Oroboros O2k-Workshop

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 Amplex™ UltraRed, mt-membrane potential with safranin, TMRM or rhodamine 123, ATP production measurement with Magnesium Green™ and Ca^{2+} uptake capacity with Calcium Green™). 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 |

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	*Welcome reception at Hotel Körbersee & **get-together:** Introduction of participants and their research interests - a welcome by Oroboros Instruments*	Schroecken
19:30	*Dinner*	

2   Tuesday, Oct 04

	Workshop 1	Weblink
07:30-08:30	*Breakfast*	
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** Chamber assembly OroboPOS service	POS Service O2k-Start
10:45-11:15	*Coffee / Tea*	
11:15-12:30	Team **2** Team **1** Chamber assembly OroboPOS service	POS Service O2k-Start
12:30-14:30	*Lunch packages / Walk & Talk* *Alternative:* individual O2k-tasks	

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	*Coffee / Tea*	

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	*Dinner*	
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	*Breakfast*	
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	*Coffee / Tea*	
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 (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	*Lunch packages / Walk & Talk*	
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	*Coffee / Tea – Take turns in your team to continue the experiment*	
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 – O_2 flux** Analysis of the hands-on experiment with permeabilized cells.	[O_2-Flux Analysis](#) [MitoPedia: DatLab](#)
19:00-20:30	*Dinner + registration for the walk to the Alpmuseum*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts	

4   Thursday, Oct 06

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:00	**Introduction to H_2O_2 measurements**	[Amplex UltraRed](#) [H_2O_2](#) [Komlodi BEC 2021.4](#) *
09:00-10:00	**Hands-on (6 teams): Standard H_2O_2 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	*Coffee / Tea – Take turns in your team to continue the experiment*	
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	**H_2O_2 data analysis: introduction and hands-on in teams**	[H_2O_2-Flux Analysis](#)

12:30-14:30	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
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	*Coffee / Tea*	
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	*Dinner*	
20:30-21:30	**SUIT Quiz**	

5   Friday, Oct 07

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	
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)** O2 background test with the TIP2k; analysis of oxygen flux; O2 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 O2 background TIP2k	TIP2k manual
10:30-11:00	*Coffee / Tea – Take turns in your team to continue the Experiment*	
11:00-12:00	**Hands-on (6 teams): Data analysis - instrumental O₂ background flux**	
12:00-12:30	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum –* *guided tour and reception: € 15*	Alpmuseum*
15:30-16:00	*Coffee / Tea*	
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**	Q-Module
	Demo experiment with yeast	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**

OROBOROS INSTRUMENTS   O2k   Mitochondria and cell research

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*
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Handler Alyssa	**US MA Needham Kropp L** - Stealth BioTherapeutics, US**
Heine Markus	**DE_Hamburg_Scheja L** - University Medical Clinic Hamburg-Eppendorf, DE*
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Timon-Gomez Alba	**AT Innsbruck Oroboros** - Oroboros Instruments, AT
Younis Awais	**UK Nottingham Lavery GG** - Nottingham Trent University, UK***

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

Acknowledgements

The Next World-Summits on Mitochondrial Physiology and Bioenergetics

Become a MiP*member* now and join our MiP*events*
   » www.mitophysiology.org

22ⁿᵈ European Bioenergetics Conference

2024 August 26-31
Congress Innsbruck, Austria
www.ebec2024.org

NextGen O2k – Applications

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