



Oxygraph-2k Manual

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A: www.bioblast.at/index.php/MiPNet19.18A_O2k-Start

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Oxygraph-2k: start high-resolution respirometry

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OROBOROS Oxygraph-2k

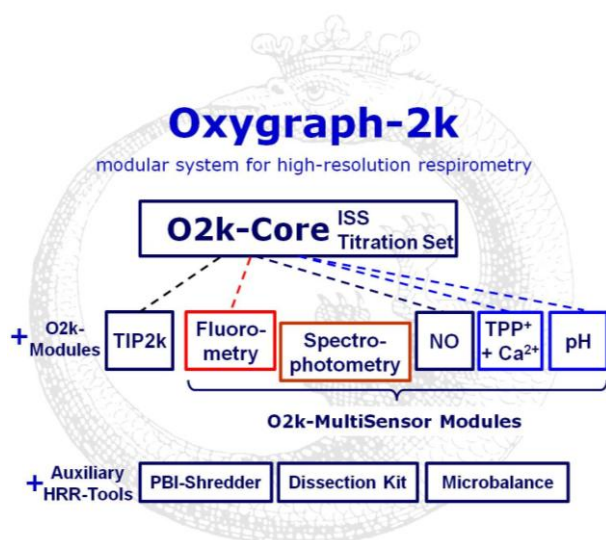
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Overview The OROBOROS Oxygraph-2k (**O2k**) provides a unique high-resolution approach to the monitoring of cellular and mitochondrial respiratory function. High-resolution respirometry (**HRR**) is indispensable at low respiratory activities, kinetic transitions and low 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 respiratory measurements at low, physiological intracellular oxygen levels and oxygen kinetics.

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 instrumental control follow standardized operating procedures (**O2k-SOP**) with automatic real-time analysis supported by the DatLab software. Scientific support complements HRR. Detailed laboratory **O2k-Protocols** on HRR provide step-by-step instructions. Numerous new **O2k-Publications** on applications of the OROBOROS Oxygraph-2k focus on critical issues of mitochondrial physiology.

While the OROBOROS Oxygraph-2k 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 Oxygraph-2k (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).

O2k-Core: OROBOROS Oxygraph-2k for HRR



The **O2k-Core** is the experimental system complete for basic high-resolution respirometry (HRR). The O2k-Core includes the O2k-Main Unit with stainless steel housing, O2k-Assembly Kit, two OroboPOS (polarographic oxygen sensors) and OroboPOS-Service Kit, the DatLab software ([DatLab 6](#)), the ISS-Integrated Suction System and the Titration Set. The O2k-Core supports all add-on O2k-Modules of the Oxygraph-2k.

1. OROBOROS USB and DatLab installation

A laptop or PC is required for data acquisition and analysis using DatLab. Minimum configuration: Pentium III, 256 MB, USB port, Windows XP or later Windows versions.

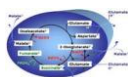


Copy the entire folder "OROBOROS O2k-Course on HRR" from the **OROBOROS USB-flash drive** to your computer. The 'OROBOROS-FileFinder' is the tool to find topics, such as all chapters of the **O2k-Manual** and **O2k-Protocols**, with corresponding DatLab demo files and templates for training with DatLab.

Open Access updates:



» <http://wiki.orooboros.at/index.php/O2k-Manual>



» <http://wiki.orooboros.at/index.php/O2k-Protocols>

DL6Install.exe

It is recommended that you exit all Windows applications before running the DatLab Installation program. 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 the control of the Oxygraph-2k, TIP2k, data acquisition & analysis.

L[⌘] Drag the DatLab icon into an appropriate position on your desktop.


2. Unpack the Oxygraph-2k

After unpacking, **store the original Box 1 (or Peli Case) and packing material for any future shipment of the Oxygraph-2k.**


- Box 1** (or the Peli Case) contains the O2k-Main Unit, including the chamber holders in a mounted position, the holders for the polarographic oxygen sensors (OroboPOS) and the POS connectors (optionally the TIP2k syringes).
- Box 2** contains standard accessories [SA], the OROBOROS USB-flash drive, optional O2k-Modules and accessories.

3. Placing the Oxygraph-2k

The Oxygraph-2k 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 temperature. Keep the space on the table below the Oxygraph-2k bottom clear to prevent any interference of ventilation beneath the O2k-Main Unit.

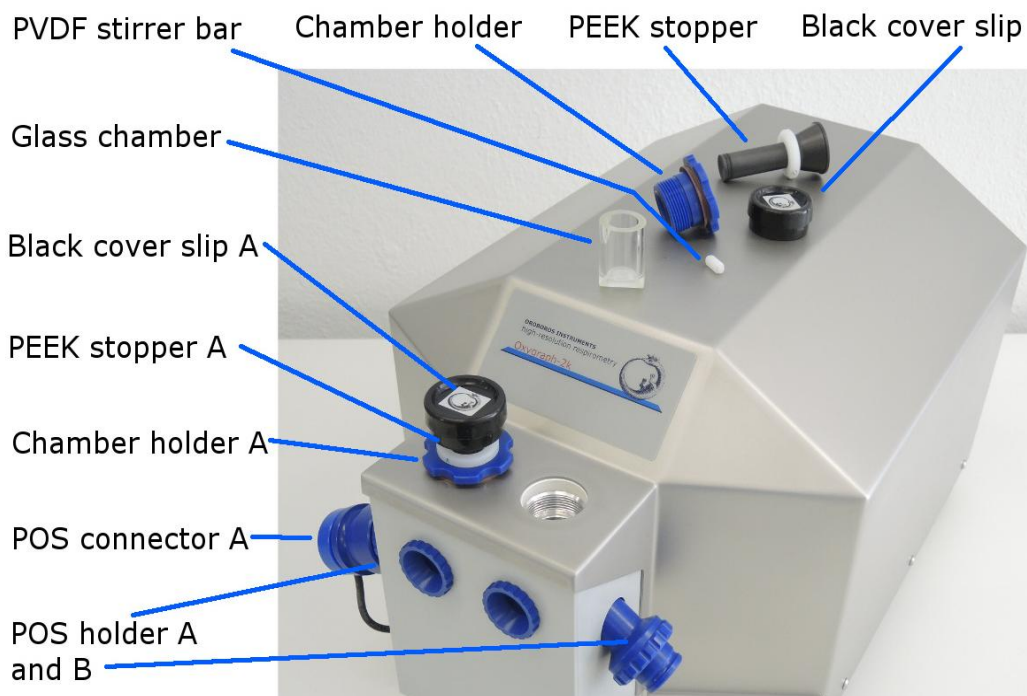
-  Avoid damage due to condensation. When placing the O2k from a cold to warm environment, let it equilibrate for at least 4 hours in the warm room before connecting to the mains.

4. Assembly of the Oxygraph-2k

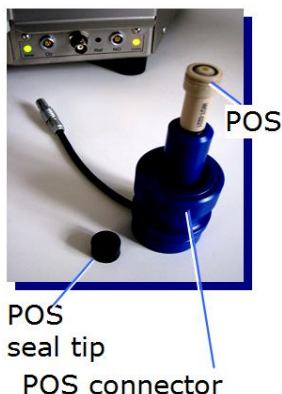
-  Uncharge yourself to avoid possible damage of the electronics. Shortly connect to electrical ground or simply touch the O2k steel housing ([MiPNet14.01](#)).

**OroboPOS assembly**

O2k-Manual B » [MiPNet19.18B POS-Service](#)



Remove the POS connectors from the POS holders by pressing the sleeve downwards against the bottom plate of the POS connector. Unplug the cable, and unscrew the chamber holder from the copper block.

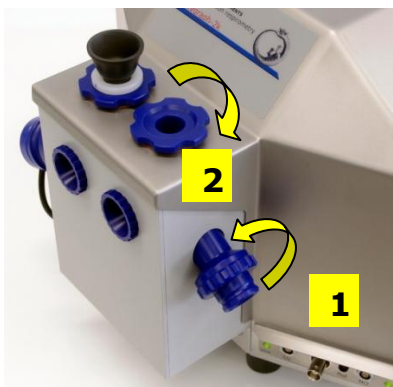


The POS are shipped dry (without electrolyte) in a Perspex housing in the OroboPOS-Service Kit. Before assembling the Oxygraph-2k, apply electrolyte and membrane, following the OroboPOS service instructions in [MiPNet19.18B](#). Clean the gold-plated pin and thread of the POS connector, using a smooth cloth wetted with distilled water, then with a cloth wetted with pure ethanol (or methanol), finally with a dry cloth.

4.1. O2k-Chamber and OroboPOS assembly

O2k-SOP

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



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 plug next to the control light of the stirrer, with the red dot on the male plug [4] facing straight upwards. Each OroboPOS head can be used on chamber A or B of the Oxygraph-2k. Note the POS number (marked on the cylindrical body of each POS) for each chamber (see Section 6.1).



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, and release the sleeve for fixing the POS in its final position.

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.

4.2. O2k-Chamber volume calibration

O2k-SOP

DatLab must be installed before calibrating the chamber volume. Connect the O2k to the mains (Section 5), 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 H₂O 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 stopper fixation rings (A) and (B) with the L-shaped Allen key (OroboPOS-Service Kit) and 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.

5. Start the Oxygraph-2k



Check the suitability of the delivered cable for your 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 and NZ: O2k-Main Power Cable\230 V\AU-NZ, 21113-01



Rear of the Oxygraph-2k Main Unit Series G.

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 Oxygraph-2k.

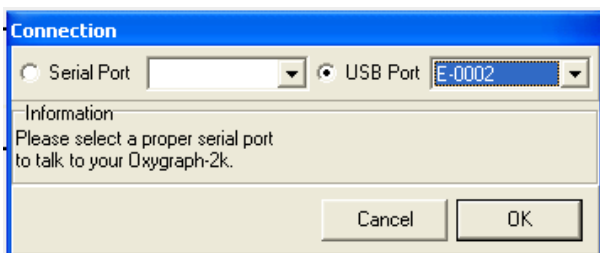
Mains on Connect the O2k with the USB cable to a laptop or PC. Switch on the O2k on the rear (Mains on/off switch: I/O). Now the four front control lights illuminate: The outer left diode (**MAINS**) is green, and temperature is regulated at 25 °C. The inner left and right diodes (**STIRRER**) are green and stirrer rotation is on. The outer right diode (**COMM**) changes to yellow if a USB connection is available, and to green after operational connection to DatLab.

6. Start DatLab

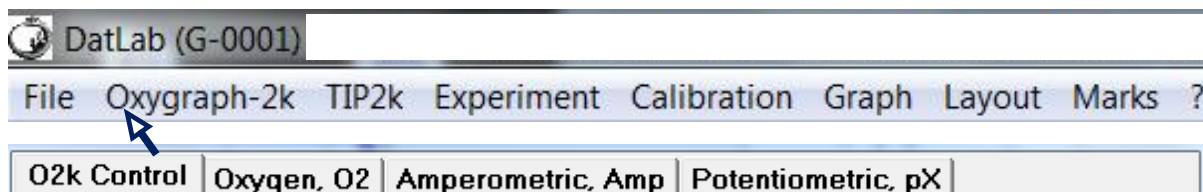


On your PC desktop, L[Ⓜ] on the icon "Oroboros DatLab" to start the program.

Either the **O2k Control** window opens automatically by default, or open O2k Control by pressing [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.

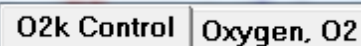


6.1. Oxygraph-2k \ O2k Control [F7]

Open the "O2k Control" window [F7] to control the Oxygraph-2k operation mode.

- ▼ Load an **O2k-Setup**, or edit 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 Oxygraph-2k. O2k Control settings of the selected setup are displayed after **Load setup**.
- Save setup** Save the O2k Control settings under a setup name. To create a new O2k-Setup, edit the name in the window, and L[⏏] left click on the **Save setup** button. O2k-Setup files can be renamed or deleted in the menu Oxygraph-2k \ Manage Setups.
- Connect to Oxygraph-2k** Connect to Oxygraph-2k sending the displayed control settings.

6.1.1. Oxygraph-2k \ Tab: O2k Control



- Power-O2k** Assign a label (Power-O2k or **P**-number) to your Oxygraph-2k. L[⏏] Left click on **Save** to save changes.
- Block temperature [°C]** L[⏏] Left click on the input window (L[⏏] double click to overwrite all digits), and enter the block (=experimental) temperature of your choice (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 with the consequence of high signal noise. Both chambers may be set at an identical stirrer speed without electronic interference.
- 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].

Light in chambers [On/Off] Toggle between On/Off to switch the light on or off when connecting. The light is switched on and off during the experiment by pressing [F10].

6.1.2. Oxygraph-2k \ Tab: Oxygen, O2

O2k Control Oxygen, O2

Gain for sensor can be changed for signal amplification in specific cases. The default is a gain of 1 V/ μ A, which is sufficient in most cases and provides a large scope for measurements under hyperoxia.

▼ Options are 1, 2, 4, or 8 V/ μ A amplifications.

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 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](#)). L[⌘] click on **Save** to save changes. Subsequently, you may L[⌘] **Cancel** to close the window, or proceed in the **O2k Control** tab. The settings are also saved when immediately changing to the O2k Control tab and connecting to the O2k.

6.1.3. Oxygraph-2k \ Tabs: Amperometric, Amp and Potentiometric, pX

Amp and pX Settings for the Amp and pX tabs are described in [MiPNet17.05 O2k-Fluorescence LED2-Module](#), [MiPNet15.05 O2k-NO Amp-Module](#), and [MiPNet15.03 O2k-MultiSensor-ISE](#).

6.2. 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**

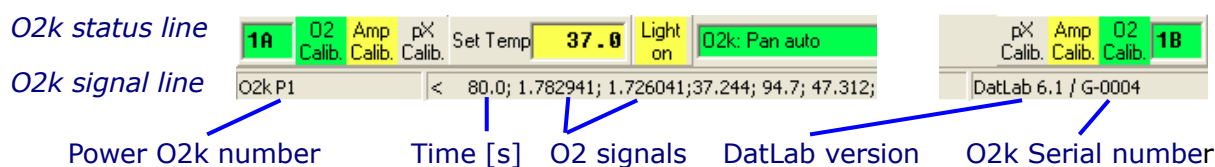
Year-Month-Day P#- (=Power-O2k P#) **O#** (=sequential number of the experiment). Extension **DLD** for **DatLab Data**.

Select a subdirectory, optionally edit the file name and L[⌘] **Save**. The DLD file is saved, and communication between DatLab and the O2k, data recording and saving are initiated.

COMM LED of the O2k-Main Unit

- **Red** O2k main power is on, no USB connection to the PC.
→ USB is not plugged in or the USB driver from Windows is not installed. This may take 1-2 min at the first connection of the O2k to the PC or laptop.
- **Orange** USB connection to the PC is established, but DatLab connection (measurement) is not started.
- **Green** USB connection to a PC is established and a Datlab measurement is in progress.
- **Green 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.

6.3. 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. Immediately after polarization of the sensors, the signal may be very high, declining slowly to a lower steady-state value. If the signal remains at 9.99 V, press [F7] and reset the gain to a lower value. L[Ⓜ] **Send** to activate the new 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.

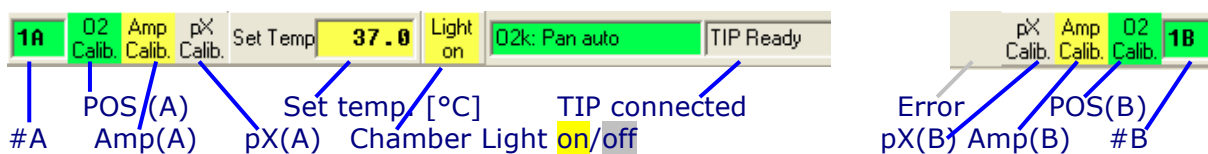
Amp (A) and Amp (B) [V] Amperometric signal from fluorescence or amperometric sensors (NO or other) after current [A] to voltage [V] conversion, chamber (A) and (B).

pX(A) [V] 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.

6.4. 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 Control tab of the O2k Control 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.


 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 record. 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.

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

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

O2k: Pan off: **Yellow** indicates that automatic panning is switched off, and the 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  click on the text. This does not influence continuous data recording.

TIP: Ready Indicates that a Titration-Injection microPump (TIP2k) is connected to the Oxygraph-2k, 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. Oxygraph control

7.1. O2k Control keys

After selection of an O2k-Setup in the O2k Control [F7] window, followed by Connect, only the following control functions are routinely required during experimental operations.

Left Stirrer On/Off [F11] Toggles between the on and off state of the stirrer, always returning to the pre-set stirrer speed.

Right Stirrer On/Off [F12] as for left stirrer (see above).

Stirrer test [F9] Start a stirrer test by L[⌘] "OK": stirring is stopped in both chambers and restarted after a selected period (default: 30 s). »[MiPNet06.03](#)

Light 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.

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, and individual control settings may be edited.

Send to Oxygraph-2k The control settings displayed in the O2k Control window are sent to the O2k, all changes of settings are saved in the protocol, displayed as Events and the status of the instrument is changed to the new settings.

7.3. File \ Save and Disconnect

To stop recording data, go to the menu **File** and select **Save and Disconnect** and confirm **Save and disconnect**, thus terminating further data acquisition.

While connected, all data are saved automatically. It is, therefore, not possible to disconnect without saving the data. Close the file, or proceed with the same file in the disconnected mode.

- Upon disconnect, the COMM light on the O2k switches from green to orange. The O2k Control window shows the last values used during operation before disconnecting.

It is important that after disconnection from DatLab the Oxygraph-2k 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 Oxygraph-2k**. Again, a default file name is given, as described above, with the next sequential number. After re-connecting, the Oxygraph-2k continues operation uninterrupted, the experimental time is reset to zero, and data recording is restarted with automatic saving to file.

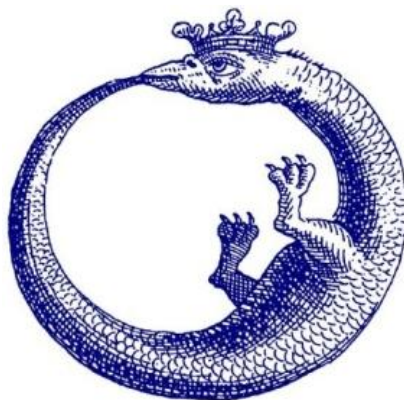
After disconnecting the communication between the O2k and DatLab, the O2k can be switched off on the rear of the O2k-Main Unit.



Further information and updated versions: *go Bioblast*

» www.bioblast.at/index.php/MiPNet19.18A_O2k-Start

Next step – O2k-Core Manual D » [MiPNet19.18D O2k-Calibration](#)



The joy of success is the next step