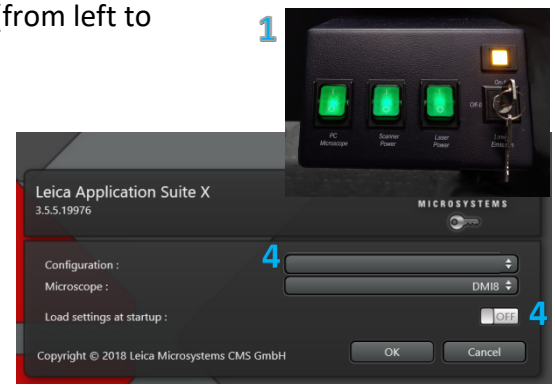
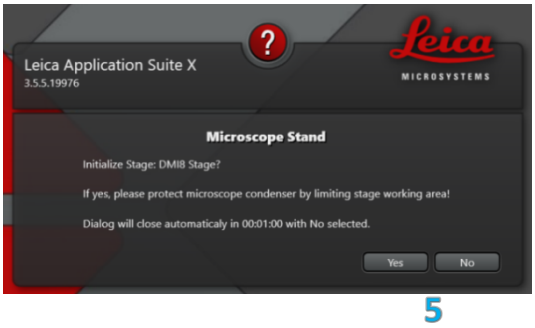
Quick start manual Leica SP8

STARTING THE SYSTEM:

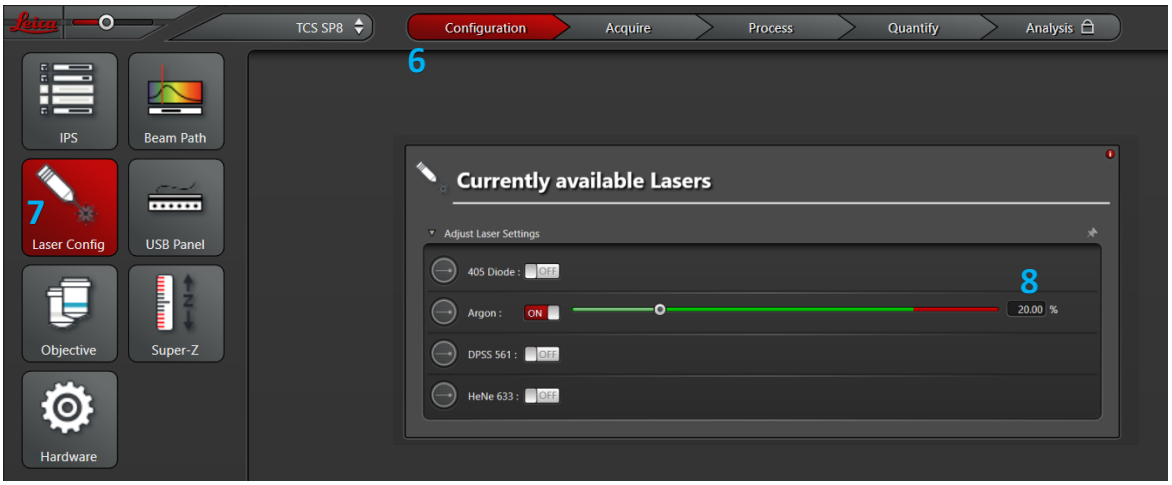
1. Turn on the system by switching the green buttons (from left to right) and turning the laser key.
2. Turn on the mercury lamp if you want to look at fluorescence through the eyepieces.
3. Start the program **LAS X** with the shortcut on the desktop.
4. Select a suited configuration. Make sure to load OIC settings at startup or switch this option off, continue with OK.
5. When asked to initialize the XY stage, make sure the microscope condenser arm is pushed backwards and the objective is at the lowest position.





ACTIVATE YOUR LASERS:

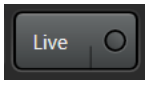
6. Click on the **Configuration** tab.
7. Click on **Laser Config**.
8. Activate the laser(s) you need by checking the box(es). If you are using the Argon laser, do NOT forget to put the digital power slider at 20% (50% for FRAP experiments).



Remember: the Argon laser has to cool down before you turn off the system!!!

BEAM PATH SETTINGS:

9. Click on the **Acquire** tab (located next to the **Configuration** tab, see 6).
10. In the top select the laser lines that you want to use.
11. Click on the shutter per laser individually to activate the laser.
12. Select the laser and their intensity by moving the sliders up or down (AOTF%, between 0-10% to begin with).
13. Activate the PMTs / HyDs (12a) with the **ON/OFF** button and click on the colored button on the left to choose a color for images (12b). ***Use HyD detector in standard mode*.**
14. Click on **None** to open a drop-down menu with emission spectra. Choose a fluorophore emission spectrum to help you setting the optimal spectral detection window.
15. Adjust the slider to optimally collect the fluorescence. Double clicking on the slider will open a window where you can enter the begin and end wavelength. **Avoid including laser wavelength.**
16. Double click on the gain value for each active detector and set the gain initially at 850V or 100% for HyD detector.
17. Choose your objective lens and place sample on the confocal.
18. Click on the **Live** button to check a live image of your sample.



Excitation / Emission	
Blue	405 / 415-480
Green	488 / 500-550
Red	561 / 570-625
FarRed	633-/645-750

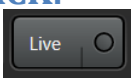
SETUP FOR ACQUISITION OF IMAGES:

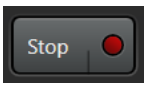
19. Click on the **Acquisition** tab if it is not already active.
20. The acquisition will be automatically on **xyz** scanning mode.
21. Imaging parameters (XY Window) can be changed by opening the drop-down window.
22. The format of your image is automatically set to 512x512 pixels and the speed is automatically chosen at 400 Hz.
23. Image and pixels dimensions are automatically updated.
24. Activate **Bidirectional X**

SEQUENTIAL ACQUISITION:

25. Position all bandpass sliders before enabling sequential scan
26. Set number of scans with "+" button
27. Select option: 'Between frames' when imaging fixed sample. Setup beam path of lasers and detectors in every scan individually.

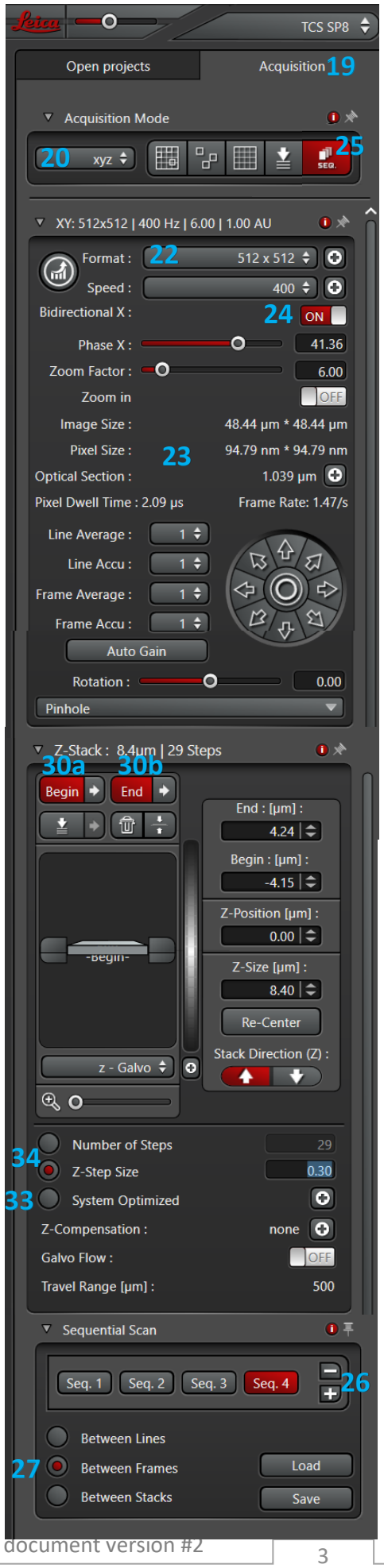
ACQUISITION OF A Z-STACK:

28. Start imaging by clicking on 

at the bottom of your screen.
29. Use the Z Position knob on the smart panel to adjust the focus plane.
30. Move to the **bottom** of your sample and set the positions of your Z-Stack by clicking **Begin** (a)**.** Go to the top of your sample and repeat for **End** (b).
31. Click on **Stop**. 

32. Use **system optimized** if you desire to obtain the optimal number of images calculated for your Z-Stack size (depending on your objective, zoom and image format).
33. If you want to adjust the number of z-steps or the z-step size then click on

Nr. of steps or **z-step size**, respectively.



SHUTTING DOWN THE SYSTEM

34. Save your data to the network transport drive (O: drive).
35. Remove your sample, if used, **clean the oil of the objectives** with 70% ethanol and lower the objective.
36. Check the microscope calendar (www.erasmusmc.nl/oic) if another user will continue after you.

WHEN THERE WILL BE ANOTHER USER:

37. **Leave the Argon laser on, if it will be used again later in the day.**
38. Leave on the other lasers, Hg lamp and heating / CO_2 only if they will be used within 2 hours.
39. Exit the LAS X software and log off from Windows.

IF YOU ARE THE LAST USER OF THE DAY:

40. **Switch off all activated lasers via Configuration tab > Laser Config.**
41. **Wait ~5 minutes for the Argon laser to cool down**
    (The fan will stop when cooling down has finished).
42. **Turn off mercury lamp.**
43. Exit the LAS X program.
44. Shut down the computer.
45. Turn off the system by turning the laser key and switching the green buttons off (from right to left).

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CONTACT INFORMATION

For direct support at the microscope **call 35813.**

For other question contact us via:

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