



O2k-Workshops

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Workshop on high-resolution respirometry & O2k-Fluorometry

2016 March 06-07
Singapore, SG

*Pre-satellite workshop:
3rd International Symposium
New Frontiers in Cardiovascular Research.
March 08-09, 2016*

Venue:

National Heart Centre Singapore
5, Hospital Drive
Singapore, 169609

Host:

Derek J. Hausenloy, PhD
Jean-Paul Kovalik, PhD
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Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD
OROBOROS INSTRUMENTS
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The 108th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with our O2k-Network Lab in Singapore. This O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS O2k** with integrated on-line analysis, introducing new features of **DatLab 6**.

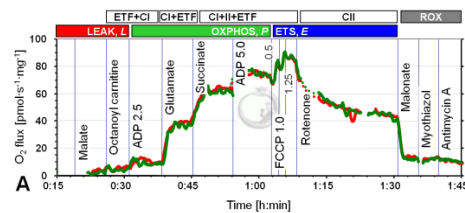
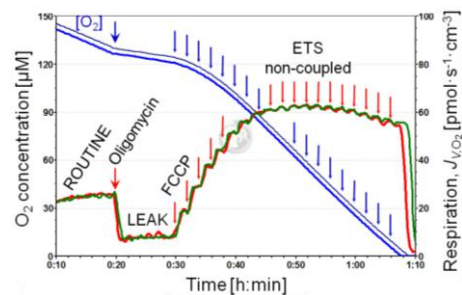
The workshop will include a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS 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 to comparing measurements of mt-membrane potential using **MultiSensor Modules** and to evaluating perspectives of HRR in mitochondrial physiology.



Program IOC

Sunday, March 06:

08:45 Registration
 09:00 – 09:15 Welcome by Derek Hausenloy
 09:15 – 09:30 **Introduction of participants:** Who is who?
 09:30 – 10:30 **Get started with the O2k.**
 10:30 Coffee break – Registration ctn.
 11:00 – 12:15 **Pros and cons of mt-preparations:** Coupling and substrate control of O₂ consumption and H₂O₂ production in homogenate, permeabilized fibres – or isolated mitochondria?
 12:15 – 12:30 Permeabilized fibre preparation – what to take care of?
 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 – 17:30 The Bioblast wiki and O2k-Network.
 17:30 – 18:00 **Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.
 18:30 O2k-Workshop dinner @ Candlenut



Monday, March 07:

08:30 – 10:30 **Experiment:** HRR and O2k-Fluorometry – respiration and extracellular H₂O₂ production.
 10:30 Coffee break
 11:00 – 12:00 **Experiment continued**
 12:00 Lunch
 12:45 – 15:30 **Data analysis**
 15:30 Coffee break
 16:00 – 16:40 **Technical support & Open innovation**
 16:40 – 18:00 **Feedback – conclusions – stay connected** as an O2k-Network Lab



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

Recommended reading

O2k-Core Manual:

»[Bioblast link](#)«

SUIT protocols for O2k 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 link](#)«

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 link](#)«

HRR and O2k-Fluorometry

»[Manual: O2k-Fluo 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. »[Bioblast link](#)«

»[O2k-Fluorometry Publications](#)«

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.

»[Bioblast link](#)«

