O2k start
High-Resolution Respirometry

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Overview The Oroboros O2k (**O2k**) 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 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 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 software, providing real-time display of oxygen concentration and respiration (oxygen flux). 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**), 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 the DatLab software. Scientific support complements HRR. Detailed laboratory **O2k-Procedures** on HRR provide step-by-step instructions. Numerous new **O2k-Publications** on applications of the Oroboros O2k focus on critical issues of mitochondrial physiology.

While the Oroboros O2k 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.
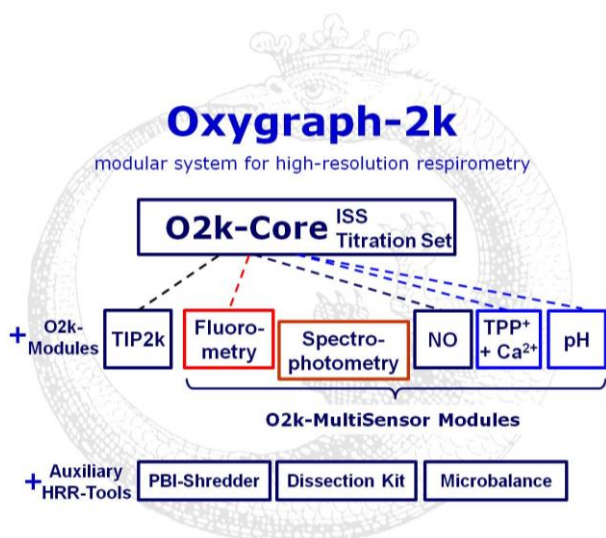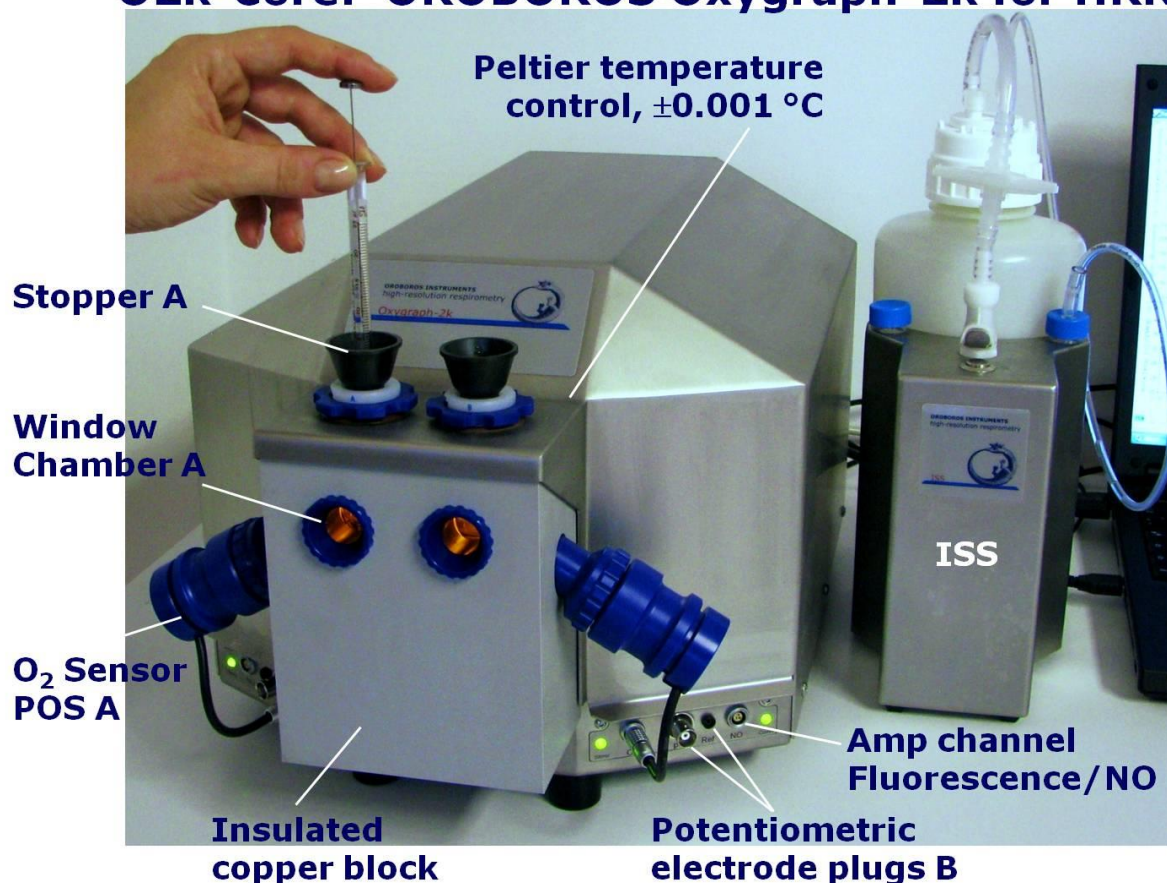
The Oroboros O2k (O2k) is the modular system for high-resolution respirometry (HRR). The O2k is the 2-chamber high−resolution respirometer for monitoring oxygen consumption with small amounts of biological samples. The modular O2k-concept yields a high flexibility for HRR, with the **O2k-Core** as the basis, and O2k-Modules supported by the O2k-Core. Available O2k-Modules are the Titration-Injection microPump (**TIP2k**) and the **O2k-MultiSensor Modules** (Fluorometry; Ion Selective Electrodes, ISE). Spectrophotometry will be available only with the NextGen-O2k.

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O2k-Core: OROBOROS Oxygraph-2k for HRR

Peltier temperature control, ±0.001 °C

Stopper A

Window Chamber A

O₂ Sensor POS A

Insulated copper block

Potentiometric electrode plugs B

Amp channel Fluorescence/NO

ISS

The O2k-Core **(Series G, compare Series H) is the experimental system complete for basic high−resolution respirometry (HRR),** including the O2k-Main Unit with stainless steel housing, O2k-Assembly Kit, two OroboPOS (oxygen sensors), and OroboPOS-Service Kit, DatLab software (**DatLab**), ISS-Integrated Suction System and O2k-Titration Set. The O2k-Core supports all add-on O2k-Modules.

1. Unpack the O2k

Box 1   (or Peli Case) contains the O2k-Main Unit with chamber holders in a mounted position, the holders for the polarographic oxygen sensors (OroboPOS) and the OroboPOS-Connector (optionally 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, optional O2k-Modules 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.

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, such as all chapters of the **O2k-Manual** and **O2k-Procedures**, with corresponding DatLab demo files and templates for training with DatLab.

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".

O2k-DatLab Install

DatLab templates

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

OROBOROS DatLab

3. Electrical 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
Current during standard operation (when temperature is stable): approx. 0.25 A

☞ 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, syringes 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

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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 hours in the warm room before connecting to the mains.

4. Start the O2k

Rear of the O2k-Main Unit Series G-H.
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.
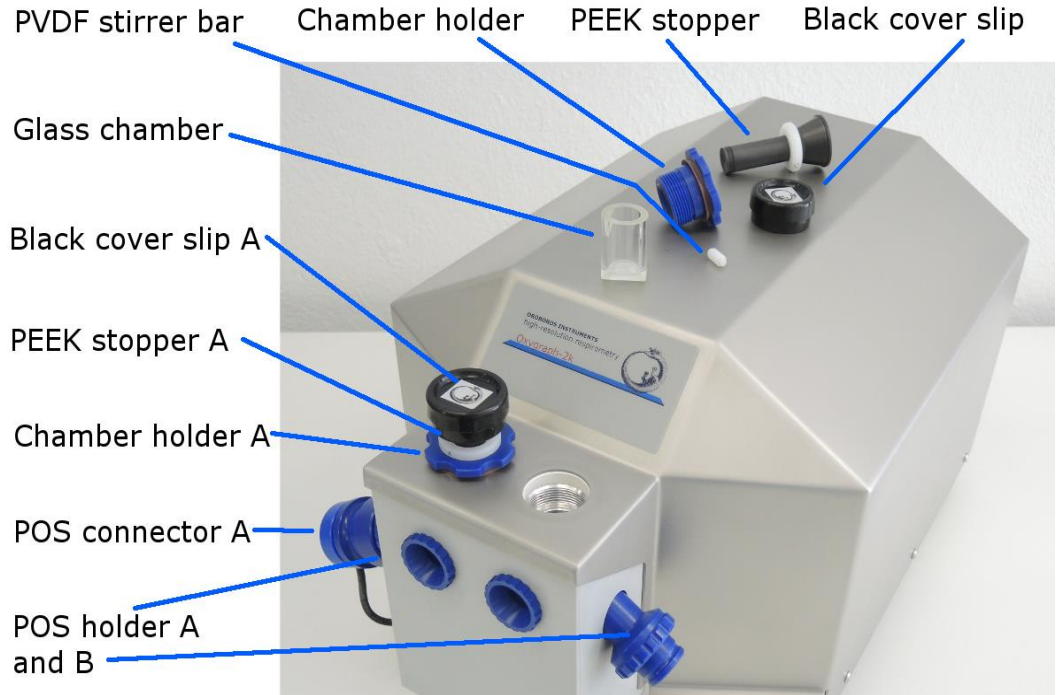
WGT quality control seal: Do not open. An intact quality control seal is required for warranty obligations related to the electronics of the O2k.

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

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

PVDF stirrer bar   Chamber holder   PEEK stopper   Black cover slip

Glass chamber

Black cover slip A

PEEK stopper A

Chamber holder A

POS connector A

POS holder A
and B

Unscrew the blue chamber holders from the O2k housing. Remove the POS connectors from the POS holders by pressing the sleeve downwards against the bottom plate of the POS connector. Unplug the cable.

5.1. O2k-Chamber and OroboPOS assembly

O2k-SOP

➲Clip A3

**Insert the glass O2k-Chamber** into the copper block of the O2k-Main Unit, then add the PVDF stirrer bar.

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.

POS

POS
seal tip

POS connector

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

O2k-Manual

The O2k-Core is electronically equipped with all plugs required for O2k-MultiSensor Modules. 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.

Left: Plugs in O2k-Series H.

Mains   pX   Ref   O2   Amp   Fluo   Stirrer

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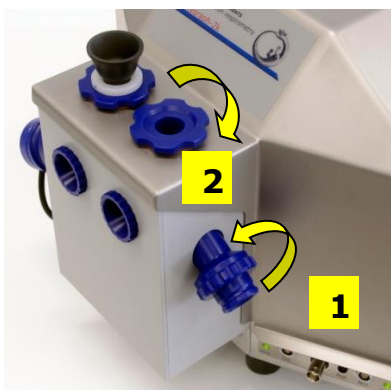
1. Screw the POS holder **1** into a position where the glass chamber with the angular cut towards the POS holder is slightly lifted upwards.

2. 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.

3. Screw a POS head onto each POS connector (finger-tight). Push a *wetted* POS seal tip (black gasket) over the POS head (arrow **3**). Position the pore centrally and do not stretch the gasket.

4. Connect the POS connector cable to the O2k-Main Unit. Insert the male plug of the cable **4** into the female O2 plug (left: O2k-Series G; compare Series H). The red dot on the male plug **4** faces straight upwards. 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).

5. The POS connector cable **4** must be in a relaxed, undistorted position. Press the sleeve downwards (arrow **5**), insert the POS with the black gasket straight into the POS holder **6**, and release the sleeve for fixing the POS in its final position.

➨Clip A2

☞**Do not rotate the POS connector** 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.

5.2. 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).

➲Clip A5

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 L-shaped Allen key (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.

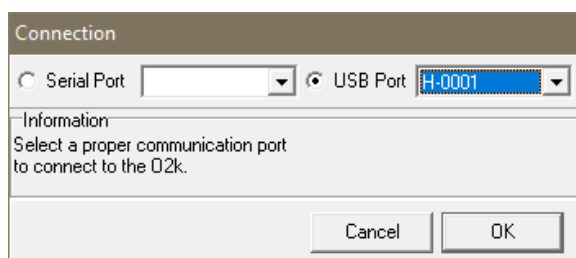7. Tighten the screws of the fixation rings (A) and (B) gently with the Allen key.

➲Clip A

6. Start DatLab

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.

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.

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6.1.   O2k \ O2k configuration

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

Oxygen sensor # The 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).

Channel label The label for the Oxygen channel is O2.

Amp and pX ☑   Select or deselect the Amp or pX channel. Enter the corresponding sensor numbers and channel labels.

6.2.   O2k \ O2k control
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 10.0 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 Fluo-LEDs.

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 is 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
- MiPNet17.05 **O2k-Fluo LED2-Module**
- MiPNet15.05 **O2k-NO Amp-Module**
- MiPNet15.03 **O2k-MultiSensor-ISE**

6.3.   Connect and save a DLD file
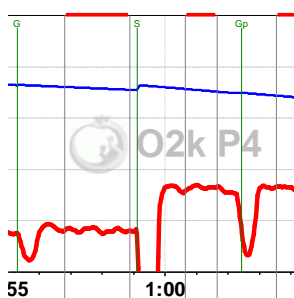
In an automatically opened Save as window, the default file name has the form: **2014-09-12 P1-01.DLD**

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.

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- 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

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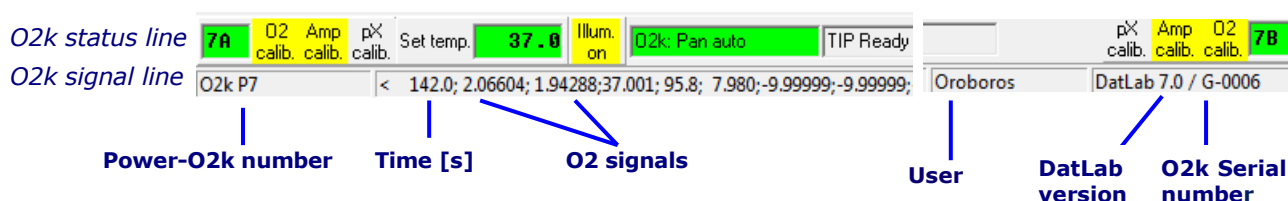
Time [s]   Time of the experiment since connecting, displayed on the *X*-axis as [h:min] or [h:min:s].

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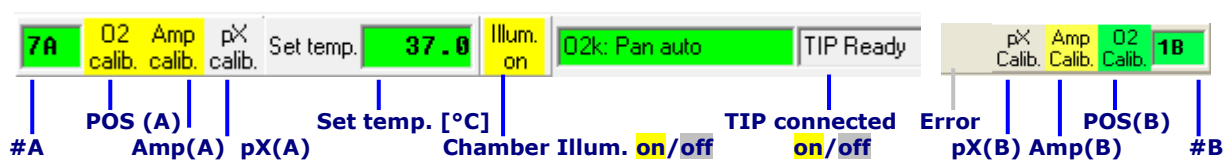
#A and #B   The labels of the left and right chamber, where # represents the Power-O2k number (P#), chosen in the O2k configuration window. The background is green if rotation of the stirrer is on, briefly yellow when the stirrer is accelerating, red if the stirrer is switched off.

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.

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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 to the DatLab protocol, after changing any of these O2k control settings.

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 <mark>COMM</mark> 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.

Further information and updated versions:
» http://wiki.oroboros.at/index.php/MiPNet19.18A_O2k-start

Next step **– O2k-Core manual D**
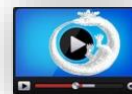» http://wiki.oroboros.at/index.php/MiPNet19.18D_O2k-calibration

The joy of success is the next step

Supplement A. Videosupport

A.1. Videosupport weblinks

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

A.2. Videosupport Oroboros USB flash drive

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

➲ **A3 Insert OroborPOS**
   OroboPOS-Connector

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

➲ **A O2k ready to use**

Videos   Johannes Aitzetmüller: fancy tree films, Innsbruck.

Supplement B. Links to O2k-Catalogue and Support

» http://wiki.oroboros.at/index.php/Oxygraph-2k
» http://wiki.oroboros.at/index.php/O2k-Core
» http://wiki.oroboros.at/index.php/O2k-technical_support_and_open_innovation

C. Contributions

[1]Erich Gnaiger: Oroboros Instruments and [2]Universitätsklinik für Visceral-, Transplantations- und Thoraxchirurgie, D. Swarovski Forschungslabor, DSL, Medizinische Universität Innsbruck; EG wrote the manual and edited the final version.

[1]Ondrej Capek: Oroboros Instruments. OC was mainly responsible for trouble shooting of test versions from DatLab 6 to DatLab 7.

[3]Christina Plattner: Biozentrum Innsbruck - Sektion für Bioinformatik, SBI, Medizinische Universität Innsbruck. CP edited the manual for DatLab 7 upgrade.

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

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