

Oroboros O2k-Workshop



Mitochondrial Physiology Network 28.02(01):1-8 (2023)

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Updates: http://wiki.orooboros.at/index.php/MiPNet28.02_IOC162_Schroecken_AT

162nd O2k-Workshop on High-Resolution Respirometry

2023 October 02-07

Schroecken, Vorarlberg, Austria



The **162nd O2k-Workshop on high-resolution respirometry (HRR)** is the **45th** International Oxygraph Course (IOC) held in Schroecken since 1988. We provide an overview of the **O2k-FluoRespirometer**, with real-time analysis by **DatLab 8 (new)** and applications of the **Titration-Injection microPump TIP2k**.

The workshop provides hands-on practice in instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) and **Instrumental quality control** which is a fundamental component of HRR.

A wide range of mitochondrial topics is covered; abstracts and experimental experiences can be presented by participants. The **Blue Book** (5th edition) and the **Mitochondrial physiology** provide a basic introduction to mitochondrial bioenergetics, and therefore we recommend reading them beforehand. A demo experiment will be performed to start a detailed discussion of protocol design. The hands-on sessions include training with SUIT protocols using HEK 293 cells. Breakout sessions will provide opportunities for practice on specific topics.

The O2k-Workshop will give an introduction to the **O2k-Applications** using **fluorescence**. The hands-on will include calibrations and data analysis with Amplex UltraRed and Safranin, demonstrating

the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, and hydrogen peroxide production or mitochondrial membrane potential, respectively.

Finally, the new applications of the **NextGen-O2k** will be presented: the Q-Module to assess coenzyme Q-redox state, NADH-Module to assess NAD-redox state and PB-Module to assess 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.



Lecturers and tutors

Baglivo Eleonora	Biomedical Pixie, Oroboros Instruments
Gnaiger Erich	CEO, Innovation Alchemist, Oroboros Instruments
Grings Mateus	Mitochondrial Jedi, Oroboros Instruments
Leo Elettra	Mitochondrial Mermaid, Oroboros Instruments
Timon-Gomez Alba	Mitochondrial Mage, Oroboros Instruments

Preliminary program

1 Monday, Oct 02

* 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
18:00-19:00	<i>Welcome reception at Hotel Körbersee & get-together:</i> Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:00	<i>Dinner</i>	

2 Tuesday, Oct 03

	Workshop 1	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to the O2k	O2k-FluoRespirometer NextGen-O2k
09:00-09:30	Introduction to DatLab 8	MitoPedia: DatLab
09:30-10:30	Introduction to oxygen calibration Hands-on (8 teams): Oxygen calibration (instrumental quality control 1) DL-Protocol (Instrumental): O2k-cleaning BeforeUse DL-Protocol (Instrumental): O2 calibration air and zero	SOP: O2k-cleaning and ISS O2k-Start SOP: POS-calibration Baglivo BEC 2022.8 *
10:30-11:00	<i>Coffee / Tea (O2ks running)</i>	
11:00-12:00	Hands-on continuation (8 teams): Oxygen calibration (instrumental quality control 1) Oxygen calibration data analysis	DL-Protocol (Instrumental): - O2 calibration air and zero
12:00-12:30	Traces presentation and discussion	
12:30-14:30	<i>Lunch packages / Walk & Talk</i>	

14:30-15:00	Traces presentation and discussion	
15:00-16:30	SUIT demo experiment SUIT chemicals, coupling control	DL-Protocol (SUIT): - SUIT-003 O2 ce D009
16:30-17:00	<i>Coffee / Tea</i>	
17:00-18:30	Discussion about the demo experiment: Experimental design, coupling control of mitochondrial respiration Other sample types and pathway control	MitoPedia: Respiratory states Mitochondrial Physiology* Blue Book *
18:30-19:00	Substrate-uncoupler-inhibitor titration (SUIT) protocols – fundamental principles Oroboros SUITbrowser: How to find a DL-Protocol (DLP)	MitoPedia: SUIT Oroboros SUITbrowser Video: How to find a DL-Protocol (DLP)
19:00-20:30	<i>Dinner</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts	

3 Wednesday, Oct 04

	Workshop 2	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Hands-on (8 teams) - O2k-experiment: Respiration with permeabilized HEK 293T cells - SUIT protocol	DL-Protocol (Instrumental): - O2k-cleaning BeforeUse - O2 calibration air
09:00-09:30	Respiration of permeabilized cells – Introduction to SUIT-008	SUIT-008 O2 ce-pce D025
09:30-10:00	Addition of biological sample to the respirometer chamber and practical aspects of chemical titrations	Addition of biological sample SOP: Hamilton microsyringes
10:00 -10:30	<i>Coffee / Tea (O2ks running)</i>	
10:30-12:30	Hands-on (8 teams) - O2k-experiment: Respiration with permeabilized HEK 293T cells - SUIT protocol	DL-Protocol (SUIT): - SUIT-008 O2 ce-pce D025 DL-Protocol (Instrumental): - O2k-cleaning AfterUse_inhibitors
12:30-14:30	<i>Lunch packages / Walk & Talk (O2ks running)</i>	
14:30-15:30	Hands-on continuation (8 teams) - O2k-experiment: Respiration with permeabilized HEK 293T cells - SUIT protocol	DL-Protocol (Instrumental): - O2k-cleaning AfterUse_inhibitors
15:30-15:45	Handling the TIP2K	TIP2k manual
15:45-16:15	Hands-on (8 teams): 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	DL-Protocol (Instrumental): - O2k-cleaning BeforeUse - Instrumental O2 background TIP2k

16:15-16:45	Introduction to instrumental O2 background (traces overview), using the TIP2k	MiPNet14.06 Instrumental O2 background
16:45-17:15	<i>Coffee / Tea - (O2ks running with TIP2k program)</i>	
17:00-18:00	Hands-on continuation (8 teams): Instrumental O2 background (quality control 2) O2 background test with the TIP2k; analysis of oxygen flux; O2 background from air saturation to zero oxygen concentration	
18:00-19:00	Data analysis - instrumental O₂ background flux Hands-on (8 teams): Data analysis - instrumental O₂ background flux	
19:00-20:30	<i>Dinner</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts	

5 Thursday, Oct 05

	Workshop 4	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:15	DatLab analysis of SUIT protocols	MitoPedia: Respiratory control ratios
09:15-10:00	Hands-on: DatLab analysis - O₂ flux Analysis of the hands-on experiment with permeabilized cells.	O₂-Flux Analysis MitoPedia: DatLab
10:00-10:30	<i>Coffee / Tea</i>	
10:30-12:30	Presentation of traces and discussion of results Take-home message	
12:30-14:30	<i>Lunch packages / Walk & Talk</i>	
14:30-15:00	Summary of SUIT-008 results and discussion	
15:00-15:45	Introduction to O2k applications Fluo-Module: AmR, mtMP, CaG, MgG	Amplex UltraRed Mt membrane potential Magnesium Green Calcium Green
15:45-16:15	Hands-on (8 teams): Fluo-Module	DL-Protocol (Instrumental): - O2k-cleaning BeforeUse
16:15-16:45	<i>Coffee / Tea (O2ks running)</i>	
16:45-17:45	Hands-on continuation (8 teams): Fluo-Module	DL-Protocol (Instrumental): - AmR calibration - Saf calibration
17:45-19:00	Breakout session - Hands on: Discussion on Fluo experiments and analysis - AmR or Saf	H₂O₂-Flux Analysis mtMP data analysis
19:00-20:30	<i>Dinner</i>	
20:30-21:30	O2k perspectives: 10+5 min presentation of abstracts SUIT Quiz	

4 Friday, Oct 06

	Workshop 3	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	O2k instrumental setup – overview with videos	O2k-Videosupport
09:00-09:45	Hands-on (split in 3 groups): a. Chamber assembly b. Volume calibration c. OroboPOS service	POS Service O2k manual
09:45-10:30	Hands-on (split in 3 groups): b. Chamber assembly c. Volume calibration a. OroboPOS service	
10:30-11:00	<i>Coffee / Tea</i>	
11:00-11:45	Hands-on (split in 3 groups): c. Chamber assembly a. Volume calibration b. OroboPOS service	
11:45-12:30	Breakout session Suggestions: Oxia, Sample Holder, sV-Module	
12:30-13:00	<i>Lunch packages</i>	
13:00-16:00	<i>Walk to the Alpmuseum – guided tour and reception: € 15 (Workshop room will be closed for packing)</i>	Alpmuseum
16:00-16:30	<i>Coffee / Tea (Workshop room will be closed for packing)</i>	
16:30-17:15	Introduction to NextGen-O2k applications Q-Module NADH-Module PB-Module	Q-Module NADH-Module PB-Module
17:15-18:00	Reference protocols 1 and 2: developing protocols for a mitochondrial snapshot	SUIT reference protocol
18:00-19:00	Oroboros Ecosystem - Tutorial on the Bioblast wiki	Bioblast O2k-Network O2k-Publications
19:00-20:30	<i>Dinner</i>	
20:30-21:30	<i>Feedback discussion: Next steps in the individual projects</i>	

6 Saturday, Oct 07

	Departure	
07:00-07:30	<i>Breakfast</i>	
08:15	Departure from Hotel Körbersee, bus departure 9:00 at Salober	

Accommodation and location

Hotel Körbersee
T +43 5519 265

<https://www.koerbersee.at/en>
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>

>4,500 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>

Acknowledgements

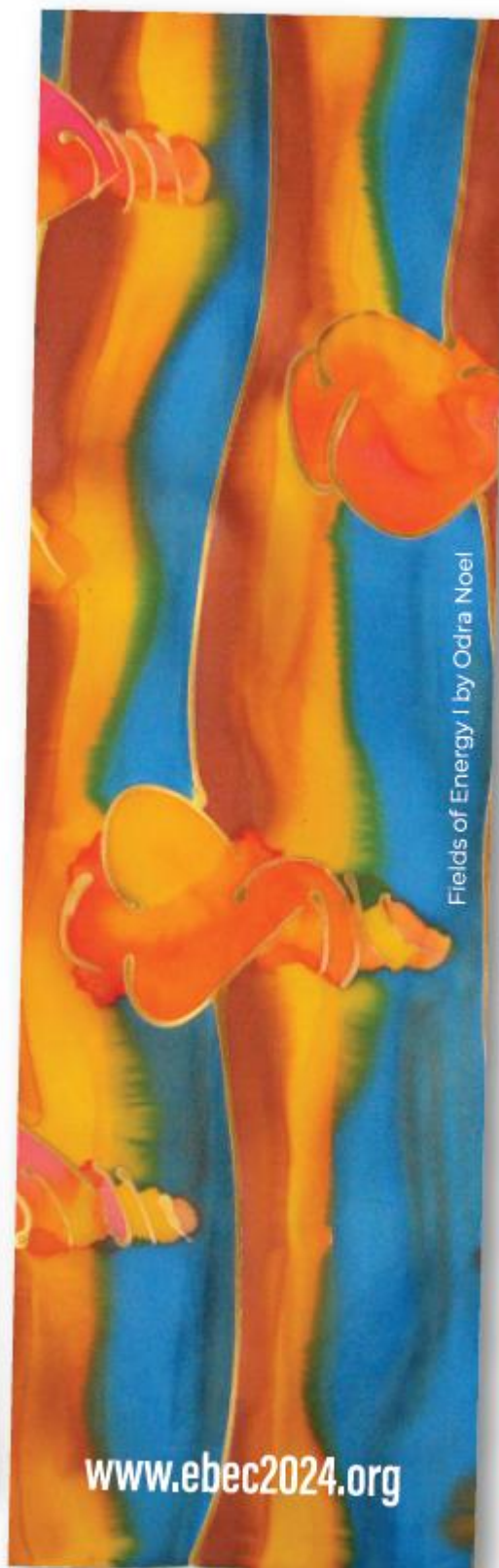
Program prepared for printing by Grings M, Cardoso LHD and Gnaiger C, Oroboros Instruments.

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**



The Next World-Summits on Mitochondrial Physiology and Bioenergetics



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](#)