

Leica Stellaris 8 Users Guide

Additional guide from the web:

<https://www.dbs.nus.edu.sg/wp-content/uploads/sites/7/2021/09/CBIS-Leica-Stellaris-User-Manual.pdf>

Leica Tutorials

<https://www.youtube.com/@LeicaMicrosystemsTutorials/videos>

Please acknowledge NIH grant S10 OD032447-01 in preprints and publications using the Stellaris (PI: Hans-Willem Snoeck). This increase chances for further grants.

AVOID THESE DAMAGING EVENTS:

- Oil dripping down objective lenses. Use oil sparingly, do not add extra when changing slides or gently wipe off oil when changing slides. Wipe the top and sides of objective when you are finished. Use only lens paper.
- Bumping Objective lens with the stage Insert or your slide. Avoid any rough handling of any part of the instrument.
- Nail polish or other substances on the objectives or on microscope components (e.g. Oil on Dry lenses)
- Detector beep and high laser intensities (less 10% is recommended, higher can be used for dim samples or far-red wavelengths).
 - Use “frame mode” to avoid detector overload beep.
- Frequently turning on and off the system / lasers.

Other Important information:

- **All data must be saved to the E: drive** under your lab group, download your data to external hard drive, backup to cloud or multiple drives. After 2 weeks please delete your data
- **4-hour reservation maximums** so that others can access the system (with exceptions, email me).
- **If you hear a beeping sound, turn down laser intensities.** Beeping means the detectors are getting too much light. WLL (White Light Laser) intensity is generally kept below 20% (It can be set higher), 405 laser is generally kept below 5% (recommend 0.5% for DAPI). Slow scanning can also send too much light to the detectors. ‘Frame mode’ can help to avoid detector overload.
- **Sometimes the system misbehaves (settings not working).** Try closing LASX and restarting computer. If this doesn’t work, you can shut down and restart the entire system.

System Startup

Look over the microscope area, make sure stage is clean. If not, email me jwm2175@.

Start up causes robotic stage to calibrate it's entire range. Nothing should be in its way or it will get crashed.

Turn on:

1. Main power (Orange switch 1), wait 2 seconds
2. Laser power (Orange switch 2)
 - a. Laser emission key is always "on" (vertical)

3. Computer

The following should automatically turn on:

- a) LED lamp (for viewing bye-eye)
- b) Microscope Control power
- c) Lightbox light (white box, right of microscope)

Turn these on if they are off.

4. Double click LASX (LEICA) Software



Avoid using the microscope controls until LASX software has booted up.

Chose "machine.xlhw" and "DMi8":



System Shut down

Are you the last user of the day? (If yes, shut down system completely)

Is there someone coming in 30 min or less? (if yes, leave system on, but sign out)

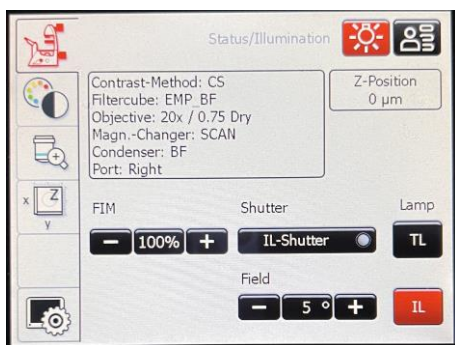
- If using oil or water immersion: gently wipe lens with lens tissue
- Lower the objective lens to lowest point
- Set to 10X objective lens
- Close LASX software
- Download your data to an external hard drive. All data > 2 weeks old may be deleted.
- Sign out of Computer:



- If you are the last user of the day (or no one using for at least 30 min):
 - shutdown computer, Turn off switches 2, and 1.

Locating your sample by-eye

- Use **Leica touchscreen** to find your sample:



- FLUOR, IL-shutter (=Incident Light (fluorescent))
- BF TL-shutter (= Bright Field Transmitted Light, i.e. bright field, or phase)
- Carefully focus up using microscope knob or the “joystick”
Joystick has course and fine buttons
- Look at the Z position, touch screen x-y-z to orient your focusing. In focus is typically ~1.40 mm from the lowest point in the focal adjustment.
- Use Z position presets

If by-eye view through oculars is completely black:

> touch screen > objective tab > press the “eye” button.

Image Acquisition Tile Scanning

* If LASX controls are not responding you can close LASX and restart the computer, or you may have to shut down and restart entire system.

Set up Acquisition settings

In Acquisition menu

If you have standard imaging settings from previous experiments:

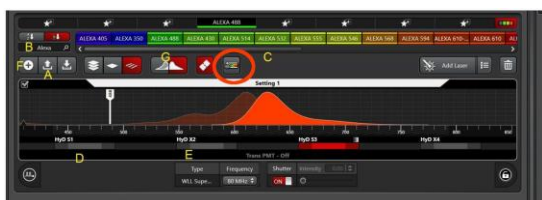
Open the previous image and choose “**apply**” to set the settings to those of the open image.

You can also save and open “.seq” settings files.

*Note that this may not save scan speed zoom and image averaging.

First time setting up your acquisition

- Set Objective to 20X dry.
- Set scan speed to 700.
- Set pixel size to 1024 x 1024. Note pixel size and zoom.
- Set line (or frame) average to 1 for now. Average of 3 will improve image quality but will slow the acquisition.
- Use the **dye assistant** (circled in red) to set your dyes/laser detector setup:



- Type in the name of each dye you will use at the ellipsis (...), e.g. DAPI, GFP, Alexa 647...

Dye Assistant will try to choose the best options for you. Generally, use no more than 2 lasers, and 2 detectors per “Channel” (=track). 1 channel per 1 dye gives the least cross-talk/bleed through but is slower. Don’t image DAPI and A488/GFP in the same channel as DAPI has a broad emission peak that extends to green. Line scanning acquisition is faster than frame.

- Accept the dye assistant settings.

Notice the channel layout. There are a lot of settings!

- Control the **laser** by clicking on the **dropped pin**
- Control the **detector** by clicking on the colored line below the spectrum of each channel:



There are 5 detectors that can all be used at once! 2 -HyD S, 2 HyD X, 1 HyD R (best for Near InfraRed).
Detection range is ~440 to 825 nm.

Lasers: There is a White light laser (WLL) that can excite at any wavelength between 440 nm and 790 nm. Add as many laser lines as you need. There is a separate 405nm laser for DAPI. The WLL and the detectors can all be tuned for emission and excitation. The dye assistant has chosen the theoretical best, brightest settings but your samples may need specific tuning.

For initial imaging:

- Set the 405 laser intensity to ~0.5
- Leave the other lasers intensities at 2.

Set laser intensity and detector gain for each channel / fluorophore

Click “**LIVE**” (live viewing). “Fast live” will allow you to view faster using a preview image (not final resolution).

Focus your image (microscope stand, joy stick, or smart dial).

==> smart dial fine or course setting can be selected.

Adjust gain and intensity using the “Smart Dials”. These can also be adjusted by clicking the laser dropped pin or the detector. Click in the window to select which image the Smart Dial controls.

Make sure you are in focus, out of focus light is much dimmer.

Repeat gain and intensity adjustments for all your dyes/images. Click the image window to activate the dials for each channel.

*Tip: If your image has bright signal across the glass that looks like shearing / scattering / diffraction rings, this might be light scattering:

Solution: Drag the left side of the detector wavelength further away from the laser (or, you can move the laser further away from the detector). Grab at the shaded region on the left of the activated detector to move it.

Save this adjustment setting so that it is not lost (either save a .seq file or capture and image), the detector and laser lines can easily be reset.

*Tip: view and assess saturated image pixels by clicking the box on upper left in the image viewing window (toggles thru viewing options). Leica calls the saturation view, “glow over” and “glow under”(pix=zero intensity). Glow under is very bright green. To view only the “glow over [=saturated]” and avoid the bright green zeros pixels: > configuration > uncheck “glow under”.

Using Navigator for Tile Scanning (very brief)

In Acquisition menu > Click the **Navigator button**, green box below (actual may look somewhat different):



Return to the acquisition menu by clicking ‘close’ or X-out (both are upper right). Navigator settings will be remembered when you click it again.

Navigator menu > “**Spiral**” (lower left) to rapidly scan across your slide. Zoom in or out with mouse wheel, drag with mouse, acquisition settings are in menus on the left. The white box is your image field.

Spiral: spiral scan through your slide. Stop any time by clicking again.

Use the Region of Interest (ROI) tools at the bottom of the screen to outline the portion of your slide that you want to image. Click a second time to complete the ROI.

Set tile merge settings using the **Stage Tab** button (lower left):

Merge images during acquisition: **ON** (or you can merge later in Mosaic Merge tab)

Set the % overlap to 10 or 15%

After acquisition, new images will appear in the Projects tab and in the Mosaic Merge tab.

Autofocus points (AF+)

Use this to set auto focus points across your tile scan.

Click the **autofocus tab** at the left to see a list of autofocus points and autofocus method. You can delete your autofocus points here.

Set autofocus settings: 30 microns and precision (1 click from max precision)

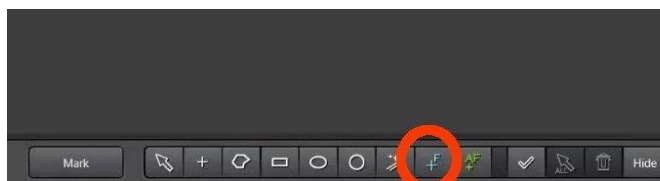
80 microns is usually too large and will take too long.

Default Autofocus method should be okay.

Manual focus points (F+)

Use this to manually set Z position during the tile scan acquisition using “set Z”.

Manual focus points are set with the “**F+**” tool at the bottom of the window. Click F+, then click on image to add a handful of manual focus points:



Set the Z for manual focus points:

Click **focus map** (lower right) to view a list of the focus points and their current Z positions. Points will have X and Y but not Z. You need to set the Z:

During Live Image, double click on a focus point number in the focus map and the stage will move to it. Manually focus with the **Smart Dial** or joystick. When in focus, click **“set Z”**. Repeat for other focus points.

You can also use autofocus here to set the Z focus. If so, make sure to **set autofocus settings. (e.g. 30 microns and 1 click from maximum precision)**.

Additional web guide for setting focus points:

https://cpb-us-e1.wpmucdn.com/sites.northwestern.edu/dist/a/560/files/2020/06/2020_DM6B-Quick-start-guide_LAS-Navigator_done.pdf

A good demonstration of LAS tile scanning is here:

<https://www.youtube.com/watch?v=nzYSE468gNQ>

Once you have set up your tile scan, you may want to **line average to 3** or **increase image “format” pixel size to get the best resolution**. Also, double check that the brightness of each channel is not over saturated and looks good.

Click Acquire (lower right) to capture your image tiles.

Good luck!

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This guide will be routinely updated and may contain errors.