User Guide for Leica SP8 Confocal Microscope

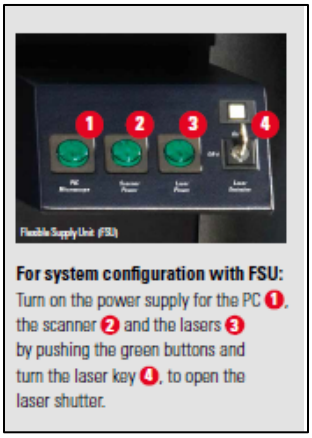
The System (Located in Biosciences 232):

2 HyD detectors, 1 PMT detector

Laser lines (UV@405 for DAPI; 3 Argon@458, 488, 514; DPSS@561; HeNe@633)

Objectives: 2.5x, 5x, 10x, 20x, 40x (oil), 63x (oil)

Super-resolution capability: HyVolution technology combines ultra-sensitive HyD detectors with Huygens deconvolution to achieve resolution down to 140 nm.



Powering on:

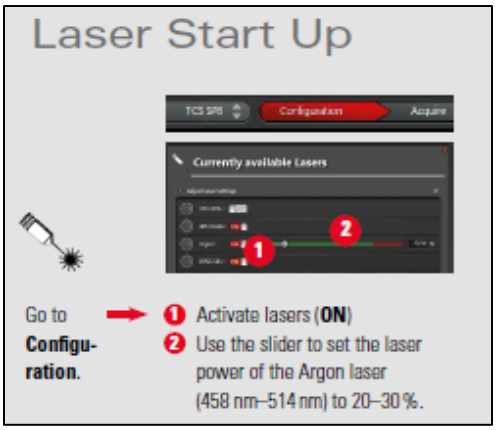
1. Turn on switches for PC microscope (1), scanner power (2), and laser power (3).
2. Turn on the laser emission key (4).
3. Turn on switches for the UV light source (5) and the power strip (6) supplying the camera.
4. Log into the user book. Enter your name, name of the PI, actual start time, and UV start time.

Shutting down:

1. Turn off Argon laser. This is done through the "Acquire" panel of the LASX software. **NOTE:** The Argon laser needs time to cool before the system is shut down.
2. If you used immersion oil, make sure you have cleaned off all objectives used during your session with lens paper.
3. Turn off the switches for the power strip (6) supplying the camera and the UV light source (5).
4. After the laser fan stops, turn off the laser emission key (4).
5. Turn off the switches for laser power (3), scanner power (2), and PC microscope (1).
6. Complete your entry in the log book by recording the time at finish and the UV out time.
7. Place completed invoice form in proper location.

Software Start-up:

1. Login with username and password.
2. Double click on LASX software icon.
3. When prompted, enter "**Machine**" for *configuration*, "**DMi8**" for *microscope*, and "**OFF**" for *load settings at start up*.
4. When prompted, answer "**Yes**" to *initialize stage*

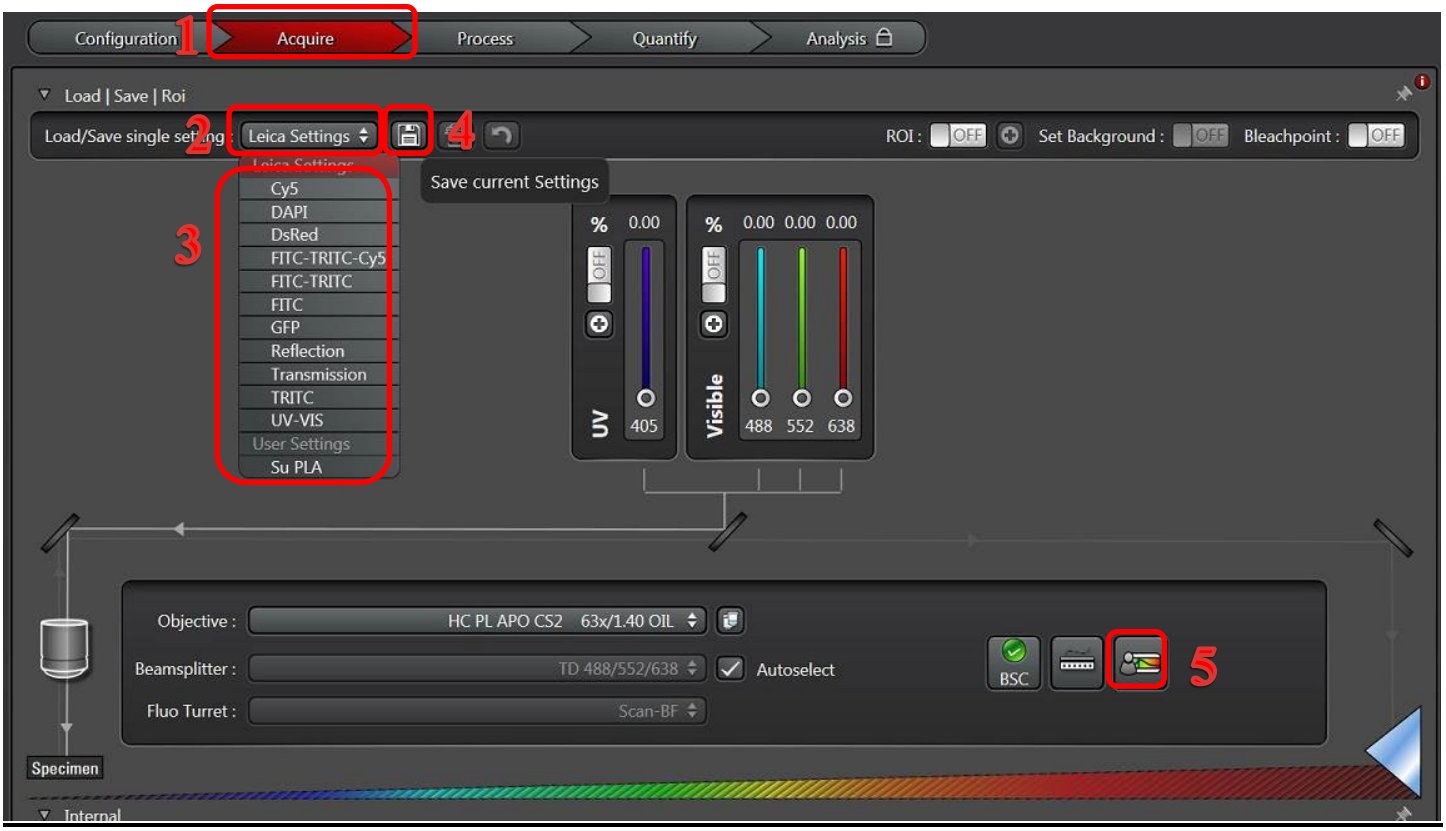


Laser Start-up:

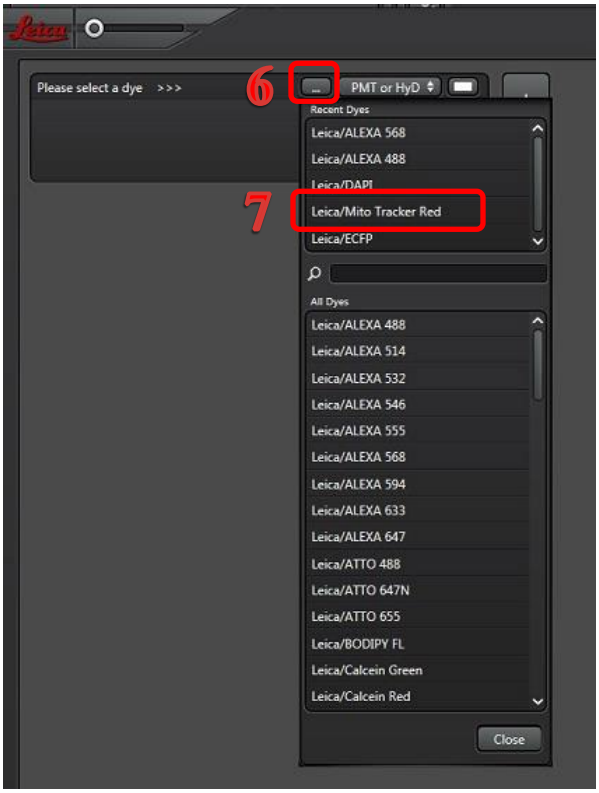
1. Click on the "Configuration" tab located in the left top panel of the software.
2. Turn on the lasers that will be utilized.
3. Use slider to set the power of the Argon laser at 25%.

Image Acquisition:

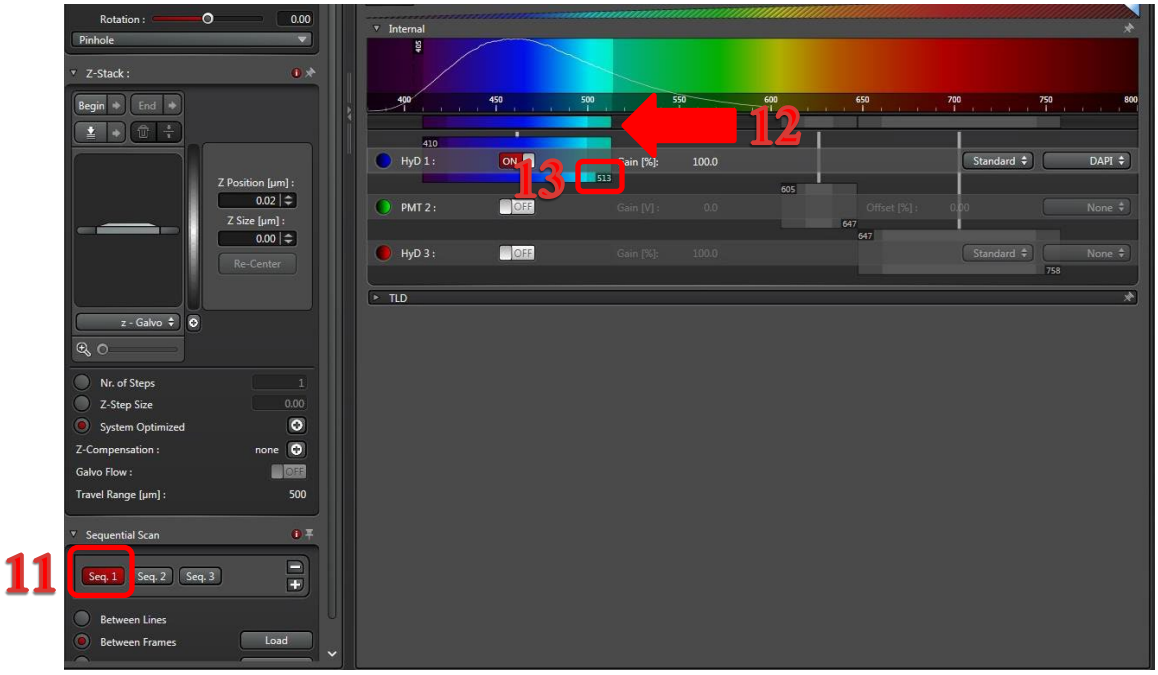
1. Click Acquire
2. One way to set your light paths is to click on Leica Settings.
3. Select the dyes that you are using.
4. You can also save your own settings. Click the save icon to create a user setting.
5. An alternative way is to click on the Dye assistant icon.



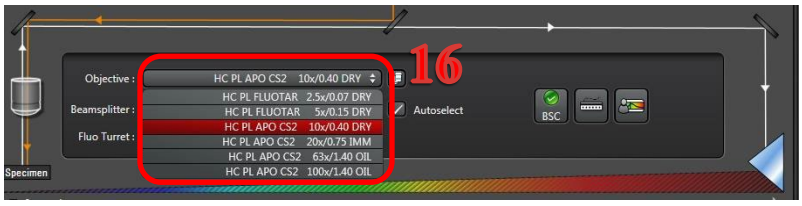
6. In the Dye assistant, click on the …box.
7. Click on the dye you are using.
8. Repeat for all the dyes you have.
9. You will now see a listing of possible light path set ups. The bottom one “Frame or track sequential” is similar to what we use on the Zeiss microscopes.
10. Click the Apply button in that window.

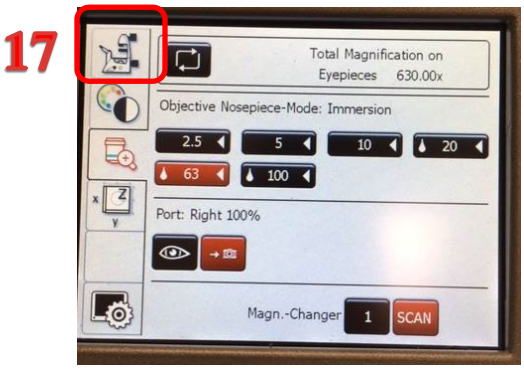


11. You can adjust your emissions filters by clicking on the Sequence you wish to adjust. Seq1 is selected in this example.
12. Click on the end of the filter and drag it to where you wish to have it.
13. The numbers on the bottom indicate the emission wavelength at that point.
14. Repeat for other Sequences.

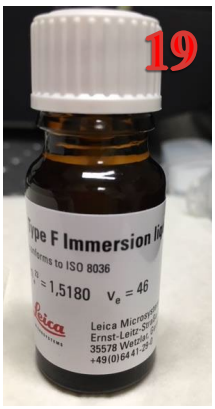


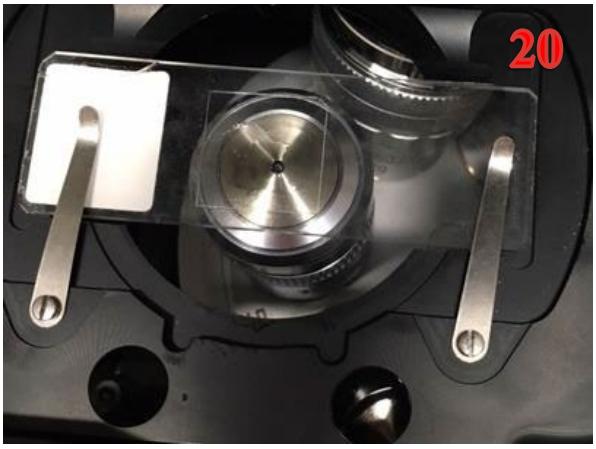
15. The objective lens can be selected from either the software or from the touch screen located on the microscope.
16. From Software: Click the Objective window and select desired objective from drop down menu.
17. From Microscope Touch Screen: Select objective icon. Then select desired objective. Selected objective is highlighted in red.

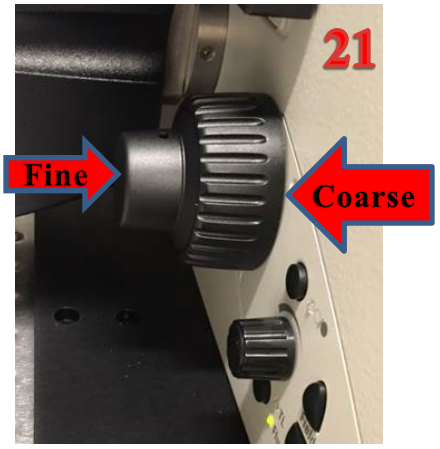




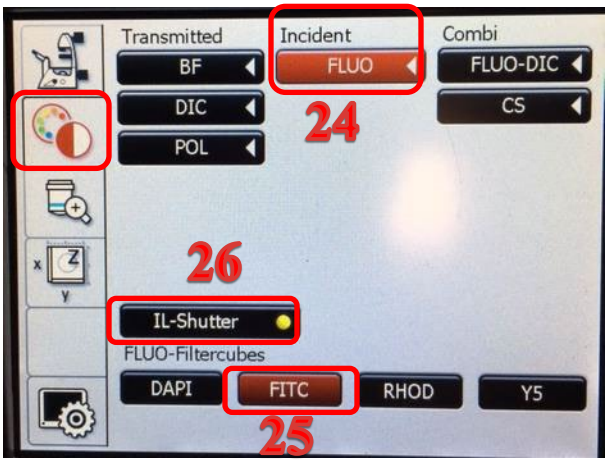
18. Clean the objective using lens paper and Sparkle.
19. Place oil on the slide or on the objective.
20. Place sample on microscope and use the clips to secure it in place.
21. Raise the objective by turning the focus knob away from you.

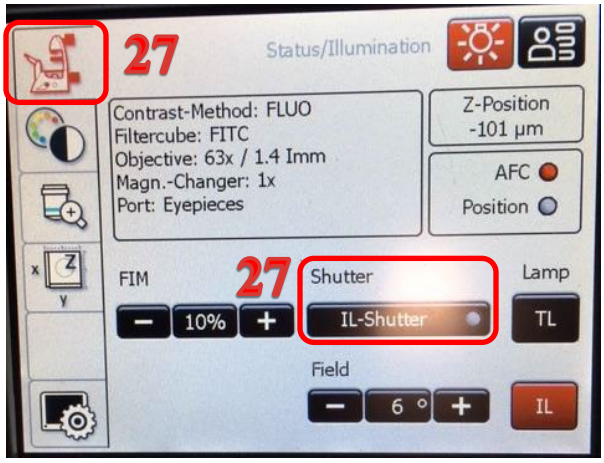




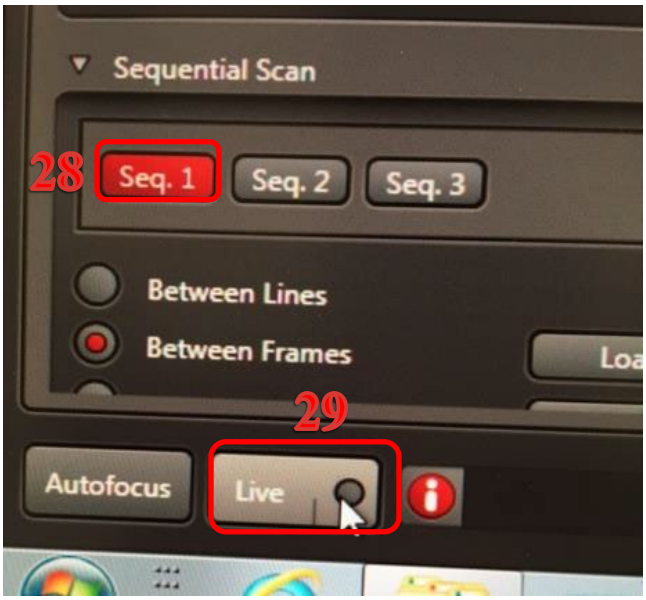


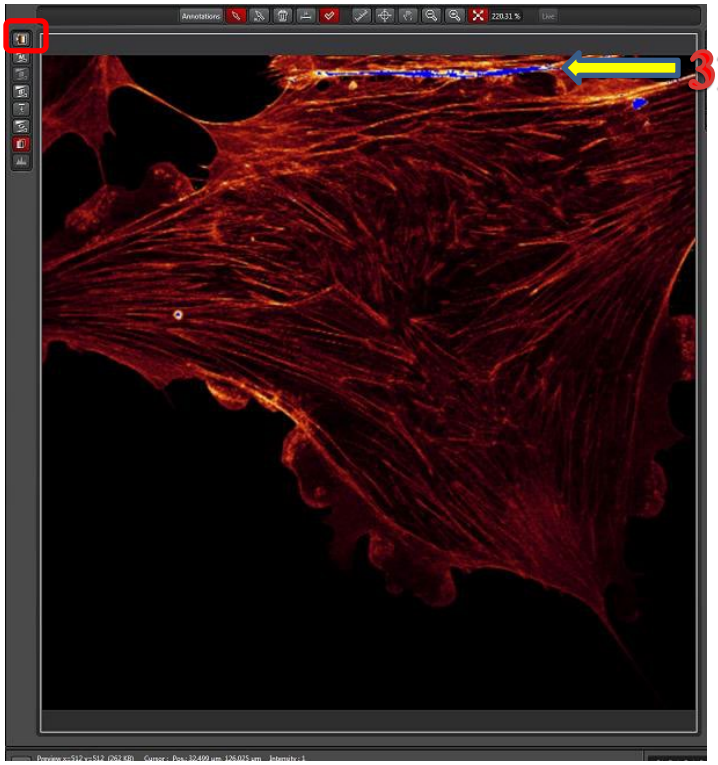
22. Use touch pad on the microscope stand to view/focus sample through the eyepiece
23. Select the color and contrast icon.
24. Under Incident select FLUO.
25. Select the FLUO-Filtercube that you want.
26. Select IL-Shutter. Yellow light indicates light is on. After you get focus, select IL-Shutter to turn off light. Gray light indicates off.
27. You can also turn the light on and off from the Microscope window.





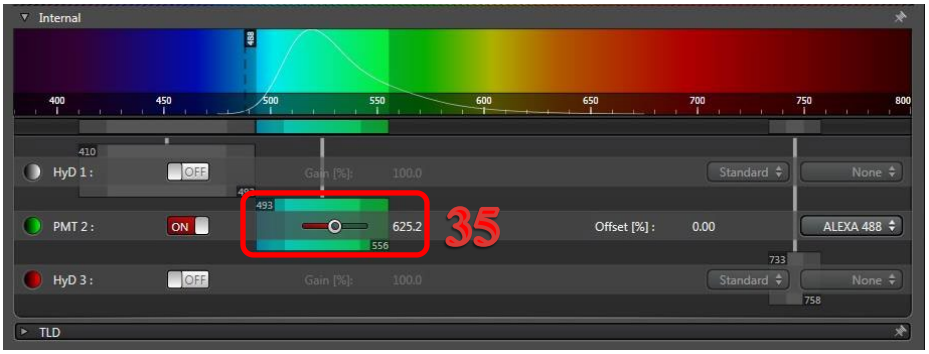
28. To optimize image acquisition parameters, select the sequence you wish to scan first. This is located under sequential scan on the left panel of the software.
29. Click Live. This is a fast XY scan.
30. On the panel surrounding the acquired image, click the LUT button.
31. Blue indicates saturation. Green indicates below detection level (not seen in example).

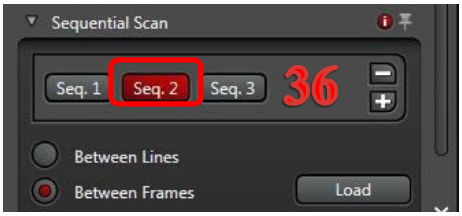




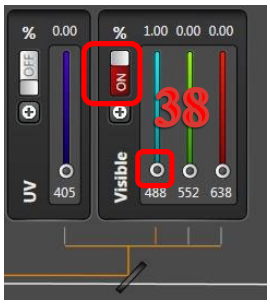
32. One way to adjust the gain is from the control box.
33. The offset can be adjusted from here too.
34. If the Smart Offset is disabled, it cannot be adjusted. This occurs when you select the HyD detector.

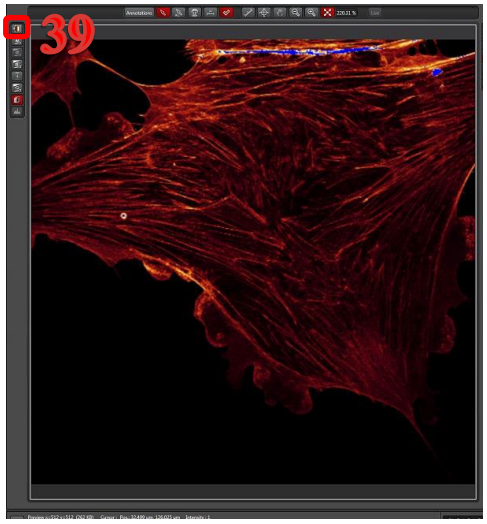
35. Another way to adjust the gain is to click on the gain in the software which brings up a slider. You can use the mouse to move the slider or the scroll bar on the mouse to adjust the gain.
36. Select the next Sequence and repeat the steps.
37. Repeat until you have gone through all your dyes.

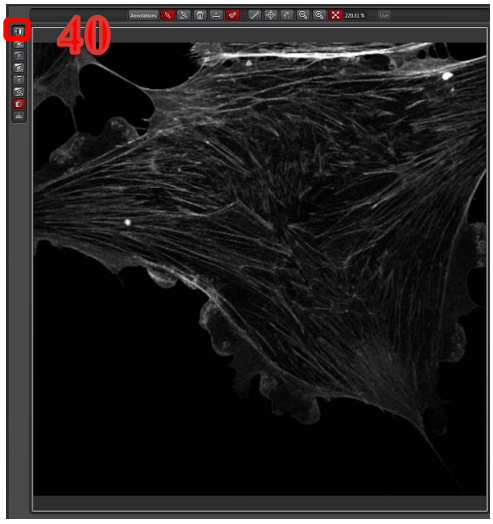




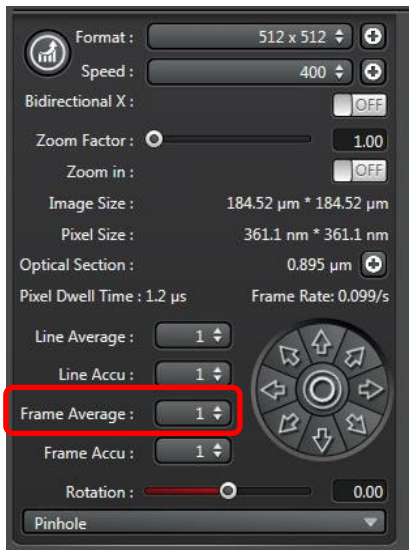
38. To adjust the laser power, click on the button of the laser you wish to adjust then either use the scroll bar on the mouse to adjust or click on the laser power % and type in number you want.
39. To get out of the LUT settings box click the box again and you get a gray scale view.
40. Click the gray scale view box to get back to a colored image view.

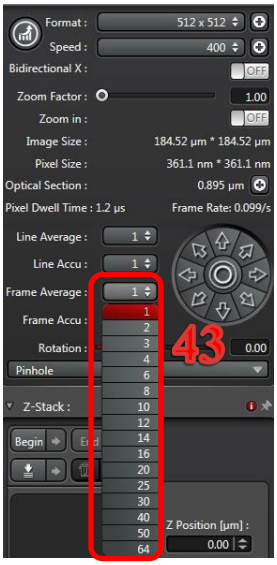


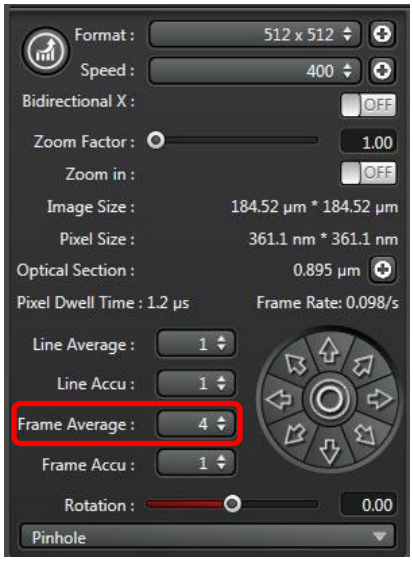




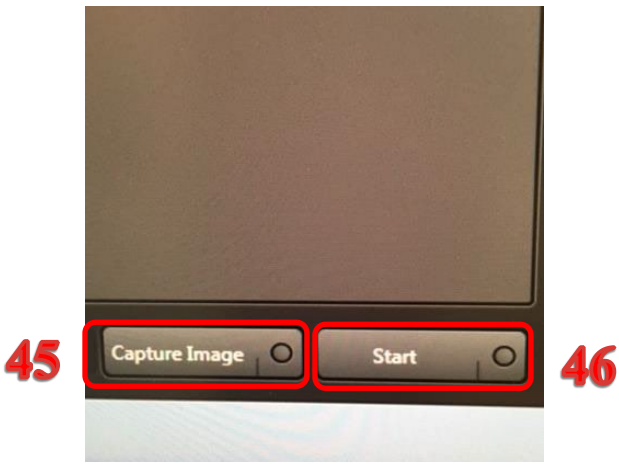
41. The Pinhole can be adjusted from both the Control Panel (A) and the software (B). A value of 1.00 airy unit (AU) typically gives the best signal/noise ratio for most fluorescent applications.
42. Go to the Frame Average window to select Frame Average.
43. Select value from drop down menu (4 is recommended).
44. See the average selected in the window. Frame average will collect sequence 1 then 2 then 3 and repeat this 4 times for the average.

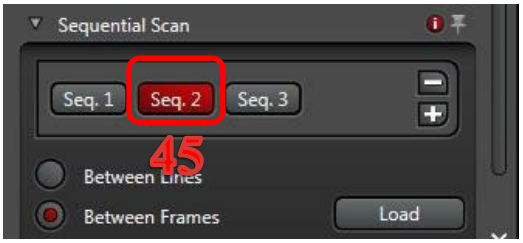




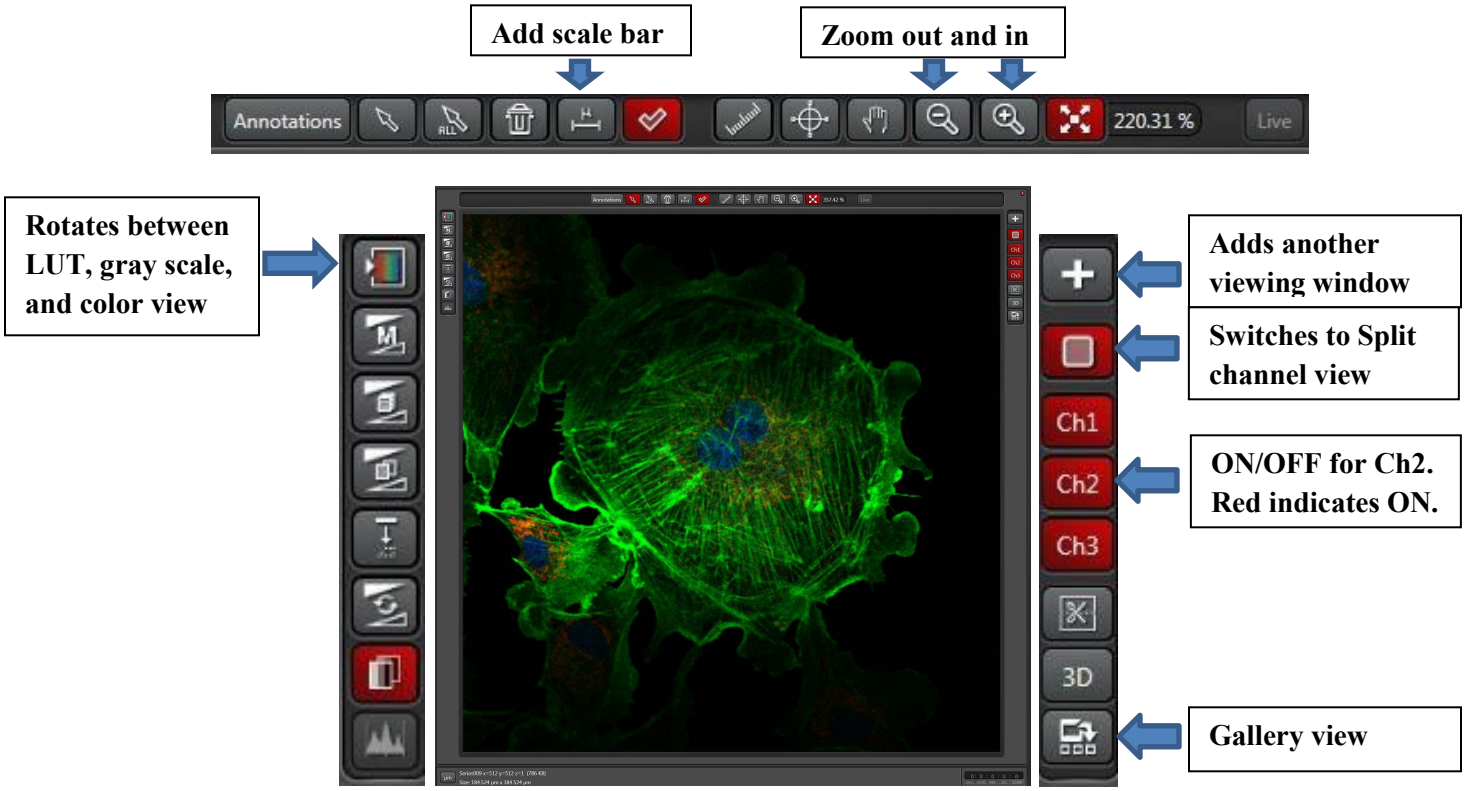


45. Click Capture to acquire a single-color image. The color will be the Sequence that is selected. In the example below, Seq 2 is selected.
46. Click Start to acquire a multi-color image.

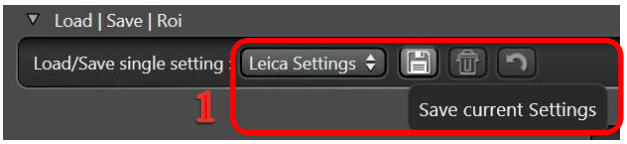




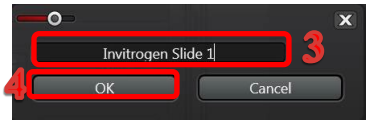
47. Acquired multicolor merged image is shown in right window of the display.

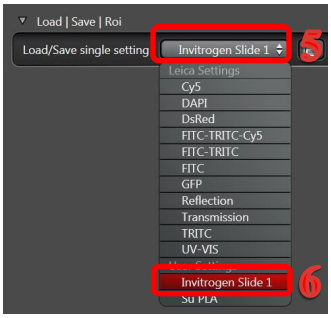


Saving Your Configuration (Imaging Settings):

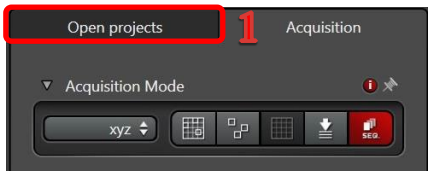


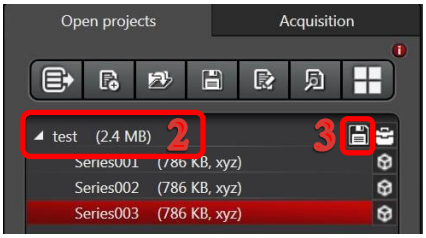
1. Next to Leica Settings window click on the floppy disc. If you hover over it you will see Save current Settings.
2. This opens up a window and you will see Default_Name_1
3. Enter a configuration name.
4. Click OK.
5. Now when you click Leica Settings you will see your configuration.
6. Highlight and click to load your configuration.

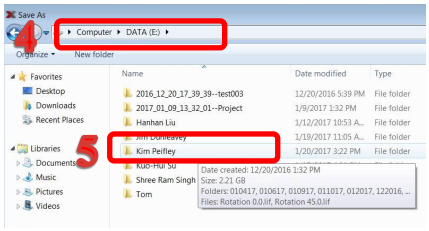




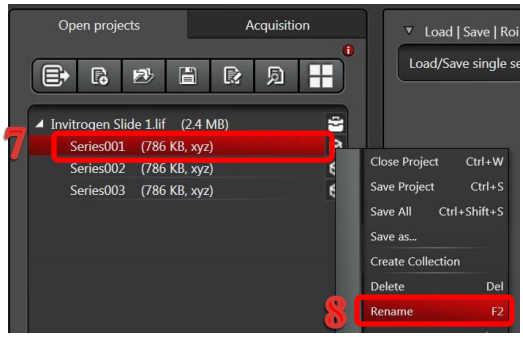
Saving Projects and Images:

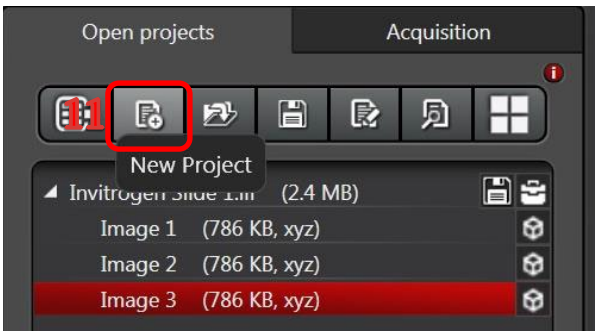




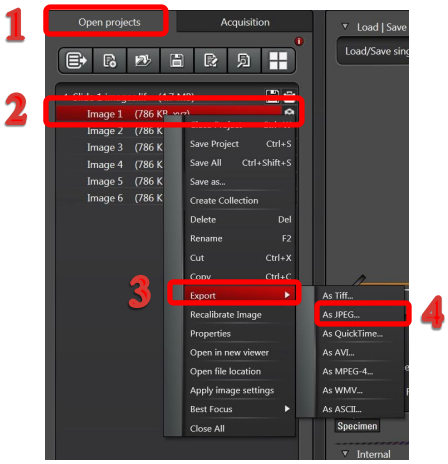


1. Click Open Projects Tab.
2. Highlight Project you wish to save.
3. Click on floppy disc icon to right of Project name.
4. Select the Data (E:) drive to save data locally.
5. Select your folder or create a folder with your name.
6. Name your project and click Save. All images acquired will be listed under this project.
7. To name your individual images, highlight each image and right click to get menu.
8. Select Rename.
9. The window will be highlighted so that you can enter the new name.
10. Hit enter to store/see the new name.
11. To create a new project, click the new project button in the menu bar.
12. Follow previous instructions to name the project.





Exporting Images:



1. Make sure you are in Open Projects tab.
2. Highlight Image you want to Export.
3. Right click for menu and select Export.
4. Select file type you wish to save image as.
5. Export to JPEG window click Browse.
6. Select the Destination file.
7. Click OK.
8. Check to make sure Destination folder shows the folder you selected.
9. Make any selections you wish to have.
10. If you want a Scale Bar make sure the “Micron Scale” box is checked.
11. Click Save.

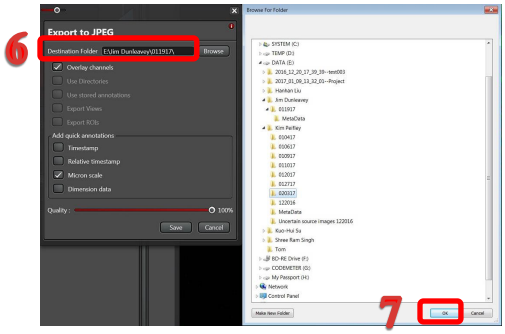
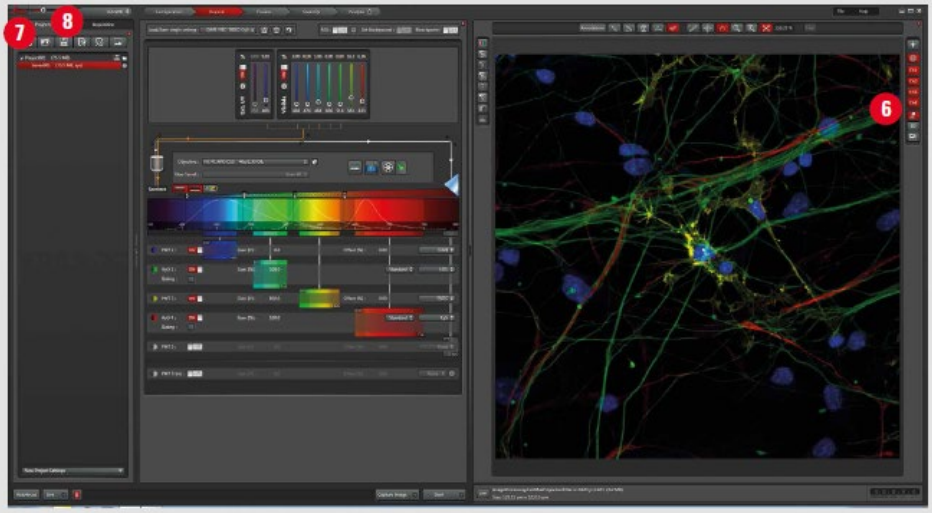


Image Acquisition of Z-stacks:

Z-Series Acquisition

1. Open **Z-Stack** Dialog.
2. Set **Focal Plane.**
3. Set **Begin-** and **End-Position.**
4. Click **System Optimized** to define the number of slices.
5. Click **Start** to acquire the z-series*.



6. Click **Max.** to generate a maximum projection.
7. To save the experiment go to tab **Project** and
8. click on **Save**.

LIGHTNING: Utilizes a combination of confocal fluorescence imaging with point spread function-based deconvolution to provide resolution down to 140 nm. In this mode, acquired images are automatically deconvolved immediately after image capture.

1. Select LIGHTNING from the tab in the upper left corner of the screen.
2. Use the sliding bar under ‘Lightning’ to increase speed or resolution. Note that increasing the resolution will also increase photobleaching due to longer scan times.
3. For optimal deconvolution, make sure to select the appropriate mounting medium (Vectashield, Permount, etc) under ‘Deconvolution Settings’.
4. Lightning Deconvolution starts automatically after image acquisition. Images captured in Lightning mode will yield an original image and a second, deconvolved image with “_Lng” at the end of the file name.