Leica Stellaris 5 upright (Bot) - 1: Start-up

How to start up and mount a sample on Leica Stellaris 5 upright confocal laser scanning microscope located at Irchel Campus, room Y42-H-79.

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INTRODUCTION

This guide from the Center for Microscopy and Image Analysis shows how to start up and mount a sample on the NEW Leica Stellaris 5 confocal laser scanning microscope. It will introduce users in the new Stellaris 5 confocal laser scanning microscope.

The most relevant new features are:

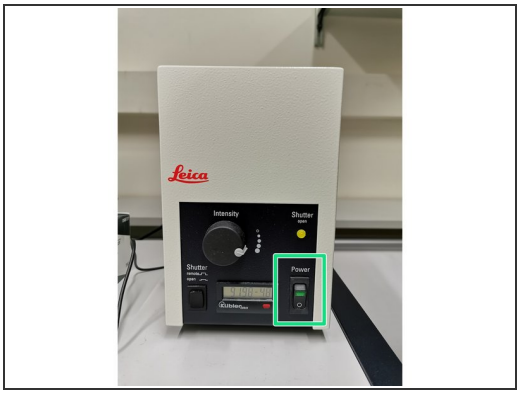
- NEW Spectrally flexible white light laser (WLL) with an extended spectral output on the red and near-infrared spectrum (485 nm up to 685 nm).
- NEW 4 Power HyD S detectors.
- NEW TauSense technology, which gives access to lifetime-based information, delivering additional insights to your experiments.
- Possibility of switching between resonant and non-resonant scanner directly within the software.

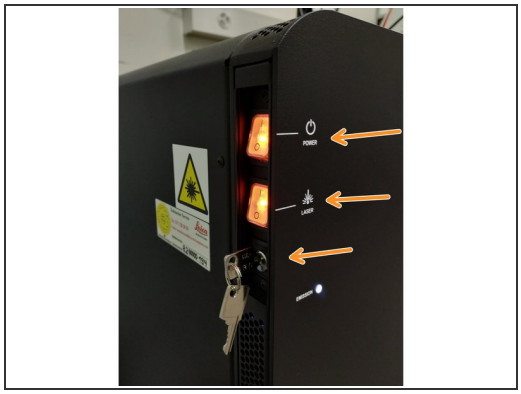
Please find more detailed information about the system here.

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Step 1 — Switching ON hardware

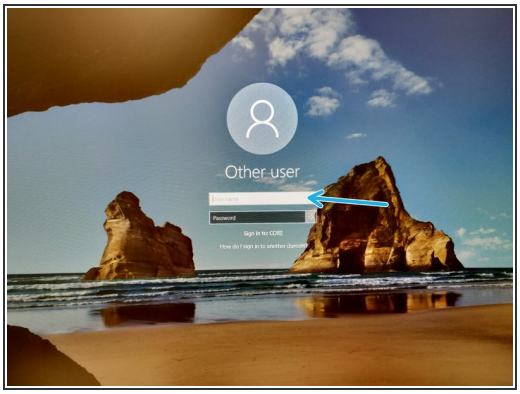






- Switch ON the **fluorescence lamp**.
  - *Once turned on, the lamp should remain ON for a minimum of 30 min.*
- Switch ON the **"Power"**, **"Laser"**, and turn the **" Emission"** key to "On-1" (control unit next to the microscope).
- Switch ON the PC.

Step 2 — Sign-in and starting "LAS X" software







- **Sign-in** with your **ZMB core** credentials.
- **Start** the "**LAS X**" software.
- Make sure **"machine.xlhw"** is selected as "Configuration", and **"DM6"** as "Microscope".
- Click "**OK**".
- Click **"Yes"** in order to **initialize the x/y stage**. *Please make sure nothing is placed currently on the stage.*

  ⓘ *x/y stage initialization is necessary to be able to use the "LAS X Navigator".*

Step 3 — Choosing an objective





- **Lower** the **stage** by keeping the "LOWER Z" button pressed on the right side of the microscope.

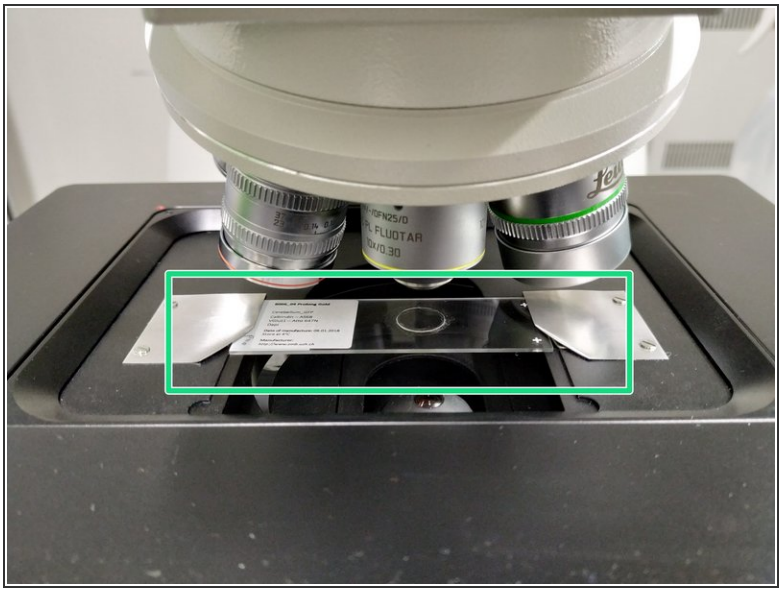
  ⚠ This is a mandatory step as it avoids possible collision of the stage and objective during exchange of inserts and/or samples.

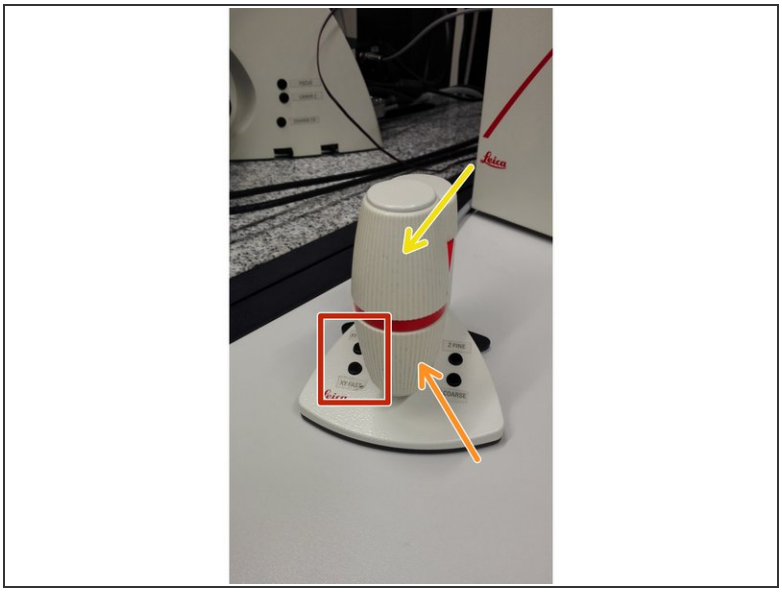
- You can now toggle between objectives within the software (drop-down menu).

- Select the **10x dry objective** in the "Aquire" tab.

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

Step 4 — Mount and position your sample





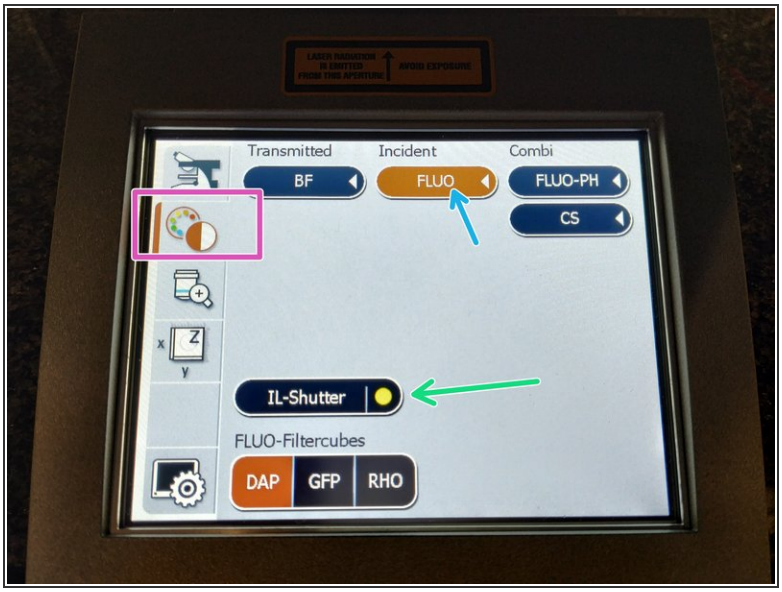
- **Slide** your sample into place with the **coverslip facing up** .
- Move your sample under the objective with the help of the external controller **"Smart Move"**.
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

Step 5 — 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: Eyepieces 100%".

Step 6 — Focus your sample



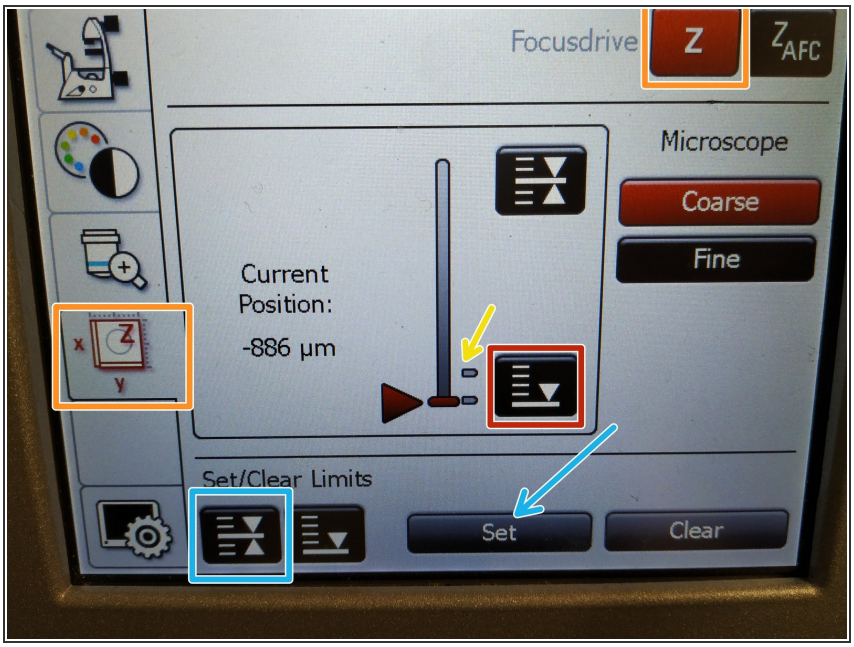


- On the touch screen at the microscope stand choose the **light path tab**.
- Click **"FLUO"** and choose an appropriate "FLUO-Filtercube" : e.g. "DAP".
- Open the **"IL -Shutter"**(if activated the dot is yellow).
- Look through the oculars and **focus your sample** by using:
  - or the z-wheel on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

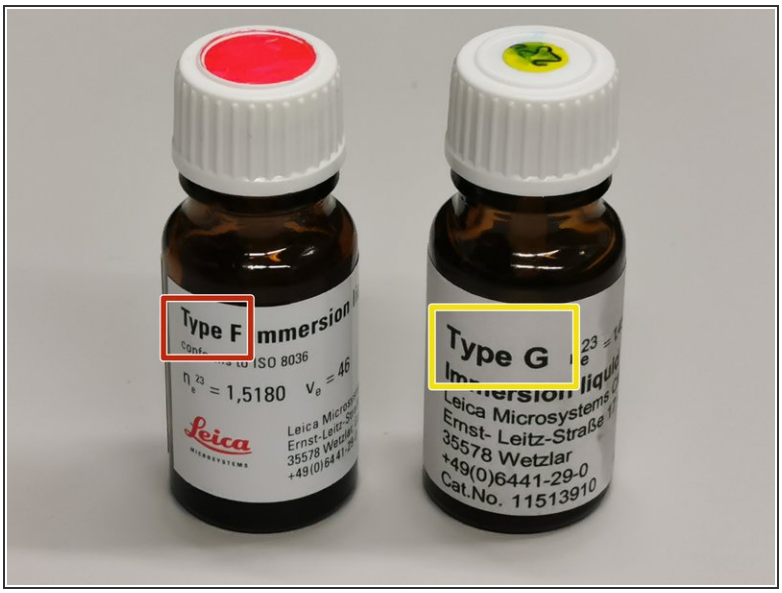
Step 7 — Optional - Save your focus position

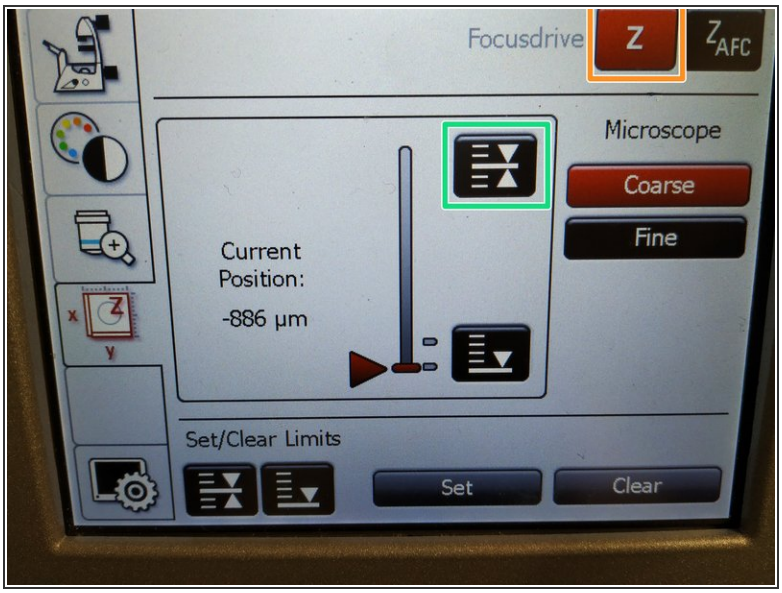


ⓘ *The storage of the focal plane is helpful in order to find the focus back if the sample or objective will be changed.*

- 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 8 — Switching to a higher magnification





- Lower the stage. Toggle within the software to the objective of choice.

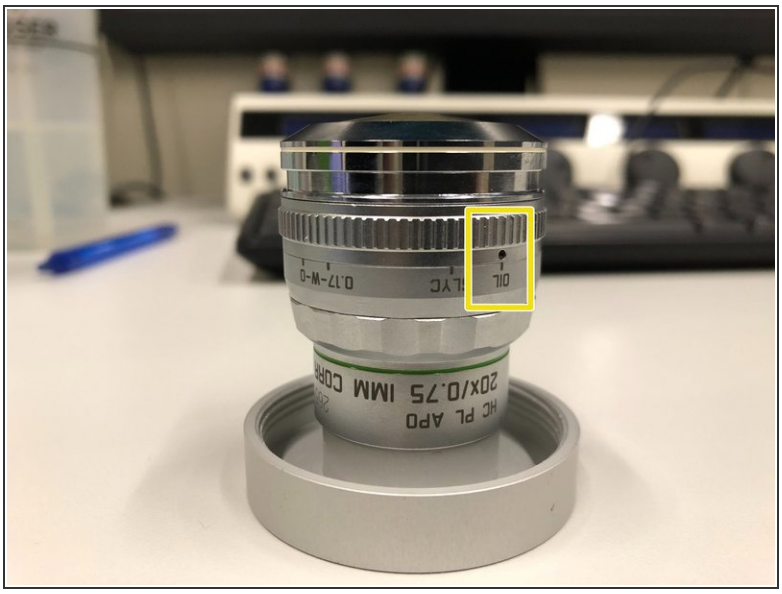
ⓘ Depending on the objective different **immersion media** will be used. Apply directly on the sample.

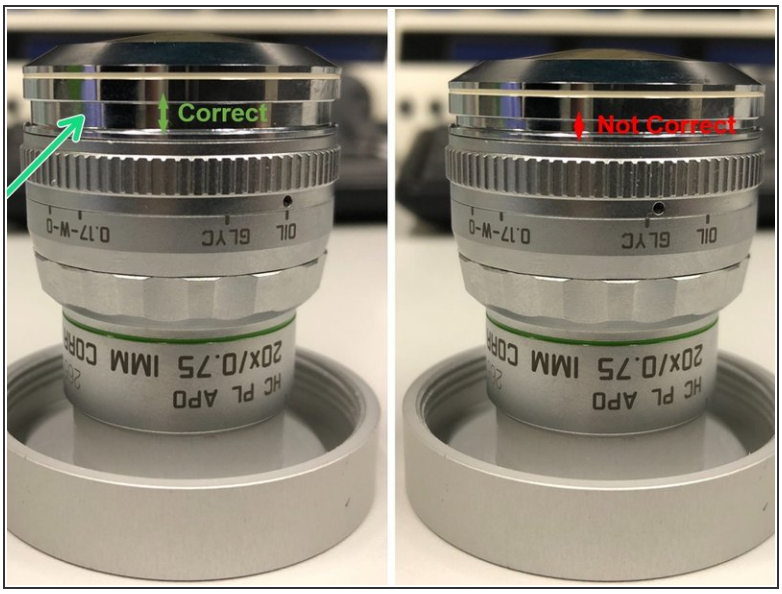
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: *"Type-G" immersion liquid* .
- "Water" objectives: *ddH2O* (always use fresh).

⚠ 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 9 — Additional information - Immersion objectives





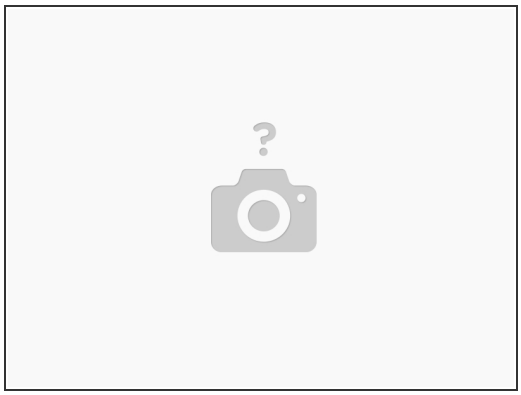
⚠ For optimal imaging performance on some objectives the **correction collar** has to be adjusted.

- **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.

Step 10 — Next steps



- You are now ready to acquire your images. Please check the corresponding follow up guides.
  - Leica Stellaris 5 - 2: Acquire an image in xy and z.