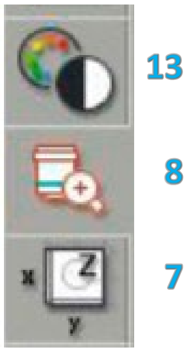
Navigator Manual Leica Stellaris5 LIA
Be311

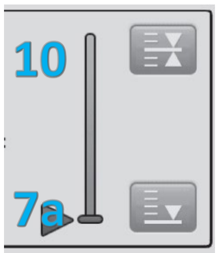
START THE SYSTEM:

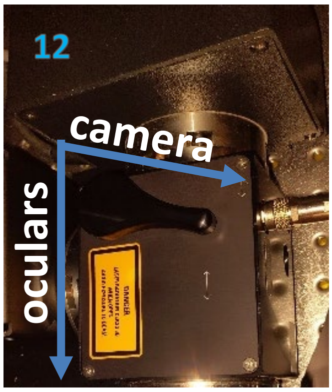
1. Start the system (buttons located on the left side of the box) in the order from **top to bottom** and turn the key. .
2. Start the **computer** (located on shelf above the microscope).
3. Switch on the **Metal-Halide lamp** (located on the desk) for the use of fluorescence by oculars or camera.
4. Start the program **LAS X** with the shortcut on the desktop.
5. Keep **load settings at startup on**, continue with OK.
6. When the LASX has fully started, click on **Laser Overview**.
   Activate the correct lasers, needed to excite your fluorophores.

FOCUS SAMPLE



7. At the touchscreen display of the microscope stand select tab: XYZ (7).
   a. Move the table to the lowest position, by holding the button (7a)
8. In the Display tab (): Choose your **objective**.
9. Place you sample underneath the microscope and if needed add some oil.
10. Bring the table back to the focus plane, by holding button 10.
11. In the Display tab (8):
    a. Make sure the light goes to the: **eyepiece**, eyepiece/camera or camera.
12. Make sure the **manual switch** at the back of the microscope towards the microscope
13. Go to the Imaging tap:
    a. To focus with for **transmission light**, choose BF and press TL-shutter
       To enable/disable the light path.
    b. Choose FLUO for using the **fluorescence light source**.
       - Filter A = blue, I3 = green, N21 = red
       - Enable/disable with button IL-shutter.
14. When you need the camera, place the manual switch (12) pointing right.





SETUP UP CHANNELS

LAS-X starts in the Acquire tab.

Follow the **Manual** or **Guided** setup of the acquisition

Guided setup with dye assistant:

15. Open the dye assistant and select the used dyes and choose the desired method.
    a. In general use: line/frame scan sequentially.
    b. Continue with step 20.

Manual setup:

16. Drag all desired fluorophores in the first region (*Setting 1*) below.
    a. Make use of the search box if a (similar) dye is not already listed.
17. Use button "+" for more channel settings.
18. Drag the spectra from the first channel to fill the new channel(s) with a single spectrum.
19. Choose **acquisition order**: stack, frame or line.

BEAM PATH SETTINGS:

Settings to start with are in general:

50% smart gain (detector) and 2% smart intensity (laser)

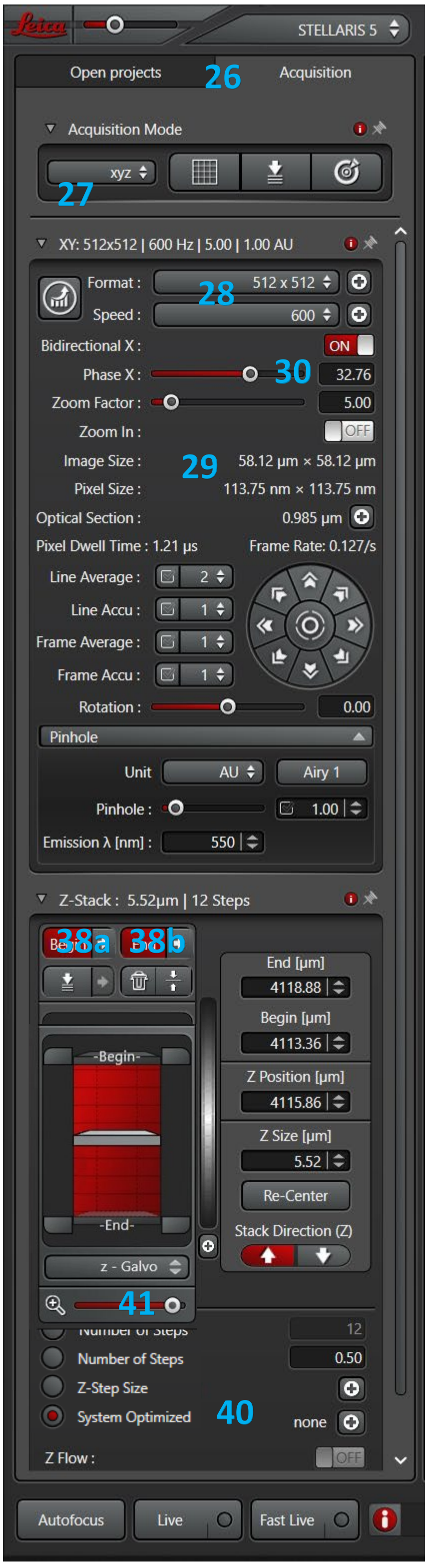
	Excitation	Emission
Blue	405 nm	415-480 nm
Green	488 nm	500-550 nm
Red	561 nm	570-630 nm
Far Red	638 nm	645-700 nm

20. Click the **laser line** to open the settings of the laser
    - Intensity of the activated laser can be changed with the **slider intensity.**
21. Double click the **colored slider** (band pass filter) to open the setting of the detector
    a. To enter the optimal detection range according to the table above.
22. If needed the color (LUT) can be changed with left mouse click. Button is only available when configuring the detector. Current color is always visible in square on the right side of slider (23).
23. Show live or fast live with buttons at the bottom of the LASX window

24. Both gain and laser can be adjusted with the smart panel, also while scanning.
    a. Click inside the desired image or setting to change setting in the correct channel.

Re-use settings

25. When settings from previously acquired images must be used:
    a. Open tab Open Projects
    b. Open a previously saved project, or browse (different button) to the project and open the image.
    c. Select the image in the list to open.
    d. Use button **Apply** or use right mouse click menu and use **Apply Settings.**
    e. Settings that won't be set an must be **changed manually** are: Image format, Scan speed, Bi-directional scanning, Zoom and Objective.



SETUP FOR ACQUISITION OF IMAGES:

26. Click on the **Acquisition** tab if it is not already active.
27. The acquisition starts automatically in XYZ scanning mode.
    a. Imaging parameters (XY module) can be changed by opening the drop-down window.
28. The format of your image is initially set to **512x512 pixels** and the speed is set at **600 Hz**.
29. Image and pixels dimensions will updated automatically.
30. **Bidirectional X** is activated by default**.**

OPTIMIZING SETTINGS:

31. Set position of all sliders (21) before optimizing settings.
32. Setup acquisition settings of lasers and detectors in every setting individually.

    <u>In short:</u>

33

33. For best effect show acquisition with LUT ”glow over/under” with button:
34. Increase laser intensity (and simultaneously decrease detector gain) for highest signal without bleaching of fluorescent signal.
35. Decrease detector gain to minimize saturation levels in the active channel.

    Controls for detector gain and laser intensity are located on the Smart panel or as sliders in the software (figure page3, 34 & 35) or can be adjusted with the Smart Pannel.

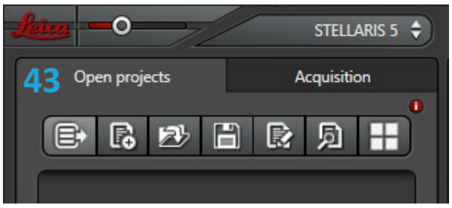
SETUP OF A Z-STACK:

36. Start imaging with button **Live** (23)
37. Use Z Position knob on the Smart panel to adjust focus plane.
38. Move to the **bottom** of your sample and set the position of Z-Stack with button **Begin** (a)**,** repeat for **End** (b).
39. Click button **Stop** (23) when live image is no longer needed.
40. Use **System Optimized** to obtain the optimal Z-Stack (depends on chosen objective, zoom and image format).

    If you want to adjust the number of z-steps or the z-step size then activate **Nr. of steps** or **z-step size** respectively and change the value.
41. Optionally change zoom to show the complete z-stack for your convenience.

EXPERIMENT

42. Acquire images with button **Start** or single channel recording with **Capture Image** button.
43. Acquired images are visible in tab Open Projects, but not yet saved.



44. With buttons on top, projects can be saved, opened, create new & more.
45. When the slide needs to be replaced (in combination with an oil objective):
    a. Lower the stage by pressing button (7a).
    b. **Recover focus** by pressing button (10).

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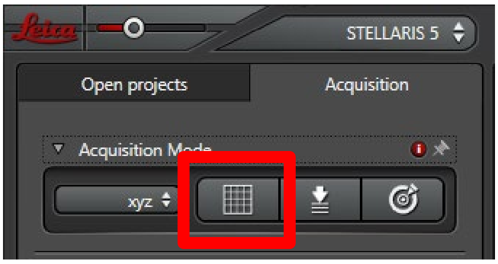


NAVIGATOR MODULE

The navigator module can be used for **multi position** acquisition or **large overview** acquisitions of slides. A new window opens when entering the navigator module.

Getting Started

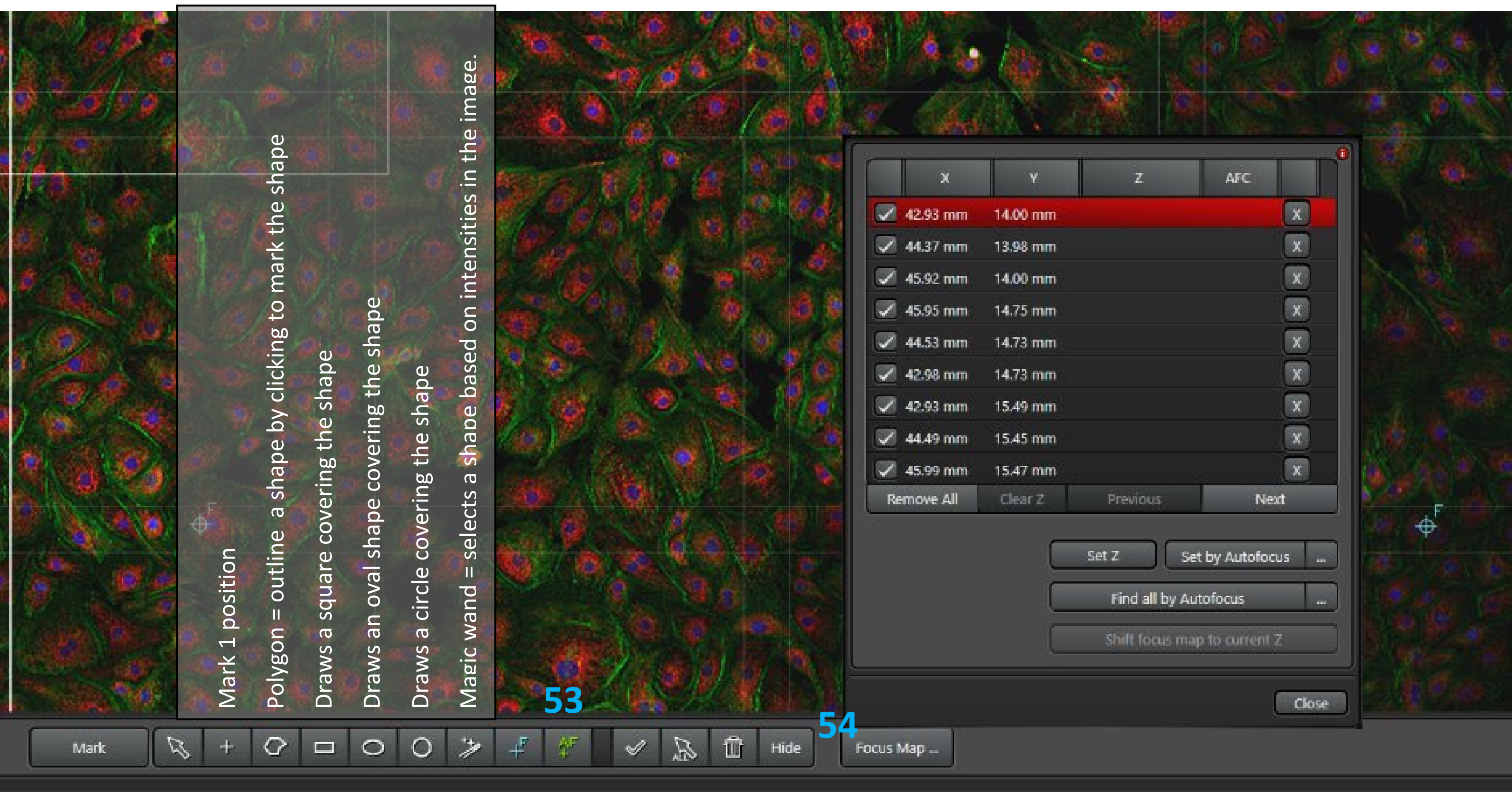
46. The Settings made in the first part of the manual are used to create the pictures, but can still be changed in the different menu's on the left.
    a. Projects = Images created are listed in the Projects folder.
    b. Beam Path = Same settings as set starting from point 24.
    c. Scanmode = Same settings as set in XYZ menu (31)
    d. Z-stack = Z-stack menu. When the Z-stack is set in the first part the settings will also be here in the Navigator.
    e. Stage = Settings for the Tile-Scans
    f. Carrier= when using a predefined sample carrier you can select the carrier in this menu.

47. To visualize you sample create a Spiral overview in a lower magnification e.g. 10x – 20x.

    a. You can make imaging faster by reducing the format and/or increasing the speed (both in the Scanmode menu)and /or scanning only 1 setting e.g. Dapi. (In the Beampath menu deselect the channels that you don't want to be scanned)
    b. Stop when you have sufficient field of view.

48. When Double Clicked in the screen you can move the stage to a specific point. The system will automatically create a picture in this position.
49. Previews will be stored in the preview list on the right. These images are only previews and will not be stored in the Project. When you want to store a preview use the button "Save Overview …". Images on the right will show you which scans and previews are made. Sometimes it can be confusing to see everything, but by unchecking the boxes you can show/not show them.

    By clicking the , the scans/previews will be removed.



50. Change to a higher magnification if more resolution is needed. The overview made with the lower magnification, will still be visible as a preview and helps to find interesting positions that you want to image. You will see the square that represents the microscope position change in size according to the chosen objective magnification.
51. To mark a position that you want to image you can use the drawing tools at the bottom of the screen.
52. You can Mark multiple positions that will be stored in the task list on the right.
    a. If you want to remove area's from the Task list select the area with the arrow in the bottom and press the bin. If you want to remove them all the Tasks press the All arrow and press the bin.
    b. By clicking  the , the tasks will also be removed.
53. Add focus points to each position to correct for the incline/decline in the sample.
54. Verify the focus points with the **Focus map.**
    a. Select the first position (line will turns red, stage will move to the stored XY coordinates)
    b. Click **Live/Fast Live** and find the focus with the Z-position knob on the Smart Pannel
    c. When in focus, press "**Set Z**"
    d. Go to the next focus point by pressing "**Next**" and go through all the focus points.
    e. When finished, press "**Close**" and stop Live scanning.

55. Make sure in the Stage menu the Merge Images during Acquisition is ON (46e)
56. If you want to keep the raw images separately stored, turn “Keep Raw Data” ON (46e)
57. Press Start to start the imaging. All images that are check in the task list will be imaged. Images will be stored in the Project folder (but project is not jet saved)
58. When you want to go back to the normal view, close the navigator with “Close Navigator”

Use the **help function** of LASX, or via the shortcut on the desktop. “getting started” information is available on this page. More detailed **step by step** information about how to setup experiments is available via the link **instructions** on the left side on the page.

SHUTTING DOWN THE SYSTEM

59. **Save your data** to the network transport drive (O: drive).
60. Bring stage to **lowest position** by pressing button (7a).
61. Remove your sample and if used, **clean the oil of the objective(s)** with 70% ethanol on a normal paper tissue.
62. Bring the **10x objective** in place.

63. Check the microscope **calendar** if another user will continue after you.

WHEN THERE WILL BE ANOTHER USER:

64. Leave the lasers on if they will be **used within 2 hours**.
65. Leave the Metal-Halide lamp on if it will be **used within 2 hours**.
66. Exit the LASX software and **log off** from Windows.

IF YOU ARE THE LAST USER OF THE DAY:

67. **Switch off all activated lasers via laser button (6)**
68. **Turn off Metal-Halide lamp.**
69. Exit the LAS X program.
70. Shut down the computer.
71. Turn off the system by turning the **laser key** and switching the yellow buttons off (from bottom to top).

CONTACT INFORMATION

For direct support at the microscope **call (010-70) 35813.**

For other questions, contact the OIC via:

Martijn de Gruiter	tel. 31105	h.degruiter@erasmusmc.nl
Marla Lavrijsen	tel. 42205	m.lavrijsen@erasmusmc.nl
Gert-Jan Kremers	tel. 43578	g.kremers@erasmusmc.nl

OIC website: www.erasmusoic.nl