Leica STED/CARS Manual

Operating the microscope for fluorescence and confocal.

1. **For CARS** turn on the detector box and CARS laser next to computer screen. **Check STED filter.**
2. **FOR STED:** switch on the STED laser on the back, **Check STED filter.**
3. Turn on the 3 green buttons, left to right, and then turn the key next to them.
4. Turn on the separate fluorescence lamp (placed under the table).
5. Turn on the PC. Log on (the password is written in the book) for Memphys users password **Memphys1234**, for Lipid users password **Lipid1234**.
6. Open LASX software, all 3 buttons in the opening dialogue box should be off for confocal and CARS, for STED-STED button is on and in configuration: **Machine-STED-only**.
7. Choose objective in the software, and add a drop of oil on the oil objective if required.
8. Using **CNG TL** transmission on the microscope-find your sample in ocular.
9. Find your sample using fluorescence (**Fluo** on the left side of the microscope), choose a filter, press the shutter button on the front. A for UV, I3 for green, N2.1 for red.
10. When the sample is located turn off the shutter before carrying on in the software.

Operating the software

1. Config=>Laser configuration => Choose WLL (min. 470 nm otherwise all wavelengths), 70 %, ON, for Argon 50%, ON
2. In **Acquire** menu set a laser wavelength and turn it up just a little bit then it becomes active.
3. Set a detector. THE LASER IS NOT ALLOWED TO BE IN THE SAME RANGE
4. Adjust Gain to 500 – 700 under PTM
5. You can add Em/Ex curves by right clicking on the lower graph for chosen dye.
6. Check Smart Offset – couple of pixels is OK
7. Turn ON the laser by pressing the ON button on top of the page in the middle.
8. Press LIVE in the bottom to the left to display the live image. Then the laser is turned ON. Press capture image to take a single image.
9. Optimize the picture in the panel to the left.
    - Choose pixel (e.g 512 x 512) or use Optimal XY for final image acquisition.
    - Zoom if needed.
    - Line Averaging Collects the average from several images.
    - Line Accumulation For STED
    - Frame averaging for not moving samples
10. Parameters of control panel can be set: in the software using the button next to the objectives.
11. If you have a sample with a week signal you can adjust the Pinhole. Then you might also have to adjust the laser intensity afterwards.
12. **For sequence**: do sequence between frames, choose sequence-start from a longer wavelength, adjust wavelengths.

13. **Z-stack** define **Beginning** and **End**. Press Start
14. Save data and project. Next time the settings can be reused: Project =>Apply settings
15. When done, **switch off lasers in software, switch off software, switch off green buttons and fluorescence lamp, turn off the detector box.**

For CARS

1. Turn on CARS laser, the laser power about 1174mW is ok. Wait 15-30 minutes for the power.
2. Check is STED filter is out. Turn on detector box before starting everything else.
3. Mount filters you need.
4. Choose objective: 10x Air, 20x Air (IMM) or 40x IRAPO water, cover sample very well.
5. After you start the software check that OPO and IR power set to 2000mW.
6. Activate the CARS pump laser, choose wave length (for lipids use 816.4nm, for water 790nm) and tune laser. CARS Stocks laser is 1064nm. Check aperture is 24 (opened). 30% for both lasers. IR power approx. 1300mW.
7. Turn on 4 CARS detectors, gain 600, adjust smart offset until only a few pixels are displayed
8. Check that there is no autofluorescence.
9. After you finish, **switch off CARS laser, other lasers, turn off detector, switch off software and 3 buttons on the right side of the table.**

For STED

1. Switch on the laser, which is behind the computer: 1) switch and wait until green light, 2) turn the key
2. Follow the procedure for operation of the microscope above (1-5), in the opening dialogue box you must select **STED on**
3. Choose objective 100x oil 1.4 NA – add immersion oil to the objective
4. Check is STED filter is in
5. STED laser is on in software, 80% power. Activate STED. Perform beam align in software.
6. For measurements set STED intensity (in laser panel) for 40% and adjust as needed.
7. STED detector HYD3, gating is on (V) for WWL laser. **Off for Argon laser**. DO not adjust gain.
8. For STED in configuration untick pixel size: system optimized (optimally 15 nm).
9. Laser (not STED) power may need to be increased as less photons are collected due to the STED effect.
10. Use photon counting mode for imaging. For image acquisition use accumulation mode.
11. After you finish**, switch off lasers, software, fluorescent lamp, 3 green buttons.**

In the case of problem contact:
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