Leica STELLARIS 8 Confocal Microscope Instructions

Start computer

1. Turn on computer on PC console
2. Connect to Wi-Fi
3. Log in with your UARK username and password

Turning on the instrument

1. Follow the sequence from 1-3 on the control box (black box under table to your right) to initialize:
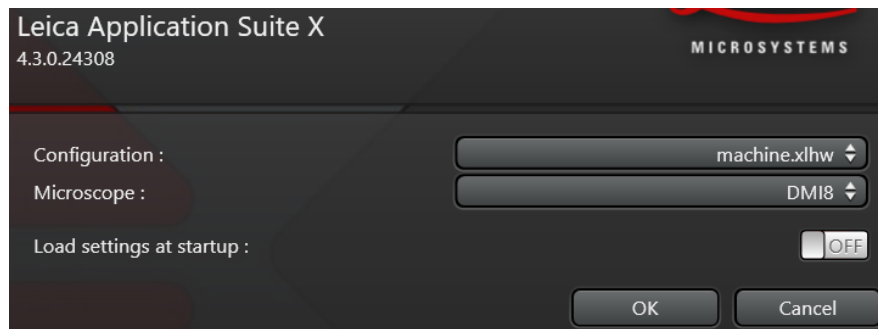
> **Scanner Power**
>
> **Laser Power**
>
> **Laser Emission** (turning key to the right)

2. Double click on **LAS X** application on the desktop.

   a) Set the parameters on the new window to the following:

    b) Click OK. Turning on settings at startup is only used the same settings will be used by the same or different user.

    c) Click **YES** when prompted to initialize stage.
    d) Wait until all the instrument finalizes loading and a new window appears.

Setting up instrument

1. Select the **Configuration tab** at the top of the window.
    a) Select **Laser Config**. This will show a secondary window "Currently available lasers."

b) Click the lasers needed (e.g., Diode 405 and/or WILL depending on the excitation required) until **ON** appears. **WILL** can be left at **85.00%**.
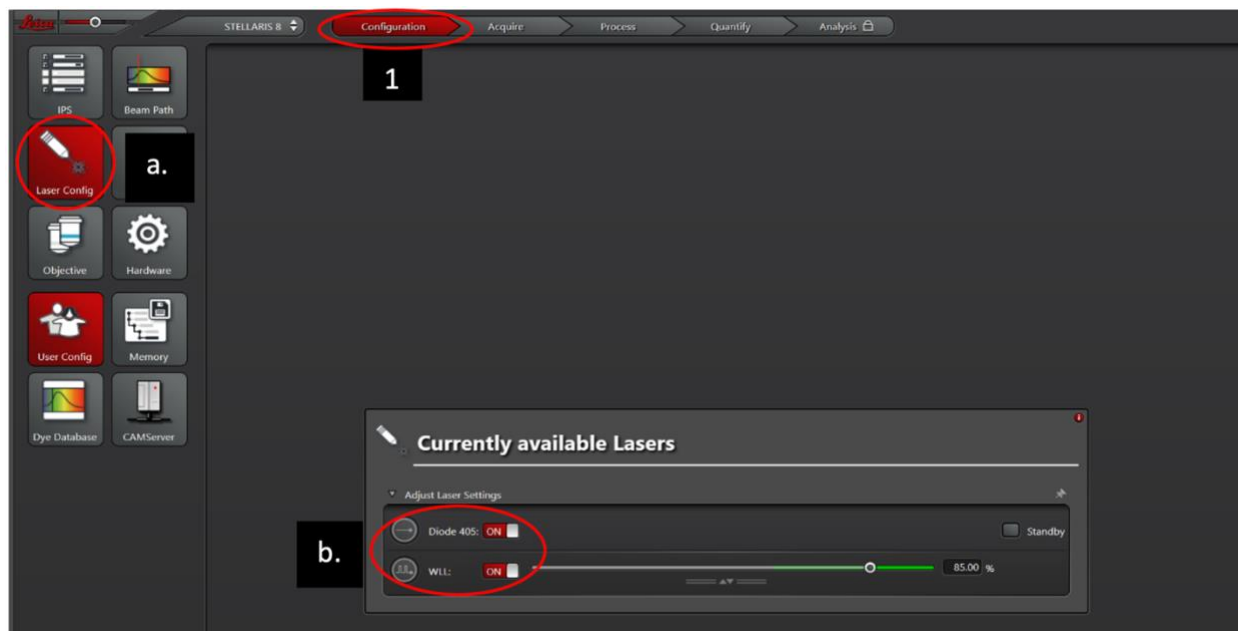
Setting up imaging parameters

1. Prepare your sample and gently place it on the stage on the microscope. It is an inverted microscope (objectives below the stage), so the coverslip (not the slide) should be close to the objective. Start at the lowest objective, 10X.

2. Use the settings on the microscope to locate your sample by using the XY knobs (to move side to side) or Z knob (to move up and down). The XY and Z knobs (Salt and Pepper shaker controls) next to the microscope. The front knob moves the X (bottom)/Y (top) plane whereas the back knob moves the Z plane. Toggle from fast/precise when needed. You can also use fluorescent filters, GFP, DAPI or Rhodamine to find your area of interest, if desired. Make sure you click the shutter to turn on/off the fluorescence light on the microscope.

   a. Adjust and focus sample by using the microscope and external touch screen panel.

   b. Under the "Transmitted" tab, click **BF** = bright-field.

   c. Click IL-Shutter to view sample (yellow = shutter open, gray = closed). Adjust using fine and coarse knobs.

   d. Close shutter.

   e. If needed, screen for fluorescence using the microscope and touch screen "Incident" tab, click **FLUO**. Click on the needed FLUO-Filtercube (e.g., GFP, DAPI, etc.). Make sure to screen quickly, as the laser will bleach the sample.

   f. View sample and adjust as needed.

g. Close shutter.

3. On the software, select the **Acquire tab** at the top of the window.

Please refer to this video before starting

Introduction to LAS X for STELLARIS: https://youtu.be/VGtTtBkoih4

a. Select excitation wavelength by either typing the fluorophore name on the **Fluorophore Search Bar** or by dragging the fluorophore from the **Fluorophore preset list** to the gridded area. The excitation wavelength will be shown as a number above a vertical line. The fluorophore spectrum will also appear as a peak. The horizontal bar (labeled as HyD S) represents the detection (emission range), which can be modified by either double clicking and typing in the range of wavelengths (spectral range) or dragging the bar. Detector bars should be no less than 10nm away from the excitation wavelength (e.g., For EYFP- 488 laser, detector should be set up from 498-540 nm).

b. Customize the color needed with the locked icon located on the bottom left corner of the laser window.

c. If bright-field is needed, click **Trans- PMT** below the wavelength bars and click "on."

d. More than one fluorophore can be added to the same grid area for simultaneous scanning.

e. For sequential scanning, click add laser to open a new setting for an additional fluorophore. Again, customize the color.

f. On the **acquisition tab** (left panel), select desired parameters for format, speed, etc. Good parameters to start are:

    i. Format: 512x512
    ii. Speed: 600
    iii. Line average: 1

Imaging

1. Press **live** or **fast-live** (bottom left-hand corner) to start scanning.

2. Use the dials on the control panel below the computer screen to adjust the quality of the image, by adjusting laser intensity and detector gain. Ideally, reduce laser intensity with the **intensity dial** (second dial from left to right) and increase **gain** (first dial from left to right). Other dials in the control panel can be used to adjust focus and zoom. Also move the **X/Y/Z planes** of the sample with the salt and pepper shakers to adjust area of interest.

3. Click **Stop** live when desired conditions are achieved, before changing to other parameters or when changing magnification.

4. If higher magnification requires changing objective, select desired objective on the objective settings on the screen. You will be warned when changing to an immersion objective. **20X is a multi-immersion objective (water, glycerol, oil), and you need to look at the collar of the objective to change to the proper immersion.** 63x is an oil immersion objective. When using any of these objectives please make sure you clean the objective **with lens paper only,** before moving to another objective. **Do not return to a non-immersion objective if sample contains residues of immersion media**.

5. Change the acquisition parameters, if needed, to acquire sharper images when capturing. Usually increasing format to size to 1024x1024, reducing scanning speed to 100, increasing line average, to 6 or frame average.

6. Press capture (right bottom corner of screen

7. For Z-scanning select area of scanning but choosing start and end points, thickness of each section, etc. Click **Start.**

8. Click on the **projects tab** (left side) rename the folder/picture in the program and at the bottom click save all. Imaging conditions will be saved on lif files. You can access them by right-click on image properties.

9. Images can be exported by right-click on the image and click "snapshot all" and transfer them into folder of choice.

10. Save your images into the edrive and then transfer them to your personal BOX folder.

Imaging multiple channels

Refer to the LAS X ImageCompass: Multichannel Configuration video for optimal imaging conditions for your particular purposes: https://youtu.be/-t2Zl0JJJ4c.

Shutting down microscope

1. Return all settings.

    a. **Acquire tab**: delete lasers/wavelengths; clean objectives with lens paper and return to 10X; return all settings in the acquisition tab to the original settings (Format 512x512, speed 400, line average 1).

    b. **Configuration tab**: turn off Diode 405 and/or WILL.

2. Shut down the desktop.

3. Once the computer is shut off, turn off the switches in reverse order: 3, 2, 1.