

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

FAT4BRAIN O2k-Workshop on High-Resolution Respirometry



2022 Oct 03 - 08

Schroecken, Vorarlberg, Austria



The **FAT4BRAIN O2k-Workshop** will be held in Schroecken, Austria, providing 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 yeast 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.



Lecturer and tutors

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: chamber assembly, volume calibration and OroboPOS service	
09:30-10:45	<u>Chamber assembly</u>	O2k-Start
10:45-11:15	<i>Coffee / Tea</i>	
11:15-12:30	<u>OroboPOS service</u>	POS Service
12:30-14:30	<i>Lunch packages / Walk & Talk</i> <i>Alternative: individual O2k-tasks</i>	
14:30-15:30	Hands-on: 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: 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 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 reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
11:30-12:30	Hands-on - 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 - O2k-experiment: Respiration with permeabilized HEK 293T 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	SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007

	DL-Protocol (Instrumental): O2k-cleaning AfterUse _inhibitors	
16:00-16:30	<i>Coffee / Tea</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: 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	Hands-on: - 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
09:00-09:30	Introduction to H₂O₂ measurements	Amplex UltraRed H₂O₂ Komlodi BEC 2021.4 *
09:30-10:00	Hands-on: H₂O₂ protocol for living cells Oxygen dependence of H ₂ O ₂ production with baker's yeast 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</i>	
10:30-11:00	Hands-on: continuation DL-Protocol (O2&AmR): SUIT-013 AmR ce D023 DL-Protocol (Instrumental): 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: 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: Instrumental O2 background (instrumental quality control 2) O2 background test with the TIP2k; analysis of oxygen flux; O2 background from air saturation to zero oxygen concentration; or for permeabilized muscle fibers in the high-oxygen range of 500 – 200 μ M DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): Instrumental O2 background TIP2k	TIP2k manual
10:30-11:00	<i>Coffee / Tea</i>	
11:00-12:00	Hands-on: 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	<i>Dinner</i>	
20:30-21:30	<i>Feedback discussion: Next steps in the individual projects</i>	

6 Saturday, Oct 08

Departure	
06:30-7:30	Breakfast
08:15 (at the latest)	Departure from Hotel Körbersee
09:00	Bus departure 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**

OROBOROS INSTRUMENTS

O2k

Mitochondria and cell research



List of participants

Participant	Institution
Dommaschk Eva-Maria	University Hospital Jena, DE

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

Accommodation and location

Hotel Körbersee
T +43 5519 265

www.koerbersee.at
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



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» www.mitophysiology.org



22nd European Bioenergetics Conference

2024 August 26-31
Congress Innsbruck, Austria
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

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Mitochondria and cell research



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