

111th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 June 13-14
Seattle, WA, USA

Venue:

South Lake Union Campus
University of Washington
850 Republican St.
Seattle, WA 98109, USA

Host:

David J Marcinek, PhD
Matthew D Campbell, PhD
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http://wiki.oroboros.at/index.php/US_WA_Seattle_Marcinek_DJ

Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD

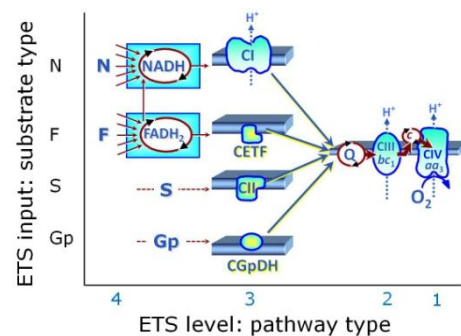
OROBOROS INSTRUMENTS

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The **111th O2k-Workshop** on high-resolution respirometry and O2k-Fluorometry is held in cooperation with one of our prominent O2k-Network Labs in Seattle. 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.



Program IOC

Monday, June 13:

08:45
09:00 – 09:15
09:15 – 09:30
09:30 – 10:00
10:00 – 10:30
 10:30
11:00 – 12:15

12:15 – 12:30
 12:30
13:15 – 15:00

15:00 – 15:30
 15:30
16:00 – 17:00
17:00 – 17:30
17:30 – 18:00
 18:30

Registration
 Welcome by David Marcinek
Introduction of participants:
 Who is who?
Get started with the O2k:
 Overview with video clips.
Human muscle biopsy preparation.
 Coffee break – Registration continued.
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?



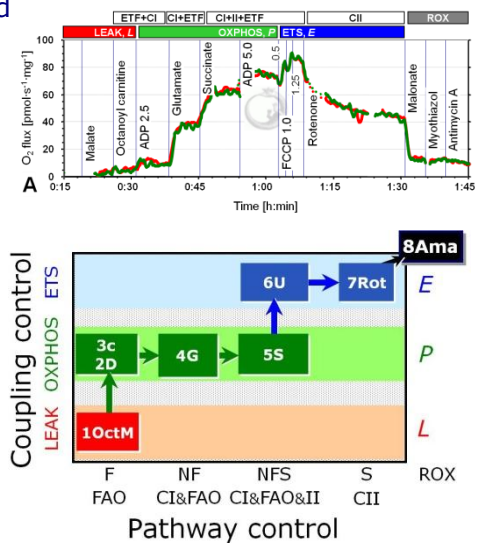
Permeabilized fibre preparation – what to take care of?
 Lunch

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

Experimental setup 1:
 OroboPOS - sensor quality control, calibration.

Coffee Break
Experimental setup 2: Calibration of O2k-Fluo Sensors
 The Bioblast wiki and O2k-Network.

Q&A session on HRR and OXPHOS analysis: Design of experimental protocol.
 O2k-Workshop dinner at Orfeo restaurant <http://orfeorestaurant.com/>



Tuesday, June 14:

08:30 – 10:30

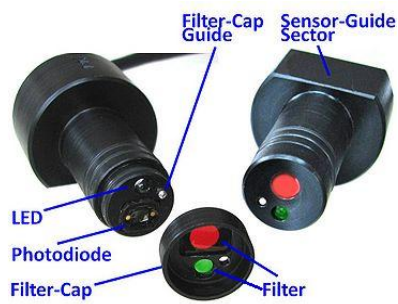
 10:30
11:00 – 12:00
 12:00
12:45 – 15:30
 15:30
16:00 – 17:00

Demo-Experiment: HRR and O2k-Fluorometry with permeabilized fibers from mouse – respiration and extracellular H₂O₂ production.
 Coffee break

Experiment continued
 Lunch

Data analysis & technical support
 Coffee break

Genevieve Sparagna: Mitochondrial supercomplexes and cardiolipin in human heart failure – public lecture at 850 Republican Orin Smith auditorium



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.

»[Bioblast link](#)«



O2k-Core Manual:

»[Bioblast link](#)«

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.

»[Bioblast link](#)«

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](#)«

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

