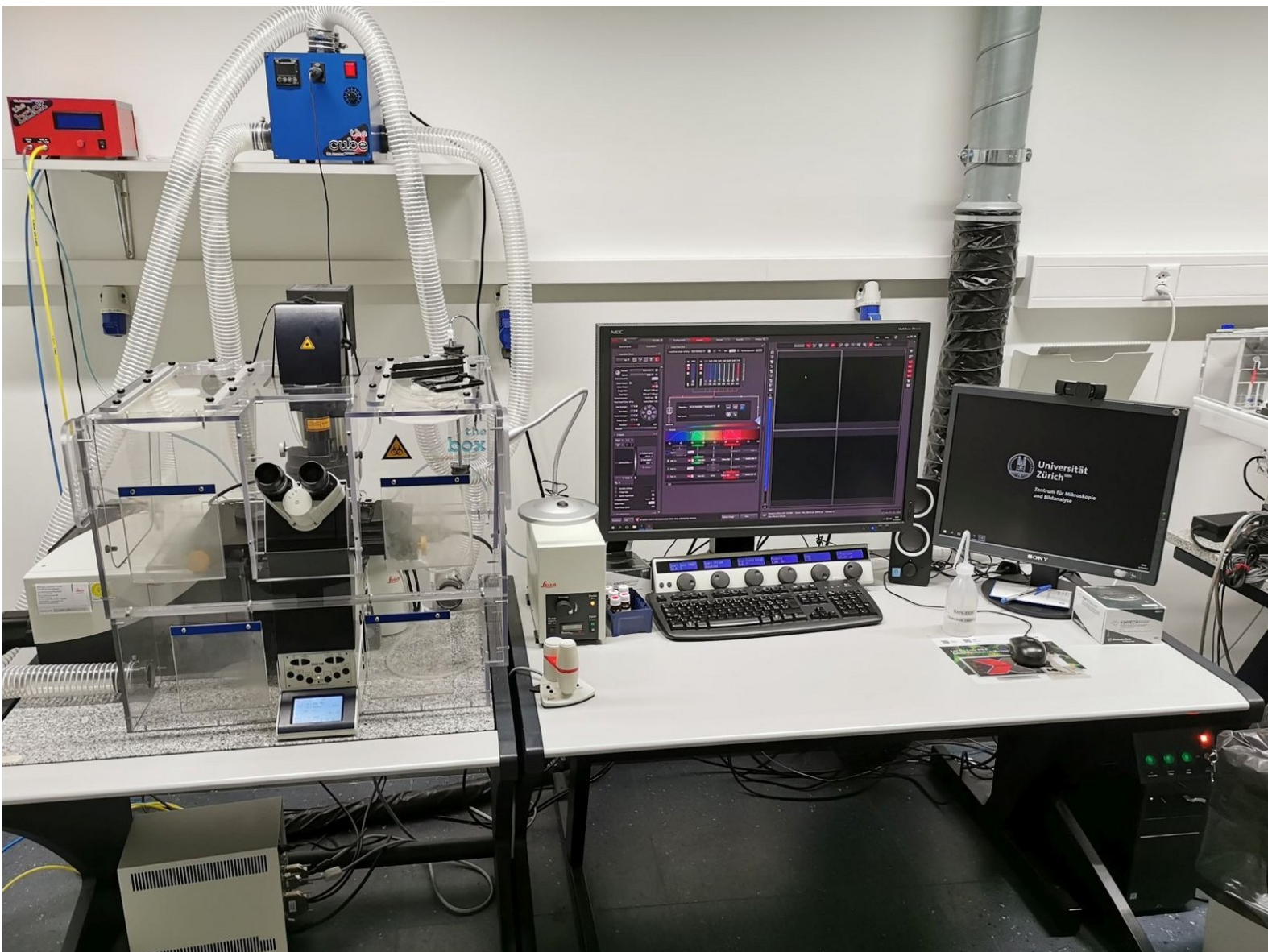


# Leica SP8 inverse (Schlieren) - Part 1: Start-up

How to start up and mount your first sample on the Leica SP8 inverse confocal laser scanning microscope located in Schlieren.

Written By: z mbstaff

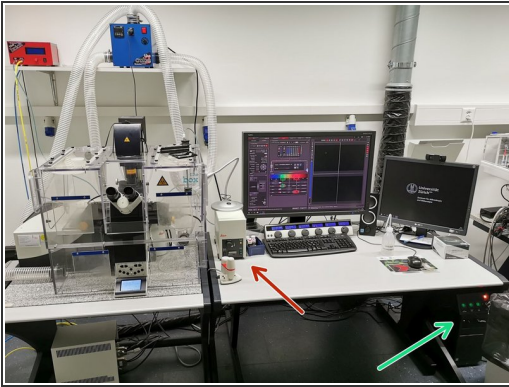


## INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis we show how to start up and mount your sample on the Leica SP8 inverse confocal laser scanning microscope located in Schlieren.

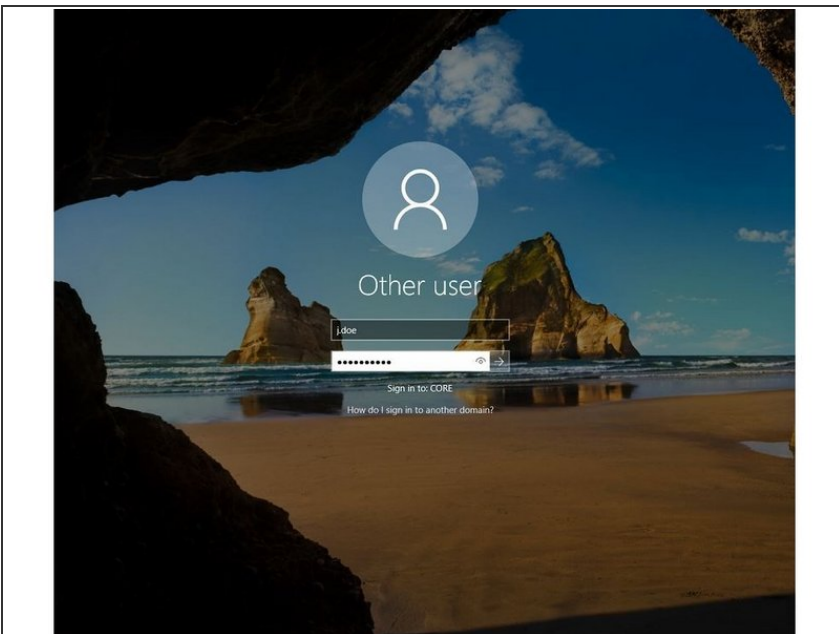
Please find detailed information about the system setup [here](#).

## Step 1 — Switching ON Hardware



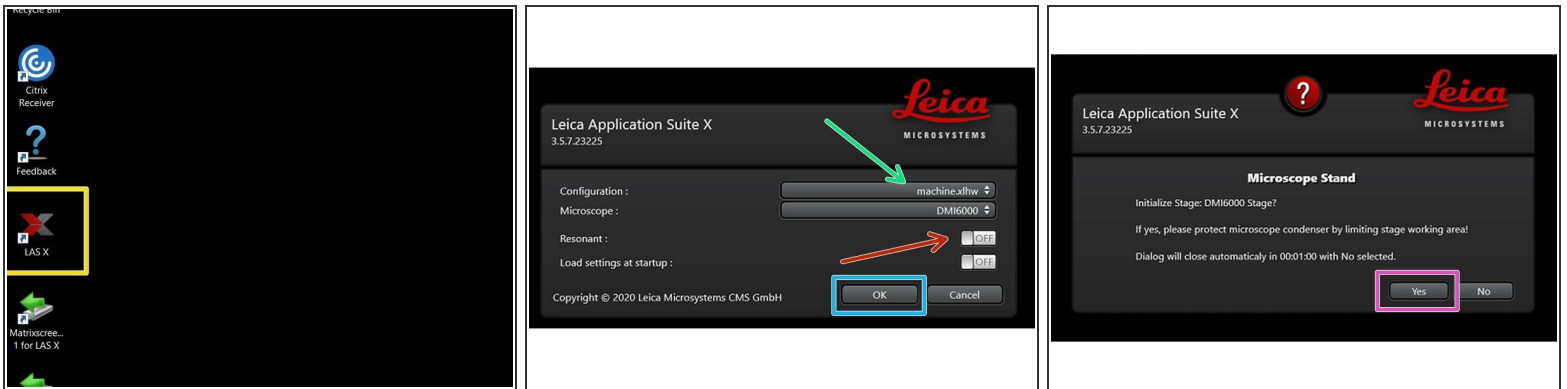
- On the right hand side of the table (main switch board):
  - Switch ON the "**PC /Microscope**", "**Scanner Power**" and "**Laser Power**".New line.
  - Turn the "**Laser Emission**" key to "On-1".
- Switch ON the **fluorescence lamp**.
  - You can vary **lamp intensity** by turning the wheel
  - Ensure "**Shutter**" button is pressed and "**Shutter open**" control light is on

## Step 2 — Sign-In



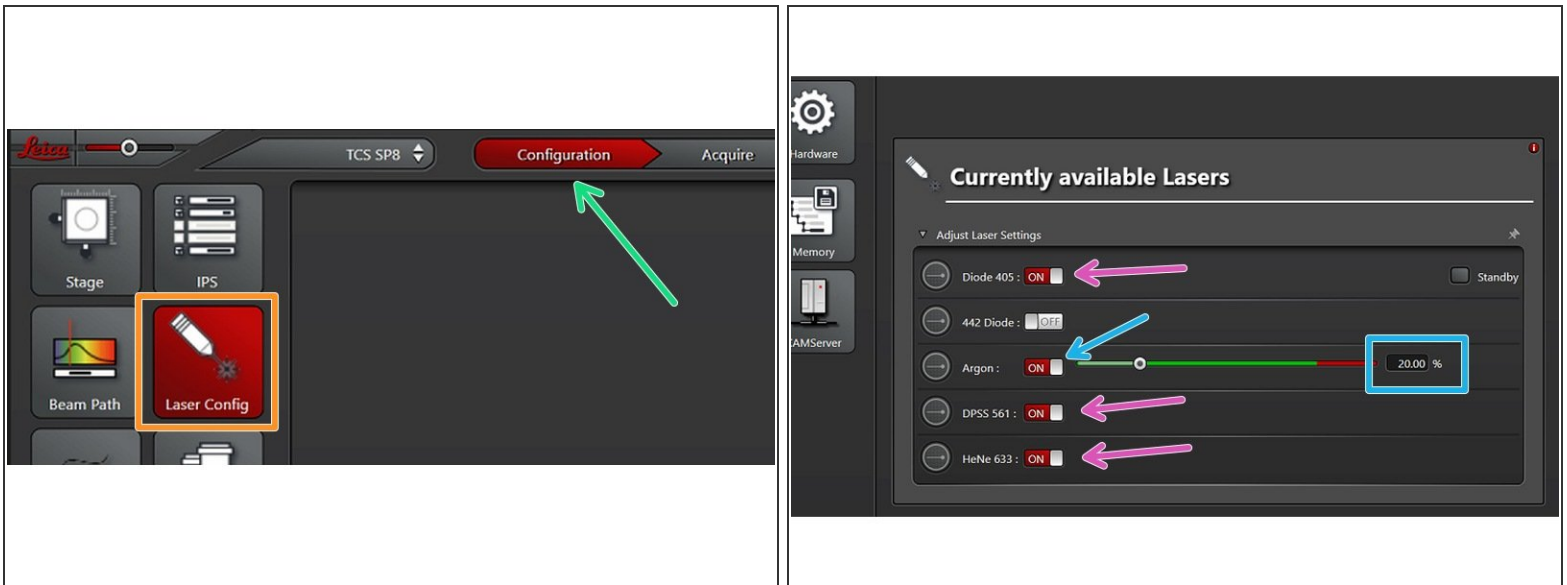
- **Sign-in** with your **ZMB core** credentials.

### Step 3 — Choose Scanner and initialize stage



- Start the "**LAS X**" software using the Desktop link.
- Select "**machine.xlhw**" as configuration (only necessary when starting software for the first time)
- Optional: enable the "**Resonant Scanner**" (this scan mode allows faster imaging but is not well suited for recordings of dim signals).
- Click "**OK**"
- Ensure that no sample is mounted on the stage and click "**YES**" when prompted to **initialize the stage**.

## Step 4 — Switch ON the lasers in the software



- Navigate to the "**Configuration**" tab
- Select the "**Laser Config**"
- Only **switch "ON"** the lasers you will need for your experiment!
- If needed "switch ON" **Argon laser** and set **power to 20%**
- Change back to "**Acquire**" tab

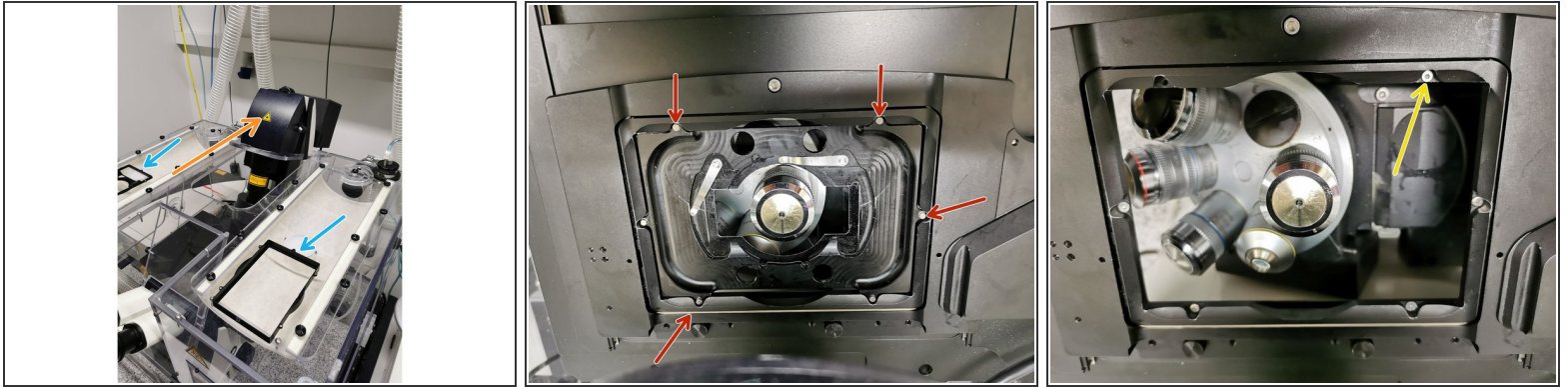


## Step 5 — Choosing an objective



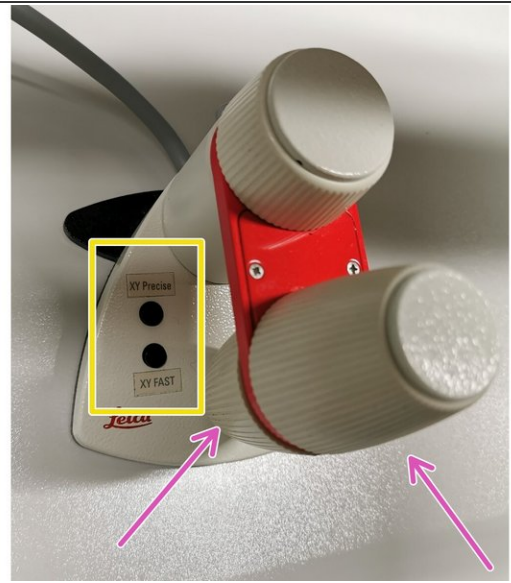
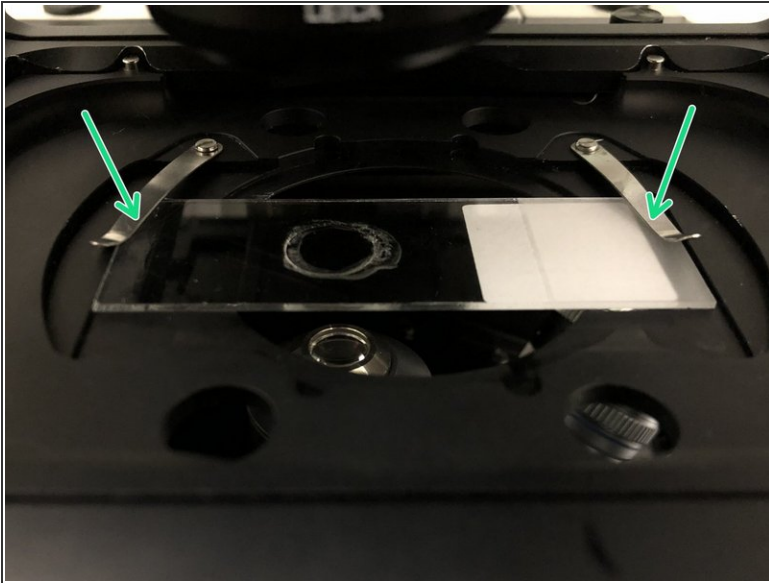
- Lower the **objective turret** by pressing the "Z" downwards button on the right side of the microscope.
- ⚠ This step avoids possible collision during placing of inserts and/or samples.
- **Select your objective** of interest from the drop down menu.
- ❗ It is recommended to start with the 10x dry objective for simplified focusing.
- ☑ Depending on the objective different **immersion media** have to be used. Apply directly on the sample not the objective!
  - Oil objectives: "Type-F" immersion liquid.
  - Glycerin objectives: either "Type-G-n23" immersion liquid (for RT measurements) or "Glycerin-n37" immersion liquid (for measurements at 37°C)."

## Step 6 — Installing a sample holder



- **Push back** the condenser arm.
  - You can find different stage inserts on top of the incubator box.
  - The stage inserts are hold in place by small **magnets**. You can remove an insert by carefully pulling against the resistance.
  - Empty stage ready to load a new insert (screws visible).
- ⚠ Ensure that not the **well plate holder** is loaded when placing a stage insert.

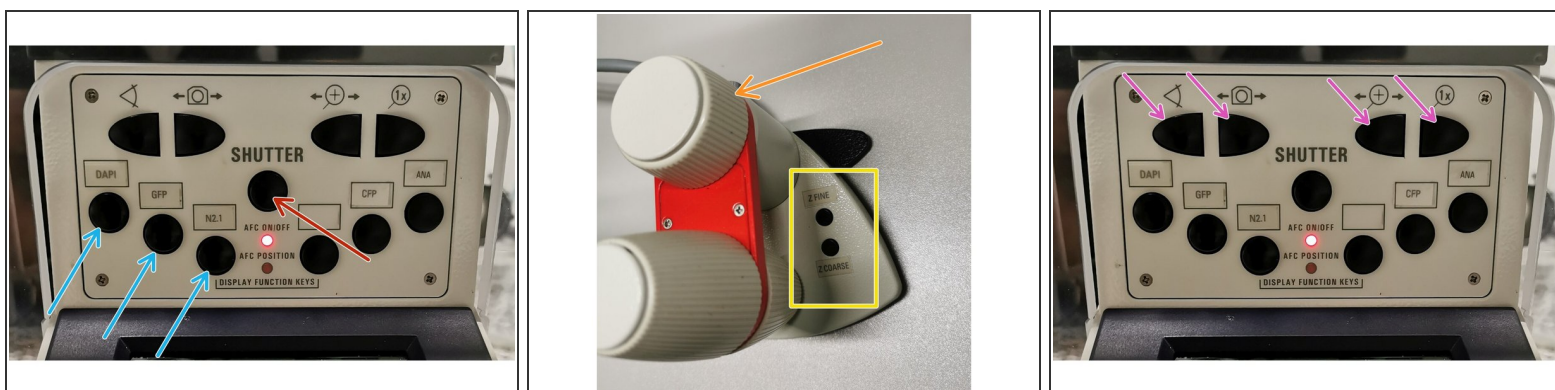
## Step 7 — Mount and position your sample



- ☑ Add a drop of immersion liquid on the coverslip when using an immersion objective (do not "drown" the complete coverslip!).
- Place your sample with the **coverslip facing down** and fix it with the two clamps .
  - ⚠ For beginners we strongly recommend to **fix only one clamp** to not break your sample/the objective when missing the focus.
- Move your sample over the objective with the help of the external xy controller (two wheels above each other).
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

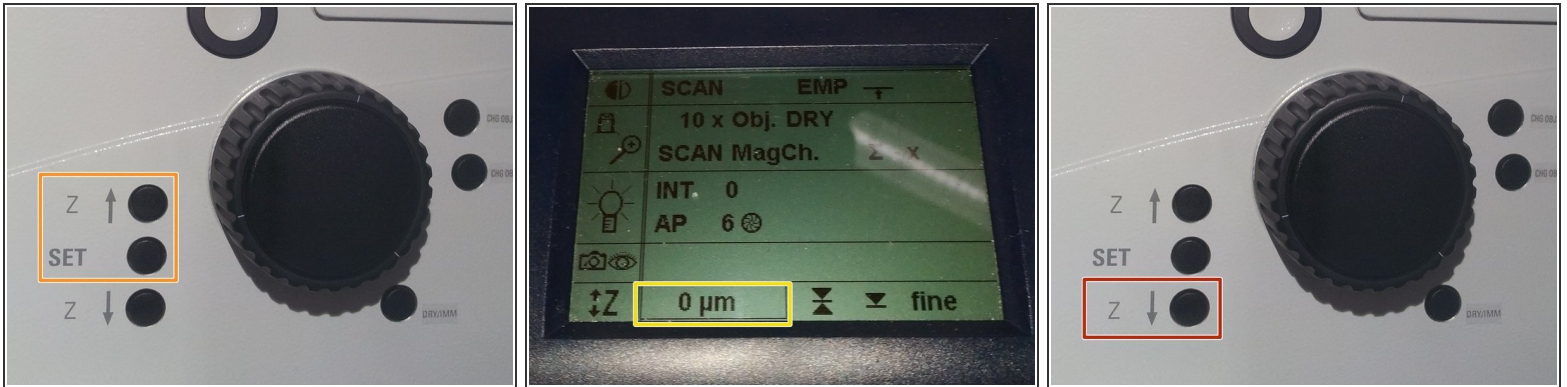


## Step 8 — Focus the sample



- **Select a filter** which allows you to bring your sample in focus (preferably a bright signal). You should hear a click when the filter is loaded.
- Press the "**Shutter**" button. You should now see some light coming out of the objective (GFP, N2.1 should be clearly visible, DAPI is difficult to see).
- Look into the ocular and **move** the external **focus wheel** until your sample gets in focus.
  - ⓘ Turning the z-wheel **clockwise** = moving objectives **upwards** (towards sample).
  - On the controller toggle between "Z FINE" and "Z COARSE" for changing the focusing speed.
  - ⚠ **Z COARSE** mode moves **very fast** and one can easily miss the focus. Double check from time to time whether you already lifting your sample!
- ⓘ If you cannot see anything at all it may be that a filter got stuck during the microscope startup.
  - Usually, this can be fixed by pressing the upper four buttons one after the other from left to right. Finally, press again the very left upper button and recheck if you can find the focus.

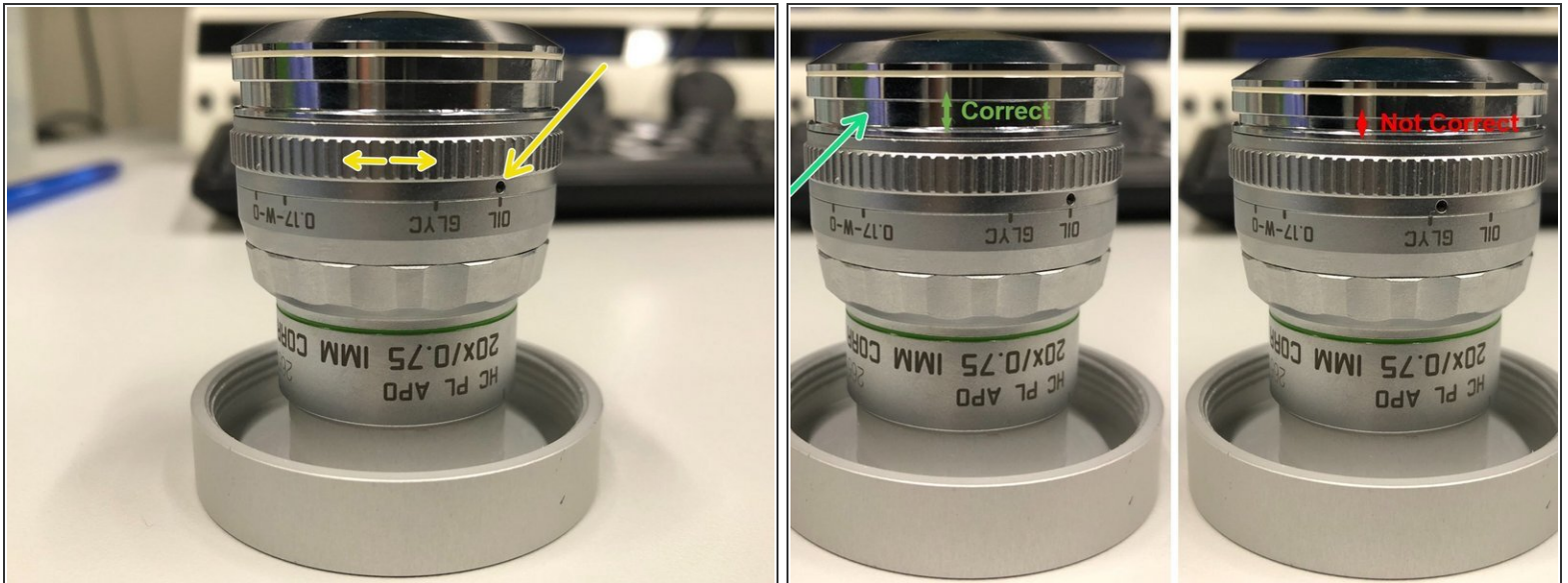
## Step 9 — Optional - Save your focus position



**i** Storing the focus position can be helpful to find back the focus if the sample or objective has to be changed.

- **Jointly** press the "SET" and upper "Z" button (right side of the microscope stand) to set the current z-position to zero.
- ⚠ "If a focus position was saved by a previous user, you have to repeat that "twice" to delete first the old saved position.
- The "Z"-position on the display should now show "0 μm".
- Press the lower "Z" to move down ( *for safe change of the objective or the sample* ).

## Step 10 — Collar rings on immersion objectives



**⚠ For optimal imaging performance of the 20x 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)).
- Ensure that the top part of the **spring-loaded front lens** is released (working position). Mandatory for all immersion objectives (20x IMM, 63x glycerin, 63x oil).