Leica STELLARIS 8 Confocal DIVE - Operating Instructions
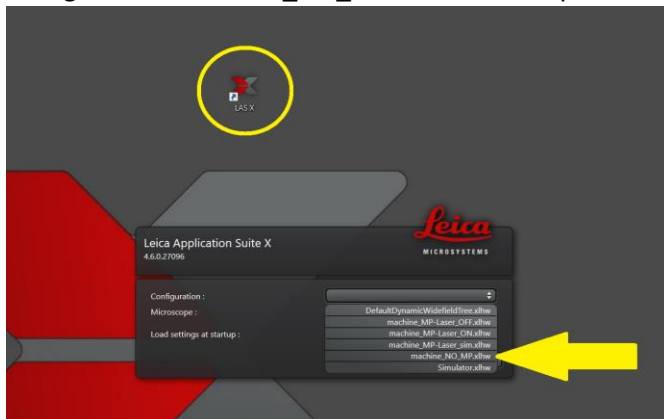
Turning the system ON:

a. Switch 1 (Label 1): Main power
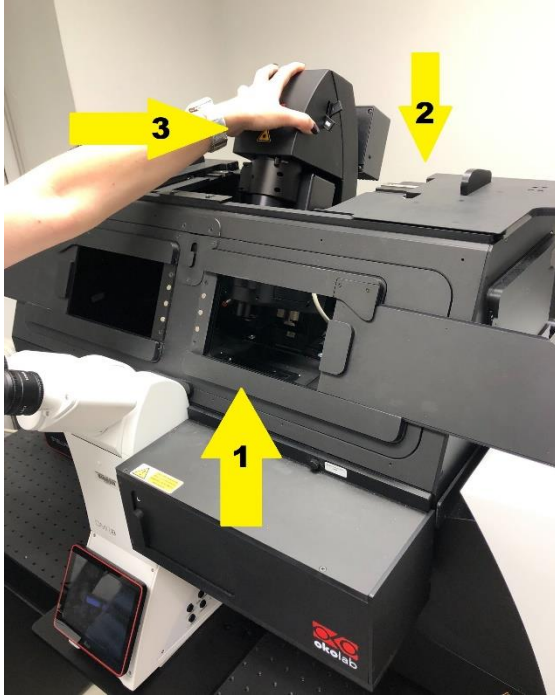b. Switch 2 (Label 2): Laser power
c. Switch 3 (Label 3): Start computer

Computer/Software:

1. Go to the E Drive and make a folder for yourself in the UVA folder if you do not have one already. You will store images here. No databases are required in this software. The images will be .lif format.
2. Log into Windows, choose your username and enter password.
3. Start the software: LAS X. In the start-up window from the drop-down menu select:
   Configuration: **machine_NO_MP** and Microscope: **DMi8**

Mount your sample:

1. In order to mount your sample you need to:

   A. Slide open the front door (1)
   B. Slide open the top door (2)
   C. Tilt the transmission arm (3)
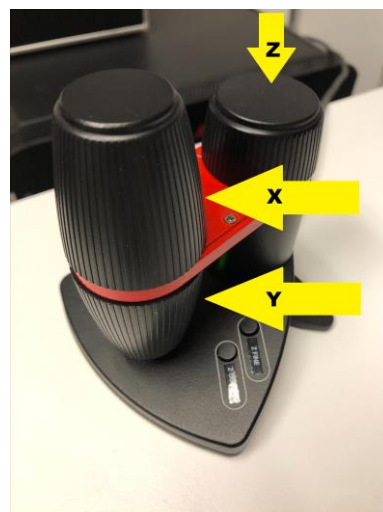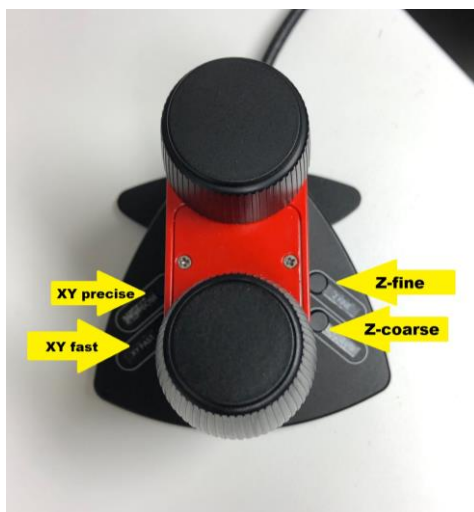   D. Mount your sample on the insert/holder

   E. Restore the arm:

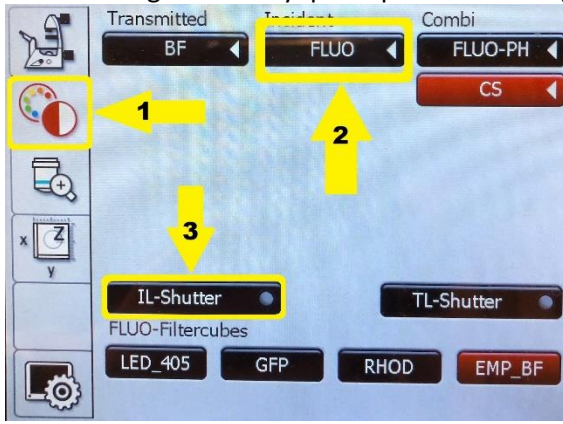## Getting Started and Image Acquisition

1. Locating your sample through the eyepiece:
   Look through the eyepiece to find your field of interest. You can use the joystick to move the stage around to locate your sample. Speed of the joystick can be regulated using the buttons on the side.

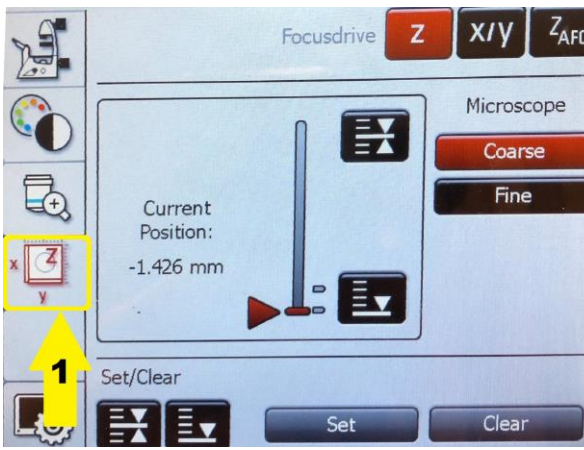Use Leica touchscreen to find your sample, use either of the following:

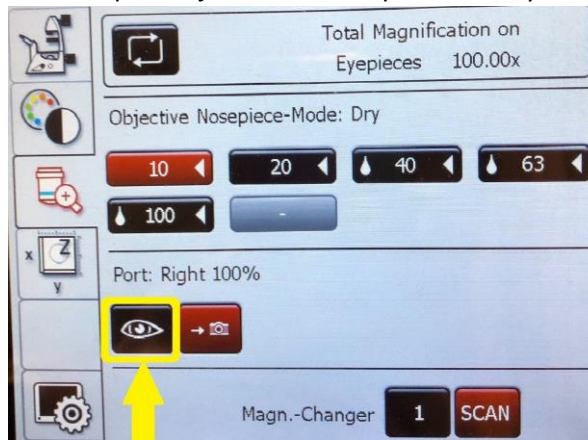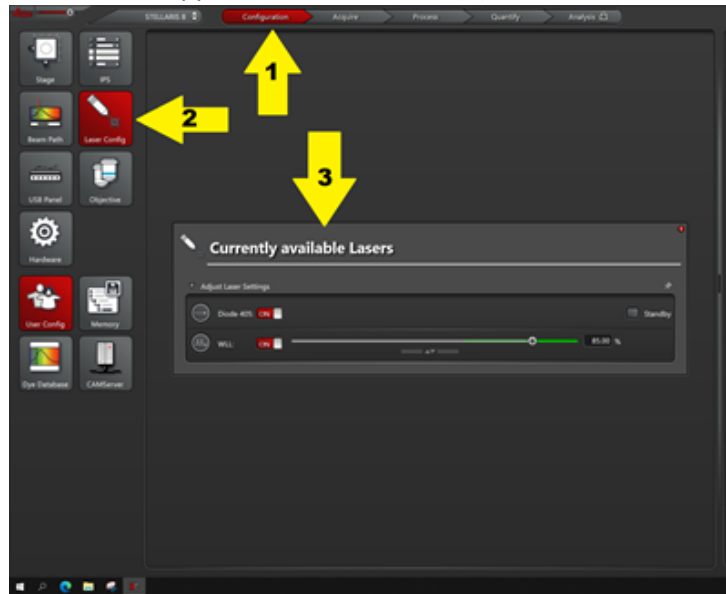| Pick the color pallet tab (1), next pick FLUO (2), to activate the light in the eyepiece pick IL-shutter (3)	Pick the color pallet tab (1), next pick BF (2), to activate the light in the eyepiece pick TL-shutter (3)

Carefully focus up using microscope knob or the joystick, pick x-y-z tab (1) to orient your focusing.
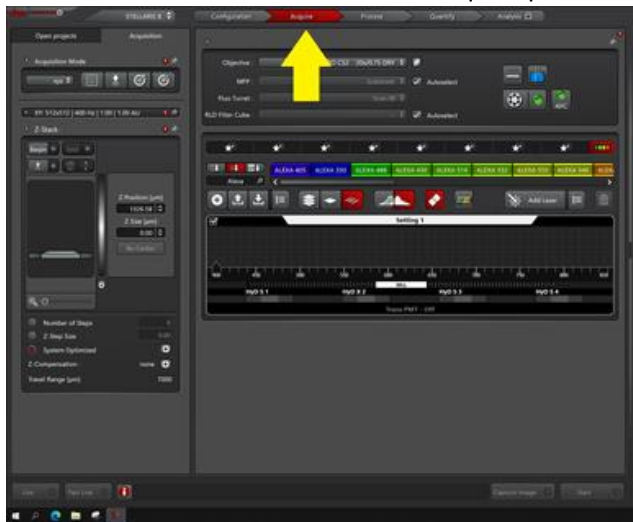

If by-eye view through oculars is completely black:
On the touch screen pick objective tab and press the "eye" button.

2. Click the configuration button (1) on the top of the screen to activate the configuration.
Click the Laser configuration icon (2) and turn on the 405 and white light laser (WLL) from the dialogue box appeared on the screen (3).
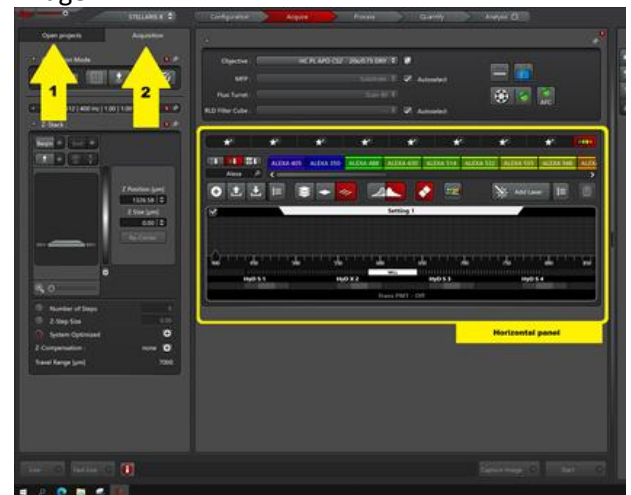

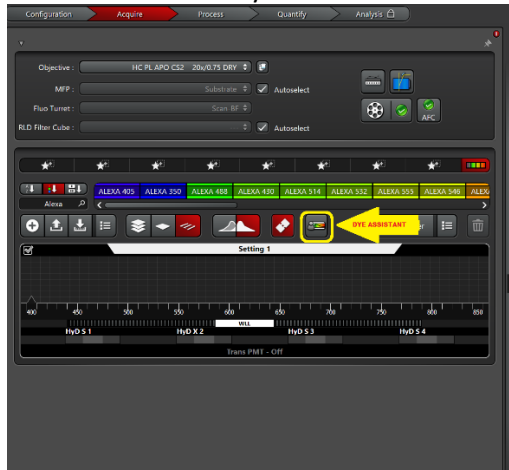3. Click the Acquire panel to image the sample.
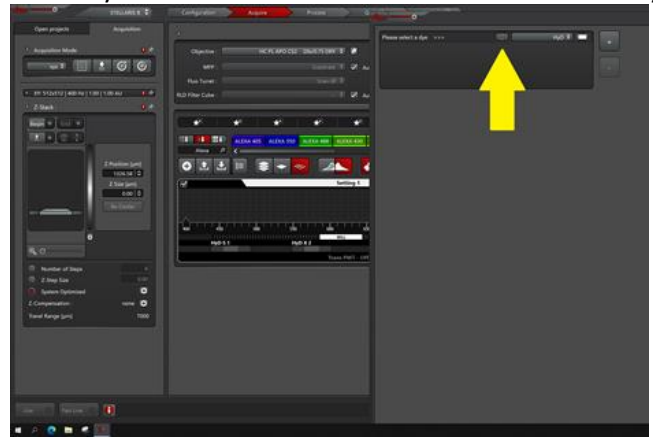

Acquire panel contains:
- Horizontal panel to select the fluorophore
- Two vertical sub-panels:
  - Open projects (1) – where you can save images
  - Acquisition (2) – where you can acquire the image.
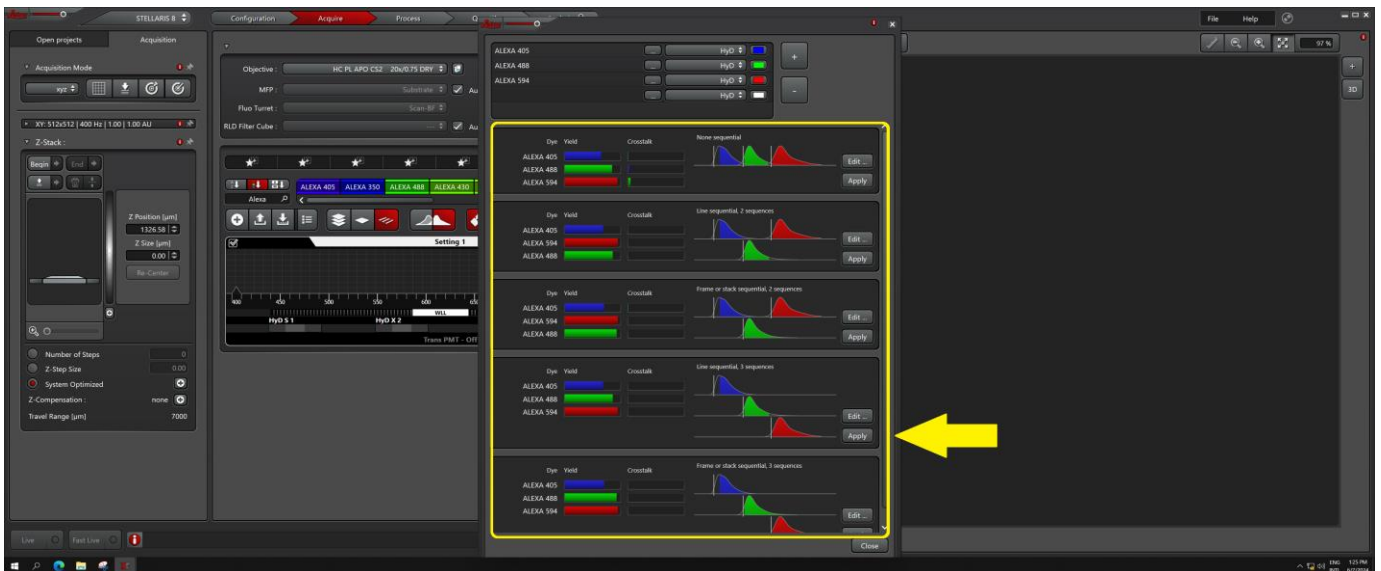
4. Click on a dye assistant icon:

5. In the dye assistant window click on: Please select a dye (…)

In the Please select your die (…) section, choose your dye(s) - click on the bar and the drop down arrow.
Then go down to the next bar for each dye until all dyes have been added. Pick the dyes, which are matching your sample.
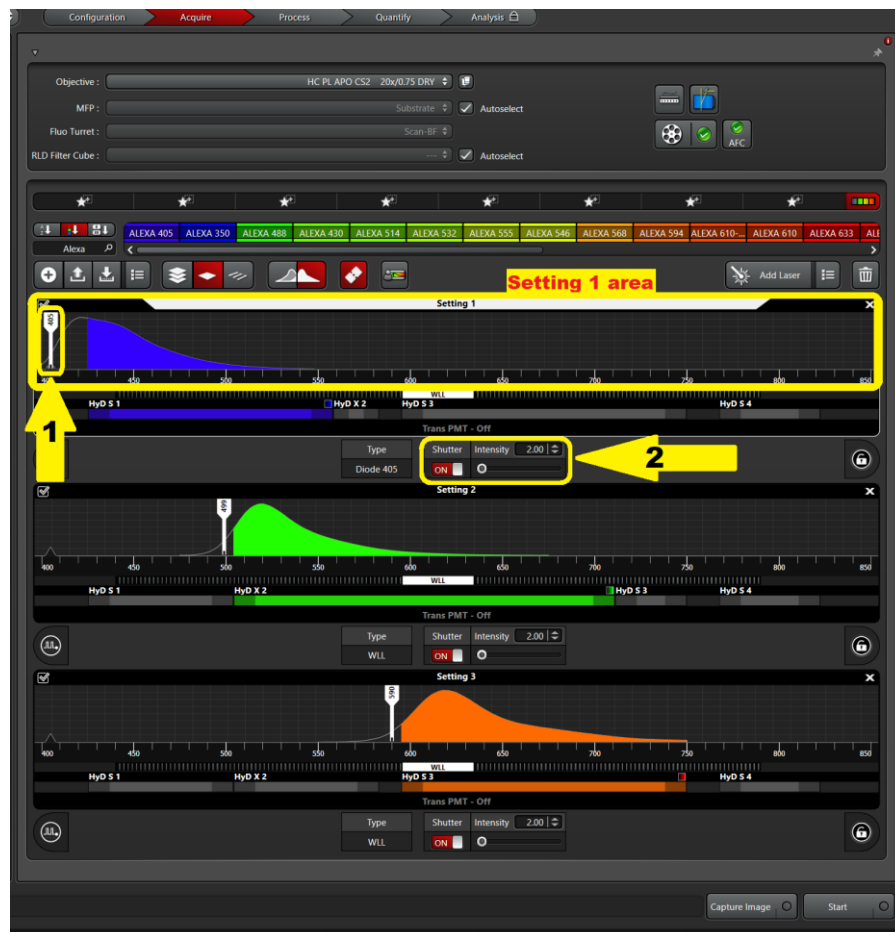
6. The dye assistant will present multiple ways to image the sample with the dyes of your choice. Pick lines switching sequential and click on Apply.

7. Now you should see the dyes of your choice with corresponding detectors.

8. Click on the "Setting 1" to activate it (Setting 1 area). To change the intensity click on the laser pin (1) and adjust intensity using the slider underneath the panel (2).

9. To adjust the gain click on the ribbon under the spectrum (1) and adjust it by using the slider underneath (2).

1. To preview your sample you can use one of the two buttons:
   Live (1) – switches on the live mode
   Fast live (2) - enables fast live mode with the settings for the fast scan, which allows you to toggle quickly back and forth between settings

The Live button will turn into a Stop button that you can press to end live imaging. NOTE: it is important to focus on the brightest area of the sample even if it is oversaturated. This will be adjusted for later on.
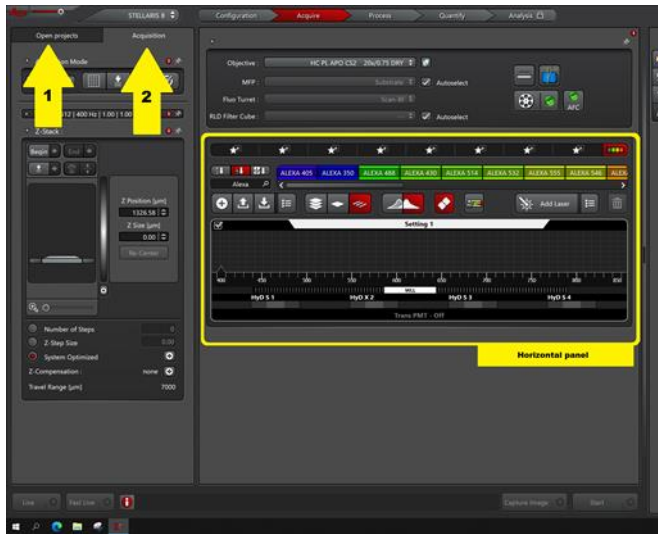
To acquire an image you can use of the two buttons:
   Capture image (3) – acquires a frame of the current stage position using all channels
   Start (4) – starts the experiment, including Z-stack and time series
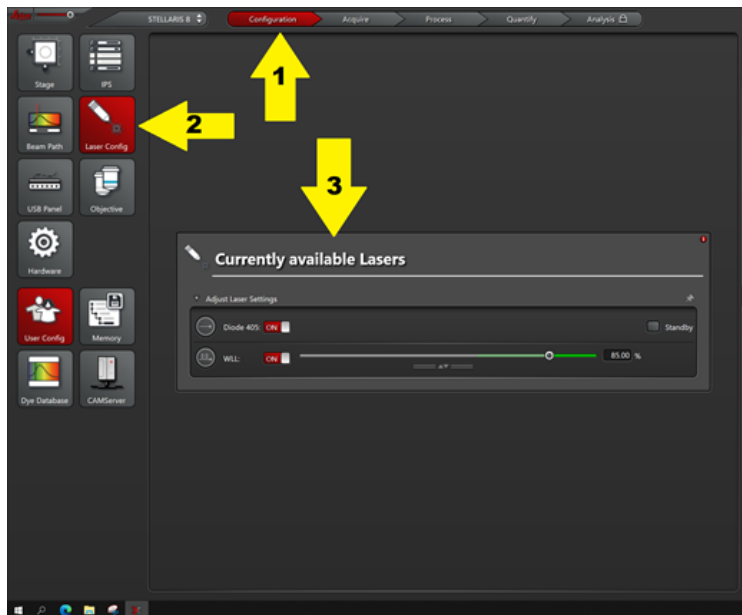
10. To save images click on Open projects (1)

Next, use one of the two floppy disc icons to save your Project (1 or 2) and save the raw data on E drive.

11. Shutting down the microscope:
   A. Lower the objective lens to the lowest point
   B. Remove the sample
   C. Clean objectives with lens paper and return to 10X
   D. Configuration tab: turn off Diode 405 and/or WLL

   E. Shut down the LAS X software
   F. Shut down the computer from the Windows menu
   G. Once the computer is shut off, turn off the switches in reverse order: 2, 1.