



Course on High-Resolution Respirometry

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64th Workshop on High-Resolution Respirometry

2011 December 8
Hyderabad, India



Satellite to the 1st Annual Conference of the Society for Mitochondrial Research and Medicine - India (1st SMRM): Mitochondria in Biology and Medicine, Hyderabad, India - <http://www.mitoindia.org>.

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The 64th Workshop on High-Resolution Respirometry (HRR) is the **First Oxygraph-2k Workshop held in India**. Participants are scientists, mostly the Ph.D. students, who want to learn in detail the experimental basis of high-resolution approaches to measure mitochondrial respiratory function. The workshop includes a basic introduction to the **OROBOROS Oxygraph-2k** with integrated on-line analysis by **DatLab 4.3** and applications of the Titration-Injection microPump **TIP2k**. Specific emphasis is placed on on-line practical demonstration of instrument and software performance, to introduce the participants into the technical and operational details of **high-resolution respirometry (HRR)**. Quality control of instrumental performance will be demonstrated by on-line calibration of the oxygen sensors, evaluation of signal stability, and perspectives of HRR in mitochondrial physiology.

Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols**. An overview is given on the **O2k-MultiSensor System**, with the TPP⁺ electrode for simultaneous measurement of mt-membrane potential and oxygen consumption. As an outlook to OROBOROS innovations, our newest perspectives will be presented on the **O2k-Fluorescence Module**, which is developed in the frame of the project *MitoCom Tyrol*. Short presentations of 'Hot topics in Mitochondrial Physiology' extend the discussions on respiratory protocols.

Programme IOC64

Thursday, December 8

09.30 - 11:00

Session 1:

Principles of high-resolution respirometry: From switching on the Oxygraph-2k to the experimental result - with help from the O2k-Manual.

11.00 Coffee/Tea break

11.30 - 13.00

Session 2:

Instrumental quality control 1: Calibration of the oxygen sensor, stability testing, and evaluation of sensitivity to measure oxygen flux.

13.00 Lunch

14.00 - 15.30

Session 3:

Experimental design 1: Coupling control and substrate control of mitochondrial respiration.

15.30 Coffee/Tea break



16.00 - 18.00

Session 4:

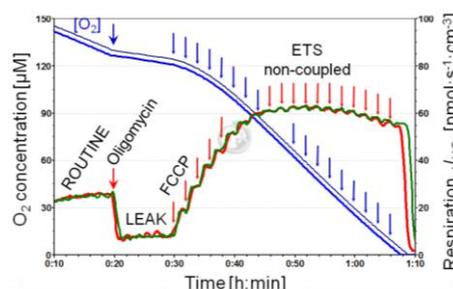
Instrumental quality control 2: Instrumental background test and on-line analysis of oxygen flux.

18.00 Coffee/Tea break

18.30 - 20.00

Session 5:

Experimental design 2: Respiratory control in intact cells, permeabilized cells and tissues, and permeabilized muscle fibres.



Specific links (Oroboros wiki Bioblast; OROBOROS USB-stick)

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|---------|----------------------------|--|
| Sess. 1 | MiPNet12.06
MiPNet12.07 | O2k-Manual: Oxygraph-2k: Start high-resolution respirometry.
O2k-Manual: DatLab 4 Guide through the menus. |
| Sess. 2 | MiPNet12.08
MiPNet06.03 | O2k-Manual: Oxygen and pX Calibration DatLab 4.3.
O2k-Protocols: Oxygen calibration and solubility in experimental media. |
| Sess. 3 | MiPNet12.11
MiPNet12.12 | O2k-Protocols: OXPHOS capacity and respiratory control in isolated mitochondria.
O2k-Protocols: Mitochondrial pathways through Complexes I+II: Convergent electron transport at the Q-junction and additive effect of substrate combinations. |
| Sess. 4 | MiPNet12.08
MiPNet14.06 | O2k-Manual: Oxygen and pX Calibration DatLab 4.3.
O2k-Protocols: Instrumental background correction and accuracy of oxygen flux. |
| Sess. 5 | MiPNet08.09
MiPNet12.15 | O2k-Protocols: High-resolution respirometry with cultured cells.
O2k-Protocols: MitoPathways: Respiratory states and flux control ratios. |

O2k-Workshop Materials and References

- Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58.
- 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.
- Hand SC, Lemieux H, Gnaiger E. Flux control ratios in isolated mitochondria. OXPHOS capacity and respiratory control in isolated mitochondria. *Mitochondr Physiol Network* 12.11.
- Gnaiger E. MitoPathways: Respiratory states and flux control ratios. *Mitochondr Physiol Network* 12.15.

Questions for the O2k-Workshop

The **O2k-Manual** and **Protocols** provides answers to many of these questions [☞ MiPNet numbers in the O2k-Compendium on the USB stick] – and you find more information on www.orooboros.at and wiki.orooboros.at

1. Oxygraph-2k assembly [☞MiPNet12.06]

How do you detect an oxygen leak in the chamber?

2. Polarographic oxygen sensor (POS) [☞MiPNet12.08]

- 2.1. Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
- 2.2. Does the POS respond to oxygen concentration, cO_2 [$\mu\text{mol}\cdot\text{dm}^{-3} = \mu\text{M}$], or partial oxygen pressure p_{O_2} [kPa]?
- 2.3. How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
- 2.4. Should the chamber be open or closed during air calibration?
- 2.5. How do you perform a zero oxygen calibration?
- 2.6. When is the oxygen calibration of a POS preferentially performed?
- 2.7. How long does it take approximately (5, 15, 30 or 45 min) to perform an oxygen calibration at air saturation, after the O2k is switched on (at experimental temperature in the range of 20 to 37 °C)?
- 2.8. Does the oxygen signal have to be stable for an oxygen calibration of the POS?
- 2.9. Can you calibrate the POS with biological sample and respiratory activity in the aqueous solution, when equilibration is performed with a gas phase in the chamber and stability of the signal is observed?

3. Cleaning the Chamber [☞MiPNet06.03]

- 3.1. Which solution should be placed in the chamber when the O2k is not in use (i.e. overnight, for a few days)?
- 3.2. What is the recommended cleaning procedure between experimental runs?

4. Instrumental background test [☞MiPNet12.09; ☞MiPNet14.06]

- 4.1. Does the oxygen signal have to be stable (constant) for setting a mark in an instrumental background test?
- 4.2. How do you define flux stability?
- 4.3. Do you need to calibrate the POS before performing an instrumental background calibration?
- 4.4. How often do you have to check the instrumental background?

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Further information

Introductory course material is available on our homepage www.oroboros.at, within the following sections:

O2k-Manual www.oroboros.at/index.php?o2k-manual
Protocols www.oroboros.at/index.php?mipnet-protocols
O2k-Publications <http://wiki.oroboros.at/index.php/O2k-Publications>

Bioblast

wiki.oroboros.at - the
information synthase for
 Mitochondrial Physiology and
 high-resolution respirometry:

<http://www.bioblast.at>



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