Leica TCS SP8 X Confocal Microscope and Leica Application Suite X software

DRAFT VERSION

Room B123b

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 X (SP8X) confocal.

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 SP8X 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 requires 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 the future.

2   Turning on the system

If you are the first user of the day:

1) Turn on the EL6000 fluorescence lamp (Fig. 1) and make sure the shutter is set to remote

2) From the main control unit turn on the PC Microscope switch (Fig. 2, (1))

3) Wait for the microscope stand to initialize and the workstation to start up

4) From the main control unit turn on the Scanner Power (Fig. 2, (2))

5) From the main control unit turn on the Laser Power (Fig. 2, (3))

6) From the main control unit turn the Laser Emission Key to ON (Fig. 2, (4))

7) Log on to the workstation with the BIU-User account

N.B. The white light laser should be fully controlled by the main control unit and the software. You should not need to touch the laser unit on the left side of the microscope.
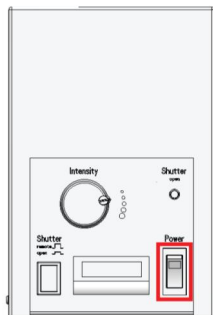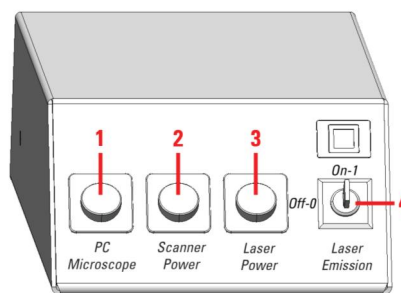
Fig. 1 EL6000   **Fig. 2 Main control unit**

3   Using the software

3.1   Starting the software

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

1) Configuration: use 'machine.xlhw' for image acquisition (or 'SimulatorSP8.xlhw' for data transfer and analysis).

2) Microscope: use 'DMI8' for image acquisition or 'DM manual-6' if you only wish to analyze existing images.

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. If any errors are indicated please inform the BIU staff even if the setup continues normally.

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.
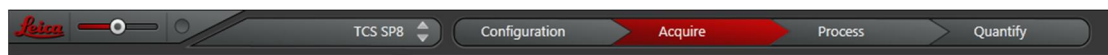
The first slider is for choosing the size of the control icons. This is followed by a drop down menu for choosing between possible confocal modes. The final element is a four tab arrow (Configuration, Acquire, Process, Quantify, and Analysis). Clicking on any one of these tabs will open a corresponding view in the software.

3.2 Confocal Modes

TCS SP8
Normal confocal imaging.

Live Data Mode
For flexible combination of imaging sequences.

HyVolution
Resolution improvement with a combination of pinhole adjustment and Huygens deconvolution.

Matrix Screener
Leica high content screening module.

3.3 Configuration
The configuration settings will allow you change system components and adjust their settings. Normally the image bit-depth and possibly the laser settings will be the only components you may want to change here. This step will also allow you to change objectives, motorized stage settings, create new dye information, etc. These should only be changed if a BIU staff representative is present.

3.4 Acquire

Turning on lasers
From the laser panel click on the button with a '+' sign. This will open the panel for turning on the 405nm and the 470-670nm white light laser.

Please note that the scanner is equipped with two hybrid detectors (HyD) and two photomultiplier tubes (PMT). The HyD's 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 you will need to turn the detectors off and it will take a few moments before they recover and you can continue.

Beam path settings

Once you have the appropriate lasers activated you will need to set up your beam path. Use the Leica factory settings as a starting point.

The Leica settings contain usual single dye settings and a few usual dye combinations. You will need to adjust these settings for your particular experiment. Once you have adjusted your settings you should store them by clicking the diskette beside the settings. Please note that you will not be able to overwrite the factory settings but should create a personalized name for your settings by, for example, adding you initials to the original name.

Acquisition mode

The acquisition mode should automatically be set to xyz scanning at 512×512pixels with a scan speed of 400 Hz.

3.5 Process

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

3.6 Quantify

Mostly for intensity measurements and plotting but also contains a co-localization tool.

3.7 Analysis

Currently not licensed.

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

Click FILE/EXIT to terminate the LAS software. Leave the PC, scanner and lasers on.

Clean the microscope and surroundings, have the lights turned on so that you can see properly. If you used the oil objectives, gently wipe the oil with the lens paper provided. **Do not rub or use ethanol.**

4.2 You are the last user of the day

To completely turn off the system:

1) Open the 'Lasers' tab (see section 3.4) and set all lasers to the off position.

2) Clean microscope and objectives used as in section 4.1

3) From the main control unit turn the Laser Emission Key to OFF

4) Exit LAS software and turn off the computer.

5) From the main control unit turn off the Laser Power

6) From the main control unit turn off the Scanner Power

7) Check that the PC has completely shut down

8) From the main control unit turn off the PC Microscope switch

9) Turn off the EL6000 fluorescence lamp

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