

NeuroTechnology Studio

Leica DMI8

User Manual



For questions or concerns contact:

Lai Ding: (617)264-5946 (781)901-6116 lding@bwh.harvard.edu

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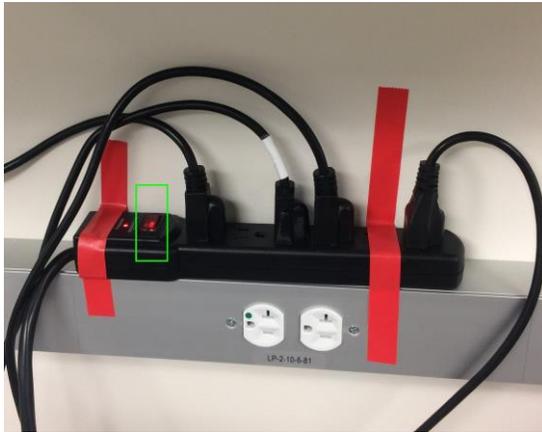
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DO NOT TOUCH ANY BUTTON/SWITCH UNLESS IT IS SPECIFIED BELOW

Start-up

1) Turn on the power strip, It is located on the wall above the power bench.



2) Wait until the display screen at the front of the microscope stand finishes startup



3) If Computer is not ON, turn on the computer.

4) Log in to your lab account on the computer:

Login to the computer using the lab account and password you were given during your training.
Remember to log off when you are finished with your session.

5) Next, open the software "LAS X"

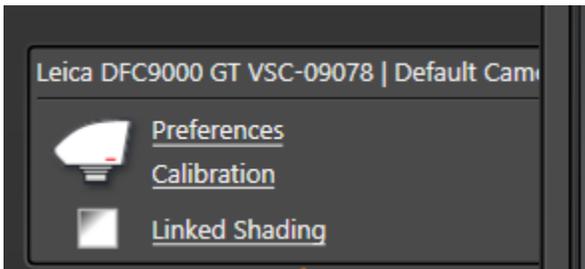


A dialog window will pop up. Make sure the selection is the same as picture below, click the button: **OK**.

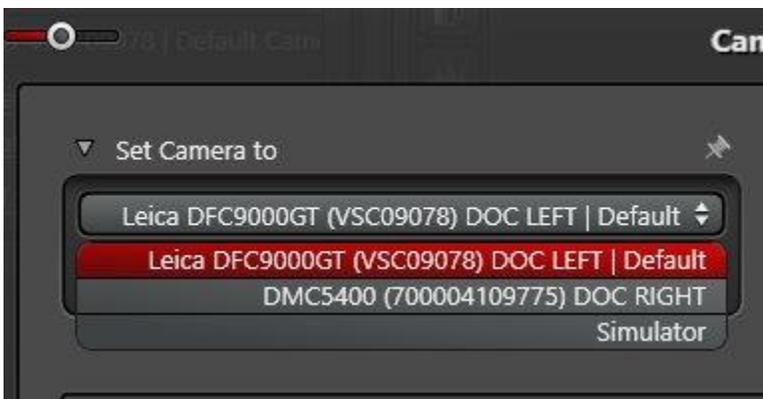


As the hardware configures, you will hear the microscope parts starting to activate. This is normal. If any error message shows up, take a picture and notice Lai Ding or Teri Zgoda immediately.

Select your camera: click “Preferences” under camera section.



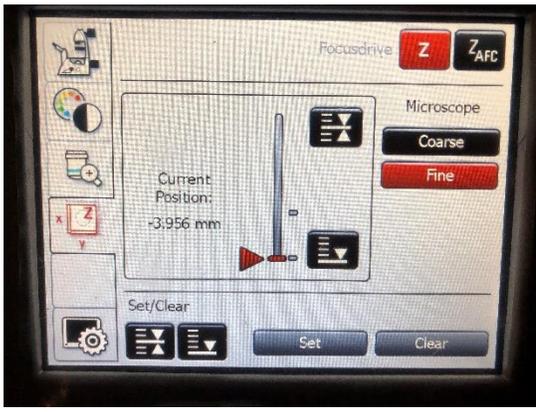
Then choose the camera under “Set Camera to” section. “Leica DFC9000GT LEFT” is for fluorescence imaging. “DMC5400 RIGHT” is for bright field color imaging



You are now ready to image! Please save the files to E: drive “User Images” folder and the corresponding lab folder. **DO NOT SAVE IMAGES UNDER C: or D: DRIVE!**

Microscope Display

Almost all these features can also be changed using the LAS X software.



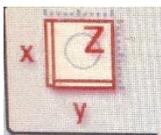
Status of current selections. Can also change from TL to IL and open/close shutter



Change light type and filter cube



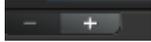
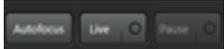
Change the objectives and light path (directed towards eyepiece, left camera port, or right camera port)



Press  to bring the objective all the way down, and  to set the focus

Channel Set-up



- To add a channel, press the **PLUS** button in the middle window. To delete a channel press the **MINUS** button 
- To change the false color of the channel, double click on the gradient rectangle 
- Determine what wavelengths you need. Select the filter cube, which will be outlined in red when active. 
- Turn on the LED source. You may have to change the wavelength it is emitting at using the dropdown below the slider. If it can't use that wavelength with that filter, the box will be grayed out with a line through it. 
- Make sure that the wavelengths in these dropdown boxes are the same throughout all the channels. This quickens the time it takes to take a photograph.
- Use the slider to increase/ decrease the light intensity
- Press the **LIVE** button in the lower left-hand corner to view your subject 

- Use the knob circled in red to adjust Z



- Use the knobs below to adjust X and Y. There is a small toggle switch circled in red below that changes between fine and coarse movement.

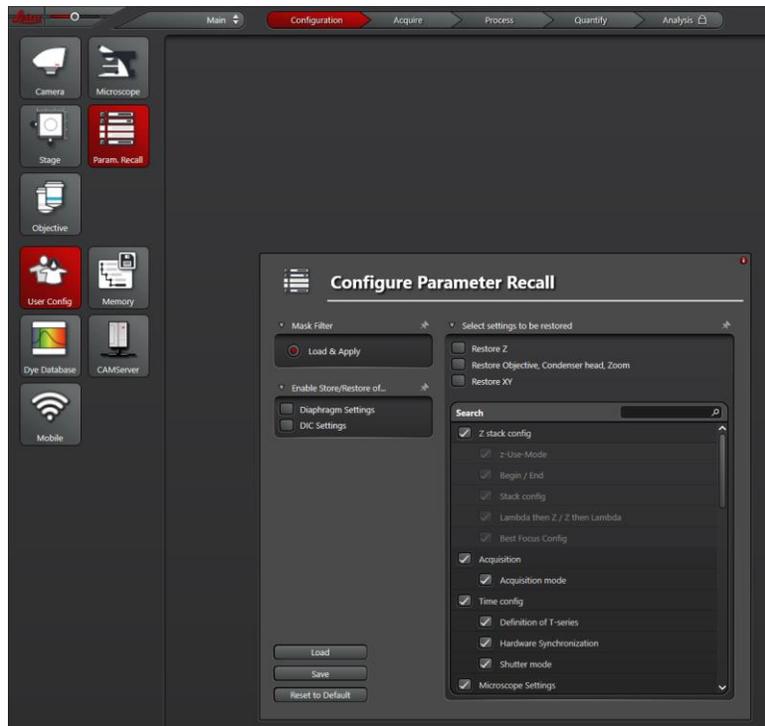


- Adjust exposure time and laser intensity for each channel.
- Under **PROJECT SETTINGS** dropdown, uncheck **SEQUENCER MODE**
- Press **SINGLE IMAGE** to photograph one single channel.
- Press **CAPTURE IMAGE** to photograph all channels.
- Press **START** to begin a z-stack or time series.

Apply Previous Settings



- Open a previous project using button circled in yellow
- Go to “Configuration” tab and “Param Recall” section, click “Load” button, under the pop-up file dialog window, select “setting.xml” file

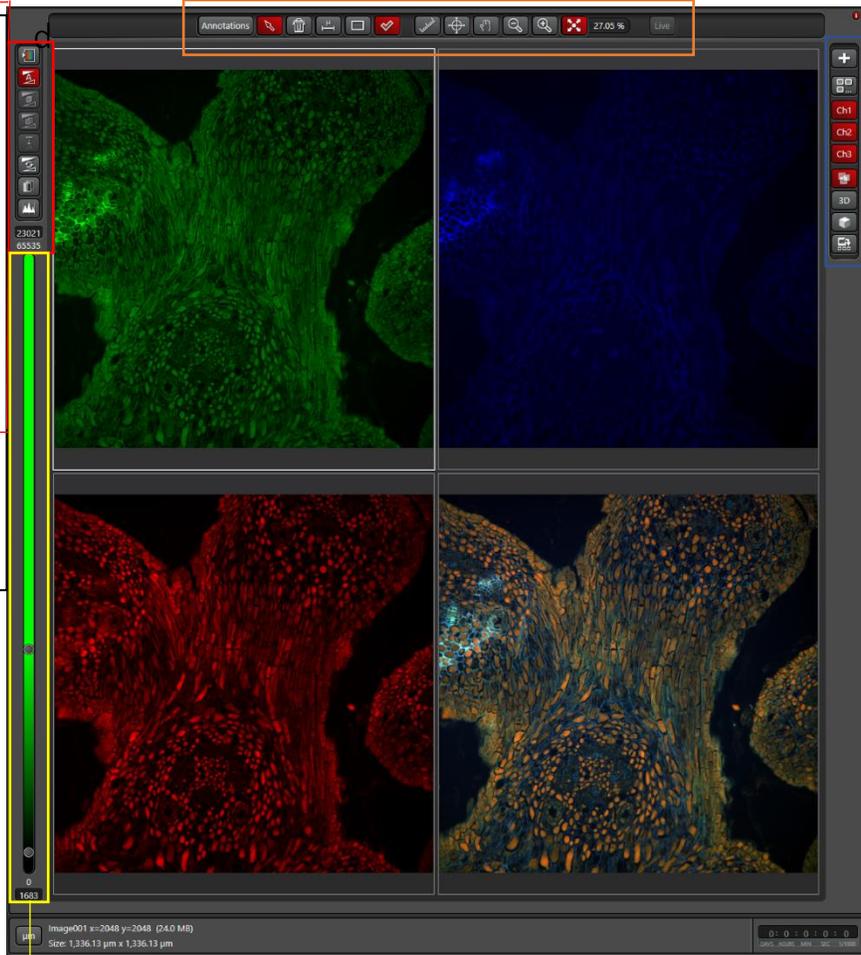


- Go back to “Acquire” tab, press the button circled in red to apply the settings

Display Window

Annotation: Add shapes or scale bars to image. Measure distance between points.

- Change Look Up Table (color, intensity, or black and white)
- Autoscale all channels
- Stack autoscale
- Channel auto scale
- Reset
- Use background LUT to scale
- Open Histogram



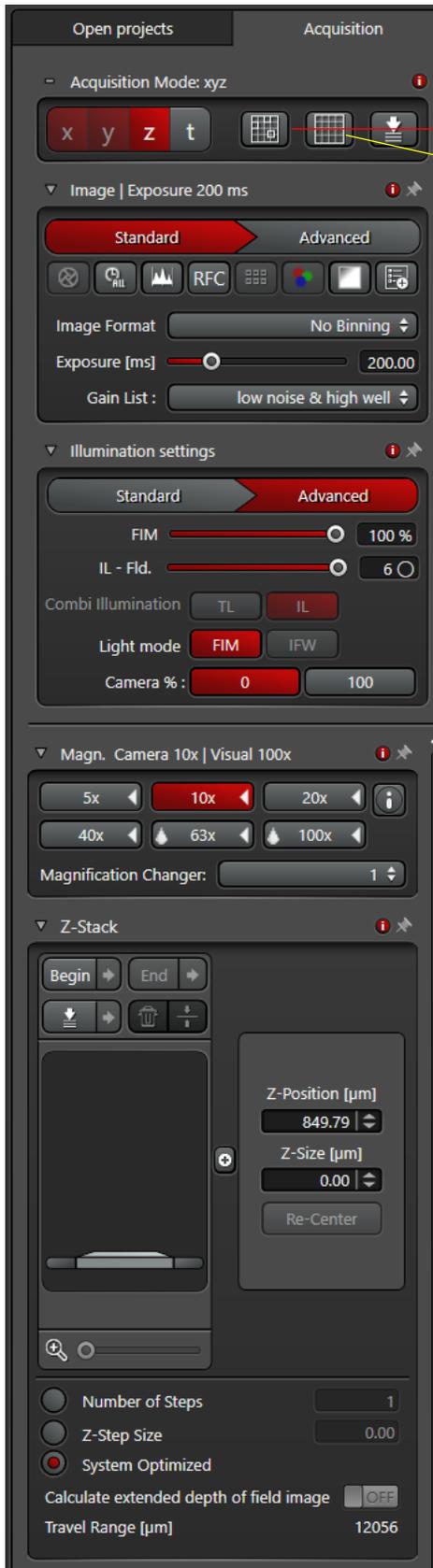
Select what channels you want to view.

This button is to view a composite of all the channels.

Open 3D viewer window. Useful only if you've taken a Z-stack.

Look up table and contrast. Moving the two circles closer together will increase contrast.

Acquisition Window



Select **Z** for Z-stack and **T** for time series.

Mark and Find
Tile Scan

Binning: select if you want a faster, quicker image with less resolution exposure here.

Longer exposure = more light = higher chance of photobleaching

FIM: fluorescence intensity manager, higher % more light

IL – FLD: field stop, should always be at 6

Camera %: (light is directed towards eyepiece) or 100 (light is directed to camera)

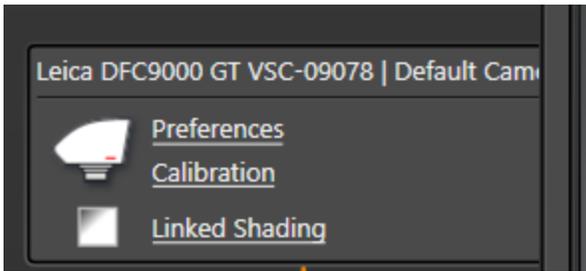
Change objective here

Keep “Magnification Changer” as “1”

Scroll through the Z to find when your subject just begins to dim and go out of focus, then press BEGIN. Go through the focus to the next point the subject starts to go out of focus and click END.

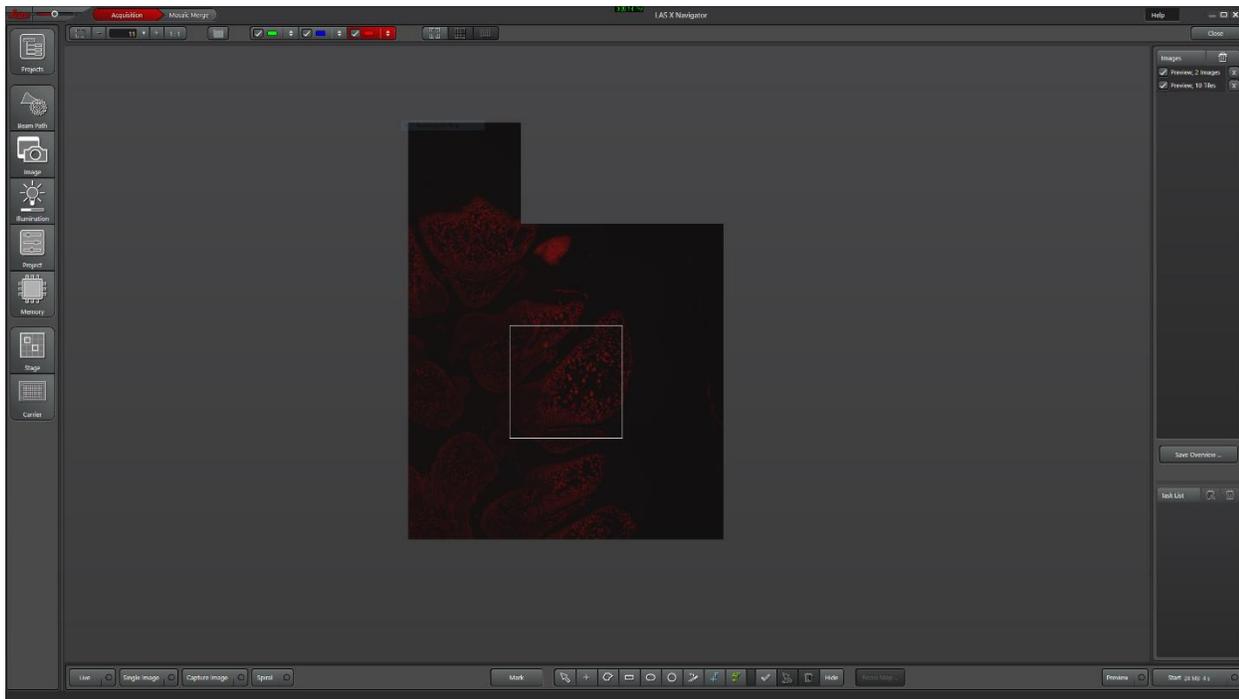
The system will provide number of steps with “optimized” Z-step size if “System Optimized” box is checked. User can assign step size or number of steps with corresponding box checked.

Navigator



- If doing a Tile Scan, click on **Link Shading** to open up a new window. Check the ACTIVATE boxes on the new window, then close. This will smooth out some of the dark edges you would see on the final tiled image.

Pressing the Navigator button  will open up a new window. Press the X to close the window and get back to the main acquisition page.



- Click LIVE to find the center of your subject and bring it in focus
- Click SPIRAL to begin a spiral overview scan. It will scan in the channel selected at the top, in this screenshot the red channel is highlighted.
 - The time it would take to scan the entire channel is displayed in the lower right corner
 - The shorter the exposure time, the faster the spiral will be
- Press STOP to end the scan

- Select what area you want to photograph using the polygonal tools in the bottom window
- Click  blue to put manual focus points on the scan area. Green is autofocus.
- Click FOCUS MAP to bring up a window displaying the points. Press live, then double click on the first point to bring it into the field of view. Focus and press SET Z, then next to bring it into the next focus point.
- Hit START to begin the tile scan.
- Once the scan is finished, click on the MOSAIC MERGE tab at the top of the window.
- Select SMOOTH BLENDING on the drop down menu, and click merge tiles.

Leica DMI8 Objective/Camera Info

Objective

FOV: Field Of View FL: Fluorescence Camera (LEFT) CC: Color Camera (RIGHT)

Objective	5X	10X	20X	40X*	63X (oil)*	100X (oil)*
N.A.	0.12	0.45	0.4	0.95	1.4	1.4
FOV_FL (mm)	2.67 x 2.67	1.34 x 1.34	0.67 x 0.67	0.34 x 0.34	0.21 x 0.21	0.13 x 0.13
FOV_CC (mm)	2.64 x 1.76	1.32 x 0.88	0.66 x 0.44	0.33 x 0.22	0.21 x 0.14	0.13 x 0.09
Working Distance	14000um	2800um	6900um	170um	140um	90um
Resolution XY @ 550nm	2.80um	0.75um	0.84um	0.35um	0.24um	0.24um
Resolution Z @ 550nm	38.2um	2.72um	3.44um	0.61um	0.43um	0.43um

***: not suitable for plastic bottom dish or multi-well plate**

Camera binning/format

Pixel Scale **FL : Fluorescence Camera (LEFT DFC9000GT)** Camera Pixel Size : 6.5um

Bin \\ Objective	5X	10X	20X	40X	63X (oil)	100X (oil)
No binning	1.3um*	0.65um*	0.325um*	0.163um*	0.103um*	65nm
2x2	2.6um	1.3um	0.65um	0.325um	0.206um	130nm*
3x3	3.9um	1.9um	0.975um	0.489um	0.310um	195nm
4x4	5.2um	2.6um	1.3um	0.65um	0.41um	260nm
8x8	10.4um	5.2um	2.6um	1.3um	0.82um	520nm

*: recommended binning setting

Pixel Scale **CC : Color Camera (RIGHT DMC5400)** Camera Pixel Size : 2.4um

Format \\ Objective	5X	10X	20X	40X	63X (oil)	100X (oil)
20MP	480nm	240nm	120nm	60nm	38nm	24nm
5MP	960nm	480nm*	240nm	120nm*	76nm	48nm
2.2MP	1450nm*	720nm	360nm*	180nm	114nm *	72nm*
4K	480nm	240nm	120nm	60nm	38nm	24nm

*: recommended format setting

Shutdown

- Make sure you save all of your files and transfer electronically. If using an external hard drive, make sure it is clean!
- Delete any sensitive data off the server
- Check to see if there is anybody else using the microscope on the schedule and log off your user account
- Press the switch on the power strip to turn off the microscope
- Cover the microscope