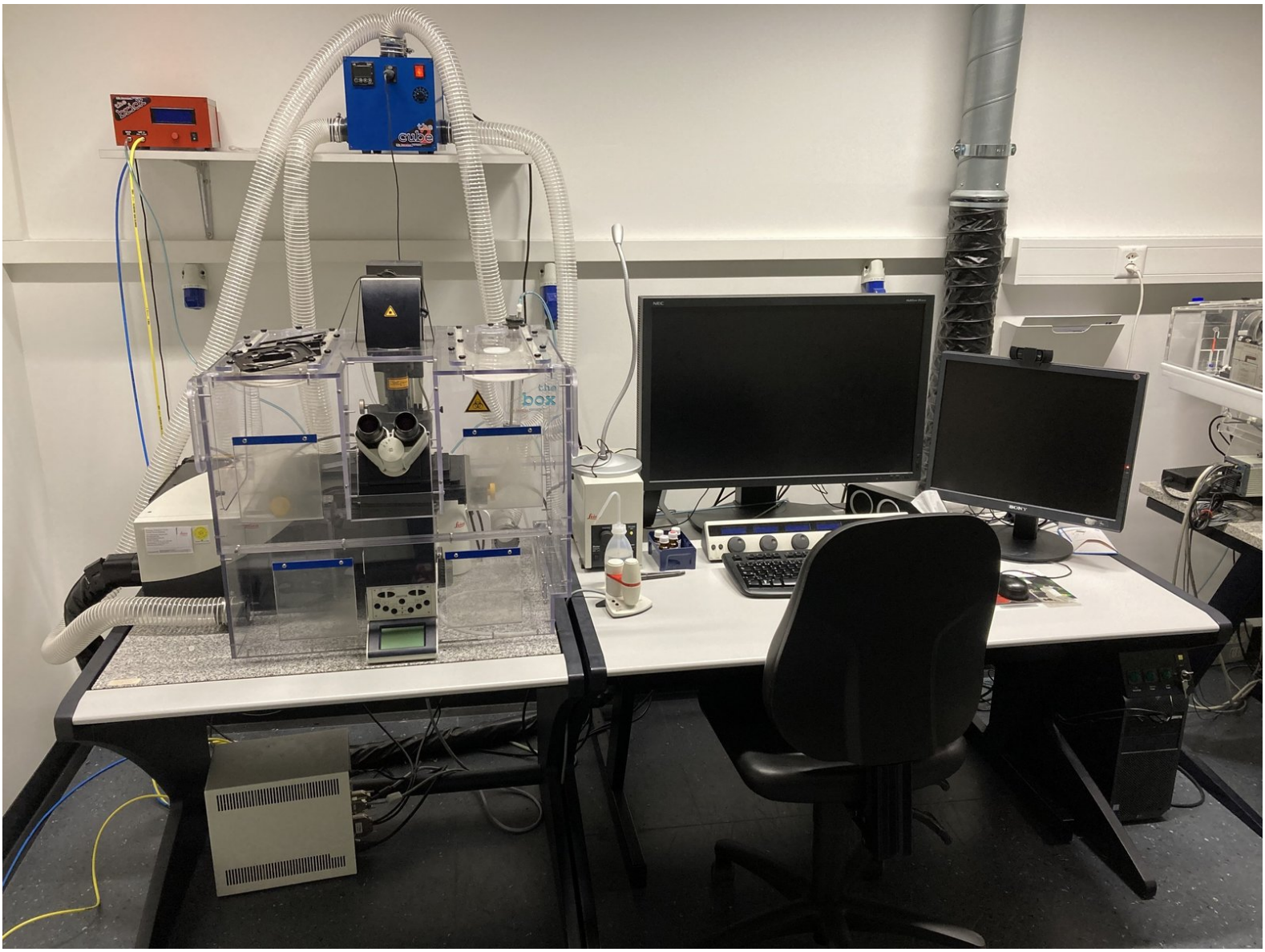
Leica SP8 Inverse (Schlieren): Live Cell Imaging

How to set up environmental control at the Leica SP8 inverse confocal laser scanning microscope located at WAD in Schlieren..

Written By: Philipp Schätzle

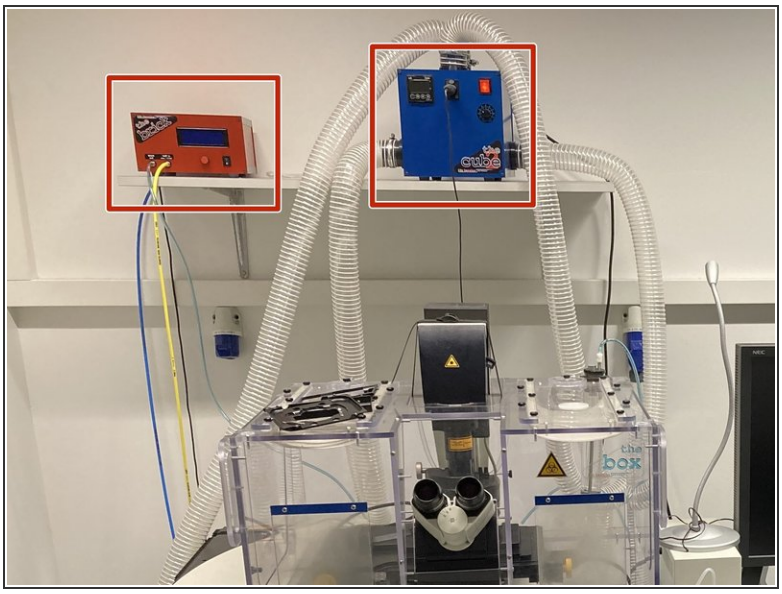


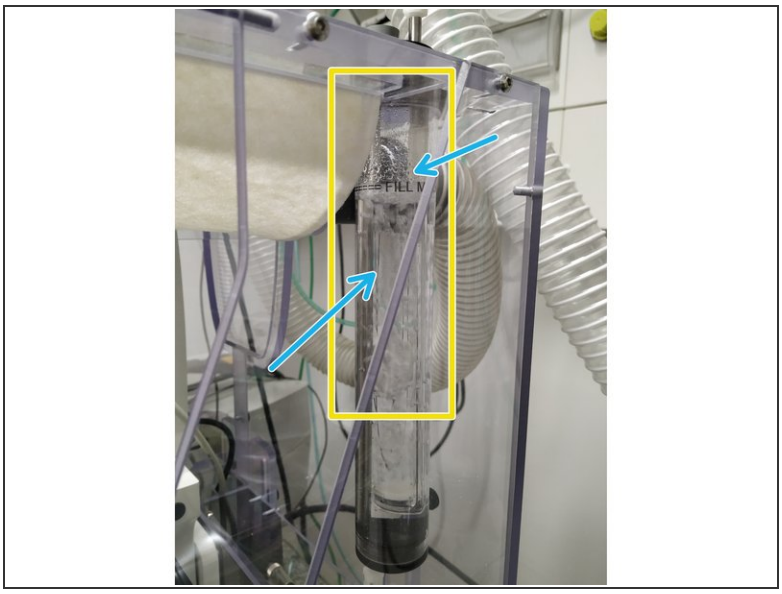
INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis we describe how to set up the environmental control system for live cell experiments at the Leica SP8 inverse confocal laser scanning microscope located at WAD in Schlieren (Wagistrasse 12, 5th floor, room WAD-12-K-105).

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Step 1 — Before you start





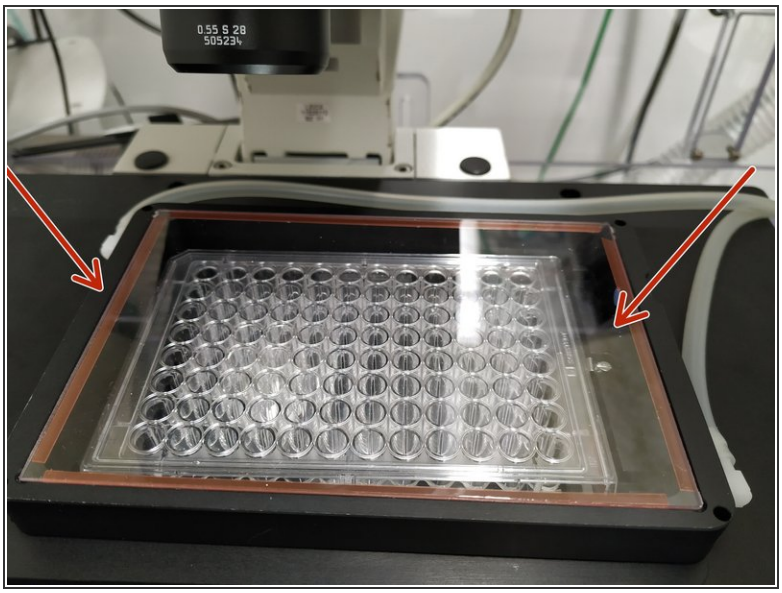
ⓘ **Start heating** at least 30 min before you start your experiment. This allows the chamber and the microscope components to heat up and avoids drift during your experiment.

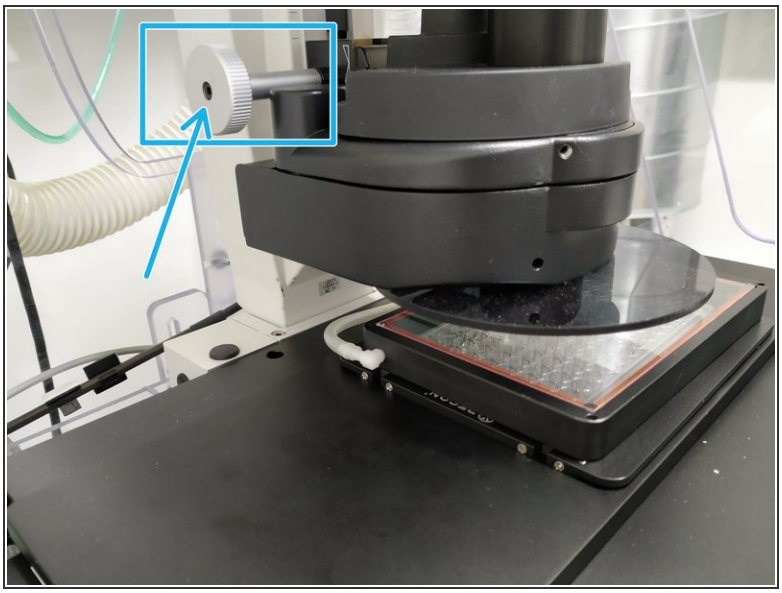
- **Identify** the following **components**:
  - **Temperature** (**cube**) and **CO2** controller (**brick**).
  - CO2 chamber (inside the box).
  - The **humidifier**.
- Make sure the **humidifier column** is filled with **water**. If not, carefully refill with **ddH2O**. Don't overfill!

⚠ Use dishes or well plates suited for imaging. Preferably glass bottom.

⚠ Make sure your media does not interfere with the imaging - e.g. use media without phenol red.

Step 2 — Mount CO2 chamber

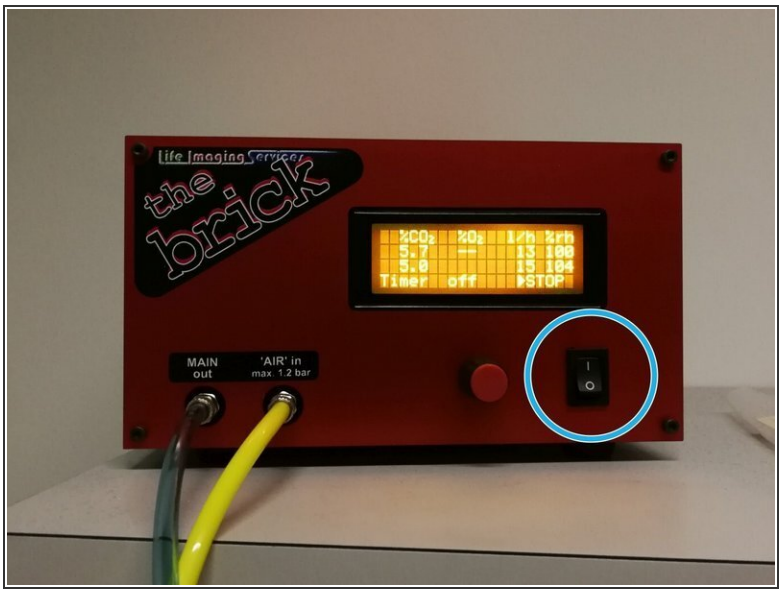




- Place the frame on top of the stage. Make sure it is covered with the glass/plastic lid.
- Adjust the condenser height in case it touches the top of the lid.

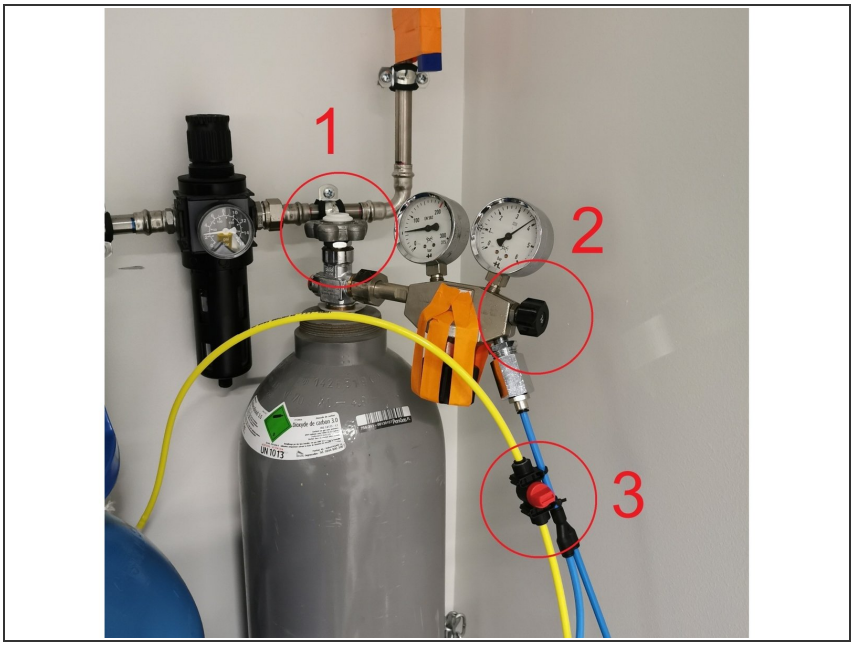
Step 3 — Activate "The Brick" (CO2 control system)





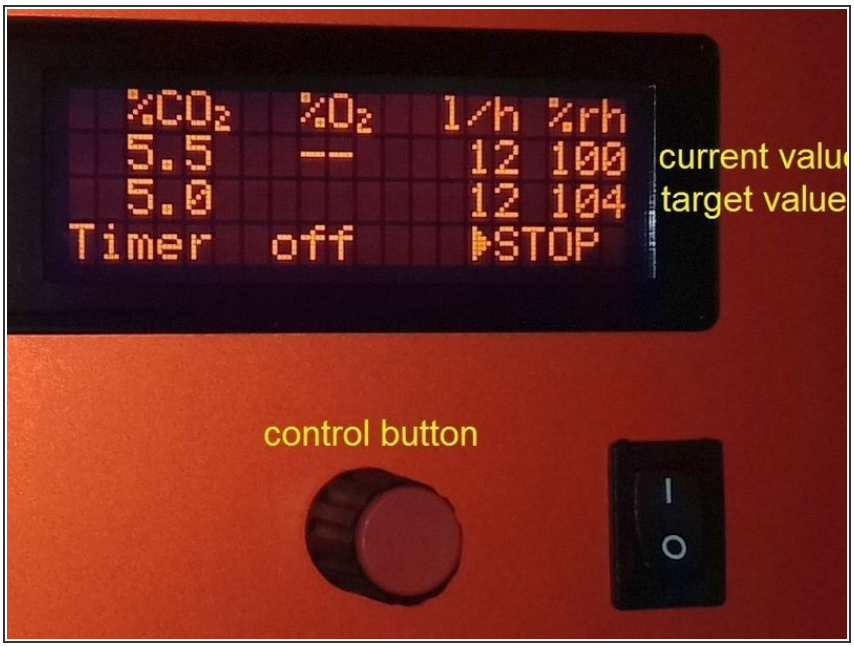
- Open the blue valve on the tubing next to the microscope.
- Switch on "The Brick".
- The **brick** needs a couple of minutes for being initialized. Wait until the unit has finished warming up (~ 1 min) before proceeding.

Step 4 — Open the CO2 and pressurized air valves



- (1) open the main valve at the CO2 bottle
- (2) open the valve at the pressure reducer
- (3) open the pressurized air valve at the yellow tubing

Step 5 — Set CO2 controller



- **5% CO2** and a **flow of 12 l/h** are set as **standard**. If you need to change it, **please set it back** at the end of your experiment!
- The second row displays the **current readings**.
- The third row indicates the **target settings**.
- For changing settings:
  - **Turn** the **control button** to **navigate** through the options/set values. The small arrow indicates where you are.
  - **Press** the **knob** to **select** the desired option - indicated by an "inverted" arrow.
  - Turn the knob to define the desired value. By pressing it again the new value is set.

Step 6 — Set temperature controller

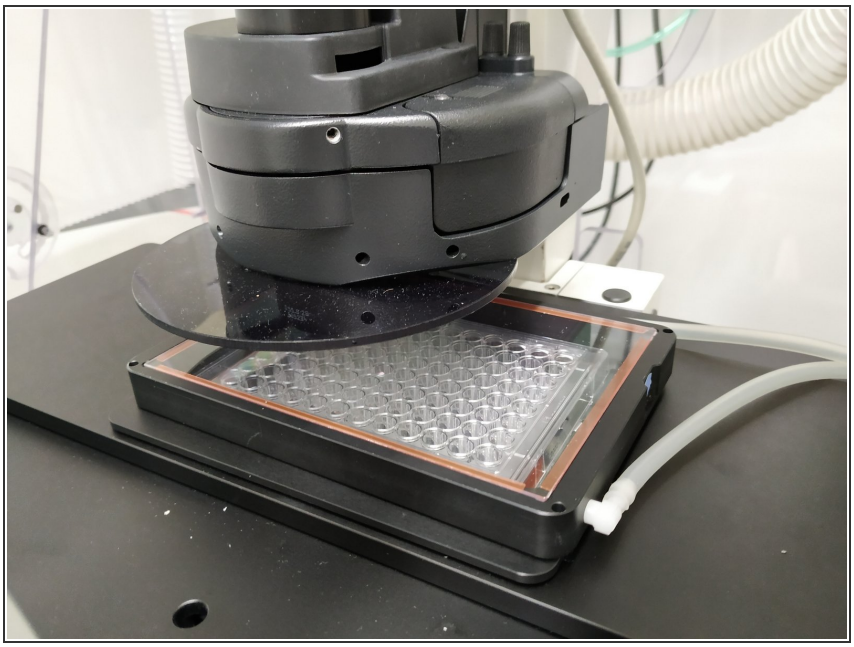




⚠ **37° C** are set as a **standard** configuration. If you change it, please **set it back** to 37° C at the end of your experiment!

- The **desired temperature** can be defined by pressing the arrow buttons.
- The yellow number (top) indicates the **current temperature** measured inside the incubation chamber.

Step 7 — Mount your sample



⚠ Wait until the **CO2** concentration and **temperature has stabilized** (approx. 30 min).

- Next mount your sample.
- Set up your imaging experiment.

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