

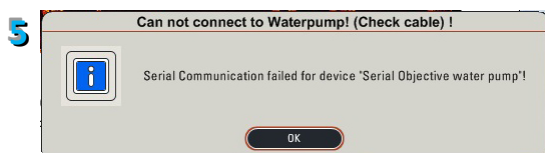
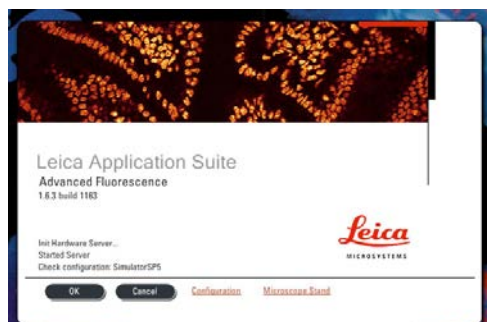
Quick start manual for Leica SP5_604

STARTING the system:

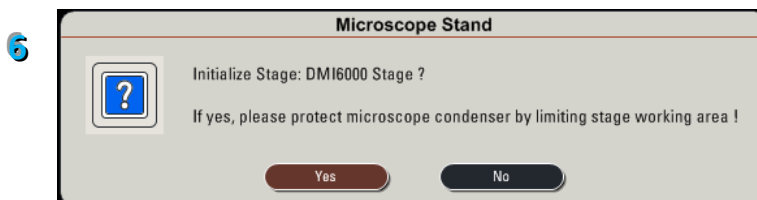
1. Turn on the system by the pushing green buttons (left to right) and turning the laser key. Turn on the mercury lamp if you want to look at fluorescence through the eyepieces.



2. Logon to Windows as LASAF User (no password).
3. Double Click on LAS AF icon on the desktop.
4. Click on OK in the LAS AF window.
5. If a waterpump error occurs, click on OK.

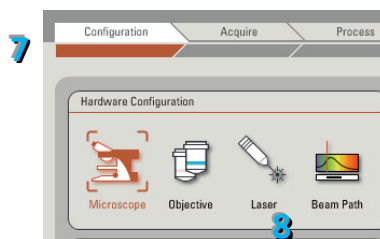


6. When asked to initializing the table, make sure the microscope condenser arm is pushed back and the objective is at the lowest position.

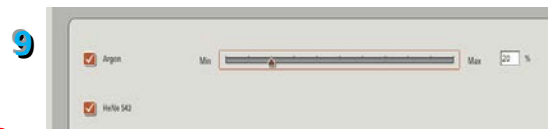


ACTIVATE YOUR LASERS:

7. Click on the **Configuration** tab.
8. Click on **Laser**.
9. Activate the laser(s) you need by checking the box(es).



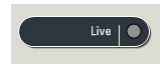
If you are using the Argon laser, do NOT forget to put the digital power slider at 20% (50% for FRAP experiments).



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

BEAM PATH SETTINGS:

10. Click on the **Acquire** tab (located next to the **Configuration** tab, see 7.).
11. Click on **UV** and **Visible** to activate the laser(s).
12. Select the laser and their intensity by moving the sliders up or down (AOTF%, between 10-20% to begin with).
13. Activate the PMTs (13a) by clicking on the **Active** button and click on the colored bar to choose a color for your images (13b). **Use the HyD detector in standard mode.**
14. 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.
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.
16. Click on **PMT** for each active detector and set the gain initially at 850V. (100% for HyD detector).
17. Click on the **Live** button to check a live image of your sample.



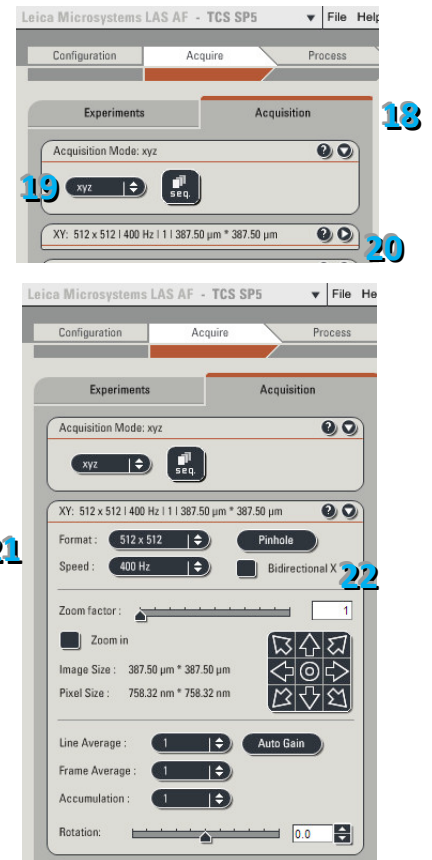
The image shows a screenshot of the "Beam Path Settings" software interface. The interface is divided into several sections:

- Top Left:** "Load/Save single setting" section with a dropdown menu for "Leica Settings", "Delete", and "Save" buttons.
- Top Right:** "ROI Scan" section with checkboxes for "ROI", "Bleach Point", and "Set Background".
- Center:** A large spectral plot showing intensity versus wavelength (nm) from 400 to 800 nm. The plot is color-coded (violet to red) and has a white curve overlaid. A vertical line is drawn at 405 nm. A blue "15" is overlaid on the plot.
- Bottom:** Three PMT (Photomultiplier Tube) control panels labeled "PMT 1", "PMT 2", and "PMT 3". Each panel has a color bar (13b), a dropdown menu for the filter (14), and an "Active" checkbox (13a). PMT 1 is set to "Leica/DAPI", PMT 2 to "Leica/ALEXA 488", and PMT 3 to "Leica/ALEXA 647".
- Left Side:** A schematic diagram (16) showing the optical path from the "Specimen" through "Objective, Eyepiece", "Spectrometer", and "Control Panel" to the "AOTF" (Acousto-Optic Tunable Filter).
- Bottom Left:** A checkbox labeled "Additional Channels" which is checked.

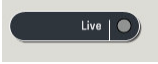
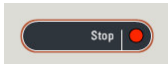
Blue callout numbers 11, 12, 13a, 13b, 14, and 15 are overlaid on the interface to highlight specific features.

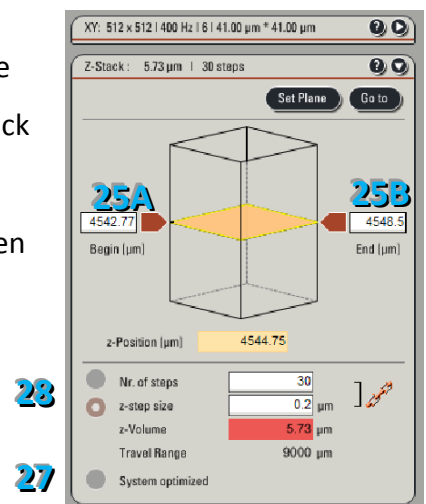
SETUP for ACQUISITION OF IMAGES:

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 displayed in 512x512 pixels. The speed is automatically chosen at 400 Hz and the image size as well as the pixel size is automatically calculated and displayed.
22. Activate **Bidirectional X**



ACQUISITION OF A Z-STACK:

23. Start imaging by clicking on  at the bottom of your screen.
24. Use the Z Position knob on the smart panel to adjust the focus plane.
25. A. Move to the bottom of your sample and set the Begin position of your Z-Stack by clicking on the **Begin arrowhead**.
25. B. Move to the top of your sample and set the End position of your Z-Stack by clicking on the **End arrowhead**.
26. Click on **Stop**. 
27. To set the number of z-steps, use **system optimized** if you desire to obtain the optimal number of image calculated for your Z-Stack size (depending on your objective, zoom and image format).
28. 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

29. Save your data to the network transport drive (O: drive).
30. Remove your sample, clean the oil of the objectives and lower the objective.
31. Check the microscope calendar (www.erasmusmc.nl/oic) if another user will continue after you.

When there will be another user:

- 32. Leave the Argon laser on, if it will be used again later in the day.**
33. Leave on the other lasers, Hg lamp and heating / CO₂ only if they will be used within 2 hours.
34. Exit the LASAF software and log off Windows.

If you are the last user of the day:

- 35. Turn off all lasers in the Las AF software (Configuration panel).**
- 36. Wait 5 minutes for the argon laser to cool down (fan will stop when cooling down has finished).**
- 37. Turn off mercury lamp**
38. Exit the LASAF software.
39. Shut down the computer.
40. Turn off the system by turning the laser key and pushing the green buttons (right to left).

