O2k-Manual: O2k Quality Control 2

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Updates: http://wiki.oroboros.at/index.php/MiPNet14.06 Instrumental O2 background

O2k Quality Control 2:

Instrumental oxygen background correction and accuracy of oxygen flux

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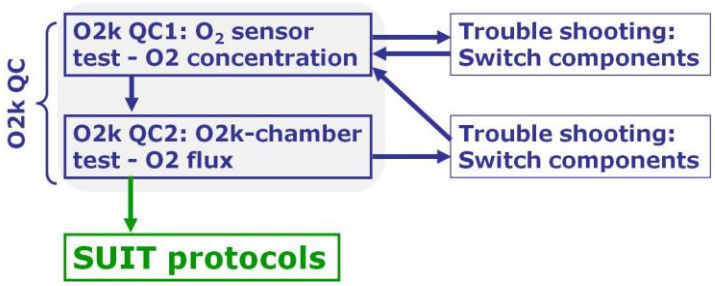
Summary: Correction for instrumental background oxygen flux is a standard in high-resolution respirometry (HRR), automatically performed by the software DatLab. Background measurements provide a quality control of instrument function. In the Oroboros O2k, background corrections are usually within a few % of experimental flux

over the entire experimental oxygen range. At minimum activities, however, even the small background effects become significant and require compliance with standard operating procedures (SOPs) described in this chapter as part two of O2k Quality Control (DatLab).

1. Introduction

For calibration of the polarographic oxygen sensor (POS) and measurement of instrumental background oxygen consumption, incubation medium without biological sample is added to the O2k-chamber at experimental conditions. In a closed chamber under these conditions, oxygen concentration ideally remains constant. In practice, however, instrumental background effects are caused by back diffusion into the chamber at low oxygen pressure, oxygen diffusion out of the chamber at elevated oxygen levels, and oxygen consumption by the POS. Determination of instrumental background constitutes an important SOP in HRR. Instrumental background oxygen flux is (i) minimized in the Oroboros O2k by instrumental design and selection of appropriate materials; (ii) is routinely tested, and (iii) background correction of oxygen flux is applied automatically by DatLab.



As an important component of quality control, instrumental background is monitored at regular intervals during a project and documented as a SOP to exclude instrumental artefacts. This SOP is implemented even in cases of high experimental oxygen concentrations when background correction is merely within 1 %-5 % of oxygen flux. Taken together, the concept of instrumental background oxygen flux and appropriate corrections are indispensable components of quality control in HRR. To obtain accurate parameters for instrumental O_2 background correction, instrumental tests are performed in which several oxygen levels are set in the closed O2k-chamber, in order to cover the whole range of the experimental oxygen regime, and background oxygen flux is measured as a function of oxygen concentration.

The instrumental background test needs to be performed after a new setup, POS service or every assembly/disassembly and volume calibration of the chambers. As an example, in Oroboros MitoFit lab the instrumental background test is performed every month to keep high quality standard.

2. Preparations

2.1. Solutions

Dithionite solution (30 mM or 10 mM, in phosphate buffer)*

Component	Final conc.	FW	Addition to 10 mL final
$Na_2S_2O_4$	30 mM	174.1	0.051 g
$Na_2S_2O_4$	10 mM	174.1	0.017 g

Phosphate buffer (50 mM, pH 8)

	Final conc.	Component	FW	Addition to 1 L final
Base	44 mM	$Na_2HPO_4 \cdot 2\ H_2O$	178.0	7.83 g
Acid	5.9 mM	$NaH_2PO_4 \cdot H_2O$	138.0	0.81 g

Dithionite solution is prepared freshly. Add 51 mg dry dithionite into a 10-mL volumetric glass flask. Add phosphate buffer up to 10 mL final. Keep the flask closed. Minimize air exposure.

*Note: Up to Version MiPNet14.06(03) a dithionite concentration of 10 mM was used. Instrumental O2 background experiments showed identical results with 10- and 30-mM dithionite stocks. However, when using new commercial bottles of dithionite, 30 mM may be a too high concentration, in which case we recommend using 10 mM dithionite.

2.2. Media

The dithionite background experiment should be performed in mitochondrial respiration medium MiR05, MiR05-Kit or MiR06 (add catalase to obtain MiR06). In many other media (including cell culture media and unbuffered water), side reactions lead to additional oxygen fluxes which interfere with the instrumental background oxygen flux. As an alternative, a strongly buffered alkaline phosphate buffer may be used (>100 mM; >pH 8). Instrumental O_2 background parameters obtained in mitochondrial respiration medium can be used for experiments with other media (e.g., cell culture media).

2.3. Calibration of oxygen sensors

» MiPNet06.03 POS-Calibration-SOP

» Run DL-Protocol/Set O2 limit

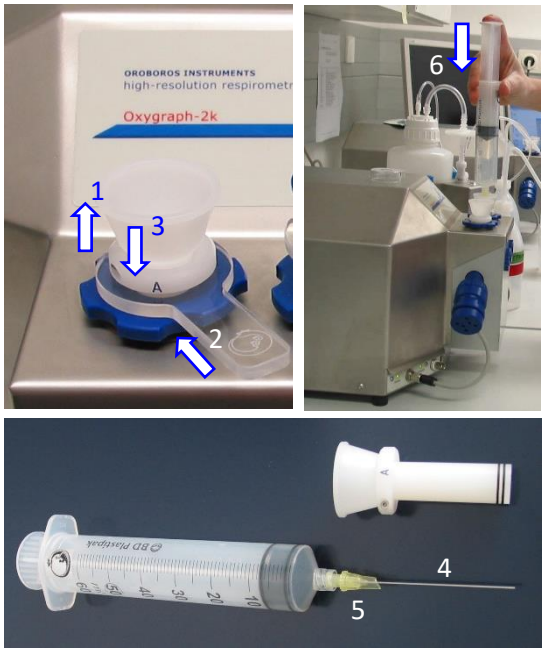
2.4. Setting oxygen concentration in the O2k-chamber

Instrumental background tests should cover the entire experimental oxygen range. Most experiments are performed at oxygen levels at or below air saturation (Gnaiger 2001), but artificially elevated, high oxygen levels are used with permeabilized fibers (Doerrier et al 2018).

H_2O_2: With MiR06 (add catalase to MiR05), oxygen concentration is easily adjusted by injecting small amounts of a H_2O_2 stock solution into the closed chamber. Oxygen levels are increased in steps of <200 µM (e.g., from air saturation up to 350 µM) to avoid formation of gas bubbles in the medium.

O_2 (gas phase): Graded levels of oxygen can be achieved in instrumental background tests with the aid of a gas phase included in the O2k-chamber, replacing air with hydrogen, nitrogen, or argon (to decrease oxygen levels), or with oxygen (to increase oxygen levels). Mass transfer between gas and liquid phases proceeds until the targeted oxygen level is reached. This process is stopped when the gas phase is eliminated by closing the chamber (Gnaiger et al 1995; Gnaiger 2008).

For increasing oxygen concentrations above 400 µM, the preferred approach is the application of a gas phase with a high oxygen content. If a calibration at air saturation was just performed, there is already an 'open chamber', i.e., a chamber with a gas phase. Insert the stopper, completely closing the chamber. Siphon off any medium extruded through the stopper capillary. Then, partially open the stopper (arrow 1), insert the stopper-spacer tool (2) and push down the stopper (3). The gas injection syringe with supplied needle (4; correct length) and spacer (5) is filled with oxygen gas. Inject a few mL of oxygen into the gas phase (6), thereby creating an elevated oxygen pressure above the stirred aqueous medium. Oxygen in the gas and aqueous phases will start rapidly to equilibrate.



Observe the oxygen signal in DatLab carefully. When the targeted oxygen concentration is nearly reached, close the chamber, thereby displacing the gas phase and stopping the equilibration process. After stabilization of oxygen flux, the first state of background flux is recorded, by marking an appropriate section of the oxygen flux (MitoPedia: Marks - DatLab). Further steps of oxygen levels towards air saturation may be achieved by shortly opening the stopper (again using the stopper-spacer tool, 2), observing the decline of oxygen concentration and closing the chamber at the targeted oxygen level. Preferentially, use dithionite as in the method described below.

The main disadvantage of intermittently opening the O2k-chamber for application of a gas phase during background experiments is the risk of inclusion of gas bubbles when closing the chamber. Elimination of gas bubbles is more difficult in O2k-MultiSensor ISE or Q-Module applications, when electrodes are introduced through inlets in the stopper and/or the shape of the stopper is conical. Importantly, in these applications, instrumental background correction is even more important since inserted electrodes add oxygen storage capacities and potential leaks.

Gas bubbles are avoided in instrumental O_2 background tests with the injection of dithionite, either using the automatic system TIP2k or manually.

3. Instrumental O2 background

Protocols for the instrumental O2 background can be found within the DatLab software, in the pull-down menu Protocols and clicking on Instrumental: Browse DL-Protocols and templates to open a folder with the library of instrumental DL-protocols. Then, select in the O2k background folder one of the following protocols:

- Instrumental_O2_background_TIP2k
- Instrumental_O2_background_manual injections
- Instrumental_high_O2_background_TIP2k
- Instrumental_high_O2_background_manual injections
- Instrumental_O2_background_sV

3.1. TIP2k in feedback control mode

Fill the TIP2k syringes with the freshly prepared dithionite solution (rinsing before the syringes 3 times with double distilled or deionized H_2O and at least once with the dithionite solution), taking care to minimize exposure of the dithionite solution to air. Use a syringe with a higher volume than both TIP2k syringes and with a long needle to fill both TIP2k syringes sequentially.

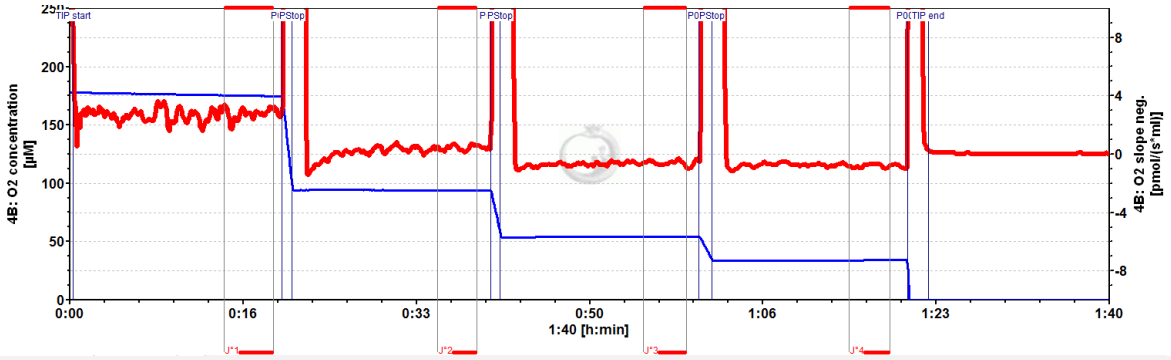
After air calibration, close the chamber either directly (air-level normoxia) or after elevating oxygen levels (hyperoxia; section 2.4). When using the 200 mm^3 syringes (with the shorter needle), the TIP2k is aligned with a mark on top of the O2k to allow a correct position of the TIP2k needles in the stopper. After closing the chamber, place wet TIP2k Filter Papers in the receptacle of the stoppers making sure they are completely in contact with the receptacle. Then, rinse the TIP2k needles with double distilled or deionized H_2O and wipe off the liquid accumulated outside the needle with a pipette tip (do not use a tissue). Press Test Start in the TIP2k program window, wipe off the extra liquid from the TIP2k needle with a pipette tip, and insert the TIP2k needles through the capillary of the stoppers. Adjust the height of the needle spacer, if needed, in order to have the syringe needle inserted into the chamber, but without touching the stirrer.

» MiPNet12.10 TIP2k-manual

The following parameters are used in the TIP2k setup file (BG Feedback program) for the instrumental background test starting at air saturation levels (DL-protocol: Instrumental_O2_background_TIP2k):

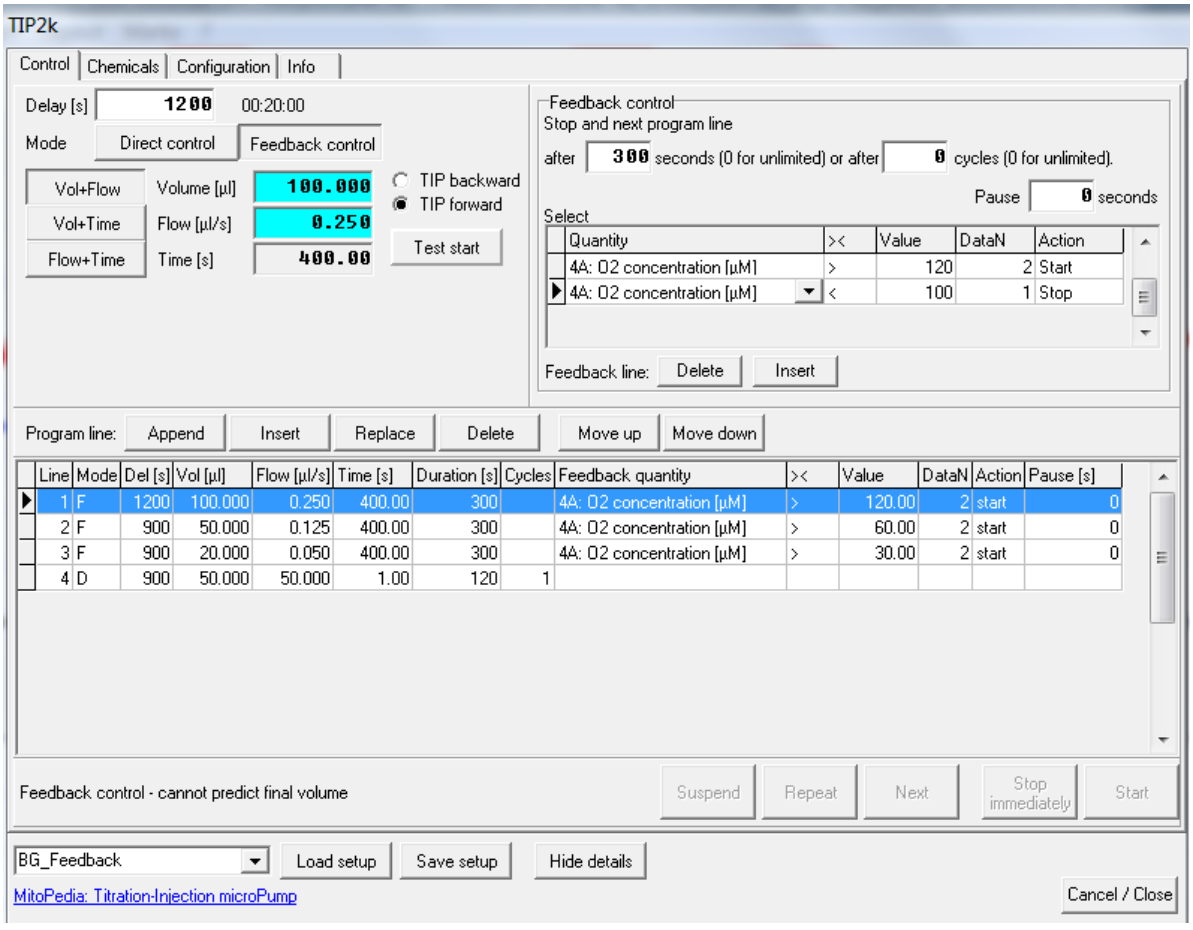
Line	Mode	Start injection if oxygen level (left chamber) is	Stop injection if oxygen level (left or right chamber) is	Flow	Delay	Interval	Volume
		µM	µM	µL·s^{-1}	s	s	µL
1	FB	>120	<100	0.250	1200	300	
2	FB	> 60	< 50	0.125	900	300	
3	FB	> 30	< 23	0.050	900	300	
4	D			50			100

The TIP2k program starts with a delay of 1200 s (20 min), to let the oxygen flux stabilize after closing the chamber, providing the first background level (J°1). Then, the first injection starts at 0.25 µL·s^{-1}. The TIP2k operates now in feedback mode while oxygen levels decline. The TIP2k stops when an O_2 concentration <100 µM is reached, and possibly overshoots by 10 µM to yield a level of about 90 µM (J°2). The 1200 s interval (20 min) is programmed as a feedback control time of 300 s plus a delay of 900 s before each subsequent injection at 0.125 µL·s^{-1} to 50 µM (J°3) and 0.050 µL·s^{-1} to 23 µM (J°4). Lowered injection speeds reduce the overshoot to 5 µM and 3 µM.



TIP2k Setup "BG_Feedback": Instrumental background oxygen flux at air saturation (176 µM; 37 °C, 600 m altitude), 90 µM, 45 µM, 20 µM. Each level was maintained for 20 minutes.

After recording the last background level (J°4 at 20 µM), a final titration of excess dithionite (100 µL) is induced in the direct control mode for zero oxygen calibration (R0) of the POS.



In the DatLab main menu, select TIP2k, TIP2k control and BG Feedback ▼ from the dropdown menu and press Load setup. Start the titration program. During operation, the TIP2k window may be closed.

3.2. Manual injections

Use a Hamilton microsyringe for manually injecting the dithionite solution. The effective concentration of dithionite decreases in the stock solution over time due to

autoxidation when small amounts of oxygen leak into the solution. The potency of the solution can be tested by injecting a small volume (2.5 µL) into the closed O2k-chamber and observing the change in oxygen concentration. The stoichiometric correction factor, *SF*, expresses the deviation of the effective dithionite concentration from the dithionite concentration added initially,

$$SF = \frac{\Delta n_{O2}(\text{eff})}{\Delta n_{O2}(\text{calc})} = \frac{\Delta c_{O2} \cdot V_{\text{chamber}}}{v_{\text{inject}} \cdot c_{Na_2S_2O_4}} \quad (1)$$

SF	Stoichiometric correction factor for dithionite concentration
Δn_{O2}(eff)	Effective change of the amount of oxygen [µmol]
Δn_{O2}(calc)	Calculated change of the amount of oxygen [µmol]
Δc_{O2}	Effective drop in oxygen concentration [µmol·dm^{-3}; µmol·L^{-1}]
V_{chamber}	Chamber volume [mm^3; µL]
v_{inject}	Injected volume of dithionite solution [mm^3; µL]
$c_{Na2S2O4}$	Dithionite concentration in the initial stock solution (approx. 19.8 mmol·dm^{-3} considering a complete consumption of oxygen originally dissolved in the aqueous solvent), irrespective of further oxygen uptake by the effectively anoxic solution. v_{inject} is the volume injected to achieve a specific drop in oxygen concentration:

$$v_{\text{inject}} = \frac{\Delta c_{O2} \cdot V_{\text{chamber}}}{SF \cdot c_{Na_2S_2O_4}} \quad (2)$$

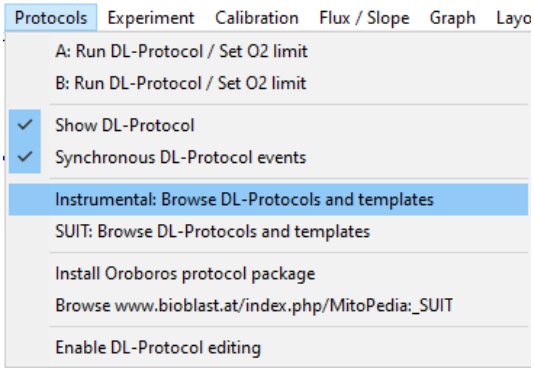
A typical value of *SF* is 0.7 in a freshly prepared stock solution. Since no accurate oxygen concentrations must be achieved for determination of an instrumental background, a value of 0.7 can be used for most purposes. When using the TIP2k in Feedback Control Mode, calculation of *SF* is not necessary.

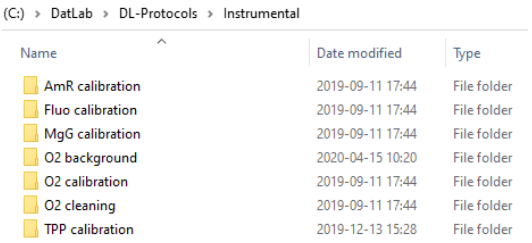
3.3. Data analysis excel template

- The Excel template "Template O2 background.xlsx" is provided for analyzing instrumental background experiments from DatLab 7.4. (https://wiki.oroboros.at/index.php/Instrumental:_Browse_DL-Protocols_and_templates).
  Use the pull-down menu Protocols and click on Instrumental: Browse DL-Protocols and templates to open a folder with the library of instrumental DL-protocols and then select in the O2 background folder the "Template O2 background.xlsx" file.
- If you did not apply a finally evaluated O2 background correction to your experimental DatLab file, you can edit the O2 background parameters directly

in the 'O2 analysis template DL7.4' provided with DatLab software (https://wiki.oroboros.at/index.php/SUIT:_Browse_DL-Protocols_and_templates)

» MiPNet24.06_Oxygen_flux_analysis_DatLab_7.4





4. Analysis of instrumental background tests

» MiPNet08.09, MiPNet10.04

5. References

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6. Acknowledgements

Mario Fasching and Timea Komlodi contributed to this MiPNet as former members of Oroboros Instruments.

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Supplement A: O_2 background parameters and accuracy of O_2 flux

A1. Oxygen consumption by the polarographic oxygen sensor

The POS yields an electrical signal while consuming the oxygen which diffuses across the oxygen-permeable membrane to the cathode. The cathode and anode reactions are, respectively,

$$O_2 + 2\ H_2O + 4\ e^- \quad 4\ OH^- \qquad (3a)$$

$$4\ Ag \quad 4\ Ag^+ + 4\ e^- \qquad (3b)$$

$$4\ Ag^+ + 4\ Cl^- \quad 4\ AgCl \qquad (3b')$$

The electric flow (current, I_{el} [A]) is converted into a voltage (electric potential, V_{el} [V]) and amplified. In the O2k, the gain, $F_{O2,G}$, can be selected in DatLab 7.4. within the O2k setup menu, with values of 1, 2, 4, or $8 \cdot 10^6$ V/A, where 1 V/µA is the basal gain at a gain setting of 1. The raw signal after amplification, R_{O2} [V], is related to the original POS current,

$$I_{el} = R_{O2} \cdot F_{O2,G}^{-1} \qquad (4)$$

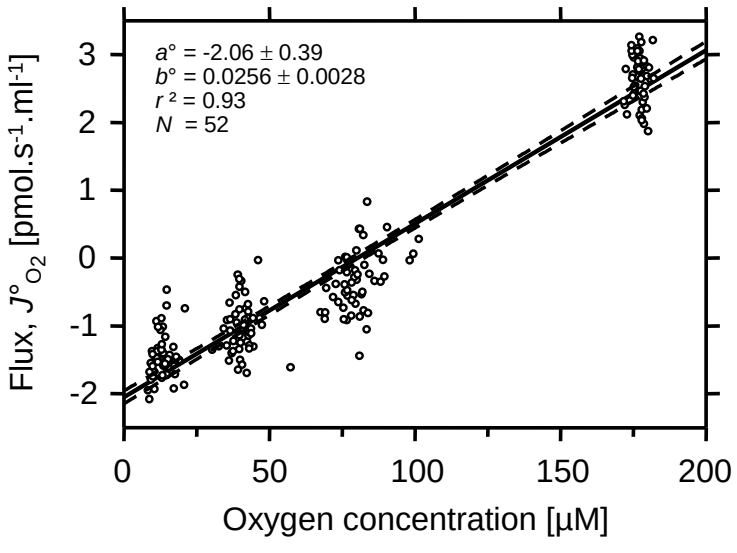


Figure A1. Instrumental background oxygen flux, $J°_{O2}$, as a function of oxygen concentration, c_{O2} [µM], in the O2k (37 °C; NaCl solution with an oxygen solubility factor of 0.92 relative to pure water). Measurements in 52 chambers (2 mL volume) of 26 different instruments. In all tests, four oxygen ranges were selected consecutively in declining order. Each oxygen concentration was maintained for 20 min, at the end of which time intervals of 200 seconds (corresponding to 200 data points at the sampling interval of 1 s) were chosen for estimating average flux at each corresponding oxygen concentration. Averages and SD were calculated for the intercept, $a°$, and the slope, $b°$, by linear regression for each individual chamber. The full and stippled lines show the linear regression and 99 % confidence intervals calculated through all data points.

R_{O2} is about 9 V (at air saturation, 37 °C, and a gain of $4 \cdot 10^6$ V/A) and is thus typically 2.2 µA under these conditions. In the cathode reaction (Eq. 3a), electric flow, I_{el} [A=C·s^{-1}], is stoichiometrically related to molar oxygen flow, I_{O2} [mol O_2·s^{-1}], through the stoichiometric charge number of the reaction, $\nu_{e^-/O2} = 4$, and the Faraday constant, F, i.e. the product of the elementary charge and the Avogadro constant (F = 96,485.53 C·mol^{-1}; Mills et al 1993).

The oxygen/electric flow ratio is (Gnaiger, 1983),

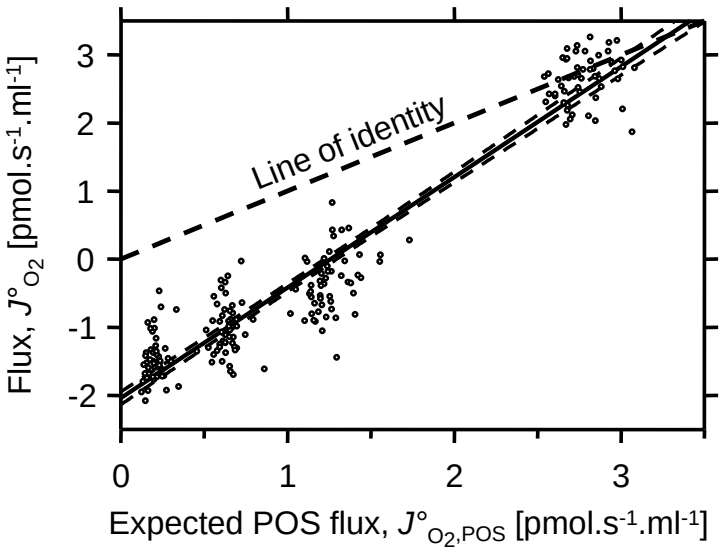
$$Y_{O2/e-} = (\nu_{e-/O2} \cdot F)^{-1} = (4 \cdot 96{,}485)^{-1}\ \text{mol}\cdot\text{C}^{-1} \quad (5)$$
$$= 2.591068\cdot10^{-6}\ \text{mol}\ O_2\cdot\text{C}^{-1}$$
$$= 2.591\ \text{pmol}\ O_2\cdot\text{s}^{-1}\cdot\mu\text{A}^{-1}$$

Oxygen consumption by the POS can be directly measured in the closed O2k-chamber at air saturation (Fig. A1), as volume-specific oxygen flux, $J_{O2}°$ [pmol·s^{-1}·cm^{-3}], and the corresponding theoretical oxygen flux in Eq. 3a can be calculated, $J_{O2,POS}$ (Fig. A2),

$$J_{O2,POS} = (R_{O2} - R_{O2,0}) \cdot Y_{O2/e-} \cdot F_{O2,G}^{-1} \cdot V^{-1} \quad (6a)$$

where $R_{O2,0}$ is the raw signal at zero oxygen (zero current), and V is the O2k-chamber volume.

Figure A2. Instrumental background oxygen flux, $J°_{O2}$, as a function of the theoretical oxygen consumption by the POS, calculated from the electrical signal (current) as a function of oxygen concentration (from data in Fig. A1). The line of identity (dashed) illustrates the full correspondence between experimental and theoretical oxygen consumption at air saturation (top right) and the increasing deviation at declining oxygen concentration owing to a linear increase of oxygen backdiffusion.



It is more convenient to relate the theoretical oxygen consumption of the POS to the measured oxygen concentration, c_{O2} [µM], using the oxygen calibration factor, $F_{O2,c}$ [µM/V],

$$J_{O2,POS} = (c_{O2} \cdot F_{O2,c}^{-1}) \cdot Y_{O2/e-} \cdot F_{O2,G}^{-1} \cdot V^{-1} \quad (6b)$$

Combining constants from Eq. 5, at a gain setting of 4 V/µA and a volume of 2 cm^3, Eq. 6 is,

$$J_{O2,POS} = (R_{O2} - R_{O2,0}) \cdot 0.3239\ \text{pmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-3}\cdot\text{V}^{-1} \quad (6c)$$
$$= c_{O2} \cdot F_{O2,c}^{-1} \cdot 0.3239\ \text{pmol}\cdot\text{s}^{-1}\cdot\text{cm}^{-3}\cdot\text{V}^{-1}$$

The full and stippled lines show the linear regression and 99 % confidence intervals. On average, signal stability was indicated by apparent oxygen fluxes close to zero during air calibration, when oxygen concentration is maintained stable by exchange with the gas phase. Average J'_{O2} amounted to 0.04 ± 0.14 pmol·s^{-1}·cm^{-3} (range from –0.28 to 0.25 pmol·s^{-1}·cm^{-3}). To express signal noise independent of these low levels of signal drift, linear regressions were calculated through these 200 second sections, and this drift was subtracted from the concentration before calculating the SD.

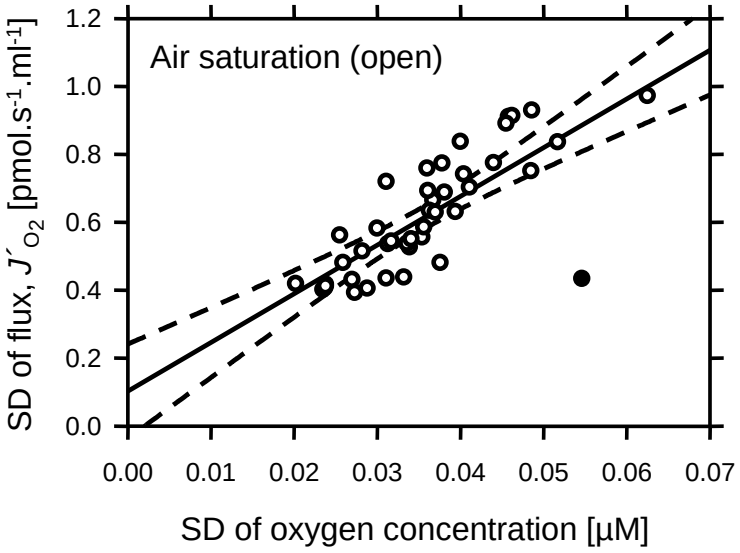


Figure A3. Noise (SD of the mean) of the apparent oxygen flux, J'_{O2}, as a function of noise (SD of the mean) of oxygen concentration, c_{O2} (180 ± 2 µM; at 95 ± 1 kPa barometric pressure), in the "open" O2k-chamber (37 °C; NaCl solution, at air saturation), over time intervals of 200 seconds (corresponding to 200 data points at the sampling interval of 1 s). Each data point (N=43) represents an independent 2-mL O2k-chamber. The SD of oxygen concentration was calculated from the raw signal without smoothing. Flux was calculated from concentration smoothed with a moving average (30 data points), using an eight-point polynomial for calculation of the slope. The outlier (full circle) corresponds to a data set with an individual spike.

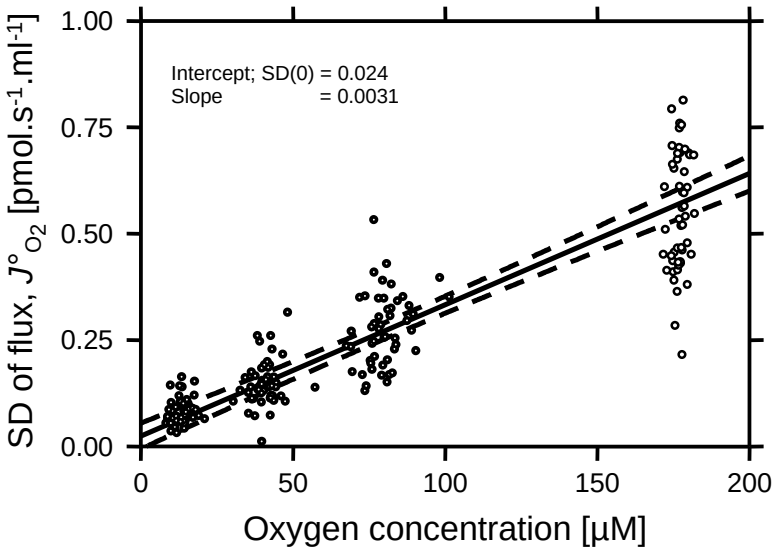


Figure A4. Noise (SD of the mean) of the instrumental background oxygen flux, $J°_{O2}$, as a function of oxygen concentration, c_{O2} [µM], in the Oroboros O2k (37 °C; NaCl solution), over time intervals of 200 seconds (corresponding to 200 data points at the sampling interval of 1 s). Each data point (N=43) represents an independent 2-mL O2k-chamber. Flux was calculated from concentration smoothed with a moving average (30 data points), using an eight-point polynomial for calculation of the slope. The full and stippled lines show the linear regression and 99 % confidence intervals. To express noise of flux independent of small changes in flux over time, linear regressions were calculated through 200 second sections, and this trend was subtracted from flux before calculating the SD.

A2: Accuracy of instrumental background tests

Instrumental background interferes with accurate measurement of respiratory oxygen flux, if background effects remain undefined. The instrumental oxygen background parameters are a property of the O2k-chamber. Any contamination of the medium causing oxidative processes (microbial respiration) is detected. Then background oxygen consumption is a property of a contaminated medium. Otherwise, instrumental background does not depend on the specific medium. Therefore, background parameters obtained in one medium can be used for another medium in the same chamber.

In a series of 52 experimental background determinations, 52 different 2-mL O2k-chambers (37 °C) were tested (O2k, Series A). The following average conditions applied:

Oxygen concentration at air saturation, c_{O2}*	= 179.9 µM
Average oxygen concentration at $J°_1$, $c_{O2,1}$	= 177.2 µM
Oxygen calibration signal at air saturation, $R_{O2,1}$	= 8.744 V (Gain 4)
Oxygen calibration signal at zero oxygen, $R_{O2,0}$	= 0.033 V (Gain 4)
Oxygen calibration factor, $F_{O2,c}$	= 20.69 µM/V
$J_{O2,POS}$ = 0.3239 x 177.2/20.69	= 2.77 pmol·s^{-1}·cm^{-3}

At air saturation in the 2-mL (cm^3) chamber, the theoretically expected oxygen consumption by the sensor is 2.77 pmol·s^{-1}·cm^{-3}, in direct agreement with the experimental result. At an average flux of 2.64 pmol·s^{-1}·cm^{-3} (±0.35 SD; *N*=52; Fig. A1), the ratio between measured and theoretically expected oxygen consumption by the POS was 0.95 (±0.12 SD; *N*=52). This possibly provides the first experimental evidence for the exact 4-electron stoichiometry in the reduction of oxygen at the cathode of the POS.

Supplement B: TIP2k in direct control mode

» MiPNet12.10 TIP2k-Manual

Fill the TIP2k syringes with freshly prepared dithionite solution. After air calibration, record the first point of the background experiment as described above.

Programming the TIP2k: Calculate the necessary injection volumes as described in Section 2.5, initially assuming *SF* = 0.7 (stoichiometric correction factor for dithionite concentration). *SF* can be calculated after the first injection and – if necessary – the TIP2k be reprogrammed for subsequent injections. Alternatively, *SF* may be determined initially:

- Set the Volume, v_{inject}, to 5 µL;
- Test start before inserting the needles, to replace the dithionite solution in the needles;
- Wait for stabilization of oxygen flux;
- Inject 5 µL and calculate *SF* using Equation (1).

Example: Oxygen level in the chamber is 160 µM. The user wants to obtain four background levels (in addition to the one recorded near air saturation). With four evenly spaced steps it is possible to reach a minimum of 20 µM reducing the oxygen concentration by 35 µM steps. The necessary injection volume, v_{inject}, to achieve the desired reduction of oxygen concentration can then be calculated from Equation (2). In the present example:

SF = 0.7

Δc_{O2} = 35 µM

$V_{chamber}$ = 2 mL

$c_{Na2S2O4}$ = 9.8 mM

v_{inject} = 10 µL

Four injections of 10 µL each should therefore bring the oxygen concentration near the desired last level of 20 µM. Optionally, with a fifth injection, zero oxygen concentration could be reached. It is recommended to use a larger excess volume for zero calibration.

Always consider the expected experimental oxygen concentration range: for an experiment at high oxygen levels, calculate injection to decrease from the initial oxygen level (e.g., 350 µM) to the final oxygen concentration (e.g., air saturation). The minimum time required between injections to obtain stable fluxes is about 10 minutes. The time course of the instrumental background should match the decline of oxygen concentration in the real experiment. Longer intervals will typically be

chosen (15 min in our example). The TIP2k can be set up in the following way:

Select Direct control and Vol+Flow

Delay [s]	0
Volume [µL]	10
Flow [µL/s]	30
Interval [s]	900
Cycles	4

Start the experiment with Start.

Supplement C: Further details

» MiPNet22.11 O2k-FluoRespirometer manual.

» MiPNet12.10 Titration-Injection microPump. TIP2k user manual.

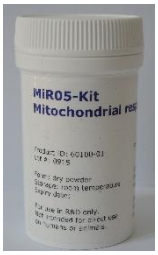
» MiPNet28.07 NextGen-O2k Series XB manual

» MiPNet28.08 Oroboros O2k Series J manual

O2k-Procedures

» MiPNet06.03 O2k Quality Control 1: Polarographic oxygen sensors and accuracy of calibration.

» MiPNet08.09 HRFR with leukemia cells: respiratory control and coupling.



» http://wiki.oroboros.at/index.php/MiPNet10.04

» MiR05-Kit: http://wiki.oroboros.at/index.php/MiR05-Kit

» O2k-Videosupport: Working with the TIP2k (Instrumental O2 background) https://www.youtube.com/watch?v=VV9pRcK076k