



## Course on High-Resolution Respirometry

IOC70. Mitochondrial Physiology Network 17.10: 1-5 (2012)

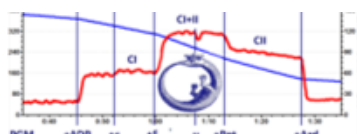
©2012 OROBOROS®  
Version 2: 2012-05-30

# O2k-Workshop Barcelona

70<sup>th</sup> International Workshop on HRR

2012 May 29–30 Barcelona, Spain

Esther Kopolovitz Center (CEK)  
IDIBAPS-Hospital Clinic de Barcelona  
Meeting rooms 1 and 2  
c/Rosello 153, 08036 Barcelona



K-Regio project *MitoCom Tyrol* funded by the Tyrolian Government and the European Regional Development Fund

**Local Organizer:** Dr. Pablo Garcia-Roves, Esther Kopolovitz Center (CEK), Barcelona, Spain  
[http://www.bioblast.at/index.php/ES\\_Barcelona\\_Garcia-Roves\\_PM](http://www.bioblast.at/index.php/ES_Barcelona_Garcia-Roves_PM)



The 70<sup>th</sup> O2k-Workshop on High-Resolution Respirometry (HRR) is the **Third Oxygen-2k Workshop held in Spain**. Participants are scientists who want to learn in detail the experimental basis of high-resolution approaches to measure mitochondrial respiratory function. Day 1 of the workshop includes a basic introduction to the **OROBOROS Oxygen-2k** with integrated on-line analysis by **DatLab 5** and applications of the Titration-Injection microPump **TIP2k**. Specific emphasis is placed on on-line practical demonstration of instrument and software performance, to introduce the participants into the technical and operational details of **high-resolution respirometry (HRR)**. Quality control of instrumental performance will be demonstrated by on-line calibration of the oxygen sensors, evaluation of signal stability, and an O2k-Demo experiment.



Day 2 presents the most recent developments on the **O2k-Fluorescence LED2-Module**. Applications are presented with Amplex Ultrared, illustrating the unique advantages of controlling oxygen concentration, and measuring oxygen consumption and hydrogen peroxide production simultaneously. The oxygen dependence of H<sub>2</sub>O<sub>2</sub>

production is shown in intact yeast cells, and **substrate-uncoupler-inhibitor titration (SUIT) protocols** with tissue homogenate, using the PBIO-Shredder. An overview is given on the **O2k-MultiSensor Modules**, with the TPP<sup>+</sup> electrode for simultaneous measurement of mt-membrane potential and oxygen consumption.

Short presentations of 'Hot topics in Mitochondrial Physiology' extend the discussions on respiratory protocols, perspectives of HRR and general topics in mitochondrial physiology.



## Programme IOC70

<b>1 Tuesday, May 29</b>		-
<b>O2k-Workshop</b>		<b>Weblink</b>
09:45	<i>Arrival and registration</i>	
	<b>Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result</b>	<a href="#">Gnaiger 2008 POS</a>
10:00-10:05	<b>Pablo Garcia-Roves:</b> Welcome to the Barcelona O2k-Workshop.	<a href="#">IOC70</a>
10:05-11:15	<b>Erich Gnaiger:</b> <b>The O2k-concept on high-resolution respirometry.</b>	<a href="#">Glossary:</a> <a href="#">Respiratory states</a>
	1. <b>Get O2k-connected with your <b>OROBOROS-USB:</b> a guided tour to the O2k.</b>	<a href="#">get O2k-Connected</a>
11:15	<i>Coffee / Tea - Get-together - introduction of the participants and their research interests</i>	<a href="#">POS Service</a>
11:45-13:00	2. <b>Oxygraph-2k: O2k-Setup and OroboPOS service.</b>	<a href="#">O2k-Start</a>
	3. <b>OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.</b>	<a href="#">O2k-Calibration</a>
	4. <b>O2k-background test and analysis of oxygen flux.</b>	<a href="#">O2k-Background</a>
13:30	<i>Lunch</i>	
15:00-16:30	<b>Erich Gnaiger:</b> <b>Experimental design - Substrate and coupling control of mitochondrial respiration: SUIT protocols.</b>	<a href="#">Gnaiger 2009 Int J Biochem Cell Biol</a>
16:30	<i>Coffee / Tea</i>	<a href="#">CI+II</a>
17:00-19:00	<b>Pablo Garcia-Roves and Erich Gnaiger:</b> <b>O2k-Demoexperiment</b>	<a href="#">Pesta 2012 Methods Mol Biol</a>
<b>Further information from the O2k-Manual and O2k-Protocols</b>		
	DatLab Guide through the menus: DL-Demo files and DL-Excel templates DatLab Analysis and Excel templates	<a href="#">DatLab 4 Guide</a>  <a href="#">DatLab4 Flux Analysis</a>
	Coupling control of mitochondrial respiration: LEAK, OXPHOS, ETS, ROX	<a href="#">mt-Respiration</a>
	Coupling Control Protocol with intact cells: ROUTINE, LEAK, ETS, ROX	<a href="#">Cell Respiration</a>
<b>2 Wednesday, May 30</b>		
<b>O2k-Fluorometry and Mitochondrial Physiology</b>		<b>Weblink</b>
10:00-11:30	<b>Erich Gnaiger:</b> <b>The O2k-Fluorometer concept</b>	-

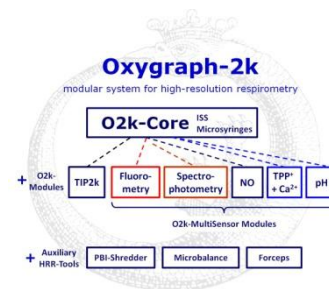
	1. The O2k-Fluorescence LED2-Module.	<a href="#">O2k-Fluorescence LED2-Module</a>
	2. O2k-Fluo application with Amplex Ultrared: Oxygen dependence of hydrogen peroxide production.	<a href="#">O2k-Fluorescence</a>
11:30	Coffee / Tea	-
<b>Hot MiP-Topics (Chair: Pablo Garcia-Roves)</b>		
12:00-12:15	<b>Perales Jose C:</b> Do we understand hepatic gluconeogenesis?	-
12:15-12:30	<b>Hernandez Maribel:</b> Mitofusin 2 (Mfn2) links mitochondrial and endoplasmic reticulum function with insulin signaling and is essential for normal glucose homeostasis.	-
12:30-12:45	<b>Claret Marc:</b> Deletion of mitochondrial fusion protein Mitofusin 2 in hypothalamic POMC neurons leads to obesity and impaired glucose homeostasis in mice.	-
12:45-13:00	<b>Villena Josep A:</b> Transcriptional control of mitochondrial function in white adipose tissue by PGC-1 co-activators.	-
13:30	Lunch	-
15:00-16:30	<b>Erich Gnaiger:</b> Permeabilized fibres vs muscle tissue homogenate	-
	1. Are permeabilized muscle fibres a valid model for measurement of hydrogen peroxide production?	-
	2. Mitochondrial respiratory function in tissue homogenate (PBI-Shredder) versus permeabilized fibres.	<a href="#">Shredder vs. Fibres</a>
	3. Respiration with succinate - paradoxes in MiP.	<a href="#">IOC66.pdf</a>
16:30	Coffee / Tea	-
17:00-18:00	<b>Erich Gnaiger:</b> Fluorometric (AmR) and potentiometric (TPP) measurement of mt-membrane potential and future perspectives O2k-MultiSensor developments.	<a href="#">O2k-MultiSensor</a>
<b>Hot MiP-Topics (Chair: Pablo Garcia-Roves)</b>		
18:00-18:15	<b>Vieira Elaine:</b> The effect of melatonin on mitochondrial respiration.	-
18:15-18:30	<b>Garrabou Gloria:</b> Oxygen consumption in mitochondrial toxicity and disease.	-
18:30-18:45	<b>Bosch Marta:</b> Mitochondrial cholesterol: A connection between caveolin, metabolism, and disease.	-
18:45-19:00	<b>Summary discussion: O2k-Workshop Feedback</b>	<a href="#">O2k-Feedback</a>

## O2k-Manual [MiPNet 17.05]

As innovation within our *open innovation* approach, the 'Manual for the O2k-Fluorescence LED2-Module' evolves as a guided tour through the Bioblast wiki

### O2k-Catalogue: O2k-Fluorescence LED2-Module

[http://www.bioblast.at/index.php/O2k-Fluorescence\\_LED2-Module#O2k-Manual:\\_O2k-Fluorescence\\_LED2-Module](http://www.bioblast.at/index.php/O2k-Fluorescence_LED2-Module#O2k-Manual:_O2k-Fluorescence_LED2-Module)



Application	Sensor	Filter set	Light intensity (LED current)	Light intensity (switch position)
<a href="#">Amplex® UltraRed</a>	<a href="#">Fluorescence-Sensor Green</a>	<a href="#">AmR</a>	2 mA	4
<a href="#">Safranin</a>	<a href="#">Fluorescence-Sensor Blue</a>	<a href="#">Saf</a>	1 mA	3
<a href="#">Magnesium green</a>	<a href="#">Fluorescence-Sensor Blue</a>	<a href="#">MgG / CaG</a>	1 mA	3
<a href="#">Calcium green</a>	<a href="#">Fluorescence-Sensor Blue</a>	<a href="#">MgG / CaG</a>		

## Participants IOC70

Name	Lab
<a href="#">Alcarraz Gema</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<a href="#">Bano Maria</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Secondary lesions due to chronic alcohol ingestion, muscle pathology group – <b>ES</b>
<a href="#">Boada Jordi</a>	Grup de Fisiopatologia Metabòlica, Dept. Ciències Mèdiques Bàsiques, Universitat de Lleida - <b>ES_Lleida_Boada J</b>
<a href="#">Bosch Marta</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Cell proliferation and signalling group - <b>ES Barcelona Bosch M</b>
<a href="#">Catalan Marc</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Secondary lesions due to chronic alcohol ingestion, muscle pathology group – <b>ES</b>
<a href="#">Claret Marc</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<a href="#">De Nigris Valeria</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<a href="#">Diaz-Ramos Angels</a>	Institute for Research in Biomedicine, IRB Barcelona - <b>ES</b>
<a href="#">Garcia-Roves Pablo M.</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES Barcelona Garcia-Roves PM</b>
<a href="#">Garrabou Gloria</a>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Secondary lesions due to chronic alcohol ingestion, muscle pathology group - <b>ES</b>
<a href="#">Gnaiger Erich</a>	DSL, Dept Visceral, Transpl Thoracic Surgery, Medical Univ Innsbruck - <b>AT Innsbruck OROBOROS INSTRUMENTS</b>

<b>Gonzalez Franquesa Alba</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES Barcelona Garcia-Roves PM</b>
<b>Hermes Albert</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Cell proliferation and signalling group - <b>ES</b>
<b>Hernandez Maribel</b>	Institute for Research in Biomedicine, IRB Barcelona - <b>ES Barcelona Zorzano A</b>
<b>Lerin Carles</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<b>Llobet Sese Laura</b>	Biochemistry and Molecular and Cellular Biology Department, Faculty of Veterinary University of Zaragoza, Laboratory Dr.Julio Montoya - <b>ES</b>
<b>Martinez Laura</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<b>Martinez Lourdes Navarro</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES Barcelona Garcia-Roves PM</b>
<b>Matias Nuria</b>	Institute of Biomedical Research of Barcelona - <b>ES</b>
<b>Mendez Andres</b>	Bellvitge Biomedical Research Institute, IDIBELL, University of Barcelona - <b>ES</b>
<b>Moren Constanza</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Secondary lesions due to chronic alcohol ingestion, muscle pathology group - <b>ES</b>
<b>Perales Jose C</b>	Bellvitge Biomedical Research Institute, IDIBELL, University of Barcelona - <b>ES Barcelona Bermudez MJ</b>
<b>Ribas Vincente</b>	Institute of Biomedical Research of Barcelona - <b>ES</b>
<b>Schneeberger Marc</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<b>Tobias Ester</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Secondary lesions due to chronic alcohol ingestion, muscle pathology group - <b>ES</b>
<b>Vieira Elaine</b>	August Pi i Sunyer Biomedical Research Institute, IDIBAPS Barcelona, Diabetes and obesity laboratory - <b>ES</b>
<b>Villena Josep A</b>	Vall D'Hebron Research Institute, VHIR Barcelona - <b>ES</b>

## Contact

Erich Gnaiger, PhD  
 Medical University of Innsbruck  
 Department of Transplant Surgery  
 D. Swarovski Research Laboratory  
 Anichstrasse 35  
 A-6020 Innsbruck, Austria  
 Email [erich.gnaiger@i-med.ac.at](mailto:erich.gnaiger@i-med.ac.at)

<http://wiki.oroboros.at>  
[www.mipart.at](http://www.mipart.at)

Schöpfstrasse 18  
 A-6020 INNSBRUCK, Austria  
 T +43 512 566796  
 F +43 512 566796 20  
 Email [instruments@oroboros.at](mailto:instruments@oroboros.at)  
 Homepage: [www.oroboros.at](http://www.oroboros.at)  
 Cooperation and Feedback in Science

