Setup for Live Imaging using the WF3 Microscope

Please ask for help if you are unsure

General considerations

Choice of Media

- Phenol Red dye in culture media has low-level auto-fluorescence particularly in the green emission wavelengths, which can interfere with the fluorescent signal, and is best avoided. There are Phenol Red free media available for fluorescent imaging.

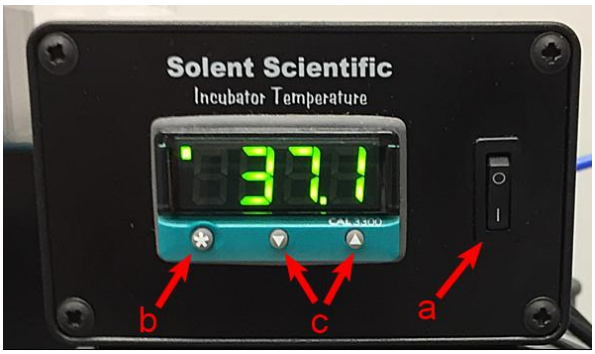
Well Liquid levels

- Imaging for several hours or more at 37^0C can cause evaporation of the culture/imaging media. Make sure each well has sufficient liquid level to compensate for this. Also filling unused wells in the same plate can be beneficial. Placing a container of water at the base of the microscope incubation chamber helps to provide a humid environment (an old pipette box with about 1cm of water is usually sufficient).

Heating

The microscope incubator chamber can be set to a specified temperature. Turn on at least 30 minutes before use for the chamber to reach temperature and equilibrate. For longer term imaging (time series) then the heat the chamber for at least 2 hours before use to prevent focus drift)

Turning on



- Make sure all incubator doors are closed
- Switch on the plug on the wall marked heating
- Turn on the heating unit to the right of the microscope (a)
- The display will show the current temperature
- Check the temperature setting by holding the "*" button for a few seconds (b)
- The display will flash with the set temperature

To adjust the temperature:

- Press and hold the "*" button for a few second and the display will flash with the set temperature (b)
- Use the up and down buttons to change the temperature (c)
- Press and hold the "*" to return to current temperature
- Please return to 37^0C after use if you have changed it

Humidity

Imaging of live samples will result in evaporation of the growth media supporting the cells. This will become a significant problem for longer term imaging at higher temperatures (eg 37_0C). This evaporation can be significantly reduced by placing a container (a base of a used pipette tip box is ideal) partially filled (1cm deep) with water inside the base of the microscope incubation chamber but away from the stage movement. If running over several days this box should be checked and filled daily. **Take care not to spill water inside the microscope incubator chamber**

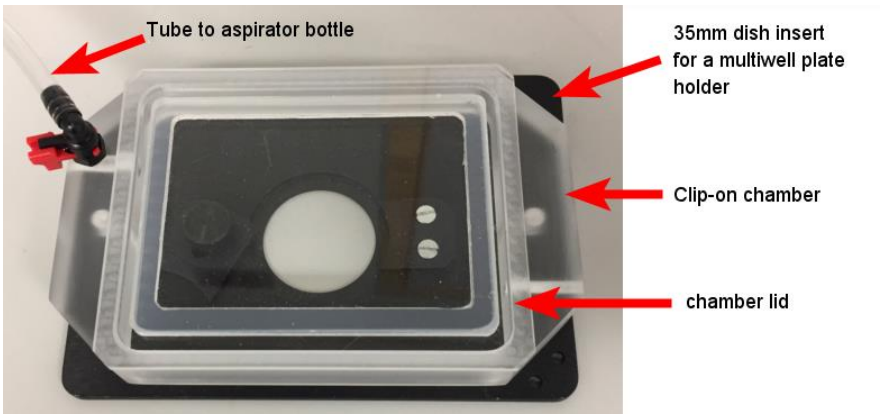
CO_2

If using CO_2 please check cylinder pressure a few days before required in case the cylinder needs replacing – see checking method below

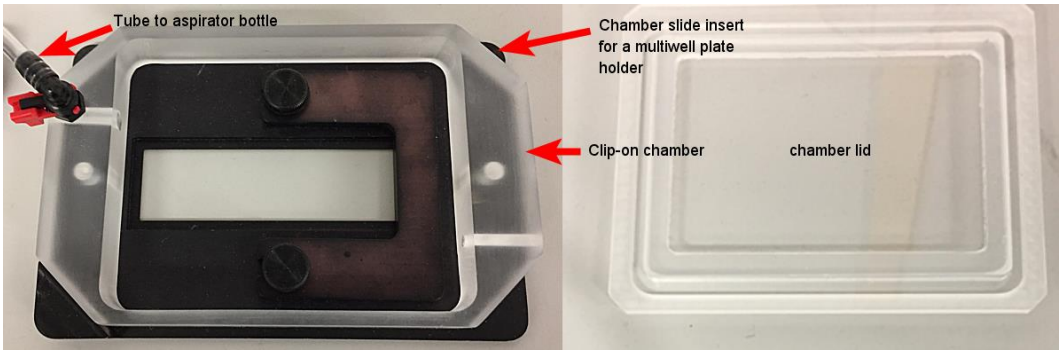
Please note that the microscope incubator chamber, as a whole unit, cannot maintain a controlled CO_2 environment. Instead, it is possible to supply a 5% CO_2/air supply to a specific sample carrier lid. There are several lid options for 35mm dishes, chamber slides and multi-well plates. Each is supplied with tubing to connect to the 5% gas supply

Lid options

- 35mm dish



- Chamber slide



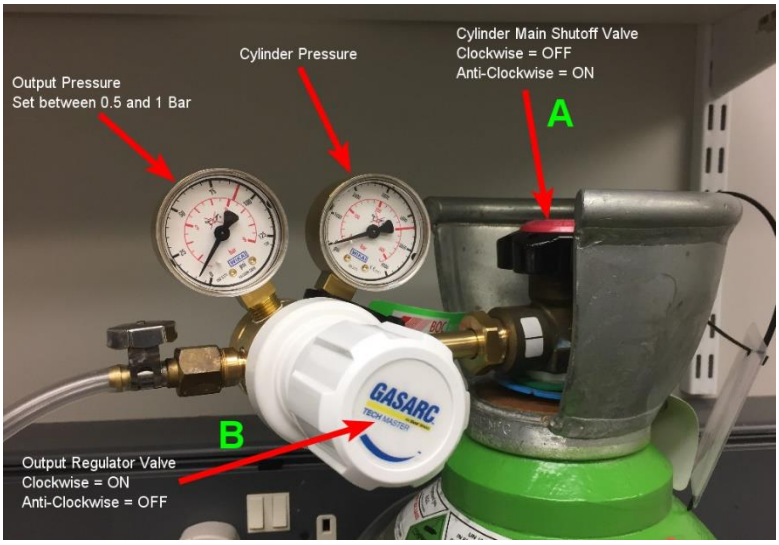
- Multi-well plate

Turning on

- Check the level of distilled water in the humidifying aspirator bottles inside the incubator chamber - should be about a cm full in each (b)
- Place the lid you are using over your sample and connect to the aspirator bottle – black connector (a)

Gas Cylinder Safety Check

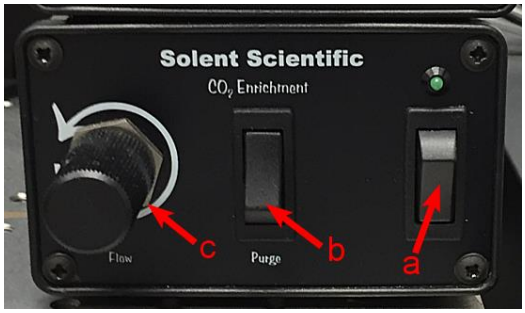
- Check gauges: (there may be some residual pressure from the last use)
  - left had gauge should be between 0 and 1 BAR
  - right hand gauge should be between 0 and last used cylinder pressure value
- Check the main cylinder valve is closed - fully clockwise (A)
- Check the pressure regulator valve is closed (B) – it should feel loose (almost fully anticlockwise) – it will feel stuck if turned too far anticlockwise
- Check visually the pipework form cylinder to CO_2 controller



Turn on CO_2 gas cylinder

- Open the main cylinder valve (A) about I turn (anticlockwise) – a positive pressure value should be displayed in the right-hand gauge showing the gas level in the cylinder (open more if no pressure showing). If pressure displayed is still zero, then the cylinder may be empty - contact FILM staff
- Then by turning the pressure regulator valve (B) slowly clockwise, increase the pressure to between 0.5 and 1 BAR as displayed in the left hand (output) gauge - a slight resistance will be felt when the valve starts to open.

Setting flow rate

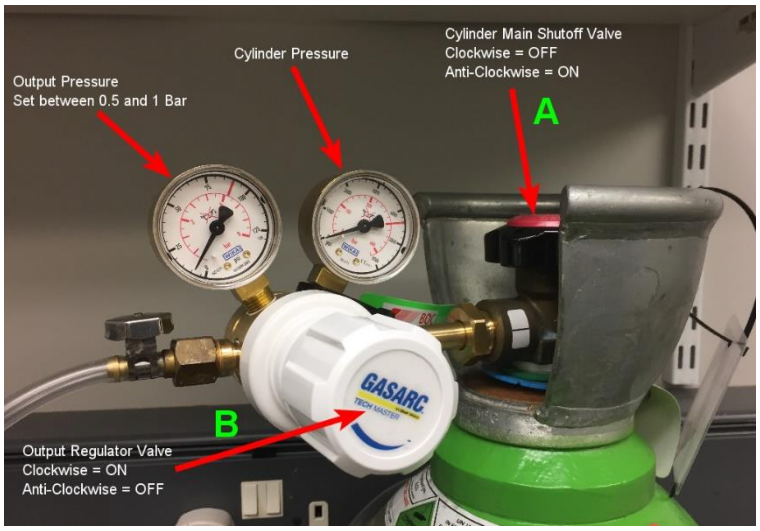


- Switch on the plug on the wall marked CO_2
- Turn on CO_2 controller to the right of the microscope (a)
- Check for bubbling inside the aspirator bottles inside the incubator
- If nothing is immediately visible, press and hold the purge button (b) on the front of the CO2 controller until bubbling appears. Release when bubbles appear.
- The flow (bubble) rate can be set (approximately 2-5 bubbles per second) by turning the flow control on the front of the CO2 unit (c)
- Check the regulator output gauge again and adjust the regulator valve as necessary to maintain 0.5 to 1 BAR

Shutting down

CO_2

- Check the main cylinder valve (A) is closed - fully clockwise
- Check the pressure regulator valve (B) is closed – almost fully anticlockwise - it should feel loose – it will feel stuck if turned too far anticlockwise



- Turn off CO_2 controller to the right of the microscope and turn off at the wall

Heating

- Return the temperature set value to 37^0C if it has been changed
- Switch off the heating unit to the right of the microscope and turn off at the wall
- Remove humidifier box if used.