Leica SP8 (Lengg): Live Cell Imaging

How to set up environmental control at the Leica SP8 confocal laserscanning microscope located at the Research Center for Children, floor C, room 4.020

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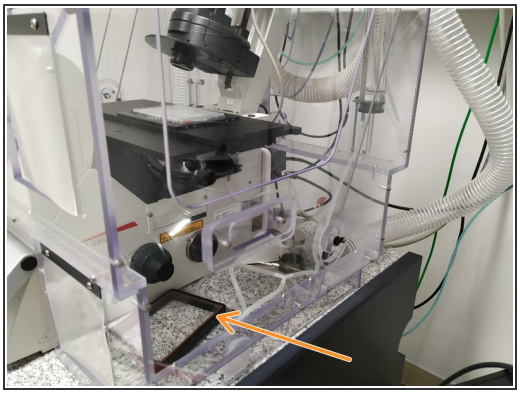
INTRODUCTION

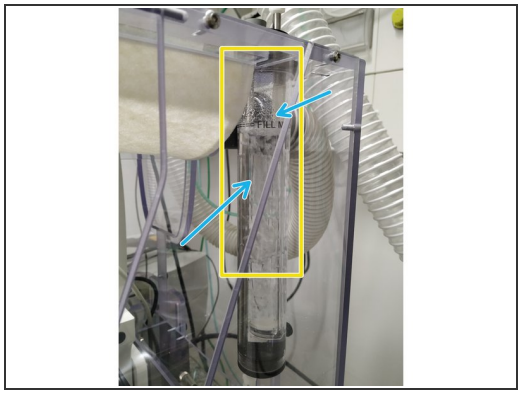
In this guide of the Center for Microscopy and Image Analysis we describe how to set up the environmental control system for live cell experiments at the Leica SP8 confocal laser scanning microscope located at Research Center for Children, Lengg

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Step 1 — Before you start







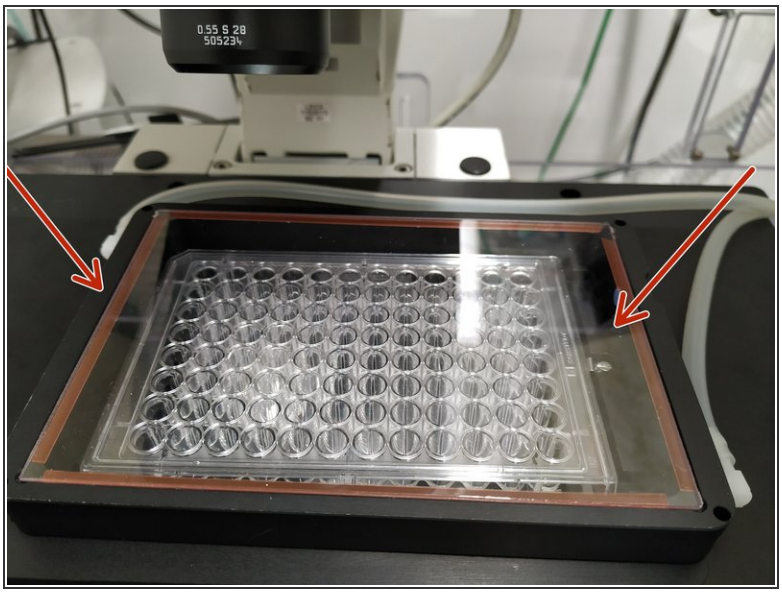
ⓘ **Start heating** at least 1 hr. before you start your experiment. This allows the chamber and the microscope components to heat up and avoids drift during your experiment.

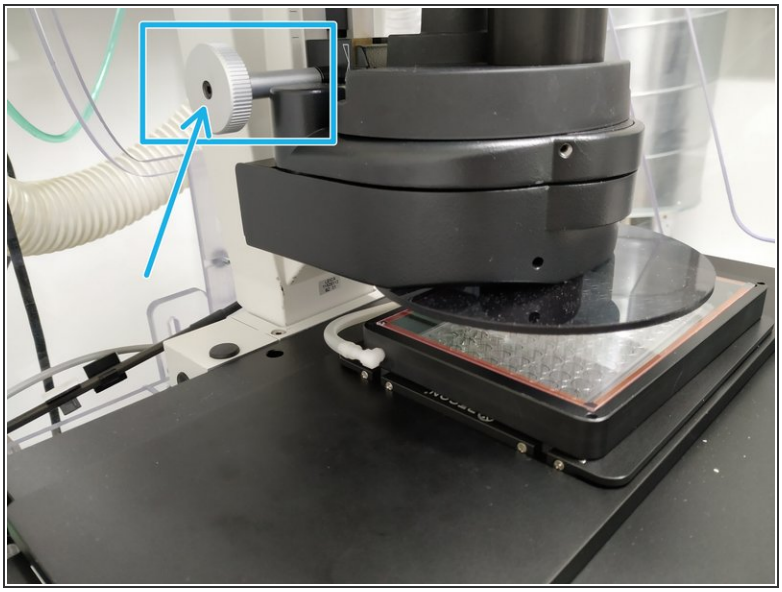
- **Identify** the following **components**:
  - **Temperature** (**cube**) and **CO2** controller (**brick**).
  - CO2 chamber (inside the box).
  - The **humidifier**.
- Make sure the **humidifier column** is filled with **water**. If not, carefully refill with **ddH2O**. Don't overfill!

⚠ 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

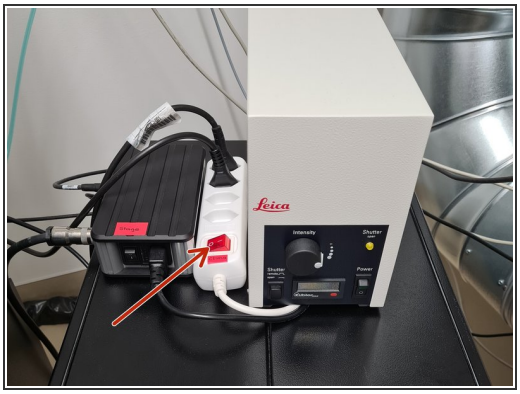




- Place the frame on top of the stage. Make sure it is covered with the glass/plastic lid.
- Adjust the condenser height in case it touches the top of the lid.

Step 3 — Switch ON environmental controls







- Turn on the **power switch**.
- The **Cube** (temperature) and **brick** (CO2) should already be turned ON. Otherwise turn them on.
- The **brick** needs a couple of minutes for being initialized.

Step 4 — Set temperature controller

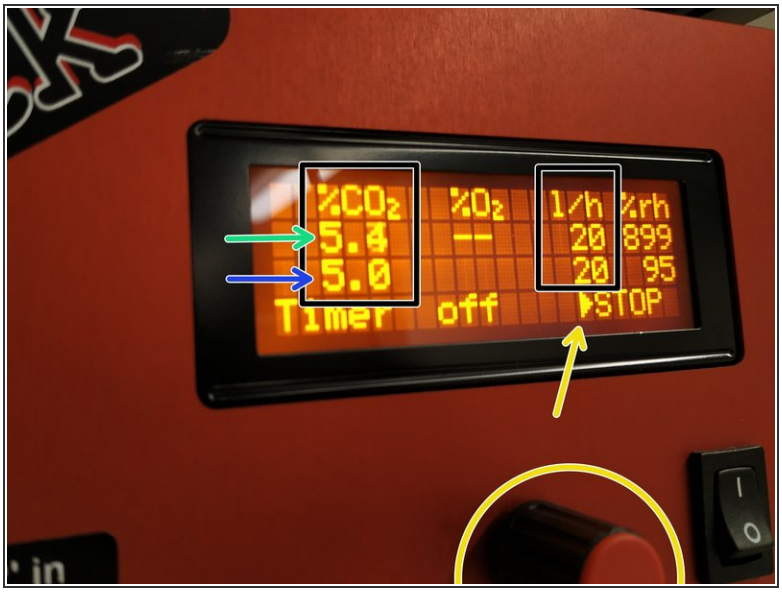




⚠ **37° C** are set as a **standard** configuration. If you change it, please **set it back** to 37° C at the end of your experiment!

- 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 5 — Set CO2 controller

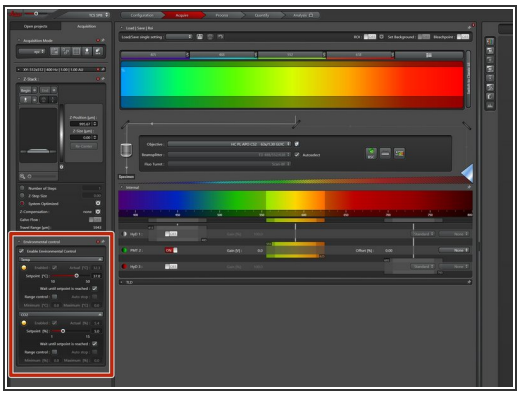


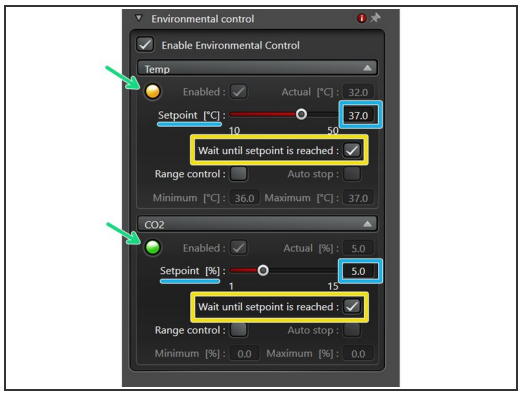


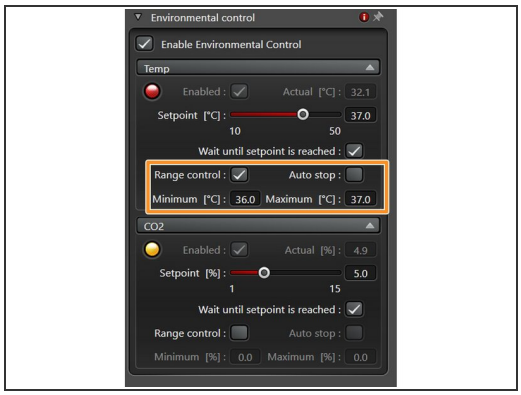
ⓘ After turning on, the gas flow should start automatically.

- **5% CO2** and a **flow of 20l/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 **chosen settings**.
- For changing settings:
  - **Turn** the **knob** 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 — Optional - Set values via the "LAS X" software



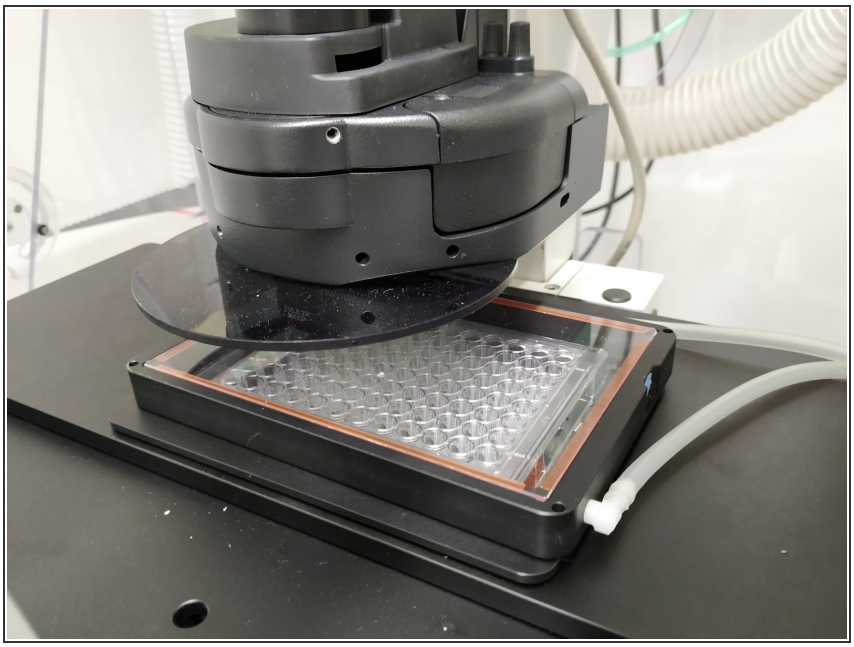




ⓘ If the hardware configuration "machine_with_ClimateControl.xlhw" has been chosen during the "LAS X" start-up, the **Temperature** and **CO2** values can also **set and logged** via the **"LAS X" software**.

- Open the **Environmental Control tab**.
- **Set** the desired temperature and CO2 value via the slider or by typing the value.
  - If the set value is reached, it is indicated by a green light. When still adjusting, the indicator light shows orange.
- Check if your time-lapse experiment should only start when the set values have been reached.
- It is also possible to **define a range** where experiments are allowed to start.
  ⓘ *If the measured value is not in the defined range it is indicated by a red indicator light, otherwise green.*

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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