

Oroboros O2k-Workshop



Mitochondrial Physiology Network 27.04(01):1-10 (2022)

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Updates: http://wiki.orooboros.at/index.php/MiPNet27.04_IOC155_Schroecken_AT

155th O2k-Workshop on High-Resolution Respirometry

2022 Oct 03 – 08

Schroecken, Vorarlberg, Austria



The **155th O2k-Workshop on high-resolution respirometry (HRR)** is the **43rd** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **O2k-FluoRespirometer**, with real-time analysis by **DatLab 7.4** or **DatLab 8 (new)** and applications of the **Titration-Injection microPump TIP2k**.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in teams. **Instrumental quality control** is a fundamental component of HRR and will be put to practical test in teams using multiple O2k.

A wide range of mitochondrial topics is covered; abstracts and experimental experiences can be presented by participants. IOC participants invariably asked for a detailed discussion of protocol design. The **Blue Book** (5th edition) and the **Mitochondrial physiology** provide a basic introduction to mitochondrial bioenergetics, complementing the training course, and therefore we recommend reading them beforehand.

The O2k-Workshop will give an introduction of the **O2k-Applications** using **fluorescence**, (ROS production measurement with AmplexTM UltraRed, mt-membrane potential with safranin, TMRM or rhodamine 123, ATP production measurement with Magnesium GreenTM and Ca²⁺ uptake capacity with Calcium GreenTM). The hands-on will include Amplex UltraRed experiments with HEK 293T

cells, demonstrating the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, and hydrogen peroxide production.

Finally, the new applications of the **NextGen-O2k** will be presented: the Q-Module to assess coenzyme Q-redox state (with a Demo-experiment), NADH-Module to assess NAD-redox state and PhotoBiology (PB) Module, which allows to detect oxygen production from photosynthesis. It is possible to join for a visit to the *Alpmuseum*, and lunch breaks provide an opportunity for relaxing *Walks&Talks*, enjoying the refreshing scenery of the secluded alpine environment or using spare time for individual practice.



Lecturers and tutors

Cardoso Luiza	Mitochondrial Wizard, Oroboros Instruments
Gnaiger Erich	CEO, Innovation Alchemist, Oroboros Instruments
Grings Mateus	Mitochondrial Jedi, Oroboros Instruments
Schmitt Sabine	Mitochondrial Detective, Oroboros Instruments

FAT4BRAIN project tutor



Cecatto Cristiane	Mitochondrial Phoenix, Oroboros Instruments
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Program

1 Monday, Oct 03

* printed in workshop materials

	Arrival	Weblink
15:00	Arrival in Bregenz: Meeting point Bregenz train station at 15:00; approx. 1 h bus drive to Schröcken and Hochtannberg (Salober); walk to Hotel Körbersee (approx. 40 min)	IOC-travel
18:30-19:30	<i>Welcome reception at Hotel Körbersee & get-together:</i> Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:30	<i>Dinner</i>	

2 Tuesday, Oct 04

	Workshop 1	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:30	O2k-Series I and XA and DatLab 7.4 and 8.0 O2k instrumental setup – overview with video clips	O2k-FluoRespirometer NextGen-O2k MitoPedia: DatLab DL-Protocols O2k-Videosupport
09:30-12:30	Hands-on (2 teams): chamber assembly, volume calibration and OroboPOS service	
09:30-10:45	Team 1 Team 2	POS Service

	Chamber assembly	OroboPOS service	O2k-Start
10:45-11:15	<i>Coffee / Tea</i>		
11:15-12:30	Team 2 Chamber assembly	Team 1 OroboPOS service	POS Service O2k-Start
12:30-14:30	<i>Lunch packages / Walk & Talk</i> <i>Alternative: individual O2k-tasks</i>		
14:30-15:30	Hands-on (6 teams): Oxygen calibration (instrumental quality control 1) DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air		SOP: O2k-cleaning and ISS O2k-Start SOP: POS-calibration Gnaiger 2008 POS
15:30-16:00	<i>Coffee / Tea</i>		
16:00-18:30	Hands-on (6 teams): 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)
18:30-20:00	<i>Dinner</i>		
20:00-21:00	DatLab analysis: Quality control and reproducibility of technical repeats		DatLab-Analysis Baglivo BEC 2022.8 *

3 Wednesday, Oct 05

	Workshop 2	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:45	Experimental design: Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states Mitochondrial Physiology * Blue Book *
09:45-10:15	Substrate-uncoupler-inhibitor titration (SUIT) protocols – fundamental principles	MitoPedia: SUIT
10:15-10:45	<i>Coffee / Tea</i>	
10:45-11:00	Oroboros SUITbrowser: How to find a DL-Protocol (DLP)	Oroboros SUITbrowser Video: How to find a DL-Protocol (DLP)
11:00-11:30	Experiment traces overview: Respiration of permeabilized cells - Measurement of oxygen	SUIT reference protocol

	consumption with Reference Protocols RP1 (SUIT-001) and RP2 (SUIT-002) DL-Protocol (O2): SUIT-001 O2 ce-pce D003 DL-Protocol (O2): SUIT-002 O2 ce-pce D007	SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
11:30-12:30	Hands-on (6 teams) - getting started with an O2k experiment: washing, stirrer test, air calibration DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air	SOP: O2k-cleaning and ISS SOP: POS-calibration
12:30-14:00	<i>Lunch packages / Walk & Talk</i>	
14:00-16:00	Hands-on (6 teams) - O2k-experiment: Respiration with permeabilized HEK 293 T cells - SUIT protocols 001 and 002 (RP1 and RP2) DL-Protocol (O2): SUIT-001 O2 ce-pce D003 DL-Protocol (O2): SUIT-002 O2 ce-pce D007 DL-Protocol (Instrumental): O2k-cleaning AfterUse	SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
16:00-16:30	<i>Coffee / Tea – Take turns in your team to continue the experiment</i>	
16:30-17:45	DatLab analysis and SUIT protocols Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	MitoPedia: Respiratory control ratios MitoPedia: SUIT
17:45-18:45	Hands-on (6 teams): DatLab analysis – O₂ flux Analysis of the hands-on experiment with permeabilized cells.	O₂-Flux Analysis MitoPedia: DatLab
19:00-20:30	<i>Dinner + registration for the walk to the Alpmuseum</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts	

4 Thursday, Oct 06

	Workshop 3	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to H₂O₂ measurements	Amplex UltraRed H₂O₂ Komlodi BEC 2021.4 *
09:00-10:00	Hands-on (6 teams): Standard H₂O₂ protocol for permeabilized cells in 7 O2ks DL-Protocol (O2&AmR): AmR calibration DL-Protocol (O2&AmR): SUIT-013 AmR ce D023	SUIT-013 AmR ce D023
10:00-10:30	<i>Coffee / Tea – Take turns in your team to continue the experiment</i>	
10:30-11:00	Hands-on (6 teams): continuation DL-Protocol (O2&AmR): SUIT-013 AmR ce D023 DL-Protocol: O2k-cleaning AfterUse	SUIT-013 AmR ce D023
11:00-12:30	H₂O₂ data analysis: introduction and hands-on in teams	H₂O₂-Flux Analysis
12:30-14:30	<i>Lunch packages / walk & talk</i> <i>alternative: individual O2k-tasks</i>	

14:30-15:50	Hands-on (6 teams): DatLab analysis - O₂ and H₂O₂ flux Summary discussion	H₂O₂-Flux Analysis
15:50-16:30	Introduction to permeabilized fibers Questionnaire	MiPNet14.14 Permeabilized Fiber Preparation
16:30-17:00	<i>Coffee / Tea</i>	
17:00-18:00	Data interpretation using SUIT protocols OXPHOS analysis: diagnosis of respiratory defects	MitoPedia: SUIT
18:00-19:00	Introduction to mitochondrial membrane potential with fluorescent dyes, mitochondrial ATP production with Magnesium Green and calcium retention capacity with Calcium Green	Mitochondrial membrane potential Safranin Magnesium Green Cardoso BEC 2021.1 * Calcium Green
19:00-20:30	<i>Dinner</i>	
20:30-21:30	SUIT Quiz	

5 Friday, Oct 07

	Workshop 4	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to instrumental O₂ background (traces overview), using the TIP2k	MiPNet14.06 Instrumental O₂ background
09:00-11:00	Hands-on (6 teams): Instrumental O₂ background (instrumental quality control 2) O ₂ background test with the TIP2k; analysis of oxygen flux; O ₂ background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 – 200 µM DL-Protocol: Instrumental O ₂ background TIP2k	TIP2k manual
10:30-11:00	<i>Coffee / Tea – Take turns in your team to continue the Experiment</i>	
11:00-12:00	Hands-on (6 teams): Data analysis - instrumental O₂ background flux	
12:00-12:30	<i>Lunch packages</i>	
12:30-15:30	<i>Walk to the Alpmuseum – guided tour and reception: € 15</i>	Alpmuseum*
15:30-16:00	<i>Coffee / Tea</i>	
16:00-16:30	MitoFit Preprint Archives and Bioenergetics Communications	MitoFit Preprint Archives Bioenergetics Communications
16:30-17:30	Oroboros Ecosystem - Tutorial on the Bioblast wiki	Bioblast O2k-Network O2k-Publications
17:30-18:30	NextGen-O2k: Q-Module Demo-Experiment Demo experiment with yeast	Q-Module Komlodi BEC 2022.3 *

18:30-19:00	NextGen-O2k: NADH-Module and PB-Module	NADH-Module PB-Module
19:00-20:30	Dinner	
20:30-21:30	Feedback discussion: Next steps in the individual projects	

6 Saturday, Oct 08

	Departure	
06:30-7:30	Breakfast	
	Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9:00 am at Salober.	

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 FluoRespirometry
 - 🕒 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**



List of participants

Participant	Institution
Balmaceda Valeria	IT Padova Viscomi C - University of Padova, IT*
Berg Katrine	DK Aarhus Boetker HE - Aarhus University, DK***
Bowden Samantha D	US LA New Orleans Rees BB -University of New Orleans, US
Chen Qian	US PA Philadelphia Barsotti RJ - Philadelphia College of Osteopathic Medicine, US*
Dommaschk Eva-Maria	University Hospital Jena, DE – FAT4BRAIN project
Dvorak Ales	CZ_Prague_Dvorak A - Charles University, CZ*
Figueirido Costa Claudio	BE_Leuven_Fransen M - University of Leuven, BE
Handler Alyssa	US MA Needham Kropp L - Stealth BioTherapeutics, US**
Heine Markus	DE_Hamburg_Scheja L - University Medical Clinic Hamburg-Eppendorf, DE*
Kuntic Marin	DE_Mainz_Daiber A - Johannes Gutenberg University of Mainz, DE*
Kropp Laura	US MA Needham Kropp L - Stealth BioTherapeutics, US**
Martensen Pia Moller	DK Aarhus_Martensen PM - Aarhus University, DK*
Palei Ana	US MS Jackson Hosler J - University of Mississippi Medical Center, US**
Pospisilova Katerina	CZ_Prague_Dvorak A - Charles University, CZ*
Seefeldt Jacob	DK Aarhus Boetker HE - Aarhus University, DK***
Strohm Lea	DE_Mainz_Daiber A - Johannes Gutenberg University of Mainz, DE*
Timon-Gomez Alba	AT Innsbruck Oroboros - Oroboros Instruments, AT
Younis Awais	UK Nottingham Lavery GG - Nottingham Trent University, UK***
Moukova Anna	CZ Olomouc Modriansky M - Palacky University, CZ*

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

Accommodation and location

Hotel Körbersee www.koerbersee.at
T +43 5519 265 hotel@koerbersee.at



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>

MitoFit Preprints



The Open Access preprint server for mitochondrial physiology and bioenergetics

» https://www.mitofit.org/index.php/MitoFit_Preprints

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>

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The Next World-Summits on Mitochondrial Physiology and Bioenergetics



MiP
Mitochondrial
Physiology
School 2023

13th MiPschool
Mitochondrial Physiology
2023 July 23-27
Oberurgl, Austria

www.mitophysiology.org

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Physiology
2023

15th MiPconference
Bioenergetics Communications on mitObesity
2023 July 27-30
Oberurgl, Austria
20 years MiPsociety

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www.ebec2024.org



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.

Contact

Erich Gnaiger, PhD
 Oroboros Instruments GmbH
 Schoepfstrasse 18
 A-6020 Innsbruck, Austria
 T +43 512 566796 F +43 512 566796 20
instruments@oroboros.at | www.oroboros.at
Mitochondria and cell research



Virtual O2k-Workshops are listed as [MitoGlobal Events](#)