IOC94 Mitochondrial Physiology Network 19.09(02): 1-6 (2014)
Updates: www.bioblast.at/index.php/MiPNet19.09_IOC94

Version 2: 2014-07-10

94th Workshop on high-resolution respirometry & O2k-Fluorometry

2014 Jun 27- Jul 02 Shanghai, China

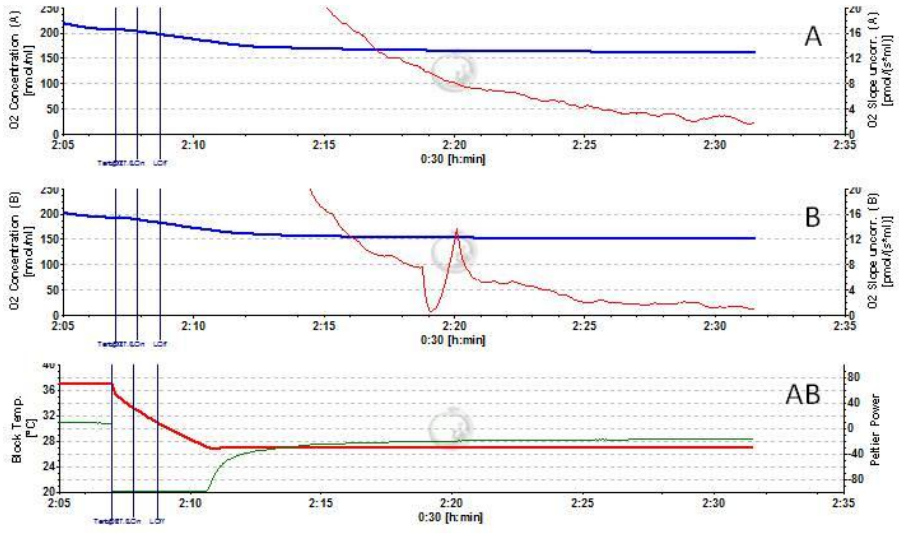
Venue:
CN Beijing Zenda
Room 105, Building 3, Block 1, Mingpu Plaza 3279 Sanlu Road, Minhang District, Shanghai, China

Contact:
Ming Cheng, ZENDA LLC, Sales manager, Wuhan Office.
Tel: 86-13554361876
Xin Yuan, ZENDA LLC, Shanghai office.
Tel: 86-15821657315
yuanxin79@gmail.com

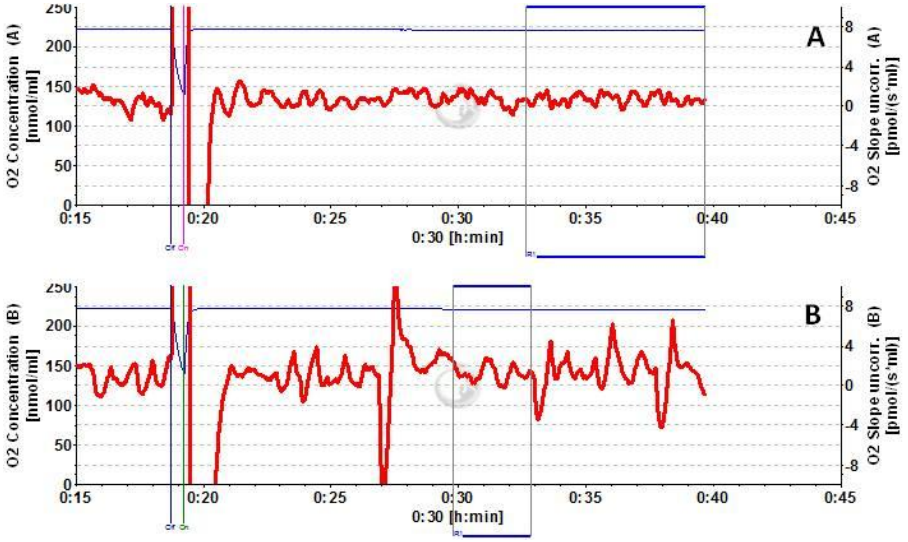
Lecturer and O2k-Demo experiments:
Erich Gnaiger, Ao.Univ.-Prof. PhD
OROBOROS INSTRUMENTS Corp
high-resolution respirometry
Schoepfstr 18, A-6020 Innsbruck, Austria - www.oroboros.at - erich.gnaiger@oroboros.at

The 94th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with our distributor ZENDAS LLC in China.

1. O2k-Workshop experiment: high-resolution respirometry in clutured insect cells

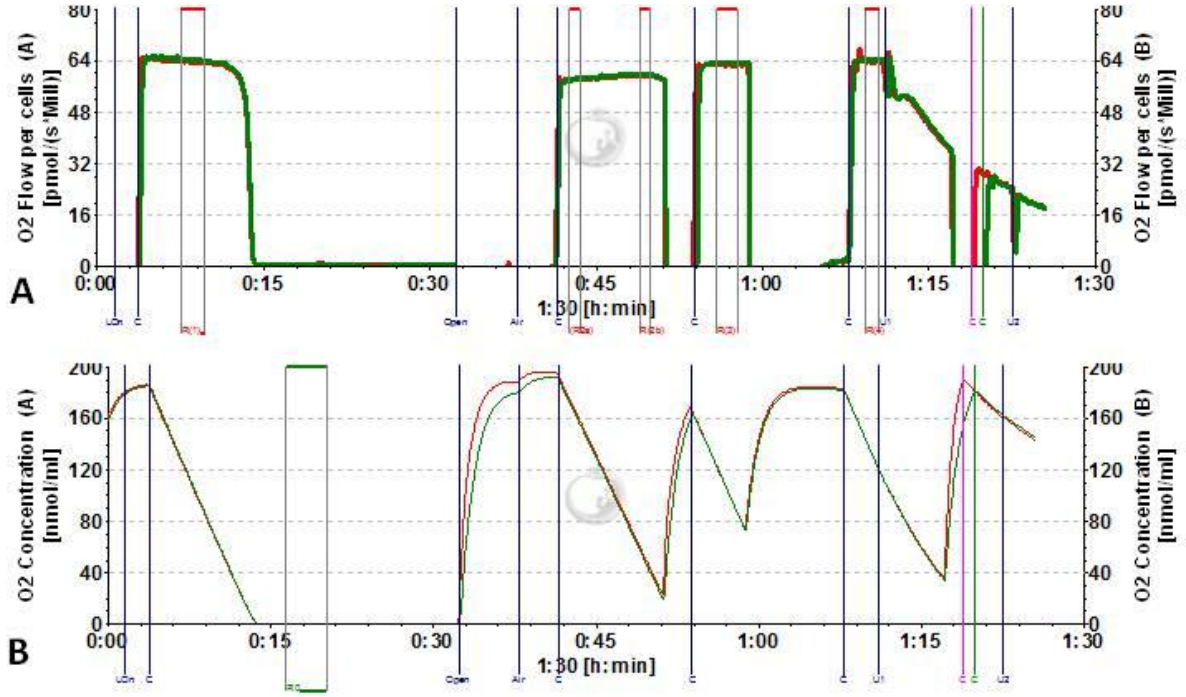


Immediately after preparation of new oxygen sensors (electrolyte and membrane application) the O2k was switched from 37 °C to 27°C (AB: red line). The oxygen signal is sensitive to temperature (blue lines; A and B for O2k-Chamber A and B, respectively), and thermal as well as oxygen gas equilibrium of 'insect cell culture medium' is reached in the 'open' chambers within c. 30 min (A and B: red lines; Slope uncorr. approach zero). Now the O2k is ready for calibration (next file). Graph layout 01. 2014-07-01 AB-01.DLD



Equilibration is continued in the 'open chamber' at 27 °C and interrupted by a 'stirrer test' [F9]: the stirrers are switched off for 30 s, with automatic re-start to 750 rpm. The signals of both sensors show a rapid response (blue traces). Equilibration of chamber A is complete (at a slope of 0.7 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$), whereas sensor B might require some prolonged equilibration. Marks R1 are used for oxygen calibration at air saturation, with raw signals (Gain 1) of 1.77 V and 1.79 V (Table 3). Graph layout 02, *N*=40 data points for slope calculation. 2014-07-01 AB-02.DLD

Cultured insect cells were provided by Leizhao Hua (Inst. Biotechnology) at a concentration of 5 million cells/ml in 'insect cell culture medium'.



Superimposed traces of the two Oxygraph-2k chambers. Graph A: Oxygen flow per million cells. Graph B: Oxygen concentration in the two chambers. The complete volume of medium in the O2k-Chambers was replaced with the cells in suspension. Before closing the chambers (C), the cell suspension was stirred for a few min in the 'open chamber' mode. Respiration (Graph A) was highly reproducible in the two chambers, stabilized immediately at 64 pmol $O_2 \cdot s^{-1} \cdot 10^{-6}$ cells with oxygen-independence until the aerobic-anoxic transition at c 15 min. Zero oxygen calibration was performed under anoxia (R0; Graph B). After 25-30 min anoxia, cell respiration was slightly depressed with a trend towards recovery, which was completed after reoxygenation (towards 1:00 hour) and respiration remained stable after another re-oxygenation to prevent anoxia. At c. 1:10 (1 hour and 10 min), a dose of FCCP was added which was probably injected at a

too high and hence inhibitory concentration, after which respiration was depressed and showed a progressive decline which was not markedly pronounced after a second FCCP titration. Graph layout 06; *N*=10 data points for slope calculation. 2014-07-01 AB-03.DLD

Sections of the experiment were marked (red bars in Graph A) for numerical analysis in Table 1.

Table 1: Marked sections of experiment 2014-07-01 AB-03.DLD showing ROUTINE respiration before anoxia, R(1), and after anoxia, R(2a) and R(2b) with a slight trend to recovery, and after full recovery following two reoxygenations, R(3) and R(4).

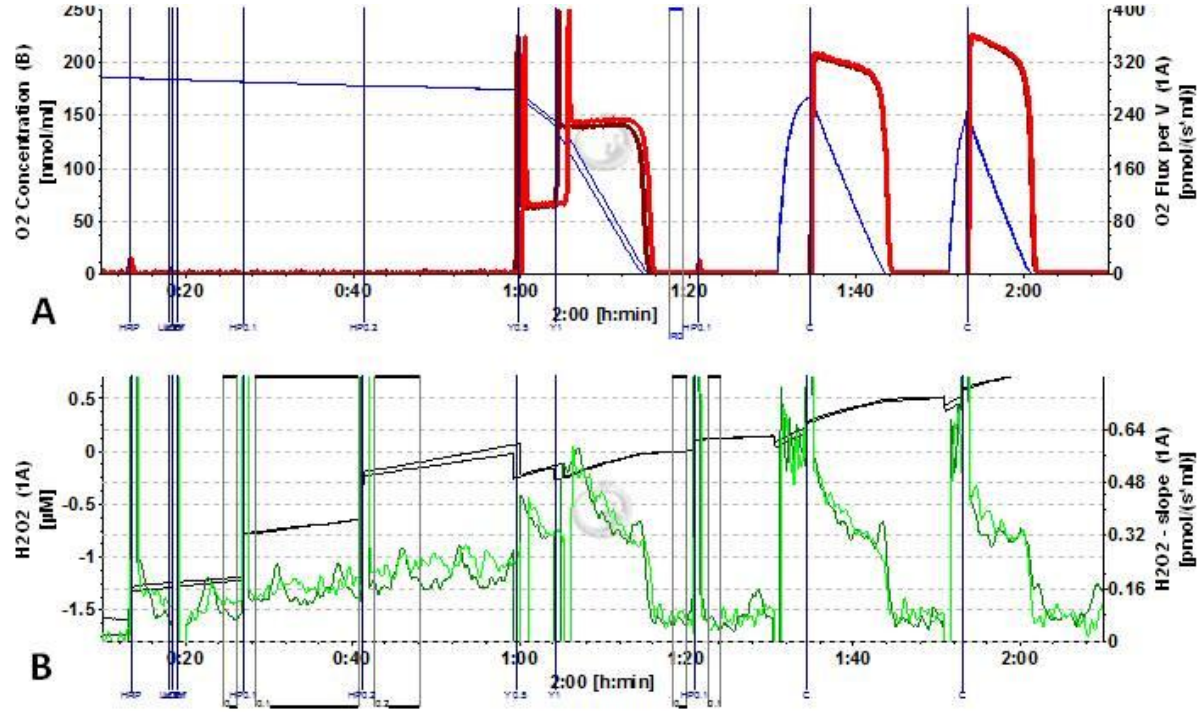
	Unit	R(1)	R(2a)	R(2b)	R(3)	R(4)
Start		00:07:33	00:42:34	00:48:52	00:55:53	01:09:14
Stop		00:09:42	00:43:28	00:49:48	00:57:42	01:10:28
N Points		65	27	29	54	37
O2 Flow per cells (A)	**pmol/(s*Mill)**	**63.7**	**58.5**	**59.4**	**62.8**	**63.7**
O2 Flow per cells (B)	**pmol/(s*Mill)**	**64.3**	**58.4**	**59.7**	**63.4**	**64.3**
O2 Concentration (A)	nmol/ml	90.5	166.2	54.4	109.8	143.6
O2 Concentration (B)	nmol/ml	91.8	164.0	51.8	110.1	143.5

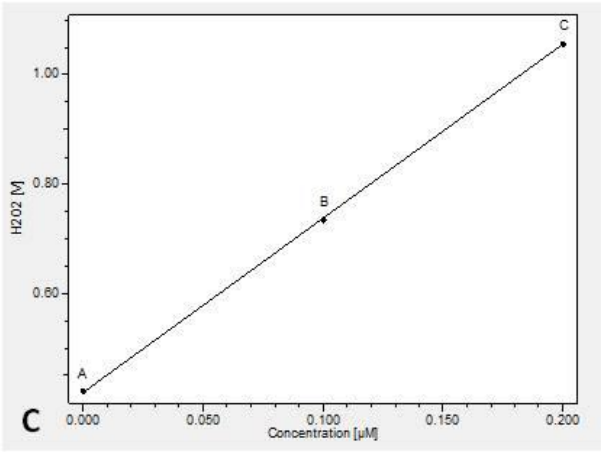
2. O2k-Workshop experiment: high-resolution respirometry and O2k-Fluorometry (hydrogen peroxide production) in yeast cells

Freeze-dried yeast cells were used on the 2nd day for a real-time experiment of HRR combined with O2k-Fluorometry.

Stirrer test (40 min) and air calibration at 37 °C with phosphate buffer solution (mark, Graph B). The raw signals (Gain 1) were 2.27 V and 2.28 V (Table 3). Closing the chamber (C) and evaluation of instrumental background oxygen flux at air saturation (mark J°1, Graph A): 2.16 and 2.38 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$), which is sufficiently close to the theoretical oxygen consumption per volume of the polarographic oxygen sensors predicted at 2.9 pmol $O_2 \cdot s^{-1} \cdot ml^{-1}$ (displayed in the DatLab oxygen calibration window [Show details]). Graph layout 02. *N*=40 data points for slope calculation. 2014-07-02 AB-01.DLD

All quality control criteria of oxygen sensor performance were met (MiPNet06.03 POS-Calibration-SOP).





Superimposed traces of oxygen concentration and respiration (Graph A) in phosphate buffer solution (PBS) and of H_2O_2 equivalents and H_2O_2 production (Graph B). During the first 1 h period, two steps were performed of H_2O_2 calibration with two titrations of 0.1 µM H_2O_2 (Graph B, showing the step changes; Graph C linear regression). Graph layout 05; *N*=10 data points for slope calculation.. 2014-07-02 AB-02.DLD

At 1:00 h, two 50 µl titrations (Y0.5 and Y1) of freshly prepared yeast suspension in PBS (20 mg W_d/ml stock; 1 mg/ml final yeast concentration) initiated stable respiratory ROUTINE states of respiration (Graph A), with R05 (Y0.5) being only 0.46 of Ra (Y1; Table 2). Stable respiration was in contrast to a continuous decline of H_2O_2 production with a discontinuity during the aerobic-anaerobic transition (Graph B). A H_2O_2 calibration was performed under anoxia (HP0.1; c 1:20 hours), showing the significantly lower response of the fluorescence signal in the presence versus absence of yeast cells. After anoxia, ROUTINE respiration increased consecutively above the initial activity levels (Table 2).

Table 2: Marked sections of respiration before and after anoxia in rehydrated yeast cells.

Averages	Unit	R0.5	Ra	Rb	Rc
Start		01:02:16	01:07:58	01:37:45	01:55:42
Stop		01:03:37	01:12:07	01:41:10	01:57:46
N Points		40	125	103	63
O2 Flux per W_d (1A)	pmol/(s*mg)	**205.0**	**225.0**	**315.4**	**345.4**
O2 Flux per W_d (B)	pmol/(s*mg)	**211.8**	**232.4**	**319.0**	**348.4**
O2 Concentration (1A)	nmol/ml	147.5	57.9	69.0	73.6
O2 Concentration (B)	nmol/ml	151.8	68.8	70.1	76.4

3. Temperature effect on air and zero oxygen calibration

Temperature exerts an influence not only on the oxygen solubility, which increases with decreasing temperature, but also on the signal of the oxygen sensor. Air and zero calibrations (Table 3) were performed during the workshop at 27 °C (2014-07-01) and 37 °C (2014-07-02).

Table 3. Calibration variables automatically recorded by DatLab: The raw signals of the two oxygen sensors at air saturation, R_1, increased by a factor of 1.27 at an increase of temperature by 10 °C, whereas the zero oxygen signal, R_0, was independent of temperature at 0.2 to 0.5% of the signal at air saturation.

Filename	POS #	Gain V/µA	$Temp_{R1}$ at calib.°C	R_1 V	R_0 V	$p_{b,R1}$ kPa	p_{O2}* kPa	p_{H2O}* kPa
				air sat. signal	zero signal	barom. pressure	air sat.	
2014-07-01 AB-02	1669	1	27.00	**1.7662**	**0.0096**	101.3	20.47	3.57
2014-07-02 AB-01	1669	1	37.00	**2.2670**	**0.0095**	100.7	19.78	6.27
2014-07-01 AB-02	1670	1	27.00	**1.7940**	**0.0059**	101.3	20.47	3.57
2014-07-02 AB-01	1670	1	37.00	**2.2769**	**0.0054**	100.7	19.78	6.27

At a standard atmospheric pressure of 100 (101.325) kPa, the oxygen concentration at air saturation in pure water is 207.3 (210.2) µM at 37 °C but 245.7 (249.1) µM at 27 °C. Insect culture medium was used at 27 °C, assuming an oxygen solubility factor of 0.89, which at a measured barometric pressure of 101.3 kPa yields an oxygen concentration for calibration at air saturation of 221.6 µM. PBS buffer at 37 °C and 100.7 kPa barometric pressure has an oxygen concentration of 198.5 µM at air saturation. All calculations are preformed automatically by DatLab.

Contribution

This O2k-Workshop is a contribution to the K-Regio project *MitoCom Tyrol*.

This project is funded by the Tyrolean Government and the European Regional Development Fund (ERDF) and is subject to the regulations of EU law as well as to the Directive of the Tyrolean Government on the Funding of Science, Research and Development.

References

Fasching M, Gnaiger E (2014) Polarographic oxygen sensors: calibration, accuracy and quality control SOP. Mitochondr Physiol Network 06.03(12): 1-8.

Fasching M, Gradl P, Gnaiger E (2014) O2k-Fluorescence LED2-Module. Mitochondr Physiol Network 17.05(05): 1-7.

Fontana-Ayoub M, Fasching M, Eigentler A, Laner V, Gnaiger E (2013) O2k-Fluorometry: Amplex® UltraRed using freeze-dried baker's yeast. Mitochondr Physiol Network 18.06.

www.oroboros.at **www.bioblast.at** - the *information synthase* for Mitochondrial Physiology and high-resolution respirometry

Recommended reading

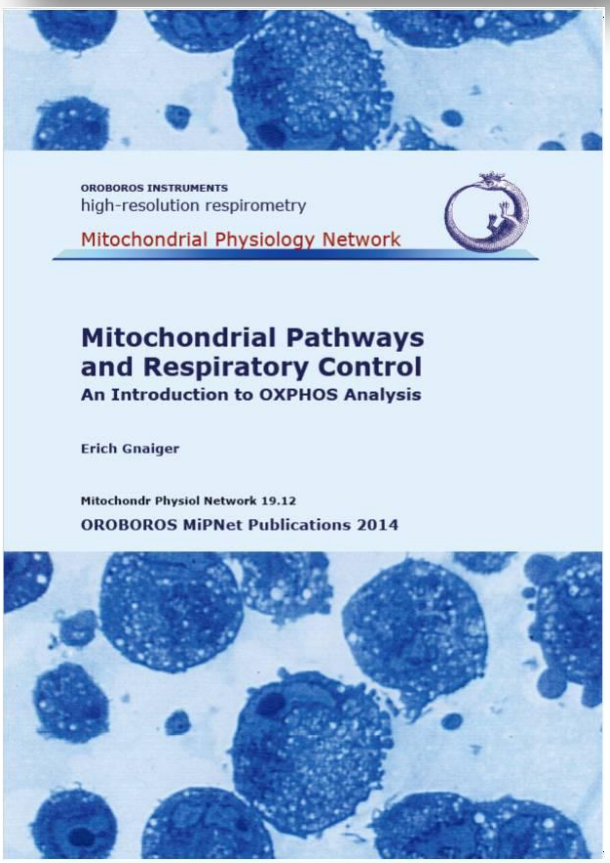
O2k-Core Manual

New: **»O2k-Core Manual.pdf**

SUIT protocols for high-resolution respirometry

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopisies of human muscle. Methods Mol Biol 810: 25-58. »Bioblast Access

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: 327-52. »Bioblast Access



HRR and O2k-Fluorometry

» Manual: O2k-Fluorescence LED2-Module

Eigentler A, Fontana-Ayoub M, Gnaiger E (2013) O2k-Fluorometry: HRR and H_2O_2 production in mouse cardiac tissue homogenate. Mitochondr Physiol Network 18.05(01): 1-6.

» O2k-Fluorometry

Mitochondrial pathways

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck: 80 pp. »Open Access - ***handout to O2k-Workshop participants***