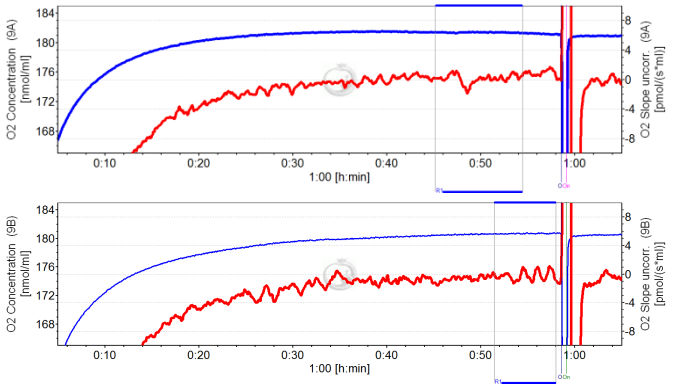
Mitochondrial Physiology Network 12.08(08): 1-8 (2014)
O2k-Manual D: www.bioblast.at/index.php/MiPNet12.08_O2k-Calibration

Version 8: 2014-03-04

Oxygen calibration by DatLab

Gnaiger E

OROBOROS INSTRUMENTS Corp
high-resolution respirometry
Schöpfstr 18, A-6020 Innsbruck, Austria
Email: erich.gnaiger@oroboros.at
www.oroboros.at



OROBOROS Oxygraph-2k

Section

Page

Summary: Accurate calibration of the oxygen sensor depends on *(1)* calibration solutions prepared at known oxygen partial pressures, as achieved in the Oxygraph-2k (O2k) at defined temperature, continuously recorded total gas pressure (barometric pressure), and thermodynamic equilibrium between the gas and aqueous phase; *(2)* high stability of the signal of the polarographic oxygen sensor (POS), tested for sufficiently long periods of time; *(3)* linearity of signal output with oxygen pressure, achieved with the POS in the range between oxygen saturation and zero oxygen pressure; and *(4)* application of accurate oxygen solubilities for aqueous solutions for the conversion of partial oxygen pressure into oxygen concentration (MiPNet06.03 with O2k-SOPs). The standard oxygen calibration procedure is described for O2k high-resolution respirometry with the automatic calibration routine by DatLab.

D

1. Experimental oxygen calibration

The polarographic oxygen sensors (OroboPOS, POS) are calibrated by a two-point calibration, routinely achieved at air saturation and zero oxygen concentration. Accordingly, static calibration involves the determination of the constant signal of the POS recorded at 0% and 100% air saturation (R_0 and R_1) under the particular experimental conditions (temperature, signal amplification by electronic gain, polarization voltage, stirring speed, medium).

1.1. Air calibration

O2k-SOP: Air saturation is achieved after cleaning by stirring the medium without sample in the chamber in contact with air, following the procedures below and in MiPNet06.03:

1. Add incubation medium into the chambers, using the experimental chamber volume and an excess to fill the injection capillary of the stopper (c. 100 mm^3). The excess volume does not have to be accurate, as long as it is above the minimum volume. Switch on the stirrers either during or after addition of the medium.

2. Insert the stoppers slowly to their volume-calibrated position (MiPNet12.06). Siphon off excess medium ejected through the injection capillary and collected in the well of the stopper. Then lift the stoppers using the stopper-spacer tool, leaving a gas volume above the liquid phase for final air equilibration (Fig. 1).

Figure 1. Stopper-spacers used for air calibration with the PVDF stopper (Chamber A) or titanium stopper (Chamber B).

The central level of the gas phase should remain above the rotating stirrer bar. No foam bubbles must be formed which block gas exchange. This gas volume has to be renewed (exchanged for air) if the medium originally was not near air saturation, to ensure a well defined p_{O_2} in the gas phase during equilibration. Equilibration is a slow process, but stability should be reached within one hour (Fig. 2).

3. After stabilization of the POS signal, R_1 must be <10 V. At a Gain of 1, the recorded signal at air saturation is about 2-3 V, and a R_1 signal of 1 V (corresponding to 2 V at Gain 2) corresponds to a signal current of the POS of 1 µA.

Continue recording for >3 min to check for signal stability. You may proceed at this point with a stirrer test [F9] (MiPNet06.03) and O2k-background control (MiPNet14.06).

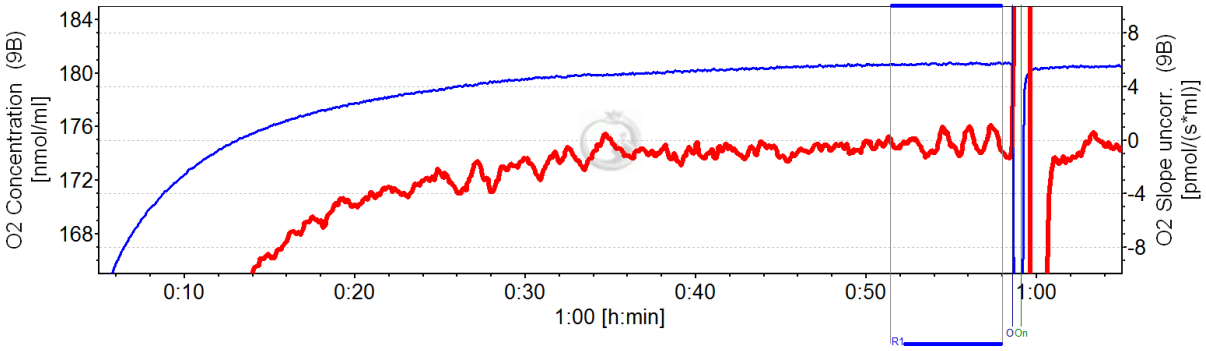


Figure 2. Oxygen concentration (blue plot; full scale 20 nmol/ml or 20 µM) over 1 h after switching on the Oxygraph-2k (Power-O2k P9, chamber B) at room temperature and 37 °C experimental temperature, with medium stirred for equilibration with a gas phase of air at 575 m altitude. The red plot is the negative slope of oxygen concentration over time [pmol·s^{-1}·ml^{-1}] on the right *Y*-axis, with zero in the middle position. A slope of zero (for 'O2 Slope uncorrected') indicates a constant O_2 signal over time. 2014-02-19 P9-01.DLD

1.2. Zero oxygen calibration

4. Zero oxygen calibration is achieved best with mitochondria or cell suspensions by allowing complete oxygen depletion. Alternatively, titrate zero solution (Na-dithionite, OroboPOS-Service Kit) into the chamber. The zero signal, R_0, is recorded after stabilization. R_0 should be <2% of the signal at air saturation, but <5% is acceptable, and most importantly the zero signal must be stable.

go Bioblast » MiPNet06.03 POS-Calibration-SOP

2. General notes on calibration in DatLab

☞ Before completing the Oxygen calibration [F5] in DatLab, oxygen concentration [µM=nmol/ml] is displayed on the basis of default calibration seetings. These default settings must be replaced by a proper calibration. Volume-specific flux [pmol·s^{-1}·ml^{-1}] is accurate only after oxygen calibration and editing the instrumental background parameters (MiPNet12.09).

DatLab calibration window: Select a single plot by clicking onto the label of the plot in the figure legend on the right of the graph. [F5] opens the calibration window for the selected channel. The selected plot determines the type of channel opened for calibration.

D

DatLab calibration: connected vs. disconnected: DatLab uses calibration values applied real-time (connected to the O2k, recording data) as default values for future experiments. When calibration values are edited in the disconnected mode, they apply only to the current file and will not be used as a new default. This allows to re-calibrate old files without overwriting the current default values for calibration. Ideally, calibration values that should be used as new defaults are applied real-time when the experiment is still running. However, if the DatLab-calibration is performed after disconnecting, these calibration parameters can be read into DatLab using the **Copy from file** function and clicking on **Calibrate and Copy to Clipboard** the next time DatLab is connected.

3. DatLab-calibration of oxygen sensors

Before disconnecting the Oxygraph-2k from DatLab, calibration information is automatically saved and available upon connecting the Oxygraph-2k, even if you exit DatLab and start the program again. This calibration information is displayed in the Edit Experiment window [F3], which is opened automatically after pressing the Connect button in the Oxygraph Control window [F7].

Application of default values does not provide accurate calibrations in general. Default calibration values must be replaced by experimental calibration values, whenever sufficient stability of the calibration cannot be assumed, or when previous calibration conditions do not apply: Calibration source: Active file,

Calibration

Source	Active file		Active file	
R1 / R0 [V]	5.2920	0.0158	4.0104	0.0138
Calib. temp. [°C]	37.0000		37.0000	
Pressure [kPa]	100.20		100.2000	
FM	0.920		0.9200	

In DatLab, the oxygen signal can be re-calibrated at any time during the experiment. The recorded raw signal, R_t, is converted to oxygen concentration, $c_{O_2,t}$ [µM], or partial pressure, $p_{O_2,t}$ [kPa or mmHg].

Calibration with DatLab merely requires *(1)* setting marks on defined calibration sections of the oxygen plot, or *(2)* retrieving calibration information from independently recorded calibration, which may be stored as default, and *(3)* information on the oxygen solubility of the medium in relation to pure water. The digitally recorded barometric pressure and temperature are automatically applied in algorithms for oxygen calibration.

3.1. Graph layout for calibration

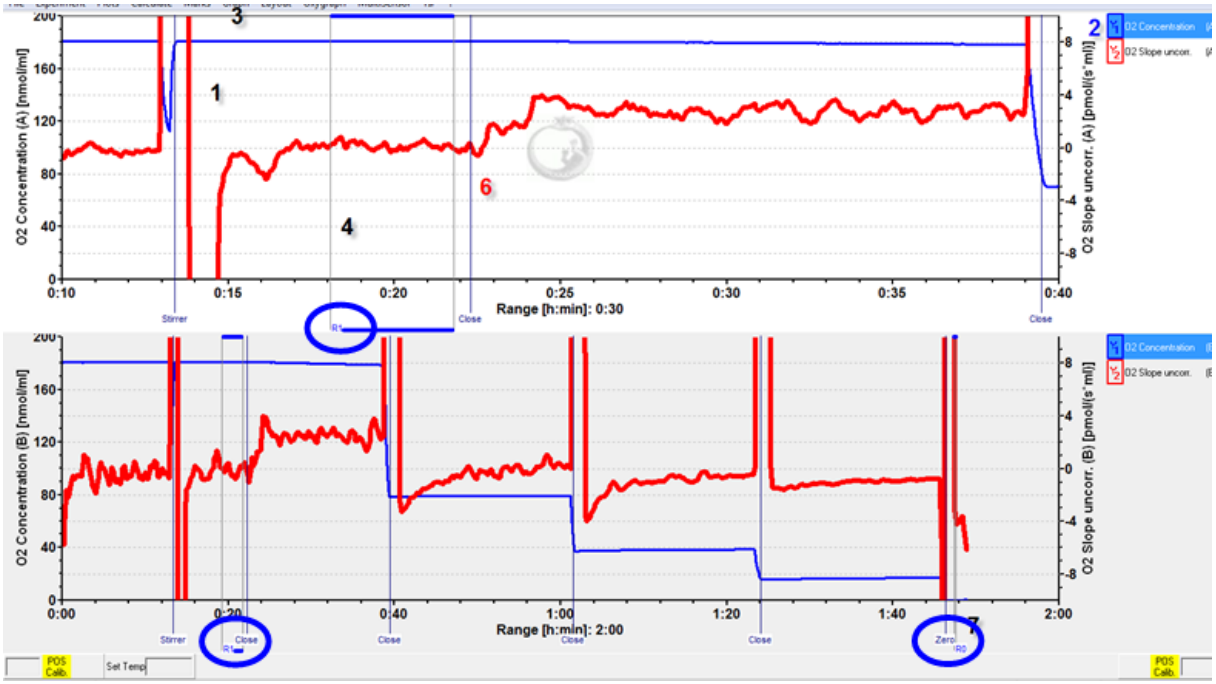
Graph Layout: "**01 Calibration Exp. Gr3-Temp**" » Supplement A

Three graphs are shown, displaying O_2 concentration and O_2 slope uncorrected for the left (A) and right (B) chamber, and temperature and Peltier power.

3.2. Mark

Mark a section of the experiment at air saturation, when signal stability is reached. This should be done real-time to save default calibration information. Corrections are possible after disconnecting from the O2k. For calibration, follow steps (1) to (7) illustrated on the following graph:

Graph Layout: "**02 Background Experiment**" » Supplement A



1. Select a graph by clicking with the left mouse button into the graph, or directly by step 2.
2. Select the oxygen signal as the active plot by clicking on Y_1 in the figure legend on the right of the graph. The active plot is highlighted.
3. Activate the "marking mode" of the curser by either selecting "Mouse Control: Mark" in the Graph menu, or pressing [Ctrl+M].
4. Set a mark: Hold [Shift], click the left mouse button and move the cursor along the time axis. Remove a section of the mark or the total mark: Holding [Shift], click the right mouse button and move the cursor along the time axis.
5. Rename the mark: Left mouse click on the bar of the mark. Rename the mark for air calibration as "R1", and the mark for zero calibration as "R0".

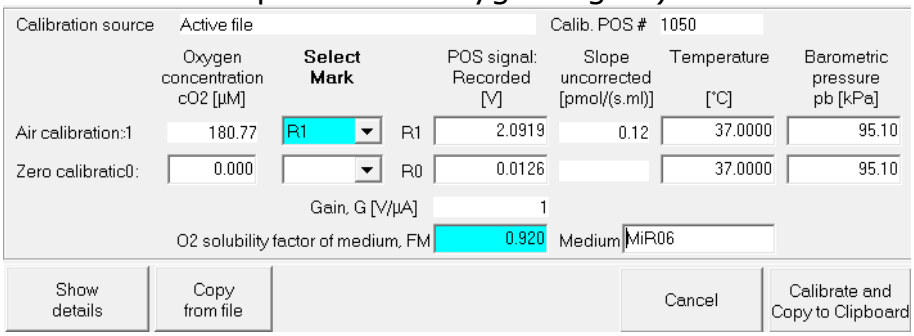
D

6. Observe events (set by [F4]) which indicate particular titrations or any events which are of interest. In this example, the Event "Close" indicates that the chamber was closed, thus terminating the air calibration phase. Events provide important guidelines for setting and editing calibration marks.
7. Multiple marks may be set.

3.3. O2 calibration [F5]: edit

Select a plot for oxygen concentration and press [F5] to open the calibration window.

O2 concentration (A): Indicates that calibration is performed for the oxygen signal in chamber A (the selected graph with the active plot of the oxygen signal).



Calibration source: The file in which the last calibration has been performed real-time with DatLab connected to the O2k.

POS #: The OroboPOS number is displayed as entered in the Oxygraph-2k Configuration window [F7] for each chamber. Usually, sensors are not switched between chambers, except for troubleshooting.

Select Mark ▼ **R1** for "Air calibration": Click on the pull down button and select the appropriate mark (R1).

▼ **R0** for "Zero calibration": Click on the pull down button and select the appropriate mark (R0). Many times the zero calibration value is used from a previous experiment.

O_2 solubility factor of medium, F_M: Enter the oxygen solubility factor of the medium, F_M, relative to pure water.
More details: » MiPNet06.03 POS-Calibration-SOP

Medium: Enter the name of the experimental incubation medium.

c1: The O_2 concentration at air saturation [µmol $O_2 \cdot dm^{-3}$ = µM = nmol$\cdot cm^{-3}$], $c_{O_2}^*$, calculated as a function of temperature, barometric pressure, and oxygen solubility factor of the medium.

c0: The known O_2 concentration at the second calibration point. The most practical c_0 is zero oxygen concentration.

Copy from file: Calibration parameters can be copied from a file in which a calibration has been performed.

Calibrate and Copy to clipboard: After clicking on this button, the entire plot of oxygen concentration is re-calibrated [µM = nmol/ml], and the corresponding negative slope or volume-specific oxygen flux [pmol·s^{-1}·ml^{-1}] is now based on this new calibration. Calibration parameters are automatically copied to clipboard for entry into the "O2-Calibration-List"

Cancel: Press cancel to exit the calibration window without saving any changes.

3.4. O2 calibration: calculations by DatLab

In the window c_1 (left), the oxygen concentration, c_{O2} [µM], is shown as calculated for air saturation under experimental conditions. If a mark is selected (▼ for R1 and/or R0) the average voltage (Raw signal [V]) recorded over the marked section is shown in the corresponding window on the right. The corresponding signal stability is displayed as the uncorrected negative slope of the signal during calibration, Slope uncor. [pmol·s^{-1}·ml^{-1}]. Temperature and barometric pressure are displayed as measured over the marked section.

Calibration values R_1 and R_0 can be edited numerically, without exerting an influence on c_1. If temperature or barometric pressure are edited, then c_1 is recalculated for the changed conditions.

In many cases, the zero calibration is performed in a different run saved as a separate file. If this zero calibration should apply to previous runs, then the corresponding R0 (Raw signal [V]) is edited numerically. Temperature and pressure do not have to be entered, since they are without influence on the calibration calculations for zero oxygen.

Reset to system default: Use only if previously entered and calculated parameters do not make sense.

Details: All oxygen calibration parameters are displayed as calculated by DatLab and as saved in clipboard upon calibration.

go Bioblast » MiPNet06.03 POS-Calibration-SOP

D

4. References

Forstner H, Gnaiger E (1983) Calculation of equilibrium oxygen concentration. In: *Polarographic Oxygen Sensors. Aquatic and Physiological Applications*. Gnaiger E, Forstner H (eds), Springer, Berlin, Heidelberg, New York: 321-333. »

Gnaiger E (1983) The twin-flow microrespirometer and simultaneous calorimetry. In: *Polarographic Oxygen Sensors. Aquatic and Physiological Applications.* Gnaiger E, Forstner H (eds), Springer, Berlin, Heidelberg, New York: 134-166. »

Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 128: 277-297. »

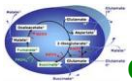
Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dykens JA, Will Y, eds) John Wiley: 327-352. »

Gnaiger E, Steinlechner-Maran R, Méndez G, Eberl T, Margreiter R (1995) Control of mitochondrial and cellular respiration by oxygen. J Bioenerg Biomembr 27: 583-596. »

Steininger C, Allerberger F, Gnaiger E (2002) Clinical significance of inhibition kinetics in *Streptococcus pyogenes* in response to penicillin. J Antimicrob Chemother 50: 517-523. »

O2k-Manual

» MiPNet12.06 Oxygraph-2k: start high-resolution respirometry.
» MiPNet12.09 Oxygen flux analysis: DatLab real-time.
» MiPNet15.03 O2k-MultiSensor system with ion selective electrodes (ISE).
» MiPNet15.05 O2k-Manual: amperometric sensors (NO).

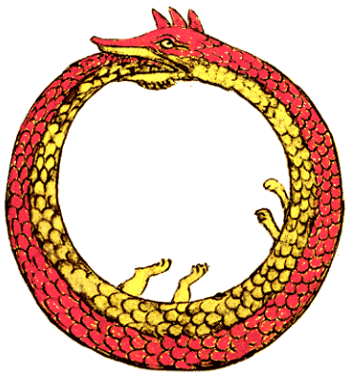


O2k-Protocols: O2k-SOPs

» MiPNet06.03 POS calibration SOP.
» MiPNet14.06 Instrumental background and accuracy of oxygen flux.

Full version: *go Bioblast*
» www.bioblast.at/index.php/MiPNet12.08_O2k-Calibration
Next step – O2k-Manual E » MiPNet12.09 O2 Flux Analysis

D



Supplement A. Graph Layout

Graph Layout: "**01 Calibration Exp. Gr3-Temp**"

Calibration experiment with temperature and Peltier power in Graph 3.

This is typically the first layout used after switching on the O2k. Oxygen concentration (blue lines, left Y-axis) and O2 slope uncorrected (red lines, right Y-axis) are displayed on the top graph for the left chamber, and below for the right chamber. The third graph (bottom) shows the block temperature on the left Y axis and the Peltier power on the right Y axis. Only when both temperature and Peltier power are constant, the chambers have reached thermal equilibrium. The next step is to observe equilibration of the oxygen signal with a defined gas phase above the stirred aqueous phase ('open' chamber; usually with air as the first step) to perform an oxygen calibration.

If anything unusual is observed (always zero flux, jumping signals), the layout "Z Trouble Shooting" should be used.

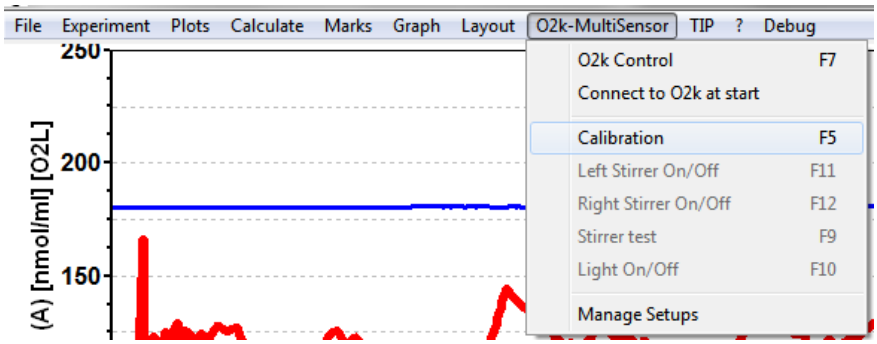
Graph Layout: "**02 Background Experiment**"

For recording O2 sensor calibration and a test for instrumental background oxygen flux.

For each chamber, 'O2 Concentration' and 'O2 Slope uncorrected' are displayed on the left and right Y-axis, respectively. 'O2 Slope uncorrected' is the negative slope of oxygen concentration (multiplied by 1000 to convert to units [pmol/ml]) over time [s]. No correction is applied for instrumental background oxygen flux. Zero flux in the 'open' chamber at air calibration indicates stability of the oxygen signal. After closing the chamber, 'O2 Slope uncorrected' deviates from zero as a function of the oxygen consumption of the polarographic oxygen sensor and of oxygen diffusion into or out of the chamber.

Supplement B. DatLab 5.2.

O2k-MultiSensor – Calibration [F5]



Pulldown menu

Linear calibration (two-point or linear regression for multiple calibration steps) for any parameter measured with the Amp or pX channel.

Example: pH Calibration

Linear calibration of pH as a function of recorded voltage is performed by a two-point calibration, using two pH calibration buffers, pX_0 and pX_1 (where, for example, pH_0 may be 7.0 and pH_1 may be 4.0; deviations of the actual pH of the calibration buffers from these values are due to experimental temperature).

See (MiPNet08.16), for further details. The corresponding raw signals are recorded, R_0 and R_1 (-0.0479 V and 5.4161 V).

The calibration factor, F, is

Eq. 6. $$F = \frac{pX_1 - pX_0}{R_1 - R_0}$$

The offset, d, is

Eq. 7. $$d = \frac{pX_0 \cdot R_1 - pX_1 \cdot R_0}{R_1 - R_0}$$

Calibration of the recorded signal at any time t, R_t, then uses the relation

Eq. 8. $$pX(t) = (R_t - a) \cdot F$$

Calibration of TPP^+ electrodes: » MiPNet15.03.