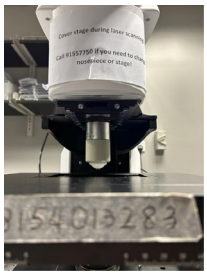
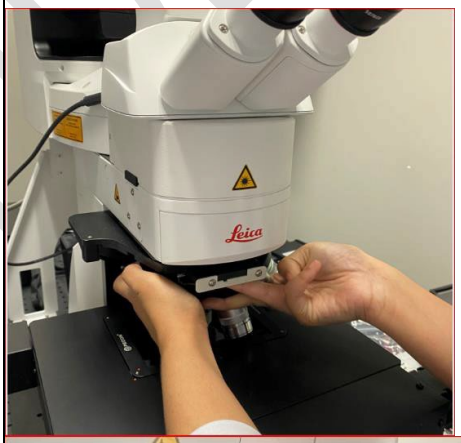
CBIS LM user manual: Leica Stellaris 8 Confocal (upright)

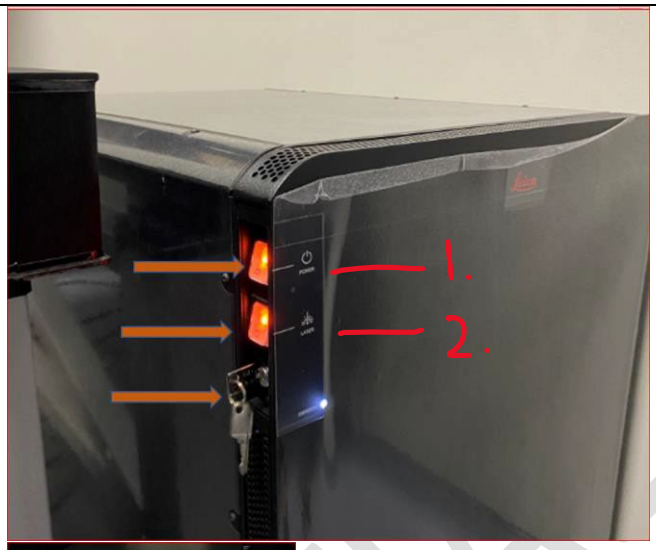
To Start

1. **Before power on the system, check the objectives are at certain distance away from the stage. Otherwise, call core staff.**
2. **Change nose piece when necessary**
    a. Six position nosepieces
        i. HC PL APO CS 10x/0.4 dry
        ii. HC PL Apo 20x/0.7 Imm Corr
        iii. HC PL APO CS 63x/1.2 H2O Corr (for coverslip)
        iv. HC APO L U-V-I CS2 63x/0.9 dipping
        v. HC PL Apo CS2 100x/1.4 Oil
    b. Single position nosepiece:
        i. HCX APO L 20x/1.0 w lens
    c. Use Allen key provided to loosen the screw before pulling out the nosepieces slowly and carefully.
    d. Slowly slot in with the nosepieces you would like to us into the holder: For the single slider, dove-tail end face to the scop body. For the 6-positoin nosepiece, the circle chip facing to the scope body.
    e. Tighten the screw.
    f. Clear any sample/adaptor from the stage.



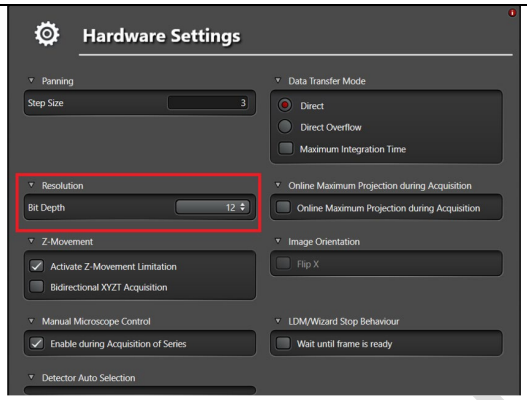


2. Switch on the system
    a. Power supply unit:
        i. Label 1: Main power
        ii. Label 2: Laser power (label 3, Laser emission key should be on already)
    b. Label 5: Computer
    c. The stage will be initialized automatically.
    d. Switch on External fluorescence light source (label 4) only when it is necessary.

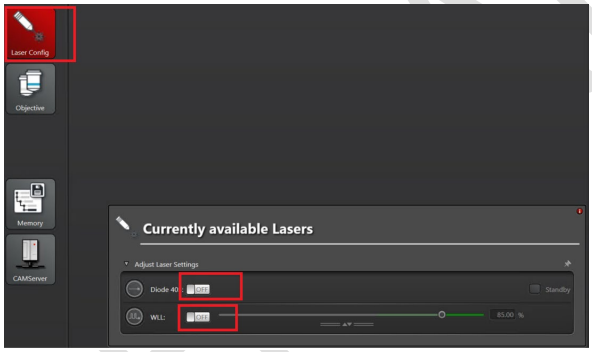


3. Log into windows and start software: LAS X. In the start-up window from the drop-down menu select:
    a. Configuration: Confocal.xihw
    b. Microscope: DM6
    c. Load settings at startup: off.
    d. Ok.

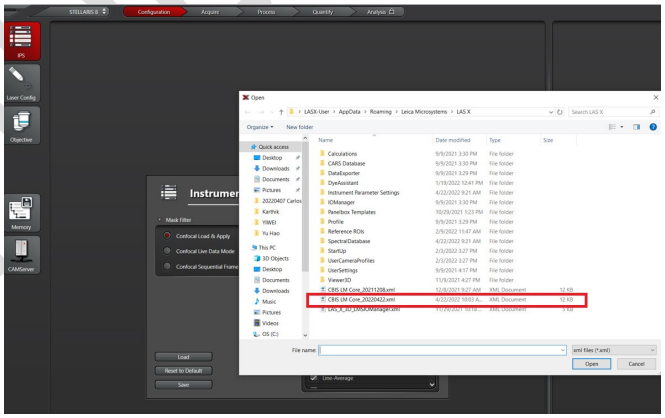
4. Configuration
   a. Select “Hardware settings” and set “Bit Depth” resolution as desired.



   b. Open Laser overview: Click on the laser you need to use. Leave the output value for WLL in the green range.

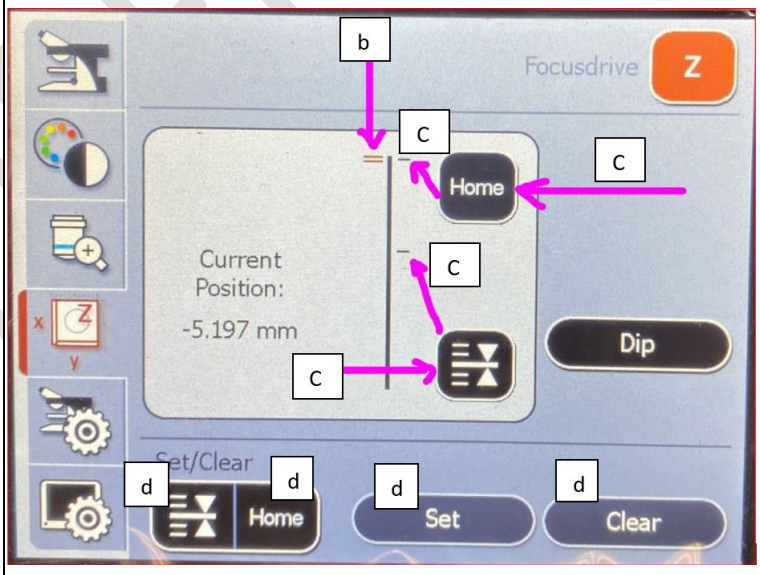


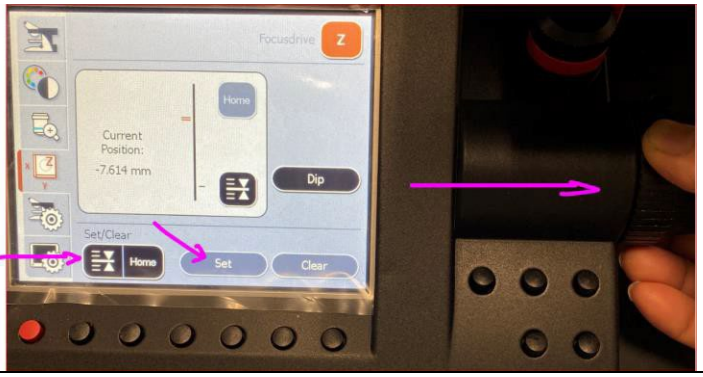
   c. Load IPS, instrument Parameter Settings in order to reuse a complete set of imaging parameters from a existing image.



5. Check touch screen before locating your sample.
    a. Home:
        i. FIM: intensity changer
        ii. Field: illumination area.
        iii. Shutter: open
    b. Choose objective: check correction ring position and apply immersion medium if necessary.
    c. Choose observation mode and filter cube.
        i. TL: transmitted light
        ii. IL/FLUO: fluorescence light:
        iii. Ti Filter A (blue), GFP (Green), N21 (Red)

6. Mount sample onto stage
    a. Leave your sample onto stage.
    b. Check current position of the objective lens: the short double lines in orange color
    c. The two single black short lines indicate the existing settings for positions of "Home" and "focus". When you press and hold on the "Home" or "Focus" position, the objective will be moving toward to the position respectively.
    d. You may set/clear the position which possibly left by previous user and may not suitable for your sample by select the "Home" and/or "Focus" icons on the bottom, then click on "set"/"clear".
    e. When you turn the fucus know clock wise, you are moving objective down and close to the sample.





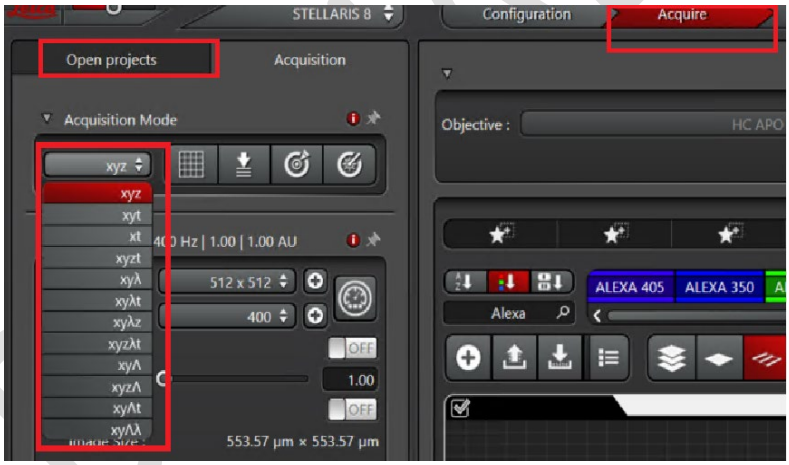
f. Watch on the sample and objective. Stop moving the objective once it touches the sample.
g. Focus sample.
h. Set current position as focus position which will be use as the reference for the bottom end of your objective lens to prevent cracking the sample slide by the objective.

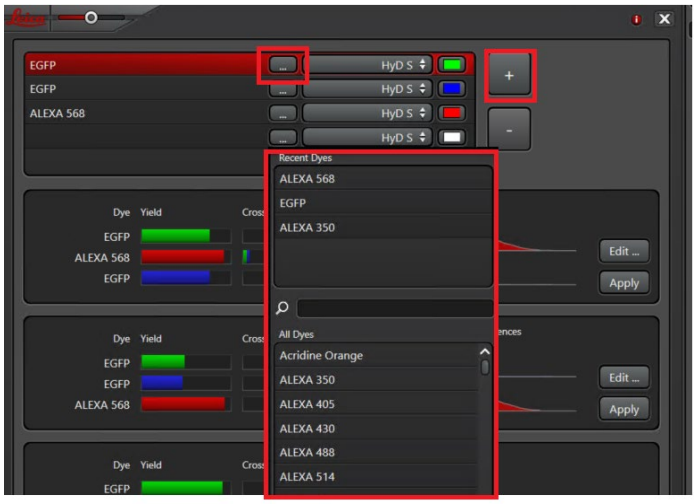
7. Setup image Light-path.
   a. Go to “Acquire”
   b. Choose acquisition mode from the dropdown list, e.g. XYZ for 3D scan
   c. If you have saved the light path settings before, you may “Open Projects” and select one image, right click mouse and “Apply image settings”.

   d. Or you may do manually setup as follows.

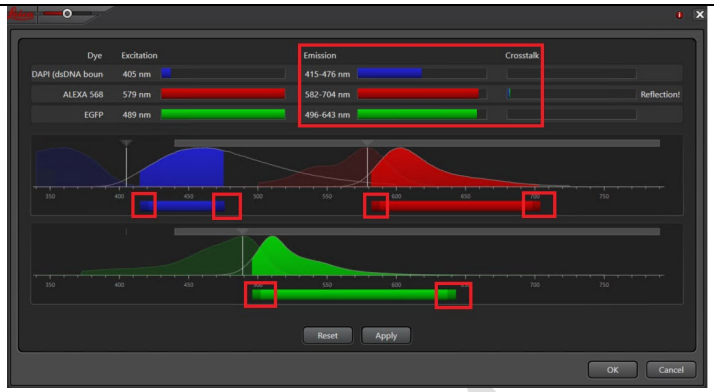
      - Select frame scan mode, Click “Open Dye Assistant” window.

      - In the Dye Assistant window, click on the dye list to choose according to your sample, add or delete the channels for your imaging experiment when necessary.
      - Compare the yield and crosstalk indication bar, and choose the one with high yield and low crosstalk.



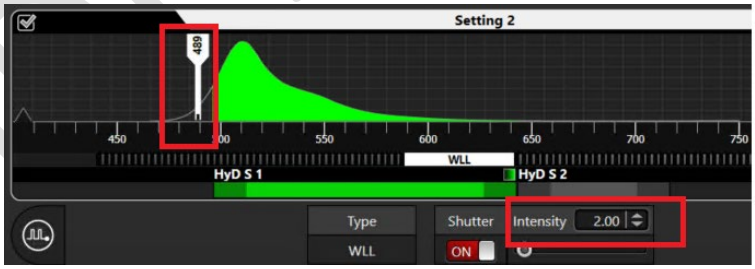


- Click on “Edit” to open the channel edit window.Drag the two ends of each color bar for each channel accordingly, while watching the emission efficiency bar on the top of the window. “Apply”.

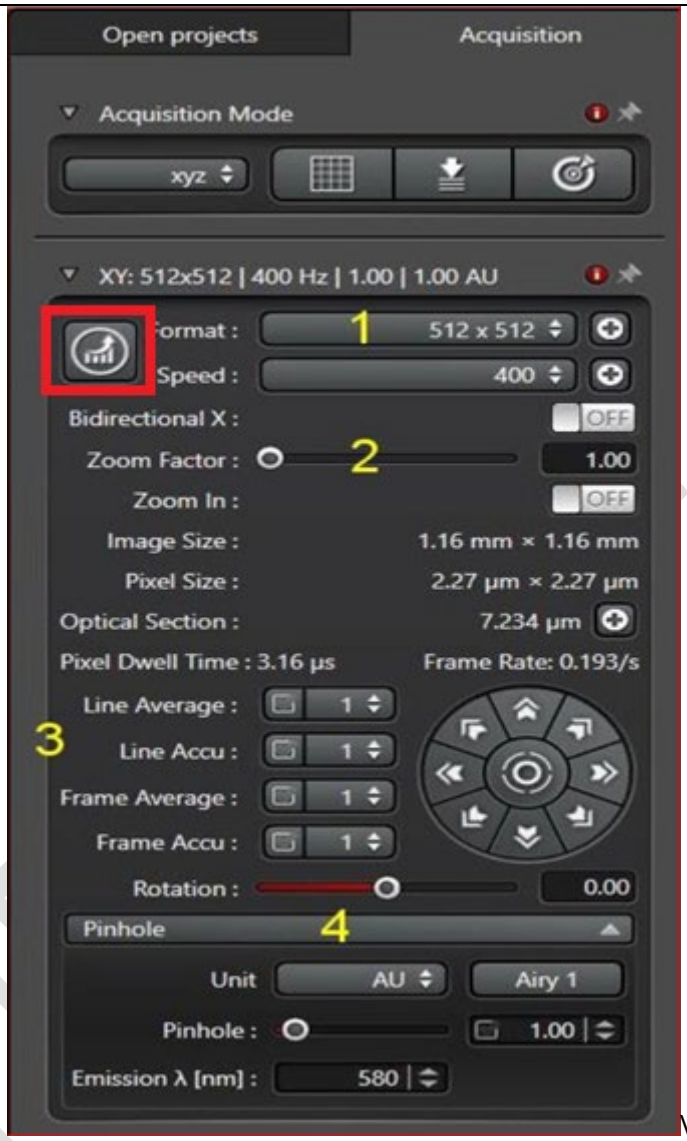


e. For each channel, 1. Activate; 2. Click on HyD left end to call HyD setting window. 3. Switch on the detector. 4. If you need to change color, single click on the color icon and double click on the color when the additional color window appears.

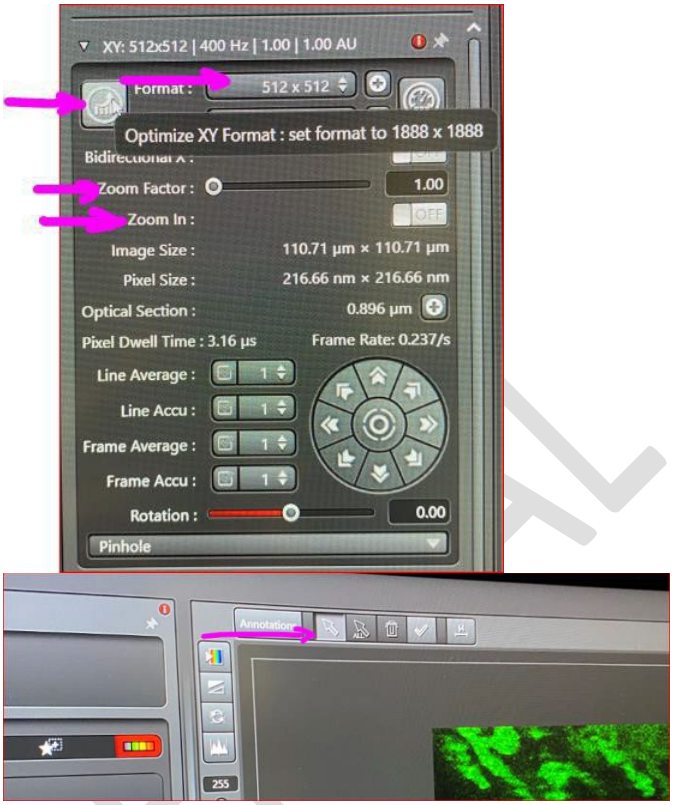
f. Click on laser, change intensity or even length (by moving the laser line around) for excitation.



8. Setup image laser scan.
    a. 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. (using the icon to optimize pixel size in order to fulfil Nyquist sampling).
        • Averaging (3) - required to give you sufficient image quality:
        o line averaging for live imaging
        o line or frame averaging for fixed cells
        • Pinhole (4) - is preset at 1AU but may be adjusted to change Z volume
    b. Choose speed: e.g. 400, which means 400Hz. The higher value gives short pixel dwell time thus higher imaging speed.



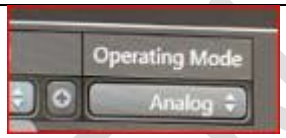
c. If you need crop image while fulfil Nyquist sampling:
   i. Click on the optimal button to apply with "Optimize XY Format".
   ii. For a small area scan with Nyquist sampling: after step i, click on "Zoom in", draw an ROI on the image.
   iii. To keep the zoom-in factor constantly: after step i and ii, define zoom factor.



9. Fast scan to preview image and fine tune imaging parameters
    a. Activate “Toggle apply to hardware” if you would like to see the immediate change of image after you change the hardware settings, such as laser and emission range.
    b. Start preview scanning by clicking on either the LIVE button or FAST LIVE (bottom of the screen.
    c. Select the channel you would like to fine tune imaging parameters, use the remote-control panel

to change gain and laser intensity to get image with optimal brightness and contrast.

    d. When you use analog scan mode, use “LUT” as reference: blue pixel: saturated signal (with maximum grey value); green pixel: intensity 0. You should keep as little as possible pixels fall turn to green or blue so you will avoid masking the weak signal or clip strong signals from the sample.

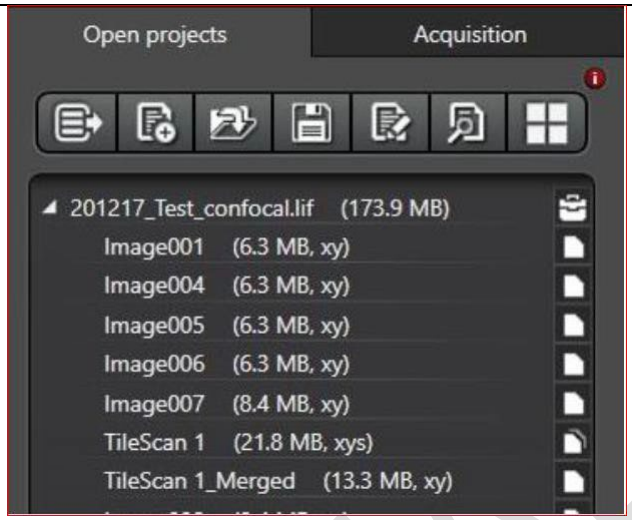


10. Z stack imaging
    a. Use “rubbish bin” to clear all setting before you start a new one.
    b. Move objective to bottom of the sample, click on “Begin”
    c. Move objective to bottom of the sample, click on “End”
    d. Set step size - the "optical section" size (z) can be read from the X-Y panel 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 to allow user to scan 2D image in the xyz acquisition mode.

11. Capturing Images
    Once the setup is complete the image is recorded using either “Capture Image” for a single image or use “Start” for an image sequence (Z-stack, tiling or time series)

12. 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 every time you capture an image to update the library or you could lose your data.



Shutdown Procedure

13. As the last user of the day
    a. Clear your sample and clean lens when necessary.
    b. Exit from LASX.
    c. **Hold "Home" to move the objective to the top position.**
    d. Transfer data to shared workstation (mapping network: \\Confocal\FV3000)
    e. Clear up the desk
    f. Turn off LED unit
    g. On the central power unit - Switch off:
        i. Laser
        ii. Power
    h. Shut down PC
14. If there is user coming later, following steps 13 a-13f and log off PPMS account.