Oroboros O2k-Workshop

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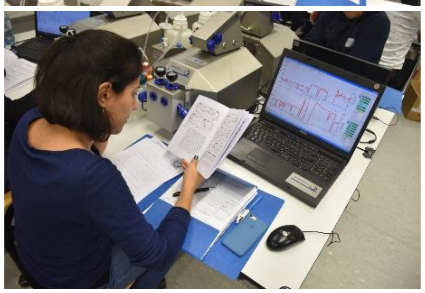
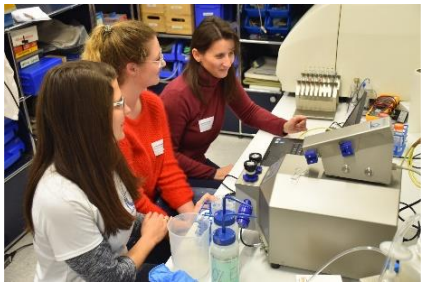
O2k-Coaching Days 154th O2k-Workshop on high-resolution respirometry

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 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.

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	*Welcome - Get-together: Introduction of participants and their research interests*		*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	*Lunch break*		
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	*Coffee / Tea*		*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	*Coffee / Tea*		*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	*Lunch break*		
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	*Coffee / Tea*		*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	*Coffee / Tea*		*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	*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** – O_2 flux	Oxygen flux analysis	*Oroboros O2k-Laboratory*
14:45-15:15	*Coffee / Tea*		*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** **H_2O_2 production with Amplex UltraRed**	O2k-FluoRespirometer O2k-Fluo Smart-Module MitoPedia: Amplex UltraRed	*Oroboros O2k-Laboratory*
09:30-10:00	*Coffee / Tea*		*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_2O_2 production of permeabilized cells: SUIT-026 protocol, focused on reverse electron transfer DL-Protocol: SUIT-026 AmR ce-pce DLPU DL-Protocol: SUIT-026 AmR mt D064	SUIT-026 DL-Protocol User (DLPU)	*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_2O_2 flux	H_2O_2 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*

List of participants

Participant	Institution
Chowdhury Soumitra	**NO_Bodo_Viswanath K** - Nord University (NO)
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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.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

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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

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