



Course on High-Resolution Respirometry

IOC99 Mitochondrial Physiology Network 20.02: 1-3 (2014)
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99th Workshop on High-Resolution Respirometry & O2k-Fluorometry

2015 March 23-24
Cape Town, ZA

Venue:

Research Unit for Exercise Science and Sports Medicine (ESSM)
Department of Human Biology
University of Cape Town

Host:

Edward O Ojuka, PhD
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Lecturers and tutors:

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The 99th O2k-Workshop on high resolution respirometry (HRR) and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with the [MiPNet Lab of Prof. Edward O Ojuka](#) in Cape Town. The O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS Oxygraph-2k** with integrated real-time analysis, introducing new features of **DatLab 6**.

The workshop will include a discussion on optimization on OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, cell culture and tissue homogenate). HRR provides information on cell respiration with simple phosphorylation 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 transport 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 on HRR in mitochondrial physiology.



Program IOC99

Monday, March 23:

08:30 Registration

09:00 – 09:10 **Edward Ojuka**
Welcome – Perspectives on pre-MiPschool Workshop and MiPschool in Cape Town

09:10 – 09:20 **Introduction of participants: Who is who?**

09:20 – 10:20 **Erich Gnaiger: Get started with the Oxygraph-2k.**

10:20 Coffee break – Registration ctn.

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

12:00 – 12:30 Q&A session on mt-preparations and coupling control

12:30 Lunch break

13:30 – 14:15 **Phosphorylation protocol for intact cells**

14:15 – 15:15 **Comprehensive OXPHOS analysis:** Simultaneous measurements of respiration and mt-membrane potential

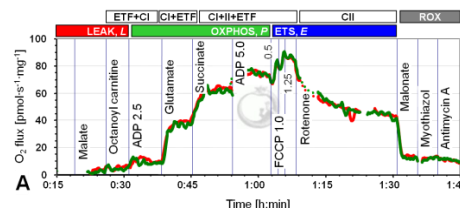
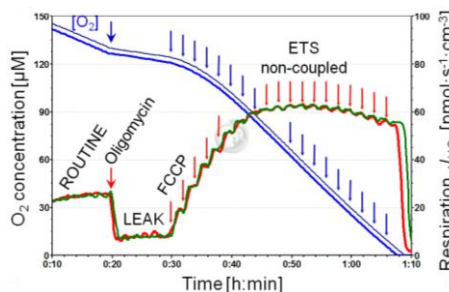
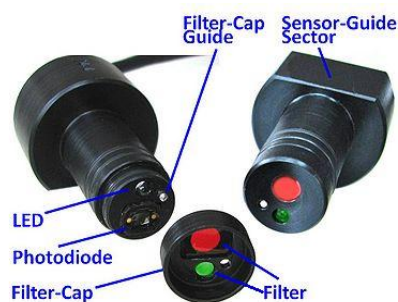
15:15 – 15:45 **Q&A session on HRR and OXPHOS analysis**

15:45 Coffee break

16:15 – 17:15 **Experimental setup 1:** OroboPOS-sensor quality control and calibration

17:15 – 18:00 **Q&A session on HRR and OXPHOS analysis**

19:30 O2k-Workshop dinner



Tuesday, March 24:

09:00 – 10:30 **Experiment:** HRR and O2k-Fluorometry with intact cells – respiration and extracellular H₂O₂ production.

10:30 Coffee break

11:00 – 13:00 **Experiment continues**
Data analysis, Trouble shooting and Conclusions

13:00 Lunch and feedback

Recommended reading

O2k-Core Manual

New: [»O2k-Core Manual.pdf](#)

SUIT protocols for 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 Access](#)

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 Access](#)

HRR and O2k-Fluorometry

» [Manual: O2k-Fluorescence 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](#)

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.

[»Open Access](#) - **handout to O2k-Workshop participants**

