



Course on High-Resolution Respirometry

IOC89 Mitochondrial Physiology Network 19.04: 1-3 (2014)
Updates: www.bioblast.at/index.php/IOC89

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89th Workshop on High-Resolution Respirometry & O2k-Fluorometry

2014 May 1-2
La Jolla, CA, USA

*Post-conference workshop: Exp Biol
San Diego, USA. 2014 April 26-30*
» experimentalbiology.org/2014/Home.aspx

Venue:

Scripps Research Institute
3545 Cray Court, San Diego CA, 92121
Parking available

Contact:

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Lecturers and tutors:

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OROBOROS INSTRUMENTS Corp

high-resolution respirometry

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The 89th Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with one of our MiPNet Labs in California, US. The O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS Oxygraph-2k** with integrated on-line analysis, introducing new features of **DatLab 5.2**.

The main focus will be a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations, 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 phosphorylation system. Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols** in HRR using the **O2k-Fluorescence LED2-Module** for simultaneous measurement of hydrogen peroxide production (Amplex red®). Discussions are extended on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TPP⁺ or TPMP⁺ (potentiometric), and on perspectives of HRR in mitochondrial physiology.

The group of Brunhilde Felding-Habermann (Scripps Research Institute) uses high-resolution respirometry since 2011 and extended the O2k-Core by the O2k-Fluorescence LED-2 Module.



Programme IOC89

Thursday, May 1:

08:45 Registration

09:00 – 09:30 **Introduction of participants:** who is who?

09:30 – 10:30 **Get started with the O2k:** set-up of the instrument.

10:30 Coffee break – Registration ctn.

11:00 – 12:30 **Pro’s and con’s of mt-preparations:** Coupling and substrate control of respiration and H₂O₂ production in tissue homogenate, permeabilized fibres – or isolated mitochondria?

12:30 Lunch

13:15 – 14:00 **Phosphorylation protocol for intact cells.**

14:00 – 15:00 **Comprehensive OXPHOS analysis:** A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.

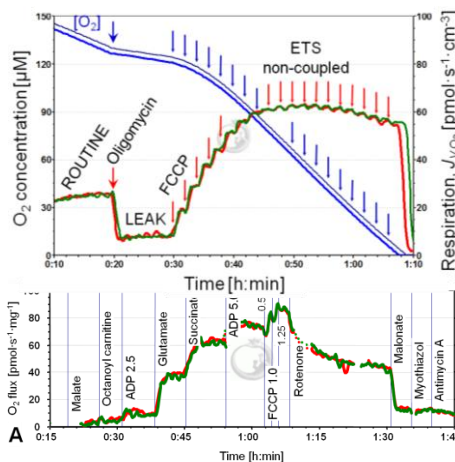
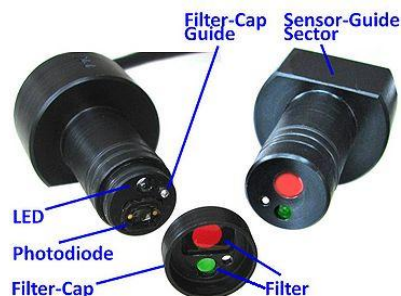
15:00 – 15:30 **Experimental setup 1:** OroboPOS - sensor quality control, calibration.

15:30 Coffee Break

16:00 – 17:00 **Experimental setup 2:** Calibration of O2k-Fluo Sensors

17:00 – 18:00 **Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.

18:30 O2k-Workshop dinner



Friday, May 2:

09:00 – 10:30 **Hands-on:** HRR and O2k-Fluorometry with breast epithelial cells (MCF-10A and MDA-MB 435) – respiration and H₂O₂ production.

10:30 Coffee break

11:00 – 12:30 **Hands-on continued**

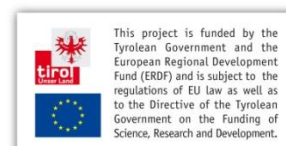
12:30 Lunch

13:15 – 15:30 **Data analysis**

15:30 Coffee break

16:00 – 18:00 **Data analysis continued feedback – conclusions.**

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



Contribution to K-Regio MitoCom Tyrol.

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 biopsies 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: 327-352.

[»Bioblast Access](#)

HRR and O2k-Fluorometry

[»Manual: O2k-Fluorescence LED2-Module](#)

Eigentler A, Fontana-Ayoub M, Gnaiger E (2013) O2k-Fluorometry: HRR and H₂O₂ 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](#)

