O2k-Protocols SOP:
O2k Quality Control 1

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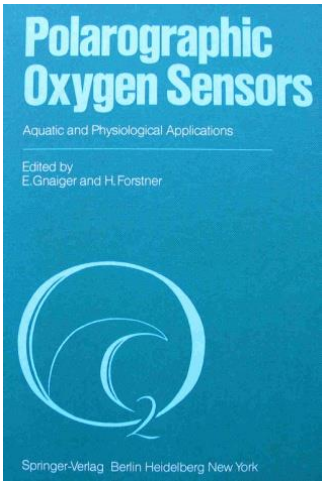
O2k Quality Control 1: Polarographic oxygen sensors and accuracy of calibration



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Summary: High-resolution respirometry (HRR) critically depends on maintenance (MiPNet19.18B) and accurate calibration of the polarographic oxygen sensors (OroboPOS, POS). Calibration errors > 10 %, as commonly encountered in the literature, cannot be accepted in HRR. Standard operating procedures (**O2k-SOP**) are described throughout the MiPNets: (*1*) Cleaning and preparation for use of O2k-chambers (MiPNet19.03); (*2*) Quality control for evaluation of proper POS function (SOP: O_2 sensor test, MiPNet06.03); and (*3*) Accurate POS calibration (MitoPedia: O2-Calibration - DatLab, MiPNet06.03). This is Part 1 of O2k Quality Control, a component of the *Oroboros Diagnostics Quality Management* (Part 2: Instrumental oxygen background correction MiPNet14.06).

1. Calibration and quality control (O2k-SOP)

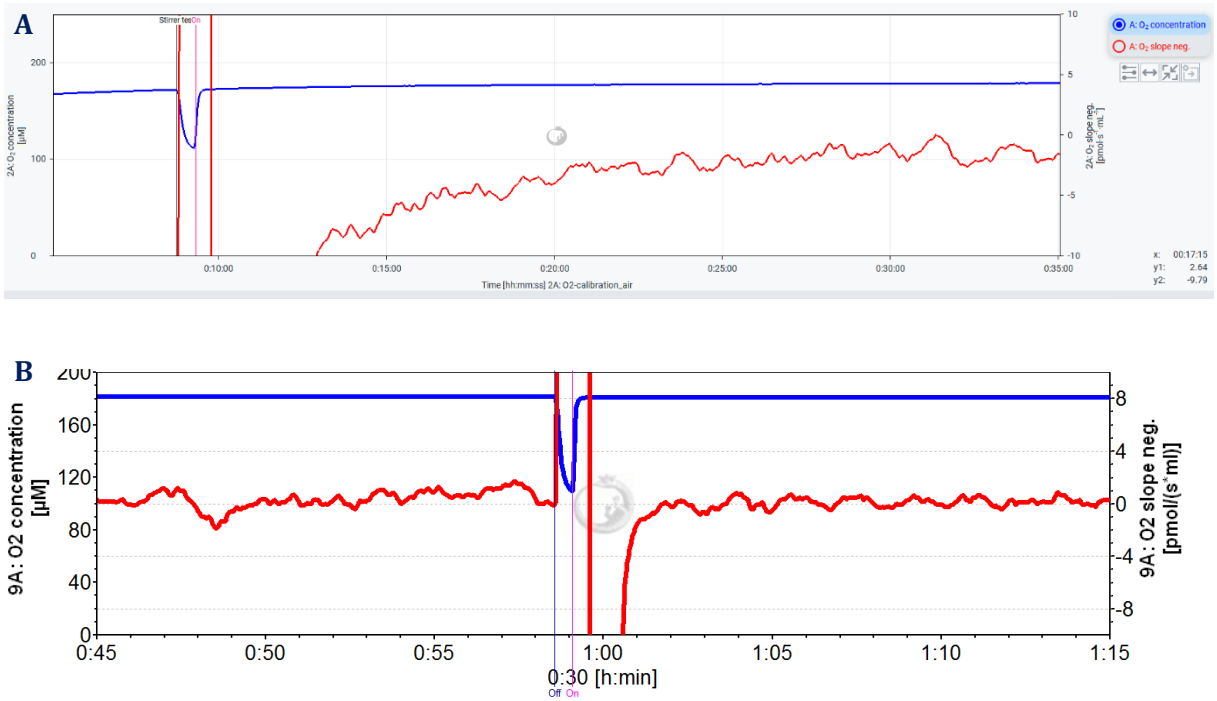
1.1. Preparation

1. Switch on the O2k, connect it to DatLab. Edit the O2k channel settings in DatLab. Clean the O2k-chambers using the DL-protocol "O2k-cleaning BeforeUse" (MiPNet19.03).
2. Increase the temperature of the stock of experimental medium to slightly above experimental temperature. Add 2.1-2.5 mL medium to each 2.0-mL O2k-chamber and 0.60 mL to each 0.5-mL O2k-chamber. This helps avoid the formation of gas bubbles and minimizes disturbance of the O2k temperature.
3. With the stirrer on (typically 750 rpm = 12.5 Hz), insert the stopper fully; check that no air bubbles are in the volume-calibrated chamber.
4. Siphon off excess medium from the top of the stopper.
5. Lift the stopper to the "open position" using the stopper spacer.
6. Start a new file in DatLab and select the DL-protocol "O2-calibration air" or "O2-calibration air and zero".

1.2. The O_2 sensor test

1. Stirrer test: When the O_2 concentration is stable but before final stabilization of O_2 slope neg., perform a stirrer test, switching both stirrers automatically off and on. The default period is 30 s, for experiments at 37 °C. At lower experimental temperature, this period should be prolonged (60 s at 25 °C).

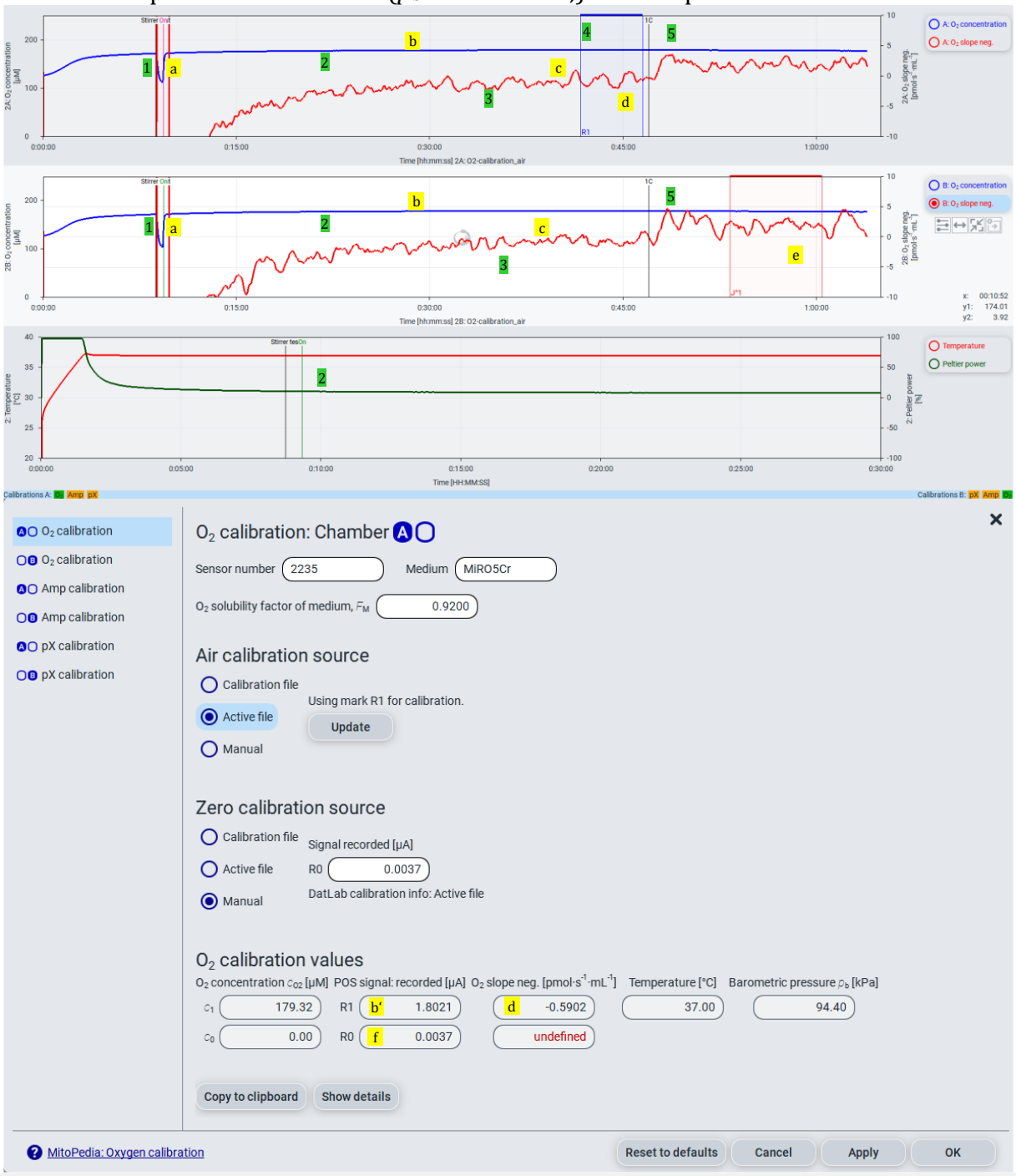
Quality control **a**: Upon automatic re-start of the stirrer (On), the increase of the oxygen signal should be rapid and monoexponential.



Stirrer test for quality control of the POS (standard 30 s) with 30 min time scale displayed with Graph Layout "02-Calibration - Background" (MiR05; 37 °C; data recording interval: 2 s. **(A) DatLab 8** slope smoothing: 80 s. 2017-02-06 P2-01.DLD; **(B) DatLab 7.4**; slope smoothing: 40 data points. 2014-02-19 P9-01.DLD

2. About 20 min are required for approximate air equilibration after temperature equilibration of the incubation medium, visualized as stabilization of the Peltier power.

Quality control **b**: The raw signal (blue trace) should be between 1 and 3 µA when at sea level up to 1000 m altitude (p_b 90 - 101 kPa,) and temperature is 25 - 37°C.



POS Quality control (DatLab 8) using the DatLab protocol (DL-Protocol) O2_calibration_air.DLP: Plot of the O_2 sensor test (above; File 2017-02-06 P2-01.DLD; time scale is 1:05 h:min) and oxygen calibration window (below).

POS Quality control (DatLab 7) using the DatLab protocol (DL-Protocol) O2_calibration_air.DLP: Plot of the 1-hour POS performance test (above; File 2014-07-24_P4-01_O2-calib.DLD; time scale is 1:10 h:min) and oxygen calibration window (below).

3. Within 40 min, the oxygen signal should be stable with O_2 slope neg. (uncorrected) close to zero.

Quality control **c**: Signal noise should be low, reflected in the noise of the O_2 slope neg. (red trace) within ± 2 (± 4 is acceptable) pmol·s^{-1}·mL^{-1} for both 2.0-mL and 0.5-mL chambers at a data recording interval of 2 s and slope smoothing 80 s selected for calculation of the slope.

4. Set a mark on the oxygen signal (R1 – the mark is automatically named R1 when using the DL-protocol) and click on Calibration to open the DatLab O_2 calibration window. Three options are available for applying O_2 calibration values:
(*1*) clicking on "calibration file" loads the values from a calibration file; (*2*) clicking on 'Active file', uses the values from mark R1 of the current file; (*3*) clicking on 'Manual', allows for the values to be entered manually. If "Active file" is selected, the R1 will be shown in **b'**. Add the O_2 solubility factor of the medium, F_M (O2 solubility factor).

Quality control **d**: The slope uncorrected should be ± 1 pmol·s^{-1}·mL^{-1} for 2.0-mL and 0.5-mL chambers if averaged across the section of the experiment marked as R1 for air calibration (**d**). The recorded POS signal should be close to the previous calibration under identical experimental conditions. Click on Apply.

5. Close the chamber and if required, perform a zero oxygen calibration (section 4), or continue with a complete instrumental O_2 background test (MiPNet14.06).

Quality control **e**: After closing the chamber, select plot Y2 and set mark J°$_1$. The O_2 slope (neg.) should be 3 ± 1 pmol·s^{-1}·mL^{-1} for the 2.0-mL chamber (MiPNet14.06) and 10.0 ± 4 pmol·s^{-1}·mL^{-1} for the 0.5-mL chamber (Figure S1). Flux values higher than 4.0 or 14.0 pmol·s^{-1}·mL^{-1} for the 2.0- or 0.5-mL chamber, respectively, may indicate a biological contamination.

Quality control **f**: The zero signal at mark R0 obtained at zero calibration (section 4) should be < 2 % of R1 (stable at < 5 % is acceptable).

2. Zero oxygen calibration

Zero calibration can be done by one of the following methods:

2.1. Zero calibration with instrumental O_2 background test: TIP2k

O2k-SOP: » MiPNet14.06 Instrumental O2 Background

2.2. Zero calibration: manual titration of dithionite (O2k-SOP)

1. Prepare "zero solution": Dissolve two spatula tips or 20 mg of dithionite (sodium hydrosulfite, Na-dithionite, $Na_2S_2O_4$; O2-Zero Powder in the OroboPOS-Service Kit) in 0.5 mL deionized water. Mix in a small vial with minimum gas space. Use fresh dithionite. Dithionite is oxidized during prolonged storage and needs to be replaced.
2. Inject 20 µL zero solution into the closed O2k-chamber using a 50 µL microsyringe.
3. Oxygen depletion is very rapid, and zero oxygen is reached within a few minutes. However, a few more minutes may be required until a stable signal is obtained, R_0 [µA].
4. Inject another 10 µL zero solution. Repeat as long as the signal responds by a further decline. Siphon off excess medium from the stopper.
5. On the final titration when no further decline is seen, the zero signal stabilizes quickly (< ± 0.2 or < ± 0.8 pmol·s^{-1}·mL^{-1} for the 2.0-mL or 0.5-mL chamber respectively).
6. Set a mark over the stable "zero" signal (R_0), to complete the two-point oxygen calibration [F5]. Select Mark R1 and Mark R0 for R_1 and R_0 in the O_2 calibration window.

2.3. Zero calibration: mitochondrial respiration

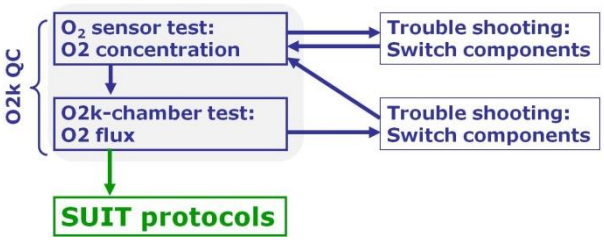
Due to the high oxygen affinity of isolated mitochondria, intact cells and tissue homogenate, residual traces of oxygen are insignificant after respiratory oxygen depletion. Therefore, you can use your experimental sample for such zero-oxygen calibration. Alternatively, prepare a stock of baker´s yeast, with 200 mg dry yeast in 2 mL physiological salt solution. Stir heavily to obtain a homogenous suspension of yeast cells and add 50 µL yeast suspension into the 2-mL chamber through the cannula of the stopper, using a microsyringe.

More details: »Gnaiger et al (1995), »Gnaiger (2001).

3. O_2 sensor test and zero oxygen calibration: when?

The O_2 sensor test should be performed:

- Every day after switching on the O2k.
- During troubleshooting procedures, when switching components between the two chambers, a quick sensor test is performed after each step (stirrer test, raw sensor signal).



- After application of a new membrane and POS Service. In some cases, the signal of the OroboPOS improves (higher signal stability, less noise, shorter response time), when leaving the O2k switched on overnight (O2k-chambers filled with deionized H_2O at 25° C with stopper in closed position and the illumination switched off).

Zero oxygen calibration should be performed:

- Every few weeks.
- When working at low oxygen levels it is recommended to measure R0 before and after each measurement using the experimental sample for zero calibration (Section 2.3).

O_2 sensor test and zero calibration are also performed at the beginning and end of the O2k-chamber test (instrumental O_2 background test).

4. O_2-Calibration list: quality control

Oroboros FileFinder: Click on the icon "O2k-Manual". Go to 'O2k-Qualtiy control and SOPs' and move to the right to open the Excel file "O2-calibration.xlsx". Save a copy of this Excel template and paste the calibration parameters into new lines sequentially for chamber (A) and (B), thus generating a data base for quality control of instrumental calibration (Gnaiger 2008). Trends over time can thus be evaluated, and possible irregularities of sensor performance are quickly recognized for intervention by sensor service.

» MiPNet19.18B POS-service

5. O_2 calibration window - details

Concentration

Calibration factor for concentration [µM·µA^{-1}]	F_c	99.7071	$F_c = (c_1 - c_0) / (R1 - R0)$
Calibration offset [µA]	a_c	0.0037	$a_c = (c_1 \cdot R0 - c_0 \cdot R1) / (c_1 - c_0)$

Pressure

	Oxygen pressure, p_{O2} [kPa]	POS signal: Current, I[µA]	Oxygen consumption by POS, J^p_{O2} [pmol·s^{-1}·mL^{-1}]
Air calibration:	p_1 18.4587	I_1 1.8022 I_1 = R1	J^p_1 2.3300 $J^p_1 = 2.591 \cdot (I_1 - a_p) / V$
Zero calibration:	p_0 0.0000	I_0 0.0037 I_0 = R0	

Calibration factor for pressure [kPa·µA^{-1}]	F_p	10.2634	$F_p = (p_1 - p_0) / (I_1 - I_0)$
Calibration offset [µA]	a_p	0.0037	$a_p = (p_1 \cdot I_0 - p_0 \cdot I_1) / (p_1 - p_0)$

O_2 solubility, S_{O2} [µM·kPa^{-1}]	9.7148	$c_{O2} = p_{O2} \cdot S_{O2}$
H_2O vapor pressure, p_{H2O}^* [kPa]	6.2749	$p_{O2}^* = (p_b - p_{H2O}^*) \cdot 0.20946$
Volume fraction of O_2 in dry air	0.20946	
O2k chamber volume, V[mL]	2.0000	
Gain	1	

O_2 calibration window – details (DatLab 8). Upon clicking 'show details' in Calibration window, oxygen calibration parameters are displayed as calculated by DatLab. (For DatLab 7, see Supplement).

In the calibration window, click on "view details" to see the oxygen calibration parameters and equations calculated by DatLab.

Concentration: Parameters are displayed for conversion of the raw signal to concentration.

Calibration factor for concentration, F_c [µM·µA^{-1}]: This is the multiplication factor, F_c, calculated to convert the recorded current (corrected for the zero signal) into oxygen concentration.

Calibration offset, a_c [µA]: This is the POS zero signal at zero oxygen concentration, which is subtracted from the current before multiplication with the calibration factor.

Pressure: Parameters are displayed for conversion of the POS signal current to partial pressure of oxygen. These are the fundamental parameters for evaluation of signal stability over periods of several months since the POS responds to partial pressure of oxygen in the medium rather than concentration.

p_1 [kPa]: p_{O_2} at air saturation, $p_{O_2}^*$, a function of temperature and barometric pressure.

p_0 [kPa]: Usually p_{O_2} at zero oxygen concentration, or any other p_{O_2} at the second calibration point, p_0.

I_1 [µA]: POS signal as a current, at air saturation.

I_0 [µA]: POS signal as a current, at zero oxygen concentration, or any other p_{O_2} at the second calibration point.

Oxygen consumption by the POS, $J^\circ_{O_2}$ [pmol·s^{-1}·mL^{-1}]: Theoretical oxygen consumption of the oxygen sensor at air saturation under experimental conditions.

Calibration factor for pressure, F_p [kPa·µA^{-1}]: This is the multiplication factor, F_p, calculated to convert the current of the POS (corrected for the zero current) into partial pressure of oxygen.

Calibration offset, a_p [µA]: This is the POS zero current, at zero partial pressure of oxygen, which is subtracted from the current before multiplication with the calibration factor.

O_2 solubility, S_{O_2} [µM·kPa^{-1}]: a function of temperature and oxygen solubility factor of the medium.

H_2O vapor pressure, $p_{H_2O}^*$ [kPa]: a function of temperature, is subtracted from the barometric pressure, p_b.

Volume fraction of O_2 in dry air: 0.20946, when multiplied with the pressure ($p_b - p_{H_2O}^*$), it yields the partial pressure of oxygen.

O2k chamber volume, V [mL]: The effective aqueous volume of the closed O2k-chamber.

Gain, G: The gain is used for current to voltage conversion. Gain is fixed to 1.

6. References

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Acknowledgements

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Supplement: POS quality control – 0.5 mL chamber (DatLab 8)

Figure S1. POS Quality control – 0.5 mL (DatLab 8) using the DatLab protocol (DL-Protocol) O2_calibration_air.DLP: Plot of the POS performance test (above; File 2023-02-02_H-0366_02.DLD; time scale is 0:35 h:min; medium MiR05; 37 °C; data recording interval: 2 s; slope smoothing: 80 s) and oxygen calibration window (below).

Supplement: O2 calibration window – details (DatLab 7)

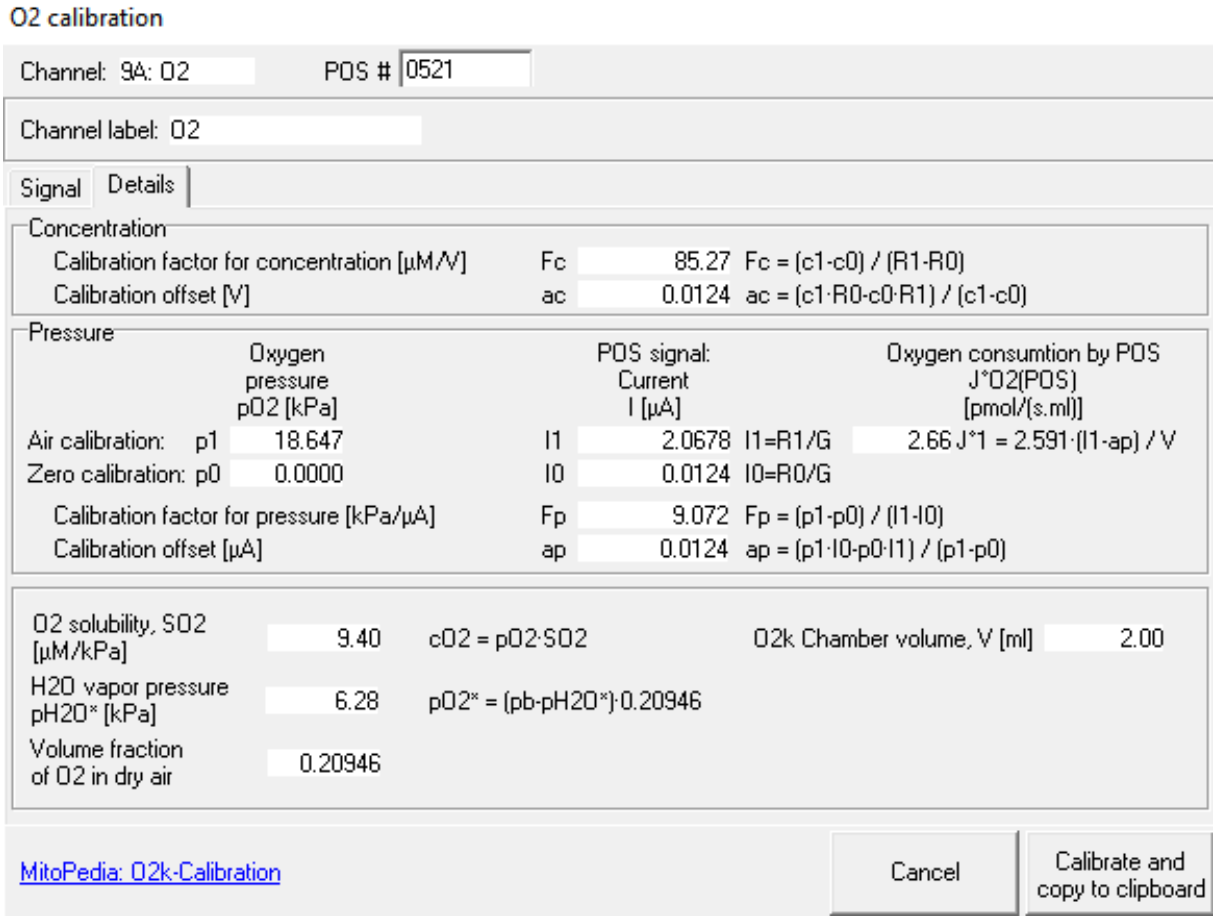

Figure S2. O_2 calibration window – details (DatLab 7). Upon clicking [F5] / Tab Details (MiPNet26.06) oxygen calibration parameters are displayed as calculated by DatLab.