Leica SP 8 STED set up and operation

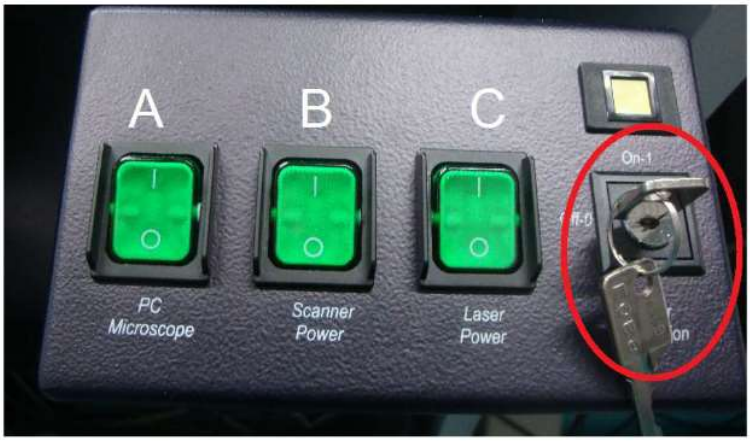
STED (super resolution only). SKIP THIS IF YOU DON'T NEED SUPER RESOLUTION

Note that you can ONLY do STED with 100x Oil and 93x Glycerol objectives. Using any other objective with STED is pointless. If you need better resolution than what 20x provides, simply use 63x Water or 93x Glycerol objectives depending on your mounting media. Also if you sample contains DAPI or Hoechst you are NOT doing STED with your sample as this could potentially damage the expensive detectors.

Turn on the laser from the power switch from the right. Don't touch the key yet. When you turn on the laser switch the **SHG READY** light will turn green for a while, before turning yellow. You can do other things as you wait for the light to turn green again. Once the light is green, you can turn the kay to **on** position.

START-UP

Turn on the computer and login. Did you do that? Ok, good. Once you have logged in start Microscope, Scanner and Lasers by pressing switches **A**, **B** and **C**. Then turn the safety key to **On-1** position.



Turn on the fluorescent lamp from its power switch if you want to see your samples with your eyes.

Turn on the software from the icon (Las X).

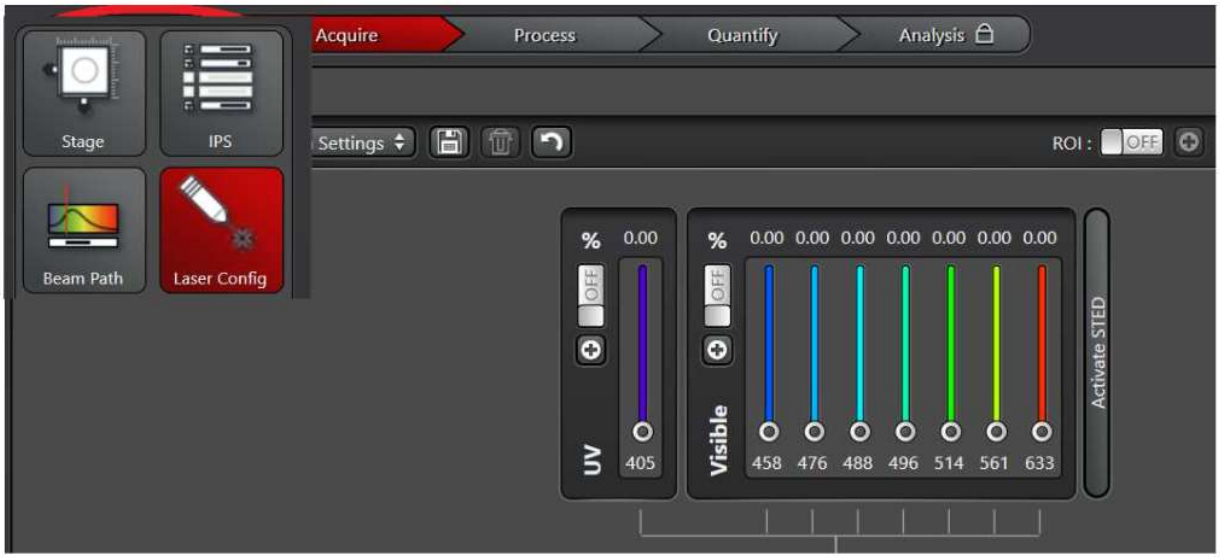
NORMAL USE: In the start-up menu chose **USE THIS machine_working NO STED.xlhw**. If the Argon laser fails to work, re-boot with **USE THIS machine_no stage.xlhw**. Note that the stage cannot be controlled from software if this option is chosen.

STED USE: In the start-up menu chose **USE THIS machine_working.xlhw**. If the Argon laser fails to work, re-boot with **USE THIS machine_no stage.xlhw**. You also need to enable STED by clicking the STED on in this menu. Scan field cannot be rotated in this mode.

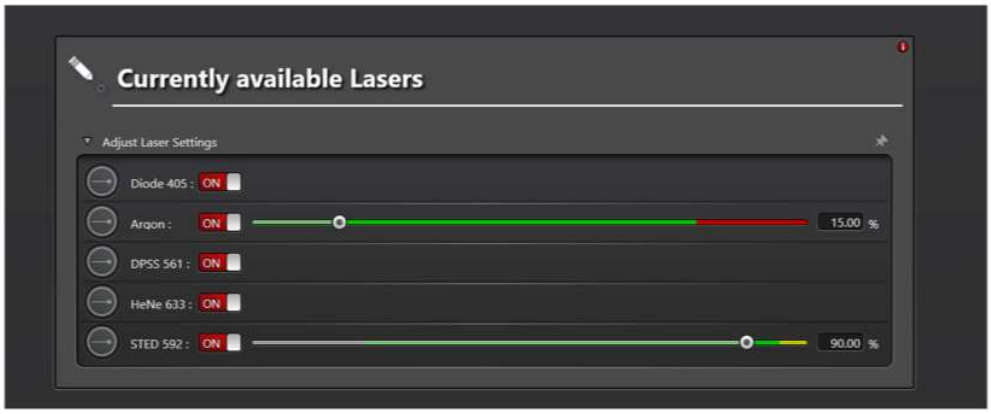
If you need autofocus , you should go to the microscope front panel, press the XYZ tab on the left column, then hit the **Z_{AFC}** tab on the top right finally toggle on the **AFC** from the **On/Off** button.

During the start-up the microscope will ask you if you want to initialize the stage. Press **yes**. **Do not press no**. Leica claims that stage is married with Argon laser and stage must be initialized for the Argon to work.

Once the microscope has started to the **Configuration** settings



Chose the **Laser Config** tab from the left and turn on the lasers you need. If you need 456 nm, 488 nm or 512 nm laser line you should set the **Argon** to 15%. If you are doing STED, you should set the **STED 592** depletion laser to 90%. (See below).



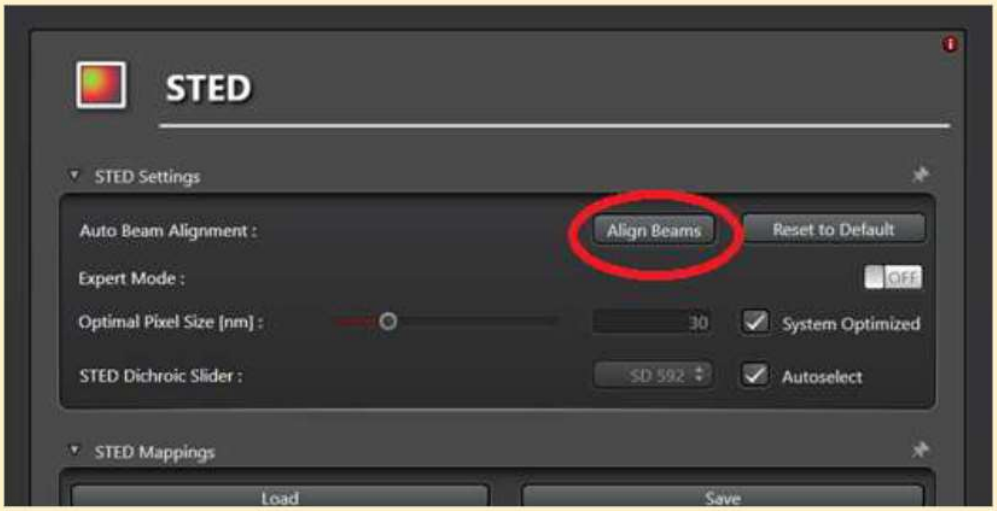
Turn on the lasers you need. Regardless of the colors you are using, turn on the 592 nm depletion laser. Remember that Argon laser gives 458 nm, 488 nm and 514 nm laser lines.

STED ONLY

Next, make sure that 93x objective is selected from the panel in front of the microscope.

Once you are certain that 93x objective is in place, hit the the **STED** tab on the left. This is a crucial part and super resolution will not work unless the calibration is performed.

In the STED tab, hit the Align Beams button. Wait until the system announces that the calibration is completed.

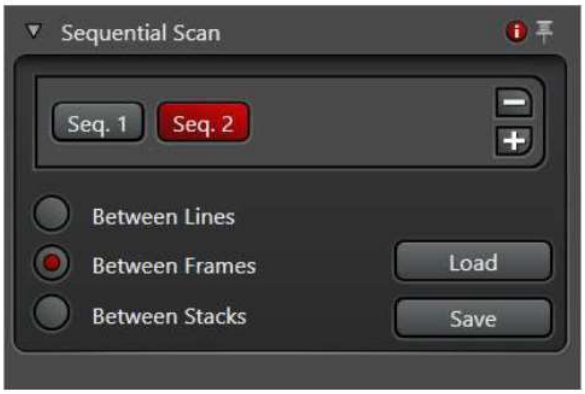


When you are done with the aforementioned steps, return to **Acquire** tab.

Sequential imaging

Turn on the **Sequential Imaging** tab from **Seq** button (A) in the Acquisition Mode menu. Hitting the icon turns it red and opens the Sequential Scan tab.

Between Lines mode should be If and only if you don't move your detection regions between sequential scans. If you have to move your detection regions between sequential scans, use **Between Frames** (for 2D or 2D+ time scans) or **Between Scans** (for 3D or 3D+time scans).



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Z-STACKS

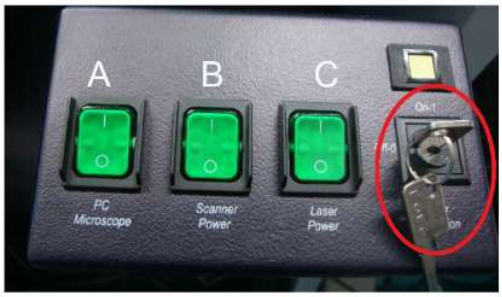
To acquire Z-stack, adjust the scanning level to above or below (it doesn't matter) of the object of your interest and click the **Begin** (A). The button will turn red to indicate that it is active. Then move the scanning plane to the other extent of the sample and press **End** (B). Now the end button should also turn red.

If you want to adjust the end/begin points, just move the scanning level and press the relevant button again (while they are red), this will update the value.

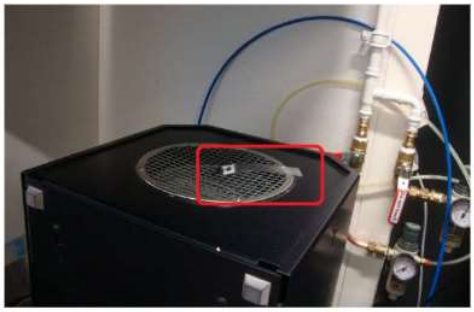
If you want to erase the stack press button C. This is recommended after you have acquired a stack. If you want to move to begin of your stack, press the arrow next to the **Begin** button. If you want to move end of your stack, press the arrow next to the **End** button. If you want to move to the middle of your stack, press the button D.

SHUTTING DOWN

1. Save your data and move it to LMU disc
2. While the data is being moved remove your sample and clean the objectives you used.
3. Check the reservation system and take note when the next user is coming. If the next user is coming within 2 hours, **leave on all the lasers they need**. Else **turn off all the lasers**.
4. Close LAS X
5. If you used the STED laser and the next person is not using it, disable and turn off the sted laser from the black box next to the microscope.
6. If the next user within 24 hours needs the microscope heated, turn on the heating and **adjust the temperature** as per instructions on **following page**.
7. If next reservation is at room temperature turn off the heating if you used it.
8. If no-one is coming within 2 hours, turn off the fluorescent lamp
9. If no-one is coming within two hours, turn off the switches **A** and **B** and disarm the lasers by turning the key to **off-0** position.



10. Wait that the laser-cooling-indicator is at 'down' position.



Once the indicator is at down position, turn off the switch **C**.

11. If you used the STED laser, turn off the 592 laser and turn the key to **off-0** position.

12. If no-one is coming within 2 hours, shut down the computer, else log off.