expert help 1195



Select Standard
Grain Expert provides configurations for all of the internationally accepted standards for grain analysis together with a wide range of statistical methods.
Grain Expert help 1196

Results and Histogram
Display the results using graphical *Histograms* - bar and pie charts available - as well as *Tabular* data in the *Grid* conveniently grouped under detail and statistic tabs for both *Fields* and *Grains*.
Image Analysis help 1127
Grain Expert help 1199

Reference Data
Add user and analysis references to make the job totally product and company specific. The references are automatically included in the report.
Image Analysis help 1140
Grain Expert help 1205

Create Report
Reports are created using *Microsoft Excel* and a suitable template. A standard template is supplied with *Grain Expert* and can be tailored to user and corporate needs.
Image Analysis help 1146
Grain Expert help 1206

Select Images to Measure

1: On the *Processing Sequence* click on *Select Images to Measure*.

2: Click on a *Thumbnail* in the *Gallery*.

3: Click to *Append* button on the *Select Images* dialog to add the selected image to the *Sequence Image List*.

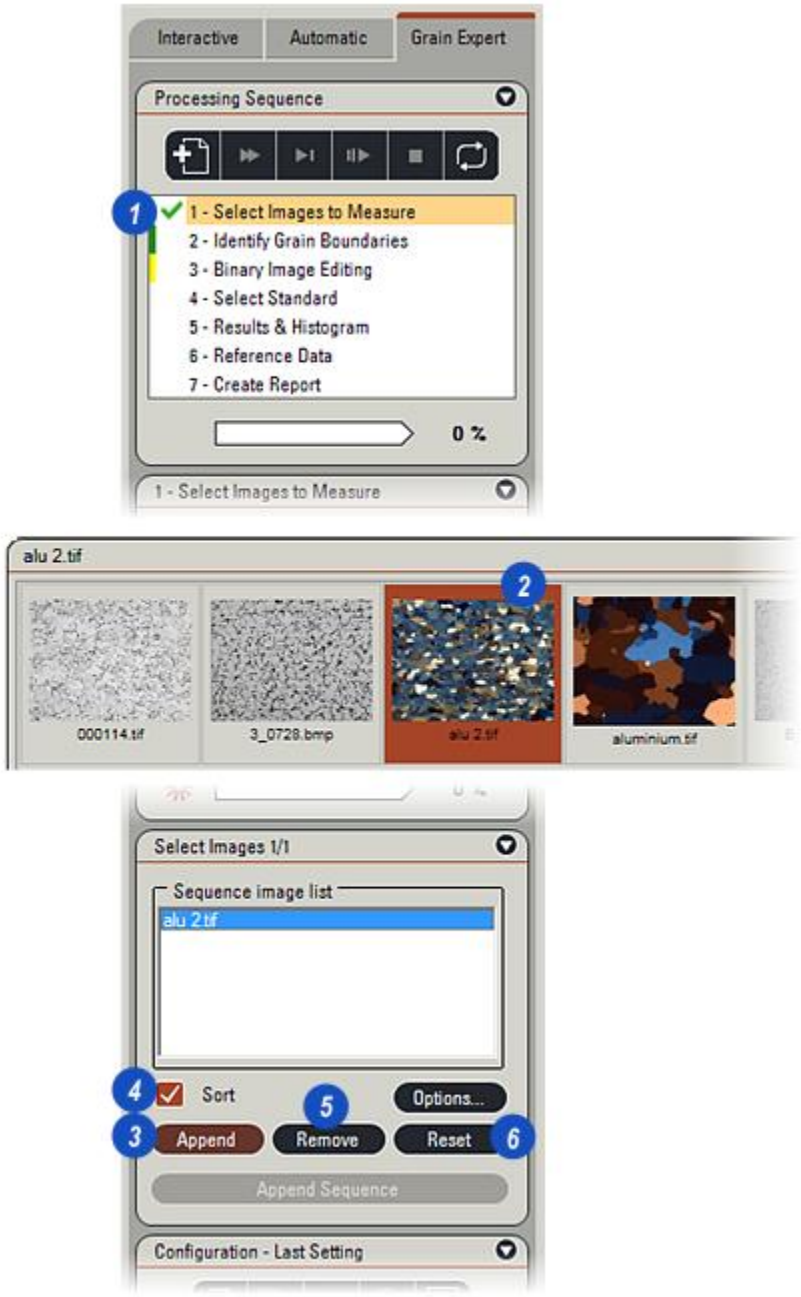
Repeat the steps to add more images to be measured as a batch.

All selected images must have the same type - for example *.bmp, .png, .jpg* - without compression, and the same resolution.

4: Images can be selected in any order from the *Gallery*. To sort them into numerical sequence click to enable the *Sort* check box.

5: Use the *Remove* button to remove a single selected image from the list, or...

6: ...click the *Reset* button to remove all images.



Additional controls on the *Select Images* panel are check boxes – click to enable, click again to disable - reached by:

1: Click on the *Options* button to reveal...

2: ...the *Process Settings* pop-up menu.

3: *Accumulate Results:* Adds the results of the current pass to those of the previous. Two or more images have to be selected.

4: *Export Report*: At the end of the analysis run an *Excel Report* is generated and displayed. Excel must be installed on the computer.

5: *Show Histogram*: Displays the *Histogram* at the end of the analysis run.

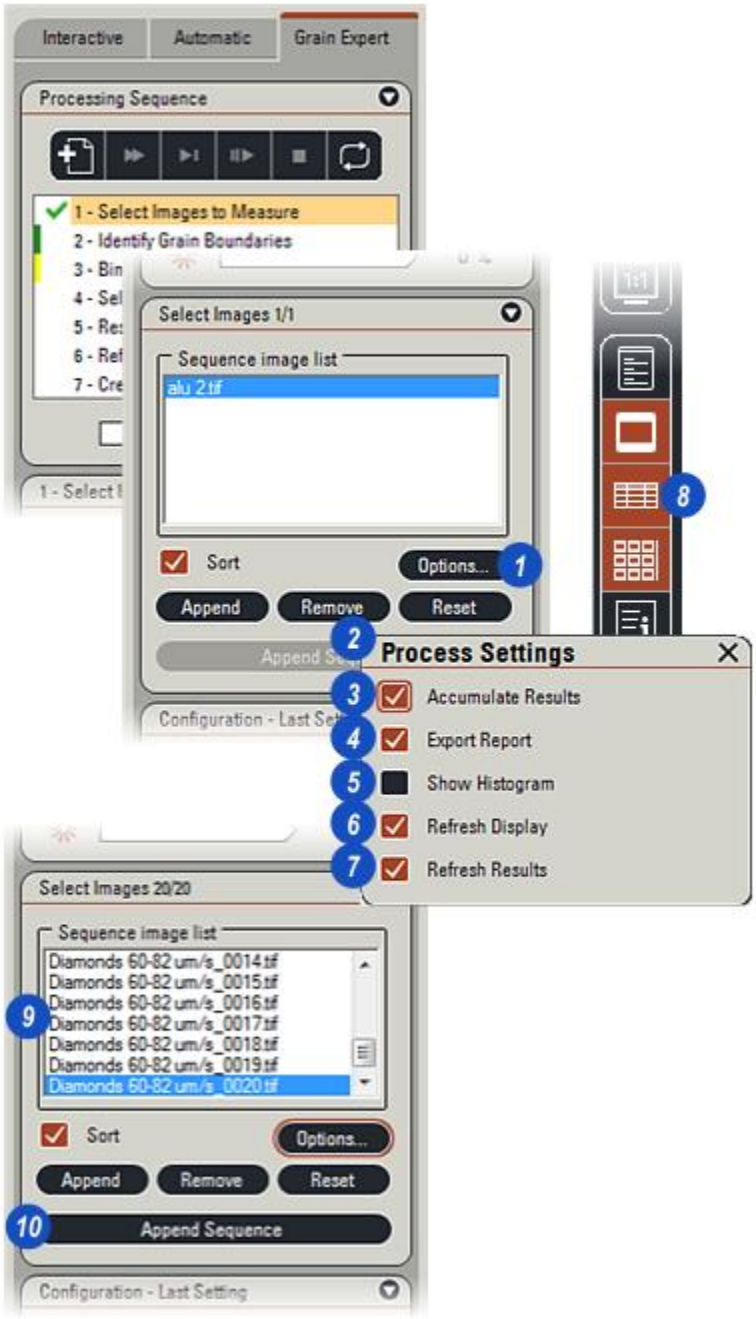
6: *Refresh Display*: Updates the display as processing proceeds independently of the *Interactive/Apply* setting on each sequence panel.

7: *Refresh Results*: Updates the *Grid* as processing proceeds. Turn the *Grid* on by clicking to enable the *Grid* display button **(8)** on the *Side Tool Bar*.

9: If multiple images are part of a sequence, the software recognises this and…

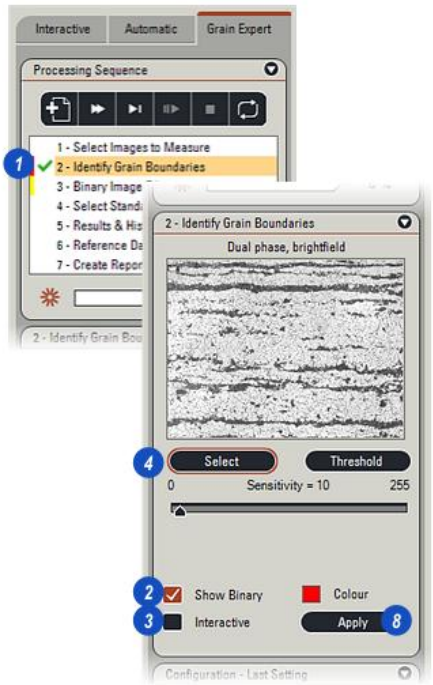
10: …the *Append Sequence* button becomes active. Click the button and the selected range is imported into the *Image List*. This avoids having to load images separately. To select a range of images in the *Gallery*, click on the first image and then holding down the *Shift* key click on the last. All of the images between will be selected.

Continued…



To select a range of images separately, click on the first image and then holding down the *Ctrl* button click on each of the others. If *Sort* is enabled, the sequence will be sorted numerically.

To speed grain boundary detection, several *Reconstruction Images* are provided with *Grain Expert*. By matching one of these to the selected specimen, a more appropriate and faster software algorithm can be used.



1: Click to select *Identify Grain Boundaries* in the *Processing Sequence* menu.

2: On the *Identify Boundaries* panel, click to enable *Show Binary* to view the processed image.

3: Click to disable *Interactive to* prevent processing whilst matching a *Reconstruction Image* to the specimen.

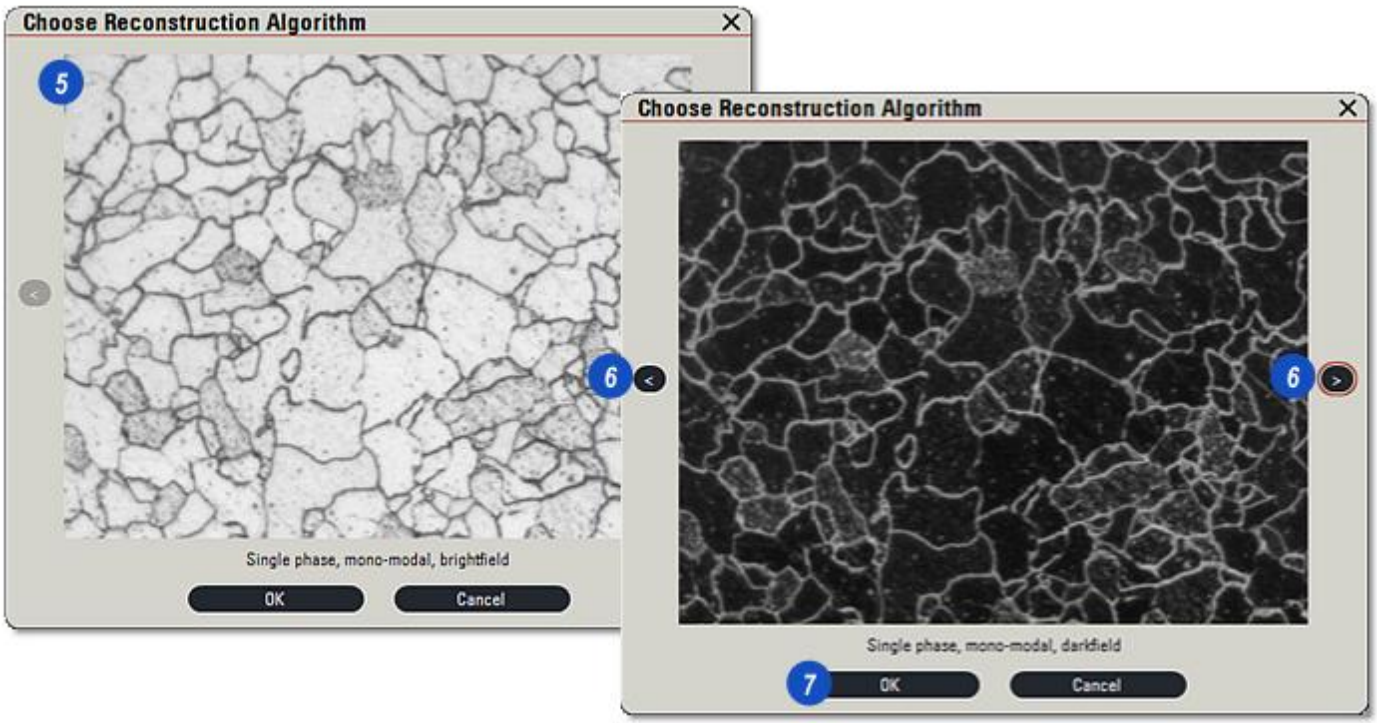
4: Click the *Select* button.

5: The first *Reconstruction Image* appears.

6: Move back and forth through the images until one appears that more closely matches the specimen than any of the others. It does not have to be identical.

7: Click *OK*.

8: Click on the *Apply* button and the initial boundary detection will appear in the binary window.

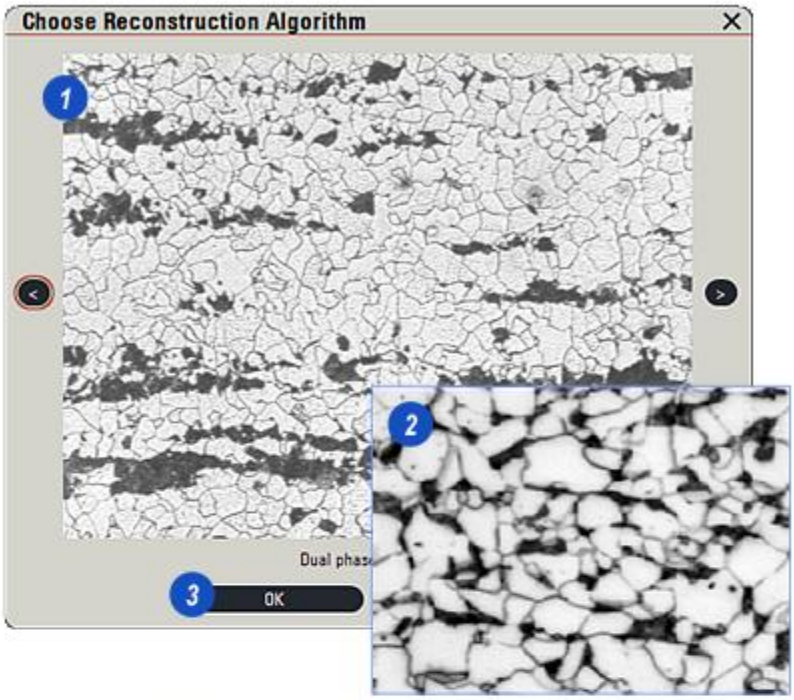


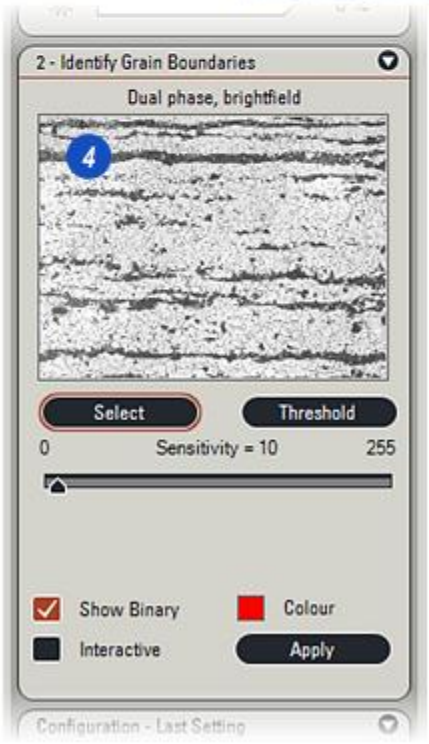
1: In the example the *Dual phase brightfield Reconstruction Image* has been chosen as quite closely matching that of…

2: …the specimen.

3: Click *OK* and…

4: …a scaled version of the *Reconstruction Image* appears in the *Identify Boundaries* window.





Grain Expert supports both greyscale and colour images, but using conventional bright- and darkfield illumination the grains will appear only as various shades of grey. Images captured using polarised light techniques will display in colour and the *Etched Polarised Reconstruction Image* should be selected.

1: Click to select *Identify Grain Boundaries* in the *Processing Sequence* menu.

2: On the *Identify Boundaries* panel, click to enable *Show Binary* to view the processed image.

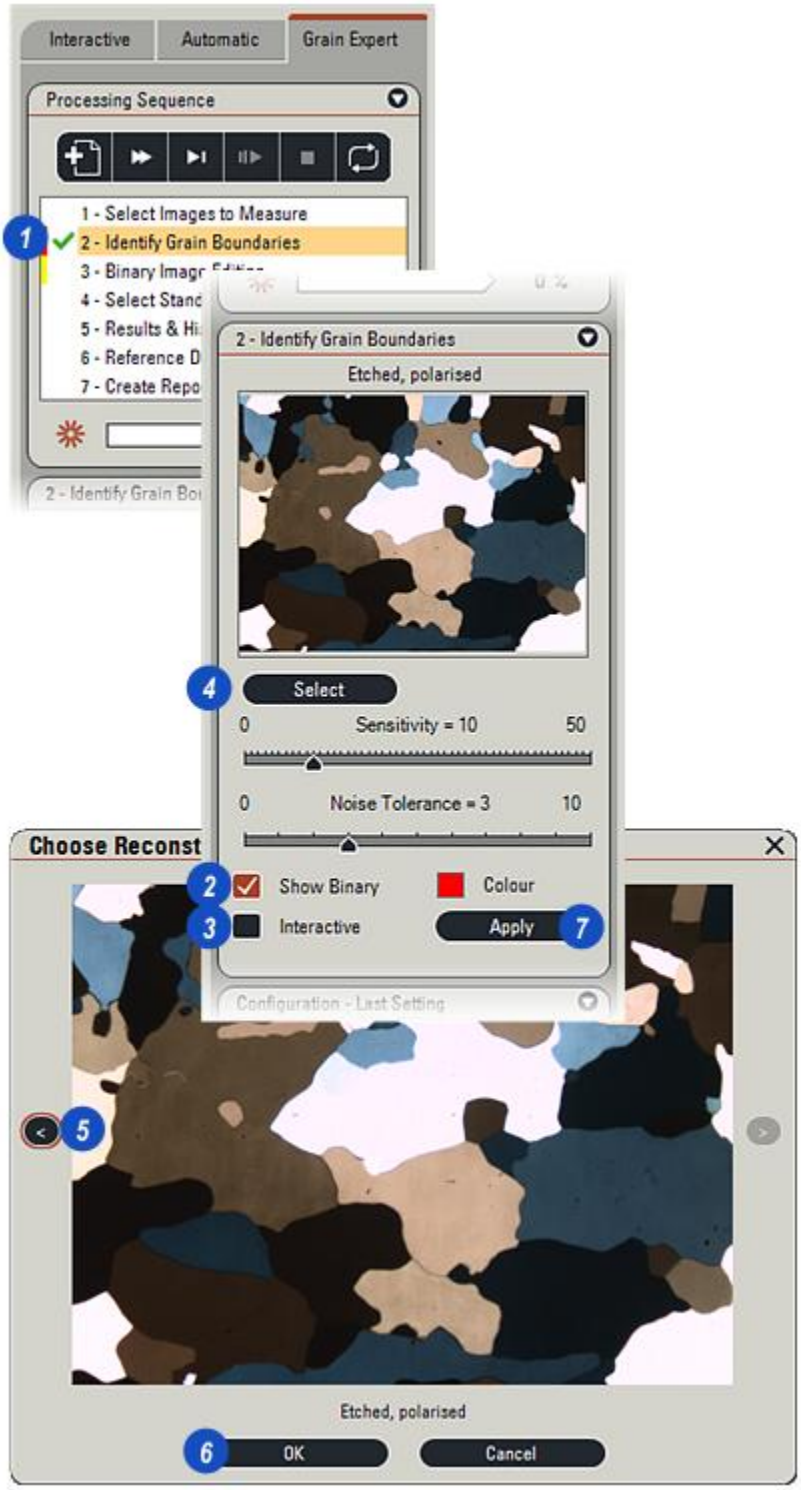
3: Click to disable *Interactive to* prevent processing whilst finding the *Reconstruction Image.*

4: Click the *Select* button.

5: The first *Reconstruction Image* appears. Move through the reconstruction samples to find *Etched, Polarised.*

6: Click *OK*.

7: Click on the *Apply* button and the initial boundary detection results will be displayed in the binary window.



Select the Boundary Colour

The colour used to show the grain boundaries can be chosen to suit both the user and the image.

1: Click on the *Colour* box on the *Identify Boundaries* panel. The *Select Color* dialog appears.

2: Choose a new colour by dragging the target mark on the *Colour Wheel*,

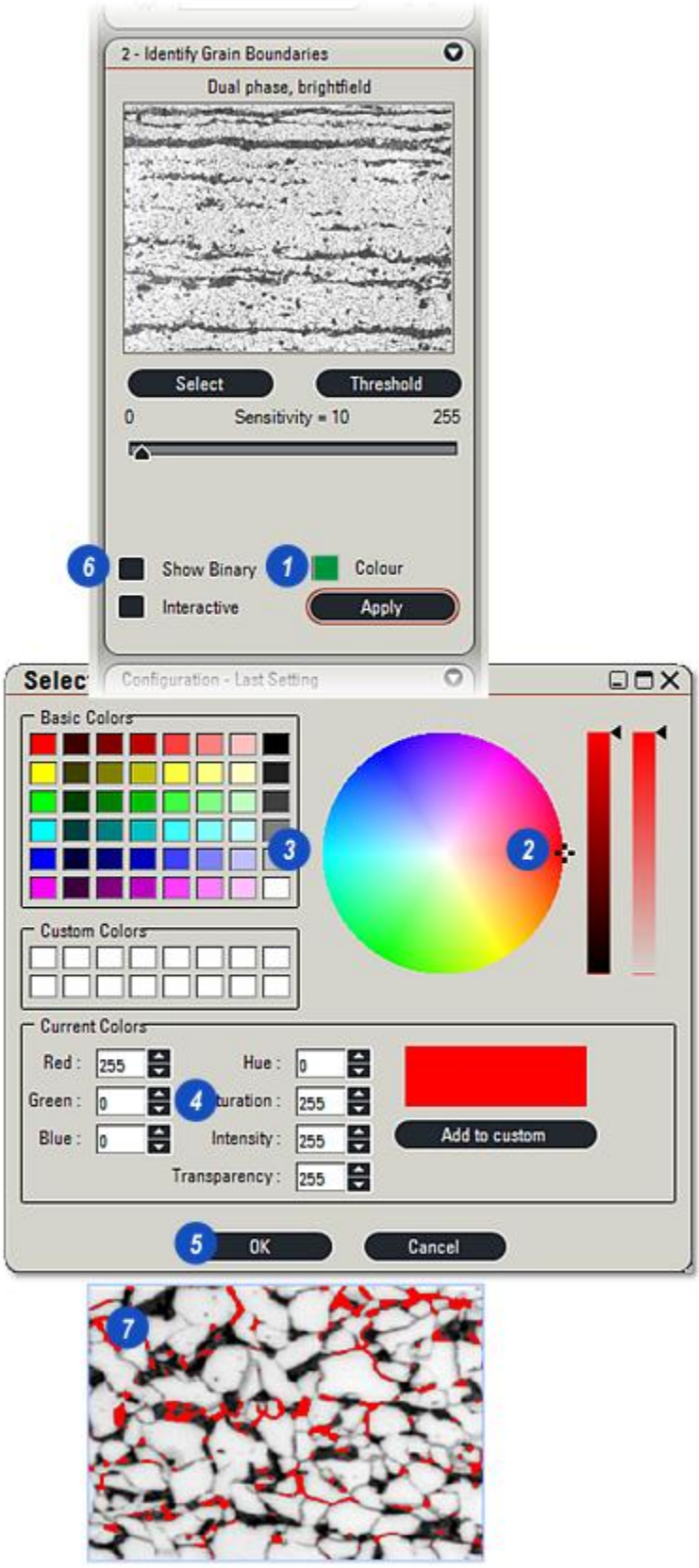
3: …clicking to select a *Swatch* or…

4: …typing *Values.*

5: Click *OK*. The new colour appears in the *Colour* box.

6: Click to enable the *Show Binary* check box and…

7: …the new colour appears on the binary output image.



The *Threshold* and *Sensitivity* controls together, serve to detect the boundaries between individual grains. Time spent at this and during the *Binary Editing* stage to identify the boundaries as closely as possible, will result in a more accurate analysis.

Set the Sensitivity to Mid-range:
This is the starting setting to provide initial boundary detection and will be adjusted later:

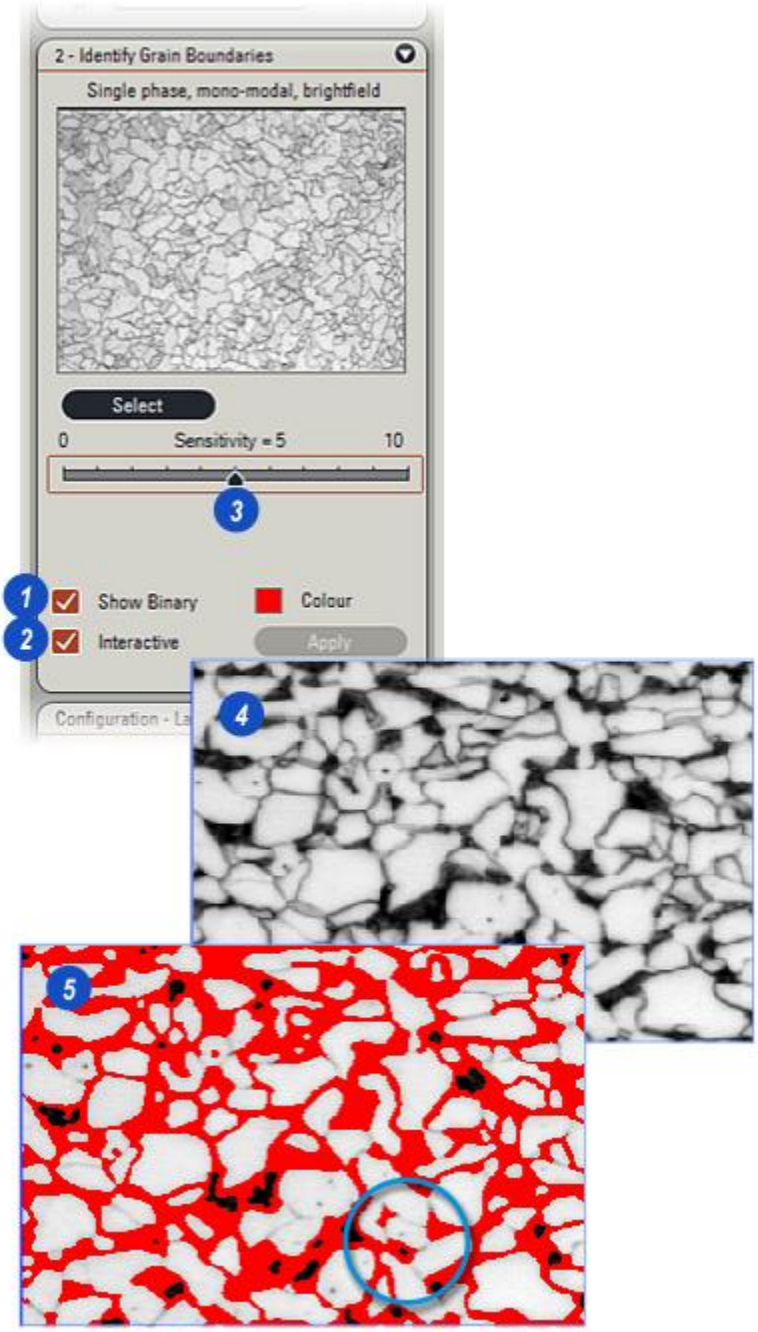
1: If necessary, click to enable *Show Binary*. This will display the binary output image with the detected boundaries shown in the selected colour.

2: Click to enable Interactive update.

3: Click, hold and drag the *Sensitivity* slider to about the mid-point on the track.

4: Part of the original image and…

5: …after the *Sensitivity* has been changed. At this stage many of the boundaries remain undetected – the original grey instead of red.



Depending upon the *Reconstruction Image* chosen, for greyscale images an additional control will be available to the user - the *Threshold* button that can fine-tune the boundary detection using a Histogram.

1: The binary image with *Sensitivity* set from the previous step.

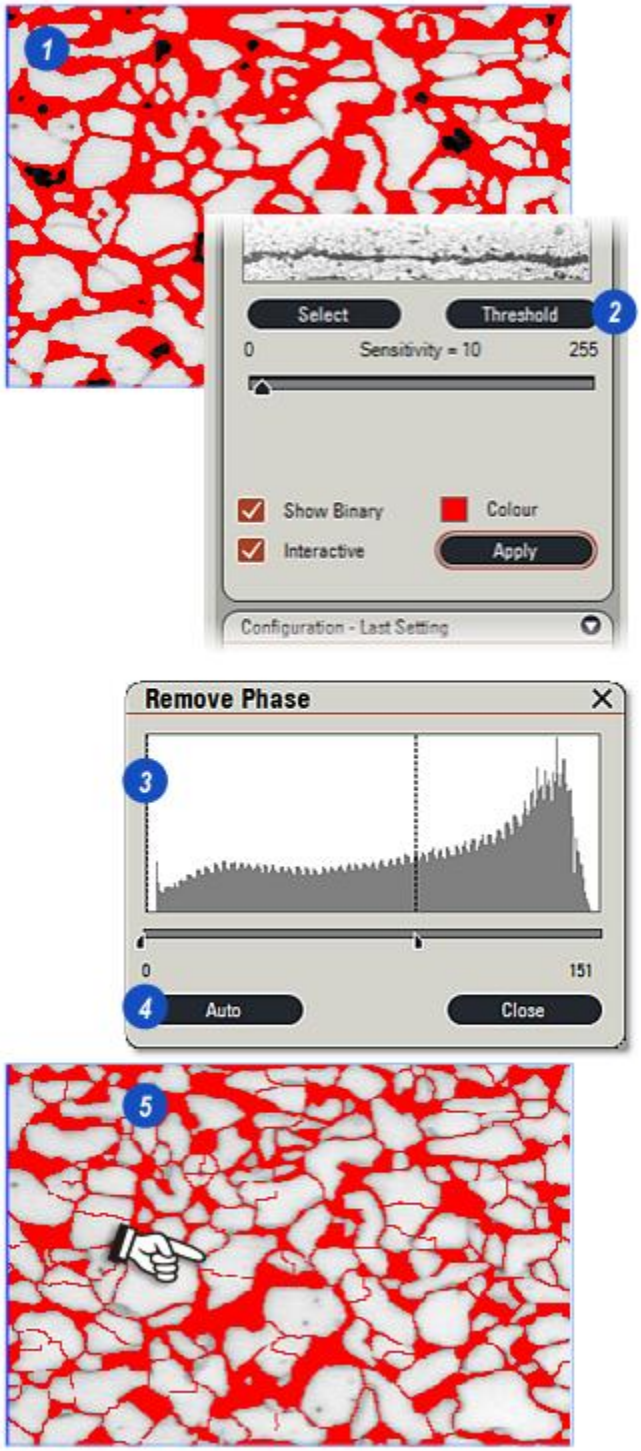
2: On the *Identify Grain Boundaries* panel, click the *Threshold* button.

3: The *Threshold Remove Phase Histogram* appears representing the image with the total black pixels (Value 0) to the left and the white pixels (Value 255) to the right.

The vertical dotted bars and the sliders beneath the *Histogram* represent the span of pixel values included in the binary image. Pixels with a value greater than *151*, the setting on the illustration, are ignored.

4: Click on the *Auto* button. This will automatically detect detail in the image that could represent grain boundaries.

5: The binary image after *Auto* detect - far more boundaries have been detected and the entire image is more refined.



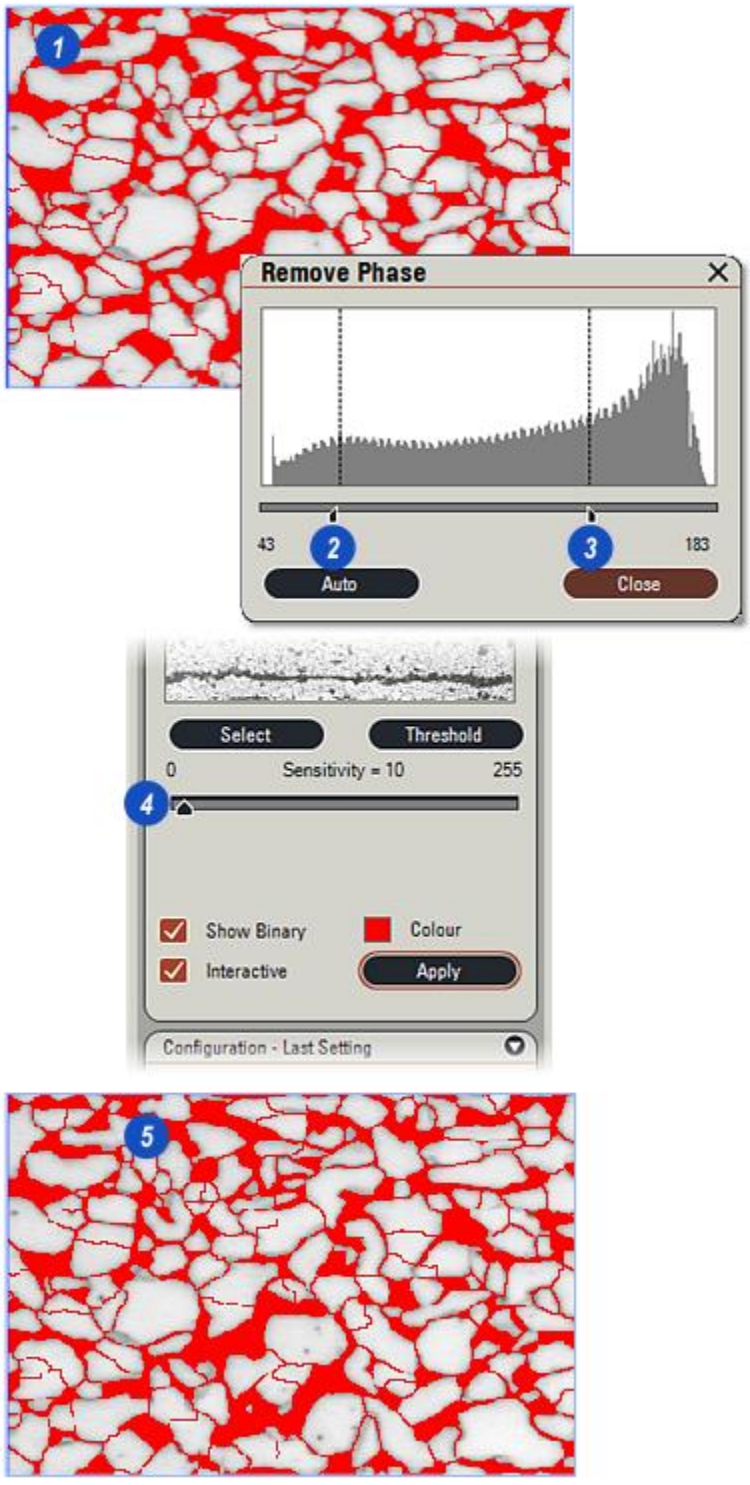
1: The binary image following the *Auto* detect stage.

The *Histogram Remove Phase* dialog can be used to refine the detection process by:

2 & 3: Dragging the *Histogram* sliders to include or reject pixel values. On the illustration, the black and dark grey range has been excluded by dragging the left slider to value 43. The mid- and light grey range has been increased by dragging the right slider to value 183. In this way it is possible to detect boundaries that the *Auto* detect did not find.

4: Complete the detection by gradually dragging the *Sensitivity* slider to the left – approaching 0 – until there is no perceptible improvement in the boundary detection.

5: The binary output image so far.



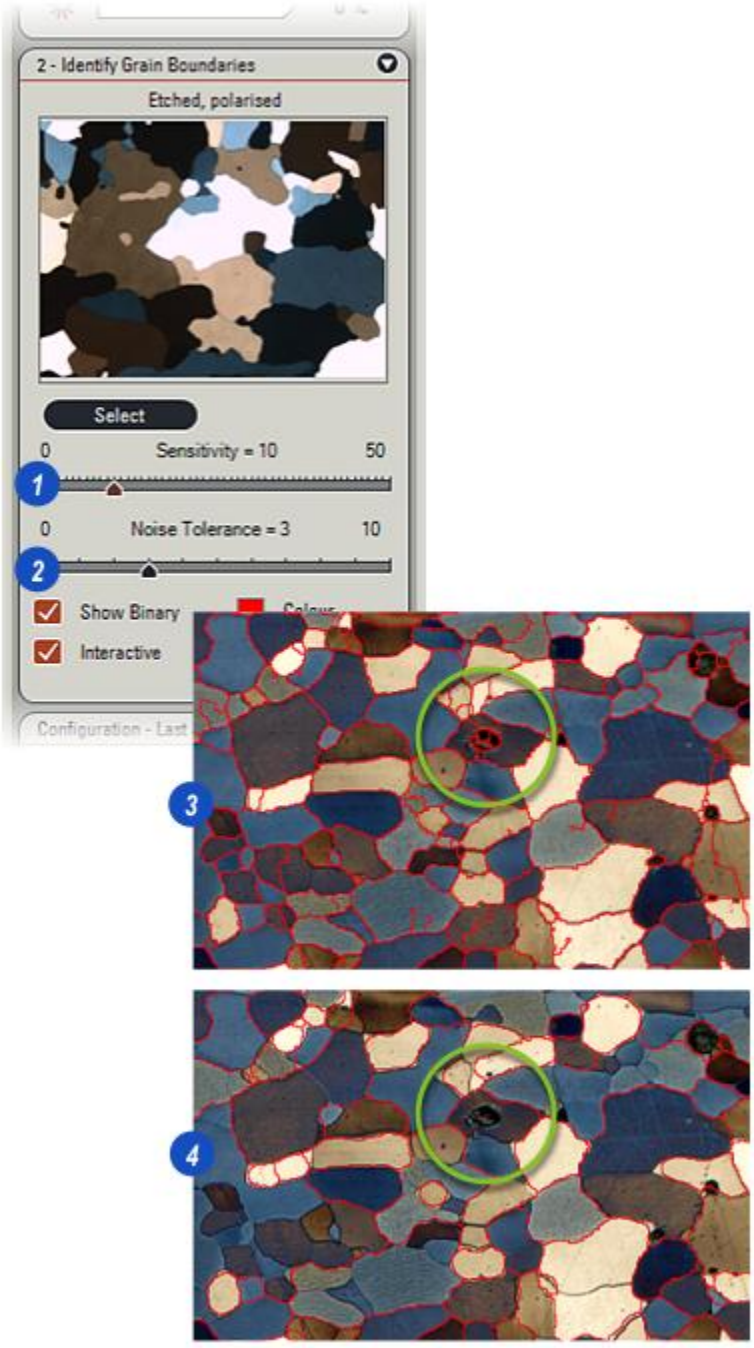
When colour images are selected, the Grain Boundaries panel has an additional control - the Noise Tolerance slider.

1: Click, hold and drag the *Sensitivity* slider to achieve the best boundary detection possible, and then refine the detection by...

2: ...dragging the Noise Tolerance slider to the left or right.

3: Part of the original image with Noise Tolerance set very low and possibly more boundary or 'edge' detection than is really necessary.

4: Noise Tolerance set too high at 7 and some boundaries have been missed. For this image a setting somewhere between the two would be appropriate and adequate.



Binary Image Editing

Binary Image Editing provides the tools for working directly on binary images to add, remove, select and de-select features. Facilities also include drawing and filling shapes as well as grouping features. Use *Binary Image Editing* to 'repair' incomplete boundaries and mark phases that should not be included in the analysis.

1: On the *Processing Sequence* menu, click on the *Binary Image Editing* entry.

2: The *Binary Image Editing* panel appears. There are two groups of buttons:

3: *Mode* and…

4: *Tools*. Some of the *Mode* buttons affect the way that the Tools behave.

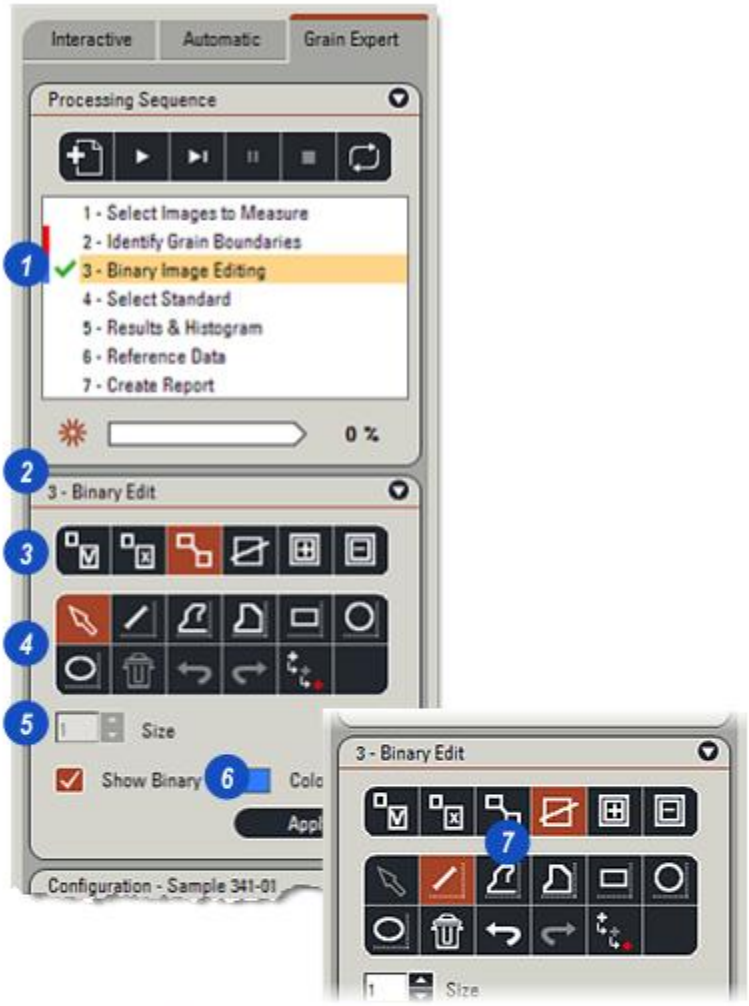
5: The *Size* window controls the line thickness for some of the Tools used for drawing.

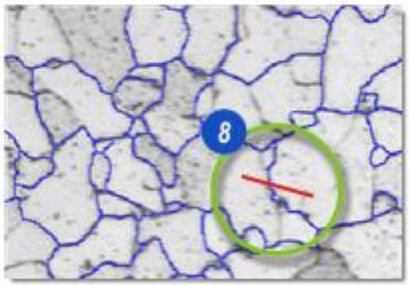
6: The colour of the binary output image is user selectable.

7: The fast-edit tool combination of *Erase* and *Draw Line*, can be used to remove detected boundaries - the image is not modified, only the binary overlay.

8: Click on the image and draw a short line intersecting the boundary to be removed.

9: The boundary is automatically detected and removed.





For detailed information about the *Binary Editing* tools and how to use them, read *Image Analysis* [1095] help.

Three *Standards* are available within *Grain Expert:*

- *ASTM E112*,
- *JIS G 0551/2* and
- *ISO 643 2003.*

Select the Standard:

1: Click on the *Select Standard* entry on the main menu.

2: The *Select Standard* panel appears.

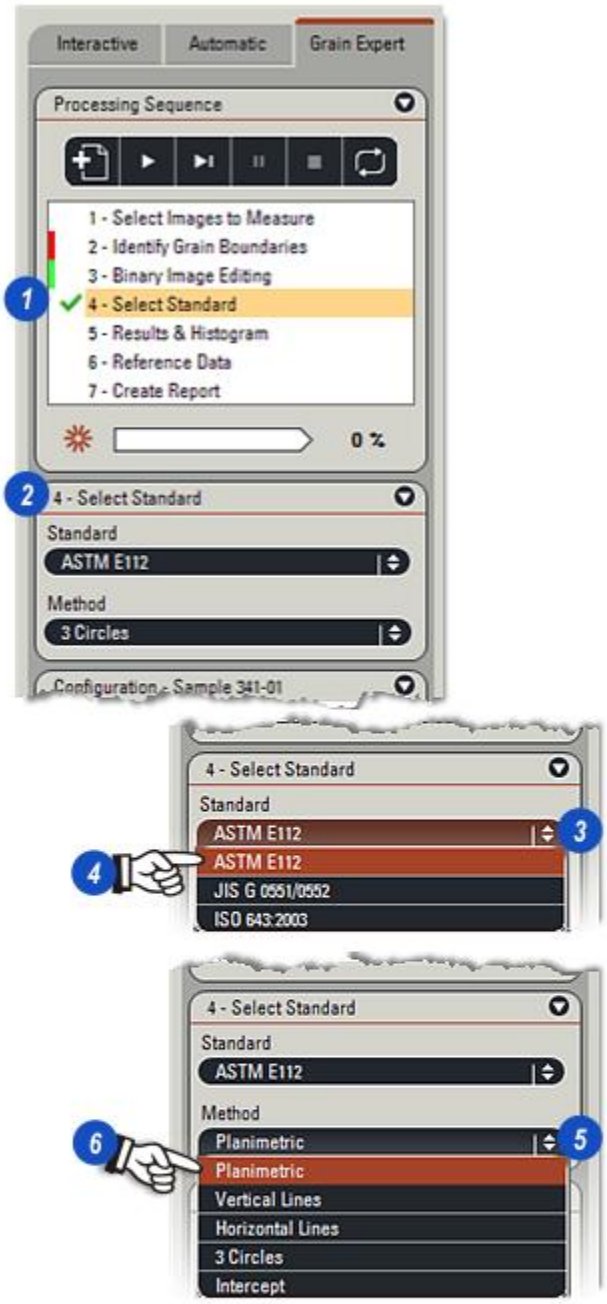
3: Click on the arrows to the right of the *Standard* header and…

4: …from the drop down list, click to select the required standard.

Choose the Method:

5: Click on the arrows to the right of the *Method* header.

6: From the drop down list, click to select the *Method* required. Examples of the *Method* structures and the display results are shown on the following pages.



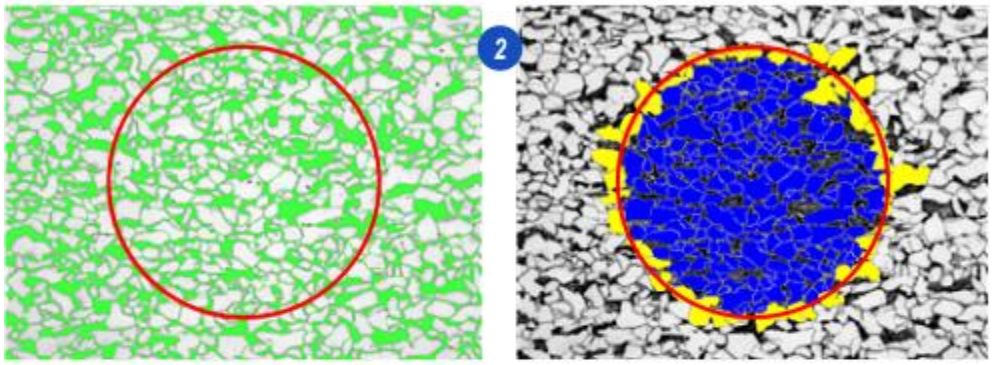
The following *Method* diagrams and result displays are applicable to the analysis of the entire *Field*:

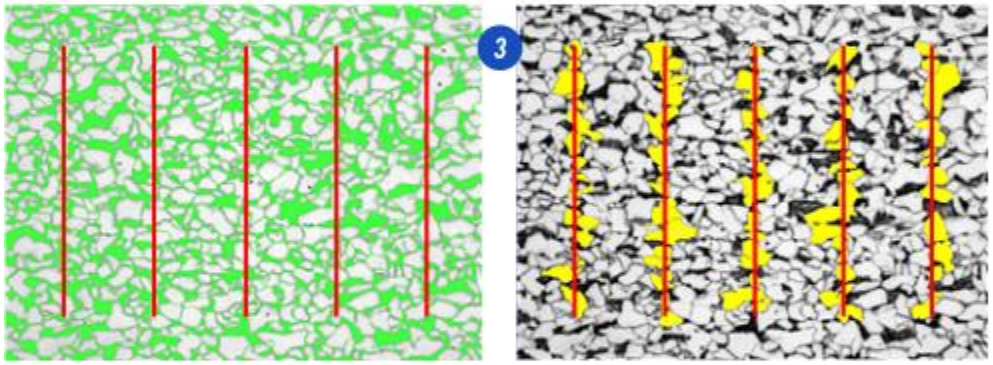
1: On the *Results and Histograms* panel, click on the arrows to the right of the *Field/Grain* header and select the *Filed* option.

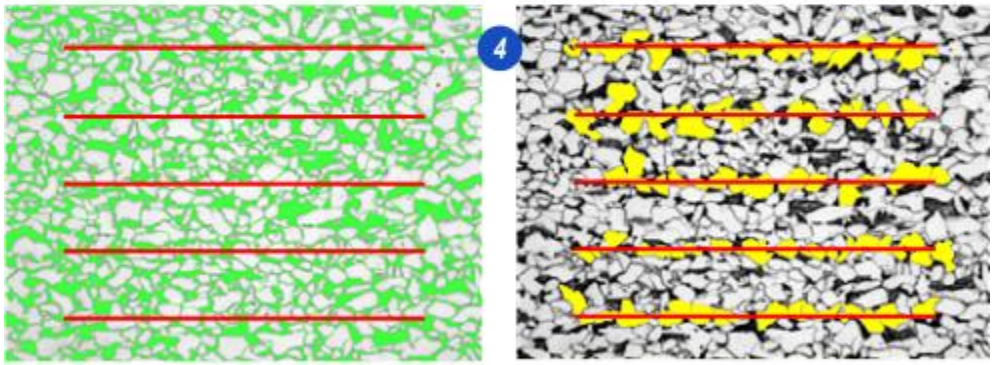
2: *Planimetric* Method.

3: *Vertical Lines* Method.

4: *Horizontal Lines* Method.







Continued from the previous page...

1: Select the *Field* option on the *Results and Histogram* panel.

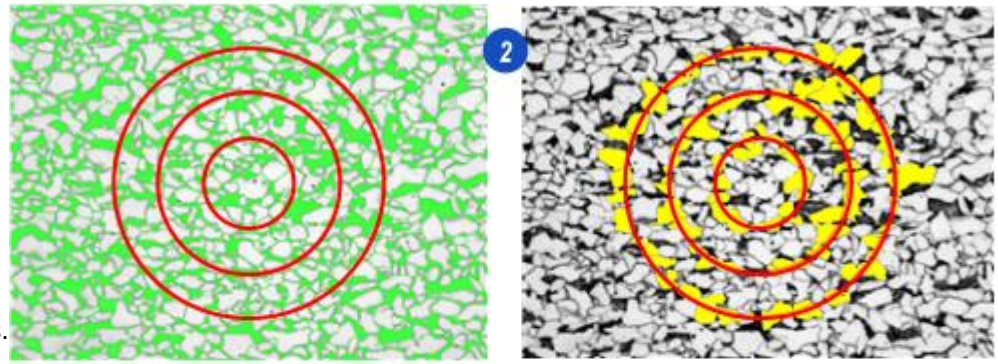
2: *3 Circles* Method and…

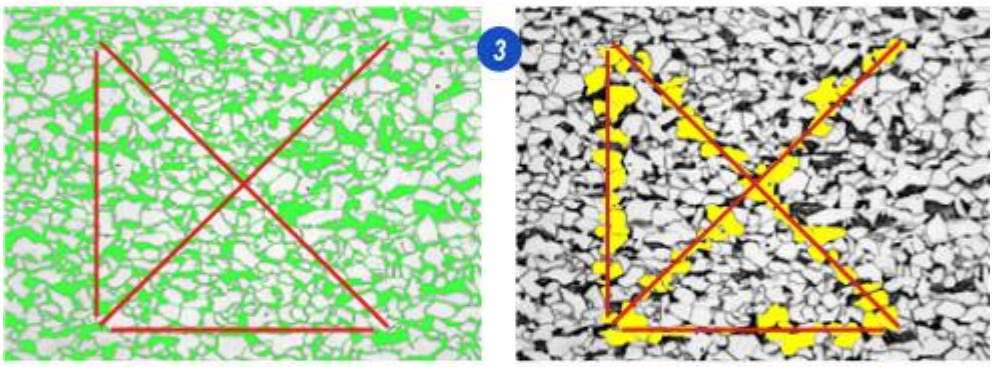
3: *Intercept* Method.

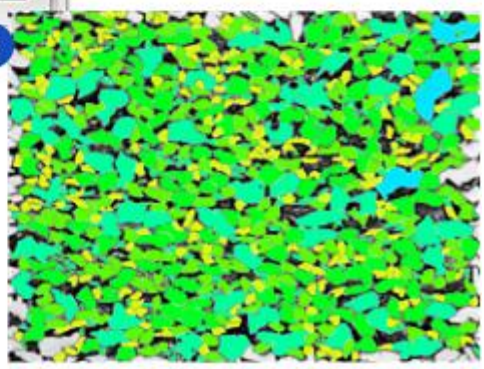
Grain Results:

4: To display the *Grain* results binary output image in which each grain size is colour coded, on the *Results and Histogram* panel click on the arrows to the right of the *Field/Grain* header and select *Grain* from the options.

5: The *Grain* results binary output image.







1: Click to select *Results & Histogram* on the *Processing Sequence* menu.

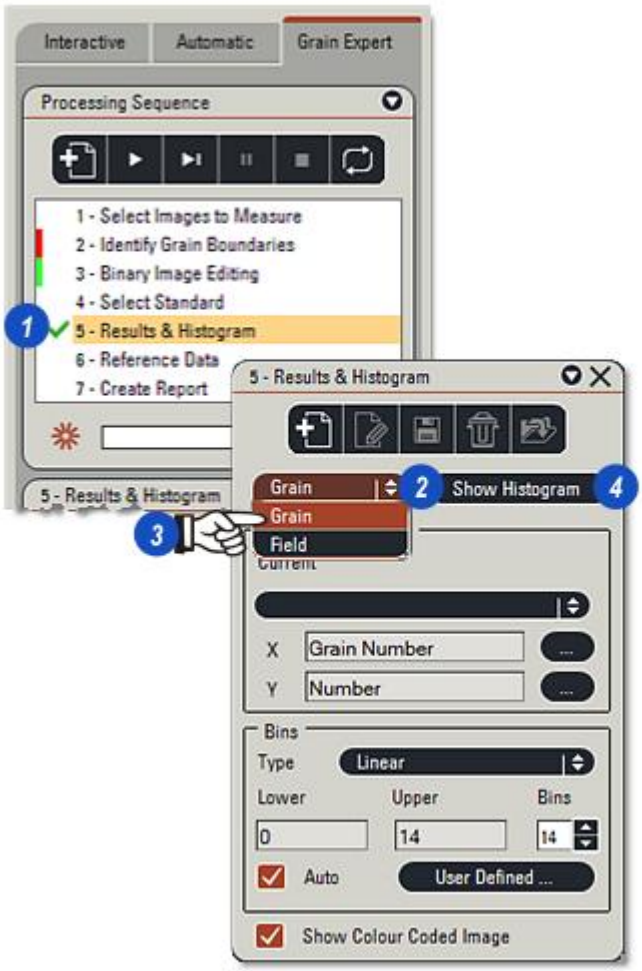
2: Click on the arrows to the right of the *Grain/ Field* header and…

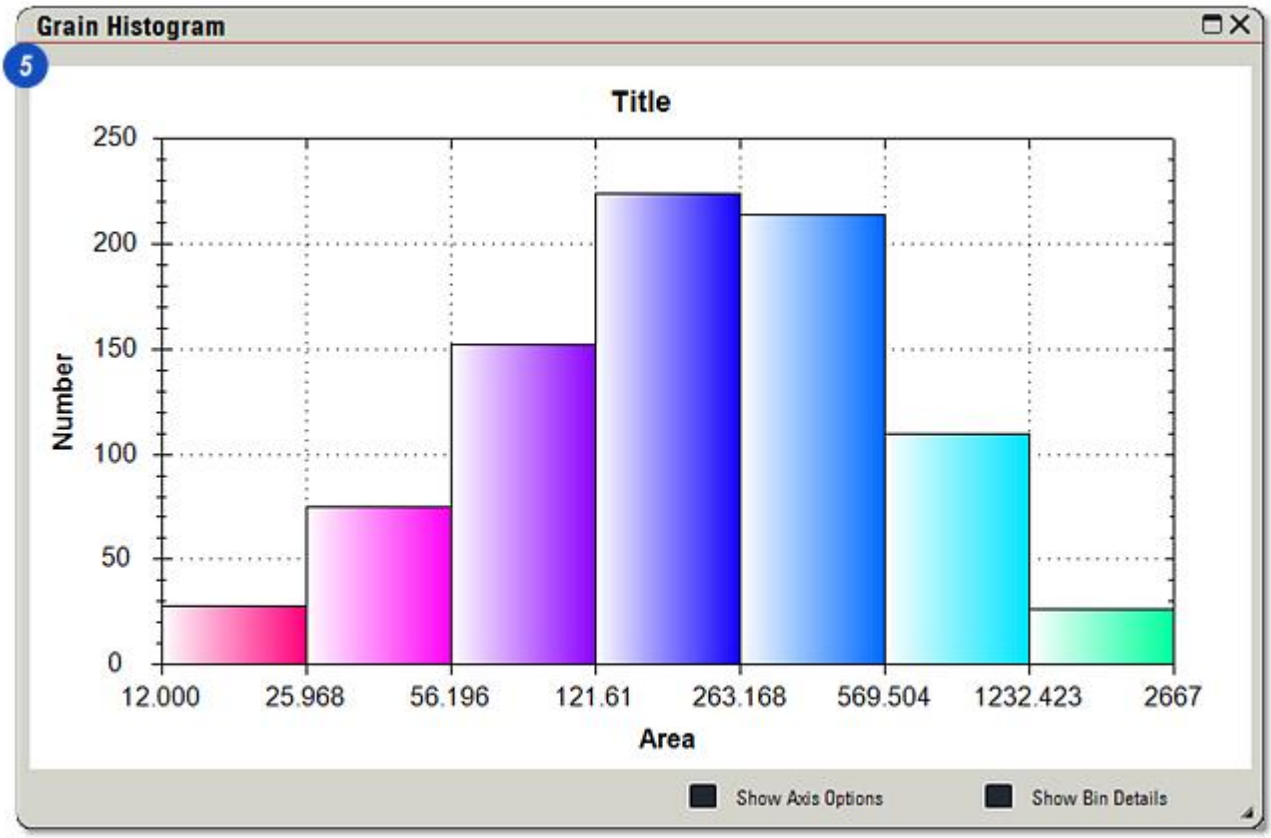
3: …from the drop-down list click on the *Grain* option.

4: Click on the *Show Histogram* button.

5: The *Grain Histogram* shows the grains and their results plotted against the parameters chosen for the *X* and *Y* axes. Selecting the *X* and *Y* axes parameters: *Go there…*

For details of other *Axis* and *Bin* options, see *Image Analysis* [1134] help.





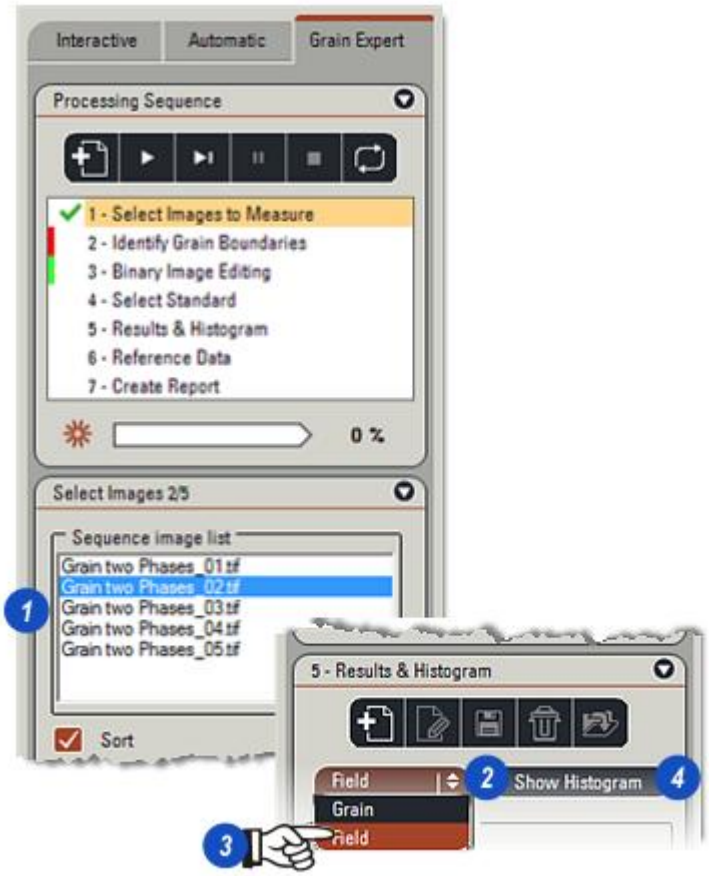
1: For this analysis 5 fields were chosen.

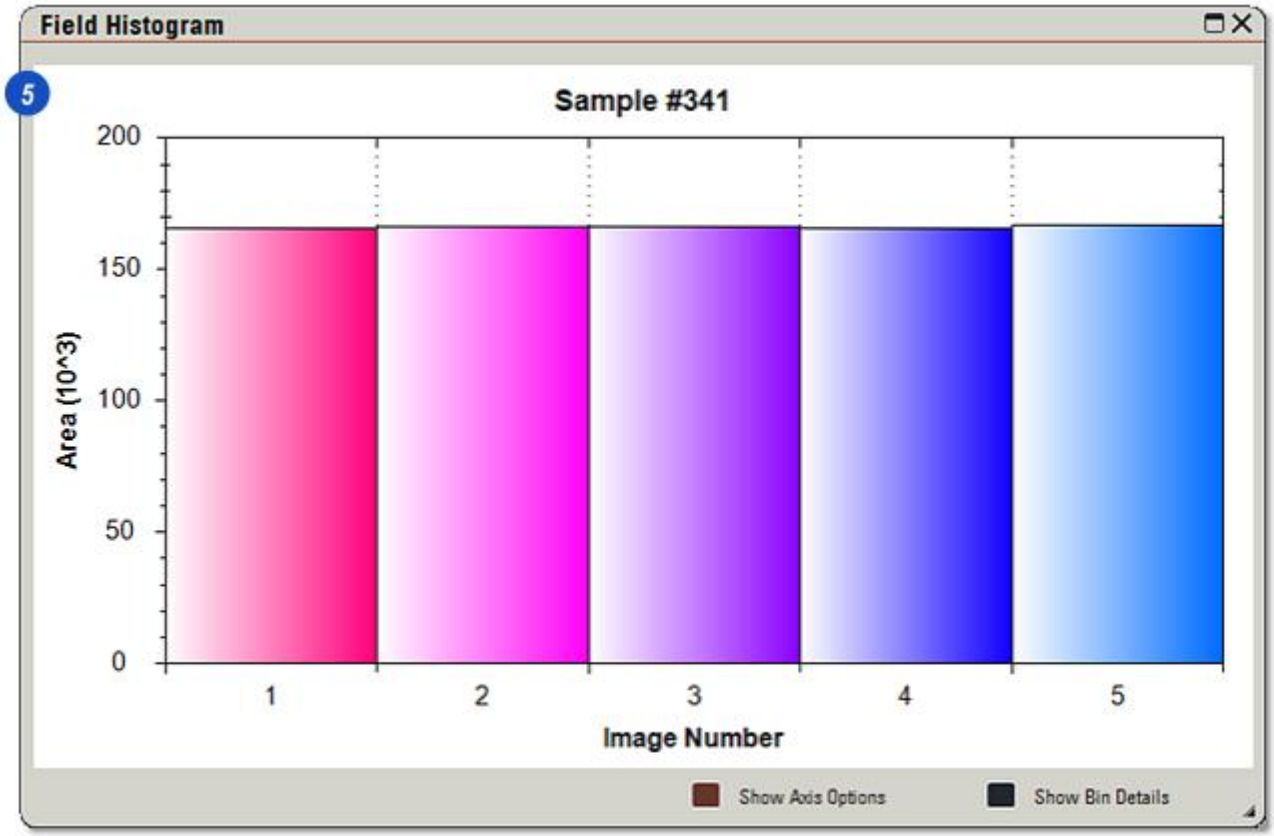
2: On the *Results and Histogram* panel, click on the arrows to the right of the *Grain/Field* header and from the options…

3: …click to select *Field*.

4: click on the *Show Histogram* button.

5: The *Field Histogram* shows the 5 fields along the *X* axis with their measured area on the *Y* axis.





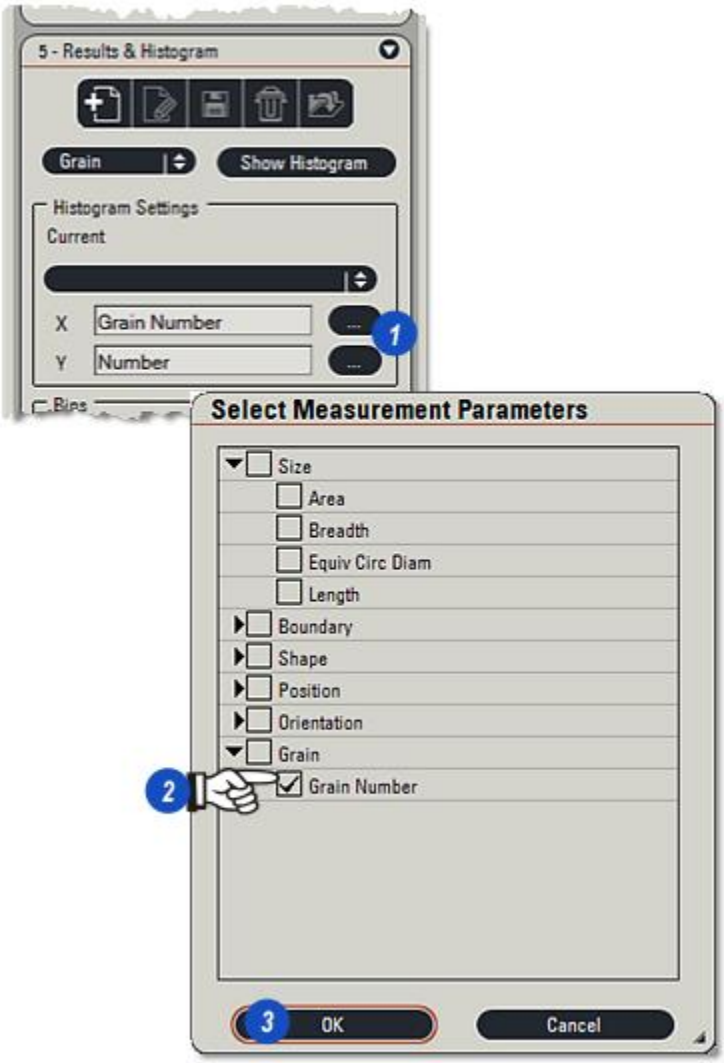
Select the Histogram Parameters

With the *Field* option selected for the *Histogram* display, only the *Y* axis parameter can be changed - the *X* axis will always read *Image Number*. For the *Grain* option both *X* and *Y* parameters can be selected.

1: Click on the browse button against the axis to be changed. If the button is greyed out the option is not available.

2: The *Select Measurement Parameters* dialog appears. The items in the list will depend upon the display option chosen and the axis to be altered. Click to select the parameter required.

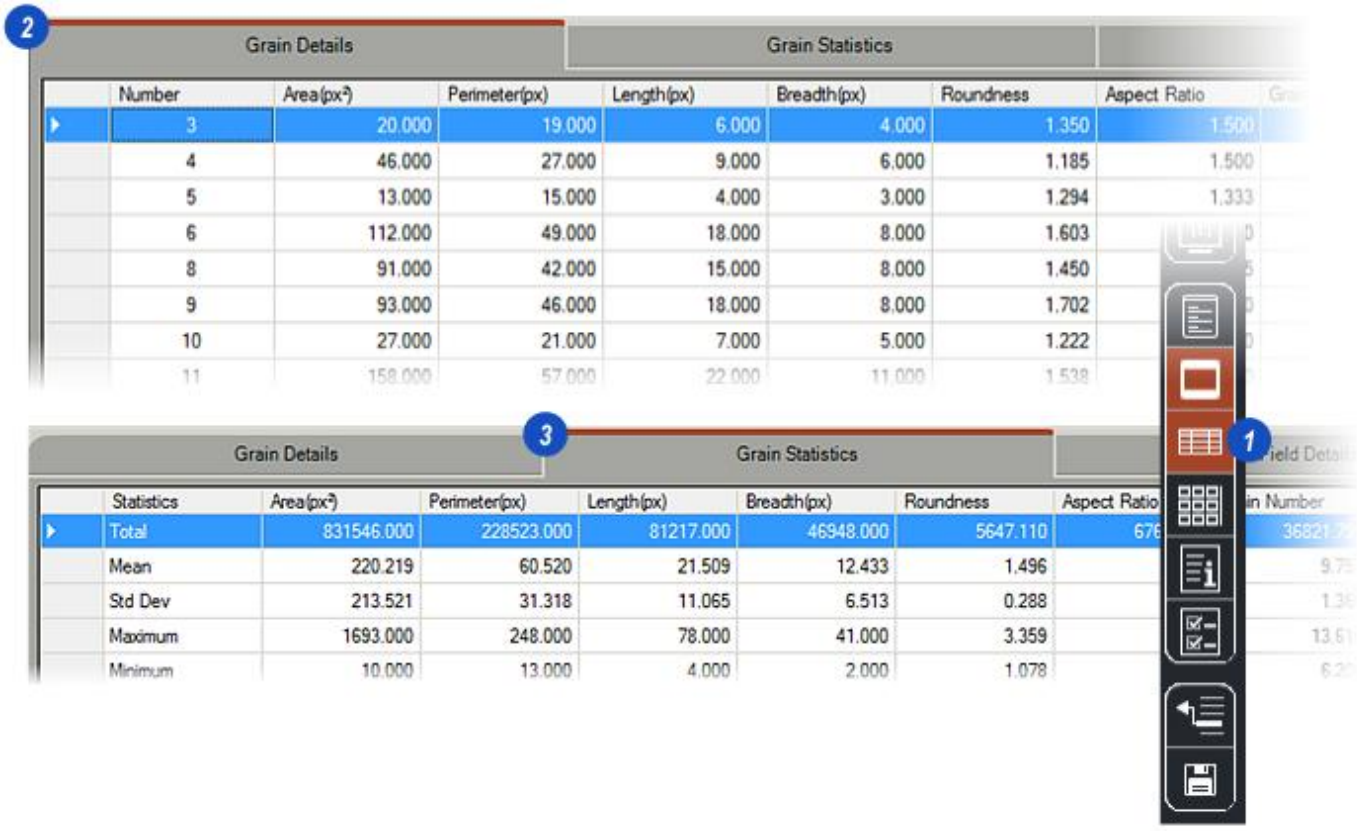
3: Click *OK*.



1: If necessary, click on the *Show Grid* button on the *Side Tool Bar* to display the results in tabular form.

Four tabs are displayed across the top of the *Grid*. To the left the *Grain* results – *Details* and *Statistics* – and to the right the *Field* results, *Details* and *Statistics* also (Shown on the following page).

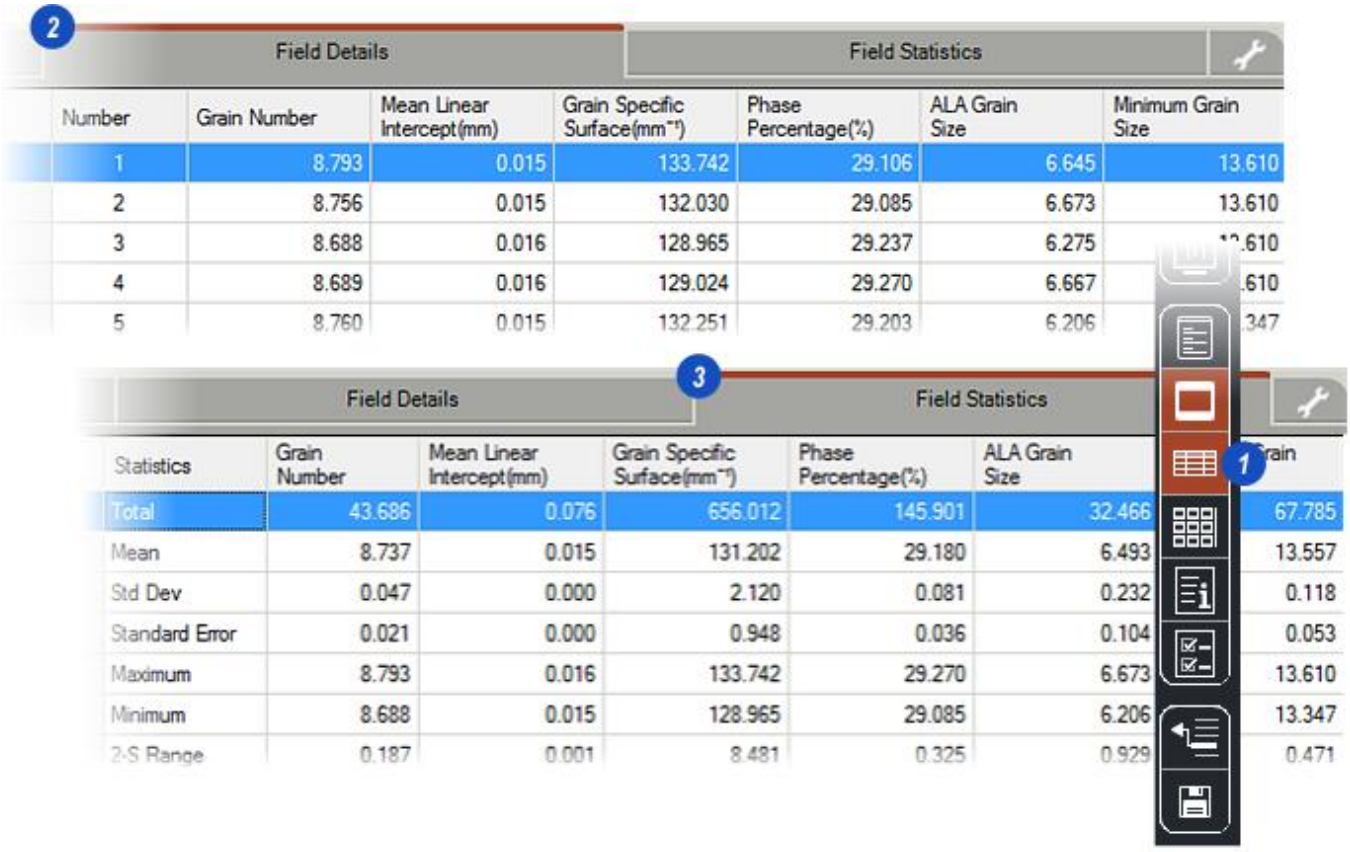
2 & 3: Click on the appropriate tab to reveal the results. The result parameters can be selected to suit the job and user: *Go there…*



1: If necessary, click on the *Show Grid* button on the *Side Tool Bar* to display the results in tabular form.

Four tabs are displayed across the top of the *Grid*. To the left the *Grain results – Details* and *Statistics* (Shown on the previous page) – and to the right the *Field* results, *Details* and *Statistics* also.

2 & 3: Click on the appropriate tab to reveal the results. The result parameters can be selected to suit rhe job and user: *Go there…*



Select the Tabular Display Parameters

1: To change the *Tabular (Grid)* display column header parameters for *Grain* or *Field*, click on the appropriate results tab.

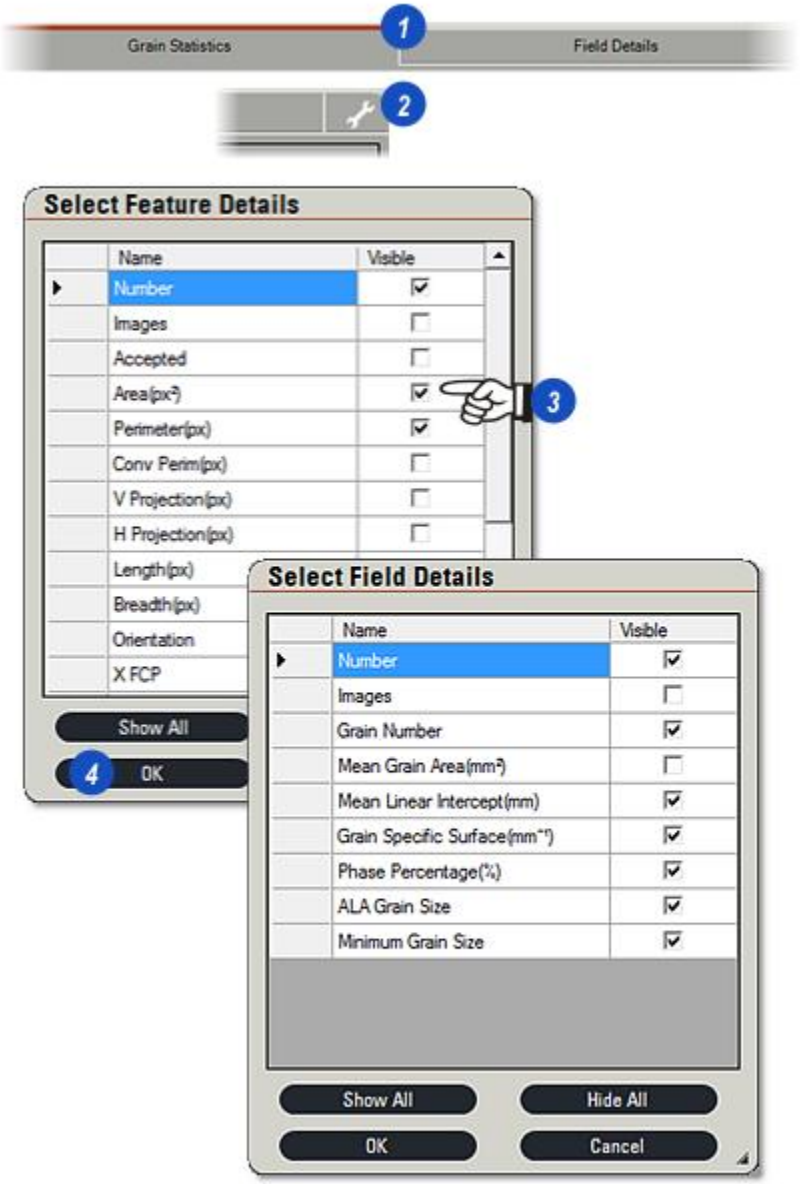
2: Click on the *Tool Tab* and, depending upon the results tab selected the *Select Details* or *Select Statistics* dialog will appear.

3: Enable the required check boxes to include the parameter required in the displayed results.

4: Click *OK*.

The *Show All* option when enabled will select all of the options.

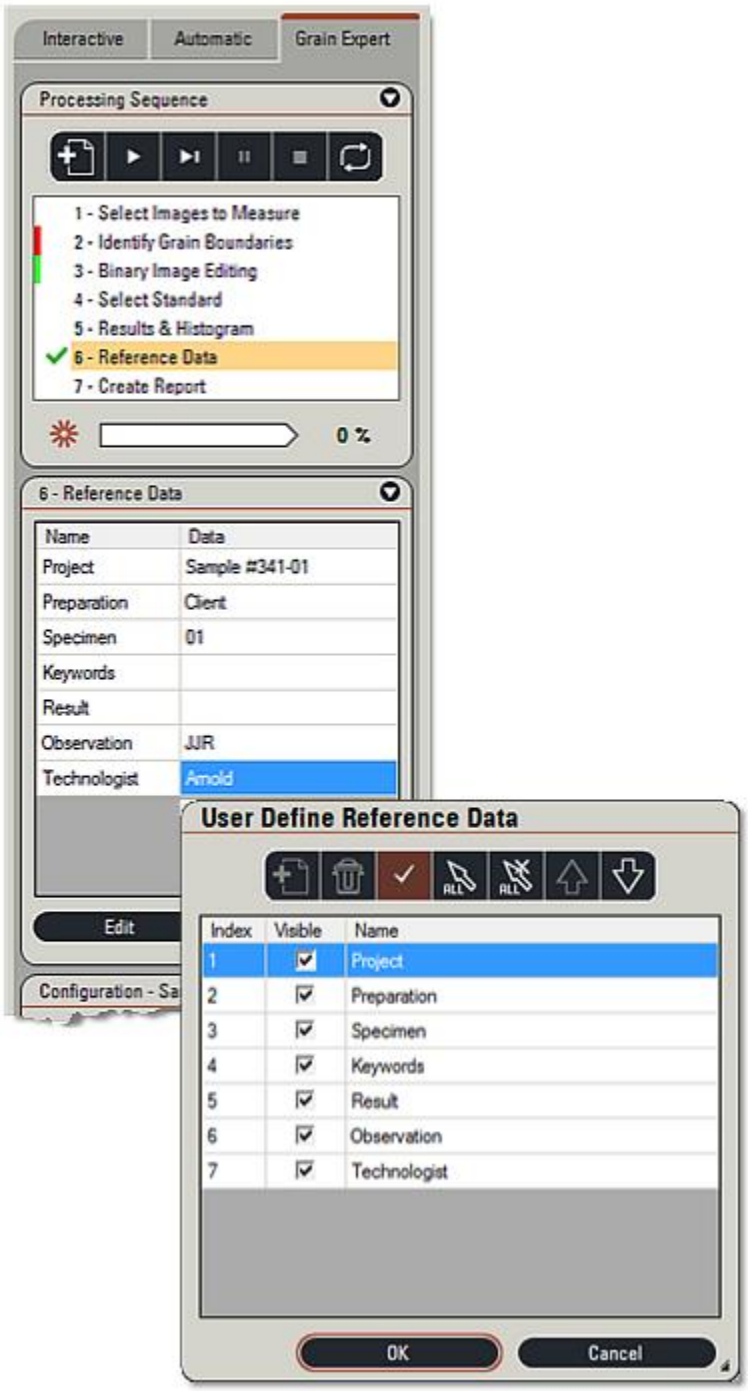
Click on the *Hide All* button to clear all of the options before starting a new range of selections.



A comprehensive range of *Data* Items can be appended to the *Grain Expert* results that will identify important details such as the *Project Name*, the *Specimen* and how it was prepared. Enter the information in the *Reference Data* dialog.

Administrators can add to the supplied list of data headings to comply with corporate demands.

For details refer to *Image Analysis* 1140 help:



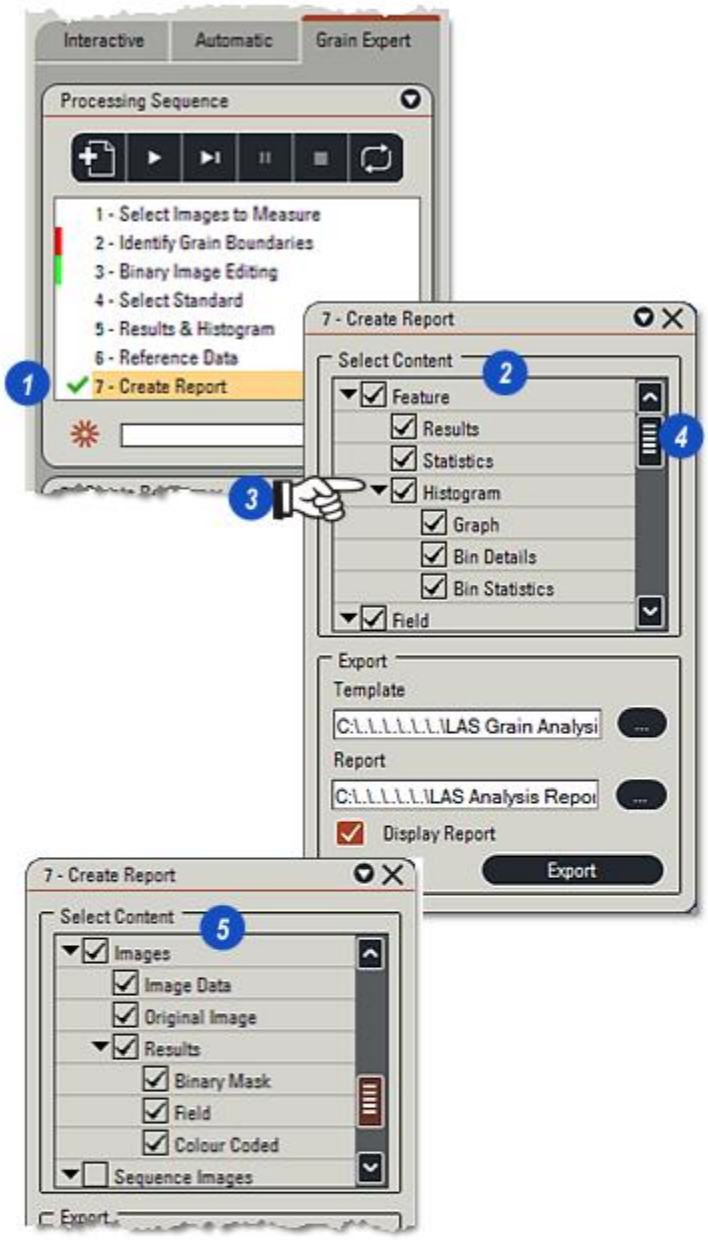
The report is created using *Microsoft Excel* which must be installed on the computer.

1: On the *Processing Sequence* menu, click to select *Create Report*.

2: The *Select Content* dialog lists all of the information and images that can be included in the report.

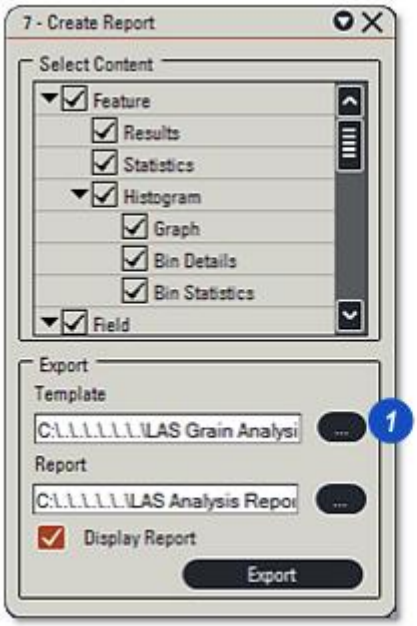
3: The list is divided into sub-sections – click on the small arrow to the left of a sub-section header to reveal all of the section options.
To include an item in the report, click to enable the check box to the right of the item.

4: The list is extensive so use the scroll bar to reveal more items **(5)**. Images tend to be large files that can make a report unwieldy, especially if it is to be transmitted electronically – by e-mail for example – so where possible keep the included images to a necessary minimum.



Select the Excel Template

The reports are created using an *Excel* template; A standard template is provided with *Grain Expert* that will be suitable for many applications. It can be modified by the user to reflect the job or corporate style. Alternatively, use any appropriate existing *Excel* template.



Locate the Template:

1: Click on the browse button to the right of the *Template* text box.

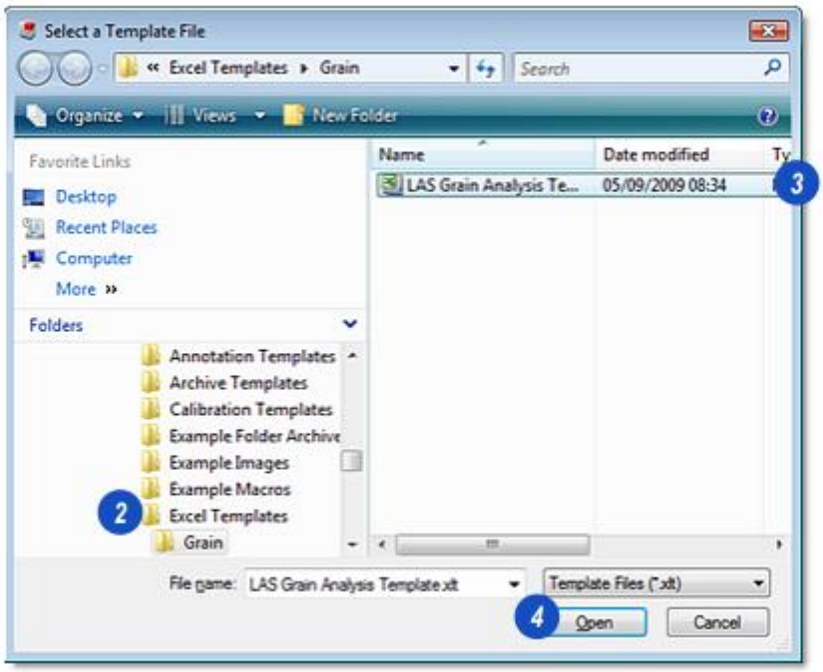
2: Navigate to the *Template* folder…

3: …and file and click to select it.

4: Click on the *Open* button.

A default template is supplied with *Grain Expert* and is located in the *Users/Excel Templates\Grain* folder with the name:
LAS Grain Analysis Expert Template.xlt.

The precise path may vary with the installed operating system.



Select the Report Save Location

1: Click on the browse button to the right of the *Report* text box.

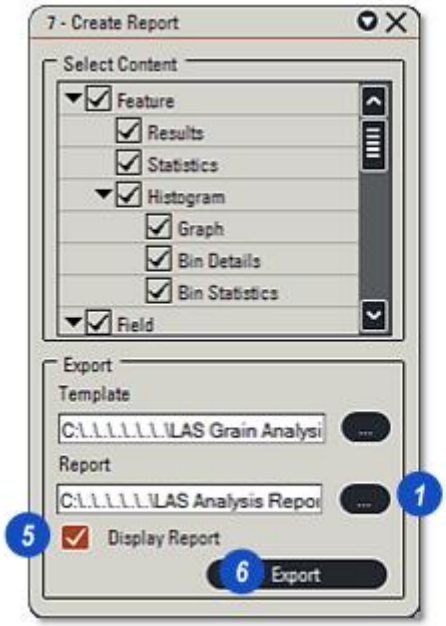
2: Navigate to the folder into which the report file will be saved.

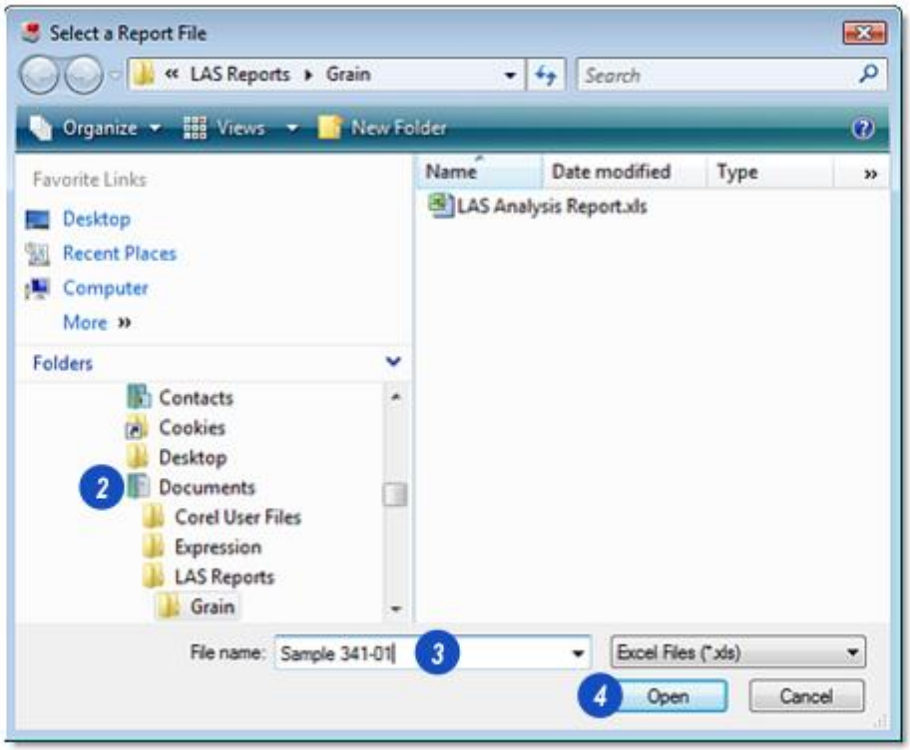
3: Type a name for the file and…

4: …click on the *Open* button.

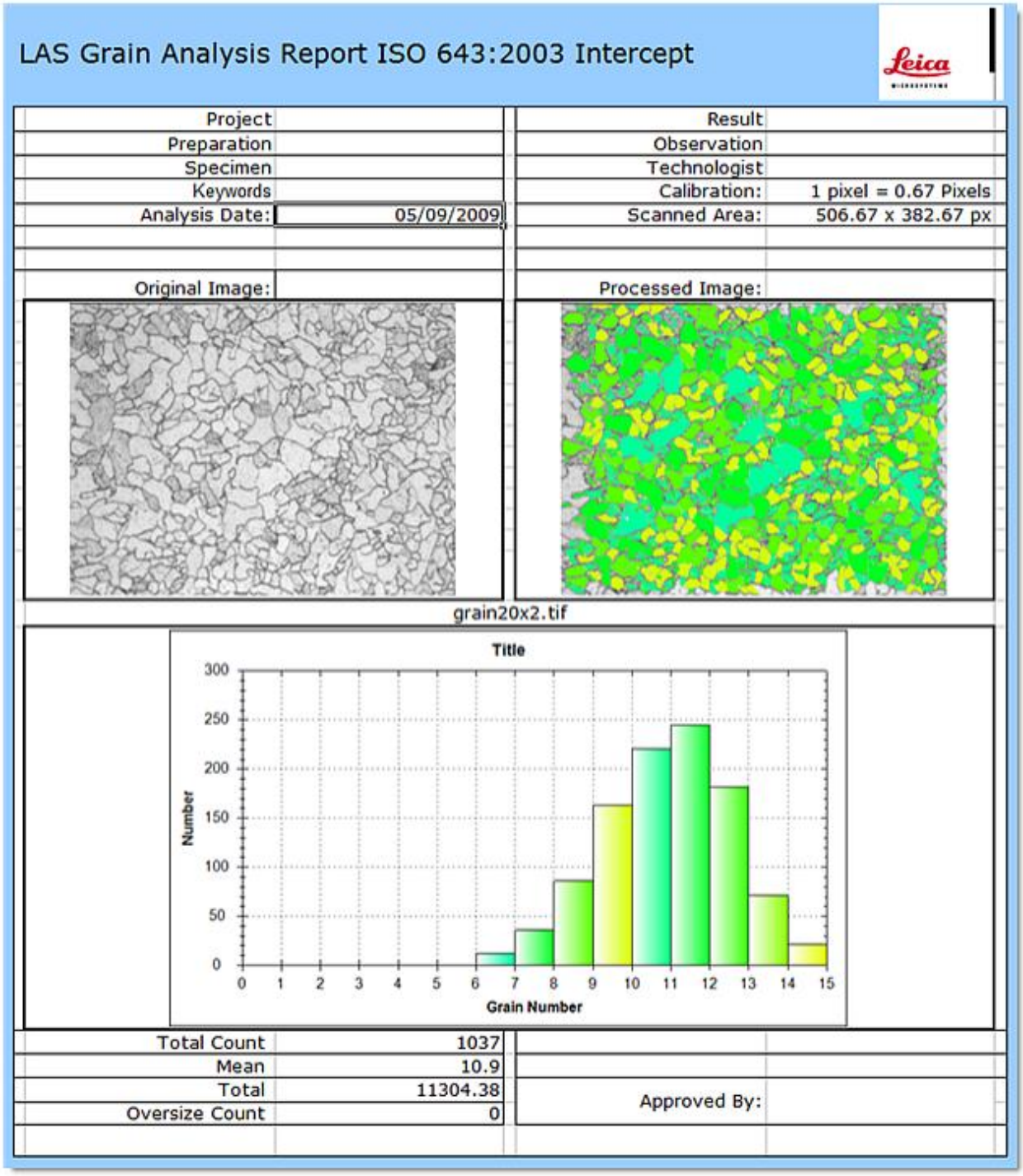
5: If the report is to be displayed as soon as it is created, click to enable the *Display Report* check box.

6: Click on the *Export* button.





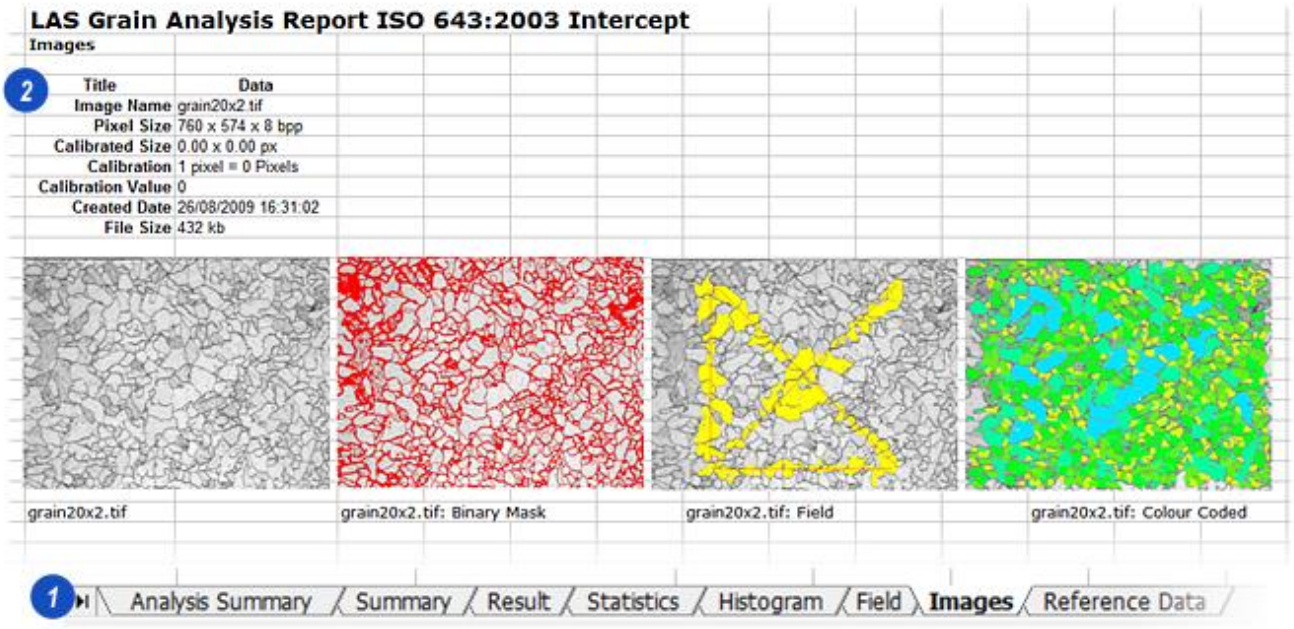
This report was created with Microsoft Excel using the the template supplied with *Grain Expert. The report can be displayed and read (but not created) using Excel Viewer.*



This report was created with Microsoft Excel using the the template supplied with *Grain Expert. The report can be displayed and read (but not created) using Excel Viewer.*

1: Depending upon the information included in the report, it is divided into sheets access by clicking the tabs along the lower edge.

2: The illustration shows the *Images* sheet.



Excel Tags:

Excel reports can be styled to suit the user by using *Tags* that can determine the results and images being displayed. Tags are simple text strings that can be typed directly into an Excel cell or copied using the *Excel Macro* feature.

More information about *Excel Tags*[425].

The Tags for *Grain Expert* have a specific format and are listed opposite. Click on the required tag for help in using it.

<*LAS AM User Data*>[440] Reference Data

<*LAS AM Results*>[1202] Grain Results

<*LAS AM Summary*>[442] Grain Summary

<*LAS AM Field Summary*>[443] Field Summary

<*LAS AM Histogram Statistics*>[446] Grain Statistics

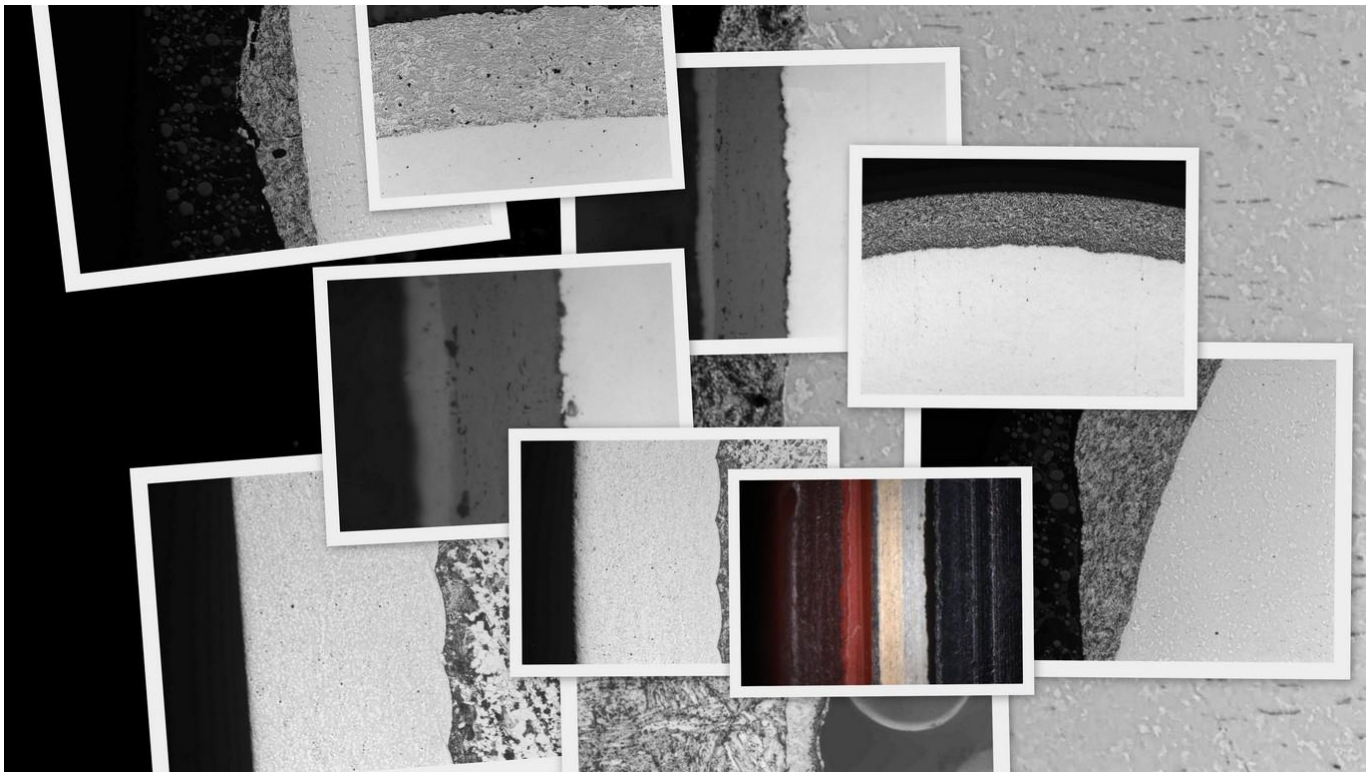
<*LAS AM Histogram Bin Data*>[447] Grain Bin Data

<*LAS AM Histogram Chart*>[445] Grain Chart Graphic

<*LAS AM Images*>[448] All Images

<*LAS AM Image*>[449] Selected Image

Layer Thickness Expert



Leica Layer Thickness Expert allows you to measure the thickness of a layer or coating on a sample at multiple positions along its length.

There are many applications where coatings of different types are used within the field of materials production. Examples include paint, chrome plating and plastic powder coatings. These are applied to the substrate surface with the intention of changing properties at the surface of the substrate material. This may be for protection from a corrosive environment (such as paint on a car) or perhaps purely for decoration.

Layers on raw products such as steel can be applied as an extension of the production process and, as such, require analysis for quality control. *Leica Layer Thickness Expert* models the standards ASTM B 487 and ISO 1463, which are test methods for the measurement of metal oxide thickness by microscopical examination of a cross-section. Users can change most parameters to suit their individual requirements and create custom reports using Excel.

Leica Layer Thickness Expert features several methods of layer identification from manual to automatic. It allows user interaction to confirm layer detection, and provides comprehensive reporting.

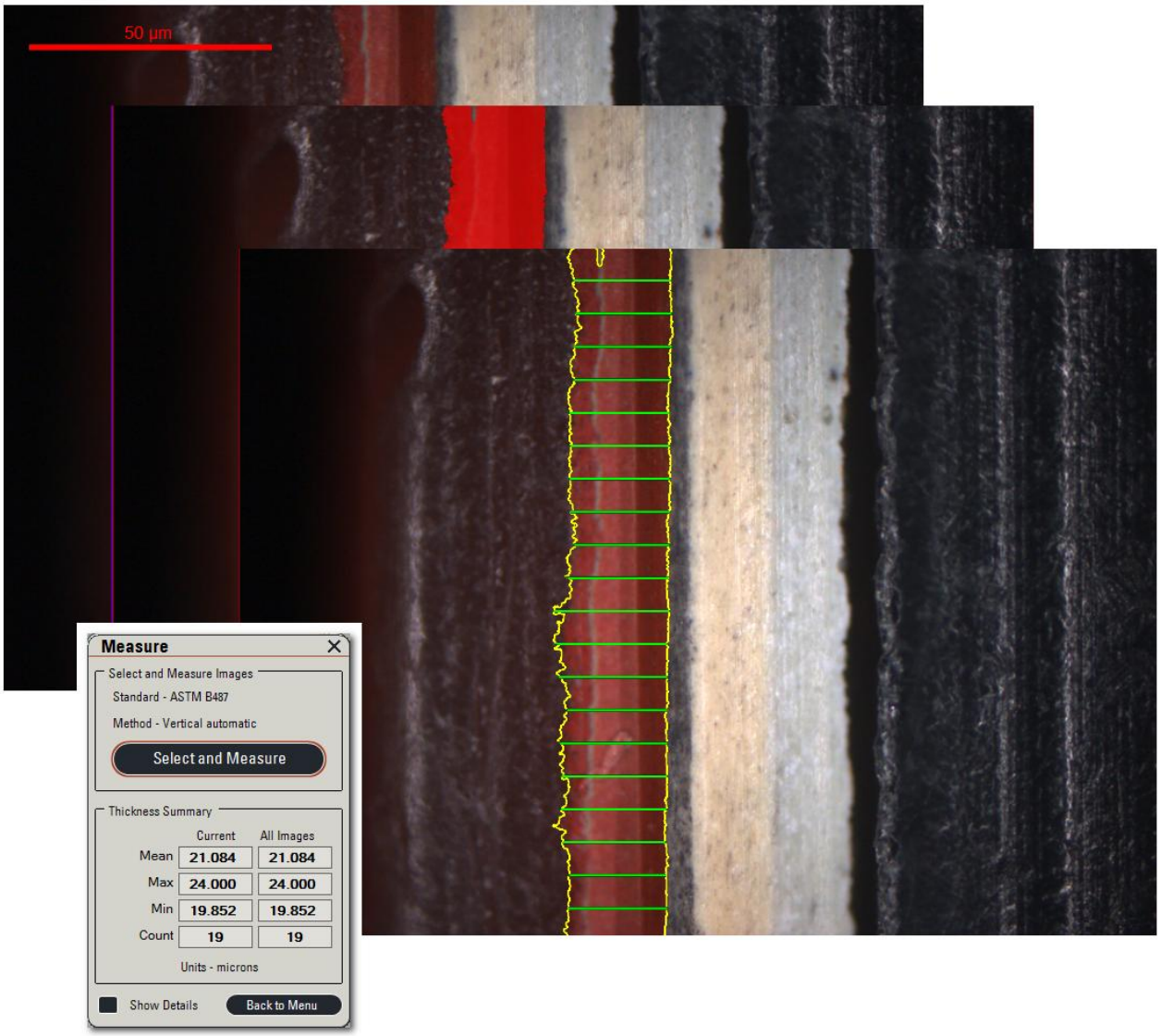
Leica Layer Thickness Expert offers a comprehensive solution for the analysis of layers and coatings of many different materials. It evaluates high-quality images provided by Leica Microscopy hardware.

Since layers can exhibit a wide variety of contrasts, *Leica Layer Thickness Expert* offers a range of techniques (from fully automatic to fully manual) for identifying a layer and calculating average thickness.

State-of-the-art image processing automatically enhances and detects the layer (the operator can customise settings and confirm results). Results may be used to qualify material to specifications determined between purchaser and manufacturer, to identify variations in manufacturing processes, or to provide data for research studies of the structure and properties of layers and coatings.

LAS intelligently integrates the latest advances in automated microscopy, computing and digital image analysis. With a wide range of applications designed specifically for materials and metallurgy laboratories, it performs routine (yet sophisticated) analytical tasks rapidly, efficiently and economically.

This common micro-imaging environment is used to provide solutions to many industry-standard and custom materials applications such as grain, steel inclusion, cast iron, phase and cleanliness analysis.



The principal areas of the user interface:

1: *Menu Bar*: For Options and Help.

2: *Workflows*: *Leica Layer Thickness Expert* is available on the Analysis Workflow.

3: *Application Panels* Tab: Click *Runner*.

4: *Prompt Message Panels*: Hints for program sequence steps.

5: *Control and Results Panels*: Used to operate the application and to show summary results.

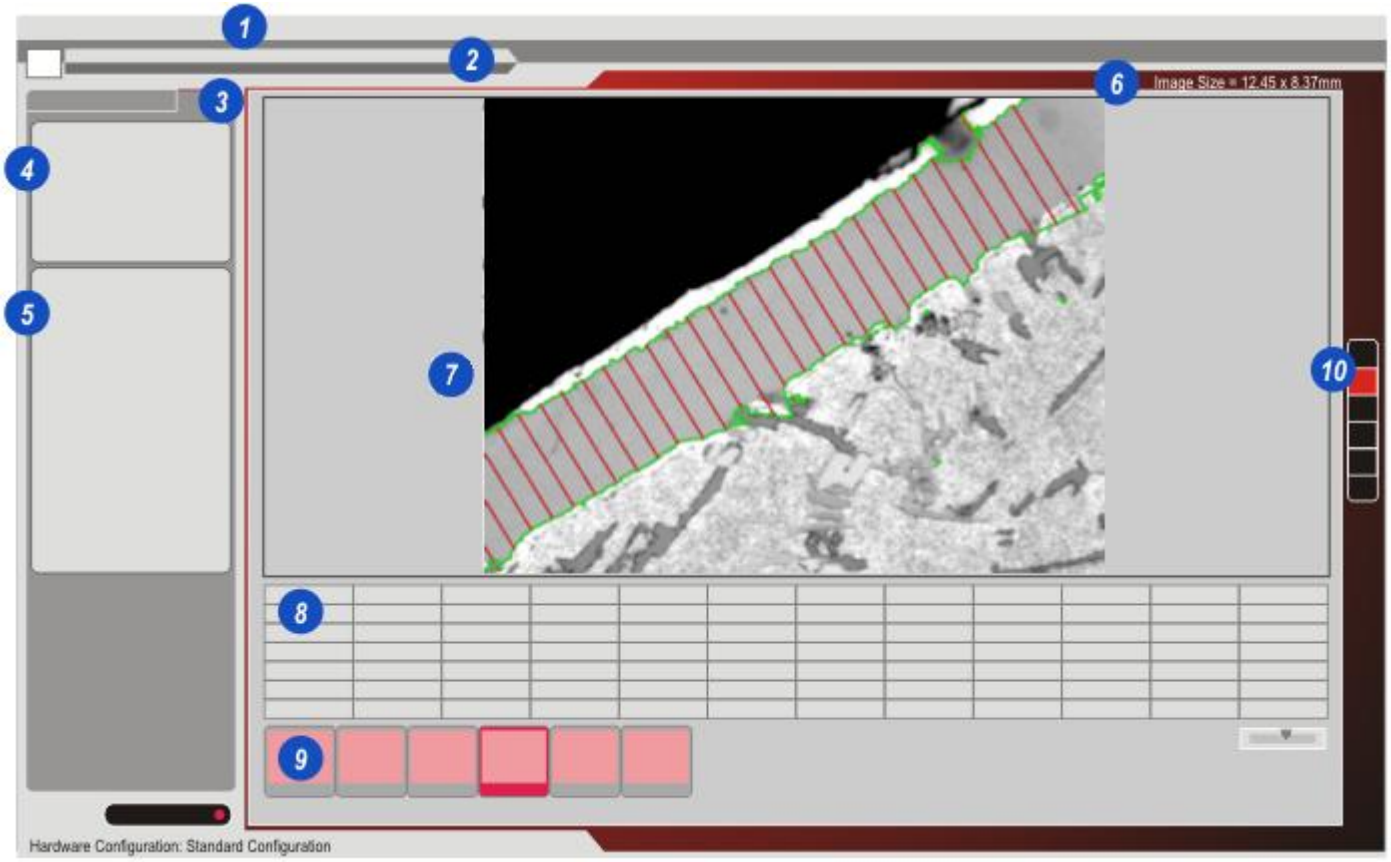
6: *Image Scaling* information.

7: *Image Viewer*: Shows original image, sometimes with the superimposed Layer Binary Overlay.

8: *Results Grid*: Not used by this application.

9: *Gallery of Image Thumbnails*: Used to select the images to measure.

10: *Side Tool Bar.*



Getting started – new users

- Check out all the links in the Help navigator to the left – plenty of help is available!
- To view the *Leica Layer Thickness Expert* help, press F1 while the application is running.

Getting started – new users of LAS

- *Leica Layer Thickness Expert* uses LAS to acquire images and is started from the **Analysis > Runner** step of LAS
- Please refer to the main LAS help for information concerning the configuration, calibration and use of LAS to acquire images.
- To view the LAS help, press F1 while LAS is running.

Supported Microscopes, Cameras, Computers and Software

Please see the *Systems Requirements* PDF file:

Start > Leica Application Suite V4 > Documents

This provides details of all supported hardware and recommends suitable computer specifications. In case of doubt, please run the *LAS PC Performance* utility and observe the recommendations it produces.

Prerequisites

LAS Image Analysis must be purchased and licensed on the same system as *Leica Layer Thickness Expert*.

LAS Runner must be licensed on the same system as *Leica Layer Thickness Expert*. This is included with the purchase of a *Leica Layer Thickness* License.

Microsoft Excel is required if you wish to customise the report template.

Image Format

Colour and Monochrome images are supported.

It is recommended that the image pixel size is in the range 1 to 3.3 Mpixel. Images with pixel sizes greater than 5 Mpixel are not supported.

Display resolution

The minimum screen resolution is 1280 x 1024. If you use a lower vertical resolution some of the control panels may be obscured. You will be able to move them to a position where they are visible, but this is rather inconvenient. Please minimise the height of the Windows task bar as this occupies some of the vertical screen space.

Image Acquisition

Please ensure that the images are acquired with the correct calibration. This will be more certain if you are using a microscope with a coded nose-piece. If not, then make sure that the objective in use is the same as that selected in LAS.

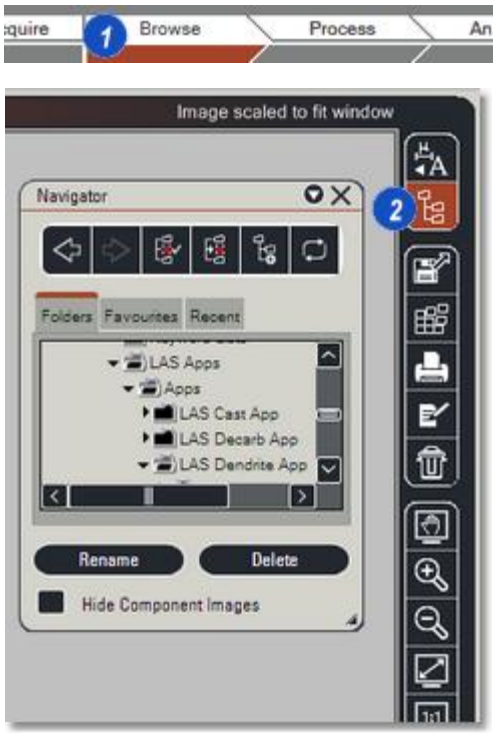
Adjust the camera exposure, gain and gamma to provide a high quality image. In particular, ensure that an HQ format is used, the value of gain = 1 and the histogram black and white levels are set to the default values.

If you are using a measurement mode that requires thresholding, i.e. all methods except the Manual Method, it is strongly recommended that the shading correction is set for all images acquired. This will ensure that the thresholding is performed uniformly over the entire image.

Calibration Units

Please ensure that all images that you measure at the same time have the same calibration for consistent results.

Make sure the Navigator panel is floating



You need to ensure that the *LAS Navigator* is floating before starting an *Expert* application. This allows you to select test images and real sample images that you have acquired, for use within the application.

1: In LAS, select the *Browse Workflow*.

2: If the *Navigator* panel is docked with the other Control Panels on the left, click the *Show Navigator* button on the *Side Tool Bar* to make it float.

3: You are now ready to start [1217] your *Expert* application.

Start Leica Layer Thickness Expert

1: Select the *Analysis Workflow*.

2: Display the *Runner* tab.

3: If necessary, click to expand the *LAS Application Selection* panel.

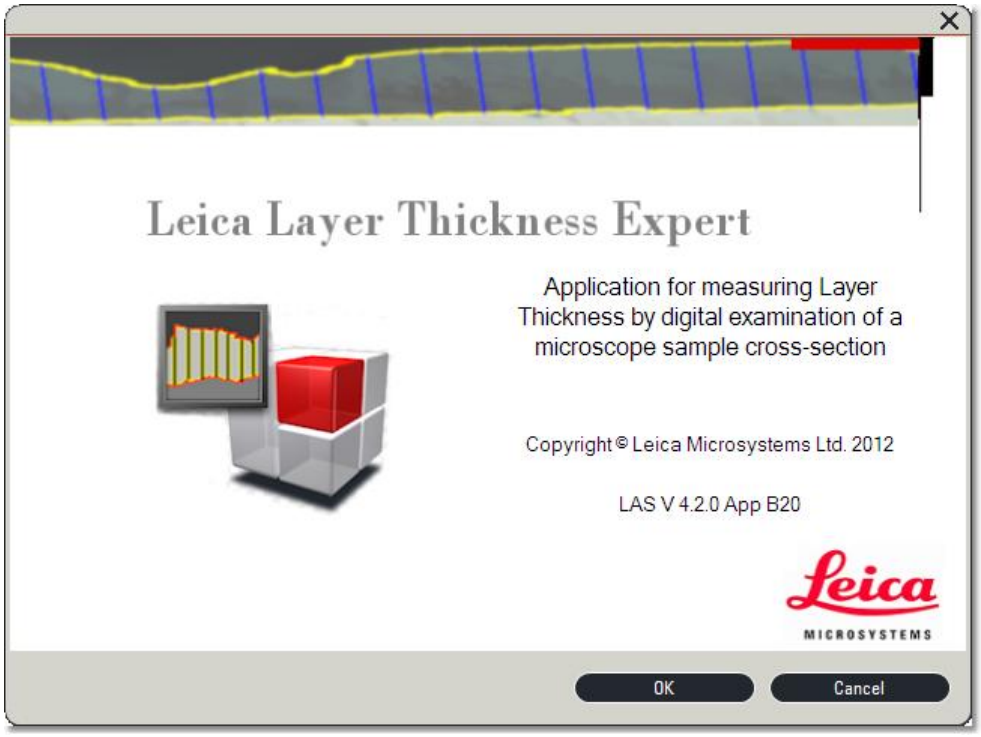
4: Click on an icon to select your chosen Expert Application.

5: Click *Run*.

6: If the *LAS Application Selection* panel is not visible click the *Application Selection* button.

When the application start-up screen appears,
do one of the following to start the application:

- Click *OK*
- Click the logo.



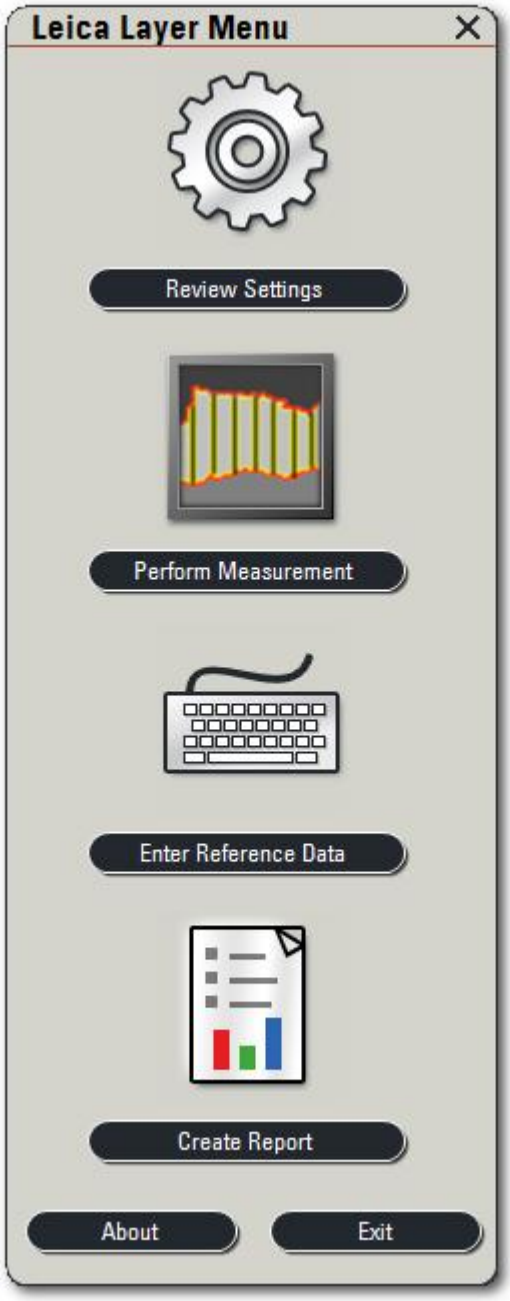
The *Leica Layer Menu* provides links to the steps you should take to analyse a Layer Thickness specimen.

Before using this menu, please ensure that the images have been acquired using LAS[1220] and stored in a known folder.

Note - it is preferable to acquire images with a resolution approximately in the range to 1 to 3 Mpixels.

Ensure that the images are correctly calibrated, particularly when using manual microscopes.

- *Review Settings*[1221]*:* Select the measurement method and the standard to use. Use default parameters or create user-defined settings. Determine the detection limits and then use the supplied or user images to test the settings.
- *Perform Measurements*[1232]*:* Uses the parameters established in *Review Settings* to detect and analyse the specimen.
- *Enter Reference Data*[1247]*:* Add comments and specimen details that will be included in the final report. This is where you can add the notes required by the standards.
- *Create Report*[1249]*:* The results and reference data are presented using a supplied Excel template that can be modified to suit user or company styles.



The first stage of Layer Thickness analysis is to acquire a selection of digital images using LAS and to save these to the computer's hard drive. The advantage of this approach is that you always have the original images available to check your results later.

To obtain precise results from *Leica Layer Thickness Expert* you need a good, representative sample of the specimen that has been processed to display optimum contrast.

- LAS acquires calibrated images by reading the magnification from the microscope and the sensor size from the camera to accurately determine the image dimensions.
- Imaging conditions (such as microscope settings and camera exposure) are automatically recorded by the software. This data is stored with the image and is useful for checking consistency.
- Images are named and acquired into a Windows folder. It's a good idea to make a note of these details.
- You can annotate images with a calibrated scale bar and labels (such as date, time, image name and description).

Please refer to the Advice and Prerequisites 1215 for the recommended image capture settings.

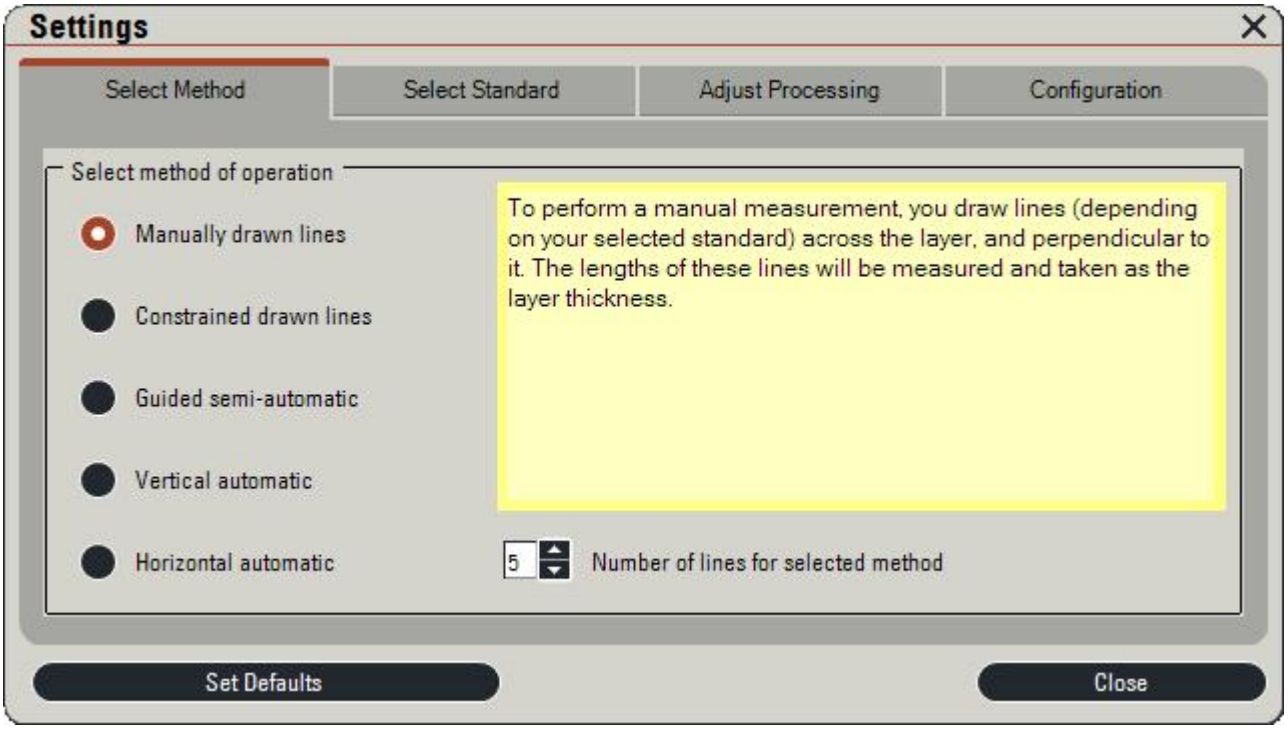
1: Click the *Review Settings* button on the *Leica Layer Menu*.

The *Settings* dialog has the following tabs:

- *Select Method* 1222: Decide which layer measurement method to use, according to the type of specimen.
- *Select Standard* 1224: Choose a standard to use.
- *Adjust Processing* 1225: Specify how image processing is applied, to help identify the layer. You can apply the settings to a test image to check that analysis is working properly, before working on a real sample image.
- *Configuration* 1231: You can define overlay colours, specify a report template, create new configuration files or load configuration files that you have previously saved.

Notes

- If you want to revert to the default settings for the application, click *Restore Defaults*. You can do this at any time before clicking *Close*.
- If you close the *Settings* dialog and save your changes, this will **overwrite** your current configuration file, giving you a new set of defaults.



Select Method

The *Select Method* tab allows you to choose the way in which measurements are made.

See Description of Methods 1223

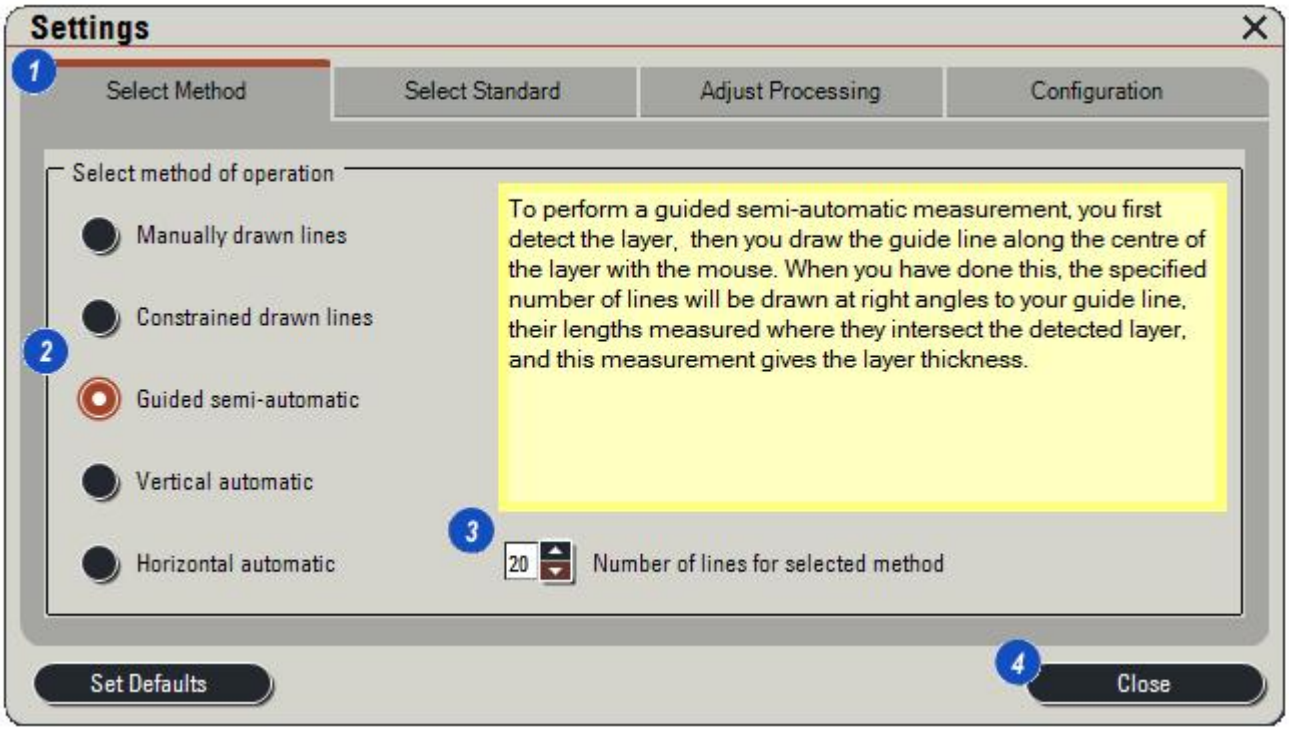
1: Display the *Select Method* tab.

2: Click a radio button to select a method.

3: Specify the number of lines to use for this method.

- For methods where you draw lines across the layer manually, this is normally in the range 5-10.
- For automatic methods, choose a number that will provide a good representation of the layer; this is normally in the range 10-100.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).



The methods available are as follows:

Manually drawn lines 1239

To perform a manual measurement, you will need to draw lines across the layer and perpendicular to it, using the mouse. *Leica Layer Thickness Expert* will then measure the lengths of these lines and take this measurement as the Layer thickness.

Constrained Drawn Lines 1240

To perform a Constrained Drawn Lines semi-automatic measurement, you must first detect the layer using image processing. The outline of the layer will appear and you draw lines across the layer and perpendicular to it, using the mouse.The drawn lines are constrained to be within the detected layer. The measurement then takes place as for manual measurement.

Guided semi-automatic 1241

To perform a guided semi-automatic measurement, you must first detect the layer and then draw a line along the centre of the layer. *Leica Layer Thickness Expert* will then draw the specified number of lines at right angles to your line, measure the lengths where they intersect the detected layer and take this measurement as the layer thickness. You will need to enter the number of lines to draw. Type a number into the text box at the bottom of the dialog.

Vertical and Horizontal Automatic 1242

To perform an automatic measurement, the layer must be oriented vertically or horizontally. You must first detect the layer and then LAS Layer Thickness will draw the specified number of lines at right angles to the selected orientation. The lines lengths are measure where they intersect the detected layer and this measurement is taken as the layer thickness. You will need to enter the number of lines to draw. Type a number into the text box at the bottom of the dialog.

Select Standard

Choose the standard to use from the following options:

- **ASTM B 487**: Standard Test Method for Measurement of Metal Oxide Coating Thickness by Microscopical Examination of a Cross Section.
- **ISO 1463**: Metallic and Oxide Coatings - Measurement of Coating Thickness - Microscopical Method

The Standard selected does not change any of the measurement methods or other aspects of the operation other than the text that is used to describe the standard.

You can enter the notes required by the standard in the Reference Data.

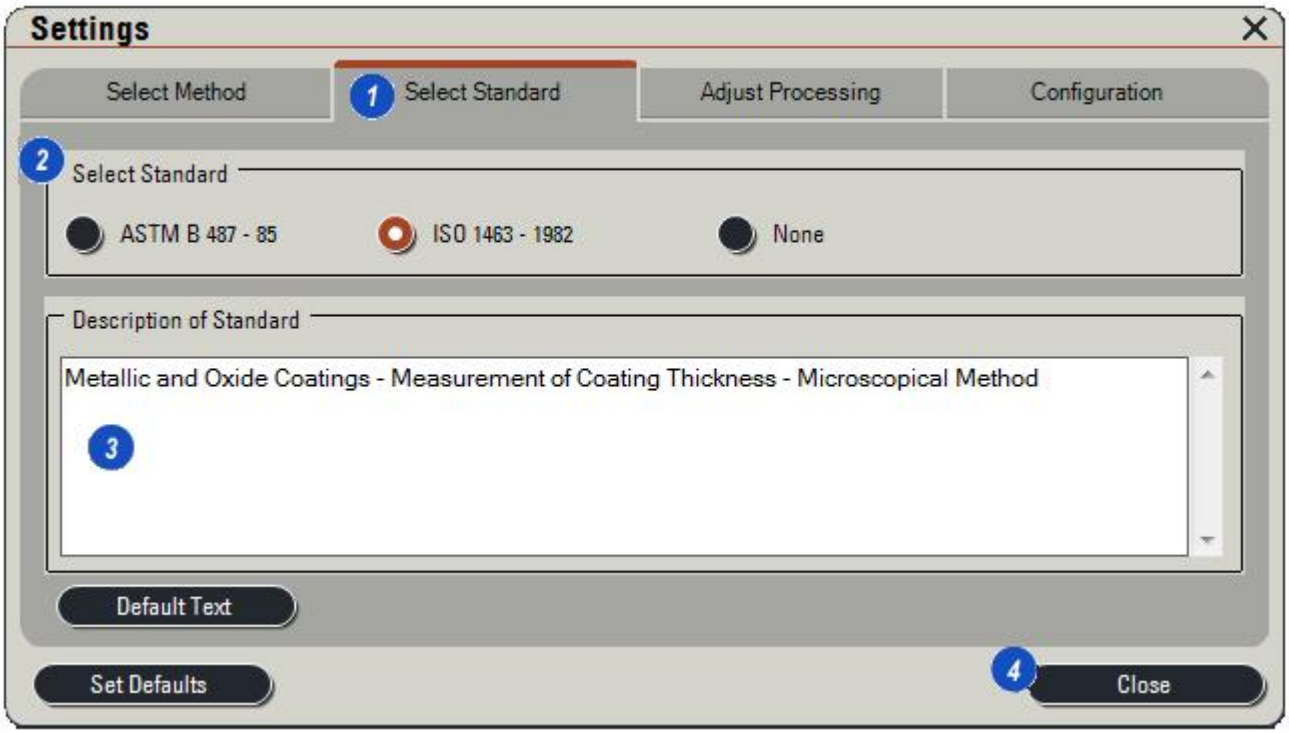
Enter Reference Data 1247

1: Display the *Select Standard* tab.

2: Click a radio button to select a standard.

3: Enter any text that you want to be included in reports that use this standard.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).



Adjust Processing

The *Adjust Processing* panel allows you to optimize the image for the best layer detection and subsequently to remove any unwanted artifacts. You can also configure the histogram showing the distribution of layer thickness.

1: Display the *Adjust Processing* tab.

The controls are grouped as follows:

2: Grey Enhance 1226

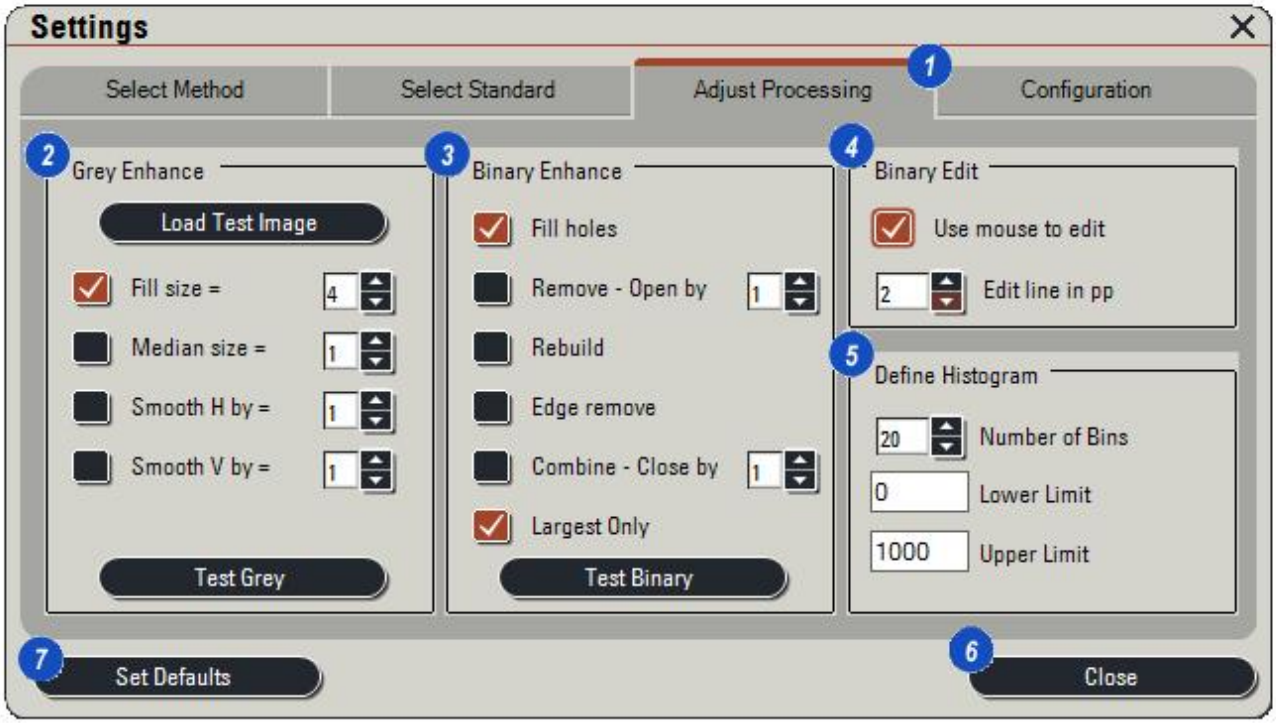
3: Binary Enhance 1228

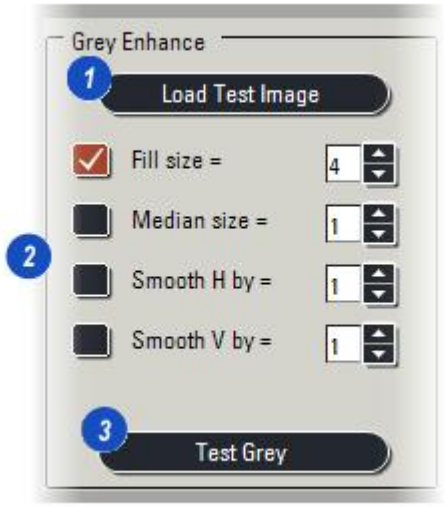
4: Binary Edit 1229

5: Define Histogram 1230

6: Make and test the adjustments then *Close* the dialog or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).

7: If you want to revert to the default settings, click the *Set Defaults* button.





Grey Enhance settings:

Fill size - this removes local detailed variations in the image and is often very effective.

Median size - this is a noise filter that removes sharp spikes in the image with minimum effect on the resolution.

Smooth H and V - smoothing functions that are suitable for horizontal or vertical layers only.

The grey (or colour) processing functions are applied to the selected image to enhance the contrast of the layer and remove spurious detail that might compromise the following threshold step. With the functions provided, you can experiment on test images to achieve acceptable results.

1: Load an image similar to those that you want to measure. See Load Test Image[1227]

2: Select the functions you want to use by enabling the check box and entering a size value. Appropriate size values are in the range 1 to 20. Larger values will increase the effect of the function but will also extend the time taken to process the image.

3: Click the *Test Grey* button to apply the selected function.

You can now examine the effect of the function. Continue to experiment until you are happy that the layer you want to measure appears with good contrast compared to the surrounding region with reduced artefacts. You will probably want to further test this result by performing the Binary Enhance[1228] step.

Note that image processing will change the appearance of the image and it may appear to have less detail as a result.

Load Test Image

The test image can be one that you have captured or one of the samples installed with the application. These are stored in:

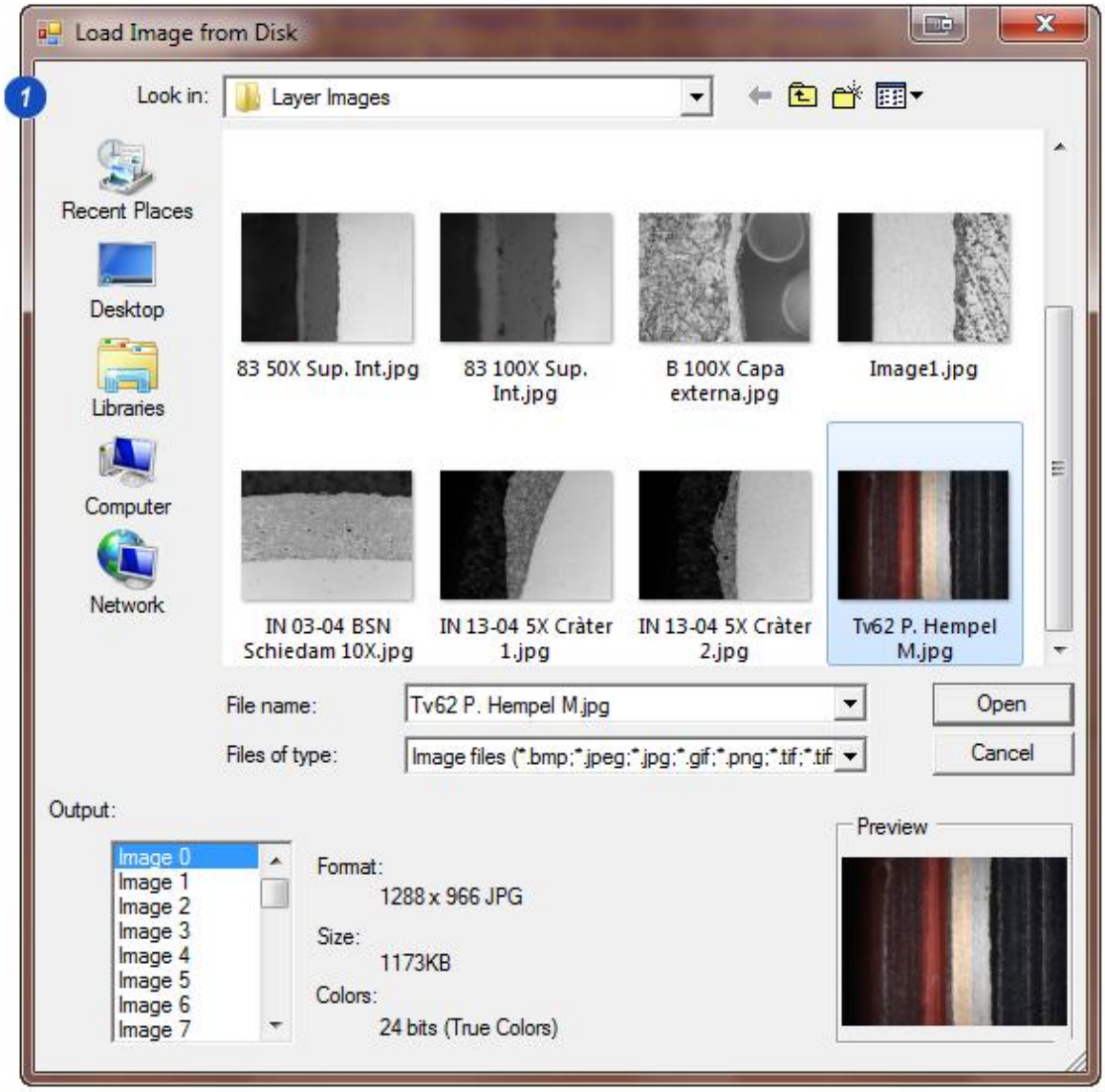
Users > Public Documents > Leica Application Suite > LAS Apps > Apps > LAS Layer App > Example Images

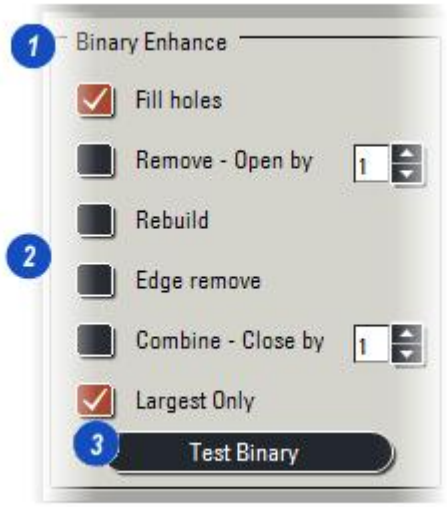
When you click the Load Test Image button:

1: The *Load Image from Disk* dialog appears.

Navigate to either the *Example Images* folder or to a user-defined folder and click to select an image.

Continued Binary Enhance 1228





Binary Enhance Functions

- *Fill holes* - Fills in empty regions in the mask that are completely surrounded by pixels.
- *Remove - Open* - Removes small regions from the mask.
- *Rebuild* - Returns the mask to its original size except where the regions have been removed. Only use in conjunction with the Remove step.
- *Edge Remove* - Deletes any part of the mask that is touching the edge of the image. As a layer often extends to the edge of an image, use this with care.
- *Combine - Close by* - joins together regions of the mask that are near neighbours.
- *Largest Only* - Selects the single largest isolated region in the mask. As this is normally the layer you want to measure, this can be a convenient function to apply.

The Binary Enhance functions are applied to the image produced by the Grey Enhance functions. You must select the test image from Grey Enhance before using the Binary Enhance functions.

The Binary image is used as a mask that defines the extent of a Layer and is used by all the semi-automatic and automatic methods. It has no effect on the Manual method.

Ideally, the result of the threshold and the binary enhancement will be a mask that exactly represents the layer. In practice, providing the mask correctly represents the edges and centre of the layer, a small amount of additional artifact is unlikely to affect the results. With the functions provided, you can experiment on test images to achieve an acceptable mask.

1: The Binary Enhance functions are shown on the panel.

2: Select the function(s) you want to use by setting the check boxes and entering a size value. Appropriate size values are in the range 1 to 20. Larger values will increase the effect of the function but will also extend the time taken to process the image.

3: Click the *Test Binary* button to perform these steps:

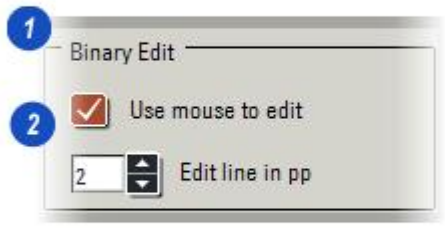
a) Apply the selected Grey Enhance function.

b) Set the grey or colour threshold. See Layer Detection 1235

c) Apply the binary selected function.

You can now examine the effect of the combined grey and binary functions. Experiment with the settings until you can obtain an accurate layer mask. If you cannot achieve this, you will need to use the Manual method.

Binary Edit Settings



If, after using the Grey and Binary Enhance functions, you find that there are still regions of the mask that do not belong to the layer, Binary Editing[1237] allows you to interact with the image using the mouse to remove or cut away these unwanted regions.

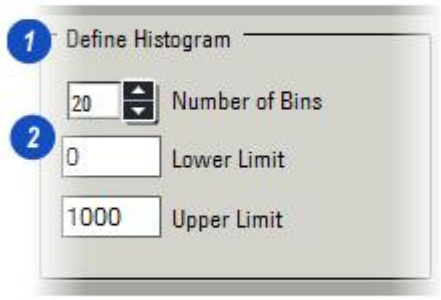
1: The Binary Edit functions are shown on the panel.

2: Select *Use mouse to edit* if you want to be able to edit the layer after the Binary Enhance step, during measurement.

When drawing lines with the Edit tool, you can set the width of the line so that it is easier to see on high-resolution images displayed in 'Fit-to-window' mode.

When you click the *Test Binary* button, the Binary Edit will be available after the Binary Enhance has taken place.

The operation of the Binary Edit tools is described in Layer Mask Editing[1236]



The application creates a histogram that shows the distribution of the layer thickness measurements accumulated over one or several images.

1: The Define Histogram functions are shown on the panel.

2: Enter the number of bins to be used depending on the detail that you want to see. These bins will be distributed between the Lower and Upper limits.

These values will be used by the histogram created when measurements are made.

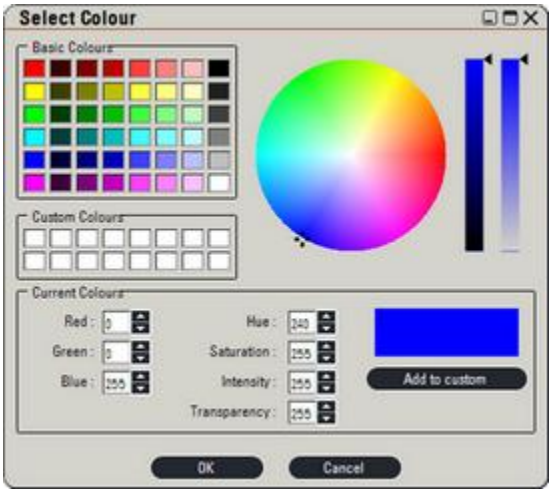
It is possible to review the histogram during the measurements. See Review Histogram 1243

Note: The Limits are assumed to be in the same units of calibration as the images measured.

The *Configuration* tab allows you to choose the Overlay Colours and Load and Save a Configuration.

1: Display the *Configuration* tab.

2: To change the colours of lines and overlays (for example to give a better contrast against the image you are working with) click on the appropriate colour swatch. Pick a new colour using the *Select Colour* dialog:

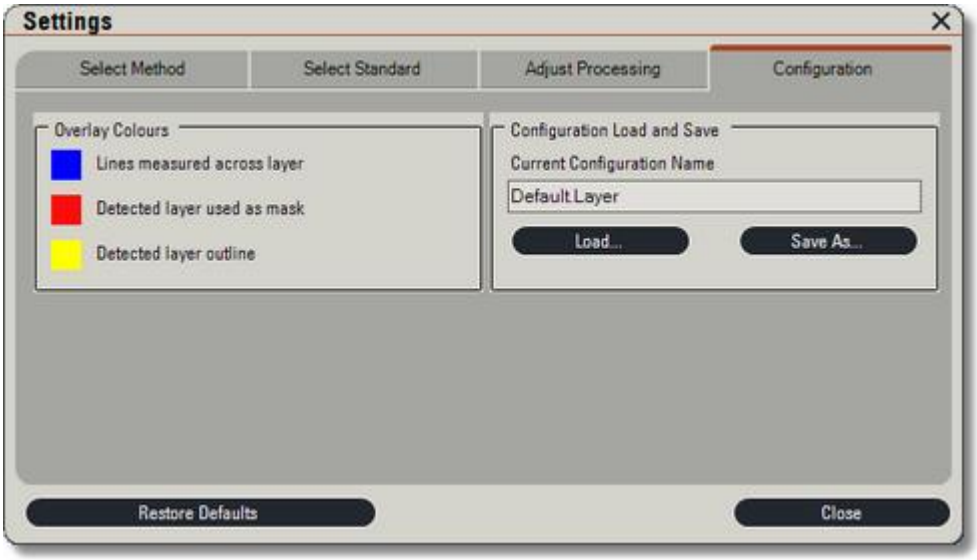


3: At this point you can click *Save As* to save all your changed settings to a named configuration file. These files are located in the following folder (and have the extension .Layer):

C:\Users\Public\Documents\Leica Application Suite \LAS Apps\Apps\LAS Layer App\Config Files

You can also *Load* existing configuration files that you have tuned to different types of specimen.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made; this will **overwrite** your current configuration file, in effect giving you a new set of defaults).



The flow chart opposite shows the sequence of steps for selecting, detecting, measuring and showing the results in *Perform Measurements.*

1: On the *Leica Layer Menu* click the *Perform Measurement* button to enter Measurement mode.

2: Click *Select and Measure*.

3: The images available in the selected folder are displayed in the *Gallery*. Select the image to measure and click *Continue*. This will be loaded and any Grey Enhancement specified in the selected Settings configuration will be applied.

4: Adjust the threshold to identify the layer. (This does not apply to the Manual method). Click *Continue*. Binary Enhancement will be applied. Depending on the Settings, you may have the chance to Edit the mask at this point.

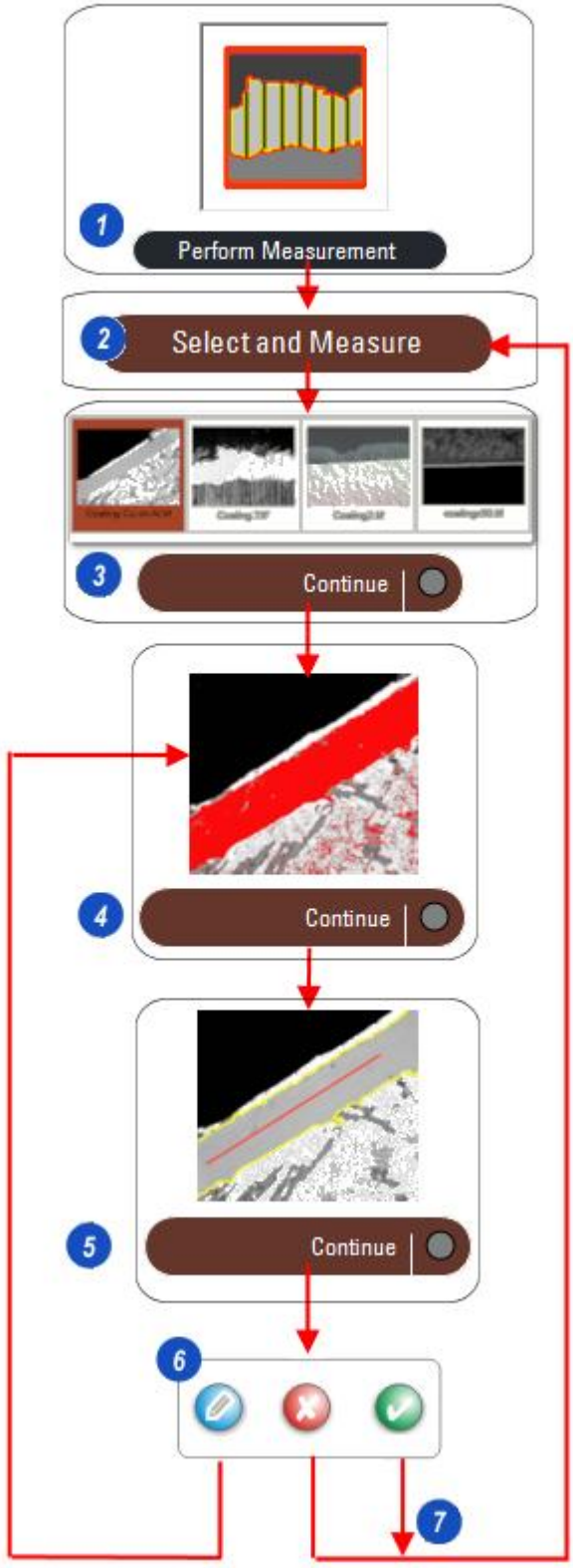
5: For the Manual and Semi-automatic methods, draw lines on the image. Click *Continue*.

6: Review the measurements you have made and choose what to do with them:

- **Edit** the detection and remove spurious lines.
- **Ignore** the image and results. Get the next image.

7: **Accept** the results and add them to any existing results. Get another image.

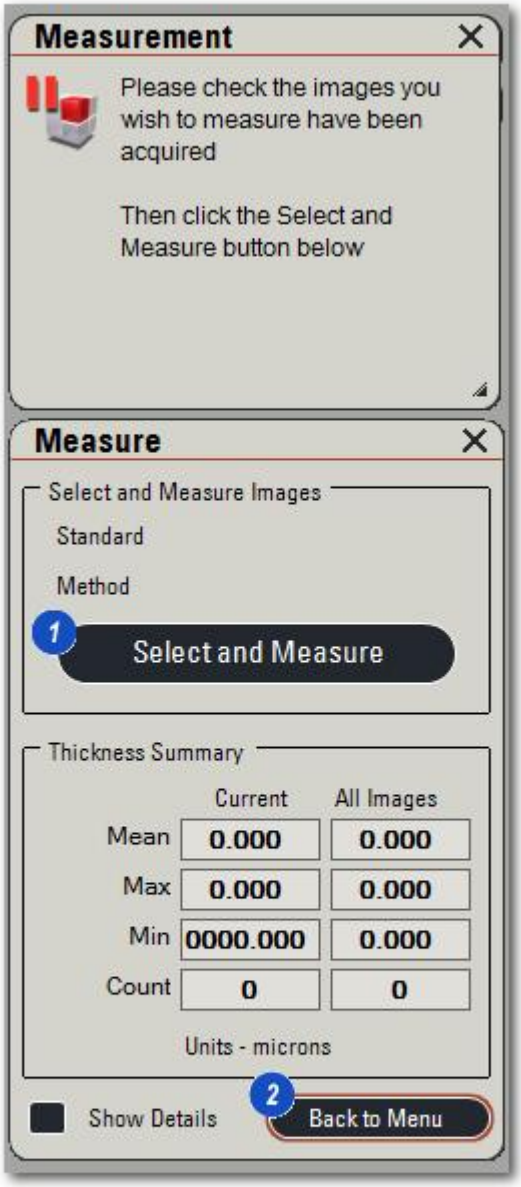
Measure another image or return to the *Leica Layer Menu*.



On the *LAS Layer Menu* click the *Perform Measurements* button. The *Measure* panel appears. Initially the Thickness Summary results are empty, indicating that no measurements have been made.

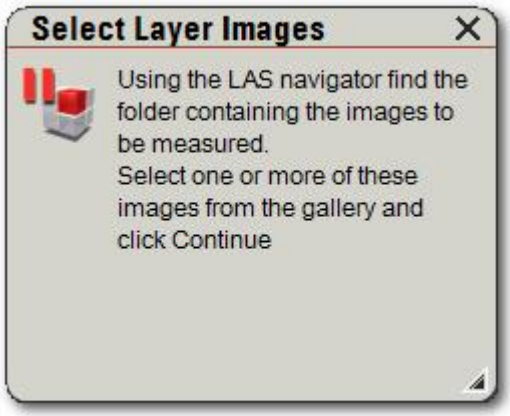
1: You should already have acquired the images you want to measure and placed them into a known folder. If this is the case, click the *Select and Measure* button.

2: If you have not acquired any layer images, click *Back to Menu* and exit the Layer application. Go to the LAS Acquire workflow step and acquire the images, then start the *Leica Layer Thickness Expert* again.



Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



1: Click on the *Show Navigator* button on the *Side Tool Bar*.

2: Move the *Navigator* to a convenient position by clicking and dragging the header.

3: Navigate to the image folder.

4: Double-click to display the available images. Click to select a single image, or Ctrl-click to select up to 10 images.

5: You can minimise the Navigator by clicking the arrow to the right of the header.

Click the *Continue* button to load the image. After the image is loaded, the Grey Enhancement will be applied.

The Layer is detected using the parameters set up by the *Review Settings dialog (Adjust Processing* 1225 tab).

The detection method used will be either Monochrome as shown to the right, or Colour, depending on the image type.

However, due to the range of contrast that can be encountered in different images, some areas that should be included in the measurements may not be detected, and some regions of the layer may be ignored. You can adjust the regions included as follows:

1: Click to enable the *Accumulate* check box.

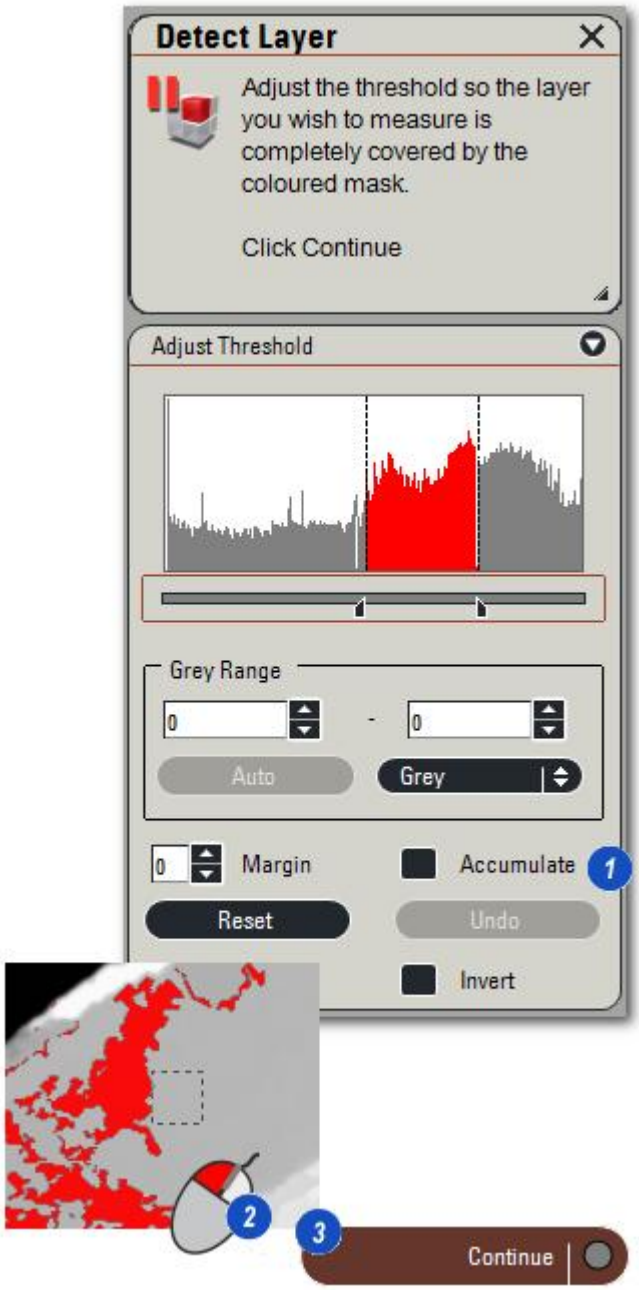
2: Drag a marquee around an area to include.

Image values contained within the marquee are added (accumulated) to the existing range and the areas are detected.

Alternatively adjust the sliders under the histogram.

For further details, see the section on Adjust Threshold in the main LAS help (LAS Image Analysis module).

3: Click *Continue* when you are satisfied with the detection. Do not be too concerned if there are some separate detect regions, as these will be rejected by the Binary Enhancement in the next step.



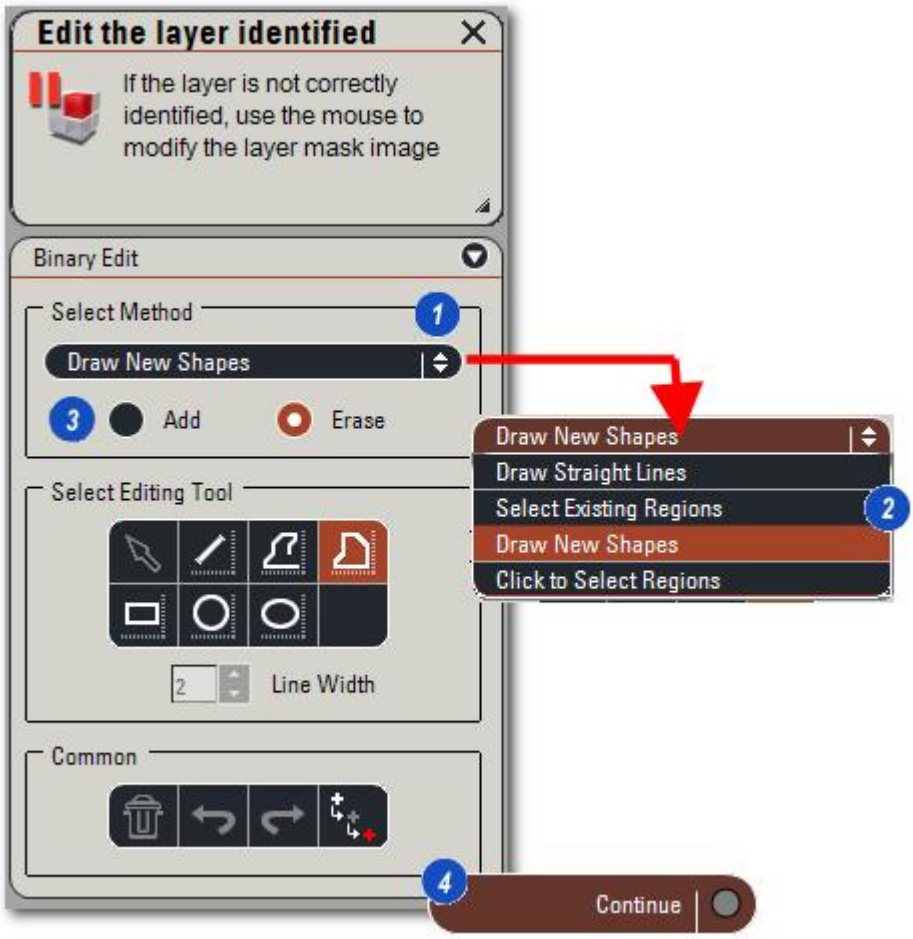
You can edit the Layer mask resulting from the Threshold and Binary Enhance steps by selecting *Review Settings* > *Adjust Processing* [1225] > *Binary Edit* [1229].

The *Binary Edit* tools allow you to optimise the Layer mask by adding or removing regions.

The panel is divided into the following sections:

- *Select Method*: Sets the drawing method - the most useful in this case being the one called Draw New Regions; this appears by default.
  - Use the *Add* and *Erase* radio buttons to decide which function you want to perform.
- *Select Editing Tool*: Displays the generic toolbox and the Line Width control. In this case the default selection is the Fill Area Tool.
- *Common:* The tools for deleting *(Trash Can)* drawn shapes, undoing and re-doing the last actions and changing the cursor colour.

For further details, see The Binary Edit Tools [1237]



Select Method:

1: Click on the arrows to the right of the header.

2: Click to select a method. *Leica Layer Thickness* uses only *Select Existing Regions* or *Click to Select Regions*.

3: Click the appropriate function radio button. Perform the editing as required.

4: Click *Continue* to move to the Line drawing step.

Common Tools

1: *Delete (Trash Can):* Click to delete the drawn shapes.

2: *Undo/Redo:* Click to undo/redo previous operations.

3: *Cursor Colour:* Click to change the cursor colour to suit the image (this toggles between black, white and red).

Drawing Tools

4: The appropriate tool is automatically selected with your chosen method:

Selection Tool:

Available when *Click to Select Regions* method is active. Use this to select individual nodules or artifacts (to deselect or reinstate them).

Position the cross-hair cursor over a nodule or artifact and left-click.

Area Tool:

Available when *Select Existing Regions* is active. The tool draws a closed irregular shape around a group of nodules or artifacts.

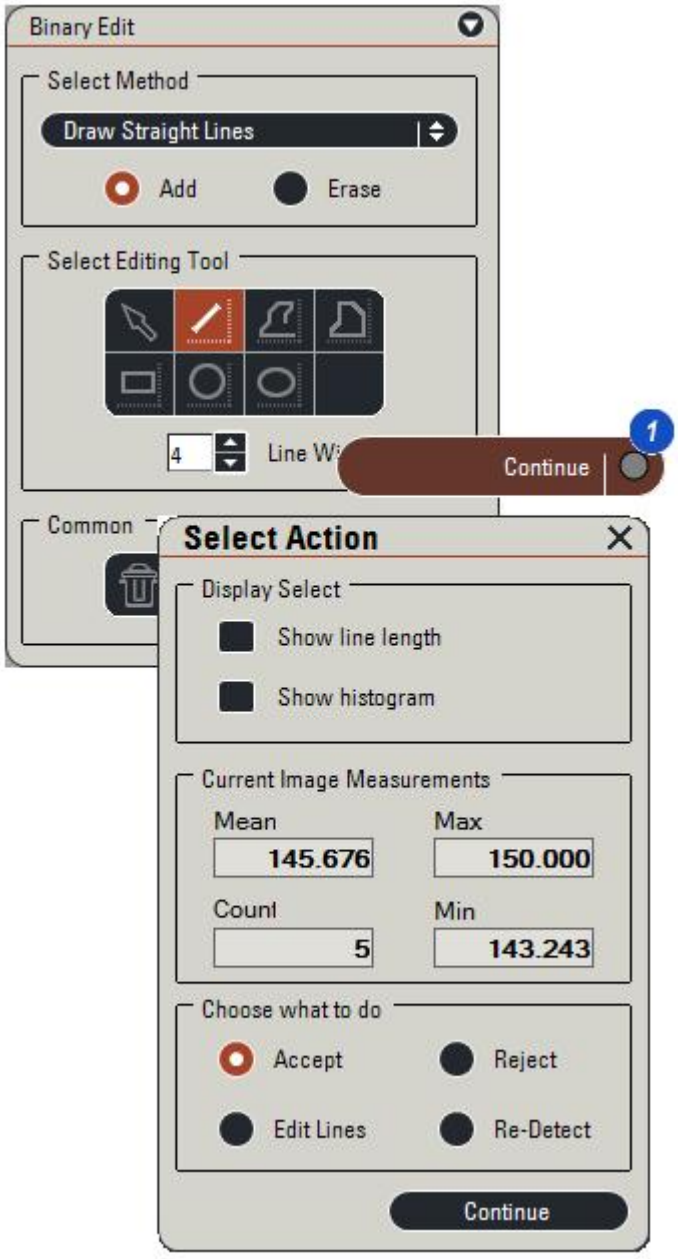
Left-click to start; Move the cursor to the next point and left-click again; Repeat around the shape; End with a right-click.

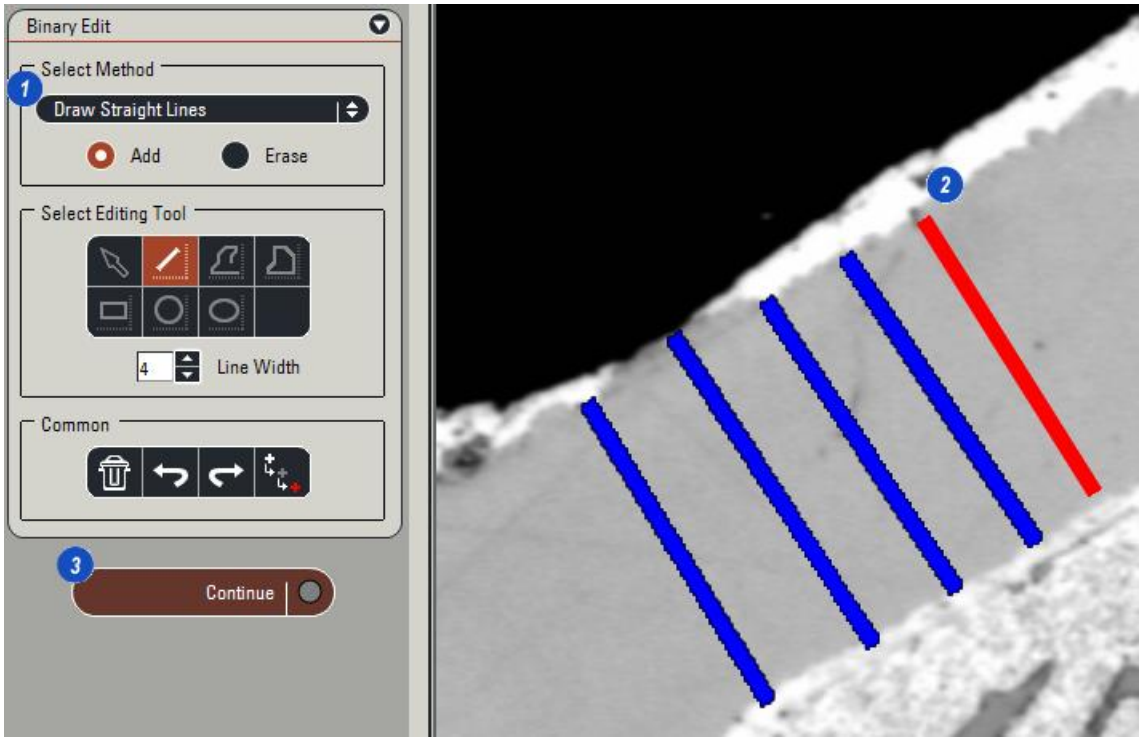
With the layer mask now ready, the lines representing the layer thickness are drawn across the layer. The way these lines are produced depends on the Measurement Method selected as described in the following topics:

- Method 1 - Manual 1239
- Method 2 - Constrained Semi-automatic 1240
- Method 3 - Guided Semi-automatic 1241
- Method 4 & 5 -Automatic 1242

When the lines are drawn as required:

1: Click *Continue* to display the *Select Action* panel.





To perform a manual measurement:

1: Check that *Draw Straight Lines* is active.

2. In the image window, draw at least 5 lines on the image. The actual number required is specified in the Settings dialog. These lines should be drawn at regular intervals, to avoid introducing bias into the averaging process, and at right angles to the coating. Accuracy is important, as *Leica Layer Thickness* measures the precise length of the line.

The width of the lines is exaggerated in the illustration.

3: Click *Continue* when you have drawn the lines to Select the next action and view the measurements.

To perform a Constrained Semi-automatic measurement:

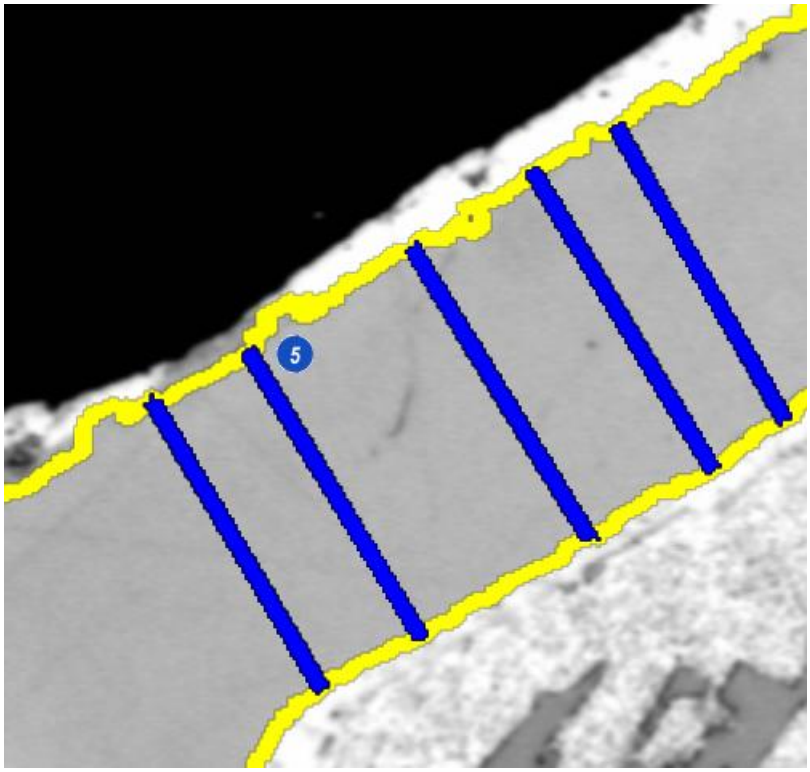
1: Check that Draw Straight Lines is active.

2. In the image window, the outline of the layer is shown (in yellow in this illustration).

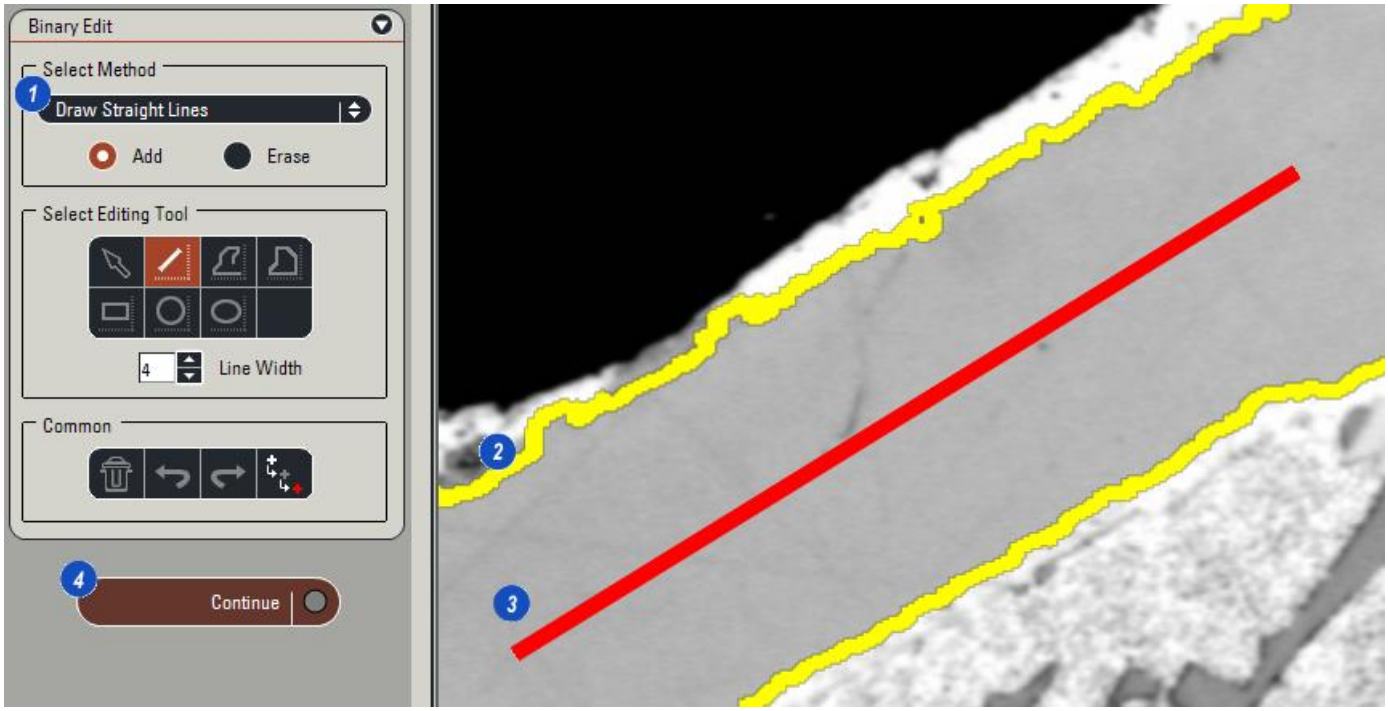
3: In the image window, draw at least 5 lines on the image. The actual number required is specified in the Settings dialog. These lines should be drawn at regular intervals, to avoid introducing bias into the averaging process, and at right angles to the coating. Make sure that the lines extend beyond the coating.

4: Click *Continue* when you have drawn the lines to Select the next action and view the measurements.

5: Lines are shown in blue where they fall on the Layer mask. If a line (or part of a line) does not fall in the mask, it is removed. *Leica Layer Thickness* only measures the part of the line that falls within the mask. Accuracy is therefore not as important as it is for manual measurement.



Method 3 - Guided Semi-automatic



To perform a Guided Semi-automatic measurement:

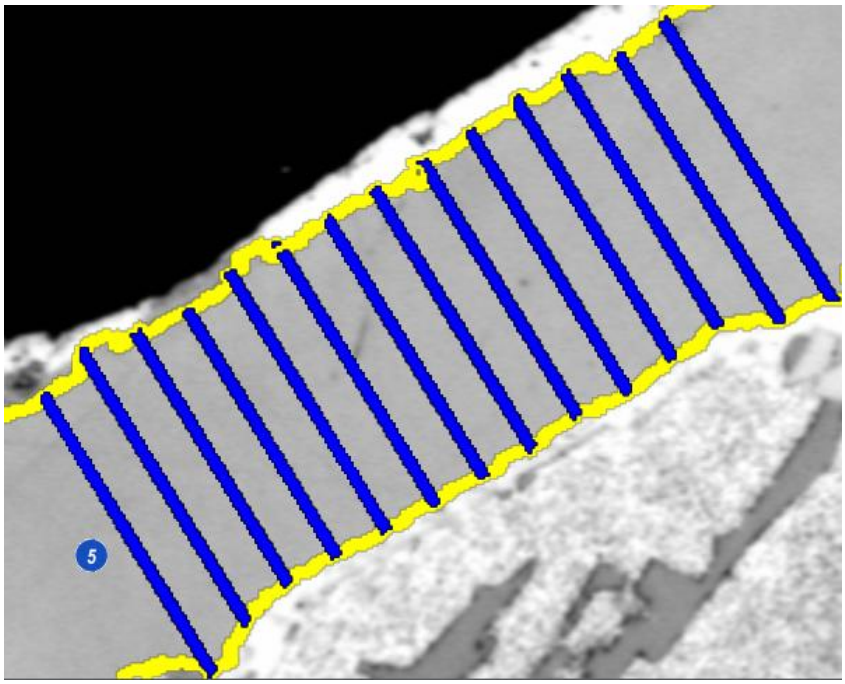
1: Check that Draw Straight Lines is active.

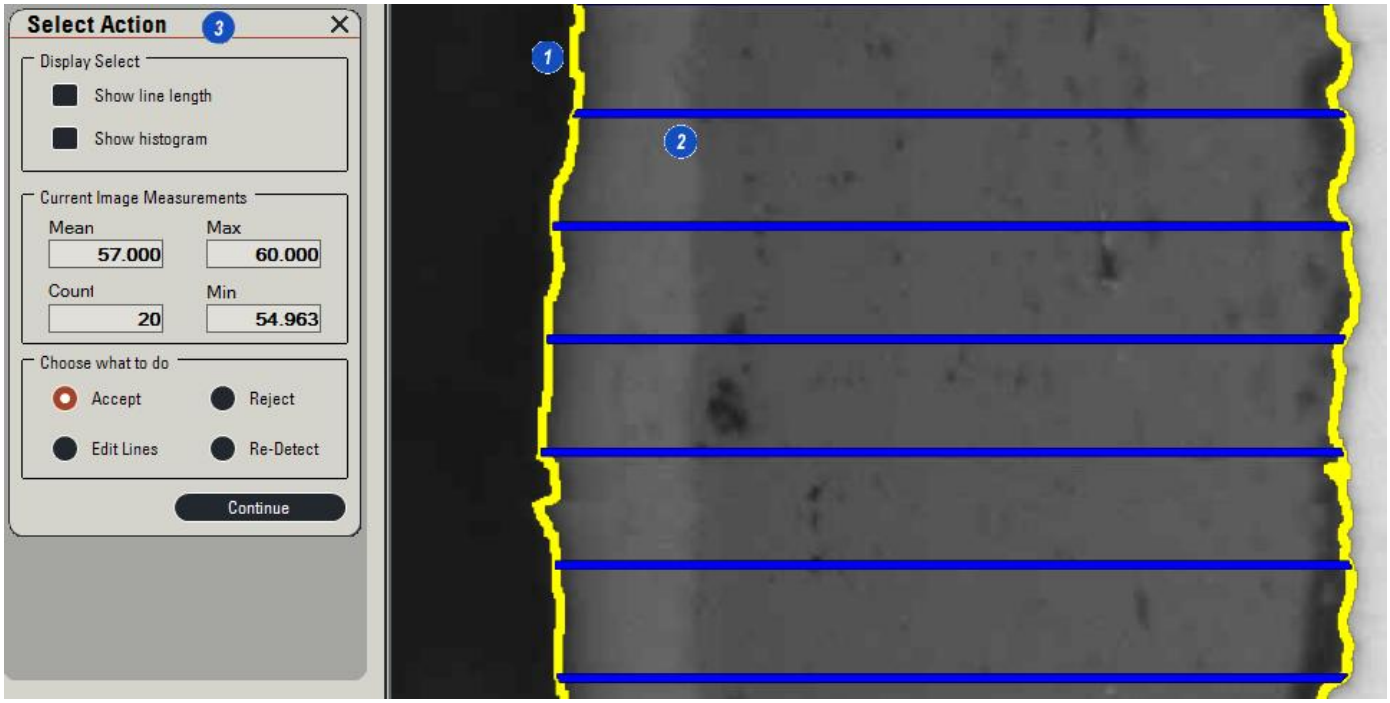
2. In the image window, the outline of the layer is shown (in yellow in this illustration).

3: In the image window, draw a single line along the centre of the Layer mask at the position where you want to measure the layer.

4: Click *Continue* when you have drawn the lines to Select the next action and view the measurements.

5: Lines are now drawn automatically, perpendicular to the centre line and only inside the mask. The number of lines is specified in the Settings dialog.





To perform an Vertical Automatic measurement:

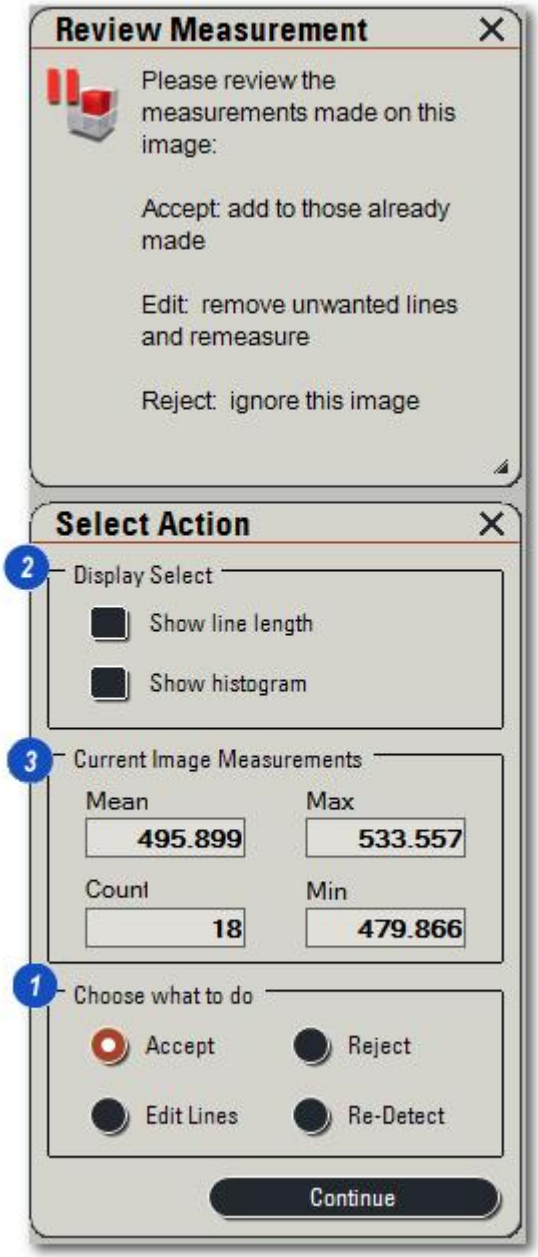
1. In the image window, the outline of the layer is shown (in yellow in this illustration).

2: In the image window, equally spaced lines are drawn horizontally across the layer mask. The number of lines is specified in the Settings dialog.

3: The *Select Action* panel is displayed immediately. Click *Continue* when you have drawn the lines to Select the next action and view the measurements.

The process for a Horizontal layer is similar.

Once the measurements have been made:



1: *Choose what to do* with the measurements:

- *Accept* - you are confirming that the measurements are correct and they are to be accumulated into any measurements made on previous images in this series. In this way the results from several images can be accumulated and averaged.
- *Reject* - you decide that this is the wrong image or it cannot be measured with the method chosen. Ignore this image and select another one. Measurements are ignored.
- *Edit Lines* - use this action if you notice that the lines are not correct and you want to edit them before remeasuring. For example, you can remove extraneous short lines that appear during an automatic measurement. Displays the Edit tools so that line fragments can be removed. See Layer Lines Editing 1244
- *Re-Detect* - using the same image go back to the mask threshold detection step and adjust the detection.

2: To help make these decisions, you can display and inspect the measurements; the options are:

- *Show line length*: The length of each line is shown as a label on the image. Note that for lines that are close, the labels may be obscured, so this feature is most suitable for vertical layers.
- *Show Histogram*: Show the length measurements for the current image graphically as a histogram. Uses the histogram settings - see Define Histogram 1230

3: A summary of the measurements for the current image is given.

4: Click the *Continue* button to confirm your selected action.

The *Binary Edit* panel is shown and its tools allow you to remove line fragments or to join lines that are split. Or you may simply want to redraw some of your original lines. An example is shown below.

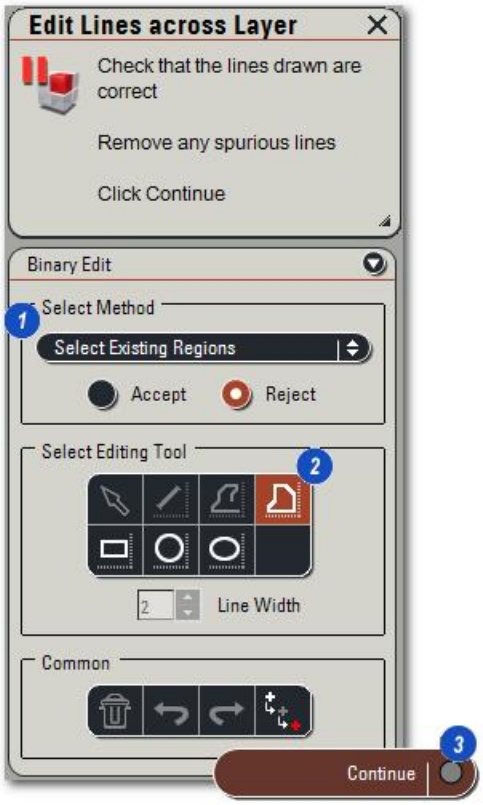
Edit lines across layer:

1: Click on the arrows to the right of the method options, click to select a method and chose to *Accept* or *Reject*.

2: Choose a suitable tool. The *Fill Area Tool* is often the easiest to use. Perform the editing as required.

3: Click *Continue* to remeasure this image and return to the Select Actions[1243] step.

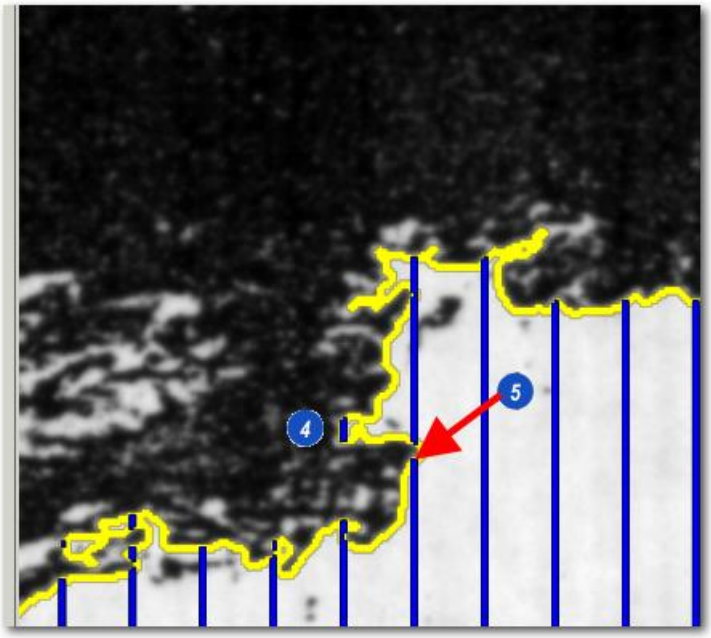
Continued Drawing Lines across the Layer[1238]



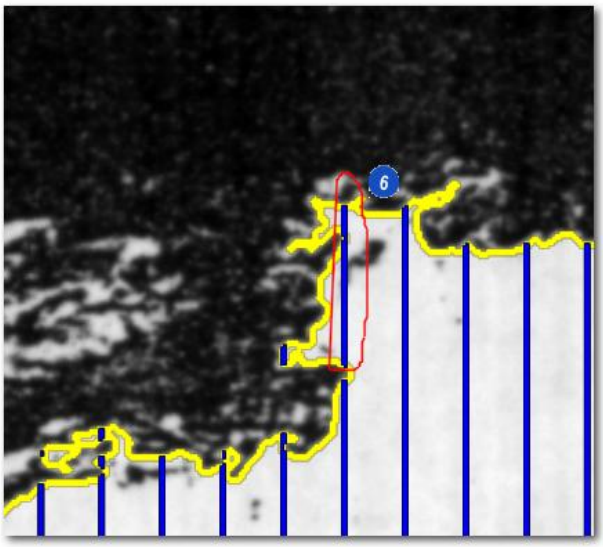
Example

4: This short fragment is not measured as lines that deviate by a long way from the mean are rejected.

5: Look closely and you will see that this line is broken.

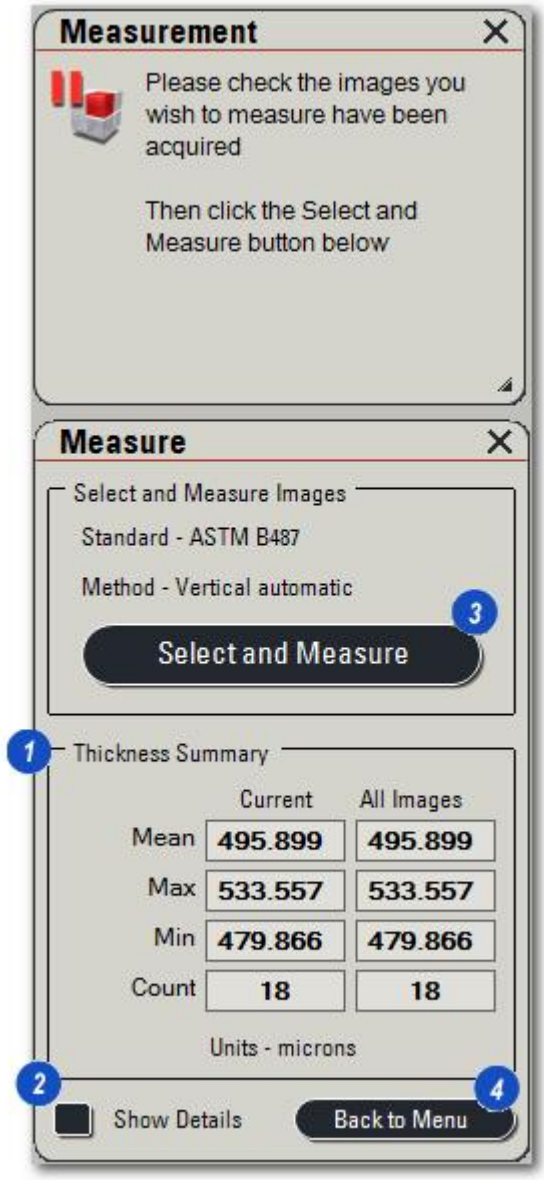


6: Use the edit tools to draw round the line and remove it. Alternatively you may think that it should be joined with the line below. To do that select the *Draw New Shapes* tool.



Summary Results

When all images have been detected and measured, a summary of the accumulated results is displayed on the *Measure* panel.



1: Summary Thickness Results for the last image measured and All images.

Mean is the average thickness

Max is the maximum thickness

Min is the minimum thickness

Count is the number of lines measured

2: Click the *Show Details* checkbox to display extra information about the current measurements. The accumulated histogram for all images measured so far is diplayed. See Detailed Results for all accepted Images 1246

3: Click *Select and Measure* to measure another image. See Select Image(s) to Measure 1234

4: Click *Back to Menu* to return to the *Application Features* menu so that you can create a report of these measurements. See Operating Leica Layer Thickness Expert 1219

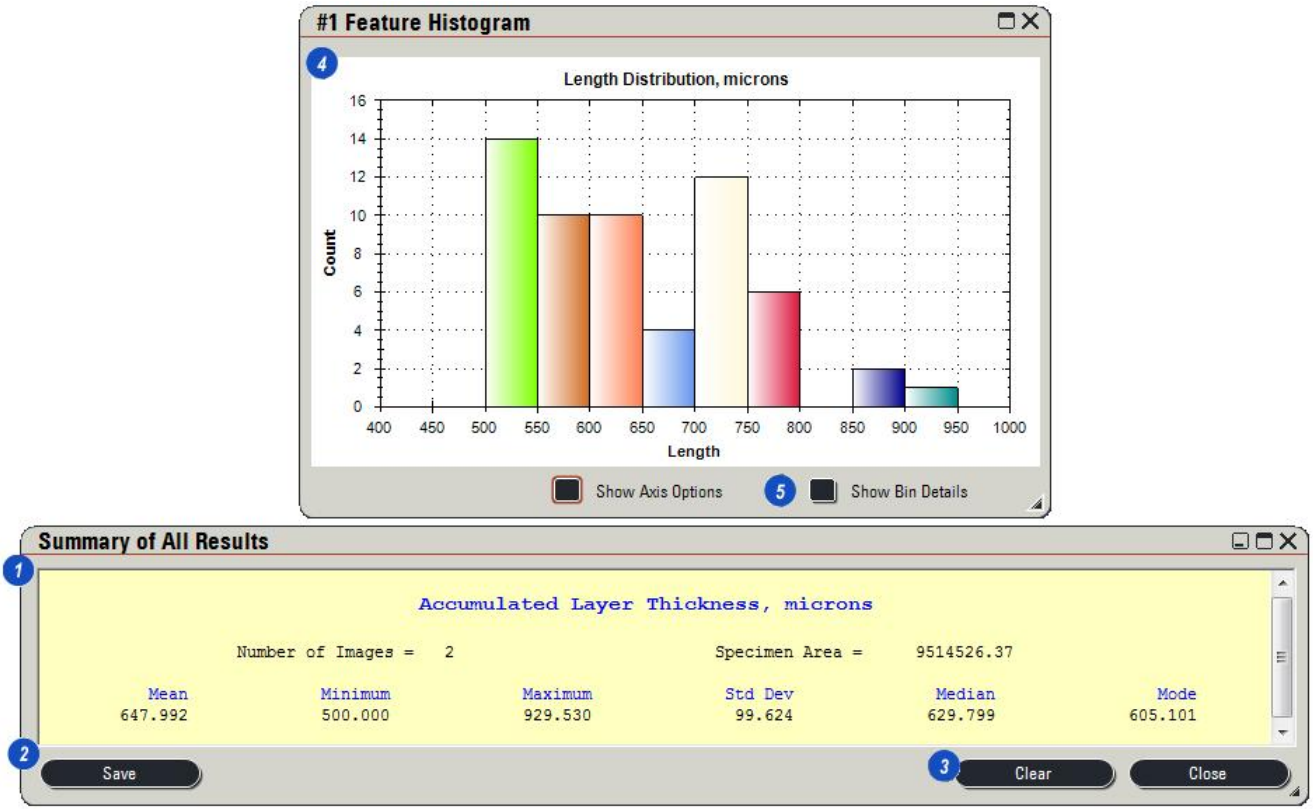
1: Selecting the *Show Details* checkbox option on the Measure dialog displays the results for the all images.

2: Save the results by clicking the *Save* button; on the Windows Save dialog navigate to the required folder and save the file in .txt format.

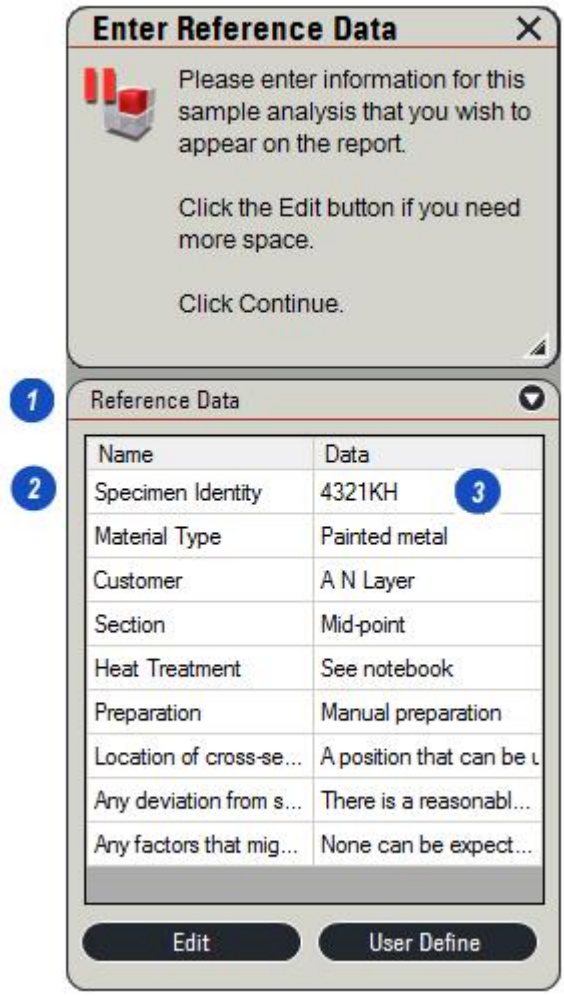
3: The *Clear* button clears the display, but this has no effect on the results (it is only the window that is cleared).

4: The *Length Distri*bution shows the variation in thickness.

5: Additional detail of the histogram data is revealed by these checkboxes.



Enter Reference Data



Users can add information about specimens and processing on the *Reference Data* panel. Some of the information is displayed on the example template installed with the application.

On the *Application Main Menu,* click *Enter Reference Data.*

1: The *Reference Data* panel appears

2: Click to select an item.

3: Click inside the text box to the right and type the data.

- For ISO 1463 you will need to enter:
  The location of the cross-section on the coated item.
  Notes and comments if required.

- For ASTM B 487 you will need to enter:
  The location of the cross-section on the coated item.
  Any deviations from the standard.
  Any factors that might influence interpretation of the reported results.

You can select the *Reference Data* headings to suit your working methods and descriptions. To do this you must run LAS as an administrator. (Right-click the desktop icon and select *Run as Administrator*.)

1: Click on the *User Define* button.

2: The *User Define Reference Data* dialog appears.

3: Click a check box to toggle the visibility of a heading.

4: The *Tools* from left to right are:

Create: A new user heading. Initially it is called Field X; Click on the name and type a more appropriate heading name.

Delete: The Trash Can removes a selected heading completely.

Tick Mark: Enables or disables visibility of the selected heading. Same as clicking the check box.

Select All: Enables all headings.

*Hide A*ll: Disables all headings.

Up/Down Arrows: Move the selected heading up or down the list.

5: Click *OK* to finish.

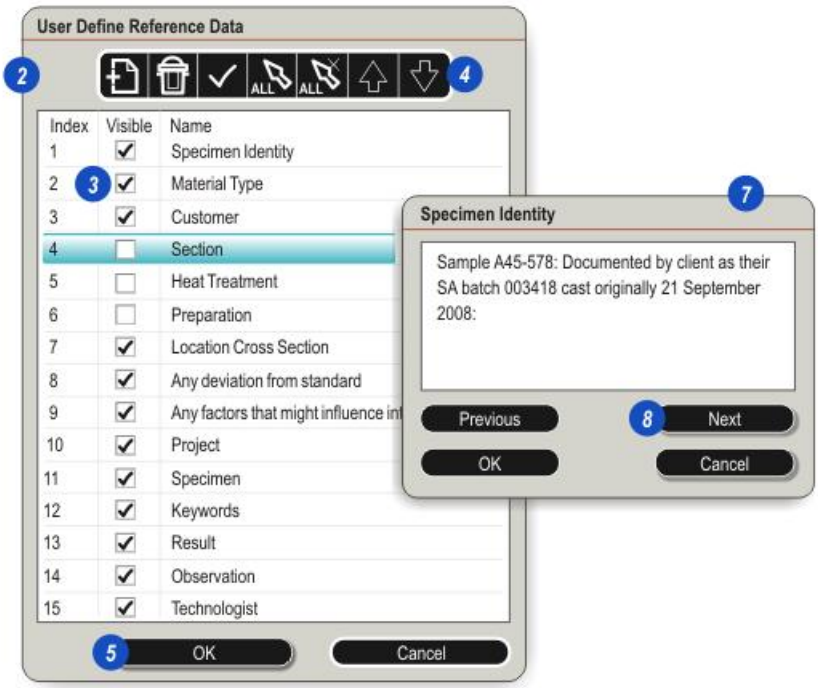
If any item of data is too long to fit inside the panel text box:

6: Click the *Edit* button.

7: A larger text box appears. Click inside the window and type the data.

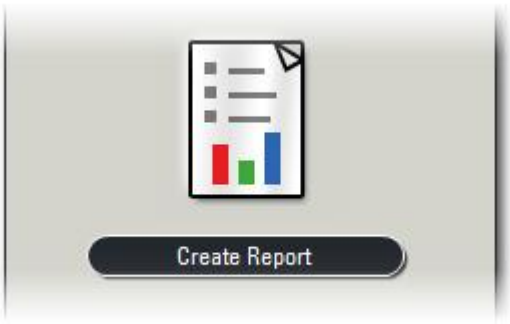
8: Scroll between the headings using the *Previous* and *Next* buttons.

Note - changing the Reference Data may require the Excel report template to be modified. After making changes to the Reference Data names, please check the report output; if necessary, modify the report template.



Create Report

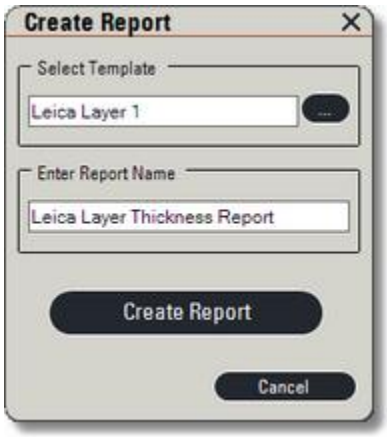
You can incorporate the measurement results into a comprehensive report using Microsoft Excel. The example template for the report is installed with the application. This template can be modified as required. You can find general techniques used for creating LAS reports in the main LAS Help.



On the *Application Main Menu* click the *Create Report* button.

1: This opens the *Create Report* panel.

2: Click on the browse button and select an Excel report template. The template will normally have an extension .xlt.



3: Click inside the *Enter Report Name* text box and type a unique name for the report.

3: Click *Create Report*.

The report will be displayed. Before you create another report with the same name, you must close the displayed report.

The example report template is located in the following folder:

C:\Users\Public\Documents\Leica Application Suite\LAS Apps\Apps\LAS Layer App

Below is an example of a Layer Thickness report using the template installed with the application.

By default, reports are stored in the following folder:

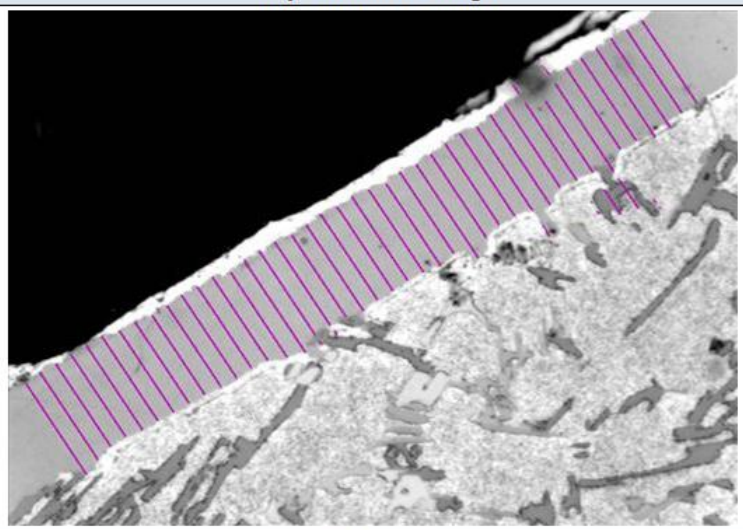
Libraries\LAS Reports\Leica Layer Thickness Reports

LAS Layer Thickness Report

Specimen Identity	ABC	Method used:	Guided semi-automatic
Material Type	Heavy and painted	Analysis Date:	03/03/2012
Customer	A B Customer	Heat Treatment	From the oven
Section	Mid-range	Preparation	Manual method

Location A position that can be deduced from the middle of the sample	Factors The factors that we can see are not ones that we may need to see
Standard used:: None Enter description or leave blank	Deviation There is some chance of a deviation in the context of the specimen

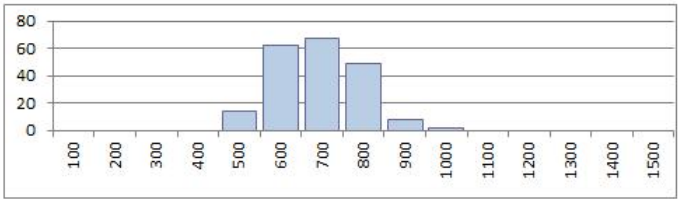
Layer Thickness Image



Layer Thickness Results

Number of Images = 0 Specimen Area = 0

Mean	Minimum	Maximum	Std Dev	Median	Mode
147.73	44.60	198.65	17.83	140.00	140.00



Approved By:	

Cast Iron Expert

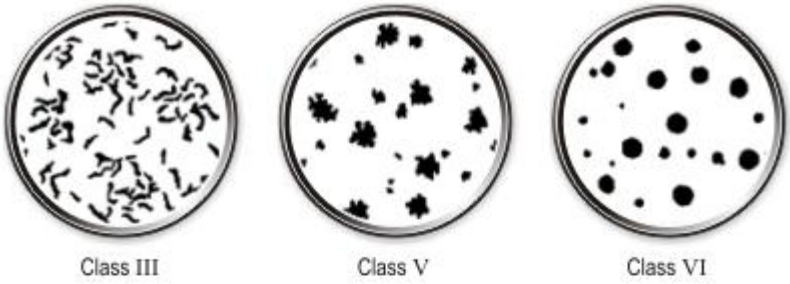
It is important to be able to control the composition and microstructure of cast iron because they have a direct effect upon its engineering properties.

The graphite, ferrite and pearlite present in cast iron largely affect those engineering properties. Since each component has characteristic qualities (size, shape and colour), they can be detected and analysed using digital imaging techniques. Determining the composition gives an understanding of the iron quality.

Leica Cast Iron Expert models the standards ASTM E247, ISO 945-2 and JIS G5502; users can change most parameters to suit their individual requirements.

The program features multiple sample analysis for both graphite and ferrite/pearlite, as well as binary image editing capabilities and comprehensive reporting.

The graphite in cast iron is classified according to shape and size; *Leica Cast Iron Expert* has been optimised to measure Class III, Class V and Class VI in ductile cast iron, as shown in the illustrations opposite.



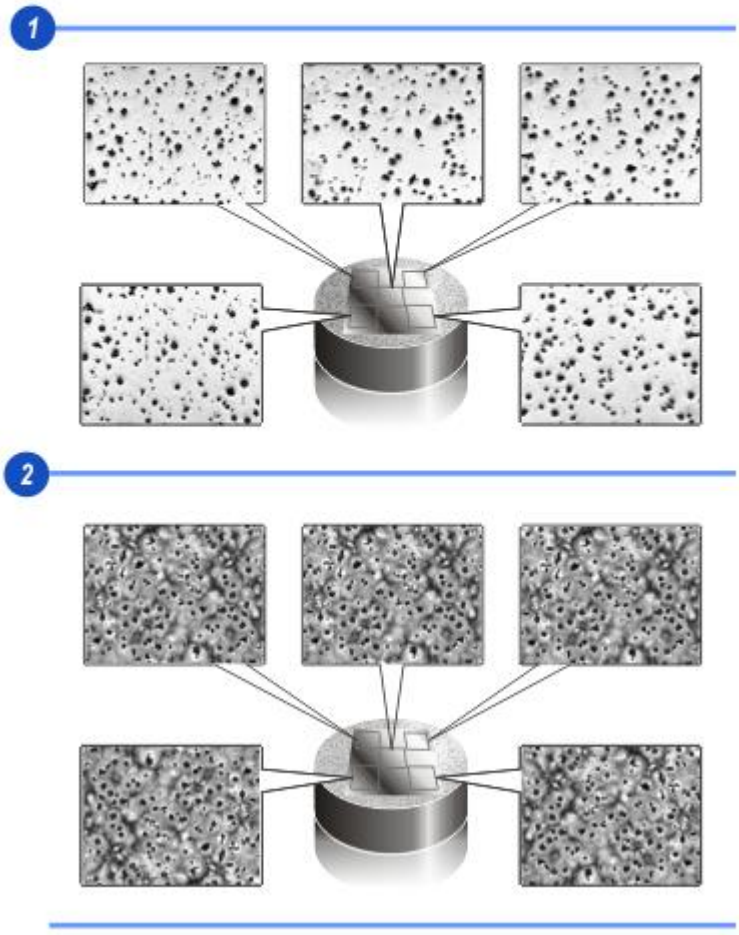
During image acquisition, the cast sample is prepared twice:

- The first preparation involves grinding and polishing to reveal the graphite nodules. Images are then captured at locations over the sample (1).
- The sample is then processed again, usually by etching, to additionally expose the ferrite and pearlite content. Another set of images (2) is then captured at the same locations as those taken for the graphite.

In combination, the two sets of images can be analysed to produce area and content measurements for all three constituents.

The analysis process depends upon contrast. However, since graphite is predominantly black and pearlite is dark grey, precisely distinguishing between the two is difficult. So, graphite is measured in the first set of images, and then graphite together with pearlite is measured in the second set. Subtraction yields separate values for both graphite and pearlite.

Ferrite, closer to white and therefore more easily detected, is measured on the second set of images.



The principal areas of the user interface:

1: Menu Bar*:* For Options and Help.

2: Workflows: Leica Cast Iron Expert is available on the Analysis Workflow.

3: Application Panels Tab: Click *Runner*.

4: Prompt Message Panels: Hints for program sequence steps.

5: Control and Results Panels: Used to operate the application and to show summary results.

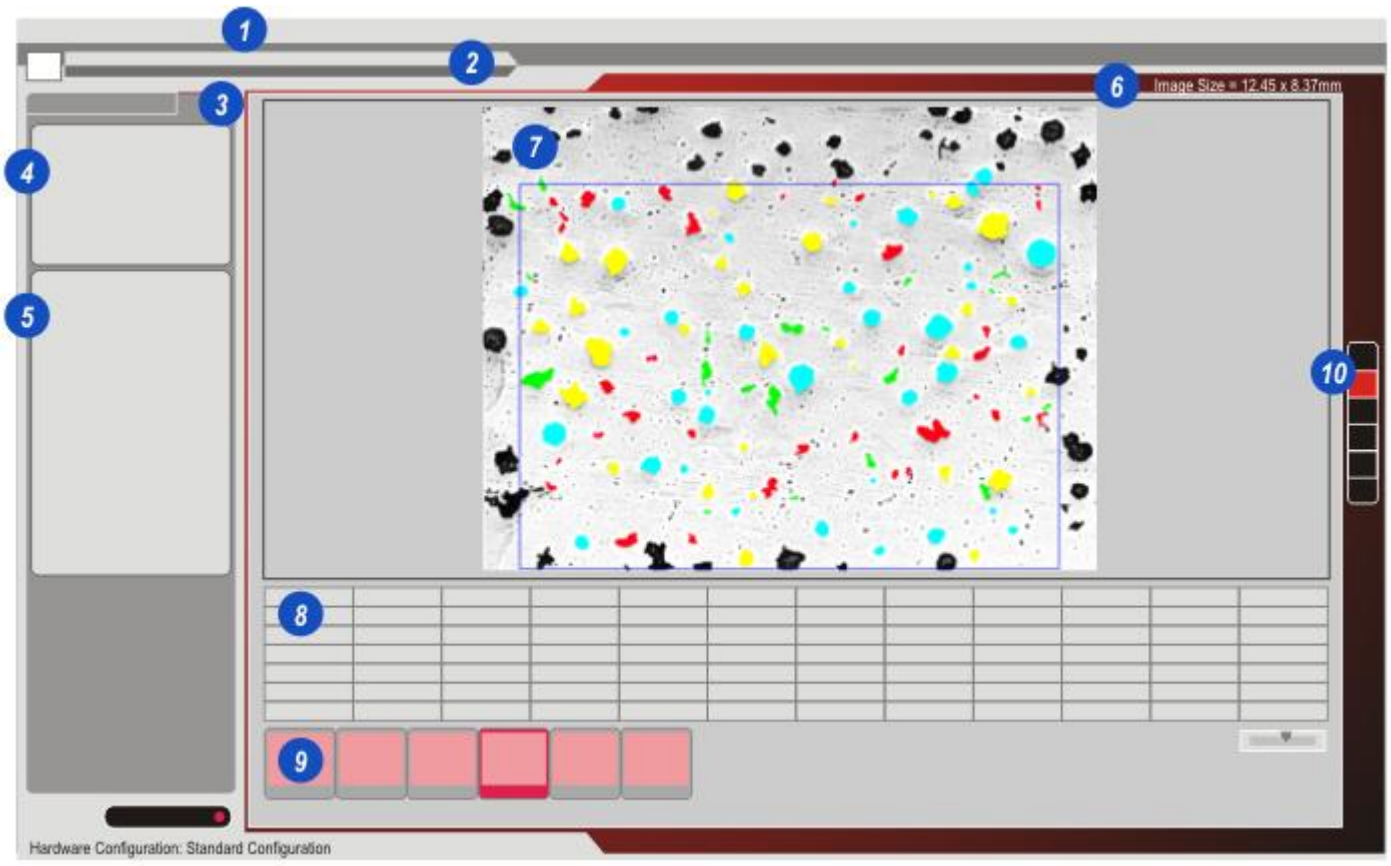
6: Image Scaling information.

7: Image Viewer: Shows original image, sometimes with the superimposed Layer Binary Overlay.

8: *Results Grid*: Not used by this application.

9: Gallery of Image Thumbnails: Used to select the images to measure.

10: Side Tool Bar.



Getting started – new users

- Check out all the links in the Help navigator to the left – plenty of help is available!
- To view the *Leica Cast Iron Expert* help, press F1 while the application is running.

Getting started – new users of LAS

- *Leica Cast Iron Expert* uses LAS to acquire images and is started from the *Analysis* > *Runner* step of LAS
- Please refer to the main LAS help for information concerning the configuration, calibration and use of LAS to acquire images.
- To view the LAS help, press F1 while LAS is running.

Supported Microscopes, Cameras, Computers and Software

Please see the *Systems Requirements* PDF file:

Start > Leica Application Suite V4 > Documents

This provides details of all supported hardware and recommends suitable computer specifications. In case of doubt, please run the *LAS PC Performance* utility and observe the recommendations it produces.

Prerequisites

LAS Image Analysis must be purchased and licensed on the same system as *Leica Cast Iron Expert*.

LAS Runner must be licensed on the same system as *Leica Cast Iron Expert*. This is included with the purchase of a *Leica Cast Iron* License.

Microsoft Excel is required if you wish to customise the report template.

Image Format

Colour and Monochrome images are supported. It is recommended that Monochrome images are used.

It is recommended that the image pixel size is in the range 1 to 3.3 Mpixel. Images with pixel sizes greater than 5 Mpixel are not supported.

Display resolution

The minimum screen resolution is 1280 x 1024. If you use a lower vertical resolution some of the control panels may be obscured. You will be able to move them to a position where they are visible, but this is rather inconvenient. Please minimise the height of the Windows task bar as this occupies some of the vertical screen space.

Image Acquisition

Please ensure that the images are acquired with the correct calibration. This will be more certain if you are using a microscope with a coded nose-piece. If not, then make sure that the objective in use is the same as that selected in LAS.

Adjust the camera exposure, gain and gamma to provide a high quality image. In particular, ensure that an HQ format is used, the value of gain = 1 and the histogram black and white levels are set to the default values.

As the measurements use thresholding, it is strongly recommended that the shading correction is set for all images acquired. This will ensure that the thresholding is performed uniformly over the entire image.

Calibration Units

Please ensure that all images that you measure at the same time have the same calibration for consistent results.

Make sure the Navigator panel is floating

You need to ensure that the *LAS Navigator* is floating before starting an *Expert* application. This allows you to select test images and real sample images that you have acquired, for use within the application.

1: In LAS, select the *Browse Workflow*.

2: If the *Navigator* panel is docked with the other Control Panels on the left, click the *Show Navigator* button on the *Side Tool Bar* to make it float.

3: You are now ready to start[1256] your *Expert* application.

Start Leica Cast Iron Expert

1: Select the *Analysis Workflow*.

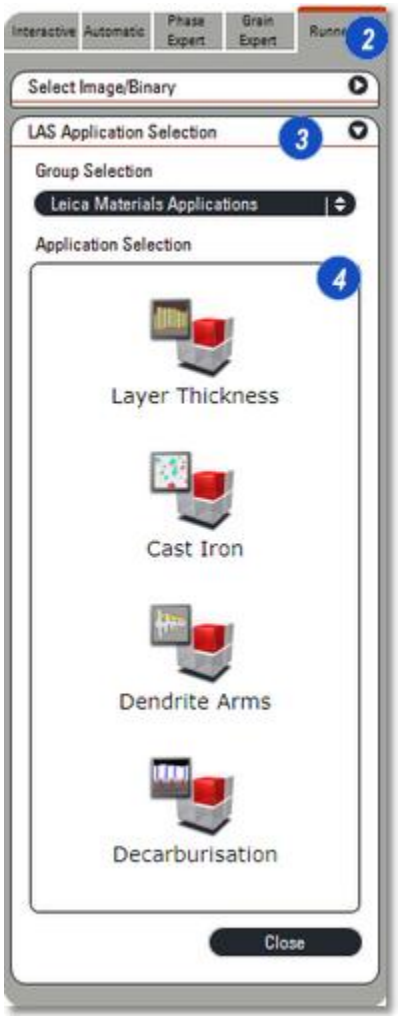
2: Display the *Runner* tab.

3: If necessary, click to expand the *LAS Application Selection* panel.

4: Click on an icon to select your chosen Expert Application.

5: Click *Run*.

6: If the *LAS Application Selection* panel is not visible click the *Application Selection* button.



When the application start-up screen appears,
do one of the following to start the application:

- Click *OK*
- Click the logo.

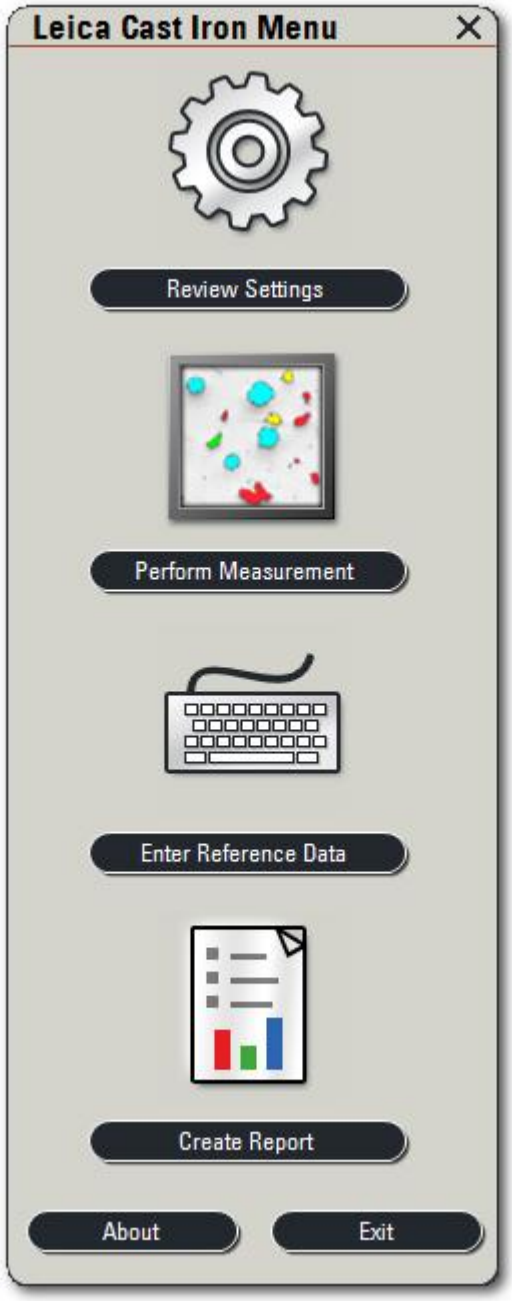
The *Leica Cast Iron Menu* provides links to the steps you should take to analyse a cast iron specimen.

Before using this menu, please ensure that the images have been acquired using LAS[1260] and stored in a known folder.

Note - it is preferable to acquire **monochrome** images with a resolution approximately in the range to 1 to 3 Mpixels. . Leica Cast Iron will convert colour images to monochrome before analysis.

- Review Settings[1261]*:* Use default parameters or create user-defined settings. Determine the detection limits and then use the supplied or user images to test the settings.
- Perform Measurements[1274]*:* Uses the parameters established in *Review Settings* to detect and analyse the specimen.
- Enter Reference Data[1286]*:* Add comments and specimen details that will be included in the final report.
- Create Report[1288]: The results and reference data are presented using a supplied Excel template that can be modified to suit user or company styles.

Click the *Review Settings* button.



The first stage of Cast Iron analysis is to acquire a selection of digital images using LAS and to save these to the computer's hard drive. The advantage of this approach is that you always have the original images available to check your results later.

To obtain precise results from *Leica Cast Iron Expert* you need a good, representative sample of the specimen that has been processed to display optimum contrast.

- LAS acquires calibrated images by reading the magnification from the microscope and the sensor size from the camera to accurately determine the image dimensions.
- Imaging conditions (such as microscope settings and camera exposure) are automatically recorded by the software. This data is stored with the image and is useful for checking consistency.
- Images are named and acquired into a Windows folder. It's a good idea to make a note of these details.
- You can annotate images with a calibrated scale bar and labels (such as date, time, image name and description).

Please refer to the Advice and Prerequisites 1255 for the recommended image capture settings.

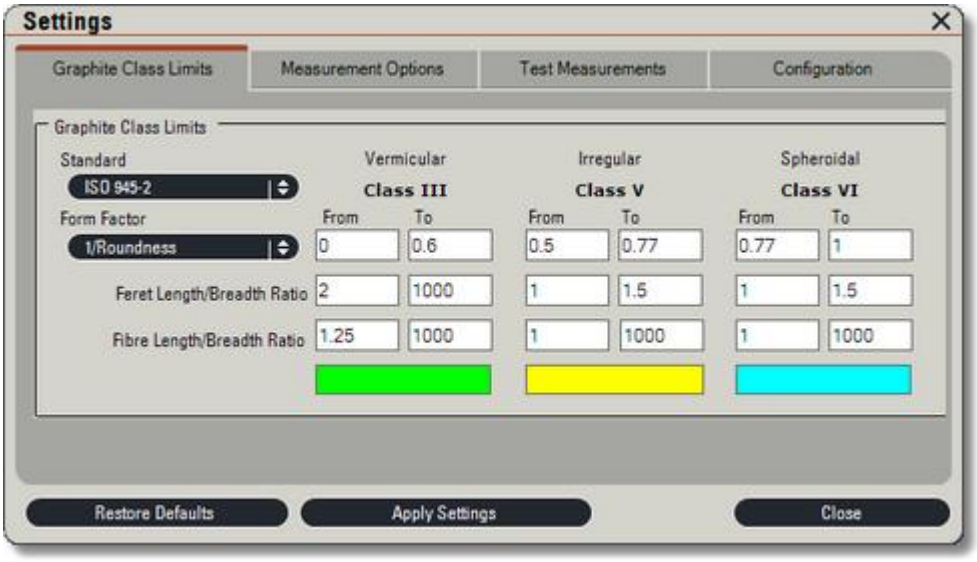
1: Click the *Review Settings* button on the *Leica Cast Iron Menu*.

The *Settings* dialog has the following tabs:

- *Graphite Class Limits*[1262]*:* You can use default values for nodule classes or set your own.
- *Measurement Options*[1263]*:* Sets the type of image processing to be applied, overlay colours, and graphite size limits.
- *Test Measurements*[1265]*:* Applies your settings to a test image so you can observe the effects of the Class Limits before processing a real image.

Notes

- If you want to revert to the default settings for the application, click *Restore Defaults*. You can do this at any time before clicking *Close*.
- If you close the *Settings* dialog and save your changes, this will **overwrite** your current configuration file, giving you a new set of defaults.



Graphite Class Limits Settings

The *Graphite Class Limits* tab allows you to adjust the shape parameters for graphite nodules (for example to meet local requirements). Normally you will not need to change these settings.

The Standard selected does not change any of the measurement methods or limits. For the types of material covered by *Leica Cast Iron Expert*, the classes are equivalent but with the class numbers changed.

Standard	Class number equivalents		
ASTM E247	IV	II	I
ISO 945-2	III	V	VI

1: Display the *Graphite Class Limits* tab.

2: Select the *Standard* to use from the drop-down menu.

3: Click the *Apply Settings* button.

4: Select the *Form Factor* (roundness algorithm) from the drop-down menu.

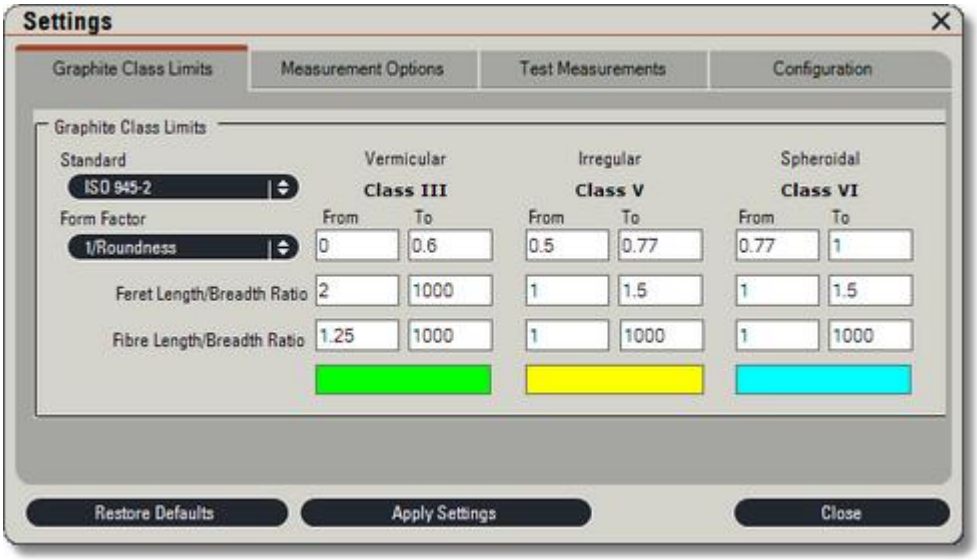
5: Click the *Apply Settings* button.

6: If required, type in new lower and upper limits for Form Factor, Feret Length/Breadth and Fibre Length/Breadth.

7: Click the *Apply Settings* button.

8: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).

You can revert to the *Default* settings at any time.



The *Measurement Options* tab allows you to adjust the processing and size parameters for graphite.

1: Display the *Measurement Options* tab.

2: If necessary, change any settings in the *Apply Processing to Images* 1264 panel.

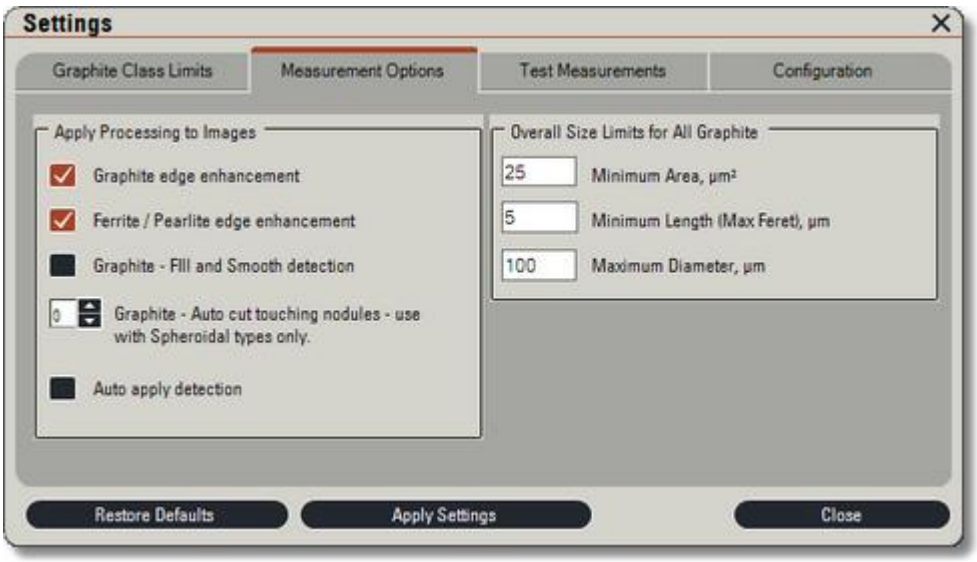
3: If necessary, change any settings in the *Overall Size Limits for Graphite* panel.

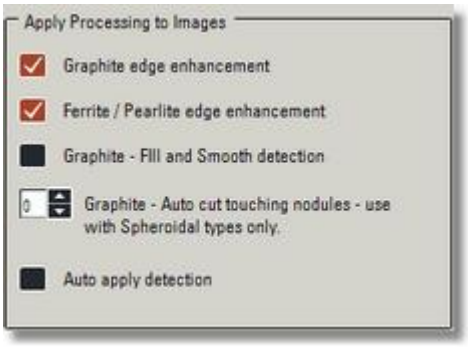
4: After each change click the *Apply Settings* button to update the values.

5: At any time, you can revert to the default settings by clicking the *Default* button and then the *Apply Settings* button.

Adjust the overlay colour to give good contrast with the image you are using.

Note that the *Maximum Diameter* setting determines the size of the 'guard' region. If you increase this value, larger nodules will be measured. If you make the value smaller, large nodules touching the edge will not be measured.



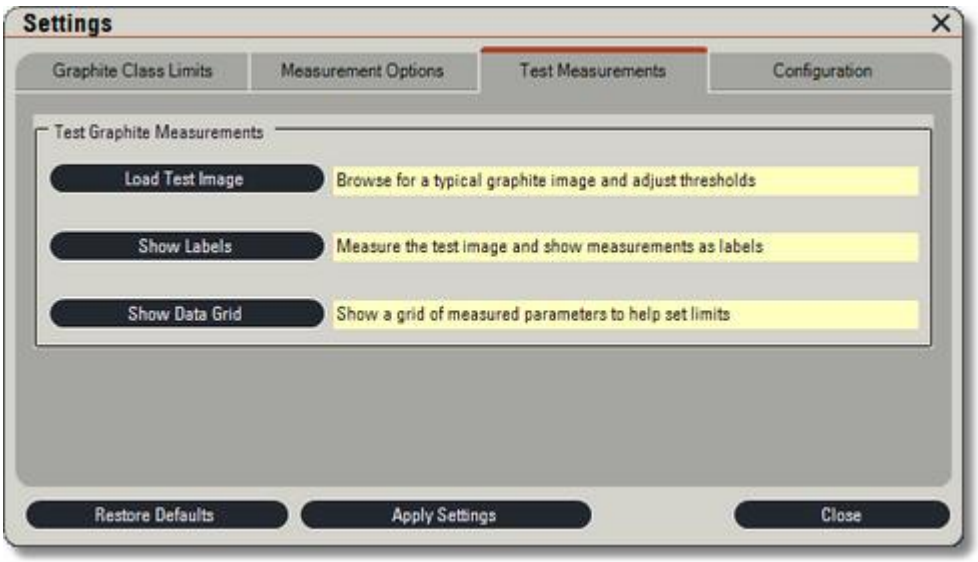


- *Graphite and Ferrite/Pearlite edge enhancement:* Both tools perform in the same way. When enabled the edges of the graphite or ferrite are sharpened, reducing the variation in the detection. Note that the image that may appear to have less detail as a result.
- *Graphite - Fill and Smooth detection*: When enabled automatically fills and detects 'holes' in the detected graphite, including them in the nodule area.
- *Graphite - Auto cut*: Specify the size at which touching features are separated and detected individually. **Only** use this setting with nodular graphite types, as it will cause multiple incorrect cuts to elongated graphite.
- *Auto apply detection*:

Once you have set the *Class Limits* and *Measurement Options*, you can use the *Test Graphite Measurements* tab to check that your detection threshold settings are at sensible values before performing measurements on your real samples.

This feature uses initial settings that are optimised for Graphite detection, rather than Ferrite/Pearlite (you can change this if you want).

- *Load Test Image* 1266: Browse for a suitable graphite image, and use it to optimise your detection threshold range.
- *Show Labels* 1270: Measures, collects and displays the data for any detected nodules on the test image, with the settings that will be used on your real samples. You can check the results by applying one of several labelling options.
- *Show Data Grid* 1271: Displays the measurement data as a grid.



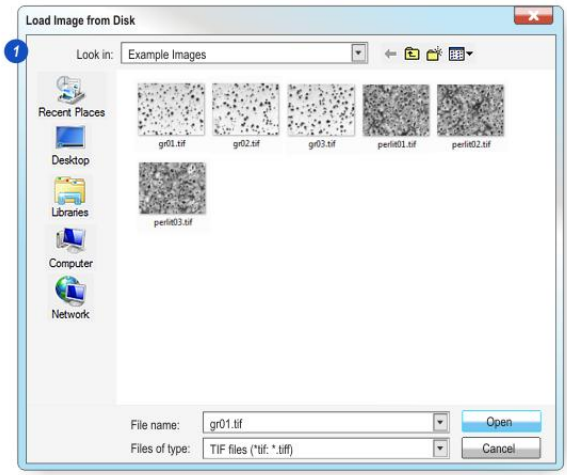
1: Click the *Load Test Image* button on the *Test Image Measurements* tab to display the *Load Image from Disk* dialog.

2: Navigate to a folder containing a suitable test image.

The test image can be one you have captured, or one of the samples installed with the application. The installed test images are in the following folder:

Users > Public Documents > Leica Application Suite > LAS Apps > Apps > LAS Cast App > Example Images

3: Select the image and click *Open*.

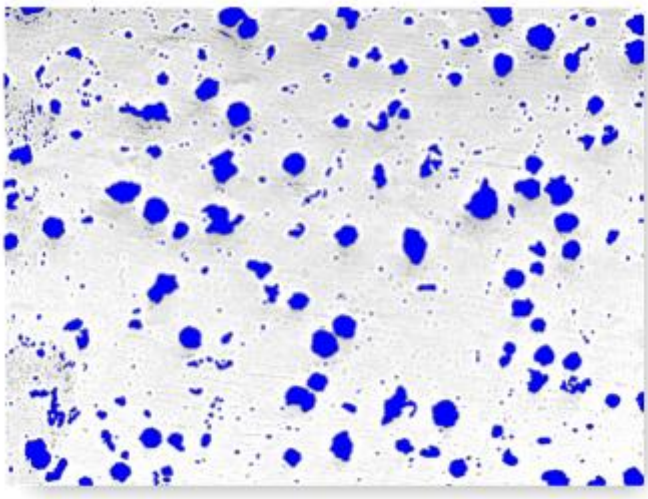


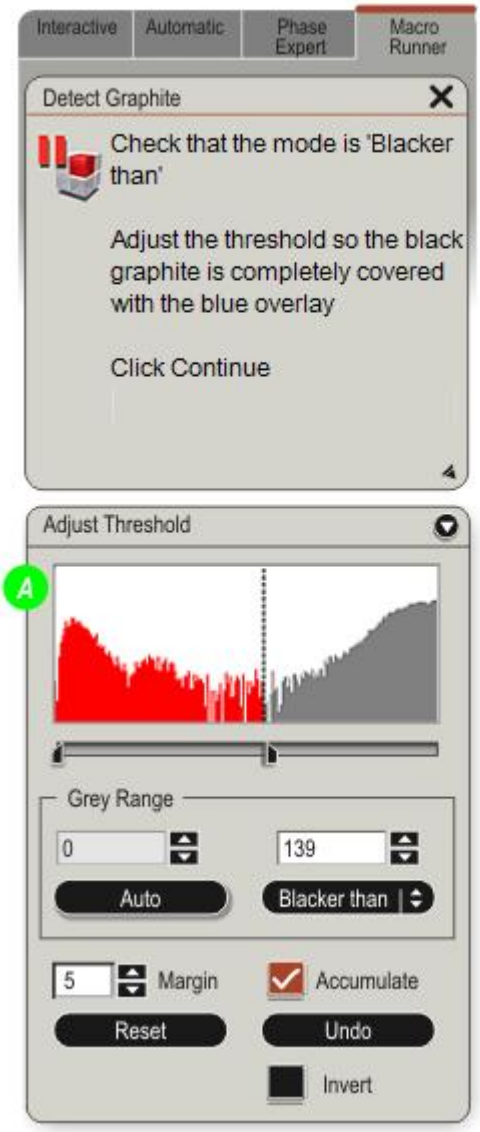
As the image opens in the LAS *Viewer*, the *Adjust Threshold* panel appears. This allows you to select a range of values to represent the required nodule (between Black = 0 and White = 255).

Graphite, for example is predominantly black so the range will be typically 0 to 140. Ferrite and Pearlite are mainly pale grey and white so the range will be closer to 200 to 255.

The threshold range is displayed in red on the *Histogram* and can be changed using the *Histogram* sliders or by using the numeric up/down arrows.

On the image, areas that fall within the threshold values are shown by a blue overlay.





Using the *Adjust Threshold* dialog you can alter the threshold value range to detect the nodules precisely.

To start the detection process:

1: If necessary, clicking on the small arrows to the right of the *Mode* header and from the drop-down menu, clicking:

- *Blacker than* for Graphite or .
- *Whiter than* for Ferrite/Pearlite.

2: Click on the *Reset* button to clear any existing detection values.

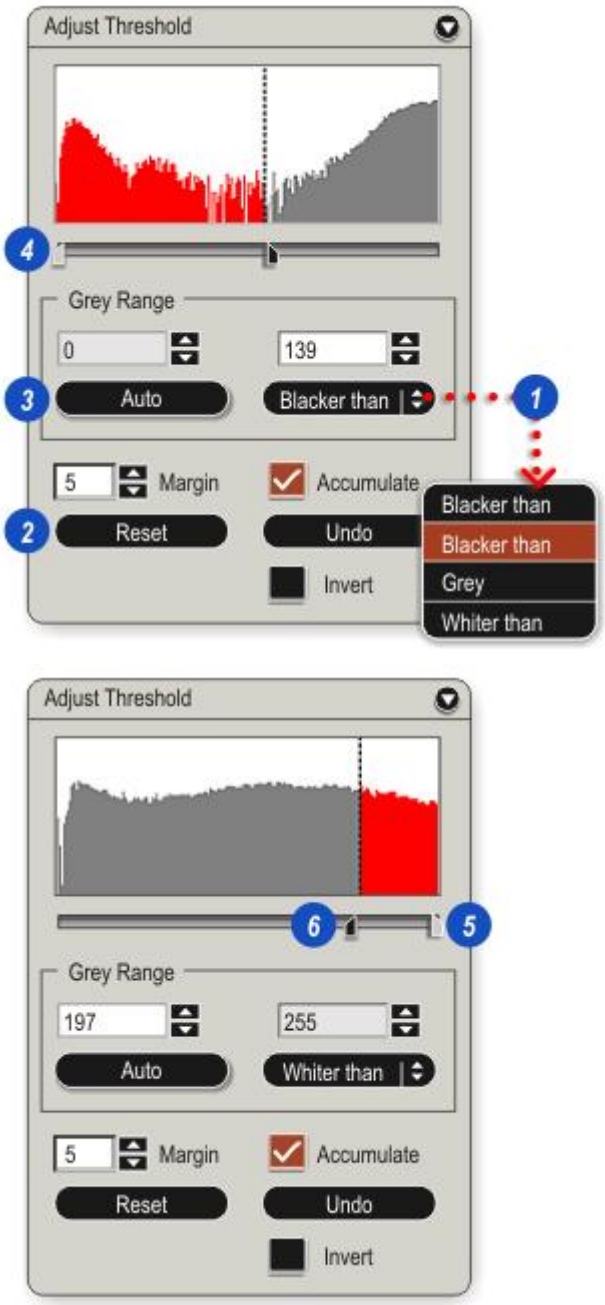
3: Click the *Auto* button. This will automatically select nodules that contain threshold values appropriate to the selected nodule type - *Graphite* or *Ferrite/Pearlite*

Often these three simple steps are sufficient to detect the nodules correctly. However, if you need to change the threshold range:

4: In *Blacker than* mode (Graphite detection) the low value (Black) is fixed at 0 with the slider and low value text box disabled.

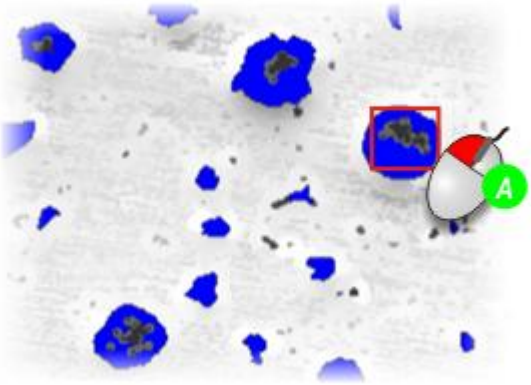
5: In *Whiter than* mode (Ferrite/Pearlite detection) the high value (White) is set at 255 and slider and text box are disabled.

6: Change the threshold and detection if required (drag the slider or change the value in the *Grey Range* field).



1: Click the *Undo* button to undo the last step or action.

2: Precise threshold values can be added to the detection range by clicking to enable the *Accumulate* check box and then dragging a marquee around the region on the image to be included. The range within the marquee will be added to the threshold and detection range.

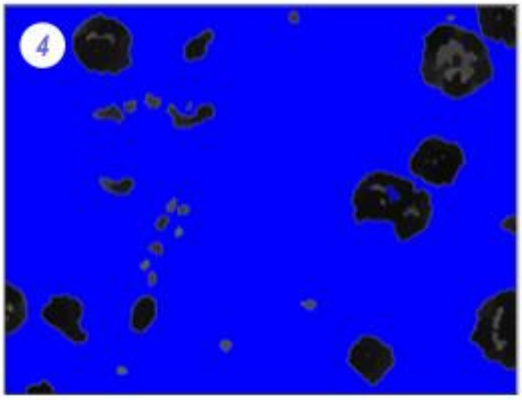


If the *Accumulate* check box is disabled only the values enclosed by the marquee will be detected and all others ignored.

3: Use the *Margin* up/down arrows to adjust the spread of selected threshold values.

For example, if *Margin* is set to *5* and a value of *12* selected from the image by drawing a marquee, then the *Margin* is added/subtracted to the selected value resulting in a range of *7* to *17 (i.e. 12 ± 5)*

4: Enable the *Invert* check box to highlight all features *except* those detected: the blue overlay is inverted. This can help you see features that have not been detected but which may be of interest.



Click to disable *Invert* and revert to the detected nodules overlay.

Click *Continue* to return to *Test Graphite Measurements*.

Clicking the *Show Labels* button on the Test Graphite Measurements[1265] tab starts the measurement process and displays the *Select Label* panel. Use this to help you tune the parameter limits.

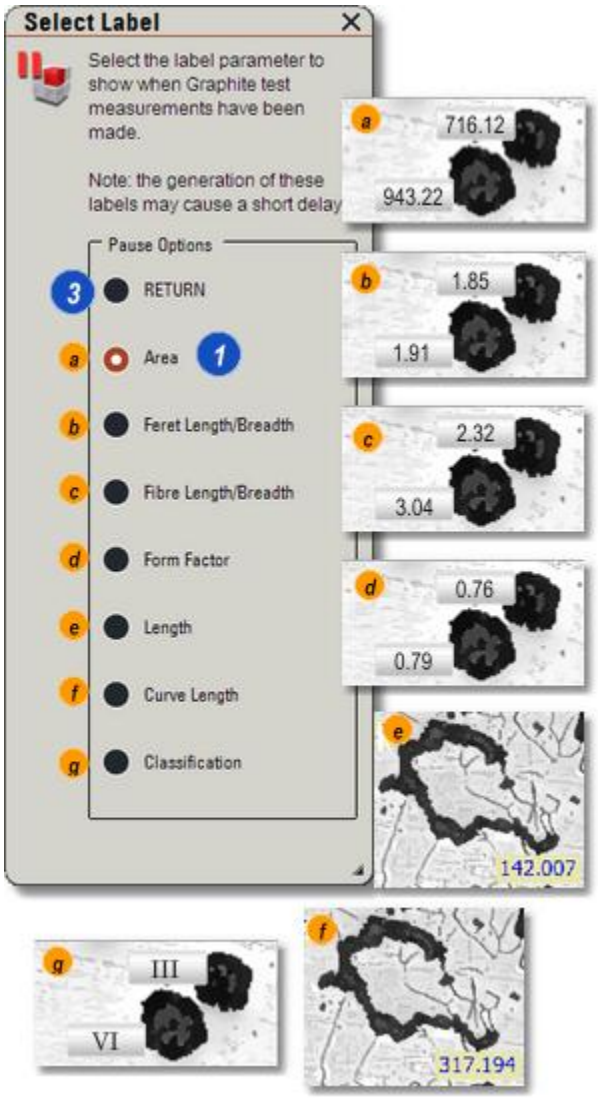
Labels will be shown clearly when the image is displayed at 1:1.

You can display one of the following parameters against each nodule:

- *Area*
- *Feret Length/Breadth* - as a ratio
- *Fibre Length/Breadth* - as a ratio
- *Form Factor* - a value representing the roundness
- *Length* - equivalent to maximum diameter (typical values: 100 - 400)
- *Curve Length* - approximates to the stretched length of long and thin shapes (typical values: 100 - 700)
- *Classification* - the Class number as a Roman numeral.

  The parameter selected here will be used on images in the final report.

  Note: For further definitions see the Appendix[1114] to the Image Analysis module.



To display a parameter label:

1: Click on a radio button.

2: Click *Continue* and the selected parameter label appears.

3: To exit the *Show Label* dialog, select the *RETURN* radio button and then click *Continue* (2).

Show Data Grid

Clicking the *Show Data Grid* button on the Test Graphite Measurements[1265] tab displays the measurement results as a grid.

1: Click on a tab to reveal results as *Details* and *Statistics* for Features, Field and Profile.

2: If required, change the feature details[1272] that are displayed.

3: Click on a row to highlight that feature's results and select it on the image.

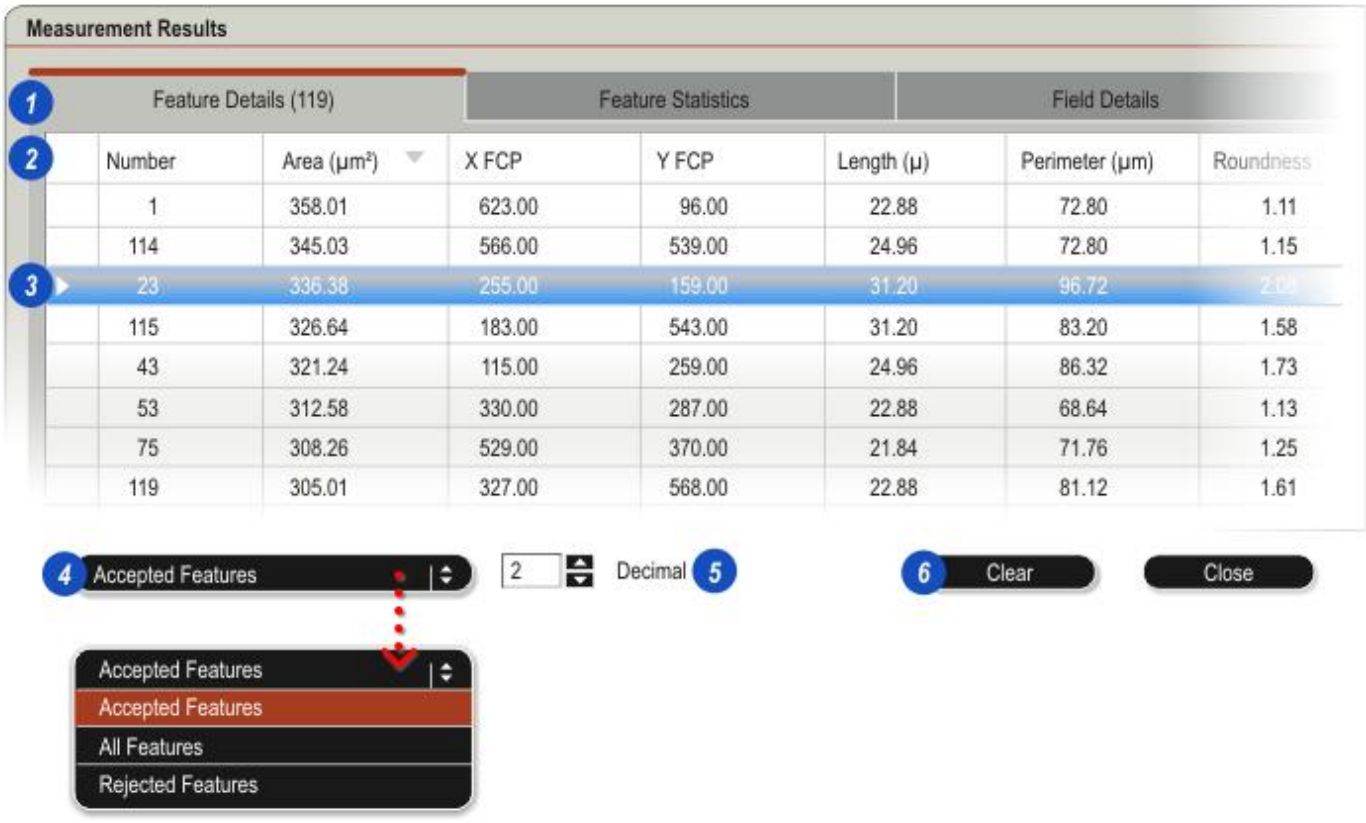
4: Use the drop-down menu at the bottom of the *Measurement Results* window to select which features are displayed. Choose from:

- *Accepted Features*
- *All Features*
- *Rejected Features*

5: Specify the number of places after the decimal point using the *Decimal* text box.

6: Click the *Clear* button to delete all results ready for a new measurement.

7: Click the *Close* button to close the *Measurement Results* window.



Change the Feature Details

You can change the features displayed in the Measurement Results grid. The feature detail options depend upon which tab is currently selected - *Details* or *Statistics*.

1: Click on the *Spanner* icon to the right of the grid.

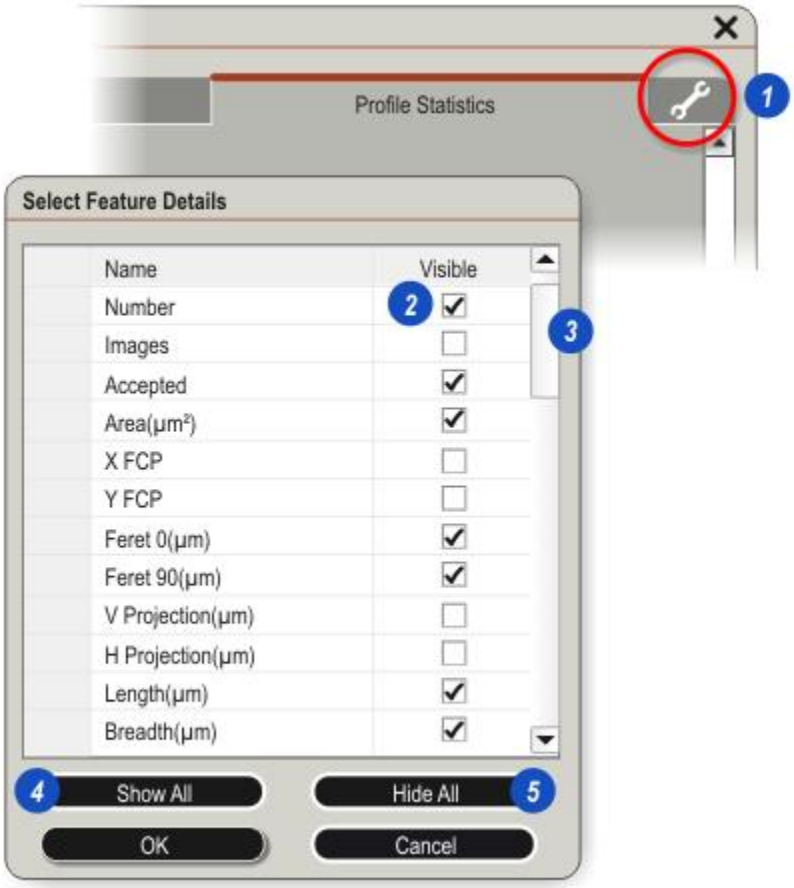
2: Enable or disable the *Visible* check box to display a parameter.

3: Reveal more options by dragging the vertical slider.

4: Click the *Show All* button to select all options.

5: Click the *Hide All* button to de-select all options.

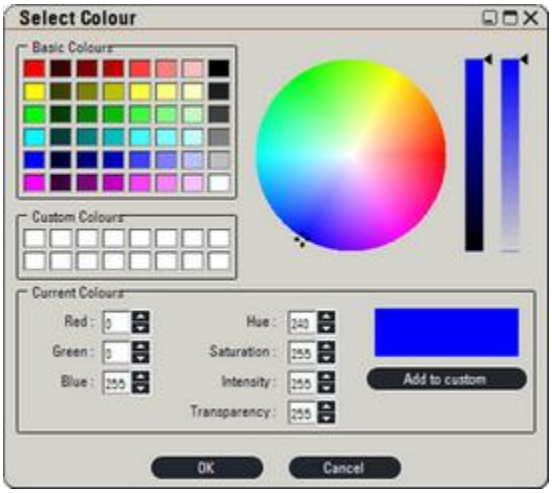
6: Click *OK.*



The *Configuration* tab allows you to choose the Overlay Colours and Load and Save a Configuration.

1: Display the *Configuration* tab.

2: To change the colours of lines and overlays (for example to give a better contrast against the image you are working with) click on the appropriate colour swatch. Pick a new colour using the *Select Colour* dialog:

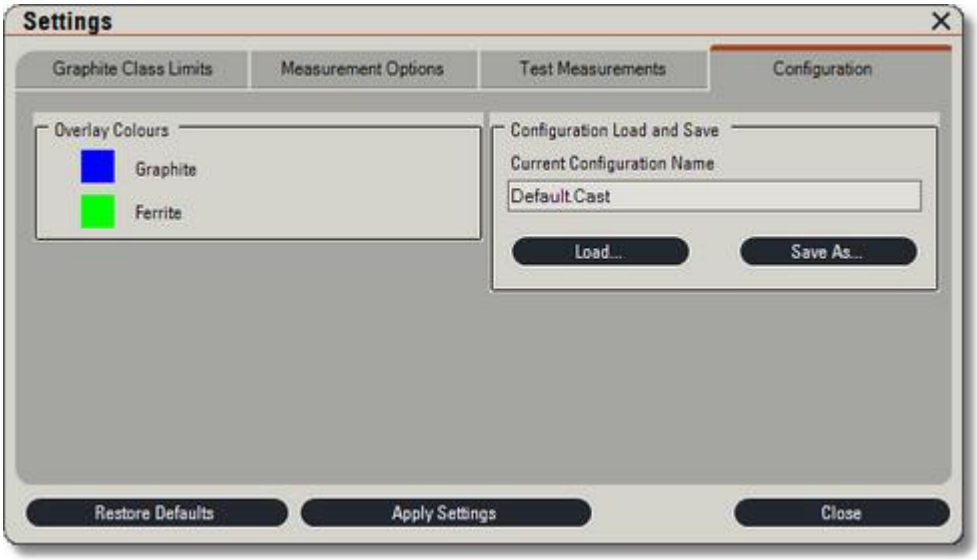


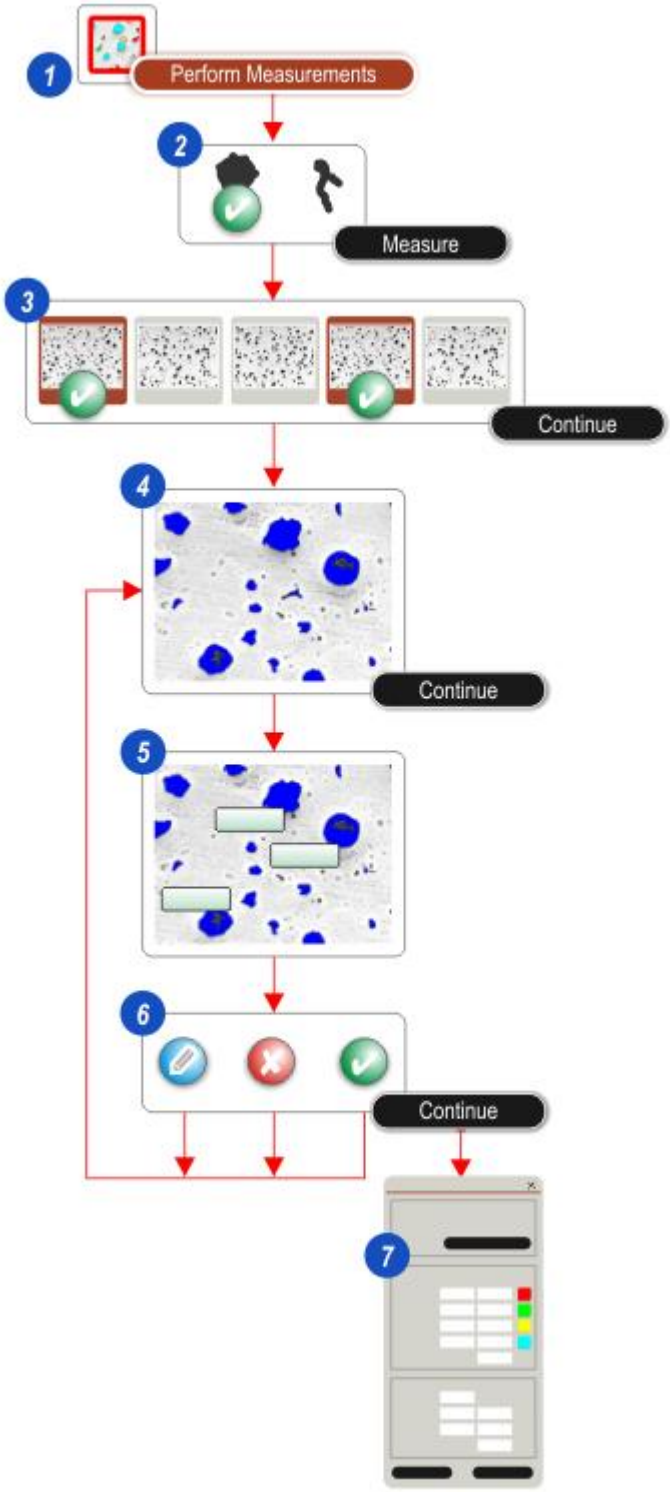
3: At this point you can click *Save As* to save all your changed settings to a named configuration file. These files are located in the following folder (and have the extension .Layer):

C:\Users\Public\Documents\Leica Application Suite \LAS Apps\Apps\LAS Layer App\Config Files

You can also *Load* existing configuration files that you have tuned to different types of specimen.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made; this will **overwrite** your current configuration file, in effect giving you a new set of defaults).





The flow chart shows the sequence of events for selecting, detecting, measuring and displaying the image results in *Perform Measurements.*

1: On the *Leica Cast Iron Menu* click the *Perform Measurements* button.

2: Select either *Graphite* or *Ferrite/Pearlite* to measure. Usually, the first detection and measurement pass is for *Graphite* which is then followed by a *Ferrite/Pearlite* pass on images from the same processed specimen.

3: The images available in the source folder are displayed in the Gallery. You can select up to 10 images to measure.

4: Apply feature detection to the image.

5: Select *Display* options.

6: Select what to do with the *Results*:

- ***Edit*** the detection and remove artifacts.
- ***Ignore*** the image and results. Get the next image.
- ***Accept*** the results and add them to any existing results. Get the next image.

7: Display the accumulated results.

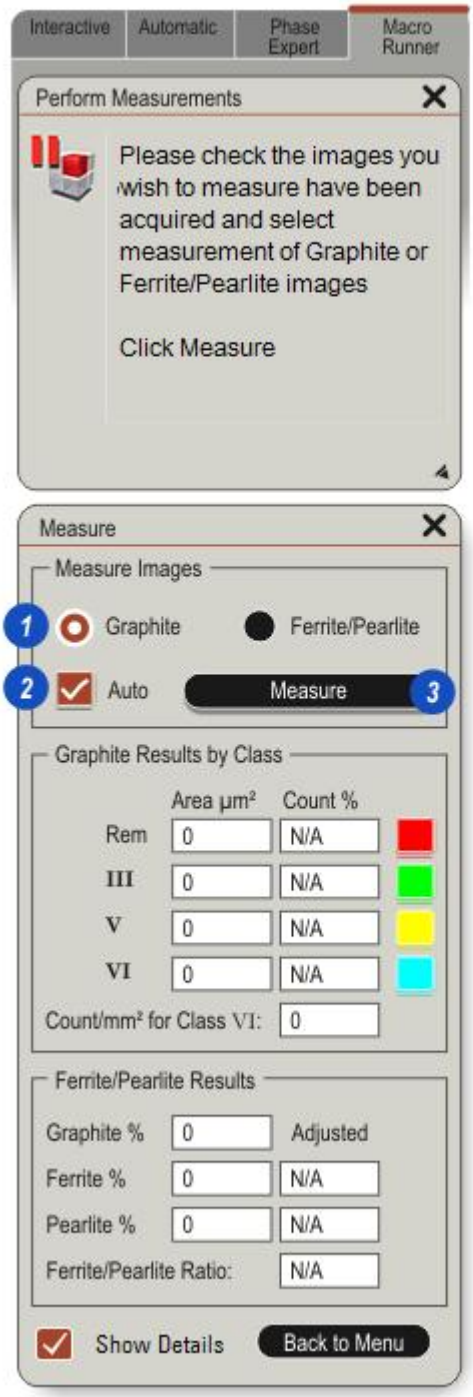
On the *Leica Cast Iron Menu* click the *Perform Measurements* button. The *Measure* panel appears.

1: Select to measure either *Graphite* or *Ferrite/Pearlite*.

2: If required, click to enable the *Auto* check box to process all the selected images without any user interaction. Only use this if the images are very consistent in their preparation and imaging.

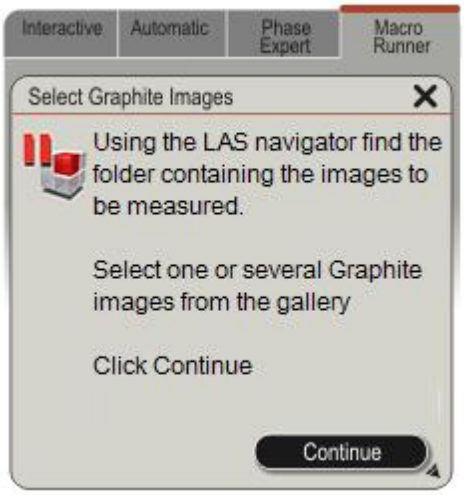
3: Click the *Measure* button.

Continued Select Images to Measure 1276



Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



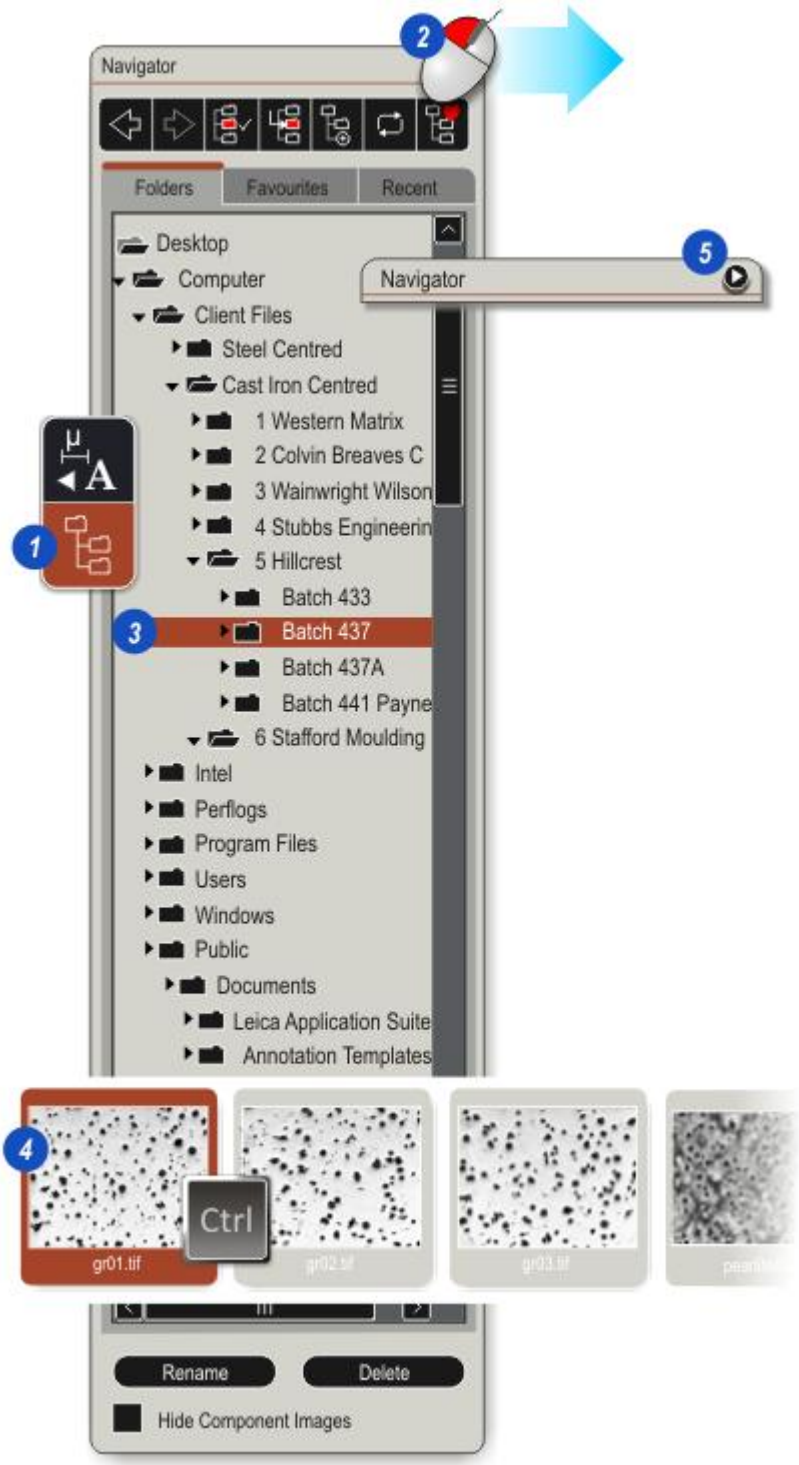
1: Click on the *Show Navigator* button on the *Side Tool Bar.*

2: Drag the *Navigator* to a convenient position.

3: Navigate to the image folder and double-click it. The available images are displayed in the *Gallery*.

4: Click to select an image, or Ctrl-click to select up to 10 images.

5: Minimise the *Navigator* by clicking the arrow to the right of the header.



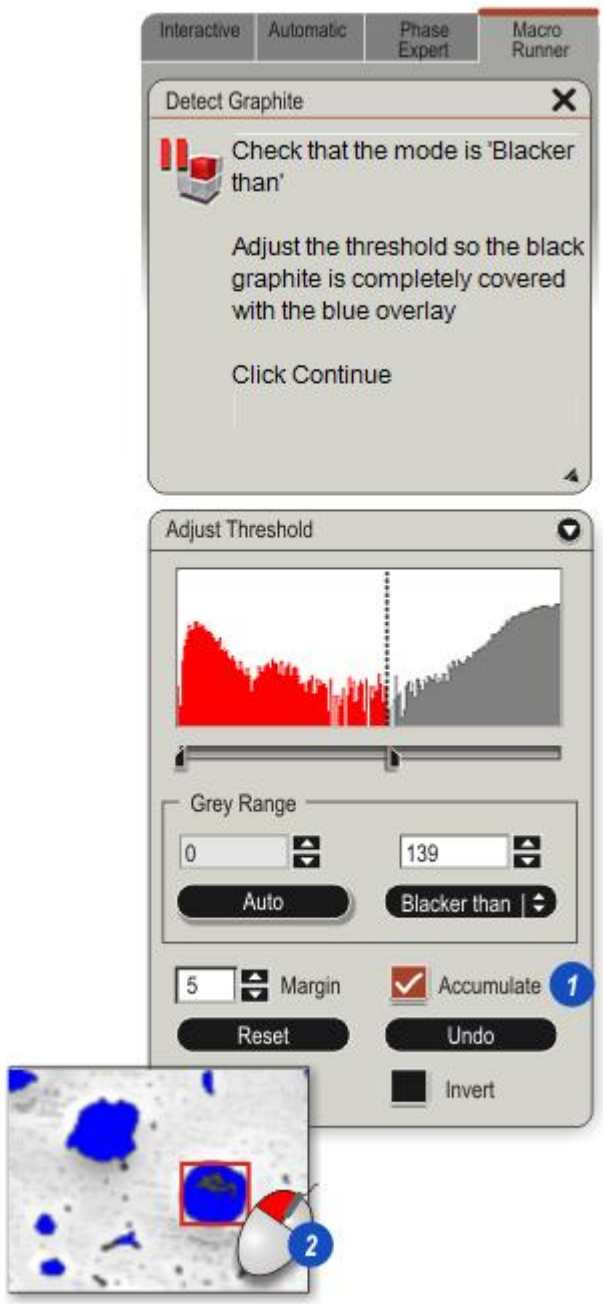
The *Graphite* or *Ferrite/Pearlite* nodules are detected using the parameters currently specified in the *Review Settings* [1261].

Due to the range of features that can be encountered in images, some regions that should be included in the measurements may not be detected. You can include those regions as follows:

1: If necessary, click to enable the *Accumulate* check box.

2: Locate an area to include and drag a marquee around it.

Image values contained within the marquee are added (accumulated) to the existing range and all regions with those values are detected.



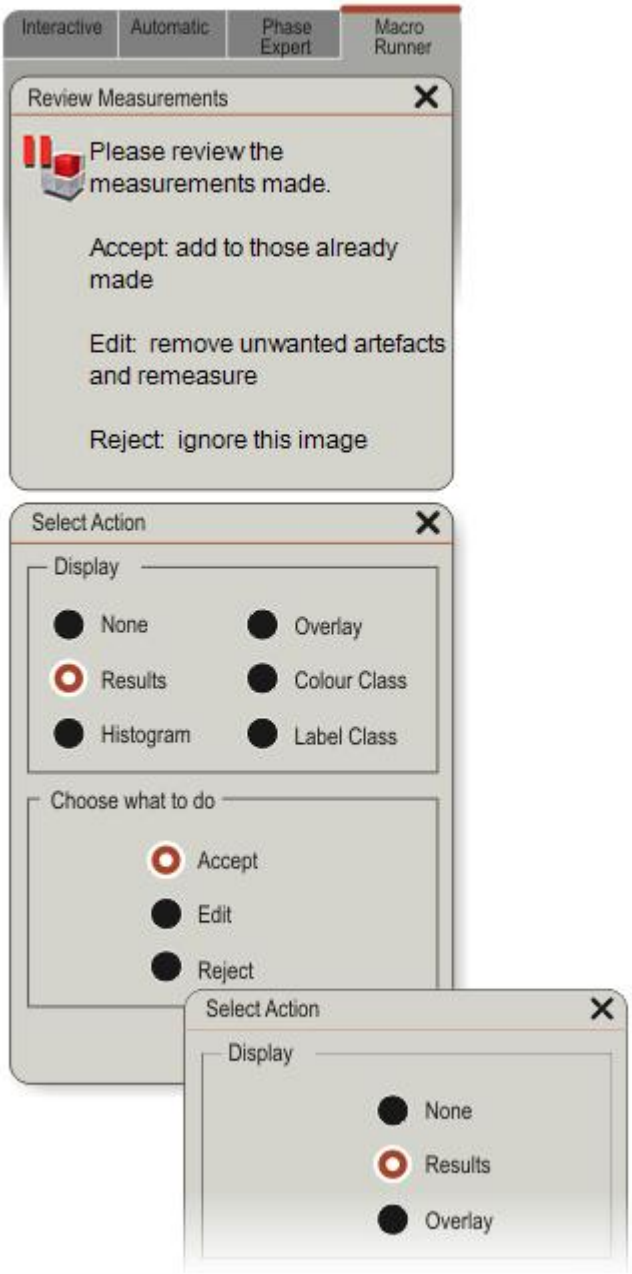
Once the measurements have been made:

1: Choose what to do with the measurements:

- *Accept*: Accepts the measurements and adds them to any previous measurements. In this way the results from several selected images can be accumulated and averaged.
- *Edit*[1283]: Launches the Edit tools so that you can select or deselect features or remove artifacts.
- *Reject*: Ignore the current measurements and check for any more images to process.

2: To help make these decisions, you can display and inspect the measurements; the options are described below.

3: Click *Continue*.



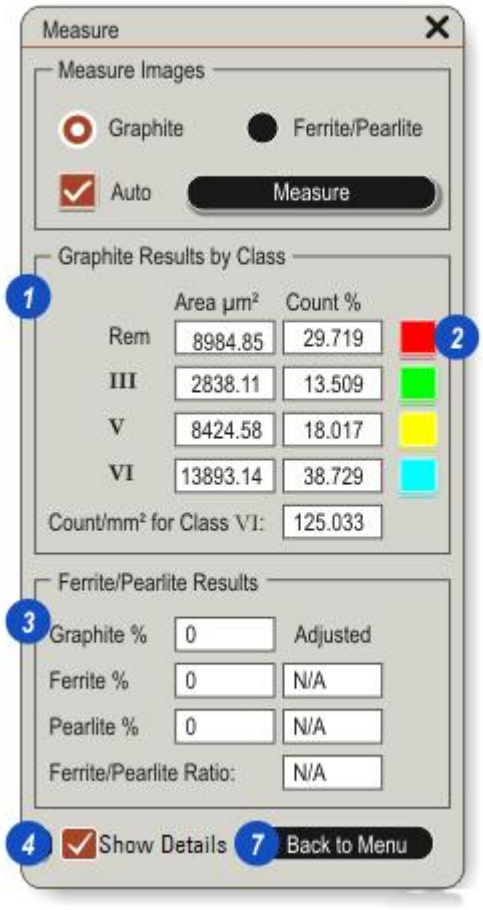
Options for Graphite

- *None*: Only the original image is displayed.
- *Results*: A list of measurement results for the current image Results for the Current Image[1280]
- *Histogram*: Show the length measurements for the current image graphically as a histogram.
- *Overlay*: Applies the detection overlay to the image.
- *Colour Class*: Displays each detected feature in the colour appropriate to its class.
- *Label Class*: Shows each detected feature labelled with the measurement parameter selected in the *Review Settings*

Limited options for Ferrite/Pearlite

- *None*: Only the original image is displayed.
- *Results*: A list of measurement results for the current image Results for the Current Image[1280]
- *Overlay*: Applies the detection overlay to the image.

When all images have been detected and measured the results are displayed on the *Measure* panel.



1: Results for the *Graphite* detection.

2: *Classes* and *Remainder are* indicated by colour swatches. These colours are used to identify features if the *Colour Class* option is chosen in *Select Actions* [1278].

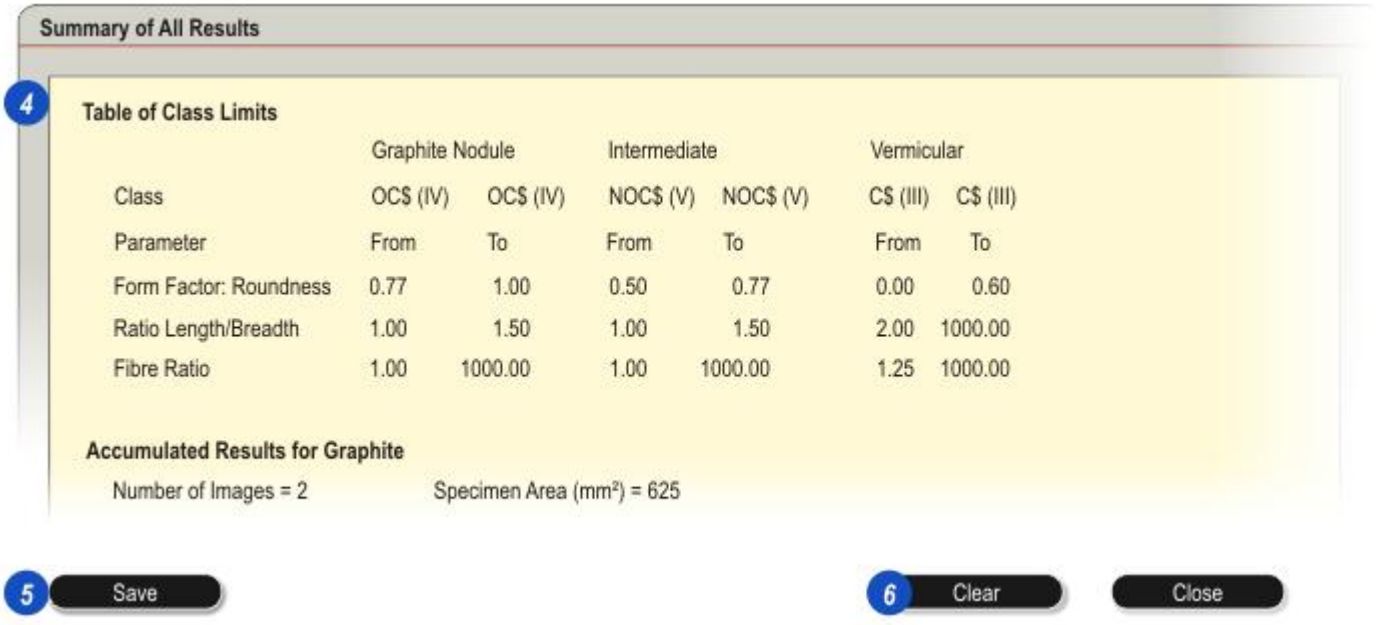
3: Results for *Ferrite/Pearlite* if these have been made.

4: Click the *Show Details* checkbox to display all current measurements and limits. The histogram that is displayed is the accumulated histogram for all images measured so far.

5: Click the *Save* button to display the *Windows Save* [1280] dialog. Navigate to the required folder and save the results in *.txt* format using an appropriate name.

6: Click the *Clear* button to clear the display.

7: Click to return to the *Application Features* menu.

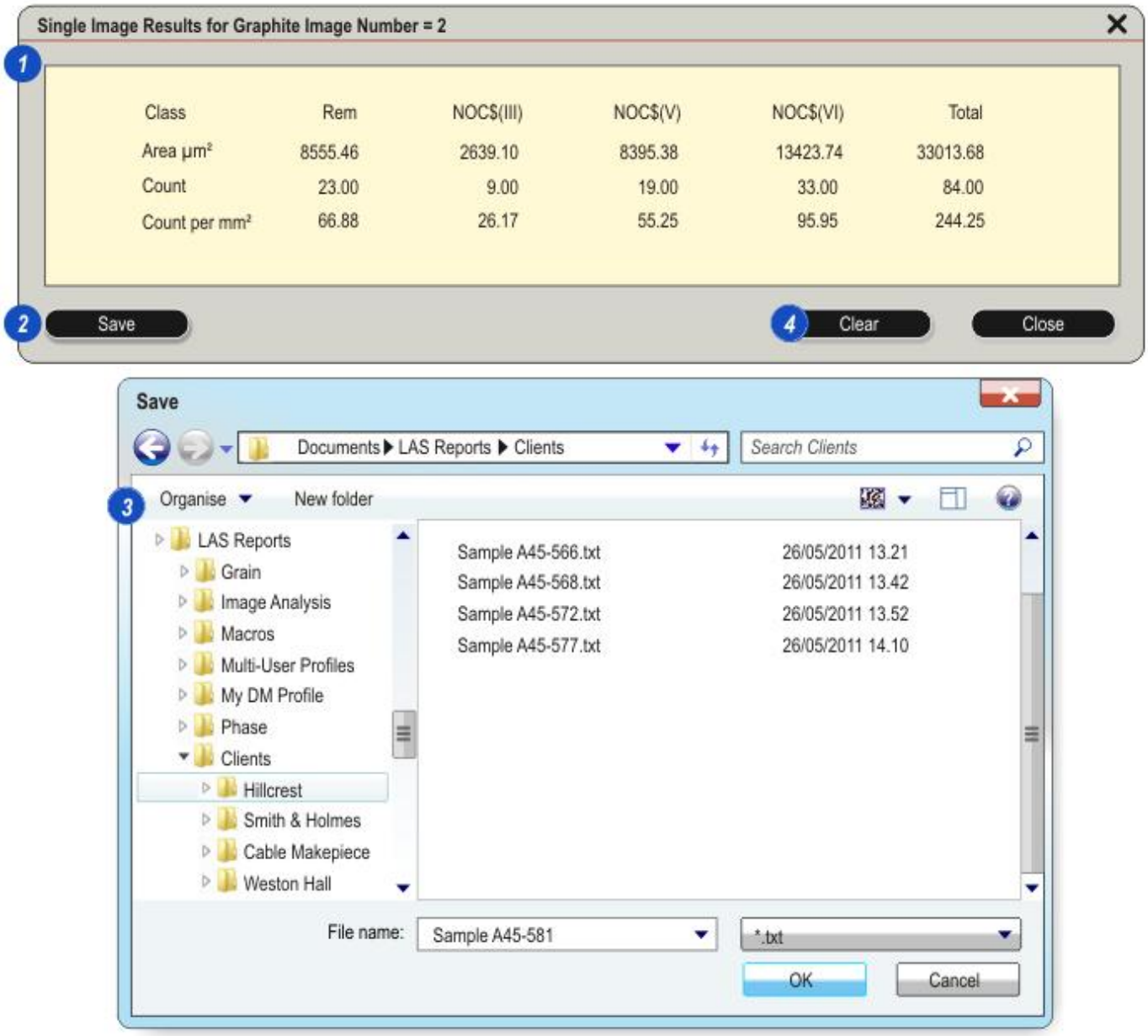


1: Selecting the *Results* option on the *Select Actions*[1278] dialog displays the results for the current image.

2: Click the *Save* button.

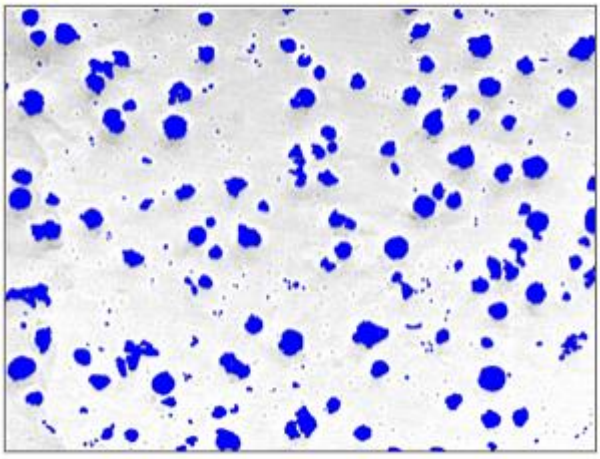
3: In the *Windows Save* dialog, navigate to the required folder and save the file in *.txt* format.

4: The *Clear* button clears the display prior to editing and re-measurement. This has no effect on the results, it is only the window that is cleared.



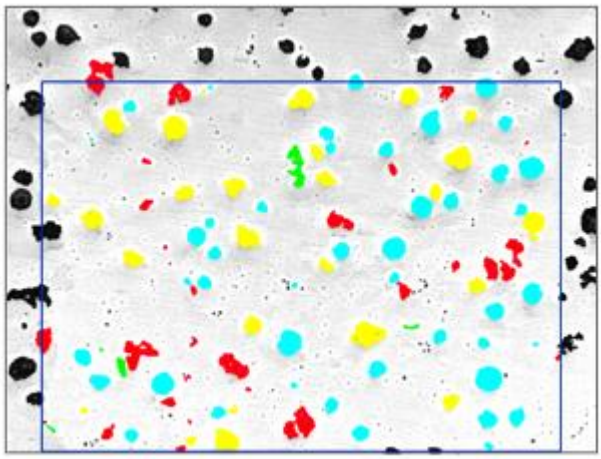
The illustrations show examples of the display options on the *Select Actions* panel:

- The *Detection* overlay shows all the detected and measured nodules.

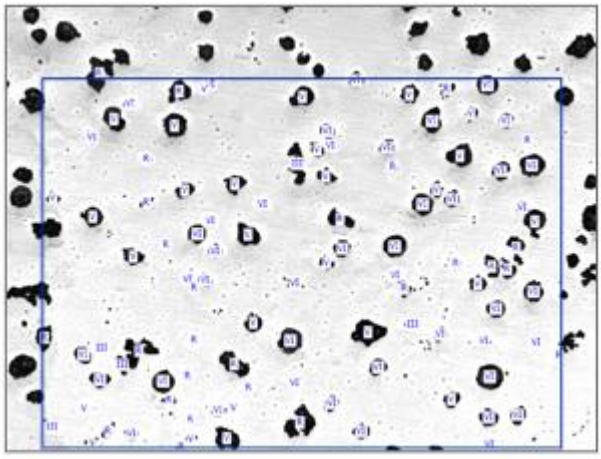


- The *Colour Class* overlay shows the detected and measured nodules coloured according to their class.

  The overlay shows the *Measure Frame*, a predefined area with a dark blue outline. Only nodules whose bottom right-hand corners fall within the frame are measured. All others are ignored.



- The *Label Class* option attaches a class label (*III*, *V* and *VI*) to each of the measured features. Those that do not fall within the selected limits are marked *Rem* (Remainder).



Note: If the labels appear to be small, this may be because the image is of high resolution. Use the Zoom and Pan controls on the *Side Tool Bar* to see these more clearly.

1: The *Histogram* displays the length distribution results graphically for Class VI (ISO).

Note - the options described here are simply for investigating the results, they are not saved.

2: Click *Show Axis Options*.

3: This reveals the *Y Axis Options*:

- *Auto:* Automatically sets the range.
- *%:* Range determined by the greatest value as a percentage of the total
- *Log:* Uses a logarithmic scale.

4: Click inside the *Title* text box and type a title for the *Histogram*.

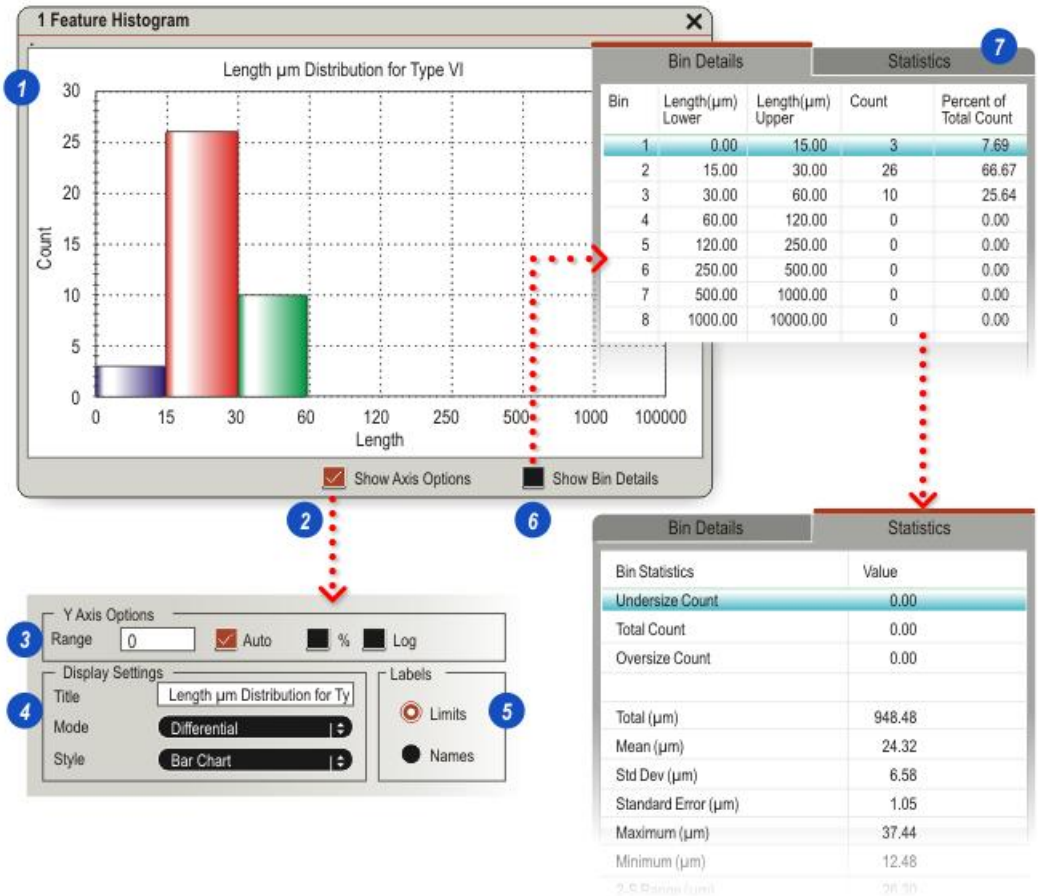
Mode and Style: Select appropriate display options from the drop-down lists.

5: *Labels* (X Axis options):

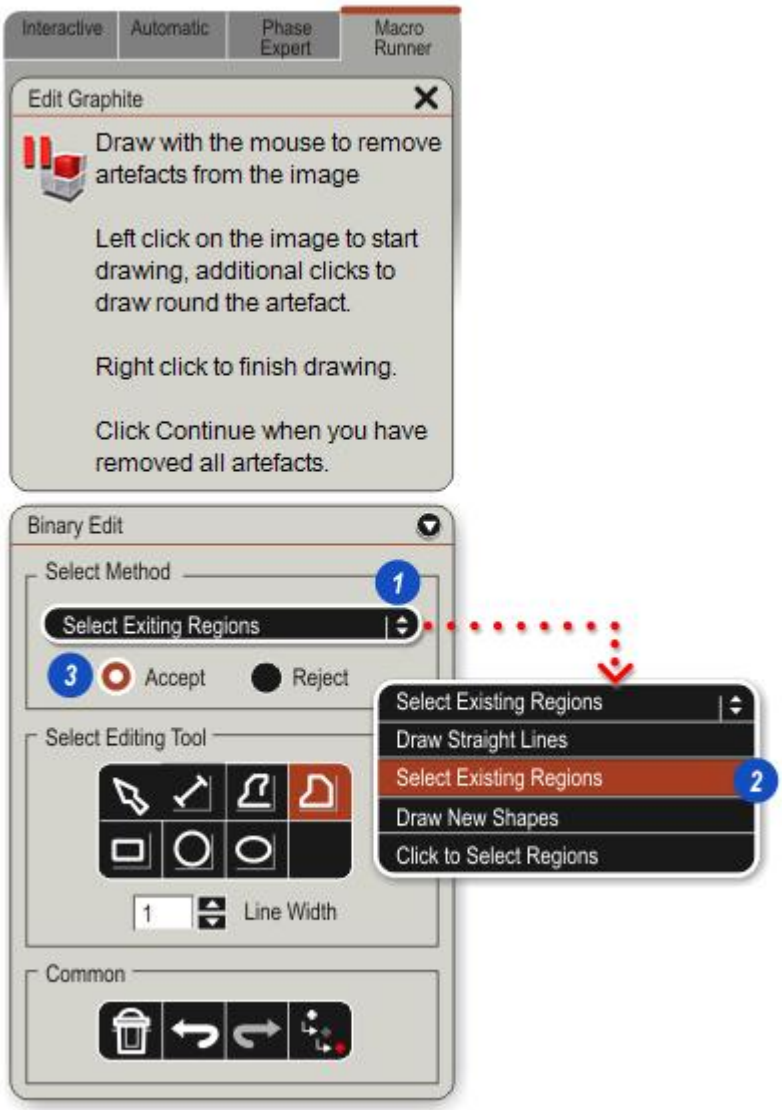
- *Limits:* Available display area divided by the total bin count.
- *Names:* Each bin sequentially numbered.

6: Click to enable *Show Bin Details* to display:

- *Bin Details:* Values for individual bins
- *Statistics:* Statistics (7) based on all measurements.



Choosing the *Edit* option in *Select Actions*[1278] opens the *Binary Edit* panel. The tools allow you to work on the detection image overlay to deselect regions that are artefacts.



The panel is divided into the following sections:

- *Select Method:* Sets the drawing method: *Leica Cast Iron* uses only two of them:
  - *Select Existing Regions:* Draw a shape to enclose nodules or artifacts to be edited.
  - *Click to Select Regions:* Used to click on individual nodules or artifacts to edit.

  Both methods have function radio buttons that determine whether the nodules or artifacts should be deselected or reinstated.
- *Select Editing Tool*: Displays the generic toolbox and the *Line Width* control. The available tools change with the editing method selected.
- *Common:* The tools for deleting *(Trash Can)* drawn shapes, undoing and re-doing the last actions, and changing the cursor colour.

Select Method:

1: Click to display the drop-down menu

2: Select a method. Leica Cast Iron uses only *Select Existing Regions* or *Click to Select Regions.*

3: Click a radio button:

- *Reject/Delete* deselects the edited nodules or artifacts so that they are not included in the measurements.
- *Accept/Keep* reinstates a previously rejected nodule or artifact.

Common Tools

1: *Delete (Trash Can):* Click to delete the drawn shapes.

2: *Undo/Redo:* Click to undo/redo previous operations.

3: *Cursor Colour:* Click to change the cursor colour to suit the image (this toggles between black, white and red).

Drawing Tools

4: The appropriate tool is automatically selected with your chosen method:

Selection Tool:
Available when *Click to Select Regions* is active. Use this to select individual nodules or artifacts (to deselect or reinstate them).

Position the cross-hair cursor over a nodule or artifact and left-click.

Area Tool:

Available when *Select Existing Regions* is active. The tool draws a closed irregular shape around a group of nodules or artifacts.

Left-click to start; Move the cursor to the next point and left-click again; Repeat around the shape; End with a right-click.

Finished Editing

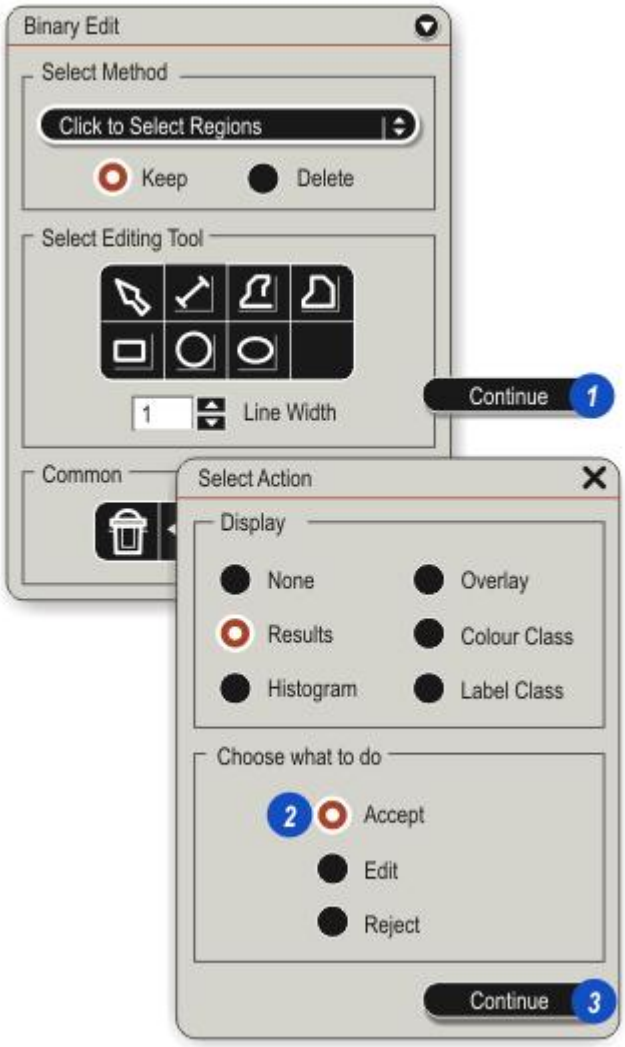
When you have finished editing:

1: Click *Continue* to return to the *Select Action* panel.

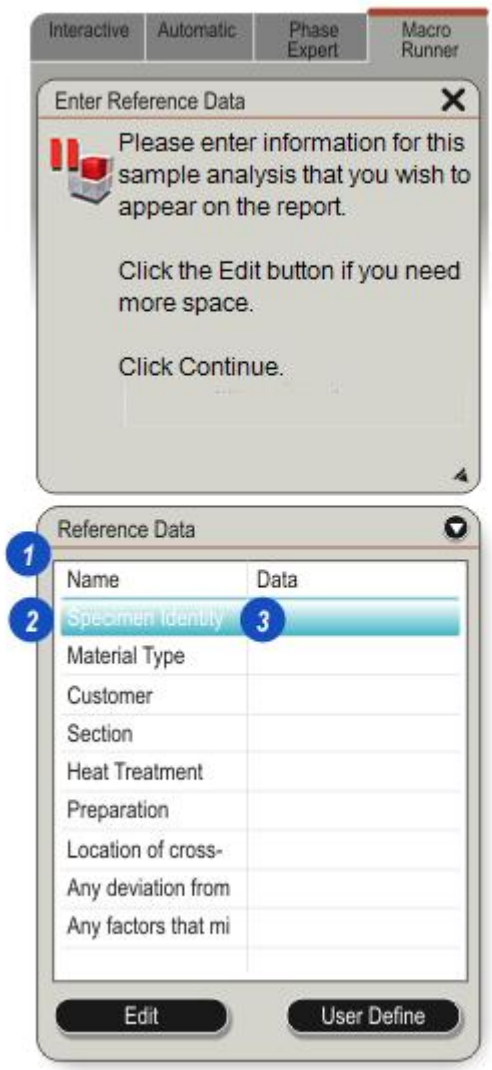
2: Click to select *Accept* (or *Reject* if the image is to be ignored).

3: Click *Continue*.

- If there are further images to process, this returns you to Feature Detection[1277]
- If all processing is complete this returns you to *Results*[1279].



You can add information about specimens and processing on the *Reference Data* panel. Some of the information is displayed on the standard reports installed with the application.



1: On the *Application Features* menu click *Enter Reference Data.* The *Reference Data* panel appears.

2: Click to select an item.

3: Click inside the text box to the right and type the data.

You can select the *Reference Data* headings to suit your working methods and descriptions. To do this you must run LAS as an administrator. (Right-click the desktop icon and select *Run as Administrator*.)

1: Click on the *User Define* button.

2: The *User Define Reference Data* dialog appears.

3: Click a *Visible* check box to show/hide that element in the *Reference Data* list.

4: The *Tools* from left to right are:

- *Create*: Adds a new user heading. The default is Field X; You can change this to a more appropriate name.
- *Delete*: Removes a selected heading completely.
- *Tick Mark*: Enables or disables the selected heading. (same functionality as the Visible check box).
- *Select All*: Enables all headings.
- *Hide All*: Disables all headings.
- *Up/Down Arrows*: Moves the selected heading up or down the list.

5: Click *OK* to finish.

If any item of data is too long to fit inside the panel text box:

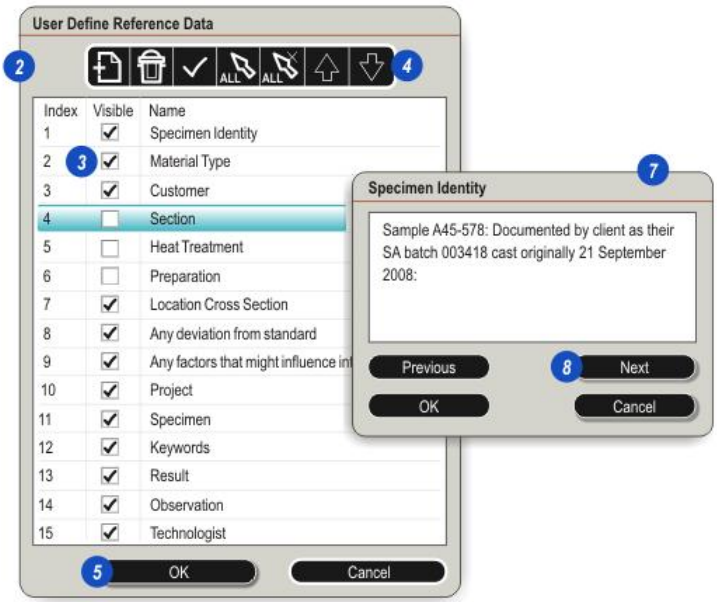
6: Click the *Edit* button.

7: Enter a heading in the resulting dialog.

8: Scroll between the headings using the *Previous* and *Next* buttons.

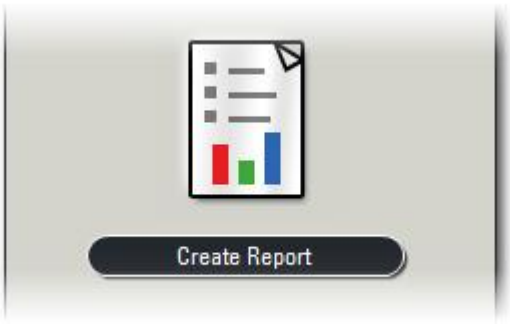
9: Click *OK* to finish editing.

Note - If you change the Reference Data, you may need to modify the Excel Report[1288] template. After making changes to the Reference Data names, please check that what you see in the report is what you wanted. If not, please modify the report template to suit.



Create Report

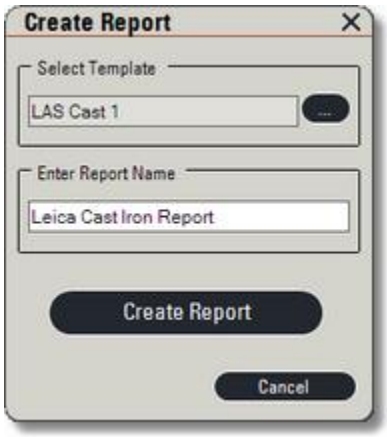
You can incorporate the measurement results into a comprehensive report using Microsoft Excel. The example template for the report is installed with the application. This template can be modified as required. You can find general techniques used for creating LAS reports in the main LAS Help.



On the *Application Main Menu* click the *Create Report* button.

1: This opens the *Create Report* panel.

2: Click on the browse button and select an Excel report template. The template will normally have an extension .xlt.



3: Click inside the *Enter Report Name* text box and type a unique name for the report.

3: Click *Create Report*.

The report will be displayed. Before you create another report with the same name, you must close the displayed report.

The example report template is located in the following folder:

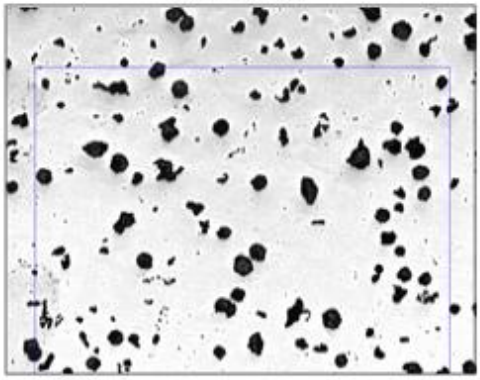
C:\Users\Public\Documents\Leica Application Suite\LAS Apps\Apps\LAS Layer App

Below is an example of a cast iron report using the template installed with the application.

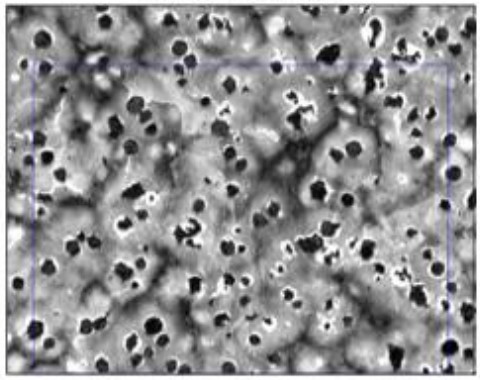
LAS Cast Iron Report

Material Type	Cast Iron	Preparation	Copper cast/grind/polish
Customer	Hillcrest	Specimen	3 of 21
Section		Technologist	MRJ
Heat Treatment	No	Analysis Date	18/02/2012

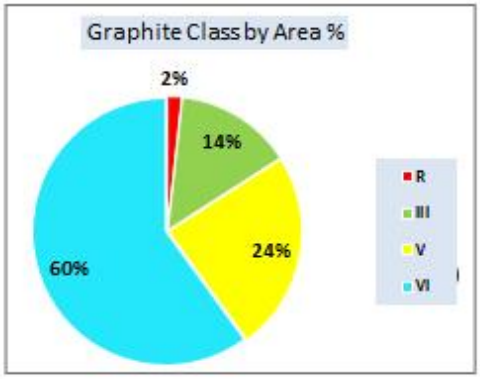
Graphite Image



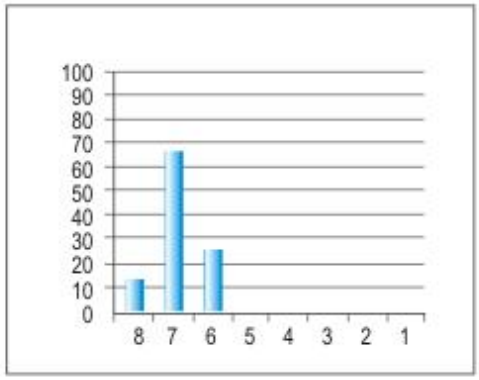
Ferrite/Pearlite Image



Graphite Images: 2



Ferrite/Pearlite Images: 2



Phase percent adjusted						Ratio	
Graphite%	0.00	Ferrite%	0.00	Pearlite%	0.00	Ferrite/P'lite	0.00

Graphite Nodule Analysis			Graphite Area, mm²			0.34
Class	R	III	V	VI	Total	
Area	1.8%	14.2%	24.1%	60.0%	35617	
Count	3.3%	6.5%	29.3%	60.9%	92.00	
Count per mm²	8.72	17.45	78.51	162.83	267.51	

Approved by:

Dendrite Arms Expert

Leica Dendrite Arms Expert helps to identify Dendrite Arms on a sample imaged by an optical microscope. It measures the Dendrite Arm spacing (or the number of gaps between arms) and produces tabulated results.

Leica Dendrite Arms Expert is fully compatible with the Leica Application Suite (LAS).

Features

- Step-by-step guided operation
- Interactive settings
- Results stored in named configurations for easy recall
- Include specimen details and reference information with results
- Manual and semi-automatic methods
- Display of Dendrite Arms over the sample digital image with the number of arms identified
- Accumulation of results over multiple images
- Display of individual results for each image
- Customisable Excel results template

Dendrite Arms Analysis

The mechanical properties of wrought materials, aluminium, copper and alloys are strongly dependent on secondary dendrite arm spacing. As this parameter decreases, so strength, ductility and elongation increase. Finer spacing makes the materials more responsive to heat treatment, giving better properties or faster treatments.

Arms initially grow at a small spacing near the tip of the dendrite. They are replaced by larger arms with increased spacing. Secondary dendrite arm spacing is controlled by solidification time.

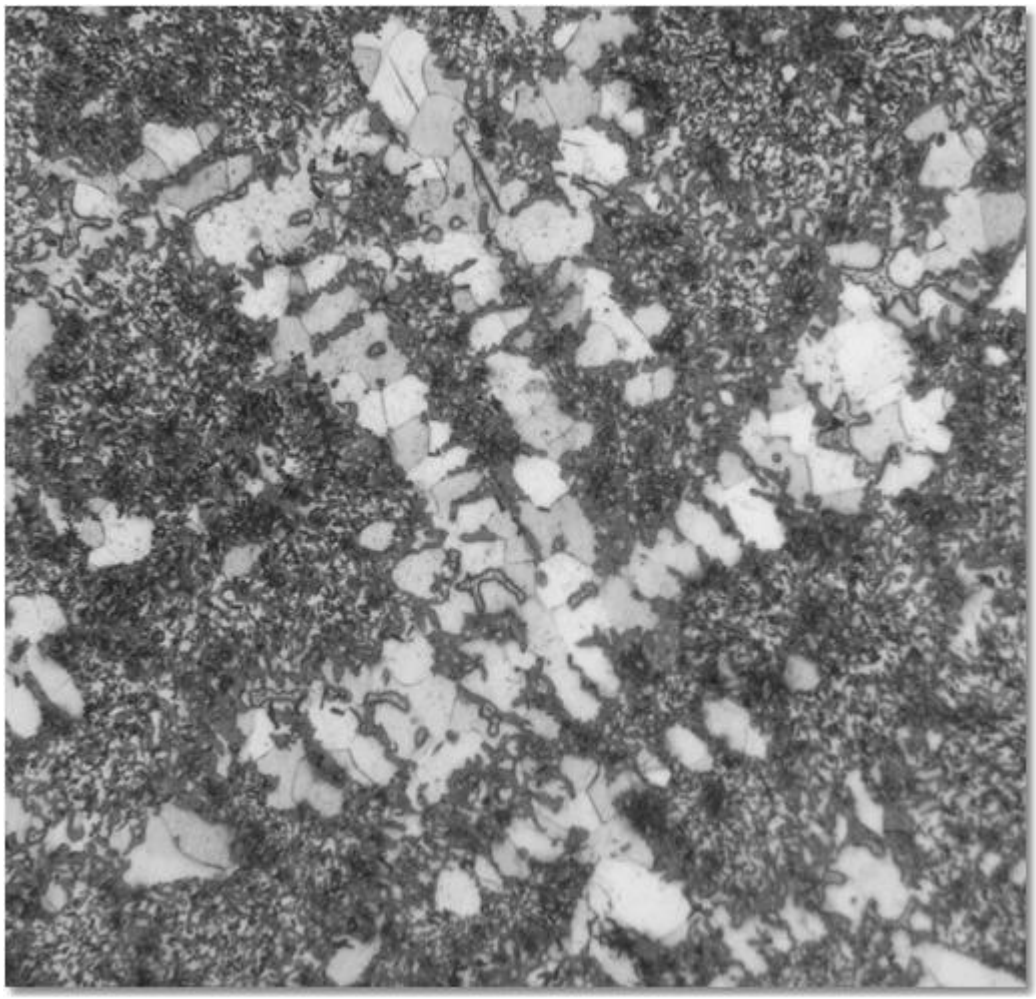
Traditional microscope methods of measuring dendrite arms are slow and require operator skill and concentration, while lacking the precision of automatic image analysis.

Leica Dendrite Arms Expert determines the dendrite arm spacing from the length of the lines and the number of arms.

- In Manual mode, you draw lines on an acquired digital image and enter the number of arms using the keyboard. This method is suitable for the widest range of material and preparation methods.

- In Semi-automatic mode, you still draw lines on an acquired digital image, but the number and width of the arms are calculated automatically. This method is probably faster than the manual method but is more dependent on the specimen type (showing clear separation of the arms) and preparation consistency (requiring a high contrast image without background artefacts).

You can analyse multiple samples quickly and consistently.



The principal areas of the user interface:

1: *Menu Bar*: For Options and Help.

2: *Workflows*: Leica Cast Iron Expert is available on the Analysis Workflow.

3: *Application Panels* Tab: Click *Runner*.

4: *Prompt Message Panels*: Hints for program sequence steps.

5: *Control and Results Panels*: Used to operate the application and to show summary results.

6: *Image Scaling* information.

7: *Image Viewer*: Shows original image, sometimes with the superimposed Dendrite Arm positions marked.

8: *Results Grid*: Not used by this application.

9: *Gallery of Image Thumbnails*: Used to select the images to measure.

10: *Side Tool Bar.*

Getting started – new users

- Check out all the links in the Help navigator to the left – plenty of help is available!
- To view the *Leica Dendrite Arms Expert* help, press F1 while the application is running.

Getting started – new users of LAS

- *Leica Dendrite Arms Expert* uses LAS to acquire images and is started from the *Analysis* > *Runner* step of LAS
- Please refer to the main LAS help for information concerning the configuration, calibration and use of LAS to acquire images.
- To view the LAS help, press F1 while LAS is running.

Supported Microscopes, Cameras, Computers and Software

Please see the *Systems Requirements* PDF file:

Start > Leica Application Suite V4 > Documents

This provides details of all supported hardware and recommends suitable computer specifications. In case of doubt, please run the *LAS PC Performance* utility and observe the recommendations it produces.

Prerequisites

LAS Image Analysis must be purchased and licensed on the same system as *Leica Dendrite Arms Expert*.

LAS Runner must be licensed on the same system as *Leica Dendrite Arms Expert*. This is included with the purchase of a *Leica Dendrite Arms* License.

Microsoft Excel is required if you wish to customise the report template.

Image Format

Colour and Monochrome images are supported.

It is recommended that the image pixel size is in the range 1 to 3.3 Mpixel. Images with pixel sizes greater than 5 Mpixel are not supported.

Display resolution

The minimum screen resolution is 1280 x 1024. If you use a lower vertical resolution some of the control panels may be obscured. You will be able to move them to a position where they are visible, but this is rather inconvenient. Please minimise the height of the Windows task bar as this occupies some of the vertical screen space.

Image Acquisition

Please ensure that the images are acquired with the correct calibration. This will be more certain if you are using a microscope with a coded nose-piece. If not, then make sure that the objective in use is the same as that selected in LAS.

Adjust the camera exposure, gain and gamma to provide a high quality image. In particular, ensure that an HQ format is used, the value of gain = 1 and the histogram black and white levels are set to the default values.

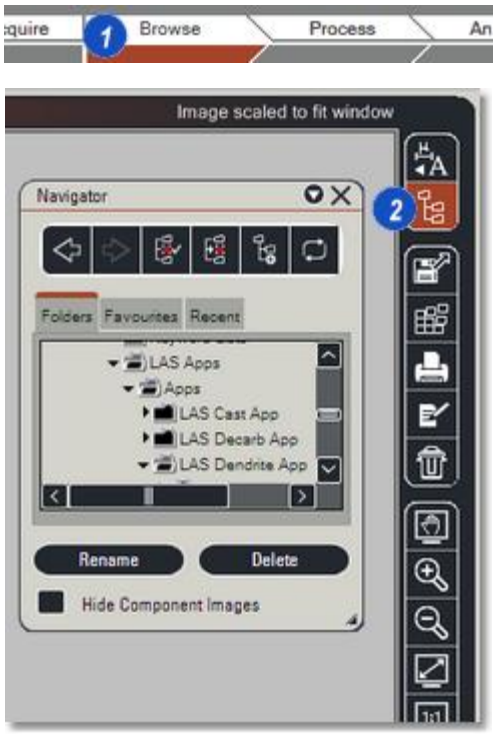
If you are using the Semi-automatic measurement mode, which requires thresholding, it is strongly recommended that the shading correction is set for all images acquired. This will ensure that the thresholding is performed uniformly over the entire image.

Calibration Units

Please ensure that all images that you measure at the same time have the same calibration for consistent results.

Make sure the Navigator panel is floating

You need to ensure that the *LAS Navigator* is floating before starting an *Expert* application. This allows you to select test images and real sample images that you have acquired, for use within the application.



1: In LAS, select the *Browse Workflow*.

2: If the *Navigator* panel is docked with the other Control Panels on the left, click the *Show Navigator* button on the *Side Tool Bar* to make it float.

3: You are now ready to start[1296] your *Expert* application.

1: Select the *Analysis Workflow*.

2: Display the *Runner* tab.

3: If necessary, click to expand the *LAS Application Selection* panel.

4: Click on an icon to select your chosen Expert Application.

5: Click *Run*.

6: If the *LAS Application Selection* panel is not visible click the *Application Selection* button.

Dendrite Arms Start-up Screen

When the application start-up screen appears, do one of the following to start the application:

- Click *OK*
- Click the logo.

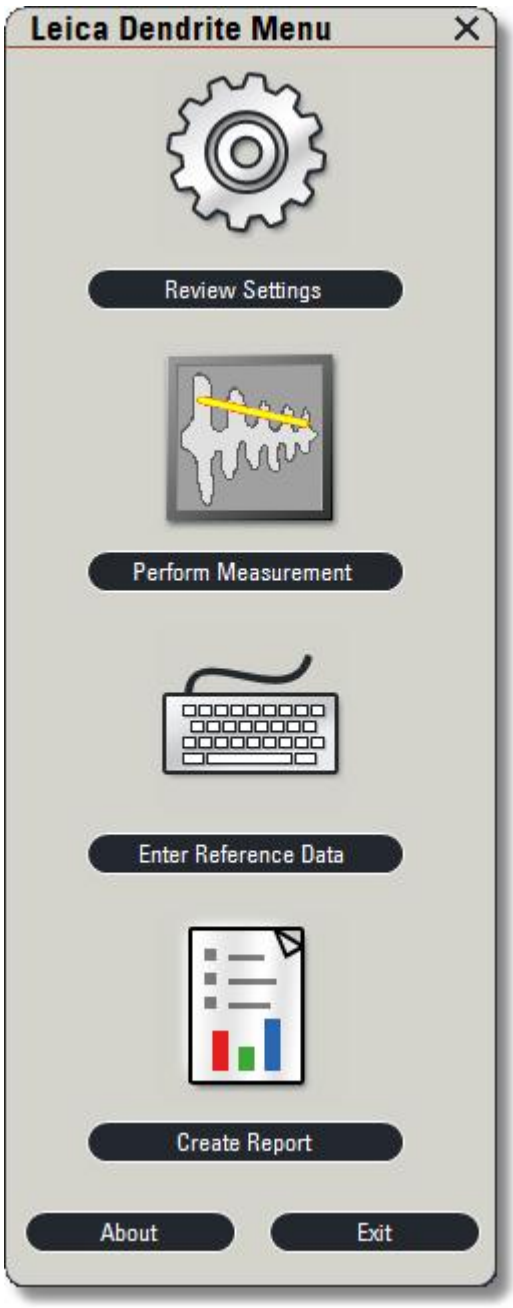
The *Leica Dendrite Menu* provides links to the steps you should take to analyse a dendrite specimen.

Before using this menu, please ensure that the images have been acquired using LAS 1299 and stored in a known folder.

Note - it is preferable to acquire images with a resolution approximately in the range to 1 to 3 Mpixels. Typically the Leica DFC400 and DFC450 cameras are suitable.

- *Review Settings* 1300*:* Use default parameters or create user-defined settings. Determine the detection limits and then use the supplied or user images to test the settings.
- *Perform Measurements* 1312*:* Uses the parameters established in *Review Settings* to detect and analyse the specimen.
- *Enter Reference Data* 1328*:* Add comments and specimen details that will be included in the final report.
- *Create Report* 1330*:* The results and reference data are presented using a supplied Excel template that can be modified to suit user or company styles.

Click the *Review Settings* button.



Acquire Images with LAS

The first stage of Dendrite Arms analysis is to acquire a selection of digital images using LAS and to save these to the computer's hard drive. The advantage of this approach is that the original images are always available to check your results later.

- To obtain precise results from *Leica Dendrite Arms Expert* you need a good, representative sample of the specimen that has been cut from the product, revealing a section perpendicular to the surface of the sample.
- Images should be processed to display optimum sharpness and contrast before acquisition. Pay careful attention to the shading correction for images that are to be measured automatically.
- LAS acquires calibrated images by reading the magnification from the microscope and the sensor size from the camera to accurately determine the image dimensions.
- Imaging conditions (such as microscope settings and camera exposure) are automatically recorded by the software. This data is stored with the image and is useful for checking consistency.
- Images are named and acquired into a Windows folder. It's a good idea to make a note of these details.
- You can annotate images with a calibrated scale bar and labels (such as date, time, image name and description).

  Please refer to the Advice and Prerequisites 1294 for the recommended image capture settings.

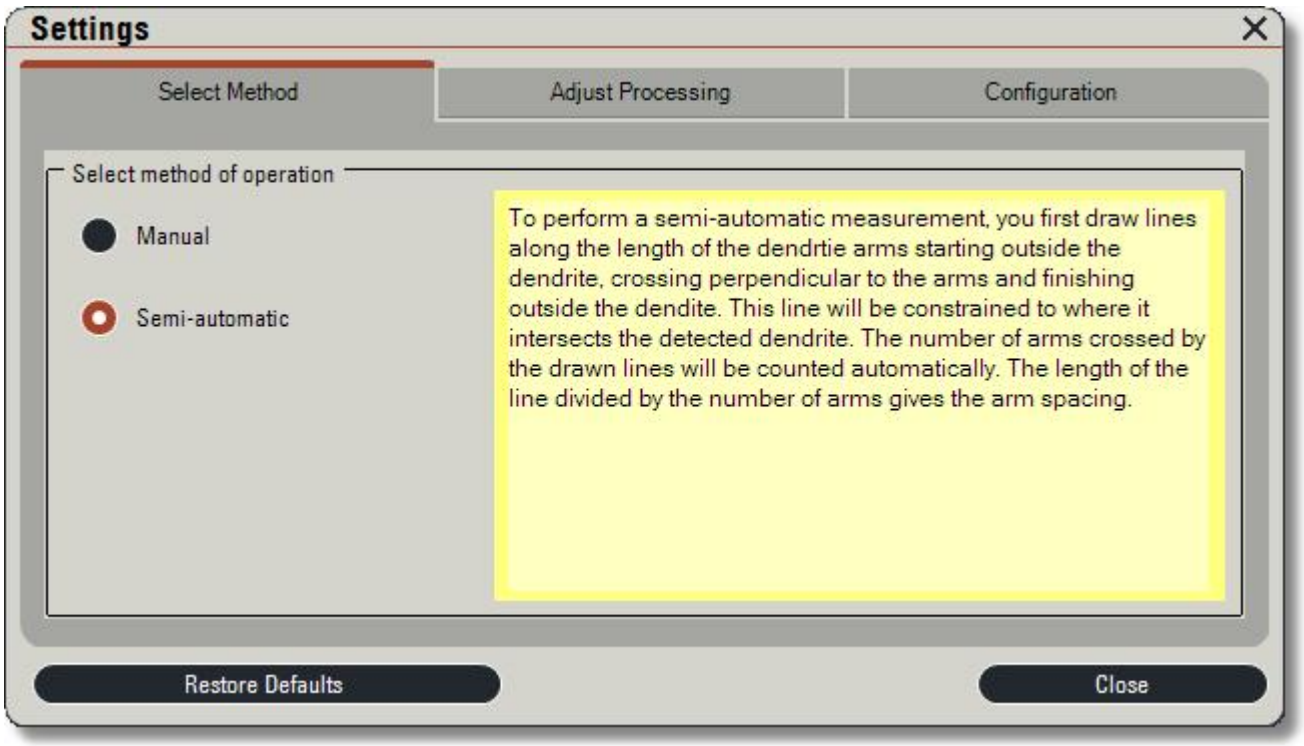
1: Click *Review Settings* on the *Leica Dendrite Menu*.

The *Settings* dialog has the following tabs:

- *Select Method* 1301: Decide which measurement method to use, according to the type of specimen.
- *Adjust Processing* 1302: Specify how image processing is applied, to help identify the dendrite arms. You can apply the settings to a test image to check that analysis is working properly, before working on a real sample image.
- *Configuration* 1308: You can define overlay colours, specify a report template, create new configuration files or load configuration files that you have previously saved.
-

Notes

- If you want to revert to the default settings for the application, click *Restore Defaults*. You can do this at any time before clicking *Close*.
- If you close the *Settings* dialog and save your changes, this will **overwrite** your current configuration file, giving you a new set of defaults.



Select Method

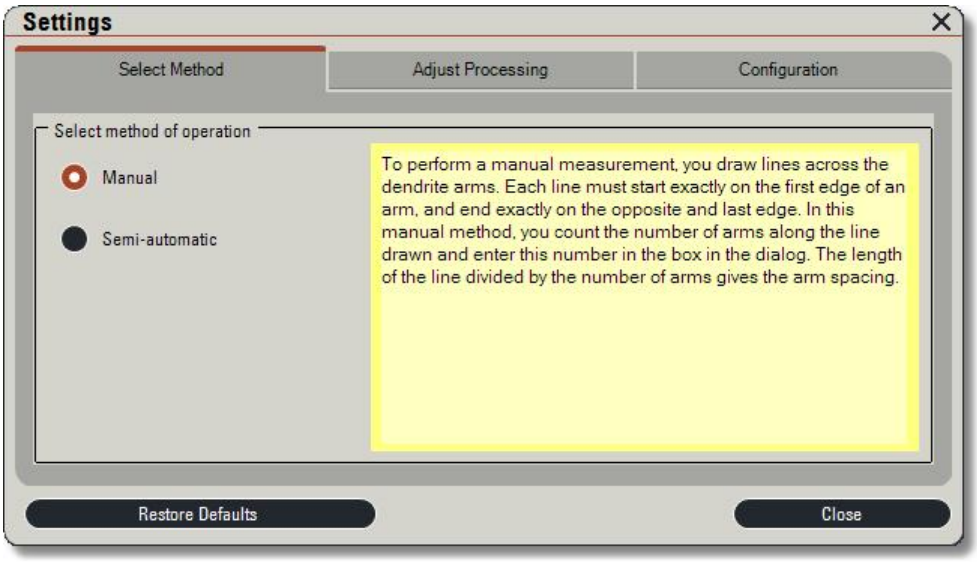
The *Select Method* tab allows you to choose the way in which measurements are made.

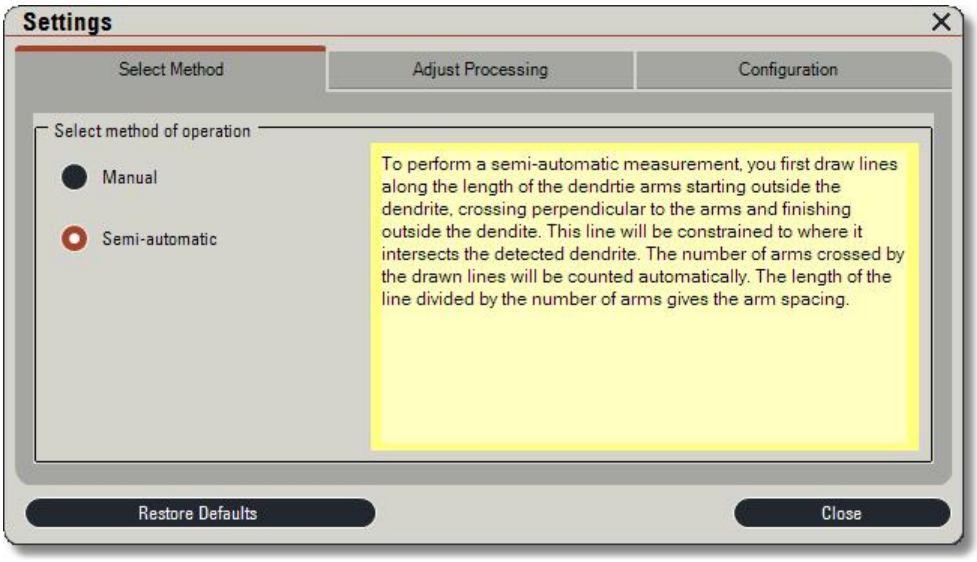
The method you select here will be used every time you perform measurements[1312], until you change the selected method.

1: Display the *Select Method* tab.

2: Click a radio button to select a method (*Manual* or *Semi-automatic*).

3: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).





Adjust Processing

The *Adjust Processing* panel allows you to optimize the image for the best dendrite arm detection and subsequently to remove any unwanted artifacts. You can also configure the histogram showing the distribution of dendrite arms.

1: Display the *Adjust Processing* tab.

The controls are grouped as follows:

2: Grey Enhance [1303]

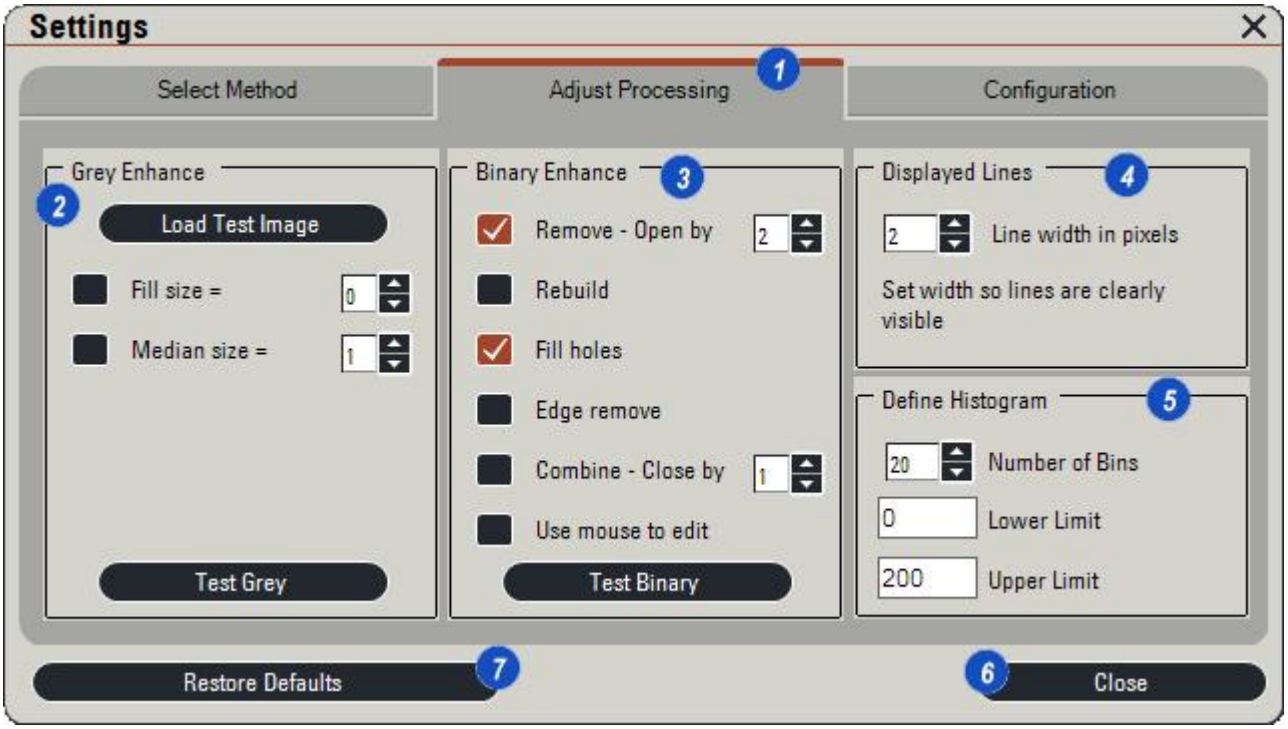
3: Binary Enhance [1305]

4: Displayed Lines [1306]

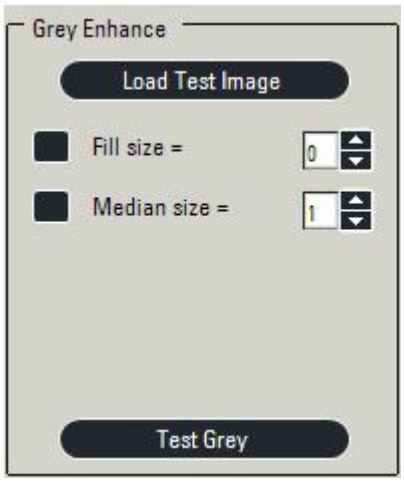
5: Define Histogram [1307]

6: Make and test the adjustments then *Close* the dialog or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).

7: If you want to revert to the default settings, click *Restore Defaults*.



Grey Enhance Settings



Grey Enhance settings

Fill size - this removes local detailed variations in the image and is often very effective.

Median size - this is a noise filter that removes sharp spikes in the image with minimum effect on the resolution.

The grey (or colour) processing functions are applied to the selected image to enhance the contrast of the image and remove spurious detail that might compromise the following threshold step. With the functions provided, you can experiment on test images to achieve acceptable results.

1: Load an image similar to those that you want to measure. See Load Test Image [1304]

2: Select the functions you want to use by enabling the check box and entering a size value. Appropriate size values are in the range 1 to 20. Larger values will increase the effect of the function but will also extend the time taken to process the image.

3: Click the *Test Grey* button to apply the selected function.

You can now examine the effect of the function. Continue to experiment until you are happy that the image you want to measure appears with good contrast compared to the surrounding region with reduced artefacts. You will probably want to further test this result by performing the Binary Enhance [1305] step.

Note that image processing will change the appearance of the image and it may seem to have less detail as a result.

Load Test Image

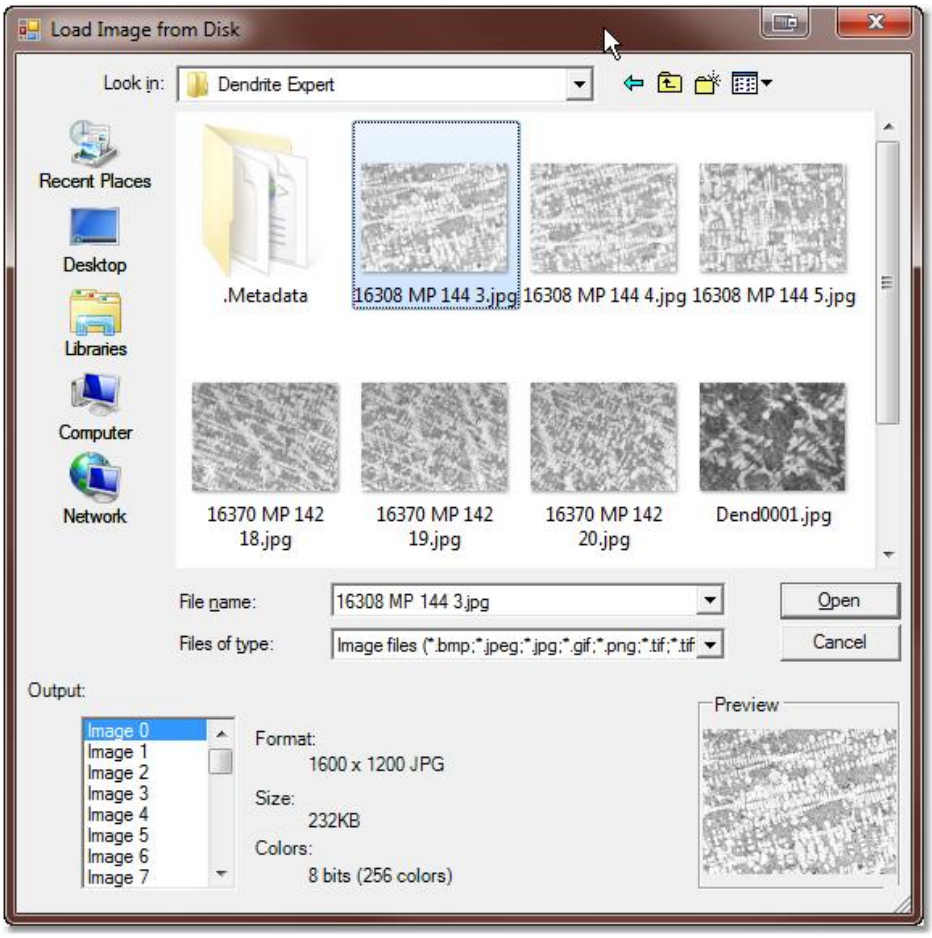
The test image can be one that you have captured or one of the samples installed with the application. These are stored in:

Users > Public Documents > Leica Application Suite > LAS Apps > Apps > LAS Dendrite App > Example Images

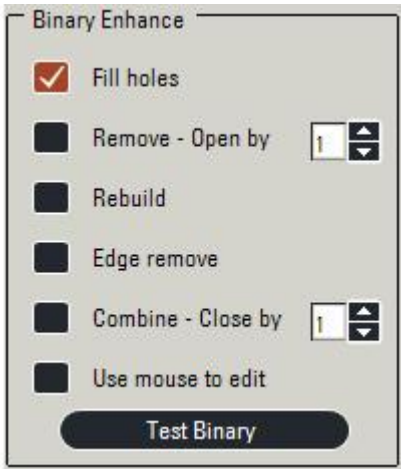
1: Click the *Load Test Image* button; the *Load Image from Disk* dialog appears.

2: Navigate to either the *Example Images* folder or to a user-defined folder and click to select an image.

Continued Binary Enhance 1305



Binary Enhance Settings



Binary Enhance Functions

Fill holes - Fills in empty regions in the mask that are completely surrounded by pixels.

Remove - Open by - Removes small regions from the mask.

Rebuild - Returns the mask to its original size except where the regions have been removed. Only use in conjunction with the Remove step.

Edge remove - Deletes any part of the mask that is touching the edge of the image. Use this with care.

Combine - Close by - joins together regions of the mask that are near neighbours.

Use mouse to edit - allows you to edit the image mask during the Measurement phase (after the Binary Enhance step). Use the Fill Area Tool to add or erase regions from the mask.

The Binary Enhance functions are applied to the image produced by the Grey Enhance functions.

Note: You must select the test image[1304] from Grey Enhance before using the Binary Enhance functions.

The Binary image is used as a mask that defines the extent of a sample and is used by the Semi-automatic[1313] method. It has no effect on the Manual method.

Ideally, the result of the threshold and the binary enhancement will be a mask that exactly represents the dendrite arms. In practice, providing the mask correctly represents the edges and centre of the image, a small amount of additional artifact is unlikely to affect the results. With the functions provided, you can experiment on test images to achieve an acceptable mask.

1: The Binary Enhance functions are shown on the panel.

2: Select the functions you want to use by setting the check boxes and entering a size value. Appropriate size values are in the range 1 to 20. Larger values will increase the effect of the function but will also extend the time taken to process the image.

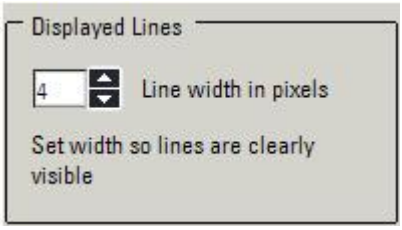
3: Click *Test Binary* to perform these steps:

a) Apply the selected Grey Enhance function.

b) Set the grey or colour threshold. See Dendrite Arm Detection[1316]

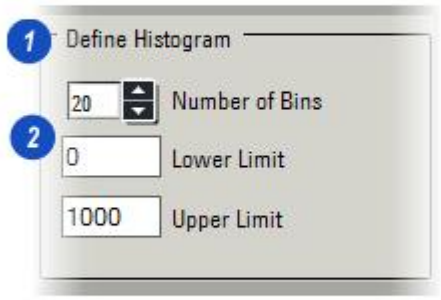
c) Apply the binary selected function.

You can now examine the effect of the combined grey and binary functions. Experiment with the settings until you can obtain an accurate mask. If you cannot achieve this, you will need to use the Manual method[1322].



Line width in pixels - Allows you to change the width of drawn lines to optimise visiblilty on your images.

Define Histogram Settings



The application creates a histogram that shows the distribution of the dendrite arm measurements accumulated over one or several images.

1: The Define Histogram functions are shown on the panel.

2: Enter the number of bins to be used depending on the detail that you want to see. These bins will be distributed between the Lower and Upper limits.

These values will be used by the histogram created when measurements are made.

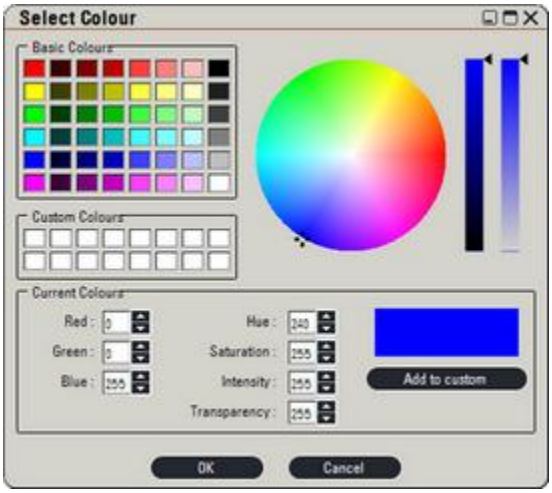
It is possible to review the histogram during the measurements. See Review Histogram 1320

Note that the Limits are assumed to be in the same units of calibration as the images measured.

The *Configuration* tab allows you to choose the Overlay Colours, set up Drawing Tools and Load and Save a Configuration.

1: Display the *Configuration* tab.

2: To change the colours of overlays (for example to give a better contrast against the image you are working with) click on the appropriate colour swatch. Pick a new colour using the *Select Colour* dialog:



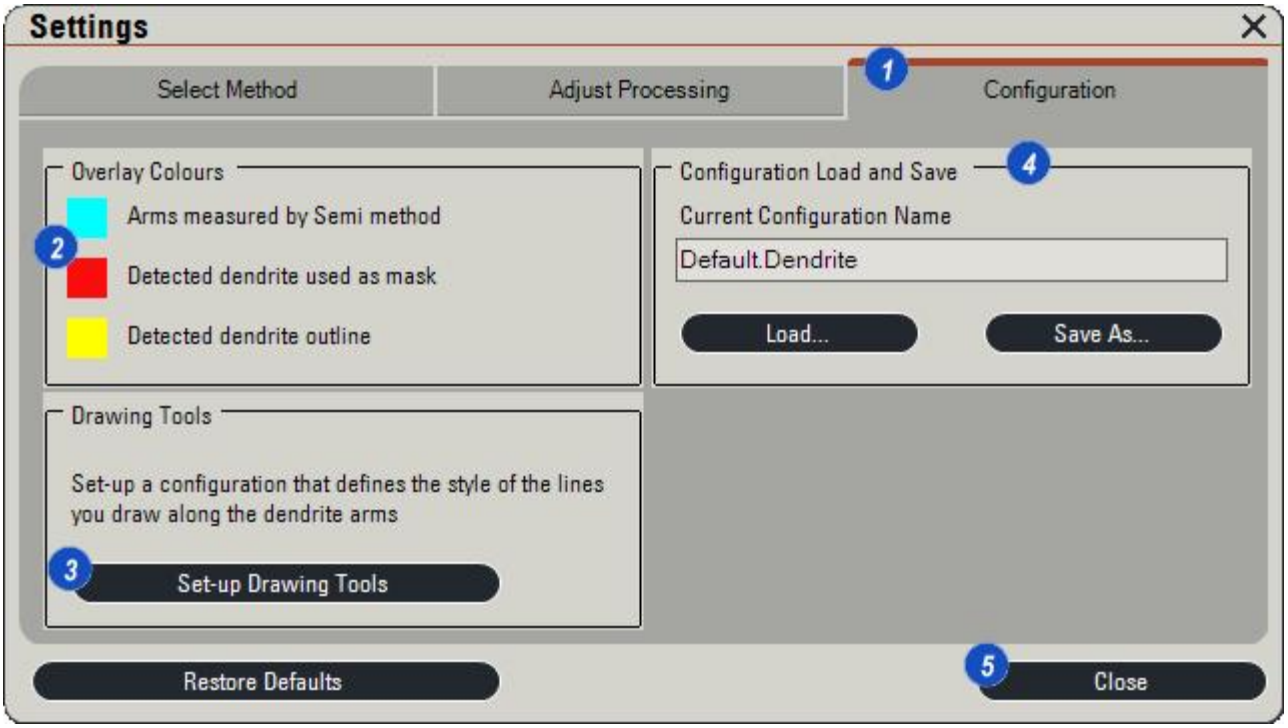
3: If necessary, set up the Drawing Tools[1309].

4: At this point you can click *Save As* to save all your changed settings to a named configuration file. These files are located in the following folder (and have the extension .Dendrite):

C:\Users\Public\Documents\Leica Application Suite \LAS Apps\Apps\LAS Dendrite App\Config Files

You can also *Load* existing configuration files that you have tuned to different types of specimen.

5: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made; this will **overwrite** your current configuration file, in effect giving you a new set of defaults).



Set Up Drawing Tools: Load Test Image

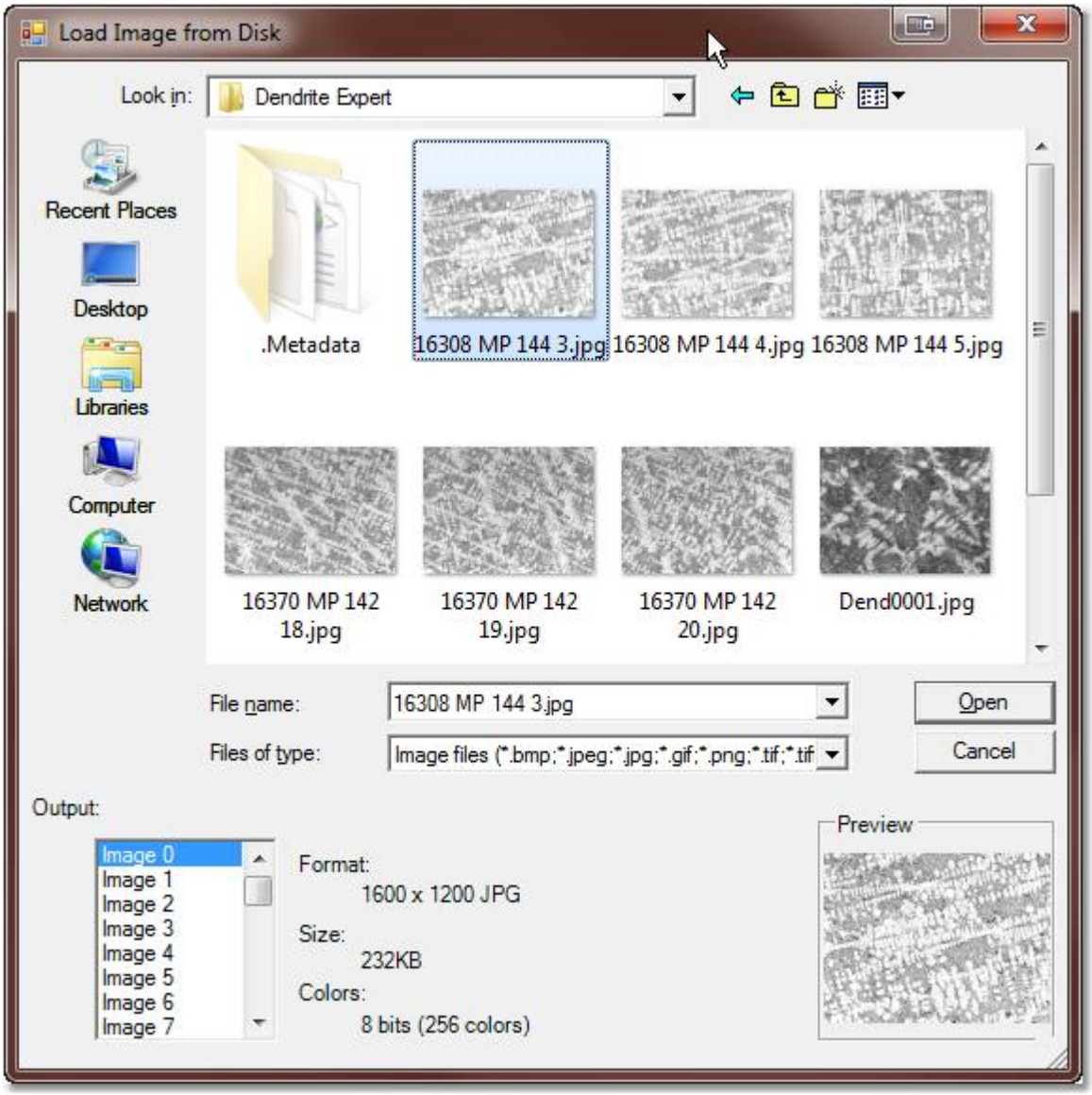
1: Click *Set-up Drawing Tools* on the *Configuration* tab to display the *Load Image from Disk* dialog.

2: Navigate to a folder containing a suitable test image.

The test image can be one you have captured, or one of the samples installed with the application. The installed test images are in the following folder:

Users > Public Documents > Leica Application Suite > LAS Apps > Apps > LAS Dendrite App > Example Images

3: Select the image and click *Open*.



To set up the line style that will be used with your images:

1: With the test image loaded in the *Image Viewer*, select the *Line - Two Point* tool.

2: Draw a line on the image.

3: Display the *Properties* dialog and set up the line properties (thickness, colour and end bar) to suit the image.

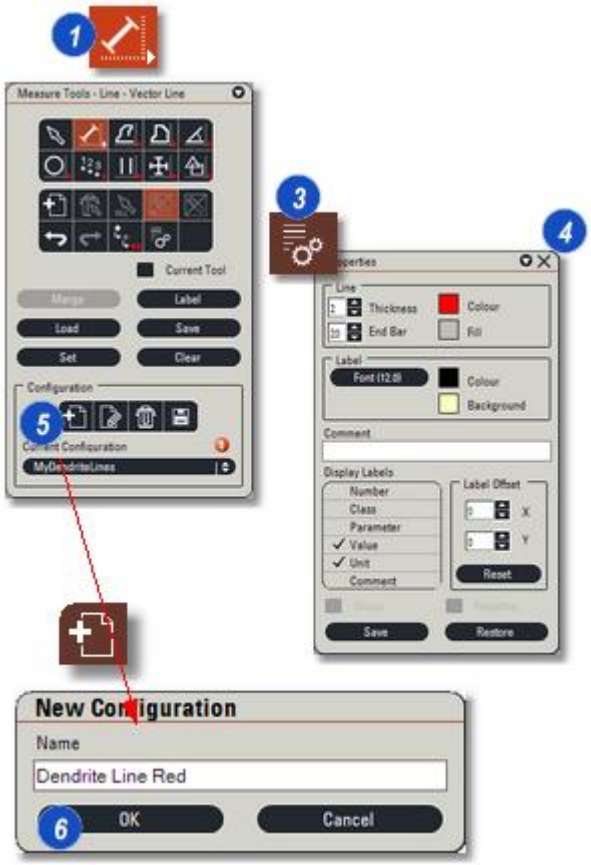
4: Close the *Properties* dialog.

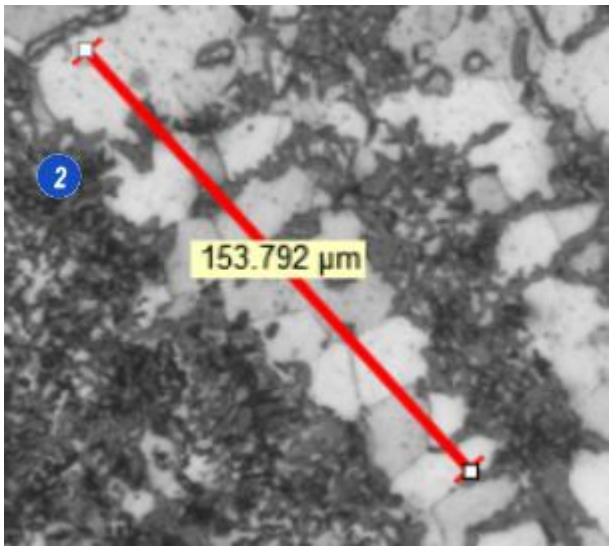
5: Click the *New Configuration* button in the *Configuration* panel.

6: Enter a *Name* for this drawing tool configuration and click *OK*.

7: The configuration will be available in the *Current Configuration* drop-down menu:

This configuration will be used every time you draw a line in *Leica Dendrite Arms Expert*, until you select a different configuration.



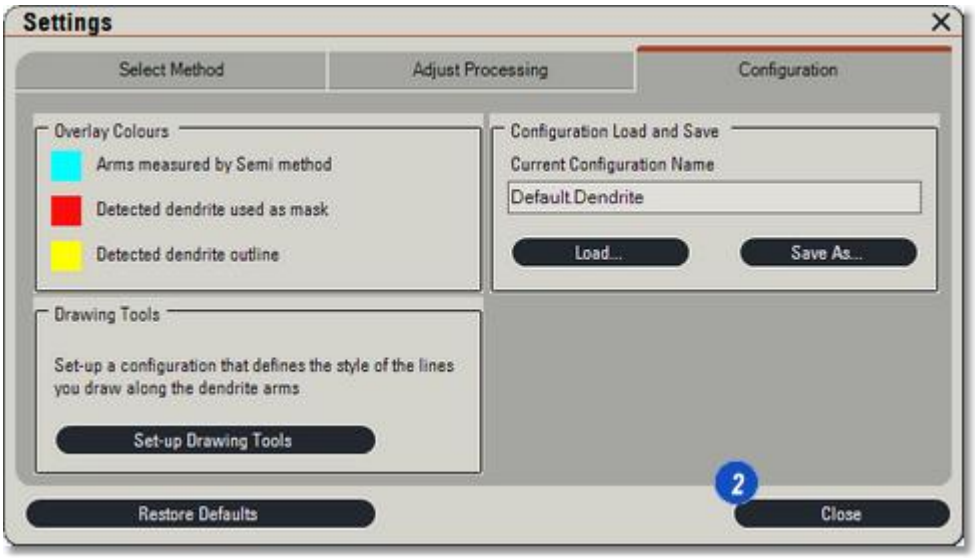


1: Click *Continue* at the bottom of the *Runner* panel.

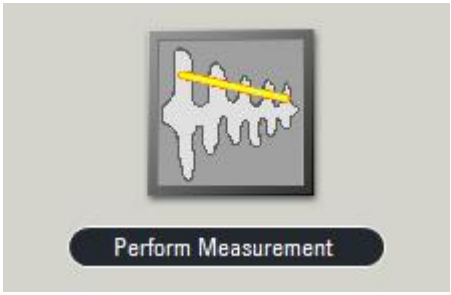
2: Click *Close* to dismiss the *Settings* dialog.

3: Click *Yes* to accept the changes to the main *Leica Dendrite Arms Expert* configuration settings.

You should now be able to see the *Leica Dendrite Menu* once more.



Overview



The basic method for selecting, detecting, measuring and showing the results is as follows:

1: Select an image to measure.

2: (**Semi-automatic method only**):

- Adjust the threshold so the dendrite regions you want to measure are completely covered by the mask.
- Binary Enhancement is applied. If you enabled *Use mouse to edit* in the Settings[1305] dialog, you will have the chance to make changes to the mask.

3: Draw lines on the image to indicate the dendrite arms.

4: Review the measurements you have made and choose what to do with them (accept, edit, re-detect or reject)

5: Repeat the above steps to measure another image, or return to the *Leica Dendrite Menu*.

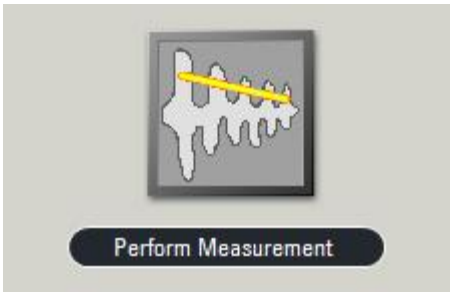
Semi-automatic Method

The Semi-automatic method uses the power of *Leica Dendrite Arms Expert* to make the process of measuring dendrite arms easier for you.

- You do not need to be quite as accurate when drawing lines on your images.
- The number of dendrite arms (or gaps) is calculated automatically.

To start the measurement process:

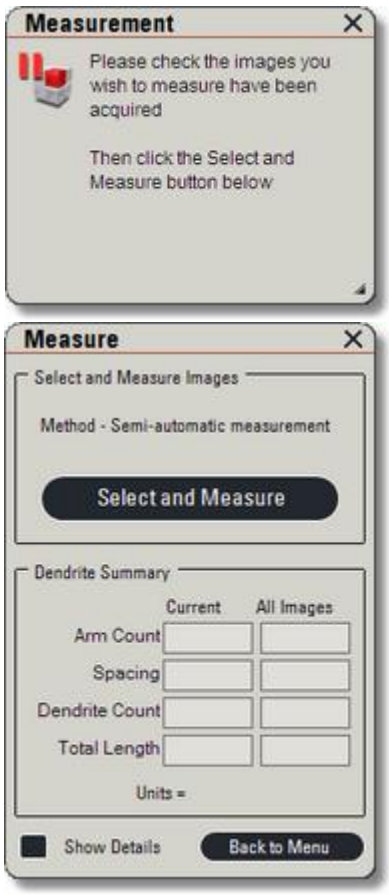
1: On the *Leica Dendrite Menu* click *Perform Measurement*.



2: Click *Select and Measure*.

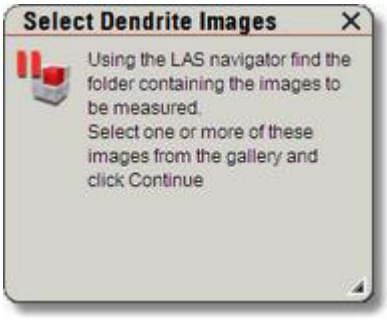
Notes:

- The *Show Details* option is only relevant after you have made some measurements; it displays the Feature Histogram[1320] and Summary of All Results[1321].
-
- The *Dendrite Summary* pane shows information about measurements already made in this session (it will be blank to start with).
-



Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



1: Click on the *Show Navigator* button on the *Side Tool Bar*.

2: Move the *Navigator* to a convenient position by dragging the title bar.

3: Navigate to and select the image folder.

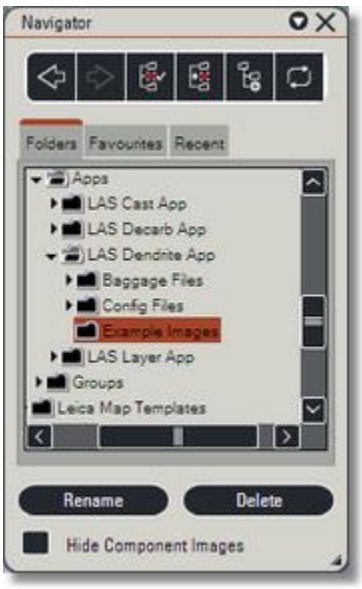
4: The available images will be displayed in the *Gallery*.

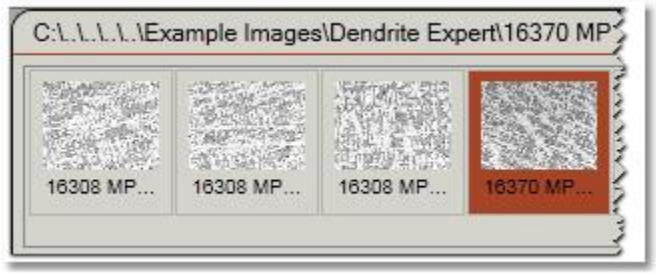
5: In the *Gallery*, click to select a single image, or Ctrl-click to select up to 10 images.

Important: If you select multiple images, ensure they are all of the same type (i.e. all mono or all colour) to ensure that the Adjust Threshold process works correctly. Note that if you only select a single image at this stage (e.g. mono) then a different image at a later stage (e.g. colour) the Adjust Threshold process works correctly.

6: You can minimise the Navigator by clicking the arrow to the right of the header.

7: Click *Continue* to load the image. Any Grey Enhancement will be applied automatically.





Dendrite Arms are detected using the parameters set up by the *Review Settings dialog (*Adjust Processing 1302 tab).

The detection method used will be either Monochrome or Colour (as shown to the right), depending on the image type.

However, due to the range of contrast that can be encountered in different images, some areas that should be included in the measurements may not be detected, and some regions may be ignored. You can adjust the regions included as follows:

1: Click to enable the *Accumulate* check box.

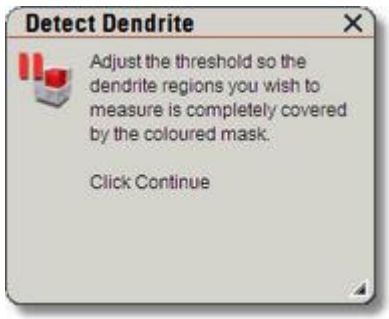
2: Drag a marquee around an area to include.

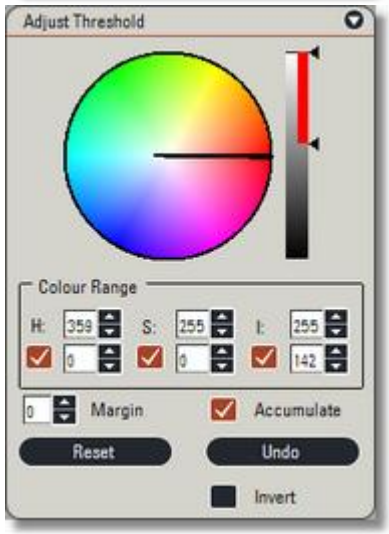
Image values contained within the marquee are added (accumulated) to the existing range and the areas are detected.

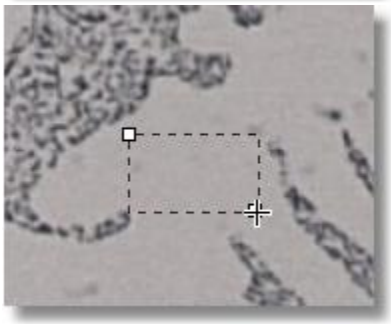
Alternatively adjust the sliders under the histogram.

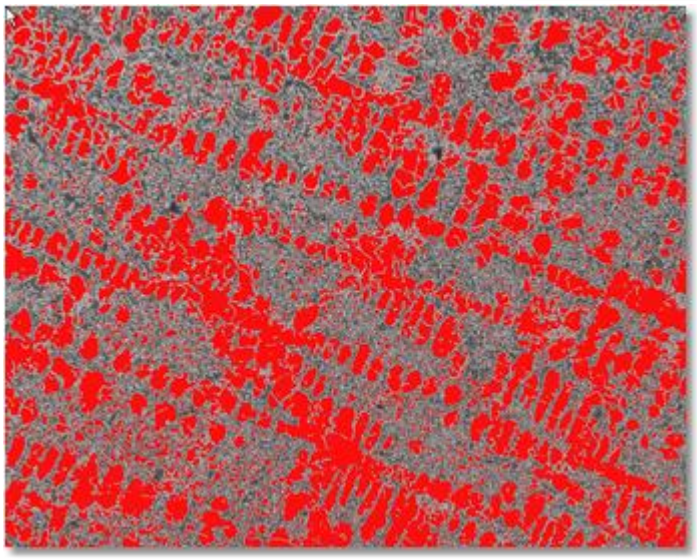
For further details, see the section on Adjust Threshold in the main LAS help (LAS Image Analysis module).

3: Click *Continue* when you are satisfied with the detection. Do not be too concerned if there are some separate detect regions, as these will be rejected by the Binary Enhancement in the next step.



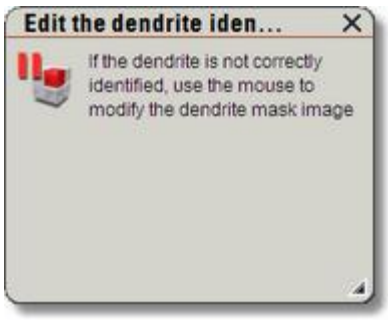






Edit Mask Using the Mouse

If you enabled *Use mouse to edit* in the Settings[1305] dialog, you can make changes to the mask. (If not, go to Draw Lines[1318].)



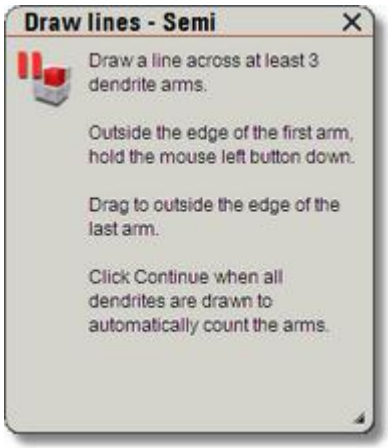
1: The *Fill Area Tool* is selected by default.

2: Enable *Add* or *Erase* as appropriate, then draw a shape around the region you want to add to (or erase from) the mask. Left-click to place points, then right-click to complete the shape.

3: Repeat to add or erase as many regions as you require to the mask.

4: When you have finished editing the mask, click *Continue*.

The image is updated in the Image Viewer, with dendrite arms highlighted in your chosen overlay colour[1308] (yellow, in our example).



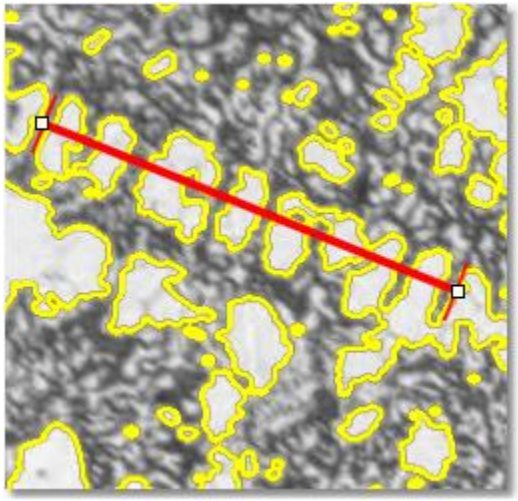
Important note

In the *Measure Tools* panel, the *Vector Line Tool* is automatically selected, **but not visible**; you can toggle between the *Vector Line Tool* and the *Selection Tool* by clicking the *Selection Tool* icon.

- *Vector Line Tool* active:

- *Selection Tool* active:

1: With the *Vector Line Tool* active, drag to draw a line across at least three dendrite arms.

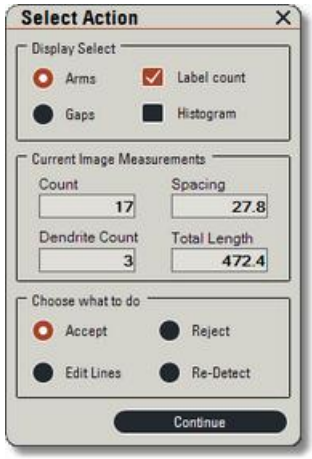


2: Repeat, until you have drawn at least three lines over different dendrites. You do not need to be as accurate as when you are using the Manual method; the arms and gaps will be calculated automatically in the next part of the process.

7: Click *Continue*.

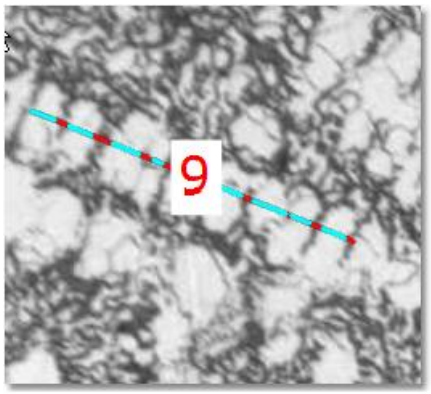
LAS Dendrite Arms Expert will calculate the number of arms covered by each line you have drawn, and display them in the *Image Viewer*.

At this point, you can review the measurements you have made and choose what to do with them. You can also display different information on the *Image Viewer*.



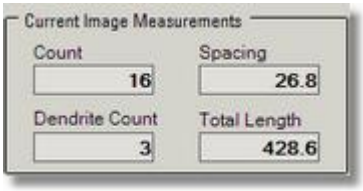
1: In the *Display Select* pane, choose from the following:

- Choose to display Arms or Gaps.
- Choose whether or not to display the Label Count; this automatically labels each line you drew with the number of arms calculated by the software:



- Choose whether or not to display the Histogram[1320].

2: In the *Current Image Measurements* pane, you will see the details that have been calculated from the lines you drew (such as Arm Count, Dendrite Count, Spacing and Total Length).



3: In the *Choose what to do* pane, select one of the following:

- Accept the results and add them to any existing results.
- Edit the detection and remove spurious lines. This takes you back to the Draw Lines[1318] step, where you can use the Selection Tool and Vector Line Tool to amend your measurements.
- Reject the image and results. Select[1315] another image.
- Re-Detect: This takes you back to the Detect Dendrite[1316] step.

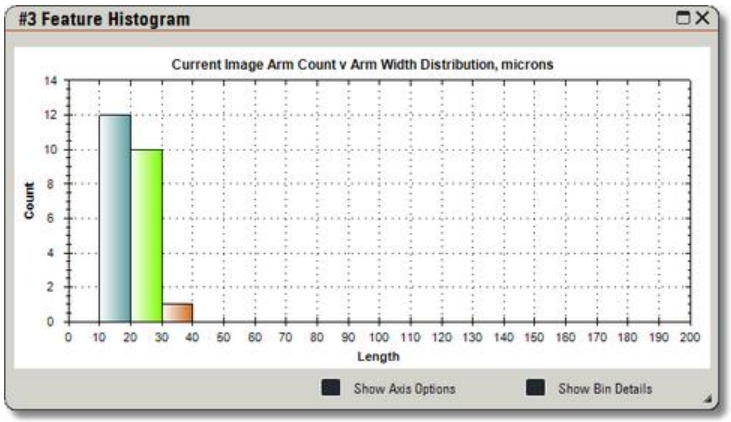
4: Click *Continue*.

This takes you back start of the Measure process, from where you can select and measure another image or return to the *Leica Dendrite Menu*.

Once you have finished measuring images, the next step is to Enter Reference Data[1328].

The histogram displays the accumulated histogram for all images measured so far.

Basic Histogram display



Axis Options

If you enable *Show Axis Options* at the bottom of the *Histogram*, you can customise the display:

Y Axis Options:

- Auto: Automatically sets the range.
- %: Range determined by the greatest value as a percentage of the total
- Log: Uses a logarithmic scale.

Display Settings:

- Title: Type in a new title for the histogram and press Enter.
- Mode: Choose from Differential, Cumulative + and Cumulative -
- Style: Choose from Bar Chart, Pie Chart and Horizontal Bar Chart

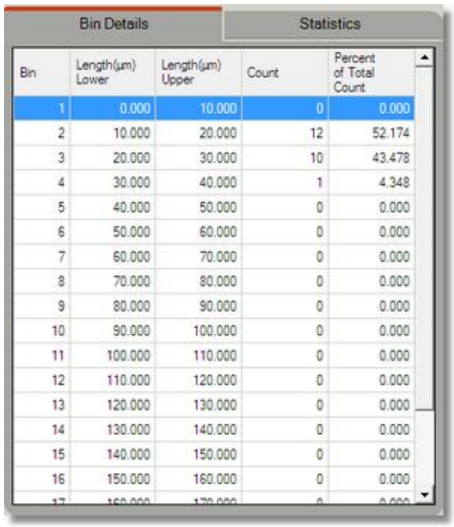
Labels:

- Limits: Length values for individual bins
- Names: e.g. Bin 1, Bin 2.

Bin Details and Statistics

If you enable *Show Bin Details*, this displays two extra tabs:

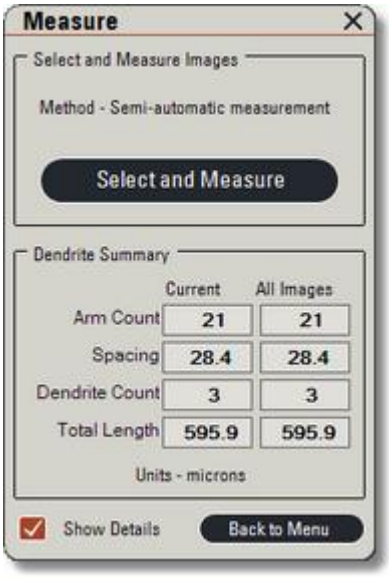
- Bin Details: Values for individual bins
- Statistics: Statistics based on all measurements.



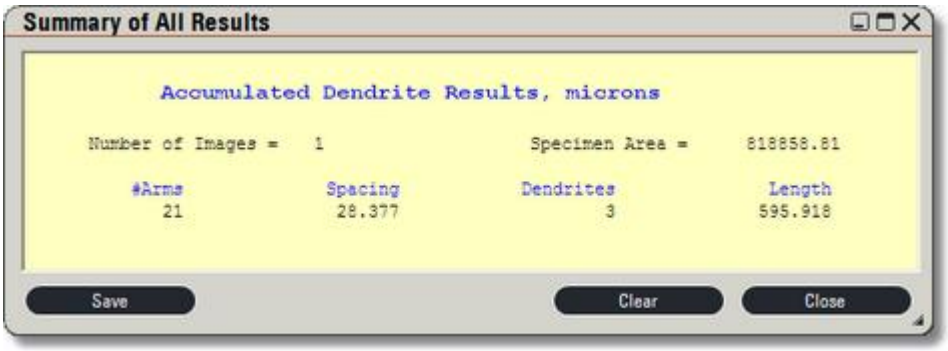
Summary of All Results

From the Measure[1315] dialog, you can display a summary of all results, for all the images you have measured in this session:

1: Enable *Show Details*.



2: The *Summary of All Results* window will be displayed:



(The *Feature Histogram*[1320] will also be displayed.)

3: Click *Save* if you want to save the contents of the *Summary* window as a text file.

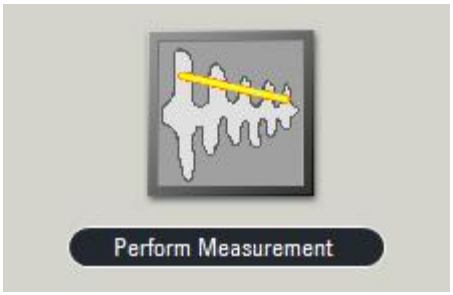
When using the Manual method:

- You must be accurate when drawing lines on your images.
- You need to count and manually enter the number of dendrite arms as you draw lines on an image.

Start the Process

To start the measurement process:

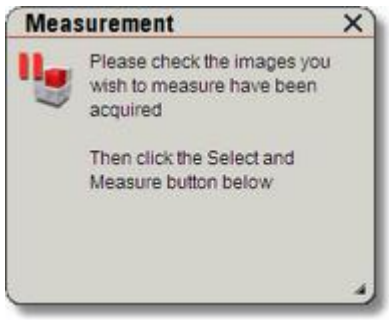
1: On the *Leica Dendrite Menu* click *Perform Measurement*.

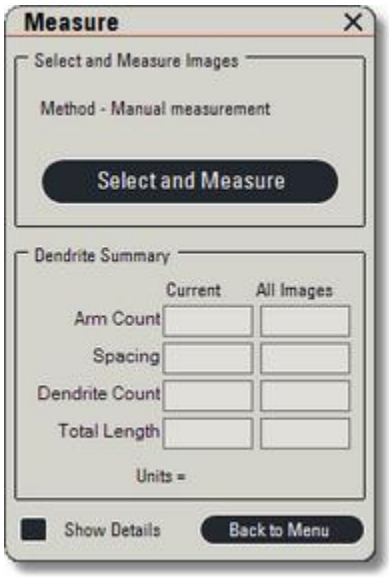


2: Click *Select and Measure*.

Notes:

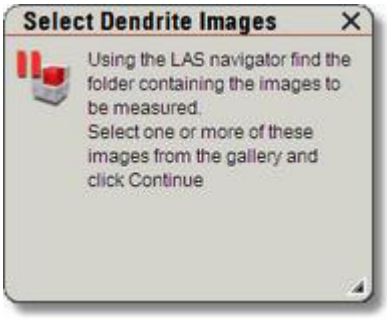
- The *Show Details* option is only relevant after you have made some measurements; it displays the Feature Histogram[1320] and Summary of All Results[1321].
- The *Dendrite Summary* pane shows information about measurements already made in this session (it will be blank to start with).





Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



1: Click on the *Show Navigator* button on the *Side Tool Bar*.

2: Move the *Navigator* to a convenient position by dragging the title bar.

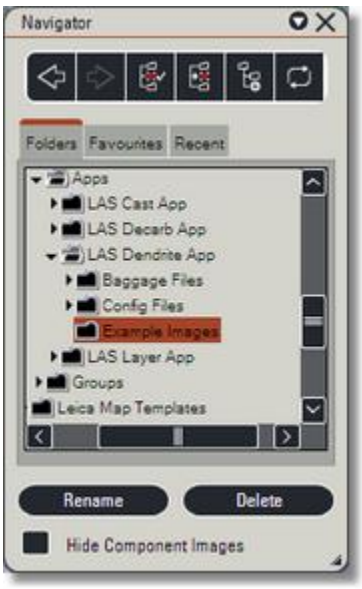
3: Navigate to and select the image folder.

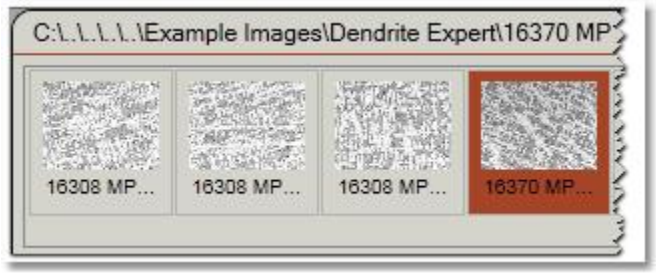
4: The available images will be displayed in the *Gallery*.

5: In the *Gallery*, click to select a single image, or Ctrl-click to select up to 10 images.

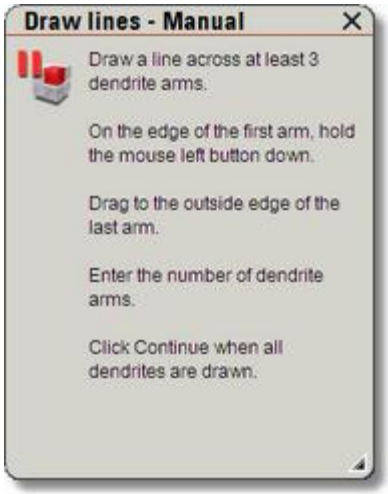
6: You can minimise the Navigator by clicking the arrow to the right of the header.

7: Click *Continue* to load the image. Any Grey Enhancement will be applied automatically.





The image appears in the *Image Viewer*.



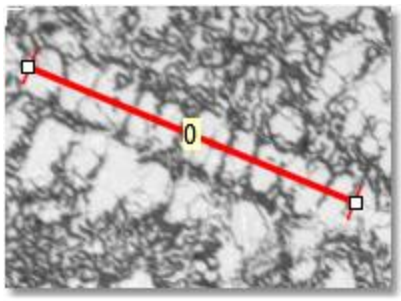
Important note
In the *Measure Tools* panel, the *Vector Line Tool* is automatically selected, **but not visible**; you can toggle between the *Vector Line Tool* and the *Selection Tool* by clicking the *Selection Tool* icon.

- *Vector Line Tool* active:

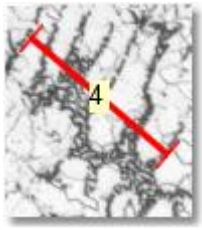
- *Selection Tool* active:

1: In the *Measure Tools* panel, enable *Show as Label*.

2: With the *Vector Line Tool* active, drag to draw a line across at least three dendrite arms.



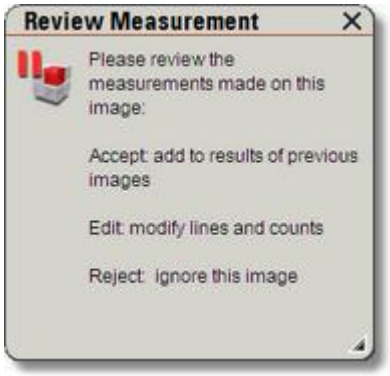
3: Immediately press the number keys corresponding to the number of dendrite arms crossed by this line (e.g. 4) then press the *Tab* key. The label will update to show the number you have entered, as will the value in the *Number of Dendrites* pane.

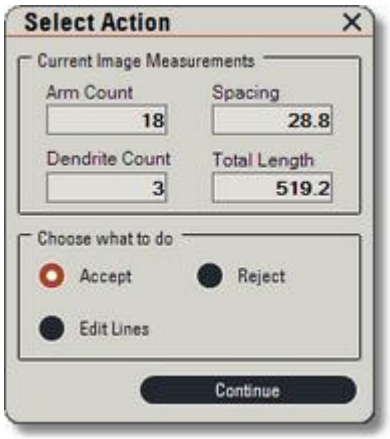


4: Repeat, until you have drawn at least three lines over different sets of dendrite arms.

5: Click *Continue*.

At this point, you can review the measurements you have made and choose what to do with them.





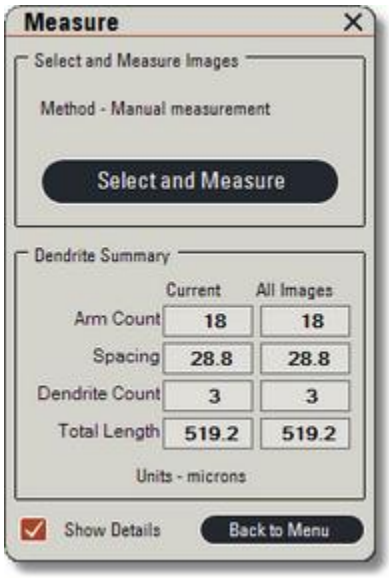
1: In the *Current Image Measurements* pane, you will see the details that have been calculated from the lines you drew (such as Arm Count, Dendrite Count, Spacing and Total Length).

2: In the *Choose what to do* pane, select one of the following:

- Accept the results and add them to any existing results.
- Edit the detection and remove spurious lines. This takes you back to the Draw Lines[1325] step, where you can use the Selection Tool and Vector Line Tool to amend your measurements.
- Reject the image and results. Select[1324] another image.

3: Click *Continue*.

This takes you back start of the measurement process, from where you can *Select and Measure* another image or click *Back to Menu* to return to the *Leica Dendrite Menu*.

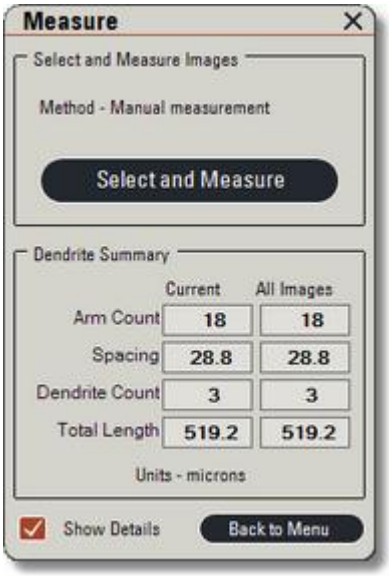


Once you have finished measuring images, the next step is to Enter Reference Data[1328].

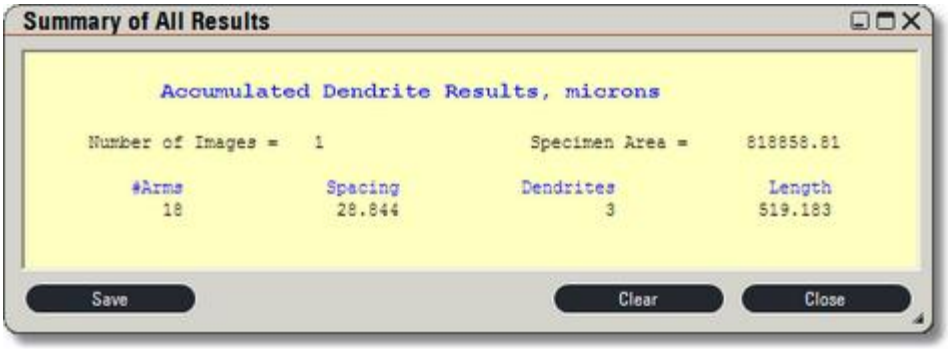
Summary of All Results

From the Measure[1324] dialog, you can display a summary of all results, for all the images you have measured in this session:

1: Enable *Show Details*.



2: The *Summary of All Results* window will be displayed:



3: Click *Save* if you want to save the contents of the *Summary* window as a text file.

Enter Reference Data

You can add information about specimens and processing on the *Reference Data* panel. Some of the information is displayed on the standard reports installed with the application.

1: On the *Leica Dendrite Menu* click *Enter Reference Data.* The *Reference Data* panel appears.

2: Click to select an item.

3: Click inside the text box to the right and type the data.

You can select the *Reference Data* headings to suit your working methods and descriptions. To do this you must run LAS as an administrator. (Right-click the desktop icon and select *Run as Administrator*.)

1: Click on the *User Define* button.

2: The *User Define Reference Data* dialog appears.

3: Click a *Visible* check box to show/hide that element in the *Reference Data* list.

4: The *Tools* from left to right are:

- Create: Adds a new user heading. The default is Field X; You can change this to a more appropriate name.
- Delete: Removes a selected heading completely.
- Tick Mark: Enables or disables the selected heading. (same functionality as the Visible check box).
- Select All: Enables all headings.
- Hide All: Disables all headings.
- Up/Down Arrows: Moves the selected heading up or down the list.

5: Click *OK* to finish.

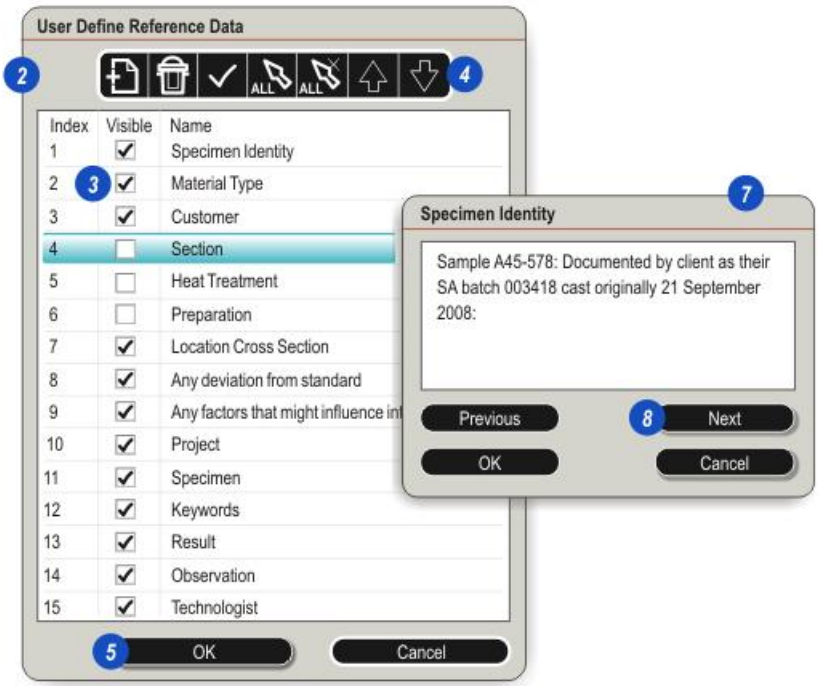
If any item of data is too long to fit inside the panel text box:

6: Click the *Edit* button.

7: Enter a heading in the resulting dialog.

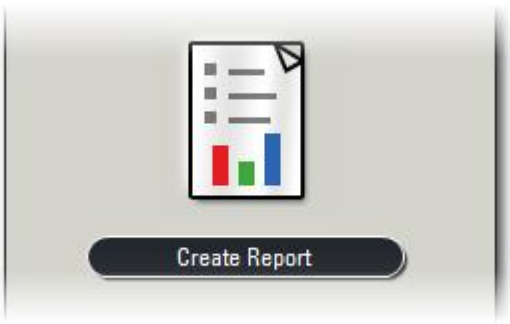
8: Scroll between the headings using the *Previous* and *Next* buttons.

9: Click *OK* to finish editing.
Note - If you change the Reference Data, you may need to modify the Excel Report[1330] template. After making changes to the Reference Data names, please check that what you see in the report is what you wanted. If not, please modify the report template to suit.



Create a Report

You can incorporate the measurement results into a comprehensive report using Microsoft Excel. The template for the report is installed with the application.



1: On the *Leica Dendrite Menu*, click the *Create Report* button to open the *Create Report* panel.

2: In the *Select Template* pane, click the browse button and select one of the standard report templates:

- Leica Dendrite Histo.xlt: For use with images measured using the Semi-automatic method
- Leica Dendrite.xlt: For use with images measured using the Manual method

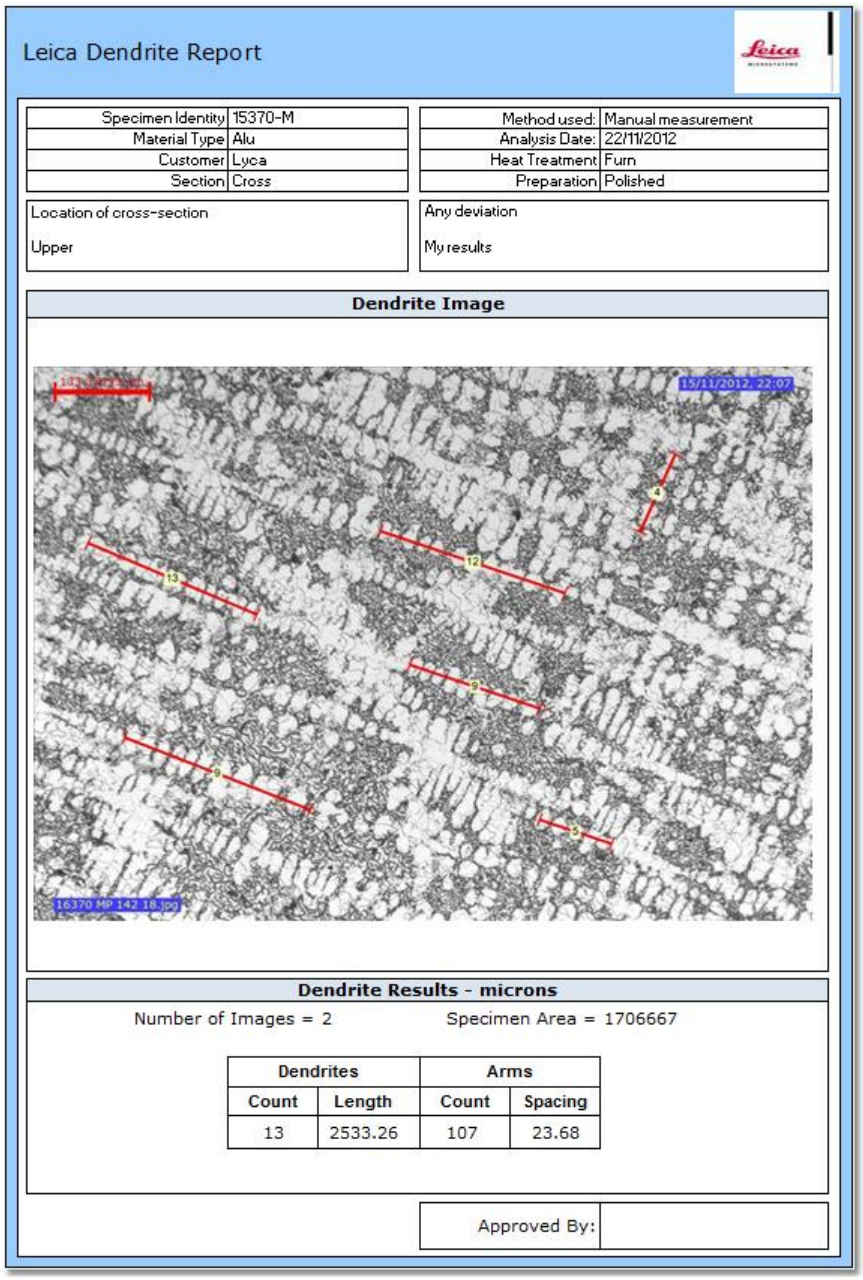
3: Enter a unique name for the report.

4: Click *Create Report*.

The report templates are located in the following folder:

C:\Users\Public\Documents\Leica Application Suite\LAS Apps\Apps\LAS Dendrite App

Below is an example of a *Leica Dendrite.xlt* Report.



Leica Dendrite Report

Specimen Identity	15370-M	Method used:	Manual measurement
Material Type	Alu	Analysis Date:	22/11/2012
Customer	Lyca	Heat Treatment	Furn
Section	Cross	Preparation	Polished

Location of cross-section Upper	Any deviation My results

Dendrite Image

Dendrite Results - microns

Number of Images = 2 Specimen Area = 1706667

Dendrites		Arms	
Count	Length	Count	Spacing
13	2533.26	107	23.68

Approved By:

Decarburisation Expert

Leica Decarburisation Expert helps identify decarburised regions of a sample imaged by an optical microscope, measures the decarburisation depth and produces tabulated results.

Leica Decarburisation Expert is fully compatible with the Leica Application Suite (LAS).

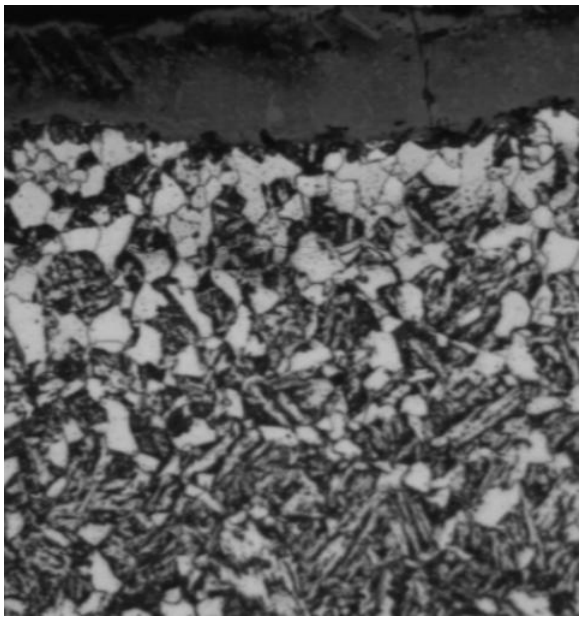
Features

- Step-by-step guided operation
- Interactive settings
- Results stored in named configurations for easy recall
- Include specimen details and reference information with results
- Simulation of standards including:
  - ASTM E1077 - Standard Test Method for Estimating the Depth of Decarburisation of Steel Specimens
  - ISO 3887 - Steel Non Alloy and Low Alloy Determination of Decarburisation Depth
  - JIS G0558 - Methods form Measuring Decarburised Depth of Steel
  - DIN 50192 - Determination of the Depth of Decarburisation
- Manual and Automatic methods
- Display of decarburisation regions on screen
- Display of profile showing the variation of decarburisation with position into the sample
- Accumulation of results over multiple images
- Display of individual results for each image
- Customisable Excel results template

Decarburisation Analysis

The depth of decarburisation is an indication of the deterioration in the metal at the surface. This may occur during processing or actually when in service, where the operating conditions are severe. Decarburisation is caused by the removal by oxidation of carbon from the surface of the product.

The depth of decarburisation can be determined visually using image analysis, by determining the quantities of carbon-bearing phases at the surface of the specimen and comparing the amount present to the unaffected interior. The brighter a region, the less carbon it contains.



Traditional microscope methods of measuring decarburisation depth are slow and require operator skill and understanding, while lacking the precision of automatic image analysis.

- In Manual mode, *Leica Decarburisation Expert* allows you to indicate the decarburisation regions by drawing lines on a digital image; the lines are measured and averaged to give the decarburisation results.

- In Automatic mode, *Leica Decarburisation Expert* determines the depth of decarburisation averaged over a defined region by interpreting the difference in the etching contrast shown as image brightness variation.

You can analyse multiple samples quickly and consistently.

See Definitions 1334

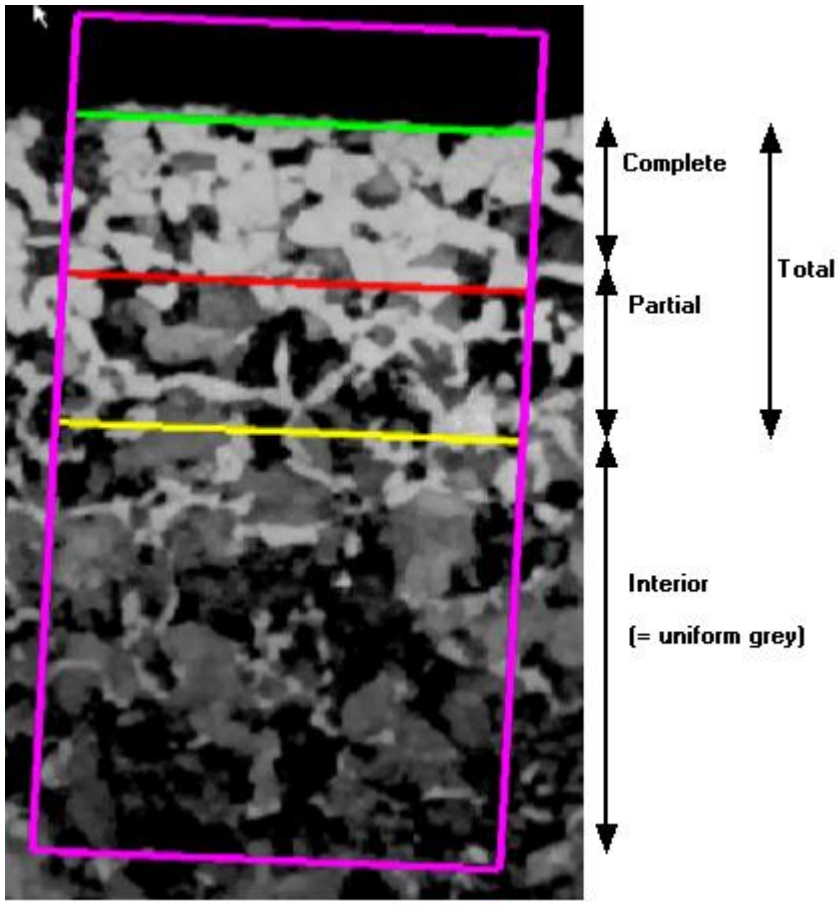
The decarburisation regions can be defined as:

- **Complete** decarburisation: Loss of carbon content to a level below the solubility of carbon in Ferrite, so that only Ferrite is present. This is the brightest region in a LAS decarburisation sample image.

- **Partial** decarburisation; Loss of carbon content at the surface of a steel specimen to a level less than the bulk carbon content of the unaffected interior but greater then the room temperature solubility limit of carbon in Ferrite. In a sample image, the grey level gradually increases with distance from the sample surface.

  You can specify where the Partial region ends using the Partial Value % 1344 setting.

- **Total** depth of decarburisation: The perpendicular distance from the specimen surface to the point in the interior where the normal carbon content is reached. This is the region furthest from the surface, where the grey level has become uniform. It represents the sum of the depths of Complete and Partial decarburisation.



Note:
Partial cut-off = (average grey level in the interior Total region) + (Partial Limit) * (average grey level in the Complete region - average grey level in the interior Total region)/100

The principal areas of the user interface:

1: *Menu Bar*: For Options and Help.

2: *Workflows*: Leica Cast Iron Expert is available on the Analysis Workflow.

3: *Application Panels* Tab: Click *Runner*.

4: *Prompt Message Panels*: Hints for program sequence steps.

5: *Control and Results Panels*: Used to operate the application and to show summary results.

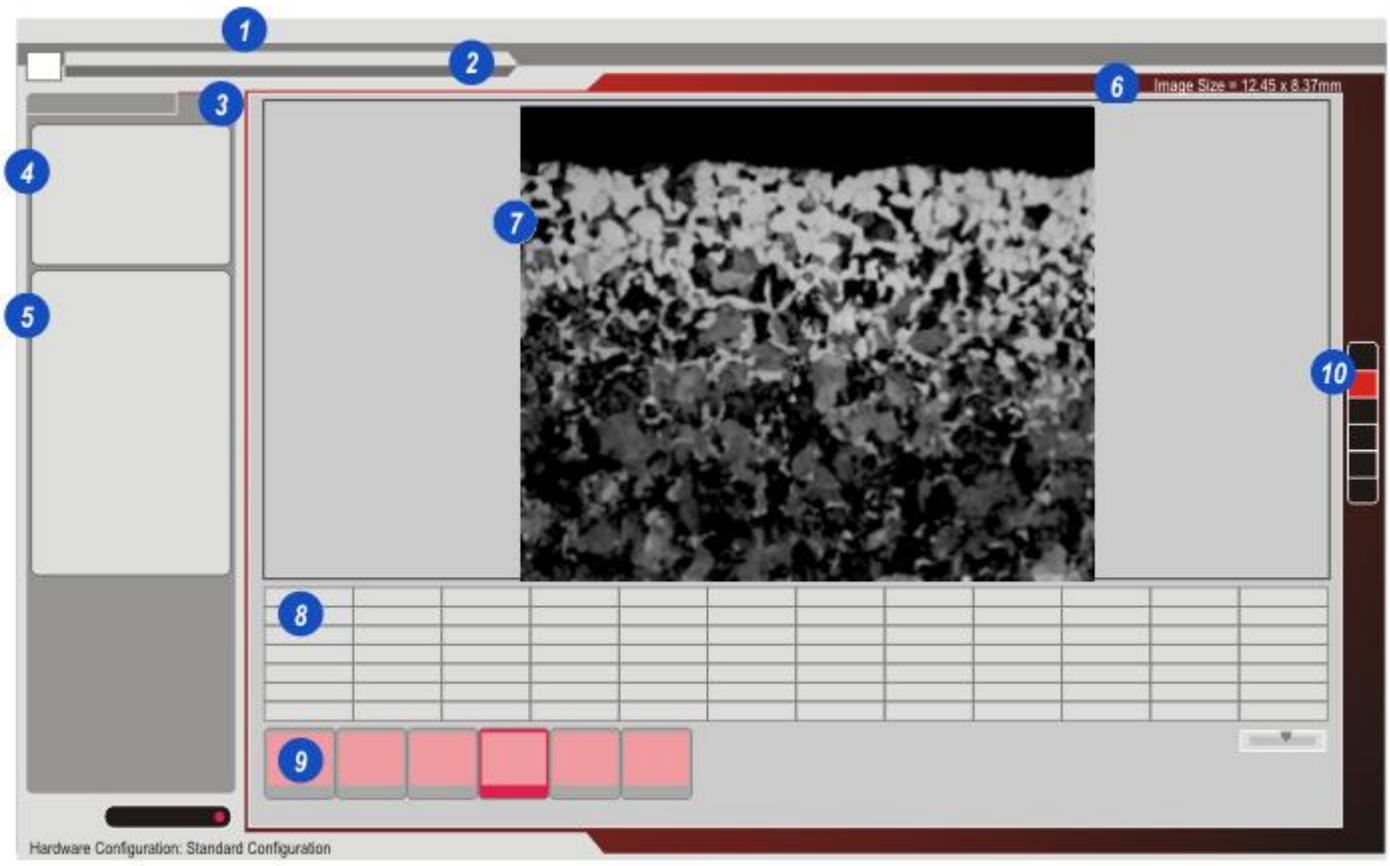
6: *Image Scaling* information.

7: *Image Viewer*: Shows original image, sometimes with the superimposed Decarburisation positions marked.

8: *Results Grid*: Not used by this application.

9: *Gallery of Image Thumbnails*: Used to select the images to measure.

10: *Side Tool Bar.*



Getting started – new users

- Check out all the links in the Help navigator to the left – plenty of help is available!
- To view the *Leica Decarburisation Expert* help, press F1 while the application is running.

Getting started – new users of LAS

- *Leica Decarburisation Expert* uses LAS to acquire images and is started from the **Analysis > Runner** step of LAS
- Please refer to the main LAS help for information concerning the configuration, calibration and use of LAS to acquire images.
- To view the LAS help, press F1 while LAS is running.

Supported Microscopes, Cameras, Computers and Software

Please see the *Systems Requirements* PDF file:

Start > Leica Application Suite V4 > Documents

This provides details of all supported hardware and recommends suitable computer specifications. In case of doubt, please run the *LAS PC Performance* utility and observe the recommendations it produces.

Prerequisites

LAS Image Analysis must be purchased and licensed on the same system as *Leica Decarburisation Expert*.

LAS Runner must be licensed on the same system as *Leica Decarburisation Expert*. This is included with the purchase of a *Leica Decarburisation* License.

Microsoft Excel is required if you wish to customise the report template.

Image Format

Colour and Monochrome images are supported.

It is recommended that the image pixel size is in the range 1 to 3.3 Mpixel. Images with pixel sizes greater than 5 Mpixel are not supported.

Display resolution

The minimum screen resolution is 1280 x 1024. If you use a lower vertical resolution some of the control panels may be obscured. You will be able to move them to a position where they are visible, but this is rather inconvenient. Please minimise the height of the Windows task bar as this occupies some of the vertical screen space.

Image Acquisition

Please ensure that the images are acquired with the correct calibration. This will be more certain if you are using a microscope with a coded nose-piece. If not, then make sure that the objective in use is the same as that selected in LAS.

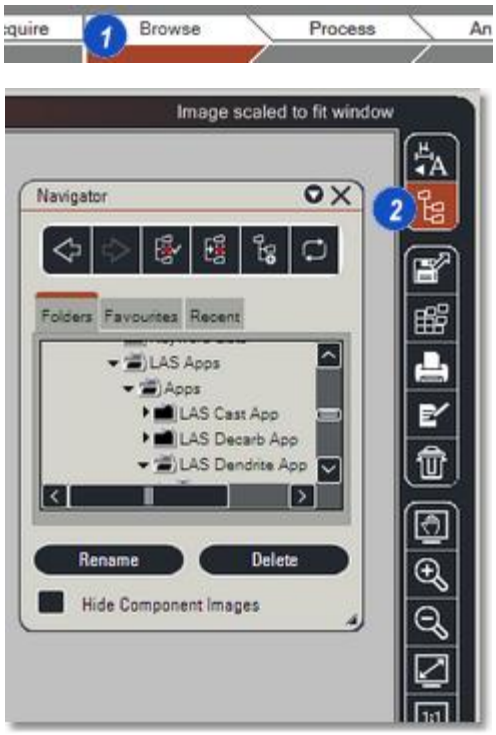
Adjust the camera exposure, gain and gamma to provide a high quality image. In particular, ensure that an HQ format is used, the value of gain = 1 and the histogram black and white levels are set to the default values.

If you are using the automatic method, it is strongly recommended that the shading correction is set for all images acquired. This will ensure that the thresholding is performed uniformly over the entire image.

Calibration Units

Please ensure that all images that you measure at the same time have the same calibration for consistent results.

Make sure the Navigator panel is floating



You need to ensure that the *LAS Navigator* is floating before starting an *Expert* application. This allows you to select test images and real sample images that you have acquired, for use within the application.

1: In LAS, select the *Browse Workflow*.

2: If the *Navigator* panel is docked with the other Control Panels on the left, click the *Show Navigator* button on the *Side Tool Bar* to make it float.

3: You are now ready to start[1339] your *Expert* application.

Start Leica Decarburisation Expert

1: Select the *Analysis Workflow*.

2: Display the *Runner* tab.

3: If necessary, click to expand the *LAS Application Selection* panel.

4: Click on an icon to select your chosen Expert Application.

5: Click *Run*.

6: If the *LAS Application Selection* panel is not visible click the *Application Selection* button.

When the application start-up screen appears, do one of the following to start the application:

- Click *OK*
- Click the logo.

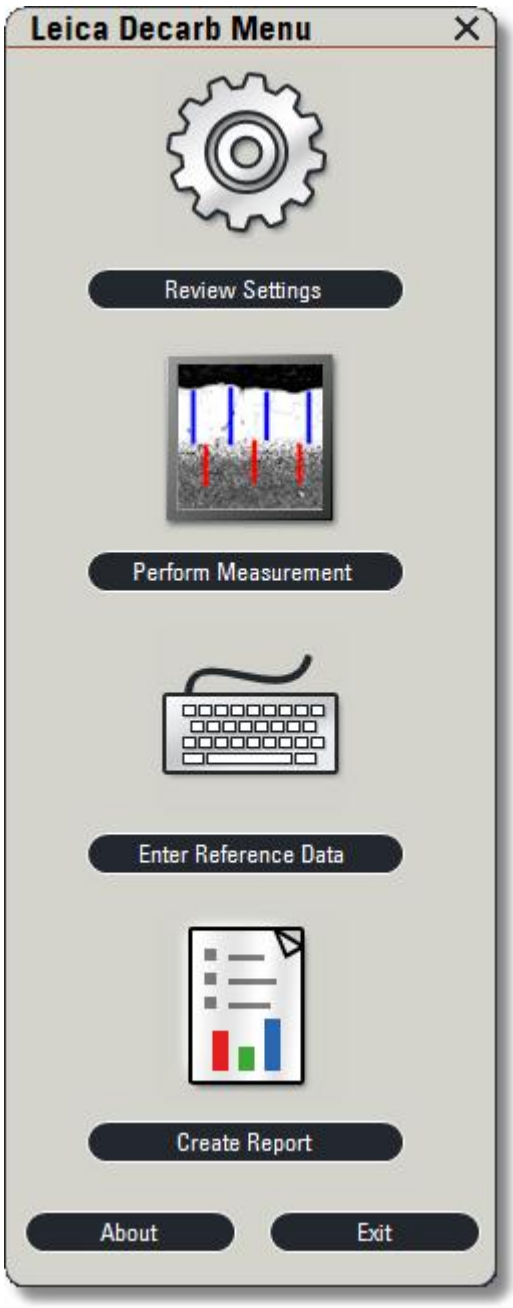
The *Leica Decarb Menu* provides links to the steps you should take to analyse a Decarburisation specimen.

Before using this menu, please ensure that the images have been acquired using LAS 1342 and stored in a known folder.

Note: It is preferable to acquire images with a resolution approximately in the range to 1 to 3 Mpixels.

Note: Ensure that the images are correctly calibrated, particularly when using manual microscopes.

- *Review Settings* 1343*:* Select the measurement method and the standard to use. Use default parameters or create user-defined settings. Determine the detection limits and then use the supplied or user images to test the settings.
- *Perform Measurements* 1350*:* Uses the parameters established in *Review Settings* to detect and analyse the specimen.
- *Enter Reference Data* 1366*:* Add comments and specimen details that will be included in the final report. This is where you can add the notes required by the standards.
- *Create Report* 1368*:* The results and reference data are presented using a supplied Excel template that can be modified to suit user or company styles.



The first stage of Decarburisation analysis is to acquire a selection of digital images using LAS and to save these to the computer's hard drive. The advantage of this approach is that the original images are always available to check your results later.

- To obtain precise results from *Leica Decarburisation Expert* you need a good, representative sample of the specimen that has been cut from the product, revealing a section perpendicular to the surface of the sample.

- The decarburisation to be measured should lie flat and in focus over the whole field of view. The sample surface should lie approximately parallel to the top of the image. The decarburisation region should occupy the upper half of the image.

- Images should be processed to display optimum sharpness and contrast before acquisition. Pay careful attention to the shading correction for images that are to be measured automatically.

LAS acquires calibrated images by reading the magnification from the microscope and the sensor size from the camera to accurately determine the image dimensions.

- Imaging conditions (such as microscope settings and camera exposure) are automatically recorded by the software. This data is stored with the image and is useful for checking consistency.

- Images are named and acquired into a Windows folder. It's a good idea to make a note of these details.

- You can annotate images with a calibrated scale bar and labels (such as date, time, image name and description).

  Please refer to the Advice and Prerequisites 1337 for the recommended image capture settings.

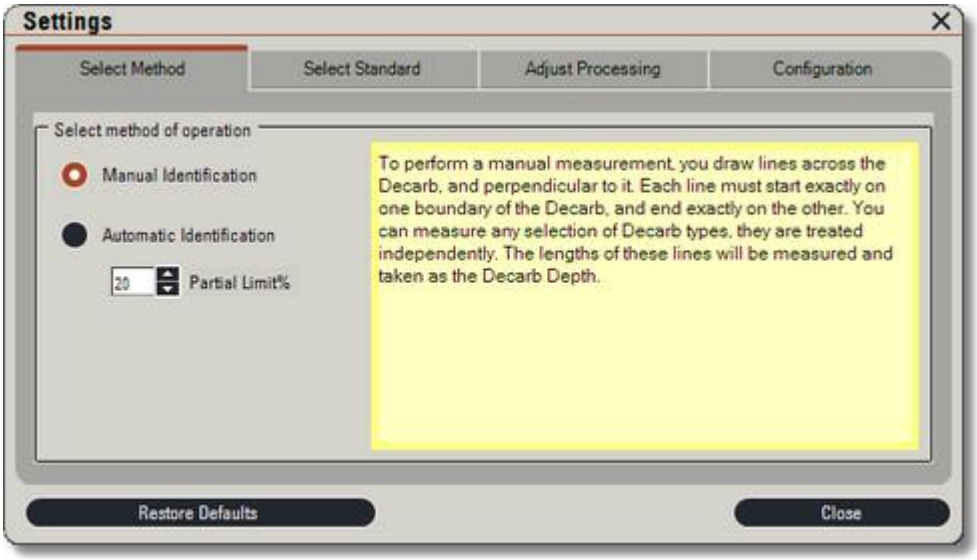
1: Click *Review Settings* on the *Leica Dendrite Menu*.

The *Settings* dialog has the following tabs:

- *Select Method*[1344]*:* Decide which measurement method to use, according to the type of specimen.
- *Select Standard*[1345]*:* Choose a standard to use.
- *Adjust Processing*[1346]*:* Specify how image processing is applied, to help identify the decarb regions. You can apply the settings to a test image to check that analysis is working properly, before working on a real sample image.
- *Configuration*[1349]*:* You can define overlay colours, specify a report template, create new configuration files or load configuration files that you have previously saved.

Notes

- If you want to revert to the default settings for the application, click *Restore Defaults*. You can do this at any time before clicking *Close*.
- If you close the *Settings* dialog and save your changes, this will **overwrite** your current configuration file, giving you a new set of defaults.



Select Method

The *Select Method* tab allows you to choose the way in which measurements are made.

The method you select here will be used every time you perform measurements[1350], until you change the selected method.

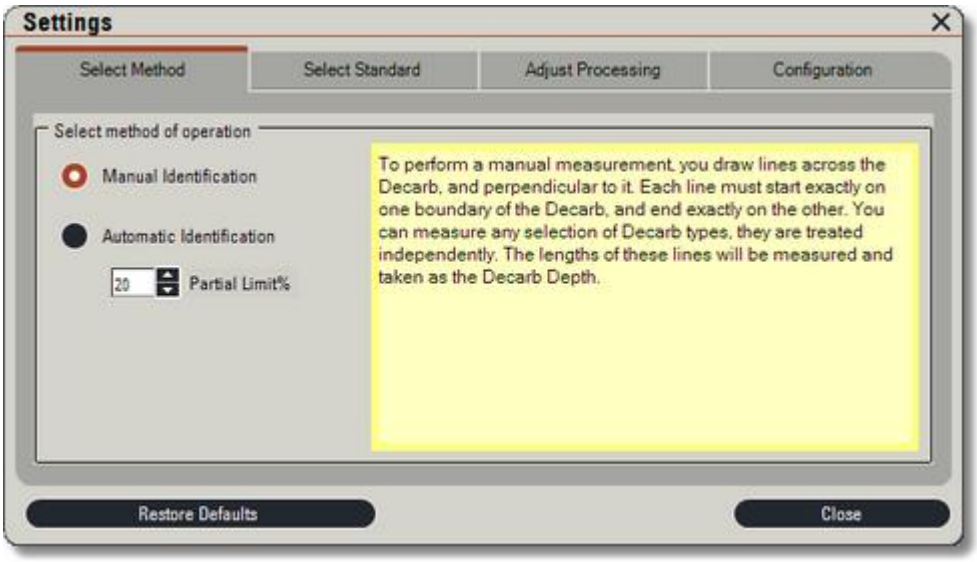
1: Display the *Select Method* tab.

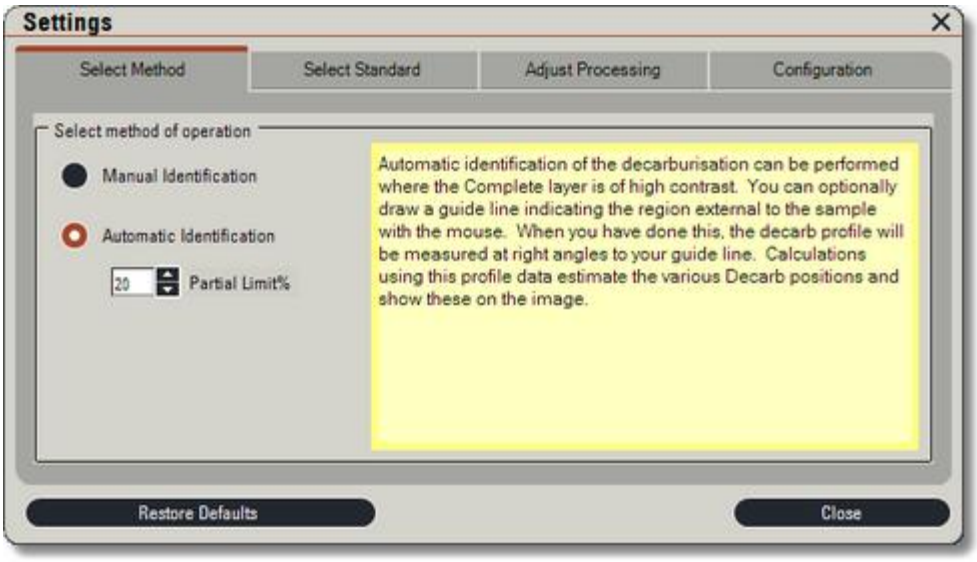
2: Select a method (*Manual* or *Automatic*).

3: Specify the *Partial Value %* to use for this method.

Use this setting to define where, for your purposes, the Partial region[1334] ends. For example, you might decide this is 10% above the uniform grey level of the Interior region of a sample.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).





Select Standard

Choose the standard to use from the following options:

- **ASTM E1077**: Standard Test Methods for Estimating the Depth of Decarburisation of Steel Specimens.
- **ISO 1463**: Metallic and Oxide Coatings - Measurement of Coating Thickness - Microscopical Method
- **JIS G0558**: Steels - Determination of depth of decarburisation
- **DIN 50 192**: Determination of Depth of Decarburisation

The Standard selected does not change any of the measurement methods or other aspects of the operation other than the text that is used to describe the standard.

You can enter the notes required by the standard in the Reference Data[1366].

1: Display the *Select Standard* tab.

2: Click a radio button to select a standard.

3: Enter any text that you want to be included in reports that use this standard.

4: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).

Adjust Processing

The *Adjust Processing* panel allows you to optimize the image for the best decarb region detection and subsequently to remove any unwanted artifacts.

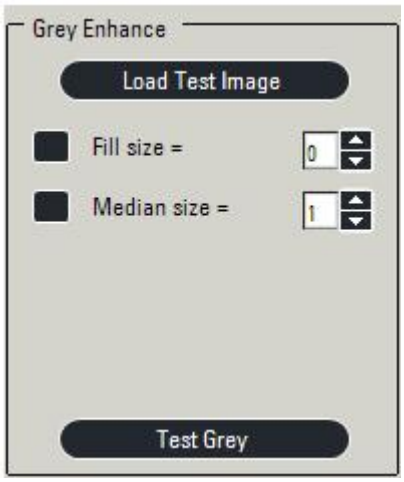
1: Display the *Adjust Processing* tab.

2: Enter the Grey Enhance Settings[1347].

3: Make and test the adjustments then *Close* the dialog or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made).

4: If you want to revert to the default settings, click *Restore Defaults*.

Grey Enhance Settings



Grey Enhance settings

Fill size - this removes local detailed variations in the image and is often very effective.

Median size - this is a noise filter that removes sharp spikes in the image with minimum effect on the resolution.

The grey processing functions are applied to the selected image to enhance the contrast. With the functions provided, you can experiment on test images to achieve acceptable results.

1: Load an image similar to those that you want to measure. See Load Test Image 1348

2: Select the functions you want to use by enabling the check box and entering a size value. Appropriate size values are in the range 1 to 20. Larger values will increase the effect of the function but will also extend the time taken to process the image.

3: Click the *Test Grey* button to apply the selected function.

You can now examine the effect of the function. Continue to experiment until you are happy that the image appears with good contrast.

Note that image processing will change the appearance of the image and it may seem to have less detail as a result.

Load Test Image

The test image can be one that you have captured or one of the samples installed with the application. These are stored in:

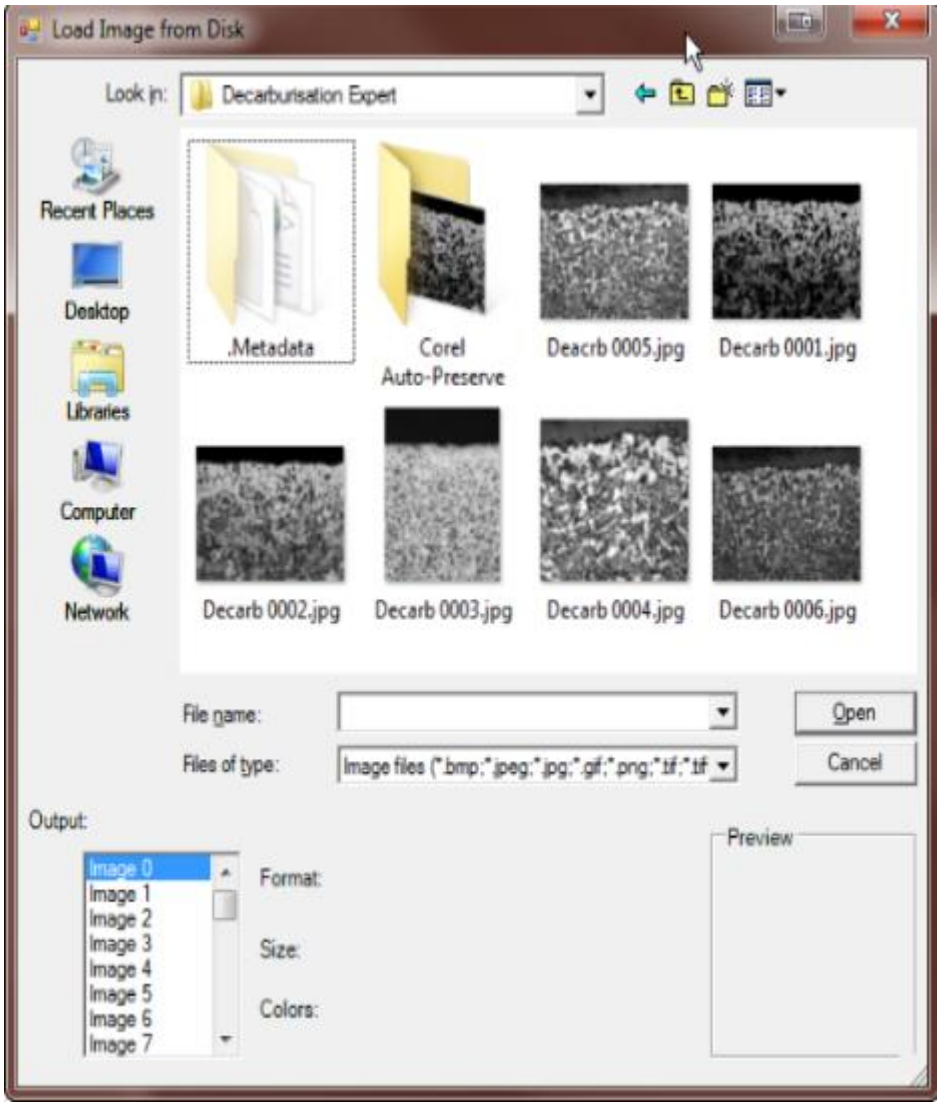
Users > Public Documents > Leica Application Suite > LAS Apps > Apps > LAS Decarb App > Example Images

When you click the *Load Test Image* button:

1: The *Load Image from Disk* dialog appears.

Navigate to either the *Example Images* folder or to a user-defined folder, click to select an image, then click *Open*.

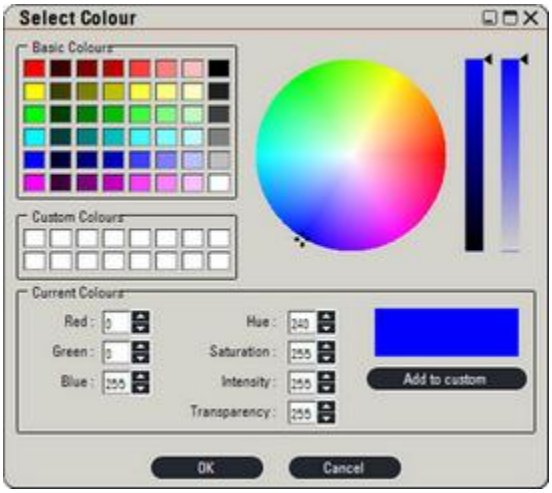
Once you have loaded a test image, adjust the grey enhance settings as described here 1347.



The *Configuration* tab allows you to choose the Overlay line width and colours, and Load and Save a Configuration.

1: Display the *Configuration* tab.

2: To change the colours of overlays (for example to give a better contrast against the image you are working with) click on the appropriate colour swatch. Pick a new colour using the *Select Colour* dialog:



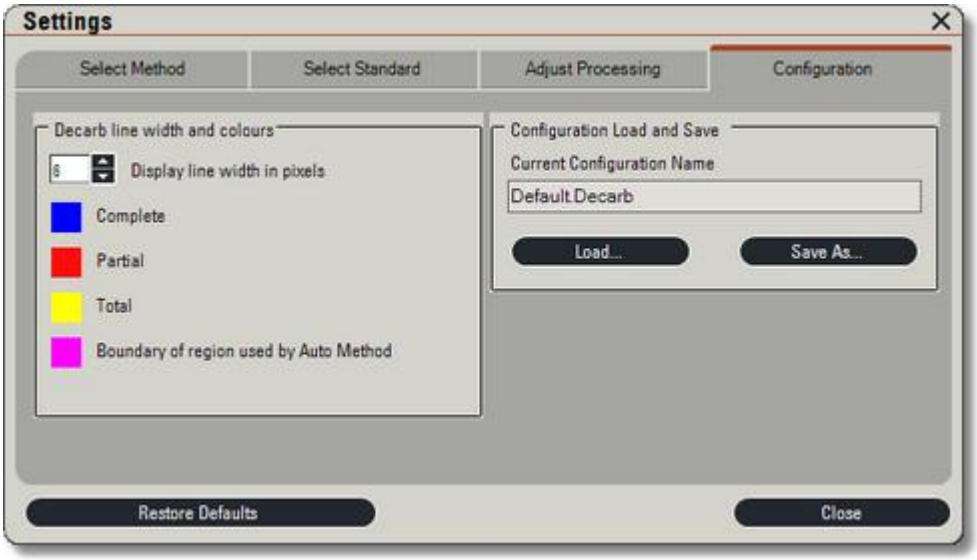
3: To change the line widths, enter a value in pixels or use the up/down arrows.

4: At this point you can click *Save As* to save all your changed settings to a named configuration file. These files are located in the following folder (and have the extension .Decarb):

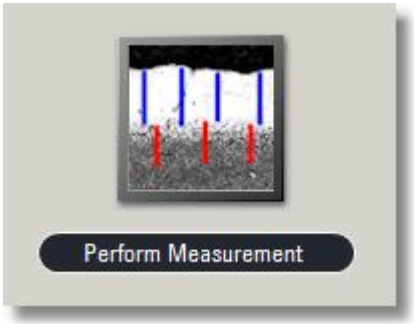
C:\Users\Public\Documents\Leica Application Suite \LAS Apps\Apps\LAS Decarb App\Config Files

You can also *Load* existing configuration files that you have tuned to different types of specimen.

5: Click *Close* or display another tab (if you close the *Settings* dialog, you will be asked if you want to save any changes you have made; this will **overwrite** your current configuration file, in effect giving you a new set of defaults).



Overview



Automatic Method

The basic method for selecting, detecting, measuring and showing the results is as follows:

1: Select an image to measure.

2: Optionally draw a line outside and parallel to the edge of the sample.

3: Allow Leica Decarburisation Expert to calculate and draw the regions automatically.

4: Review the measurements and choose what to do with them (re-measure, edit, accept or reject).

5: Repeat the above steps to measure another image, or return to the *Leica Decarburisation Menu*.

Start the Automatic Process 1351

Manual Method

The basic method for selecting, detecting, measuring and showing the results is as follows:

1: Choose whether to measure Complete, Partial or Total regions.

2: Select an image to measure.

3: Draw lines on the image to indicate the region you chose above.

4: Review the measurements you have made and choose what to do with them (change Decarb type, draw more lines, accept measurements, reject image).

5: Repeat the previous step as necessary until you have drawn lines on as many Decarb types as you require.

6: Repeat the above steps to measure another image, or return to the *Leica Dendrite Menu*.

Start the Manual Process 1359

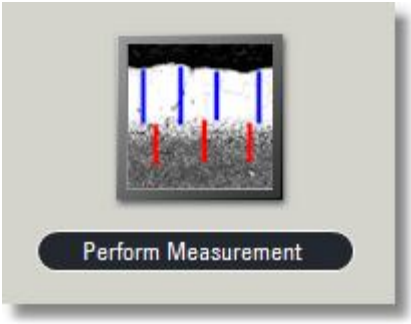
Automatic Method

The Automatic method uses the power of *Leica Decarburisation Expert* to make the process of measuring the decarburisation regions easier for you.

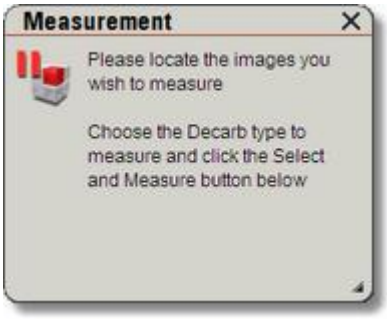
- The process is entirely automatic, unless you choose to draw an optional line to denote the edge of the sample (e.g. if the sample is not horizontal on your image, or you only want to measure using part of the image).

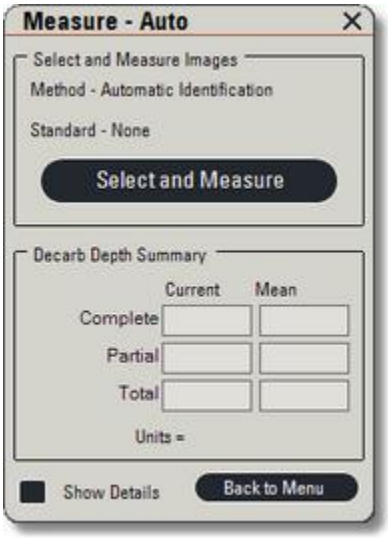
To start the measurement process:

1: On the *Leica Decarb Menu* click *Perform Measurement*.



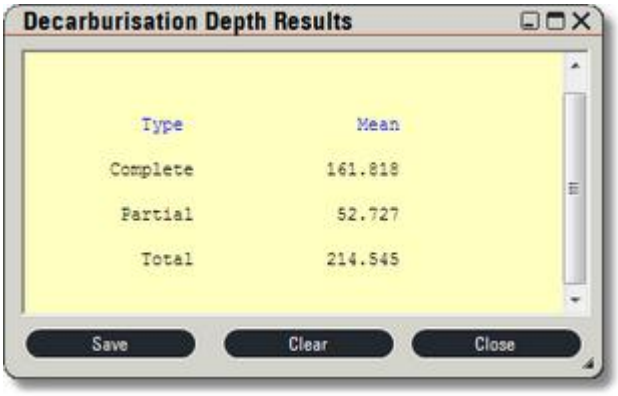
2: Click *Select and Measure*.



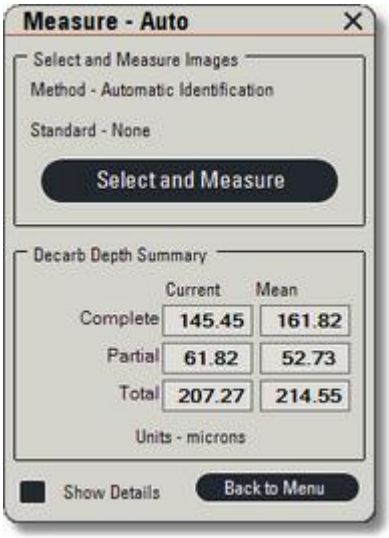


Notes:

- The *Show Details* option is only relevant after you have made some measurements; it displays information about measurements already made in this session.

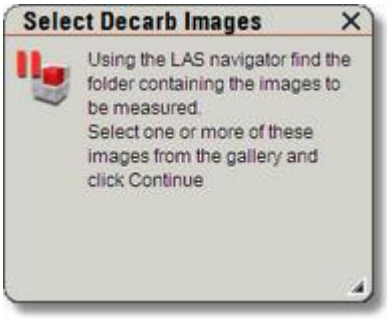


- The *Decarb Depth Summary* pane shows information about any measurements already made in this session (it is blank to start with).



Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



1: Click on the *Show Navigator* button on the *Side Tool Bar*.

2: Move the *Navigator* to a convenient position by dragging the title bar.

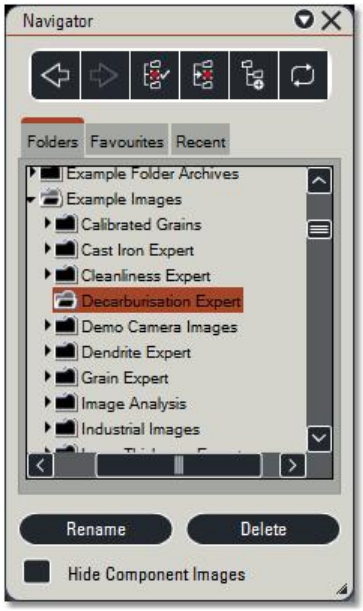
3: Navigate to and select the image folder.

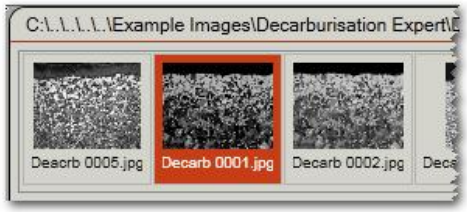
4: The available images will be displayed in the *Gallery*.

5: In the *Gallery*, click to select a single image, or Ctrl-click to select up to 10 images.

6: You can minimise the Navigator by clicking the arrow to the right of the header.

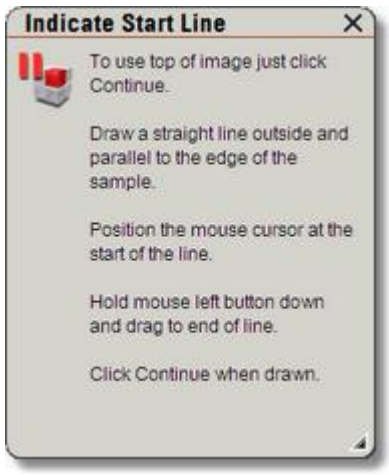
7: Click *Continue* to load the image. Any Grey Enhancement will be applied automatically.





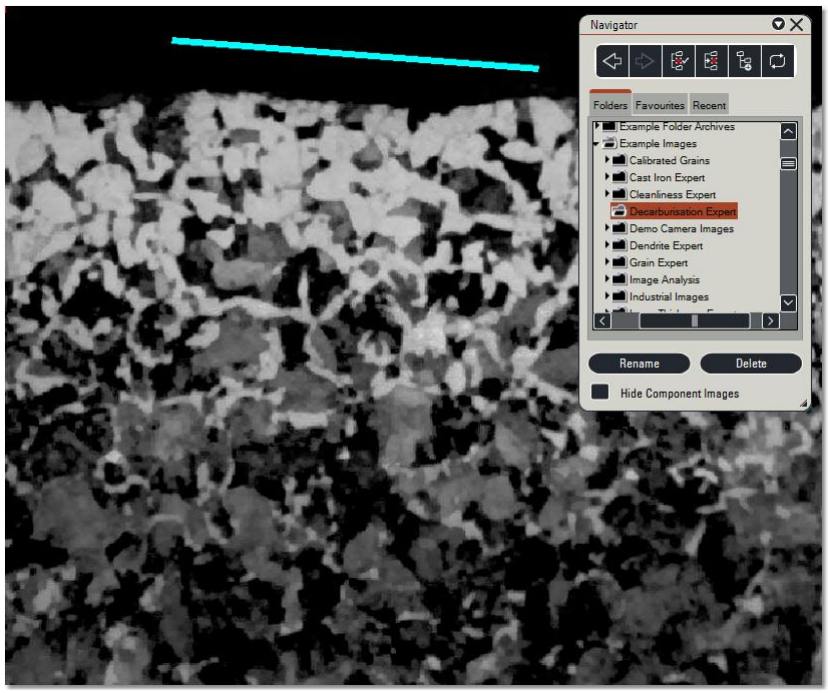
Indicate Start Line (Optional)

(Optional step) If your image is not straight, or you only want to measure Dacarb depth using part of your image:



1: Draw a line outside and parallel to the edge of the sample.

Note that the Line tool is selected automatically for you at this stage:



Generate the measurements

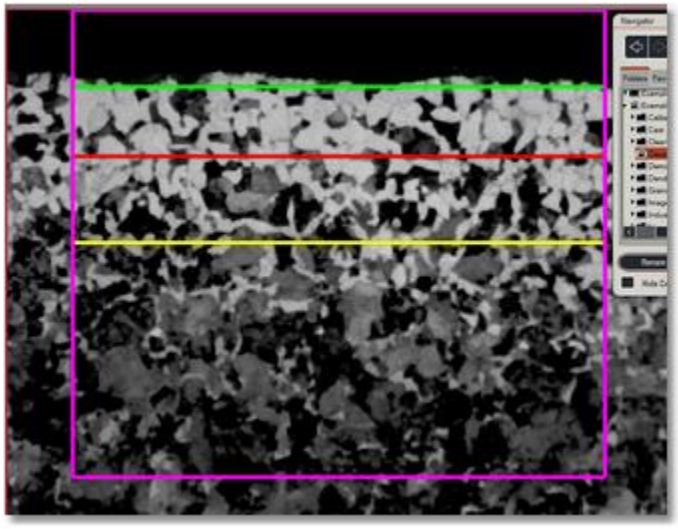
If you are happy for Leica Decarburisation Expert to perform all measurements completely automatically (or if you have drawn an optional Start Line [1354] and are ready to proceed):

1: Click *Continue*.

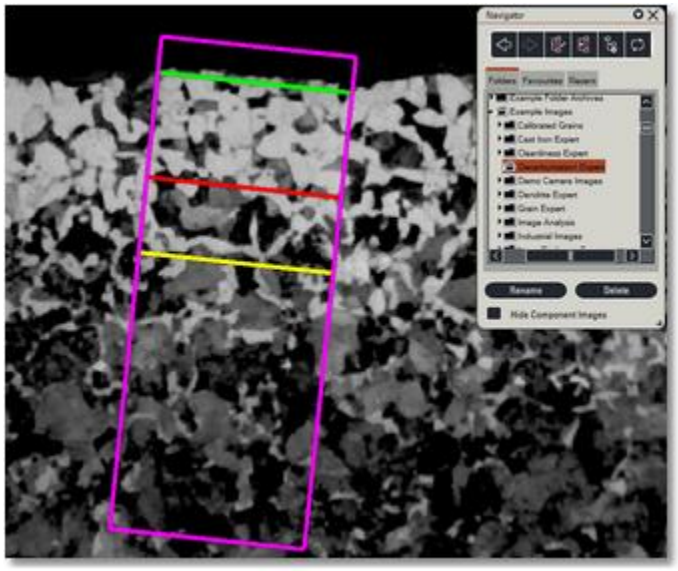
2: *Leica Decarburisation Expert* will take a few moments to calculate the Decarb regions

3: Results depend on whether you chose to draw an optional start line. See the example images on the right.

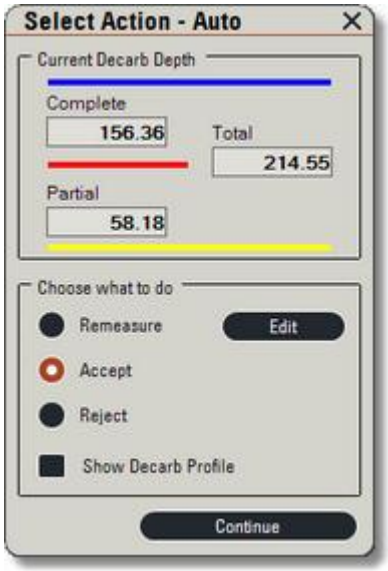
Top line drawn automatically



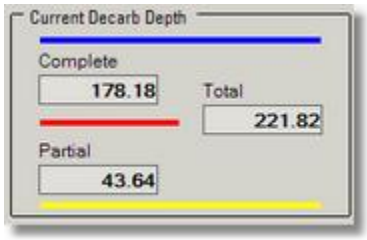
User-drawn top line



At this point, you can review the measurements you have made and choose what to do with them.



1: In the *Current Decarb Depth* pane, you will see the regions that have been calculated automatically:



2: In the *Choose what to do* pane, select one of the following:

- *Remeasure* the Decarb regions; this takes you back to Indicate Start Line (Optional) [1354]
- Edit [1357] the regions manually
- *Accept* the results and add them to any existing results
- *Reject* the image and results. Select [1353] another image.
- *Re-Detect*: This takes you back to Indicate Start Line (Optional) [1354]
- *Show Decarb Profile* [1358]: Displays the Feature Histogram (which depicts the currently calculated Decarb Distribution in microns)

3: When you have completed any resulting steps, click *Continue*.

This takes you back to Start the Process [1352], from where you can select and measure another image or return to the *Leica Decarb Menu*.

Once you have finished measuring images, the next step is to Enter Reference Data [1366].

Edit Calculated Regions

If you want to make adjustments to the calculated Decarb regions:

1: Enter new values in the *Current Decarb Depth* pane, or use the up/down arrows to adjust the values.

As you adjust the values, the boundary lines will be redrawn on the image, so you can check your changes.

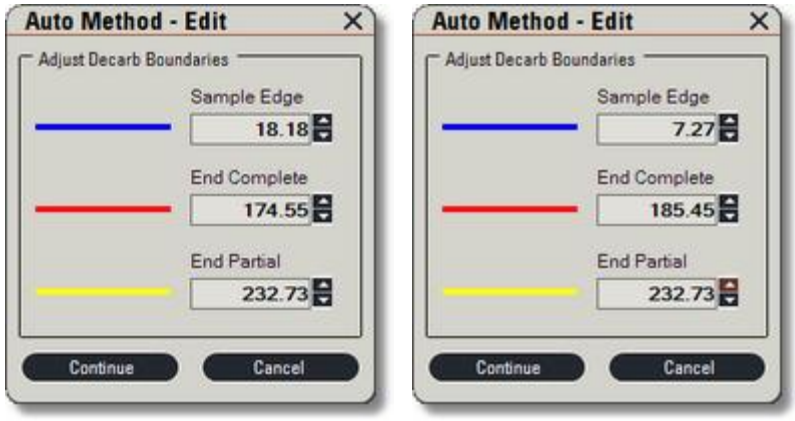
Note: The values you enter are relative to the Start Line.

2: At this point, you can enable Show Decarb Profile[1358].

3: Click *Continue* at the bottom of the *Auto Method - Edit* dialog.

This takes you back to Review Measurements[1356].

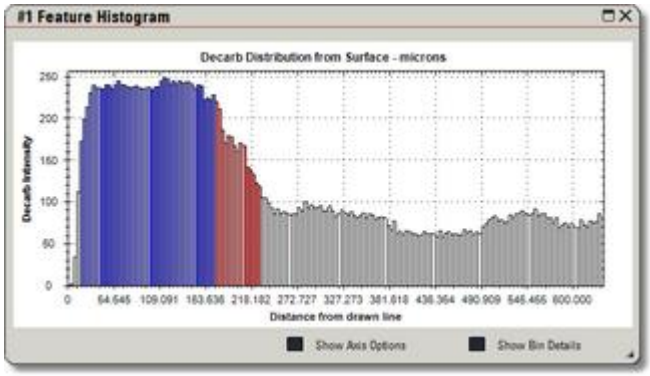
Editing the Adjust Decarb Boundaries settings



Current Decarb Depth, before and after editing

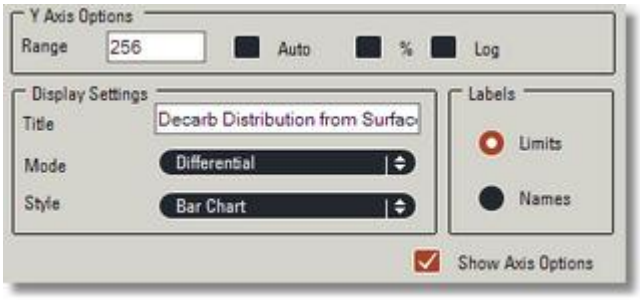
The histogram displays the profile of decarburisation variation for the current image.

Basic Histogram display



Axis Options

If you enable *Show Axis Options* at the bottom of the *Histogram*, you can customise the display:



Y Axis Options:

- *Range*: If not set to Auto, this is best set to 256 (total number of grey levels)
- *Auto*: Automatically sets the range
- *%*: Range determined by the greatest value as a percentage of the total
- *Log*: Uses a logarithmic scale

Display Settings:

- *Title*: Type in a new title for the histogram and press Enter.
- *Mode*: Choose from Differential, Cumulative + and Cumulative -
- *Style*: Choose from Bar Chart, Pie Chart and Horizontal Bar Chart

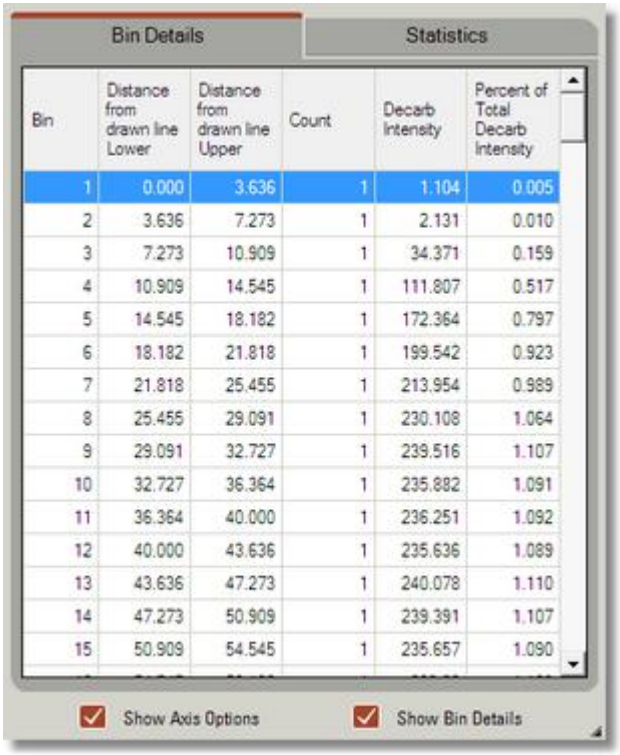
Labels:

- *Limits*: Length values for individual bins
- *Names*: e.g. Bin 1, Bin 2.

Bin Details and Statistics

If you enable *Show Bin Details*, this displays two extra tabs:

- *Bin Details*: Values for individual bins
- *Statistics*: Statistics based on all measurements.



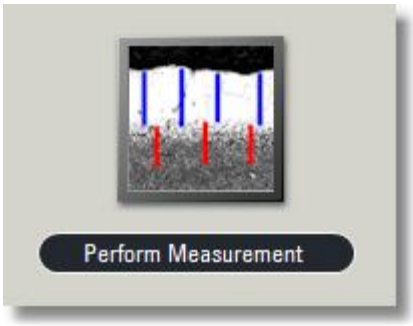
Manual Method

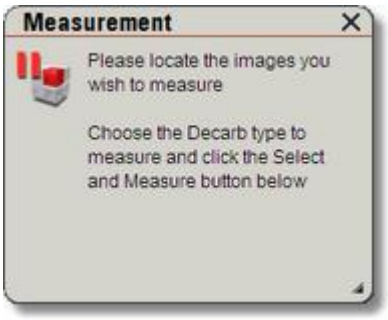
The Manual method allows you to draw measurement lines representing the various Decarburisation regions.

- Use your judgement to measure regions on samples that may not be suited to the Automatic[1351] method.

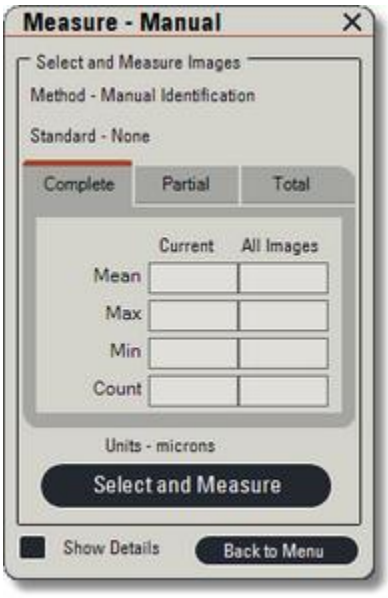
To start the measurement process:

1: On the *Leica Decarb Menu,* click *Perform Measurement*.





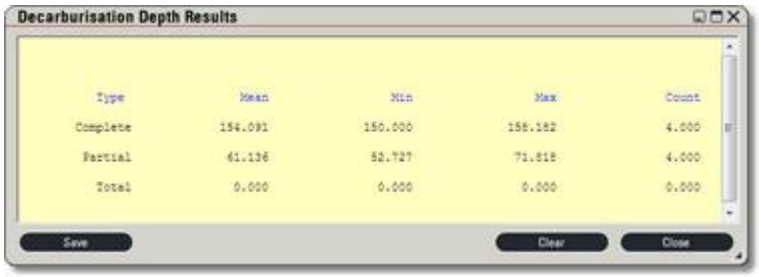
2: In the *Measure - Manual* dialog, click a tab to select which Decarb measurements you want to take first (*Complete*, *Partial* or *Total*). You will have the opportunity to measure other Decarb regions later in the process.



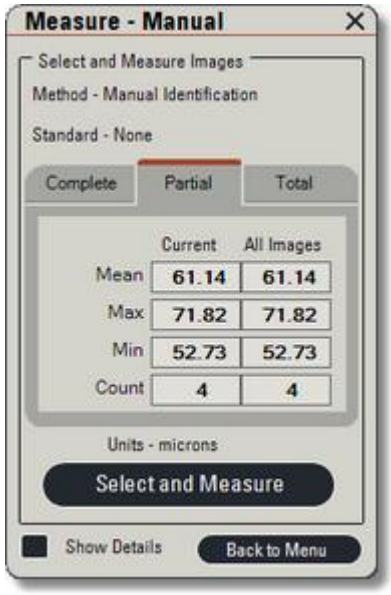
3: Click *Select and Measure*.

Notes:

- The *Show Details* option is only relevant after you have made some measurements; it displays information about measurements already made in this session.

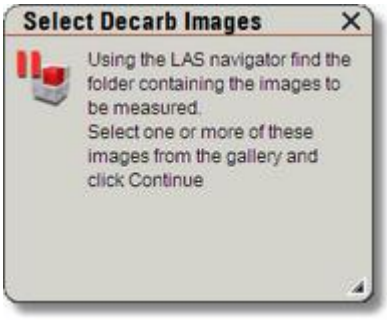


- The *Decarb Depth Summary* pane shows information about any measurements already made in this session (it is blank to start with).



Select Images to Measure

Use the *LAS Navigator* to select the source folder for the images to be measured.



1: Click on the *Show Navigator* button on the *Side Tool Bar*.

2: Move the *Navigator* to a convenient position by dragging the title bar.

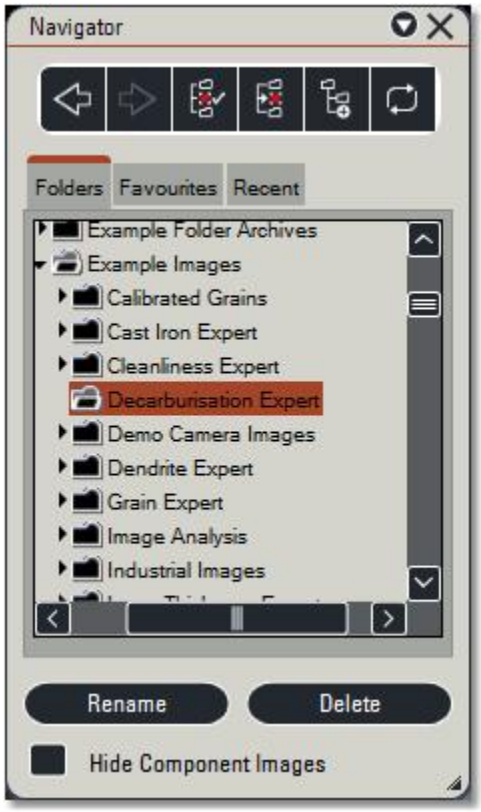
3: Navigate to and select the image folder.

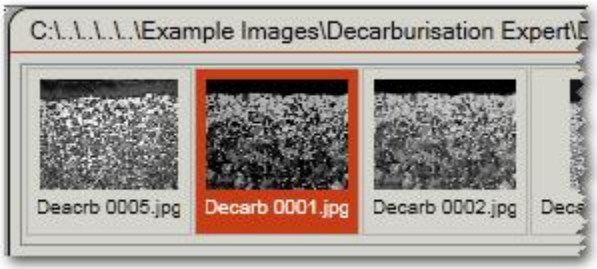
4: The available images will be displayed in the *Gallery*.

5: In the *Gallery*, click to select a single image, or Ctrl-click to select up to 10 images.

6: You can minimise the Navigator by clicking the arrow to the right of the header.

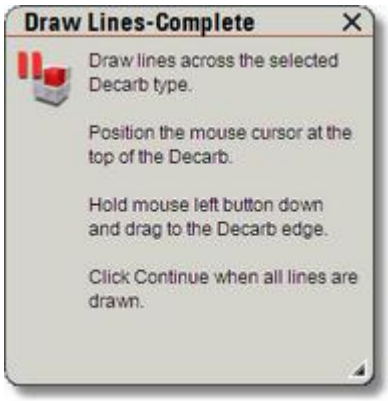
7: Click *Continue* to load the image. Any Grey Enhancement will be applied automatically.





Once you have selected an image to measure:

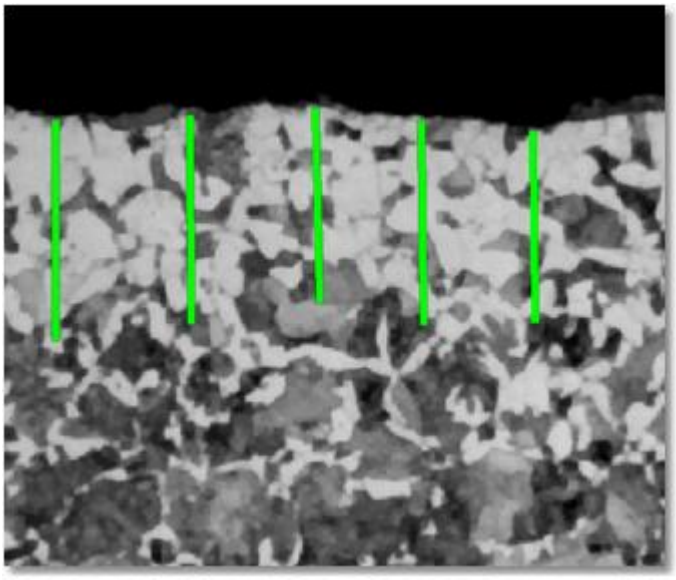
- The image appears in the *Image Viewer*.
- The title of the *Draw Lines* window reflects the Decarb region you chose to measure in the previous[1361] step:



- The *Binary Edit* panel is displayed, with the following enabled by default:
  - *Method*: Draw Straight Lines
  - *Editing Tool*: Line Tool

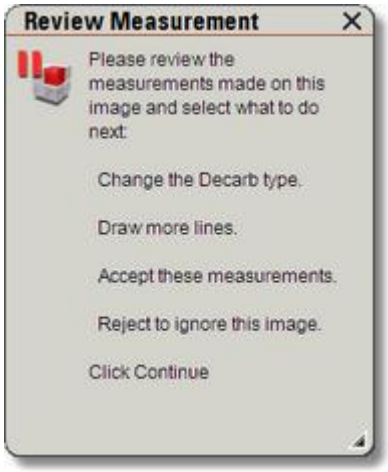
1: Draw some lines on the image that represent your chosen Decarb region. The lines should be perpendicular to the edge of the sample.

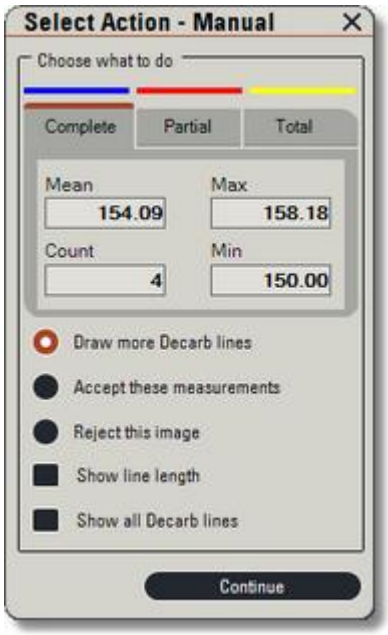
For example, here's a typical Complete region:



2: Click *Continue*.

Once you have drawn a set of lines for a Decarb region, you should review the measurements.



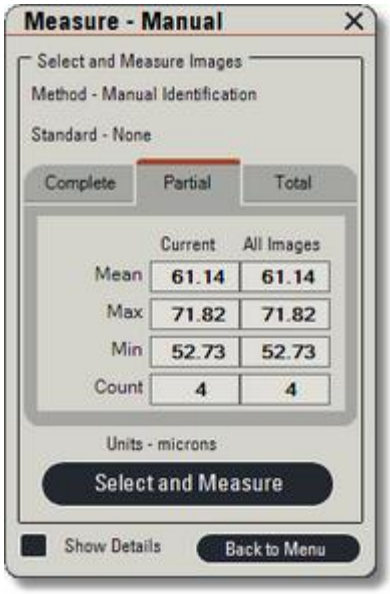


1: Choose one of the following actions:

- Change the Decarb type[1364] to draw lines for a different Decarb region on the same sample image; click on another tab (e.g. Partial) to do this
- *Draw more Decarb Lines* of the same Decarb type, to increase the accuracy of your measurements for the current region
- *Accept these measurements* (all Decarb regions) for this image; this takes you back to Select Images to Measure[1361] from where you can go back to the *Leica Decarb Menu* or select another image
- *Reject this image*: you will be returned to Start the Process[1360]

2: Once you have made your selection, click *Continue*.

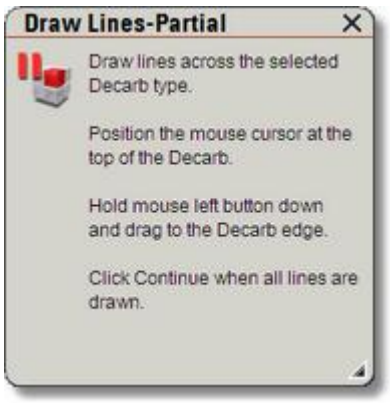
3: If you have finished measuring all your images for this session, on the *Measure - Manual* dialog click *Back to Menu*.



If you have chosen to draw lines for another Decarb region on the same image:

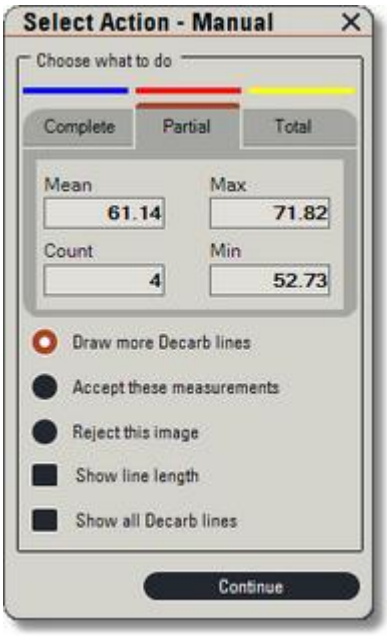
1: Ensure you have clicked a different tab on the *Select Action - Manual* dialog and clicked *Continue*.

2: Draw lines [1362] on the image that represent the next Decarb region.

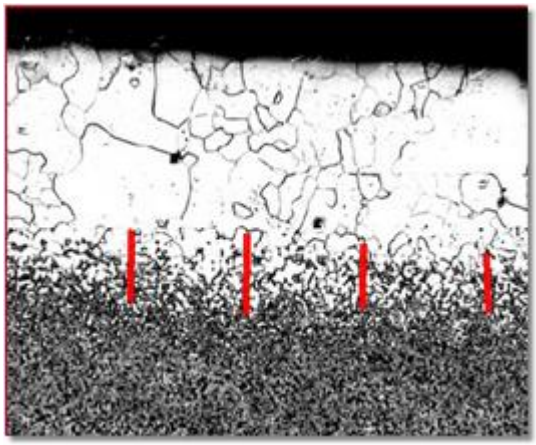


2: Click *Continue*.

- The *Select Action - Manual* dialog will display your measurements for the new Decarb region:

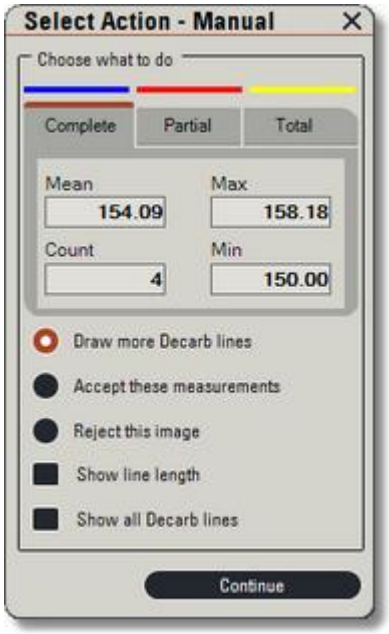


- The *Image Viewer* will display drawn lines. For example, here's a typical Partial region:

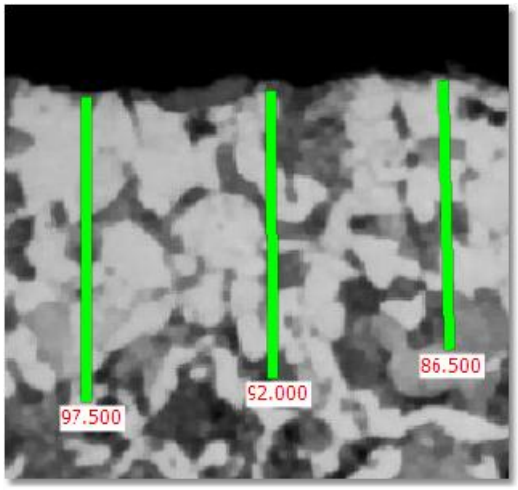


Back to Review Measurements [1363]

The Select Action dialog provides some extra display options:

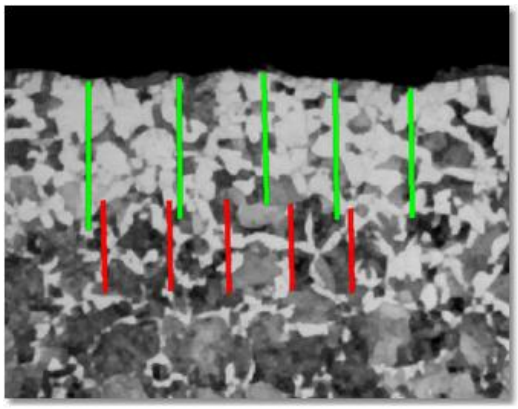


- *Show Line Length:* Dimensions will be overlaid on the *Image Viewer*:



- *Show all Decarb Lines:* If you have drawn lines for more than one Decarb region on this image, they will be displayed

  For example, the image below shows Complete and Partial lines:



Enter Reference Data

You can add information about specimens and processing on the *Reference Data* panel. Some of the information is displayed on the standard reports installed with the application.

1: On the *Leica Decarb Menu* click *Enter Reference Data.*

The *Reference Data* panel appears.

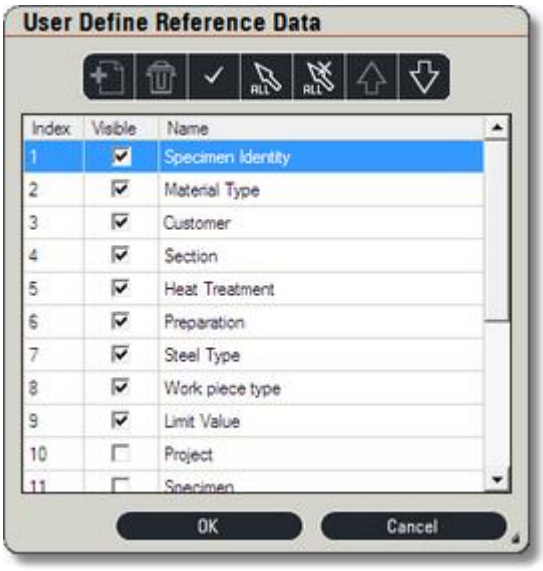
2: Click to select an item.

3: Click inside the *Data* text box to the right and type the data.

You can select the *Reference Data* headings to suit your working methods and descriptions. To do this you must run LAS as an administrator. (Right-click the desktop icon and select *Run as Administrator*.)

1: Click on the *User Define* button.

The *User Define Reference Data* dialog appears:



3: Click a *Visible* check box to show/hide that element in the *Reference Data* list.

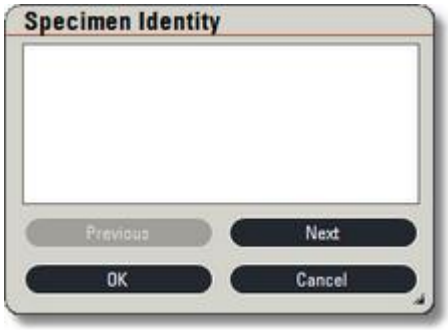
4: The *Tools* from left to right are:

- *Create:* Adds a new user heading. The default is *Field X*; You can change this to a more appropriate name.
- *Delete:* Removes a selected heading completely.
- *Tick Mark:* Enables or disables the selected heading. (same functionality as the *Visible* check box).
- *Select All:* Enables all headings.
- *Hide All:* Disables all headings.
- *Up/Down Arrows:* Moves the selected heading up or down the list.

5: Click *OK* to finish.

If any item of data is too long to fit inside the panel text box:

6: Click the *Edit* button.
7: Enter a heading in the resulting dialog.



8: Scroll between the headings using the *Previous* and *Next* buttons.

9: Click *OK* to finish editing.

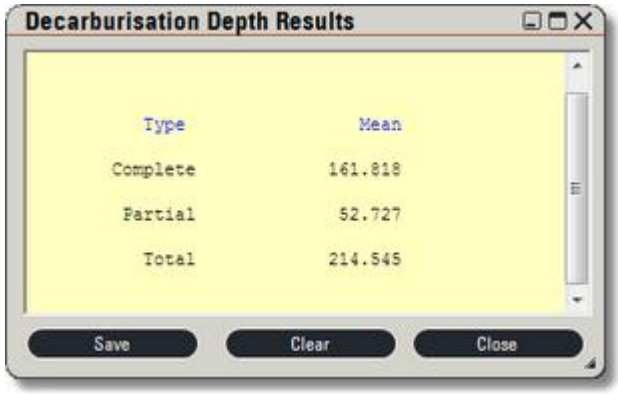
Note - If you change the Reference Data, you may need to modify the Excel Report[1368] template. After making changes to the Reference Data names, please check that what you see in the report is what you wanted. If not, please modify the report template to suit.

Create a Report

You can incorporate the measurement results into a comprehensive report using Microsoft Excel. The template for the report is installed with the application.

1: On the *Leica Dendrite Menu*, click the *Create Report* button to open the *Create Report* panel.

The measurements summary will be displayed:



2: In the *Select Template* pane, click the browse button and select one of the standard report templates:

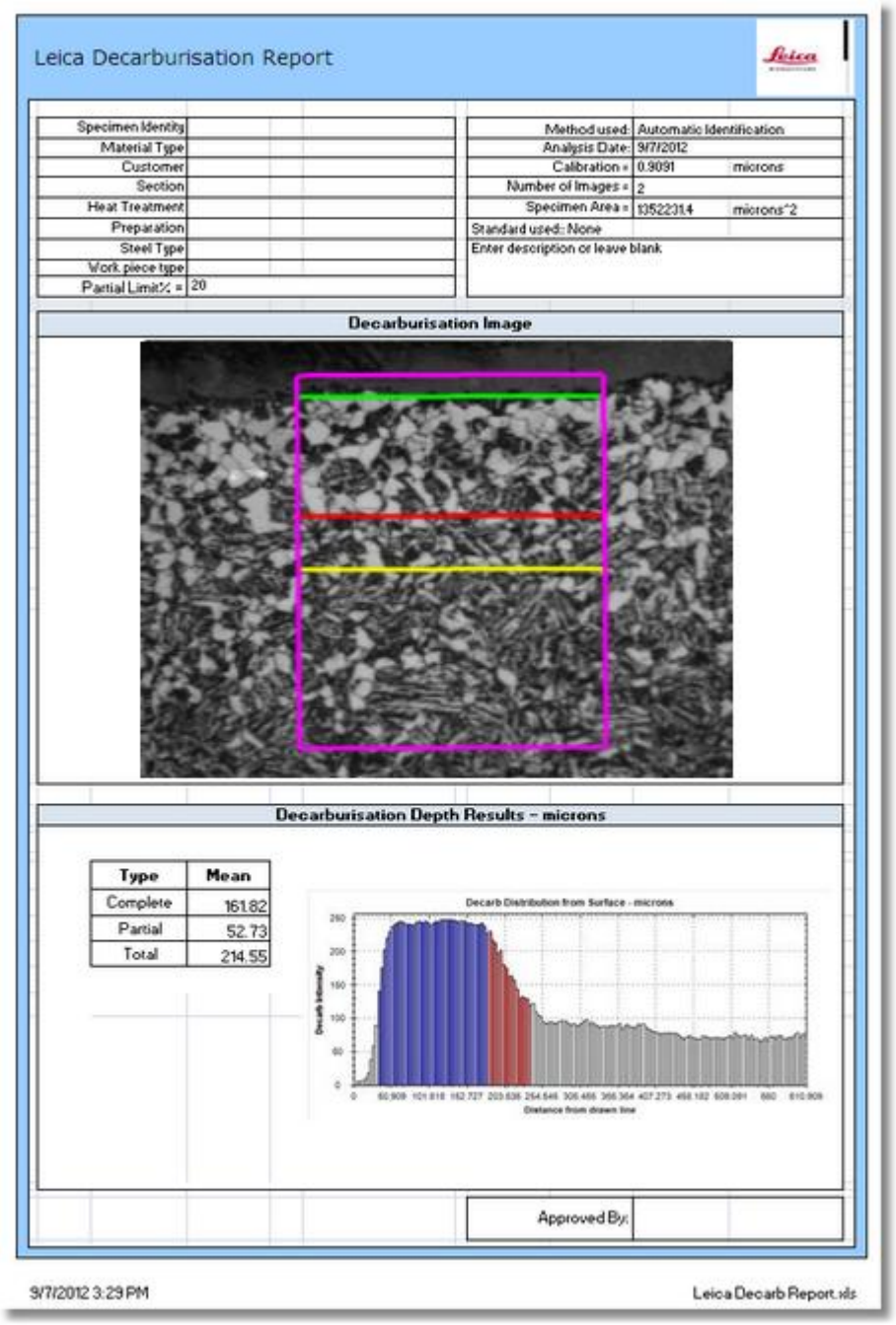
- Leica Decarb Profile.xlt: For use with images measured using the Automatic method
- Leica Decarb.xlt: For use with images measured using the Manual method

3: Enter a unique name for the report.
4: Click *Create Report*.

The report templates are located in the following folder:

C:\Users\Public\Documents\Leica Application Suite\LAS Apps\Apps\LAS Decarb App

Below is an example of a *Leica Decarb Profile.xlt* Report.



Leica Decarburisation Report

Specimen Identity	
Material Type	
Customer	
Section	
Heat Treatment	
Preparation	
Steel Type	
Work piece type	
Partial Limit% =	20

Method used:	Automatic Identification	
Analysis Date:	9/7/2012	
Calibration =	0.9091	microns
Number of Images =	2	
Specimen Area =	13522314	microns^2
Standard used:: None		
Enter description or leave blank		

Decarburisation Image

Decarburisation Depth Results – microns

Type	Mean
Complete	161.82
Partial	52.73
Total	214.55

Approved By:	

9/7/2012 3:29 PM

Leica Decarb Report.xls

Reticule

Reticule is an optional module that allows a precise pre-defined measuring grid – a Reticule - to be overlaid on a live image.

- Software generated: Completely independent of eyepiece reticules.
- A fast and reliable method of selecting the best grid to suit the application.
- A reticule can be captured and merged with the image.
- Fixed and Scalable versions available from a comprehensive Library.
- Reticule patterns are stored as standard svg format allowing users to design and create to their own special requirements.

LAS Reticule has a wide and varied range of precision uses:

- Item counting and distribution.
- Comparison and location.
- Sizing.
- Volume estimation.
- Assessing image scale.

Reticule Fast Track

Reticule Fast Track is a checklist of the steps to take to get *LAS Reticule* operational quickly:

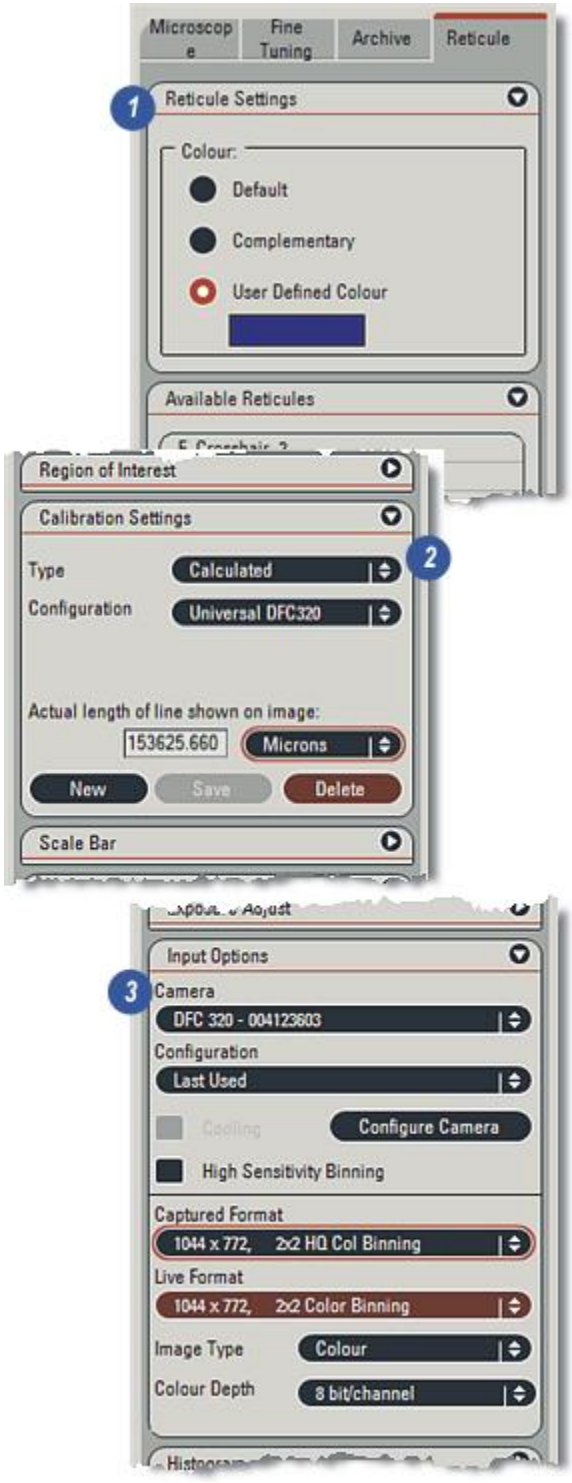
1: Select and load the required *Reticules* from the *Library:* *Go there...* 1372

2: *Calibrate* the microscope: *Go there...* 318

3: Select the best live image format to suit the application and hardware. Good resolution but with an acceptable refresh rate is the essential aim: *Go there...* 290

If using a Stereo microscope the zoom is adjusted, the *Reticule* drawings adjust in size but due to image shift are no longer in the correct position. Use the AX-Carrier option to correct this,

Most suitable for use with a microscope having automatic magnification readout.



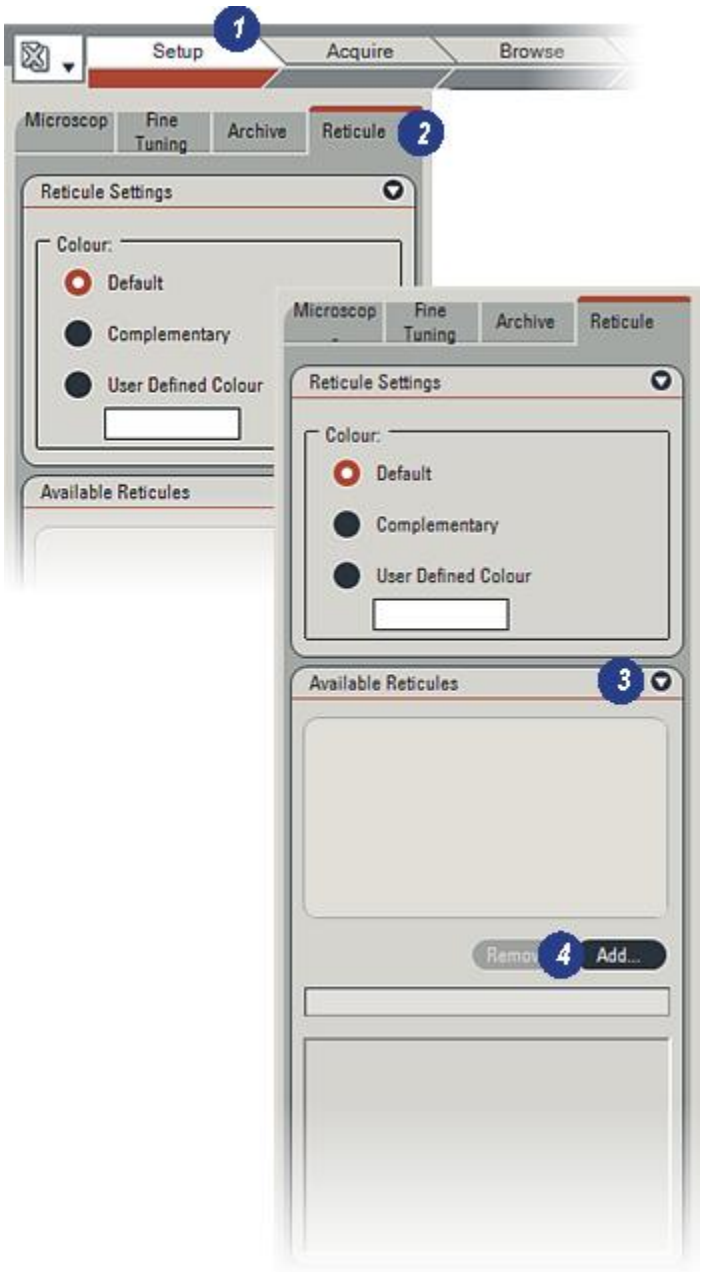
The first task is to select the styles required from the *Reticule Library* and attach them to the *Reticule* software.

1: Click to select the *Setup Workflow* and…

2: Select the *Reticule* tab. If it is not present the module is not installed or is not licensed.

3: On first use the *Available Reticules* window will be empty, so to select the styles…

4: …click on the *Add* button.



1: The Widows navigator dialog opens with the *Reticule Files* folder automatically selected.

2: A single style can be selected simply by clicking on its file name, but for multiple selections, hold down the *Ctrl* key on the keyboard whilst clicking individual files. The illustration shows 11 files selected and highlighted.

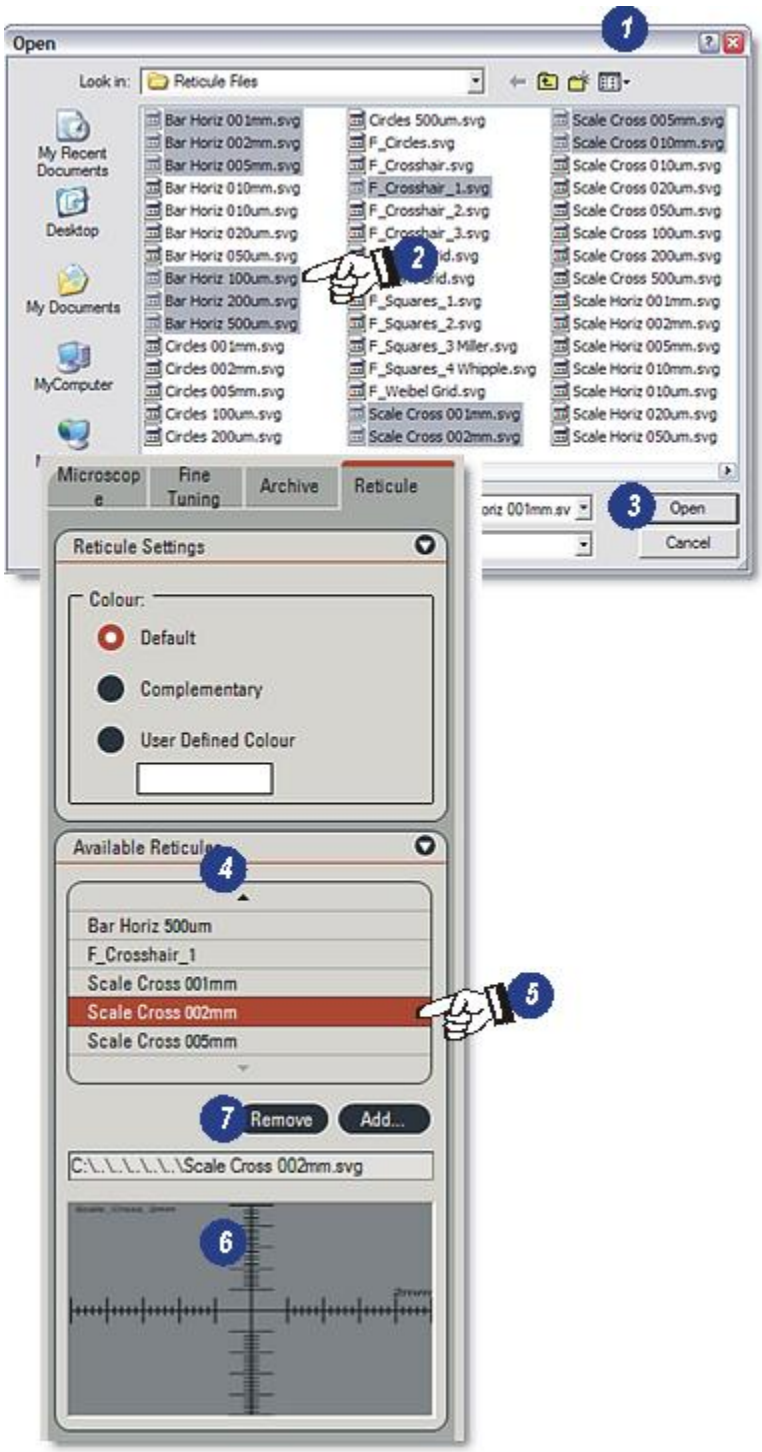
3: Click on the *Open* button.

4: The selected reticule files appear in the *Available Reticules* window.

5: Select an individual style by clicking its entry. It is highlighted and…

6: …displayed in the viewer.

7: To delete a reticule style, click to select it in the list (5) and then click the *Remove* button. If a style is inadvertently deleted it can be recovered from the library. *See previous page…* 1372



Fixed reticules are non-movable and not scalable. They are always centered in and displayed to fit within the image window - 'fit to window' where the window is the live image size.

For example, a crosshair displayed across the centre of the image window remains fixed and unchanged when the image is moved (by moving the camera or panning), or if the magnification is changed.

Examples here show:

1: *Weibel Reticule.*

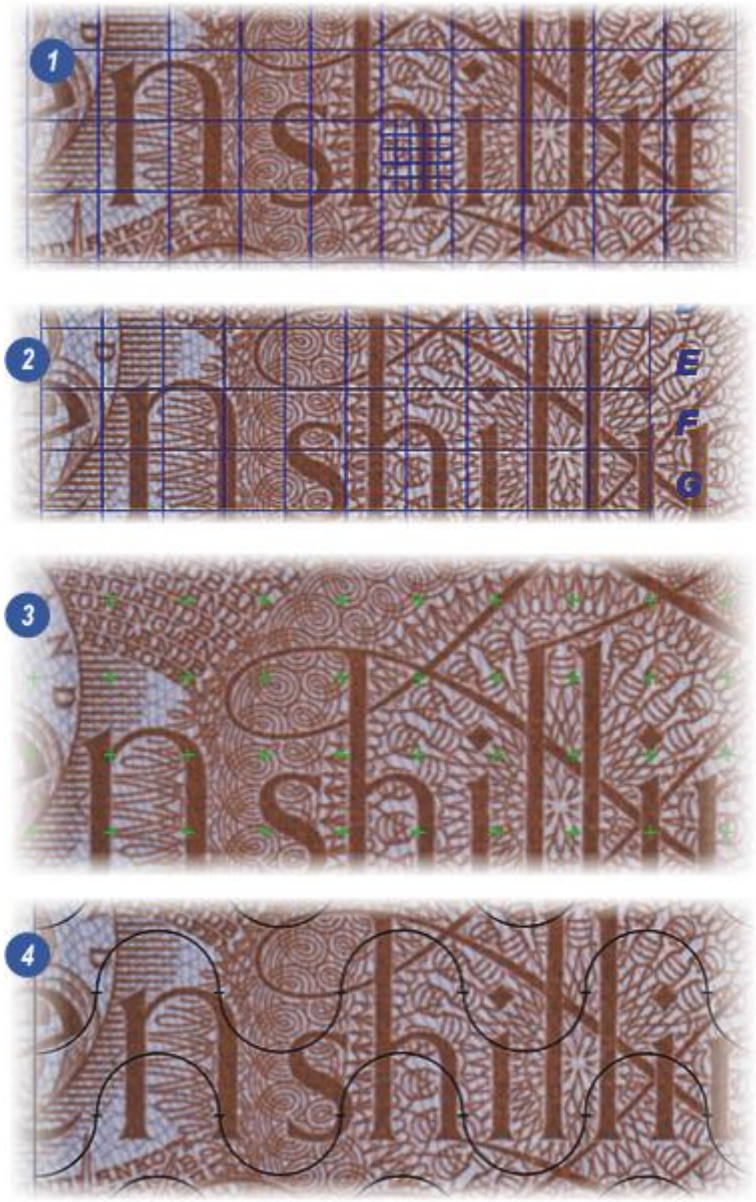
2: *Squares Reticule* one of several variations and styles.

3: A *Point Grid Reticule.*

4: *Mertz Reticule.*

Fixed Reticules are defined in pixels.

Mertz, *Point* and Weibel are examples of stereological grids.



Reticule Types: Scalable

Scalable Reticules have an absolute size associated with them and are defined in physical units for example millimetres. They can be positioned (moved) and rotated by the user; but cannot be re-sized manually.

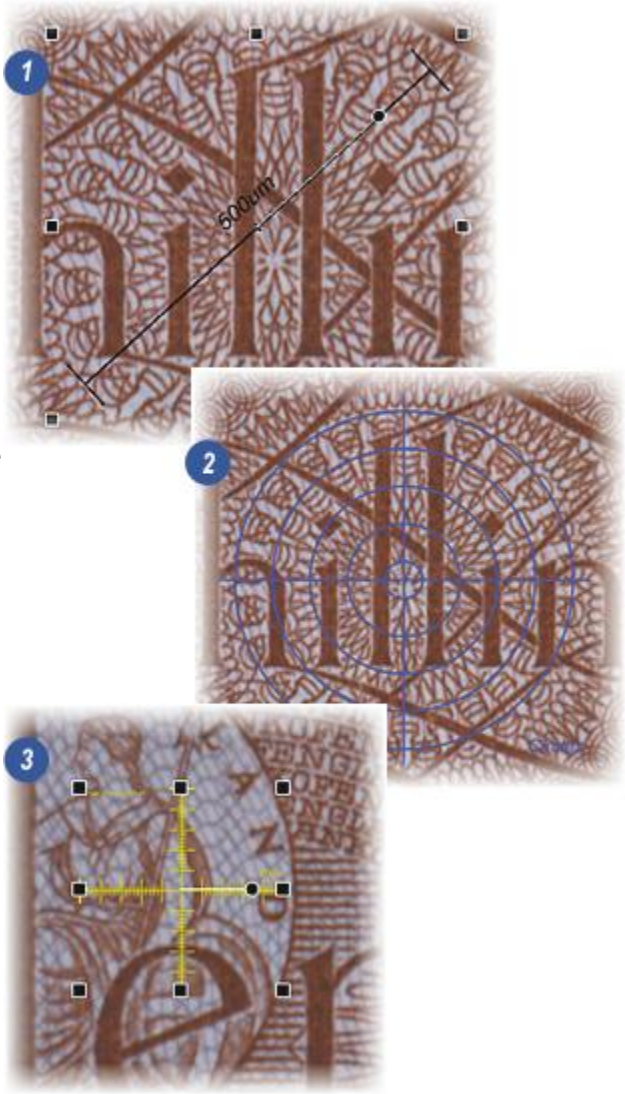
If the image is moved or zoomed, the centre of a *Scalable Reticule* will remain over the feature in the image where the user positions it and it will re-size to be correctly scaled to the image. The scaling information is obtained by reading it automatically from the connected microscope. So, for example, a rectangular reticule placed over a feature will *remain* centred over the feature and scaled to the image when the image zoomed in or out.

Scalable Reticule examples are:

1: Horizontal Bar 500µm

2: Circles 500µm

3: Cross 200µm.



There are three colour options for the reticule which are selected on the *Reticule Settings* panel:

Default: Displays the reticule in the colour defined by the Reticule definition file.

Complementary: Automatically determines the display colour based upon the average hue of the image to maintain a good contrast.

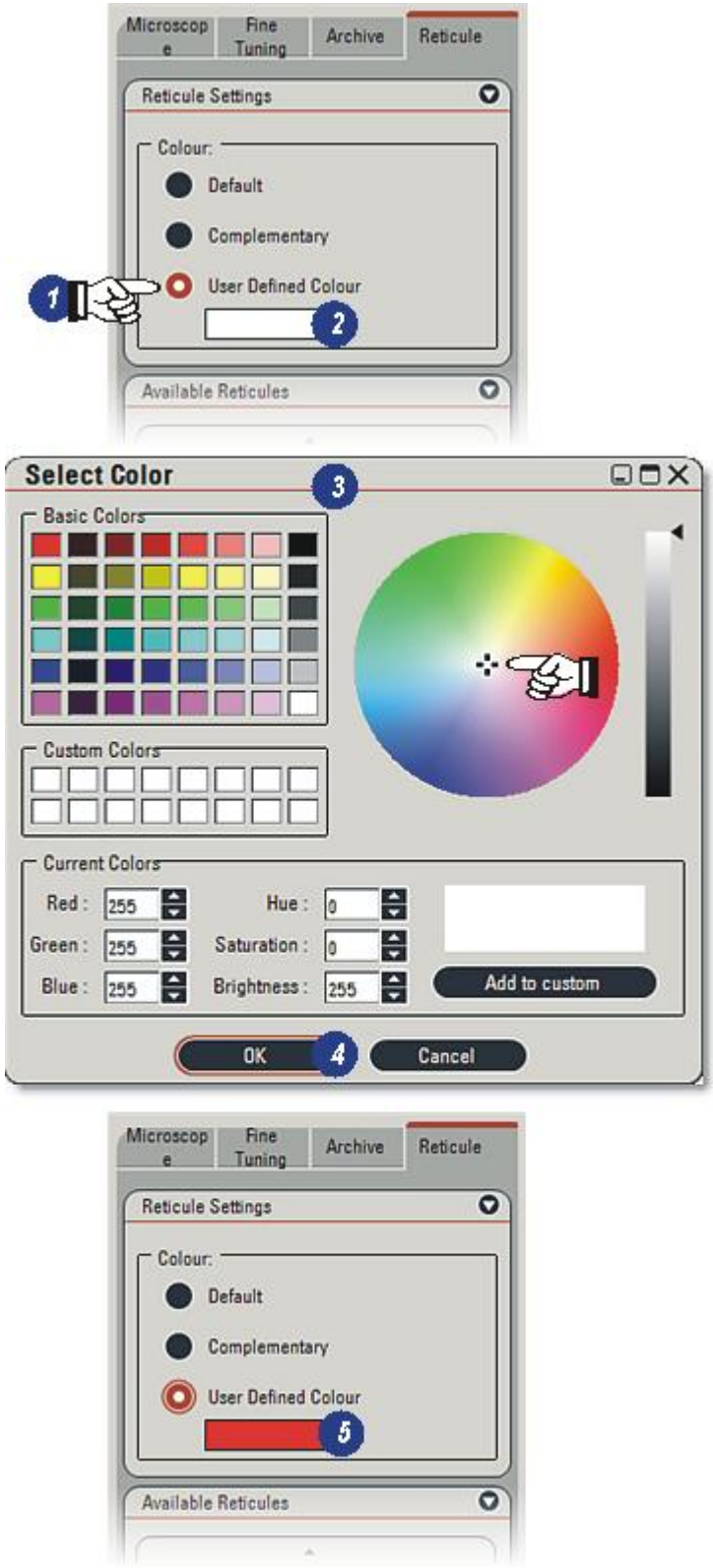
User Defined: Allows the user to select the display colour as follows:

1: Click to enable the *User Defined Colour* radio button.

2: Click in the *Selected Colour* window and…

3: …the Windows *Select Color* dialog appears. Choose a colour from the wheel by clicking and holding the 'target' and dragging it to the desired colour, or clicking to select a *Basic Color* in the matrix. Precise colour selection can be made by typing the red, green and blue (RGB) values in the text boxes, and a colour can be saved by clicking the *Add to custom* button.

4: Click *OK* and the selected colour appears on the *Reticule Settings* panel **(5).**



Apply Reticule

The reticule style to be used is selected on the *Acquire Workflow*:

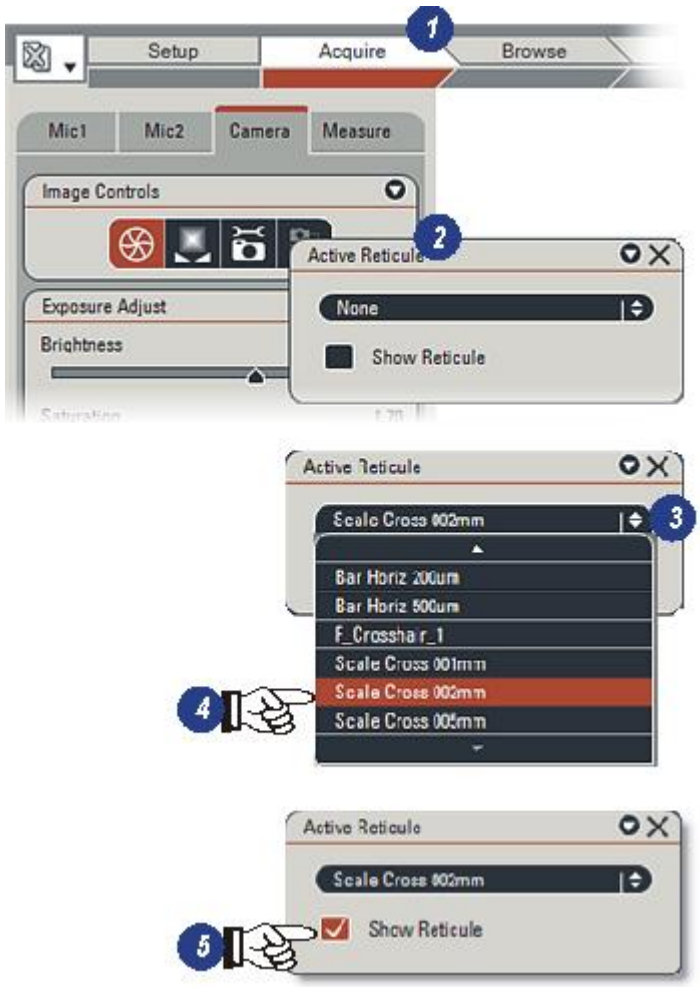
1: Click to select the *Acquire* Workflow and the *Camera* tab.

2: The *Active Reticule* control panel selects the desired reticule and determines if it is to be displayed or not. Like most other control panels, it can be moved to any part of the screen by clicking and holding on the header bar and dragging it to the required position. Return it to the normal location by clicking the 'X' on the header.

3: Click on the small arrow to the right of the *Active Reticule* list box and all of the reticule styles attached to the module appear.

4: Click to select the style required.

5: Click to enable the *Show Reticule* checkbox and the reticule will appear on the live image.

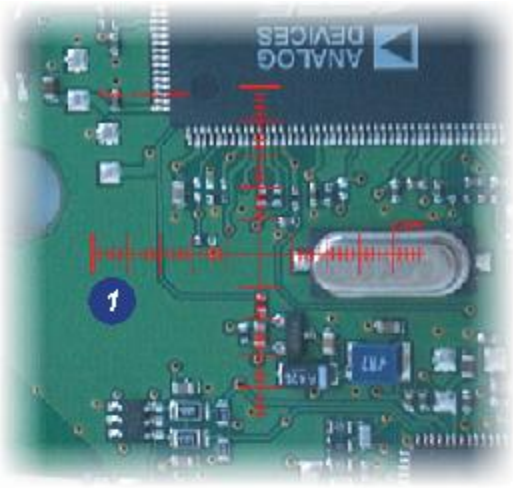


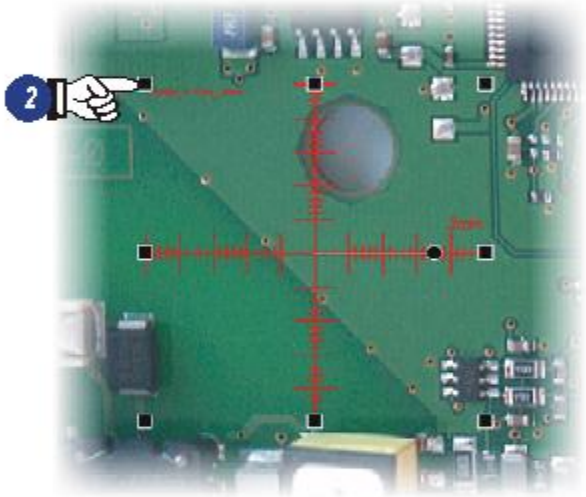
1: The selected reticule in the chosen colour on the live image.

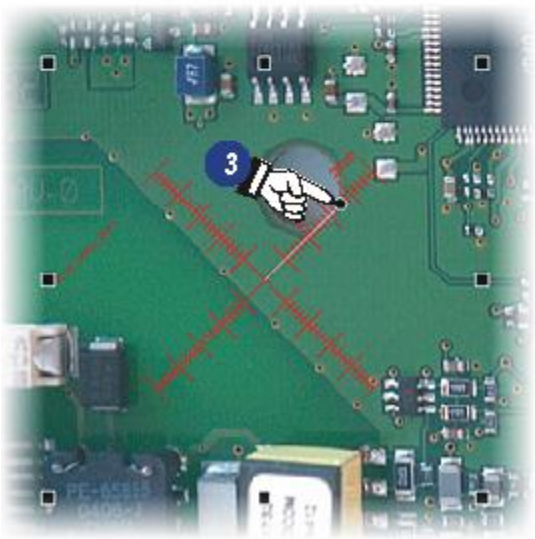
2: Move the reticule by clicking on it (8 'handles' appear) and dragging it to the desired location.

3: A *Scalable Reticule* can be rotated to any angle by clicking, holding and dragging on the *Rotate* 'handle'.

Images captured from the *Acquire Workflow* by either clicking on the *Acquire Image* button or pressing function key F3 on the keyboard, will merge the reticule with the image. To capture without the reticule, either switch it off with the *Show Reticule* checkbox or make the capture from the *Browse Workflow*.



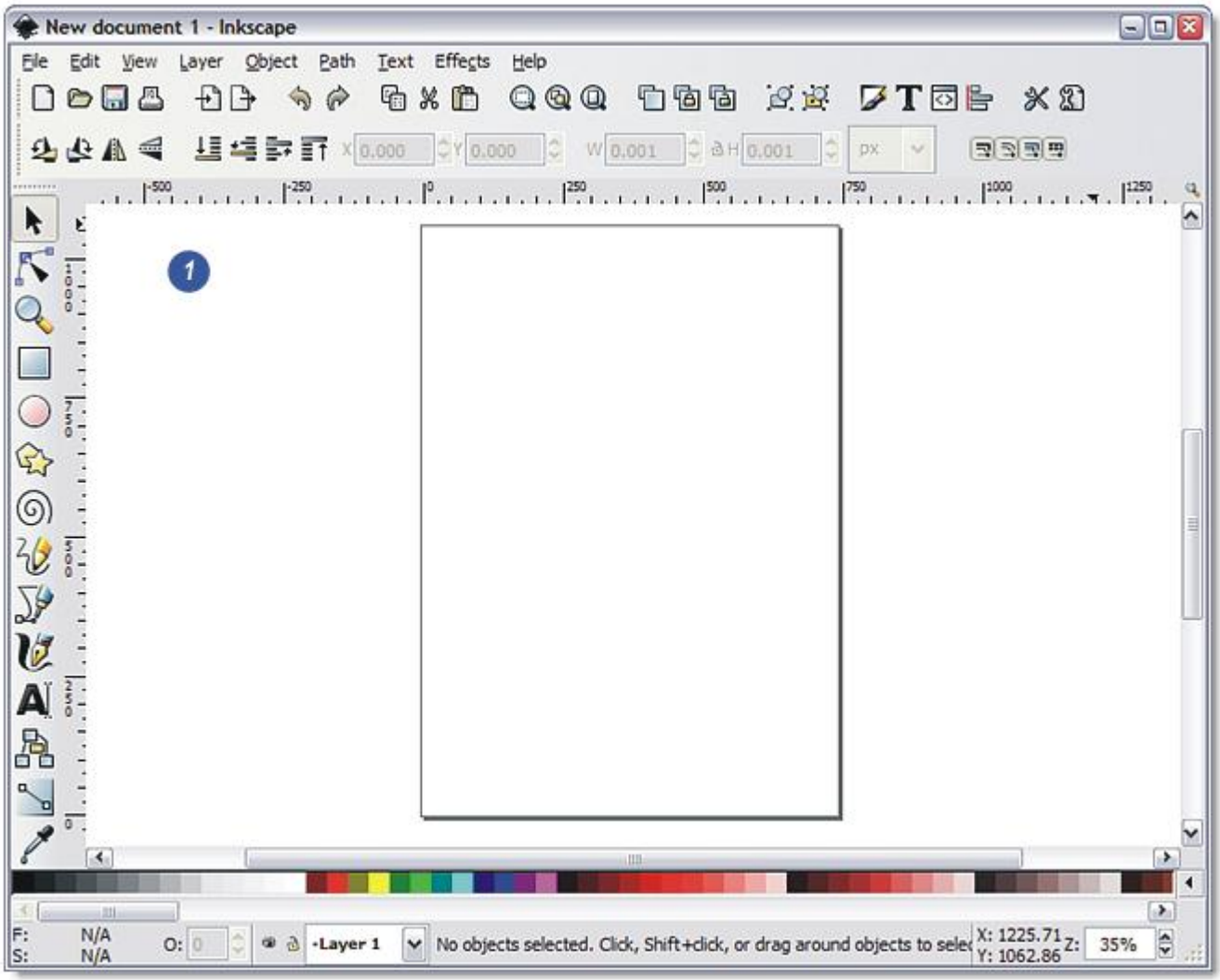




There are several vector graphic drawing packages available that are capable of creating svg *Reticule* files. An open source application - download and use the program free of charge but do not use it for commercial gain - called Inkscape (Google for the latest releases), will produce *Reticules* to import into LAS.

This and the following page is a general guide; Open source software is constantly changing and improving so some experimentation will be necessary.

- Download and install *Inkscape*.
- Run the program.
- From the *File* option, select *Document Properties* and set the default units to pixels (px), the *Canvas Size* to *Custom 640 x 640* (adjust as necessary to suit the LAS *Viewer* size and camera)
- Click *Fit Page to Selection.*



- Select a shape from the toolbar and draw it on the canvas area. The shape must not extend beyond the canvas.
- Use the various tools and dialogs to change line weights and colours.
- Save the drawing using the navigation dialogs and the Plain svg format.
- Open the file in a simple text editor - Notepad or Wordpad. Find the lines that specify:

width="680px" and height="680px"

...and make sure the 'px' suffix is present. LAS needs these units. Save the file which will be a fixed reticule.

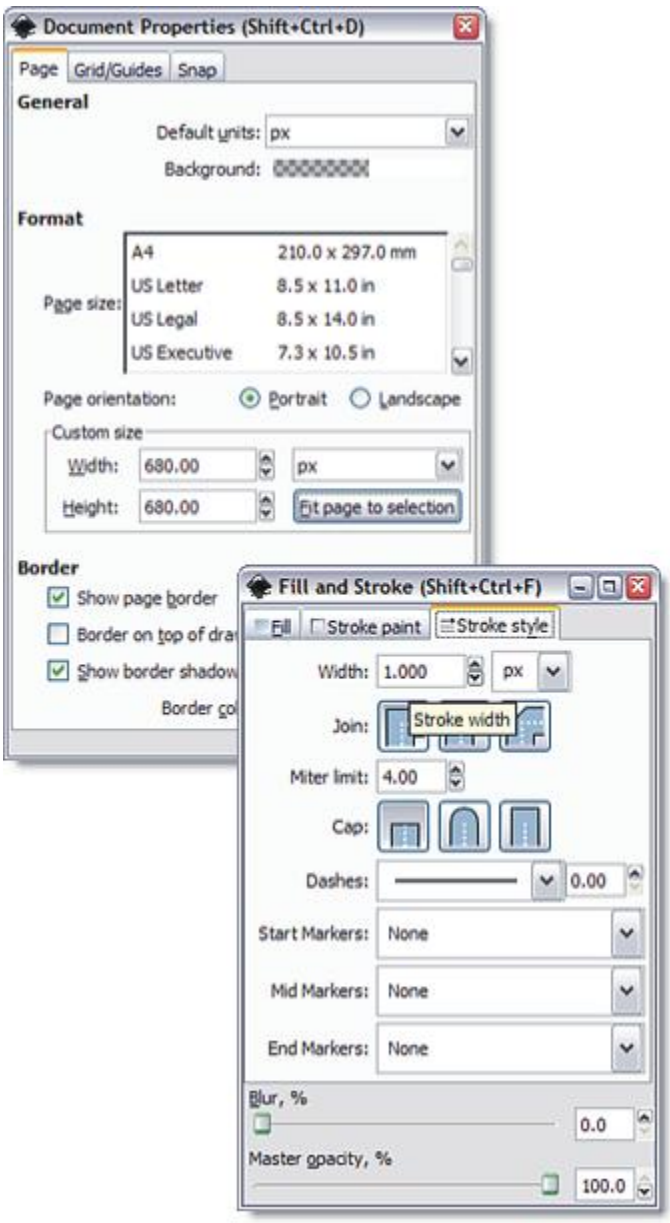
- To create a *Scalable Reticule* with the file open in the text editor and replace the width and height lines with:

width="0.2mm" height="0.2mm"
viewBox="0 0 680 680"

...where 0.2mm is the scale required. Do not change the pixel size. Save the file.

- Launch LAS and import the 'personalised' *Reticule* in the normal way: *Go there...* 1372

To determine just how *Reticule* files are constructed, open a *Reticule Library* file in *Notepad* and check that newly created files have the same structure.



Web Sharing

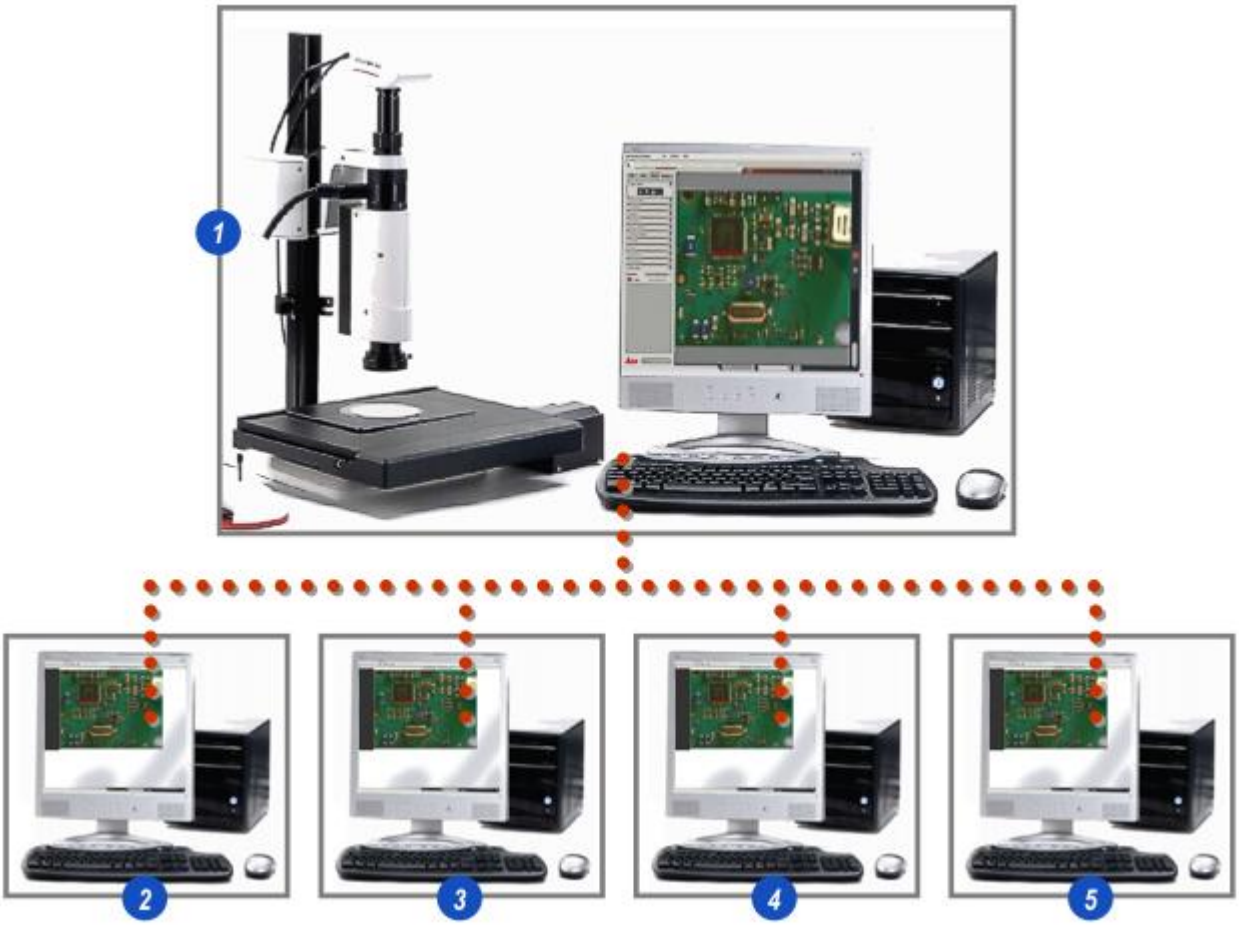
Leica Application Suite *Web Sharing* module streams live images across a local network so that they can be seen in real time by other users.

In the illustration, the *Master PC* **(1)** running Leica Application Suite with *Web Sharing* installed and enabled, streams the microscope image to the local network. Other users – *Clients* – on the network view the image in real time using only free software, *Internet Explorer* with the *Silverlight* plugin.

On the illustration the Clients are numbered **2** through to **5** but almost any number of clients can view the image simultaneously.

Web Sharing is an optional module, free to evaluate for 60 days; After that a chargeable license is required.

- Suitable for Local Area or Virtual Private networks, wired or wireless.
- Only the Master PC needs Leica Application Suite and the *Web Sharing* software.
- No special hardware required - only a standard DFC camera on the Master microscope.
- Other users – Clients – need only *Internet Explorer* with Microsoft *Silverlight* plugin to view the images.
- With the appropriate projection hardware, a Client can be used for large screen viewing in lecture halls and seminars.
- Three image size options – 640 x 480, 800 x 600 and 1024 x 768 pixels.
- Clients can see *Scale Bar* and *Master Cursor* movements, especially useful for pointing to areas of interest.
- *Image Capture* feature for Clients so that they can have a permanent, printable record on their own computer.



The *Web Sharing* launch panel is on the *Camera* tab of the Acquire Workflow.

1: Click on the *Acquire* Workflow.

2: If necessary, click on the *Camera* tab.

3: Click on the small arrow to the right of the *Web Sharing* header to expand the panel.

Both the *Cursor* (and *Scale Bar* if it is enabled) on the master monitor are available to the Clients.

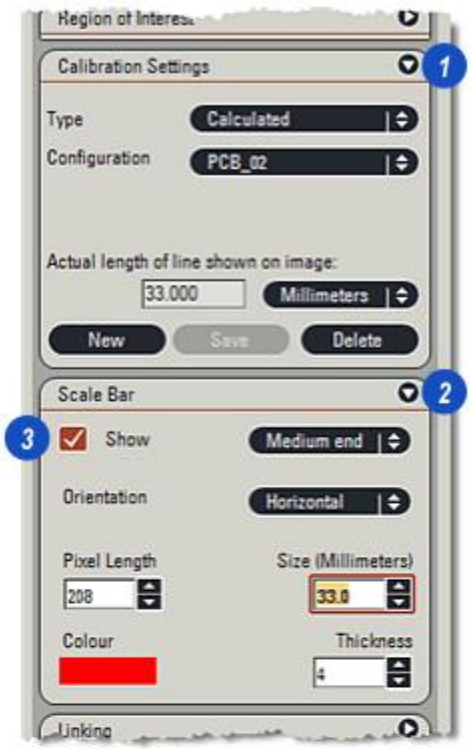
If the Master Cursor is moved - to point out a particular feature for example - the movement will be reflected on the Client monitors. If the Scale Bar is moved to a new position, this too will change position on the Client display.

If it is intended that a *Scale Bar* is visible:

1: Click the small arrow to the right of the *Calibration Settings* header and…

2: …also on the *Scale Bar* header to access the settings and in particular to enable the *Scale Bar* by clicking the *Show* button **(3)** to reveal the Scale Bar **(4)**.

When *Web Sharing* is active, most of the other LAS functions are disabled to ensure rapid refresh at the Client monitors, so the *Scale Bar* setup can only be carried out with Web Sharing disabled.



Scale Bar setup details: *Go there…* 77

Calibration: Process > Calibration: *Go there...* 318

The camera settings for both *Live* and *Captured* formats, affect the speed at which *Web Sharing* images are downloaded to the Client monitors.

The *Live* format affects the refresh rate and the *Captured* format the download speed if the Client decides to save the image. Images saved at a Client machine will have the same resolution as the Master Camera Captured Format.

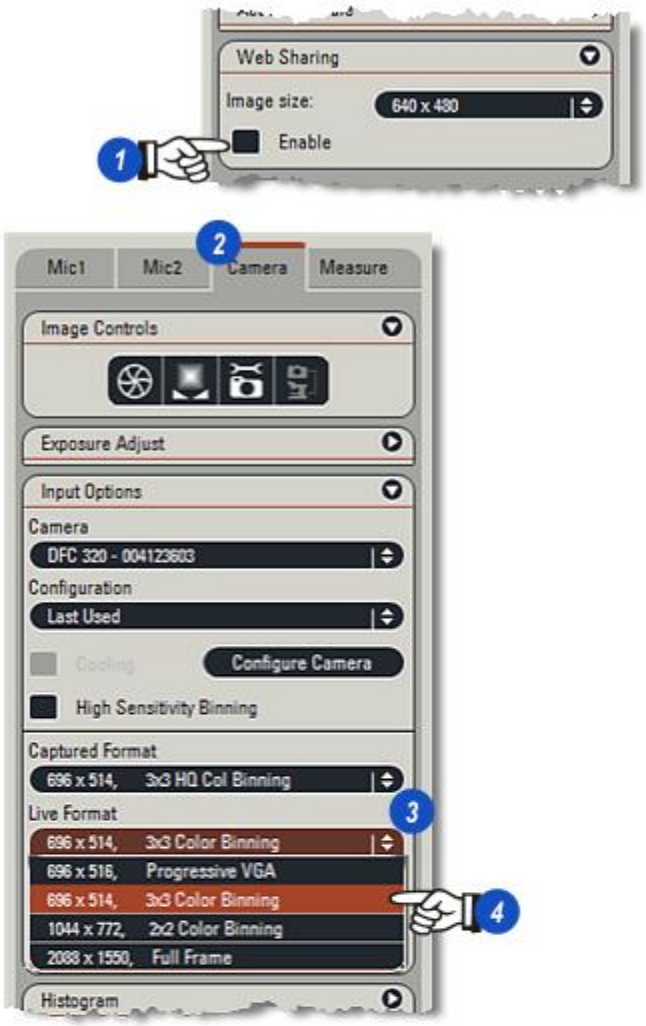
Before enabling *Web Sharing* check the camera formats; Start with a fairly low resolution – *696 x 514: 3 x 3 Color Binning* – for example, and increase the resolution if the refresh rate allows or more detail is required at the Client monitors.

1: Make sure that *Web Sharing* is disabled.

2: Click on the *Camera* tab on the *Acquire Workflow.*

3: Click on the small arrows to the right of the *Captured* and *Live Format* headers and…

4: …from the drop down menus choose one of the lower settings.



More detail about camera formats: *Go there...* 290

Continued… 1388

Usually, *Web Sharing* streams the current live image, but it is possible to temporarily disable the camera and instead stream images that have previously been captured.

The compression type of the stored image does not matter, but the resolution should be at least 1024 x 768.

The first step is to disable the camera and replace it with the *Demo Camera* loaded automatically with LAS Version 3.0 onwards. If Leica Application Suite is running, close it down.

1: On the *Desktop Task Bar* click on the *Start* button. The illustrations show Windows XP layout but Vista is very similar.

2: Click on the *All Programs* arrow.

3: Move the cursor to highlight *Leica Application Suite* in the list of programs.

4: On the sub-menu move the cursor to *License LAS* and click the option.

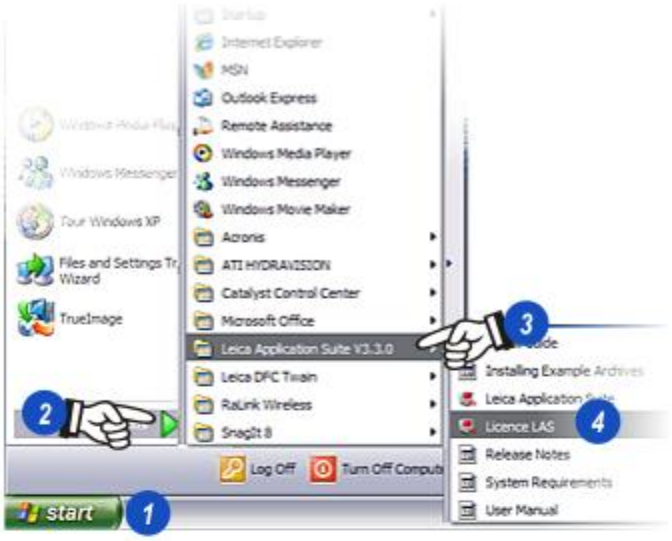
5: The *LAS Framework* will load but instead of launching the *User Interface* will stop when the *Main Menu* bar appears. Click on *Options*.

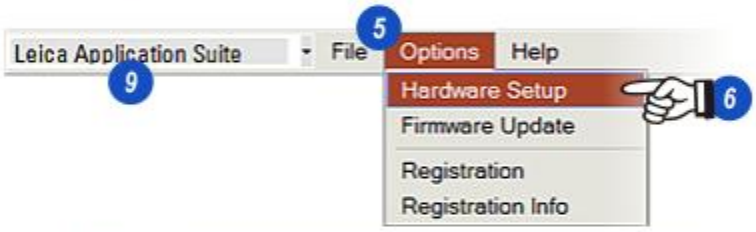
6: From the drop down menu click to select *Hardware Setup.*

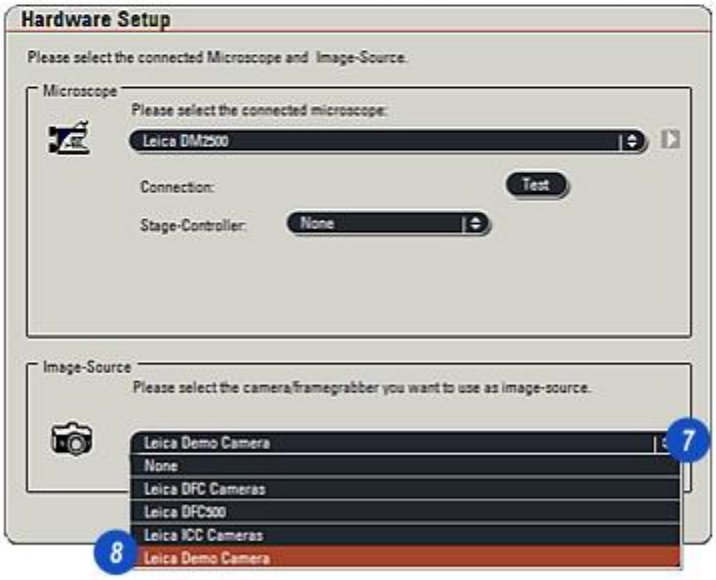
7: When the *Hardware Setup* dialog appears, click on the small arrows to the right of the Image Source header.

8: From the list of option click to select *Demo Camera* and then click *Save*.

9: Launch the program by clicking on the *Leica Application Suite* header.







With LAS running:

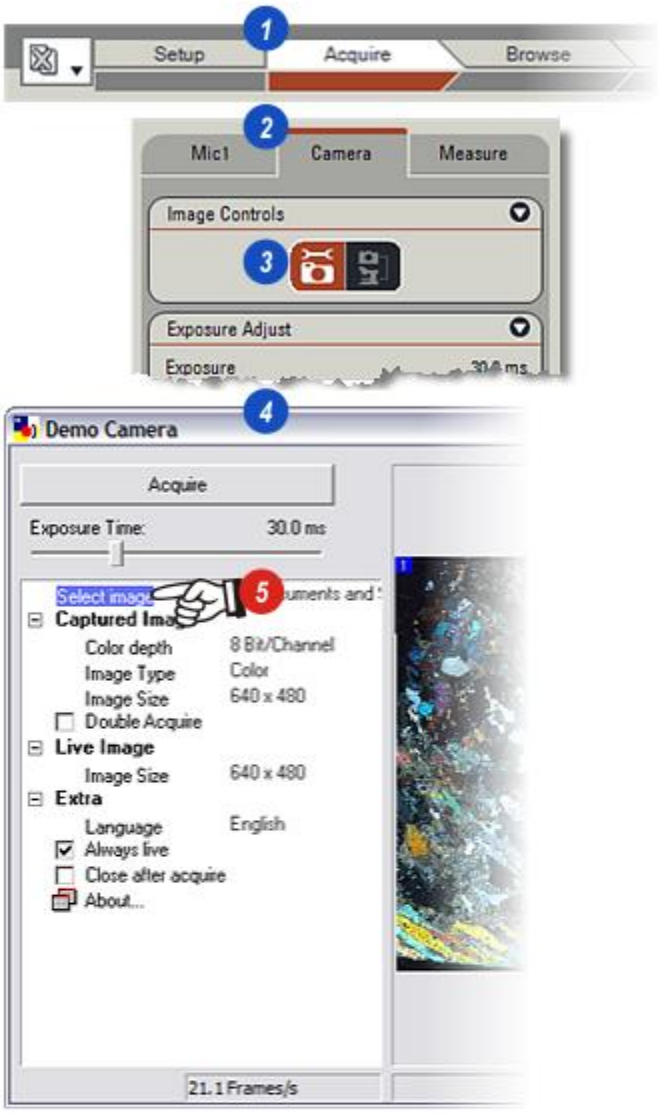
1: Click on the *Acquire Workflow*.

2: Display the *Camera* tab to reveal the camera controls.

3: Expand *Image Controls* panel and click the *Camera Twain* icon.

4: The *Demo Camera* dialog is displayed.

5: Select the image to be shared by *right-clicking* the *Select image* caption.



1: On the *Select Image File* dialog navigate to the folder that contains the image to be shared and…

2: …click the arrow to the right of the *Files of Type* window and chose the file compression type from the list by clicking it.

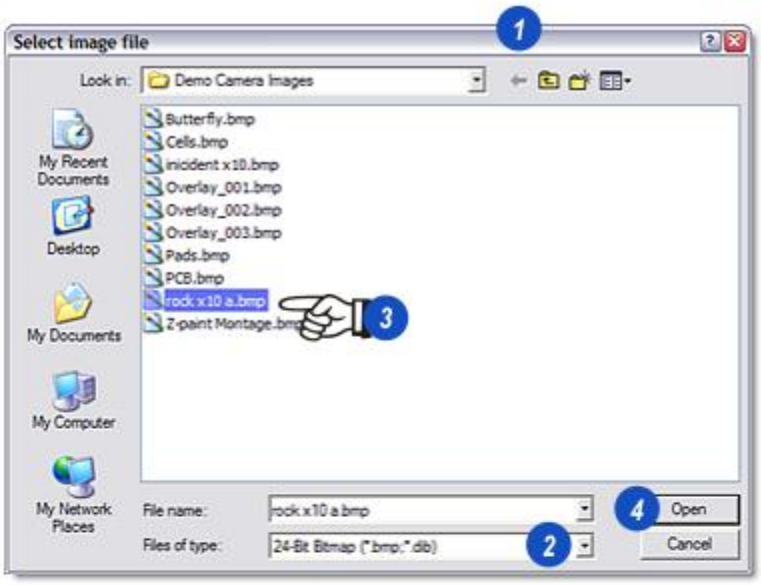
3: Click to select the image.

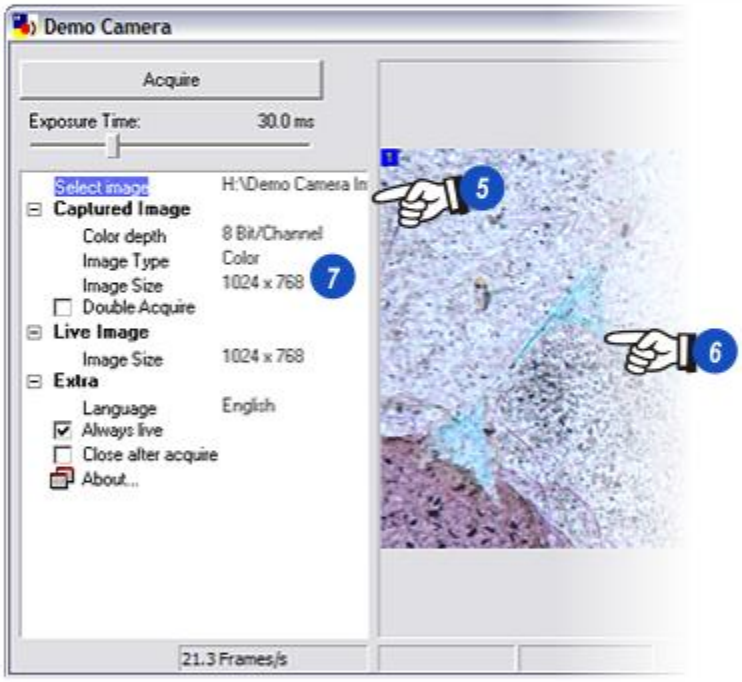
4: Click *Open*.

5: The *Demo Camera* dialog re-appears with the path of the chosen image and…

6: …the image itself displayed.

7: The image resolution is display under the *Captured Image* details - it should be at least 1024 x 768.





Close the *Demo Camera* dialog and follow the normal Web Sharing procedure from here. *Go there...* 1388

Note: Selecting a different folder during this process is only temporary; the default Demo Camera images folder is always controlled by the Preferences 63 setting.

1: If necessary, expand the *Web Sharing* panel by clicking on the small arrow to the right of the header.

2: *Web Sharing* has three size options – that is the size that the clients will see on their monitors. Larger sizes are slower to download so the refresh rate at the client will also be slower. The actual refresh rate depends upon the network hardware and the number of active users.

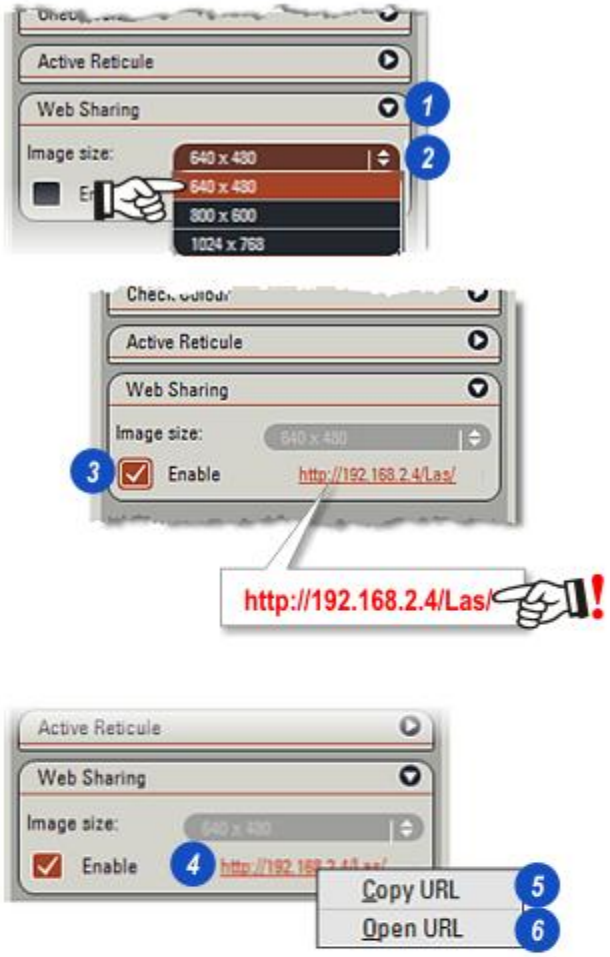
Check that Web Sharing is disabled – the *Enable* checkbox is blank – and click the arrows to the right of the header and from the drop down list click to select the required size.

3: Click to enable *Web Sharing*.

4: When the software successfully loads the *Master Address* appears on the panel. The Address is unique to the Master but can change every time LAS is started. Right click on the address to reveal options to:

5: *Copy* the address *(URL)* to the clipboard from where it can be saved or attached to an e-mail for example, or…

6: …*Open* address *(URL)* to test the network connection by sending the image back to the Master for display.



Client Check Network Connection

A client can check if he is connected to the Master by sending the Master Address and waiting to see if there is a response. The process called 'pinging' is carried out at the *Command* level.

1: Hold down the keyboard *Windows* key and at the same time press the *R* key or…

2: …click on the *Start* button and from the *Start Menu…*

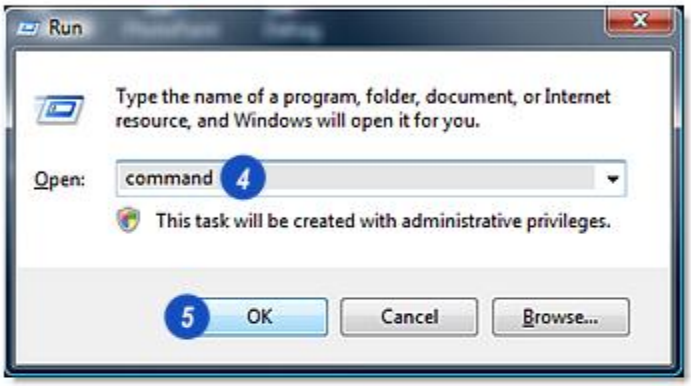
3:… click on the *Run* option.

4: On the *Run* dialog type the word *'command'* in the text box and…

5: …click *OK.*

6: In the *Command* window against the > prompt type *'ping'* and then the numeric part of the master address – in the example 192.168.2.4. Do not include the *http* or the */Las/* parts of the string.

7: If connection is made successfully the details will be displayed in the window. An unsuccessful connection will report *'Address not found'*.



Clients do not need LAS or *Web Sharing* software to receive images; All that is required is *Microsoft Internet Explorer* Version 6, 7 or 8 with the *Microsoft Silverlight* plugin. Currently (January 2009) no other browser supports *Silverlight*.

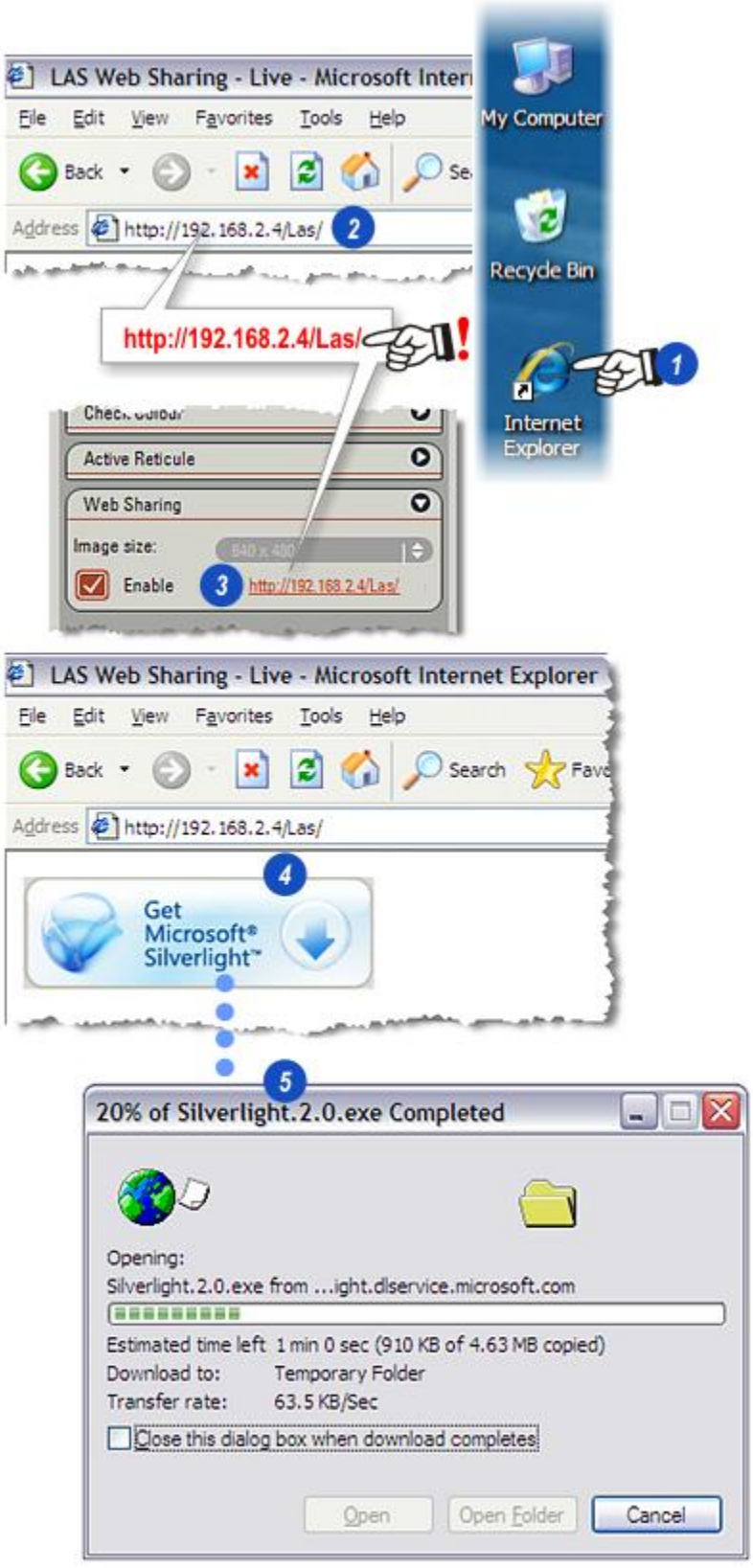
1: Launch *Internet Explorer* either from a desktop shortcut or from *Program Files*.

2: The illustration shows Internet Explorer 6 with the entire address string supplied by the Master *Web Sharing* module **(3)** typed (or copied) into the address text box. Ensure that the last forward slash (*/*) is included.

4: If *Silverlight* is not installed the link to Microsoft *Silverlight* appears. Click on the arrow to…

5: …download the plugin (4.6Mbytes) and follow the onscreen instructions to install it.

Silverlight is automatically installed with Internet Explorer versions 7 and 8.



1: The illustration shows Internet Explorer Version 7 with the live image from the Master in the *Web Sharing* window.

2: The *Connection Status* – either buffering or connected - is shown on the side panel with…

3: …and indicator in the top right hand corner – yellow whilst buffering and green when connected.

4: The Master Cursor will appear if it is within the LAS Viewer area and also the Scale Bar if it is enabled. If the Master user moves the cursor or the *Scale Bar* the new positions are displayed at the Client display.

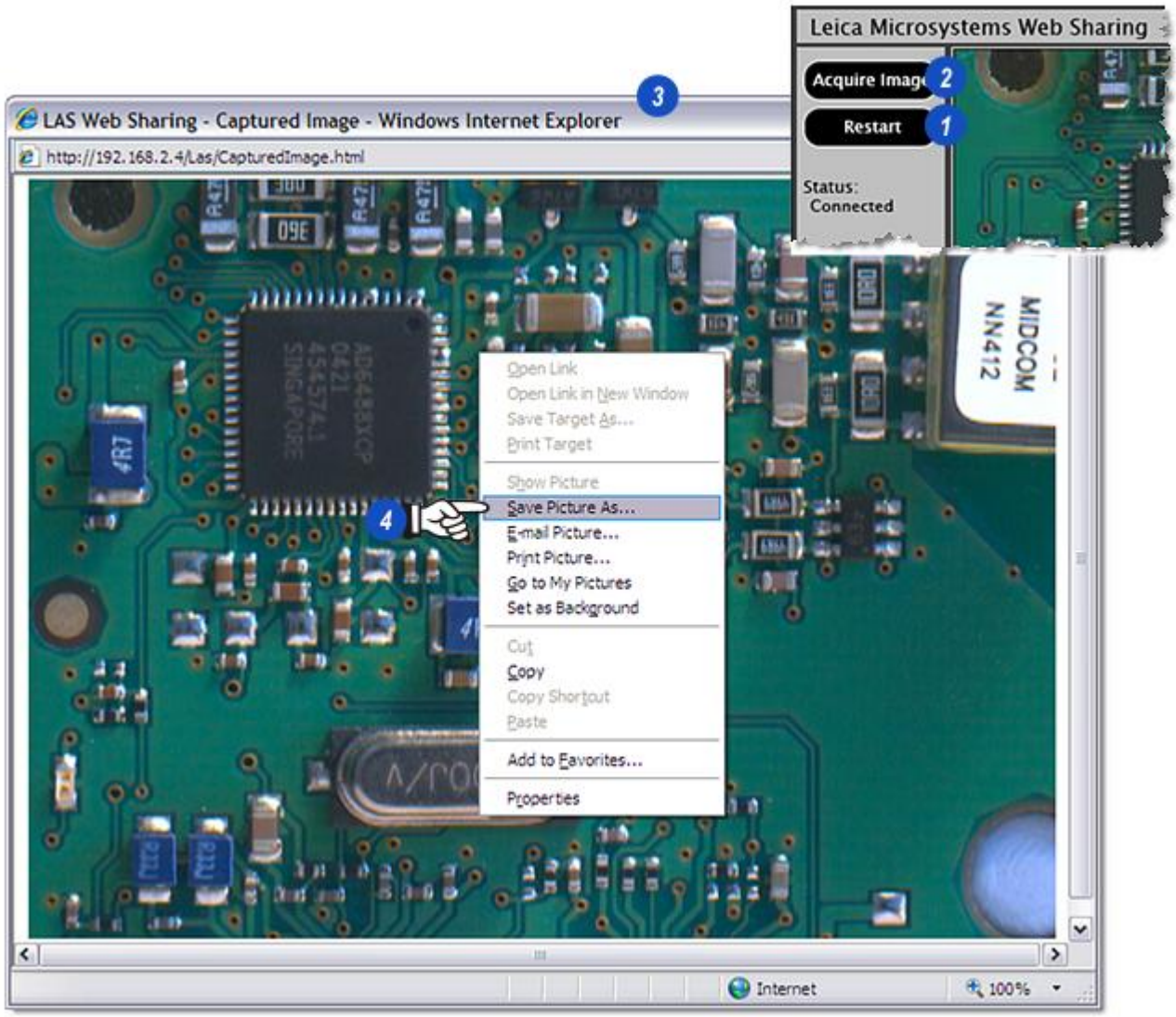
If connection is temporarily lost or the Master goes 'off line' to change images:

1: …click *Restart* to refresh the connection and current image.

2: To copy the image click the *Acquire Image* button and…

3: …when a new *Captured Image* window appears…

4: …right click on the image and from the menu click to select *Save Picture As…*

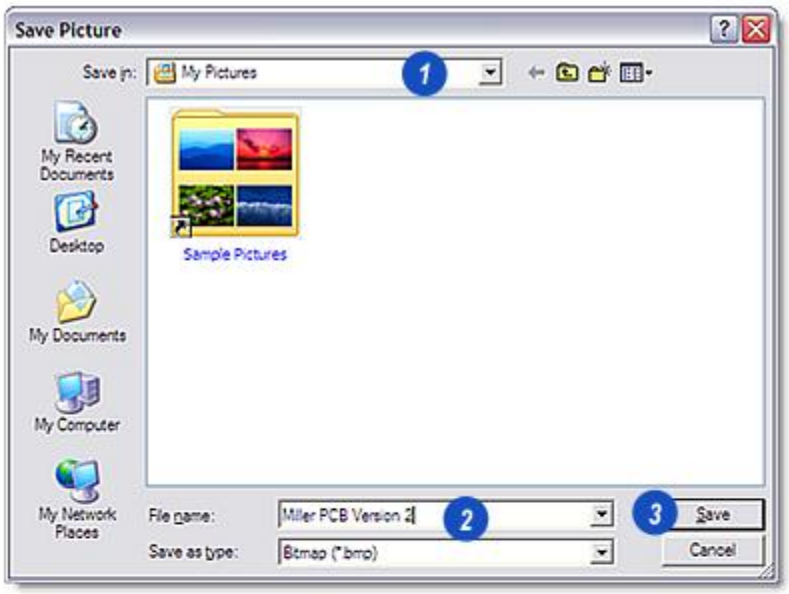


1: On the *Save Picture* dialog, navigate to the folder in which to save the image.

2: Type a name for the image and…

3: Click on the *Save* button.

Images are saved only in the *Windows bmp* format.



Multi-User Package

This is an optional module for DM Motorised microscopes. It has to be installed and licensed before it can be used.

Using *Multi-User Package*, each user of the microscope can create unique profiles and store them for retrieval later. A profile comprises the microscope hardware set up together with individual settings such as the light source and intensity for a specific combination of objective and contrast method.

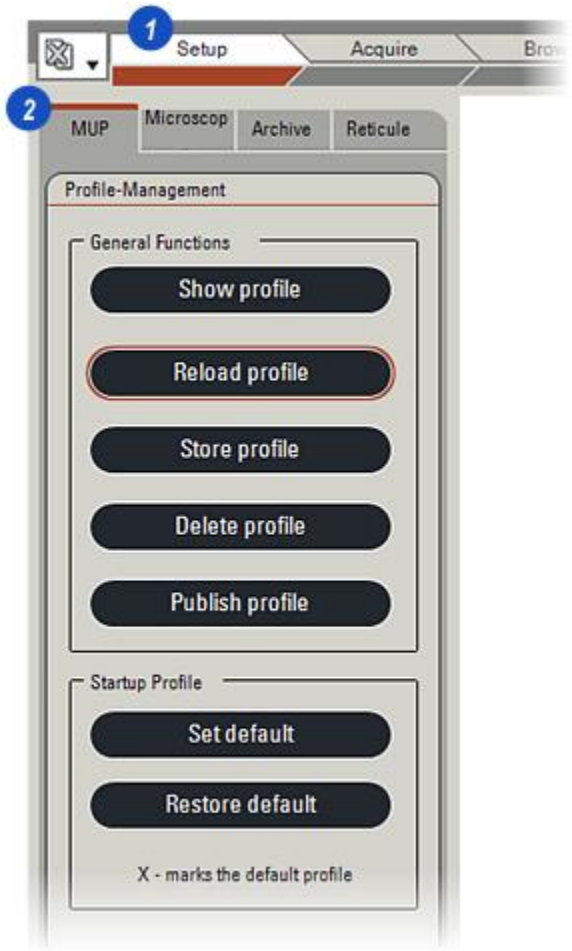
Both Administrators and Standard Users can create profiles but Administrators have an added facility in that they can make their profiles 'public' and available to all other microscope users. Standard Users can access only their own profiles.

Both Administrators and Standard Users can nominate a *Default Profile* from their Profile List that is automatically loaded when the microscope is switched on. If a *Default* has not been selected then the last set up used is loaded.

The *Multi-User Package* is available by:

1: Clicking on the *Setup Workflow* and...

2: ...on the *MUP* tab.



The *Multi-User* software recognises users by their Windows log-in so an Administrator or Standard User can be identified, the appropriate control panel displayed and access allowed to the proper *Profile List.*

Standard Users:

The first time a Standard User opens the *Multi-User Package* an empty *Profile List* is created. After a task has been carried out on the microscope, the settings can be saved as a profile by clicking the *Store profile* button.

1: The *Store new profile* dialog appears.

2: Click in the *Profile name* text box and type a unique name for the profile.

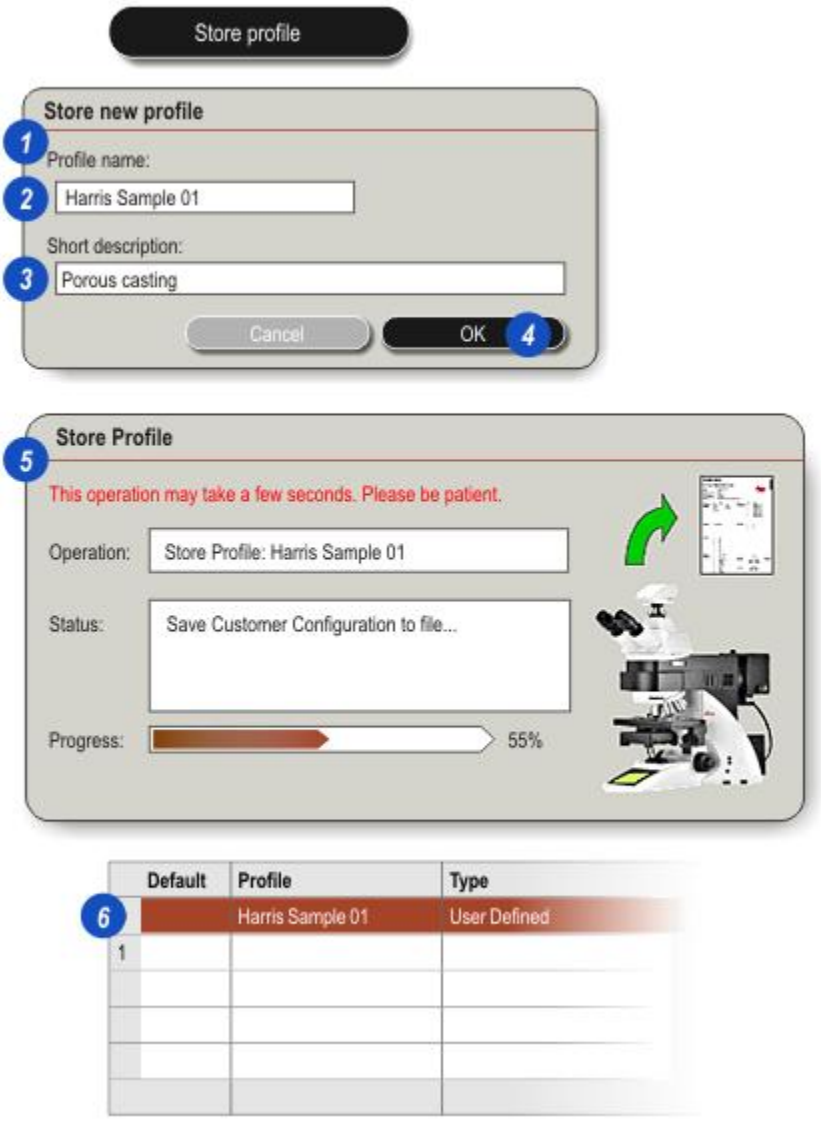
3: Click in the *Short description* text box and enter a relevant note about the settings.

4: Click *OK.*

5: The *Store Profile* message appears showing progress.

6: The profile is stored and appears in the *Profile List.* The *Type* entry, *User Defined* indicates that this profile is still private and owned by the user.

The user can then make changes to the microscope and store those as a separate profile which will also be added to the *List*. So, a 'library' of microscope settings are built up that can be re-loaded at will, saving the users time and guaranteeing consistency.



Reload a profile: The microscope settings stored in a profile can be quickly re-loaded by:

1: Click on the required profile in the *Profile List.*

2: Click the *Reload profile* button.

3: The *Reload* progress indicator appears - there are usually sounds from the microscope as well if objectives, filters and focus are being changed.

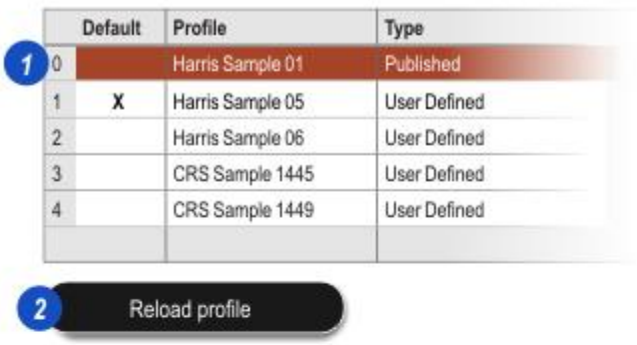
Delete a profile: To remove a profile from the *List*:

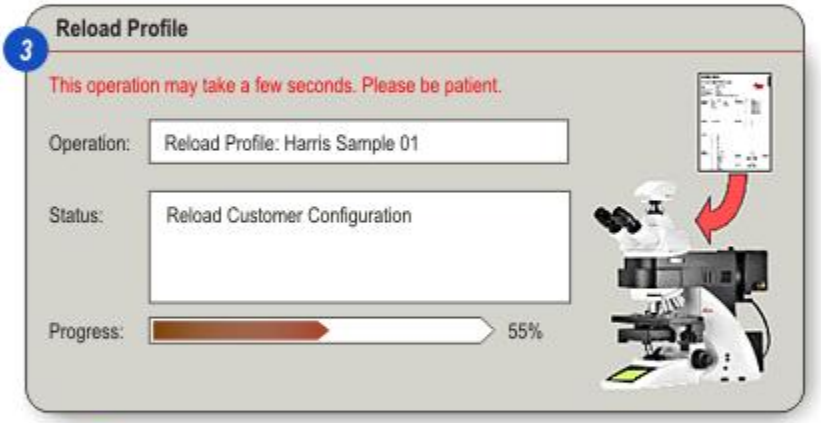
1: Click on the profile to be deleted in the *List*.

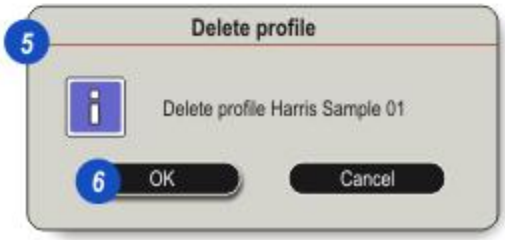
4: Click the *Delete profile* button.

5: Confirm or cancel the deletion on the dialog.

6: Click *OK*. The deletion cannot be reversed.







Select a Profile: To be used as the *Default* switch on settings:

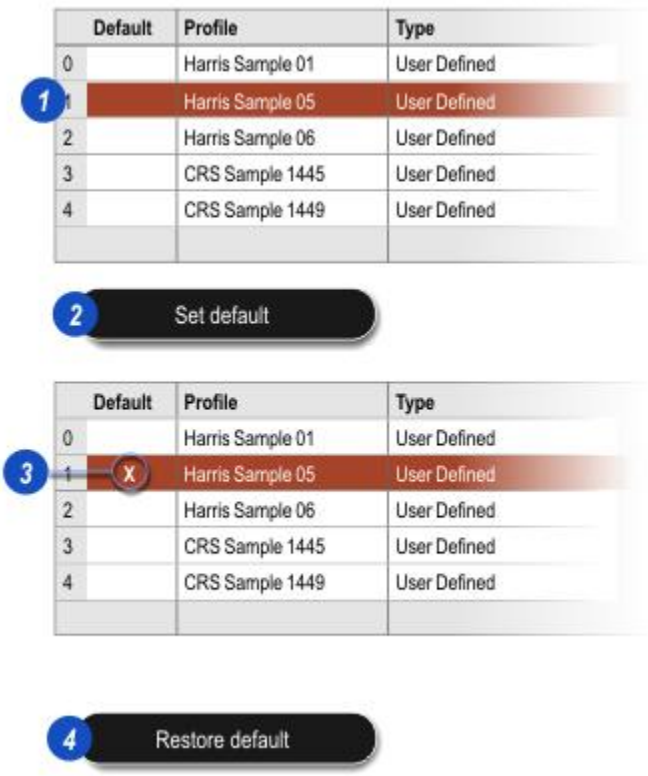
1: Click in the *Profile List* to select the profile to be used as *Default*.

2: Click the *Set default* button.

3: An **'X'** appears in the *Default* column on the *Profile List*.

4: To remove the *Default* selector, click the *Restore default* button.

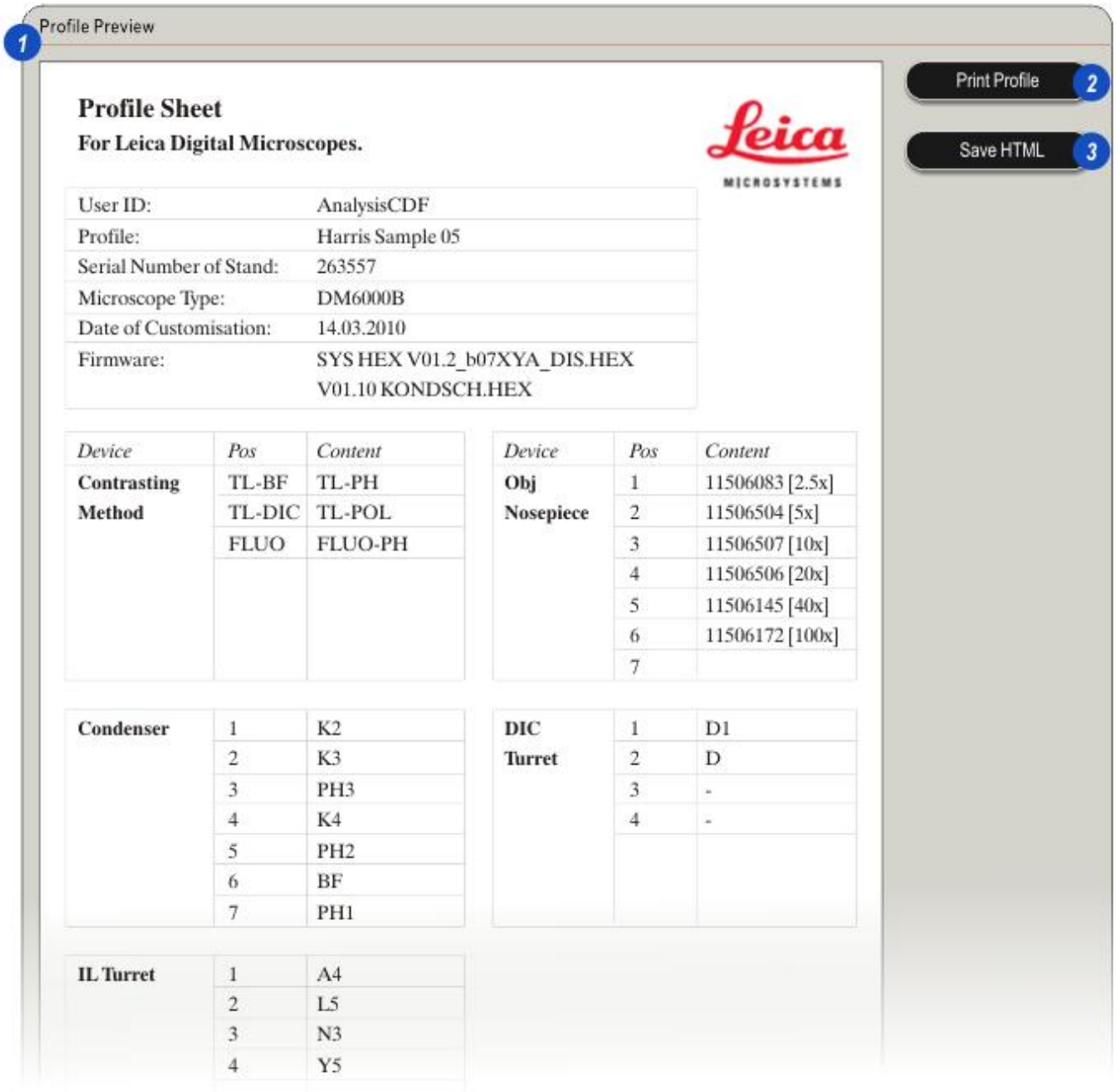
If a profile is not selected as the *Default*, the settings last used by the user will be loaded to the microscope at switch on.



Profile settings can be displayed by clicking the *Show profile* button - the *Profile Preview* **(1)** appears.

2: Print the settings by clicking the *Print Profile* button.

Profile data is stored in HTML format in a folder created by Leica Application Suite. Copy the HTML to a destination of choice by clicking the *Save HTML* button **(3)** and then navigating to the required folder.



Profile Sheet

For Leica Digital Microscopes.

Leica MICROSYSTEMS

User ID:	AnalysisCDF
Profile:	Harris Sample 05
Serial Number of Stand:	263557
Microscope Type:	DM6000B
Date of Customisation:	14.03.2010
Firmware:	SYS HEX V01.2_b07XYA_DIS.HEX V01.10 KONDSCH.HEX

Device	*Pos*	*Content*
Contrasting Method	TL-BF	TL-PH
	TL-DIC	TL-POL
	FLUO	FLUO-PH

Device	*Pos*	*Content*
Obj Nosepiece	1	11506083 [2.5x]
	2	11506504 [5x]
	3	11506507 [10x]
	4	11506506 [20x]
	5	11506145 [40x]
	6	11506172 [100x]
	7	

Condenser	1	K2
	2	K3
	3	PH3
	4	K4
	5	PH2
	6	BF
	7	PH1

DIC Turret	1	D1
	2	D
	3	-
	4	-

IL Turret	1	A4
	2	L5
	3	N3
	4	Y5

Profile data is stored in HTML format in a folder created by Leica Application Suite.

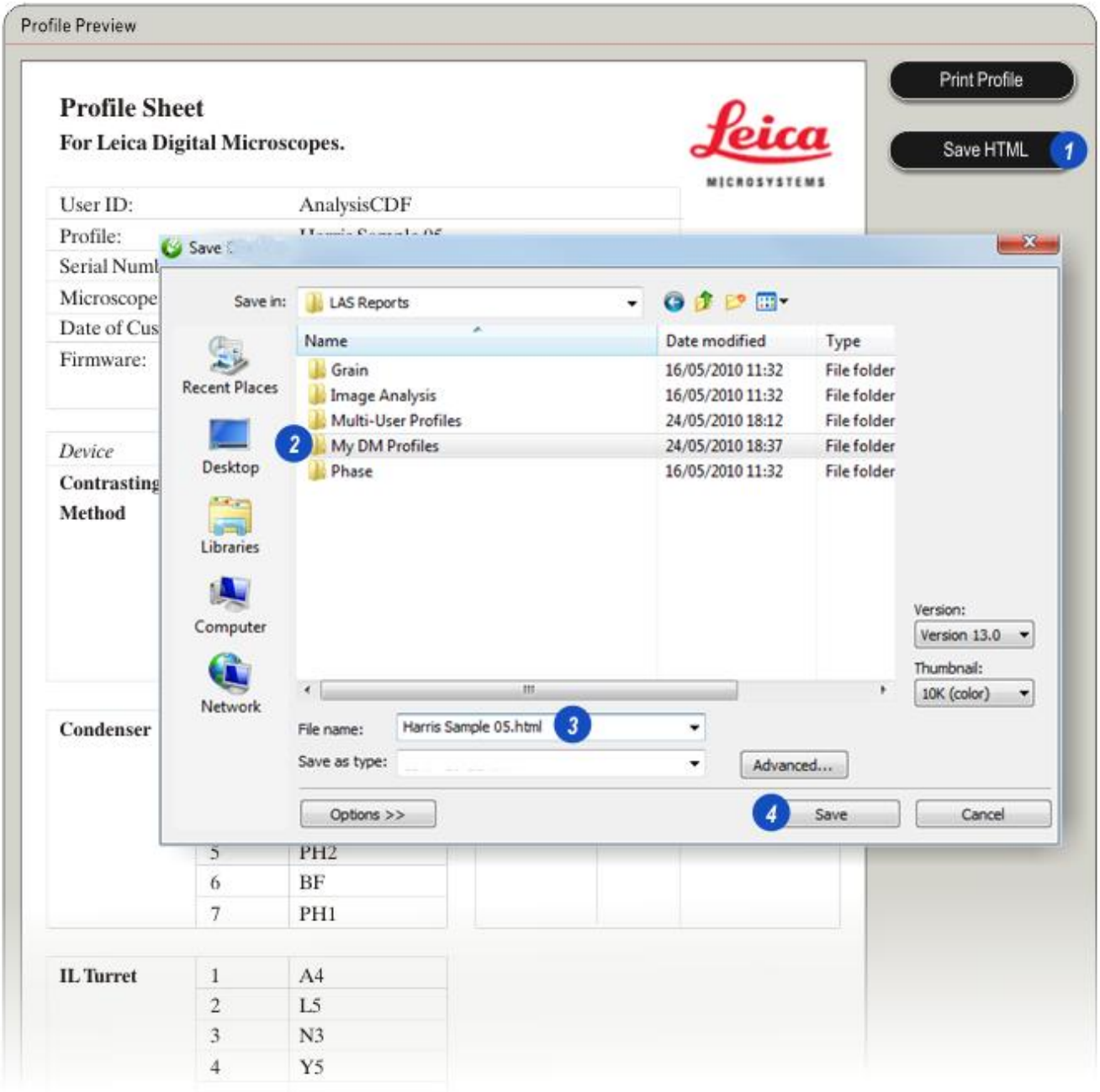
The file can be copied to a folder of the users choice and from there can be distributed to other users, for example by e-mail. Copy the HTML to a destination of choice by:

1: Clicking the *Save HTML* button...

2: ... and then navigating to the required folder.

3: Give the copy file a name and...

4: ...click *Save*.



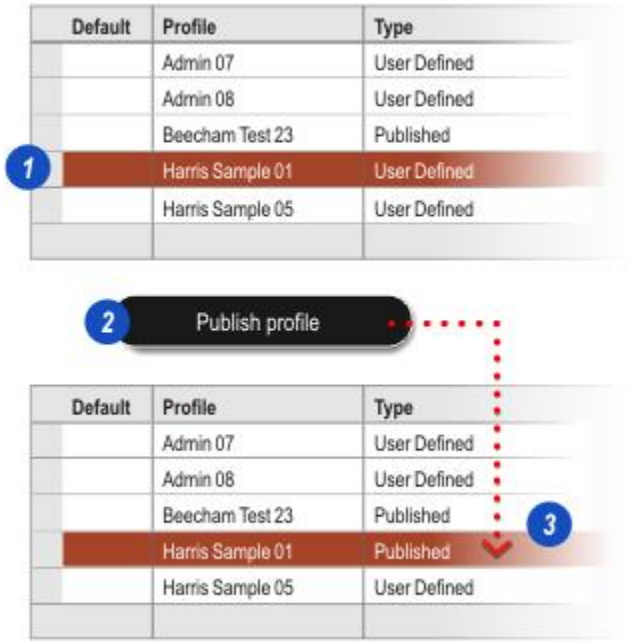
Administrators have the same tools as Standard Users plus the facility to publish any profile from their own list.

Publishing makes a profile available for sharing among all the other computer users.

1: From the *Administrator's Profile List* the profile to be published is clicked to select it.

2: Click the *Publish profile* button.

3: The profile becomes available to all users and is marked as such with the *'Published'* label.



Leica Microsystems (Switzerland) Ltd.
Stereo and Macroscope Systems
CH 9435 Heerbrugg
Switzerland

Telephone: +44 1223 411411
FAX +44 1223 210692
Hotline: +44 1223 401824
di.support@leica-microsystems.com
www.microscopy-imaging.com

Leica
MICROSYSTEMS