



## O2k-Workshops

**IOC110** Mitochondrial Physiology Network 21.04(01):1-3 (2016)  
Updates: [http://wiki.oroboros.at/index.php/MiPNet21.04\\_IOC110\\_Melbourne\\_AU](http://wiki.oroboros.at/index.php/MiPNet21.04_IOC110_Melbourne_AU)

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# Workshop on high-resolution respirometry & O2k-Fluorometry

**2016 April 12-13**  
**Melbourne, AU**

*Pre-conference workshop:*  
*6<sup>th</sup> Exercise and Sport Science Australia*  
*Conference.*  
*April 14-16, 2016*

**Venue:**

Building P  
Institute of Sport, Exercise and Active Living (ISEAL)  
Victoria University  
Ballarat Road  
Melbourne, Australia

**Host:**

Nigel K. Stepto, PhD  
David Bishop, Prof.  
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[www.bioblast.at/index.php/AU\\_Melbourne\\_Stepto\\_NK](http://www.bioblast.at/index.php/AU_Melbourne_Stepto_NK)

**Lecturers and tutors:**

Erich Gnaiger, Ao.Univ.-Prof. PhD  
Carolina Doerrier, PhD  
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The 110<sup>th</sup> O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with one of our prominent O2k-Network Labs in Melbourne. 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**.

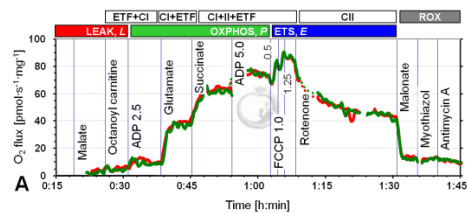
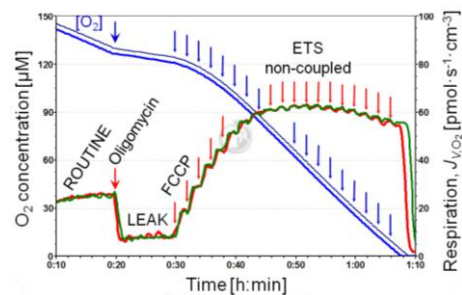
The workshop will include a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS 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 on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TPP<sup>+</sup> or TPMP<sup>+</sup> (potentiometric), and on perspectives of HRR in mitochondrial physiology.



# Program IOC

## Tuesday, April 12:

08:45 Registration  
 09:00 – 09:15 Welcome by David Bishop  
 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 **Pro’s and con’s 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



## Wednesday, April 13:

08:30 – 10:30 **Experiment:** HRR and O2k-Fluorometry with permeabilised fibres from rat – respiration and extracellular H<sub>2</sub>O<sub>2</sub> 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](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](#)«

Eigentler A, Fontana-Ayoub M, Gnaiger E (2013) O2k-Fluorometry: HRR and H<sub>2</sub>O<sub>2</sub> production in mouse cardiac tissue homogenate. *Mitochondr Physiol Network* 18.05(01):1-6.

»[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](#)«

