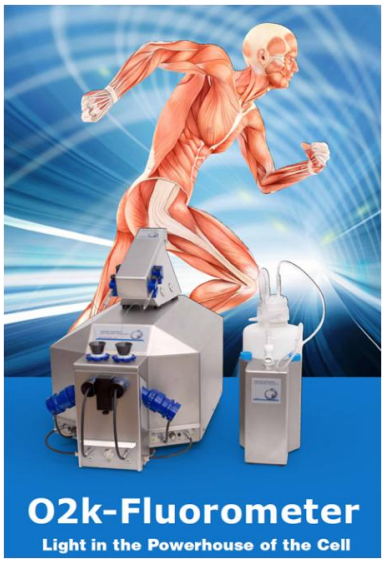
IOC104 Mitochondrial Physiology Network 20.05(03):1-4 (2015)
Updates: http://wiki.oroboros.at/index.php/MiPNet20.05_IOC104
Version 03: 2015-08-09

104th Workshop on O2k high-resolution respirometry & O2k-Fluorometry 2015 August 09-10 Greenville, NC, USA



Pre-conference workshop:
MiP*school* Greenville 2015
August 10-14

Venue:
East Carolina Diabetes & Obesity Institute
East Carolina University
East Carolina Heart Institute Building at ECU
4th floor, Robotics Conference Room

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Assisted by the Greenville team:
Peter Chien-Te Lin, Lauren Reese, Terence E Ryan, Cheryl Smith, Cody D Smith

This O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is held in cooperation with a prominent O2k-Network Cluster: US NC Greenville East Carolina Univ. The O2k-Workshop includes a basic training on instrumental quality control with the **OROBOROS O2k**, presents new features of **DatLab 6**, and introduces a new approach of experiments with a cryopreserved mitochondrial reference sample (**MitoFit test**).

MitoFit
mitochondria fit&well

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-Fluo 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

Day 1 - Sunday, August 09

08:45	Registration
09:00 – 09:15	Welcome
09:15 – 09:30	**Introduction of participants:** who is who?
09:30 – 10:30	**Get started: O2k assembly** with O2k-video support.
10:30	*Coffee break – Registration ctn.*
A. 11:00 – 12:15	**A1, A2, A3: Hands-on: O2k assembly and OroboPOS service** – alternating groups.
B. 11:00 – 12:15	**Comprehensive OXPHOS analysis:** Simultaneous measurement of respiration and mt-membrane potential: solving a puzzle.
12:15 – 12:30	**Demo: permeabilized fibre preparation** – what to take care of.
12:30	*Lunch*
13:15 – 14:15	**Instrumental quality control:** OroboPOS test, O_2 and H_2O_2 calibration, instrumental background.
A. 14:15 – 15:30	**A practical approach: DatLab features for instrumental quality control and calibration SOP** (O2k-demo).
B. 14:15 – 15:30	**B1, B2, B3: Hands-on instrumental quality control** (6 O2k).
15:30	*Coffee Break*
16:00 – 17:00	**O2k-Fluorometer demo:** Respiration and H2O2 production in cells (freeze-dried baker's yeast)
17:00 – 17:30	**Darrel Neufer:** Sharing our experience as an O2k-Network Lab.
17:30 – 18:00	**Discussion with Chuck Hoppel and Erich Gnaiger on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.
18:30	*O2k-Workshop dinner*

Day 2 - Monday, August 10

08:30 – 08:40	**Start the O2k experiment** (O2k-demo).
08:40 – 09:00	**Introduction:** HRR and O2k-Fluorometry with cryopreserved cells (MitoFit proficiency test) / permeabilized fibres – respiration and H_2O_2 production.
09:00 – 09:05	**MitoFit proficiency test:** start with cryopreserved cells.
A. 09:05 – 10:30	**SUIT protocol for OXPHOS analysis with permeabilized cells** (O2k-demo).
B. 09:05 – 10:30	**B1, B2, B3: Hands-on with permeabilized cells** (6 O2k or O2k-Fluorometers).
10:30	*Coffee break*
A. 11:00 – 11:30	**Cleaning SOP** (O2k-demo).
B. 11:00 – 11:30	**B1, B2, B3: Cleaning SOP for O2k-Chambers and microsyringes** (4 O2k, 2 O2k-Fluorometers).
11:30 – 12:00	**DatLab analysis: O2k-configuration for mark statistics.**
12:00	*Lunch*
12:45 – 13:45	**DatLab analysis: Excel templates.**
A. 13:45 – 15:00	**DatLab analysis of the O2k-demo experiment**.
B. 13:45 – 15:00	**B1, B2, B3: DatLab analysis of hands-on experiments.**
15:00 – 15:30	**Chuck Hoppel: Experimental summary – from instrumental performance to laboratory quality control .**
15:30	*Coffee break*
16:00 – 16:40	**Technical support**
16:40 – 18:00	**Feedback – conclusions – stay connected** as O2k-Network Reference Laboratories

List of participants

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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_2O_2 production in mouse cardiac tissue homogenate. Mitochondr Physiol Network 18.05(01):1-6. »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«

This O2k-Workshop is part of the K-Regio project **MitoFit**.

www.bioblast.at
- the *information synthase* for Mitochondrial Physiology and O2k high-resolution respirometry