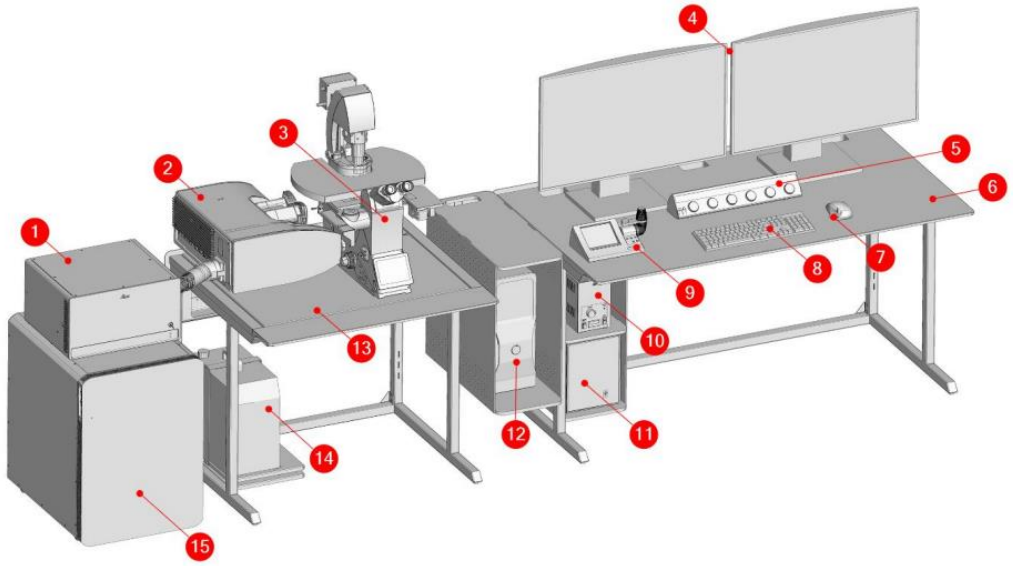
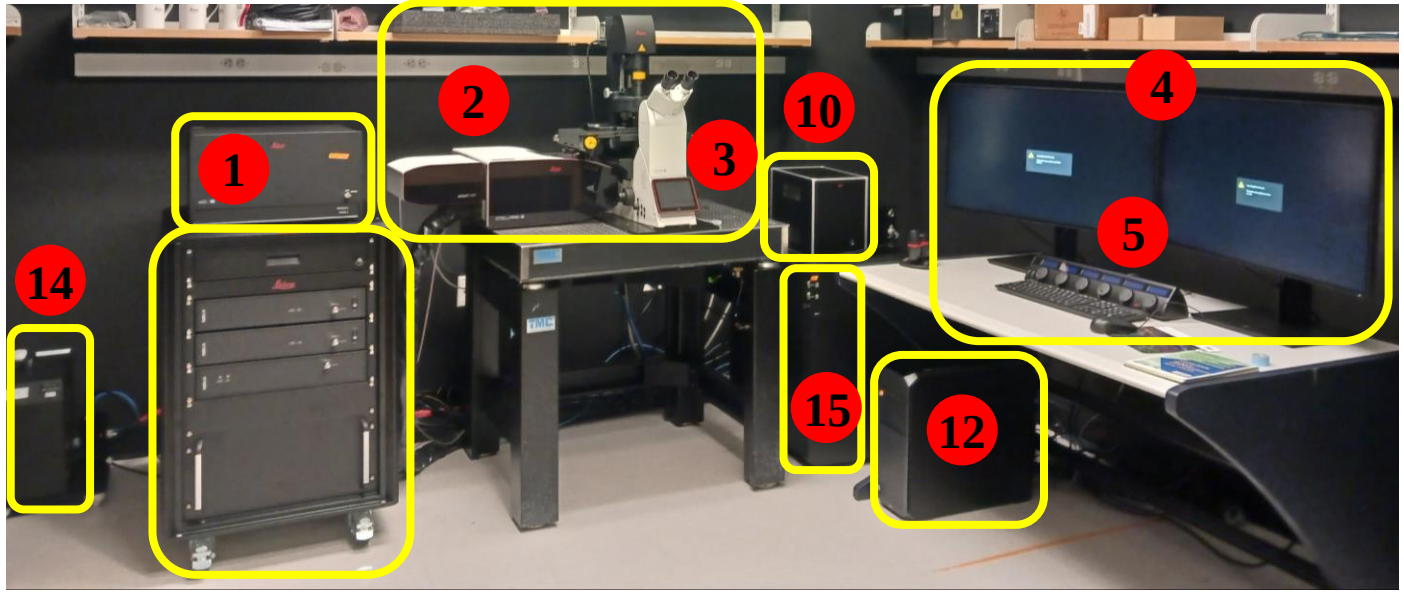
Leica STELLARIS 8 Confocal/FLIM/tauSTED Operating Instructions

System Overview (Confocal Application)

Hardware information

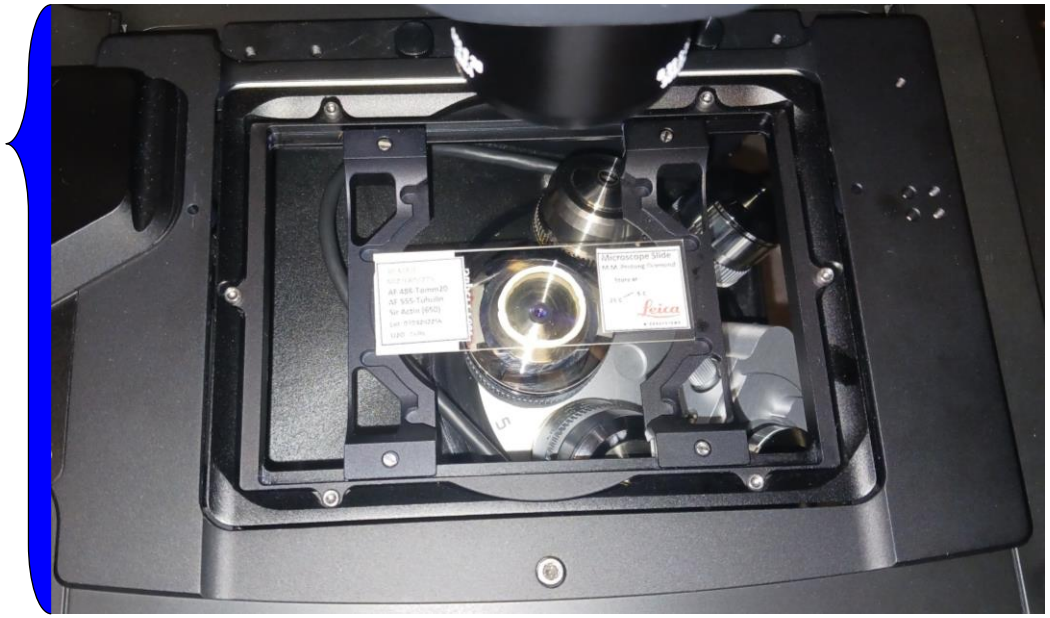




1	White Light Laser (WLL)	**10**	External light source
2	Scan Head	**12**	Workstation
3	Microscope	**14**	External cooling (chiller)
4	Monitor(s)	**15**	Supply Unit
5	Control panel		

Sample mounting and fluorescence observation through eyepieces

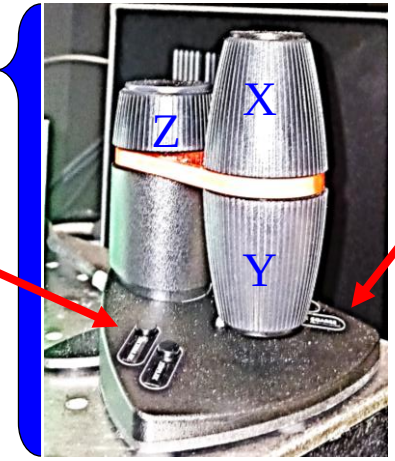
1. Tilt the illumination column.
2. Verify that the correct sample holder stage inset is correctly secured in the XY stage.



3. If using immersion lens, clean the lens and apply proper immersion.
4. Never clean dry lens!
5. If suspected dirty, or if immersion gets onto a dry lens, contact KCCI staff immediately!

Sample mounting and fluorescence observation through eyepieces

6. Secure your sample, thin coverslip Facing Down. The objectives cannot focus through a glass slide. If using immersion lens, quickly bring the immersion to contact with the sample using coarse/fast Z-focus (and XY-stage) adjustment on the "Smart Move" controller.



7. Using the Aperture (aperture diaphragm) or Field (field diaphragm) keys, the motorized diaphragms can be changed at any time. The display on the Touch Screen changes accordingly.

System Start-up

1. Turn on the power (wait for few seconds) and laser button as shown in the figure 1.

*Note: Do not touch the key.

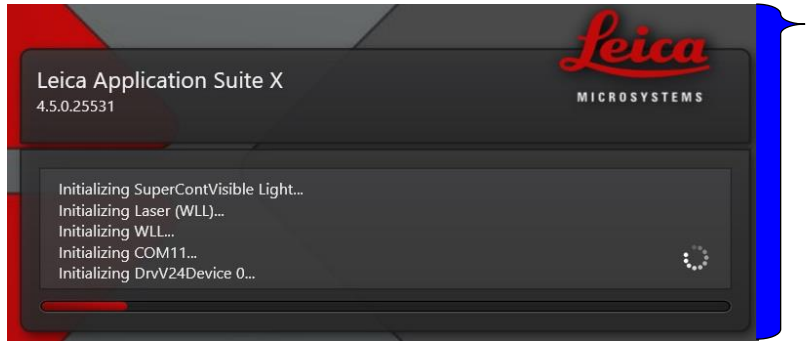
2. Turn ON the computer and wait.
3. Log into the computer with your own KCCI user account.
4. Record the starting time at the KCCI website

5. Start the system by double clicking the LAS X icon on the desktop.

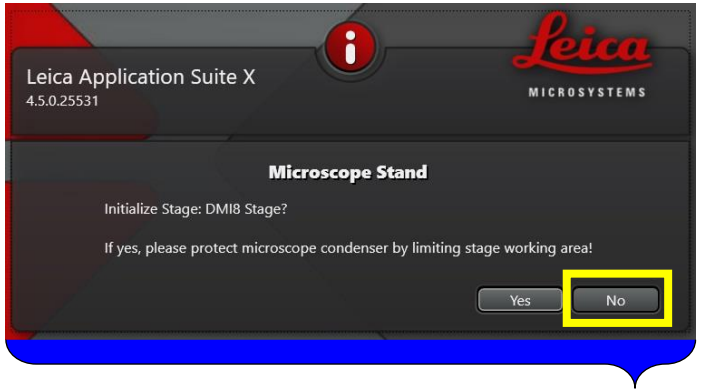
***Note:** The LAS X software is used to control all system functions and acts as the link to the individual hardware components. Image acquisition, image analysis and image processing are carried out using LAS X.

6. Select the Machine as configuration and DM18 in the Microscope section as shown.
7. Always OFF for the confocal usage.

8. Now start LAS X by clicking the OK button.



9. Wait Until the software initialization completes .



10. Select No, if you are NOT using for tiling, etc. For Tiling you should initialize the stage.

11. Wait for the LAS X Graphical User Interface window.

Using the Microscope Touchscreen

On touch screen click the highlighted icon  for basic microscope settings. Select the status/illumination menu using light source icon. Adjust the field diaphragms using + and – keys.
FIM: Fluorescence intensity manager.
IL: Incident light (Fluo: Fluorescence light)
TL: Transmitted light

Contrast Tab or Modality Tab: 

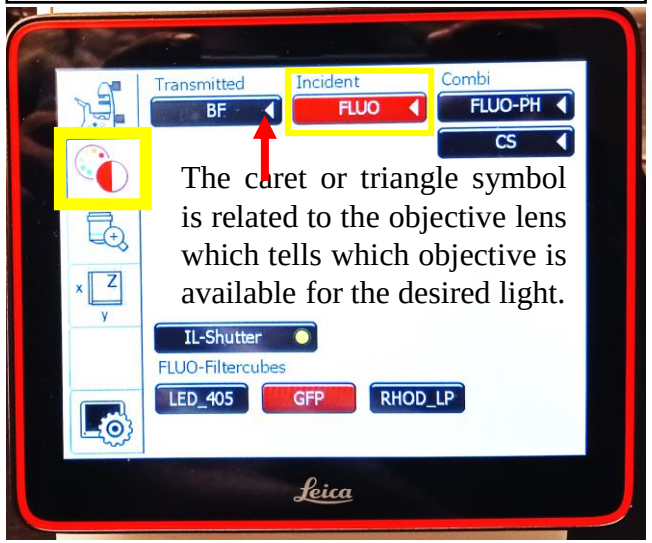
Transmitted light: Bright field (BF)
Incident light: Fluorescence (FLUO)
Combination: Fluorescence-Phase (FLUO-PH) and (CS)

The shutter can be operated using the buttons on the touch screen.

Click the FLUO for fluorescence incident light method
The available filter cubes are displayed. Select the desired cube using the corresponding button.

To see on the screen, use these settings.

To see through the ocular, use these settings.



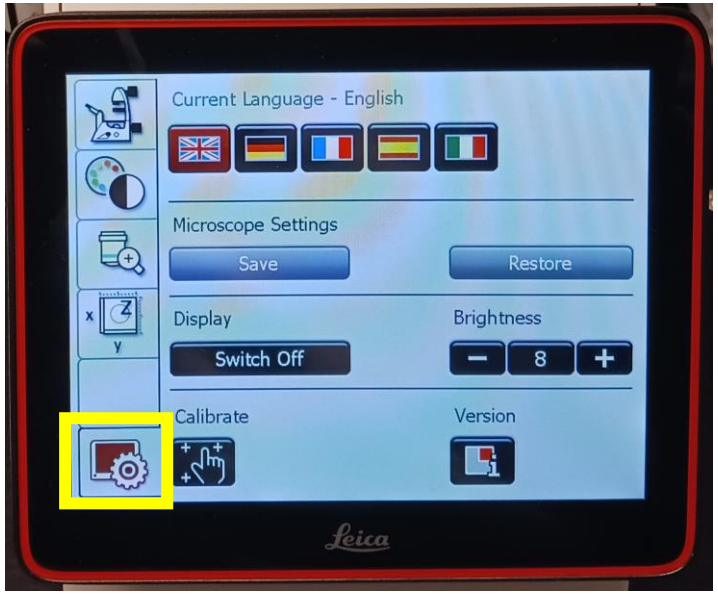
Using the microscope touchscreen

Objective Tab

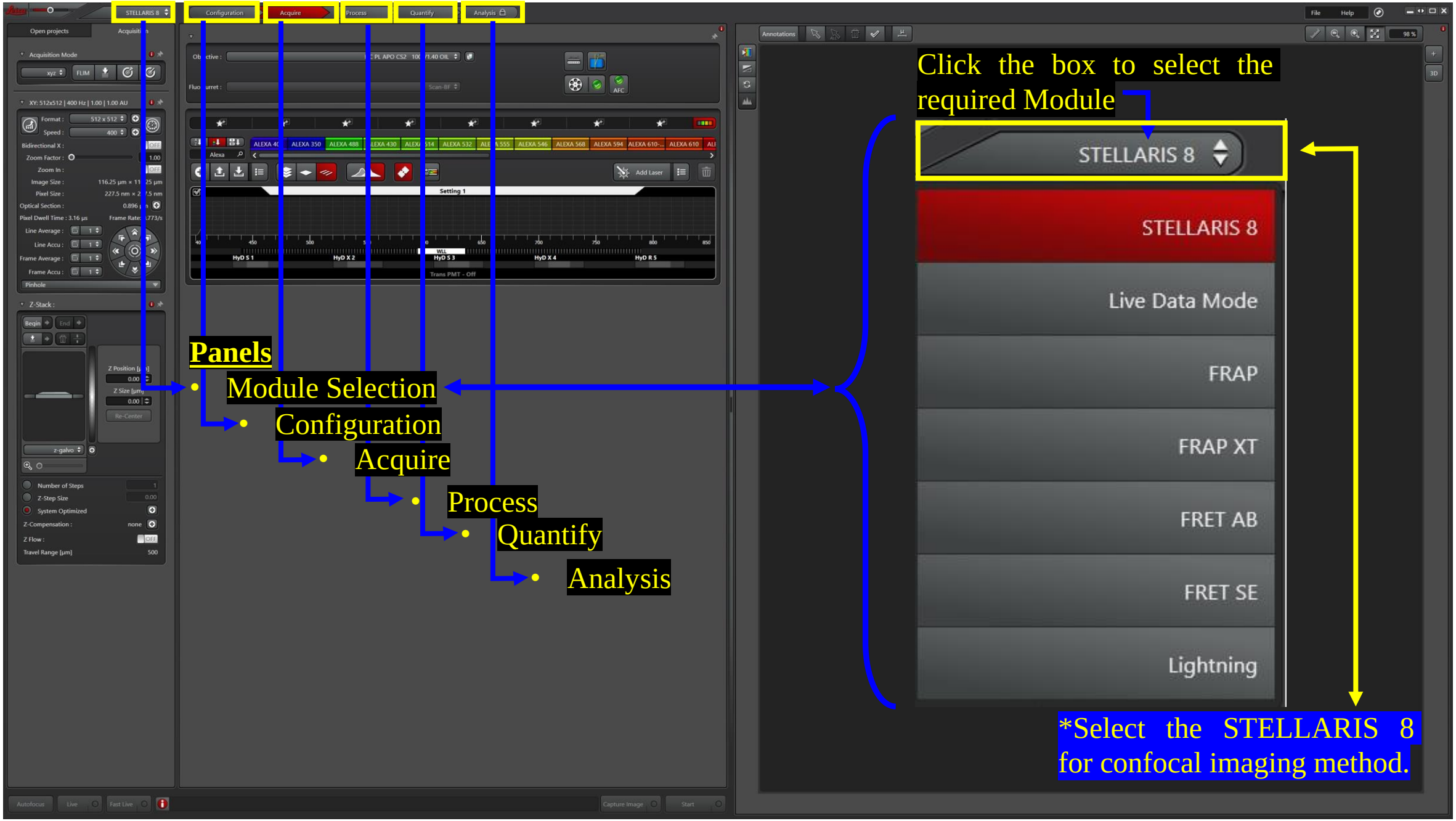
1. Shows the total magnification on the eyepieces.
2. Available objectives: Here 5.

   Click the objective button from the touch screen and select the required objective.

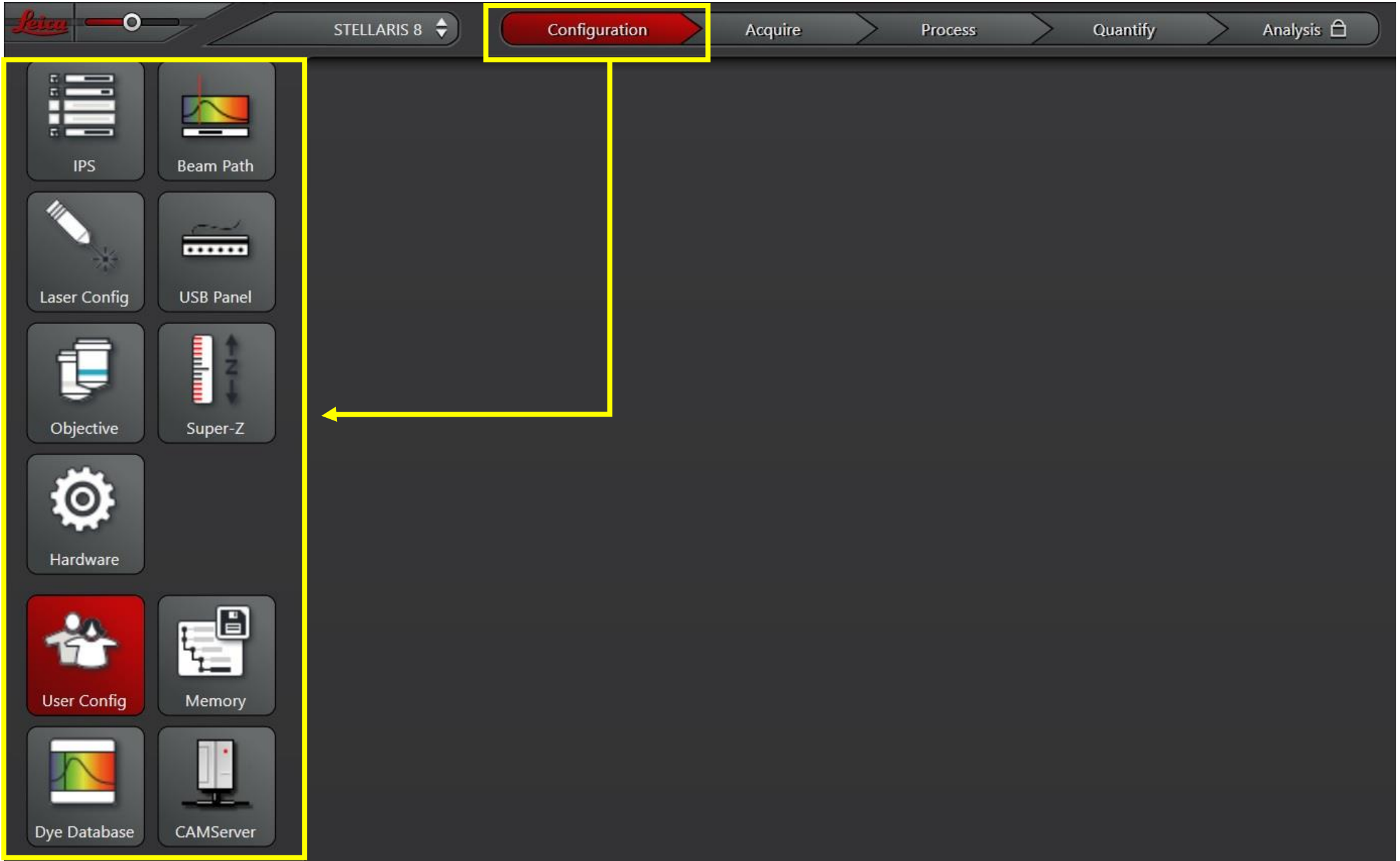
   - Use only Type F immersion liquid for the objective if required.
3. Next part of the panel shows whether the light is going to the eyepieces or to the camera.



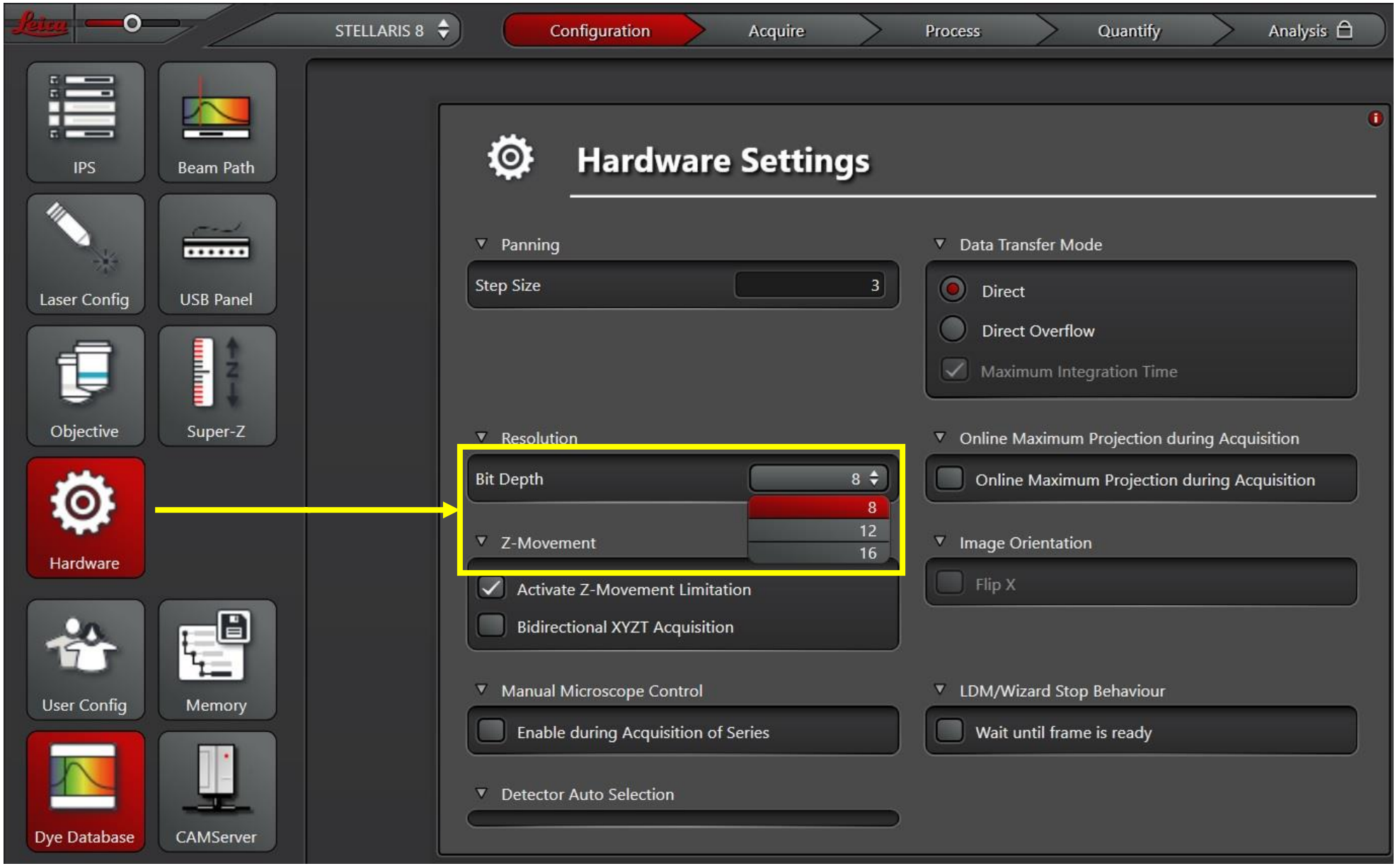
Structure of the LAS X Graphical User Interface



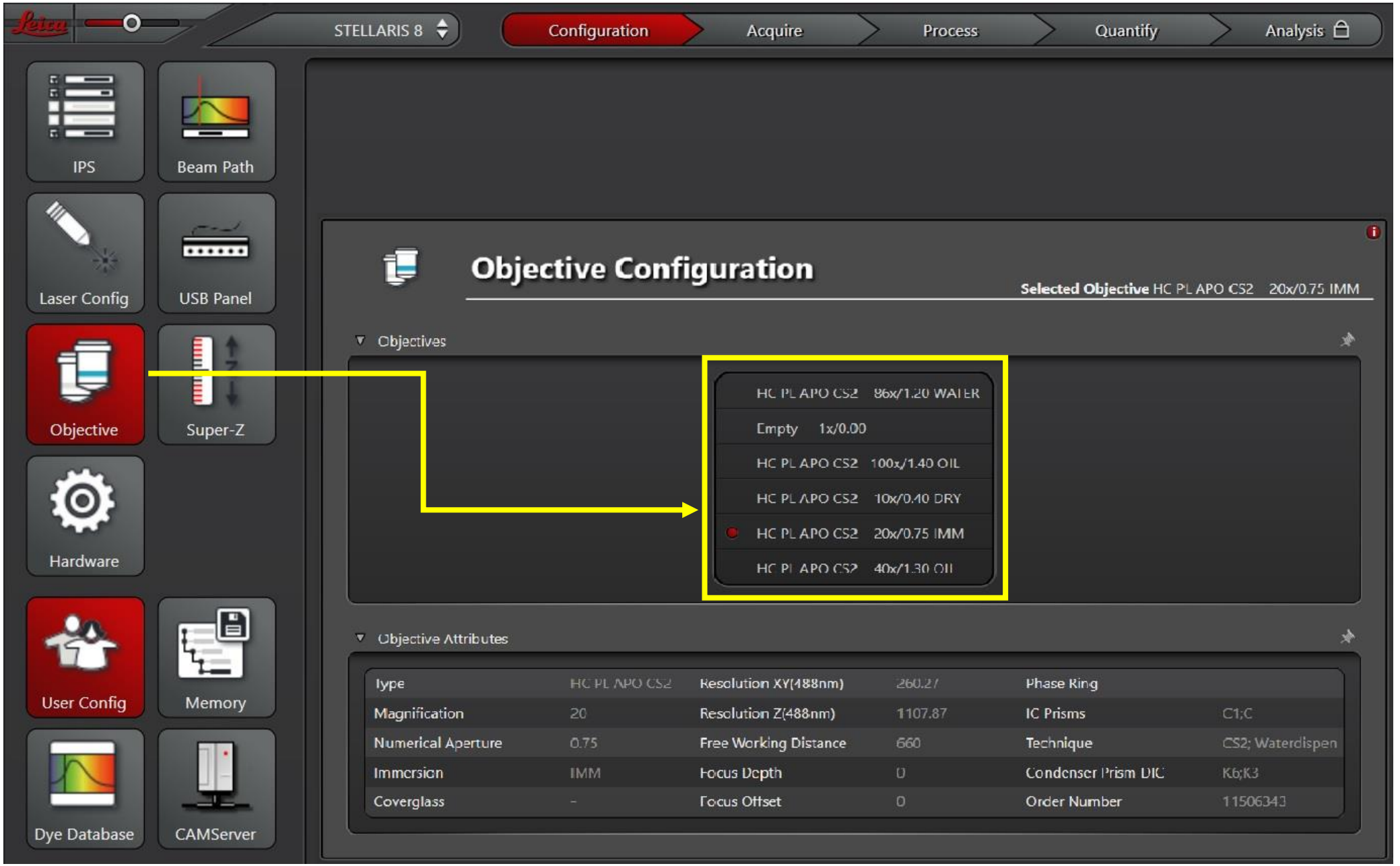
Click the configuration button to activate the configuration



Click the Hardware button to select the Panning, Resolution etc.

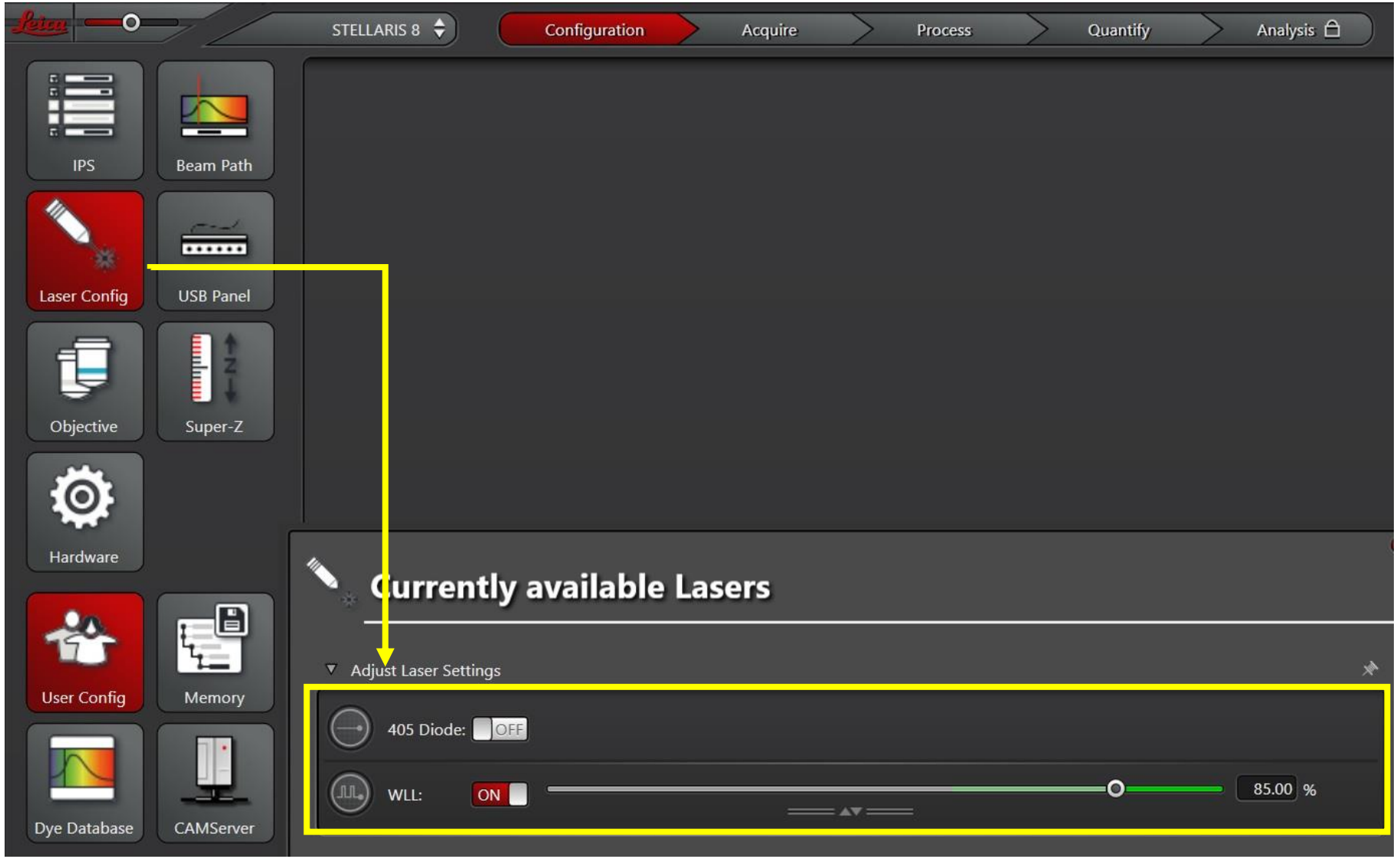


Click the Objective Icon to select the desired objective the configuration panel.

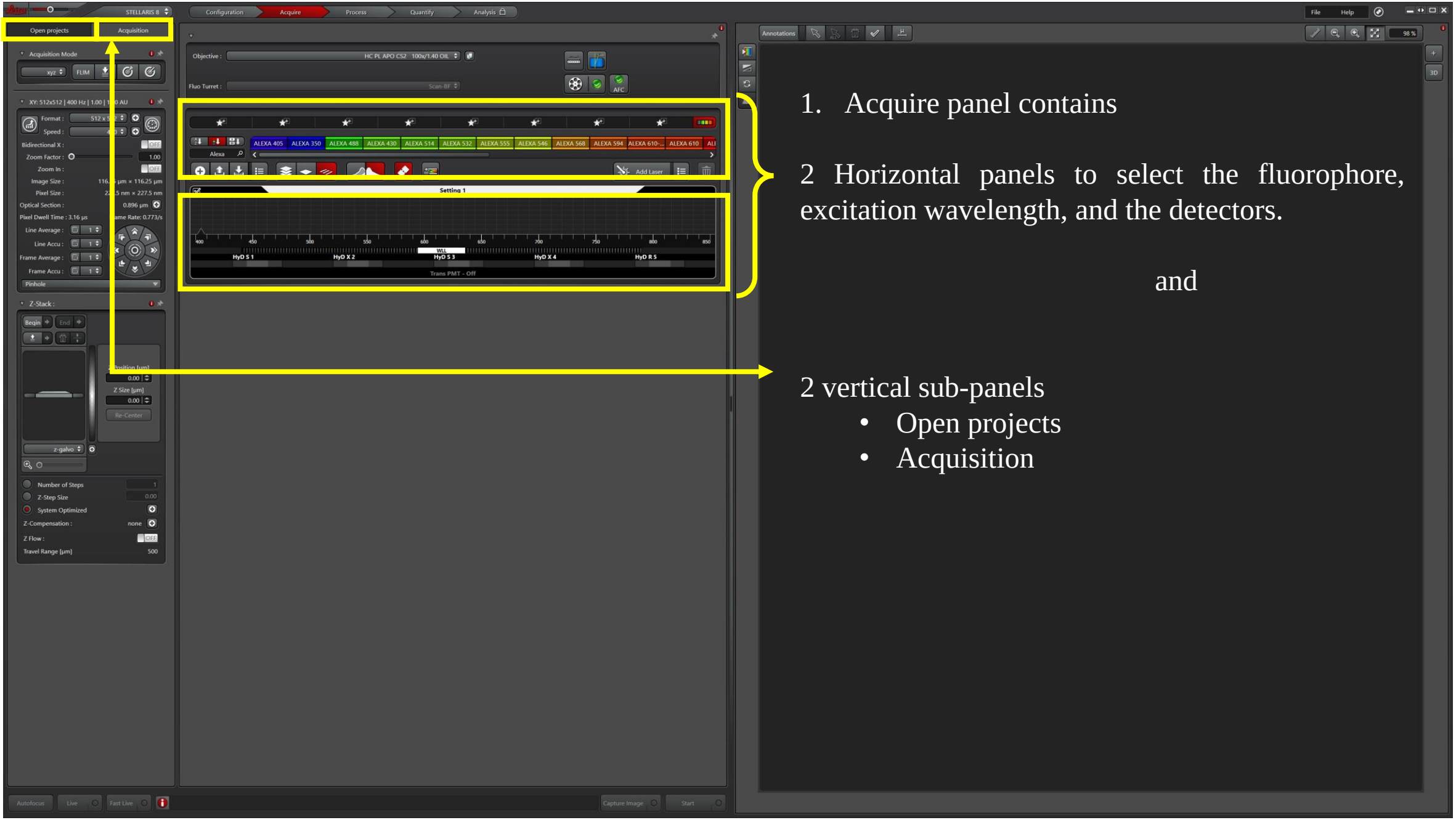


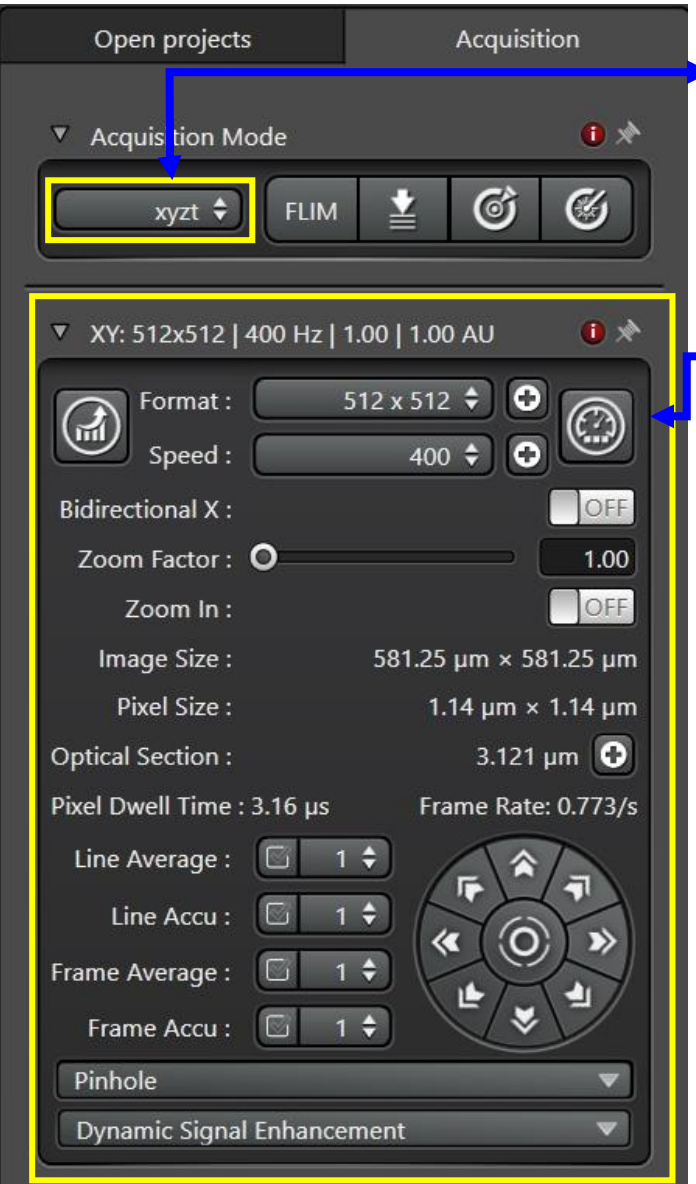
To select the required fluorophore, click the Dye Database.

Click the Laser configuration icon and turn on the white light laser (WLL) from the dialogue box appeared on the screen.



Click the Acquire panel to image the specimen. The Acquire panel is shown in the figure.

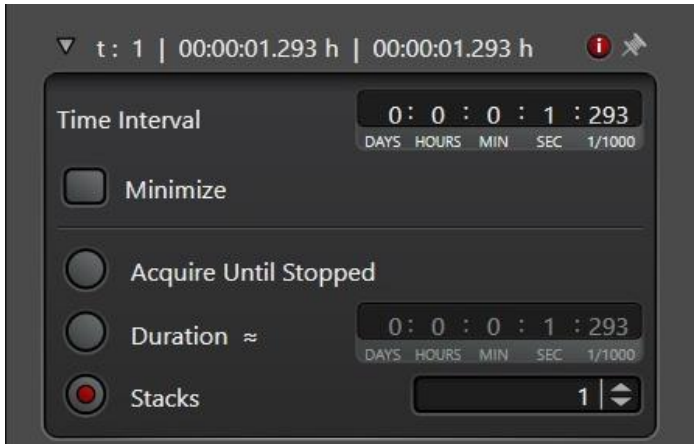




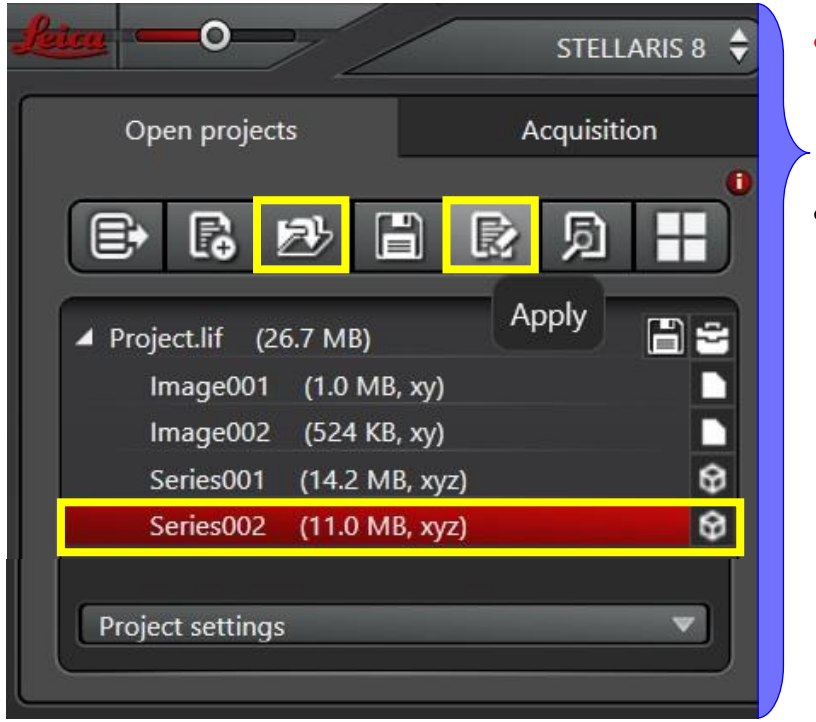
- Choose the acquisition mode from the dropdown list.
- Use the XY panel to set the image format, zoom, line averaging, pinhole.

Z-stack imaging

Time-lapse



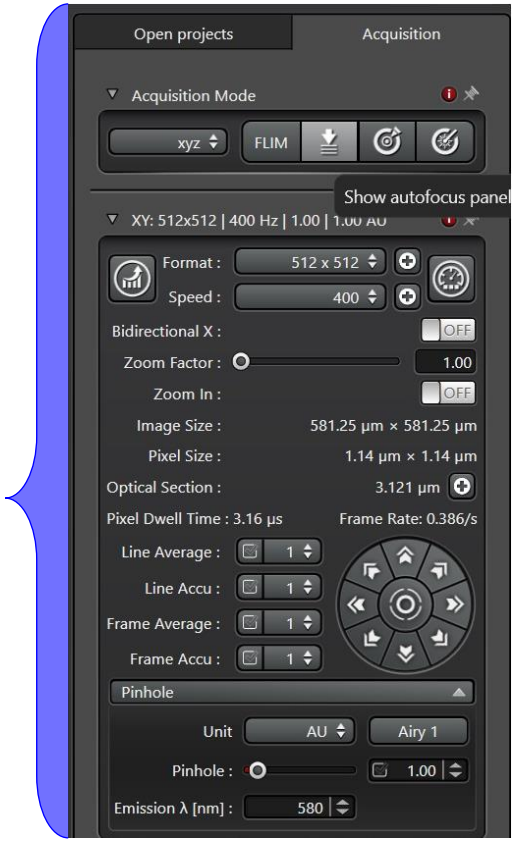
Loading your imaging method

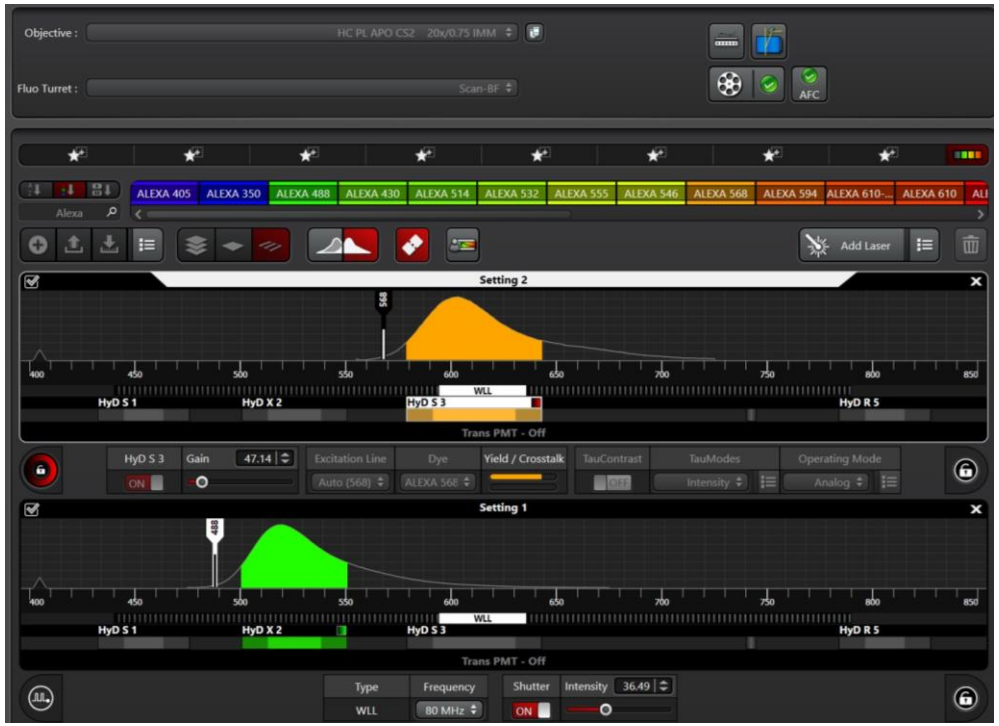


- Click the Acquisition panel under Acquire and click the Live to check the specimen or Capture Image button to record the image.

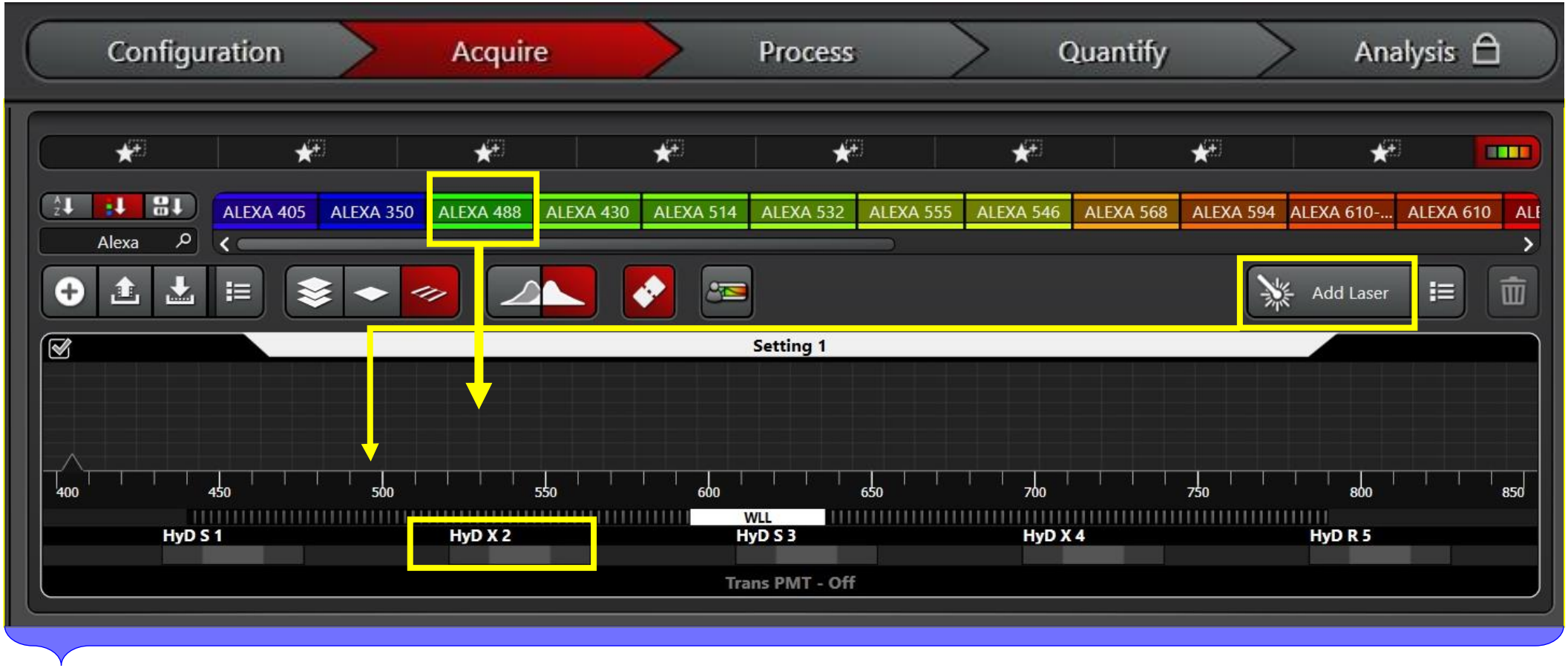
- NOTE: The method must be set up by the KCCI staff and you must be trained by the KCCI staff to use the method.

- The easiest way of loading a method is to open a previously saved image (‘lif’) under the Acquire ‘Open Projects” and then click the ‘Apply’ button to load the imaging method.



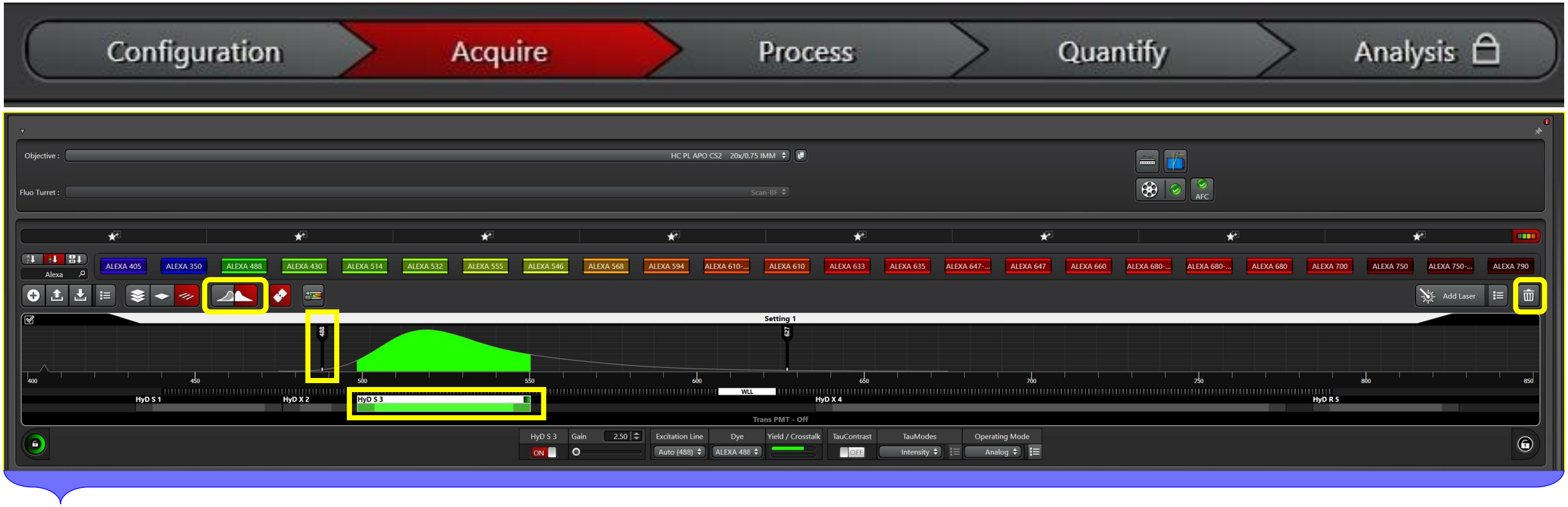


Acquire panel: Selection of the detectors, Dye, and Excitation wavelength.



- To select the fluorophore from the available top ribbon, hold the required dye button and drag it to Setting 1 (bottom panel).
- The particular hybrid detector will appear at the bottom of the selected fluorophore.

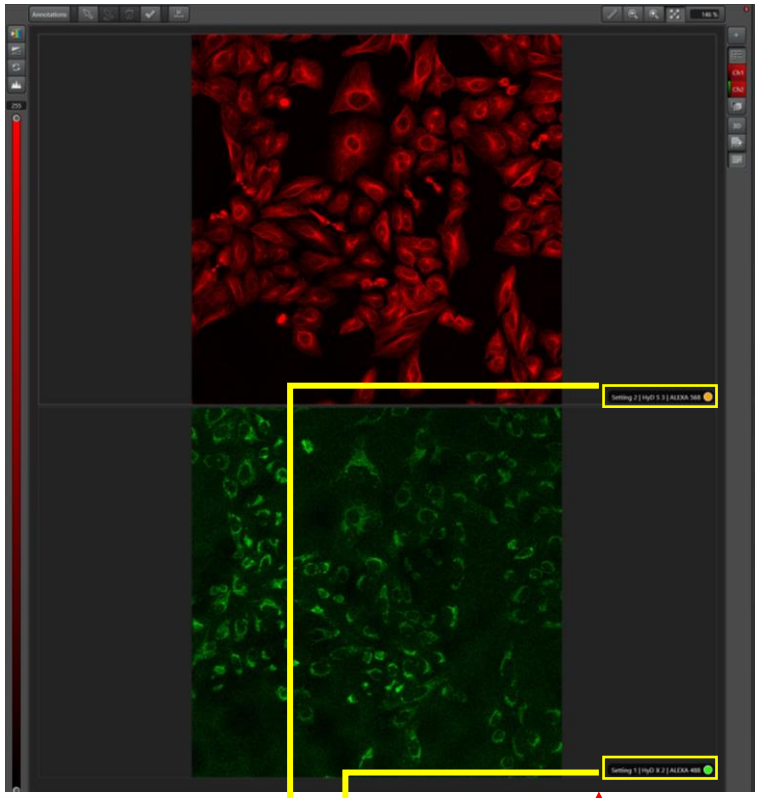
Acquire panel: Selection of the detectors, Dye, and Excitation wavelength.



- To remove the unwanted fluorophore active the detector ribbon (below the fluorophore) from the setting 1 panel and click the delete button.
- To remove the unwanted excitation wavelength, click the excitation line from the setting 1 panel and click the delete button.

Multi-fluorophores: Sequential Imaging Method

To image the additional fluorophore, create a duplicate panel by right click on the setting 1 panel. Delete the already occupied parameter via Reset (Right click on the panel and choose Reset). Select the fluorophore by dragging it to the duplicate panel and suitable excitation wavelength from the 'Add Laser' option.



2 different image panels indicates the selection of 2 different fluorophores (ALEXA 568 and ALEXA 488) and the 2 different PMTs (HyD S and HyD X).

Autofocus

Uncheck here to deactivate the particular channel.

Live

Click the Live to locate the specimen and Capture Image button (available at the boron of the Acquire panel) to record the desired selected field of view.

Fast Live

Capture

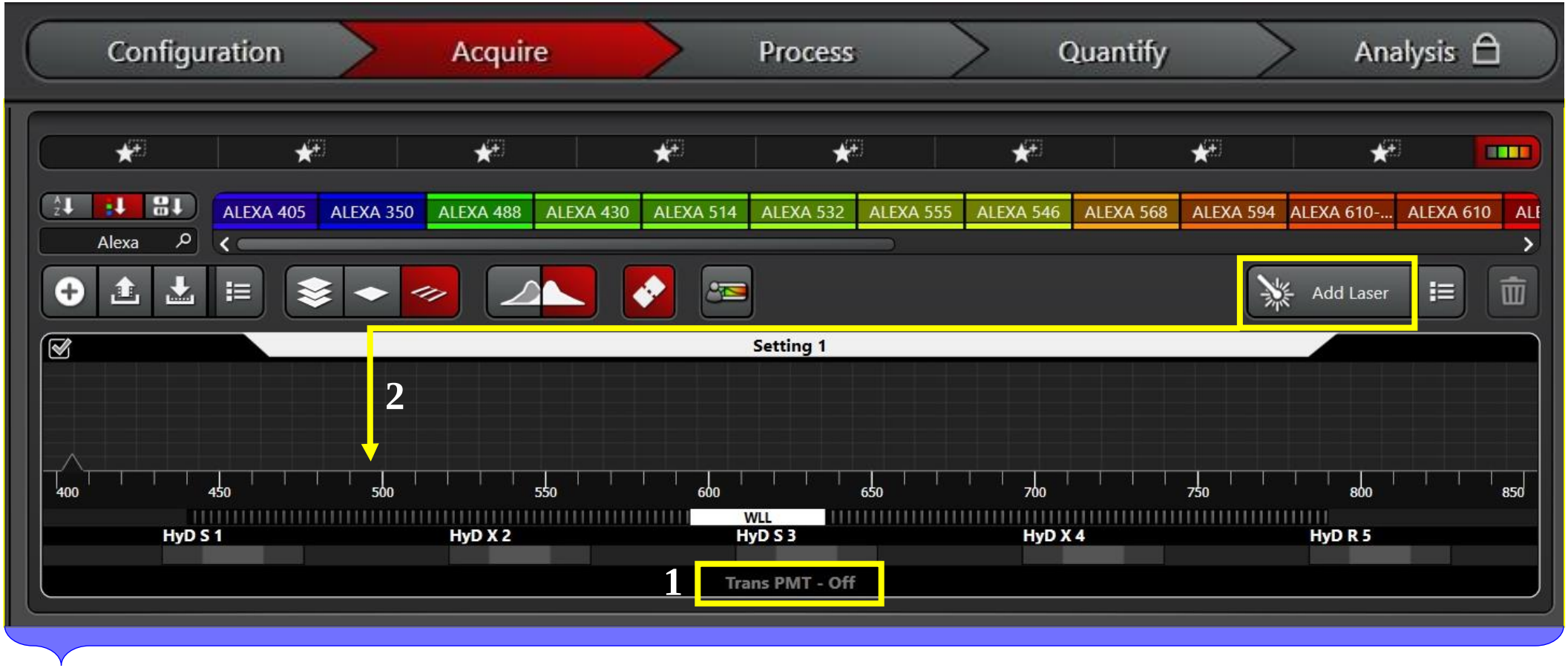
Click the ribbon under the spectrum to see the detector parameters (e.g. Gain).

Start

Click the Laser excitation line to see the excitation laser paratmers.

If No signal at the detector window tune the laser and detector parameters (excitation intensity or Gain)

Bright Field

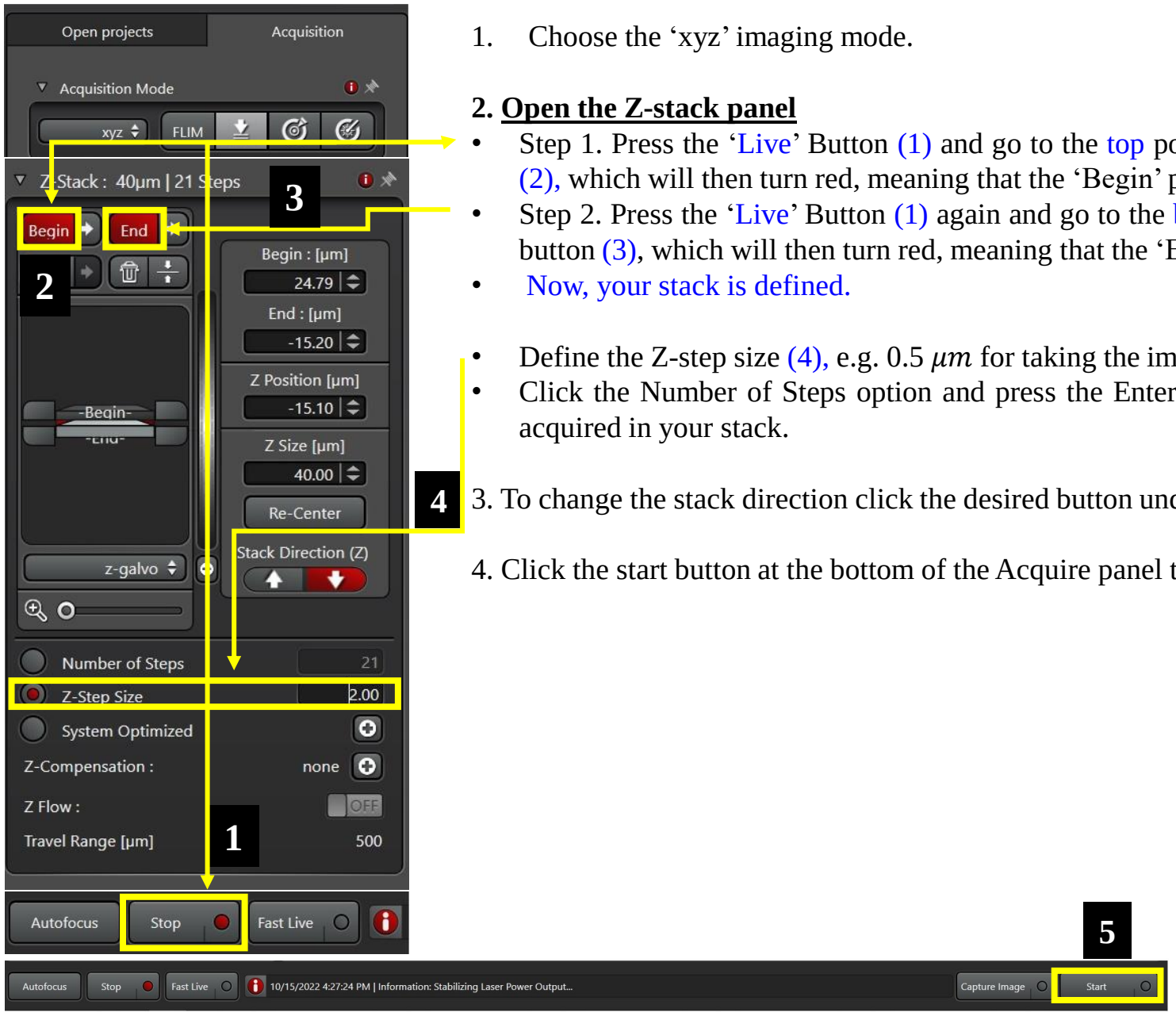


- Turn ON the Trans PMT.

  *Note: Do not select the dye in the case of Bright Field.
- Drag the Add Laser button to the setting 1 panel to select the excitation light. Make sure the white light laser is ON.
- Click Live to view the specimen and click Capture to record the image.

Take a Z-Stack

*Note: Z-stack is different from Z-galvo



1. Choose the 'xyz' imaging mode.

2. <u>Open the Z-stack panel</u>

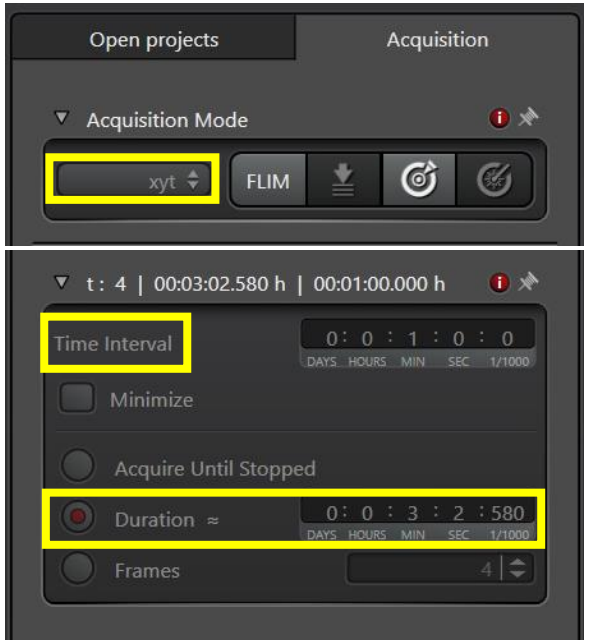
- Step 1. Press the 'Live' Button (1) and go to the top position of your stack using Z-focus. Click the 'Begin' button (2), which will then turn red, meaning that the 'Begin' position is locked in the top position of your stack.
- Step 2. Press the 'Live' Button (1) again and go to the bottom position of your stack using Z-focus. Click the 'End' button (3), which will then turn red, meaning that the 'End' position is locked in the bottom position of your stack.
- Now, your stack is defined.

- Define the Z-step size (4), e.g. 0.5 μm for taking the image sections every half micron.
- Click the Number of Steps option and press the Enter Key. You will be able to see the number of sections to be acquired in your stack.

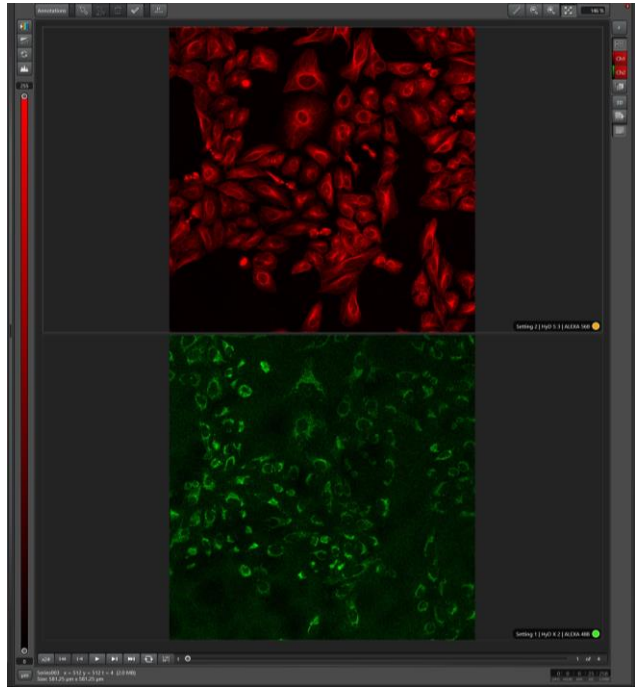
3. To change the stack direction click the desired button under the 'Stack Direction' (5) option.

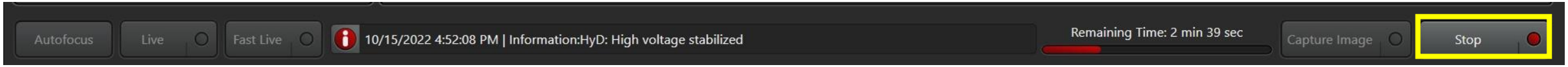
4. Click the start button at the bottom of the Acquire panel to image the specimen.

Take a Time-lapse



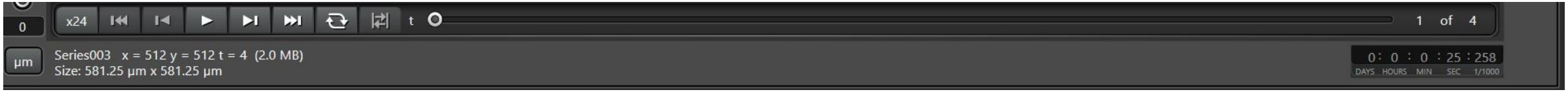
- 2-D Time-lapse: Choose the ‘xyt’ imaging mode.
- Open the ‘time-lapse’ panel,
- Define the ‘Time Interval’, e. g. 1 minute for taking images every 1 minute.
- Define the ‘Duration’, e. g. 3 minutes for taking time-lapse images for 3 minutes.
- Click the ‘Start button’ (at the bottom of the Acquire panel) to start the acquisition





Video play button

Remaining Time



Number of Frames

Shutdown the system

1. Save the data and transfer the data to KCCI/data server
2. Stop the record time at the KCCI website.
3. Turn OFF the laser from the software.
4. Close the software and shutdown the system.
5. Wait.
6. First turn OFF the Laser button and then Power button.

*Note: Remember, do not touch the key.