



## Oxygraph-2k Manual

Mitochondrial Physiology Network 19.01(A01): 5-18 (2014)  
A: [www.bioblast.at/index.php/MiPNet19.01A\\_O2k-Start](http://www.bioblast.at/index.php/MiPNet19.01A_O2k-Start)

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# Oxygraph-2k: start high-resolution respirometry

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**OROBOROS Oxygraph-2k**

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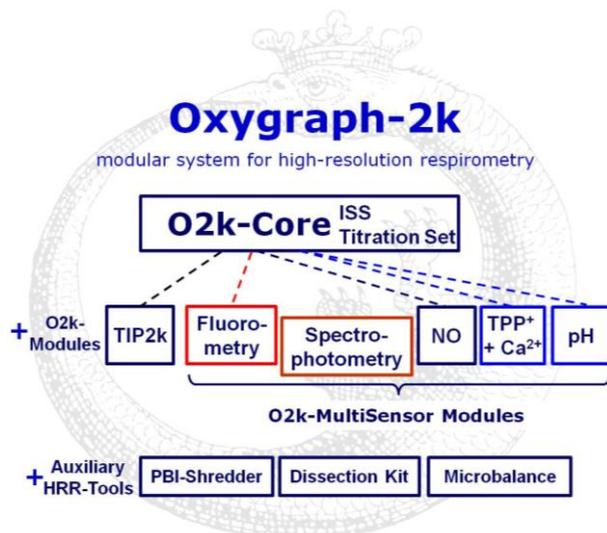


**Overview:** The OROBOROS Oxygraph-2k (**O2k**) provides a unique high-resolution approach to the monitoring of cellular and mitochondrial respiratory function. High-resolution respirometry (**HRR**) is indispensable at low respiratory activities, kinetic transitions and low oxygen levels. High resolution 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. Daily new O2k-Publications on applications of the OROBOROS Oxygraph-2k focus on critical issues of mitochondrial physiology.

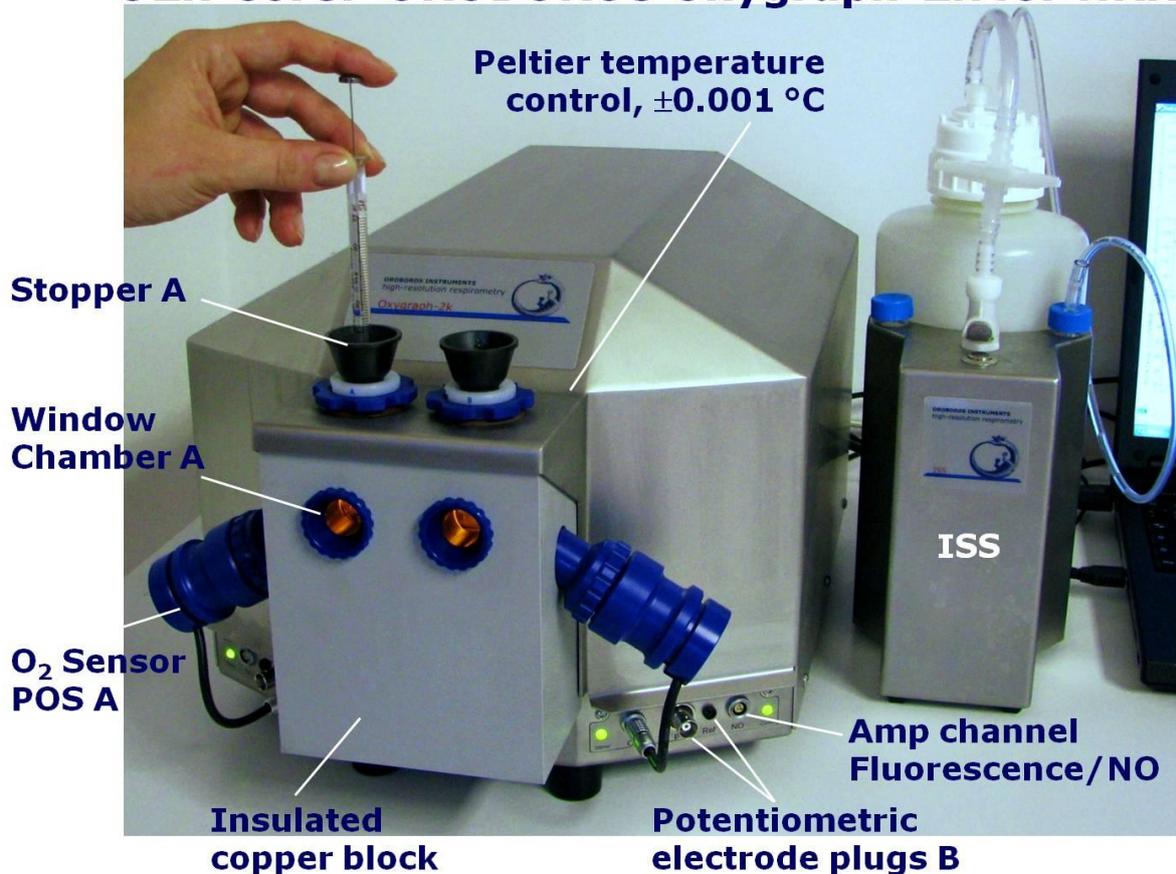
While the OROBOROS Oxygraph-2k provides the instrumental basis for high-resolution respirometry, successful operation at high accuracy up to the limit of detection depends on a professional application by the technician, scientist or student. Participation in a hands-on introductory **O2k-Workshop** 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

A



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, 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-stick 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, Windows 7.



Copy the entire folder "OROBOROS O2k-Course on HRR" from the **OROBOROS USB-Stick** 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:



» [www.orooboros.at/?O2k-Manual](http://www.orooboros.at/?O2k-Manual)



» [www.orooboros.at/?O2k-Protocols](http://www.orooboros.at/?O2k-Protocols)

**DL5Inst.exe:** It is recommended that you exit all Windows applications before running the DatLab Installation program. In the OROBOROS-FileFinder', click the OROBOROS symbol or 'O2k-DatLab Install'. Read the short instruction windows, and proceed by clicking **Next**. By default, DatLab is installed to "C:\DatLab".



Installation is quick and easy and generates this icon on your screen: Orobos DatLab for the control of the Oxygraph-2k, TIP2k, data acquisition & analysis.



Drag the DatLab icon into an appropriate position on your screen.

## 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 the standard accessories [SA], the OROBOROS USB-Stick incl. DatLab software and documentation, and 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 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

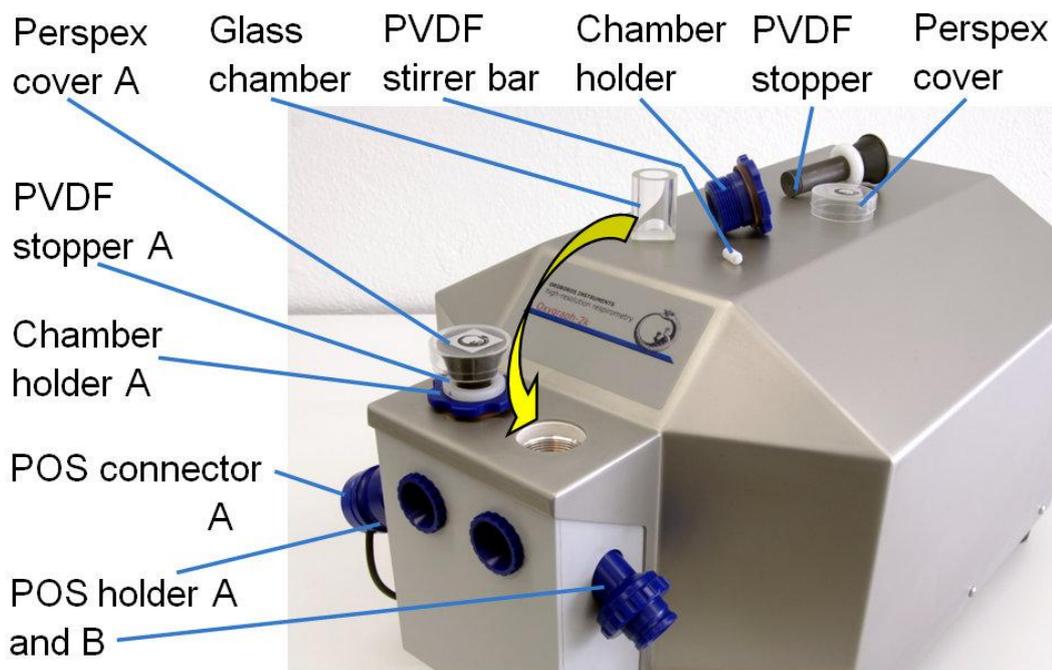


Uncharge yourself to avoid possible damage of the electronics. Shortly connect to electrical ground or simply touch the O2k metal housing ([MiPNet14.01](#)).



**OroboPOS assembly:**

**O2k-Manual B** » [MiPNet19.01B 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.



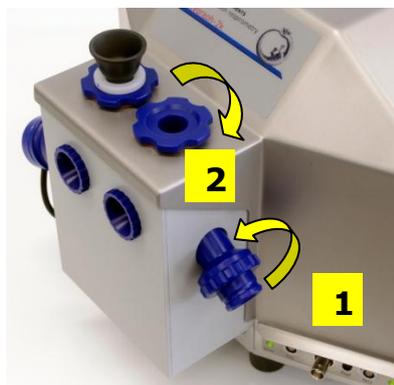
POS seal tip  
POS connector

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 [MiPNet19.01B](#). 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-Glass 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 (finger-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, avoiding bending or torsions of the cable. Insert the male plug of the cable [4] into the female plug next to the control light of the stirrer. The red dot on the male plug [4] has to face straight upwards when inserting the plug. 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 into each chamber.

2. Add accurately 2.1 ml 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  $\mu$ l when a small 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. The fixation rings (A) and (B) are fixed by gently tightening the screws with the Allen key.

## 5. Start-up the Oxygraph-2k

-  Check the suitability of the delivered cable for your power and plug in the mains power cable.

The O2k is delivered with one of three different types:

- US: O2k-Main Power Cable\120 V\US-CA, 21112-01
- Europe: O2k-Main Power Cable\230 V\Europe, 21111-01\*
- Australia and New Zealand: O2k-Main Power Cable\230 V\AU-NZ, 21113-01

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

Connect the O2k with the USB cable to a laptop or PC.



Rear of the Oxygraph-2k main unit Series E.

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 red when the default stirrer setting is OFF. The outer right diode (**COMM**) is yellow as long as the O2k is not operationally connected to DatLab.

**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.

## 6. Start-up DatLab



On your PC screen, click 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. O2k-MultiSensor - Configuration



**OroboPOS #:** 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](#)).

- ✓ **Oxygraph control at start [On/Off]:** Should be "On" (✓) if the PC is used with the Oxygraph-2k. Switch the option to "Off" for a PC used separately for data analysis.

**Save Configuration:** Click on **Save configuration** to save changes. Subsequently, you may click on **Cancel** to close the window, or proceed in the **Control** window. The settings are also saved when immediately changing to the **Control** window.

### 6.2. O2k-MultiSensor – Control [F7]

In the "O2k-MultiSensor" menu [F7], **Control** the Oxygraph-2k operation mode. Temperature, gain

settings, polarization voltage, stirrer speed, data recording interval and settings used upon connect (stirrer power and light) are edited either step-by-step, or by ▼ loading an O2k-Setup. The default is "O2k-Setup 25 °C" or the O2k-Setup which was applied last when the PC was connected to the Oxygraph-2k. Settings of selected setups are displayed after **Load setup**. Activate the displayed control settings by clicking on the **Connect to Oxygraph-2k** button.

**Block temperature [°C]:** Click on the input window (double click to overwrite all digits), and enter the block temperature of your choice (range 2.0 to 47.0 °C).

**Gain for oxygen sensor:** Can be changed for signal amplification in specific cases. The default is a gain of 1 V/μA; ▼ options are 1, 2, 4, or 8 V/μA amplifications. In particular, use a low gain if sections of the experiment are performed at high oxygen above air saturation, when the maximum voltage is close to 10 V or higher (offscale). Use a higher gain at low signal output when the maximum voltage obtained during an experiment is <3 V (relevant only at low temperature).

**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.

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

**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 activate or deactivate the stirrer after connection. 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 after connection. Independent of this On/Off setting, the light is switched on and off during the experiment by pressing [F10].

**Save setup:** Save the Oxygraph control settings under a setup name. To create a new O2k-Setup, edit the name in the window, and click on the **Save setup** button. O2k-Setup files can be renamed or deleted in the menu Oxygraph \ Manage Setups.

**Connect to Oxygraph-2k:** Click on **Connect to Oxygraph-2k**.

### 6.3. Connect and save DLD file

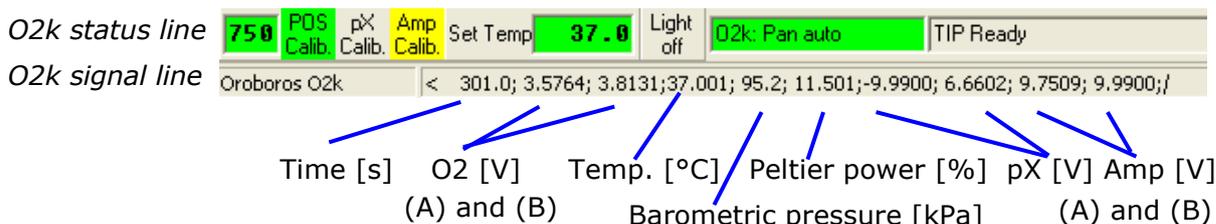
In an automatically opened **Save** window, the default file name has the form: **2014-02-12 P1-01.DLD**

**Year-Month-Day P#-** (=Power-O2k P#) **0#** (=sequential number of the experiment). Extension **DLD** for **DatLab Data**.

Select a subdirectory and edit the file name at this stage. Then click on the **Save** button. The DLD file is saved, and the communication is set up between DatLab and the O2k. Data recording and saving is initiated automatically.

Successful connection is checked on the 'Comm' diode of the O2k Main unit, which changes to green, and in the O2k status and signal lines at the bottom of the screen.

### 6.4. O2k signal line



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

**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. Click

on the Send button to activate the new gain setting and to obtain a signal  $<10$  V.

**Temp. [ $^{\circ}$ C]:** Block temperature, continuously recorded.

**Light on/off:** When the windows are closed with optical sensors, the internal light is not visible but must be switched off, and **Light on** gives a warning that the light should be switched off.

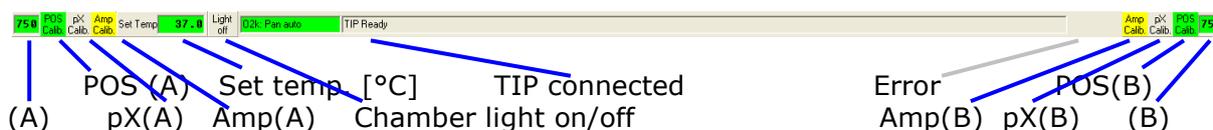
**Barom. pressure [kPa]:** Barometric pressure, continuously recorded.

**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 [Amp] 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). No number appears in systems without multi-sensor electronics. -9.99 indicates that no electrode is connected to the electronics.

## 6.5. O2k status line



**A and B:** The labels of the left and right chamber. The background is **green** if rotation of the stirrer is on, **yellow** if the stirrer is accelerating, **red** if the stirrer is switched off.

**POS 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. Click on POS Calib. to open the calibration window (left or right).

**POS 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 Oxygraph Control window, green if the measured temperature is within  $\pm 0.002$   $^{\circ}$ C of the set temperature, but yellow if the difference is more than  $\pm 0.002$ .

**Light off / Light on:** 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 clicking on the button.

**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*) 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 clicking 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 the TIP2k see ([MiPNet12.10](#)).

**ERROR:** Blinks red if an error has occurred. In this case, click on ERROR to open the Error message window. In this window, click on **"Clear errors"** to remove the blinking error signal.

## 7. Oxygraph control

### 7.1. O2k control keys

After selection of an Oxygraph-Setup in the O2k-MultiSensor Control [F7] window, followed by Connect, only the following three 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 clicking "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*.

**i** *Automatic events* are set on the plots and saved to the DatLab protocol, after changing any of these O2k controls.

### 7.2. Control [F7] with the O2k connected

During operation of the Oxygraph-2k, the O2k control key [F7] provides fast access to the Oxygraph 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 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. Disconnect

**Save file and Disconnect:** DatLab must be disconnected from the Oxygraph-2k before closing a DatLab file and re-starting data acquisition in a new file, or before opening another file. Press [F7], click on **Save file 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 O2k control keys of DatLab are inactivated, and the COMM light on the O2k switches from green to yellow. You may now switch off the O2k on the rear of the O2k-Main Unit.

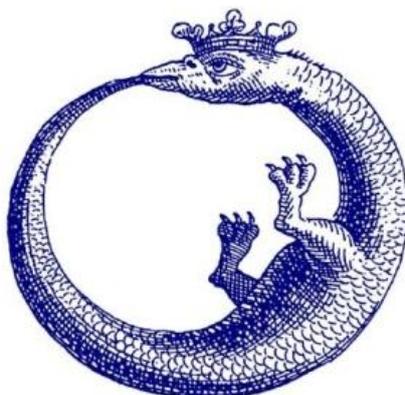
It is important that after disconnection from DatLab the Oxygraph-2k continues to regulate temperature at the same set point, and stirring speed and light in the chamber remain constant, but no data are recorded until reconnection with DatLab.

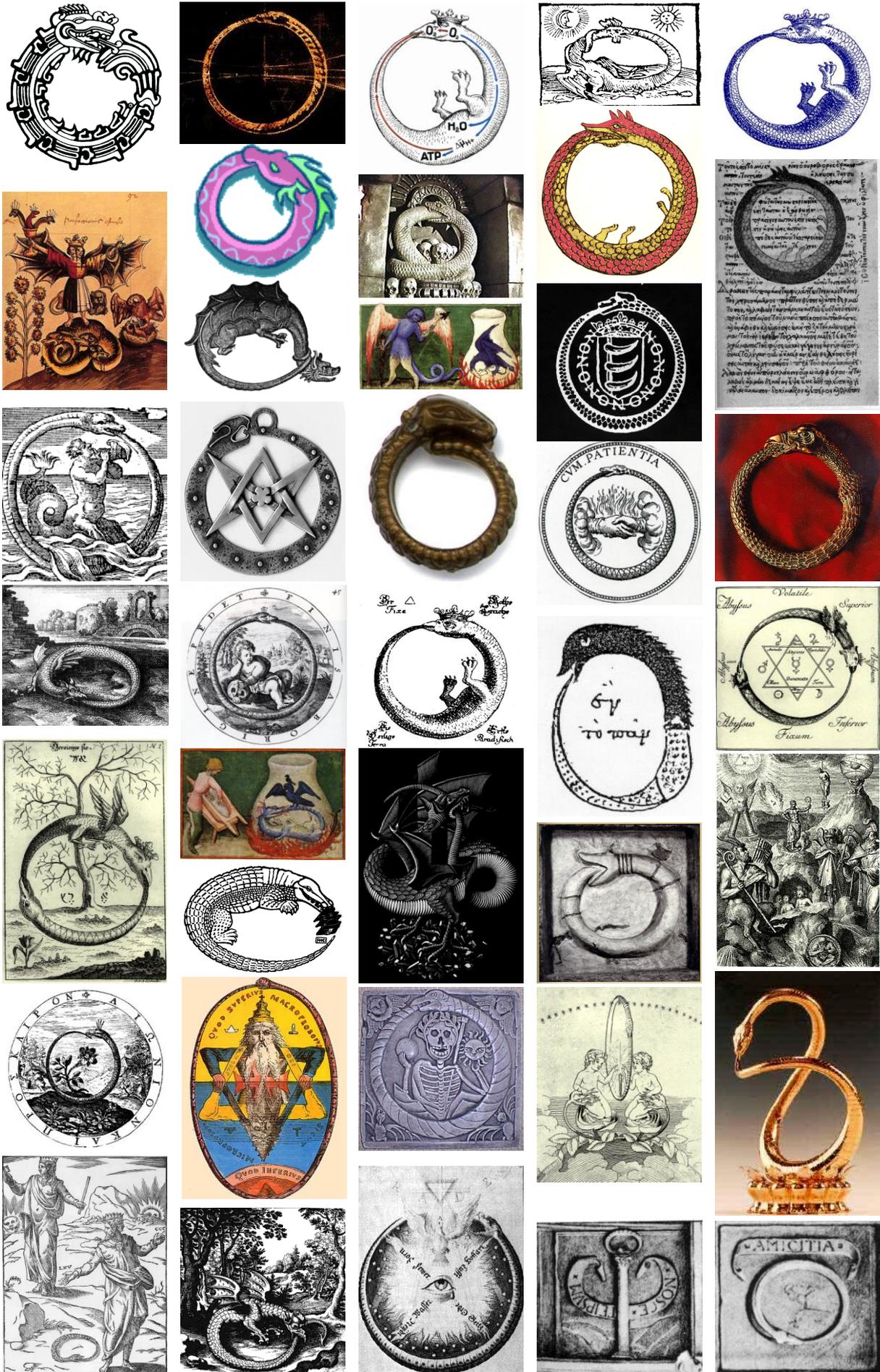
After closing the file, the Oxygraph Control window opens automatically or can be opened by pressing [F7]. Check the Oxygraph Control settings and click on **Connect to Oxygraph-2k**. Again, a default file name is given, as described above, with the next sequential number. After editing and saving the new data file name, the experimental time is reset to zero, data recording is restarted with automatic saving to file, and the Oxygraph-2k continues operation uninterrupted.



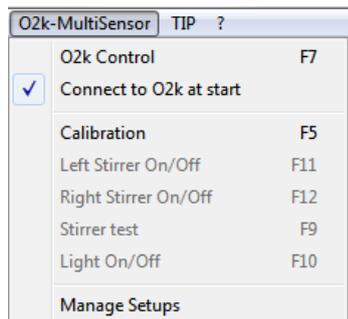
**Full version: go Bioblast**

» [www.bioblast.at/index.php/MiPNet19.01A\\_O2k-Start](http://www.bioblast.at/index.php/MiPNet19.01A_O2k-Start)





## Supplement A: Selected windows in DatLab 5.2

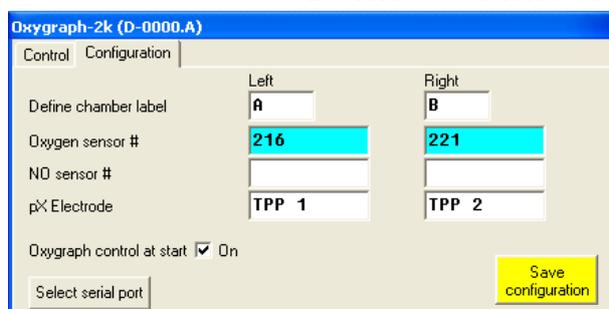


✓ Connect to O2k at start: The **O2k Control** window opens automatically by default.

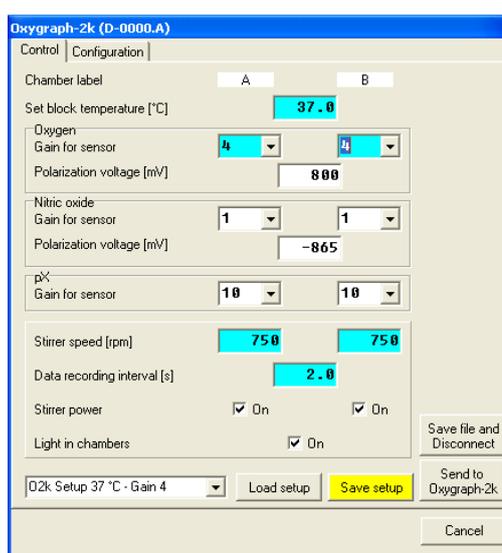
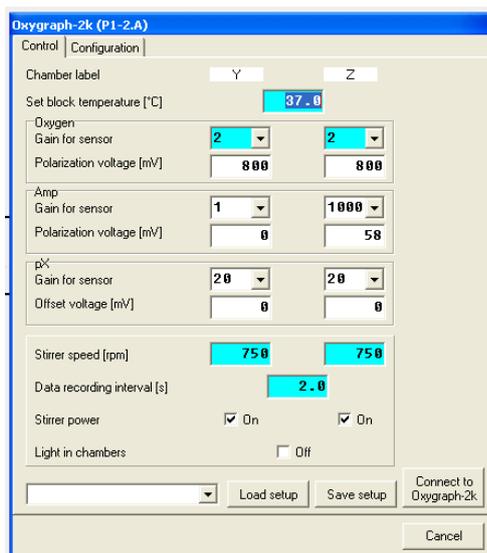
In DatLab 5, default file names have the form: **2014-02-12 AB-01**

**Year-Month-Day AB-** (=chamber A and B) **01**  
(=sequential number of the experiment).

### O2k-MultiSensor configuration window



**Define chamber label:** In the “Oxygraph” menu, **Configuration** is used to label the left and right chambers (default A and B). Chamber labels are used in file names (appended to the date), in axis labels and protocols. When operating more than a single Oxygraph, it is recommended to label the chambers of the second Oxygraph as C and D, etc. New: Use sequential number of **Power-O2k: P#**.



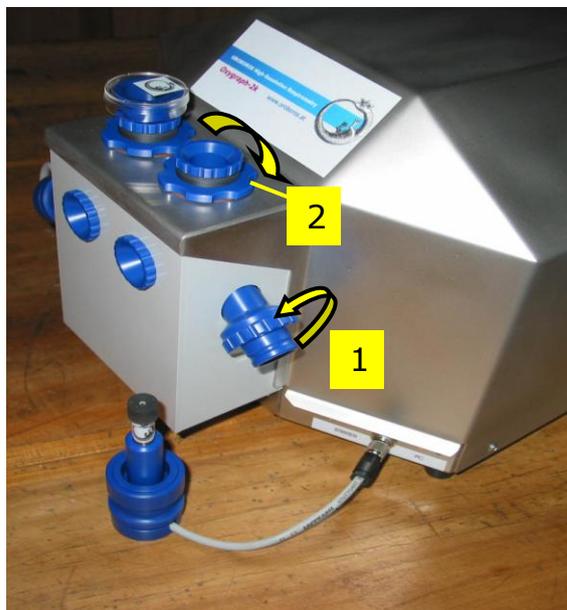
**O2k-MultiSensor control window** before connecting to DatLab (left) and after **Connect to Oxygraph-2k** during data acquisition (right). **Save setup** is emphasized after changing any settings, which may be saved under any Setup name (click on **Save setup**, or which may be activated without saving by a click on **Send to Oxygraph-2k**).

## Supplement B: O2k Series A to C

### O2k-chamber with titanium stoppers

Insert the Glass chamber into the copper block of the main unit, and add the PEEK 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 tightly to the copper block, using the lower screw (2). Unscrew the POS holder (1) stepwise and follow any downwards movement of the glass chamber by screwing down the lower screw of the chamber holder (arrow 2). When a slight backwards movement of the POS holder cannot be followed by any further downwards movement of the chamber holder (2), screw the POS holder (1) tightly clockwise, thus securing the glass chamber in a fixed position.



- 3 Clean the POS connector and screw the POS head onto the POS connector. Then apply a membrane to the POS (polarographic oxygen sensor; see [MiPNet19.01B](#)), and add a POS seal tip with the pore centrally positioned.

- 4 Then insert the POS connector into the POS holder while pressing the sleeve downwards (arrow 4), and release the sleeve for fixing the POS in its final position.



- 5 Connect the POS cable to the main unit, avoiding unnecessary bending and torsions of the cable. Insert the male plug of the POS cable (5) into the female plug positioned between the two control lights of the Oxygraph-2k (left chamber: between the MAINS and STIRRER lights; right chamber: between the STIRRER and COMM lights). The ridge on the male plug (5) has to face accurately downwards when inserting the plug,

before rotating the connector screw and fixing it finger tight.



**6** Slip the fixation ring onto the top screw of the chamber holder, with the conical side facing upwards and the flat bottom downwards.

**7** Screw the top screw of the chamber holder into the bottom screw (2). The pressure exerted by the fixation ring will maintain the top screw in the position required for maintaining a calibrated volume.



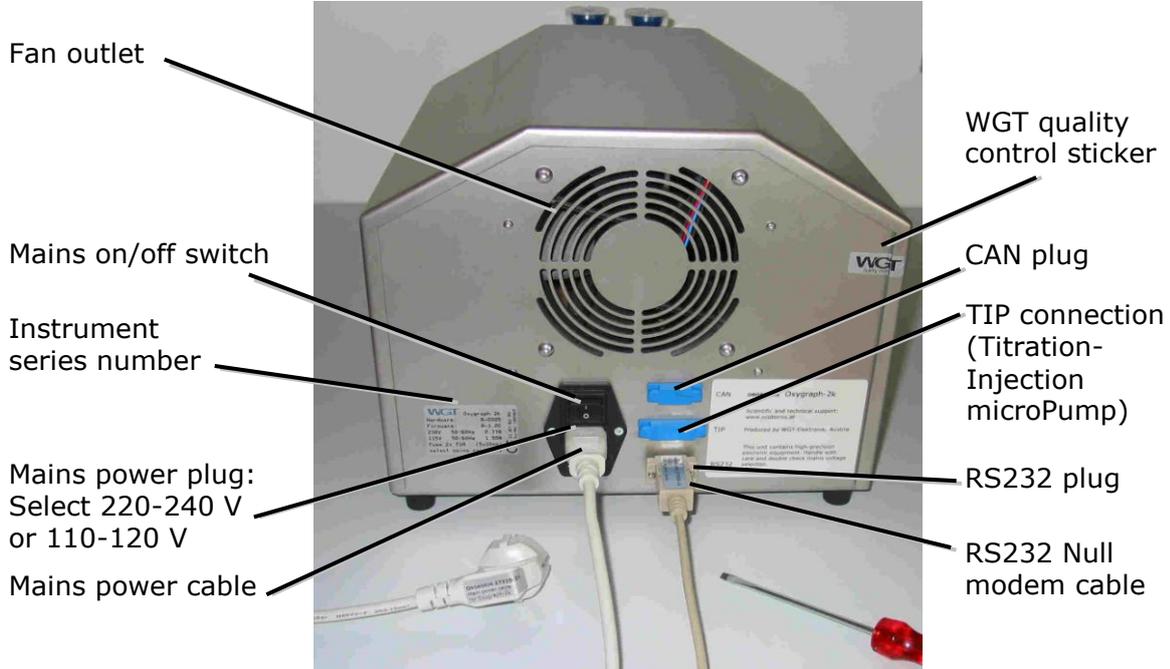
## 2.2. Chamber volume calibration

The standard chamber volume is 2 ml. Dry the chamber first. To do so, unscrew the top (6) of the chamber holder and remove it together with the fixation ring (5) to dry the space between the chamber holder and the glass chamber. Remove all droplets from the glass chamber. When re-inserting the top screw of the chamber holder (6), compress the fixation ring only slightly by rotating the screw inwards. Put the dry PEEK stirrers into the chambers. Then add 2.1 ml into the chamber for calibration of a chamber volume of 2.0 ml (more accurately, the volume of the titanium capillary is 0.085 ml or 85  $\mu$ l when a small meniscus of water is seen on top of the capillary). The volume of the titanium capillary is not part of the effective chamber volume.

Take care to insert the tip of the pipette fully into the glass cylinder to make sure that the entire volume is added properly. Otherwise, liquid may be lost between the top of the glass cylinder and the blue POM chamber holder. Then insert the titanium stopper with dry capillary (the W-ring may be moistened), pushing the stopper until it presses firmly onto the top screw of the chamber holder. Now rotate the top screw of the chamber holder further inwards (6), while pressing down the stopper firmly, until all air is removed from the chamber, and the capillary fills up until a small droplet appears on the top of the titanium capillary of the stopper. This marks the volume calibration position.

**Start-up (O2k Series A-D)**

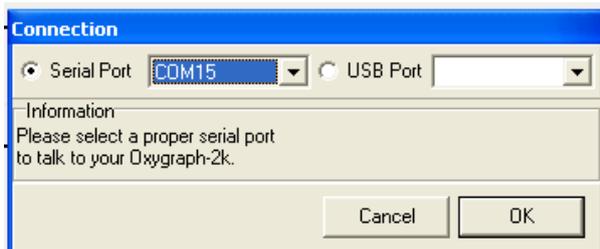
Use the RS232 cable to connect the Oxygraph-2k to your PC. To start the Oxygraph-2k, switch on the Mains of the Oxygraph-2k on the rear (Mains on/off switch).



**Series B:** Rear of the Oxygraph-2k main unit



**Series A:** Rear of the *Oxygraph-2k* main unit



Select  "Serial Port" in the Connection window and select the active serial port from the corresponding drop down menu. The active serial port is the COM port to which the RS232 cable is connected (COM1, COM2, ...).

Restart the program after changing the serial port.