Setup for Live Imaging using the LS1 Microscope

13/10/2021

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

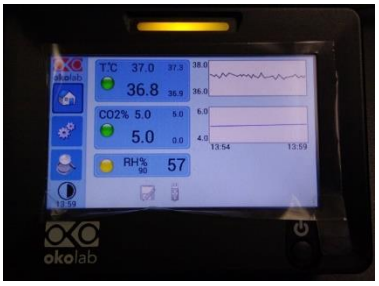
- Imaging for several hours or more at 37^0C can cause evaporation of the culture/imaging media. Make sure the dish has sufficient liquid level to compensate for this. Also filling the water reservoir in the stage top incubator can be beneficial.

Heating

The microscope stage top incubator can be set to a specified temperature. Turn on at least 15 minutes before use to allow the chamber to reach temperature and equilibrate. For longer term imaging (time series) pre-heat the chamber for at least 1 hour before use, to prevent focus drift).

Turning on

- Switch on the plugs on the wall marked CO_2 and incubation, the touch screen display of the control unit will light up.



To adjust the temperature:

- Tap the at 'T^0C' and set the temperature set point to the desired value.

To Adjust CO2 concentration:

- Tap 'CO2%' and set the CO_2 set point, **set to zero if CO_2 is not used.**

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 (e.g., 37^0C). This evaporation will be significantly reduced by using the humidity control, make sure the water level in the humidifier bottle is high enough. To fill the bottle with sterile water, unplug the cable first. Additionally, you can fill the water reservoir in the stage-top incubator (see below). **Take care not to spill water inside the microscope, let FILM staff know if this should happen.**

CO_2

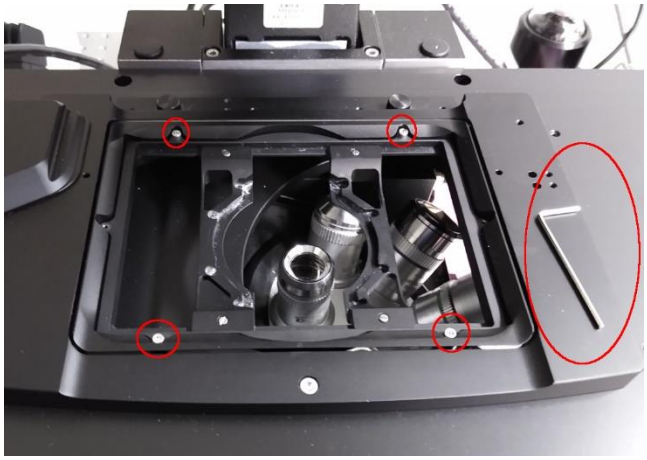
If using CO_2, please check cylinder pressure a few days before required in case the cylinder needs replacing – see checking method below

Setting up the stage-top Incubator

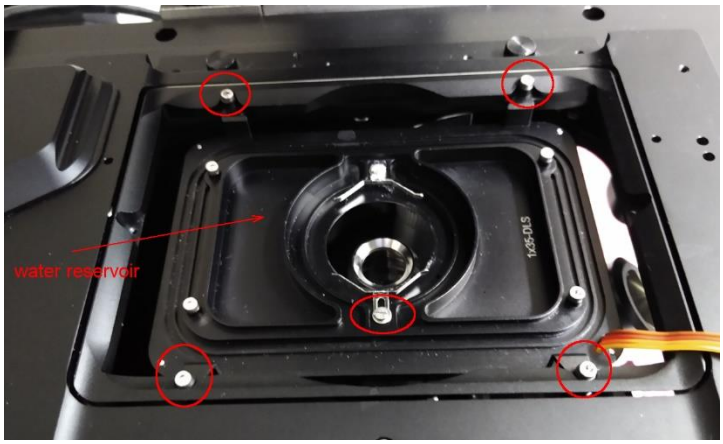
- First start the microscope and initialise the stage before fitting the incubation chamber, otherwise the wires might get tangled. Tilt the illumination pillar backwards.

- Choose the ‘machineDLS–Okolab.xlhw’ configuration on startup in the Leica software, if you want to log temperature and CO2 parameters, or the machineDLS.xlhw if this is not required.

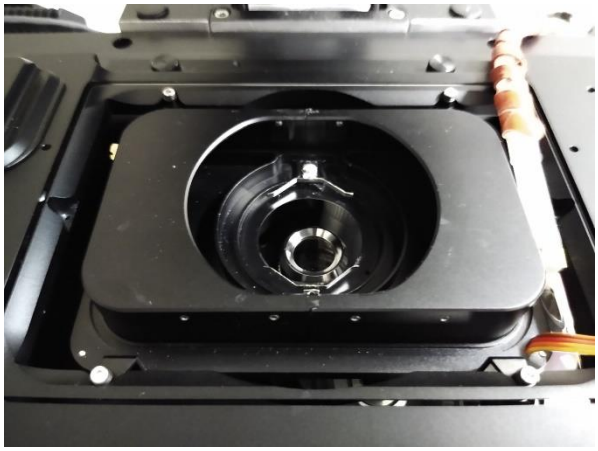
- If the universal stage insert is currently mounted on the microscope stage, this can be removed by loosening the 4 screws with the Allen key. Important, do not completely unscrew these screws, A few turns will release the stage and you will be able to remove the sample holder with part of the screws still in the thread. Be careful not to lose the screws!



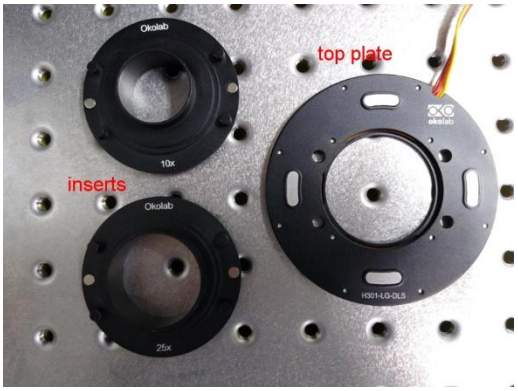
- Mount the incubator base in the galvo stage and fasten the 4 screws. You can adjust the little spring brackets to better fit the 35 mm dish (use screwdriver). Fill the water reservoir if needed. Take care not to spill water inside the microscope, let FILM staff know if this should happen.



- Add the upper part of the incubator chamber so that it aligns with the lower part, the two parts lock together. The gas supply tube should be attached to the right side. There is a temperature probe (green wire) which can be inserted into the chamber through a small hole to measure sample temperature.

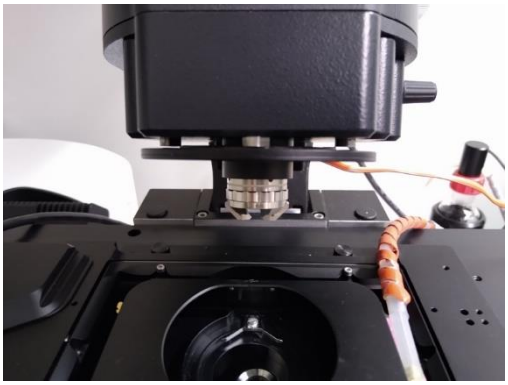


- Fit the required insert (10x or 25x) that matches the detection objective into the round top plate. The 20x objective fits the 10x insert.



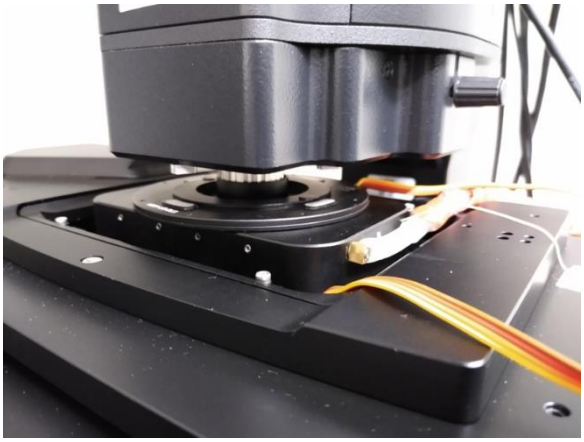
- With the illumination pillar still tilted backwards, attach the top plate to the condenser so that it surrounds the objective.

- Carefully bring the illumination pillar back to the vertical until it is in the correct position.



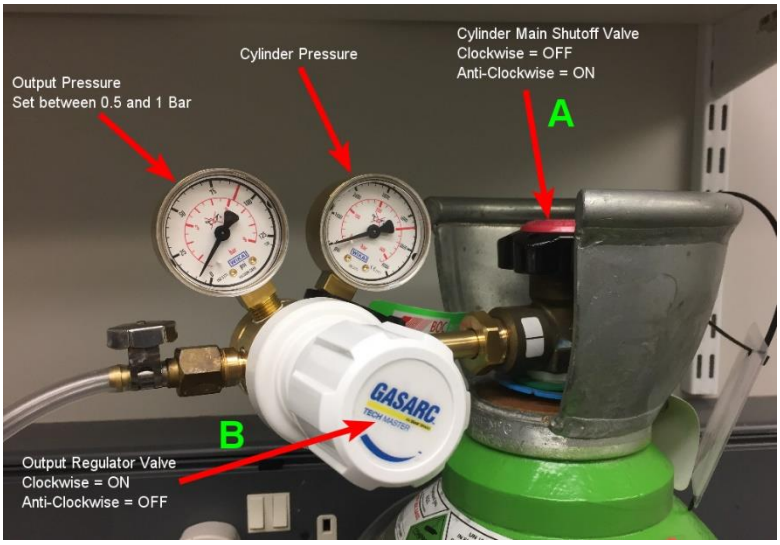
- Then lower the top-plate away from the magnets. It should sit on the incubator housing and be free to move sideways when the stage moves. This is the position during imaging.

- For easy access to the sample, you can attach the top-plate back to the condenser and tilt the arm backwards.



Gas Cylinder Safety Check

- Check gauges: (there may be some residual pressure from the last use)
  - left had gauge should be between 0 and 1.5 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 1.4 BAR as displayed in the left hand (output) gauge - a slight resistance will be felt when the valve starts to open (not more than 1.5 BAR).

Temperature display

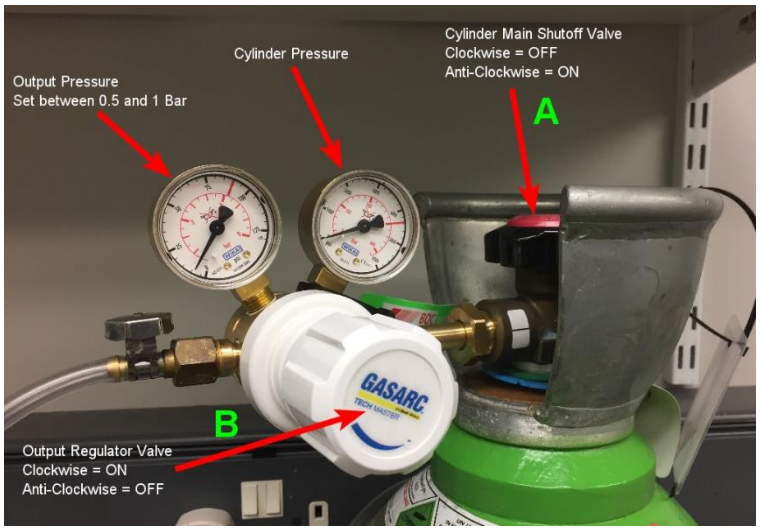
The temperature, CO2 concentration and humidity will be displayed on the control unit. A yellow dot indicates normal operation, green dot ideal operation and a red dot an error. The unit beeps if an error is detected (e.g. CO2 pressure too low, this can be muted on the display). A more detailed view including the sample temperature can be displayed when tapping the magnifying tab.

Shutting down

CO_2

- Turn off CO_2 controller to the right of the microscope and turn off at the wall
- Close the in-line safety clips (rooms 408 and 409)

- 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



Heating

- Switch off the control unit (incubation) (on/off switch) and turn off at the wall