



O2k-Workshops

IOC Mitochondrial Physiology Network 21.04(01):1-3 (2015)
Updates: http://wiki.orooboros.at/index.php/MiPNet21.04_IOC_Melbourne_AU

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Workshop on O2k high-resolution respirometry & O2k-Fluorometry

2016 April 12-13
Melbourne, AU

Pre-conference workshop:
6th 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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http://www.bioblast.at/index.php/AU_Melbourne_Stepto_NK

Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD
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The 108th 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⁺ or TPMP⁺ (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 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.

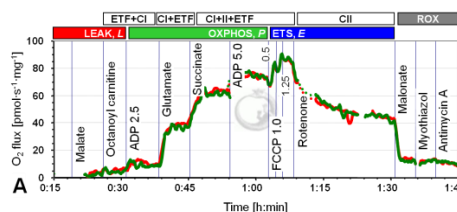
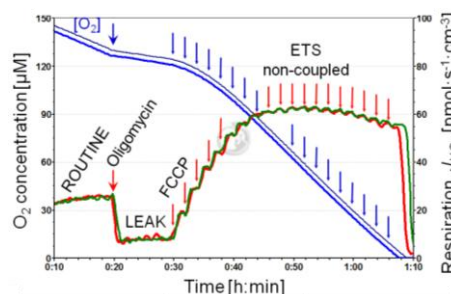
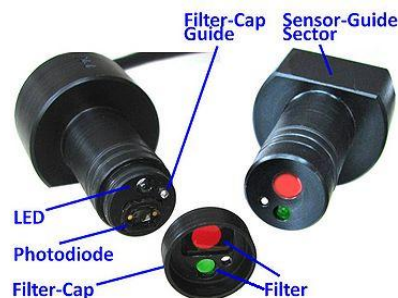
15:30 Coffee Break

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



Wednesday, April 13:

08:30 – 10:30 **Experiment:** HRR and O2k-Fluorometry with permeabilised fibres from rat – 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.

»[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](#)«

