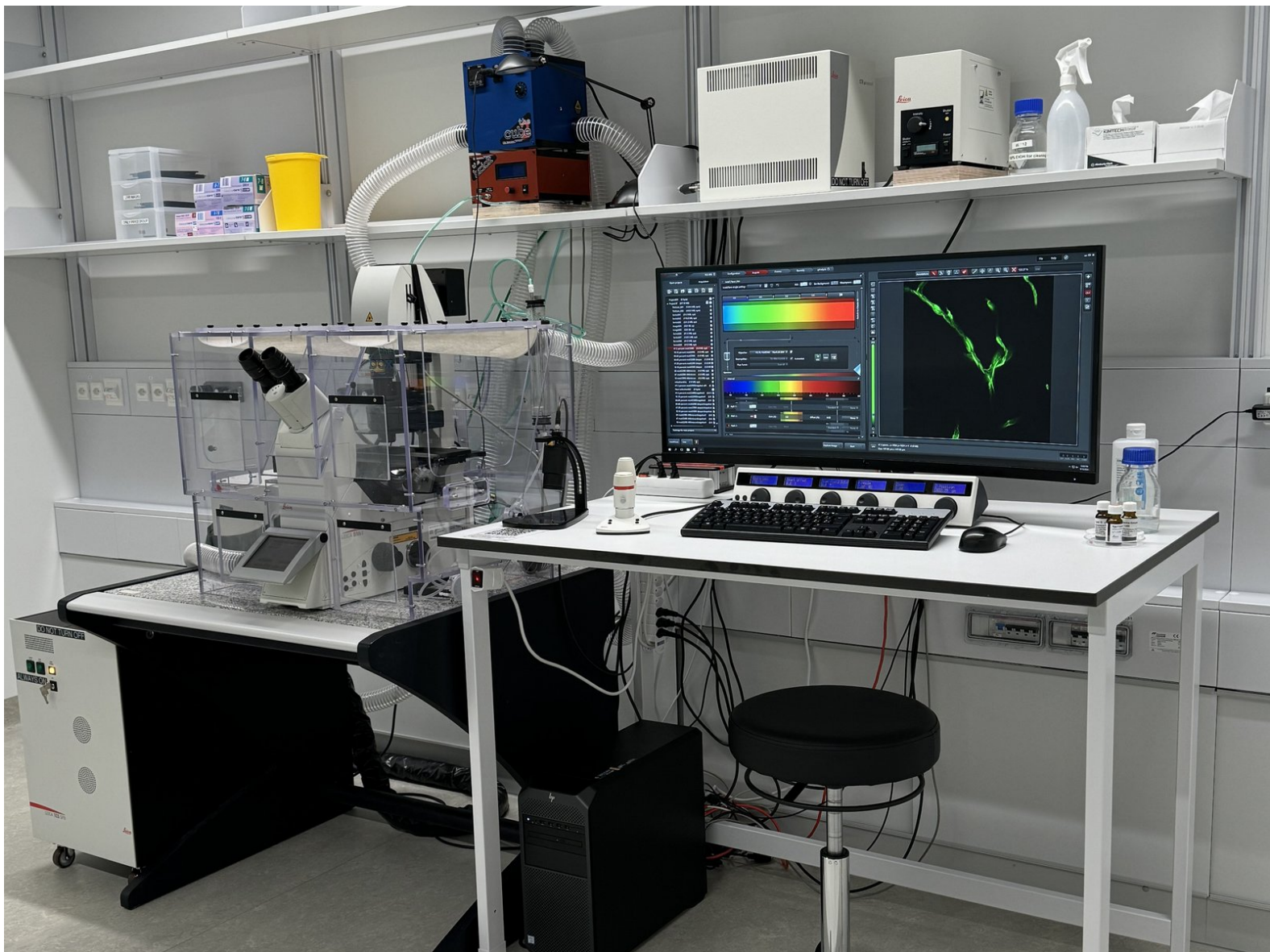


Leica SP8 (Lengg) - 1: Start up

How to start up Leica SP8 confocal laserscanning microscope located at the Research Center Children's hospital.

Written By: Urs Ziegler



INTRODUCTION

How to start up Leica SP8 confocal laserscanning microscope located at the Research Center Children's hospital.

Please find detailed information about the system setup [here](#).

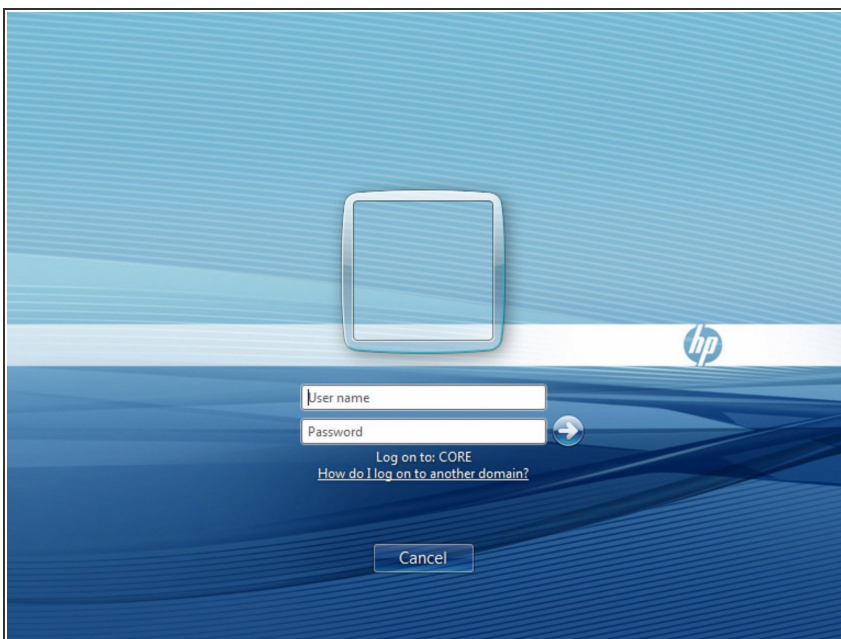
Step 1 — Switching ON Hardware



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

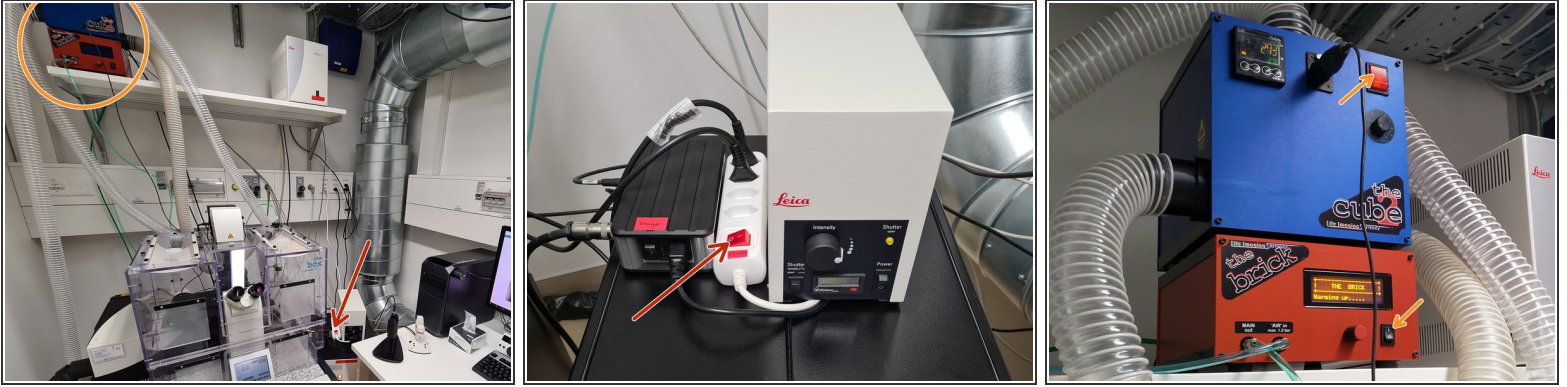
- The corresponding microscope control box can be found on the shelf above the microscope.
- Switch ON the **fluorescence lamp**.
- Press the **main power switch** mounted with a magnet on the top of the computer.
 - ⓘ PC, scanner, lasers will be switched on. If not,
 - check on the control unit that 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



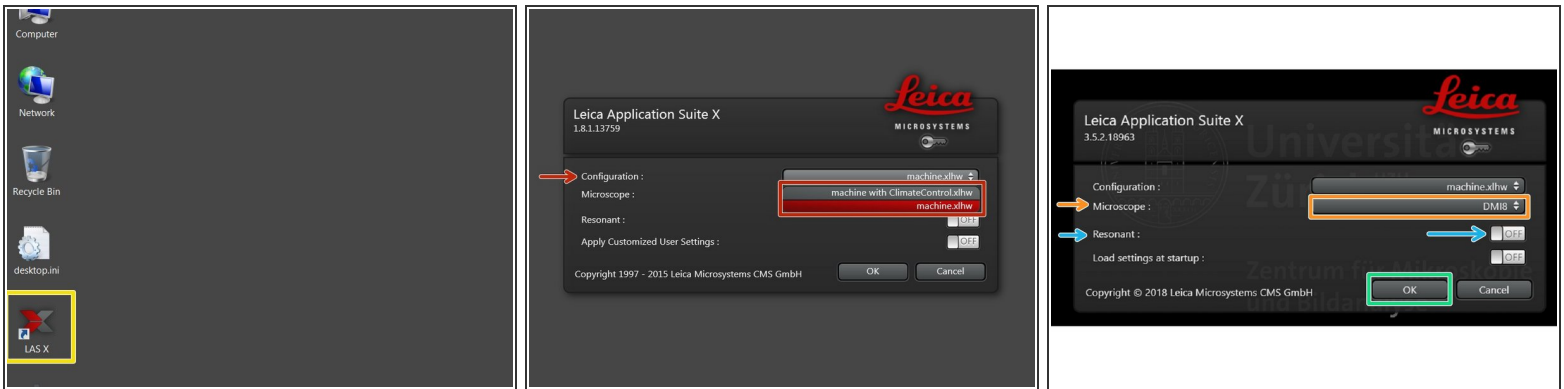
❗ ***If you would like to perform live cell imaging, please refer to the dedicated guide for more detailed instruction.***

● In brief, Turn ON the power switch.

● Cube (temperature) and brick (CO₂) are already turned on.

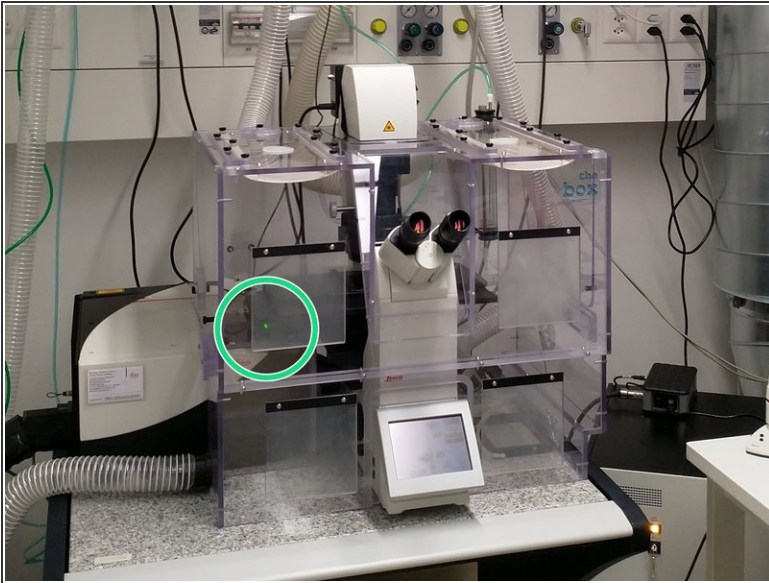
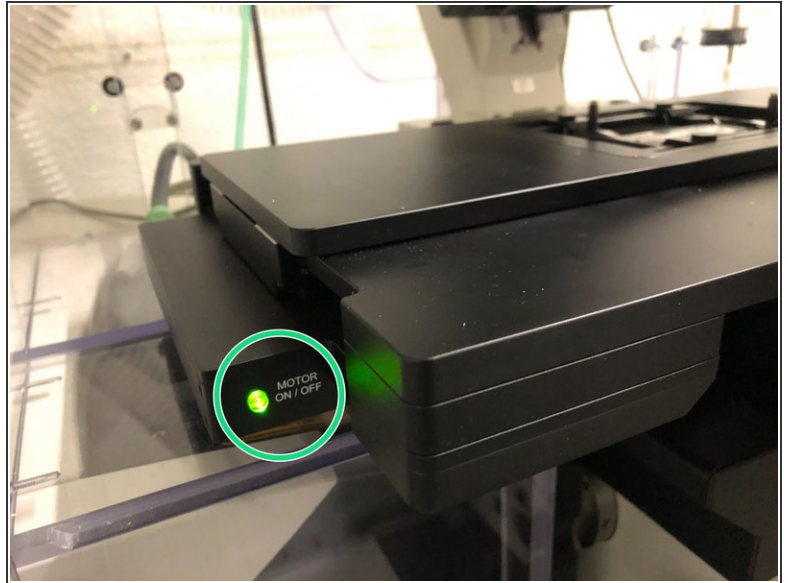
⚠ **37° C and 5% CO₂ are set as a standard. If you need to change it, please set it back to 37° C and 5% CO₂ at the end of your experiment!**

Step 4 — Starting up the "LAS X" software



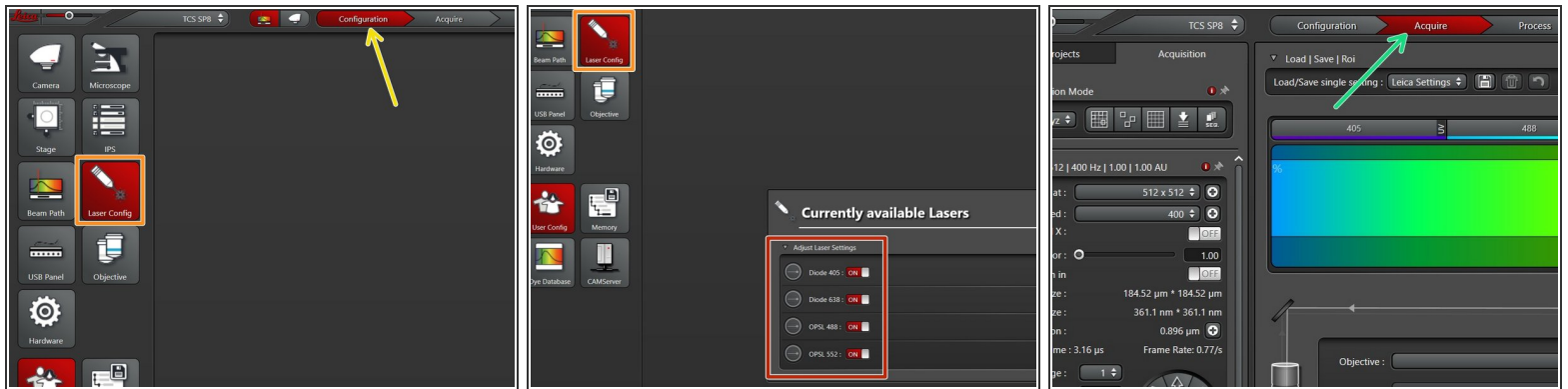
- Start the **"LAS X"** software.
- Select the appropriate **"Configuration"**.
 - "machine with ClimateControl.xlhw" if environmental control is applied and needs to be logged via the software. *Make sure the needed components have been switched on (see dedicated guide for live cell imaging).*
 - "machine.xlhw" 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



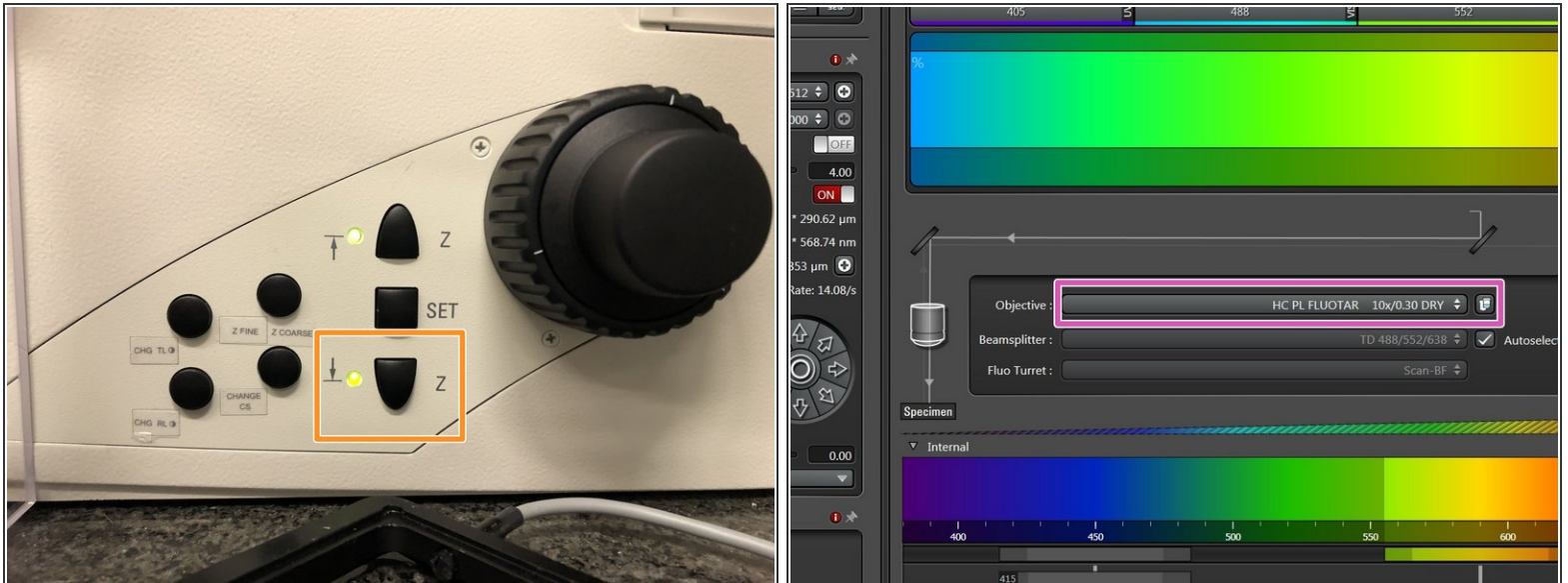
- ❗ **Please note:** The x/y stage at this microscope does not need any initialization to function!
- The **indicator light** "MOTOR ON/OFF" at the stage (left hand side) must be **green** continuously.
 - ⚠ **Flashing green light** at the stage indicates the stage was disengaged by touching or manual movement. Press the green light button to activate it again.

Step 6 — Switch ON the lasers in the software



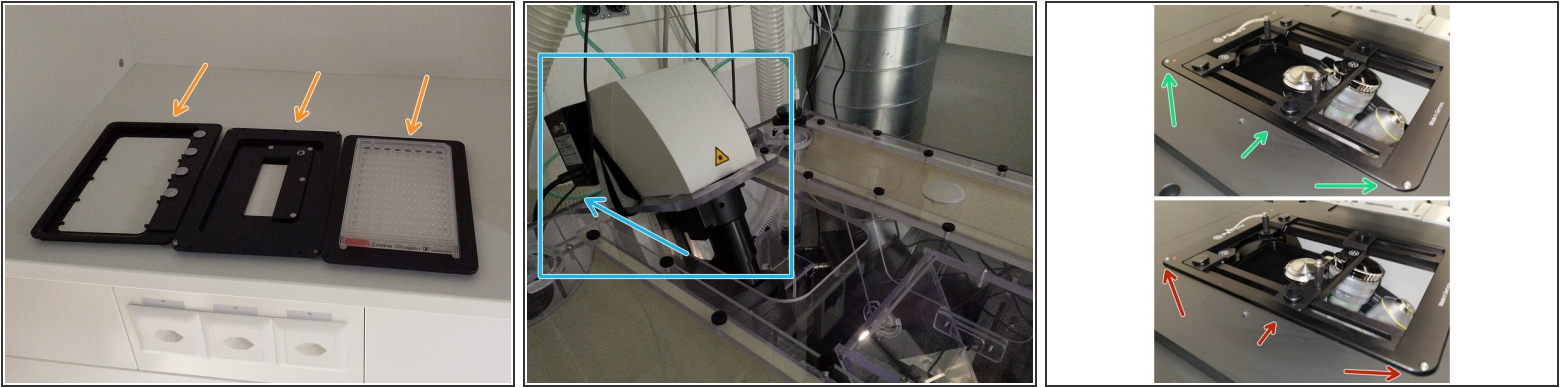
- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you will need.
- Go back to **"Acquire"**.

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



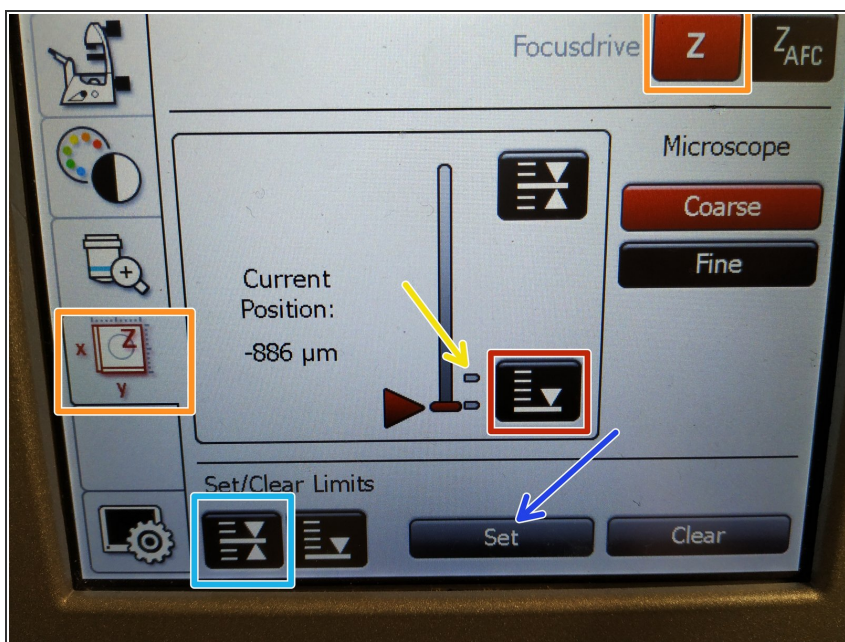
- Insert your sample with the **coverslip facing down**.
 - ❗ *Adjust the variable clamping range and moveable brackets to properly fix your sample.*
- **Move** your sample over the objective with the help of the **Joystick**.
- With the little **slider knobs** on both sides of the lower wheel you can 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



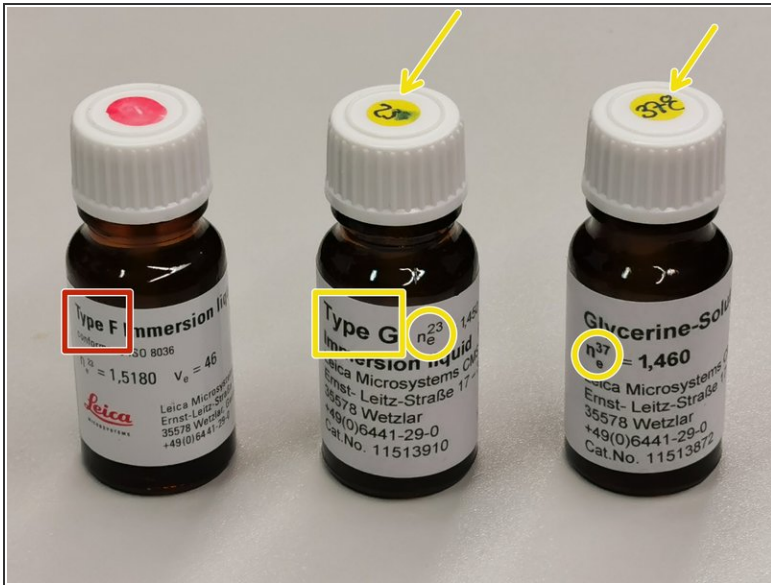
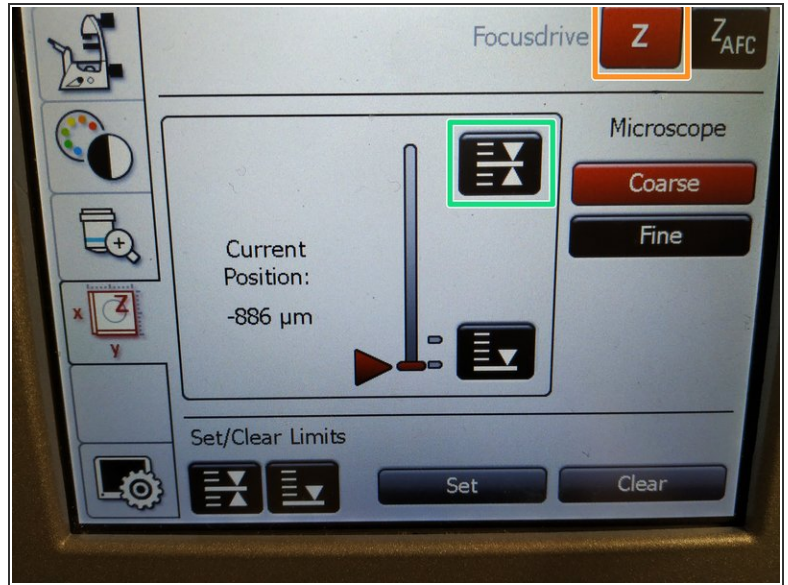
- On the touch screen at the microscope choose the **light path tab**.
- Click "**FLUO**" and choose an appropriate "FLUO-Filtercube" e.g. "DAPI".
- **Open the "IL-Shutter"** (if activated the dot is yellow).
- Look through the oculars and **focus your sample** by using:
 - the **focus wheel** on the microscope stand,
 - or the z-wheel on the **external controller (Smart Move)**.
- ① 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



- ① *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.
- ① 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

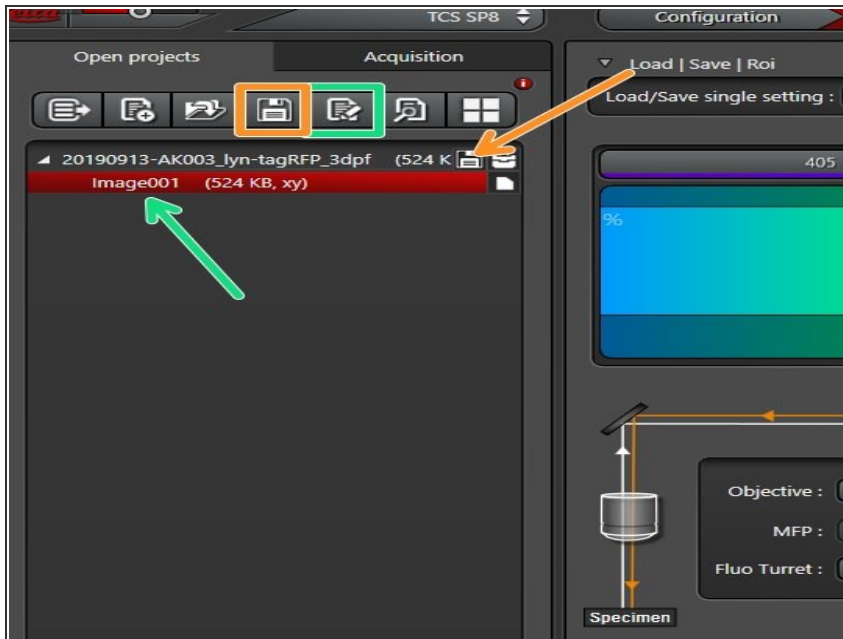


⚠ For some objectives the **correction collar** has to be adjusted.

- **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.