

LCAM startup manual, Leica SP8 confocal microscope (A2.40)

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Information microscope: <http://www.lcam-fnwi.nl/facilities/leica-sp8/>

Before using the Leica SP8 microscope, the user (and co-workers) should have had the official intake discussion with the LCAM-staff, succeeded the confocal training course & exam and had an individual training at the Leica SP8 by LCAM staff.

Start-up procedure

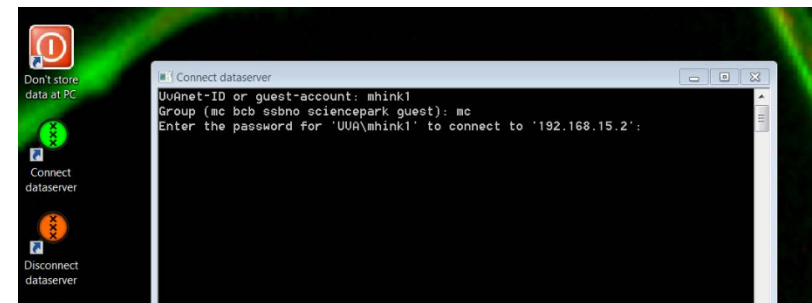
1. Switch on the computer/microscope, scanner and lasers using the three green Leica table switches. First, the **PC/Microscope**. Wait until the computer has started and the Windows login screen is visible. Then activate the **Scanner Power** and third the **Laser Power**. Then turn the key **ON** to unblock emission of the lasers.



2. Log into PC-Leica using User: **LCAM-User** Password: **imcb**

3. When you want to use the climate control turn on the Oko Touch unit and Bartels Pump Controller at the sides of the microscope.

4. Never store data on the local harddisk, all data should be stored at the dataserver. Data present on the local PC will be deleted without further notice. Contact Mark Hink in order to get access from your office computer. Login using the green **Connect dataserver** icon at the desktop: Type your userID (UvAnetID), group and password. After succesful login a network drive U:\ will be visible where you should store your data.



5. Check visually if there are lids or holders placed directly next to the motorized table. Put them to the side of the Okolab box such that the table can move around freely during initialization without crushing a holder.

6. Start the LAS X software. After some initialization you are asked to select the Configuration. Select for the configuration: **Machine** or when you want to have software control of the temperature and CO₂: **Machine +Environmental control**. Select Microscope: **DM18**, Load settings: **Off** and press **OK**.

7. The software will ask you if you want to initialize the motorized table. After you have removed any holders/lids next to the table (see point 5) press **YES**.



Visual inspection of your sample

1. Start by checking the selected objective. In the LasX software or at the front panel of the microscope body one can select the wished objective. Check by visual inspection if the lens is clean. If not, clean it using lens-tissue and some ethanol from the plastic bottle. Do not use other paper types and do not press or rub! Be aware that the microscope LCD screen shows all lenses as IMM (Immersion). This means the 63x oil imm. and the 63x water imm. show the same name. This is why it's better to use the LasX software to select the objectives.
2. Put the correct immersion liquid on top of the objective (air, water or oil). A small bottle of MilliQ water can be found inside the incubator as well as a small red-labeled bottle of Leica oil. Note we have two types of Leica oil: for Room Temperature (21°C) and 37°C. In case an oil bottle is empty: **NEVER use the immersion oil from other brands (Zeiss/Nikon/Olympus)** but contact Ronald or Mark for a refill.
3. To activate the internal light of the Okolab incubator box click on the foot pedal (by hand or if you're flexible by foot).
4. For a visual inspection of your sample in transmission light mode you have to activate **BF** or **POL** at the microscope screen and open the light shutter by clicking on **TL-Shutter** button.
5. For controlling the table position you can use the table controller having 3 knobs for X (top front), Y (top bottom) and Z (top back). All knobs can be operated in *fine* or *course* mode by pressing these buttons at the controller.
6. For a visual inspection of your sample in fluorescence mode you have to switch on the fluorescence light source using the **Power** button at the unit (normally switched on already at system startup).

You can choose from four different fluorescence filter-blocks on the front of the microscope

DAPI, Alexa405, ... (Blue)

FITC, GFP, Alexa488, ... (Green)

CFP/YFP..... (Cyan plus Yellow)

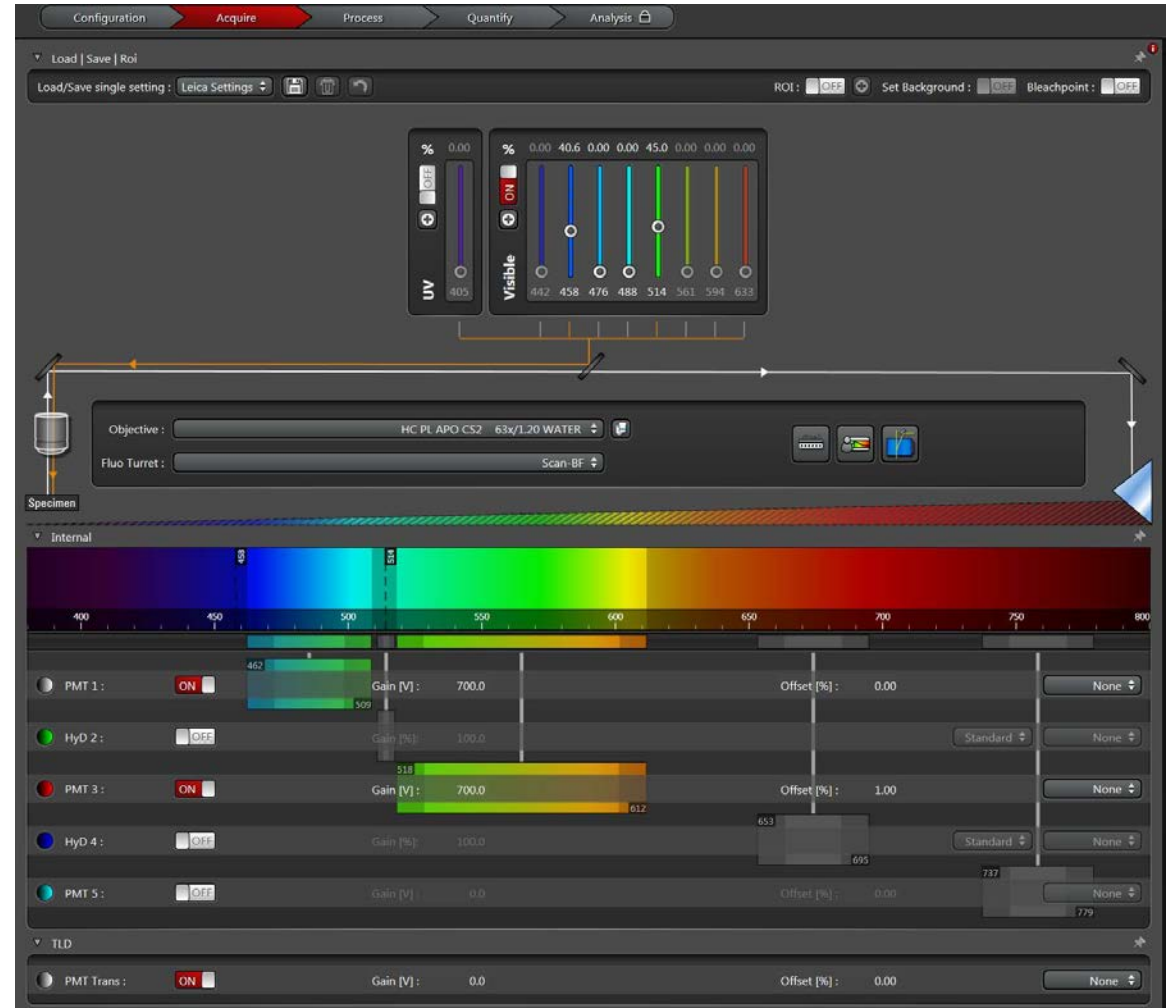
Rhod, TRITC, Cy3, Alexa543, .. (Red)

Activate this by pressing **FLUO**, **IL-Shutter** and selecting the cube of the choice

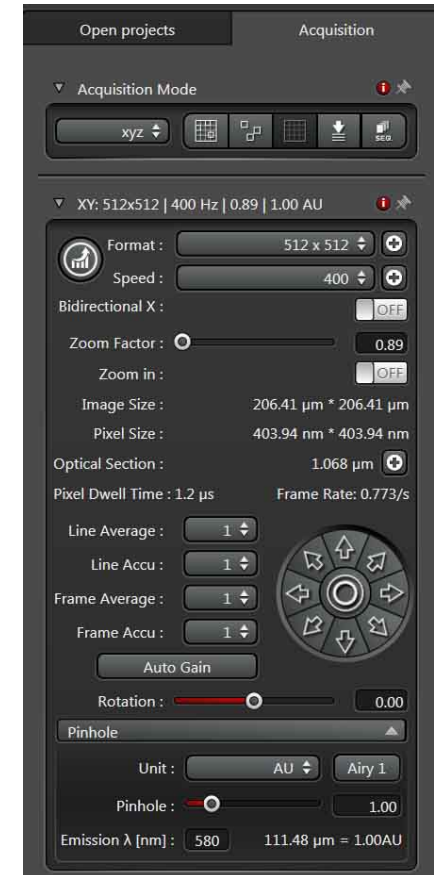


Basic handling of the confocal microscope

1. Open in the LasX software the **Configuration** tab. Click on the **Laser Config** button and switch on the laser lines you want to use (**ON/OFF** toggle switches) and adjust the main output of these lasers (Typically 20%).
2. Via the **USB Panel** button you can define the functions of the knob bar that is present in front of the computer screen.
3. For quantitative measurements: Click at the **Hardware** button and change the **Resolution Bit depth** to **12 bit**
4. Goto the **Acquire** tab. Goto the z-“stack menu (at the left side) and change “Z-piezo” to “Z-Wide”.
5. Goto the **Acquire** tab. Activate in the main window the UV and/or Visible lasers via the **ON/OFF** toggle switches and adjust the power you want to use for each sample via the sliders.
6. Select in the window below those detectors you would like to use via the **ON/OFF** toggle switches. The HyD detectors are more sensitive than the PMTs but remember they will shut down if the signals of your sample are too strong. You can also activate the transmission image detector by activating the **TLD** in the window below.
7. Adjust the spectral detection window for each of the activated detectors such that you will detect the fluorescence of the sample but **WILL NOT** detect the laser lines (indicated by dashed lines in the color spectrum). You can do this by moving and reshaping the colored boxes behind the detector name and squeeze/move the boxes of other/non-used detectors away. Also adjust the gain of each PMT detector (note: HyD settings are fixed) by the sliders or typing in a value (stay below 950 V). Do the same for the offset (typically around 0).



8. Goto the **Acquisition** menu at the top left, and adjust the **Acquisition mode** (typically *XYZ*).
9. Adjust Format (*512x512*), Speed (*400*), Bidirectional (*OFF*), Zoom factor (*1*), Zoom in (*ON*), Line average (*1*) and Rotation (*0*).
10. For optimal confocal imaging set the **Pinhole** to *1 Airy Unit*. One can optimise the pinhole diameter by filling in your favourite emission wavelength and press the *Airy1* button.
11. Use the **Live** button at the bottom to quickly adjust the position and maybe focus of your sample and take the final image using the **Capture Image** button. For more complicated image series (e.g. time series, z-stacks or FRAP) you should use the **Start** button.
12. Goto the **Open projects** menu at the top left. Click at an image or project folder you would like to save, Right-click at it and select **Save As**. Now browse to your folder at the datasever and store the data here (Note: All image data at PC-Leica will be deleted).
13. More advanced imaging options will be discussed during your individual Leica SP8 training and can be checked in the digital Leica manual, available via a shortcut at the desktop. Here also some application notes with protocols are available how to use the SP8 for applications like FRAP & FRET.
14. To enhance the mercury lamp lifetime: Please turn it off when you are scanning overnight.
15. Always inform the LCAM contact persons of the Leica SP8, Ronald or Mark, in case of microscope or network issues or when more sophisticated imaging is required.



Switching off procedure of the confocal microscope

1. If there is no one using the confocal microscope directly after your session goto the **Configuration** tab and deactivate the active lasers via the **Laser Config** button using the ON/OFF toggle switches.
2. Close the LasX software.
3. If you have used climate control: Power off the Oko Touch unit and Bartels Pump controller.
4. Disconnect the link to your folder at the dataserver using the red **Disconnect dataserver** icon at the desktop.
5. If there is someone using the confocal microscope directly after your session (check at www.lcam-fnwi.nl -> Bookings) then only clean the lenses with a tissue. First remove the oil, do not rub! In a second step clean with a little alcohol, again do not rub and move the paper only in 1 direction!
6. If you switch off the microscope completely first turn the laser key to the **OFF** mode at the green table switches, then switch off the **Laser Power** button (right green buton). **Now wait 3 minutes** if you have used the air-cooled Argon laser (458/488/514) to cool it completely.
7. Switch off the **Scanner Power**, using the middle green button.
8. Shutdown the computer and **WAIT** until this has been completed.
9. Switch off the **PC/Microscope** with the green table switch.
10. Clean the microscope, the labspace around it and put recombinant waste in the appropriate yellow bins:
 - Sharp/Glass in the yellow containers with white caps.
 - Solid material in the larger yellow open cans.
 - Discard fluid recombinant waste (growth and microscopy medium) in the wetlab or special WASTE bottles.
 - Paper should be discarded in the standard black waste bins in the room.