



Course on High-Resolution Respirometry

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73rd Workshop on High-Resolution Respirometry

**2012 January 15-16
Kolkata, India**

Satellite to the Neurocon 2013 - International Conference on Neurodegenerative and Neurodevelopmental Disorders: Translational Aspects, Kolkata, India

>> <https://sites.google.com/site/neuroconkolkata/home/pre-conference-workshop>

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The 73rd Workshop on High-Resolution Respirometry is the **Third Oxygraph-2k Workshop held in India** (following Katra and Hyderabad). Participants are scientists, mostly Ph.D. students, who will be introduced into the experimental basis of OXPHOS Analysis. High-resolution respirometry provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS Analysis is extended to evaluate coupling efficiencies and OXPHOS capacities with carbohydrate versus fatty acid substrates, and to diagnose defects in respiratory complexes of the electron transfer system and the phosphorylation system. Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols** for simultaneous measurement of respiratory function, mitochondrial membrane potential, and hydrogen peroxide production.

The O2k-Workshop includes a basic introduction to the **OROBOROS Oxygraph-2k** with integrated on-line analysis by **DatLab 5.1** and applications of the Titration-Injection microPump **TIP2k**. An overview is given on the **O2k-MultiSensor System**, with the TPP⁺ electrode for simultaneous measurement of mt-membrane potential and oxygen consumption. Further, the new **O2k-Fluorescence LED2-Module** will be presented. 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.

Programme IOC73

Tuesday, January 15

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.

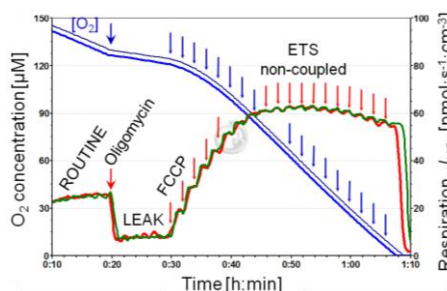
13.00 Lunch

14.00 - 15.30

Session 3:

Experimental design 1: Respiratory control in intact cells, permeabilized cells and tissues, and permeabilized muscle fibres.

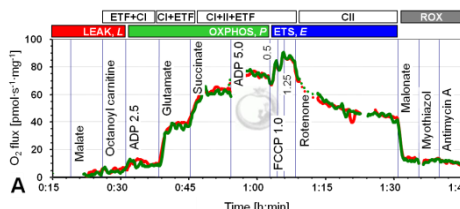
15.30 Coffee/Tea break



16.00 - 18.00

Session 4:

Experimental design 2: Coupling control and substrate control of respiration and mt-membrane potential in mouse brain.



Wednesday, January 16:

09:30 - 11:00

Session 5:

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

11.00 Coffee/Tea break

11.30 - 13.00

Session 6:

High-resolution respirometry and O2k-Fluorometry: A demo experiment.

13.00 Lunch

14.00 - 14.15

Session 7:

The corporate concept of OROBOROS INSTRUMENTS.

14.15 - 15.30

Session 8:

O2k-Fluorescence: Oxidative stress and hydrogen peroxide production. Dependence on oxygen concentration and metabolic state.

15.30 Coffee/Tea break

16.00 - 18.00

Session 9:

Summary on OXPHOS-Analysis. From intact cells to isolated mitochondria.

O2k-Workshop Suggested Reading

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.

Lemieux H, Semsroth S, Antretter H, Hoefler D, Gnaiger E (2011) Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart. *Int J Biochem Cell Biol* 43: 1729-1738.

Gnaiger E (2012) Mitochondrial Pathways and Respiratory Control. An Introduction to OXPHOS Analysis. *Mitochondr Physiol Network* 17.18. OROBOROS MiPNet Publications, Innsbruck: 64 pp.

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

O2k-Manual www.orooboros.at/index.php?O2k-Manual

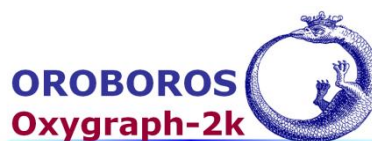
Protocols www.orooboros.at/index.php?MipNet-Protocols

O2k-Publications <http://wiki.orooboros.at/index.php/O2k-Publications>

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