

Leica TCS SP5 Confocal Live Cell Imaging Set Up

User Guide

LSU Health Sciences Center-Shreveport
Research Core Facility-Microscopy

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Rules for live cell imaging

1. When you reserve the calendar, **leave 2 hours extra time for the system to cool down for the next user**. For example: If you reserved the calendar from 8am to 5pm, you have to stop the experiment at 3pm. This allows the microscope, especially objectives, to cool down, so there won't be focus drift for the next user who may not need live imaging environment.
2. For fixed sample imaging users, if you find that the system is not cooled down by the previous user, please inform core staff immediately.
3. **When you reserve the calendar, type in “live cell”**.
4. If culture media spills out, wipe it with tissue or lens paper and clean with ethanol. We have ethanol in our lab if you need any.
5. If you plan to do live cell imaging with virus infection, please inform core staff ahead of time, we need to evaluate whether it can be brought to the microscope room for Biosafety reasons.
6. Long time (>24h) live cell imaging must be done on weekends.

The Cube Temperature Control



The Brick Gas Mixer



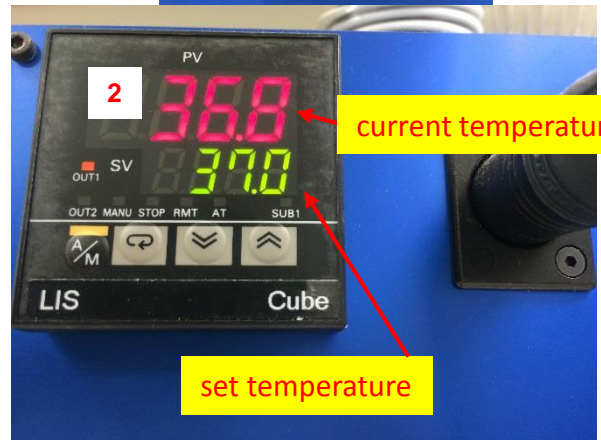
Leica floating table
air tank

Leica air tank

Leica CO₂ tank

Turn on the Controllers

1. Press the red power button to turn on The Cube. The Cube should be turned on at least 4 hours before the experiment. **It takes up to 4 hours for the entire system to warm up.**
2. The Cube controls the temperature. Red: current temperature, yellow: set temperature.
3. Increase the fan speed when warming up the system. Decrease the fan speed when the temperature reaches the set temperature and is ready for experiment.
4. The Brick is only switched on after the cube is turned on and the temperature reaches 37°C (or your desired temperature).
5. Press the black power button to turn on the Brick 1 hours prior to starting your experiment. Also open the CO₂ and air tanks after turning on The Brick.
6. Open the CO₂ tank and the air tank through turning the wheels in the direction indicated on the metal wheels.



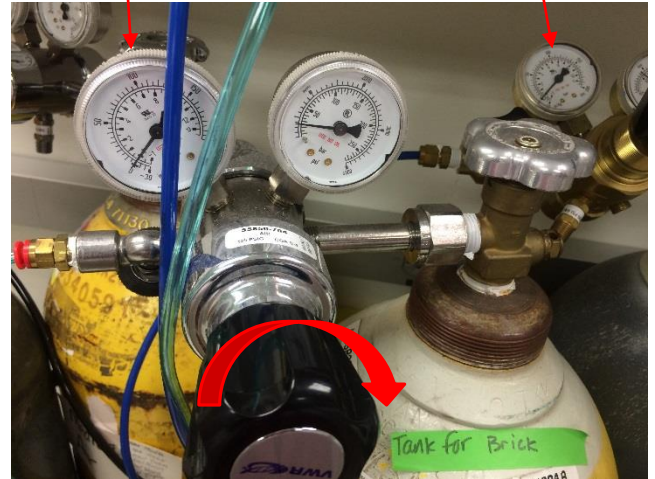
Open the tanks

- Adjust the **output pressure to 50 psi** on both tanks through turning the black plastic knob clockwise. (If CO₂ does not reach 5% on the brick screen, increase the output pressure to 60 psi so that the output pressure is high enough to maintain a 5% CO₂ in the microscope box.)

Note: There are two pressure meters on each tank. The one on the left is the output pressure, and the one on the right is the tank pressure. **Set the output pressure to 50 psi (not the tank pressure).** When the gas level in the tank is low, the tank pressure will drop. Please notify the staff when tank pressure is low.

Set to 50 psi

Set to 50 psi



- Turn the red knob on The Brick to navigate in the **Main Menu**, and press the knob to select the desired options.
- Select **Start** and enter the start window.



10. In the **Start** menu, first row: name, second row: current value, third row: set value.
11. The **Timer** can be set as high as 99h.
12. When values are set, select **STOP** to return to the **Main Menu**.
13. In the Main Menu, navigate and select **Resume** with the red knob. This will resume your settings in the Start menu.
14. Usually you do not need to do any calibration. The staff will do it once a year or two.



Name
Current Value
Set Value

Note: If you do not select Resume, your settings in the Start menu cannot be saved.

Shut down the system

1. Power off The Brick and The Cube.
2. Turn the black knob counter clockwise to decrease output pressures from both tanks and then close the tanks by turning the metal wheel.
3. **A \$50 penalty will be applied if the tanks were left on after an experiment is done.**



Close the tank

decrease the output pressure

The Brick and The Cube system for live cell imaging attached to the Leica Confocal microscope at LSU Health Sciences Center-RCF is provided by Life Imaging Services (Switzerland).

Get factory user manual from Life Imaging Services
<http://www.lis.ch/contact2.html>

The Brick model that we have at LSU Health Sciences Center-Shreveport-Research Core Facility cannot set humidity value. The Max humidity settings under Preference is default to 1, and the machine will passively increase the humidity to 70%.