Leica SP8 inverse (Irchel) - 1: Start-up

How to start up Leica SP8 confocal laserscanning microscope located at the IMLS at Irchel Campus, Room Y55-K-41.

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INTRODUCTION

How to start up and mount your first sample on the Leica SP8 confocal laserscanning microscope located at the IMLS at Irchel Campus, Room Y55-K-41.

Please find detailed information about the system setup here.

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Step 1 — Switching ON Hardware - fluorescence and PC







- **"(1)"** Switch ON the **fluorescence lamp**.
- **"(2)"** Switch on the **PC**.

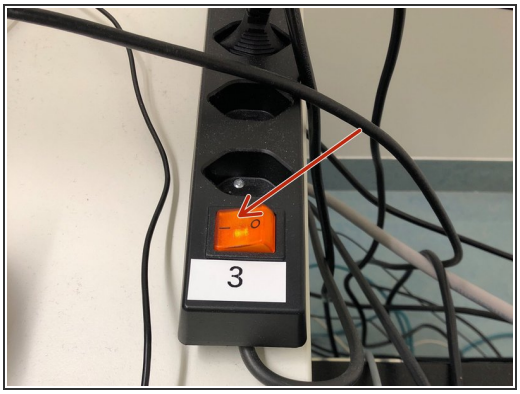
Step 2 — Sign-In



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

Step 3 — Switching ON Hardware - Microscope components







- **"(3)"** Press the **main power switch** mounted on the right hand side of the table.

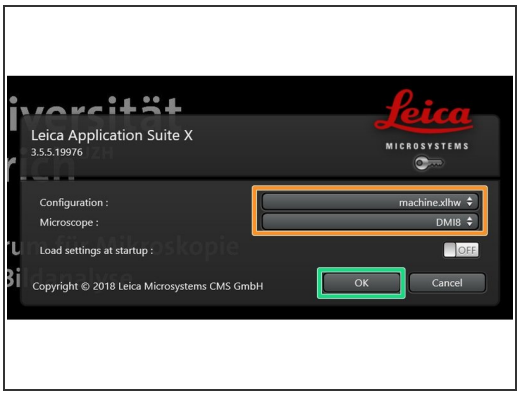
  ⓘ Scanner, lasers and microscope will be switched ON. If not, "

  - check on the **supply unit** that the "Scanner Power" and "Laser Power" are switched on, and the "Laser Emission" key turned to "On-1",
  - check that the **microscope control box** is switched on.

⚠ Please **wait** for at least **1 min** before continuing with next step.

Step 4 — Starting up the "LAS X" Software



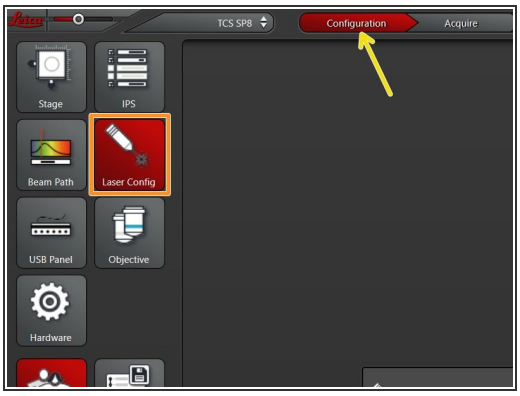


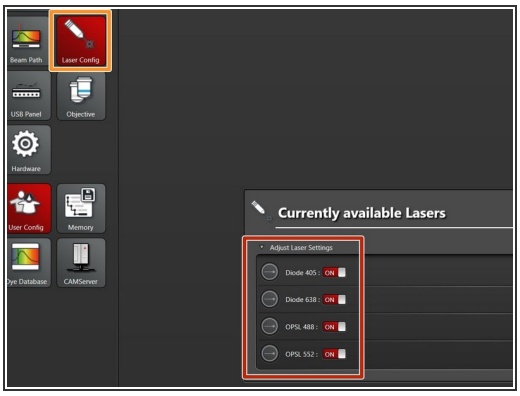


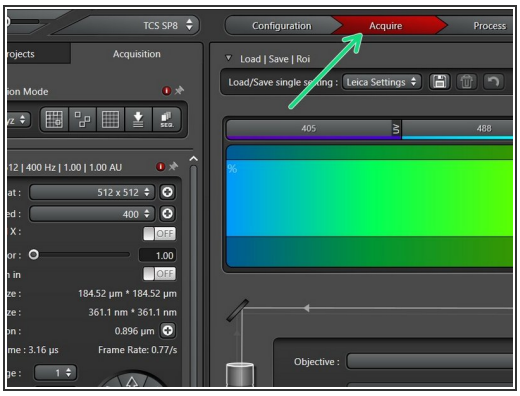
- Start the **"LAS X"** software.
- Make sure **"machine.xlhw"** is selected for "Configuration", and **"DMI8"** as "Microscope".
- Click **"OK"**.
- Click **"Yes"** in order to **initialize the x/y stage**. *Please make sure nothing is placed on the stage.*

  An x/y stage initialization is necessary in order to use the Tilescanning, Mark and Find and the Navigator function.

Step 5 — Switch ON the lasers in the software



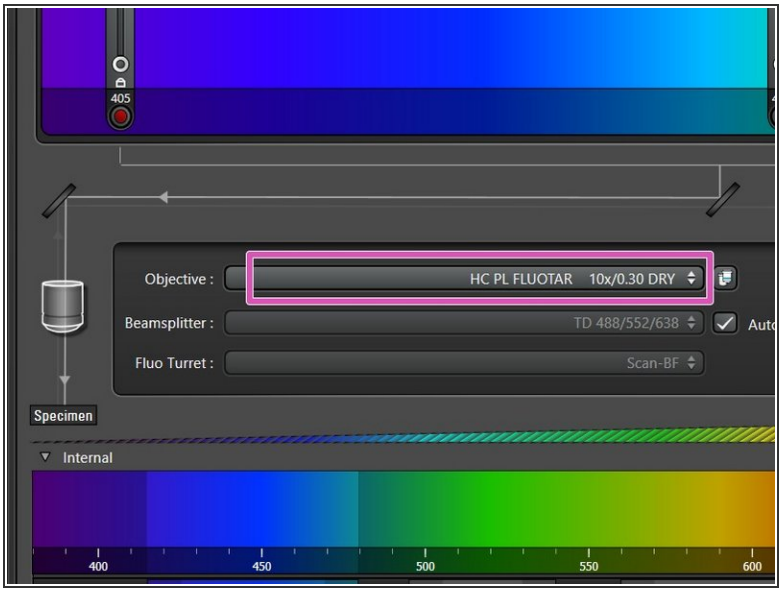




- Go to **"Configuration"**.
- Select **"Laser Config"**.
- **Switch "ON"** the lasers you will need.
- Go back to **"Acquire"**.

Step 6 — Choosing an objective





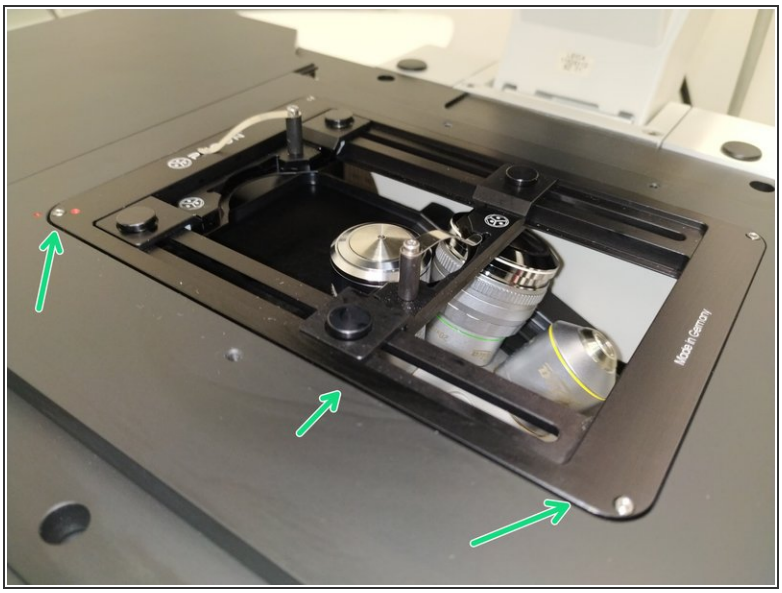
- **Lower** the **objective turret** by pressing the downwards "Z" button on the right side of the microscope.

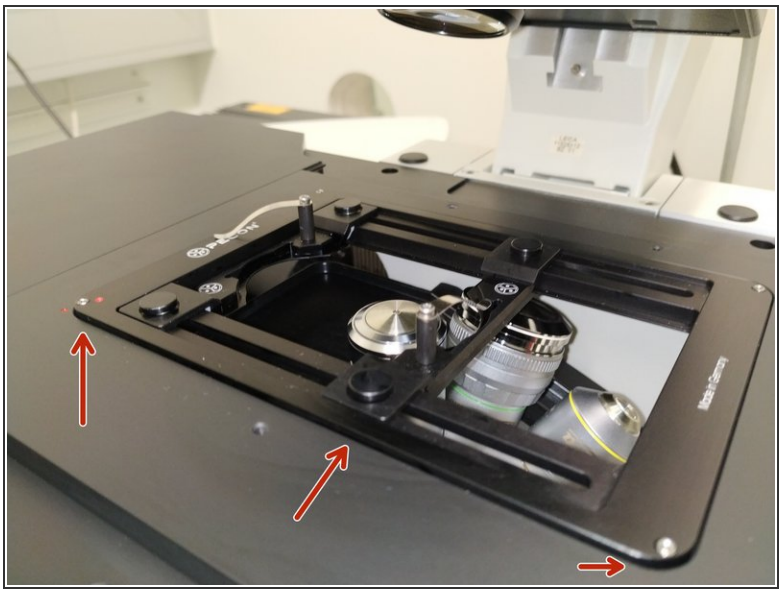
⚠ This is a **mandatory step** as it avoids possible collision of the objectives and stage during exchange of inserts and/or samples.

- 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 7 — Check stage insert

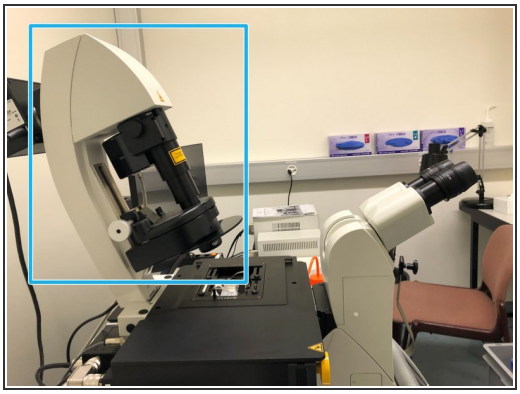


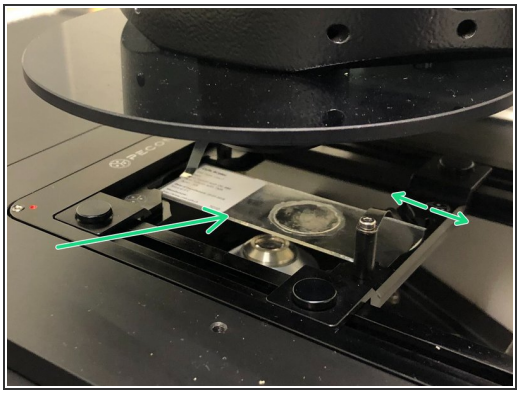


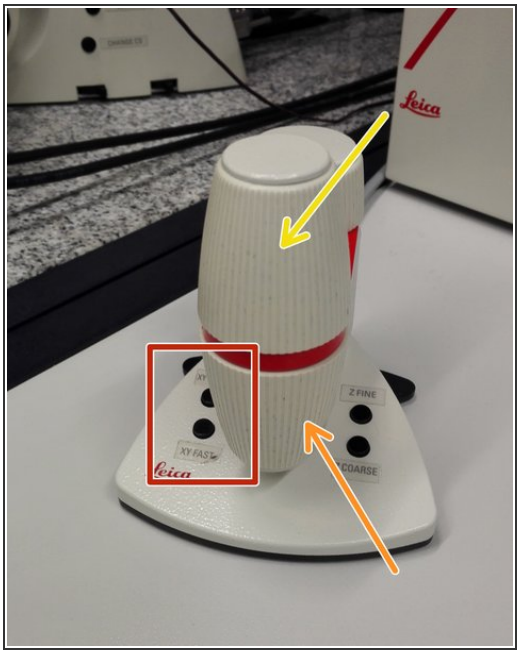
⚠ Make sure that the stage insert is correctly inserted and flat.

- Here correct and flat.
- Here not inserted correctly (stage not flat and shaky).

Step 8 — Mount and position your sample





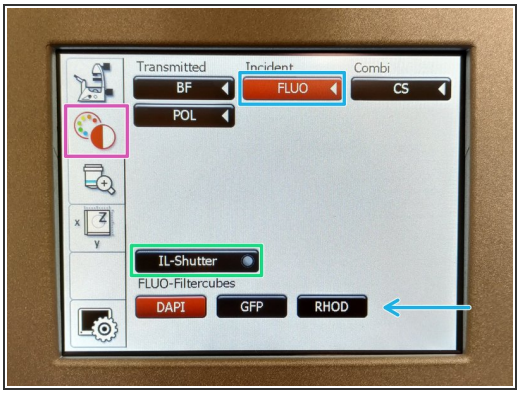


- **Push** the condensor arm of the microscope to the **back**.
- Insert your sample with the **coverslip facing down**.

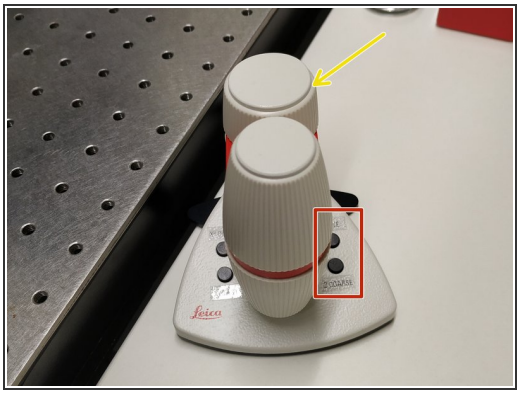
  Adjust the variable clamping range and moveable brackets to properly fix your sample.
- **Move** your sample above the objective with the help of the external controller **"Smart Move".**
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

Bring back condenser arm to its straight position.

Step 9 — Focus your sample





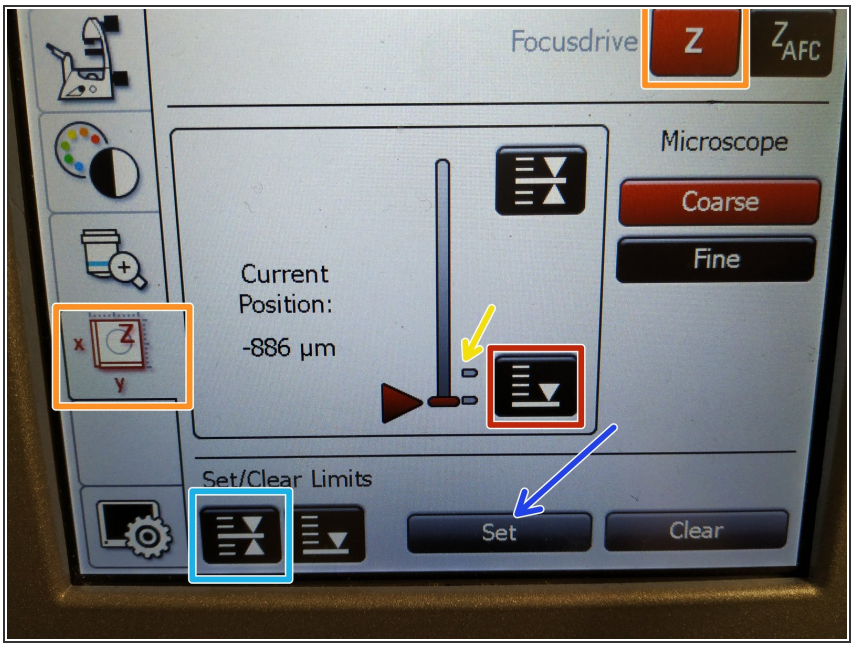


- On the touch screen at the microscope stand choose the **light path tab**.
- Click "FLUO" and choose an appropriate "FLUO-Filtercube" : e.g. "DAPI".
- **Open** the **"IL -Shutter"** (if activated the dot is yellow).
- Look through the oculars and focus your sample by using:
  - the **focus wheel**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

  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 10 — Optional - Save your focus position

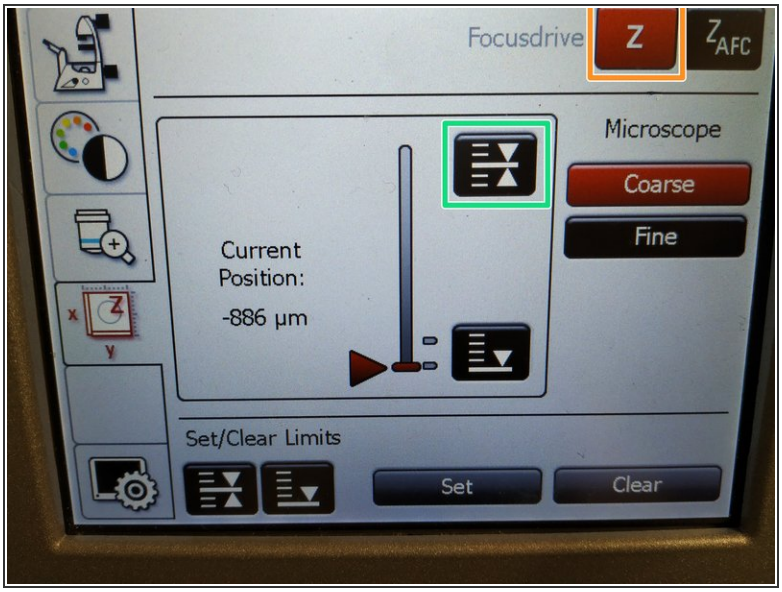


The storage of the focal plane is helpful in order to find the focus back if the sample or objective will be changed.

- 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 11 — 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 .*

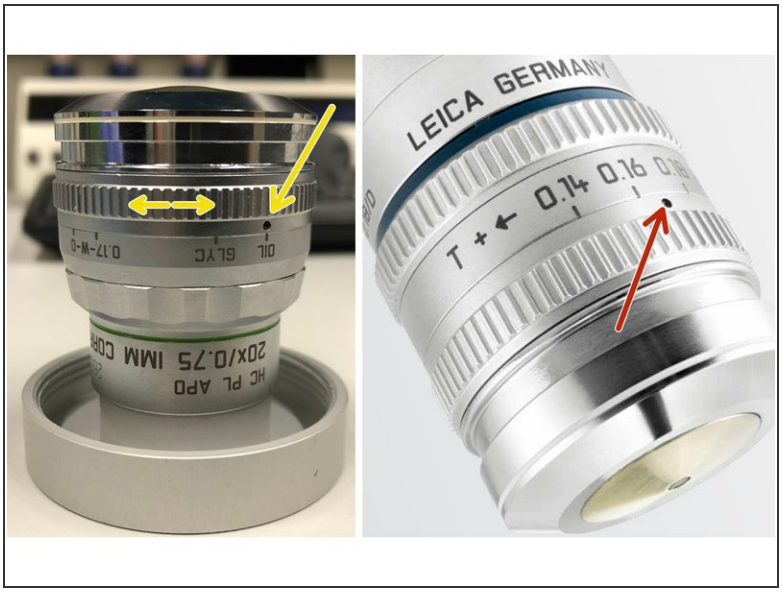
Please further consider the additional information in the next step to guaranty proper image acquisition.

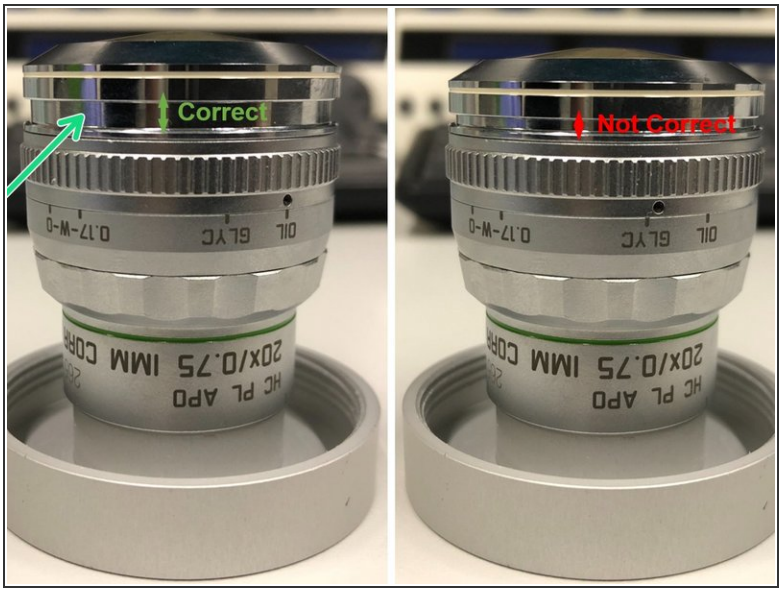
- **Mount** your sample again and press the **"Upper Focus Limit"** button.

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

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

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 **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). *Mandatory for all immersion objectives.*

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