Setup for Live Imaging using the HCF1 Microscope

Please ask for help if you are unsure

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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.

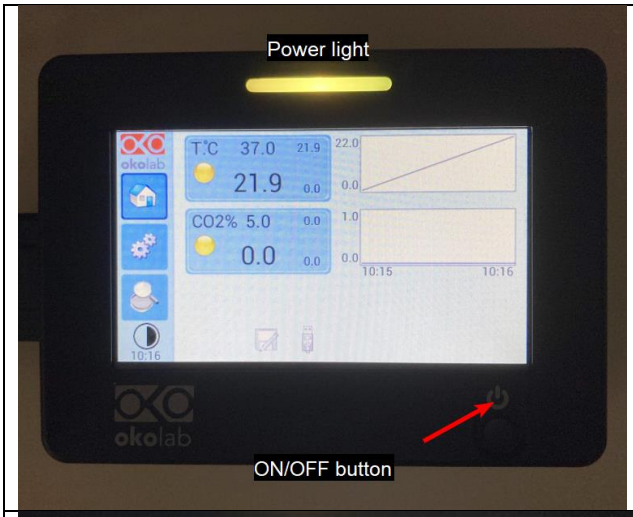
Well Liquid levels

- 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

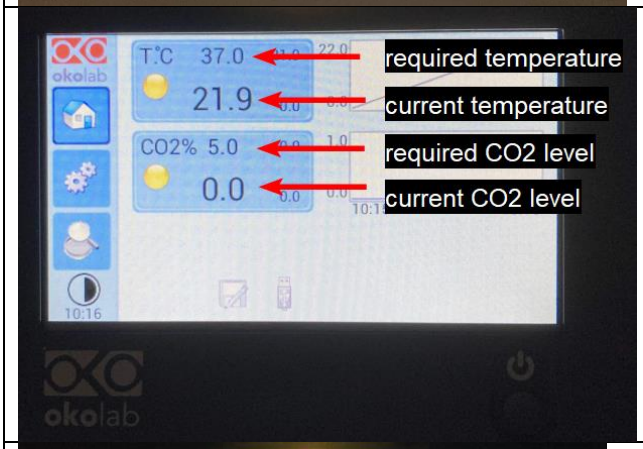
The HCF2 can provide heating to the chamber. Switch on at least 30 minutes before use to allow the chamber to reach temperature and equilibrate. For longer term

imaging (time series) then pre-heat the chamber for at least 2 hours before use, to prevent focus drift).

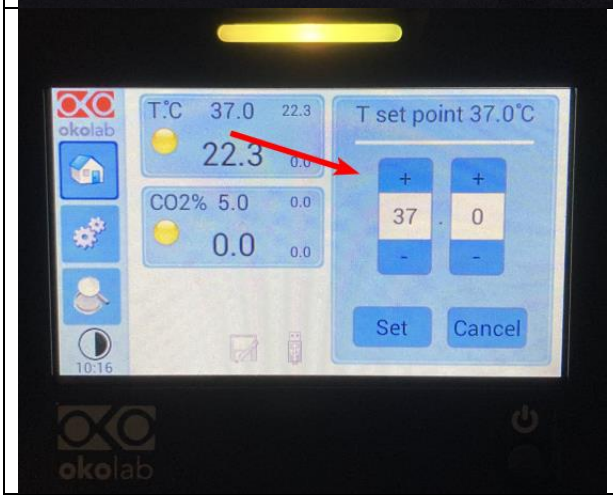


Turning on

- Start up the microscope in the usual way
- Make sure all incubator doors are closed and that the temperature probe is resting on the stage (white wire with metal end)
- Turn on the okolab environmental control unit by pressing and holding the on/off button for a few seconds



- The display will show the required and current temperature and CO2 levels



Adjust the temperature:

- Press the temperature display and adjust as necessary

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^{0}C). 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

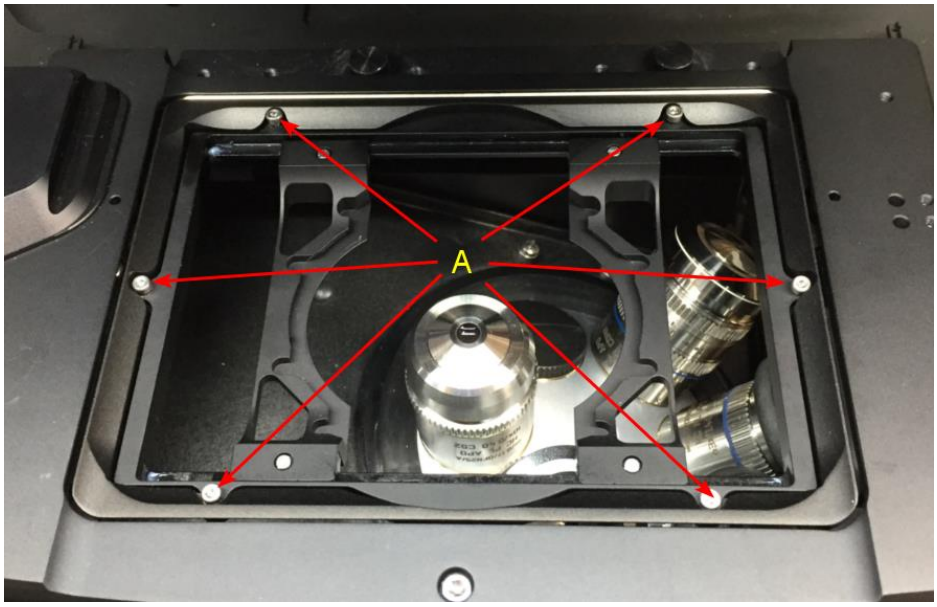
CO_2

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 or lid. There are several lid/insert options for 35mm dishes, chamber slides and multi-well plates – see above.

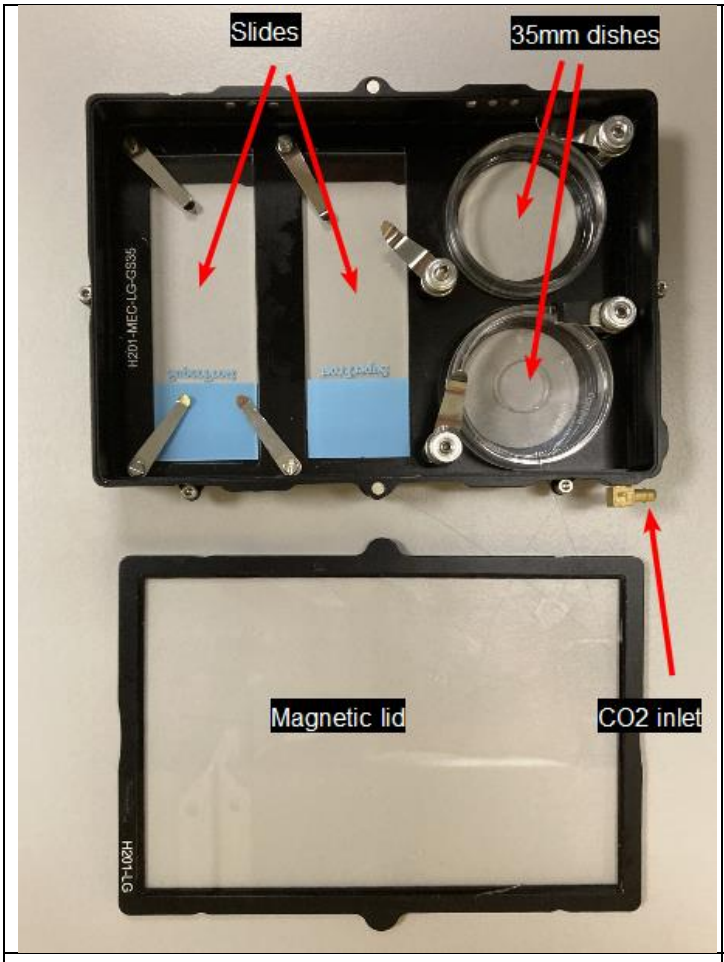
Stage Inserts

Both the sample holders for multi-well plates and for the multi-format samples are retained by a series of small screws (A). To change the sample holder, loosen these screws (4 turns) with the Allen key provided. **Important, do not completely unscrew these screws** Just 4-5 turns will release the stage and allow its removal with part of the screws still attached in their threads. **Be careful not to lose these screws!** Swap over the holder and lightly tighten the screws.

DO NOT OVER TIGHTEN SCREWS



- 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



Multi-format sample holder for chamber slides and 35mm dishes

- Ensure the condensor head has sufficient clearance above the stage
- Insert the holder into the the stage and tighten fixing screws
- Insert sample
- Fill the epmty positions with slides/dishes to maintain a closed environment
- Place on magnetic lid
- Connext the tubing from the aspirator bottle to the CO2 inlet

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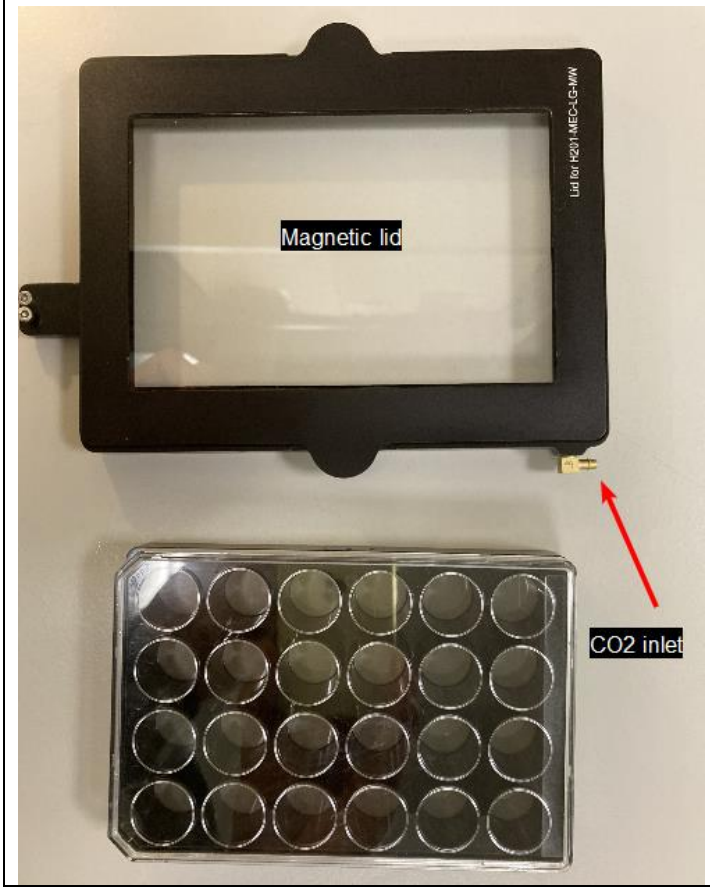


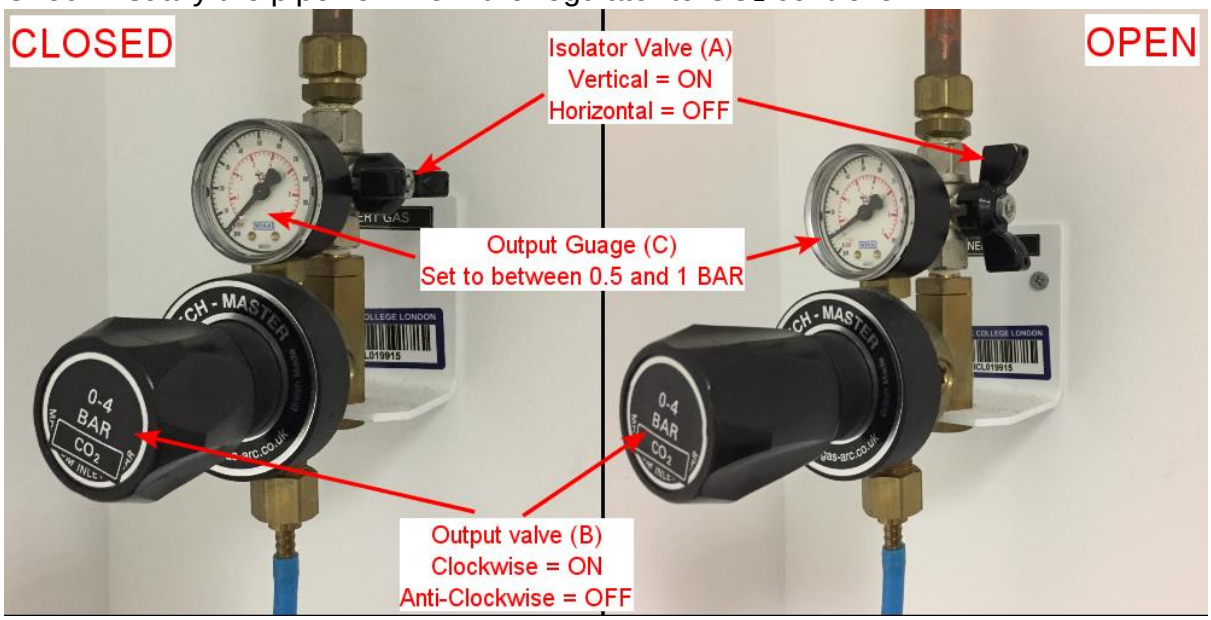
Multi-Well format sample holder

- Ensure the condensor head has sufficient clearance above the stage
- Insert the plate holder into the stage and tighten fixing screws
- Insert multiwell plate
- Place on magnetic lid
- Connext the tubing from the aspirator bottle to the CO2 inlet

NB Please note that it is not possible to image the outer rows and columns of any 96 well plate format (or outer edges of other plate formats) using objectives above x10 as the stage will make contact with the objectives.

Gas Safety Check

- Check gauge (C): (there may be some residual pressure from the last use)
    - output gauge should be between 0 and 1 BAR
- Check the main isolator valve (A) is closed – it should be in the horizontal position.
- Check the output regulator valve (B) is closed – almost fully anticlockwise (it should feel loose, but it will feel stuck if turned too far anticlockwise)
- Check visually the pipework from the regulator to CO_2 controller



Turn on CO_2 gas

- Turn the main isolator valve (A) to the vertical position.
- Then by turning the output 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.
  Important – do not set pressure above 1 bar or the tubing may separate.

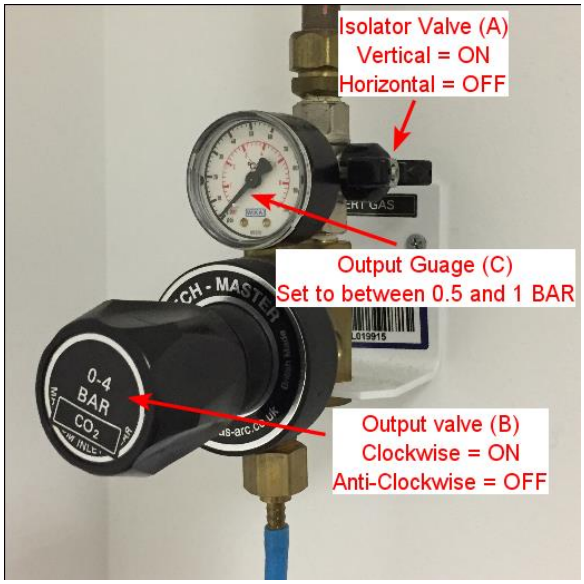
Adjust the CO2:

- Press the CO2 display and adjust as necessary

Shutting down

CO_2

- Turn the main isolator valve (A) to the Horizontal closed position.
- Close the output regulator valve (B) – almost fully anticlockwise - it should feel loose, but it will feel stuck if turned too far anticlockwise



Heating

- Remove humidifier box if used.
- Turn off the okolab environmental control unit by pressing and holding the on/off button for a few seconds until the turn ff system window appears.