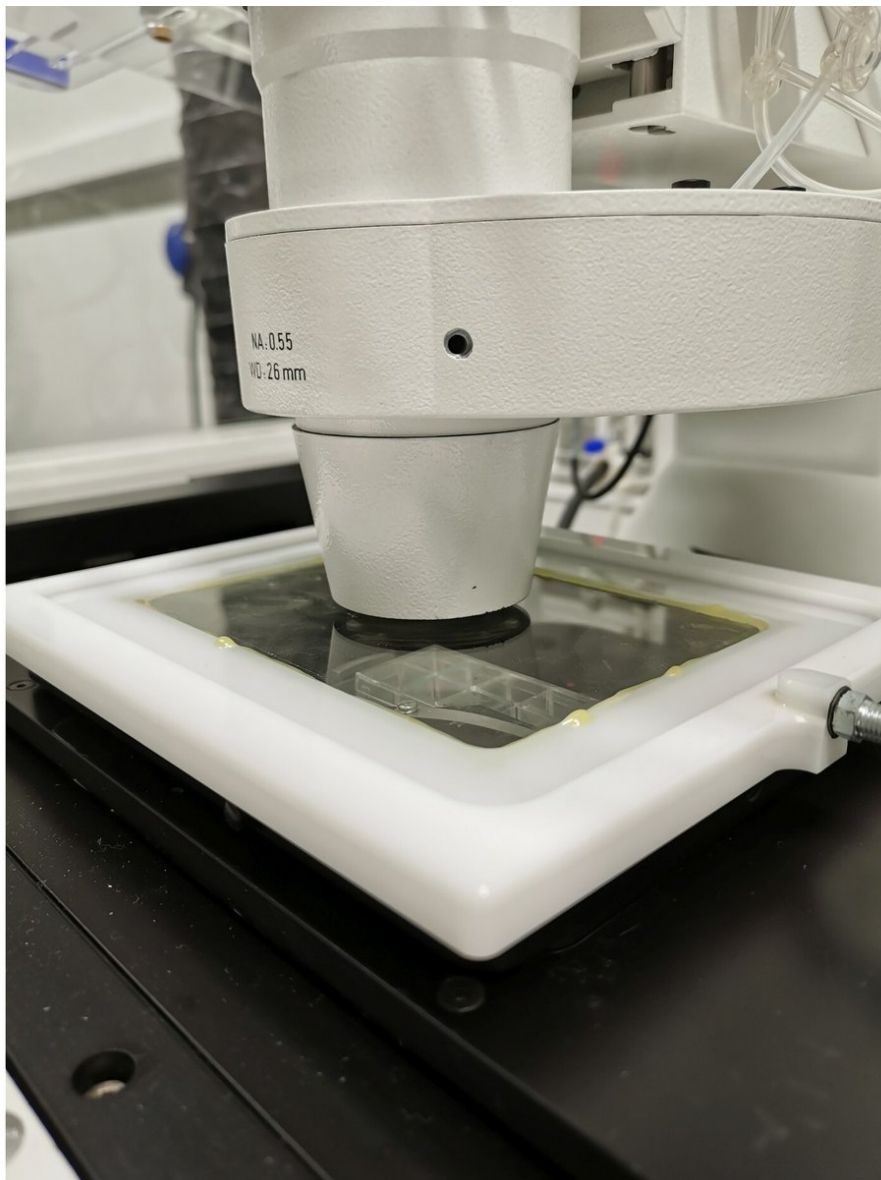


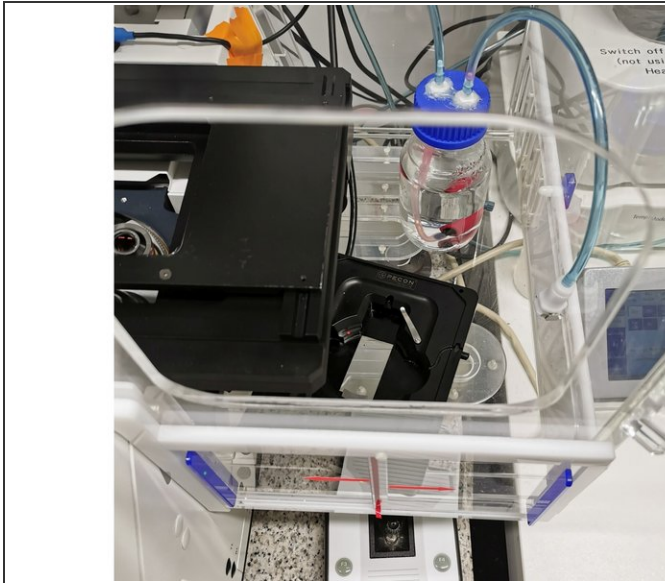
# Zeiss Axio Observer (Schlieren) - Part X: Stage incubator for live imaging

How to set up the Zeiss Axio Observer (Schlieren) for live imaging with the stage incubator and CO2 supply.

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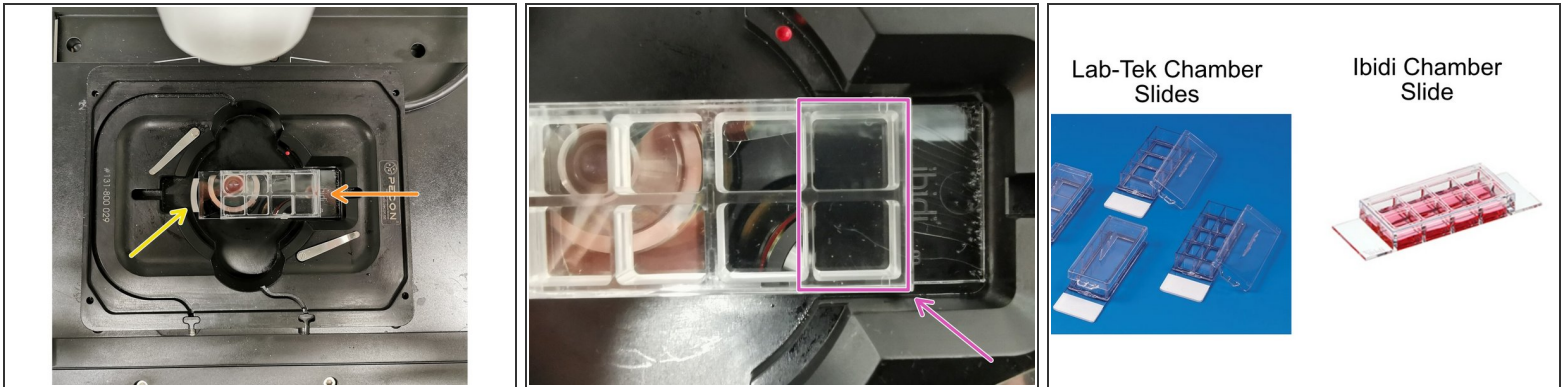


## Step 1 — Install stage incubator



- Remove installed stage insert
- The stage incubator is located on the right inside the incubator box when not used.
- Install stage incubator on stage
- ❗ Incubator will be positioned on stage but does not slide in as the other stage inserts

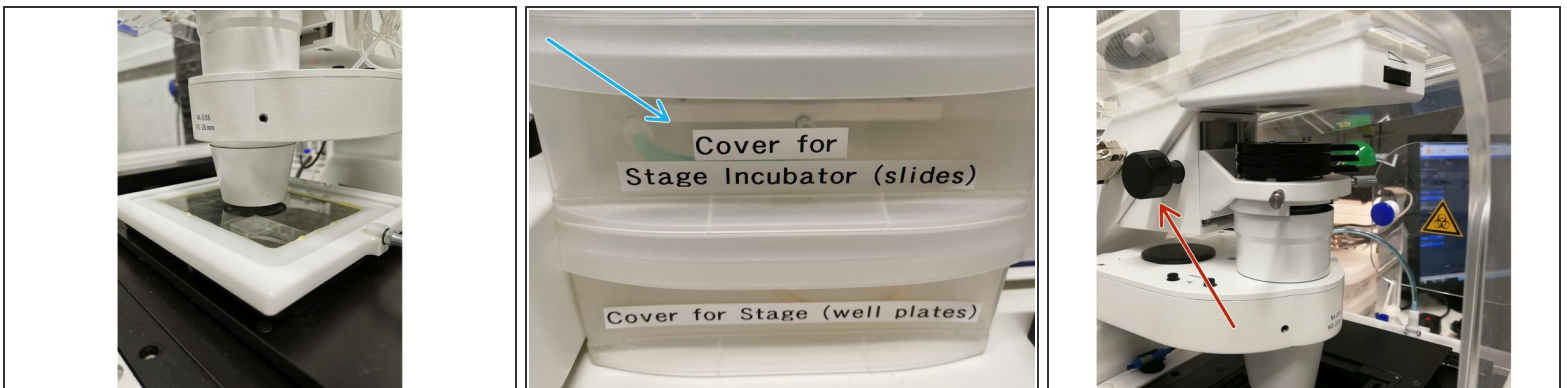
## Step 2 — Loading a slide chamber



**⚠ Only slide chambers or small round cell culture dishes can be loaded.**

- The slide chamber is held in place by a spring loaded tab.
- Load the sample by pressing the slide against the spring loaded tab and make sure it is positioned planar.
- The design of the Ibidi chamber slide allows only acquisition of 6 of the 8 wells. Lab-Tek slides work better in this sense.

## Step 3 — Stage incubator lid

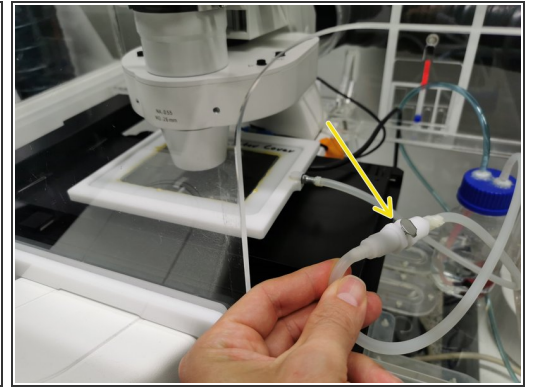
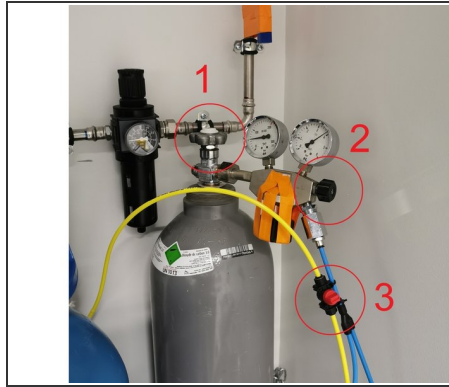
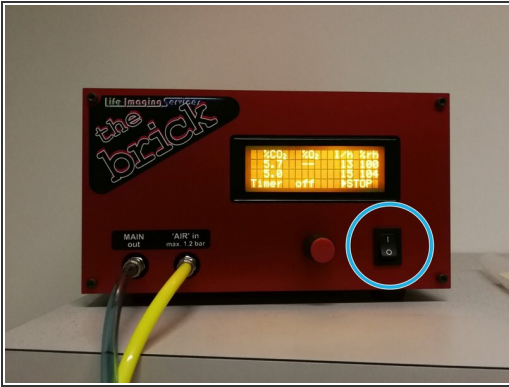


- The stage incubator should be covered by the corresponding lid.
- Make sure you are using the right lid which you can find in a drawer box on the table.

**⚠ Ensure you have enough distance between lid and condensor.**

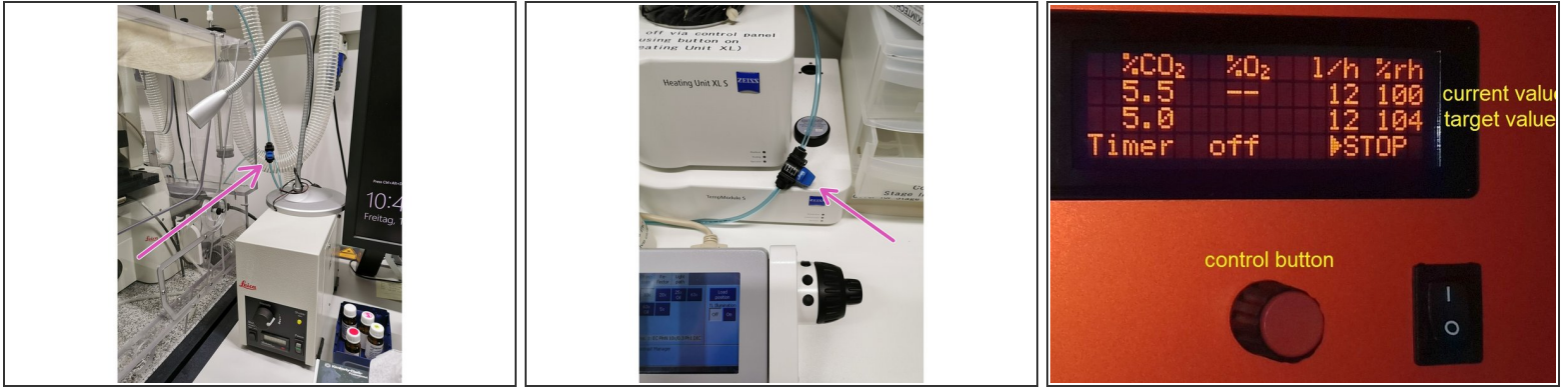
- the condensor can be moved up/down by turning this wheel

## Step 4 — Stage incubator with CO2 supply



- Switch on the "Brick" device (on a shelf above the confocal) and wait until it has finished warming up (~ 1 min)
- Open the main valve at the CO2 bottle (1), the valve at the pressure reducer (2) and the valve at the yellow tube (3).
- Connect the air supply of the lid with the water bottle inside the incubator box. Ensure the air supply tube is not getting stuck while the motorized table is moving.
- Check if you can see air bubbles in the water bottle. If this is not the case proceed with next step...

## Step 5 — Trouble shooting



- If there are no air bubbles visible in the water bottle ensure the valve at the SP8 confocal is closed and the one at the Zeiss is open.
  - The "Brick" CO<sub>2</sub> controller should be ready to go after pressing the black power button (after the ~1 min warm-up time has past). Settings may be adjusted using the control button via button turning & pressing. The default 12l/h setting works fine for the stage incubator.
- ⚠ The working distance of the 63x oil objective is too small for the stage incubator. If your cells are in focus and you reach the edge of chamber you will lift up the incubator by the objective. Please avoid this because it can break the objective!