Imperial College London

Facility for Imaging by Light Microscopy

Observing Life As It Happens

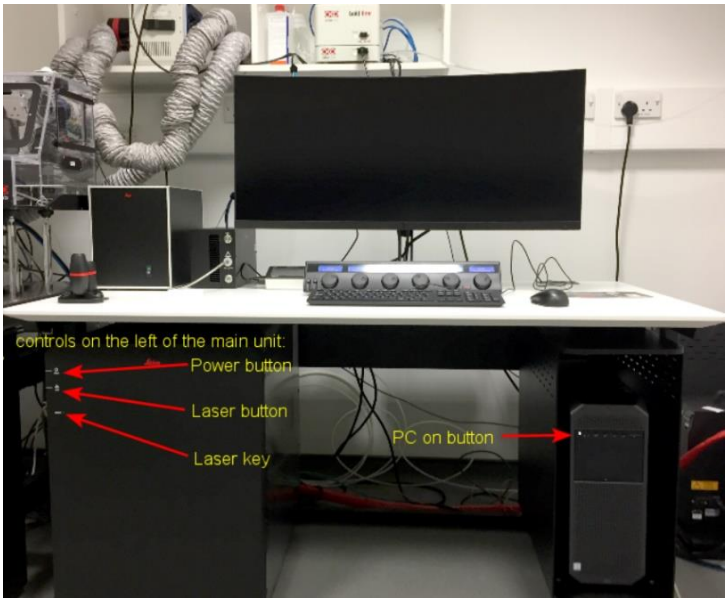
QUICKSTARTGUIDE: HCF3 - CONFOCAL LEICA Stellaris 8 inverted

(ICTEM 312)

NB All users must have completed the on-line “Introduction to Laser Safety” from Imperial College safety department

Contents

Startup Procedure



- Start PC
- On the central power unit - Switch on:
    - Power
    - Laser
    - turn on Laser Emission Key

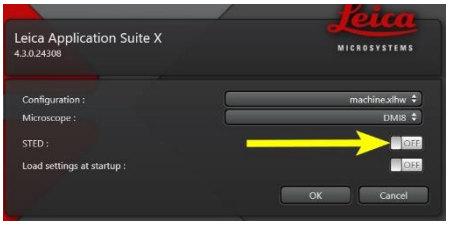
- !!! IMPORTANT !!! The microscope stage will auto calibrate (move) – to avoid trapped fingers DO NOT LOAD A SAMPLE UNTIL THE MICROSCOPE HAS FINISHD STARTING UP

- Login (IC network account)
- Wait until the TFT screen on the front of the microscope has finished booting
- Start LEICA Application Software “LAS X” on desktop

Software Setup

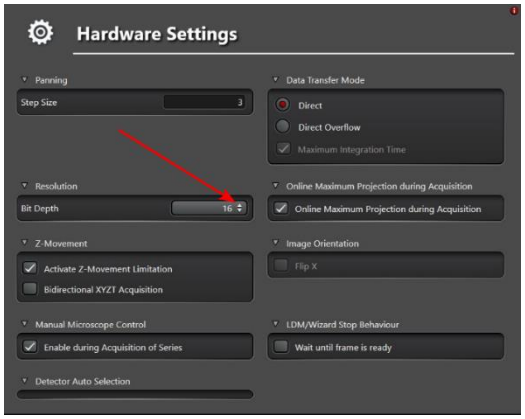
There are two possible hardware configurations for confocal: with or without environmental control

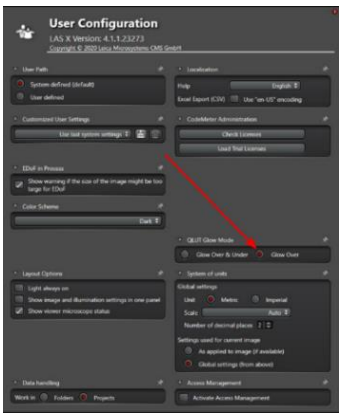
In the start-up window from the drop-down menu select machine.xlhw
NB. make sure the STED option is deselected



“Configuration” tab

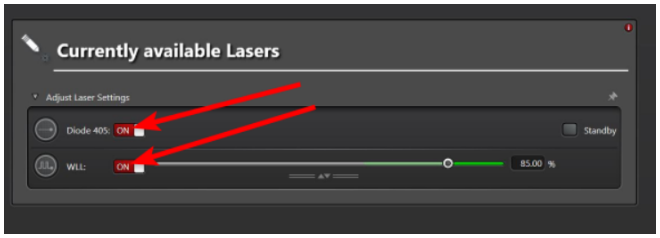
Select "Hardware" and set the required Bit Depth – 16 Bit



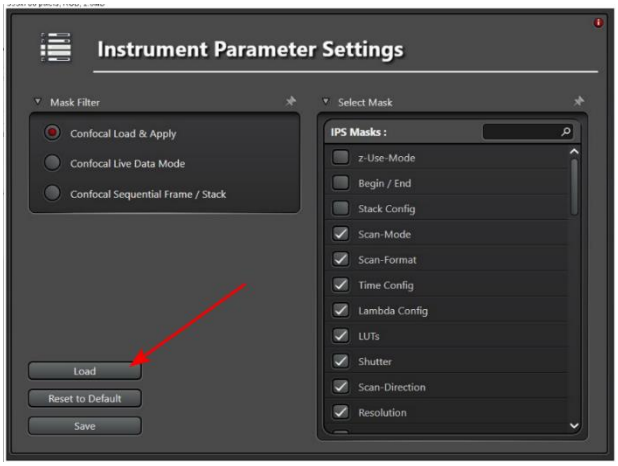


Change the range indicator lookup table to Glow Over in the “User Configuration”

Select "Laser Config" and turn on the 405 Diode and WLL (white light) lasers



Re-Using settings



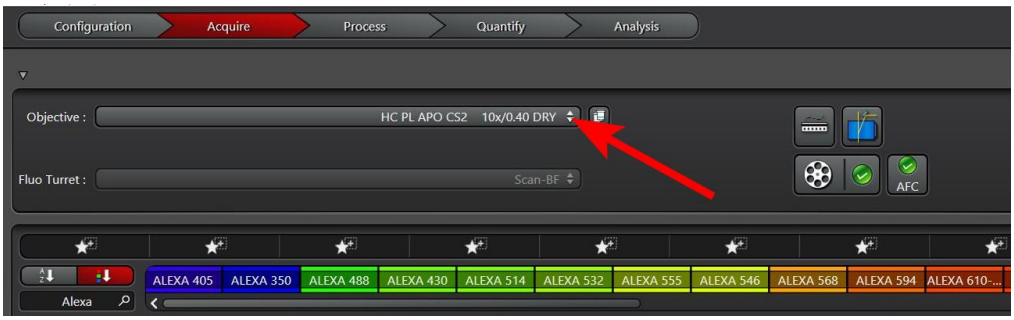
In the **Configuration** tab:

- Select IPS
- Select Load
- Select All_Users.xml from the E:\ drive
- Then applying or loading experimental settings will load all image settings

Microscope Controls

Changing Objectives

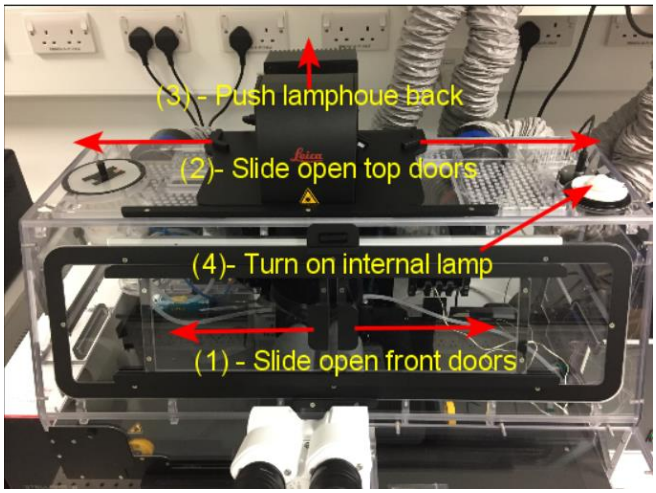
Select the “Acquire” tab and in the main setup window:



- Click on the objective and a list will appear allowing you to select the correct objective
- This will also bring up another window if changing to or from oil or water immersion
- You will need to click yes on this window to move the objective before adding oil/water
- NB - clean the oil objective and sample before clicking yes and moving to the dry objectives

Microscope chamber

Loading and changing samples:



The chamber light can also be controlled with a footswitch once turned on

TFT Touch screen on the microscope stand

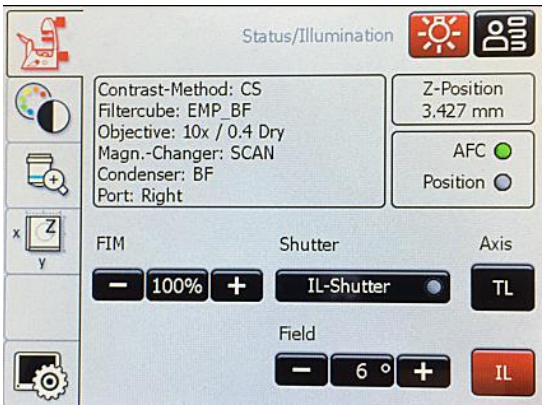
Contrast tab (1)
- select viewing method (2):

Fluorescence (Fluo) (2).

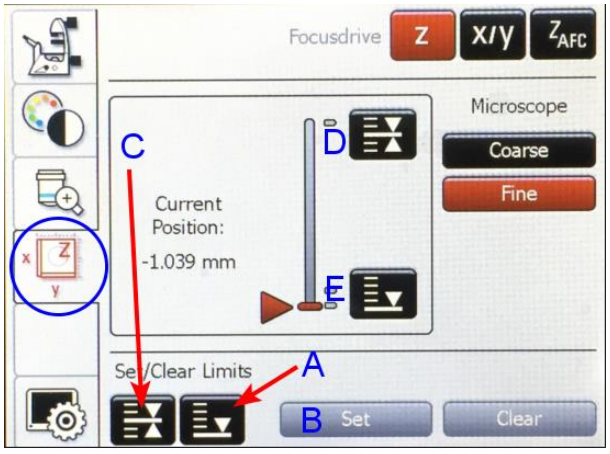
- open IL-Shutter (3)
- This system uses a multi-cube – all channels are on together (4)
- LED brightness can be controlled with the knob on the left side of the microscope stand

Brightfield (BF) or Differential interference contrast (DIC)

- see next window



To Adjust the Brightfield use the microscope tab, open the brightfield (IL) shutter, and adjust intensity and condenser aperture, using the controls on the left side of the microscope stand



Setting the Focal plane

The “load sample” and “sample focus” positions can be recorded to make changing to oil immersion objectives easier (start software first)

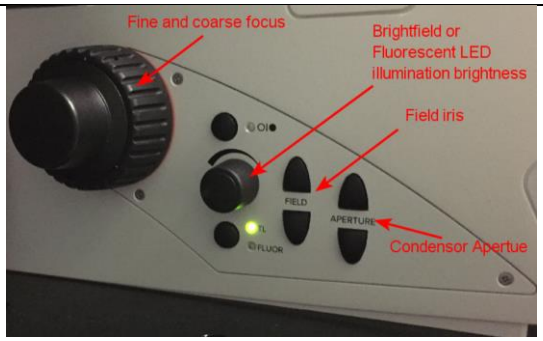
<u>Focus at low magnification (the 10x objective)</u>

On the microscope TFT panel, select the x,y,z tab. Wind down the focus to its lowest position, press button A followed by the set button B. Place the sample on the stage and focus. Press button C followed by the set button B

<u>Changing to oil objective</u>

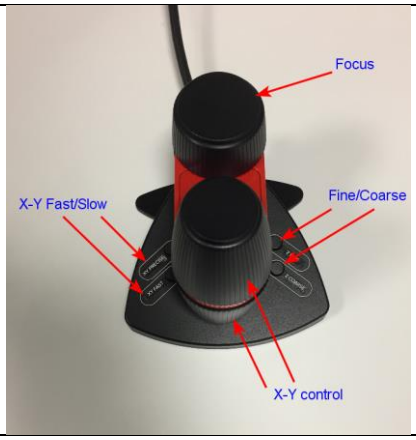
Press button E to lower the objective, remove sample, change to the oil objective, add oil, and replace the sample. Pressing D will bring the objective back up into focus

Microscope stand controls



There are controls on the left-hand side of the microscope stand to control:

- The Brightfiled lamp or the Fluorescence LED intensity (which ever method is currently selected)
- Condenser and field Iris
- Fine and coarse focus

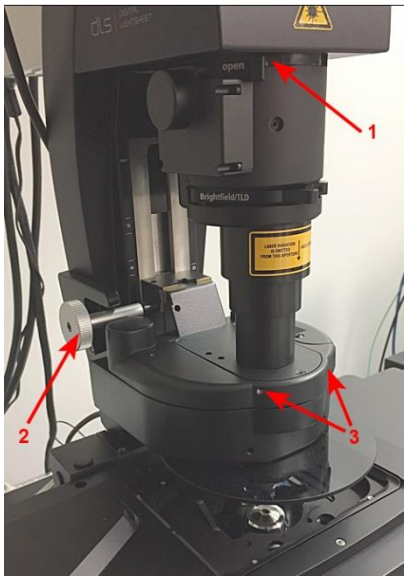


Stage controller:

- Focus (Coarse / fine)
- X-Y movement (fast / slow)

Köhler illumination

- Select a Low power objective (x10 or x20)
- Switch on brightfield
- Look through the eyepiece, if necessary, adjust the eyepieces for your eyes
- Adjust the brightness if necessary (TFT panel / left side of microscope stand)
- Focus on your sample
- Open the condenser aperture iris (TFT panel)
- Fully close the field iris above the condenser (1)
- Looking down the eyepiece, focus the black edges of the field iris with the large silver focus wheel (2)
- If necessary, centre the field iris with the Köhler screws (3) - Allen keys to adjust them are found on the back of the condenser (right side)
- Re-open the illumination iris so that the black ring just disappears from your field of view



Software / Image Acquisition

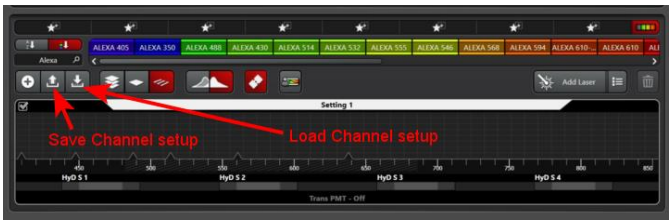
Select “Acquire” tab and select the desired acquisition mode eg XYZ

Channel Setup – Loading an existing setup

Load settings (Acquire” tab):

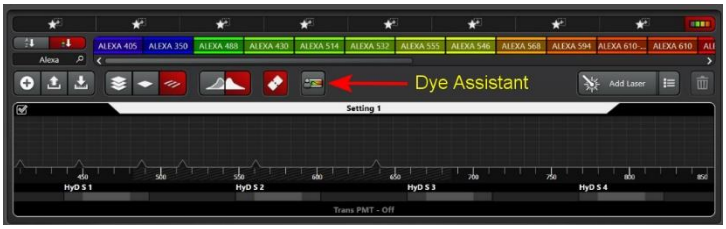
- Either, from a previously saved Image. Open the saved image/library in the “projects” tab and select the image. Then click on the APPLY icon on the menu above (NB. The objective, pixel number, bit depth and zoom and averaging are not re-loaded and may need re-setting).

- Or use the Load channel setup option from the main setup window to open a previously created and saved channel setup



Channel Setup - Automatic using the “Dye Assistant”

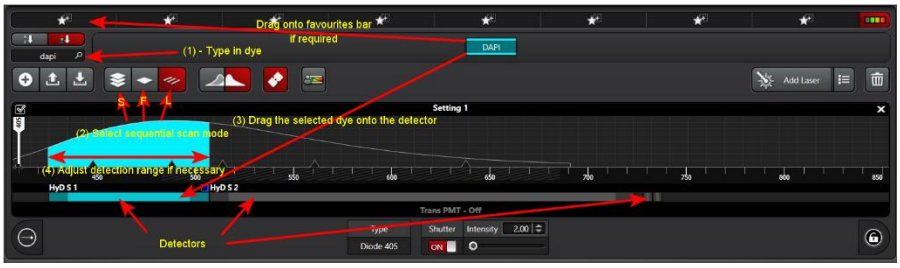
- select the Dye Assistant setup button



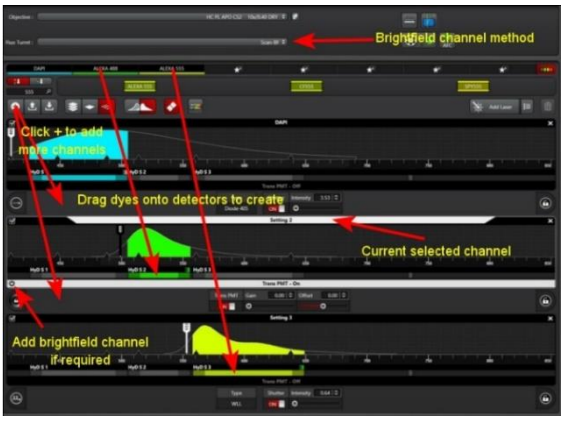
- a new window will open allowing channel selection by fluorophore.
- clicking on the "..." button will load a search window allowing dye to be selected
- repeat adding channels as necessary (a new line will automatically appear for each new channel)
- Select the sequential scan method of choice (usually Line sequential) by pressing apply. This will setup the channels and detectors automatically

Channel Setup – New manual setup

Searching for fluorophores, and dragging/dropping into channels



1. Type in dye into the search box
2. Select sequential method (Stack - between stacks, Frame - between frames, Lines - between lines)
3. Drag dye onto a detector (it will auto select the detectors)
4. Adjust detector range if required



Add further tracks/dyes as required using the (+) symbol

Setting Intensity

- Start preview scanning by clicking on either the LIVE button or FAST LIVE (bottom of the screen)
  - Live – scans all channels at scan capture settings (size, averaging, etc – can be quite slow).
  - Fast Live - scans only the currently active channel (512 format and no averaging). This is good for setting up.
  -
- Select a channel in the Channel setup window by clicking on the “Setting” panel and use the control panel to optimally set the Smart Gain, Smart Intensity, Zoom and Focus. Repeat for each channel.

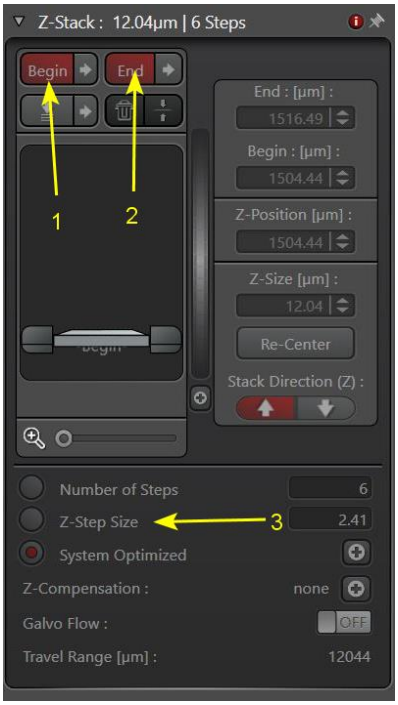
- Use the “Range Indicator” LUT to  help with this.

Image Format

Use the XY panel to Set

- Image format (1) 1024 x 1024 is typical but depends on image requirements
- zoom factor (2) - (If required, move the Zoom Area using the arrow icons, NB Zooms above 4 results in empty magnification at 1024 x 1024 pixels)
- Averaging (3) - required to give you sufficient image quality:
  - line averaging for live imaging
  - line or frame averaging for fixed cells
- Pinhole (4) - is preset at 1AU but may be adjusted to change Z volume

Z-Stacks

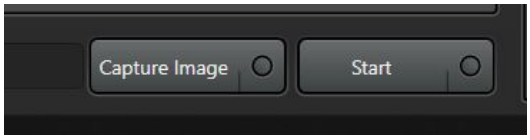


- Adjust focus in "live" mode to start of Z- stacck and select Begin (1)
- Adjust focus in "live" mode to end of Z- stacck and select End (2)
- Set step size - the "optical section" size (z) can be read from the X-Y panel or the "+" button will allow Nyquist settings to be applied

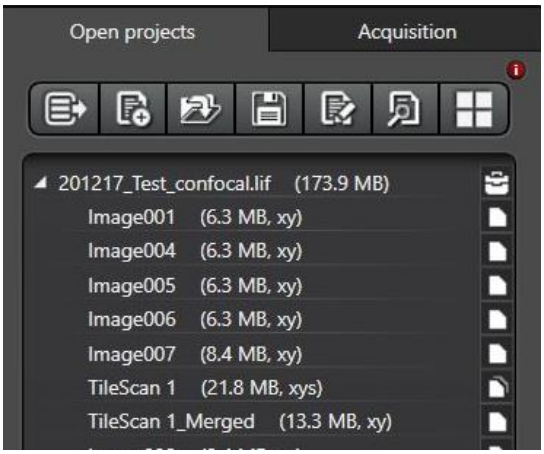
When finished with Z stack mode there is a "Trash" button to remove the stack settings

Capturing Images

Once the setup is compplete the image is recoreded using either “Capture Image” for a single image or use “Start” for an image sequence (Z-stack, tiling or time series)



Saving Data



- Images are stored in a library (.lif file format)
- Every time Capture Image or Start is pressed the image is added (but NOT SAVED) to the Library in the “Open projects” tab.
- Right clicking on the individual image names allows renaming or deleting.
- To save the images click the “Save” icon above.
- The first time it will prompt for a location and the Library name.
- Then every time you capture an image press the “Save” icon to update the library or you could lose your data.

Setting Environmental Control

This unit can be controlled either manually (independent of the software) or through the software using machine-Climate-Control.xlhw option when starting LAS-X, however, the unit needs to be turned on before running this software option.

Switching On

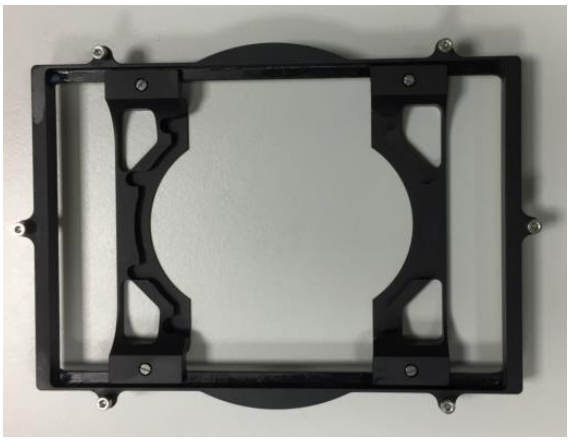
- Make sure chamber is closed
- Turn on the controller on the desk by pressing and holding the “On” button for about 3 seconds
- Press the T^{0}C button to adjust the temperature
- Press the CO_2% button to set CO_2

Turning off

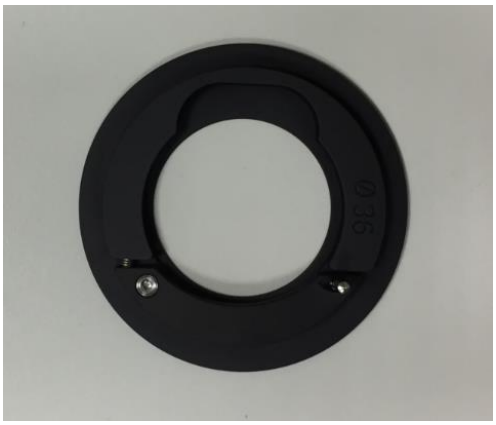
- Turn off the controller on the desk by pressing and holding the “On” button for about 3 seconds

Sample Holders

Universal Holder
Slides and 35mm dishes



35mm dish insert
for the universal holder



Multiwell plate holder

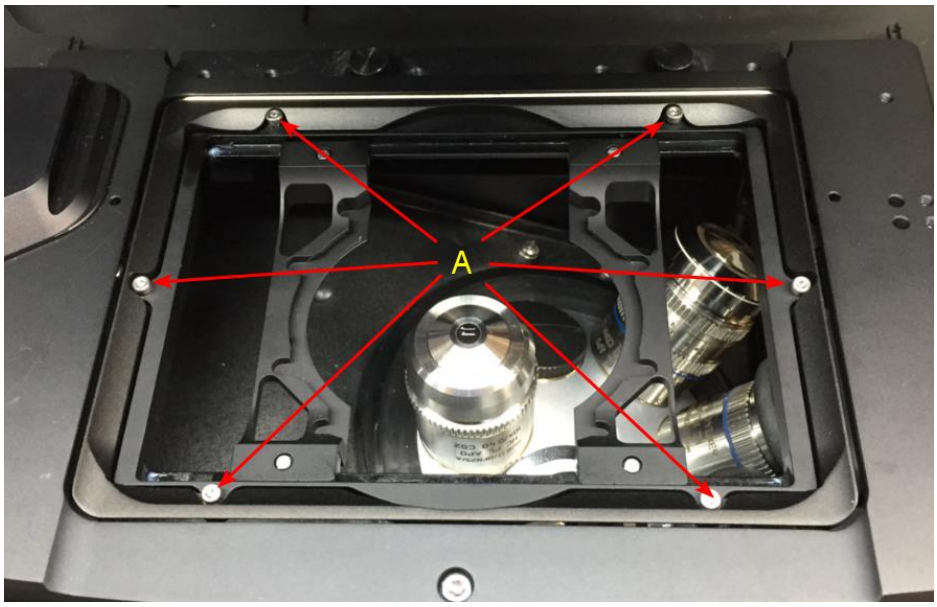
Environmental lid
for multiwell plate holder

Multi-format holder
with environmental lid

Changing Sample Holders

The sample holders are retained by a series of small screws (A). To change the sample holder, loosen these screws (4 turns) with the Allen key provided. **Important, do not completely unscrew these screws** Just 4-5 turns will release the stage and allow its removal with part of the screws still attached in their threads. **Be careful not to lose these screws!** Swap over the holder and lightly tighten the screws.

DO NOT OVER TIGHTEN SCREWS



Shutdown Procedure

- In the Configuration tab and laser config, **switch off lasers**
- Close LASX
- Update booking if necessary.
- Remove your samples & clean objective lenses with fresh lens tissue
- Clear up the desk
- Save files onto the server
- On the central power unit - Switch off:
  - Laser Key
  - Laser
  - Power
- Shut down PC or sign out for next user