Leica SP8 (Balgrist Campus) - 1: Start up

How to start up Leica SP8 confocal laserscanning microscope located at the Balgrist Campus, Room Microscopy 3 W.112

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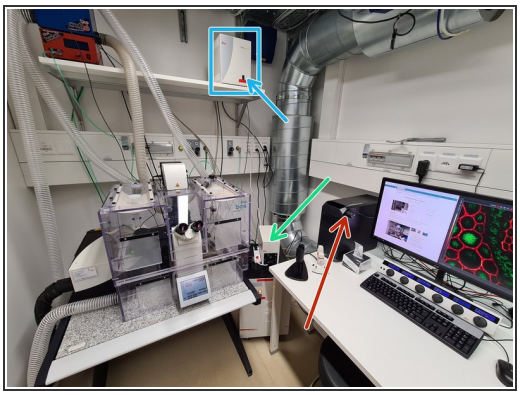
INTRODUCTION

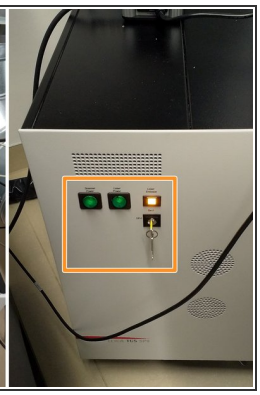
How to start up and mount your first sample on the Leica SP8 confocal laser scanning microscope located at the Balgrist Campus, Room Microscopy 3 W.112.

If you are not familiar with the location at Balgrist Campus, read How to get to the microscope at Balgrist Campus.

Please find detailed information about the system setup here.

Step 1 — Switching ON Hardware






⚠ The microscope stand is always on for stability reason. Never turn it off!

- *The corresponding microscope control box can be found on the shelf above the microscope.*

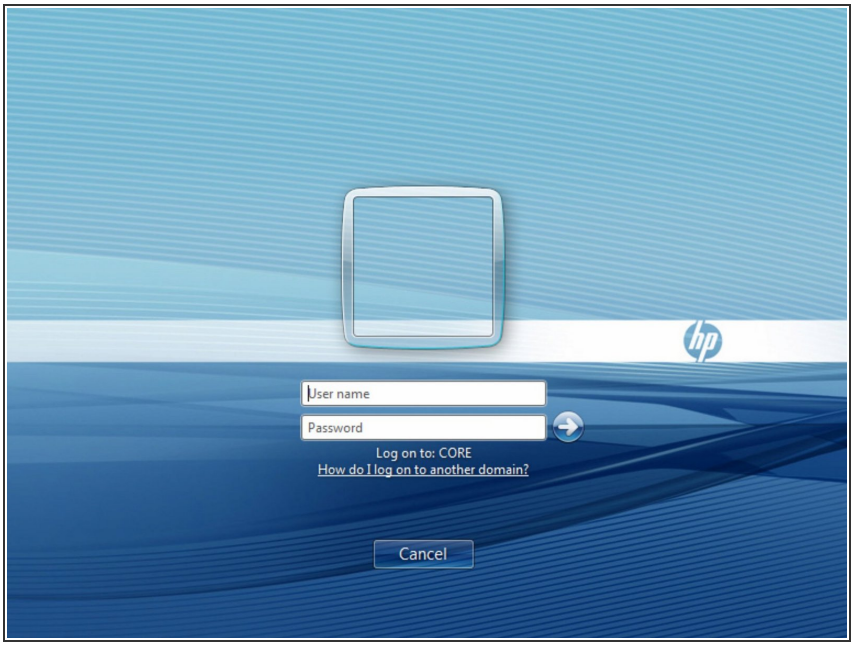
- Switch ON the **fluorescence lamp**.

- Press the **main power switch** mounted with a magnet on the top of the computer.

ⓘ *PC, scanner, lasers will be switched on. If not,*

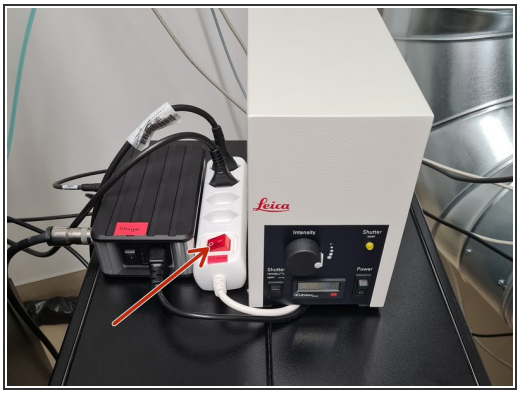
- check on the control unit that the "Scanner Power" and "Laser Power" are switched on, and the "Laser Emission" key turned to "On-1".

Step 2 — Sign-in



ⓘ **Sign-in** with your **ZMB core** credentials.

Step 3 — Optional - Only for live cell imaging and environmental control

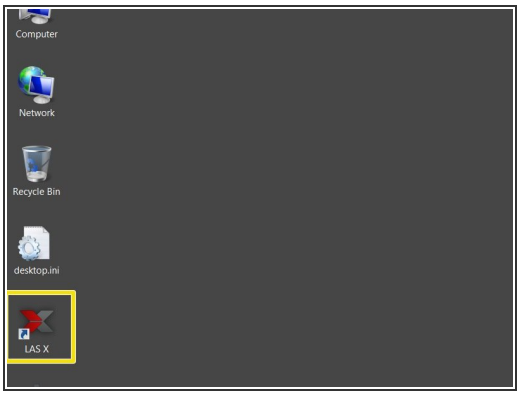



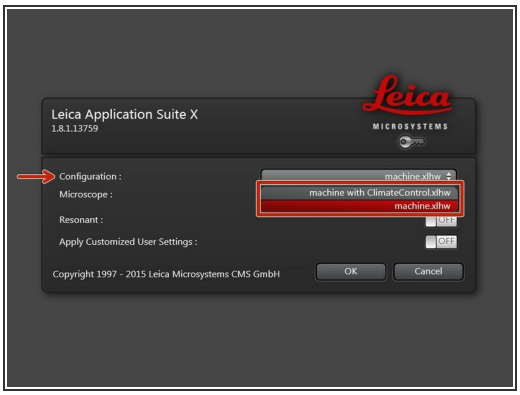
If you would like to perform live cell imaging, please refer to the dedicated guide for more detailed instruction.

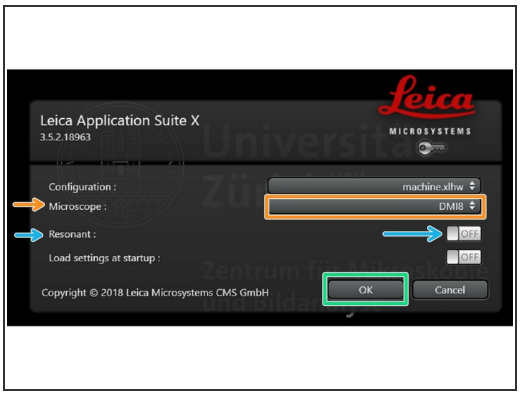
- In brief, Turn ON the power switch.
  - Cube (temperature) and brick (CO2) are already turned on.

⚠ 37° C and 5% CO2 are set as a standard. If you need to change it, please set it back to 37° C and 5% CO2 at the end of your experiment!

Step 4 — Starting up the "LAS X" software

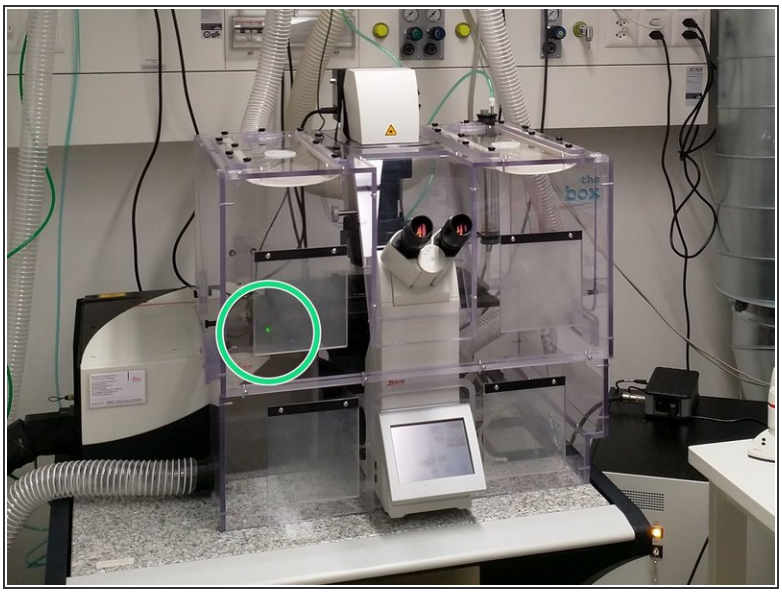


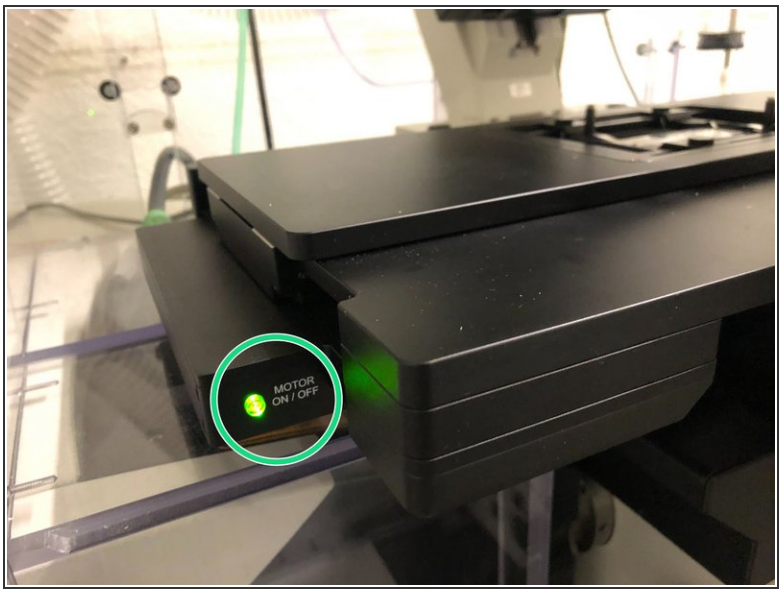




- Start the **"LAS X"** software.
- Select the appropriate **"Configuration"**.
  - "machine with ClimateControl.xlhw" if environmental control is applied and needs to be logged via the software. *Make sure the needed components have been switched on (see dedicated guide for live cell imaging).*
  - "machine.xlhw" for standard room temperature (RT) measurements. *Please note, environmental control can be also applied here (however, here temperature and CO2 will be not logged via software).*
- Make sure **"DMI8"** is selected as **"Microscope"**.
- Select either "Resonant" (ON) or non-"Resonant" (OFF) scanning mode.
- Click **"OK"**.

Step 5 — X,Y - Stage



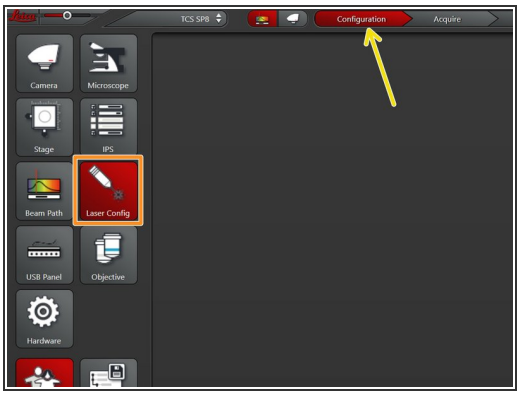


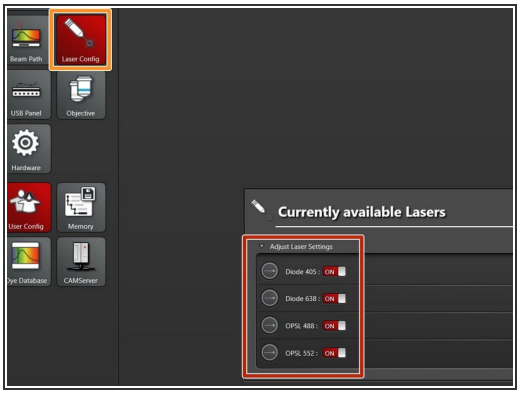
Please note: The x/y stage at this microscope does not need any initialization to function!

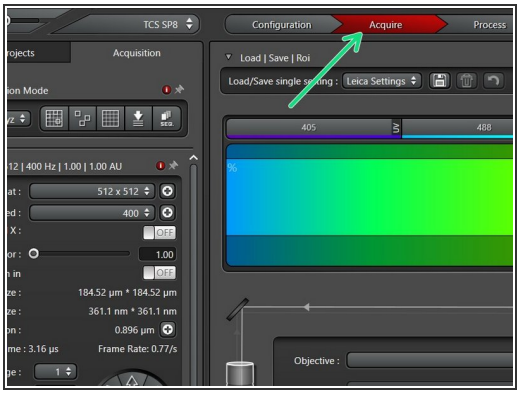
- The **indicator light** "MOTOR ON/OFF" at the stage (left hand side) must be **green** continuously.

⚠ **Flashing green light** at the stage indicates the stage was disengaged by touching or manual movement. Press the green light button to activate it again.

Step 6 — Switch ON the lasers in the software

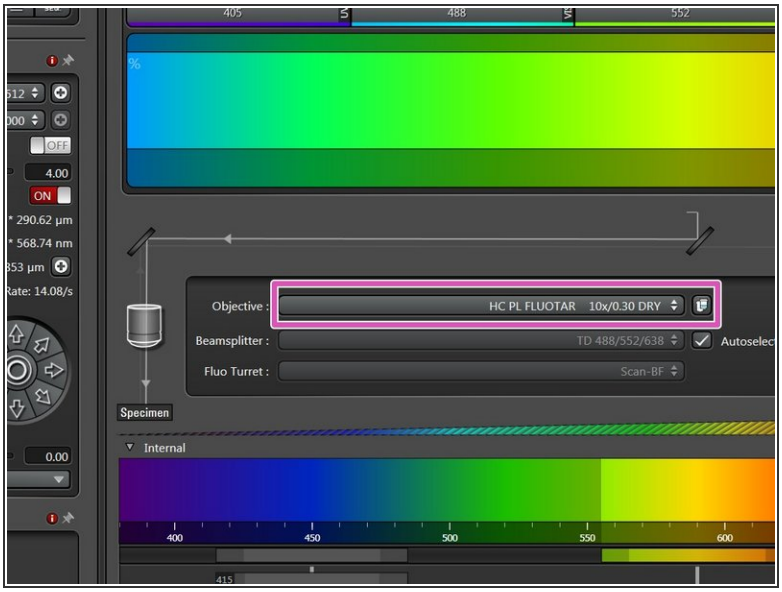






- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you will need.
- Go back to **"Acquire"**.

Step 7 — Choosing an objective



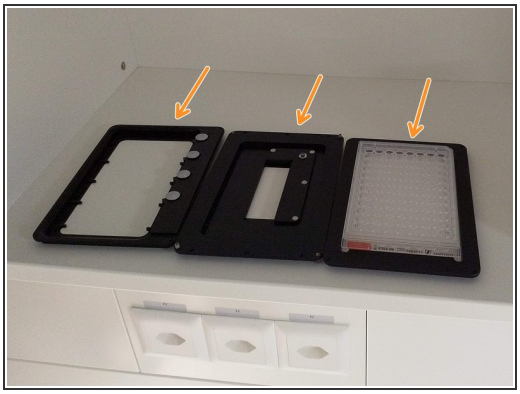
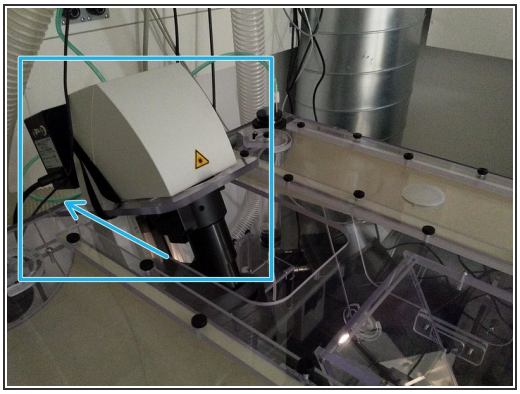
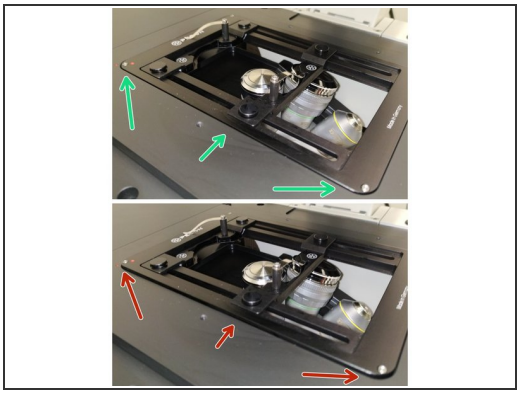
- **Lower** the **objective turret** by pressing the downwards "Z" button on the right side of the microscope.

⚠ This is a **mandatory step** as it avoids possible collision of the objectives and stage during exchange of inserts and/or samples.

- You can now toggle between objectives within the software (drop-down menu).
- Select the **10x dry objective**.

ⓘ *In order to facilitate the focusing it is recommended to start with the 10x dry objective.*

Step 8 — Installing the appropriate sample holder

Usually, a **universal insert** for slides and petridishes is already installed.

- Additional **stage inserts** can be found in the cupboard.

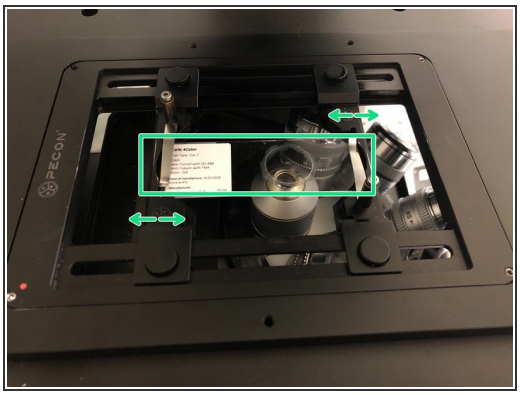
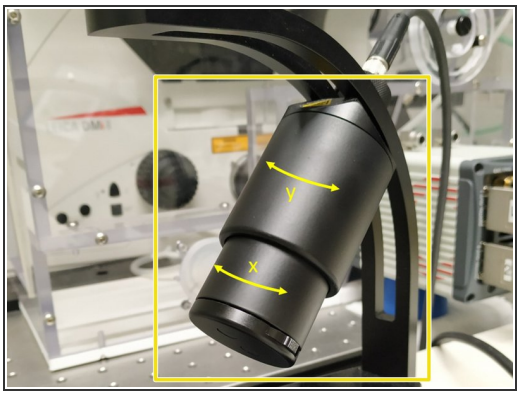
- **Push** the condenser arm of the microscope to the **back** and, if necessary install a different stage insert.

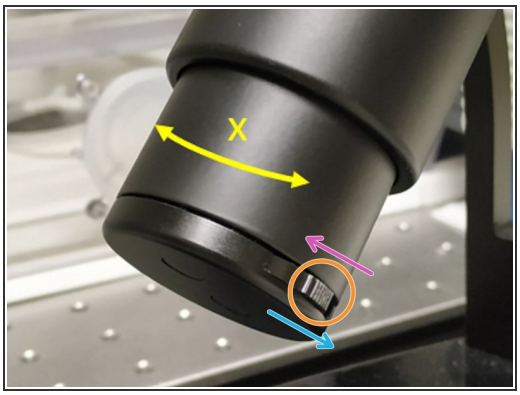
Make sure that the stage insert is correctly inserted and flat.

- Here correct and flat.
- Here not inserted correctly (stage not flat and shaky).

The stage might disengage while installing the stage insert. Press the flashing green light button again as already mentioned previously.

Step 9 — Mount and position your sample



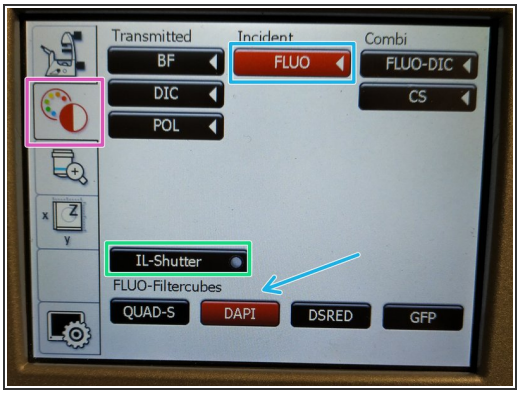
- Insert your sample with the **coverslip facing down**.

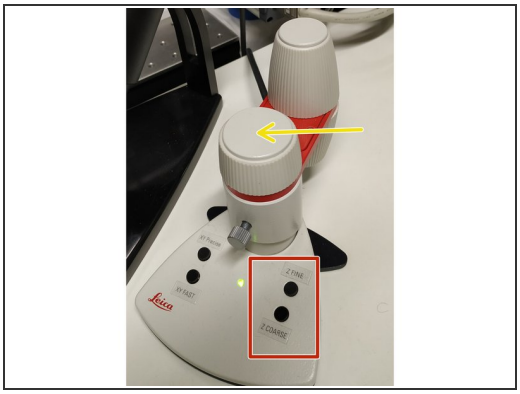
  Adjust the variable clamping range and moveable brackets to properly fix your sample.

- **Move** your sample over the objective with the help of the **Joystick**.
- With the little **slider knobs** on both sides of the lower wheel you can change between fast and slow movement.
  - Fast movement - pressing the knobs downwards.
  - Slow movement - pulling the knobs towards you.

Bring back the condenser arm to its straight position.

Step 10 — Focus your sample



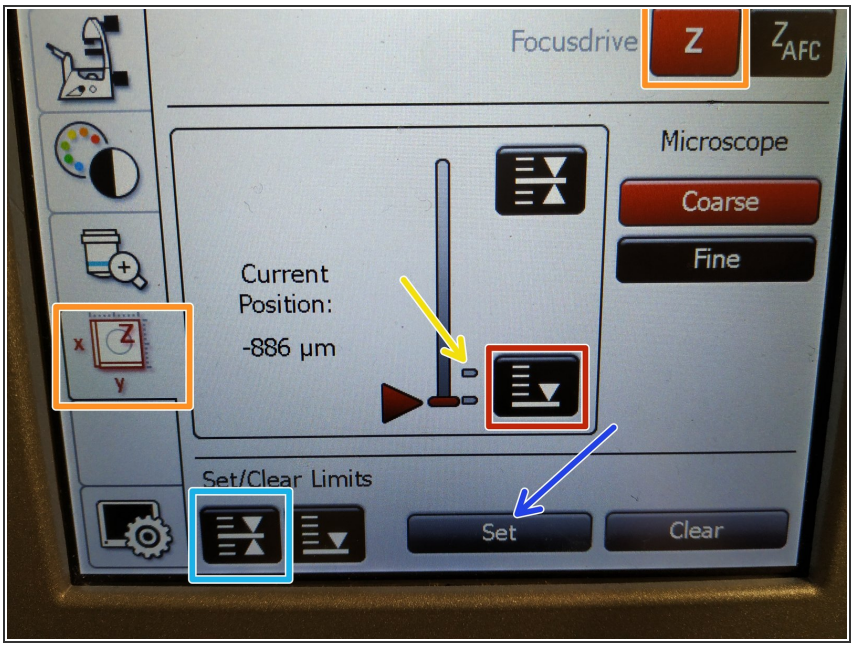


- On the touch screen at the microscope choose the **light path tab**.
- Click **"FLUO"** and choose an appropriate "FLUO-Filtercube" e.g. "DAPI".
- **Open** the **"IL-Shutter"** (if actived the dot is yellow).
- Look through the oculars and **focus your sample** by using:
  - the **focus wheel** on the microscope stand,
  - or the z-wheel on the **external controller (Smart Move)**.

  Moving objectives upwards (towards sample) turn z-wheels clockwise/away from you. Moving objectives downwards (away from sample) turn z-wheels counter-clockwise/towards you.

  - Toggle between "Z FINE" and "Z COARSE" directly on the Smart Move.

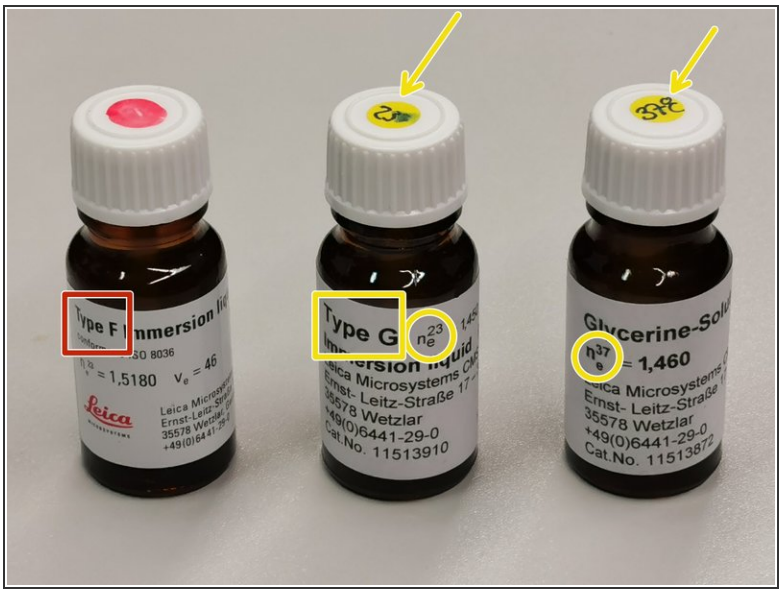
Step 11 — Optional - Save your focus position

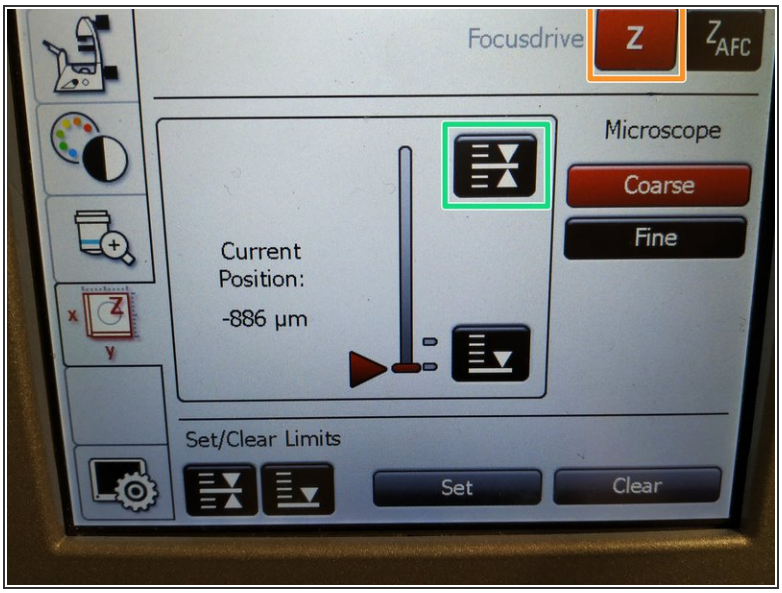


The storage of the focal plane is helpful in order to find the focus back if the sample or objective will be changed.

- To save your current focus position select the **"xyz tab"** and the **"Focusdrive Z"** on the touchscreen of the microscope.
- Click the **"Upper Focus Limit"** button.
- Press **"Set"**.
  - If done successful you will see an upper marker line appearing.
- Press the **"Lower Limit"** button in order to move down *(for safe change of the objective or the sample)*.

Step 12 — Switching to a higher magnification





- Remove your sample and toggle within the software to the objective of choice.

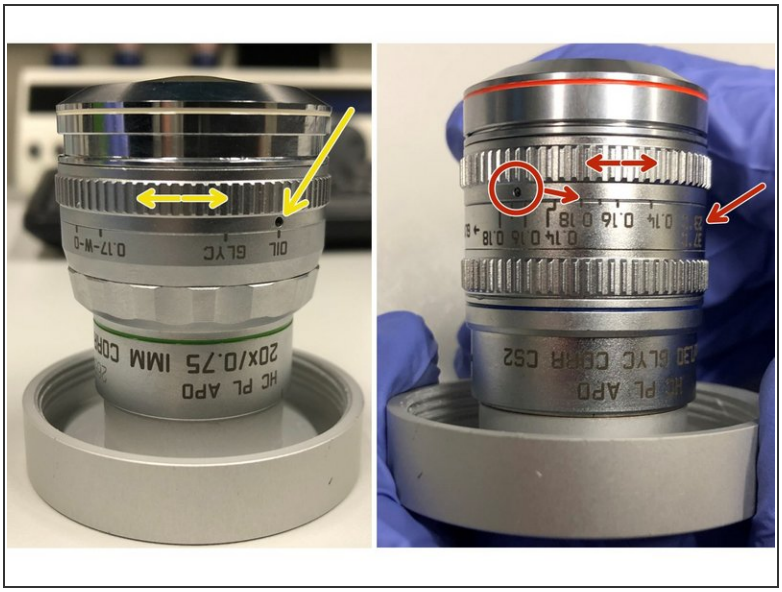
ⓘ Depending on the objective different **immersion media** will be used. Apply either on the sample or directly to the objective.

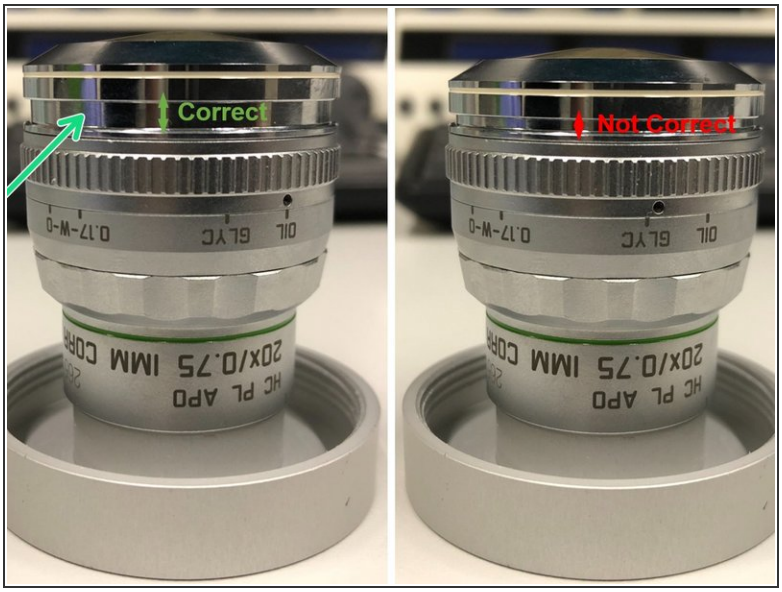
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: either *"Type-G" immersion liquid* (for RT measurements) or *"Glycerin" immersion liquid* (for measurements at 37°C).
- "Water" objectives: *ddH2O* (always use fresh).

⚠ Please further consider the additional information in the next step to guaranty proper image acquisition.

- **Mount** your sample again and press the **"Upper Focus Limit"** button.
- Focus your sample as described previously.

Step 13 — Additional Information - Immersion objectives





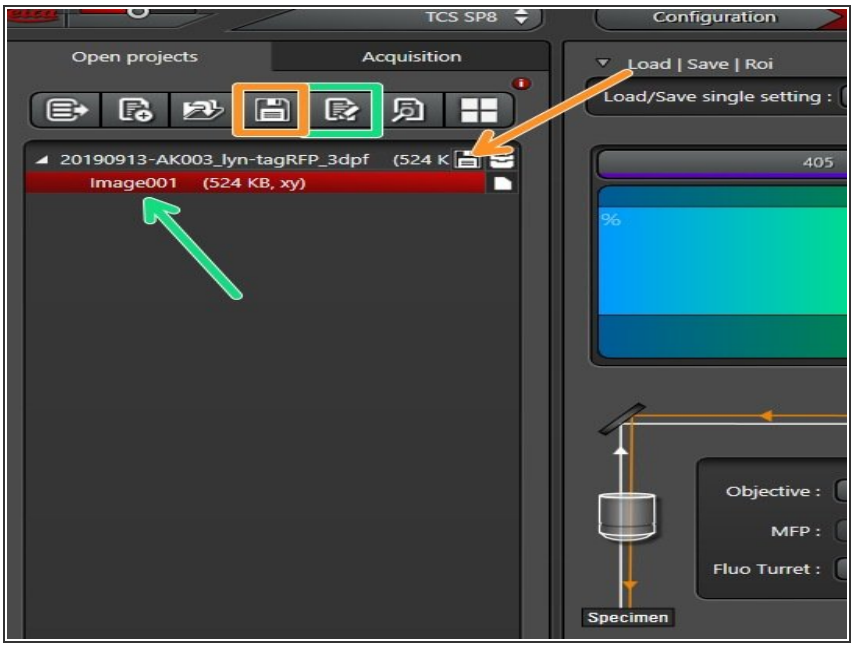
⚠ For some objectives the **correction collar** has to be adjusted.

- **20x IMM** (multi-immersion - Oil, Glycerin or Water) needs to be set to the corresponding **immersion media** ("OIL", "GLYC" or "0.17-W" (with cover glass) or "W-0" (without cover glass)).
- **63x glycerol** you can correct for the **cover glass thickness** (0.14-0.18 mm). Standard is usually 0.17 mm.

- Make sure that the cap of the **spring-loaded front lens** is released (working position).

⚠ Please, DO NOT remove the objectives for adjustment. They can be also accessed on the system.

Step 14 — Storing your data



Update: System connected to Network again.

- Please save your data from now again on your core storage (network path: \\files.core.uzh.ch\).

Please follow the instructions on our webpage how to access your data.

- Imaging settings: you can load settings from already acquired/stored data.