Induction to the Leica SP8 confocal microscopes at the Cambridge Advanced Imaging Centre

This guide/induction document is compiled from a number of sources and largely following these documents:

https://micr.med.wayne.edu/pdfs/leica_sp8_users_manual.pdf

https://media.bcm.edu/documents/2017/c8/oivm-leica-sp8-user-guide.pdf

https://zmb.dozuki.com/Guide/Leica+SP8+Falcon+and+STED+-+Live+cell+imaging/136

Question and comments:

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Kevin O'Holleran, Cambridge Advanced Imaging Centre, ko311@cam.ac.uk

Induction Videos from U Massachusetts Medical School

Focussing your sample:

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=1idu4j3x9k

LASX - Basic setup

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=wxzxmv4cwt

LASX - Dye assistant

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=kucog833yx

LASX - Imaging setup

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=gmggr7dp0s

LASX - Setting up z Stack

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=okc8nvcceb

LASX - Setting up time lapse imaging

https://www.umassmed.edu/scope/training/confocal-training/leica-sp8-confocal-training/?wvideo=qoalctqd9j

Health & Safety

BSL-1

The CAIC rooms are classified as **biosafety level 1.** Amongst other things that means:

No eating drinking smoking, not even during long sessions!

The **confocal microscopes** are classified as **lasers class 1**, safe under reasonable foreseeable conditions of operations.

Usage of **wet lab space** outside for sample preparations is **only allowed after booking with CAIC staff**

Induction & User Rights

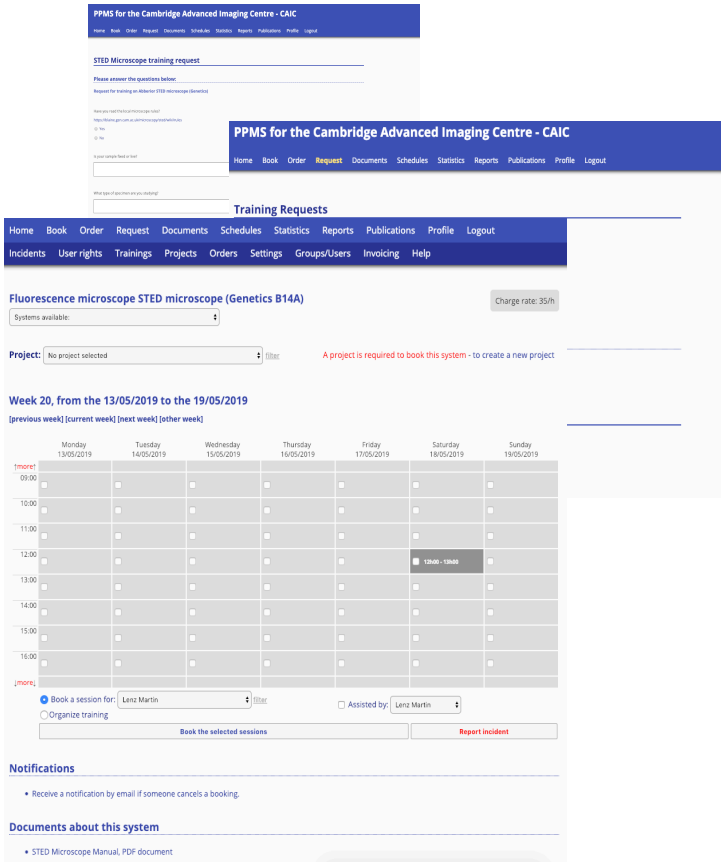
Microscopes can be **booked after successful induction**

Inducted user **get 'Novice' status** with access to microscope during office hours (Mon – Fri, 9-5pm)

Full access ('**Autonomous**') can be granted upon request and after some experience

The user **is not able to book** microscope **before becoming a member of a project** (see next page)

Bookings



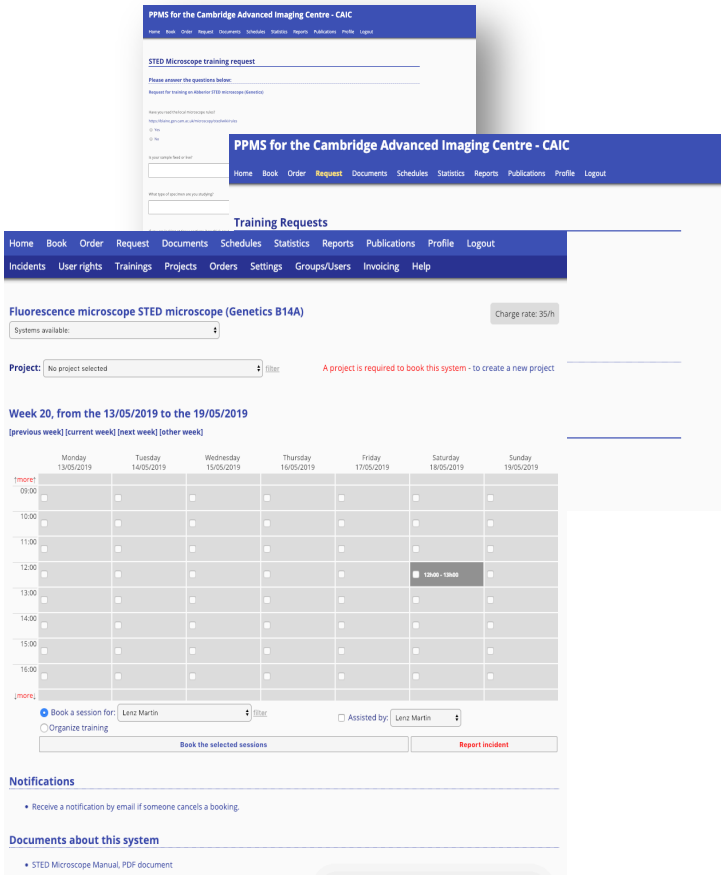
Bookings for the microscopes must be made at

https://ppms.eu/cam-caic

Project membership is necessary to book a microscope.

A user can **either create a new personal project** (including grant code for charging purposes) or can be **added to an existing project** by the project administrator.

Microscope Bookings



Confocal microscopes can be booked in **30 min increments.**

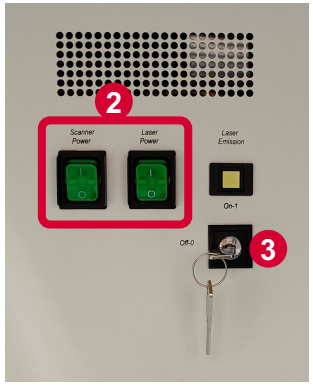
Current usage charge: **£25 per hour**

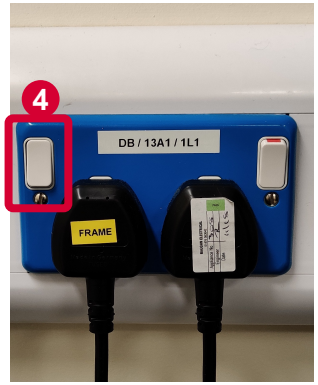
Cancellations: free of charge up to **24h** before the booking, **then 25%** of the original charges unless the time slot is booked by another user

Maximum 3h usage per user per day during peak hours

Booking **up to 14 days** in advance

Start-up procedure, SP8 Core





Before turning on the microscope make sure the 10x objective is selected and facing upward.

Turn on the power switch ❶ for epifluorescence light source if you are planning to use it. This lamp is used to visualize fluorescence in the microscope eyepieces.

Turn on Laser Power and Scanner Power Switches (❷ green switches) then turn Laser Key ❸ to on state on the large unit under the table.

Turn on microscope stand with the wall switch ❹. The stage will go through an initialization routine.

Turn on workstation ❺ located under the table.

Start-up procedure, SP8 Advanced

Before Turning on the microscope make sure the 10x objective is selected and facing upward

Turn on the power switch ❶ for epifluorescence lamp if you are planning to use it. This lamp is used to visualize fluorescence in the microscope eyepieces.

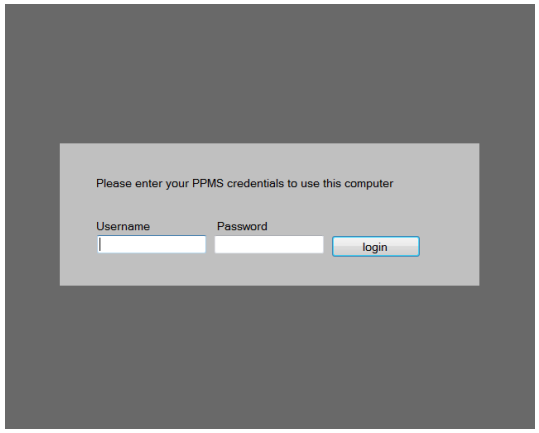
Turn on Laser Power, Scanner Power Switches, and PC Microscope (❷ green switches) then turn Laser Key ❸ to on state.

Login

If necessary, sign-in to the computer using the following credentials:

User: TCS_User

Password: *will be provided*



After approximately 1 min the screen will be displaying another authentication window.

Sign-in using your PPMS (booking system) credentials.

Overview Microscope Stand

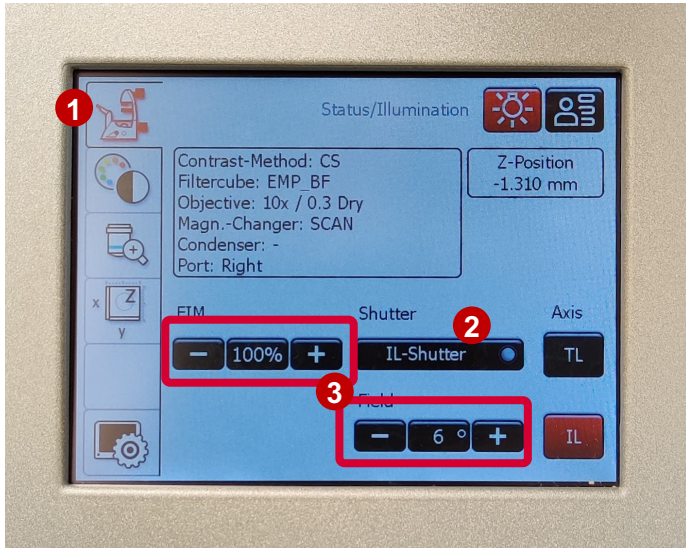
The microscope frame is equipped with standard controls like focusing knobs ❶ on the left and right side of the stand

Additional control is possible using the external ‘salt-and-pepper’ controller ❷.

The main mode of controlling functionalities on the microscope stand is the front touch panel ❸.

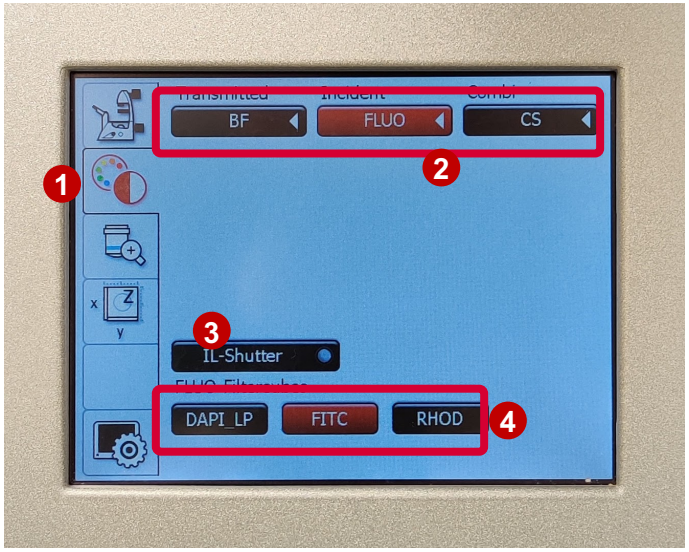
To change the intensity of the light source used for the eye piece use the control on the left ❹. This can either be the white light or the fluorescence lamp. If the eyepieces are dark, try turning it to increase light intensity.

Touch Panel, Microscope Tab



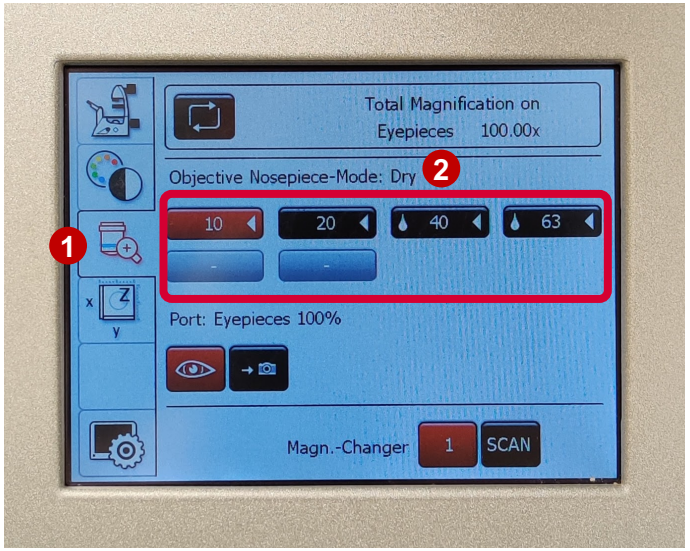
1. Microscope tab: This screen highlights the aperture opening and the intensity of the transmitted light.
2. TL Shutter (Transmitted Light Shutter) toggles the shutter between open and closed for brightfield illumination.
3. Intensity and Aperture adjustment.

Touchpanel, Dichroic Tab



1. Dichroic colour tab
2. Switching between brightfield (BF) or fluorescence (FLUO).
3. IL-Shutter opens and closes the fluorescence shutter and the TL-shutter opens and closes and the shutter for transmitted light. Only one shutter control will be visible
4. Dichroic cubes allow to preview DAPI (blue), FITC (green) or RHOD (red) through the eyepieces by touching the appropriate buttons

Touchpanel, Objectives Tab



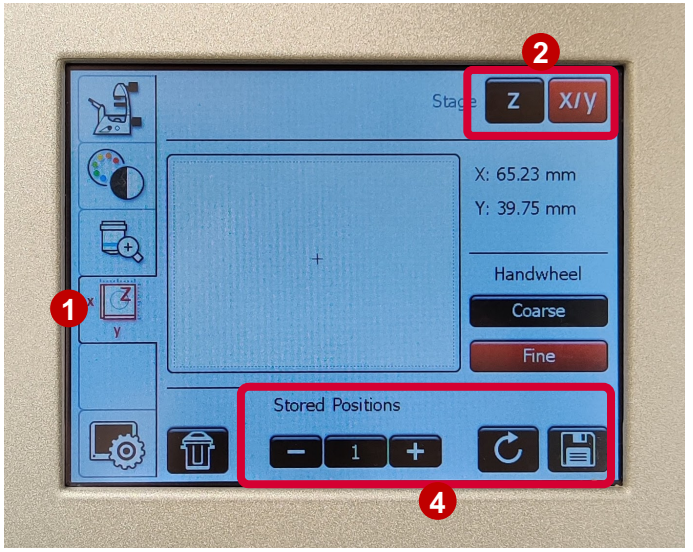
Available objective lenses:
10x, long working distance
20x, high NA ,short working distance
40x (oil immersion)
63x (oil immersion)
(sometimes) 40x (water)

1. **Objective tab:** This tab allows to switch between different objective lenses by selecting the appropriate lens
2. **Available lenses** fitted in the microscope.

Never switch from oil immersion lens (40x or 63x) directly to lower magnification dry lenses since a drop of oil will be left on the sample and smear on the objective.

If this happens let us know about it, so we can clean the lens. When you suspect any of the objective lenses to be dirty, let us know.

Touchpanel, Stage Tab



1. Positioning Tab
2. Switch between z and x/y positioning

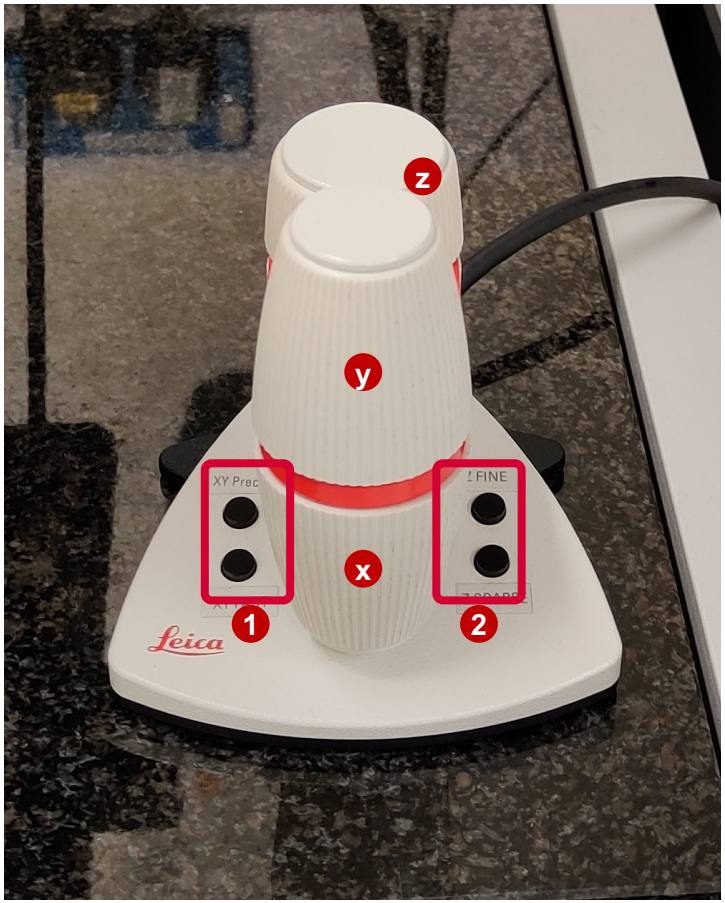
Z positioning of the sample

3. Line indicates the approximate z focus position for ‘normal’ mounting, glass bottom dish or microscope slide and adapts to the selected objective

X/y positioning of the sample

4. Allows to save and retrieve stored positions

XYZ controller

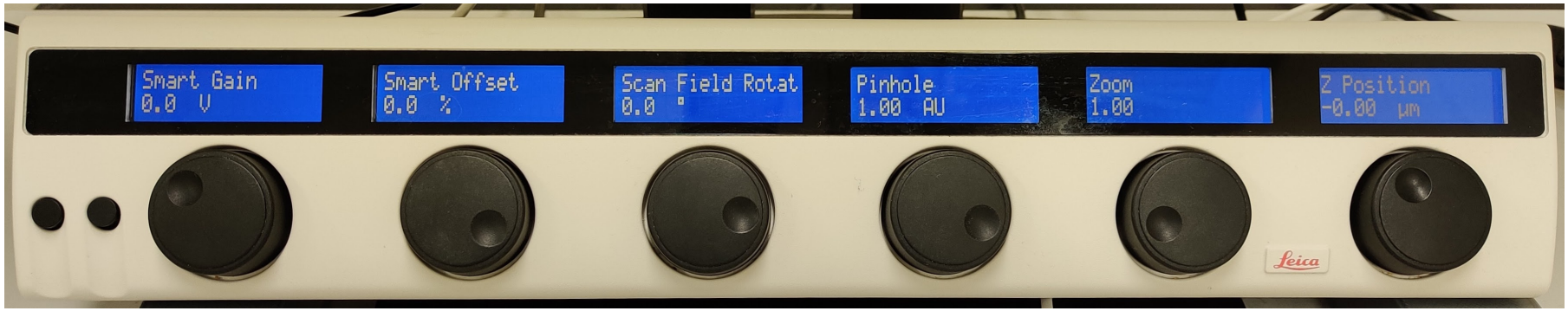


To move the stage in the X/Y/Z plane, the ‘salt and pepper’ controller can be used.

The top knob (Y) moves the stage in the Y plane and the bottom knob (X) moves the stage in the X plane. You can toggle between precise and fast by pressing the buttons 1 on the left side of the base.

The back knob controls the Z movement. You can toggle between coarse and fine control in the Z plane by using the buttons 2 on the base on the right side.

Controller Panel

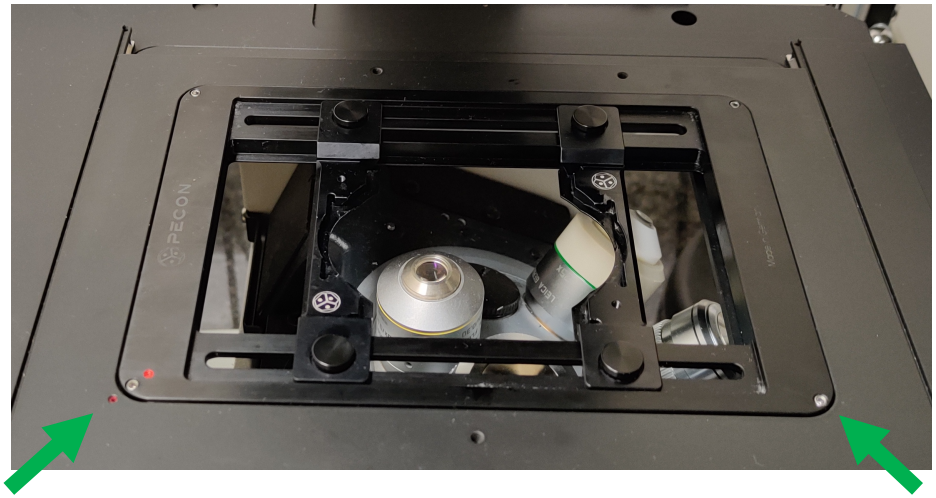


The controller panel allows to change some of the parameter for image acquisition.

All settings can also be made in the software. The standard configuration is:

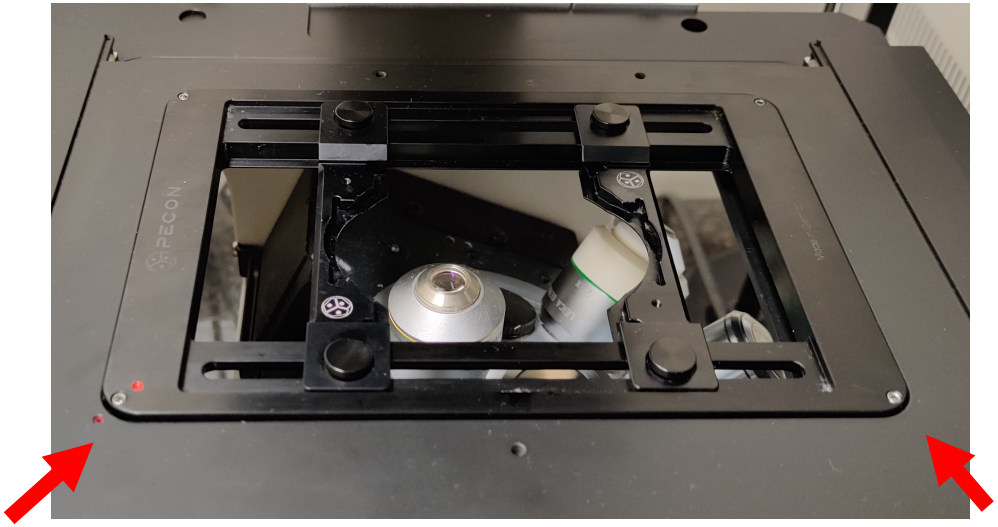
1. Gain of the selected detector
2. Scan filed rotation
3. Pinhole size in Airy Units
4. Acquisition Zoom
5. Focus position

Stage Insert



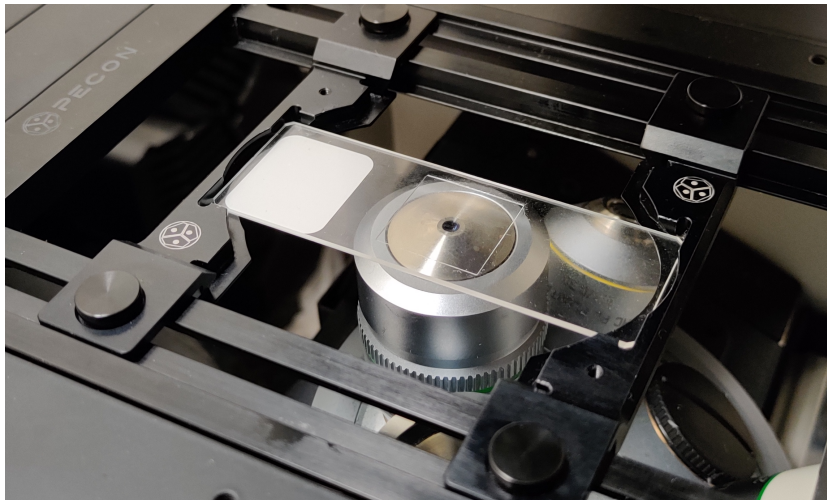
Make sure the stage insert is correctly inserted and sitting flat

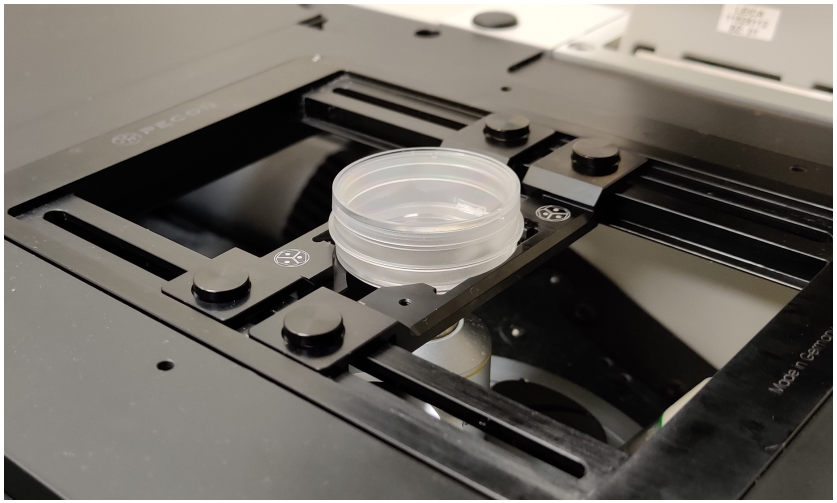
Stage insert **flat and stable**



Stage insert **not flat and shaky**

Placing the Specimen, Focusing Hints





These are inverted microscopes with the objectives below the specimen. If using a slide with attached coverslip, you must turn it upside down to image.

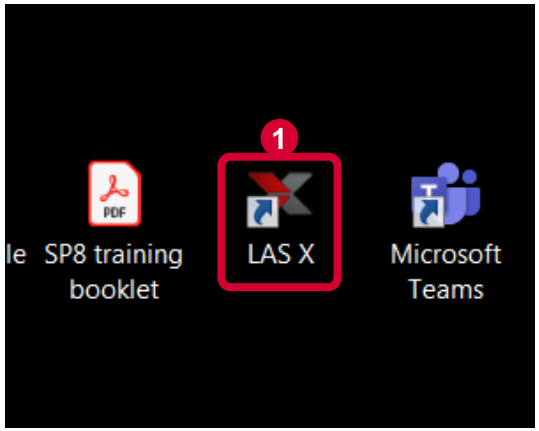
If using a plate or dish, the bottom thickness of the plate is critical. It must be a specialized glass-bottomed dish, ideally with a thickness of #1 or #1.5.

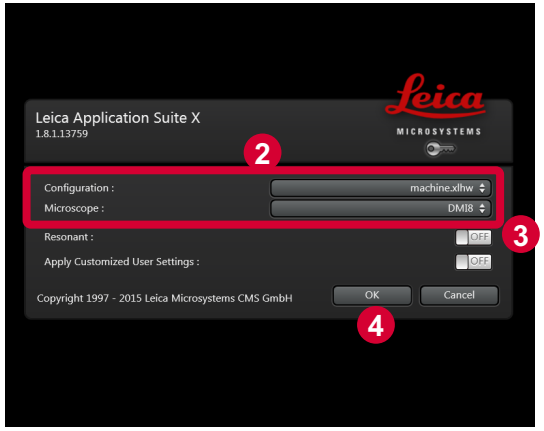
Use the objective's specific immersion media (oil or water). Use only one small drop of immersion.

Use the X and Y knobs of the stage controller to move the desired region directly over the objective.

Make sure the scan head is tilted all the way forward, otherwise you will see no image during the acquisition phase.

Software Startup, SP8 Advanced





Start the confocal software LAS X by double clicking the icon 1 on the desktop

Select the appropriate configuration and microscope: 2

machine.xlhw

DMI8

To use the resonant scanner activate the button 3

Turn off Apply Customized User Settings to start LAS X with the default settings.

Press OK an follow on-screen instructions 4

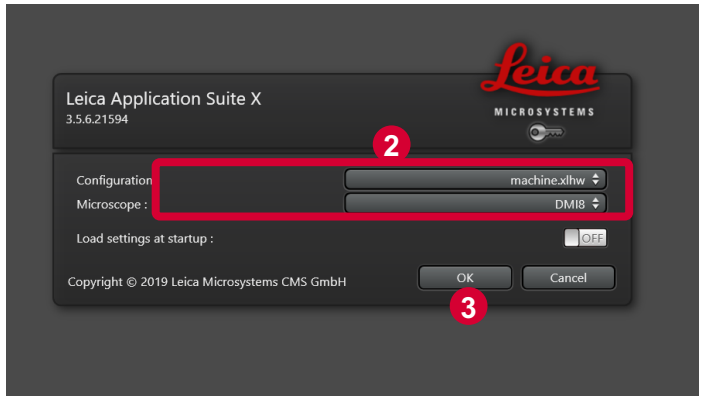
Software Startup, SP8 Core

Start the confocal software LAS X by double clicking the icon 1 on the desktop

Select the appropriate configuration and microscope:

machine.xlhw 2

DMI8



Turn off Apply Customized User Settings to start LAS X with the default settings.

Press ok an follow on-screen instructions 3

General Layout of the LASX software

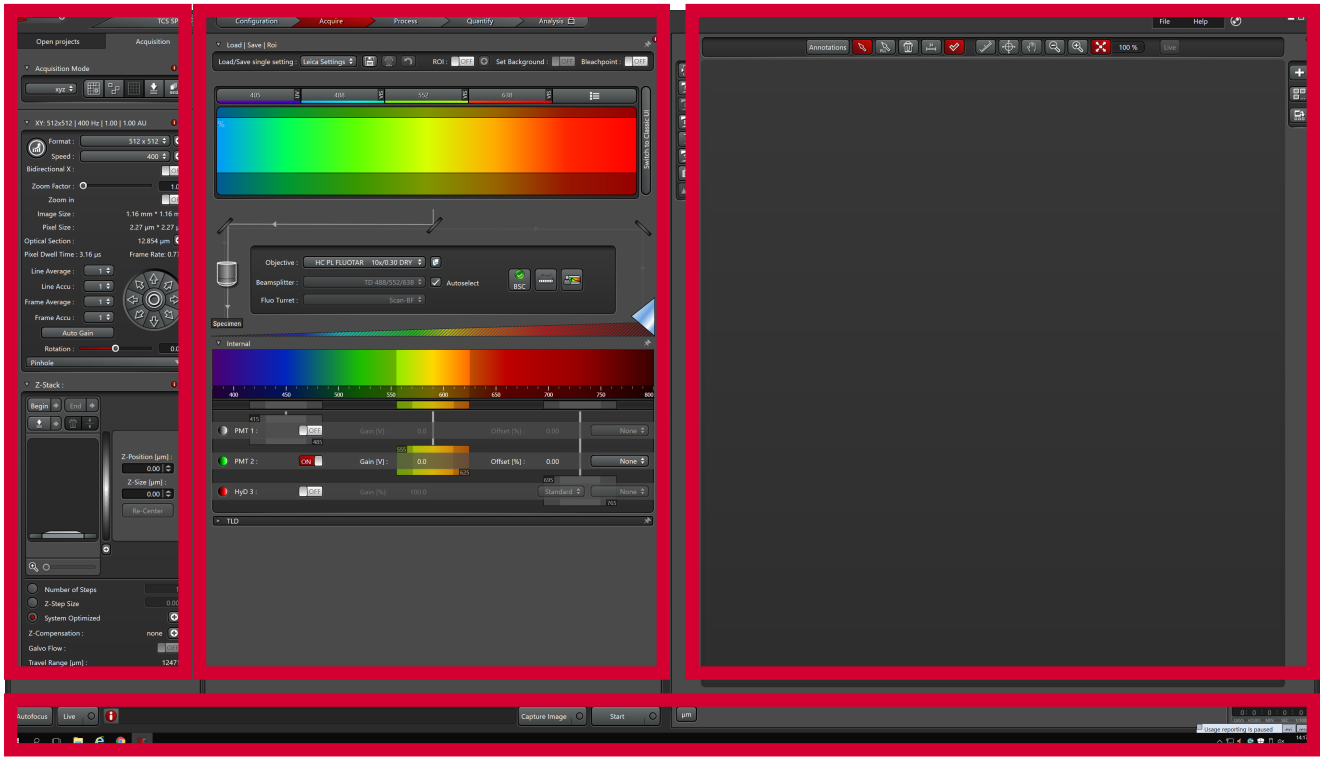


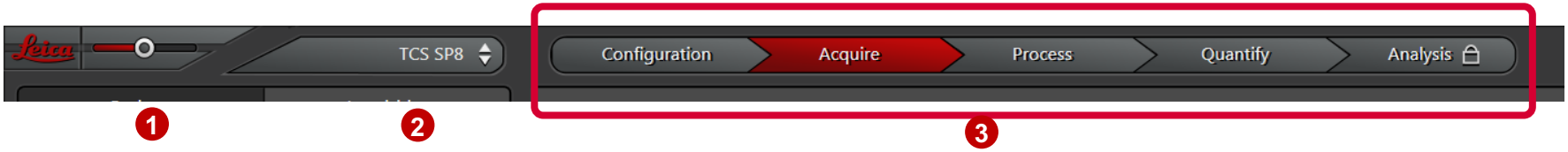
Image Acquisition Settings
These functions control the static acquisition settings for image collection. Settings such as Frame Size, Scan Speed, Averaging, Zoom …

Light Path Configuration
This area is where we set our beam path configuration and control our detector settings to optimize our fluorescence signal..

Image Display
This area is where the image will be displayed. Settings here allow the user to control how the image is viewed on screen.

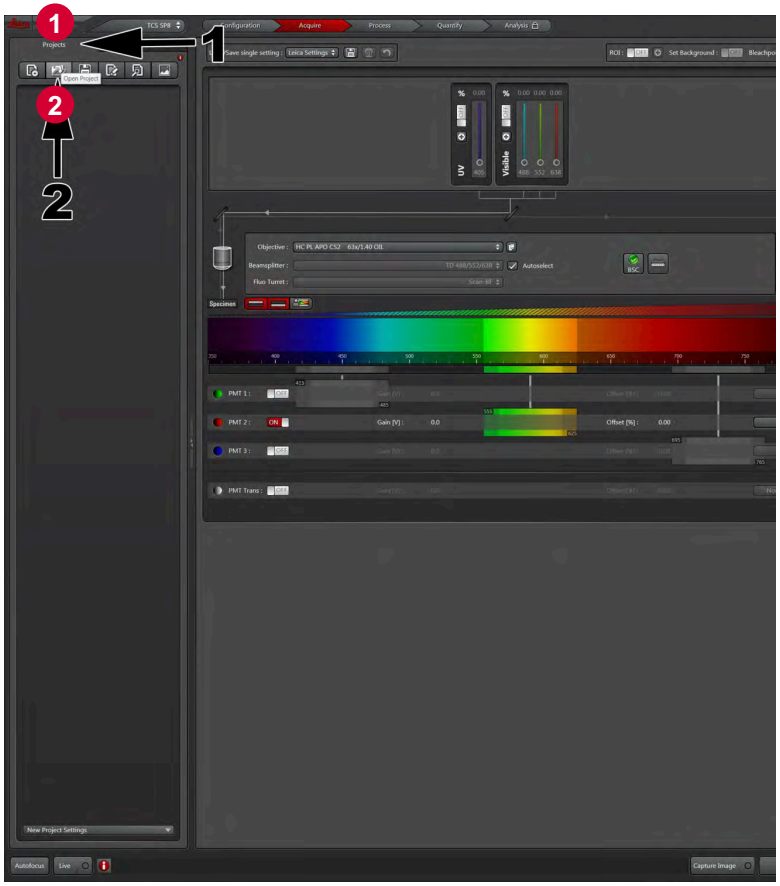
Scan Action Functions These functions start/sto scanning and initiate our experiment acquisition.

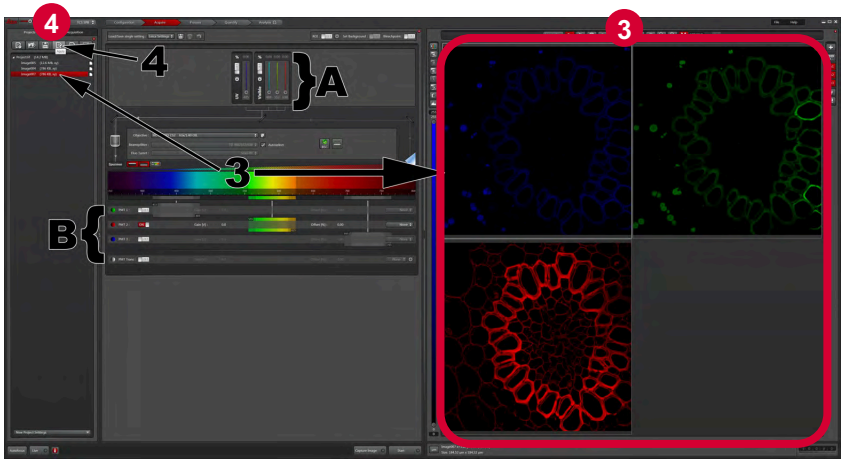
Top Banner



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

Use previous settings





The easiest way to set up the settings is to reuse the settings from a previous project.

Go to Projects tab 1

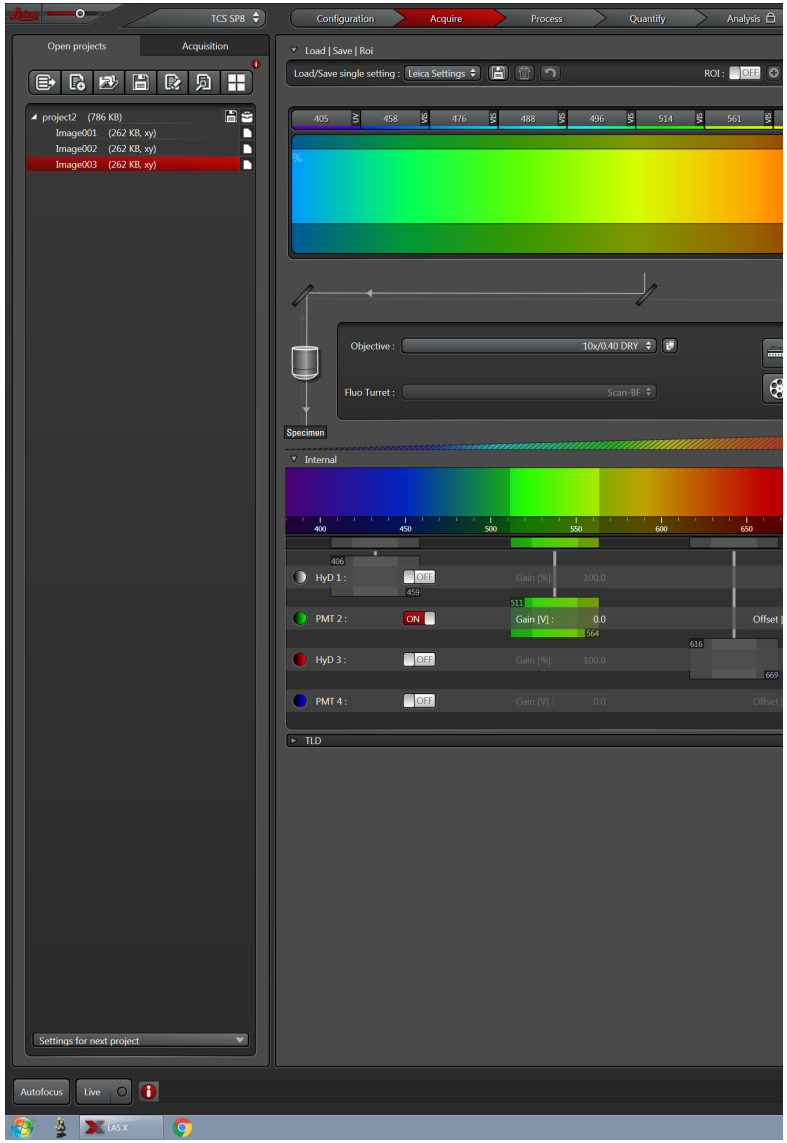
Click the open button 2 to load a previously collected file. Find your file containing a picture you have already captured and confirm.

Preview the pictures by clicking through the list. The preview of the picture can be seen on the right side of the screen 3

In order to reuse the settings from a picture, select the picture and then hit the apply button on top 4

The following settings will be copied:
Laser Power, detector and filter settings, gain, offset, pinhole and zoom factor will be set to the values from the saved image

Saving your data



Select the project and click the save Button or right click on the project name.

Always save as **.lif format** (Leica Image File). This format can be opened in Fiji & ImageJ (with BioFormats) and it will include all images from the project as well as meta data and **allows to reuse the settings from this file.**

To save individual images in various image formats (TIFF, PNG, …) right click on the image name and save in your desired file format.

Don't save scientific images as JPEG !!!

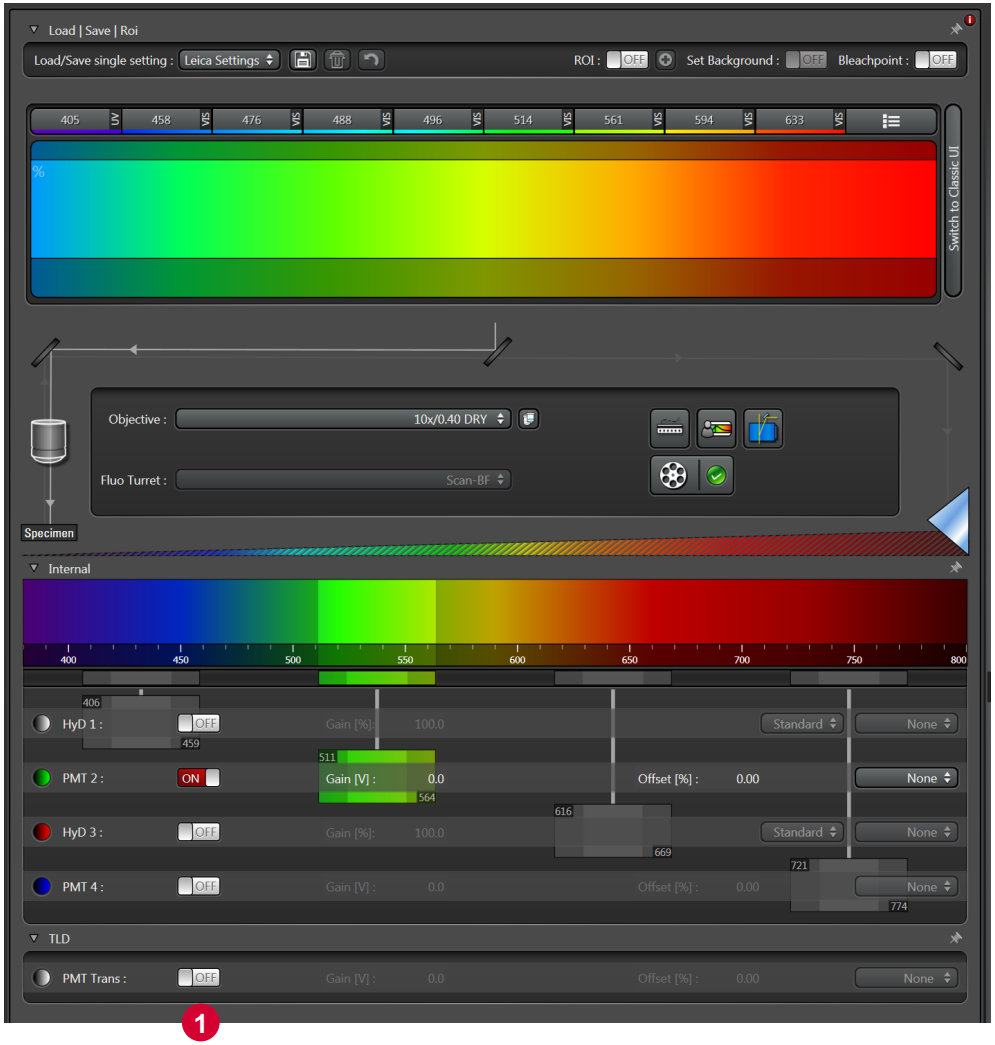
Save the data on the **local drive first** (D: or E:) before transferring them to your cloud storage. Create a folder for your group locally and store your data there.

No USB drives & sticks are allowed to be plugged in on the microscopy computer.

Then **copy your data to a cloud server of your choice (Gdrive, OneDrive, Dropbox, etc.)** or use the CAIC storage server. The CAIC storage server can be accessed from designated computer in the CAIC microscopy suite.

Data on the microscope computer is not backup, so make sure that important data is copied to another computer/server before you leave the computer.

Adding Transmitted Light Image



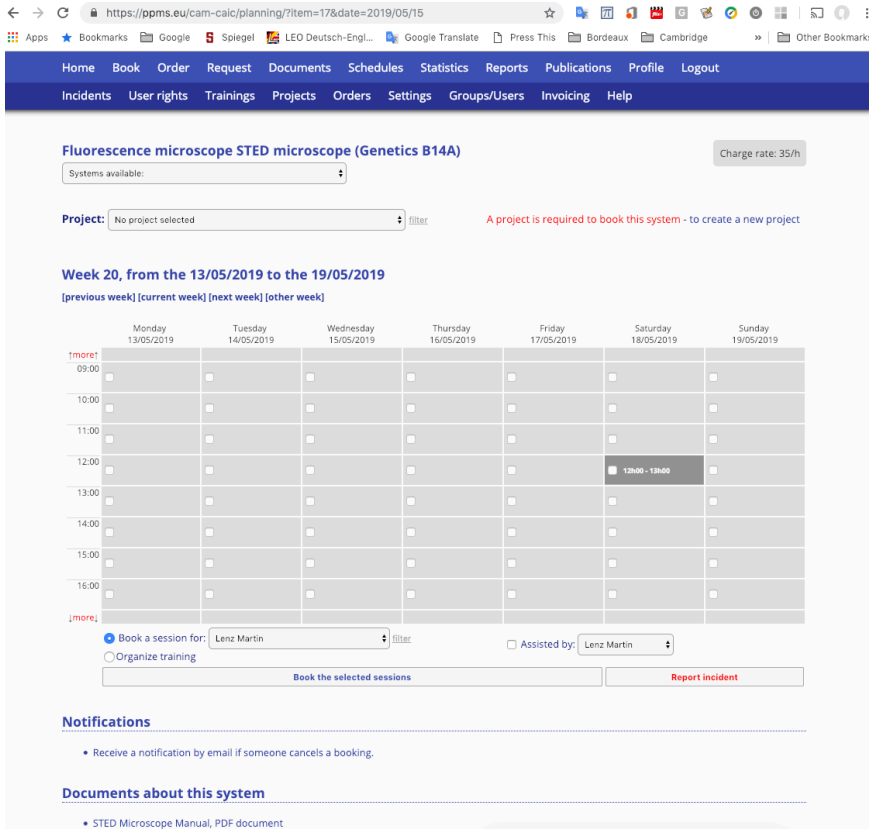
To add a transmitted light image to your acquisition turn on the PMT Trans detector 1 in one of your sequences.

If you are using the 488 nm laser choose this sequence.

Once PMT Trans is turned on, when you begin a LIVE scan, you should see an additional image box appear on the right with your other colours.

Adjust the Gain the same way you would for the other channels.

Ending your imaging session



Log into the PPMS system, select the appropriate web calendar and check when the next user is booked on the microscope.

For the microscope:

If the next user is booked on the same day, leave the microscope in standby mode.

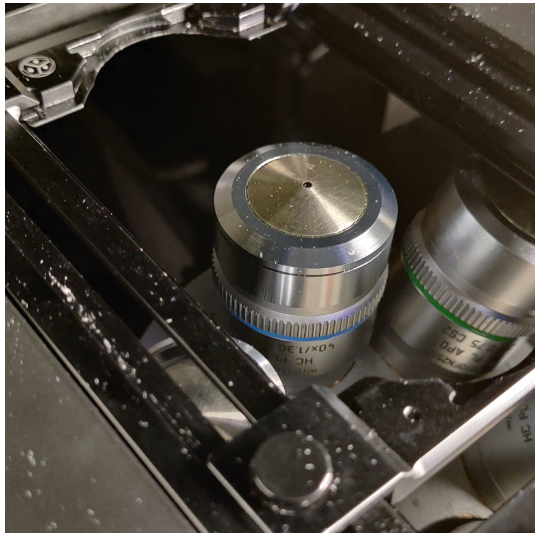
If the there is no other user booked for the rest of the day, shutdown the microscope completely.

For the fluorescence lamp:

If the next user on the microscope is booked within 1 hour, leave the lamp switched on.

If no user has booked the microscope within 1 hour turn the fluorescent lamp off.

Oil objectives

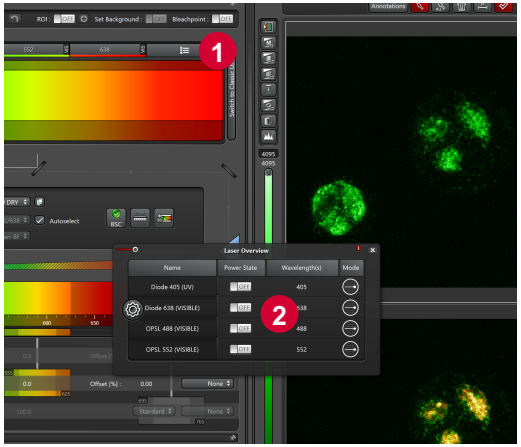


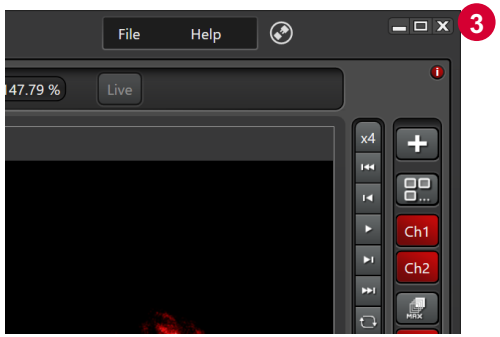
This only applies to the oil and water immersion objectives !

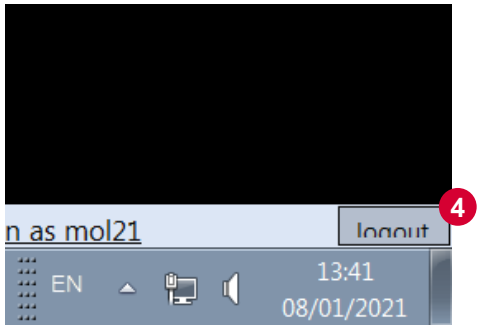
At the end of your imaging session please remove any excess oil from the objectives using lens tissue in a single wipe.

The objective lens does not need to be cleaned, just excess oil/water removed.

Stand-by procedure, SP8 Core







Make sure the 10x objective is selected and facing upward.

Open the laser control panel ❶ and turn off the lasers ❷

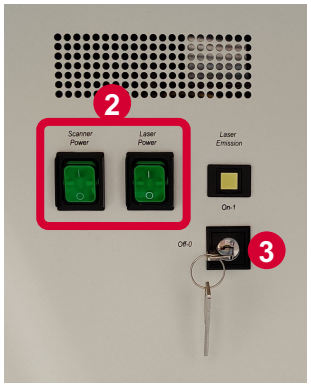
Close the LASX software by closing the window ❸

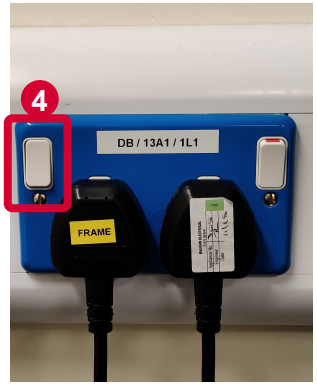
Log out of the PPMS system ❹ (bottom right)

Depending on when the next user is booked, either leave the epi-fluorescent lamp on or off (see page before)

The rest of the hardware stays switched on

Shut down procedure, SP8 core





Before turning off the microscope make sure the 10x objective is selected and facing upward.

Follow steps 1 -3 from standby procedure

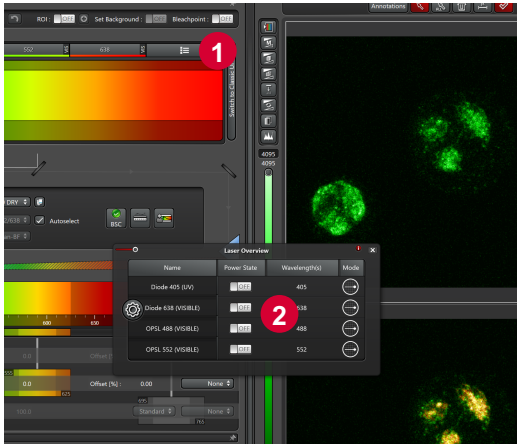
Shut down workstation.

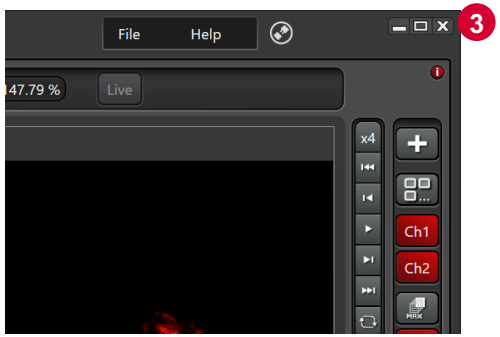
Turn off the power switch ❶ for epifluorescence light source if necessary (see page ‘Ending your session’).

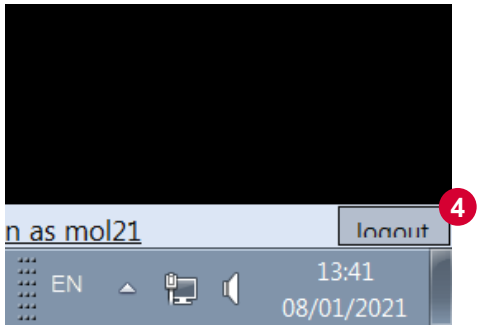
Turn off Laser Power and Scanner Power Switches (❷ green switches) then turn Laser Key ❸ to off state on the large unit under the table.

Turn off microscope stand with the wall switch ❹.

Stand-by procedure, SP8 Advanced







Make sure the 10x objective is selected and facing upward.

Open the laser control panel 1

Set the Argon-Ion laser power to 0 and turn off all the lasers. 2

Close the LASX software by closing the window 3

Log out of the PPMS system 4 (bottom right)

Depending on when the next user is booked, either leave the epi-fluorescent lamp on or off (see page' Ending your imaging session')

The rest of the hardware stays switched on

Shut down procedure, SP8 Advanced

Before Turning off the microscope make sure the 10x objective is selected and facing upward

Follow steps 1 -3 from standby procedure

Shut down workstation (normal Windows shutdown)

Turn off the power switch ❶ for epifluorescence light source if necessary (see page ‘Ending your session’).

When the computer is off, turn off Laser Power, Scanner Power Switches, and PC Microscope (❷ green switches) then turn Laser Key ❸ to off state.