

OROBOROS O2k-Workshop



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Updates: http://wiki.oroboros.at/index.php/MiPNet22.04_IOC120_Barcelona_ES

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

**2017 March 20
Barcelona, ES**

Venue:

Barcelona Science Park
Room 1 Tower D
R+D+I Towers building
C/ Baldri Reixac 4-8
08028 Barcelona

Host:

Antonio Zorzano, Prof., Dr.
Institute for Research in Biomedicine (IRB Barcelona)
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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 liver mitochondria – 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.	

Lecturers and tutors

Name	Institution
Gnaiger Erich	CEO, OROBOROS INSTRUMENTS
Doerrier Velasco Carolina	OROBOROS INSTRUMENTS
Sebastián David	Institute for Research in Biomedicine (IRB Barcelona)

Participants

Participant	Institution
Barros Susana ***	ES Barcelona Zorzano A: IRB Barcelona (ES)
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Granado Serrano Ana Belén *	ES Lleida Boada J: IRB Barcelona (ES)
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Koenig Magdalena	Goethe University, Frankfurt (DE)
Lyakhovich Alexander	Research Institute VHIR, Barcelona (ES)
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Tei Bismark Newton	Department of Chemistry, University of Cape Coast (GH)
Thinnes Anna	Goethe University, Frankfurt (DE)
Zagmutt Caroca Sebastián	University of Barcelona (ES)

*Asterisk indicate the number of O2k instruments in the participant's lab.

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](#)«



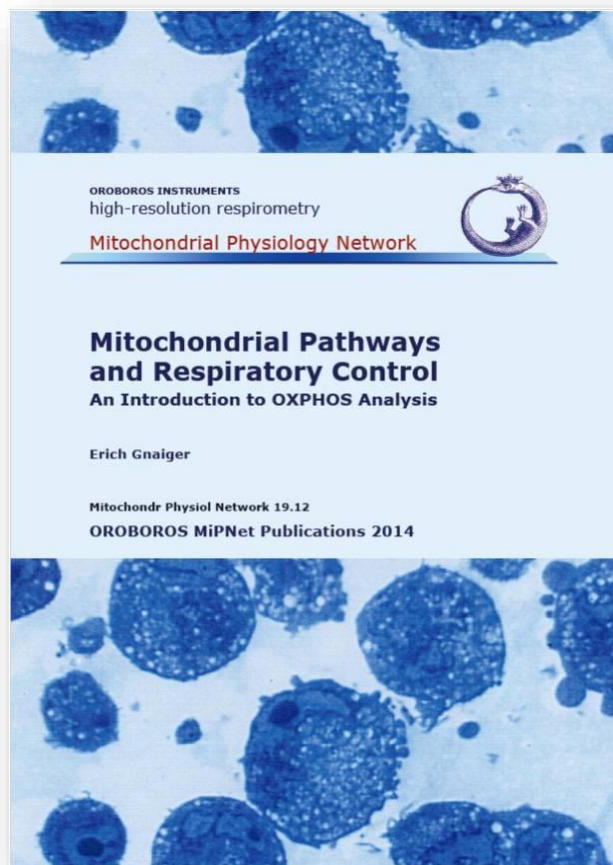
SUIT protocols for O2k high-resolution respirometry

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](#)«



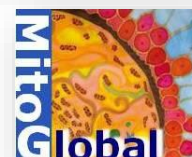
HRR with brain tissue

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
MITO EAGLE: 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