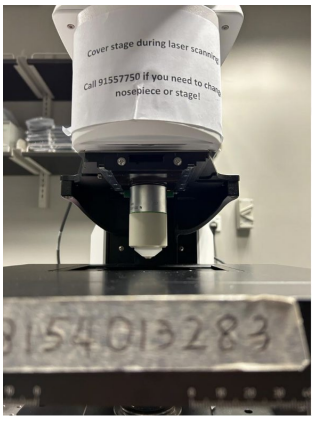
Leica Stellaris 8 Confocal_ Basic

To Start

1. **Before power on the system, check the objectives are at certain distance away from the stage. Otherwise, call core staff.**

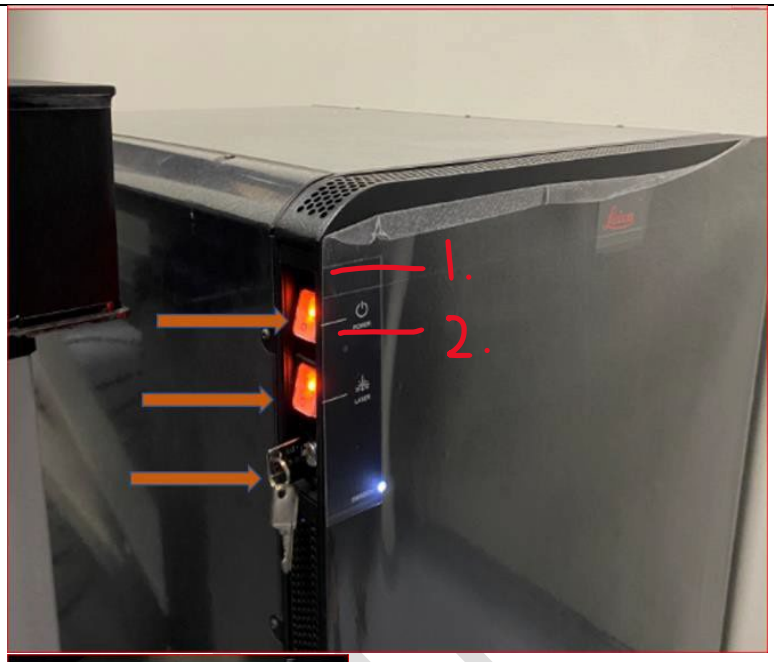


2. **Change nose piece when necessary.**
   a. Six position nosepieces
      i. HC PL APO CS 10x/0.4 dry
      ii. HC PL Apo 20x/0.7 Imm Corr
      iii. HC PL APO 20x/0,75 CS2 WD0.65
      iv. HC PL APO CS 63x/1.2 H2O Corr (for coverslip)
      v. HC APO L U-V-I CS2 63x/0.9 dipping
      vi. HC PL Apo 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.

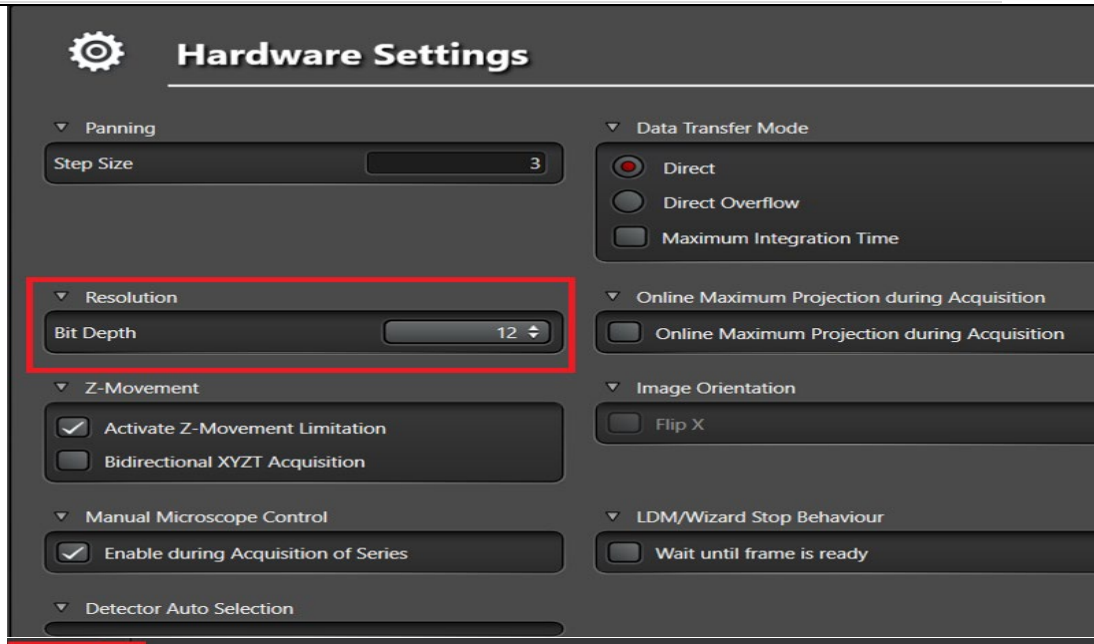
e. Tighten the screw.
f. Clear any sample/adaptor from the stage.

3. Switch on the system
   a. Power supply unit:
      i. Label 1: Main power
      ii. Label 2: Laser power (make sure 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. You must leave the light source on for 15min before you switch it off. Vise versa).

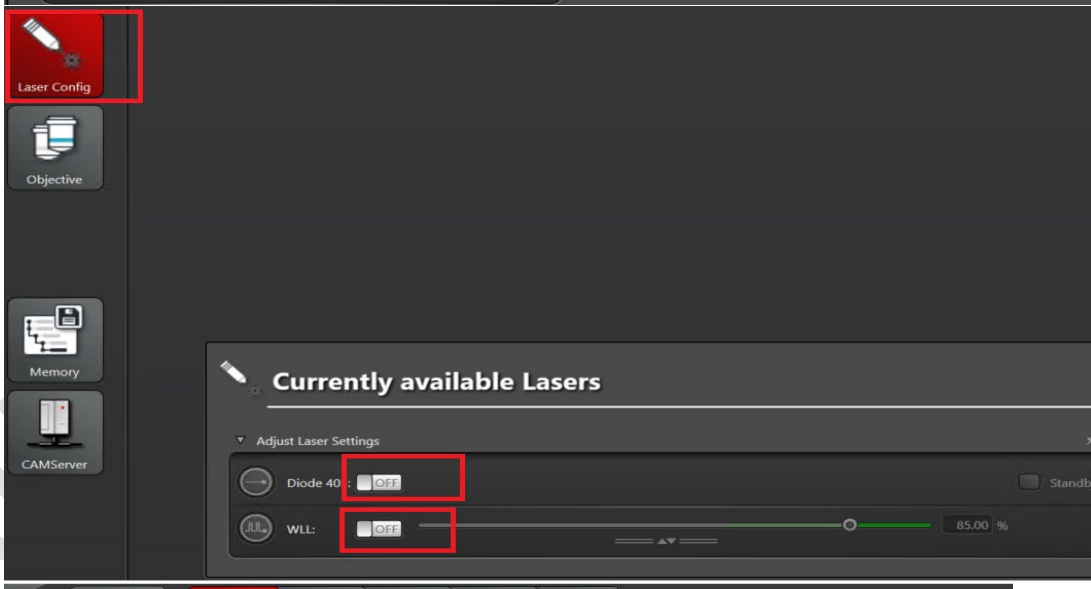


4. Log into windows and start software: LAS X. In the start-up window from the drop-down menu select:
   a. Configuration: Confocal.xlhw
   b. Microscope: DM6
   c. Load settings at startup: off.
   d. Ok.

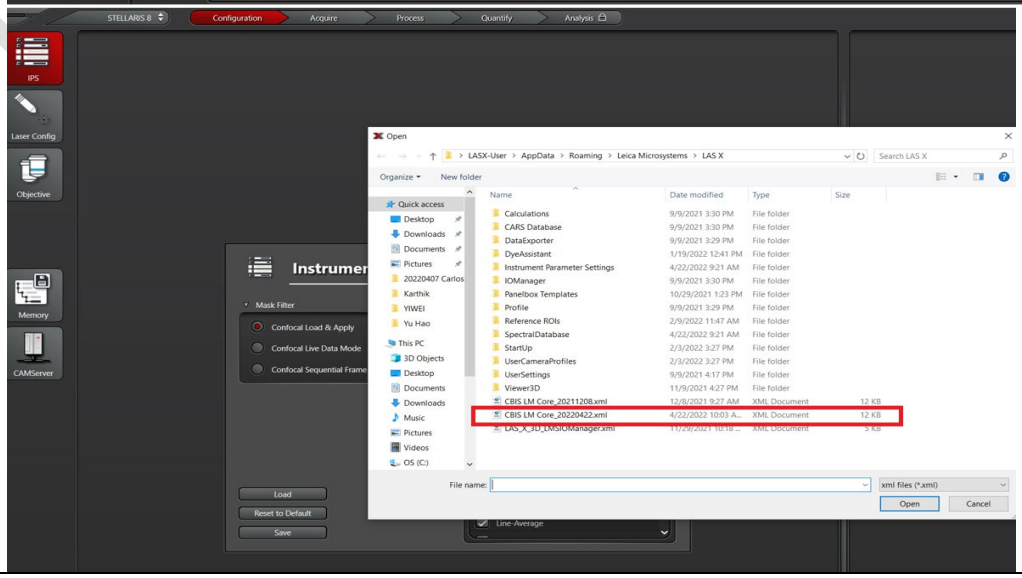
5. Configuration
   a. Select “Hardware settings” and set “Bit Depth” resolution as desired.



   b. Open Laser overview: Click on the laser you need to use. Leave the output value for WLL in the green range, i.e. <85%.



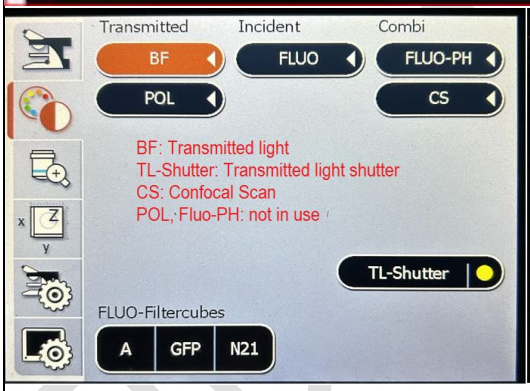
   c. Load IPS, instrument Parameter Settings to reuse a complete set of imaging parameters from an existing image.

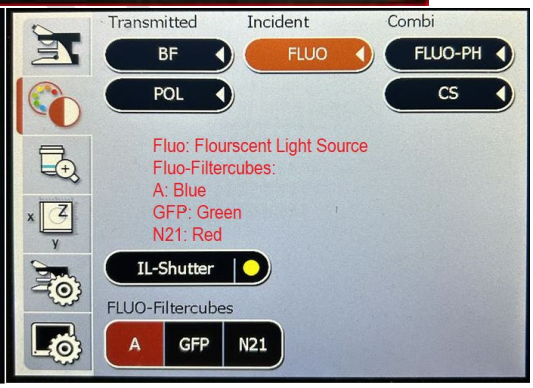


6. Using touch screen after loading sample.
    a. Choose **objective**. check correction ring position and apply immersion medium if necessary.

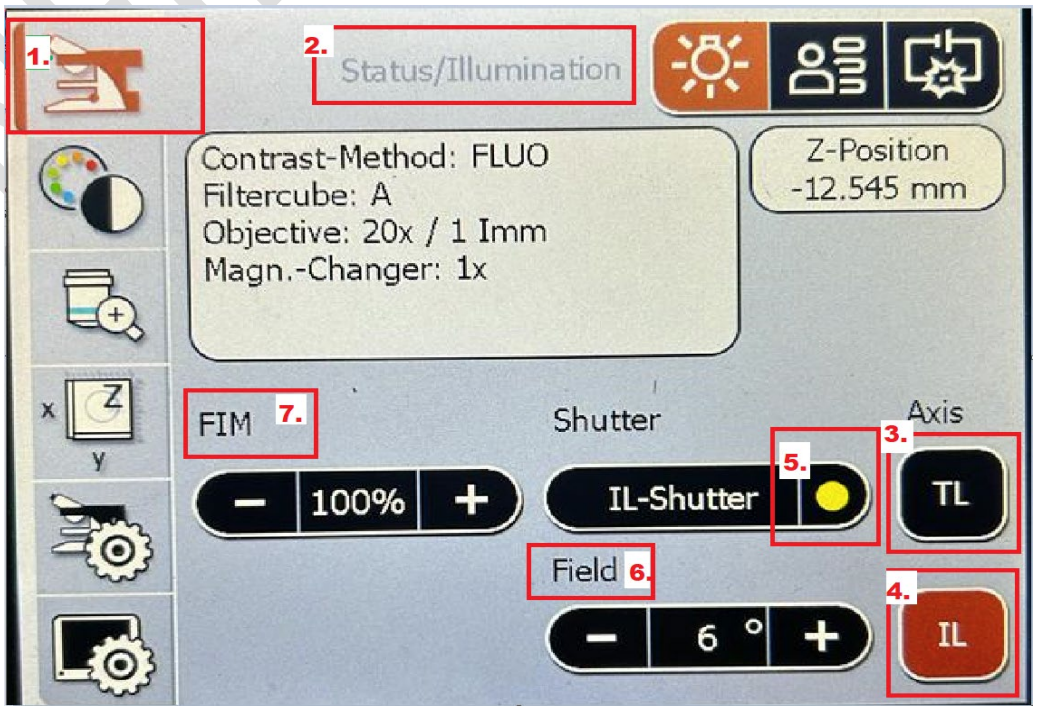
    b. Choose **Port**: click on the "**eye**" mode to guide the signal to eyepieces (the picture showing the camera mode instead).

    c. Choose observation mode.





    d. To adjust illumination light intensity/area: in the Home (1) Status/Illumination (2):
    e. TL (3): for transmitted light source.
    f. IL (4): for epi-fluorescent LED light source.
    g. IL/TL-shutter (5): to on/off shutter
    h. Field (6): change illumination area size
    i. FIM (7): to change intensity of light.



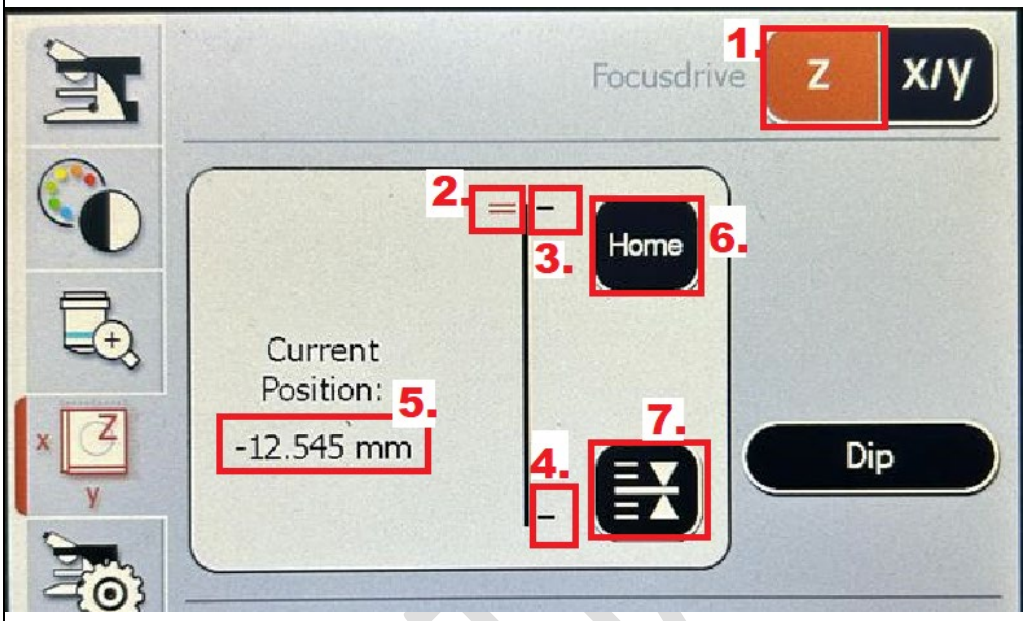
7. Focus sample with reference to Focus drive (1) on the touch screen.

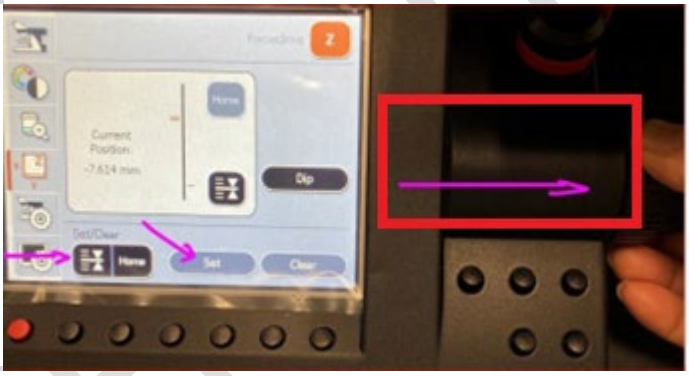
   a. The objective position is indicated by the "=" (2) with a value (5). When you use the manual focus knob, turning clockwise of the knob will bring objective down.

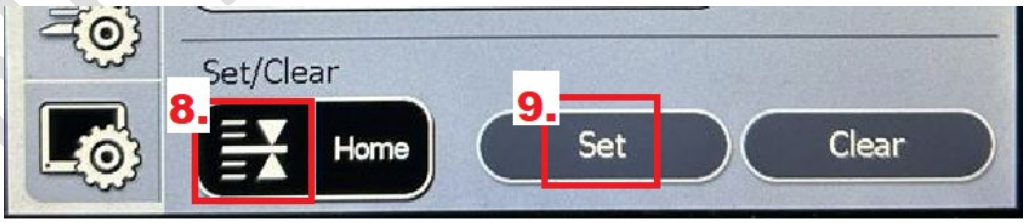
   b. The "-" lines (3) and (4), indicate the existing set position for "Home (6)" and "focus (7)". When keep holding on "Home (6)" or "Focus (7)" brings the objective toward to the positions respectively.

   c. Watch on the sample and objective. Stop moving the objective once it touches the sample.

   d. Focus sample by using the focus knob.

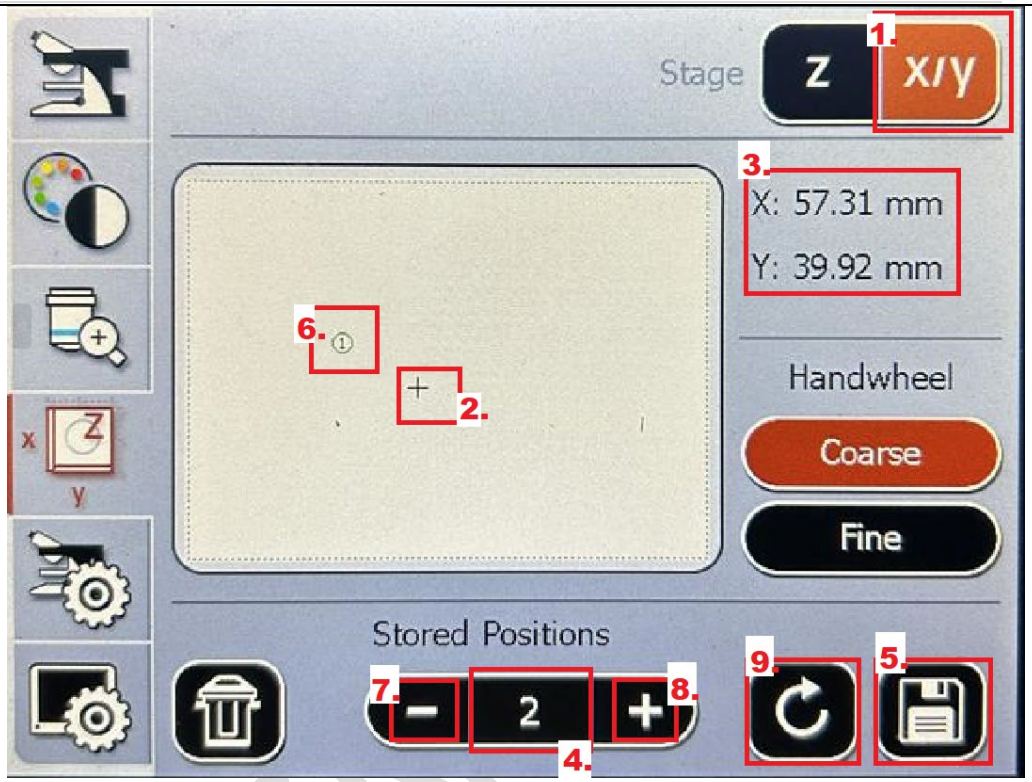






You may update the focus position based on your sample by select the " "Focus" (8) on the bottom, then click on "set" (9) /"clear" accordingly, to prevent cracking of the sample slide by the objective.

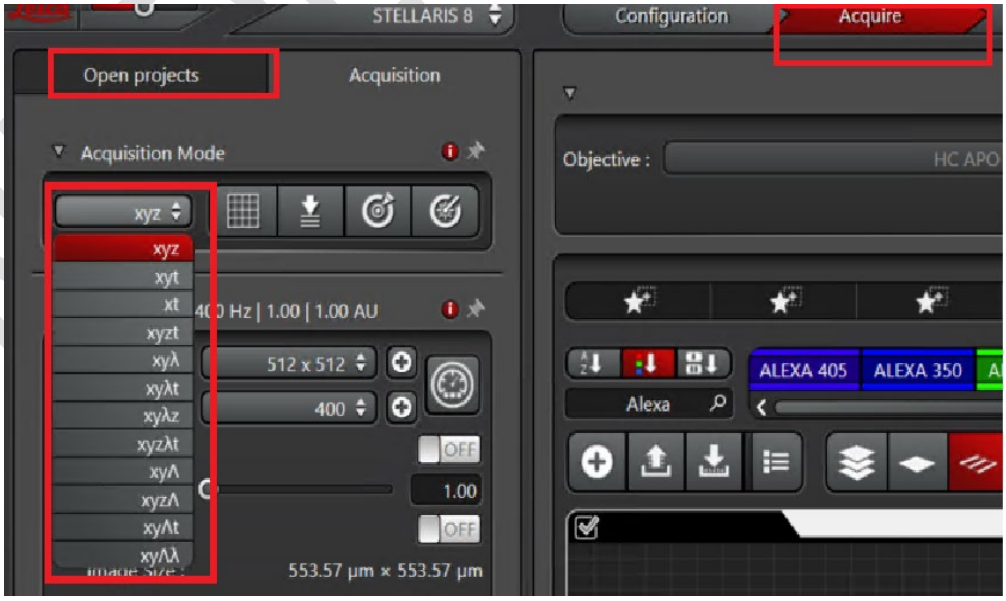
8. Use X/Y stage (1) to register sample position when it is necessary.
    e. "+" (2) indicates current stage position with a value of (3).
    f. You may confirm the registration of this position (4) by clicking on "save" (5).
    g. An example is shown on the picture. There is one position registered previously (6) indicated by the position number in a black circle on the display.



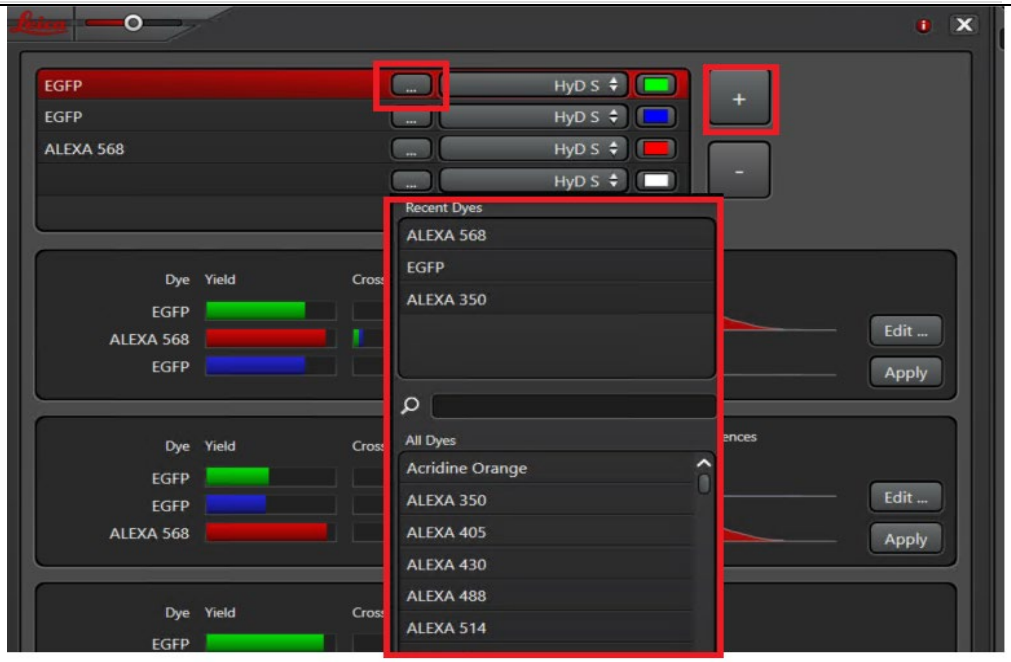
Use "-" (7) or "+" (8) together with refresh button (9) to move to different positions for registered samples, for direct observation or the multiple stage position imaging setup in confocal mode.

9. 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. r one can manually set up Light path/channels as follows.

    ➢ Select frame scan mode, Click "Open Dye Assistant" window.



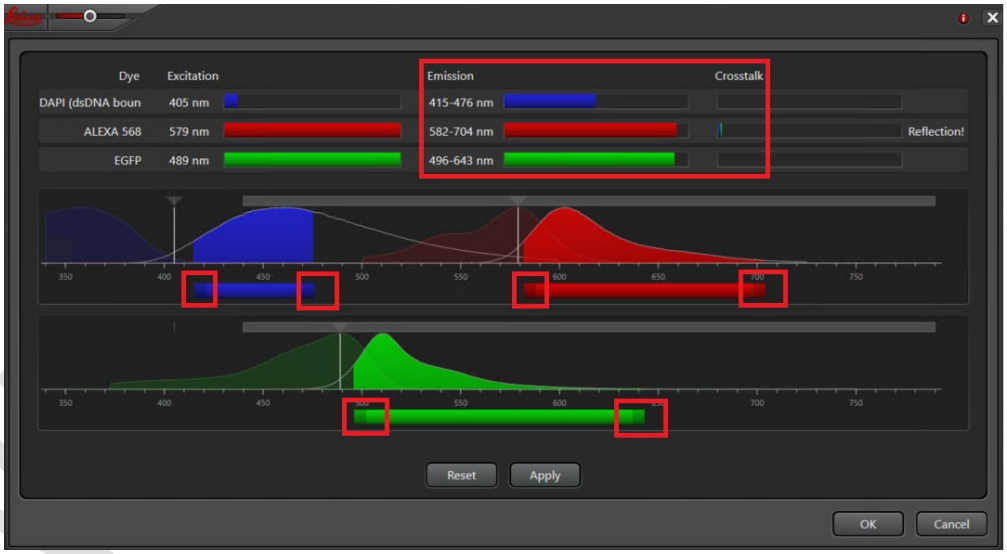
- In the Dye Assistant window, click on the dye list to choose according to your sample, add or delete the channels for your imaging experiment when necessary.



- Compare the yield and crosstalk indication bar, and choose the one with high yield and low crosstalk.

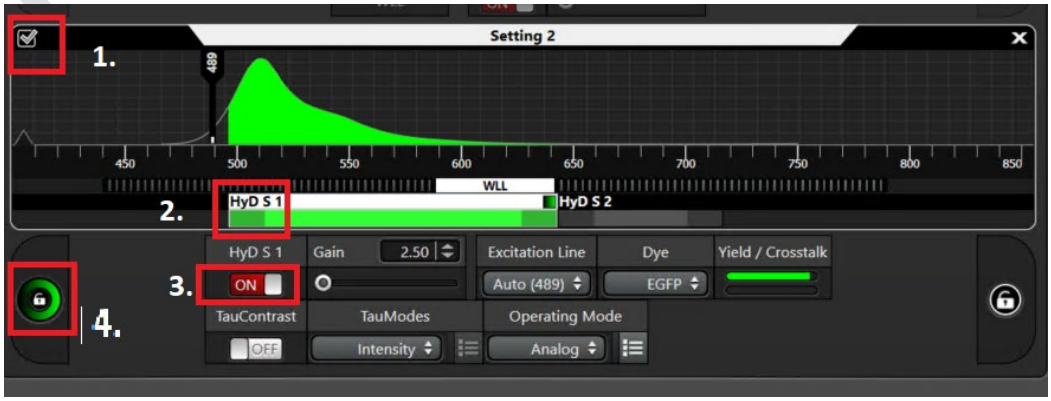
- Click on “Edit” to open the channel edit window.

- 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,

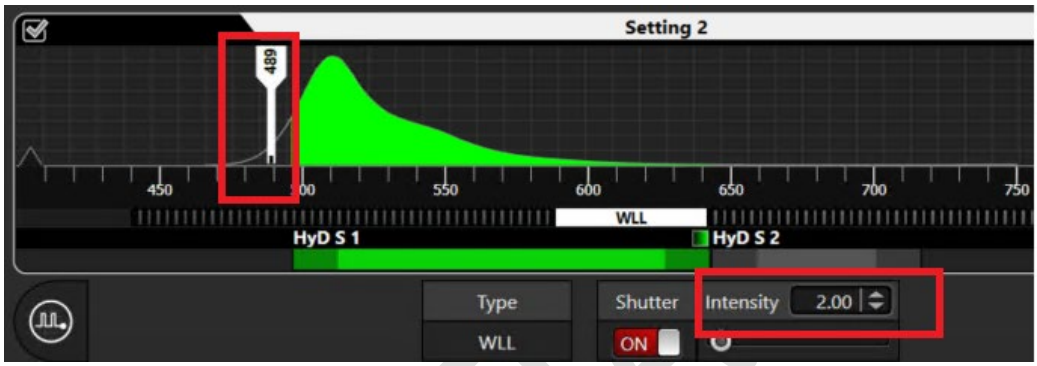
- Activate the channel (1);
- Click on HyD left end (2) to call HyD setting window.
- Switch on the detector (3)
- For normal imaging, select Analog Operating Mode. For high dynamic intensity sample, use “counting mode” instead.



- Single click on the color icon (4) and double click on the color when the

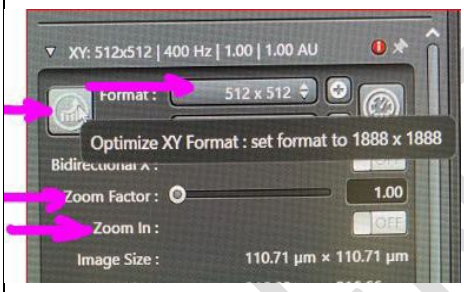
additional color window appears to assign the color to this channel's signal.

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



10. Setup image laser scan using the XY panel:
    a. Image format (1) 1024 x 1024 is typical but depends on image requirements.
    b. Choose speed: e.g. 400, which means 400Hz. The higher value gives short pixel dwell time thus higher imaging speed.
    c. Using Optimal icon to set pixel size based on Nyquist sampling.

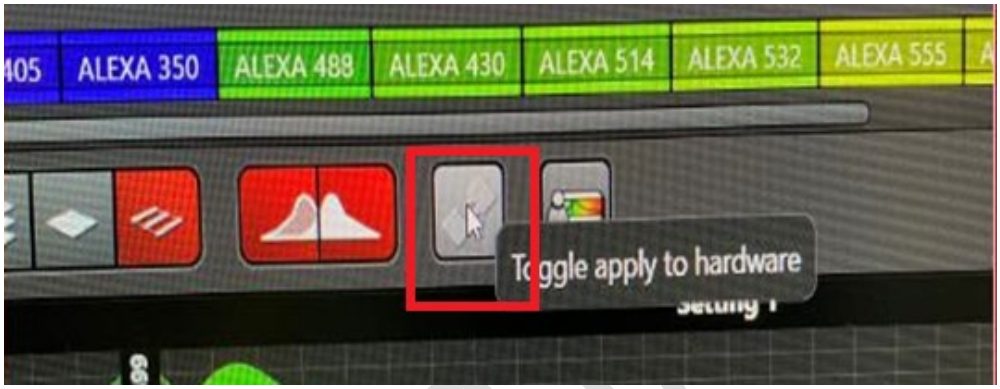
    d. ROI scan
    - Turn on " Zoom In" ,draw an ROI on the image.
    - Enter zoom factor to keep constant zoom in area.
    - Using optimal icon to optimize pixel size



    e. Averaging (3) - required to give you sufficient image quality:
    - line averaging for live imaging
    - line or frame averaging for fixed cells

    f. Pinhole (4) - is preset at 1AU but may be adjusted to change Z volume

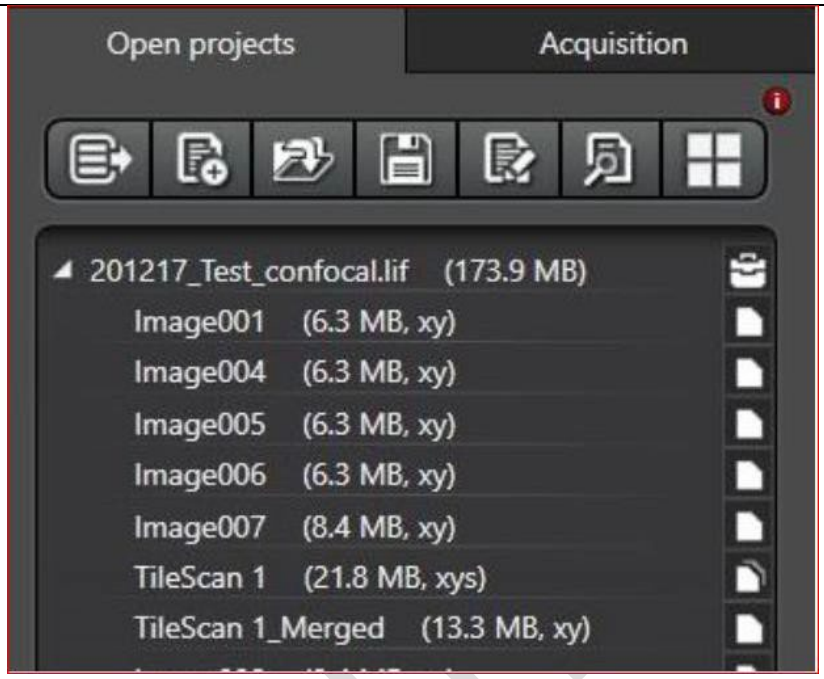
11. Setup image laser scan using the XY panel:
    - Start preview scanning by using LIVE button or FAST LIVE (bottom of the screen) and fine tune imaging parameters.
    - Activate “Toggle apply to hardware” if you would like to see the immediate change of image after you change the hardware settings, such as laser and emission range.
    - Select the channel, fine tune laser, Gain settings to get image with optimal brightness and contrast.
    - Use “LUT” as reference: blue pixel: saturated signal (with maximum grey value); green pixel: intensity 0. Pay attention to if the “Autocontrast” has been applied.



12. Z stack imaging
    a. Use “rubbish bin” to clear all settings before you start a new one.
    b. Register bottom and top position for the sample by clicking on “Begin” and “End” respectively. “Z-size” shows the whole stack size.
    c. 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.

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)

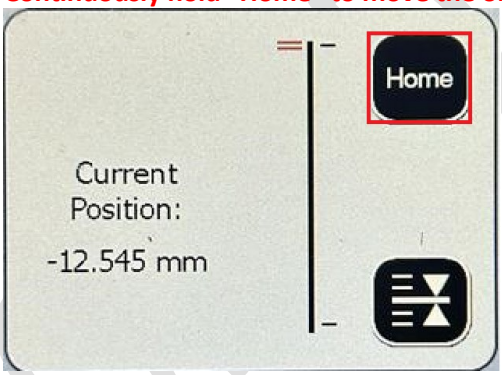
12. Saving Data
    - Images are stored in a library (.lif file format)
    - Every time Capture Image or Start is pressed the image is added (but NOT SAVED) to the Library in the "Open projects" tab.
    - Right clicking on the individual image names allows renaming or deleting.
    - To save the images click the "Save" icon above every time you capture an image to update the library or you could lose your data.



Shutdown Procedure

As the last user of the day

a. Clear your sample and clean lens when necessary.
b. Exit from LASX.
c. **Continuously hold "Home" to move the objective to the top position.**



d. Transfer data to shared workstation (mapping network: \\Confocal\FV3000-2)
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

If there is user coming later, following steps 13 a-13f and log off PPMS account.