Leica SP8
Basic Image Acquisition
Laser Scanning Confocal

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General Information
Available Lasers:
• 405nm
• 488nm
• 552nm
• 638nm

Available Objectives:
• 2.5x Dry
• 5x Dry
• 10x Dry
• 20x Multi-Immersion
• 63x Oil
• 100x Oil
3 Color Image Acquisition
This is the acquisition work area.
1. On the top of the work area - Click Configuration.
2. Click the Laser Configuration button.
3. Turn on all the lasers.

The lasers available on this microscope are: 405nm, 488nm, 552nm, 638nm.
4. Click Acquire.
5. One way to set your light paths is to click on Leica Settings.
6. Select the dyes you are using.
7. You can also save your own settings. Click the save icon to create a user setting.
8. An alternative way is to click on the Dye assistant icon.
9. In dye assistant click on the … box.
10. Click on the dye you are using.
11. Repeat for all the dyes you have.
12. 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.
13. Click the Apply button in that window.
14. You can adjust your emissions filters by clicking on the Sequence you wish to adjust. Seq1 in this example.
15. Click on the end of the filter and drag it to where you wish to have it.
16. The numbers on the bottom indicate the emission wavelength at that point.
17. Repeat for other Sequences.
Alternative way to change emission filter settings:

Click on the end of the emission filter and a window will appear. You can type in the number or use the arrows to adjust the filter.
There are two ways to select the objective:
1. From the software: Click the Objective window and select from the drop down menu.
2. From the microscope touch screen: Select the Objective Icon. Then Select the objective you want. The selected objective is highlighted in red.
If you click on the objective icon next to the objective window it will open up the Objective Configuration window. This will give you the details of the selected objective.
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.
Stage movement and Focus controls:

- Selecting Coarse or Fine movement in Z
- X movement
- Y movement
- Z movement

Selecting Coarse or Fine movement in XY
If focus seems to be moving too little:
• On the touch screen select the X,Y, Z icon.
• Under Microscope select Coarse.
To View Sample Through the Eyepiece

Using the touch pad on the microscope stand:
22. Select the color and contrast icon.
23. Under Incident select FLUO.
24. Select the FLUO-Filtercube you want.
25. Select IL-Shutter. Yellow light indicates light is on.
26. 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.

If you do not see any light coming from the objective see the next page to troubleshoot.
If you do not see any light coming from the objective.

- Check the light source box. The Intensity is likely too low. Turn it up. The second white dot is usually good enough.
- On the microscope check the side of the condenser arm. It should be Brightfield not Digital Lightsheet.
On the Microscope Touch Screen:
- Select the Objective Window.
- Check the “Port:” section – it should be the Eyepiece icon in red not the camera icon.
If you have a very small viewing area:
- Select Microscope icon on the touch pad.
- Adjust the Field by pressing the + button.
- A field of 6° is good.
Control Panel
Located beneath the monitor.
When you hit either high or low limit of any control you will hear beeps.

- **Smart Gain:** Adjust the gain.
- **Smart Offset:** Adjust the offset.
- **Scan Field Rotate:** Rotating the field of view.
- **Pinhole:** Adjust pinhole
- **Zoom:** Adjust the Zoom.
- **Z Position:** Focus adjustment and to set Z stack.
Scan Field Rotate Example:
Scan Rotation of $0^\circ$ [top] and $45^\circ$ [bottom].
This can be done from the Control Panel [A] or software [B].
Back on the software:
28. Select the Sequence you wish to scan first.
29. Click Live. This is a fast XY scan.

Note: Scan and reaction to adjustments are slower than you may be used to on the Zeiss microscopes. Be patient.
30. Click the LUT button.
31. Blue is saturation. Green is below detection [not seen here].
32. One way to adjust the gain is from the control box.
33. The offset can be adjusted from here too.
34. If you see Smart Offset disabled it means you cannot adjust the offset. This is usually 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 the Control Panel [A] or from the software [B].
Selecting the Frame Average:
42. Go to the Frame Average window
43. From the drop down menu select 4.
44. See the average you selected in the window.

Frame average will collect sequence 1 then 2 then 3 and repeat this 4 times for the average.
If you wish to Select the Line Average instead:
42. Go to the Line Average window
43. From the drop down menu select 4.
44. See the average you selected in the window.

Line average will create Sequence 1 4 times for the average and then repeat for other sequences.
45. Click Start to acquire a multi-color image.
46. Click Capture Image to get a single color image. The color will be the Sequence that is selected. In this case Seq. 2.
Contrast adjust the window that is selected and highlighted in white. In this example blue.

47. Image in Split Channel View.

A. Create another viewing window so you can view multiple images.
B. Merge Channels.
C. Ch1 turn off and on. Red is on.
D. Ch2 off and on.
E. Ch3 off and on.
F. Will show split channels as well as a merged channel window.
G. Gallery view.
A. Create another viewing window so you can view multiple images.
B. Split Channels.
C. Ch1 turn off and on. Red is on.
D. Ch2 off and on.
E. Ch3 off and on.
F. Gallery view.
To Save Your Configuration:

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 the configuration.
Saving Projects and Images
Saving Image:
1. Click Open Projects Tab.
2. Highlight Project you wish to save.
3. Click on floppy disc icon to right of Project name. If you hover the mouse over it you will see Save Project.
4. Select the Data (E:) drive to save data locally.
5. Select your folder or create a folder with your name.
6. Click on folder to open.
7. Create folder for today’s work.
8. Click on that folder to open it.
8. Name your project.
9. Click Save.
10. The project is now named. In this example Invitrogen Slide 1. All images acquired will be listed under this project.
11. To name your image highlight image and right click to get menu.
12. Select Rename.
13. Window will be highlights so you can type in new name.
14. Hit enter to see name.
15. To create a new project click the new project button in the menu bar.
16. Follow previous instructions to name the project.
17. This shows all the projects and images named for one imaging session.
To export 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. In Export to JPEG window click Browse.
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.
If you want to make sure the image was saved you can check the folder you selected.
This is the .jpeg image showing scale bar that was selected in Step 10.
Zoom/Crop an Image
There are two ways to zoom or crop image.

Control Panel:

- Adjust the dial to the Zoom you want and take image. You can do this while scanning an image or without scanning.
- When you compare an image Zoom of 1 [A] to Zoom of 2.88 [B] you can see it sets the zoom from the center of the image.
- You can also Zoom out. Maximum zoom out is 0.75 [C].
Via the software:

1. Click on Zoom in to turn on.
2. You can now go to the image and draw a zoom window wherever you want. Green box.
3. To return to zoom of 1.0 click the reverse arrow.
Z Stack Imaging and Maximum Intensity Projection
1. Select Sequence.
2. Click Live.
3. Using the Z position dial on the control panel, change the focus to the point you wish to start imaging.
4. Click Begin.
5. Change focus to the point you wish to stop imaging.
6. Click End.
7. Click Stop.

Note: Focus has to be changed from the control panel. If you use the focus knob on the microscope it will not register that with the software.
8. Select the way you want Z stack acquired: Set number of steps, Set Z step size [slice size], System optimized.

9. Z Step Size was selected to set the slice size. Z-Step size 1µ so there will be 15 steps.
10. Click Start.
11. You can see the remaining time it will take to acquire the Z stack in the bottom menu bar.
12. You can also follow the Z stack progress on the bar on the right size of the image.
13. When viewing your Z stack image you can move through the slices using the bar on the right.
14. To get Maximum Intensity Projection click the box below the last channel button.
15. This is your maximum intensity projection image.
16. To rotate image click the 3D button. This will open a whole new window.
17. Set your parameters.
18. Click Save Image
19. For orthogonal view click the Scissors Icon to the right of the image.
20. To turn off the Z stack click the Trash can icon.
Tiling
1. Set up your image like normal.
2. In Acquisition Mode window click on the icon for Define Tilescan Experiment.
3. If the Stage window does not open automatically, click the arrow next to Stage.
4. Set Field size.
5. Slick Start.
6. The remaining time to acquire the tile is listed next to the start button.
7. You can also follow the progress of the tile from the slider at the bottom of the image window. In this image it is now collecting tile 3 of 9.
8. Clicking on this icon allows you to see one position of the tile in split mode.
9. Use the slide bars to move around the full tile image.
This is the full tile image in merge.
You can turn off Tilescan 2 ways:
1. In Acquisition Mode window click on the icon for Define Tilescan Experiment.
2. In the Stage Window click the Trash Icon aka Clear All. Field Size Window Should now be blank.