

113th Workshop on high-resolution respirometry & O2k-Fluorometry

2016 August 08-09
Havana, CU

Venue:

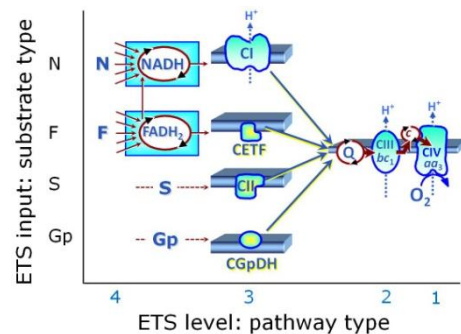
Centro de Investigación y Desarrollo de Medicamentos - CIDEM
Ave. 26, No. 1605 Boyeros y Puentes Grandes
CP 10600, La Habana, Cuba

Host:

Pardo Andreu Gilberto L, PhD
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Lecturer and tutor:

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The **113th O2k-Workshop** on high-resolution respirometry and O2k-Fluorometry is held in cooperation with our first O2k-Network Lab in Cuba. 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, 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.

Programme

1 Monday, Aug 08

*printed in workshop materials

Workshop Day 1		Weblink
08:00	<i>Registration</i>	
09:00-09:15	A welcome by Dr Andreu Gilberto Pardo and OROBOROS INSTRUMENTS	
09:15-09:30	Introduction of participants and their research interests	IOC113
09:30-11:00	Get started with the O2k: Overview with video clips.	O2k-Manual
11:00	<i>Coffee break – Registration continued</i>	
11:30-12:30	Pro's and con's of mt-preparations: Coupling and pathway control of O ₂ consumption and H ₂ O ₂ production in homogenate, permeabilized fibres – or isolated mitochondria?	
12:30-14:00	Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay	The Blue Book* SUIT reference protocol
14:00	<i>Lunch</i>	
14:45-15:30	Experimental setup 1: OroboPOS - sensor quality control, calibration.	
15:30-16:30	Demo-Experiment: High-resolution respirometry (and H ₂ O ₂ production) with freeze dried baker's yeast.	Amplex Yeast HRR yeast RA*
16:30	<i>Coffee break</i>	
17:00-18:00	Q&A session on HRR and OXPHOS analysis: Design of experimental protocol	
18:00-22:00	<i>O2k-Workshop Dinner and Welcome Party</i>	

2 Tuesday, Aug 09

Workshop Day 2		Weblink
08:30-11:00	Demo-Experiment: high-resolution respirometry with homogenized tissue of rat brain	Burtscher (2015) Mitochondrion
11:00	<i>Coffee break</i>	
11:30-14:00	Experiment continued	
14:00	<i>Lunch</i>	
14:45-16:00	Data analysis & technical support	
16:00	<i>Coffee break</i>	
16:30-17:30	The Bioblast wiki and O2k-Network, Feedback & Conclusions	
17:30-19:30	<i>Farewell activity</i>	

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](#)«



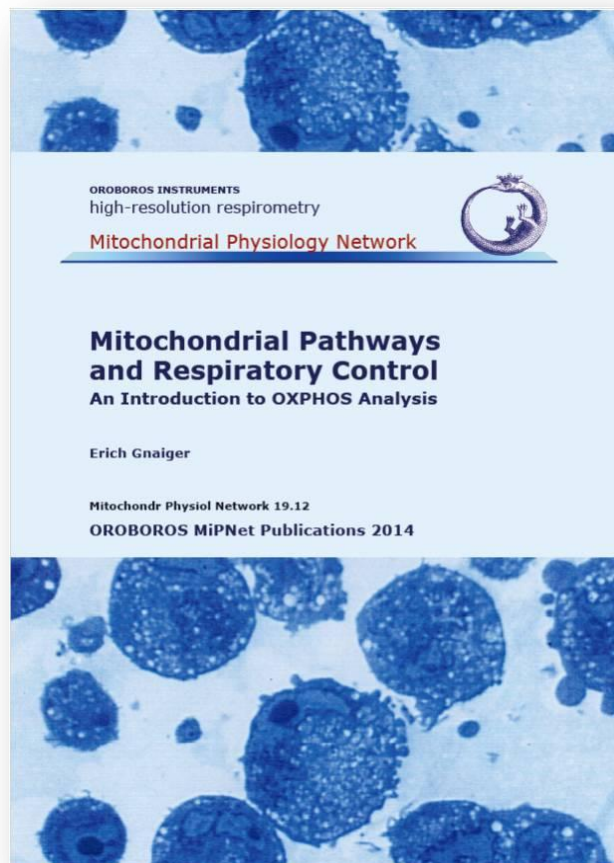
SUIT protocols for O2k high-resolution respirometry

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](#)«

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.

»[Full text in Bioblast](#)«



HRR with brain tissue

Burtscher J, Zangrandi L, Schwarzer C, Gnaiger E (2015) Differences in mitochondrial function in homogenated samples from healthy and epileptic specific brain tissues revealed by high-resolution respirometry. Mitochondrion 25:104-12. »[Bioblast link](#)«



COST Action CA15203 Mitochondrial fitness mapping

MITOEAGLE: Evolution - Age - Gender - Lifestyle - Environment

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



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