

## 120<sup>th</sup> 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.  
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[wiki.oroboros.at/index.php/ES\\_Barcelona\\_Zorzano\\_A](http://wiki.oroboros.at/index.php/ES_Barcelona_Zorzano_A)

### Lecturers and tutors:

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The **120<sup>th</sup> 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, FADH<sub>2</sub>, succinate and  $\alpha$ -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](http://wiki.oroboros.at/index.php/MIPNet22.04_IOC120_Barcelona_ES)

## Programme

### 1 Monday, Mar 20

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

## 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.

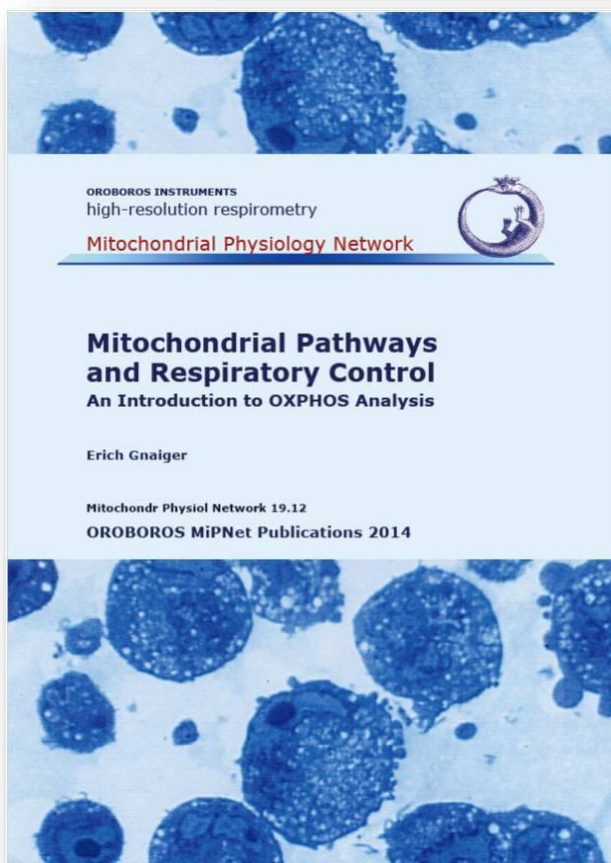
»[Full text in Bioblast](#)«

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](#)«

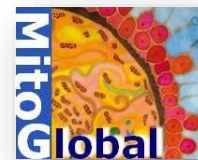




**COST Action CA15203 Mitochondrial fitness mapping**

**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](http://www.mitofit.org)