



## O2k-Workshops

**IOC108** Mitochondrial Physiology Network 21.05(1):1-3 (2016)  
Updates: [http://wiki.oroboros.at/index.php/MiPNet21.05\\_IOC108\\_Singapore\\_SG](http://wiki.oroboros.at/index.php/MiPNet21.05_IOC108_Singapore_SG)

©2016 OROBOROS®  
Version 02: 2016-03-04

## Workshop on high-resolution respirometry & O2k-Fluorometry

**2016 March 06-07**  
**Singapore, SG**

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

### Venue:

National Heart Centre Singapore  
Level 9 teaching room (near lift block C)  
5, Hospital Drive  
Singapore, 169609

### Host:

Derek J. Hausenloy, PhD  
Jean-Paul Kovalik, PhD  
[d.hausenloy@ucl.ac.uk](mailto:d.hausenloy@ucl.ac.uk), [jean-paul.kovalik@duke-nus.edu.sg](mailto:jean-paul.kovalik@duke-nus.edu.sg)

### Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD  
**OROBOROS INSTRUMENTS**  
high-resolution respirometry  
Schoepfstr 18, A-6020 Innsbruck, Austria - [www.oroboros.at](http://www.oroboros.at)  
[erich.gnaiger@oroboros.at](mailto:erich.gnaiger@oroboros.at);



The 108<sup>th</sup> 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 Jean-Paul Kovalik  
 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 O<sub>2</sub> consumption and H<sub>2</sub>O<sub>2</sub> 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.

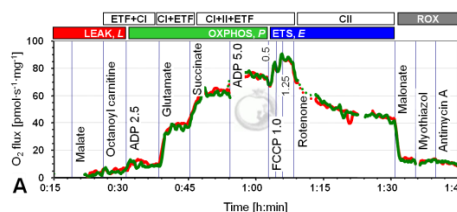
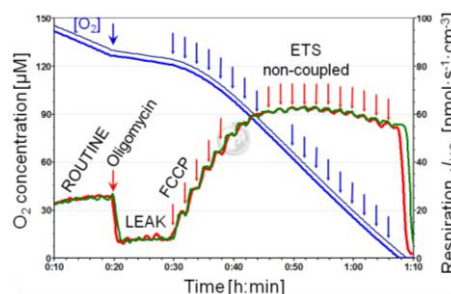
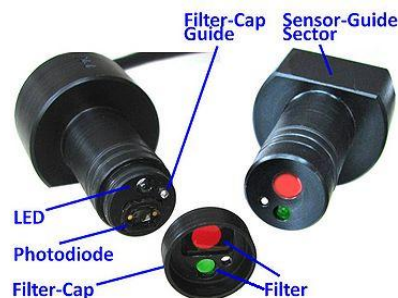
15:30 Coffee Break

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 at PappaSan, Dorsett Hotel



## Monday, March 07:

08:30 – 10:30 **Experiment:** HRR and O2k-Fluorometry – respiration and extracellular H<sub>2</sub>O<sub>2</sub> production with intact cells.

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](http://www.orooboros.at) [www.bioblast.at](http://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](#)«

Fontana-Ayoub M, Fasching M, Eigentler A, Laner V, Gnaiger E (2015) O2k-Fluorometry: Amplex® UltraRed using freeze-dried baker's yeast. *Mitochondr Physiol Network* 18.06(04):1-4 » [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](#)«

