

# O2k-Manual: O2k-FluoRespirometer



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Updates: [https://wiki.orooboros.at/index.php/MiPNet22.11\\_O2k-FluoRespirometer\\_manual](https://wiki.orooboros.at/index.php/MiPNet22.11_O2k-FluoRespirometer_manual)

## O2k-FluoRespirometer manual

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O2k-FluoRespirometer

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## Overview



The **Oroboros O2k-FluoRespirometer** provides a unique high-resolution approach to the monitoring of cellular and mitochondrial respiratory function. High-resolution respirometry (HRR) is indispensable for application of SUIT protocols at controlled oxygen levels and when measuring low respiratory activity or kinetic transitions. High-resolution is required for analysis of pathological effects causing reduced respiration (mitochondrial and metabolic diseases, ischemia-reperfusion injury, oxidative stress, apoptosis, aging). The O2k-FluoRespirometer enables simultaneous measurement of respiration and fluorometric signals ( $\text{H}_2\text{O}_2$  flux, mt-membrane potential,  $\text{Ca}^{2+}$ , ADP-ATP phosphorylation) for analysis of biopsies with limited amounts of sample (diagnosis of genetic and acquired mitochondrial defects in pediatric patients, diabetes); cell cultures with limited cell numbers or low activity; mutants with diminished respiratory capacity; chemical oxidation rates and antioxidant capacities; and oxygen kinetic measurements at low, physiological intracellular oxygen levels.



**The Oroboros O2k is the modular 2-chamber system for high-resolution respirometry (HRR).**

### O2k-FluoRespirometer:

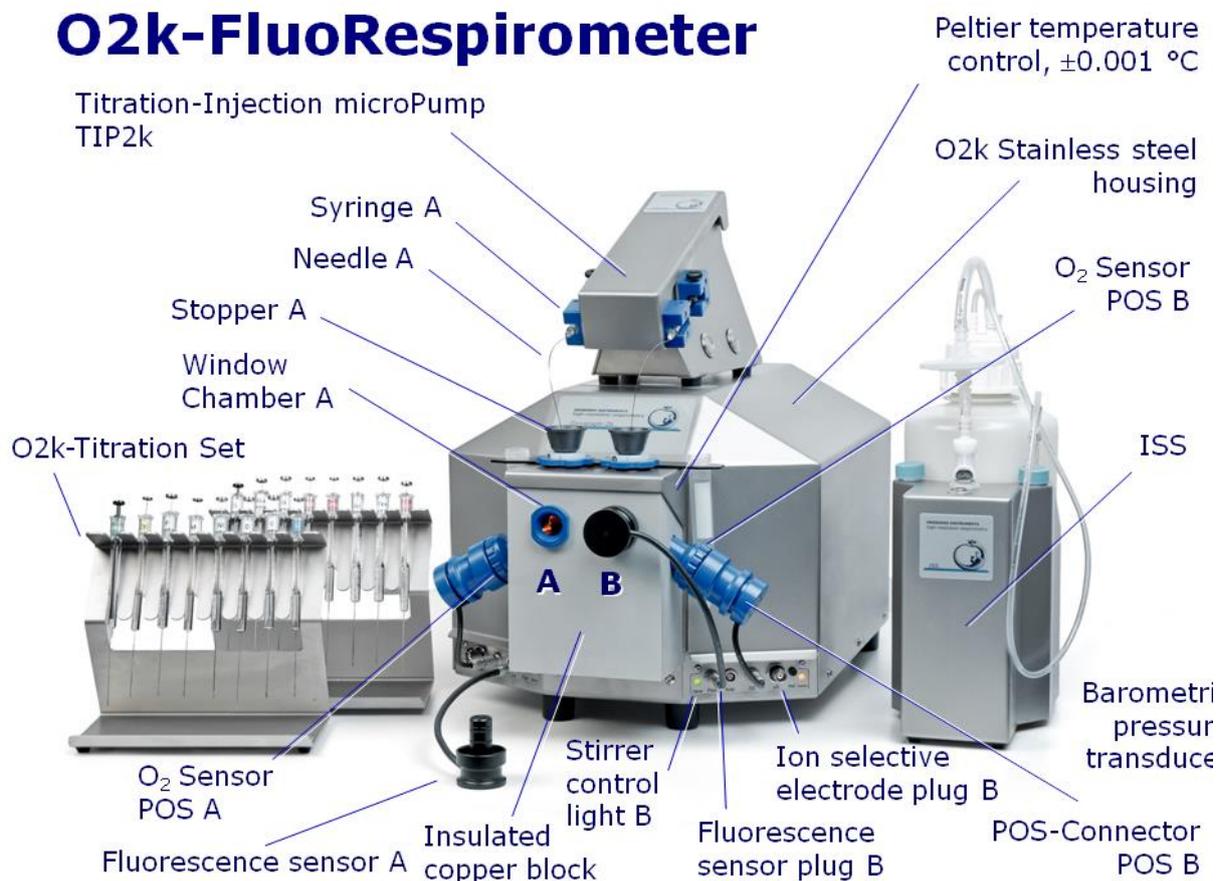
Simultaneous monitoring of oxygen consumption and fluorescence with small amounts of samples (isolated mitochondria to permeabilized tissues and cells). **Included:** O2k-Titration Set, Titration-Injection microPump (TIP2k), Integrated Suction System (ISS). **Add-on O2k-Modules:** high flexibility to expand the spectrum in HRR.

HRR with the Oroboros O2k-FluoRespirometer is based on a radically different systems approach to polarographic measurement of oxygen dynamics. HRR rests on tightly connected instrumental features and concepts integrated by the DatLab 7 software, providing real-time display of oxygen concentration and respiration (oxygen flux), and a fluorometric signal and its time derivative (slope). The mechanics, critical selection of materials, and state-of-the-art electronics provide the basis for signal stability of an optimized polarographic oxygen sensor ([OroboPOS](#)) and [Smart Fluo-Sensors](#) in an electronically controlled thermal environment with high temperature stability. Calibration and quality control follow standardized operating procedures ([O2k-SOP](#)) with automatic real-time analysis supported by DatLab. Scientific support complements HRR. Detailed laboratory [O2k-Procedures](#) provide step-by-step instructions, integrated as DL-Protocols into DatLab 7. Numerous new [O2k-Publications](#) on applications of the Oroboros O2k focus on critical issues of mitochondrial physiology.



While the Oroboros O2k-FluoRespirometer provides the instrumental basis for high-resolution respirometry, successful operation at high accuracy up to the limit of detection depends on a professional application by the technician, scientist or student. Participation in virtual [O2k-Workshop](#) is recommended to save time and gain from the experience obtained over many years of application in various settings.

## O2k-FluoRespirometer



The [O2k-FluoRespirometer](#) is the complete system for high-resolution respirometry (HRR), including the [O2k-Main Unit](#) with stainless steel housing, 2 PVDF Stirrer-Bars, 2 PEEK O2k-Stoppers, 2 OroboPOS-Connectors, [2+1 OroboPOS](#) (1 spare oxygen sensor), [OroboPOS-Service Kit](#), [4 Smart Fluo-Sensors](#), [DatLab](#) software ([DatLab](#)), [ISS-Integrated Suction System](#), Titration-Injection microPump ([TIP2k](#)) and [O2k-Titration Set](#).



### O2k-Manual user information

» [MiPNet07.08 User information](#)

» [O2k-Manual](#)

## 1. Unpack the O2k-FluoRespirometer

[Box 1](#) (or [Peli Case](#)) contains the O2k-Main Unit, the [OroboPOS-Connectors](#) without polarographic oxygen sensors (POS) and the TIP2k syringes. After unpacking, **store the original Box 1 (or Peli Case) and packing material for any future shipment of the O2k-Main Unit.**

[Box 2](#) contains standard accessories [SA], the Oroboros USB-flash drive and any additional accessories.

## 2. Oroboros USB-flash drive and DatLab

A laptop or PC is required for data acquisition and analysis using DatLab. Minimum configuration: Intel-Core-2 or equivalent CPU, 2GB RAM, USB port, Windows XP or later Windows versions. Recommended: Intel i5 or equivalent CPU, 4GB RAM, Windows 10, SSD. For the proper display of DatLab on your computer, please make sure the "Language settings" are set to English.



Copy the folder "**Oroboros O2k-Course on HRR**" from the **Oroboros USB-flash drive** to your computer. The 'Oroboros-FileFinder' is the tool to find topics and open the files. Updates of the [O2k-Manual](#) and [O2k-Procedures](#) are posted on our website.

### DL7Install.exe



It is recommended that you exit all computer applications before running the DatLab installation programme. In the 'Oroboros-FileFinder', left click the Oroboros symbol or 'O2k-DatLab install'. Read the short instruction windows and proceed by left click on **Next**. By default, DatLab is installed to "C:\DatLab".



Installation is quick and easy and generates this icon on your desktop: **Oroboros DatLab** for control of the O2k, TIP2k, data acquisition & analysis.

### Running DL7



For data safety reasons and optimal DatLab performance, it is strongly recommend to operate your DatLab files, DL-Protocols and DatLab templates on the PC's local hard drive (HDD).

**Make sure the 'Sleep' mode of your computer is disabled during data acquisition to avoid errors of data transmission.**

**MitoPedia: O2k-Open-Support** is integral to the O2k-Manual, where specifically advanced sections on high-resolution respirometry are included:



- » [MitoPedia: O2k-Respirometry](#)
- » [MitoPedia: O2k-FluoRespirometry](#)
- » [MitoPedia: O2k hardware](#)
- » [MitoPedia: DatLab](#)
- » [MitoPedia: SUIT](#)

## 3. Electric and lab space requirements

Max. power input: 120 W

Current at 100-120 V: max. 1.0 A

Current at 220-240 V: max. 0.5 A

Power consumption during standard operation (when block temperature is stable at 37 °C and ambient temperature of 22 °C): approx. 24 W



Check the suitability of the delivered cable for the mains power (230/120 V) and socket. The O2k is delivered with one of three different cable types:

**EU:** O2k-Main Power Cable/230 V/Europe, 21111-01

Note: Use O2k-Main Power Cable/230 V/Europe (Hybrid German/French plug CEE7/7, Type E+F) only for a compatible socket.

**DK:** Some Danish sockets will also accept the CEE7/7 plugs, but there is no grounding connection with these plugs without male ground pin. Exchange for an appropriate Danish power cable.

- CH: A different main power cable or adapter is required.  
 US: O2k-Main Power Cable/120 V/US-CA, 21112-01  
 AU/NZ: O2k-Main Power Cable/230 V/AU-NZ, 21113-01

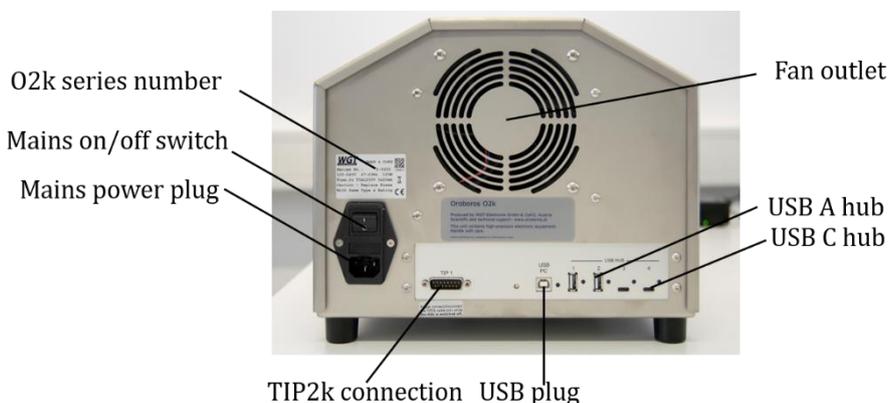


The O2k requires a minimum of lab space (0.5 m bench space) next to an external PC or laptop, with sufficient space for the ISS, O2k-Titration Set and handling. For maximum signal stability and optimal temperature control, the O2k should be placed in a position in which it is not exposed to direct sunlight and sudden changes of room temperature. Keep the space on the table below the O2k bottom clear to

prevent any interference of ventilation beneath the O2k-Main Unit.



Avoid damage due to condensation. When moving the O2k from a cold to warm environment, let it equilibrate for at least 4 h in the warm room before connecting to the mains.



**The O2k series number, Mains on/off switch, mains power plug, TIP2k connection, two USB A hubs (5 V, max. 0.5 A) and two USB C hubs (5 V, max. 1.5 A) are on the rear of the O2k-FluoRespirometer (Series I).** For previous series please see previous versions of the Manual ([MiPNet22.11 O2k-FluoRespirometer manual](#)).

**WGT quality control seal:** Do not open. An intact quality control seal is required for warranty obligations related to the electronics of the O2k.

## 4. Start the O2k

Installation:

1. Switch on the laptop or PC and wait for it to finish starting up
2. Install DatLab.
3. Connect the O2k to the electricity supply
4. Connect the O2k with the USB cable to the laptop or PC.
5. Switch on the O2k at the rear (Mains on/off switch: I/O). Now the four front control lights illuminate: The outer left diode (**MAINS**) is green, and temperature is regulated at 25 °C. The inner left and right diodes (**STIRRER**) are green and stirrer rotation is on. The outer right diode (**COMM**) changes to yellow if a USB connection is available
6. Start DatLab. The outer right diode (**COMM**) changes to green

Daily use:

1. Switch on the laptop or PC and wait for it to finish starting up
2. Switch on the O2k at the rear (Mains on/off switch: I/O).
3. Start DatLab.

In case of any problems (with the connection or temperature control) switch off the O2k and wait ~ 8-10 s before switching on the instrument again.

## 5. Assembly of the O2k-FluoRespirometer



Dispel yourself of static charge to avoid possible damage of the electronics. Shortly connect to electrical ground or simply touch the O2k steel housing. For details, see: [MiPNet14.01 ESD-damage](#)

### 5.1. Instrument overview

The O2k-FluoRespirometer is electronically equipped with all required plugs for sensors: in addition to the **O2** channel, the **Amp** channel is available with separate **Fluo** plugs in O2k-Series H-I. The **pX** channel has a separate **Ref** plug for the reference electrode.

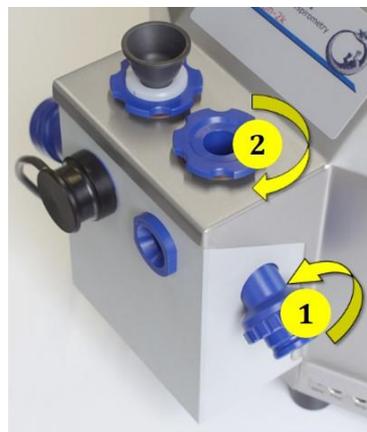
### 5.2. O2k-chamber assembly

Two 2-mL [O2k-Chambers](#) are supplied in the O2k-Service box with blue chamber- and POS holders (mounted on the O2k-Main Unit). Two 0.5-mL O2k-Chambers with black chamber- and POS holders are in the [O2k-sV-Module](#) box.



[Clip A2](#)

1. Insert the glass chamber into the copper block of the O2k-FluoRespirometer and add the [PVDF stirrer bar](#). Please keep the stirrer away from magnetic fields.
2. Screw the [POS holder](#) (1) into a position where the glass chamber with the angular cut towards the POS holder is slightly lifted upwards.
3. Screw the [chamber holder](#) loosely to the copper block ([arrow 2](#)). Loosen the POS holder stepwise (counterclockwise, slow successive quarter turns; [arrow 1](#)) and follow any downwards movement of the glass chamber by screwing down the chamber holder ([arrow 2](#)). When a slight backwards movement of the POS holder (1) cannot be followed by any further downwards movement of the chamber holder (2), then screw the POS holder (1) clockwise (tight), thus securing the glass chamber in a fixed position.



### 5.3. POS assembly

The POS are shipped dry without electrolyte, in a Perspex housing, in the O2k-Service box. Before assembling the O2k, apply electrolyte and membrane, following the OroboPOS service instructions.



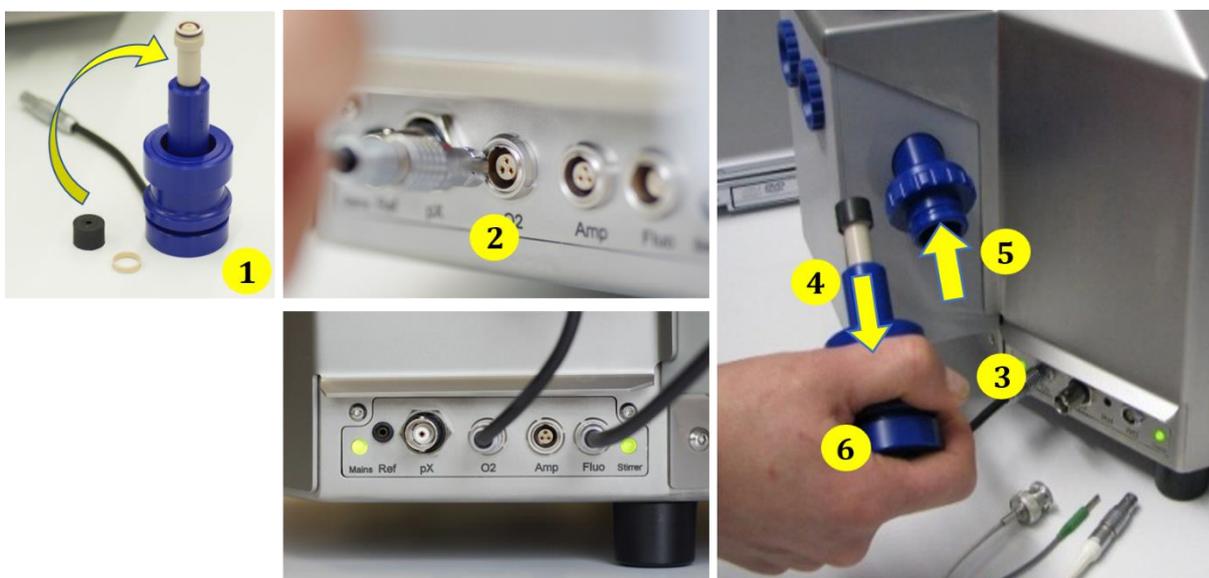
**Go to: POS assembly**  
» [MiPNet19.18B POS-service](#)



[Clip A3](#)

1. Screw a POS head onto each [POS connector](#) (finger-tight). Push a *wetted* POS seal tip (black gasket) over the POS head (arrow 1). Position the pore centrally and do not stretch the gasket. Each POS head can be used on either O2k-chamber A or B. Note the POS number (marked on the cylindrical body of each POS) for each chamber (see Section 6).
2. Connect the POS connector cable to the O2k-Main Unit. Insert the male plug of the cable (2) into the female O2 plug. The red dot on the male plug has to accurately face straight upwards when inserting the plug.
3. After inserting the plug of the POS connector cable to the O2k-Main Unit, the POS connector cable (3) must be in a relaxed, undistorted position. Press the sleeve downwards (arrow 4) insert the POS with the black gasket straight into the POS holder (5) and release the sleeve for fixing the POS in its final position.

**!** Do not rotate the POS connector (6) after it is attached to the POS holder in order to prevent damage of the black gaskets. The gaskets provide a tight seal against the glass chamber that has sharp edges. After sensor service, the POS needs some time to stabilize while the O2k is switched on, from a few hours up to a run overnight.

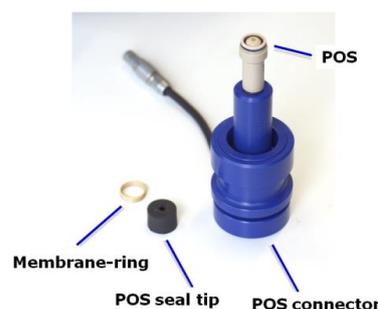


#### 5.4. O2k-chamber disassembly

1. Remove the POS connectors from the POS holders by pressing the sleeve downwards against the bottom plate of the POS connector. Unplug the cable.
2. Unscrew the blue chamber holders and the POS holder from the O2k housing.
3. Carefully insert your finger (with gloves on) into the chamber and then gently take out the glass chamber from the copper block of the O2k-FluoRespirometer.

#### 5.5. Checking the O2k-chamber and POS assembly

The O2k assembly can be tested during the Instrumental O<sub>2</sub> background test, which requires full attention during performance. An overnight test can be done to rule out media leakage out of the O2k-chambers or gas getting into the O2k-chambers. To test if the assembly is properly done, follow the steps:



1. Add MiR05 (or H<sub>2</sub>O) to the O2k-chamber and close it by gently pushing the stopper downwards. Siphon off excess medium from the top of the stopper using the [ISS](#). Check that any air bubbles are removed from the chamber.
2. Decrease the oxygen concentration down to ~ 50 μM (using [Dithionite](#) or N<sub>2</sub> injection); see: [Setting the oxygen concentration](#). When using H<sub>2</sub>O do not use dithionite!
3. Let the O2k recording overnight with the [chamber closed](#) and illumination switched off, at 25 °C.
4. The oxygen concentration should decrease over time due to the O<sub>2</sub> consumed by [OroboPOS](#). In this case, the chamber is assembled properly.
5. If the oxygen concentration increases over time and/or air bubbles are observed in the chamber, a reassembly of the chamber is needed.

### 5.6. O2k-chamber volume calibration

DatLab must be installed before calibrating the chamber volume. Connect the O2k to the electricity supply (Section 4), connect the O2k with DatLab (Section 6), and make yourself familiar with the O2k control keys (stirrer; Section 7.1). The standard chamber volume is 2.0 mL (0.5 mL with the [O2k-sV-Module](#); corresponding values below in parentheses).



1. Dry the chamber. Remove all liquid droplets from the glass chamber. Place a dry PVDF stirrer bar into each chamber.
2. Add accurately 2.07 mL (0.54 mL) of H<sub>2</sub>O into the chamber for calibration of a chamber volume of 2.0 mL (0.5 mL).  
The volume of the capillary of the stopper (black [PEEK](#)) is not part of the effective chamber volume. (The volume of the stopper capillary is [0.065 mL or 65 μL \(0.04 mL or 40 μL\)](#) when a tiny meniscus of water is seen on top of the capillary.) When titrating 2.07 mL (0.54 mL), insert the tip of the pipette to the wall of the glass chamber to add the entire volume. Liquid must not be lost between the top of the glass cylinder and the blue POM chamber holder.
3. Switch on the stirrers (Section 7.1).
4. Loosen the [Volume-Calibration Rings](#) (A) and (B) with the screwdriver allen wrench (OroboPOS-Service Kit). Push them slightly downwards in the direction of the O-rings.
5. Insert the stopper with dry capillary (the O-rings should be moistened) and push it downwards until the gas phase fully extrudes through the capillary of the stopper, while the fixation ring is fully pushed onto the chamber holder.
6. Gently push the stopper further downwards until the capillary fills up and a small droplet appears on the top of the capillary. This marks the volume calibration position.
7. Tighten the screws of the calibration rings (A) and (B) gently.



### 5.7. Smart Fluo-Sensor assembly



The [Smart Fluo-Sensors](#) are shipped in a separate box as part of the O2k-FluoRespirometer. Smart Fluo-Sensors are precalibrated with sensor-specific memory and direct input into DatLab 7.4.

**⚠ The Smart Fluo-Sensors cannot be used with O2k-Series up to Series G.**

1. Connect the [Smart Fluo-Sensor](#) cable to the **Fluo** plug. Similar to the POS (2), insert the male plug of the cable into the female **Fluo** plug. The red dot on the male plug faces straight upwards. Each Smart Fluo-Sensor can be used on either O2k-chamber A or B.
2. The blue frame of the chamber window and the Smart Fluo-Sensor are specially designed to only connect at a specific orientation, when the unbowed notches align (7). In this optimal position, the Smart Fluo-Sensor is carefully inserted in the window opening until the sensor covers the chamber window without gap (8a,b). In this position, the cable routing is horizontal (9).

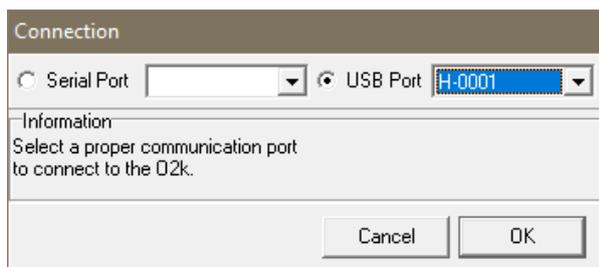


## 6. Start DatLab 7

On your PC desktop, left click on the icon “Oroboros DatLab” to start the programme. Enter or ▼ select a username. Press F7 to open the **Connection** window. Directly after set-up, select a **USB** port in the **Connection** window (ensure that the O2k is connected to the PC via the USB cable). If several O2ks are connected to one PC, the serial numbers of all O2ks are shown, ▼ select one. Press **Cancel** for disconnected operation of DatLab.

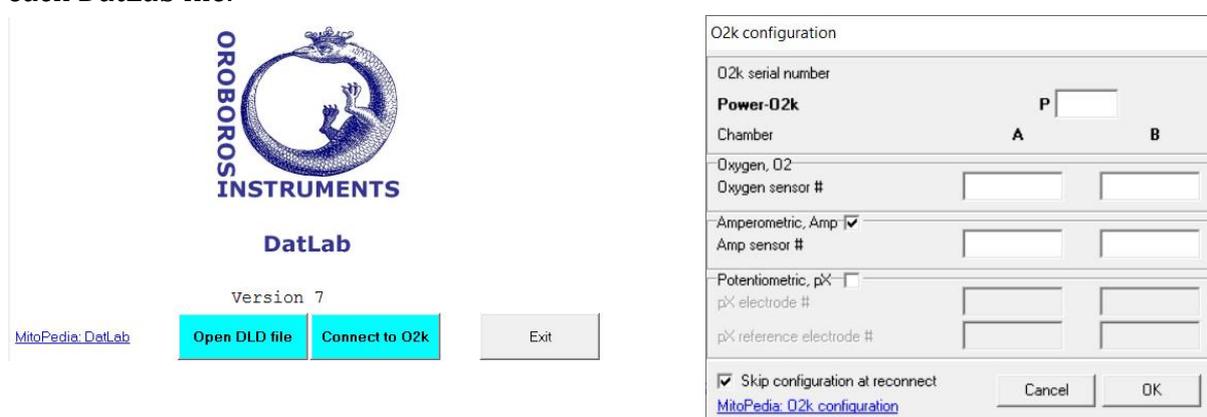


[Clip A5](#)



### 6.1. Oroboros O2k \ O2k configuration

Click **Connect to O2k** to open the O2k configuration window. In the configuration window, you can assign a label (**Power-O2k** or **P-number**) to your O2k. This is especially recommended if you have more than one O2k. The P-number is automatically saved in each DatLab file.



In the window you can further configure the oxygen, fluorescence, and potentiometric channels.

**Oxygen, O2** is the label for the [oxygen channel](#), which is always active and cannot be deselected. Enter the **Oxygen sensor #**, which can be found on the side of the POS, for chambers A and B. Usually, sensors should not be switched between chambers, except for troubleshooting. The individual sensor number is included in the protocol for generating a database of calibration values ([MiPNet06.03](#)).



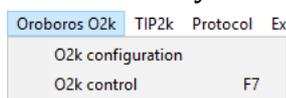
**Amperometric, Amp** is the label for the [fluorescence channel](#) (or an [Amp signal](#)). The channel must be selected  for data recording.

The **Smart Fluo-Sensor #** and LED excitation can be found on the inside of the sensor. The signal of the Smart Fluo-Sensor is recorded in the “**Amperometric, Amp**” channel. For H-series and higher, the serial number is **automatically detected** and set in “**Amp sensor #**” if the Smart Fluo-Sensor is connected to the O2k.



» [wiki.oroboros.at/index.php/O2k\\_configuration](http://wiki.oroboros.at/index.php/O2k_configuration)

**Potentiometric, pX** is the label for a [potentiometric channel](#) which is used for the ion selective electrodes, the NO and the H<sub>2</sub>S sensitive electrode. The channel must be selected  for data recording. The serial # of the electrodes must be entered manually as they are **not automatically detected**.



The O2k configuration window can also be opened during a measurement or in an open file by clicking in the menu bar “Oroboros O2k”.

## 6.2. Oroboros O2k \ O2k control \ Tab: System

Open the **O2k control** \ **System** tab by clicking in the menu bar “Oroboros O2k” or pressing **F7** to set the operation mode of the O2k.



**Load setup** ▼ is an alternative to editing settings step-by-step. The default is **O2k-setup** **25 °C** or the O2k-setup which was last applied when DatLab was connected to the O2k. O2k control settings of the selected setup are displayed after left click on the **Load setup** button.

**Save setup:** Save the O2k control settings. To create a new O2k-setup, edit the name, and left click on **Save setup**.

**Block temperature [°C]:** Left click on the input window (left double click to overwrite all digits). Enter the block (=experimental) temperature (range 4.0 to 47.0 °C at room temperature; 2 °C at lower ambient temperature).

**Stirrer speed [rpm]:** The default is 750 rotations per minute (12.5 Hz), which may be varied between 100 and 900 rpm for each chamber independently. The signal level and signal stability increase with stirrer speed in the region of low rpm but uncoupling of the magnet occurs at very high stirring speeds causing high signal noise.

**Data recording interval [s]** can be set between 0.2 s and 10 s, identical for both chambers. The default of 2 s is optimal for most applications. Do not decrease the data recording interval unless necessary, since at low data recording intervals, the apparent noise of flux is increased, and real-time information is reduced.

**Stirrer power**  **On/Off:** Toggle between On/Off to set the stirrer on or off when connecting. Independent of this On/Off setting, the left and right stirrers are switched on and off during the experiment by pressing **F11** (left) and **F12** (right).

**Illumination in chamber**  **On/Off:** Toggle between On/Off to switch the illumination on or off when connecting. It is switched on and off during the experiment by pressing **F10**. Illumination is different from the light intensity of the Smart Fluo-Sensors.

**Connect to O2k** or **Send to O2k** to activate the displayed control settings.

## 6.3. Oroboros O2k \ O2k control \ Tab: Oxygen, O2

**Gain for sensor:** The default is a gain of 1 V/μA. This is sufficient in most cases and provides the full scope for measurements at all oxygen levels.

▼ Amplification options are 1, 2, 4, or 8 V/μA, for signal amplification in specific cases (previous O2k-Series).

**Polarization voltage [mV]:** The default for the POS is 800 mV, which is maintained in all routine experiments. Any changes affect the calibration. The voltage can be modified in the range of – 2,000 to + 2,000 mV for scanning the plateau region of the polarographic oxygen sensor, or for different types of sensors.

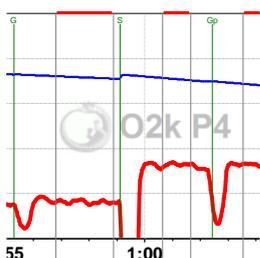
**Oxygen sensor #:** The number of the polarographic oxygen sensor is shown here for chambers A and B. To change them, left click on **Configuration** and enter the sensor number.

## 6.4. Oroboros O2k \ O2k control \ Tab: Amperometric and Potentiometric

**Amp and pX** Settings for the Amp and pX tabs are described in:

- » [MiPNet15.05 O2k-NO Amp-Module](#)
- » [MiPNet15.03 O2k-MultiSensor-ISE](#)

6.5. Connect and save a DLD file



In an automatically opened **Save as** window, the default file name has the form: **2020-10-17 P1-01.DLD**

**Year-Month-Day P#-##.DLD:** P#=**P**ower-O2k; ## =sequential number of the experiment; extension **DLD** for **DatLab Data**.

Select a subdirectory, optionally edit the file name and left click on **Save**. This initiates data recording.

**COMM LED of the O2k-Main Unit**

- **Yellow** USB connection is available, but DatLab connection (measurement) is not started.
- **Green** USB connection to a PC is established and a Datlab measurement is in progress.
- **Red flashing (1 s on/1 s off):** USB connection was lost during a Datlab measurement (USB communication error or USB unplugged). If Datlab reconnects automatically, the COMM LED remains green without flashing.

6.6. Experiment

After connecting to the O2k the **Edit experiment** window pops up. Edit experimental code, sample information, medium, and additional information. Entries can be edited at any time during real-time or post-experiment analysis. All related results are recalculated instantaneously with the new parameters.

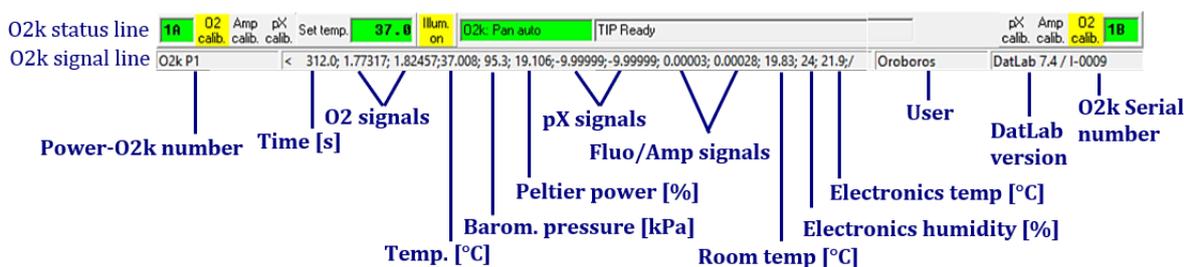
6.7. O2k-signal line

**Time [s]:** Time of the experiment since connecting, displayed on the X-axis as [h:min] or [h:min:s].

**O2(A) [V] and O2(B) [V]:** Oxygen raw signal of chamber (A) and (B), which is converted to oxygen concentration by calibration, as displayed in the plot O2 concentration.



If the oxygen signal displays 9.99 V, the amplifier has reached saturation. If the signal remains at 9.99 V, press **F7** and set the gain to 1. Left click on **Send** to activate the gain setting and to obtain a signal <10 V.



**Temp. [°C]:** Block temperature, continuously recorded.

**Barom. pressure [kPa]:** Barometric pressure, continuously recorded.

**Peltier power [%]:** Operative Peltier power applied for temperature regulation of the O2k-block, continuously recorded.

**Amp (A) and Amp (B) [V]:** Amperometric signal from amperometric sensors (Fluo, NO or other) after current [Amp] to voltage [V] conversion, chamber (A) and (B).

**pX(A) and pX(B) [V]:** Potentiometric raw signal of ion sensitive electrodes for chamber (A) and (B).

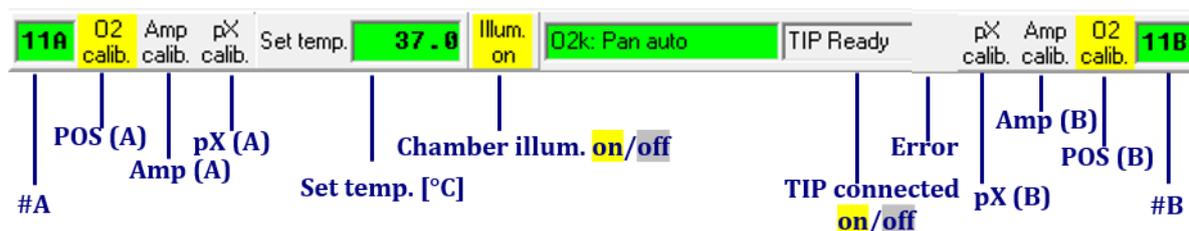
**Room Temp [°C]:** Signal from internal temperature sensor recording the room (environmental) temperature.

**Electronics Humidity [%]:** Signal from internal sensor recording the electronics humidity.

**Electronics Temp [°C]:** Signal from internal temperature sensor recording the electronics temperature.

**User:** The user name which was entered in the first window is shown on the right side of the O2k-signal line (next to the DatLab version).

### 6.8. O2k-status line



**#A and #B:** The labels of the left and right chamber, where # represents the Power-O2k number (P#), chosen in the [O2k configuration](#) window. The background is **green** if rotation of the stirrer is on, briefly **yellow** while the stirrer is accelerating, **red** if the stirrer is switched off.

**O2 calib.** Yellow indicates that the POS has not been calibrated in the present record. Calibration values are used as default from files previously saved while DatLab was connected. Left double-click on **O2 calib.** to open the calibration window (left or right).

**O2 calib.** Green indicates that the POS has been calibrated within the file. It can be recalibrated any time when connected or disconnected to the O2k.

**Set temp.** **37.0** indicates the temperature setting sent from the O2k control window, **green** if the measured temperature is within  $\pm 0.002$  °C of the set temperature, but **yellow** if the difference is more than  $\pm 0.002$  °C.

**Illum. on / Illum. off** indicates whether the chamber illumination (not to be confused with light sources for fluorescence) is on (**Illum.on**, highlighted in yellow) or off (**Illum.off**, grey). Toggle by a left double-click. When the windows to the glass chambers are closed with optical sensors, the illumination is not visible and must be switched off.

**O2k: Pan auto** indicates that automatic panning is on (**green**) with the O2k connected to DatLab. The time range is maintained while the time axis always shows the currently recorded data. The value of the offset (minimum time) increases stepwise as experimental time proceeds.

**O2k: Pan off:** **Yellow** indicates that automatic panning is off. Plots are shown in static display. This allows for manually panning backwards to observe previous sections of the experiment at a given time range. In this mode, the actual experimental time may be off-scale. Toggle between **Pan auto** and **Pan off** by a left click on the text. This does not influence continuous data recording.

**TIP: Ready** indicates that a Titration-Injection microPump (TIP2k) is connected to the O2k and is ready to start [F8](#). For TIP2k instructions see [MiPNet12.10](#).

**ERROR:** Blinks red if an error has occurred. In this case, left click on ERROR to open the Error message window. In this window, left click on [Close](#) to remove the blinking error signal.

**Warning:** Blinks red if a limit was set for the oxygen concentration and the oxygen concentration in the O2k-chamber reaches a value below that limit or if there is a problem with the USB connection.

## 7. O2k control

### 7.1. O2k control keys

After selection of an O2k setup in the **O2k control** **F7** window, followed by left click on **Send to O2k**, only the following control functions are routinely required during experimental operations.

**Stirrer A on/off** **F11** toggles between stirrer on/off in the left O2k-chamber, returning to the pre-set stirrer speed.

**Stirrer B on/off** **F12** as for stirrer A (see above).

**Stirrer test** **F9**: Start a stirrer test by left click on **OK**: stirring is stopped in both O2k-chambers and restarted after a selected period (default: 30 s). »[MiPNet06.03](#)

**Illumination on/off** **F10** switches the illumination in both chambers simultaneously on or off.

**i** **Automatic events** are set on the plots and saved.

**Manage setups** for renaming or deleting O2k-setups.

### 7.2. O2k control **F7** with the O2k connected

The O2k control key **F7** provides fast access to the **O2k control** window.

**Load setup:** After selecting ▼ a setup, it must be loaded.

**Send to O2k:** Individual control settings may be edited before left click on **Send to O2k**. All changes of settings are saved in the protocol. An event is set automatically, and the O2k-status is changed to the new settings.

### 7.3. File \ Save and disconnect

To stop recording data, go to the menu **File**, select **Save and disconnect**. Confirm **Save and disconnect** (all data are saved automatically), thus terminating further data acquisition. **Close** the file or edit the same file in the disconnected mode.

Upon disconnect, the **COMM** light on the O2k switches from green to yellow. The **O2k control** window shows the last values used during operation before disconnecting.

Importantly, after disconnection from DatLab the O2k continues to operate at constant experimental temperature, stirring speed and light in the chamber. No data are recorded until reconnection with DatLab. After closing the file, the **O2k control** window opens automatically or can be opened by pressing **F7**. Check the O2k control settings and left click on **Connect to O2k**. Again, a default file name is given, as described above, with the next sequential number. After re-connecting, the O2k continues operation uninterrupted, the experimental time is reset to zero, and data recording is restarted with automatic saving to file.

After disconnecting the communication between the O2k and DatLab, the O2k can be switched off on the rear of the O2k-Main Unit.

## 8. O2k calibration, DL-Protocols and analysis

### 8.1. Calibration



**Instrumental DL-Protocols** provide real-time guides to calibrations.

**O2** » [O<sub>2</sub> calibration - DatLab](#)

**Amp** » [Amp calibration - DatLab](#)

**pX** » [PX calibration - DatLab](#)

### 8.2. Performing an experiment with DL-Protocols

Protocols	Experiment	Calibration
	A: Run DL-Protocol / Set O2 limit	
	B: Run DL-Protocol / Set O2 limit	

Run a DL-Protocol for O2k-chamber A and B.

In the same window, the **Lower O2 limit [μM]** can be defined, with or without using a DL-Protocol.

The sequence of events and marks of the selected DL-Protocol is shown in the DL-Protocol window. During an experiment, events are set by clicking on the next event shown in yellow. Mark information is added automatically to marks set after the corresponding event. Events and marks are highlighted in green when properly set according to the DL-Protocol. An erroneous sequence of events or marks is indicated by highlighting the respective events and marks in red.

A set of current DL-Protocols can be found on the **Oroboros USB-flash drive** (...\\Oroboros O2k-Course on HRR\\O2k\\DL-Protocols) and can be accessed via the 'Oroboros-FileFinder'. The section 'O2k Manual' lists Instrumental DL-Protocols; the section 'O2k Protocols' lists SUIT DL-Protocols).

Further details:

» [Run DL-Protocol/Set O2 limit](#)

» [Library of substrate-uncoupler-inhibitor-titration \(SUIT\) protocols](#)

Use the [SUITbrowser](#) to find the best [SUIT](#) protocol for your research questions.

Further details:

» [SUITbrowser](#)

### 8.3. Flux analysis

» [Flux / Slope](#).



Further information and updated versions:

» [MiPNet22.11 O2k-FluoRespirometer manual](#)

Links to O2k-catalogue and support

» [O2k-FluoRespirometer](#)

» [O2k-Open Support](#)

## 9. O2k storage

After use, clean the chambers ([MiPNet19.03](#)) and switch off the O2k. Note that leaving the main unit switched on over long periods of time shortens the lifespan of the OroboPOS (and is an unnecessary waste of electricity). If the O2k-chambers are filled with 70% EtOH, you can store the O2k for several months, with the OroboPOS in place.

Further information on cleaning and storage:

» [MiPNet19.03 O2k-cleaning and ISS](#)

## 10. Author contributions

Gnaiger E is responsible for the project and instrumental development and preparation of the MiPNet. Komlodi T, Schmitt S, and Cecatto C contributed to the preparation of the MiPNet. Gradl L developed the DatLab software versions 3 to 7 and contributed to the concept of quality control in DatLab 7. Gradl P is responsible for the logistics of electronic and mechanical development of the O2k.

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