Oxygraph-2k: Start High-Resolution Respirometry

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OROBOROS Oxygraph-2k

Overview: The OROBOROS Oxygraph-2k 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. Such high

resolution is required in particular for the analysis of pathological effects causing reduced respiration (mitochondrial and metabolic diseases, ischemia-reperfusion injury, oxidative stress, apoptosis, aging); 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.

High-resolution respirometry with the Oroboros Oxygraph-2k is based on a radically different systems approach to polarographic measurement of oxygen dynamics. High-resolution respirometry rests on several tightly connected instrumental features and concepts integrated by the DatLab software, providing on-line 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, in an electronically controlled thermal environment with high temperature stability. Calibration and instrumental control follow standardized procedures, with highly automatic on-line analysis by the DatLab software. Scientific support complements the approach of high-resolution respirometry. Detailed laboratory protocols on HRR provide step-by-step instructions, which are complementary to the scientific publications on a wide range of applications of the OROBOROS Oxygraph-2k with a focus on 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 course is recommended to save time and gain from the experience obtained over many years of application in various settings.
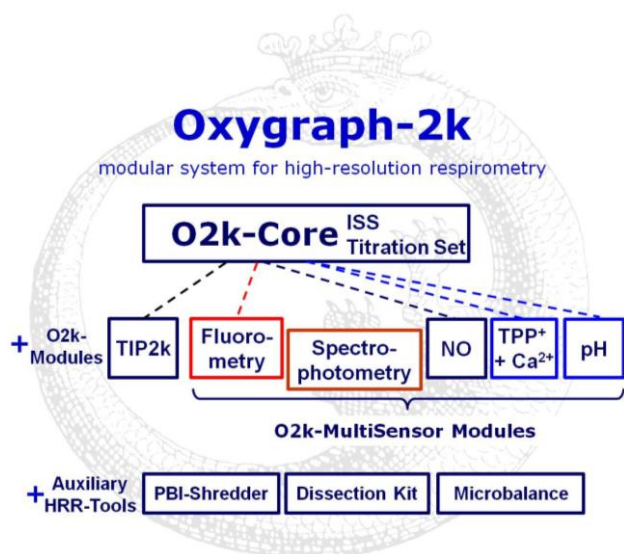
The OROBOROS Oxygraph-2k (O2k) is the modular system for high-resolution respirometry (HRR). The O2k is the 2-chamber high-resolution respirometer for monitoring oxygen consumption with small amounts of biological samples. The modular O2k-concept yields a high flexibility for HRR, with the O2k-Core as 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 OroPOS (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 PC is required for data acquisition and analysis using DatLab. Minimum configuration: Pentium III, 256 MB, 1 RS232 port, Windows 2000, Windows XP, Windows 7.

Copy the entire folder "OROBOROS O2k-Course on HRR" from the **OROBOROS USB-Stick** to your computer. Use the 'O2k-FileFinder' as a tool to find topics within the O2k-building blocks and open specific files, such as all chapters of the **O2k-Manual** and **O2k-Protocols**, with corresponding DatLab demo files and templates for off-line training of DatLab.

Open Access updates:
- www.oroboros.at/?O2k-Manual
- www.oroboros.at/?O2k-Protocols

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

Installation is quick and easy and generates this icon on your screen: Oroboros DatLab for the control of the Oxygraph-2k, TIP2k, Data Acquisition & Analysis.

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

2.   Unpacking the Oxygraph-2k

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

Box 1 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.
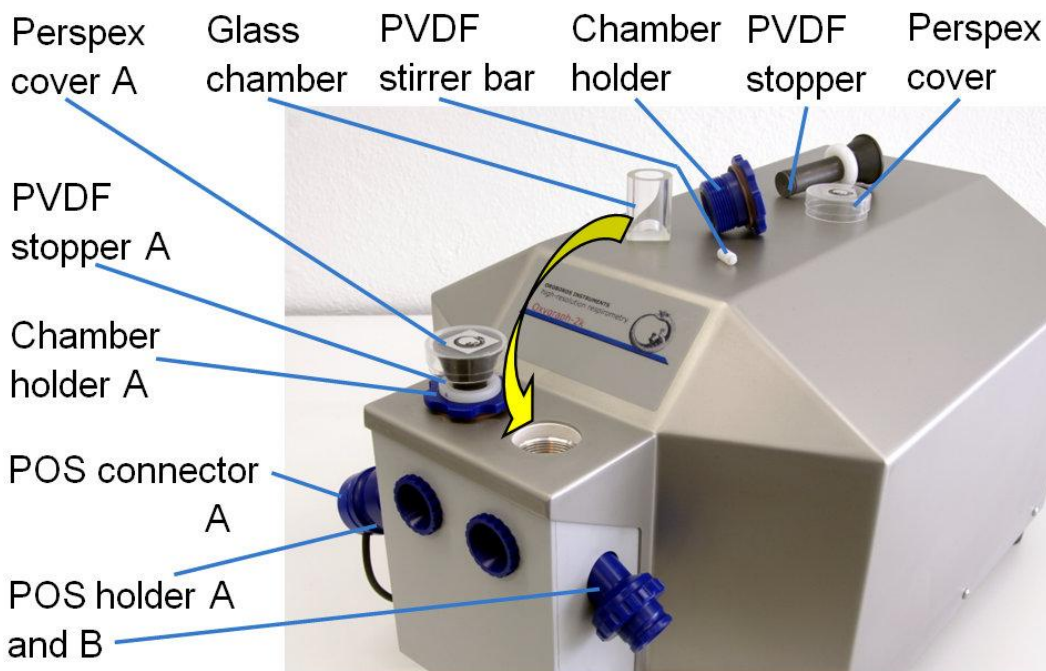
3.   Placing the Oxygraph-2k

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

4.   Assembly of the Oxygraph-2k

☞ Before touching the connector of the OroboPOS, uncharge yourself to avoid possible damage of the electronics in the POS connector. Do so by shortly connecting to electrical ground or simply by touching the O2k metal housing (MiPNet14.01).

Perspex cover A   Glass chamber   PVDF stirrer bar   Chamber holder   PVDF stopper   Perspex cover

PVDF stopper A

Chamber holder A

POS connector A

POS holder A and B

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

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 MiPNet08.04. 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), and finally with a dry cloth.

4.1. O2k-Glass chamber and POS assembly
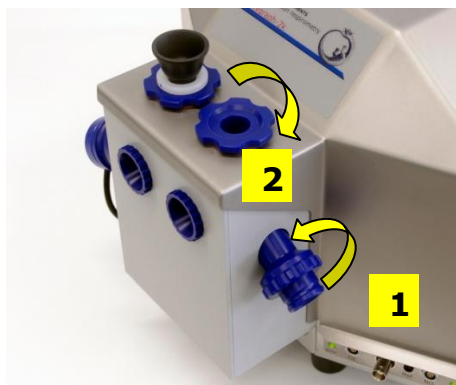
Insert the glass chamber into the copper block of the O2k-Main Unit, then add the PVDF stirrer bar.

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 (counterclockwise, not more than successive quarter turns; arrow 1) stepwise 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, with the pore positioned centrally. Do not strech the gasket across the POS head.

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 down the POS number (marked on the cylindrical body of each POSof each chamber (see Sec. 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. Chamber volume calibration

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. Dry the chamber first. To do so, remove all liquid droplets from the glass chamber. Place a dry PVDF stirrer into each chamber. Then add 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 µl when a small meniscus of water is seen on top of the capillary). The volume of the capillary of the stopper (white PVDF or black PEEK) is not part of the effective chamber volume. When titrating 2.1 ml,

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. Switch the stirrers on (Section 7.1). Loosen the fixation rings (A) and (B) of the stoppers with the L-shaped Allen key (OroboPOS-Service Kit) and push them slightly downwards in the direction of the O-rings. Then insert the PVDF 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 towards the chamber holder. Gently push the stopper further downwards until the capillary fills up and a small droplet appears on the top of the capillary of the PVDF stopper. This marks the volume calibration position, in which the fixation rings (A) and (B) are fixed by gently tightening the screws with the L-shaped Allen key.

5. Starting-up the Oxygraph-2k

☞ Check the suitability of the delivered cable for your power outlets before connecting 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

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

connection with these plugs because on the plug a male ground pin is required. Exchange for an appropriate Danish power cable.

Use the USB cable to connect the Oxygraph-2k to your PC. The serial RS232 port is not required with DatLab 5.

To start the Oxygraph-2k, switch on the Mains of the Oxygraph-2k on the rear (Mains on/off switch: I/0). The four control diodes on the front of the Main unit are now illuminated: The left diode (MAINS) is green, and the system is set to the default temperature of 25 °C. The left and right diodes (STIRRER) are red, indicating that the default stirrer setting is *OFF*. The right diode (PC) is yellow, indicating that the Oxygraph-2k has not yet received an operational connection to DatLab.
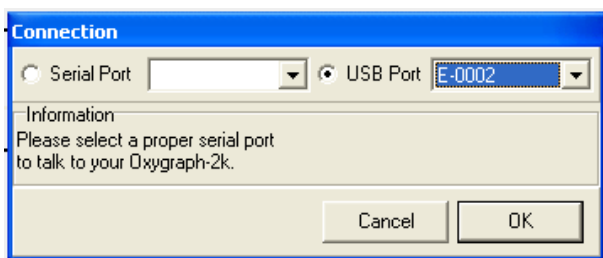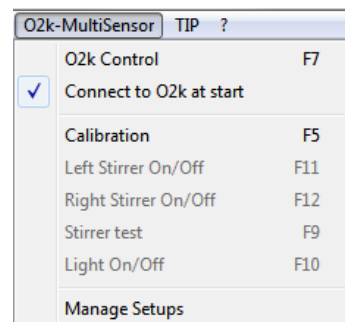
WGT **Quality Control Seal**: An intact quality control seal is required for warranty obligations related to the electronics of the Oxygraph-2k. **Do not open**.

6. Starting-up DatLab

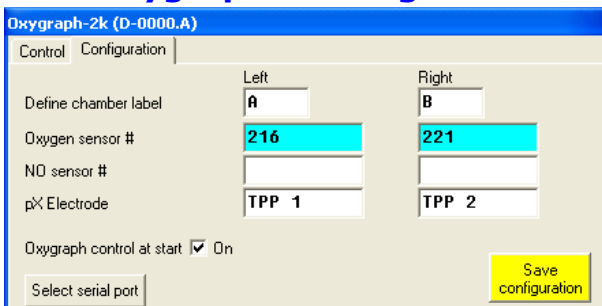On your PC screen, click on the icon "Oroboros DatLab" to start the program.

Oroboros DatLab.

✔ Connect to O2k at start: The O2k Control window opens automatically by default.
Alternatively, open O2k Control by pressing [F7].

O2k-MultiSensor	TIP	?
O2k Control		F7
✔ Connect to O2k at start		
Calibration		F5
Left Stirrer On/Off		F11
Right Stirrer On/Off		F12
Stirrer test		F9
Light On/Off		F10
Manage Setups		

Connection

○ Serial Port [▼] ⦿ USB Port [E-0002 ▼]

Information
Please select a proper serial port to talk to your Oxygraph-2k.

[Cancel]   [OK]

To connect the O2k to DatLab (the USB cable is installed), 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 off-line operation of DatLab.

6.1. Oxygraph - Configuration

Oxygraph-2k (D-0000.A)

Control Configuration

	Left	Right
Define chamber label	A	B
Oxygen sensor #	216	221
NO sensor #		
pX Electrode	TPP 1	TPP 2

Oxygraph control at start ☑ On

[Select serial port]   [Save configuration]

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.


Oxygen sensor #: The polarographic oxygen sensor (OroboPOS) number is entered for each chamber. Usually, sensors should not be switched between chambers, except for instrumental diagnostic checks. The individual sensor number is included in the protocol for generating a database of calibration values.

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

Save Configuration: Click on <mark>Save configuration</mark> 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. Oxygraph – Control [F7]
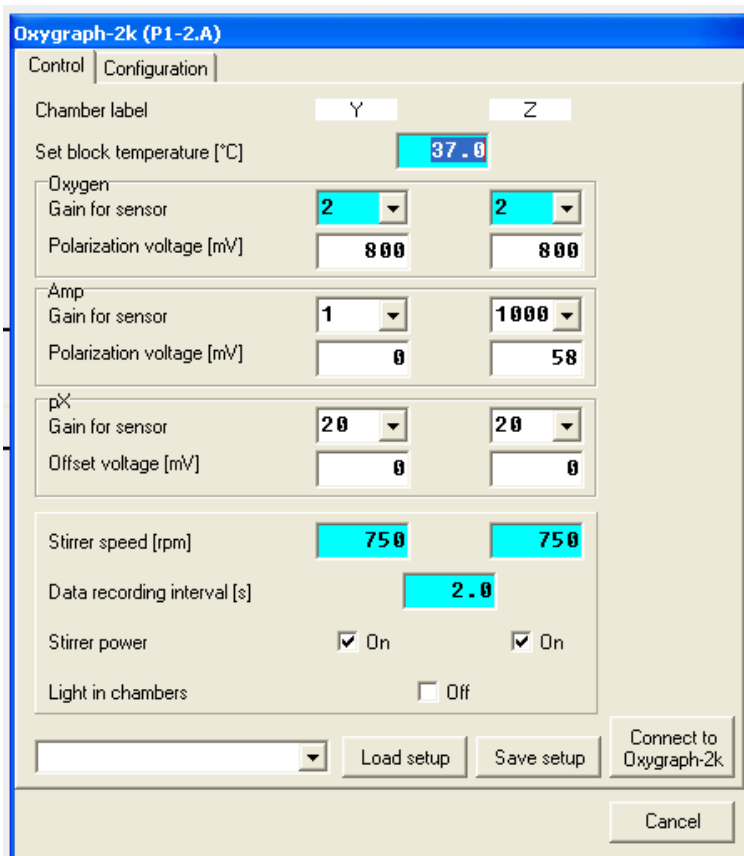
In the "Oxygraph" menu, Control is used to control the Oxygraph-2k operation mode, which is set to default at start. These settings are edited either step-by-step, or by ▼ selecting an O2k-Setup.

The new controls for the O2k (temperature, gain settings, polarization voltage, stirrer speed and data recording interval) and settings used upon connect (stirrer power and light) are displayed only after <mark>Load setup</mark>.

The initial default is "O2k-Setup 25 °C". Subsequently, the default is the O2k-Setup which was applied last when the PC was connected to the Oxygraph-2k.

Settings of various setups may be viewed at this stage. Activate the displayed control settings by clicking on the Connect to Oxygraph-2k button.

Step-by-Step Setup of O2k Control: Chamber label: The chamber labels are displayed in the Control window, and can be edited in the Configuration or Experiment Edit [F3] windows.

Set 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 45.0 °C).

Gain for oxygen sensor: Can be changed for signal amplification in specific cases. The default is a gain of 2 V/µA; ▼ options are 1, 2, 4, or 8 V/µA amplifications. In particular, use a lower 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 (e.g. at low temperature).

Polarization voltage [mV]: The default for the POS is 800 mV, which is maintained in all routine experiments. Any changes will effect the calibration. The voltage can be modified in the range of −2,000 to +2,000 mV for specific applications, particularly for scanning the plateau region of the polarographic oxygen sensor, or with different types of sensors. An identical voltage applies to both chambers.

Amp and pX: The settings available in the Amp and pX panels are described in **O2k-Fluorescence LED2-Module MiPNet17.05**, **O2k-NO Amp-Module MiPNet15.05**, and **O2k-MultiSensor-ISE MiPNet15.03.**

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, always identical for both chambers. Rigorous tests revealed that data recording intervals <1 s do not increase the information. The default of 2 s is an optimum 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 on-line 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: After clicking on Connect to Oxygraph-2k, recording starts writing data into a specific DatLab data file.

6.3. Connect and Save DLD File

In an automatically opened Save window, a default file name is suggested in the form: **2010-07-27 AB-01**

Year-Month-Day AB- (=chamber A and B) **01** (=sequential number of the experiment). The extension **DLD** stands for **D**at**L**ab **D**ata.

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 Oxygraph-2k through the serial interface. Data recording and saving is initiated automatically.

Successful connection can be checked on the 'Comm' diode of the Oxygraph-2k, which now must be on in green, and in the O2k status and signal lines at the bottom of the screen.

6.4. Edit Experiment [F3]

While the Oxygraph equilibrates to the new control values, as apparent from the displayed signals, the "Edit Experiment" window is opened automatically after Connect and Save. This window contains information for the experimental protocol and calibration parameters. All entries can be edited at any time during the experiment by pressing [F3], and all related results will be re-calculated instantaneously with the new parameters.

Reset to system default: Initially, the "Edit Experiment" window displays information from the experiment saved last in the on-line mode. The Reset buttons are used to reset these entries to system default values.

Cancel: Click on Cancel if you want to proceed quickly with the experiment, and edit experimental details later.

Experimental code: Is defined (10 digits) to collectively group a series of experiments. The File Search function[Ctrl+F] lists all files with identical experimental code within a selected directory.

Chamber label: The left and right chamber of the Oxygraph-2k can be edited here, without effect on the current file name.

Sample: An abbreviated label for the specific sample used in each chamber is entered here for the protocol. ("Medium" indicates that no sample has been added into the chamber, typical for calibration experiments.)

Unit: ▼ Select one of the units commonly used to express the concentration or amount of sample in the Oxygraph assay.

Million cells▼
mg   ▼ - mg of protein, wet weight or dry weight.
Units   ▼ - a respiratory reference flux [pmol·s^{-1}·ml^{-1}], to normalize flux to a respiratory reference state; or units of enzyme activitiy, to which respiratory oxygen flux is related to.

Concentration: If you apply a concentration of sample in the assay (e.g. Million cells per ml, or mg mitochondrial protein per ml), then enter the sample concentration. The corresponding amount of sample is calculated on the basis of the chamber volume.

Amount: If you add a known amount of sample into the sample chamber (e.g. a known weight of a biopsy sample), then enter the sample amount. The corresponding sample concentration is calculated on the basis of the chamber volume shown above.

Medium: Edit the name of the incubation medium used in the Oxygraph chambers.

Chamber volume: The default is 2.00 ml. It is important to define the actually used effective volume of the Oxygraph chamber, since this entry for the volume is used in further calculations of oxygen flux.

Background correction: Edit the parameters for the background correction of oxygen flux, based on a linear relation between background oxygen flux and oxygen concentration. Parameters can be copied from another DatLab file, so that they have to be edited only once in a series of experiments.

$a°$ Intercept [$pmol·s^{-1}·ml^{-1}$]. The default is -2.00.

$b°$ Slope. The default is 0.025.

In many applications, background correction based on the default parameters provides sufficient accuracy of respiratory oxygen flux. Background correction is applied to the plots for oxygen flux, except when Slope uncorrected is selected.

Importantly, when the chamber is open for equilibration with a gas phase (air) for calibration or for zero calibration, then the plot "Uncorrected slope" should be selected to evaluate signal stability by observing the zero slope of the oxygen signal.

Further information: MiPNet12.09 and MiPNet14.06.

Flux derivation N: ▼ Select the number of data points (N = 40 to 5 in intervals of 5) used to calculate the slope through a polynomial fit, as a basis for the plot of flux. A high value of N yields a highly smoothed curve, whereas a low value of N improves time resolution.

Data recording interval [s]: The data recording interval is displayed (read only); it is selected in the Oxygraph Control window [F7]. A short data sampling interval increases the time resolution and noise of flux, which may be compensated for by selecting a low value of N for flux derivation.

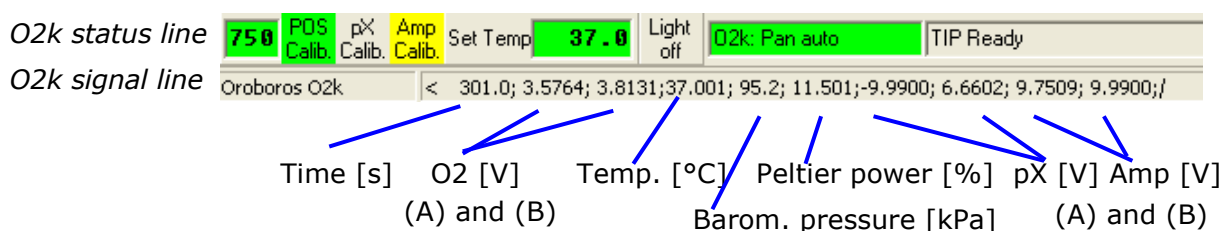**Calibration:** Information is displayed (read only), according to the last on-line calibration MiPNet12.08.

☞ ☞ Before performing an Oxygen calibration [F5], the oxygen concentration [µM=nmol/ml] is displayed on the basis of default calibration seetings. These default settings must be replaced by a proper calibration (MiPNet12.08). The (negative) slope is not corrected for

instrumental background, whereas the oxygen flux per volume is corrected on the basis of default settings for instrumental background correction. Volume-specific flux is displayed in units of $pmol \cdot s^{-1} \cdot ml^{-1}$ and, like the oxygen concentration, is accurate only after oxygen calibration and editing the instrumental background parameters (MiPNet12.09).

Comments   - are written for display and printing in the 'View Protocol' window.

Save   - to save the changes and close the 'Edit' window.

6.5. O2k-Signal Line

O2k status line

O2k signal line

Time [s]   O2 [V]   Temp. [°C]   Peltier power [%]   pX [V] Amp [V]

(A) and (B)   Barom. pressure [kPa]   (A) and (B)

Time [s]:   Time of the experiment since connecting, displayed on the *X*-axis as [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. [°C]: Block temperature, continuously recorded, but not displayed as a plot in the standard layout.
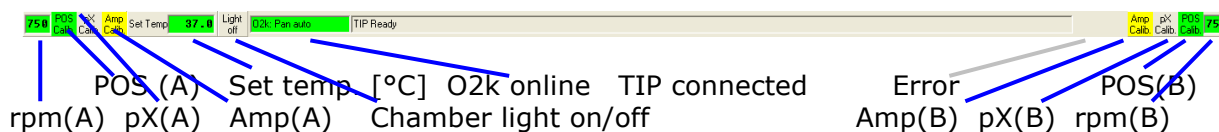
Barom. Pressure [kPa]: Barometric pressure, continuously recorded, but not displayed as a plot in the standard layout.

Peltier Power [%]: Operative Peltier power applied for temperature regulation of the Oxygraph-2k block, continuously recorded, but not displayed as a plot in the standard layout.

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.

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

6.6. O2k-Status Line

POS (A)   Set temp. [°C] O2k online   TIP connected   Error   POS(B)
rpm(A) pX(A)   Amp(A)   Chamber light on/off   Amp(B) pX(B) rpm(B)

750 rpm(A) and (B): Indicates the set stirrer speed [rpm] in the left and right chamber, respectively. The background is green if rotation power is on the set speed, yellow if the stirrer is accelerating, and red (*Off*) 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 on-line. 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 on-line or off-line.

Set temp. 37.0: Indicates the temperature setting sent from the Oxygraph Control window, green if the measured temperature is within ±0.02 °C of the set temperature, but yellow if the difference is more than ±0.02.

Light off / Light on: Indicates wheter 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 Oxygraph-2k 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 (*Start at*) is moved upwards as the experimental time proceeds.

O2k: Pan off: Yellow indicates that the Oxygraph-2k is connected to DatLab, but automatic panning is switched off, and the plots are shown in static display. This allows for manually panning the time axis 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 and Disconnect

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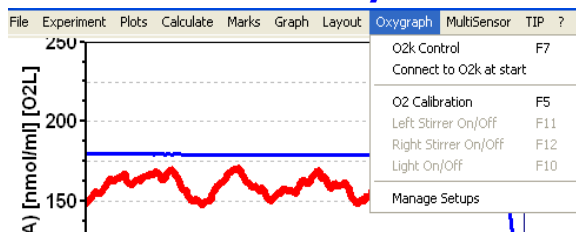
After selection of an Oxygraph-Setup in the Oxygraph Control [F7] window, followed by Connect, only the following three control functions are routinely required during experimental operations.

Calibration [F5]: Opens the calibration window for the curentyl active plot, see DatLab Oxygen and MultiSensor Calibration **MiPNet12.08**.

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]: Opens a window to enter the duration of a stirrer test (Default: 30 s). After clicking "OK", stirring will be deactivated in both chambers and reactivated after the chosen time.

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 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 before Connect to Oxygraph-2k.

Send to Oxygraph-2k: The control settings displayed in the Control window are sent to the Oxygraph-2k, 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 off-line mode. Upon disconnect, the O2k control keys of DatLab are inactivated, and the COM diode on the Oxygraph-2k switches from green to yellow. You may now switch off the Oxygraph-2k with the mains switch 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.

Appendix: O2k Series A to C

Glass chamber and OroboPOS assembly (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.
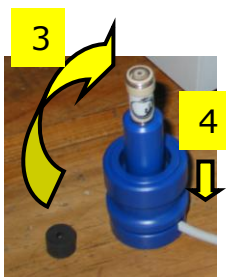

3 Clean the POS connector and screw the POS head onto the POS connector. Then apply a membrane to the POS (polarographic oxygen sensor; see MiPNet08.04), and add a POS seal tip with the pore centrally positioned.

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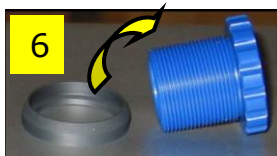
6 Slip the fixation ring onto the top screw of the chamber holder, with the conical side facing upwards and the flat bottom downwards.

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.
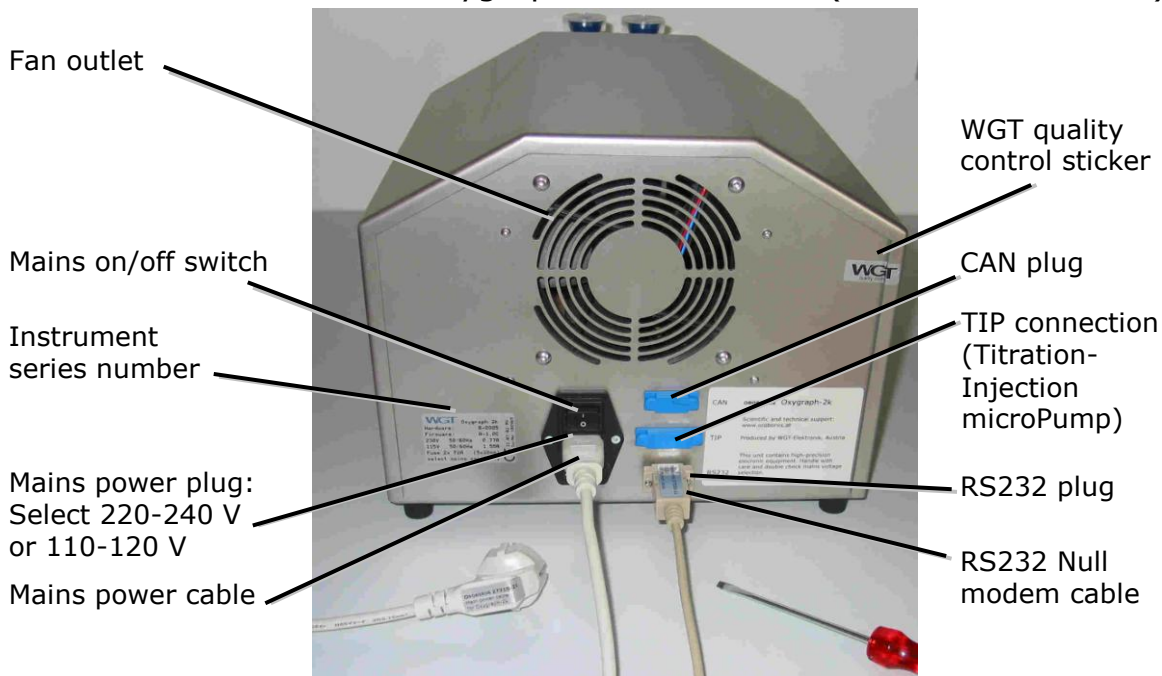

2.2.   Chamber volume calibration

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 slighly 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 µl 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.
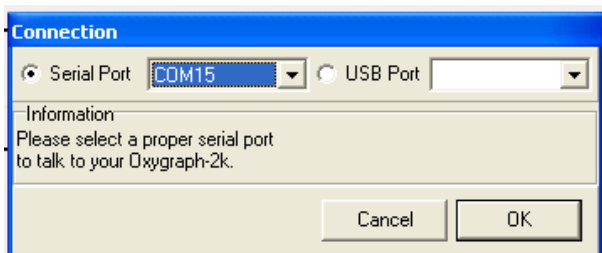
Start-up (O2k Series A-D)

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).

Fan outlet

WGT quality control sticker

Mains on/off switch

CAN plug

Instrument series number

TIP connection (Titration-Injection microPump)

Mains power plug: Select 220-240 V or 110-120 V

RS232 plug

Mains power cable

RS232 Null modem cable

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

Fan outlet

WGT quality control sticker

Mains on/off switch

CAN plug

Instrument serial number

Mains power plug: Select 220-240 V or 110-120 V

RS232 plug

RS232 Null modem cable

Mains power cable

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.