Setup for Live Imaging using the HWF1 Microscope

Please ask for help if you are unsure

General considerations

Choice of Media

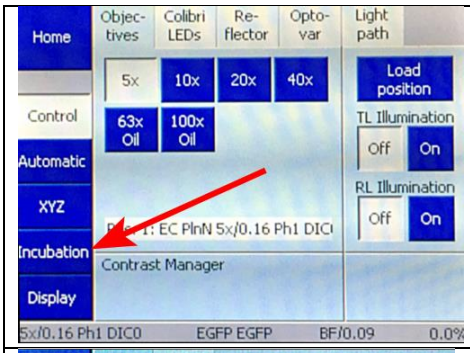
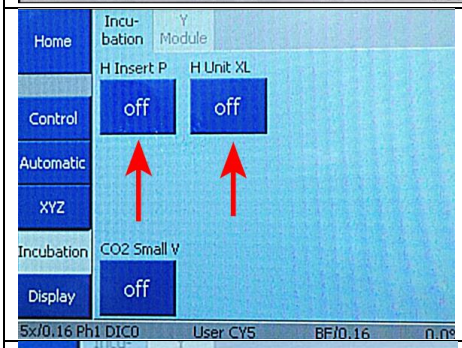
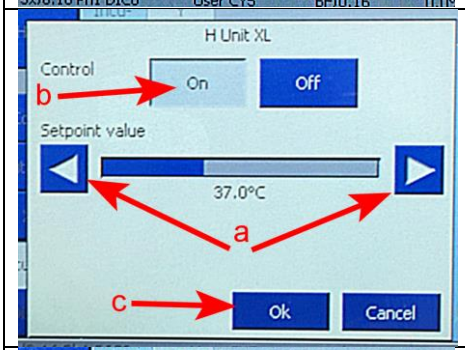
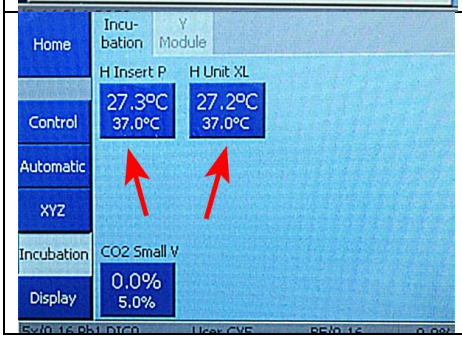
- Phenol Red dye in culture media has low-level auto-fluorescence particularly in the green emission wavelengths, which can interfere with the fluorescent signal, and is best avoided. There are Phenol Red free media available for fluorescent imaging.

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 HWF1 can provide heating to both the chamber (H Unit XL) and also to a heated stage insert for 35mm dishes (H Insert P – see Stage Inserts). Each unit can be set to a specified temperature on the TFT control screen. Switch on at least 30 minutes before use to allow the chamber to reach temperature and equilibrate. For longer term imaging (time series) then pre-heat the chamber for at least 2 hours before use, to prevent focus drift).

<table>
<tr>
<td> 
</td>
<td>Turning on
• Start up the microscope in the usual way
• Make sure all incubator doors are closed.
• On the TFT screen, select “Microscope” and then “Inkubation” (A) (if Inkubation is not visible then re-start the system).</td>
</tr>
<tr>
<td> 
</td>
<td>• The display will show the temperature of the H unit XL and the H Insert P as OFF
• To Switch on, press the OFF button to open the temperature controls for the required unit(s).</td>
</tr>
<tr>
<td> 
</td>
<td>Adjust the temperature:
• Select the required temperature (a) and then press ON (b). Then press the OK button (c).</td>
</tr>
<tr>
<td> 
</td>
<td>• The display will now show the current and the required set temperatures of each unit.
• Press the Control button to return to the objective display</td>
</tr>
</table>

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

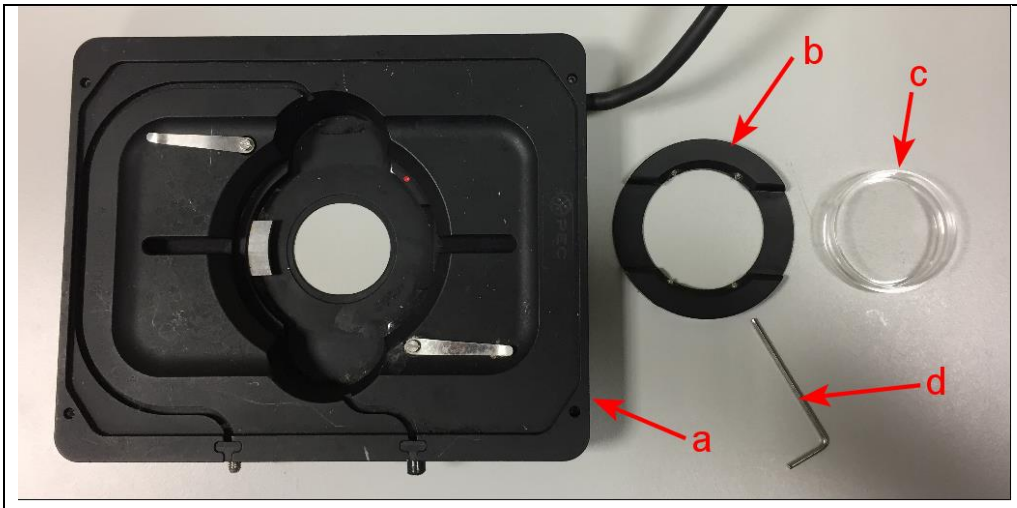
If using CO_2 please check cylinder pressure a few days before required in case the cylinder needs replacing – see checking method below

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 CO_2 to a specific sample carrier lid. There are several lid/insert options for 35mm dishes, chamber slides and multi-well plates.

Stage Inserts

H Insert P

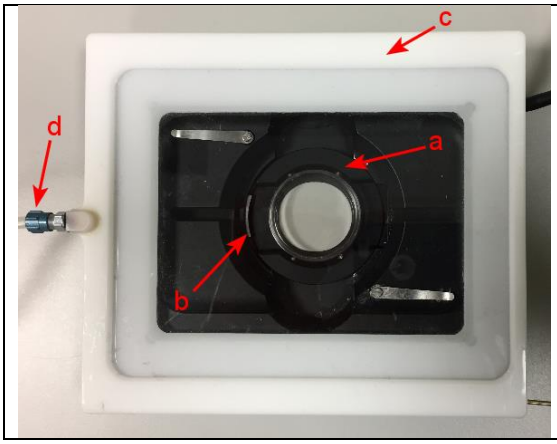
This is a heated stage insert that replaces the standard slide of multi-well plate inserts



a.

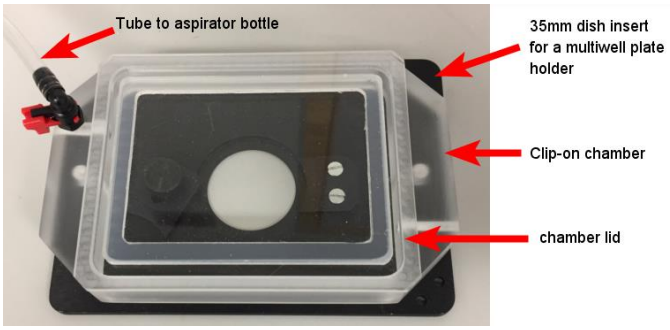
a. Heated stage
b. 35mm dish ring clamp
c. 35mm dish Alan key
d. Spring plate

Loosen the screws on the ring clamp and on a flat surface, place the 35mm dish into the clamp. Tighten screws to just hold the dish (DO NOT OVER TIGHTEN).

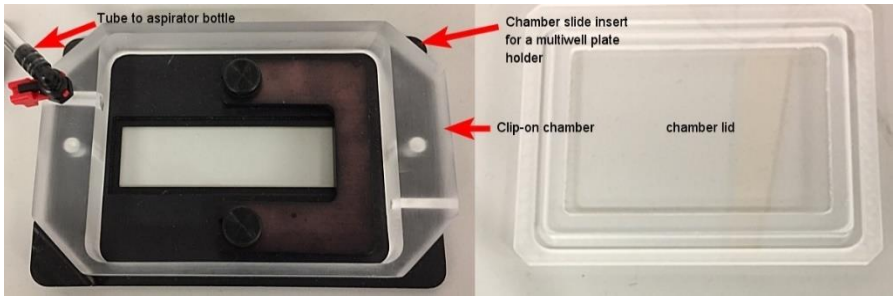
	Inset the clamp and dish (a) into the stage by sliding the clamp against the spring plate (b). Place the glass lid on top Attach the tubing to the CO2 aspirator bottle

Other options

- 35mm dish and slide assembly unit that fits the multi-well plate holder



- Chamber slide



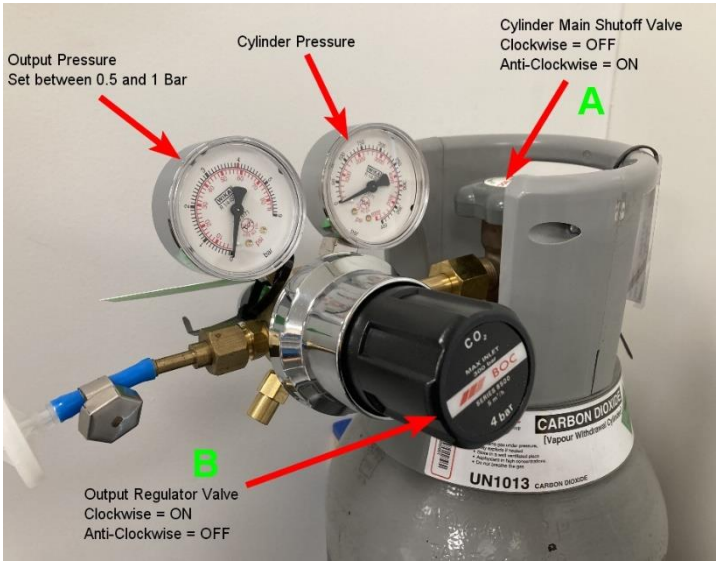
- Multi-well plate lid for normal multiwell plates

Turning on

- Check the level of distilled water in the humidifying aspirator bottle inside the incubator chamber - should be about 2 cm full and gas should be bubbling from the aspirator
- Insert sample carrier and place the lid you are using over your sample and connect to the aspirator bottle

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

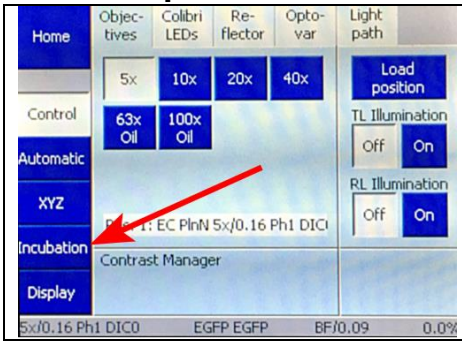


- Check visually the pipework from cylinder to CO_2 controller

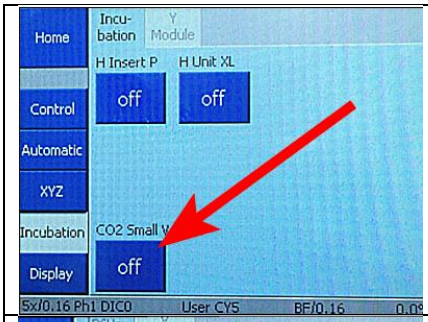
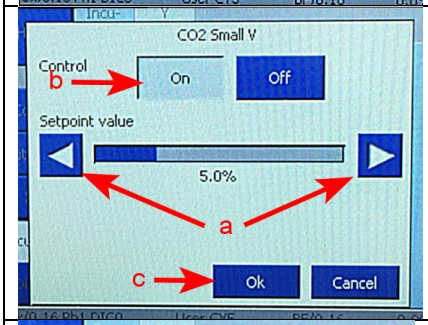
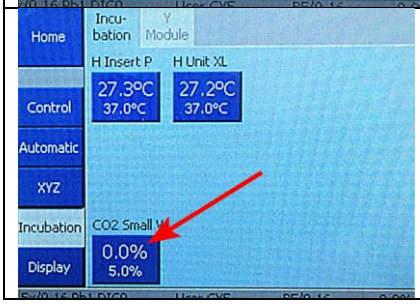
Turn on CO_2 gas cylinder

- 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.

Microscope CO2 Controller



- Start up the microscope in the usual way
- Make sure all incubator doors are closed.
- On the TFT screen, select “Microscope” and then “Inkubation” (A) (if Inkubation is not visible then re-start the system as above).

	• The display will show the CO2 Small V as OFF • To Switch on, press the OFF button to open the CO2 control panel
	**To adjust the CO2:** • Select the required temperature (a) and then press ON (b). Then press the OK button (c).
	• The display will now show the current and the required set CO2 values. • Press the Control button to return to the objective display

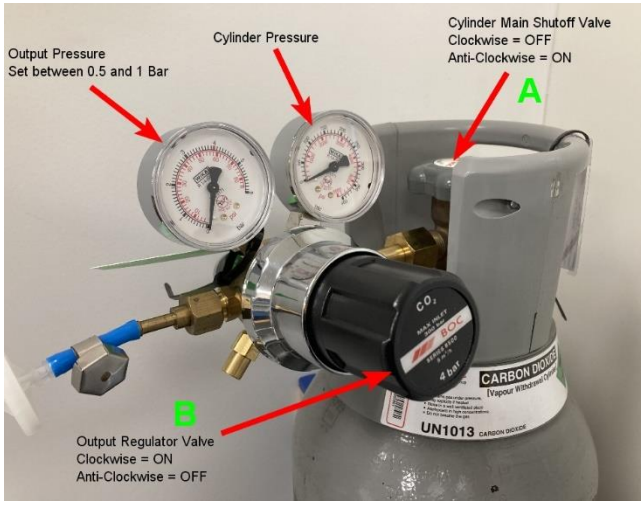
Check flow rate

- Check for bubbling inside the aspirator bottles inside the incubator
- Check the regulator output gauge again and adjust the regulator valve as necessary to maintain 0.5 to 1 BAR

Shutting down

CO_2

- Close the main cylinder valve (A) - fully clockwise



- Turn the pressure regulator valve (B) almost fully anticlockwise - it should feel loose – it will feel stuck if turned too far anticlockwise
- Turn off CO_2 on the TFT controller to the right of the microscope

Heating

- Switch off the heating unit on the TFT controller
- Remove humidifier box if used.