

Oroboros O2k-Workshop



Mitochondrial Physiology Network 27.03(02):1-8 (2022)

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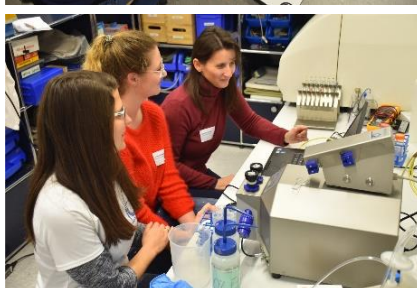
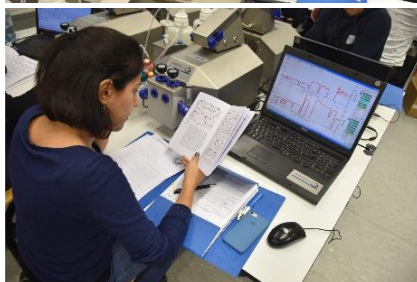
Updates: https://wiki.orooboros.at/index.php/MiPNet27.03_IOC154_Innsbruck_AT

O2k-Coaching Days 154th O2k-Workshop on high-resolution respirometry



2022 Aug 01st-04th: O2k-FluoRespirometer

2022 Aug 01st -05th: O2k-FluoRespirometer and NextGen-O2k
Innsbruck, Tyrol, Austria



O2k-Coaching Days is a training course which provides a fundamental introduction to **high-resolution respirometry (HRR)** with the Oroboros O2k. It will give an overview of the **O2k**, including real-time analysis with **DatLab 8** and applications of the **Titration-Injection microPump TIP2k**. Hands-on sessions, using one O2k per participant, range from instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**), instrumental quality control system, to respirometry experiments with substrate-uncoupler-inhibitor (SUIT) protocols using HEK 293T cells as the biological sample. Many optimized SUIT protocols are available as DL-Protocols and will be shown at the Coaching Days, as well as the [SUITbrowser](#), which helps you find the best SUIT protocol for your specific research questions. The [Blue Book](#) and [Mitochondrial Physiology](#) provide a basic introduction to mitochondrial bioenergetics, complementing the training course, and therefore we recommend reading them beforehand.

The O2k-Coaching Days - Advanced 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.

Finally, the **O2k-Coaching Days - NextGen-O2k all-in-one** will present new applications of the NextGen-O2k: the Q-Module to assess coenzyme Q-redox state, NADH-Module to assess NAD-redox state and PhotoBiology (PB) Module, which allows to detect oxygen production from photosynthesis. The 154th workshop is a unique opportunity to learn about the new developments in HRR.



Lecturers and tutors

Baglivo Eleonora	Biomedical Pixie, Oroboros Instruments
Cardoso Luiza	Mitochondrial Wizard, Oroboros Instruments
Garcia-Souza Luiz	Mitochondrial Adventurer, Oroboros Instruments
Gnaiger Erich	CEO, Innovation Alchemist, Oroboros Instruments
Grings Mateus	Mitochondrial Jedi, Oroboros Instruments
Schmitt Sabine	Mitochondrial Detective, Oroboros Instruments

Program

1 Monday, Aug 01

	O2k Basic – quality control	Weblink	Room
08:30-09:00	<i>Welcome - Get-together: Introduction of participants and their research interests</i>		MiPart
09:00-09:20	OroboPOS service and O2k instrumental setup - overview with videoclips	O2k-FluoRespirometer O2k-Videosupport	Oroboros O2k-Laboratory
09:20-10:40	Hands-on (2 groups) OroboPOS service and O2k instrumental setup	POS Service O2k-Start	Oroboros O2k-Laboratory
10:40-11:00	DatLab 7.4 and 8 overview	MitoPedia: DatLab DatLab 7 Innovations	MiPart
11:00-11:30	Instrumental quality control 1: oxygen calibration	Gnaiger 2008 POS SOP: O2-calibration	MiPart
11:30-12:30	Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: O2-calibration	Oroboros O2k-Laboratory
12:30-13:30	<i>Lunch break</i>		
13:30-14:00	Hands-on: Instrumental quality control 1: oxygen calibration (continuation). DatLab analysis DL-Protocol: O2 calibration air		Oroboros O2k-Laboratory

14:00-14:30	Instrumental quality control 2: Instrumental O2 background – overview with videoclips	SOP: O2 background TIP2k manual	Oroboros O2k- Laboratory
14:30-15:30	Hands-on: Instrumental quality control 2: Instrumental O2 background DL-Protocol: Instrumental O2 background TiP2k		Oroboros O2k- Laboratory
15:30-16:00	<i>Coffee / Tea</i>		MiPart
16:00-17:45	Hands-on: Instrumental quality control 2 (continuation). DatLab analysis. DL-Protocol: Instrumental O2 background TiP2k		Oroboros O2k- Laboratory

2 Tuesday, Aug 02

O2k Basic – SUIT protocols		Weblink	Room
08:30-09:10	Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: O2-calibration	Oroboros O2k- Laboratory
09:10-10:10	Introduction to substrate-uncoupler-inhibitor titration (SUIT) protocols – fundamental principles. SUIT reference protocol: RP1&RP2	MitoPedia: SUIT	Oroboros O2k- Laboratory
10:10-10:25	SUITbrowser: how to find the best SUIT protocol for your research questions.	Oroboros SUITbrowser	Oroboros O2k- Laboratory
10:25-10:30	Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2 calibration air	SOP: O2-calibration	Oroboros O2k- Laboratory
10:30-10:45	<i>Coffee / Tea</i>		MiPart
10:45-12:45	Hands-on: O2k-experiment: Respiration of permeabilized cells: measurement of oxygen consumption with the reference protocol RP1 (SUIT-001) and RP2 (SUIT-002). DL-Protocol: SUIT-001 O2 ce-pce D003.DLP DL-Protocol: SUIT-002 O2 ce-pce D007.DLP	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007	Oroboros O2k- Laboratory
12:45-13:00	Hands-on: O2k-cleaning after use DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Oroboros O2k- Laboratory
13:00-14:00	<i>Lunch break</i>		
14:00-14:30	Hands-on: O2k-cleaning after use (continuation) DL-Protocol: O2k-cleaning AfterUse		Oroboros O2k- Laboratory
14:30-15:30	DatLab analysis: Introduction and new features Hands-on: Individual DatLab analysis – O ₂ flux	Oxygen flux analysis	Oroboros O2k- Laboratory
15:30-16:00	<i>Coffee / Tea</i>		MiPart
16:00-17:30	DatLab analysis summary		MiPart

3 Wednesday, Aug 03

	O2k Basic – SUIT protocols and proficiency test	Weblink	Room
08:30-10:00	MitoFit proficiency test Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: O2-calibration	Oroboros O2k- Laboratory
10:00-10:30	<i>Coffee / Tea</i>		MiPart
10:30-12:15	MitoFit proficiency test Hands-on: O2k-experiment: Respiration of permeabilized cells: measurement of oxygen consumption with the reference protocol RP1 (SUIT-001) and RP2 (SUIT-002). DL-Protocol: SUIT-001 O2 ce-pce D003.DLP DL-Protocol: SUIT-002 O2 ce-pce D007.DLP	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007	Oroboros O2k- Laboratory
12:15-12:45	Hands-on: O2k-cleaning after use DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Oroboros O2k- Laboratory
12:45-13:45	<i>Lunch break</i>		
13:45-14:00	Hands-on: O2k-cleaning after use (continuation) DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Oroboros O2k- Laboratory
14:00-14:45	Hands-on: DatLab analysis – O ₂ flux	Oxygen flux analysis	Oroboros O2k- Laboratory
14:45-15:15	<i>Coffee / Tea</i>		MiPart
15:15-15:30	O2k-Applications - overview	O2k Applications	MiPart
15:30-16:00	The Bioblast wiki, Bioenergetics Communications, O2k-Network and MitoEAGLE	MitoFit Preprint Archives O2k-Network www.bioblast.at	MiPart
16:00-17:00	Proficiency test: DatLab analysis summary		MiPart

4 Thursday, Aug 04

	O2k Advanced - Fluo	Weblink	Room
08:30-09:00	Hands-on: Instrumental quality control 1: oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: O2-calibration	Oroboros O2k- Laboratory
09:00-09:30	Introduction to FluoRespirometry and H₂O₂ production with Amplex UltraRed	O2k-FluoRespirometer O2k-Fluo Smart-Module MitoPedia: Amplex UltraRed	Oroboros O2k- Laboratory
09:30-10:00	<i>Coffee / Tea</i>		MiPart

10:00-10:30	Hands-on: Amplex UltraRed calibration DL-Protocol: AmR calibration.DLP	MitoPedia: Amplex UltraRed	Oroboros O2k- Laboratory
10:30-12:15	Hands-on: O2k-experiment: Respiration and H ₂ O ₂ production of permeabilized cells: SUIT-026 protocol, focused on reverse electron transfer DL-Protocol: SUIT-026 AmR ce-pce DLP DL-Protocol: SUIT-026 AmR mt D064	SUIT-026 DL-Protocol User (DLP)	Oroboros O2k- Laboratory
12:15-12:45	Hands-on: O2k-cleaning after use DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Oroboros O2k- Laboratory
12:45-13:45	Lunch break		
13:45-14:00	Hands-on: O2k-cleaning after use (continuation) DL-Protocol: O2k-cleaning AfterUse	SOP: O2k-cleaning and ISS	Oroboros O2k- Laboratory
14:00-14:45	Hands-on: DatLab analysis – H ₂ O ₂ flux	H₂O₂ flux analysis	Oroboros O2k- Laboratory
14:45-15:15	Coffee / Tea		MiPart
15:00-16:00	Other Fluorespirometry applications – Mitochondrial membrane potential with safranin and other dyes, ATP production with Magnesium Green, Ca ²⁺ retention capacity with Calcium Green	Mt membrane potential Magnesium Green Calcium Green	MiPart
16:00-17:00	DatLab analysis summary		MiPart
17:00-17:30	Feedback & conclusions		MiPart
17:30	Farewell activity		MiPart

5 Friday, Aug 05

	O2k Advanced – NextGen all-in-one	Weblink	Room
09:00-10:00	Introduction to the Q-Module	Q-Module	Oroboros O2k- Laboratory
10:00-10:45	Hands-on: Getting started with the Q-Module Polishing the electrodes (Q-stopper) Assembly of the reference electrodes	MiPNet24.12 NextGen-O2k: Q-Module O2k-Videosupport Q-Module	Oroboros O2k- Laboratory
10:45-11:00	O2k-Demo experiment: Cyclic voltammetry Instrumental quality control for the Q-Module	MiPNet24.16 DatLab 8.0: CV-Module	Oroboros O2k- Laboratory
11:00-11:30	Coffee / Tea		MiPart
11:30-12:30	O2k-Demo experiment: Respiration and Q-redox state of permeabilized cells: coupling control protocol SUIT-006	SUIT-006 Q ce-pce D073	Oroboros O2k- Laboratory

	DL-Protocol: SUIT-006 Q ce-pce D073		
12:30-13:30	Lunch break		
13:30-14:30	Hands-on: DatLab analysis – Q-redox state	MiPNet24.12 NextGen-O2k: Q-Module	Oroboros O2k-Laboratory
14:30-15:00	The NextGen O2k all-in-one: NADH-Module	NADH-Module MiPNet26.12 NextGen-O2k: NADH-Module	MiPArt
15:00-15:30	Coffee / Tea		MiPArt
15:30-16:30	O2k-Demo experiment: PhotoBiology: Photosynthesis measurement with the O2k	PB-Module MiPNet26.11 NextGen-O2k: PB-Module	Oroboros O2k-Laboratory
16:30-17:00	Feedback & conclusions		MiPArt
17:00	Farewell activity		MiPArt

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 respirometry
 - 🕒 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
Chowdhury Soumitra	NO_Bodo_Viswanath K - Nord University (NO)
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Massop Jur	NL Wageningen Keijer J - Wageningen University and Research (NL) ***
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	AT_Innsbruck_Roach T - University of Innsbruck (AT)
Shaboodien Sara	ZA Cape Town Maarman GJ - Stellenbosch University (ZA)*
Van Rinsum Alexia	NL Wageningen Keijer J - Wageningen University and Research (NL) ***
Weidinger Adelheid	AT Vienna Kozlov AV - Ludwig Boltzmann Institute for Traumatology (AT)***
	NL Wageningen Keijer J - Wageningen University and Research (NL) ***
Zhang Deli	NL Wageningen Keijer J - Wageningen University and Research (NL) ***

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

Venue and Accommodation

Oroboros O2k-Laboratory

Schoepfstrasse 18, 6020 Innsbruck

> [How to get there](#)

Hotel suggestion:

Basic Hotel Innsbruck

> <https://www.basic-hotel.at/en/>



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.orooboros.at/index.php/O2k-Manual>

O2k-Procedures – <http://wiki.orooboros.at/index.php/O2k-Procedures>

>4,200 **O2k-Publications** – <http://wiki.orooboros.at/index.php/O2k-Publications: Topics>

Acknowledgements

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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>

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](#)