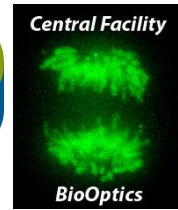


Olympus cellSens Live/TIRF Unit



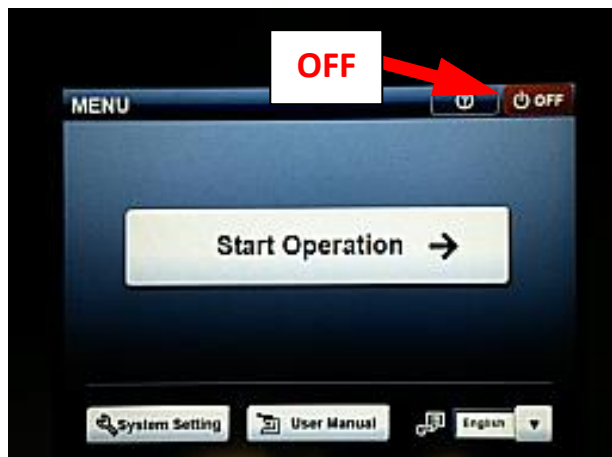
Version 5 (March 2021)

Startup (Widefield Mode)

- Switch on power strips #1-3 (behind the monitor/lasers) *in the order as they are labeled.*



- Power on the PC (labeled #4)
- Power on the touch panel of the microscope (button #5 on the rear side of the panel)
Important: exit the software (button OFF in the touch window) before powering off with #5



Unmotorized components

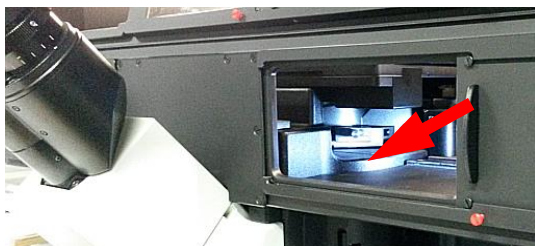
- For viewing through the eyepiece, pull out the laser safety ocular shutter:



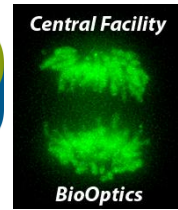
- Push the light path selector slider (on the rear side) to the left (position "WF").
In the right position ("WF/TIRF"), widefield images can be acquired at 50% of the LED power.



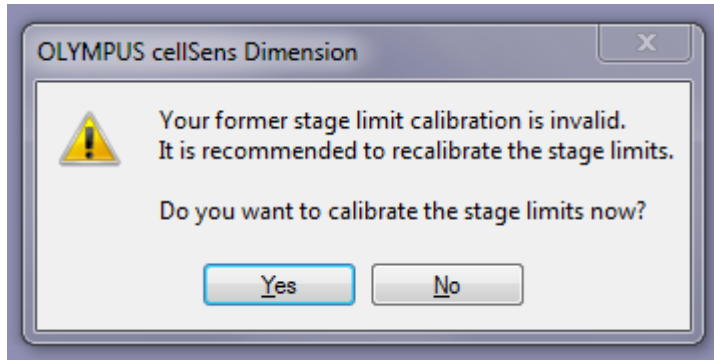
- The DIC prism snaps in two positions (push it in to have it in the light path).



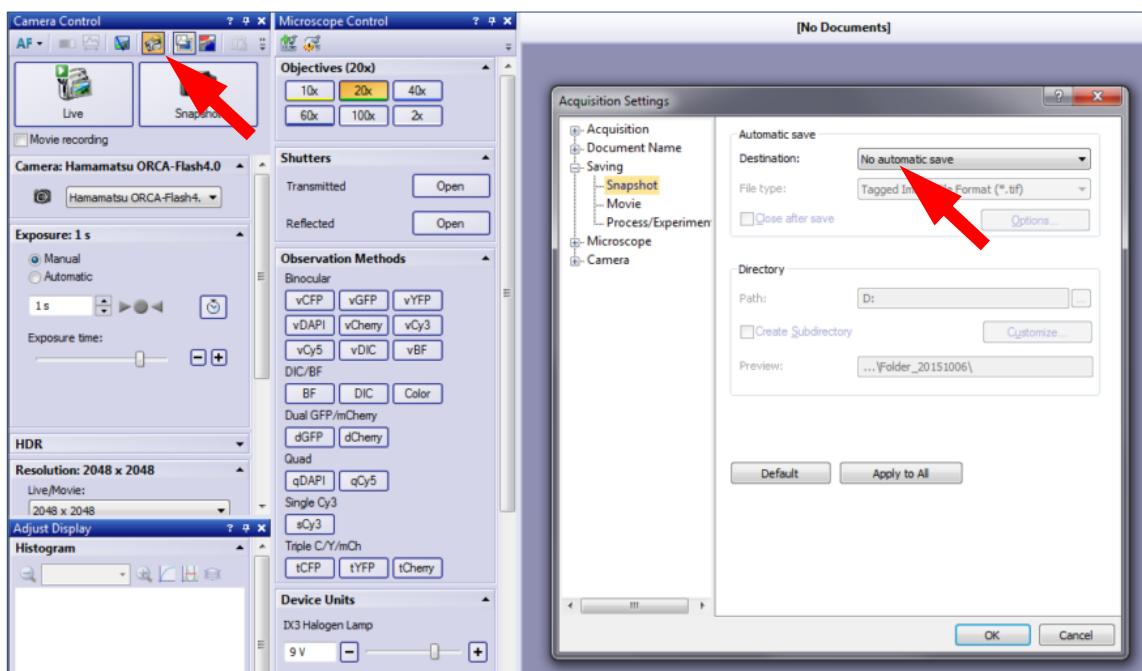
Olympus cellSens Live/TIRF Unit



- Start the cellSens software. If you did not turn on the TIRF laser power strip, a corresponding error message will pop up, which can be ignored for widefield imaging. You will be prompted to calibrate the xy stage. Confirm all steps by clicking "OK", "Next" and "Finish", respectively (do not calibrate the z focus drive).



- Acquisition settings. Open the settings dialog by clicking the icon in the Camera Control panel (see below). In the dialog opening, navigate to "Saving". Select an autosaving option ("No automatic save" or "File system"). If "File system" is chosen, choose a directory on drive D: to save your images. *Click the "Apply to All" button to activate your choice for all types of images (Snapshot, Movie, Process/Experiment).*



Shut Down

If another user has booked a slot after you (on the same day):

- Wipe all used oil objectives with lens cleaning paper
- Turn SpectraX intensities down to zero
- Close all doors of the environmental control box

Full shut down:

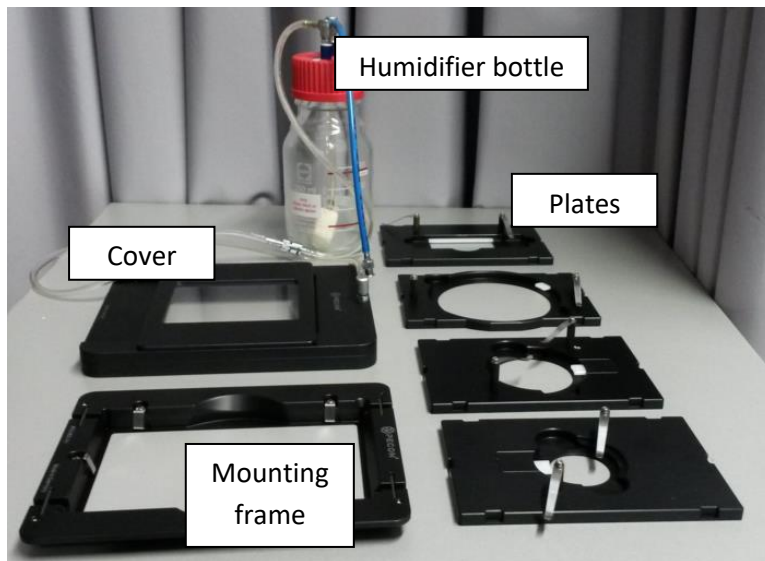
- Wipe all used oil objectives with lens cleaning paper
- Exit the cellSense software, shut down the PC
- **Exit the touch panel software with the OFF button**, then power off the panel with button #4



- Switch off the power strips behind the monitor/lasers (in the order as they are labeled)
- Close all doors of the environmental control box

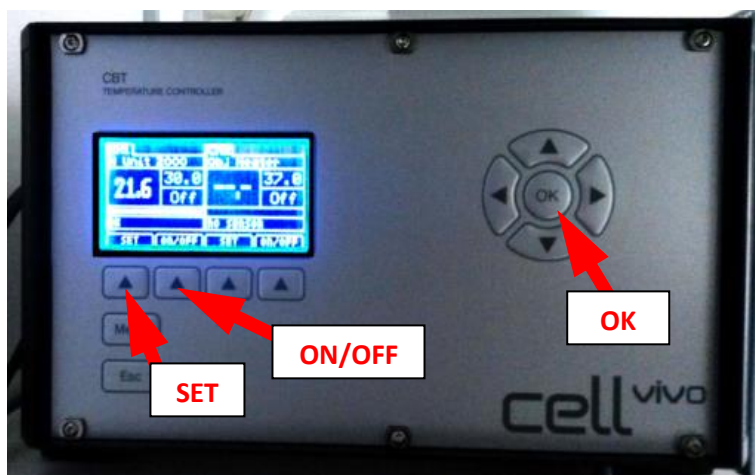
Stage Inserts

There are standard Olympus inserts for slides, 35 mm dishes and multiwell plates. For imaging under controlled CO₂ and humidity, the click-in system from PECON system is provided (together with a humidifier bottle; see below). Insert one of the plates (for 35, 60 or 100-mm dishes, chamber slides or multiwell plates) into the universal mounting frame. Make sure the the insert is firmly pressed against the two stainless steel springs in the xy stage frame.



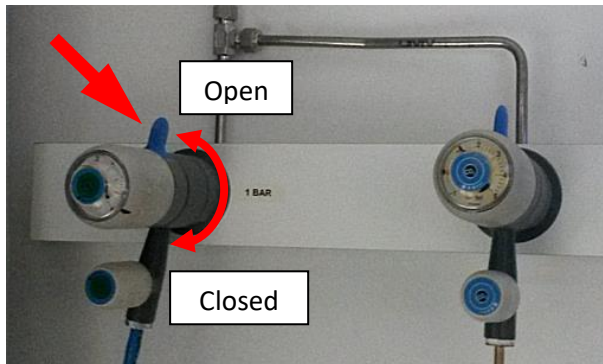
Temperature Control

The cellVivo temperature controller for the large environmental control box and its heater can be switched on and off independently from the remaining system. To activate heating, press the second button from the left ("CH1", "ON/OFF"). To select a different temperature, press the first button from the left ("SET"). Confirm you choice by pressing "OK".

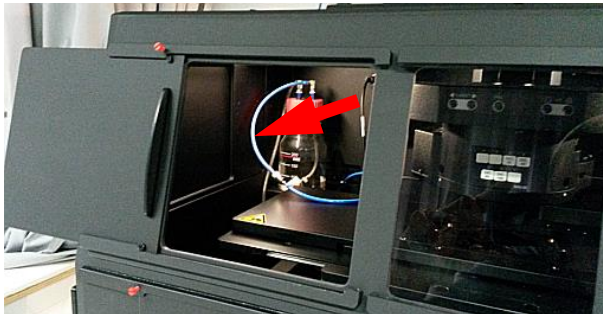


CO₂ and Humidity

- Open the main valve mounted on the wall behind the microscope (blue handle with ON/OFF position, not to be confused with the pressure regulator valve. Turn clockwise to open, counter clockwise to close). The pressure regulator valve should indicate a pressure of about 1 bar.



- Check the fill level of the humidifier bottle in the environmental control box. It should contain between 50 and 100 mL water. To resplenish (deionized, sterile/autoclaved water only), disconnect the luer connector of the blue tubing (connecting the CO₂ controller with the bottle). Reinstall the bottle and connect the transparent tubing to the cover of the Pecon CO₂ insert.



PeCon CO₂ Controller

- Power switch on the rear side.
- Press the "display" button to switch the display between the measured ("real") and set ("nom") CO₂ concentration.
- Use the "+" or "-" buttons to set the desired concentration.
- Turn on the flow by pressing the "valve" button. The LED on the button is then either green (flow through the system) or red (no flow). Note that the system does not pump continuously. The LED should turn green in more or less regular intervals. Pressing the "valve" button again turns it off (the LED remains permanently dark).
- Repeated pressing the "pump" button sets the pump/flow speed (values between 1-5; use 2 or 3).
- Do *not* change the knob of the CO₂ reducing valve.