O2k-FluoRespirometer manual

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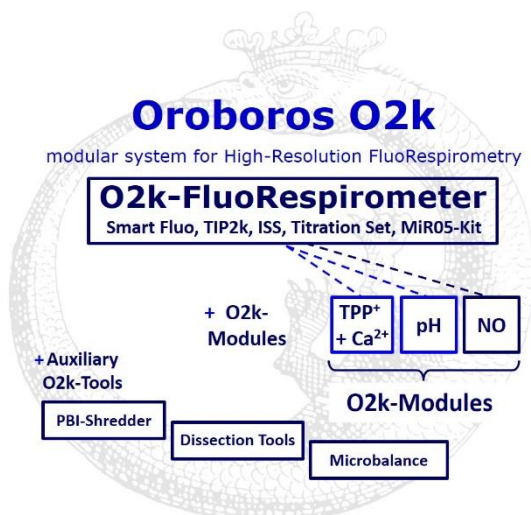
O2k-FluoRespirometer

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 low respiratory activities, kinetic transitions, and controlled oxygen levels. High-resolution is required for analysis of pathological effects causing reduced respiration (mitochondrial and metabolic diseases, ischemia-reperfusion injury, oxidative stress, apoptosis, aging). HRR is unique for simultaneous measurement of respiration and fluorometric signals (H_2O_2 flux, mt-membrane potential, 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 activities; mutants with diminished respiratory capacity; chemical oxidation rates and antioxidant capacities and oxygen kinetic measurements at low, physiological intracellular oxygen levels.

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 of 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 a hands-on introductory **O2k-Workshop** is recommended to save time and gain from the experience obtained over many years of application in various settings.

⮫Clip A

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.
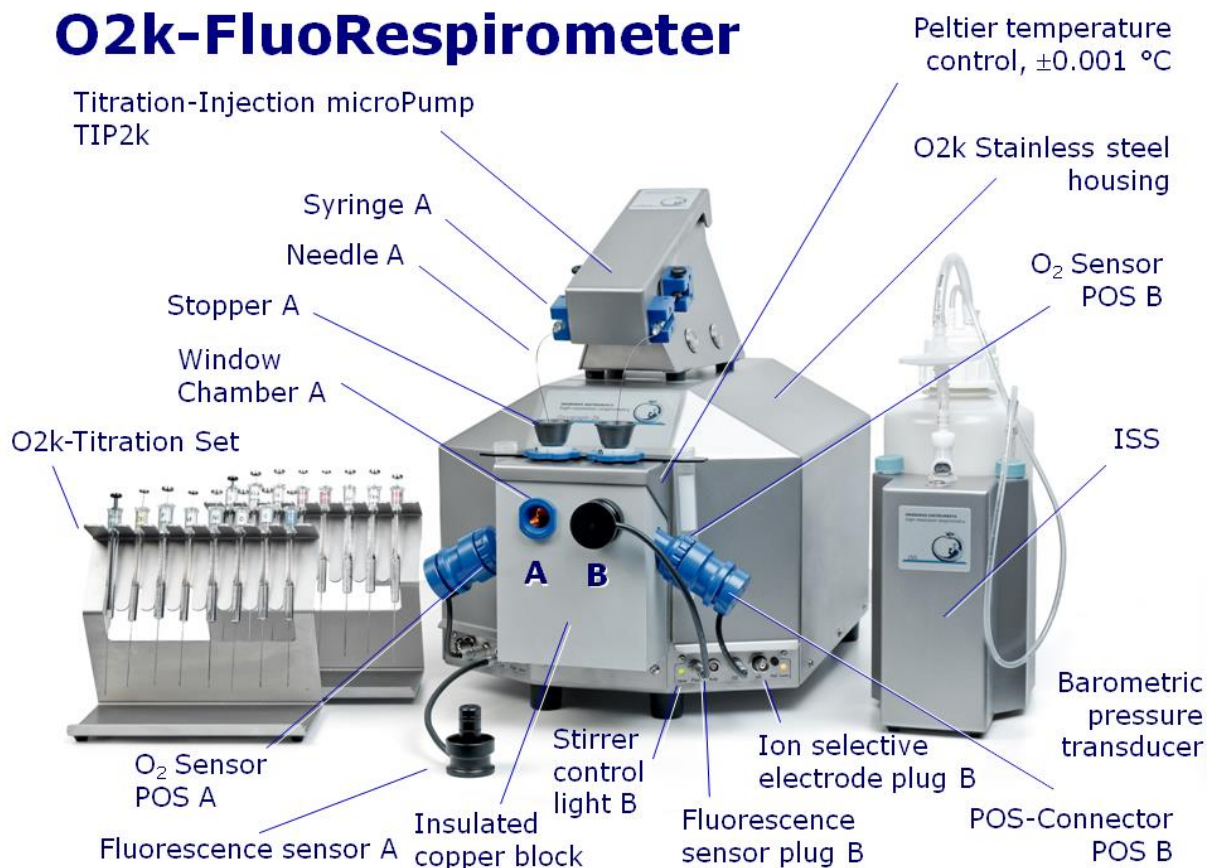
O2k-Manual user information

PLEASE STUDY THIS MANUAL

» wiki.oroboros.at/index.php/MiPNet07.08_User_information

» wiki.oroboros.at/index.php/O2k-Manual

O2k-FluoRespirometer

Titration-Injection microPump TIP2k
Peltier temperature control, ±0.001 °C
Syringe A
Needle A
Stopper A
Window Chamber A
O2k-Titration Set
O2k Stainless steel housing
O₂ Sensor POS B
ISS
A B
O₂ Sensor POS A
Fluorescence sensor A
Stirrer control light B
Insulated copper block
Ion selective electrode plug B
Fluorescence sensor plug B
Barometric pressure transducer
POS-Connector POS B

The **O2k-FluoRespirometer** **is** **the** **complete** **system** **for** **high-resolution respirometry (HRR),** including the O2k-Main Unit with stainless steel housing, O2k-Assembly Kit, 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.

1. Unpack the O2k-FluoRespirometer

Box 1   (or Peli Case) contains the O2k-Main Unit with the O2k-Chambers in a finally mounted position, the OroboPOS-Connectors without polarographic oxygen sensors (OroboPOS) 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 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 7, SSD.

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-Protocols 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', L⊕ click the Oroboros symbol or 'O2k-DatLab install'. Read the short instruction windows, and proceed by L⊕ 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.

⟲Clip A1

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

» wiki.oroboros.at/index.php/MitoPedia:_O2k_hardware

» wiki.oroboros.at/index.php/MitoPedia:_DatLab

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 consumtion 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 placing 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.

Rear of the O2k-FluoRespirometer.
New features are the USB hub 2.0 with 4 external ports, a plug for an external Pt1000 thermistor, and an optional extension through an RS485 serial port.

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

Mains on   Connect the O2k with the USB cable to a laptop or PC. Switch on the O2k on the rear (Mains on/off switch: I/0). 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, and to green after operational connection to DatLab.

5. Assembly of the O2k-FluoRespirometer

☞ Uncharge yourself to avoid possible damage of the electronics. Shortly connect to electrical ground or simply touch the O2k steel housing.

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 plug in O2k-Series H. The pX channel has a separate Ref plug for the reference electrode.
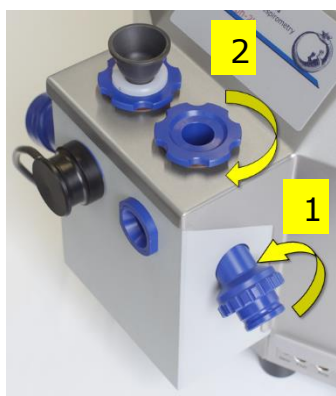
5.2. O2k-Chamber assembly

1. Insert the glass chamber into the copper block of the O2k-FluoRespirometer and add the PVDF stirrer bar.

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.

⟳ClipA2

3. Screw the chamber holder loosely to the copper block (arrow 2). Loosen the POS holder stepwise (counterclockwise, not more than 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. O2k OroboPOS assembly

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

Go to: OroboPOS assembly
» MiPNet19.18B_POS-service

Membrane-ring
POS seal tip
POS connector

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 OroboPOS head can be used on O2k-Chamber A or B. Note the POS number (marked on the cylindrical body of each POS) for each chamber (see Section 6).
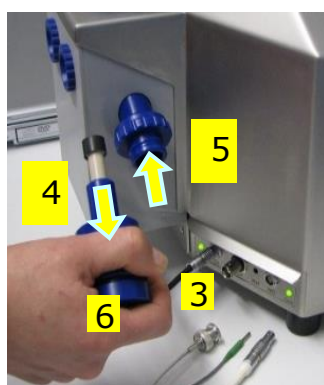
2. Connect the POS connector cable to the O2k-Main Unit. Insert the male plug of the cable 2 into the female O2 plug. The red dot on the male plug 2 has to face accurately straight upwards when inserting the plug.

O2k-Series G (upper) and H (lower).
» MiPNet19.18 (O2k-Series G) and MiPNet19.01 (up to O2k-Series F)

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.

↪Clip A3

☞**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 volume calibration

O2k-SOP: DatLab must be installed before calibrating the chamber volume. Connect the O2k to the mains (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.

1. Dry the chamber. Remove all liquid droplets from the glass chamber. Place a dry PVDF stirrer bar into each chamber.

2. Add accurately 2.1 ml of H_2O into the chamber for calibration of a chamber volume of 2.0 ml (more accurately, the volume of the stopper capillary is 0.085 ml or 85 µl when a tiny meniscus of water is seen on top of the capillary).

The volume of the capillary of the stopper (black PEEK) is not part of the effective chamber volume. When titrating 2.1 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 the stirrers on (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 of the stopper. This marks the volume calibration position.

7. Tighten the screws of the fixation rings (A) and (B) gently.

➲ClipA4

For maintenance or replacement of the glass chambers refer to **Supplement B.**

5.5. Smart Fluo-Sensor assembly


Green LED, ex. 525 nm

Blue LED, ex. 465 nm

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.

☞ **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 OroboPOS 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 O2k-Chamber A or B.
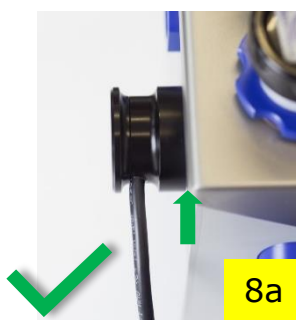
2. The blue frame of the chamber window and the Smart Fluo-Sensor are specially designed to only connect at a specific orientation, when the unbowed notches align 7. In this optimal position, the Smart Fluo-Sensor is carefully inserted in the window opening until the sensor completely and gapless covers the chamber window 8a,b. In this position, the cable routing must be horizontal 9.
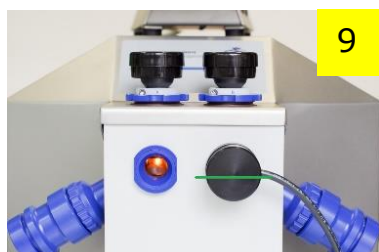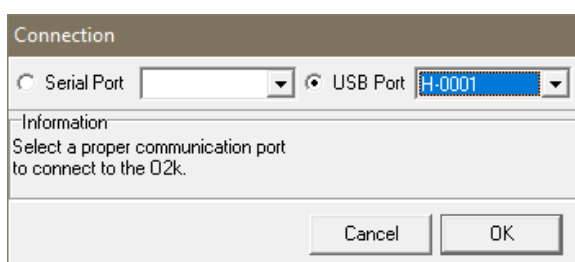
6. Start DatLab 7

On your PC desktop, L⁀ on the icon "Oroboros DatLab" to start the programme. Enter or ▼ select a user name. Click Connect to O2k to open the O2k configuration window, or press F7.

⮕Clip A5

With the USB cable connected, select ⊙ USB port in the Connection window. If several O2ks are connected to one PC, the serial numbers of all O2ks are shown to select one ▼. Press Cancel for disconnected operation of DatLab.

6.1. Oroboros O2k \ O2k configuration

Power-O2k Assign a label (Power-O2k or P-number) to your O2k.

Oxygen sensor # The OroboPOS number of the polarographic oxygen sensor is entered 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).

Smart Fluo-Sensor # The number and LED excitation can be found on the insde. The signal of the Smart Fluo-Sensor is recorderd in the "**Amperometric, Amp**" channel. Only when a Smart Fluo-Sensor is connected, the serial number is **automatically detected** and set in "**Amp sensor #**".
» wiki.oroboros.at/index.php/O2k_configuration

Channel labels **Oxygen, O2** is the label for the Oxygen channel, which is always active and cannnot be deselected.

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

Potentiometric, pX is the label for a potentiometric channel. The channel must be selected ☑ for data recording.

6.2.   Oroboros O2k \ O2k control

O2k control

System | Oxygen, O2 | Amperometric, Amp | Potentiometric, pX |

6.2.1. O2k control \ Tab: System

Open the O2k control \ System tab 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 applied last when DatLab was connected to the O2k. O2k control settings of the selected setup are displayed after L⤸ left click on the Load setup button.

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

Block temperature [°C] L⤸ Left click on the input window (L⤸⤸ double click to overwrite all digits). Enter the block (=experimental) temperature (range 2.0 to 47.0 °C).

Stirrer speed [rpm] The default is 750 rotations per minute (12.5 Hz), which may be varied between 300 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. Both chambers may be set at an identical stirrer speed.

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 and F12.

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.

6.2.2. 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, L⌖ click on Configuration and enter the sensor number.
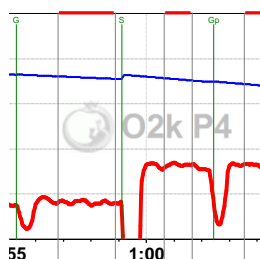
6.2.3. O2k control \ Tabs: Amperometric and Potentiometric

Amp and pX Settings for the Amp and pX tabs are described in
- MiPNet15.05 **O2k-NO Amp-Module**
- MiPNet15.03 **O2k-MultiSensor-ISE**

6.3. Connect and save a DLD file

In an automatically opened Save as window, the default file name has the form: **2017-07-21 P1-01.DLD**

Year-Month-Day P#-##.DLD: P#=Power-O2k; **##** =sequential number of the experiment; extension **DLD** for **D**at**L**ab **D**ata.

Select a subdirectory, optionally edit the file name and L⌖ 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.

6.4. 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.

6.5. O2k-signal line

O2k status line — **11A** | O2 calib. | Amp calib. | pX calib. | Set temp. | 37.0 | Illum. on | O2k: Pan auto | TIP Ready | — pX calib. | Amp calib. | O2 calib. | **11B**

O2k signal line — O2k P11 | < 278.0; 2.14858; 2.67149;37.001; 95.3; 3.939;-9.99999;-9.99999; 1.45696; 1.37142; | Oroboros | DatLab 7.1 / H-0009

Labels: Power-O2k number | Time [s] | O2 signals | pX signals | User | Fluo/Amp signals | DatLab version | O2k Serial number | Peltier power [%] | Barom. pressure [kPa] | Temp [°C]

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. L⬚ 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).

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

Ext Temp [°C] Signal from external PT1000 temperature sensor (optional). "50.000" is shown if no sensor is connected.

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

6.6. O2k-status line

11A | O2 calib. | Amp calib. | pX calib. | Set temp. | 37.0 | Illum. on | O2k: Pan auto | TIP Ready | — pX calib. | Amp calib. | O2 calib. | **11B**

Labels: #A | POS (A) | Amp(A) | pX(A) | Set temp. [°C] | Chamber Illum. **on**/off | TIP connected **on**/off | Error | pX(B) | Amp(B) | POS(B) | #B

#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 when 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 real-time.

L⊕⊕   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 re-calibrated 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 ±0.002 °C of the set temperature, but yellow if the difference is more than ±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 L⊕⊕ 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 L⊕ 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, L⊕ click on ERROR to open the Error message window. In this window, L⊕ Close to remove the blinking error signal.

7. O2k control

7.1. O2k control keys

After selection of an O2k setup in the O2k control F7 window, followed by L⟨ 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 L⟨ 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.

ⓘ **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 L⟨ loaded.

Send to O2k Individual control settings may be edited before L⟨ 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 L⌘ 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

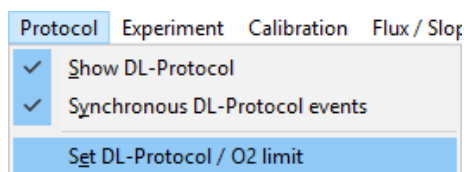☞ **Instrumental DL-Protocols** provide real-time guides to calibrations.

O2   wiki.oroboros.at/index.php/O2_calibration_-_DatLab.

Amp   wiki.oroboros.at/index.php/Amp_calibration_-_DatLab.

pX   wiki.oroboros.at/index.php/PX_calibration_-_DatLab.

8.2. Performing an experiment with DL-Protocols

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

Protocol	Experiment	Calibration	Flux / Slop
✓ Show DL-Protocol			
✓ Synchronous DL-Protocol events			
Set DL-Protocol / O2 limit			

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).

On the Oroboros homepage, a DL-Protocol library is prepared.

Further details:
» http://wiki.oroboros.at/index.php/DL-Protocols.

8.3. Flux analysis

» http://wiki.oroboros.at/index.php/Flux_/_Slope.

Further information and updated versions:
»wiki.oroboros.at/index.php/MiPNet22.11_O2k-FluoRespirometer_manual

Links to O2k-catalogue and support

» wiki.oroboros.at/index.php/Oroboros_O2k
» wiki.oroboros.at/index.php/O2k-FluoRespirometer
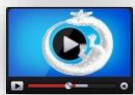» wiki.oroboros.at/index.php/O2k-technical_support_and_open_innovation

The joy of success is the next step

Supplement A. Videosupport

Videos Johannes Aitzetmüller: fancy tree films, Innsbruck.

Videosupport weblinks

» **O2k-Videosupport**
http://wiki.oroboros.at/index.php/O2k-Videosupport

Videosupport directly on Oroboros USB flash drive

➲ **A O2k ready to use:**
A_DL7_O2k ready to use.mp4

➲ **A1 Oroboros flash drive and DatLab 7 installation:**
A1_DL7_OROBOROS flash drive and DatLab 7 installation.mp4

➲ **A2 Insert O2k-Chamber:**
A2_Chamber service_Insert O2k-Chamber.mp4
O2k-Core (O2k-Main Unit), O2k-Chamber, Stirrer-Bar\white PVDF\15x6 mm, OroboPOS-Holder, O2k-Chamber Holder

➲ **A3 Insert OroborPOS:**
A3_DL7_Insert OroboPOS.mp4
OroboPOS-Connector

➲ **A4 O2k-Chamber volume calibration:**
A4_Chamber service_O2k-Chamber volume calibration.mp4
Volume-Calibration Ring, Stopper\black PEEK\conical Shaft\central Port, O-ring\Viton\12x1 mm

➲ **A5 DL7_Start DatLab7:**
A5_DL7_Start DatLab7.mp4

Supplement B. O2k-Chamber disassembly

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.

C. Contributions

[1]Erich Gnaiger: Oroboros Instruments and [2]University hospital of Visceral, Transplant and Thoracic Surgery, D. Swarovski research laboratory, DSL, Medical University Innsbruck; EG wrote the manual and edited the final version.

[2]Andras Mészáros: University hospital of Visceral, Transplant and Thoracic Surgery, D. Swarovski research laboratory, DSL, Medical University Innsbruck. AM wrote and edited the manual. Taking, editing photos and images.

[3]Lukas Gradl: software security networks – ssn, Innsbruck. LG is the programmer of DatLab 7 and previous DatLab versions 3 to 6, and contributed to the concept of quality control in DatLab 7.

[4]Philipp Gradl: WGT-Elektronik GmbH & Co KG, Kolsass. PG is responsible for the logistics of electronic and mechanical development of the O2k.