OROBOROS O2k-Workshops

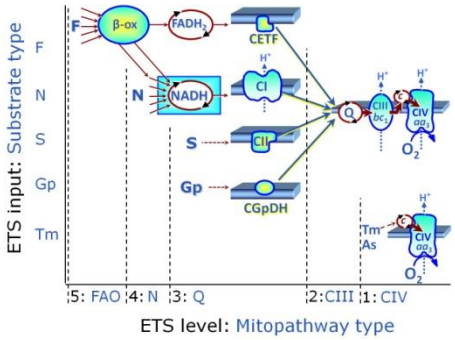
Mitochondrial Physiology Network 21.18(02):1-4 (2016)
Version 02: 2016-09-26
Updates: http://wiki.oroboros.at/index.php/MiPNet21.18_IOC117_Kuala_Lumpur_MY

117th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 September 26-30
Kuala Lumpur, MY

Host institution:
Prof. Dr. Norwahidah Abdul Karim
PPUKM
Bangunan Pra-Klinikal
Bilik Seminar Jabatan Biokimia, Level 17
MY Kuala Lumpur Abdul Karim N

OROBOROS distributor:
SAUJANA SAINTIFIK Sdn. Bhd.
1st Floor, 1F-08, IOI Business Park,
NO 1, Persiaran Puchong Jaya Selatan,
Bandar Puchong Jaya, 47170 Puchong,
Selangor Malaysia,
Kuala Lumpur, MY



Lecturer and tutor:
Erich Gnaiger, Ao.Univ.-Prof. PhD
OROBOROS INSTRUMENTS
Schoepfstr 18, A-6020 Innsbruck, Austria - www.oroboros.at
erich.gnaiger@oroboros.at

Assisted by:
Prof. Dr. Suzana Makpol (PPUKM)
Nor Ashikin Bt Sidek (SAUJANA SAINTIFIK)

The **117th O2k-Workshop** on high-resolution respirometry and O2k-Fluorometry is held in cooperation with our exclusive distributor in Malaysia. This O2k-Workshop presents a basic introduction to the **OROBOROS Oxygraph-2k** with integrated real-time data analysis. We introduce the new software **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, $FADH_2$, 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 to measurement of mt-membrane potential using Safranin (fluorometric), and on perspectives of HRR in mitochondrial physiology.

Programme

1	Monday, Sep 26
	Workshop Day 1
15:00	**Local arrangements**
15:30-17:30	O2k instrumental setup with the distributor.

2	Tuesday, Sep 27
	Workshop Day 2
09:00-09:45	**Welcome**, introduction of participants. **Videoclips:** Overview of O2k setup.
09:45-10:30	Hands-on: Oxygen sensor service and O2k setup.
10:30	*Coffee break*
10:45-12:00	**Instrumental quality control 1**: O_2 calibration and the O2k quality control system.
12:00	*Lunch break*
13:00-15:30	**O2k-Fluo experiment:** Simultaneous measurement of oxygen consumption (O2k-Core) and H_2O_2 production (O2k-Fluo LED2-Module) in intact cells (yeast).
15:30	*Coffee break*
15:45-17:30	**DatLab guide** through the menus and DatLab O_2 flux analysis: the new DatLab 7 version.

3	Wednesday, Sep 28
	Workshop Day 3
09:00-10:00	**O2k-Fluo and hydrogen peroxide production:** from isolated mitochondria to tissue homogenate and intact cells.
10:00-10:30	**Getting started** with an O2k experiment: washing, stirrer test, air calibration.
10:30	*Coffee break*
10:45-12:00	**Experimental set-up:** Calibration of O2k-Fluo Sensors.
12:00	*Lunch break*
13:00-15:30	**O2k-experiment:** Neuroblastoma – intact cells (ce) vs. permeabilzed cells (pce, mt-preparation).
15:30	*Coffee break*
15:45-17:30	**SUIT protocol** and DatLab analysis with Excel templates: Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor.

4	Thursday, Sep 29
	Workshop Day 4
09:00-10:30	**O2k-Fluo and mt-membrane potential:** calibration, SUIT protocols, problems and solutions.
10:30	*Coffee break*
10:45-12:00	**O2k-technical support and the OROBOROS website.** **Instrumental quality control 2**: O_2 background test with the TIP2k – automatic run over lunch.
12:00	*Lunch break*
13:00-15:30	O_2 background analysis. **Open questions – the modular O2k concept**: From O2k-Core applications to advanced O2k-MultiSensor analysis.
15:30	*Coffee break*
15:45-17:30	**Open questions – mitochondrial physiology:** experimental design and SUIT protocols.

5	Friday, Sep 30
	Workshop Day 5
09:00-10:30	**Open questions – DatLab analysis.**
10:30	*Coffee break*
10:45-12:00	**Summary – feedback - next steps.**

List of participants

Participant	Institution
A Rahim Noor Baitee	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Ab Rani Nazirah	**MY Kuala Lumpur Abdul Karim N:** Department of biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Abd Halim Mohamad Kamarul Nizam	**MY Puchong Selangor Saujana Saintifik**
Abd Rasid Ahmad Fais	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Abdul Karim Norwahidah	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Alias Ekram	**MY Kuala Lumpur Abdul Karim N:** Department of biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Damanhuri Mohd Hanafi	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Gunasekaran Gheeta	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Jubri Zakiah	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Kamarul Hakim Luqmanul Hakim	**MY Puchong Selangor Saujana Saintifik**
Kamaruzaman Mohd Muslim	**MY Puchong Selangor Saujana Saintifik**
Makpol Suzana	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Mardzukee Nurshafiqah	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Rosaidee Siti Nor Rodhiah	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Sidek Nor Ashikin	**MY Puchong Selangor Saujana Saintifik**
Wan Yusof Wan Junizam	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Zainal Abidin Nur Diyana	**MY Puchong Selangor Saujana Saintifik**
Zainul Abidin Siti Nur Ain	**MY Puchong Selangor Saujana Saintifik**
Zainul Azlan Hazirah	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Zakaria Fazaine	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)
Zakaria Siti Norasyikin	**MY Kuala Lumpur Abdul Karim N:** Department of Biochemistry, UKM Medical Centre, Kuala Lumpur (MY)

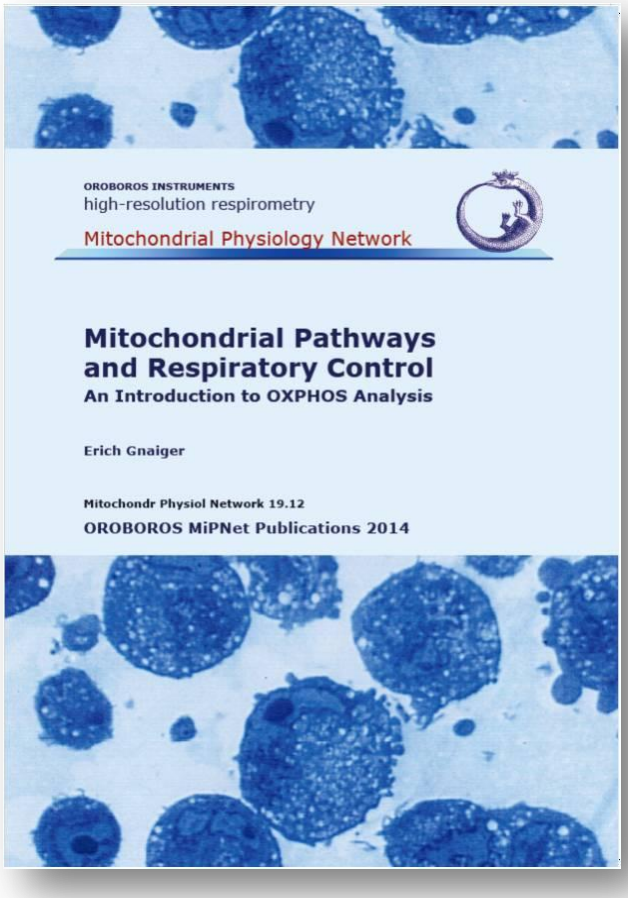
Recommended reading

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«



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«

Krumschnabel G, Eigentler A, Fasching M, Gnaiger E (2014) Use of safranin for the assessment of mitochondrial membrane potential by high-resolution respirometry and fluorometry. Methods Enzymol 542:163-81. »Bioblast link«

»O2k-Fluorometry Publications«

COST Action CA15203 Mitochondrial fitness mapping
MITOEAGLE: Evolution - **A**ge - **G**ender - **L**ifestyle - **E**nvironment

Contribution to K-Regio project **MitoFit.**
The project MitoFit is funded by the Land Tirol within the program K-Regio of Standortagentur Tirol. www.mitofit.org

Tirol
Standortagentur