

# 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](http://wiki.orooboros.at/index.php/MiPNet28.02_IOC162_Schroecken_AT)

## 162<sup>nd</sup> O2k-Workshop on High-Resolution Respirometry

2023 October 02-07

Schroecken, Vorarlberg, Austria



The 162<sup>nd</sup> O2k-Workshop on high-resolution respirometry (HRR) is the 45<sup>th</sup> 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** (5<sup>th</sup> 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

<a href="#">Baglivo Eleonora</a>	Biomedical Pixie, Oroboros Instruments
<a href="#">Gnaiger Erich</a>	CEO, Innovation Alchemist, Oroboros Instruments
<a href="#">Grings Mateus</a>	Mitochondrial Jedi, Oroboros Instruments
<a href="#">Leo Elettra</a>	Mitochondrial Mermaid, Oroboros Instruments
<a href="#">Timon-Gomez Alba</a>	Mitochondrial Mage, Oroboros Instruments

## Preliminary program

### 1 Monday, Oct 02

\* printed in workshop materials

	Arrival	Weblink
15:00	<b>Arrival in Bregenz:</b> 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)	<a href="#">IOC-travel</a>
18:00-19:00	<i>Welcome reception at Hotel Körbersee &amp; <b>get-together:</b></i> Introduction of participants and their research interests - a welcome by Oroboros Instruments	<a href="#">Schroecken</a>
19:00	<i>Dinner</i>	

### 2 Tuesday, Oct 03

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

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

### 3 Wednesday, Oct 04

	<b>Workshop 2</b>	<b>Weblink</b>
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	<b>Hands-on (8 teams) - O2k-experiment:</b> Respiration with permeabilized HEK 293T cells - SUIT protocol	DL-Protocol (Instrumental): - O2k-cleaning BeforeUse - O2 calibration air
09:00-09:30	<b>Respiration of permeabilized cells – Introduction to SUIT-008</b>	<a href="#">SUIT-008 O2 ce-pce D025</a>
09:30-10:00	<b>Addition of biological sample to the respirometer chamber and practical aspects of chemical titrations</b>	<a href="#">Addition of biological sample</a> <a href="#">SOP: Hamilton microsyringes</a>
10:00 -10:30	<i>Coffee / Tea (O2ks running)</i>	
10:30-12:30	<b>Hands-on (8 teams) - O2k-experiment:</b> 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 &amp; Talk (O2ks running)</i>	
14:30-15:30	<b>Hands-on continuation (8 teams) - O2k-experiment:</b> Respiration with permeabilized HEK 293T cells - SUIT protocol	DL-Protocol (Instrumental): - O2k-cleaning AfterUse _inhibitors
15:30-15:45	<b>Handling the TIP2K</b>	<a href="#">TIP2k manual</a>
15:45-16:15	<b>Hands-on (8 teams): Instrumental O2 background (instrumental quality control 2)</b> 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	<b>Introduction to instrumental O2 background</b> (traces overview), using the TIP2k	<a href="#">MiPNet14.06 Instrumental O2 background</a>

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

#### 4 Thursday, Oct 05

	<b>Workshop 3</b>	<b>Weblink</b>
07:30-08:30	<i>Breakfast</i>	
<b>08:30-09:15</b>	<b>DatLab analysis of SUIT protocols</b>	<a href="#">MitoPedia: Respiratory control ratios</a>
<b>09:15-10:00</b>	<b>Hands-on: DatLab analysis – O<sub>2</sub> flux</b> Analysis of the hands-on experiment with permeabilized cells.	<a href="#">O<sub>2</sub>-Flux Analysis</a> <a href="#">MitoPedia: DatLab</a>
10:00-10:30	<i>Coffee / Tea</i>	
<b>10:30-12:30</b>	<b>Presentation of traces and discussion of results</b> Take-home message	
12:30-14:30	<i>Lunch packages / Walk &amp; Talk</i>	
<b>14:30-15:00</b>	<b>Summary of SUIT-008 results and discussion</b>	
<b>15:00-15:45</b>	<b>Introduction to O2k applications</b> Fluo-Module: AmR, mtMP, CaG, MgG	<a href="#">Amplex UltraRed</a> <a href="#">Mt membrane potential</a> <a href="#">Magnesium Green</a> <a href="#">Calcium Green</a>
<b>15:45-16:15</b>	<b>Hands-on (8 teams): Fluo-Module</b>	DL-Protocol (Instrumental): - O2k-cleaning BeforeUse
16:15-16:45	<i>Coffee / Tea (O2ks running)</i>	
<b>16:45-17:45</b>	<b>Hands-on continuation (8 teams): Fluo-Module</b>	DL-Protocol (Instrumental): - AmR calibration - Saf calibration
<b>17:45-19:00</b>	<b>Breakout session – Hands on: Discussion on Fluo experiments and analysis - AmR or Saf</b>	<a href="#">H<sub>2</sub>O<sub>2</sub>-Flux Analysis</a> <a href="#">mtMP data analysis</a>
19:00-20:30	<i>Dinner</i>	
<b>20:30-21:30</b>	<b>O2k perspectives: 10+5 min presentation of abstracts</b> <b>SUIT Quiz</b>	

**5 Friday, Oct 06**

	<b>Workshop 4</b>	<b>Weblink</b>
07:30-08:30	<i>Breakfast</i>	
<b>08:30-09:00</b>	<b>O2k instrumental setup</b> – overview with videos	<a href="#">O2k-Videosupport</a>
<b>09:00-09:45</b>	<b>Hands-on</b> (split in 3 groups): <b>a.</b> Chamber assembly <b>b.</b> Volume calibration <b>c.</b> OroboPOS service	<a href="#">POS Service</a> <a href="#">O2k manual</a>
<b>09:45-10:30</b>	<b>Hands-on</b> (split in 3 groups): <b>b.</b> Chamber assembly <b>c.</b> Volume calibration <b>a.</b> OroboPOS service	
10:30-11:00	<i>Coffee / Tea</i>	
<b>11:00-11:45</b>	<b>Hands-on</b> (split in 3 groups): <b>c.</b> Chamber assembly <b>a.</b> Volume calibration <b>b.</b> OroboPOS service	
<b>11:45-12:30</b>	<b>Breakout session</b> 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>	<a href="#">Alpmuseum</a>
16:00-16:30	<i>Coffee / Tea (Workshop room will be closed for packing)</i>	
<b>16:30-17:15</b>	<b>Introduction to NextGen-O2k applications</b> <b>Q-Module</b> <b>NADH-Module</b> <b>PB-Module</b>	<a href="#">Q-Module</a> <a href="#">NADH-Module</a> <a href="#">PB-Module</a>
<b>17:15-18:00</b>	<b>Reference protocols 1 and 2:</b> developing protocols for a mitochondrial snapshot	<a href="#">SUIT reference protocol</a>
<b>18:00-19:00</b>	<b>Oroboros Ecosystem - Tutorial on the Bioblast wiki</b>	<a href="#">Bioblast</a> <a href="#">O2k-Network</a> <a href="#">O2k-Publications</a>
19:00-20:30	<i>Dinner</i>	
<b>20:30-21:30</b>	<i>Feedback discussion: Next steps in the individual projects</i>	

**6 Saturday, Oct 07**

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



## Accommodation and location

**Hotel Körbersee**  
T +43 5519 265

<https://www.koerbersee.at/en>  
[hotel@koerbersee.at](mailto: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](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

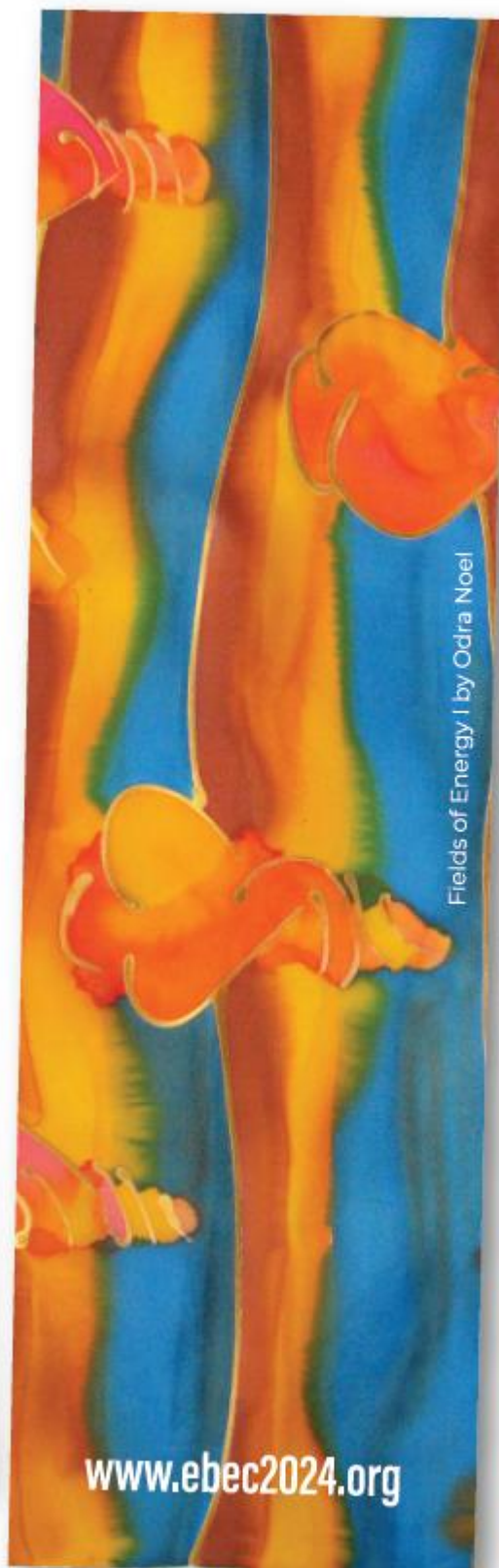
Program prepared for printing by Grings M, Cardoso LHD and Gnaiger C, Oroboros Instruments.

### 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-Summit on Bioenergetics



## 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.

### Contact

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**Mitochondria and cell research**



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