

# Oroboros O2k-Workshop



Mitochondrial Physiology Network 27.10(01):1-6 (2022)

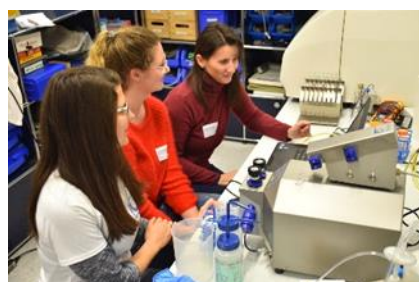
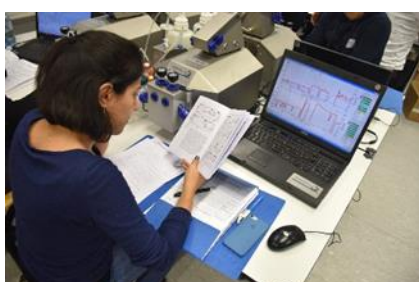
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Updates: [https://wiki.orooboros.at/index.php/MiPNet27.10\\_IOC156\\_Ramat\\_Gan\\_IL](https://wiki.orooboros.at/index.php/MiPNet27.10_IOC156_Ramat_Gan_IL)

## 156<sup>th</sup> O2k-Workshop on high-resolution respirometry



2022 Nov 28<sup>th</sup>-30<sup>th</sup>: O2k-FluoRespirometer  
Ramat Gan, Israel



The 156<sup>th</sup> O2k-Workshop on high-resolution respirometry and O2k-Fluorometry will be held in cooperation with the Mitochondrial Research Laboratory (Yardeni's lab) in Sheba Medical Center, Ramat Gan, Israel.

The O2k-Workshop will include lectures providing an overview of **high-resolution respirometry (HRR)** with the Oroboros O2k with diverse sample types and DatLab protocols to investigate pathway and coupling control of mitochondrial respiration. The workshop will also will give an introduction of the **O2k-Applications using fluorescence**, such as ROS production measurement with Amplex™ UltraRed, mt-membrane potential with safranin, TMRM or rhodamine 123, ATP production measurement with Magnesium Green™ and Ca<sup>2+</sup> uptake capacity with Calcium Green™.

Hands-on sessions will provide training in substrate-uncoupler-inhibitor titration (SUIT) protocols with cells and permeabilization of plasma membrane with digitonin, tissue homogenate and isolated mitochondria. The hands-on session will also include, besides respirometry, the use of the Smart Fluo-Module with real-time analysis using DatLab 7.4. The 156<sup>th</sup> workshop is a unique opportunity to learn about the new developments in HRR.

### Host and Venue

Tal Yardeni, PhD

Principal Investigator

Mitochondrial Research Laboratory,

Sheba Medical Center (SMC), Ramat Gan, Israel

[https://wiki.orooboros.at/index.php/IL\\_Ramat\\_Gan\\_Yardeni\\_T](https://wiki.orooboros.at/index.php/IL_Ramat_Gan_Yardeni_T)

### Lecturers and tutors

<a href="#">Cardoso Luiza</a>	Mitochondrial Wizard, Oroboros Instruments
<a href="#">Gnaiger Erich</a>	CEO, Innovation Alchemist, Oroboros Instruments

## Program

### 1 Monday, November 28<sup>th</sup>

Time	Talk/hands-on session	Weblink	Room
09:00-09:15	<i>Welcome - Get-together: Introduction of participants and their research interests</i>	<a href="http://www.bioblast.at">O2k-Network www.bioblast.at</a>	Lecture room
09:15-09:45	<b>The Oroboros Ecosystem and the O2k FluoRespirometer</b>		Lecture room
09:45-10:30	<b>Introduction to substrate-uncoupler-inhibitor titration (SUIT) protocols and sample types</b>  More than coupling control: exploring pathway control with mitochondrial preparations	<a href="#">MitoPedia: SUIT</a>	Lecture room
10:30-10:45	<i>Coffee break</i>		
10:45-11:15	<b>Hands-on: Instrumental quality control 1: oxygen calibration</b>  DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	<a href="#">Gnaiger 2008 POS SOP: O2-calibration</a> <a href="#">SOP: O2k-cleaning and ISS</a>	Laboratory
11:15-12:00	<b>The world as a laboratory: Exploring mitochondrial fitness in human populations in extreme environments – high altitude and high latitude</b>	<a href="#">The World as a Laboratory</a>	Lecture room
12:00-13:00	<i>Lunch break</i>		
13:00-13:20	<b>DatLab 7.4 overview</b>	<a href="#">MitoPedia: DatLab</a> <a href="#">DatLab 7 Innovations</a>	Laboratory
13:20-16:00	<b>Hands-on (2 O2ks): Digitonin test – Determination of the optimum digitonin concentration for permeabilization of plasma membrane</b>  DL-Protocol (O2): SUIT-010 O2 ce-pce D008 DL-Protocol (Instrumental): O2k-cleaning AfterUse	<a href="#">SOP Hamilton microsyringes</a> <a href="#">MiPNet09.12 O2k-Titrations</a> <a href="#">SUIT-010 O2 ce-pce D008</a> <a href="#">Video: How to perform an experiment with a SUIT DL-Protocol (DLP)</a>	Laboratory

### 2 Tuesday, November 29<sup>th</sup>

Time	Talk/hands-on session	Weblink	Room
09:00-09:15	<b>O2k-Applications - overview</b>	<a href="#">O2k Applications</a>	Lecture room
09:15-09:30	<b>Introduction to FluoRespirometry and applications</b>	<a href="#">O2k-FluoRespirometer</a> <a href="#">O2k-Fluo Smart-Module</a>	Lecture room

09:30-10:30	<b>H<sub>2</sub>O<sub>2</sub> production</b> - Amplex UltraRed <b>Mitochondrial membrane potential</b> - safranin and other dyes <b>ATP production</b> - Magnesium Green <b>Ca<sup>2+</sup> retention capacity</b> - Calcium Green	<a href="#">MitoPedia: Amplex UltraRed</a> <a href="#">Mt membrane potential</a> <a href="#">Magnesium Green</a> <a href="#">Calcium Green</a>	Lecture room
10:30-10:45	<i>Coffee break</i>		
10:45-11:15	<b>Hands-on:</b> Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	<a href="#">SOP: O2-calibration</a>	Laboratory
11:15-11:30	<b>SUITbrowser:</b> how to find the best SUIT protocol for your research questions.	<a href="#">Oroboros SUITbrowser</a>	Laboratory
11:30-12:00	<b>Hands-on:</b> Oxygen calibration (continuation) and calibration with H <sub>2</sub> O <sub>2</sub> DL-Protocol: O2 calibration air DL-Protocol: AmR calibration		Laboratory
12:00-13:00	<b>Hands-on (2 O2ks):</b> O2k-experiment - Respiration and H <sub>2</sub> O <sub>2</sub> flux of permeabilized cells: DL-Protocol: SUIT-026 AmR ce-pce D087	<a href="#">SUIT-026 AmR ce-pce D087</a>	
13:00-14:00	<i>Lunch break</i>		
14:00-14:30	<b>Hands-on:</b> O2k-cleaning after use (continuation) DL-Protocol: O2k-cleaning AfterUse		Laboratory
14:30-15:30	<b>DatLab analysis:</b> Introduction and new features <b>Hands-on: Individual DatLab analysis</b> - O <sub>2</sub> and H <sub>2</sub> O <sub>2</sub> flux	<a href="#">Oxygen flux analysis</a>	Laboratory

### 3 Wednesday, November 30<sup>th</sup>

Time	Talk/hands-on session	Weblink	Room
09:00-09:45	<b>Hands-on:</b> Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	<a href="#">SOP: O2k-cleaning and ISS</a> <a href="#">SOP: O2-calibration</a>	Laboratory
09:45-10:15	<b>Hands-on: Calibration with safranin</b> DL-Protocol: Saf calibration		Laboratory
10:15-11:15	<b>Hands-on (1 O2k):</b> O2k-experiment - Respiration and mt membrane potential with safranin – tissue homogenate: DL-Protocol: SUIT-006 Fluo mt D034	<a href="#">SUIT-006 Fluo mt D034</a>	Laboratory
11:15-11:30	<i>Coffee break</i>		
11:30-12:30	<b>Hands-on (1 O2k):</b> O2k-experiment - Respiration and mt membrane potential with safranin – isolated mitochondria: DL-Protocol: SUIT-006 Fluo mt D034	<a href="#">SUIT-006 Fluo mt D034</a>	Laboratory
12:30-13:30	<i>Lunch break</i>		
13:30-14:00	<b>Hands-on:</b> O2k-cleaning after use DL-Protocol: O2k-cleaning AfterUse	<a href="#">SOP: O2k-cleaning and ISS</a>	Laboratory

14:00-15:00	<b>Hands-on: DatLab analysis</b> – O <sub>2</sub> flux and mt membrane potential	<a href="#">Oxygen flux analysis</a>	Laboratory
15:00-15:30	<b>Final discussion and feedback</b>		Laboratory

## O2k-Workshop: OUR COMMON AIMS

- **Mitochondrial physiology:**  
Study mitochondrial function in the **context** of cell physiology and pathology
- **Instrumental performance – the O2k:**
  - 🌀 Learn **high**-resolution respirometry
  - 🌀 Gain **hands-on** experience
  - 🌀 Extend to O2k-**Multi**Sensor applications
- **Excellence in research:**
  - 🌀 Instrumental **quality** control
  - 🌀 Experimental design for **innovation**
  - 🌀 Data analysis meeting superior **standards**

OROBOROS INSTRUMENTS

O2k

Mitochondria and cell research



## More detail?

Gnaiger E (2020) **Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis.** 5th ed. Bioenerg Commun 2020.2. <https://doi.org/10.26124/bec:2020-0002>



Gnaiger E et al – MitoEAGLE Task Group (2020) **Mitochondrial physiology.** Bioenerg Commun 2020.1. <https://doi.org/10.26124/bec:2020-0001.v1>

**O2k-Manual** – <http://wiki.oroboros.at/index.php/O2k-Manual>

**O2k-Procedures** – <http://wiki.oroboros.at/index.php/O2k-Procedures>

**>4,200 O2k-Publications** – <http://wiki.oroboros.at/index.php/O2k-Publications: Topics>

## Acknowledgements

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## Contact

Luiza Cardoso, PhD and Erich Gnaiger, PhD  
 Oroboros Instruments GmbH  
 Schoepfstrasse 18  
 A-6020 Innsbruck, Austria  
 T +43 512 566796 F +43 512 566796 20  
[instruments@oroboros.at](mailto:instruments@oroboros.at) | [www.oroboros.at](http://www.oroboros.at)  
**Mitochondria and cell research**



Virtual O2k-Workshops are listed as MitoGlobal Events

## NextGen O2k - Applications



**Find solutions to**

- Cancer
- Obesity
- Diabetes
- Aging
- Cardiovascular
- Neurodegeneration
- Exercise physiology
- Environmental physiology
- **PhotoBiology**
- **Algal biotechnology**

**»explore**

- O<sub>2</sub> consumption
- Q-redox state
- NAD(P)H redox state
- Oxygen dependence
- Hypoxia and O<sub>2</sub> kinetics
- H<sub>2</sub>O<sub>2</sub> production
- mt-Membrane potential
- ATP production
- pH, Ca<sup>2+</sup>, NO<sup>•</sup>
- Photosynthesis
- Dark respiration
- Light-enhanced respiration

Oroboros - as a driving force in mitochondrial physiology - extends the analytical and diagnostic power of high-resolution respirometry by integration of NADH- and Q-redox monitoring in the **NextGen-O2k**. We aim at establishing the Oroboros quality control management for dissemination to our worldwide O2k-Network laboratories. This will become an effective contribution to address the acute *reproducibility crisis* of scientific investigation. In the spirit of Open Science and global networking, we will enable data sharing across projects and institutions in an Open Access database on mitochondrial physiology and pathology, to resolve the *inflation crisis* and ultimately the *value-impact crisis* of present academic publication. This will support key developments in mitochondrial medicine. In addition, we expand our business to algal biotechnology and ecology with the NextGen-O2k PhotoBiology-Module, widening our focus from medicine to environment and climate.

## Bioenergetics Communications



The Open Access journal for publishing scientific and technical advances in bioenergetics and mitochondrial physiology as **Living Communications**

» <https://www.bioenergetics-communications.org>

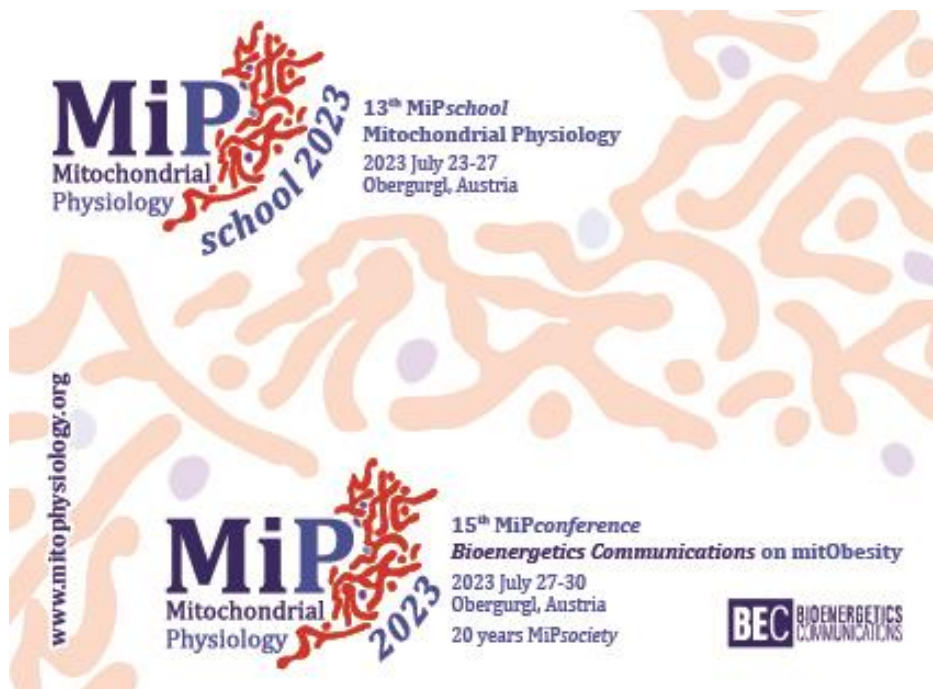
## MitoFit Preprints



The Open Access preprint server for mitochondrial physiology and bioenergetics

» [https://www.mitofit.org/index.php/MitoFit Preprints](https://www.mitofit.org/index.php/MitoFit%20Preprints)

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22<sup>nd</sup> European Bioenergetics Conference

2024 August 26-31  
 Congress Innsbruck, Austria  
[www.ebec2024.org](http://www.ebec2024.org)

