Imperial College London

Facility for Imaging by Light Microscopy

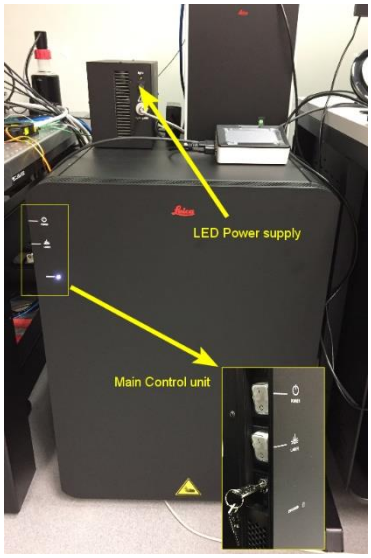
Observing Life As It Happens

QUICKSTARTGUIDE: LS1 - CONFOCAL LEICA Stellaris 5 inverted

(SAFB410)

Contents

Startup Procedure



- Start PC – Wait until the login screen appears
- Then on the central power unit - Switch on:
    - Power
    - Laser
    - turn on Laser Emission Key
    - Turn on LED Power supply
- 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

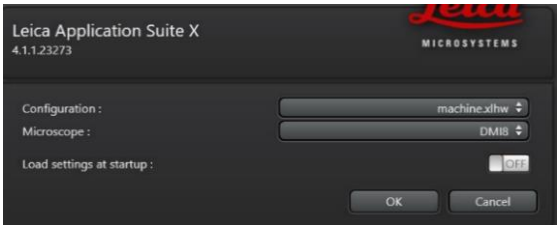
Software Setup

There are two possible hardware configurations: Light Sheet and Confocal determined by which condenser is installed (labelled either LightSheet or Confocal on the condenser housing). The confocal condenser needs to be installed if a brightfield image is required.

Very Important!! - CONDENSERS MAY ONLY BE CHANGED BY FILM STAFF

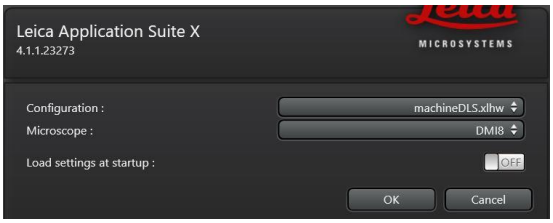
If the confocal condenser is installed:

- In the start-up window from the drop-down menu select either:
    - machine.xlhw – use without environmental control
    - machine – Okolab.xlhw – use with environmental control - (NB Heating unit must be on before starting)



If the Light Sheet (LightSheet) condenser is installed:

- In the start-up window from the drop-down menu select either:
    - machineDLS.xlhw – use without environmental control
    - machineDLS – Okolab.xlhw – use with environmental control - (NB Heating unit must be on before starting)

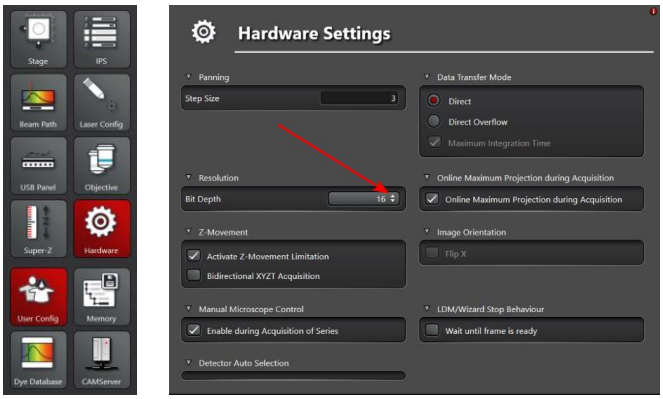


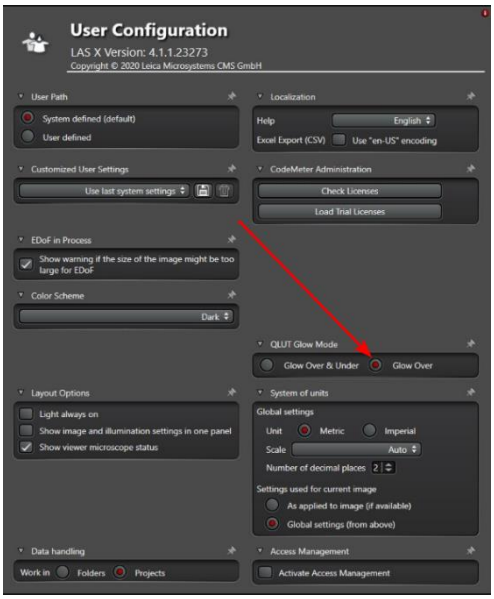
Stage Calibration

If the stage has not been calibrated since switching on, then a message window will appear asking if you want to calibrate the stage. If tiling, multi-position or using the “navigator” option is required, then the stage must be calibrated. Important - If you choose YES, the stage will automatically move to the extreme of its range, so make sure the condenser turret is raised to prevent damage and do not put your sample on the stage until the calibration has completed

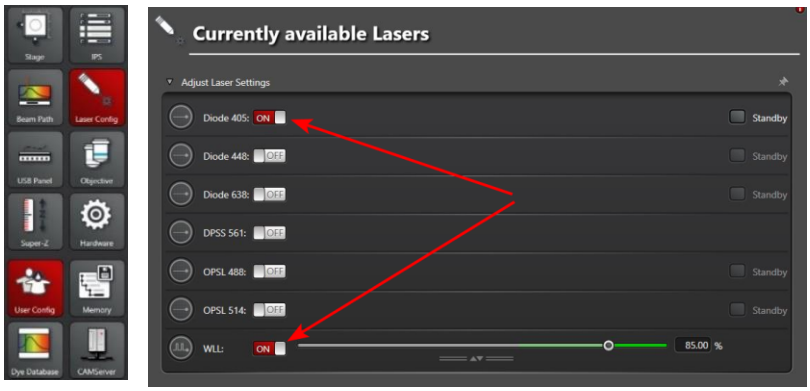
“Configuration” tab

- Select "Hardware" and set the required Bit Depth – 16 Bit
- Change the range indicator lookup table to Glow Over in the “User Configuration”





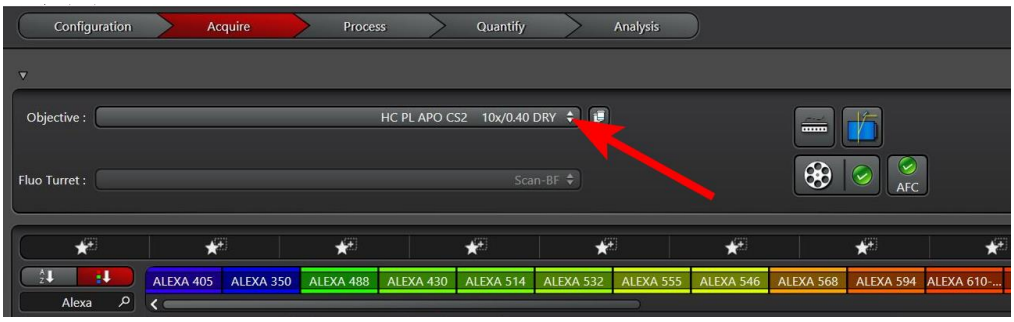
- Select "Laser Config" and turn on the 405 Diode and WLL (white light) lasers



Microscope Control

Changing Objectives

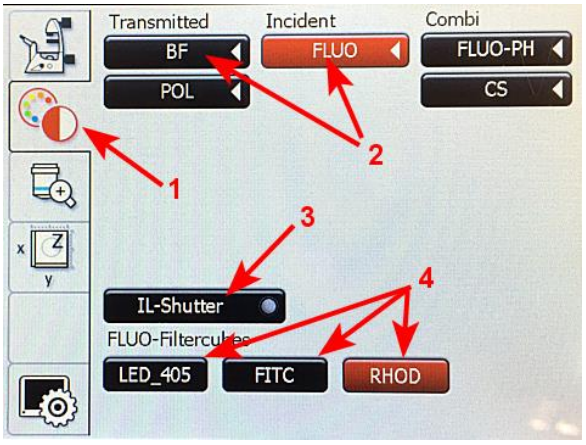
Select the “Acquire” tab and in the main setup window:



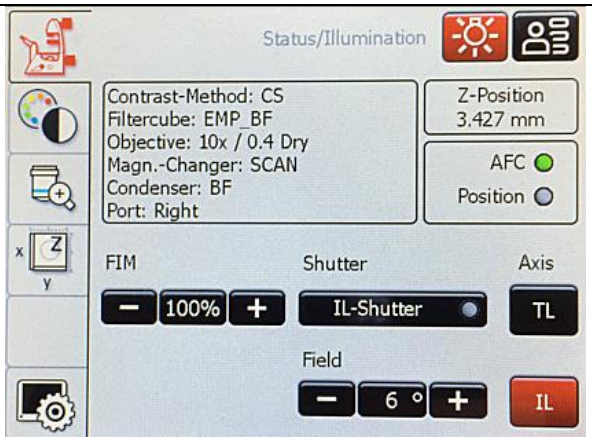
- Click on the objective and a list will appear allowing you to select the correct objective
- This will also bring up another window if changing to or from oil or water immersion
- You will need to click yes on this window to move the objective before adding oil/water
- NB - clean the objective or specimen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

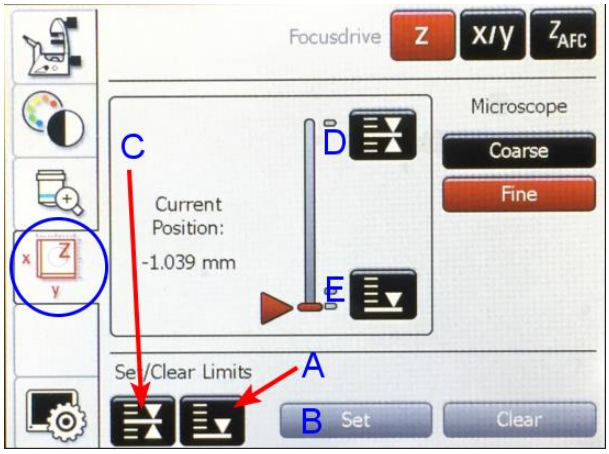
TFT Controls

Touch screen on the microscope stand

<table>
<tr>
<td> 
</td>
<td>Contrast tab (1)
- select viewing method (2):

Fluorescence (Fluo) (2).
• open IL-Shutter (3)
• select required fluorescence channel (4)
• LED brightness can be controlled with the knob on the left side of the microscope stand

Brightfield (BF)
• see next window</td>
</tr>
<tr>
<td> 
</td>
<td>To Adjust the Brightfield use the microscope tab
(1) open the brightfield shutter, and adjust intensity (2) and condenser aperture (3),
Make sure the Brightfield mode is selected at illumination arm!
  </td>
</tr>
</table>



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)

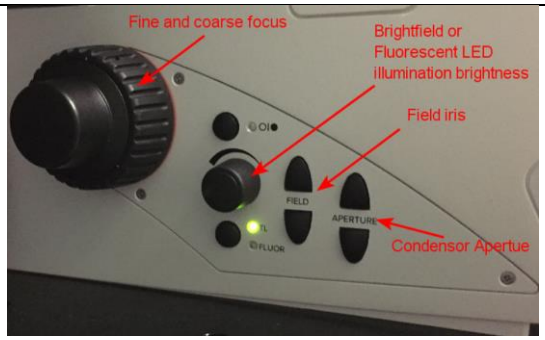
Focus at low magnification (the 10x objective)

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

Changing to oil objective

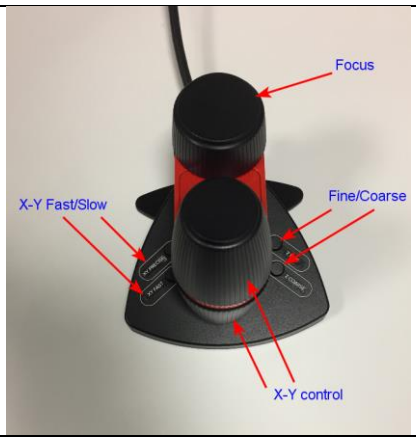
Press button E to lower the objective, remove sample, change to the oil objective, add oil, and replace the sample. Pressing D will bring the objective back up into focus

Microscope stand controls



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

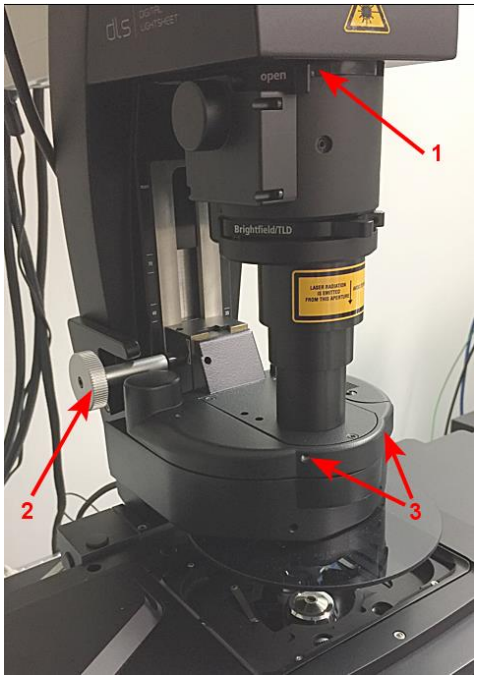
- The Brightfiled lamp or the Fluorescence LED intensity (which ever method is currently selected)
- Condenser and field Iris
- Fine and coarse focus



Stage controller:

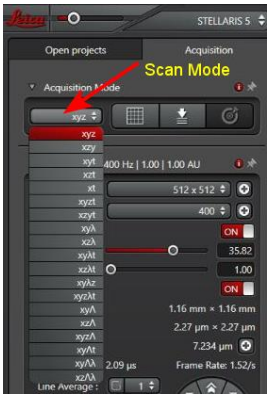
- Focus (Coarse / fine)
- X-Y movement (fast / slow)

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)
- 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 choose the desired acquisition mode e.g. XYZ

Channel Setup – Loading an existing setup

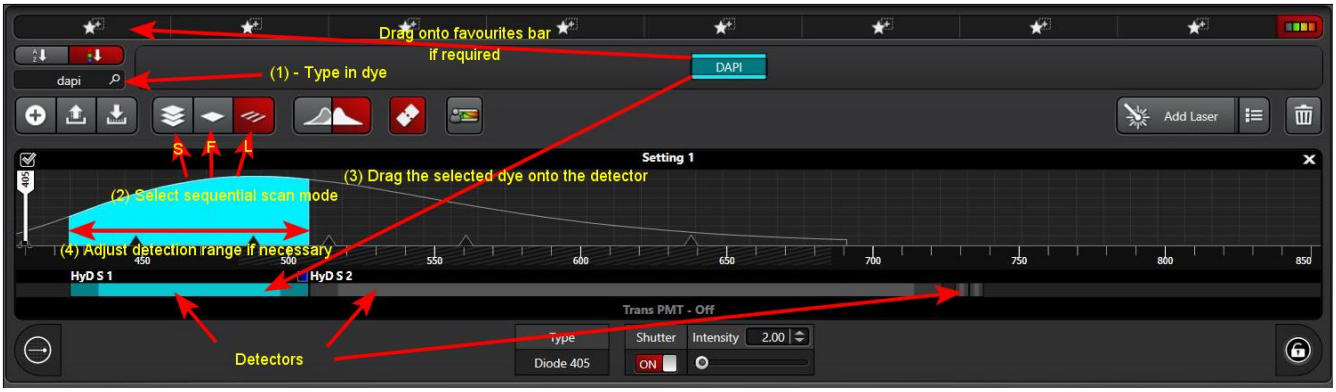
Load settings (Acquire” tab):

- 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).

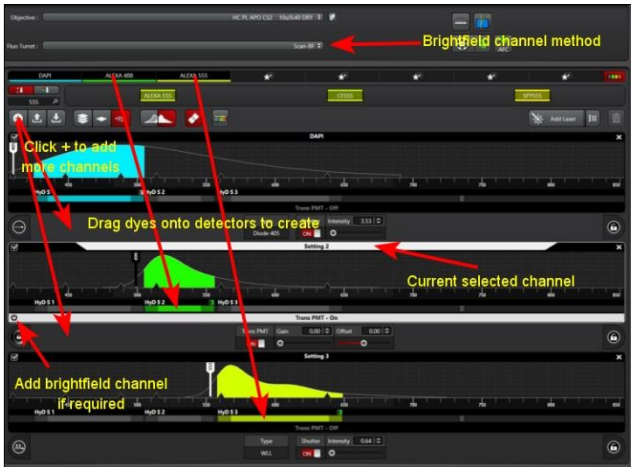
- Or use the Load channel setup option from the main setup window to open a previously created and saved channel setup

Channel Setup – New manual setup

Searching for fluorophores, and dragging/dropping into channels



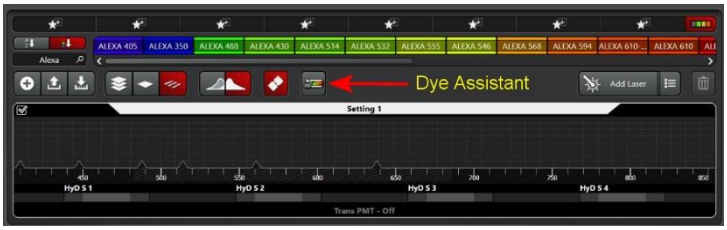
1. Type in dye
2. Select sequential method (S - between stacks, F- between frames, L- between lines)
3. Drag dye onto detector (it will auto select the detectors)
4. Adjust detector range if required



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

Channel Setup - 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

Setting Intensity

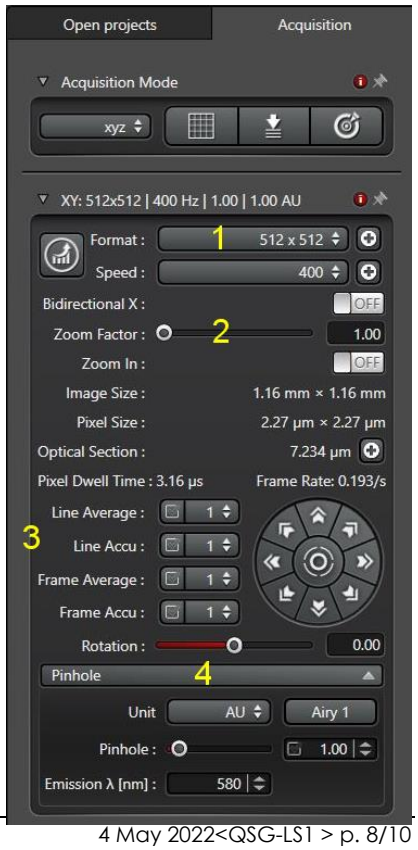
- Start preview scanning by clicking on either the LIVE button or FAST LIVE (bottom of the screen)
    - Live – scans all channels at scan capture settings (size, averaging, etc).
    - Fast Live - scans only the currently active channel (512 format and no averaging)

- Select a channel either in the Channel setup window or by clicking on the channel in the displayed live image and use the control panel to optimally set the Gain, Intensity, Zoom and Focus. Repeat for each channel.
- Use the “Range Indicator” LUT to help with this.

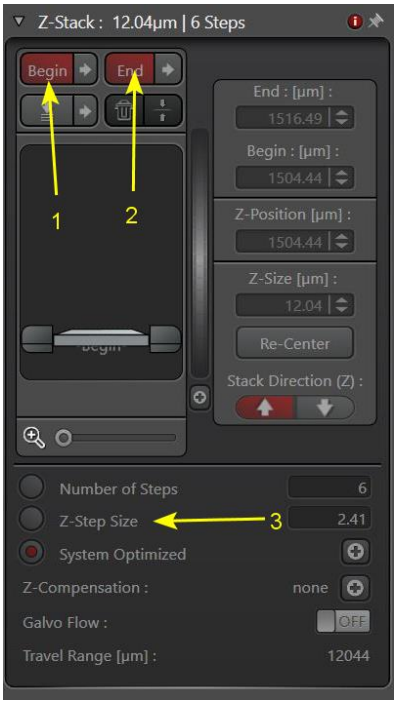
Image Format

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



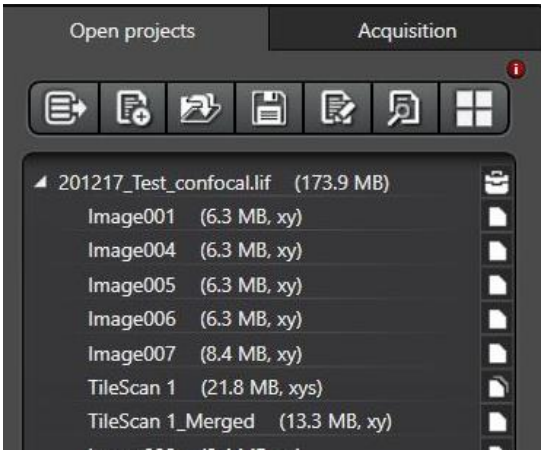
- Adjust focus in "live" mode to start of Z- stacck and select Begin (1)
- Adjust focus in "live" mode to end of Z- stacck and select Begin (2)
- Set step size - the "optical section" size (z) can be read from the X-Y panel or the "+" button will allow Nyquist settings to be applied

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.

Shutdown Procedure

- 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
- Turn off LED unit
- On the central power unit - Switch off:
    - Laser Key
    - Laser
    - Power
- Shut down PC