

DMi8 Station 1, 2 Users Guide

YouTube tutorial for Leica DMi8 microscopes

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

LASX navigator tutorial

<https://youtu.be/nzYSE468qNQ>

AVOID THESE DAMAGING EVENTS:

- Excessive Oil (oil running down objective). Gently wipe oil from lens top and side.
- Bumping Objective Lenses with the stage Insert or your sample (glass slide)
- Getting nail polish or other damaging substances on the objectives, or on any microscope components (e.g. **oil on dry lenses**)
- Any rough handling of any part of the instrument
- Turning off the mercury lamp before it has warmed up (< 15 min).
- Excessive light to the camera.
- Return to 10X lens when finished

System Startup, turn on:

Look over the microscope area. Make sure stage and objectives look okay. Send a note to me (jwm2175@) if not.

1. Microscope power (1), wait 2 seconds.
This causes the stage to move across its full range.
2. Mercury lamp power (2)
3. Computer (if not on already) (3)
4. Login to the computer and start the **LASX (LEICA) Software**
Please use your UNI to login.

It's best not to use the microscope controls until you start LASX software.

Shut down

- Gently wipe any oil from lens and side of lens with lens tissue.
- Lower the objective lenses to lowest point.
- **Set to the 10X objective lens.**
- Close LASX software.
- Download your data to an external hard drive. All data > 2 weeks old may be deleted.
 - No data can be stored on the C: drive. Temporarily store it to the E: drive.
- Sign out of system.

If no one has reserved microscope in the next 30 min:

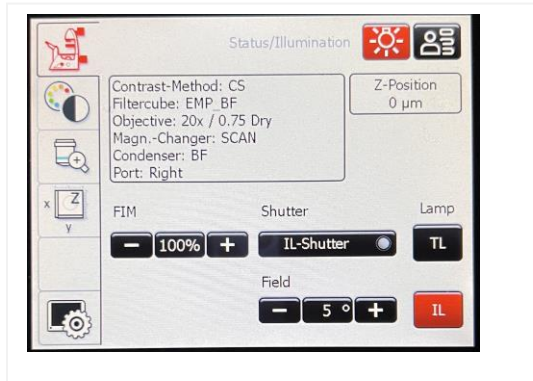
- Turn off switches 2, and 1 (If another user is coming in 30 min, you can leave the system on).
- Shut down the computer.
 - Ideally lamp should **warm up 15 min** before turning it off, and cool down 15 min before restarting.

Locating your sample by-eye (once LASX software has started)

- Use **Leica touchscreen** to find your sample by:
- FLUOR, IL-shutter (=Incident Light (fluorescent))
- BF TL-shutter (=Transmitted Light, i.e. bright field, or phase)

- Focus should initially be at zero or negative numbers.
- 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” seems to be about _____ mm on DMi station 1.

The touchscreen:



if you can't see fluorescence by-eye, go to objective tab on touch screen and press the eye button.

Image Acquisition Tile Scanning

* Note: sometimes the LASX settings get messed up. You may have to restart software or close windows for it to fix itself.

Set up Acquisition settings

In Acquisition menu

If you have typical imaging settings from previous experiments, open the previous image and choose “**apply**” (upper left, “projects” menu) to set the settings to those of the open image.

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

*Note that this may not apply all of the image settings

First time setting up your acquisition

- Set Objective to 20X dry.
- Set image acquisition settings.
- The Fluorescence cube colors are:
 - DAPI
 - A488/GFP
 - Y3 (Cy3/Rhodamine/Alexa568)
 - Y5 (Cy5/Alexa 647)
- Choose 8 bit or 16 bit camera pixel depth (the number of gray scale levels). I recommend 16 bit for quantitation, most people use 8 bit (click on camera icon to see the bit depth).

Click “**LIVE**” (live viewing).

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

Set exposure and gain of camera so that the image looks good

In general, try to limit the amount of excitation light hitting your samples. Samples can bleach easily.

*Tip: view saturated and black 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”. Glow under is very bright green and hard on the eyes. To view only the “glow over [=saturated]” go to:
> configuration > uncheck “glow under”.

Leica Navigator for Tile Scanning (very brief)

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

(You can 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)

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

Click Spiral and allow it to 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 set the ROI. Acquisition fields will be shown as a gray grid.

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. Both Merged and unmerged should be there. Delete un-merged if you are happy with the merge.

A good demonstration of LAS tile scanning is here:
<https://www.youtube.com/watch?v=nzYSE468qNQ>



Focus points

Use this if you want to adjust the focus (Z) plane during tile scanning. You can manually tell the software the location of in focus fields, which are called, focus points. You can also use “autofocus”, but I think most DMi 1 and 2 users set manual focus points.

Manual focus points

Manual focus points are set with the “F” tool at the bottom of the window (circled red). Click on this and then click on a few regions of your proposed scan. You shouldn’t need a lot.



Click **focus map** (lower right). This will be a list of the focus points and their current Z positions. Live Image your sample, double click on a focus point (one the numbers) and the stage will move to it (X-Y). Manually focus with the knob or joystick. When in focus, click “**set Z**”. Repeat this for multiple focus points.

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

Now that your tile scan is set up:

Check one more time that the brightness of each channel is not over saturated and has a nice histogram.

Click Acquire (lower right) to capture your image tiles or set up RFC (see below).

Relative Focus Correction (RFC)

If different fluorescence channels appear out of focus with each other, you can apply RFC. This will adjust the relative focus of the image when you switch fluorescent colors according to how you set the ‘store Z position’ for each Fluor.

Click RFC so that it is red (image at right, green box)

Go through each channel, find the best focus, click “store Z position (image green box, lower right).

When you have stored each Z-position, click “Apply Relative Focus Position” (image small green box in the middle).

Go back to your original Fluorescence Color and focus your image.

Now when you acquire all fluorescence channels, LAS X will apply this adjustment to each channel.



Video demonstration of Relative Focus Correction (RFC):

https://www.youtube.com/watch?v=40zy_S90LDs

John Murray jwm2175@ cumc.columbia.edu

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This guide will be routinely updated and may contain errors.