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The O2k-Fluo LED2-Module

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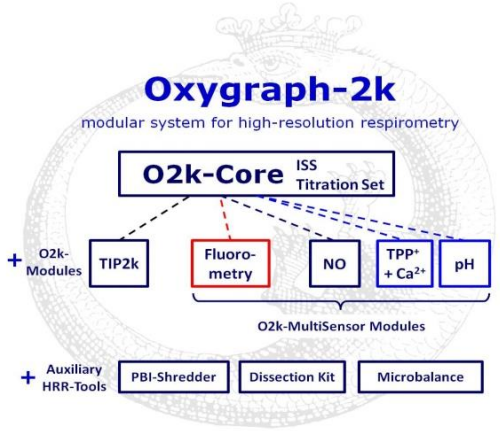


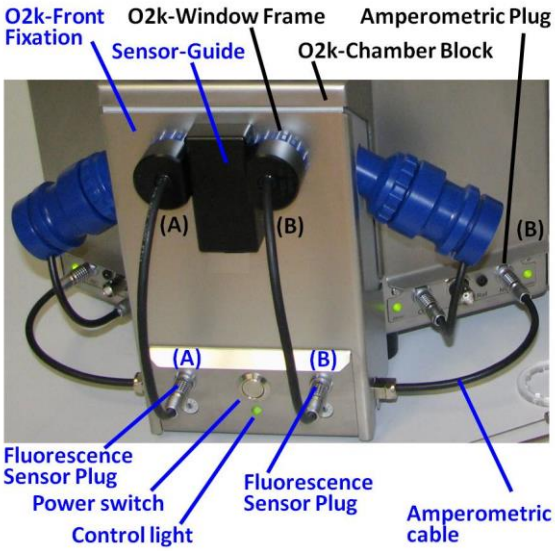
The **O2k-Fluo LED2-Module** is a modular extension of the O2k-Core (Series E-H)*. A growing number of fluorescence markers enables determination of diverse mitochondrial processes in addition to oxygen consumption, including generation of H_2O_2, ATP production, mitochondrial membrane potential and Ca^{2+}, extended by user innovation.

1. Components of the O2k-Fluo LED2-Module

The O2k-Fluo LED2-Module includes two pairs of optical sensors. Each of the four 'Fluo-Sensors' has a light-emitting diode (LED), a photodiode, a Filter-Cap, and three filter sets which can be exchanged for applications of various fluorophores. The Fluo-Control Unit is mounted to the O2k-FluoRespirometer with the O2k-Front Fixation and can be easily attached or removed. If connected to the amperometric O2k-MultiSensor channels, the signals and corresponding fluxes are recorded by DatLab simultaneously with O_2 concentration and O_2 flux.

2. Setup of the O2k-Fluo LED2-Module

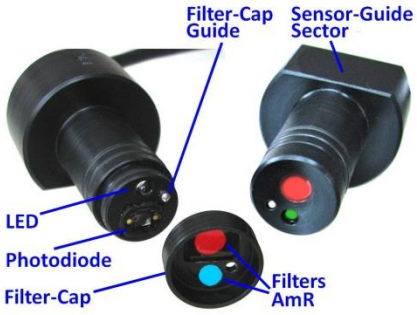
1. Switch off the O2k power switch at the rear of the O2k.
2. Remove both blue O2k-Window Frames by placing the O2k-Window Tool around the outer rim of the window frame and unscrewing counter clockwise.
3. Pull the Sensor-Guide ('nose') from the O2k-Front Fixation of the Fluo-Control Unit.
4. Align the Fluo-Control Unit with the O2k-Chamber Block. The Fluo-Power cables are placed in the middle below the O2k-Main Unit from front to rear. Attach the Fluo-Control Unit to the O2k-Chamber Block with the O2k-Window Frames and fix them tightly with the O2k-Window Tool.



5. Reattach the Sensor-Guide to the O2k-Front-Fixation.
6. Unplug the O2k-Power cable at the rear of the O2k and connect it to the female plug of the Fluo-Control Unit. Insert the male plug of the Fluo-Control Unit into the main socket at the rear of the O2k.
7. Connect the cables at the side of the Fluo-Control Unit to the 'Amp' plugs on the O2k-Main Unit.* It is not necessary to dismount the Fluo-Control Unit for basic HRFR when no fluorescence signal is recorded.

*The Amp plugs are labeled "NO" (nitric oxide) in Series D-E.

3. The Fluorescence-Sensors



Standard configuration:

Two Fluorescence-Sensors Green: 525 nm, Filter-Cap for H_2O_2 measurement with Amplex® UltraRed and mt-membrane potential with TMRM.

Two Fluorescence-Sensors Blue: 465 nm, Filter-Cap for measurement of mt-membrane potential with safranin. A different filter is used for measurement with Magnesium green® or Calcium green®.

Filter-Caps: The Filter-Cap can be exchanged for each Fluo-Sensor for applications with different fluorescent dyes.

Dismounting: Pull the Filter-Cap straight from the Fluo-Sensor. The Filter-Cap Guide prevents rotational movements.

Replacing filters: Remove the two filters and store them in the filter box labeled for this filter set. Insert the filters from the selected filter set: The round filters fit to the round window of the filter cap and cover the LED, the rectangular filters fit into the rectangular window of the filter cap and cover the photodiode.

Mounting: Hold the Fluo-Sensor and Filter-Cap in a vertical position. Align the Filter-Cap with the Filter-Cap Guide, the small steel rod protruding from the sensor. Press the Filter-Cap onto the sensor without any rotational movements.

Connect the Fluo-Sensors to the O2k: Insert the Fluo-Sensor into the window of the O2k-Chamber as far as possible, aligning the Sensor-Guide Sector with the Sensor-Guide of the O2k-Front Fixation. Connect the cable of the Fluo-Sensor to the Fluo-Sensor Plug of the Fluo-Control Unit.

Remove the Fluo-Sensors: Remove the Sensor-Guide, grab the Fluo-Sensor on its body near the O2k-Chamber window and rotate the Fluo-Sensor while pulling it out. Never pull on the cable. Replace the Sensor-Guide.

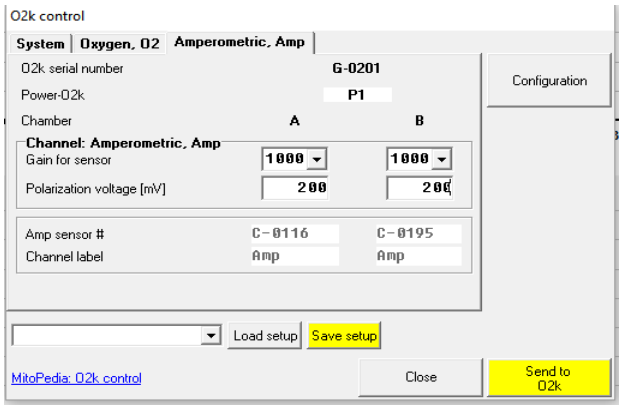
4. Stoppers

Use only black stoppers in conjunction with optical measurements. Black PEEK stoppers are now used for all HRR applications in general.

» MiPNet19.18A: Calibration of the O2k-Chamber volume, identical for black PEEK and white PVDF stoppers. During optical measurements, place black Cover-Slips on top of the O2k-Stoppers to prevent any light from penetrating into the O2k-Chamber through the injection ports.

5. Electronic and DatLab settings

1. Switch on the power of the O2k-Main Unit (rear).
2. Press the power switch on the front panel of the Fluo-Control Unit. Check that the control lights are on.
3. Start DatLab and connect to the O2k. In the O2k configuration window, tick Amperometric, Amp and define the sensors for documentation.



Control of LED-intensity: Open the Oxygraph-2k / O2k control window ([F7]), Tab: Amperometric, Amp and select the light intensity of the LED of each Fluo-Sensor (Chamber A and B) by setting the appropriate value for "Polarization voltage [mV]" (0 to 2000).

For Fluo-Module Series A, use Position 9 (Variable) on both sides of the Fluo-Control Unit.

If the Polarization voltage is >0 and the Fluo-Control Unit is switched on, the indicator light on the Fluo-Control Unit is green. If the current is zero (the LED is not used), but the Fluo-Control Unit is switched on, the indicator light is red. Vary the light intensity while the Fluo-Sensors are placed outside the chamber to observe the change in light intensity. Do not look directly into the LED to protect your eyes.

Amplification: The gain for the amperometric (Amp) channel is set in the in the field "Gain for sensor" (1, 10, 100, or 1000). The gain amplifies the Amp raw signal [V] which can be recorded in the range -10 to +10 V. Click Send to O2k to apply the new settings.

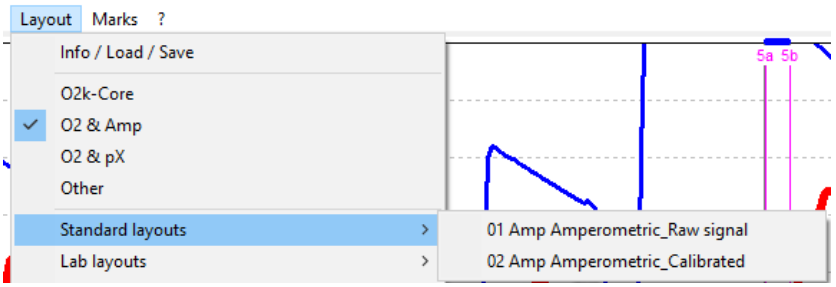
6. Suggested application-specific settings

Application	Sensor	Filter set	Light intensity Amp voltage	Gain
Amplex® UltraRed	Fluorescence-Sensor Green	AmR	100 - 500	1000
TMRM	Fluorescence-Sensor Green	AmR	100 - 500	1000
Safranin	Fluorescence-Sensor Blue	Saf	200 for >2 µM; 500 or higher for <2 µM safranin	1000
Magnesium green	Fluorescence-Sensor Blue	MgG / CaG	300	#
Calcium green	Fluorescence-Sensor Blue	MgG / CaG		#

#The optimum amplification for Magnesium green® and Calcium green® depends on the concentration of the fluorophore, which may vary widely in different applications. Therefore, no recommendation for the gain is given.

4. Close the O2k-Chamber by fully inserting the stoppers without trapping any gas bubble. A gas phase disturbs the optical signal by reflections.
5. Insert the Fluo-Sensors into the windows of the O2k-Chambers.
6. Switch off the illumination of the chambers [F10].
7. Place black Cover-Slips on top of the O2k-Stoppers.
8. Select an appropriate graph layout to observe the change in the Amp signal when changing the light intensity (Amp voltage) or amplification (Gain).

Layout / Reference layouts / O2 & Amp:



Two standard layouts are available in DatLab for the amperometric channel: **01 Amp Amperometric_Raw signal** and **02 Amp Amperometric_Calibrated**. The amperometric slope is the time derivative of the calibrated signal, multiplied by the slope amplification factor, in units [mV(conc. Unit during calibration)/s]. If the signal is calibrated in µM (nmol/ml), the unit of the slope will be [pmol·ml^{-1}·s^{-1}].

01 Amp Amperometric_Raw signal displays the raw voltage (including amplification) as recorded by the O2k at a given gain setting.

02 Amp Amperometric_Calibrated displays the signal after calibration with the parameters set in the Calibration window of the Amp channel.

9. Each channel can be labelled. Avoid long names. The default unit is [µM].

Go to Graph / Select plots (tab: Amperometric, Amp) to define the slope unit. The default unit for the slope is set by DatLab depending on the unit selected for the calibrated signal (slope factor 1000). After changing the slope unit, all values for the slope plot are automatically recalculated.

10. Select the Gain setting to obtain a maximum voltage below 10 V. If the maximum observed raw signal is 9 V in an initial experiment, then the gain should be reduced to avoid "off scale" (>9.99 V). If the maximum

recorded raw signal is lower than 1 V (e.g. 0.2 V), the gain should be increased to avoid limitation of resolution by digital noise.

7. Calibration

11. During air calibration of the OroboPOS in the absence of a biological sample, the stoppers are partially inserted in the 'open' position with a gas phase above the stirred medium. The time for gaining a stable oxygen signal can be used to thermally equilibrate the Fluo-Sensors inserted to the chamber windows. In the open position, the optical signal is disturbed by the gas phase. Remove the Fluo-Sensors for visual checks of the O2k-Chamber for short periods of time only.
12. Optical calibrations are made after addition of the sample. The O2k-Chambers must be closed.
13. Switch off the O2k-Chamber light [F10].
14. Place black Cover-Slips on top of the stoppers, and remove them only briefly during titrations.
    Different fluorescence applications require specific calibration procedures (e.g. safranin versus H_2O_2). For some applications (H_2O_2 production) the slope of the fluorescence signal is the relevant parameter.

More details:
» http://wiki.oroboros.at/index.php/O2k-Fluo_LED2-Module

8. References

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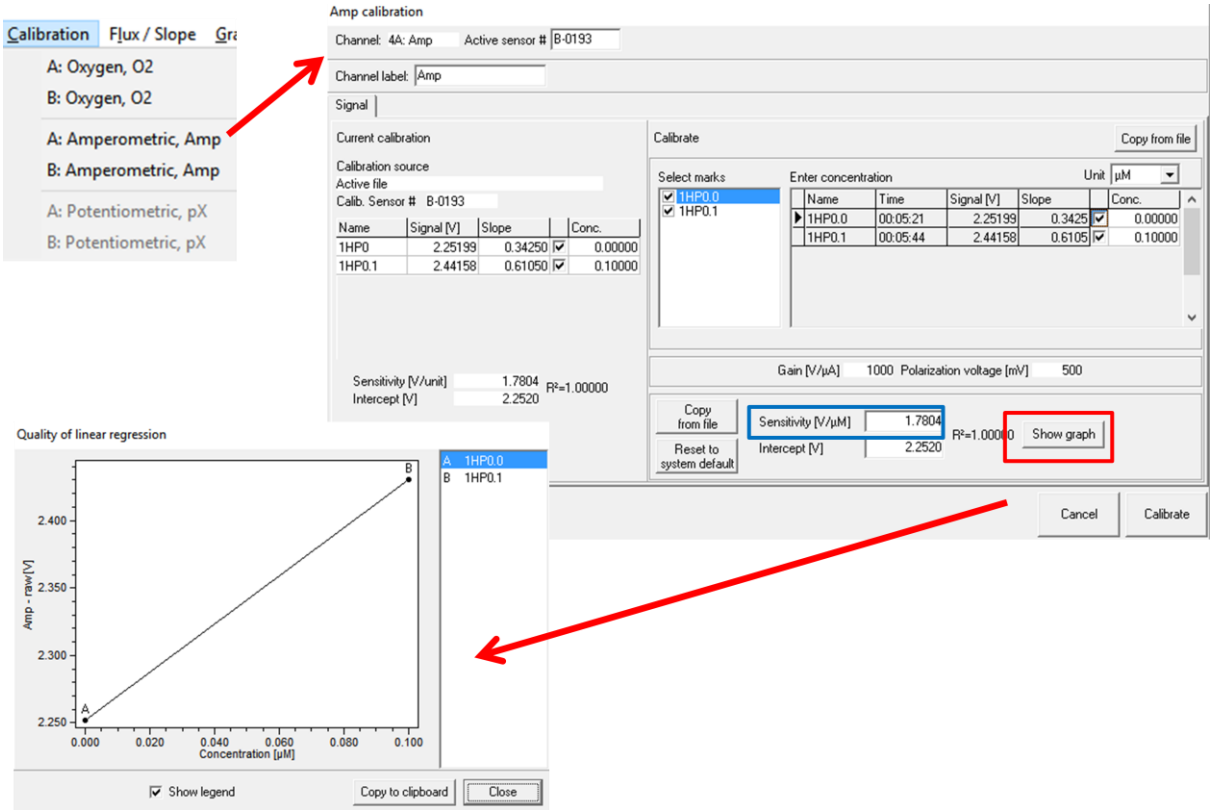
http://wiki.oroboros.at/index.php/O2k-Demo_experiments#O2k-Demo_O2k-MultiSensor

Supplement A: Amplex red and calibration with H_2O_2 titrations

Marks - Value Mark sections of the calibration experiment before and after H_2O_2 titrations. Edit the marks. The "Concentration" in the Mark information' window is used to assign a numerical value to the mark. Enter the concentration of H_2O_2 titrated into the O2k-Chamber.

Calibrate H_2O_2 concentrations are automatically retrieved from the marks set on the fluorometric signal, a background slope correction is available, and the linearity of calibration can be viewed in a graph.



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http://www.bioblast.at/index.php/MitoCom