Quick operation manual for Leica SP8 Confocal/Multiphoton Microscope
**Turning ON the system**

1- Turn on the Fluorescent light source (number 1)

2- Turn On the NDD power supply.

3- Turn On the PC power. *(wait 5 second)*

4- Turn On the scanner power. *(wait 5 second)*

5- Turn On Laser power.

6- Turn the Key to ON position.

7- Turn on the control panel by switching the toggle switch ON. *(the switch in on the back)*

**Starting the LAS X software:**

- Log in with your unique name and level one password

- On the desktop, double click on the “LAS X” icon

- Wait until the “Application Suite X” box opens.

- Click on the configuration drop box.
Choose an appropriate setting from the drop box menu for your imaging need.

- Use the first option if you need STED.
- Use the second option for confocal and WLL/FLIM.
- Use the third option if you need multiphoton imaging.

- If your project requires, you can turn ON “STED” or “Resonant scanner” at this point. Otherwise you can leave them “off”.
- Press OK and wait for few minutes until software loads up.
- You will receive a message, asking, if you want to initialize the stage, make sure that there is no sample, or any object, on the stage then you can choose “yes”.

While software is loading do not click any buttons or change the objective
- When the software opens, you can choose the objective you need, by clicking the “objective” icon on the “acquisition” page. Please ask if you do not know which objective is best for your sample.

- You can also change the objectives from touch screen controller of the Microscope.

- If you move from dry lens to immersion lens the software gives you a warning. If you are using oil or water lens you should make sure that the objective and the sample is completely cleaned before switching to a dry lens.

**Loading Your samples:**

- Lower the objective by pressing and holding the “Z down” knob on the right hand side of the scope.

- Load your sample (coverslip faces down)

- Use the objective’s specific immersion media (oil or water). Use only one small drop of immersion.

- Use the X and Y knobs of the stage controller to move the desired rejoin directly over the objective.
- Press the “Z up” knob on the right hand side of the scope. (if the objective does not move up, use the focus knob to raise the objective).

- Use the appropriate light source to find your sample.
  - For fluorescence:
    - click the filter wheel on the touch screen.
    - Click the “fluo” tab
    - Choose the filter cube you need. A4 for blue, LS for green, N21 for red.
    - Click IL-shutter to open the fluorescence shutter.
  - For Bright Field, click the “BF” or “DIC” tab on the touch screen.

- Find and focus your sample.
Setting up for Confocal Imaging:

- LAS X main window, general functions

  - The main window opens in the Acquire mode. This window has three portions: scan parameters, Laser and light path, and image panel
  - Context sensitive help can be accessed by clicking on the red ‘i’ icon in the respective panel; to open the complete version of the online help click on the “help” button in the top right corner of the window.
- in the main page click the “configuration” tab.
- Click on “Laser”
- Turn on the required lasers for your imaging need, by clicking the checkbox.
  - 405 laser for Dapi
  - WLL (470nm-670nm). when it is checked, the power should be automatically set at %70. If not, manually select %70.
  - If you are going to use Argon laser, when you check the box you should hear a click and you should hear Cooling fan running. Then choose %30 power.
- Click the “acquire” tab (acquisition)
- Check out the “acquisition mode box”, acquisition mode “xyz” should be selected.
- **Acquisition Rate**
  - The acquisition rate window selects the acquisition scan parameters, including the number of pixels in the image and the scan speed, via pull-down menus. We recommend 400 Hz as an initial rate.
  - The scan orientation may be controlled using the rotation slider. The image area may also be translated in the directions indicated by the rosette arrows. Large lateral translations at low zoom will move the scan range off axis and are therefore not recommended. Move the stage instead.
- **Pinhole**
  - There is only one pinhole. Its diameter is determined by the longest emission band and defaults to 1 Airy disk. It may be modified by clicking and dragging on the pinhole button. You can start by clicking “Airy 1” tab.
Channel set up:

- From the “acquisition mode box”, click on the SEQ icon.
- The Sequential Mode menu appears at the end of the acquisition parameters window
- Click the “load” icon
- Go to the local D: drive
- Find and open the “sequential scan folder”
- From the list choose the sequence that matches your dyes or the one that is very close to it.
- (if you don’t see the any sequential scan that matches your fluorophores, you can ask the staff member who is managing the instrument to create a new sequential scan for you)
- After loading the sequential scans, you should check each sequence, to make sure that it was loaded correctly. The correct sequence will have one laser line and one detector selected. You can check the detection range by double clicking the detector slider. (the minimum range should be 10 nm above the excitation line.

Leica SP8 has 5 internal detectors:
1-HYD1 (high sensitivity)
2-PMT2 (standard sensitivity) also can be used for FLIM
3-PMT3 (standard sensitivity)
4-PMT4 (standard sensitivity) also can be used for FLIM
5-HYD5 (high sensitivity)

- if you need to change the displayed color of any channel, you can click on the color box, then select the color you need and double click on that.

Image Acquisition:

- click the “live” icon,
- you should see live image, using the HYD detectors and if you have PMT, you should select the channel window and increase the gain on the control panel until you see an image.
- Use the “Z position” on the control panel or the focus knob to find the brightest plane of your sample.

![Gain Z position](image)

- Change to the sequence between frame from the sequential scan box.
- It is time to adjust the brightness and contrast of your image one sequence at a time.
- Click on the LUT tab.

The look-up table LUT used to display the results may be changed using the button at the top left of the display window. The first click selects a range-finding LUT for the active window that can be used to set gains and offsets. The second click changes the display in all active windows. A third click returns the LUT to the normal type.

![Change LUT](image)

LAS AF uses green for below range, blue for above range, and red for within range.

- After adjusting the brightness and contrast of each channel we can capture the final image.
- Click ‘Capture Image’ to collect final scan
**How to save an Image:**

- Click on the “open project” tab
- Right click on the project and select rename
- Rename the project to today’s date
- Right click on the project again and click Save as
- Save the project as “lif” file format on the D: drive in the “user folder”
- If you are acquiring a large data set (z stack or tile scan) make sure you open new project and rename it accordingly.
Programming a Z Stack

1. With Live acquisition going, adjust the focus to the top of the stack. Use the Confocal Controller, or the microscope controller fine focusing knob. Fine adjustments may be made using the clickable wheel.
2. Click on Set Start.
3. Re-adjust the focus to the bottom of the stack.
4. Click on Set End.

The number of steps and the step size will be automatically computed based on the longest wavelength emission band. It can be modified by clicking on Nr. of Steps or z-step size.

The diagram indicates the size and spacing of the stack and the current stage position. It may be re-scaled for better viewing using the slider provided.

Clicking on the double-vertical arrow button at the top moves the stage to the middle of the stack.

Clicking on the garbage can icon will delete the stack. This should be performed before creating a new stack.

Use of the z-Galvo stage or the nosepiece for vertical movement is controlled by the pull-down menu. The z-Galvo stage is faster.

Leave Galvo Flow off.
Shutting Down:

1- Save and transfer files (Mbox, google drive, ….)
2- Go to configuration, and uncheck all the lasers.
3- Exit the software.
4- Shutdown the computer.
5- Turnoff control panel box (#7)
6- Turn the key to off position (#6)
7- Turn off number 5
8- Turnoff number 4
9- Turn off number 3
10- Turn off number 2
11- Turn off number 1 the light source