Leica SP8 Falcon (Irchel) - Part 1: Start-up

How to start up and mount your first sample on the Leica SP8 FALCON confocal laser scanning microscope located at the Irchel Campus, room Y42-H-81.

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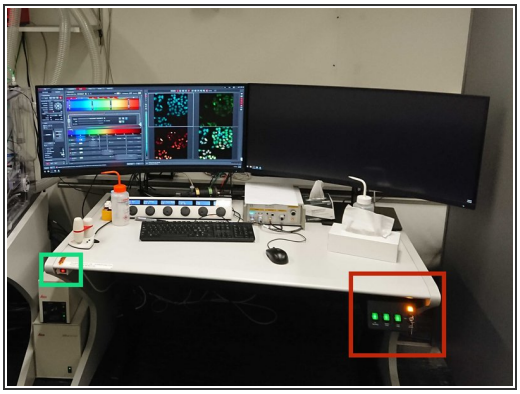


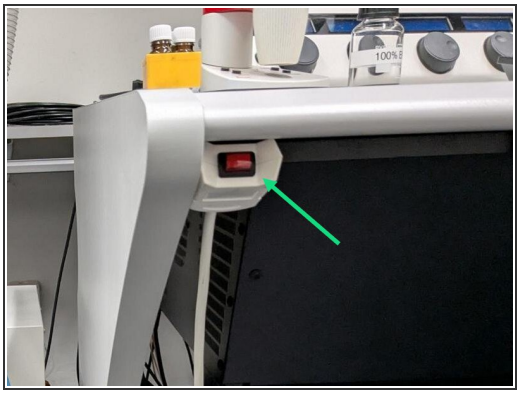
INTRODUCTION

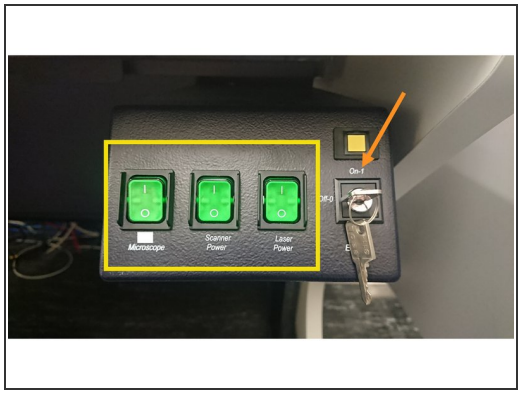
How to start up and mount your first sample on the Leica SP8 Falcon confocal laser scanning microscope located at the Irchel Campus, room Y42-H-81.

Please find detailed information about the system setup here.

Step 1 — Switching ON Hardware

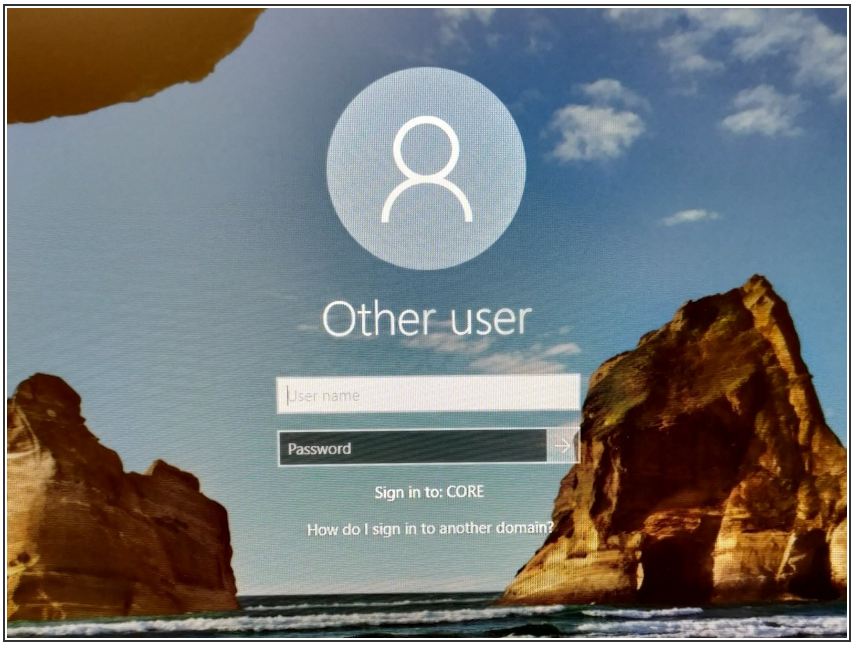






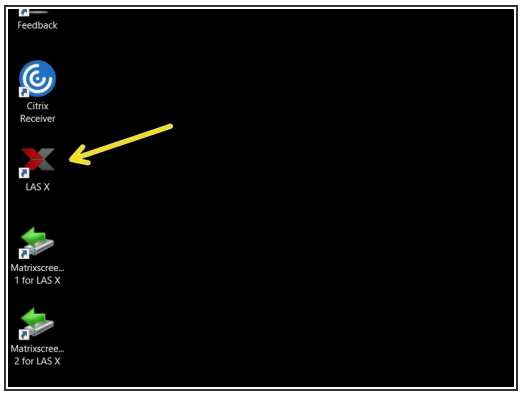
- Switch on the red button (underneath the table on the left).
  - ⓘ *Turns on PC, monitors and fluorescence lamp.*
- On the right side of the table:
  - Switch ON the **"Microscope"**, **"Scanner Power"** and **"Laser Power"** switches.
  - Turn the **"Laser Emission"** key to **"ON-1"**.

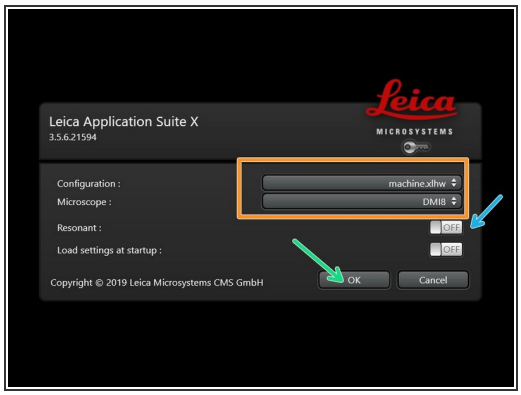
Step 2 — Sign-In



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

Step 3 — Starting up the "LAS X" Software

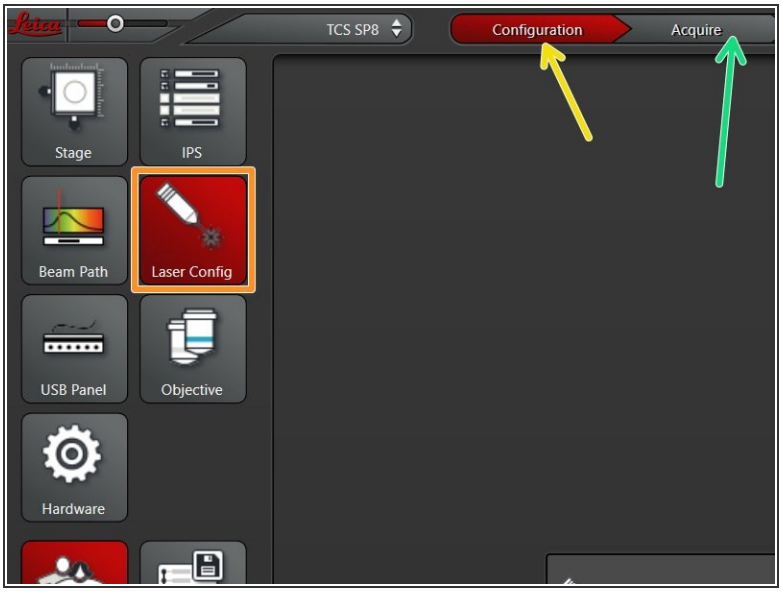


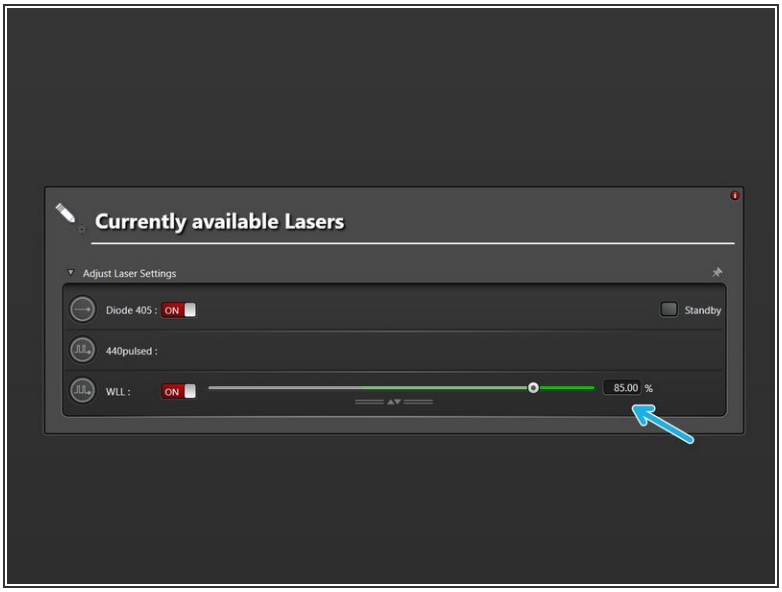




- Start the **"LAS X"** software. Select:
  - **"machine.xlhw"** for "Configuration",
  - and **"DMI8"** as "Microscope".
- Select either "Resonant" (ON) or non-"Resonant" (OFF) scanning mode.
  - ⓘ *Use "Resonant" scanner for fast acquisition and/ or live imaging. However, not advised for FLIM!*
- Click **"OK"**.
- Click **"Yes"** to **initialize the x/y stage**. *Make sure nothing is placed on the stage.*
  - ⓘ *An x/y stage initialization is necessary to use the Navigator function.*

Step 4 — Switch ON the lasers in the software





- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you will need.
  - When "ON", the WLL should be at 85% by default.
- Go back to **"Acquire"**.

Step 5 — Choosing an objective





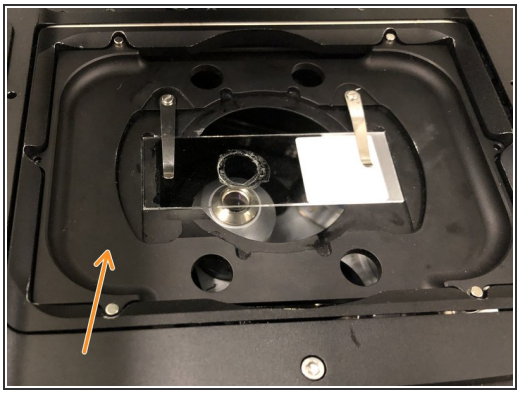
- **Lower** the **objective turret** by pressing the "Z downwards" button on the right side of the microscope.

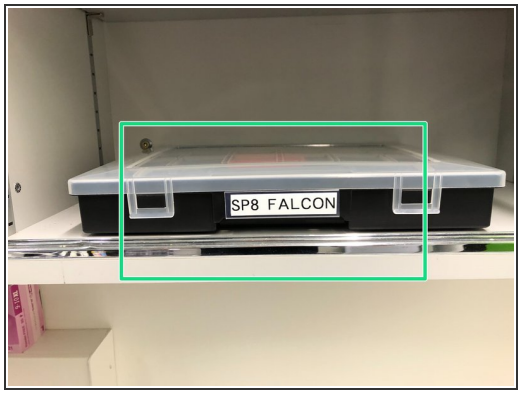
  ⚠ This step avoids possible collision during placing of inserts and/or samples.

- Select the **10x dry objective** via the "LAS X" software.

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

Step 6 — Check stage insert



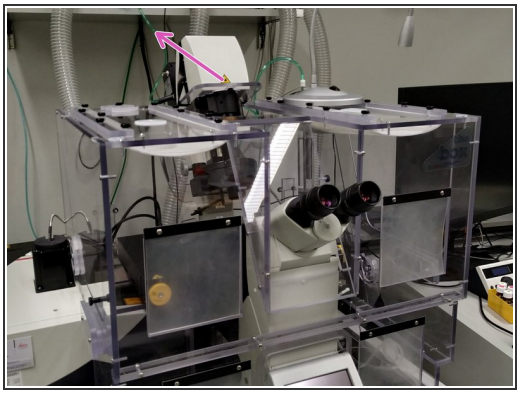


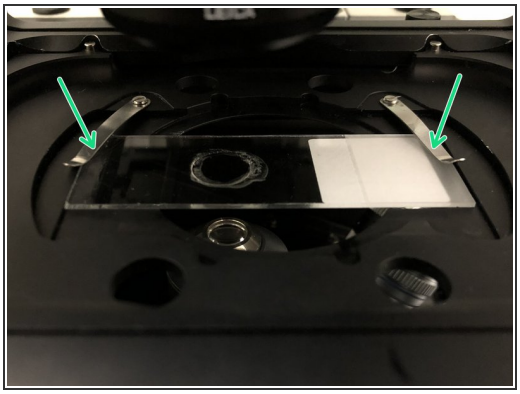


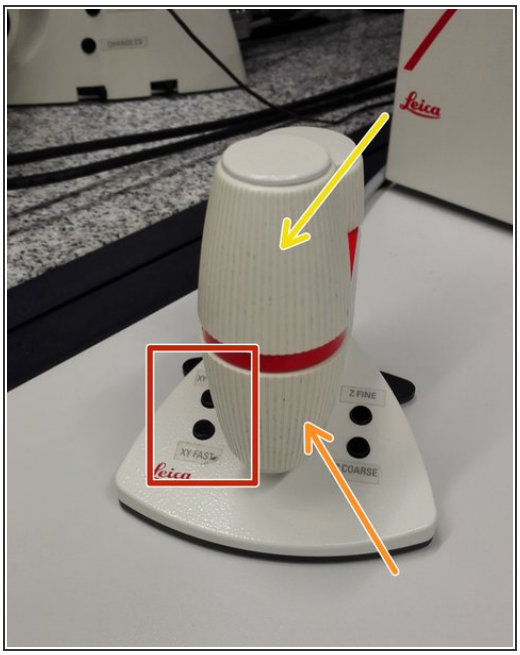
- Choose the appropriate sample holder:
  - The depicted stage insert is usually placed at the microscope.
  - Other holders (eg. 96 well plates) are stored in a box on a shelve behind the microscope.

ⓘ *If necessary, stage inserts can be easily exchanged as they are held by magnets.*

Step 7 — Mount and position your sample



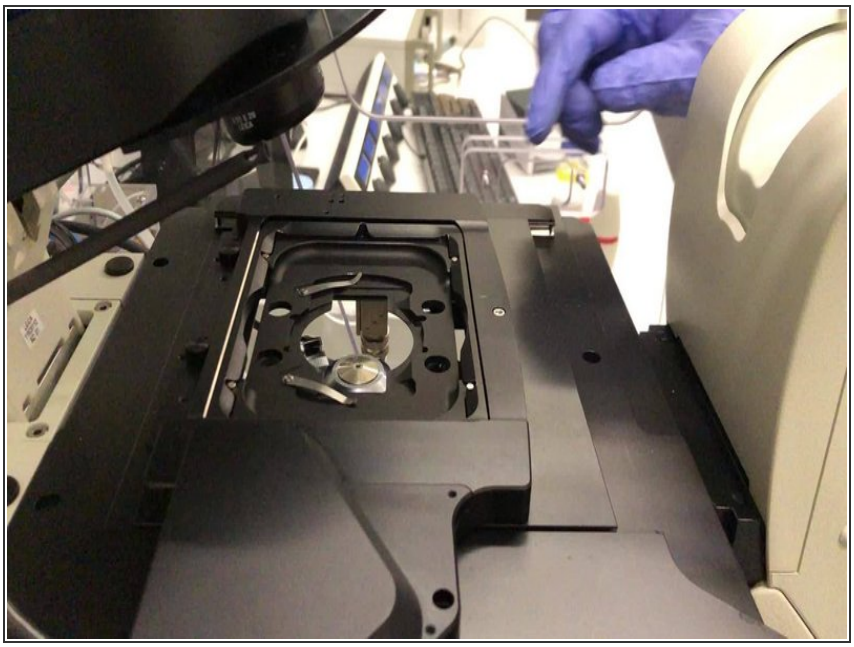




- **Push** the condensor arm of the microscope to the **back**.
- Insert your sample with the **coverslip facing down** and fix it with the two springs.
- **Move** your sample above the objective with the help of the external controller **"Smart Move".**
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

☑ Bring back condenser arm to its straight position.

Step 8 — Optional - Exchanging stage inserts

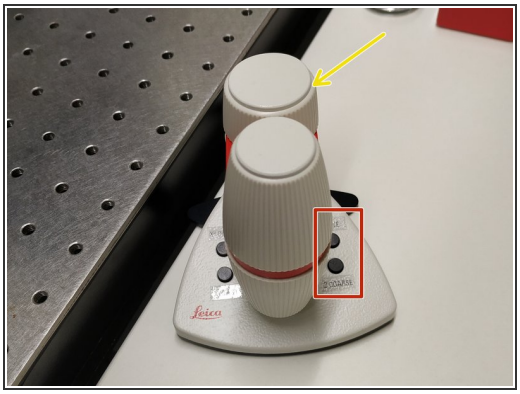


- To exchange a stage insert **Push** the condensor arm of the microscope to the **back**.
- Remove the stage insert and place the appropriate one.

Step 9 — Focus your sample

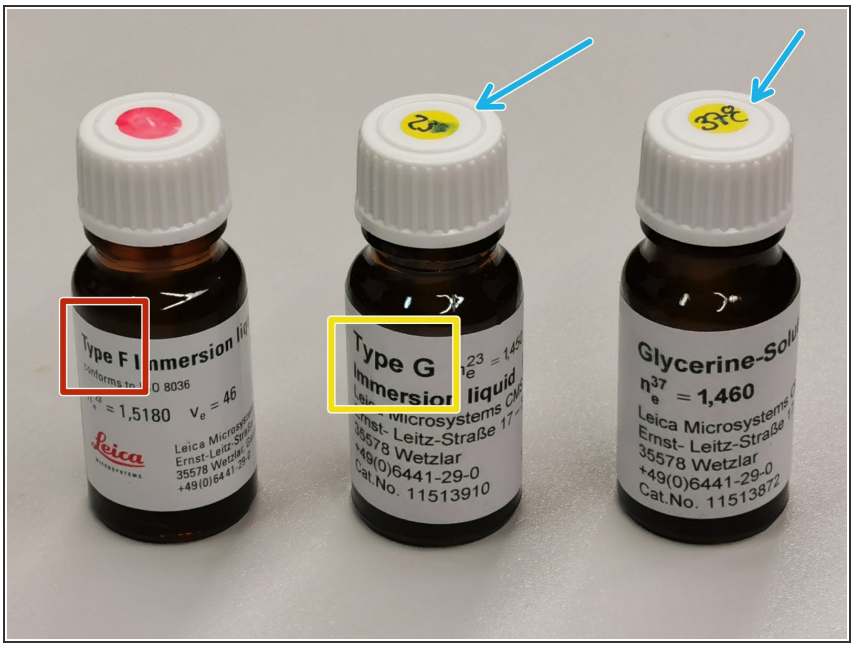






- On the touch screen at the microscope stand choose the **light path tab**.
- Click **"FLUO"** and choose an appropriate "FLUO-Filtercube" : e.g. "GFP".
- **Open** the **"IL -Shutter"** (if activated the dot is yellow).
- Look through the oculars and focus your sample by using:
  - the **focus wheel** on the microscope stand,
  - or the z-wheel on the **external controller ("Smart Move")**.
  - ⓘ Turn z-wheels clockwise to move objectives upwards (closer to the sample). Turn z-wheels counter-clockwise to move objectives downwards (away from sample).
  - Toggle between "Z FINE" and "Z COARSE" directly on the Smart Move.

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.

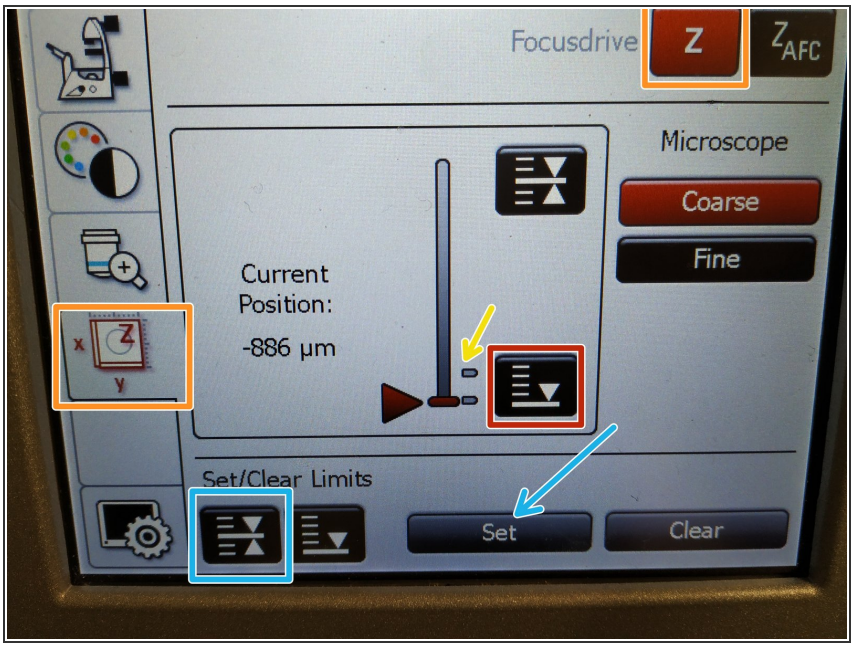
- Oil objectives: *"Type-F" immersion liquid.*
- *Glycerin objectives*: *"Type-G" immersion liquid .* For room temperature use the **23°C** glycerin media. For live cell imaging the **37°C** one.
- Water objectives : Use fresh double destiled water.

You can move (back and forth) the condenser arm for ease of access.

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

- **Focus** your sample as described previously.

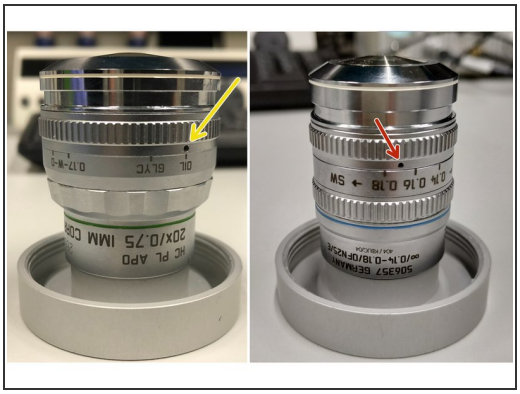
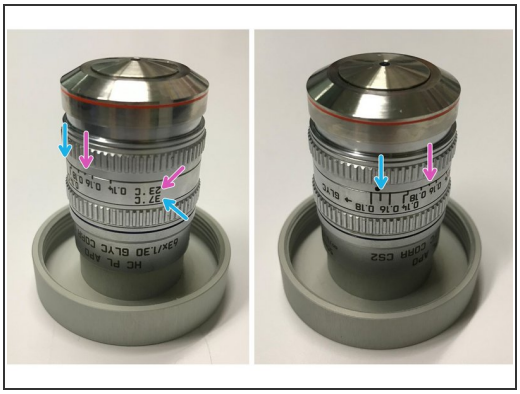
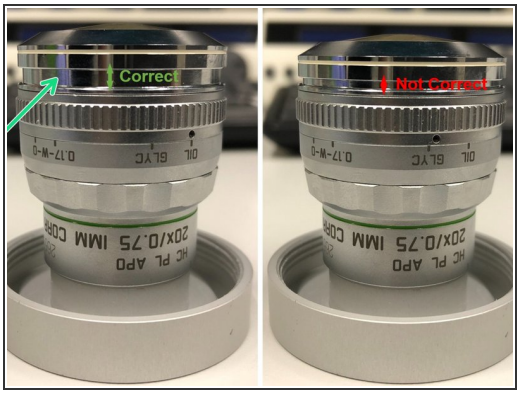
Step 11 — Optional - Save your focus position



ⓘ *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 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 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)).

ⓘ **40x water** and **63x glycerol** you can correct for the **cover glass thickness** (0.14-0.18 mm). Standard is usually 0.17 mm.

- 40x water you can adjust for the correct **cover glass thickness**.
- 63x glycerol you can adjust for the **cover glass thickness** of the corresponding **temperature**. Upper row for 23°C with the indicated 0.17mm.
  - Lower row for 37°C and indicated 0.17mm.

- Make sure that the cap of the **spring-loaded front lens** is released (working position). *Mandatory for all immersion objectives.*

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