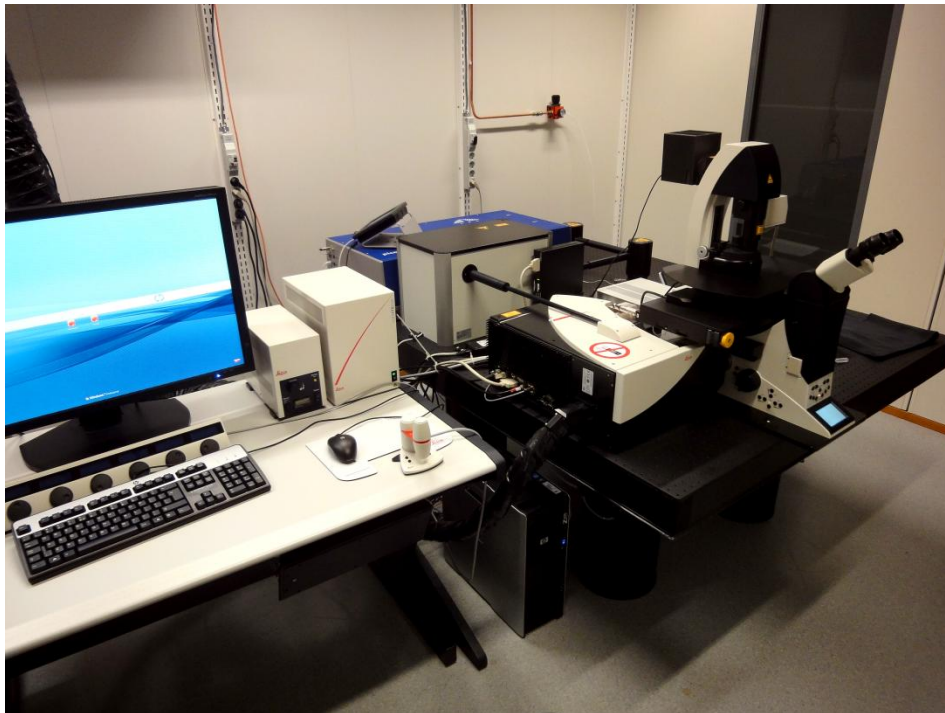


Leica TCS SP8 CARS Microscope and LAS AF 3 Software

For Confocal and CARS Imaging

DRAFT VERSION

Room B116a



User Guide

Biomedicum Imaging Unit (BIU)
University of Helsinki

www.biu.helsinki.fi

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1 General

1.1 Instrument

This user guide covers the use of the Leica TCS SP8 (SP8) confocal. The SP8 is equipped with a tandem scanner for both fast and high resolution imaging.

1.2 Reservations

Common reservation rules

Instructions for Scheduler online reservation system can be found on the BIU web page: <http://www.biu.helsinki.fi/reservations.htm>. You can make your SP8 reservations three weeks in advance. If you need to cancel your reservation, you have to do it two hours before your reservation starts. Unused reservations will also be charged. Make sure you are familiar with the use of Scheduler and the BIU fees and user policy (<http://www.biu.helsinki.fi/fees.htm>).

When you make reservations, please always remember to indicate in the Event name field which lasers you will use during your imaging. This enables the users before you to turn off those lasers that are no longer needed on that day, thus extending the lifetime of the very expensive lasers.

If you want to extend your session and there are no reservations after you, you can make a new reservation right after the current one. It is not possible to make a single reservation that extends beyond 24:00. In such a case reservation can be extended by starting a second one at 00:00.

1.3 Billing

The billing is based ONLY on the reservations made in Scheduler. There is a separate fee for the training session (2h).

1.4 Unauthorized use

BIU periodically checks the logins of the computer. If your logon/off times exceed your reserved time in Scheduler, BIU will consider that as unauthorized use of the instrument and ask for an explanation. For unauthorized use of the instrument, BIU may issue a warning. After three warnings, BIU may revoke the user's license. Also, misuse/neglect of the instrument may lead either to a warning or cancellation of the user license and the repair costs may be charged.

1.5 Users' mailing list

All registered users are automatically added to the users' mailing list. Please read those e-mails to know what is going on!

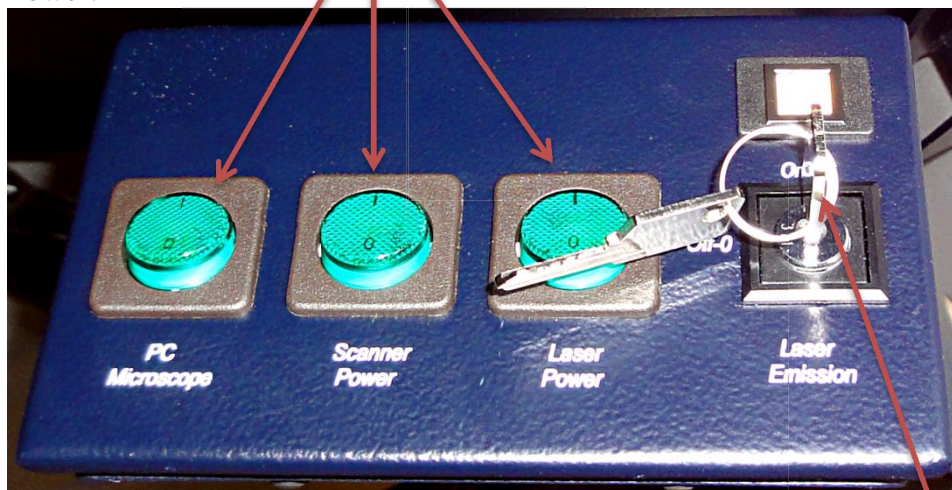
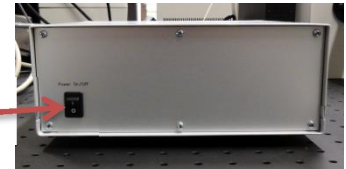
1.6 Acknowledging BIU

Whenever you are using instruments and/or services maintained/provided by BIU, you are expected to acknowledge BIU in your publications and inform BIU about the papers once they have been accepted for publication. This is extremely important as we must regularly report on the services provided by the BIU and number of publications has a direct impact on the level of service and equipment we are able to offer in future.

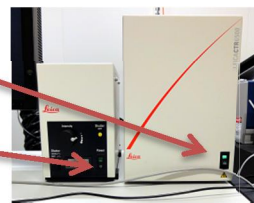
2 Turning on the system

If you are the first user for the day:

- 1) Turn on the external NDD detectors. Do this even if you do not plan to use them as they will not be detected if they are turned on after the scanner and someone else may want to use them that day.
- 2) Turn on the three main switches (PC Microscope, Scanner Power, and Laser Power):



- 3) Check that the fluorescent light source and microscope controller were automatically turned on (if not, turn them on now).
- 4) Activate the lasers by turning the "Laser Emission" key if needed (= if you are not doing exclusively CARS/MP work).
- 5) Turn on the system PC and log in.



3 Using the software

3.1 Starting the software

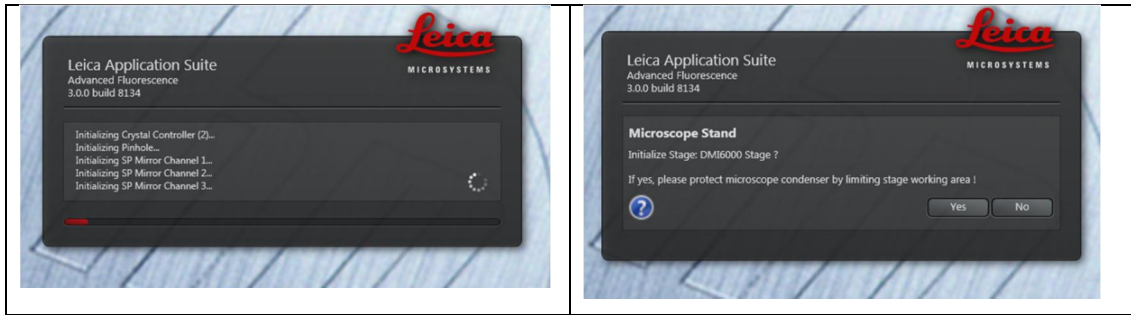
Once you are logged in double click on the LAS AF icon to start the software. After a moment you will be asked to configure the system for your session:



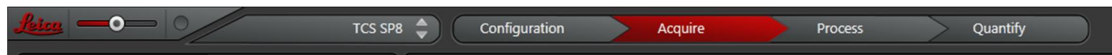
- 1) Configuration: options are “machine” (used for normal confocal work), “CARS” (used for MP and CARS protocols), and “Simulator SP8”.
- 2) Microscope: options are DMI 6000 (used normally) or “manual DM-6” (if you do not want to initialize the microscope, for example you only wish to analyze existing images).
- 3) Resonant: whether or not you wish to use the resonant scanner. Normally this will be off for fixed samples and on for “fast” live-cell etc. imaging.

NB. Once you have accepted these settings you will not be able to modify them during your software session. The only way to change these settings is to quit the software and restart it.

Once you have made your choices click “OK” to initialize the system. You will see a list of initialized components followed by a query about initializing the motorized stage. This should be done, especially if you plan to do multipoint image acquisition. However, before initialization you must check that nothing is limiting the movement of the stage and you have no sample in the holder. Ignoring this may cause extensive damage to the optics and stage of the microscope!



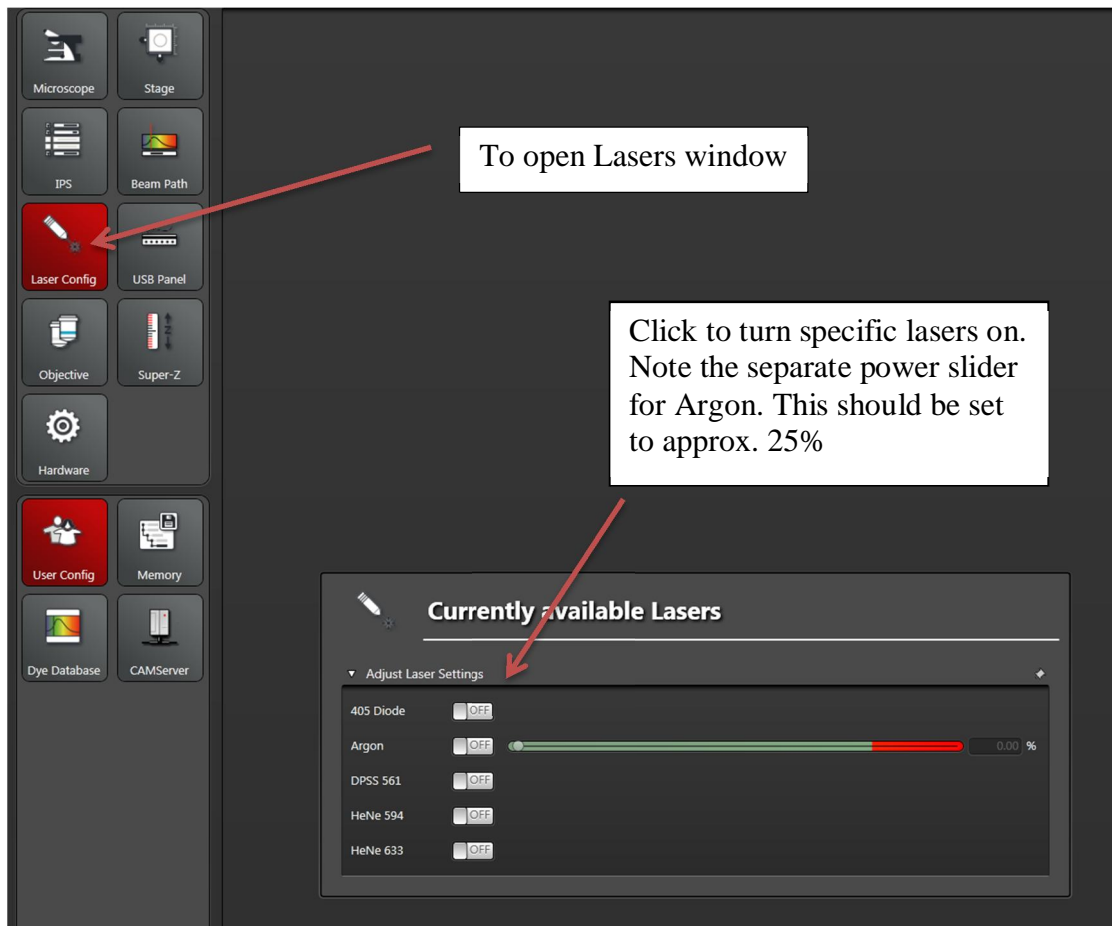
Once the initialization of system components is over The main LAS software window will open. At the top of the LAS software window you have a banner for choosing between the main software functionality.



The first slider is for choosing the size of the control icons. This is followed by a drop down menu for choosing between “normal” confocal mode (“TCS SP8”) and the various assisted modules (FRAP, FRET etc.). The final element is a four tab arrow (Configuration, Acquire, Process and Quantify). Clicking on any one of these tabs will open a corresponding view in the software.

3.2 Configuration

Normally you will only need this tab to turn on and off lasers.



3.3 Acquire

See separate guide for Leica TCS SP5 LAS AF software. Please note that the scanner is equipped with two hybrid detectors (HyD). These are extremely sensitive. When you use them for the first time you should always check that their gain is set to minimum. If too much light reaches them they will automatically shut down. If this happens it will take a few moments before they recover and you can continue.

3.4 Process

Contains general image editing, adjusting and filtering tools. Also contains a tool for dye separation.

3.5 Quantify

Mostly for intensity measurements and plotting but also contains a colocalization tool.

4 Ending your session

Always start by checking the reservation system to see if there are still other users of the SP8 confocal. If yes proceed to step 4.1, otherwise step 4.2.

4.1 There are users after you on the same day

Open the “Configuration” tab and set the Argon power slider to minimum.

Click FILE/EXIT to terminate the LAS software. Close all open windows and exit the user profile you are using but do not turn the computer off.

Leave the PC, scanner and lasers on. Clean up the objectives; have the lights turned on so that you can see properly. Use lens paper but **do not rub**.

4.2 You are the last user of the day

To completely turn off the system:

- 1) Open the “Configuration” tab, set argon laser power to minimum and set all lasers to the off position.
- 2) Turn the “Laser emission” key to the off position.
- 3) Exit LAS software and turn off the computer.
- 4) Turn of the external NDD:s
- 5) Turn off epifluorescent lightsource.
- 6) Clean objectives, wipe objectives but do rub!
- 7) Turn off scanner and PC power.
- 8) Wait at least 10 minutes, then turn off laser power.

Clean up whatever mess or spills may have occurred during your session.