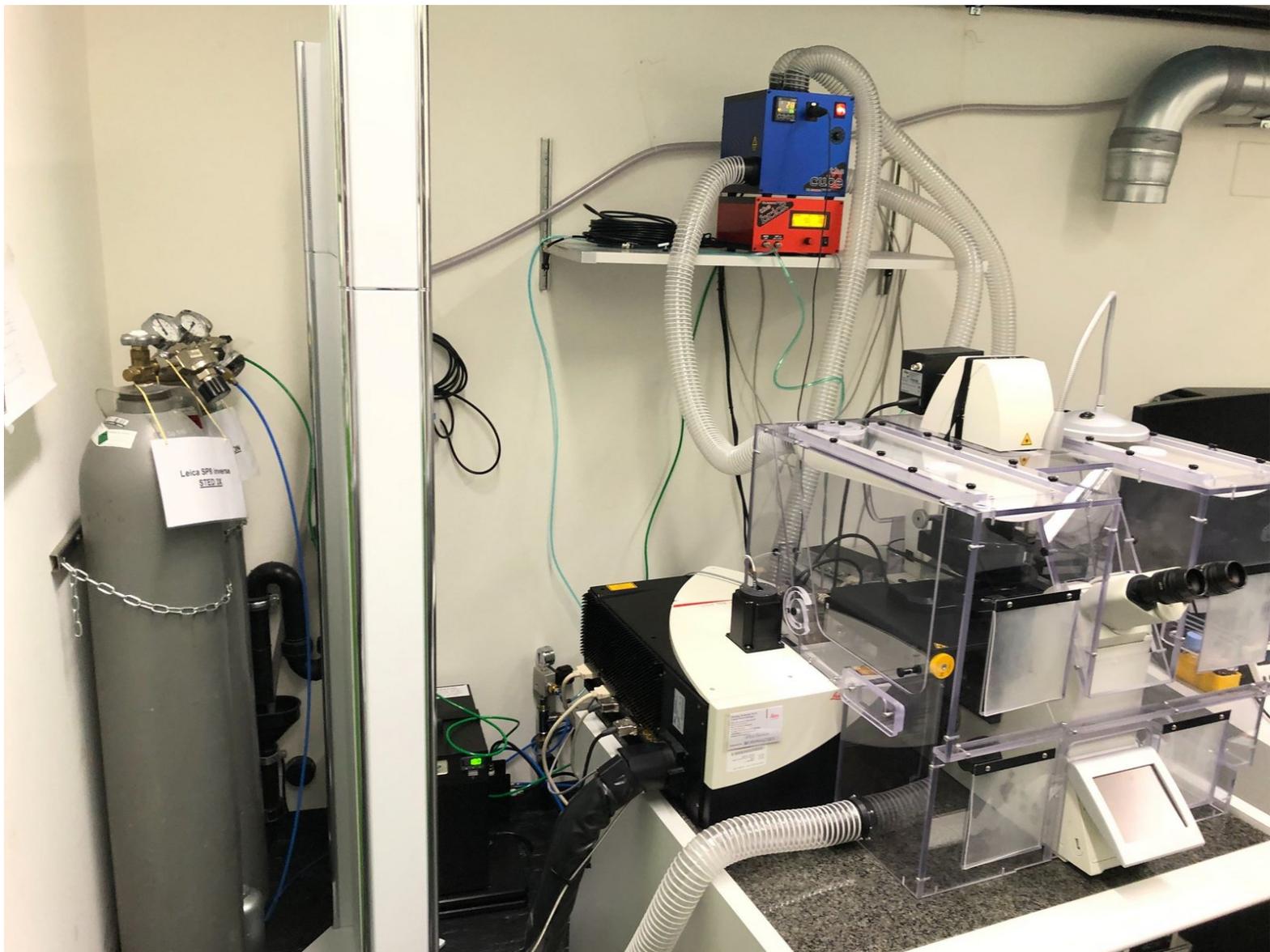


Leica SP8 Falcon and STED - Live cell imaging

How to start up the Leica SP8 Falcon and STED hardware to perform live cell imaging on the at the Center for Microscopy and Image Analysis, Room Y24-H-81.

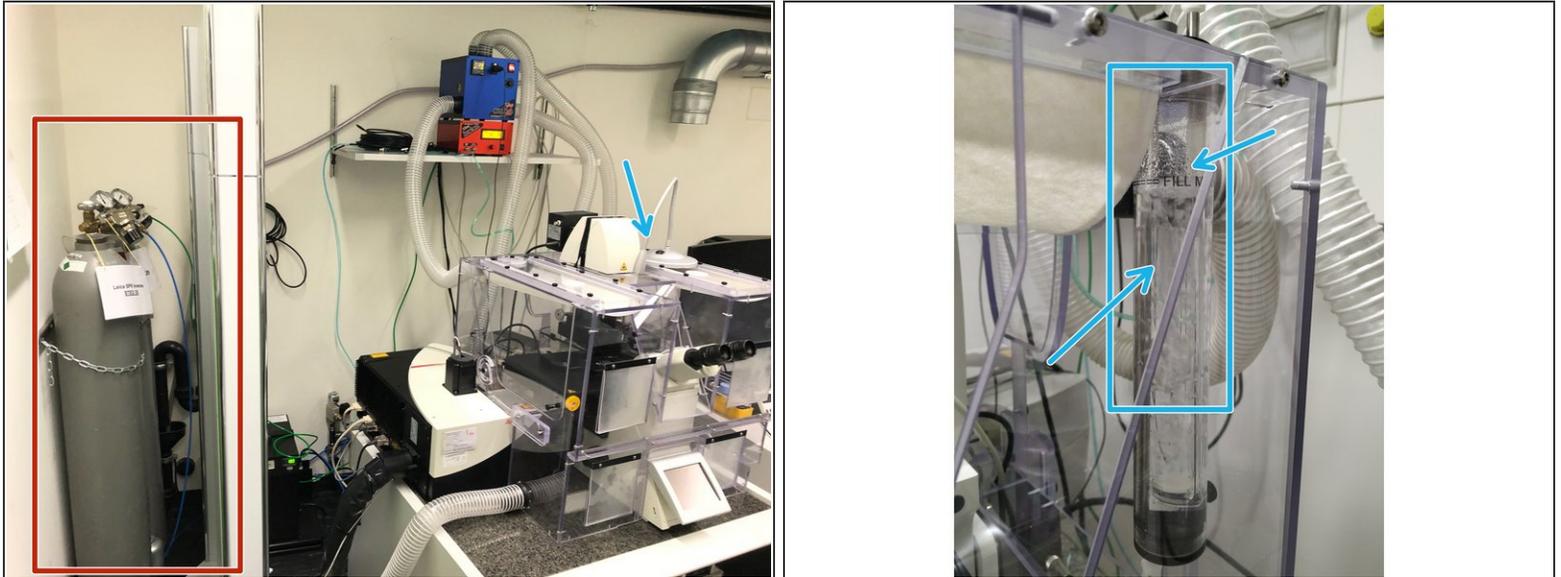
Written By: Joana Raquel Delgado Martins



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 microscopes located at UZH, Irchel, room Y42-H-81.

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 major drift during your experiment.
- Gas bottles for the SP8 Falcon and STED can be found on the left at the entrance of the room.
 - ⚠ For the **SP8 STED** only the CO2 bottle is necessary.
 - ⚠ For the **SP8 Falcon** both CO2 and N2 bottles are needed.
- **Humidifier:** Make sure the **humidifier column** is filled with water. If not, carefully refill **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 — Switch on the Temperature controller - Cube



- **Switch ON** the temperature controller (**the cube**).
- ⓘ *For the SP8 STED: The temperature controller can be found underneath the microscope.*
- Define the **desired temperature** using the arrows.
- The yellow number (top) indicates the **current temperature** measured inside the incubation chamber.

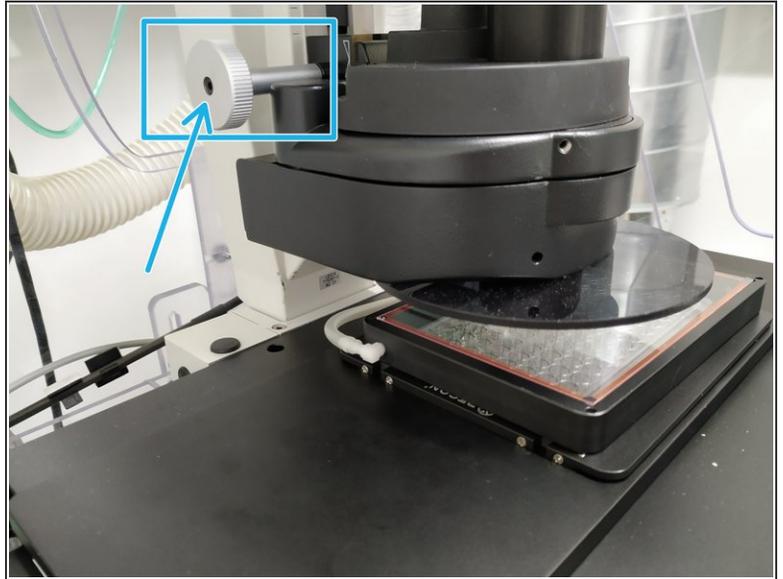
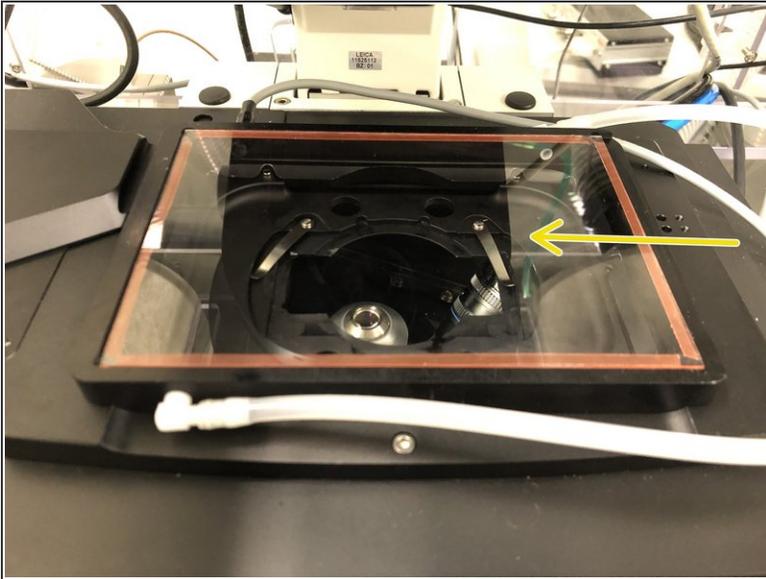
Step 3 — Mount table top CO2 chamber



i You will find boxes containing the **necessary accessories** on these cupboards.

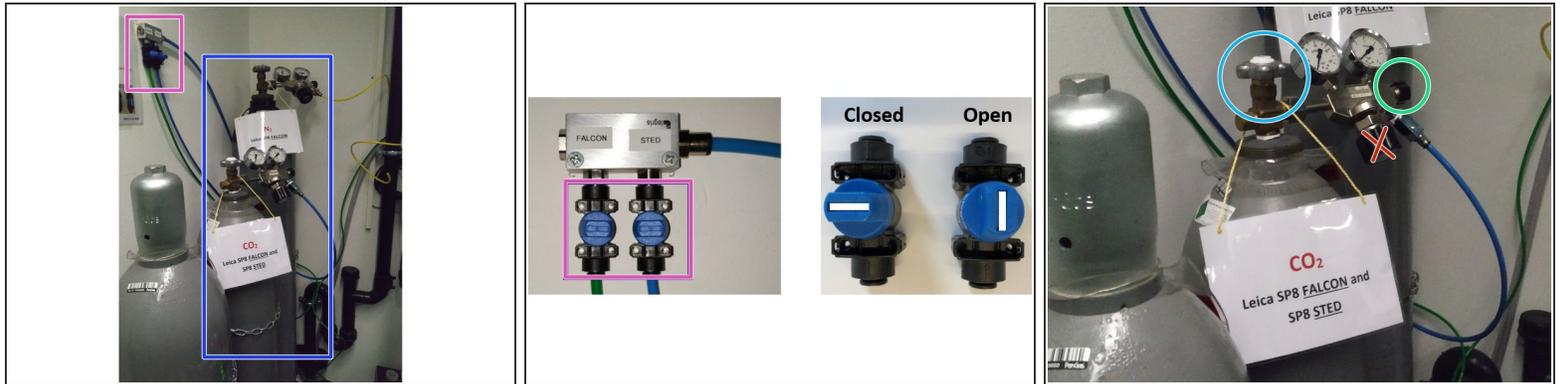
- Here for the **SP8 Falcon**,
- and here for the **SP8 STED**.
- Take the appropriate cover for the CO2 chamber from box.

Step 4 — Mount table top CO2 chamber



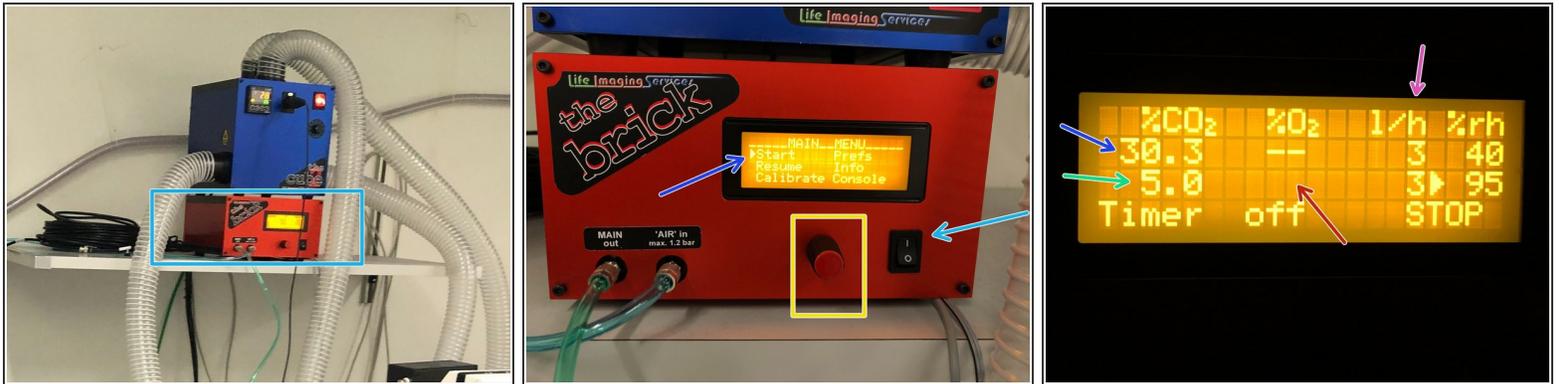
- You will find the frame inside the incubation chamber.
- Place the frame on top of the stage.
- ☞ There are 2 small metal pins on the frame and two opposing holes in the stage in order to hold it in place.
- Slide in the glass/plastic lid to cover the frame.
- Adjust the condenser height in case it touches the top of the lid.

Step 5 — Open CO2 bottle



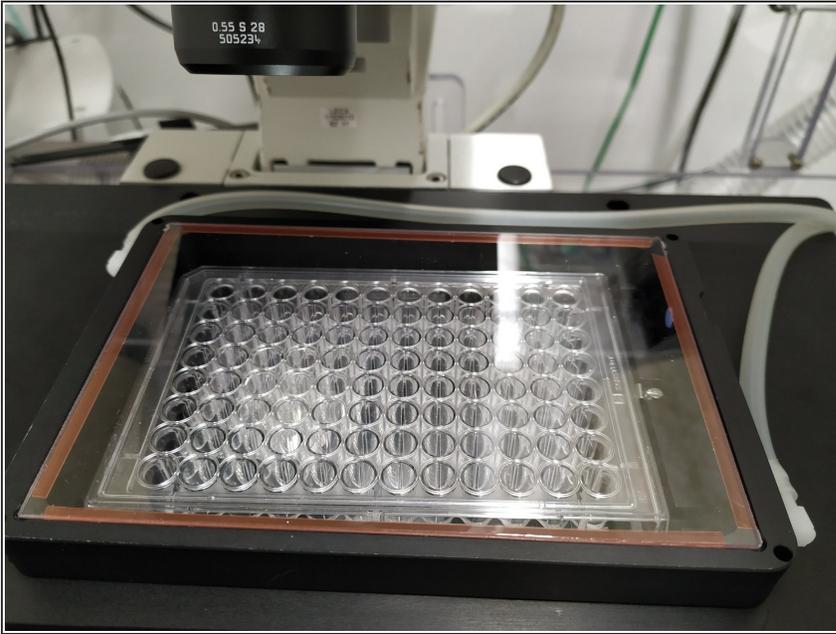
- **N2** (only necessary for SP8 Falcon) and **CO2** gas bottles (both SP8 Falcon and SP8 STED).
- To **open** the gas bottles:
 - **CO2** - Open the wall mounted distribution valves labeled "SP8 Falcon" or "SP8 STED" as needed.
 - **CO2 and N2** - Gently open the main tap of the gas bottle.
 - As well as the small knob on the right.
 - Please DO NOT CHANGE the pressure control tap!

Step 6 — Switch on the CO2 controller



- **Switch ON** the CO2 controller (**the brick**).
- ❗ *For **SP8 STED**: The CO2 controller can be found on top of the WLL/STED laser box (left hand side of the microscope system)*
- **Turn** the knob to navigate through the menu options. Small arrow next to the option will show where you are.
- **Press** the knob to select the desired option, in this case "Start".
- Use the knob to navigate through the options. The upper row gives the readings inside the chamber.
- The lower row indicates the defined settings. In this case CO2 is set to 5% and relative humidity (%rh) should be set to 95%.
- **ONLY for SP8 Falcon**: Set **O2** to **20%** for **normoxia** (usually it settles at 18.9-19%).
- Adjust the **flow** - **20 l/h** maintains the right CO2 concentration inside the chamber.

Step 7 — Mount your sample



⚠ Wait until the CO₂ concentration and temperature **stabilize** inside the chamber (approx. 30 min).

- Once temperature and CO₂ have stabilized you can place your sample/cells.
- You can now start setting up your experiment!