



Course on High-Resolution Respirometry

IOC99 Mitochondrial Physiology Network 20.02(02): 1-3 (2015)
Updates: http://wiki.oroboros.at/index.php/MiPNet20.02_IOC99

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Version 02: 2015-03-30

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
Department of Human Biology
University of Cape Town
edward.ojuka@uct.ac.za
www.bioblast.at/index.php/ZA_Cape_Town_Ojuka_EO

Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD
Verena Laner, Mag.

OROBOROS INSTRUMENTS Corp
high-resolution respirometry
Schoepfstr 18, A-6020 Innsbruck, Austria - www.oroboros.at
erich.gnaiger@oroboros.at; verena.laner@oroboros.at;



The 99th O2k-Workshop on high resolution respirometry (HRR) and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with the [O2k-Network 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 O2k-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.

10:50 – 12:00 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?

12:00 – 12:30 Ali Sameh S (Zewail City for Science and Technology): Sharing our experience as an O2k-Network Lab

12:30 Lunch break

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

14:15 – 15:15 Comprehensive OXPHOS analysis: A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.

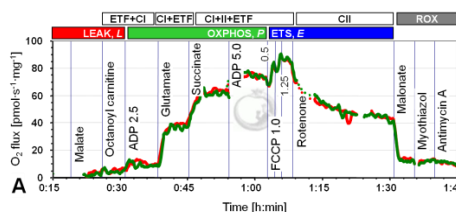
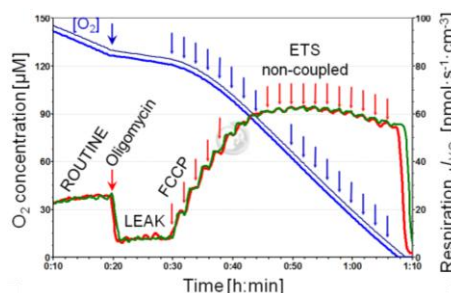
15:15 – 15:45 Experimental setup 1: OroboPOS - sensor quality control, calibration

15:45 Coffee break

16:15 – 17:15 Experimental setup 2: Calibration of O2k-Fluo Sensors

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 – 12:30 Experiment continued

12:30 – 13:00 Data analysis, technical service and conclusions

13:00 Lunch and feedback

Participants

Participant	Institution
Abdel-Rahman Ali Engy	ET Giza Ali SS : Zewail City of Science & Technology (ET)
Ali Sameh S	ET Giza Ali SS : Zewail City of Science & Technology (ET)
Andrew Brittany	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Bajpeyi Sudip	US TX El Paso Bajpeyi S : University of Texas at El Paso (TX, US)
Benade Janina	ZA Stellenbosch Benade J : Stellenbosch University (ZA)
Bezuidenhout Nicole	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Danzil Joseph	ZA Stellenbosch Essop F : Stellenbosch University (ZA)
Driescher Natasha	ZA Stellenbosch Essop F : Stellenbosch University (ZA)
Feldmann Daneil	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
George Siddiqah	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Govender Jenelle	ZA Stellenbosch Engelbrecht AM : Stellenbosch University (ZA)
Kohn Tertius	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Kriel Jurgen	ZA Stellenbosch Loos B : Stellenbosch University (ZA)
Leith David	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Louw Roan	ZA Potchefstroom Van Der Westhuizen F : North-West University (ZA)
Maarman Gerald	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Madlala Hlengiwe	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Meldau Surita	ZA Johannesburg Meldau S : National Health Laboratory Service (ZA)
Mendham Amy	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Ojuka Edward O	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Sishi Balindiwe JN	ZA Stellenbosch Sishi BJN : Stellenbosch University (ZA)
Smith James	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Tasneem Adam	ZA Cape Town Tasneem A : University of Cape Town (HICRA) (ZA)
Tsang Alvin Ming-Yun	US NC Greenville Brown DA : East Carolina Diabetes & Obesity Institute (NC, US)
Van Der Westhuizen Francois	ZA Potchefstroom Van Der Westhuizen F : North-West University (ZA)
Van Dyk Hayley	ZA Potchefstroom Van Der Westhuizen F : North-West University (ZA)
Webster Christopher	ZA Cape Town Ojuka EO : University of Cape Town (ESSM) (ZA)
Wolf Luisa	DE Regensburg Wolf L : University of Regensburg (DE)

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.**

