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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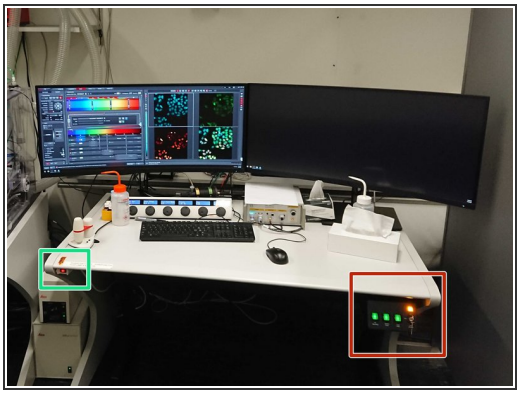
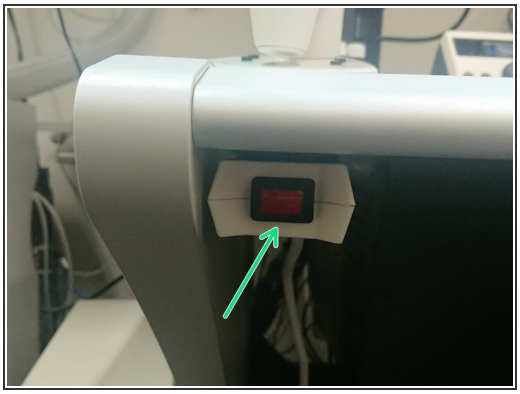
INTRODUCTION

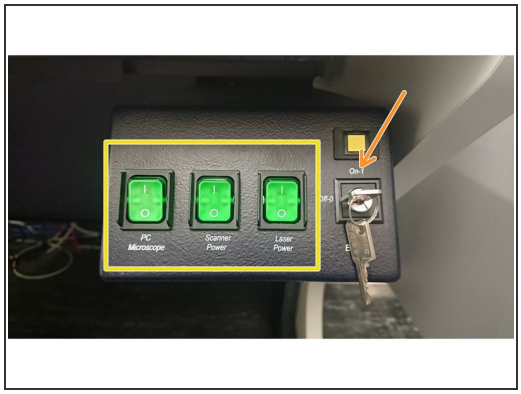
How to start up and mount your first sample on the Leica SP8 Falcon confocal laser scanning microscope located at the Irchel Campus, room Y42-H-81.

Please find detailed information about the system setup here.

Step 1 — Switching ON Hardware

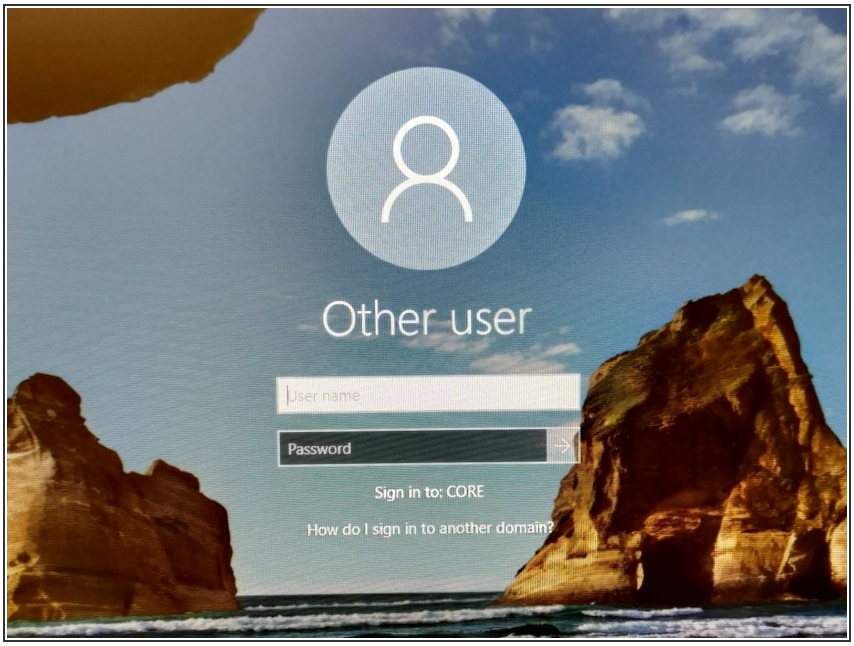






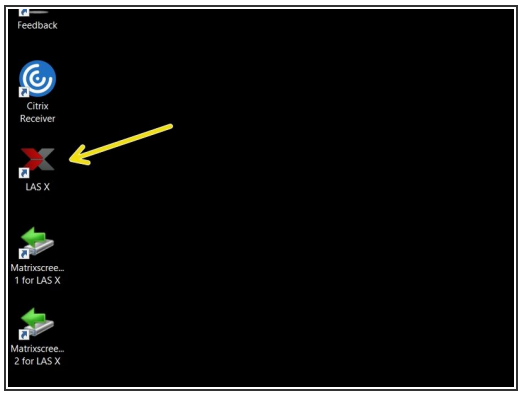
- Switch on the red button (underneath the table on the left).
  - *Turns on fluorescence lamp and monitors.*
- On the right side of the table:
  - Switch ON the **"PC/Microscope"**, **"Scanner Power"** and **"Laser Power"** switches.
  - Turn the **"Laser Emission"** key to **"ON-1"**.

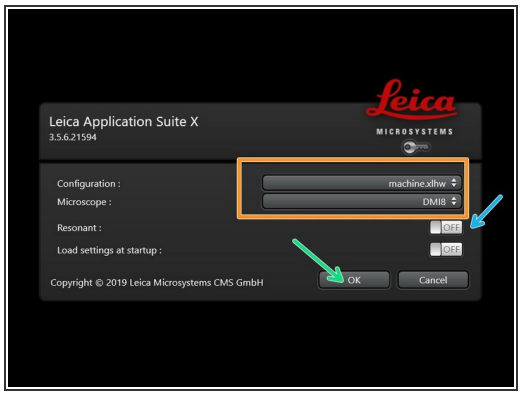
Step 2 — Sign-In



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

Step 3 — Starting up the "LAS X" Software



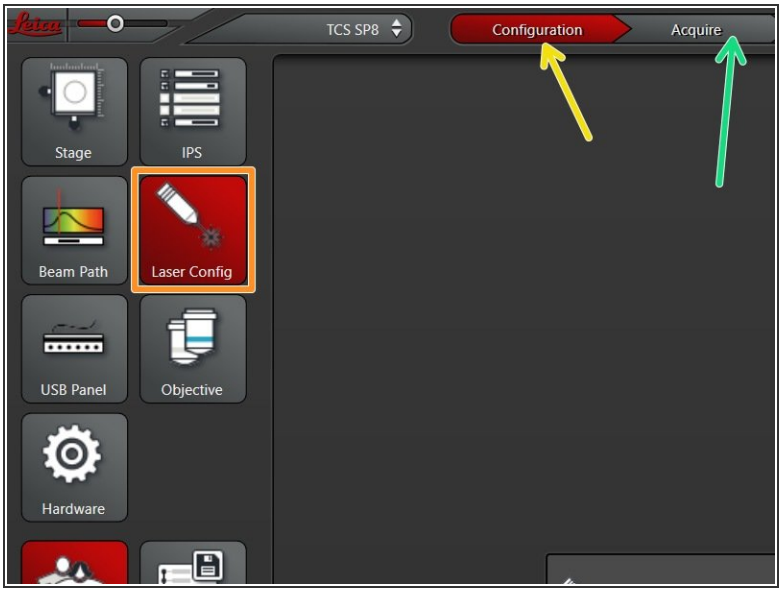


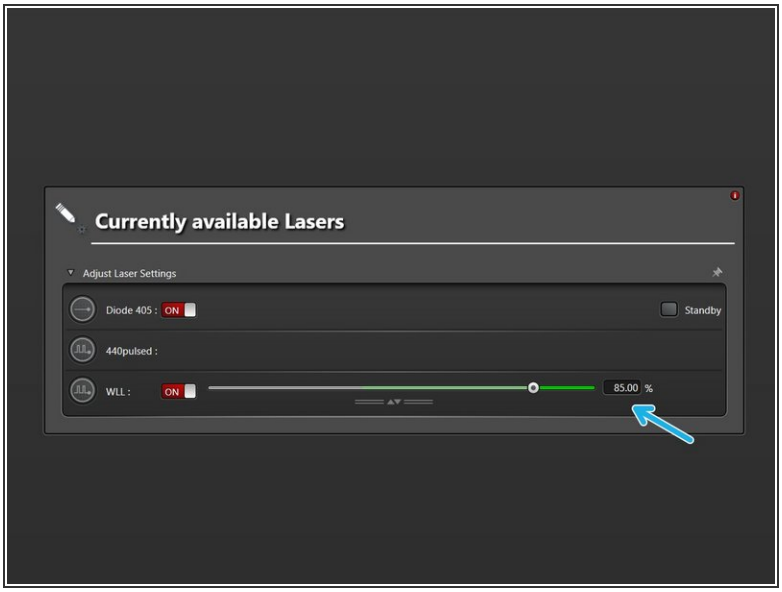
- Start the **"LAS X"** software. Select:
  - **"machine.xlhw"** for "Configuration",
  - and **"DMI8"** as "Microscope".
- Select either "Resonant" (ON) or non-"Resonant" (OFF) scanning mode.

  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

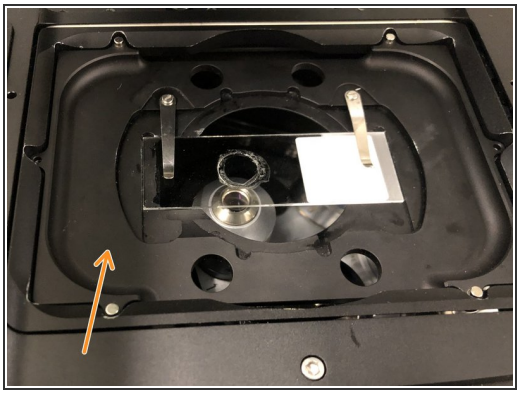
- **Lower** the **objective turret** by pressing the "Z downwards" button on the right side of the microscope.

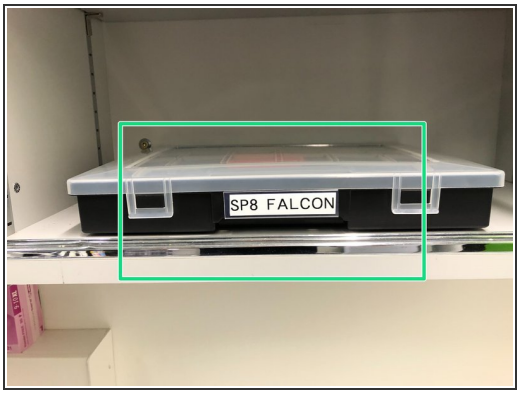
  ⚠ This step avoids possible collision during placing of inserts and/or samples.

- 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

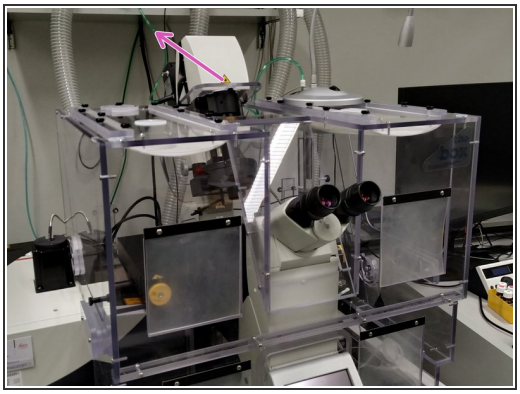


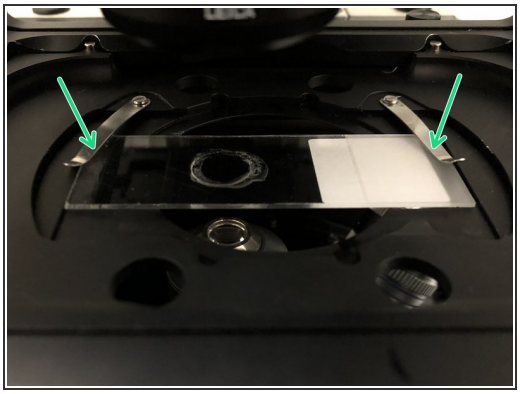


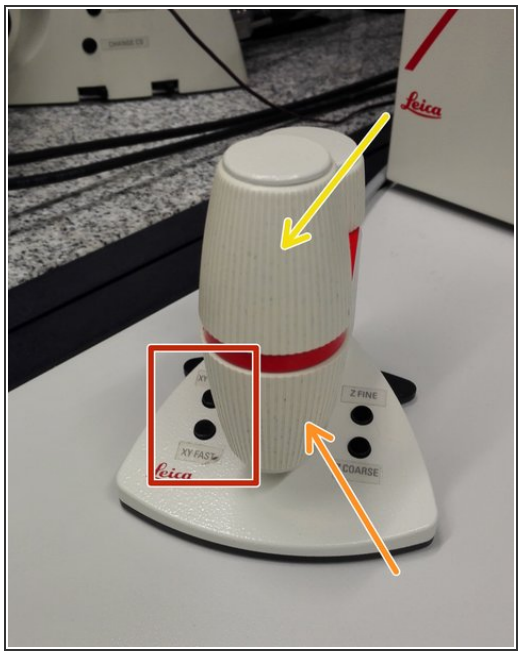
- Choose the appropriate sample holder:
  - The depicted stage insert is usually placed at the microscope.
  - Other holders (eg. 96 well plates) are stored in a box on a shelve behind the microscope.

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

Step 7 — Mount and position your sample



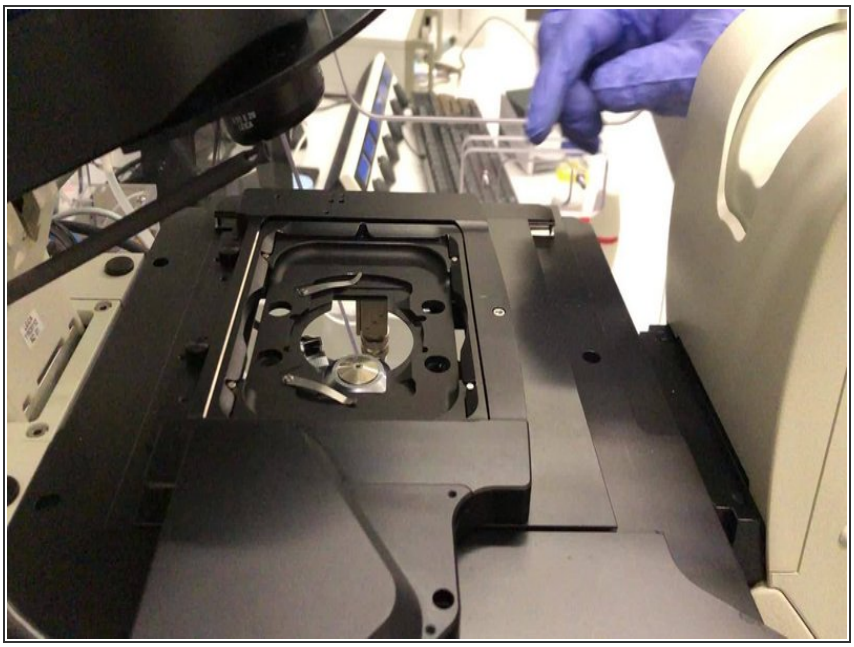




- **Push** the condensor arm of the microscope to the **back**.
- Insert your sample with the **coverslip facing down** and fix it with the two springs.
- **Move** your sample above the objective with the help of the external controller **"Smart Move".**
  - Movement in y-direction.
  - Movement in x-direction.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

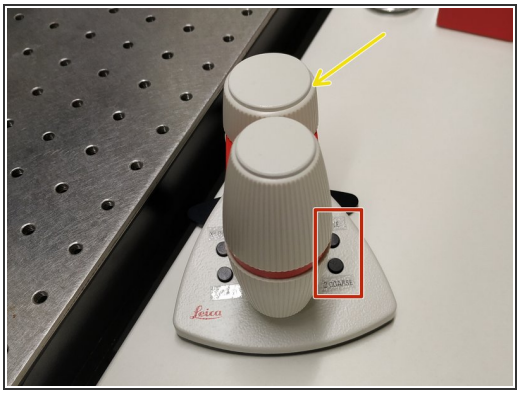
Bring back condenser arm to its straight position.

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

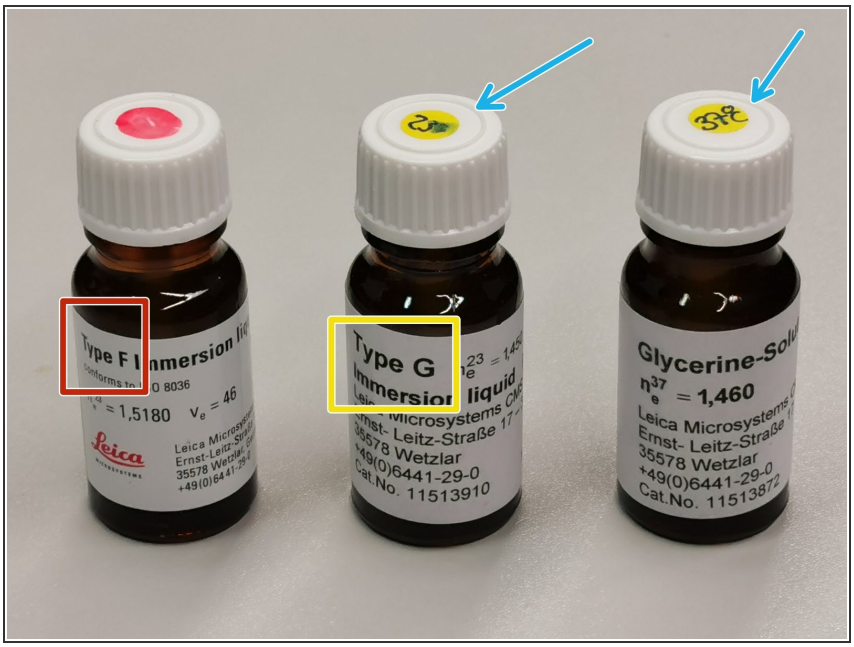


- On the touch screen at the microscope stand choose the **light path tab**.
- Click **"FLUO"** and choose an appropriate "FLUO-Filtercube" : e.g. "GFP".
- **Open** the **"IL -Shutter"** (if activated the dot is yellow).
- Look through the oculars and focus your sample by using:
  - the **focus wheel**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

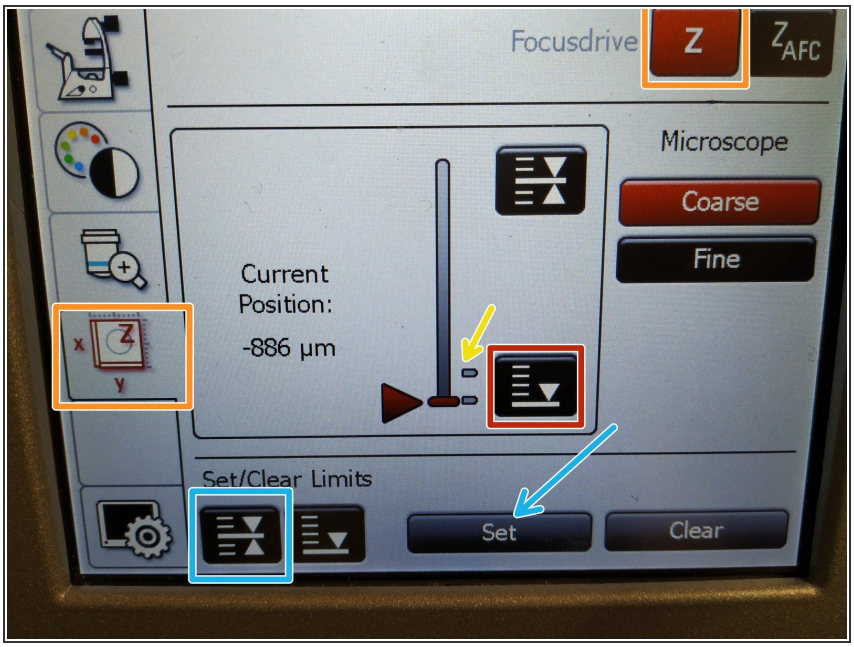
- Oil objectives: *"Type-F" immersion liquid.*
- *Glycerin objectives*: *"Type-G" immersion liquid .* For room temperature use the **23°C** glycerin media. For live cell imaging the **37°C** one.
- Water objectives : Use fresh double destiled water.

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.

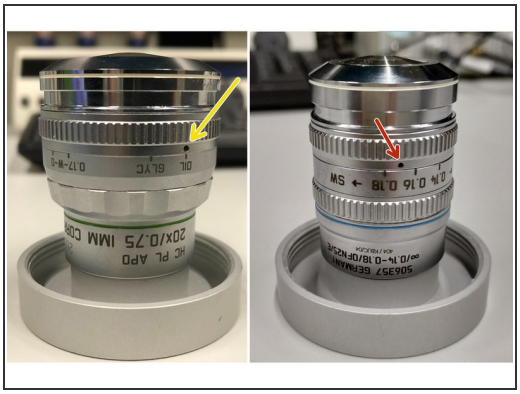
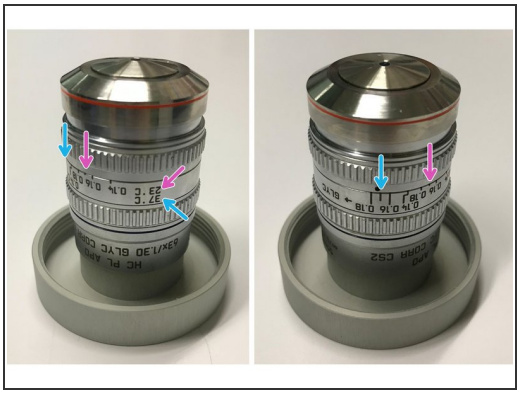
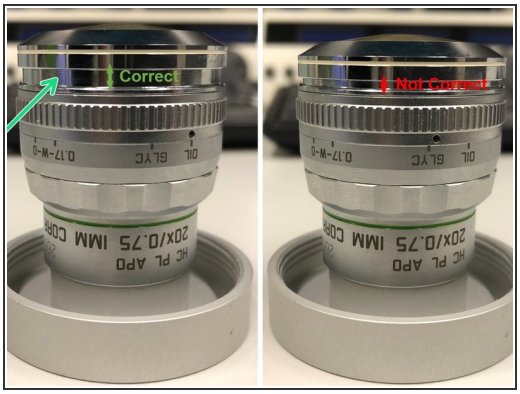
Step 11 — Optional - Save your focus position



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.