O2k-Manual:O2k-Fluo Smart-Module

O2k-Fluo Smart-Module manual

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1. Overview

The **Oroboros O2k-Fluo Smart-Module** provides a unique approach to the simultaneous monitoring of cellular and mitochondrial functions assessed by fluorometry and high-resolution respirometry (HRR), which is indispensable for experiments at controlled oxygen levels and optimizes obtaining multiple results with a minimum of sample. By simultaneous measurement of a fluorometric signal, the O2k-Fluo Smart-Module enables analysis of H_2O_2 flux (Amplex UltraRed), mt-membrane potential (safranin, TMRM or rhodamine 123), mitochondrial Ca^{2+} uptake (Calcium Green), and mitochondrial ATP production (Magnesium Green). The software DatLab 8 provides real-time display of oxygen concentration and respiration (oxygen flux), and the fluorometric signal and its time derivative (slope).

O2k-Manual user information
» MiPNet07.08 User information - PLEASE STUDY THIS MANUAL
» O2k-Manual

For previous series of the O2k-FluoRespirometer, including O2k-Fluo Smart-Module use with DatLab 7.4, please see this Manual: MiPNet22.11 O2k-FluoRespirometer manual.

2. O2k-Fluo Smart-Module

The Smart Fluo-Sensors are pre-calibrated with sensor-specific memory and direct input into DatLab (version 7.4 or 8). Each sensor contains an LED light and a photodiode to detect the fluorescence. The O2k-Fluo Smart-Module is provided with two pairs of Smart Fluo-Sensors, a pair with a green LEDs (GN), excitation peak of 525 nm, and a pair with a blue LEDs (BU), excitation peak of 465 nm.

The Smart Fluo-Sensors are shipped in a separate box, as part of some O2k-Packages or as a module to upgrade instruments from O2k-Series H and higher or NextGen-O2k Series XA and higher.

⚠️ **The Smart Fluo-Sensors cannot be used with O2k-Series G and earlier.**

Green LED, ex. 525 nm

Blue LED, ex. 465 nm

2.1. Exchanging a filter set of the Smart Fluo-Sensor

The Smart Fluo-Sensors are provided with filters appropriate for the use of (1) Amplex UltraRed - AmR, (2) safranin - Saf or (3) Magnesium Green and Calcium Green – MgG/CaG. The filter sets can also be used for other probes, and should be used with the respective Fluo-Sensor as described in the table:

| Filter Set	Fluo sensor	Probes
AmR	Green (GN)	Amplex UltraRed, TMRM
Saf	Blue (BU)	Safranin, Rhodamine 123
MgG/CaG	Blue (BU)	Magnesium Green, Calcium Green

The Fluo-Sensors Blue (BU) are provided with the Filter set "Saf" inserted. To switch for MgG/CaG, or if any filter gets damaged or misplaced, follow these procedures to replace/exchange the filter set:

1. Remove the Filter cap (see image at the right), which is held in place by an O-ring. The Filter cap should be pulled out while facing down, in order to not displace the filters.
2. Put the filters back into the appropriate box, or discard them, if damaged.
3. With the help of forceps with smooth edges, gently put the new filters into the respective places of the Filter cap (circular for LED, rectangular for photodiode). Take care not to put more than one filter, or to bend/fold the filters.
4. Keeping the Filter cap with filters on the bench, attach the Fluo-Sensor from above, following the mounting guide – a silver pin on the Fluo-Sensor, which should enter the small hole on the Filter cap.

2.2. *Smart Fluo-Sensor assembly*

1. Connect the Smart Fluo-Sensor cable to the Fluo/PB port. (1). The red dot on the plug should be pointing straight up. Each Smart Fluo-Sensor can be used on either O2k-chamber A or B.
2. Insert the sensor into the front window of the O2k-glass chambers: The blue frame of the chamber window and the Smart Fluo-Sensor are specially designed to only connect at a specific orientation, when the flat edges align (2). With these aligned, the Smart Fluo-Sensor is carefully push in until the sensor fully covers the chamber window frame and is flush against the O2k-Main Unit (3a,b). In this position, the cable routing is horizontal (4).
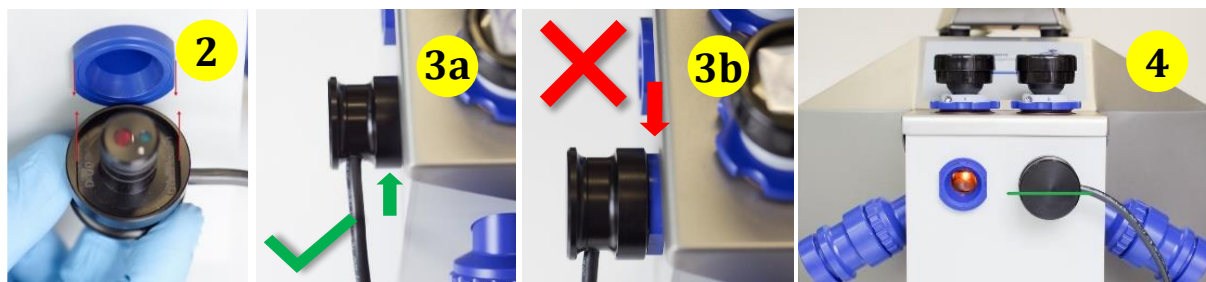
This manual is complemented by our O2k-Videosupport:
» O2k-Videosupport - FluoRespirometry

3.   DatLab 8

In this manual, only the information on DatLab 8 use related to the O2k-Fluo Smart-Module is presented. For general information on the software, please check these manuals:

» MiPNet28.07 NextGen-O2k Series XB manual
» MiPNet28.08 Oroboros O2k Series J manual

3.1. Settings

To edit the settings of the Fluo channel at the "Start recording" window, select the tab Channel and sensor settings on the left bar.
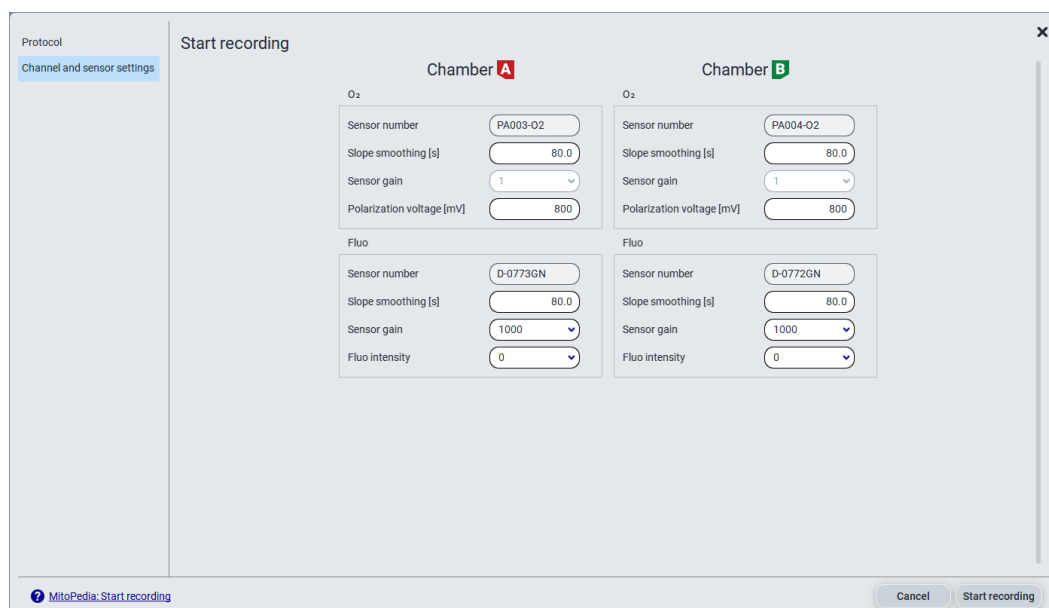
The settings can also be edited by selecting on the top menu **O2k** and then O2k Control (active) (F7). In Edit channel settings fill the required information by clicking on the Fluo tab:

Fluorometric, Fluo is the label for the <u>fluorescence channel</u>.

Sensor number: The Smart Fluo-Sensor serial number and LED excitation can be found on the inside of the sensor. If the Smart Fluo-Sensor is connected to the O2k, the serial number is automatically detected by the Oroboros O2k and set in "Sensor number". If the serial numbers are not automatically recognized, unplug the sensors and plug them into the O2k again.

Enter the following settings:

Sensor gain: Select a gain of 1, 10, 100 or 1000.

Polarization voltage: Select a polarization voltage [mV].

Once a polarization voltage higher than 0 mV is selected and applied, the LED of the Fluo sensor lights up.

3.2. Layouts

To display different combinations of plots with standardized layouts, select in the upper menu **Layouts**, select "Standard layouts," then "Fluorometric," and select one of the 4 layouts according to needs.
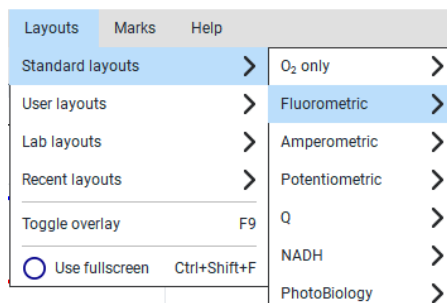
In all layouts available, graphs 1 and 2 show data from chamber A, while graphs 3 and 4 show data from chamber B. The black traces in graphs 2 and 4 show the fluorescence signal, while the green traces show fluorescence slope (variation in fluorescence over time), either raw or calibrated, depending on the layout selected.

DatLab 8 protocols for fluorescence are pre-set with one of these layouts.
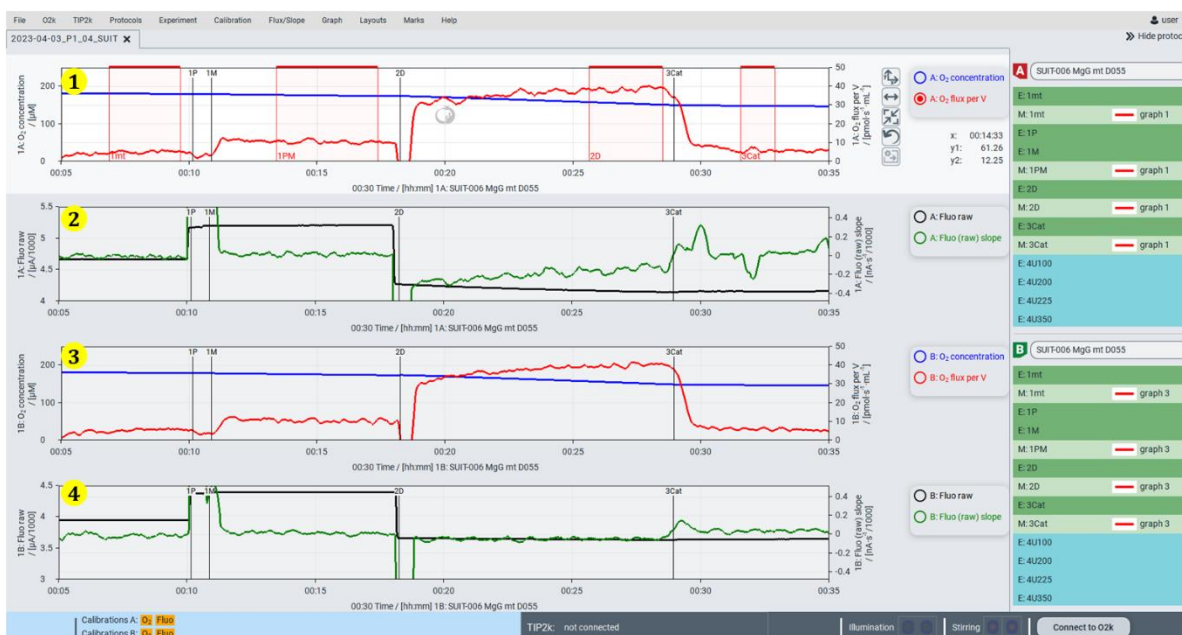
3.3.   Calibration

In DatLab 8, click on Calibration and then Calibration in the upper menu, or press F5, to open the calibration window. Select the channels and chambers to be calibrated.
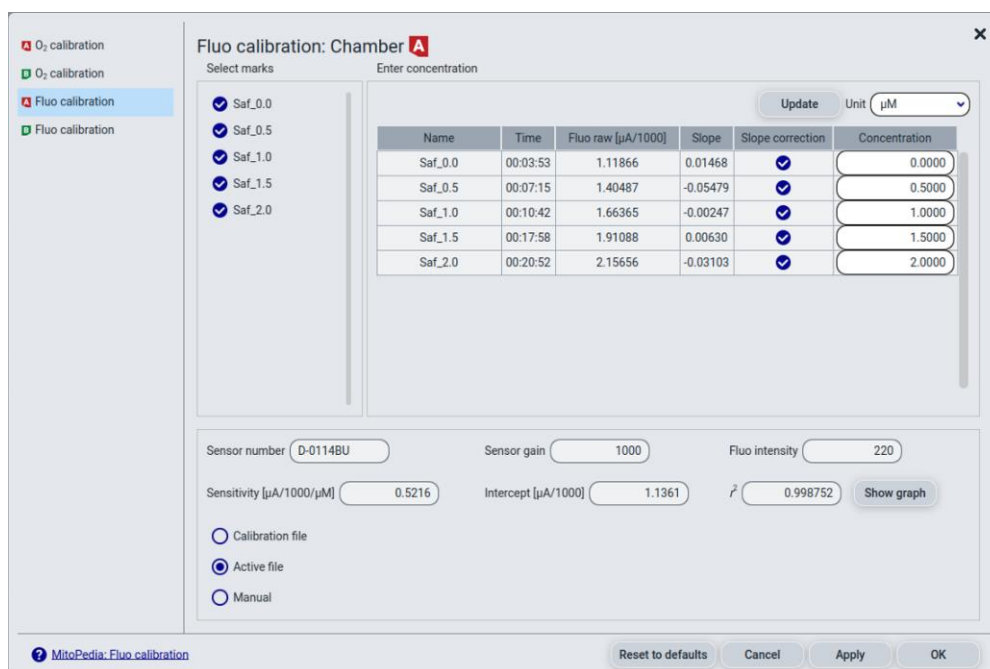
The status bar at the bottom left side of the screen/window provides information on the calibrations. Orange indicates that the channel has not been calibrated during the current run. Green indicates that the channel has been calibrated during the current run. The calibration from the previous run is maintained, although the indicator will appear in orange.

4.   DL-Protocols and data analysis

Select a SUIT protocol for FluoRespirometry:
   » MitoPedia: SUIT FluoRespirometry recommended protocols
   » SUITbrowser
   » O2k-VideoSupport: How to find a DL-Protocol

Data analysis:
   » O2k-Manual: Data analysis with DatLab

5.   Further information and links

Further information and troubleshooting:
   » O2k-Fluo Smart-Module
   » Troubleshooting: fluorescence module

Updated versions:
   » MiPNet28.09 O2k-Fluo Smart-Module manual

Links to O2k-catalogue and support
>>O2k-Products
>>O2k-Open Support

6.   Author contributions

Cardoso LHD, Timón-Gómez A and Grings M prepared the MiPNet. Gnaiger E is responsible for instrumental development of the O2k-Fluo Smart-Module.

7.   Acknowledgements