ImperialCollegeLondon

FacilityforImagingby LightMicroscopy

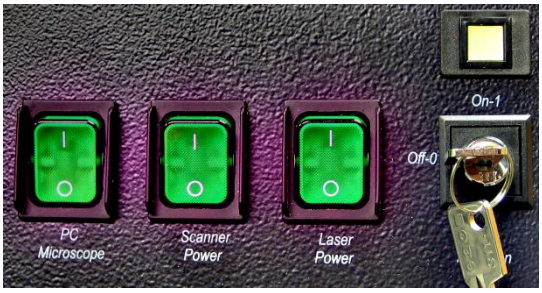
ObservingLifeAsItHappens

QUICKSTARTGUIDE:
CONFOCAL6:
LEICASP8
inverted
(SAFB408)

Contents

Startup Procedure

- On the main control panel Switch on (left to right):
  - PC Microscope
  - wait 15-20 seconds
  - Scanner Power
  - Laser Power and turn the Laser Emission Key to ON



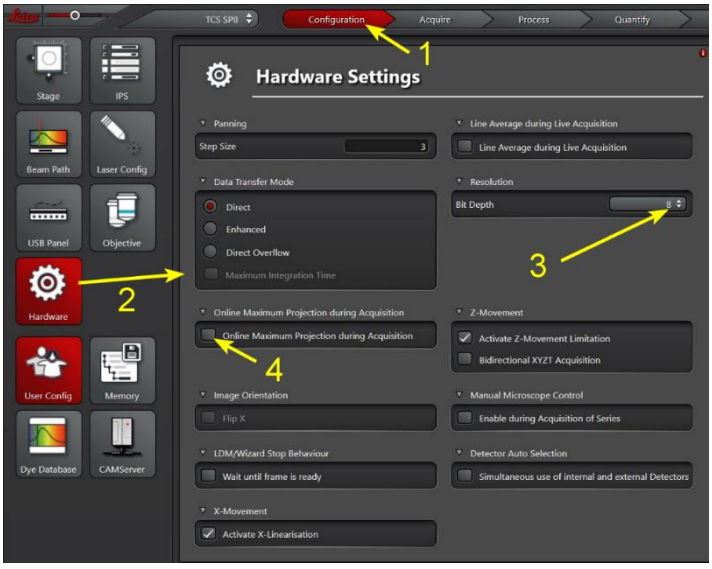
- During startup the stage will calibrate (move) – Important - Do not put your sample on the stage until the start-up procedure has completed
- Login (IC network account)
- start LEICA Application Software “LAS X” on desktop
- In the start-up window select the required configuration from the drop-down-menu:
  - machine_No Heater – use without environmental control
  - machine Heater – use with environmental control
  - select Resonant mode if required

Configuration Tab

Select **"Hardware"** (2)

Set required Bit Depth – 16 Bit (3)

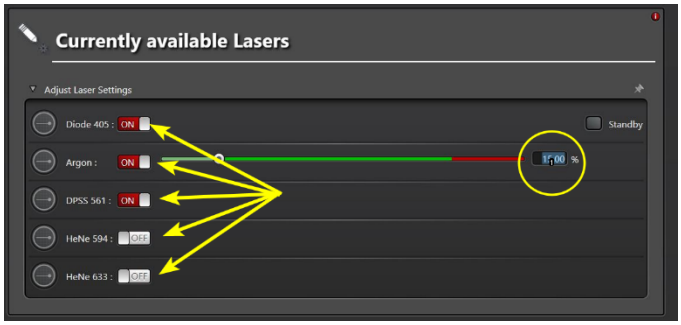
Option to display MIP during capture (4).



Select **"Laser Config"** and turn on the lasers you need

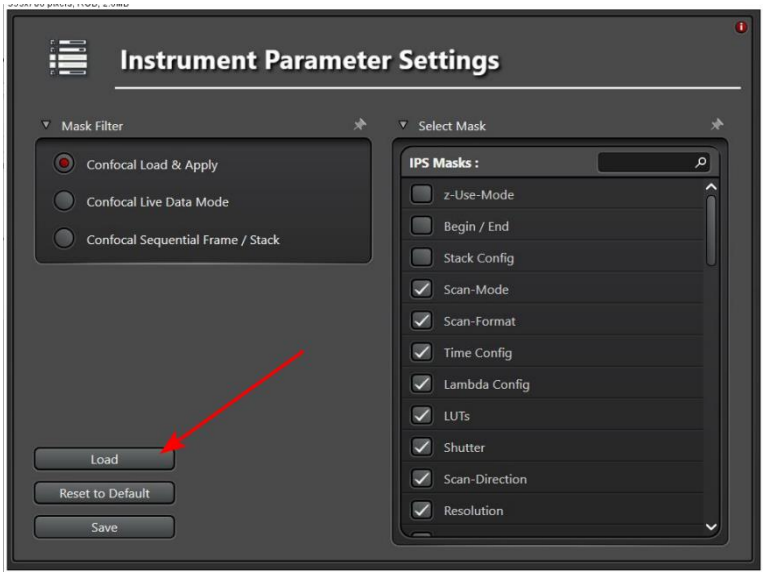
- Set the percent power on the Argon laser to 15% for normal imaging.

For photobleaching higher power may be used (NB do not use HyD detectors).



The re-use functionality

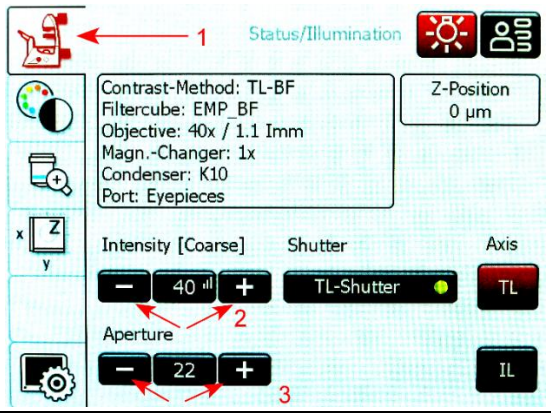
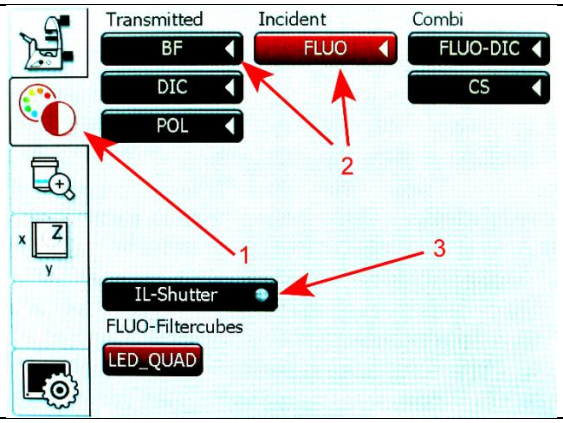
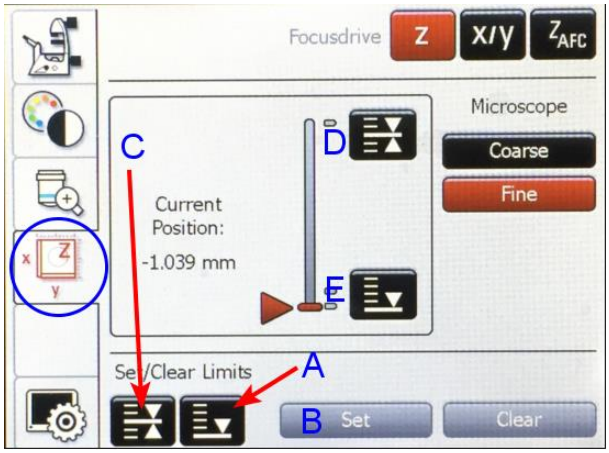
- Select "**IPS"**
- Select Load
- Select **All_Users.xml** from the D:\ drive – do not save just load



Then applying or loading experimental settings will load all image settings correctly

Microscope Control

The TFT screen on the front of the microscope:

Brightfield Control	Fluorescence/Contrast
	
Brightfield window (1) is used to adjust brightfield shutter, intensity(2) and condenser aperture (3),	Contrast/fluorescence window (1) is used to select brightfield or fluorescence mode (2) and also fluorescence shutter.
	**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

Changing Objectives

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

LED System

This microscope is fitted with a Quad cube which allows simultaneous viewing in multiple channels

On the TFT front panel of the microscope

Choose fluorescence

Open shutter

Select all or individual channels using the select buttons

Turn on (and adjust) the individual channels on the LED box

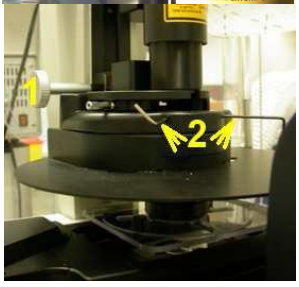
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

!! Please remember to switch off after viewing and at the end of your session!!

Köhler illumination

- Select 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
- Looking down the eyepiece, focus the black edges of the field iris with the large silver focus wheel (1)
- If necessary, centre the field iris with the Köhler screws (2) 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 Acquisition tab

- Choose the desired acquisition mode eg XYZ

Load Settings

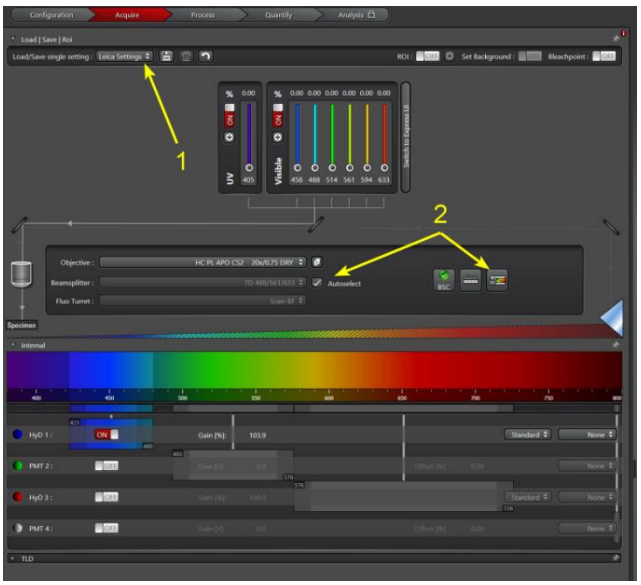
Either from a previously saved Image:

- Re-load the same settings as used in a previous image. Open the image library and select the image, then click on the APPLY icon above (check the pixel number, bit depth and zoom and averaging as these are not always re-loaded).

Or:

- use the pre-set channel setup from the scrolling list (1)
- setup your own settings manually
- create a new multichannel protocol from the advanced setup button (2) – NB make sure the autoselect button is checked.

Advanced Setup Method



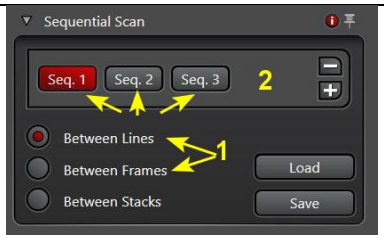
- a new window will open allowing channel setup by fluorophore.
- clicking on the "..." button will load a search window allowing fluorophore selection
- repeat adding channels as necessary (a new line will automatically appear for each new channel)
- finally you can select the scanning mode - normally line-sequential mode (*) is recommended to prevent bleed-through of signal
- a brightfield image can also be captured by checking the PMT trans button after selecting the channel you want it added to (see Sequential scan window "Seq" window below).

There are two types of detector PMT and HyD

PMT: standard detector - reasonable sensitivity, uses both Gain and Offset controls

HyD: high sensitive detector, has only Gain control

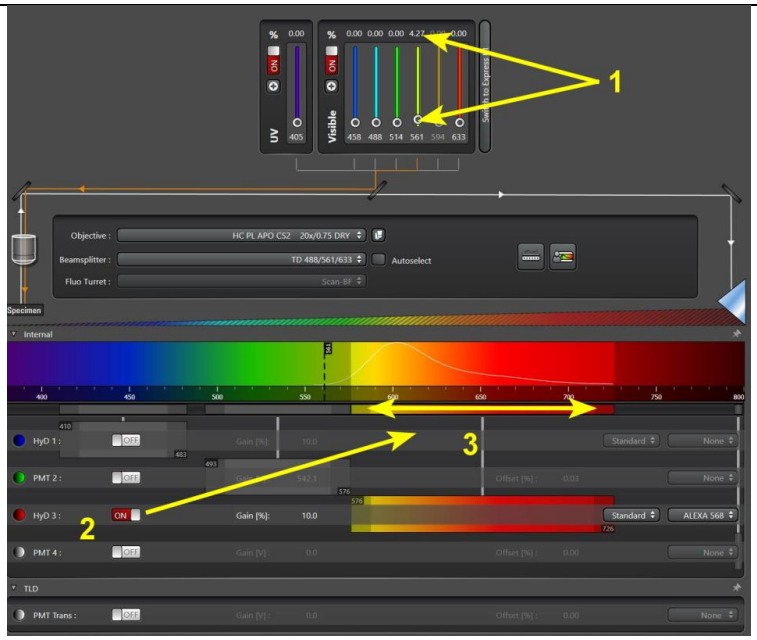
Detector selection can be changed depending on signal of flurophore



Sequential scan window (Seq):

Some setups will allow switching channel capture between lines or frames, others setups, only between frames (1).

To edit a particular channel setup or laser power, select the "Seq" number (2) for that channel



Laser power (1), detector (2) and detection bandwidth (3) can all be changed

Setting Intensity

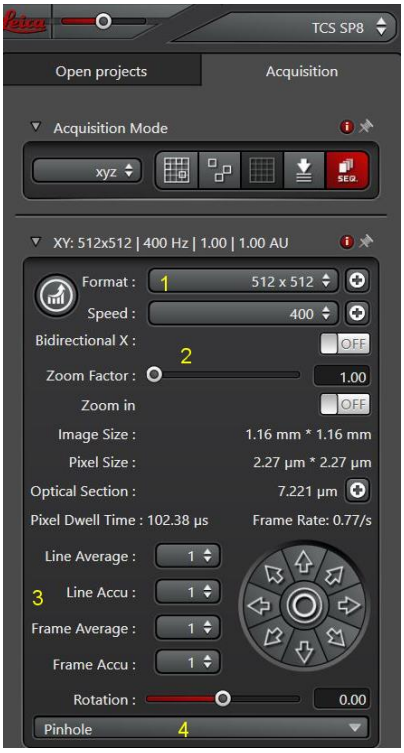
- If sequential scanning, select channel and sequential mode (line/frame) in the Sequential scan window
- start preview scanning clicking on LIVE (bottom of the screen)
- use the control panel to change Gain, Offset, zoom and focus

- Fine-focus using the Z POSITION control
- Change the screen LUT to the “Range Indicator”
- if more than one image window displayed select channel in window to change

- Increase SMART GAIN until a few single blue dots appear (saturated pixels)
- Decrease SMART OFFSET until a few green dots appear (black pixels)
- repeat for all channels

All these settings can then be saved in the Sequential scan window for re-use later.

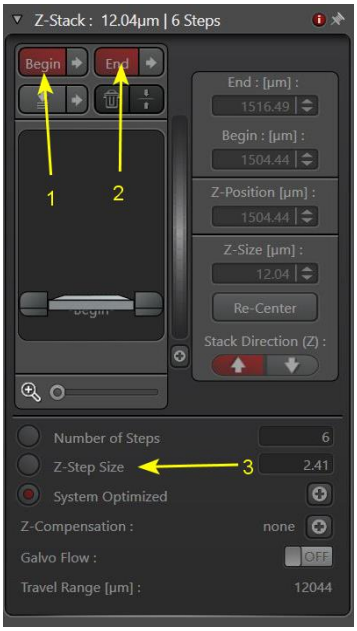
Image Format



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)
- 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

Time Series

- Select xyt or xyzt on acquisition tab from the drop down list
- expand the time panel
- adjust time interval and duration

Acquire Image

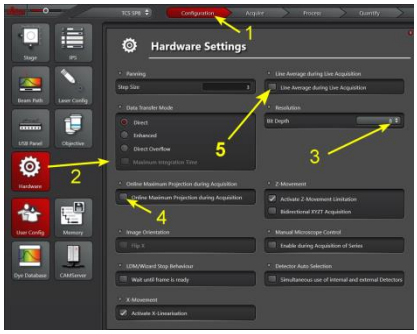
- The CAPTURE button (bottom of the screen) - captures a single image, as defined by sequential, x, y, and λ settings
- The START button (bottom of the screen) - captures a series of images , as defined by channels, x, y, z, t and λ settings

Saving Data

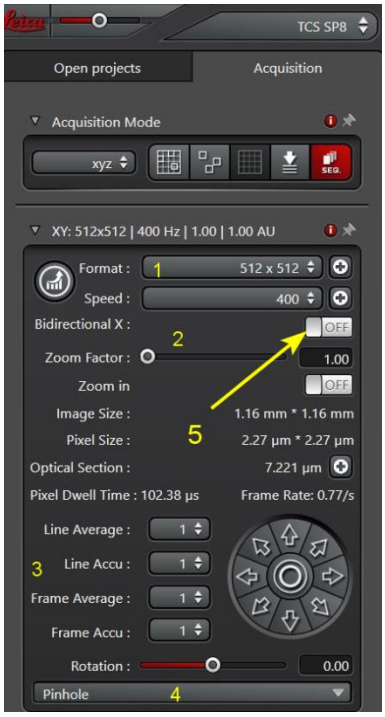
- Images are stored in a library (.lif file format)
- Every time Capture or Start is pressed the image is added (but NOT SAVED) to the Library in the EXPERIMENT tab.
- Right clicking on the individual images allows renaming or deleting.
- To save the images click the “Save” icon above.
- The first time 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.

Resonant Imaging Mode

- Select Resonant mode at start-up in configuration window
- Select "machine_heater" configuration if environmental control is required



- Under "hardware settings select the "Line averaging in Live mode"(5 - above).
- Use "Bidirectional X" (5 - right) to increase speed. NB in bi-directional mode the phase shift has to be corrected manually to align alternate scan lines



Shutdown procedure

- Check if anyone is booked after you within 2 hours
- Update booking if necessary.

If someone is booked within two hours:

- Close the software (no need to turn off lasers)
- Update booking if necessary.
- Remove your samples & clean objective lenses with fresh lens tissue and close incubation chamber
- Clear up the desk
- Save files onto the server
- Log off

If nobody is booked within two hours or you are the last user:

- **Turn off lasers in software**
- Turn “LASER KEY” to off
- **IMPORTANT leave the LASER cooling button on the main control panel ON for cooling for 5-10min!**
- Remove your samples and make sure the LEDs are off.
- Clean objective lenses with fresh lens tissue and close incubation chamber
- Save files onto the server
- Clean up the desk
- Shut down computer and switch off SCANNER and PC STAND on the Main Control Panel
- 5-10 minutes after turning the LASER KEY, switch off LASER cooling button