

110th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 April 12-13
Melbourne, AU

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://wiki.oroboros.at/index.php/AU_Melbourne_Stepto_NK

Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD
Carolina Doerrier, PhD

OROBOROS INSTRUMENTS

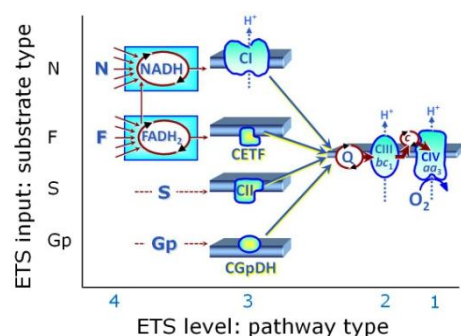
high-resolution respirometry
Schoepfstr 18, A-6020 Innsbruck, Austria - www.oroboros.at
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Pre-conference workshop:
6th Exercise and Sport Science
Australia Conference.
April 14-16, 2016

The 110th O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is held in cooperation with one of our prominent O2k-Network Labs in Melbourne. This O2k-Workshop presents a basic introduction to the **OROBOROS Oxygraph-2k** with integrated real-time data analysis. We introduce new features of **DatLab 7** and the concept of a quality control system including the MitoFit interlaboratory proficiency test.

HRR provides information on cell respiration with basic coupling control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria), to evaluate coupling efficiencies and OXPHOS capacities with electron transfer into the Q-junction converging from NADH, FADH₂, succinate and α -glycerophosphate (N,F,S,Gp), to diagnose defects in respiratory electron transfer system pathways and the 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.



Programme

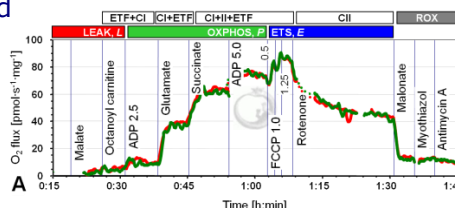
Tuesday, April 12

08:45 Registration
 09:00 – 09:15 Welcome by David Bishop
 09:15 – 09:30 Introduction of participants: Who is who?
 09:30 – 10:00 Get started with the O2k: Overview with video clips.
 10:00 – 10:30 Human muscle biopsy preparation.



10:30 Coffee break – Registration ctn.

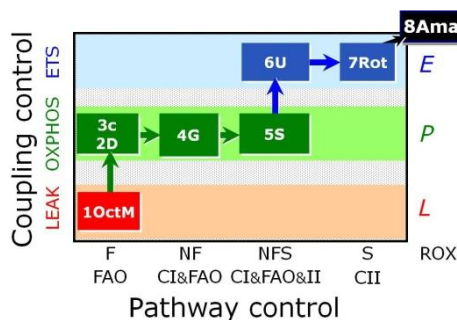
11:00 – 12:15 Pro’s and con’s of mt-preparations: Coupling and pathway control of O₂ consumption and H₂O₂ production in homogenate, permeabilized fibres – or isolated mitochondria?



12:15 – 12:30 Permeabilized fibre preparation – what to take care of?

12:30 Lunch

13:15 – 15:00 Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways.



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.

18:30 O2k-Workshop dinner

Wednesday, April 13

08:30 – 10:30 Experiment: HRR and O2k-Fluorometry with permeabilised fibres – 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

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.

»[Full text in Bioblast](#)«



O2k-Core Manual:

»[Full text in Bioblast](#)«

SUIT protocols for O2k high-resolution respirometry

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. »[Full text in Bioblast](#)«

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. »[Full text in Bioblast](#)«

HRR and O2k-Fluorometry

»[Manual: O2k-Fluo LED2-Module](#)«

Makrecka-Kuka M, Krumschnabel G, Gnaiger E (2015) High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate and isolated mitochondria. Biomolecules 5:1319-38. »[Bioblast link](#)«

»[O2k-Fluorometry Publications](#)«



COST Action CA15203 Mitochondrial fitness mapping

MITO EAGLE: Evolution - Age - Gender - Lifestyle - Environment

Contribution to K-Regio project **MitoFit**.

Funded in part by the Land Tirol. www.mitofit.org

