Leica SP8 inverse (IPZ) - Part 1: Start-up

How to start up Leica SP8 confocal laser scanning microscope located at the IPZ at Institute of Parasitology, Room PV-10.55.

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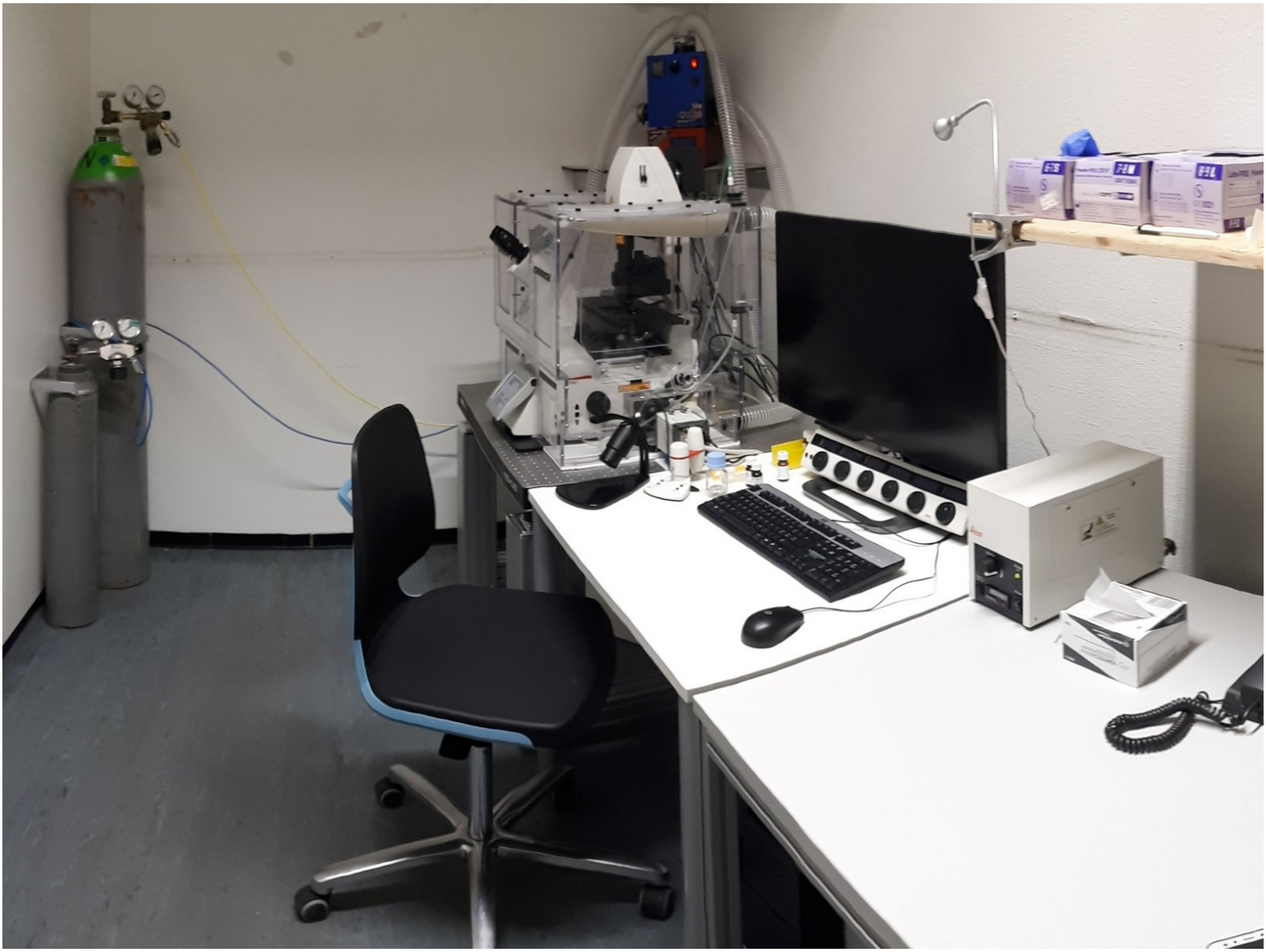

INTRODUCTION

How to start up and mount your first sample on the Leica SP8 confocal laser scanning microscope located at the IPZ at Institute of Parasitology, Room PV-10.55.

Please find detailed information about the system setup here.

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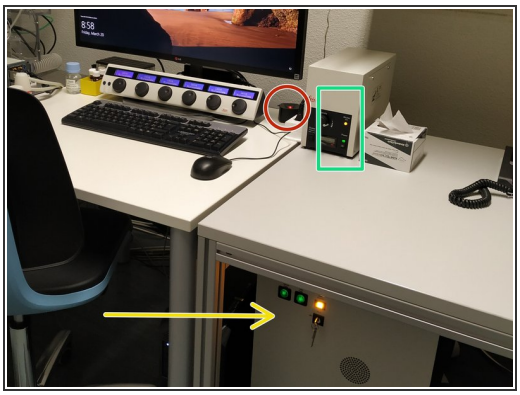
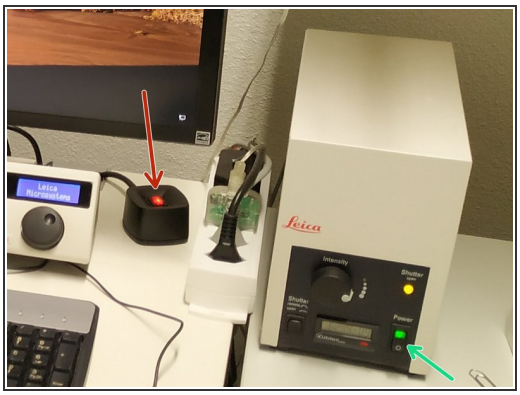
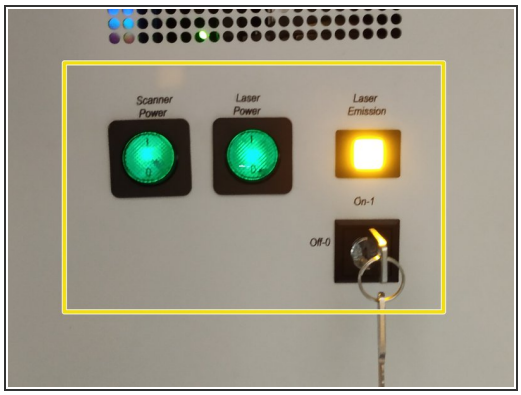
Step 1 — Switching ON Hardware





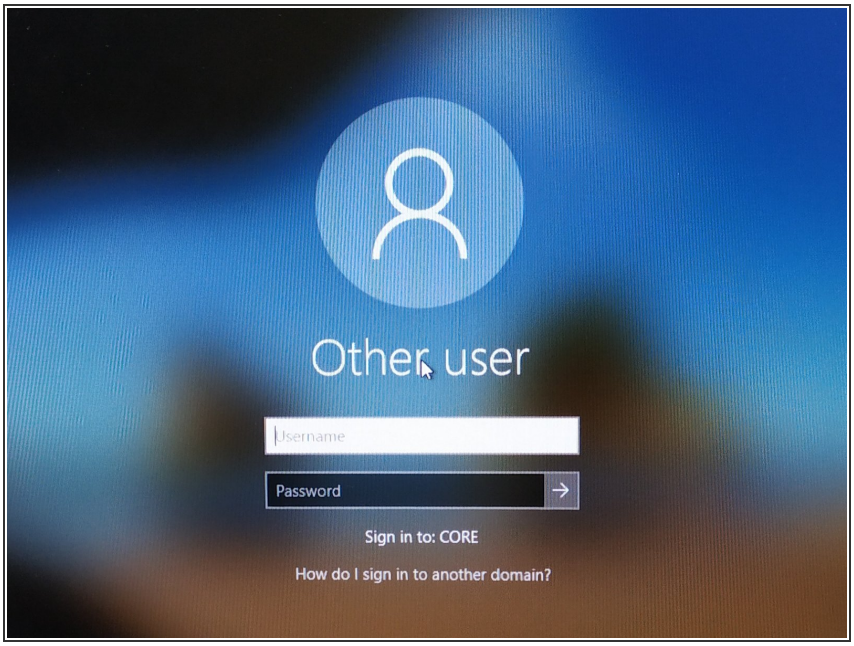


⚠ The microscope stand is always on for stability reasons. Never turn it off.

ⓘ *The corresponding microscope control box can be found underneath the microscope table.*

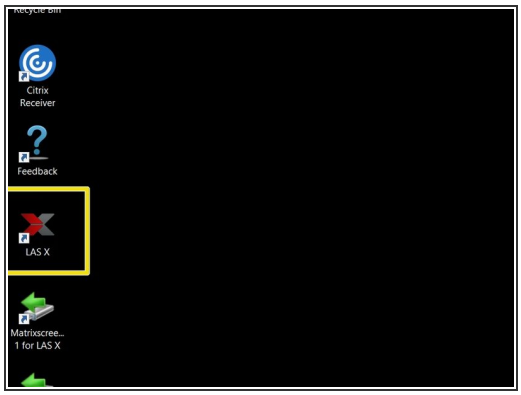
- Switch ON the **fluorescence lamp**.
- Switch ON the **"Scanner Power"**, **"Laser Power"**, and turn the **"Laser Emission" key** to "On-1" (control unit underneath the table).
- Switch ON the **power knob** (on the PC table).

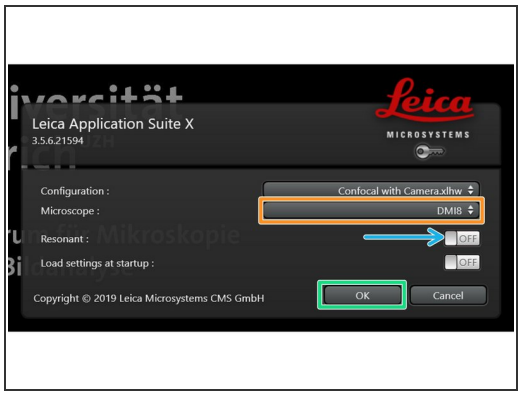
Step 2 — Sign-in



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

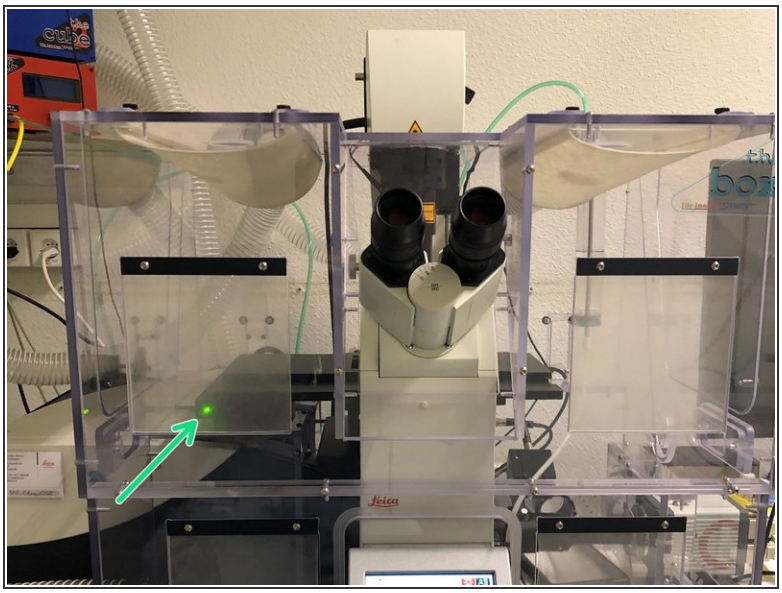
Step 3 — Starting up the "LAS X" Software

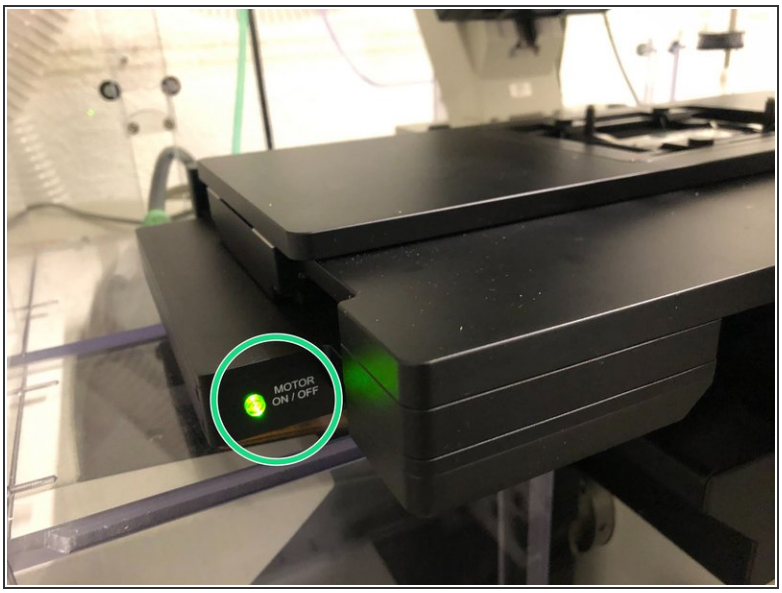




- Start the **"LAS X"** software.
- Select the appropriate **"Configuration"**.
  - "Confocal with Camera and Incubator.xlhw" if environmental control is needed. *Make sure the needed components have been switched on.*
  - "Confocal with Camera.xlhw" for standard room temperatur (RT) measurements.
  - "DefaultDynamicWidefieldTree.xlhw" for only widefield/camera option.
- Make sure "DMI8" is selected as **"Microscope".**
- Select either "Resonant" (ON) or non-"Resonant" (OFF) scanning mode.
- Click **"OK".**

Step 4 — X,Y - Stage



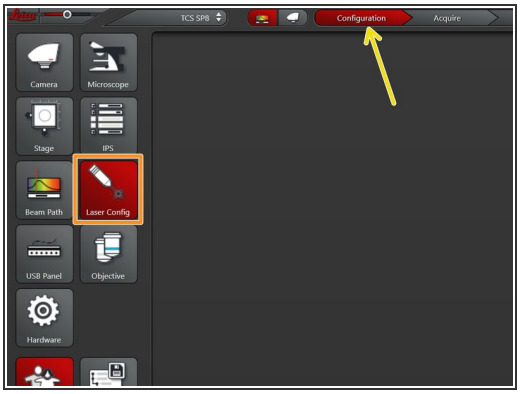


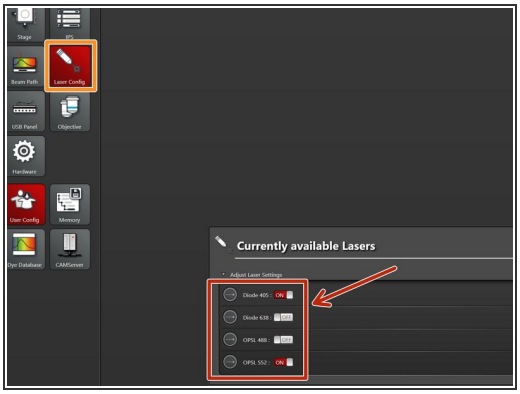
Please note: the x/y stage at this microscope does not need any initialization to function!

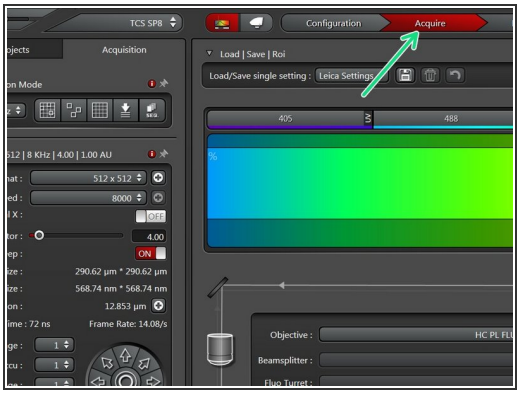
- The **indicator light** "MOTOR ON/OFF" at the stage (left hand side) must be **green** continuously.

  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 5 — Switch ON the lasers in the software

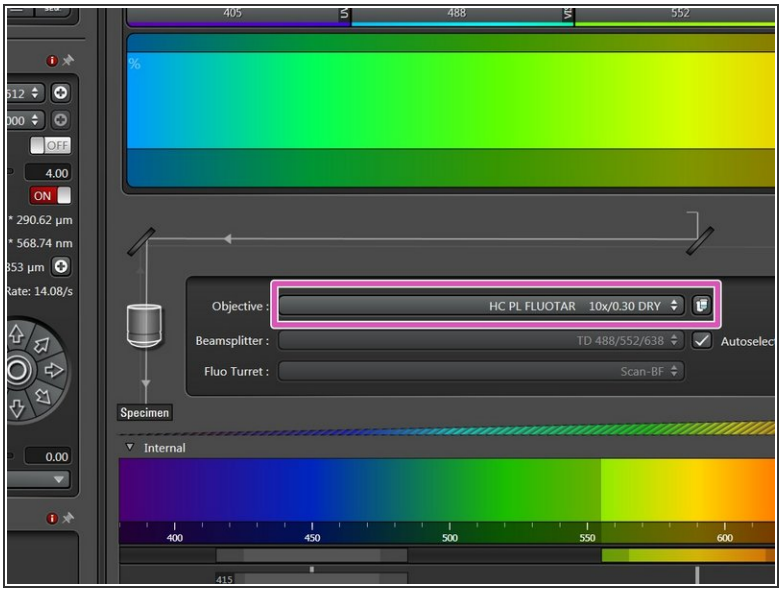






- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you will need.
- Go back to **"Acquire"**.

Step 6 — Choosing an objective



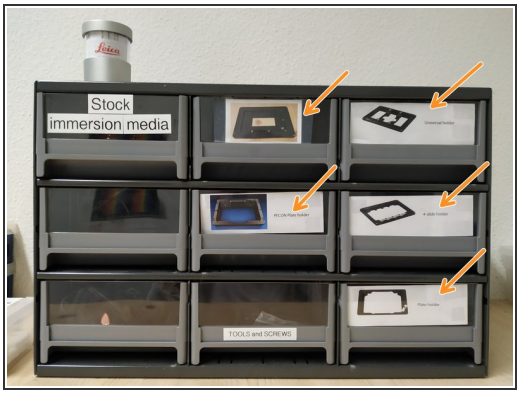
- **Lower** the **objective turret** by pressing the downwards "Z" button on the right side of the microscope.

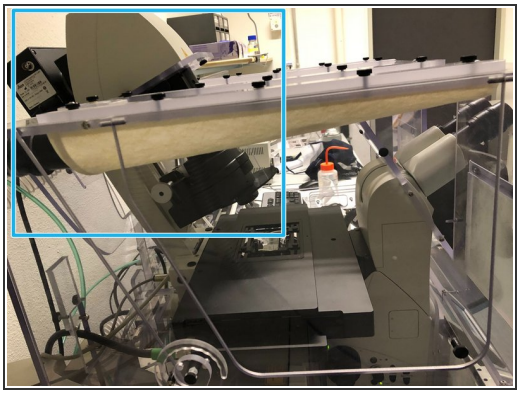
⚠ This is a **mandatory step** as it avoids possible collision of the objectives and stage during exchange of inserts and/or samples.

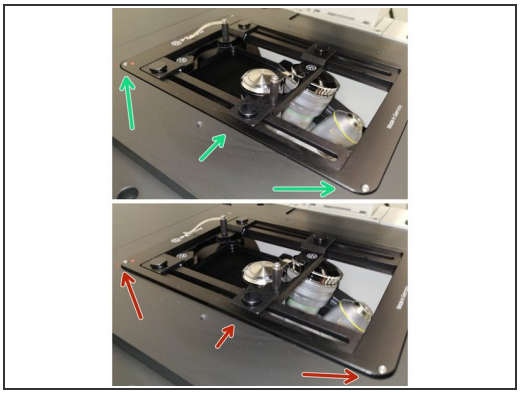
- You can now toggle between objectives within the software (drop-down menu).
- Select the **10x dry objective**.

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

Step 7 — Installing the appropriate sample holder







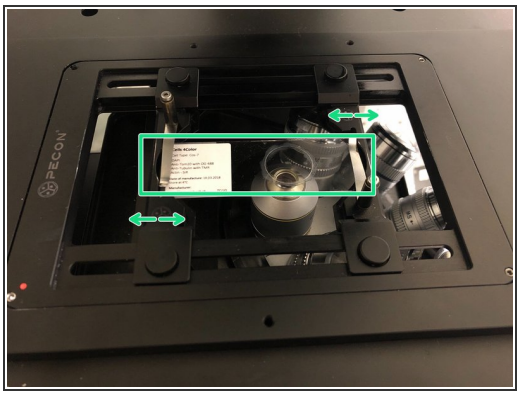
- You will find different **stage inserts** in the box on the shelf.
- **Push** the condensor arm of the microscope to the **back** and install the chosen sample holder.

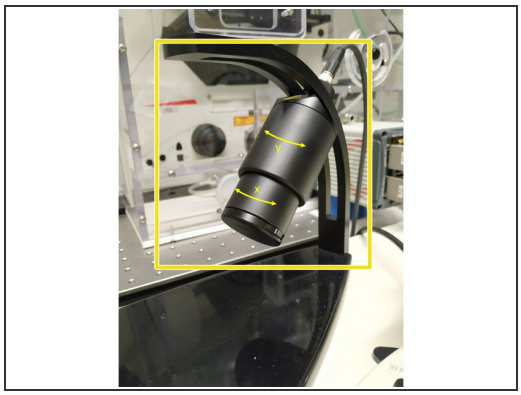
⚠ Make sure that the stage insert is correctly inserted and flat.

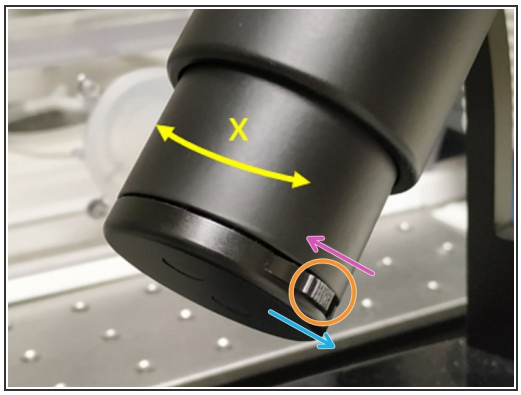
- Here correct and flat.
- Here not inserted correctly (stage not flat and shaky).

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

Step 8 — Mount and position your sample



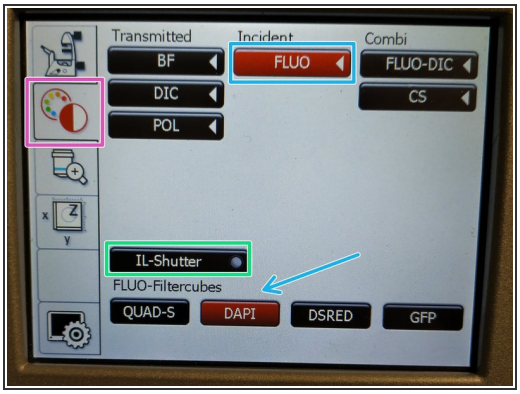


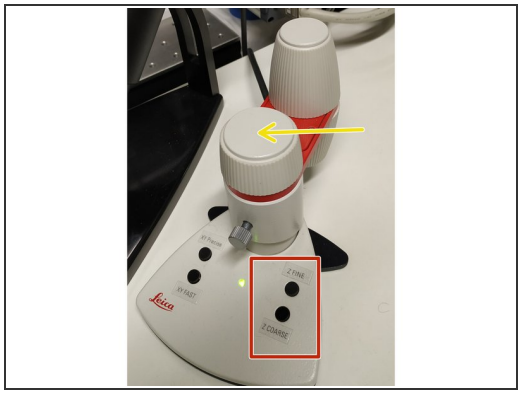


- Insert your sample with the **coverslip facing down**.
  - *Adjust the variable clamping range and moveable brackets to properly fix your sample.*
- **Move** your sample over the objective with the help of the **Joystick**.
- With the little **slider knobs** on both sides of the lower wheel you can change between fast and slow movement.
  - Fast movement - pressing the knobs downwards.
  - Slow movement - pulling the knobs towards you.

Bring back condenser arm to its straight position.

Step 9 — Focus your sample



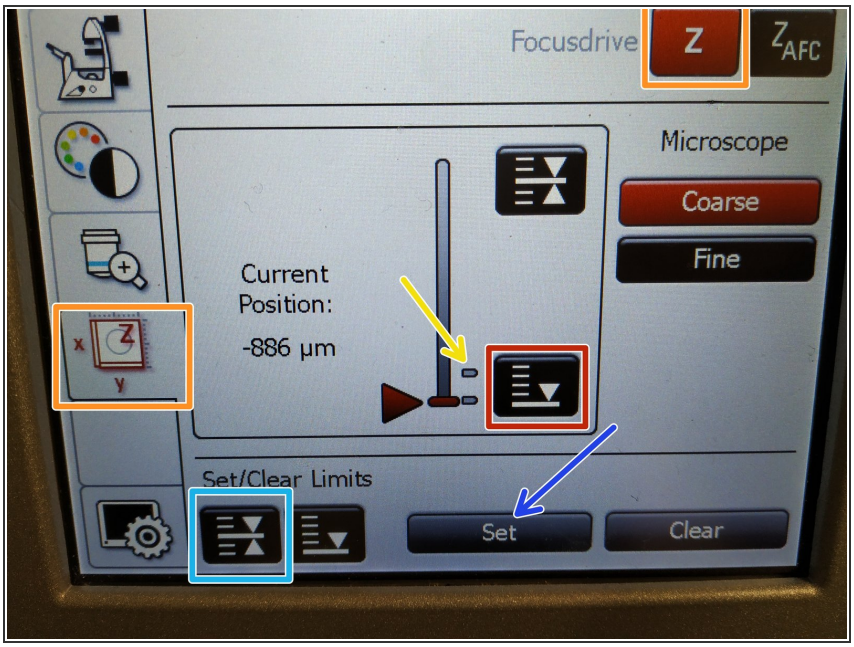


- On the touch screen at the microscope choose the **light path tab**.
- Click **"FLUO"** and choose an appropriate "FLUO-Filtercube" e.g. "DAPI".
- **Open** the **"IL-Shutter"** (if actived the dot is yellow).
- Look through the oculars and **focus your sample** by using:
  - the **focus wheel** on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

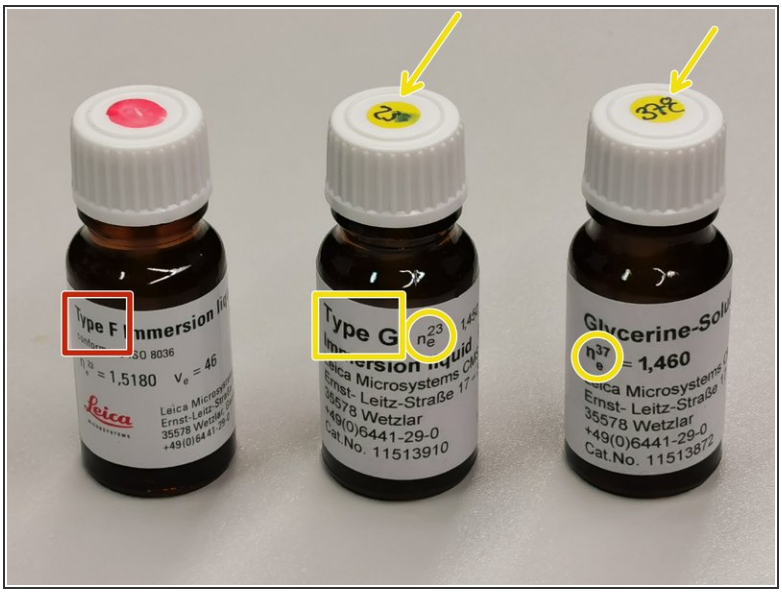
Step 10 — Optional - Save your focus position

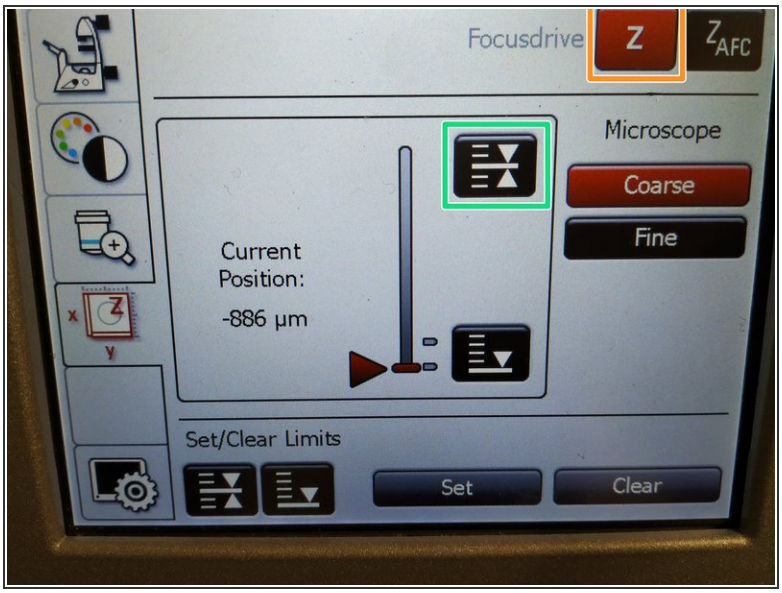


The storage of the focal plane is helpful in order to find the focus back if the sample or objective will be changed.

- 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 11 — Switching to a higher magnification





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

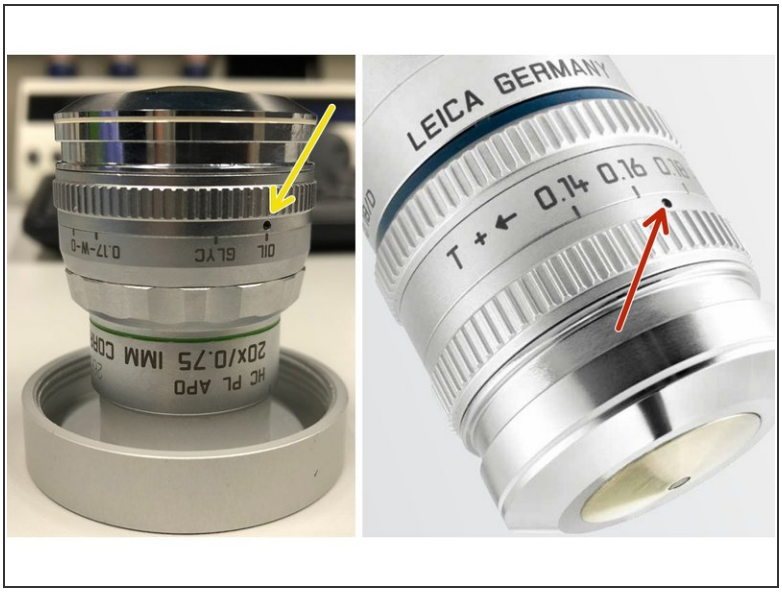
ⓘ Depending on the objective different **immersion media** will be used. Apply either on the sample or directly to the objective.

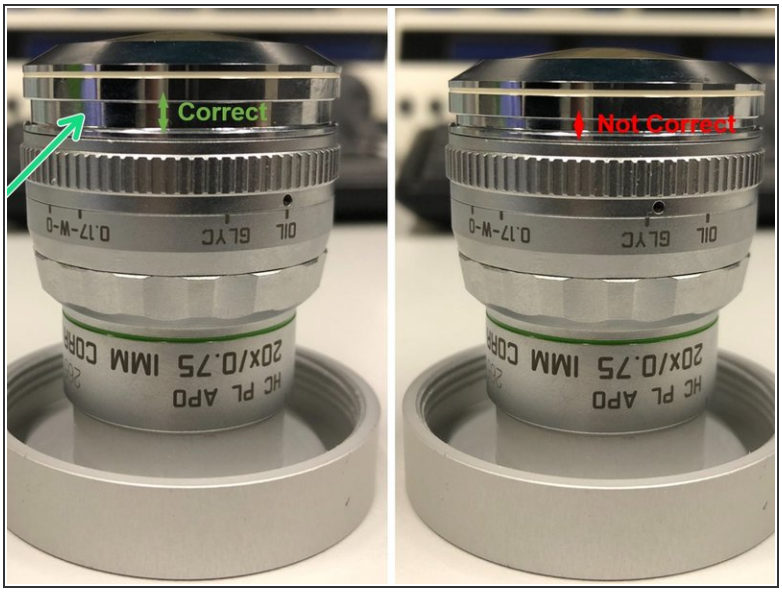
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: either *"Type-G" immersion liquid* (for RT measurements) or *"Glycerin" immersion liquid* (for measurements at 37°C).
- "Water" objectives: *ddH2O* (always use fresh).

⚠ 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 12 — 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 water** 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.