

Oroboros O2k-Workshop



Mitochondrial Physiology Network 27.10(02):1-6 (2022)

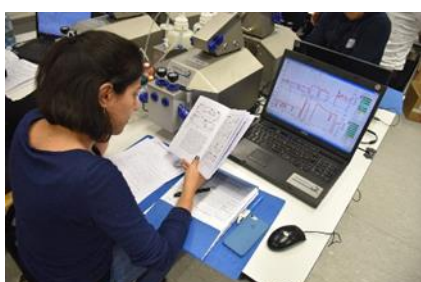
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Updates: https://wiki.orooboros.at/index.php/MiPNet27.10_IOC156_Ramat_Gan_IL

156th O2k-Workshop on high-resolution respirometry



2022 Nov 28th-30th: O2k-FluoRespirometer
Ramat Gan, Israel



The 156th 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 Ca²⁺ 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 Fluor-Module with real-time analysis using DatLab 7.4. The 156th 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

Lecturers and tutors

Cardoso Luiza	Mitochondrial Wizard, Oroboros Instruments
Gnaiger Erich	CEO, Innovation Alchemist, Oroboros Instruments

Program

1 Monday, November 28th

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

2 Tuesday, November 29th

Time	Talk/hands-on session	Weblink	Room
09:00-09:15	O2k-Applications - overview	O2k Applications	Lecture room
09:15-09:30	Introduction to Fluorespirometry and applications	O2k-Fluorespirometer	Lecture room

		O2k-Fluo Smart-Module	
09:30-10:30	H₂O₂ production - Amplex UltraRed Mitochondrial membrane potential - safranin and other dyes ATP production - Magnesium Green Ca²⁺ retention capacity - Calcium Green	MitoPedia: Amplex UltraRed Mt membrane potential Magnesium Green Calcium Green	Lecture room
10:30-10:45	<i>Coffee break</i>		
10:45-11:15	Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2-calibration	Laboratory
11:15-11:30	SUITbrowser: how to find the best SUIT protocol for your research questions.	Oroboros SUITbrowser	Laboratory
11:30-12:00	Hands-on: Oxygen calibration (continuation) and calibration with H ₂ O ₂ DL-Protocol: O2 calibration air DL-Protocol: AmR calibration		Laboratory
12:00-13:00	Hands-on (2 O2ks): O2k-experiment - Respiration and H ₂ O ₂ flux of permeabilized cells: DL-Protocol: SUIT-026 AmR ce-pce D087	SUIT-026 AmR ce-pce D087	
13:00-14:00	<i>Lunch break</i>		
14:00-14:30	Hands-on: O2k-cleaning after use (continuation) DL-Protocol: O2k-cleaning AfterUse		Laboratory
14:30-15:30	DatLab analysis: Introduction and new features Hands-on: Individual DatLab analysis – O ₂ and H ₂ O ₂ flux	Oxygen flux analysis	Laboratory

3 Wednesday, November 30th

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

12:30-13:30	Lunch break		
13:30-14:00	Hands-on: O2k-cleaning after use DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Laboratory
14:00-15:00	Hands-on: DatLab analysis – O ₂ flux and mt membrane potential	Oxygen flux analysis	Laboratory
15:00-15:30	Final discussion and feedback		Laboratory

General lecture – Monday Nov 28th, 11:15-12:00:

The world as a laboratory: Exploring mitochondrial fitness in human populations in extreme environments – high altitude and high latitude.

Are mitochondria of Inuit living in the cold less coupled to produce more heat than those of Caucasians? Is OXPHOS capacity of active Inuit hunters and Sherpas at high altitude increased relative to western-life style lowlanders? What can we learn from comparative mitochondrial physiology?

- Gnaiger E et al. (2015) Mitochondrial coupling and capacity of oxidative phosphorylation in skeletal muscle of Inuit and caucasians in the arctic winter.
<https://doi.org/10.1111/sms.12612>
- Mishmar D et al. (2003) Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci U S A 100:171-6.
- Horscroft JA et al. (2017) Metabolic basis to Sherpa altitude adaptation. Proc Natl Acad Sci U S A 114:6382-7.
<https://doi.org/10.1073/pnas.1700527114>
- [https://wiki.oroboros.at/index.php/The World as a Laboratory](https://wiki.oroboros.at/index.php/The_World_as_a_Laboratory)



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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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

- O₂ consumption
- Q-redox state
- NAD(P)H redox state
- Oxygen dependence
- Hypoxia and O₂ kinetics
- H₂O₂ production
- mt-Membrane potential
- ATP production
- pH, Ca²⁺, NO⁻
- 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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