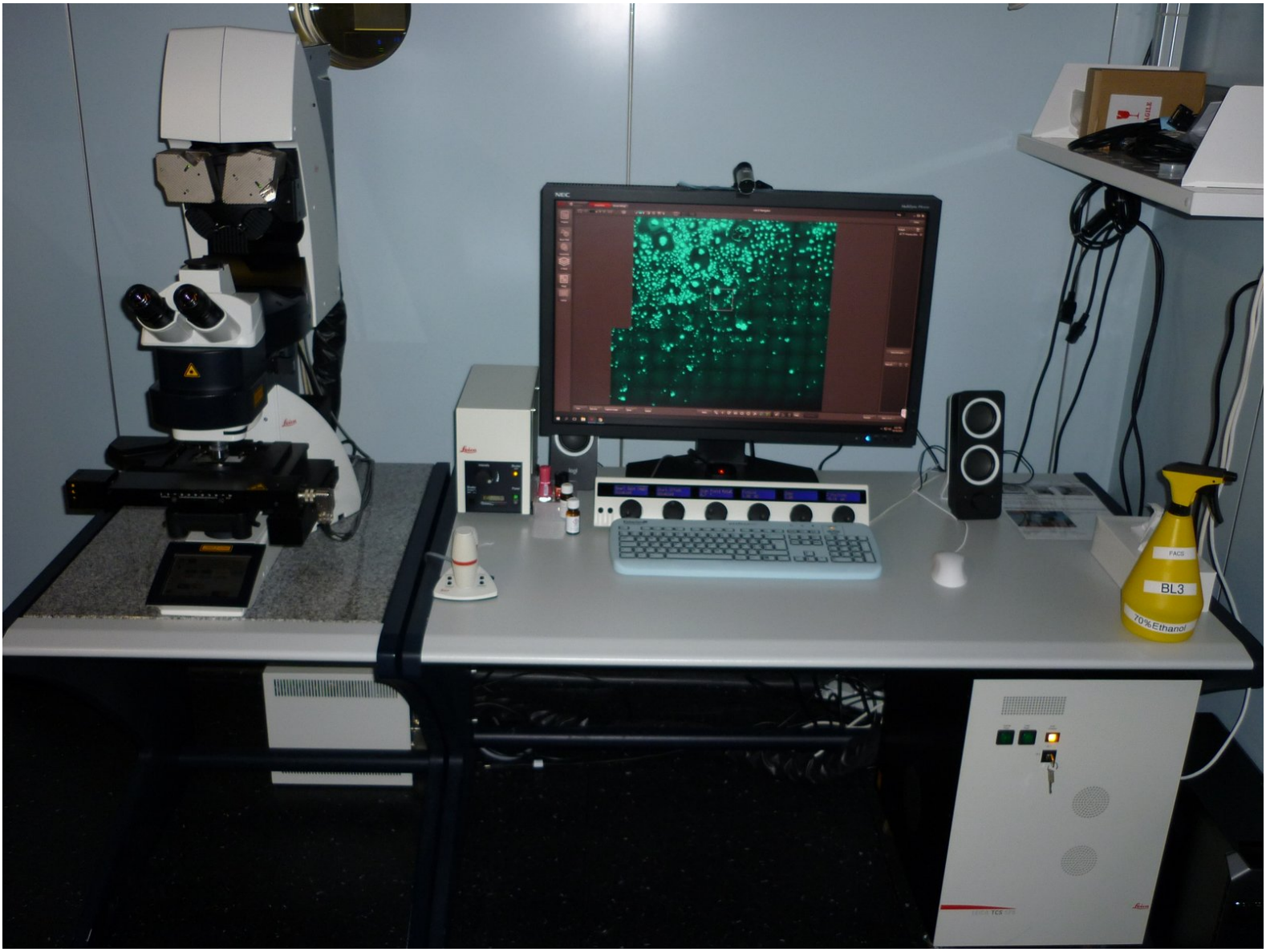
Leica SP8 upright BL3 (Irchel) - 1: Start-up

How to start up and mounting sample on Leica SP8 upright confocal laser scanning microscope located at Irchel Campus, at the Medical Virology (Y36-M-92).

Written By: Caroline Aemisegger



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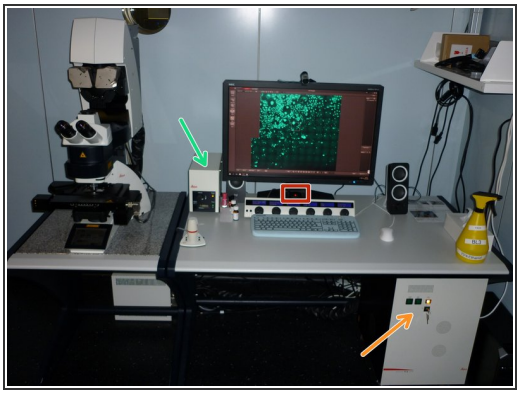
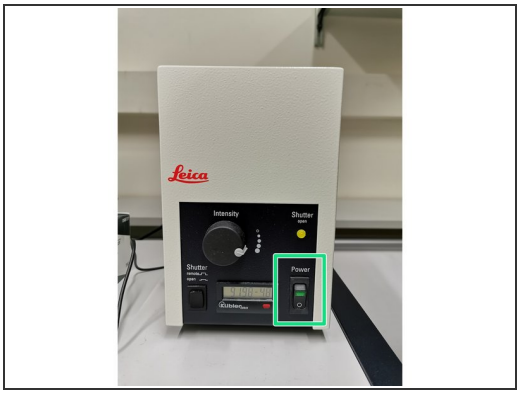
INTRODUCTION

How to start up and mount your sample on the Leica SP8 upright confocal laser scanning microscope located at Irchel Campus in the Medical Virology (Y36-M-92).

Please find detailed information about the system setup here.

Step 1 — Switching ON hardware

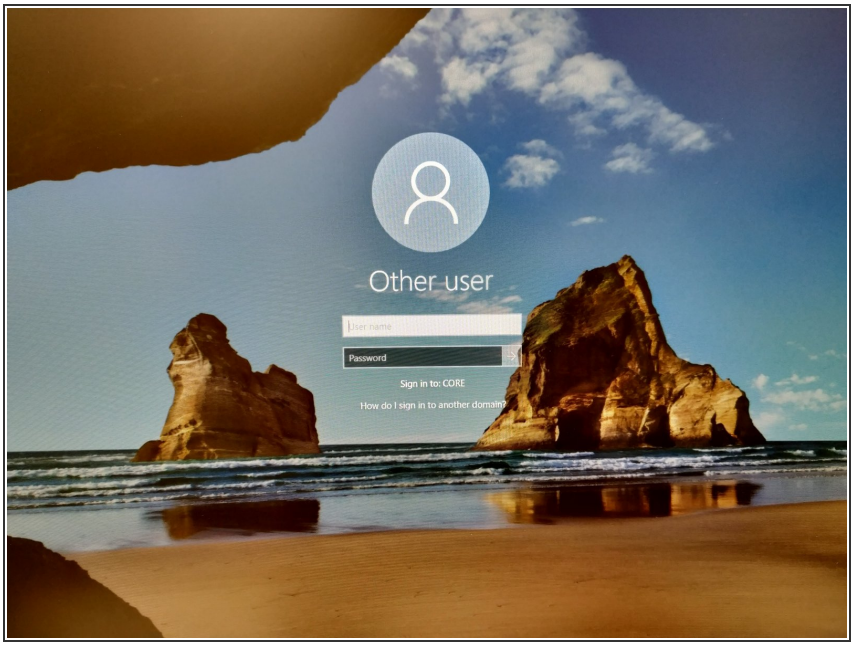




- Switch ON the **fluorescence lamp**.
- Switch ON the **"Scanner Power"**, **"Laser Power"**, and turn the **"Laser Emission" key** to "On-1" (control unit underneath the table).
- Switch ON the **power knob** (on the PC table).

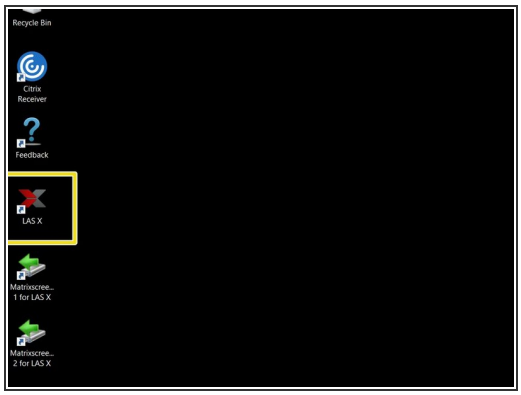
The PC and microscope will be switched on.

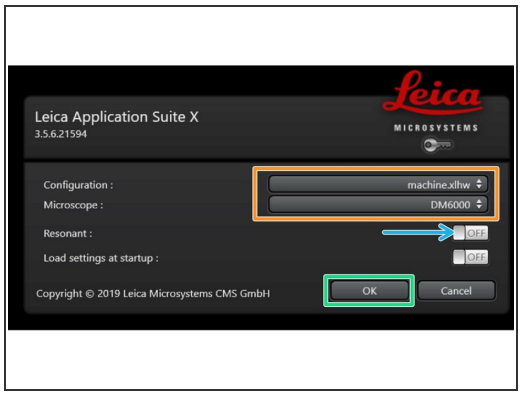
Step 2 — Sign-in

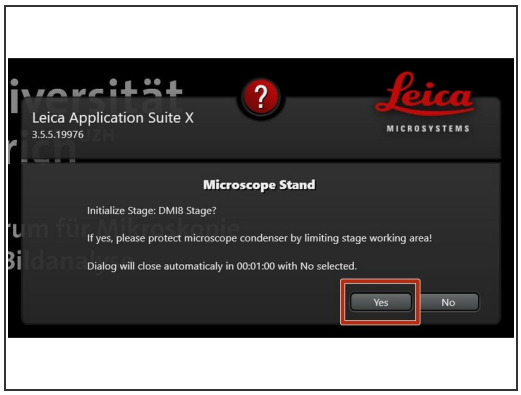


- **Sign-in** with your **ZMB core** credentials.

Step 3 — Starting up the "LAS X" Software

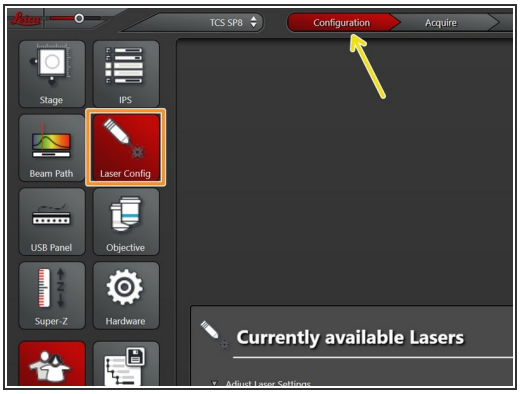


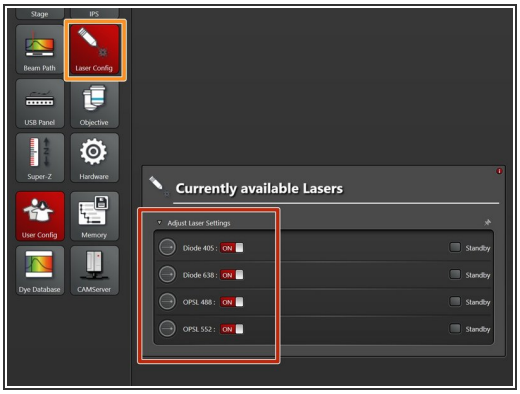


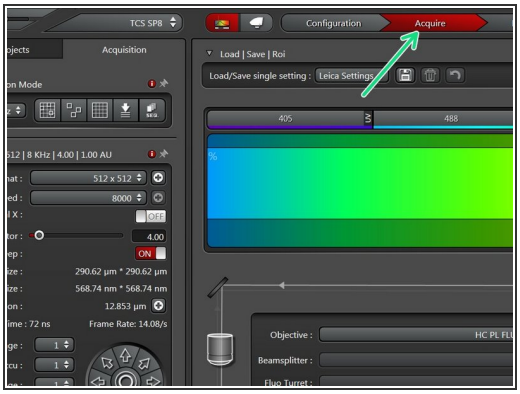


- **Start** the "**LAS X**" software.
- Make sure **"machine.xlhw"** is selected as "Configuration", and **"DM6000"** as "Microscope".
- Select either "Resonant" (ON) or non-"Resonant" (OFF) mode.
- Click "**OK**".
- Click "Yes" in order to **initialize the x/y stage**. *Please make sure nothing is placed currently on the stage.*

Step 4 — Switch ON the lasers in the software

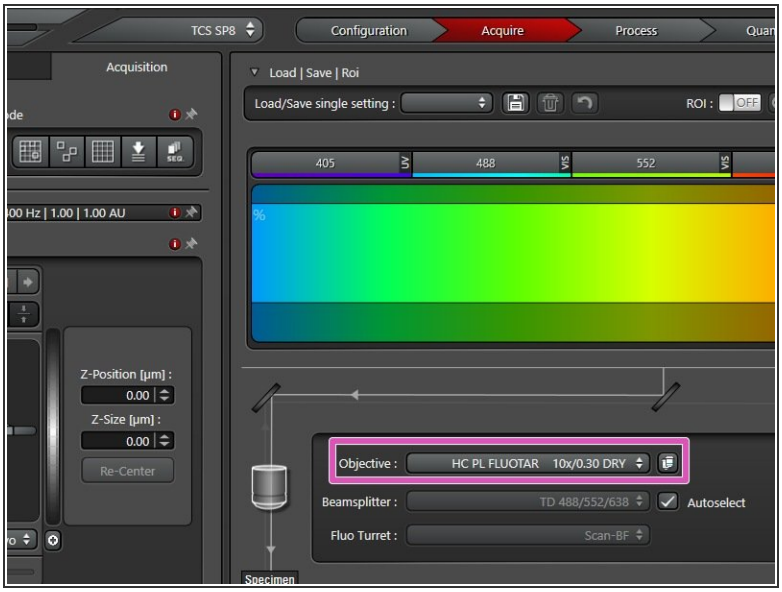






- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch ON** the lasers you will need.
- Go back to "**Acquire"**.

Step 5 — Choosing an objective



- Lower the stage by pressing the "LOWER Z" button on the right side of the microscope.

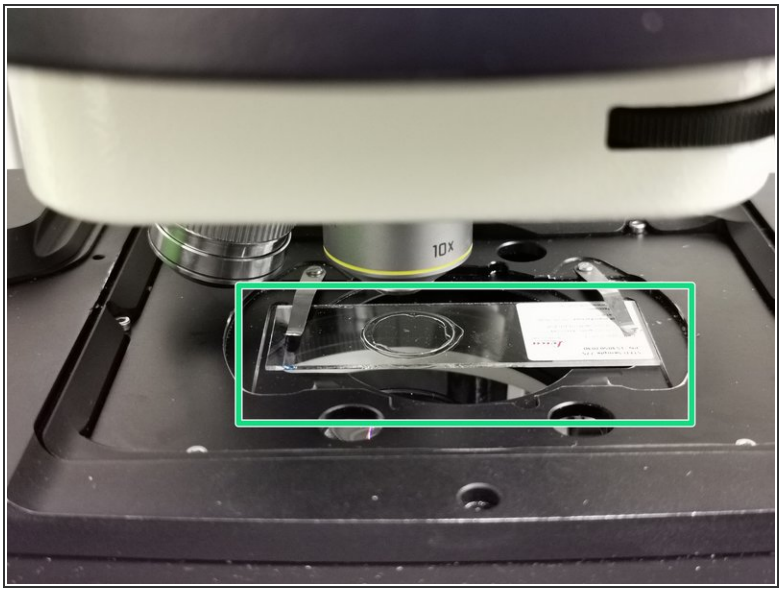
  ⚠ This is a mandatory step as it avoids possible collision of the stage and objective during exchange of inserts and/or samples.

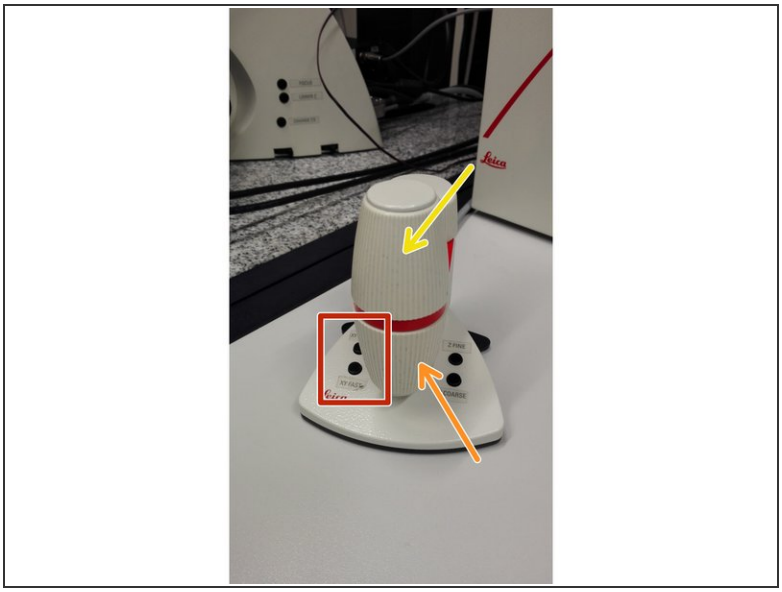
- You can now toggle between objectives within the software (drop-down menu).

- Select the **10x dry objective** .

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

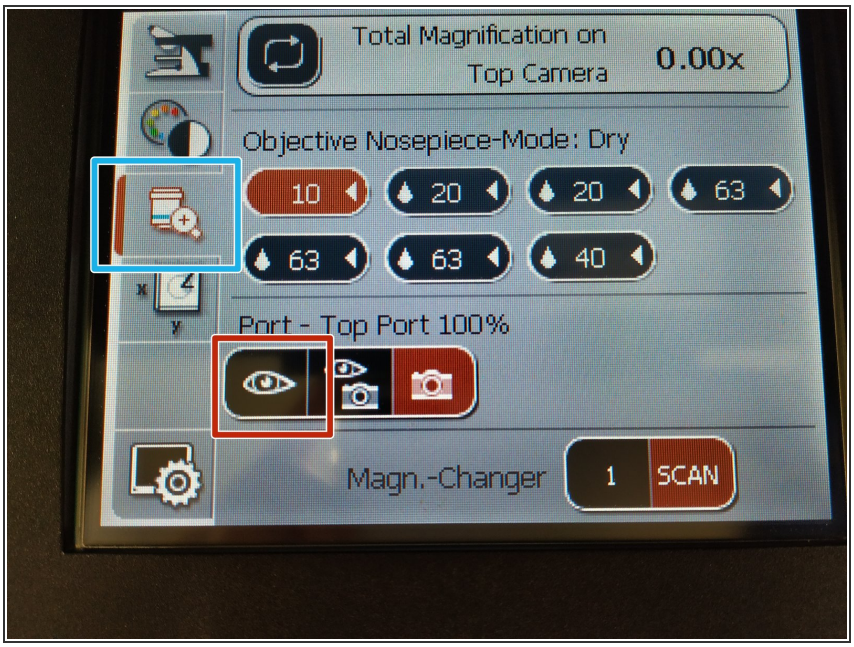
Step 6 — Mount and position your sample





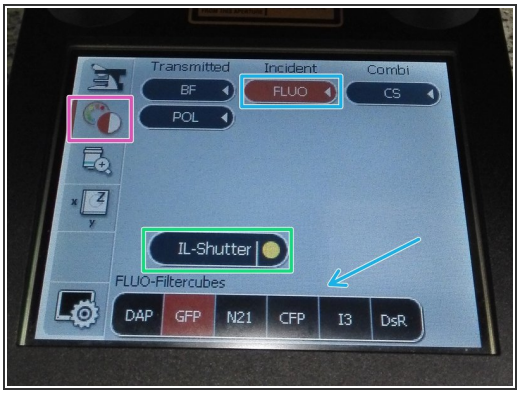
- **Insert** your sample with the **coverslip facing up** and fix it with the two springs.
- Move your sample under the objective with the help of the external controller **"Smart Move"**.
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

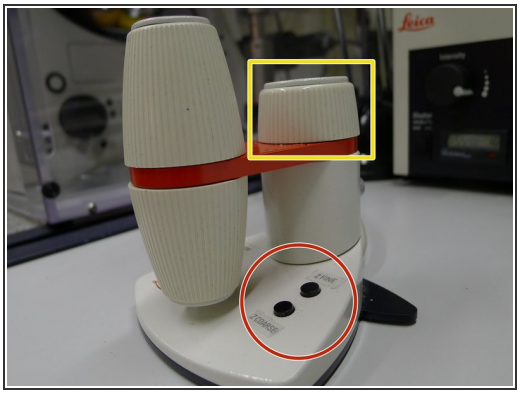
Step 7 — Choose the Eyepiece light path



- On the touch screen at the microscope stand choose the **objective tab**.
  - Make sure the **eyepiece** is selected as the "Port - Top Port 100%".

Step 8 — Focus your sample



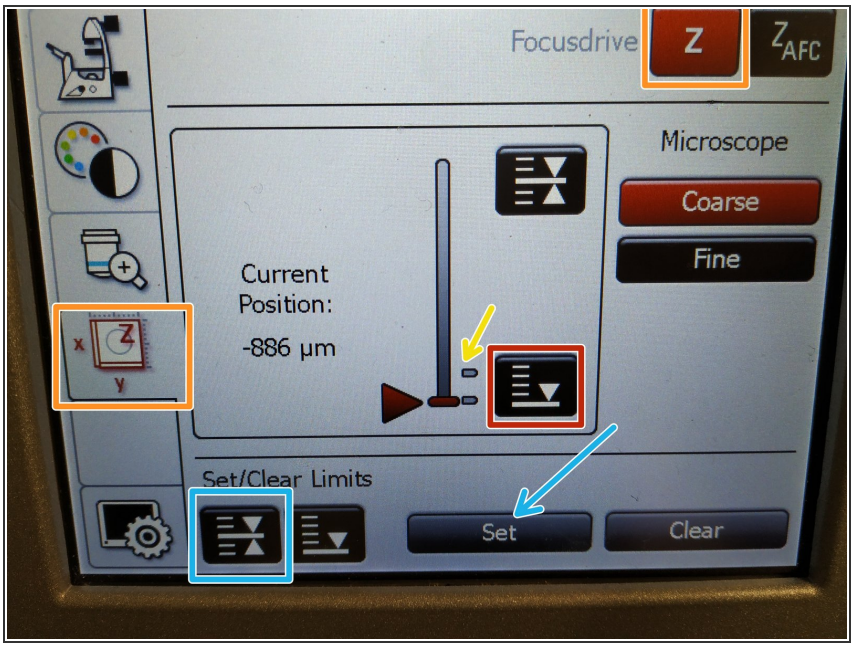


- On the touch screen at the microscope stand choose the **light path tab**.
- Click "FLUO" and choose an appropriate "FLUO-Filtercube" : e.g. "GFP".
- Open the "IL -Shutter" (if activated the dot is yellow).
- Look through the oculars and focus your sample by using:
  - the focus wheel on the microscope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

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

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

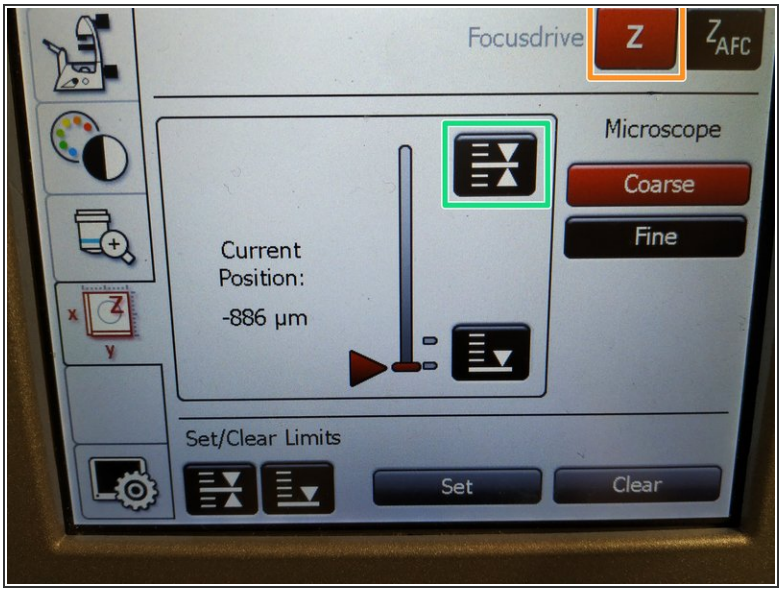
Step 9 — Optional - Save your focus position



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 successfully 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 10 — Switching to a higher magnification



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

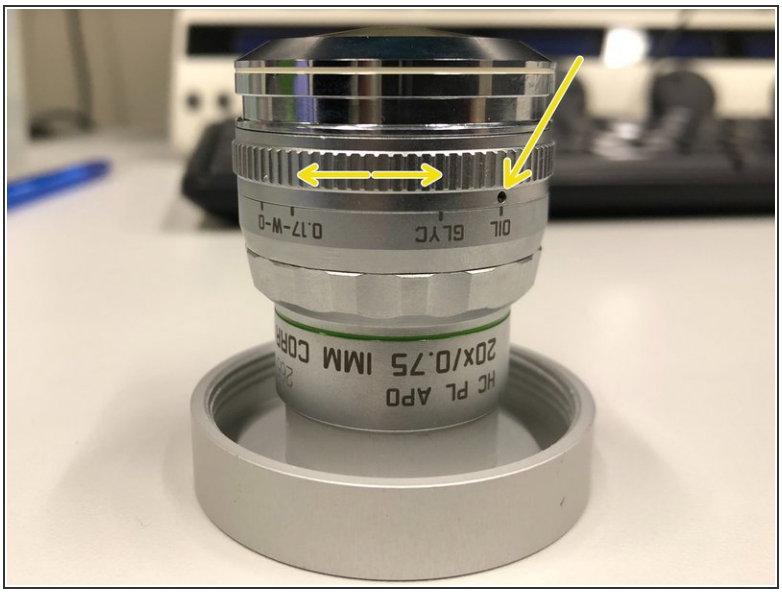
ⓘ Depending on the objective different **immersion media** will be used. Apply directly on the sample.

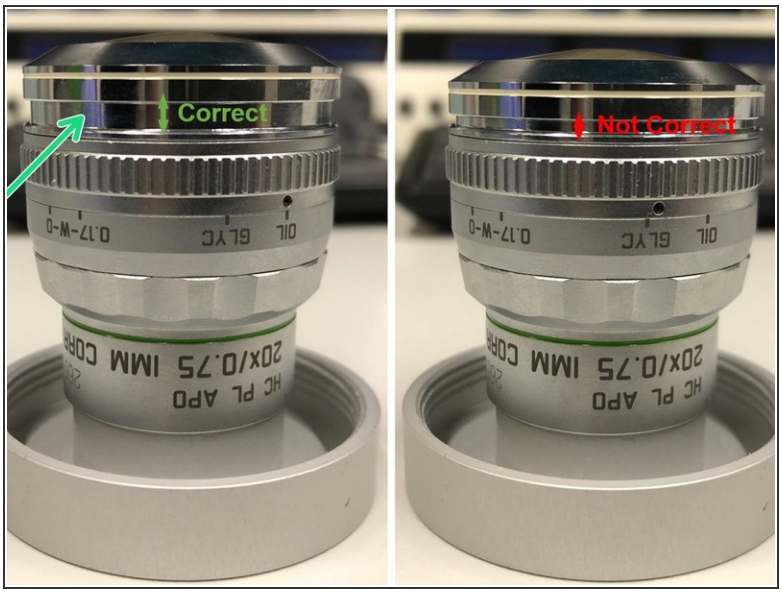
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: *"Type-G" immersion liquid* .
- "Water" objectives: *ddH2O* (always use fresh).

⚠ 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 11 — 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)).

- 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.