

120th Workshop on high-resolution respirometry & O2k-Fluorometry

**2017 March 20
Barcelona, ES**

Venue:

Institute for Research in Biomedicine (IRB Barcelona)
Facultat de Biologia, Universitat de Barcelona
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Barcelona, Spain

Host:

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wiki.oroboros.at/index.php/ES_Barcelona_Zorzano_A

Lecturers and tutors:

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The **120th O2k-Workshop** on high-resolution respirometry is held in cooperation with the O2k-Network Lab at **Institute for Research in Biomedicine (IRB Barcelona)**. This O2k-Workshop presents a basic introduction to the **OROBOROS Oxygraph-2k (O2k)** 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 HRR using the **O2k-Fluorescence LED2-Module** for simultaneous measurement of hydrogen peroxide production (Amplex red®). Mitochondria and cell research with the O2k provides the basis for a global network connected by the O2k-technology. The quality control system presented for high-resolution respirometry provides an important milestone on the way too much broader goals.

This O2k-Workshop takes place prior to [MITOEAGLE 2017 Barcelona ES](#).

A separate registration is required:

wiki.oroboros.at/index.php/MIPNet22.04_IOC120_Barcelona_ES

Programme

1 Monday, Mar 20

Workshop Day 1	Weblink
08:45 <i>Registration</i>	
09:00-09:15 Welcome by Prof. Antonio Zorzano.	
09:15-09:30 Introduction of participants and their research interests.	IOC120
09:30-10:00 Get started with the O2k: Overview with video clips.	O2k-Manual
10:00-11:30 Comprehensive OXPPOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay.	The Blue Book* SUIT reference protocol
11:30-12:00 <i>Coffee/tea break</i>	
12:00-13:00 Principles of high-resolution respirometry: From switching on the O2k to the experimental result – oxygen sensor calibration and quality control.	POS-calibration-SOP
13:00-14:00 O2k-Fluorometry and OXPPOS analysis: Amplex red assay of H₂O₂ production and design of experimental protocol.	O2k-Fluorometry
14:00-15:00 <i>Lunch</i>	
15:00-17:30 Demo-Experiment: HRR and O2k-Fluorometry with permeabilized cells – respiration and H ₂ O ₂ production applying the SUIT reference assay: Protocol driver for RP1&RP2.	RP1 RP2
17:30-18:30 Protocol driver for Marks and data analysis.	
18:30-20:00 The Bioblast wiki, O2k-Network and feedback discussion.	

Recommended reading

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.



»[Full text in Bioblast](#)«

O2k-Core Manual:

»[Full text in Bioblast](#)«

**O2k-Fluo
LED2-**

Module 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.

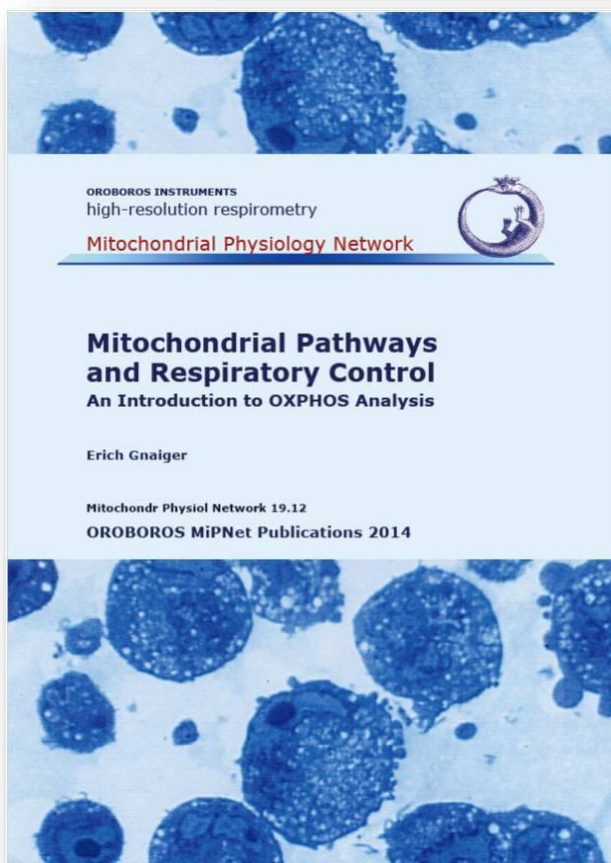
»[Full text in Bioblast](#)«

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](#)«

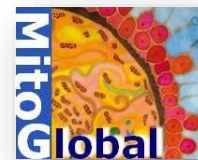




COST Action CA15203 Mitochondrial fitness mapping

MITOEAGLE: Evolution - Age - Gender - Lifestyle - Environment

Invitation to join the global network.



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