Leica Stellaris 5 inverse (USZ) - 1: Start-up

How to start up and mount a sample on the Leica Stellaris 5 inverse confocal laser scanning microscope located at USZ, Schmelzbergstrasse 12 , 8091 Zürich.

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INTRODUCTION

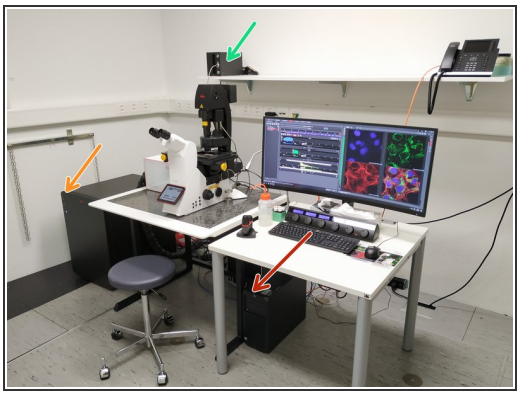
In this guide of the Center for Microscopy and Image Analysis we show how to start up and mount a sample on the NEW Leica Stellaris 5 inverse confocal laser scanning microscope located at the USZ, Schmelzbergstrasse 12 , CH-8091 Zürich.

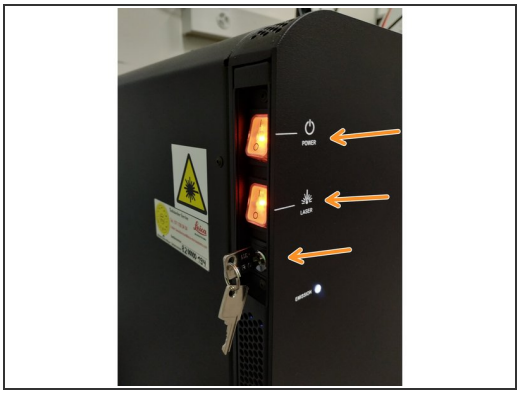
It will introduce users in the new Stellaris 5 confocal laser scanning microscope. The most relevant new features are:

- 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 3 Power HyD S detectors.
- NEW TauSense technology, which gives access to lifetime-based information, delivering additional insights to your experiments.

Please find more detailed information about the system here.

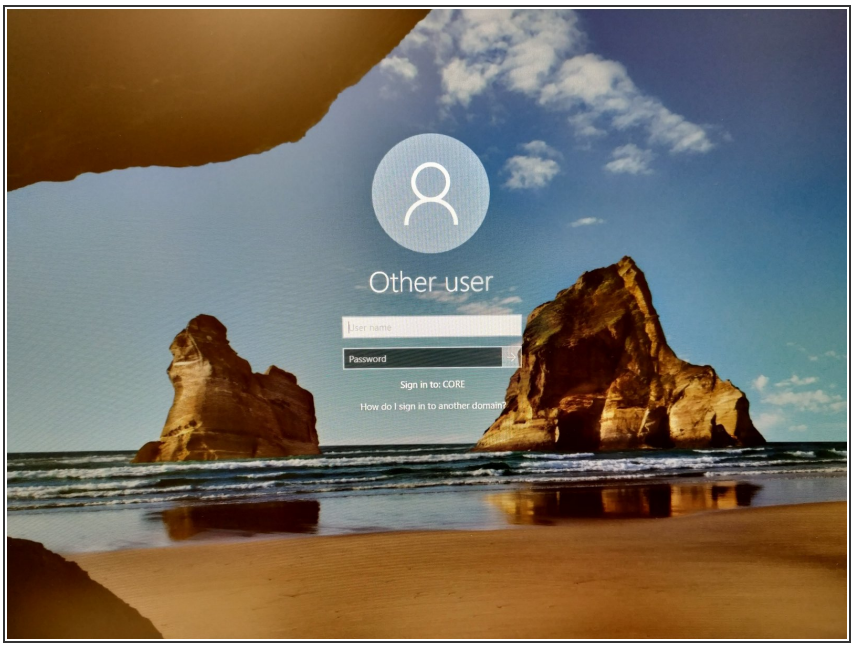
Step 1 — Switching ON hardware





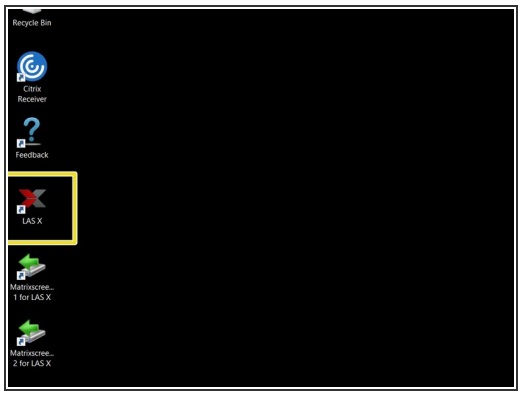
- Switch ON the **"Power"**, **"Laser"**, and turn the **"Emission"** key to "On-1" (control unit next to the microscope).
  - The LED (for fluorescence observation via the oculars) switches on automatically. No action needed.
- Switch ON the PC.

Step 2 — Sign-In



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

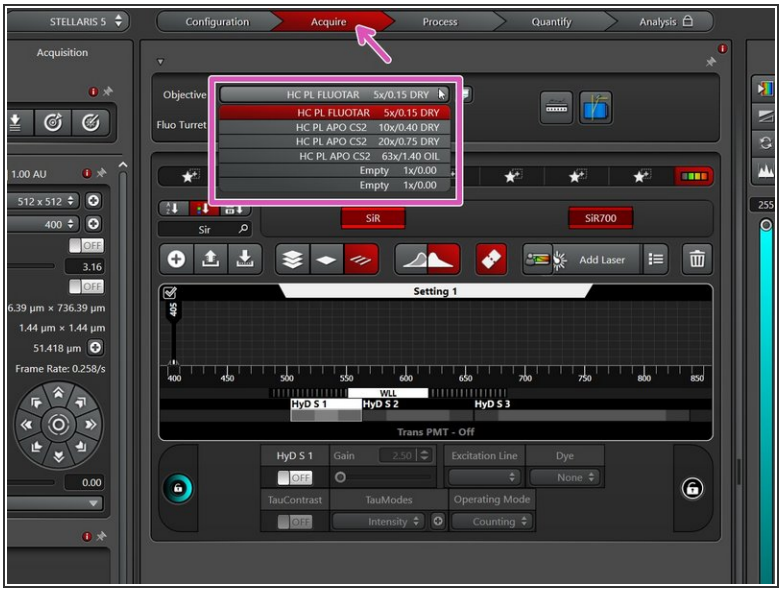
Step 3 — Starting "LAS X" software



- **Start** the "**LAS X**" software.
- Make sure **"machine.xlhw"** is selected as "Configuration", and **"DMI8"** 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 4 — Choosing an objective



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

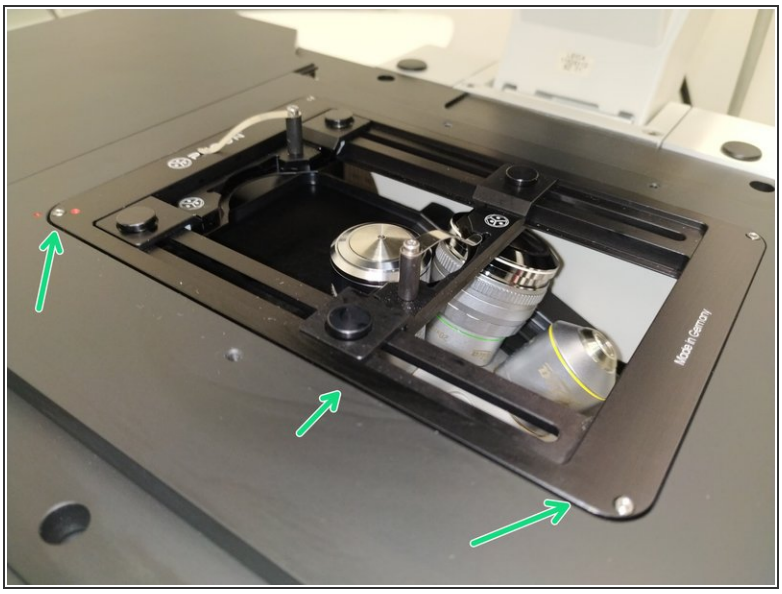
  ⚠ This is a mandatory step as it avoids possible collision of the stage and objective during exchange of inserts and/or samples.

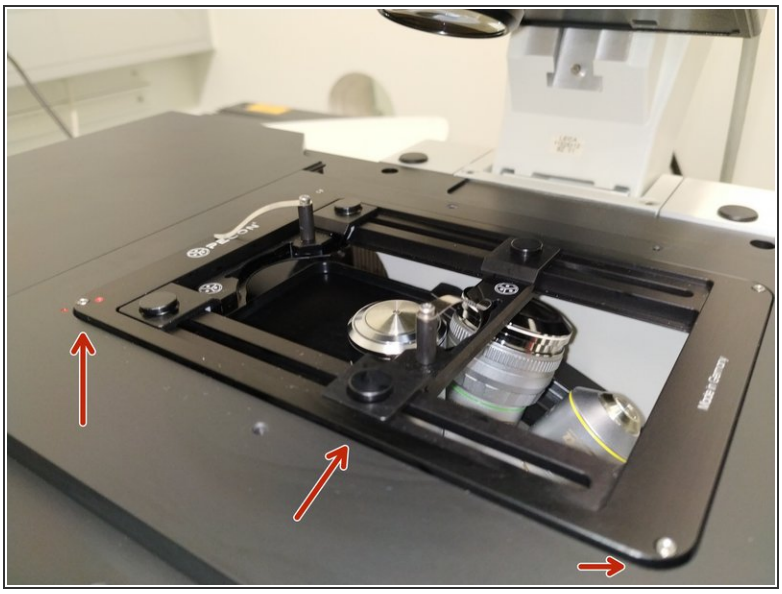
- In the **"Acquire" tab** you can now toggle between objectives (drop-down menu).

- Select the **5x dry objective** for easy sample navigation and focusing.

  ⓘ *In order to facilitate the focusing it is recommended to start with a low magnification dry objective.*

Step 5 — Check stage insert

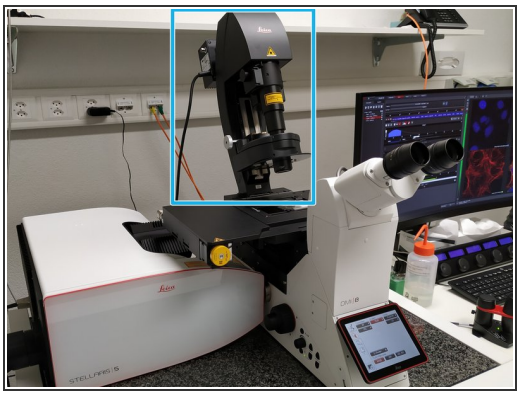
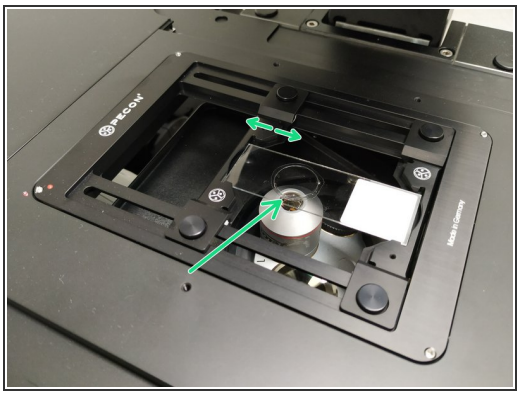


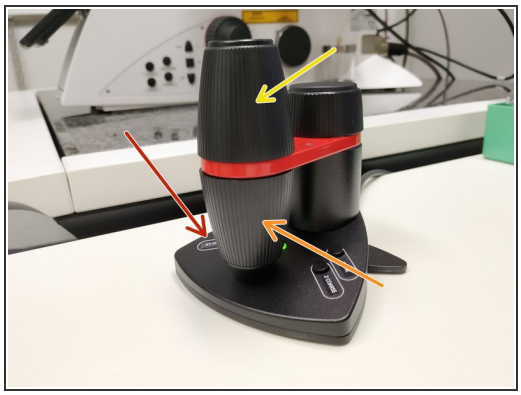


⚠ Make sure that the stage insert is correctly inserted and flat.

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

Step 6 — Mount and position your sample

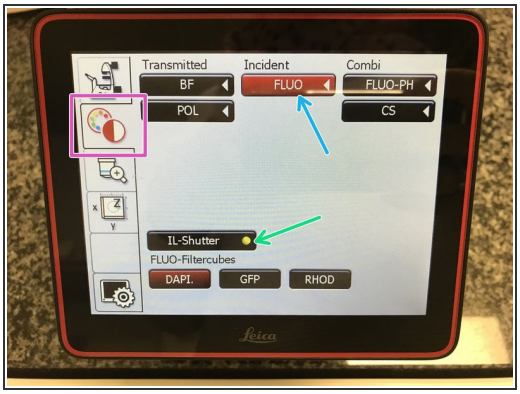



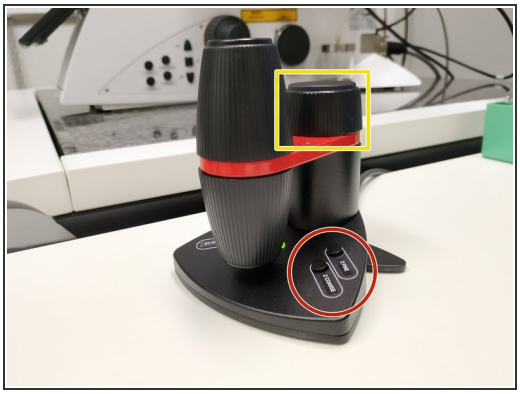


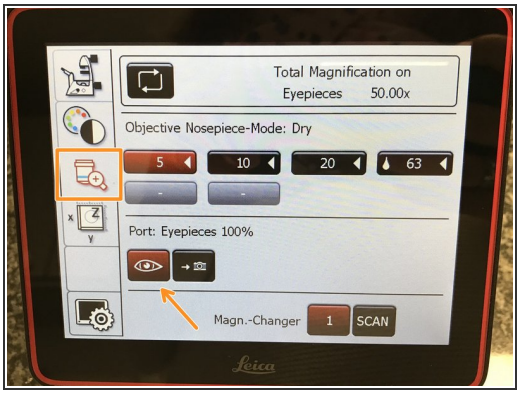
- Push the condenser arm of the microscope back.
- Insert your sample with the **coverslip facing down** .
  - *Adjust the variable clamping range and moveable brackets to properly fix your sample.*
- Move your sample under the objective with the help of the external controller **"Smart Move"**.
  - Movement in y-direction.
  - Movement in x-direction.
  - Toggle between coarse movement "XY Fast" and slow movement "XY Precise".

Bring back condenser arm to its straight position.

Step 7 — 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 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.

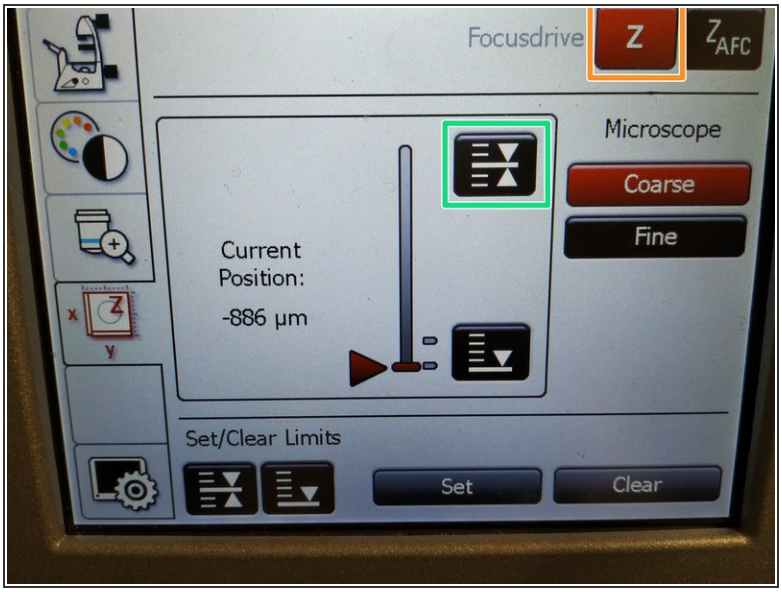
  - Toggle between "Z FINE" and "Z COARSE" directly on the "Smart Move".
- If you cannot see any signal, make sure that the light path is directed to the eye pieces.

Step 8 — 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 9 — Switching to a higher magnification



- Lower the objectives, 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.

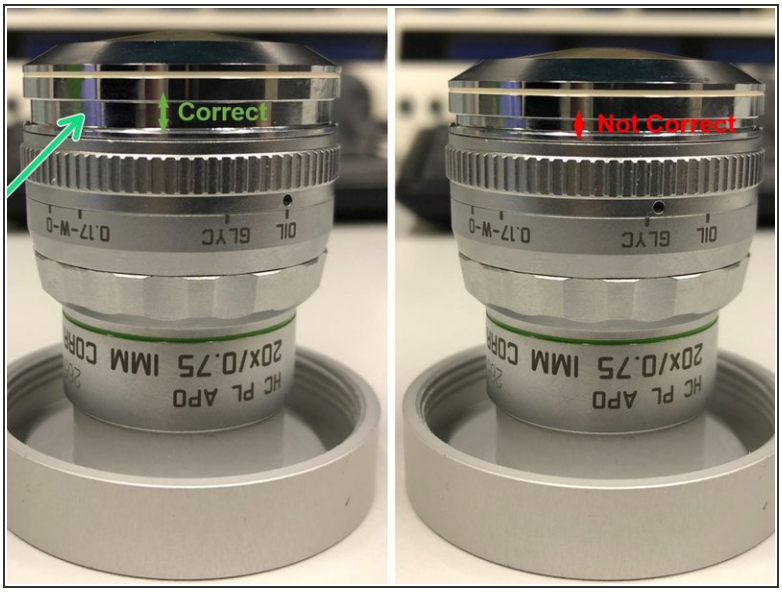
- Oil objectives: *"Type-F" immersion liquid*.
- "Glycerin" objectives: *"Type-G" immersion liquid*.
- "Water" objectives: *ddH2O* (always use fresh).

- **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 10 — Additional information - Immersion objectives



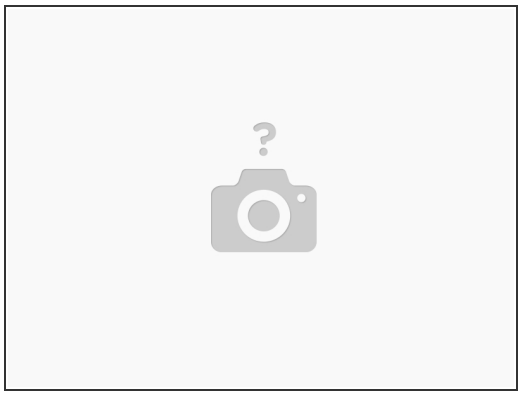
⚠ For optimal imaging performance on some objectives the **correction collar** has to be adjusted.

- **20x IMM** (multi-immersion - Oil, Glycerin or Water) needs to be set by moving the black dot 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 note: Displayed objective just an example, not exactly same as installed on system) .*

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

Step 11 — 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