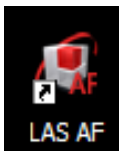
Quick start manual Leica SP5

STARTING THE SYSTEM:

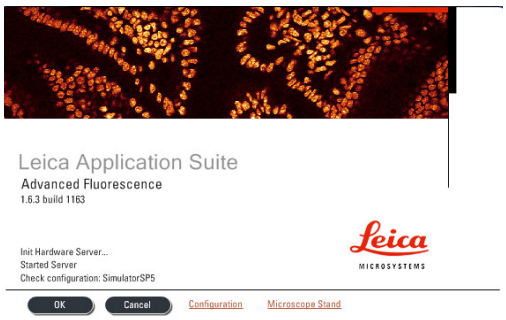
1

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. Logon to Windows as TCS User (no password).
   Double Click on LAS AF icon on the desktop.
4. Click on OK in the LAS AF window.

3

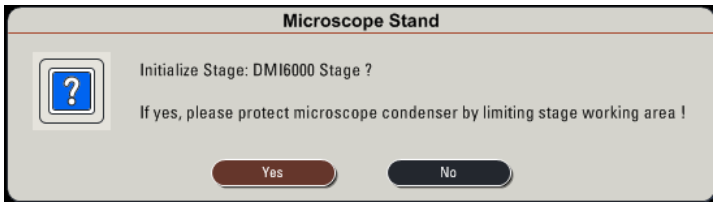


4



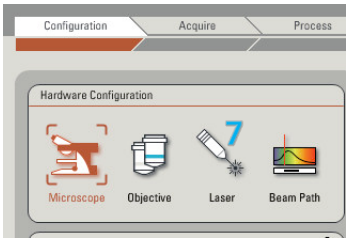
5. When asked to initializing the table, make sure the microscope condenser arm is pushed back and the objective is at the lowest position.

5



ACTIVATE YOUR LASERS:

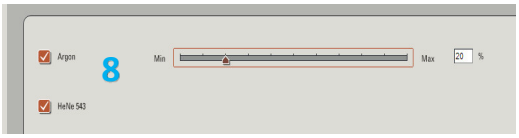
6



6. Click on the **Configuration tab**.
7. Click on **Laser**.
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).

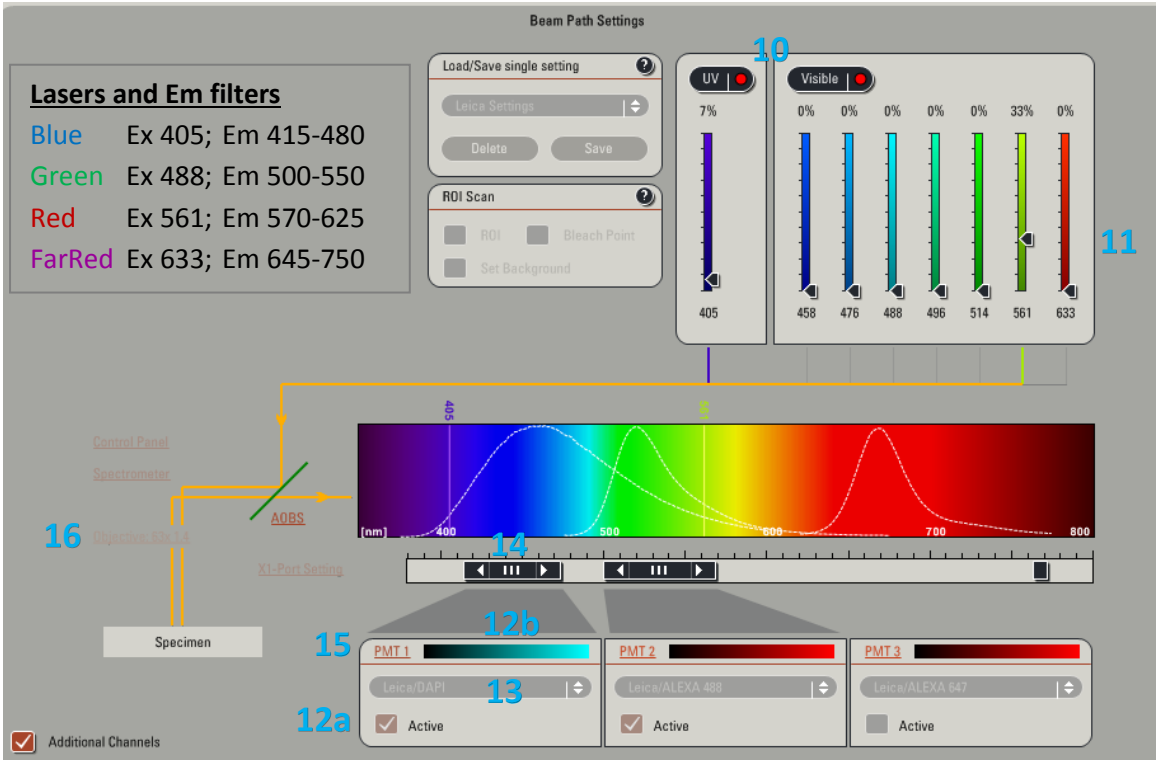
8



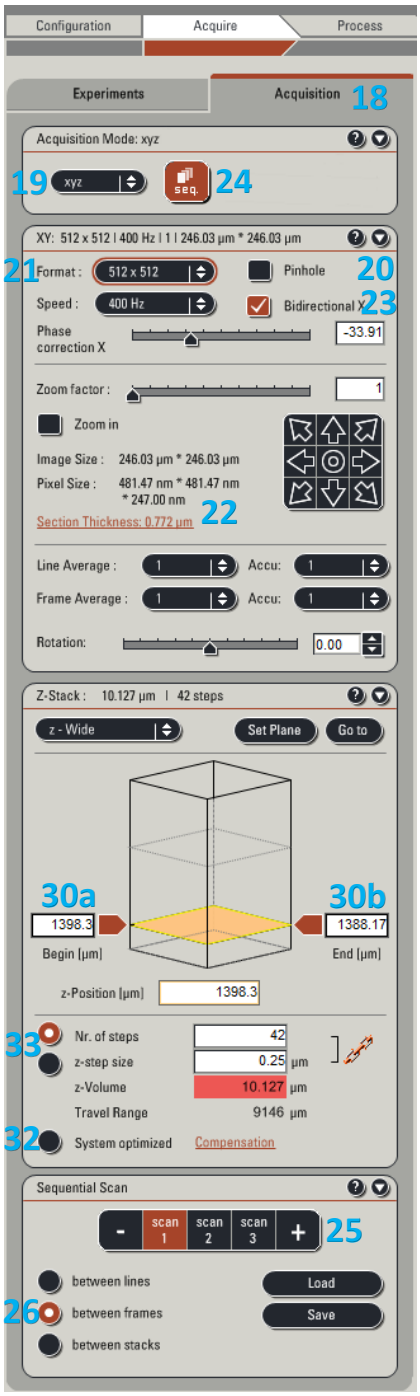
Remember: the Argon laser has to cool down before you turn off the system!!!

BEAM PATH SETTINGS:

9. Click on the **Acquire** tab (located next to the **Configuration** tab, see 6).
10. Click on **UV** and **Visible** to activate the laser(s).
11. Select the laser and their intensity by moving the sliders up or down (AOTF%, between 10-20% to begin with).
12. Activate the PMTs (12a) by checking the **Active** button and click on the colored bar to choose a color for your images (12b). ***Use the HyD detector in standard mode*.**
13. Click on **None** to open the drop-down menu with fluorophore emission spectra. Choose a fluorophore emission spectrum to help you setting the optimal spectral detection window.
14. 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 wavelengths**
15. Click on **PMT** for each active detector and set the gain initially at 850V. (100% for HyD detector).
16. Choose your objective lens and place sample on the confocal
17. Click on the **Live** button to check a live image of your sample. 



SETUP FOR ACQUISITION OF IMAGES:

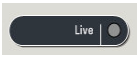


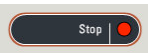
18. Click on the **Acquisition** tab if it is not already active.
19. The acquisition will be automatically on xyz scanning mode.
20. Imaging parameters (XY Window) can be changed by opening the drop-down window.
21. The format of your image is automatically set to 512x512 pixels and the speed is automatically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

SEQUENTIAL ACQUISITION:

24. Position all bandpass sliders before enabling sequential scan
25. Set number of scans with "+" button
26. Select option: 'Between frames' when imaging fixed sample.
27. 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 the **arrowhead** (a)**,** repeat for **top** (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 AF software and log off from Windows.

IF YOU ARE THE LAST USER OF THE DAY:

40. Turn off all lasers in the LAS AF software (Configuration panel).

41. Wait ~5 minutes for the Argon laser to cool down
(fan will stop when cooling down has finished).

42. Switch off mercury lamp.

43. Exit the LAS AF software.
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).

45

CONTACT INFORMATION

For direct support at the microscope **call 31105.**

For other question contact us via:

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