Mitochondrial Physiology Network 21.10(01):1-8 (2016)
Version 01: 2016-09-27 ©2016 OROBOROS
Updates: http://wiki.oroboros.at/index.php/MiPNet21.10_IOC115_Schroecken_AT

115th International Workshop on HRR and O2k-Fluorometry

2016 October 03-08
Schröcken, Vorarlberg, Austria

The **115th Workshop on High-Resolution Respirometry (HRR)** is the **36th** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **Oxygraph-2k and O2k-Fluorometer**, with real-time analysis by **DatLab 7 (*new*)** and applications of the **TIP2k**. O2k-Demo experiments show the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, hydrogen peroxide production or mt-membrane potential.

HEK 293T cells are used as a biological reference sample, which can be stored on dry-ice – introducing the MitoFit Proficiency Test.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. A wide range of mitochondrial topics is covered; abstracts and experimental experiences are presented by participants.

IOC participants invariably asked for a detailed discussion of protocol design. The **Blue Book** provides a basic introduction to mitochondrial physiology and is complemented by overview presentations with examples, including **DatLab Analysis** of demo files. **Instrumental quality control** is a fundamental component of HRR and will be put to the practical test in teams using seven O2k (14 chambers). The **O2k-MultiSensor** and particularly O2k-Fluorometry has become an integral part of the O2k-Workshop. Optimization of protocol design for various O2k-MultiSensor applications helps to critically evaluate basic principles of mitochondrial physiology. You will also see the **Titration-Injection microPump TIP2k** with feedback-control in action and practice its simple and automatic operation.

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. Join for a visit to the *Alpmuseum*.

Lecturers and tutors

Gnaiger Erich	CEO, OROBOROS INSTRUMENTS & Medical University of Innsbruck
Doerrier Carolina	Chief Science Officer, OROBOROS INSTRUMENTS
Dikova Valentina	Scientific Assistant, MitoFit project, D. Swarovski Research Laboratory, Medical University of Innsbruck
Laner Verena	Chief Operating Officer, OROBOROS INTRUMENTS
Neves Pedro	Scientific Assistant, OROBOROS INSTRUMENTS
Weber Anja	PhD student, Department of Urology, Medical University of Innsbruck

Guest lecturer

Lundby Carsten	Center for Integrative Human Physiology, University of Zurich, CH

Programme

1 Monday, Oct 03

*printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; approx. 1 hour 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 IOC115
19:30	*Dinner*	

2 Tuesday, Oct 04

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**O2k instrumental setup** – overview with video clips		O2k-Manual
09:30-11:30	**Hands-on (10 groups)**		
	O2k instrumental setup	**OroboPOS service**	
09:30-10:15	Groups 1-5	Groups 6-10	O2k-Start
10:15	*Coffee / Tea*		
10:45-11:30	Groups 6-10	Groups 1-5	POS Service
11:30-12:30	**Applications of the O2k:** DatLab guide through the menus		Gnaiger 2008 POS O2k-Calibration

12:30	*Lunch packages/ Walk & Talk* *alternative:* individual O2k-tasks	
14:30-15:30	**Oxygen calibration (instrumental quality control 1) and cell respiration** (Demo-Experiment)	O_2-Flux Analysis
15:30	*Coffee / Tea*	
16:00-18:00	**Hands-on (7 groups): Oxygen calibration and cell respiration** Advanced groups: Cell respiration and simultaneous measurement of H_2O_2 production.	Yeast reference assay
18:30	*Dinner*	
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats	POS-Calibration-SOP O2 background

3 Wednesday, Oct 05

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Experimental design:** Pathway and coupling control of mitochondrial respiration	Cells: CCP Coupling control state Glossary: Respiratory states SUIT protocols
10:00	*Coffee / Tea*	
10:30-11:30	**O2k-Demo experiment**: Respiration of permeabilized cells: Measurement of oxygen consumption (O2k-Core) with RP1 and RP2.	SUIT reference protocol
11:30-12:00	**Hands-on (7 groups) - getting started with an O2k experiment:** washing, stirrer test, air calibration	O2k-Calibration
12:00	*Lunch packages / Walk & Talk* *alternative:* individual O2k-tasks	*The Blue Book p 56**
14:00-16:00	**Hands-on (7 groups) - O2k-experiment** Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 7 Power-O2k	SUIT Reference Protocols
16:00	*Coffee / Tea* *Registration for the walk to the Alpmuseum @ Verena*	
16:30-17:45	**DatLab analysis and SUIT protocols** Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	DatLab Flux Analysis
17:45-18:45	**DatLab analysis: hands-on in teams** Analysis of the hands-on experiment with permeabilized cells.	
19:00	*Dinner*	
20:30-21:15	**O2k perspectives:** 10+5 min presentations of abstracts 1-3	IOC115 Abstracts

4 Thursday, Oct 06

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-09:00	**From isolated mitochondria to tissue fibres and tissue homogenate preparation:** The PBI-Shredder. Demonstration	MiPNet17.03 Shredder vs Fibres
09:00-10:00	**Instrumental quality control 2: Instrumental O2 background.** Demo-experiment using the TIP2k	O2 background TIP2k User Manual
10:00	*Coffee / Tea*	
10:30-12:00	**Hands-on: O2 background test,** with the TIP2k or manually**.** - for isolated mitochondria and cells in the range from air saturation to zero oxygen concentration; - for permeabilized muscle fibres in the high-oxygen range of	

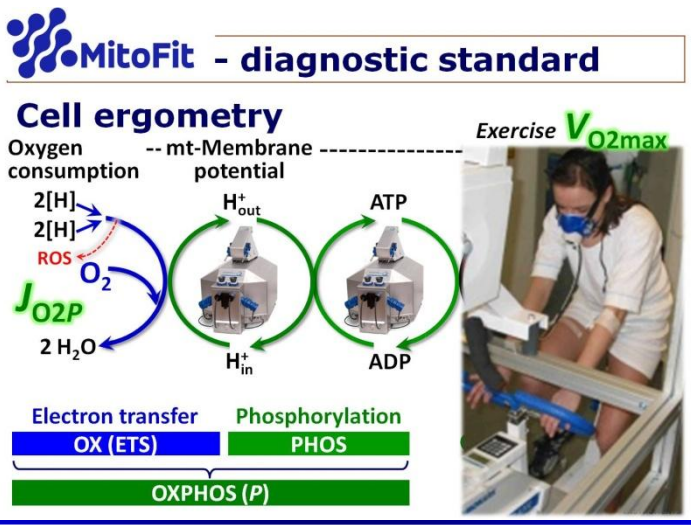
	500 – 200 µM.	
12:00	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:00	**Carsten Lundby (CH): Exercise and mitochondrial function.**	
15:00-16:00	**DatLab analysis: hands-on in teams**	DatLab Flux Analysis
16:00	*Coffee / Tea*	
16:30-17:15	**DatLab analysis: summary discussion**	
17:15-18:00	**OXPHOS analysis: diagnosis of respiratory defects**	
18:30	*Dinner*	
20:00	*Feedback discussion: Next steps in individual projects*	

5 Friday, Oct 07

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Coupling control protocol for intact cells in 7 O2ks** Advanced groups: CCP for intact cells with measurement of H_2O_2	
10:00	*Coffee / Tea*	MiPNet18.10 O2kvsMultiwell*
10:30-12:00	**Data analysis**	The Blue Book pp 43-57*
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum - guided tour and reception: € 15.-*	Alpmuseum
15:30	*Coffee / Tea*	
16:00-17:00	**Working groups: elaborate answers to the 'Questions for the O2k-Workshop'** - *come prepared*	IOC-Questions*
17:00-17:45	**IOC-questions - discussion of 'Answers'; O2k-technical service and the MitoFit proficiency test**	O2k-Technical support
17:50-18:45	**The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science:** beyond the O2k-Network to MITOEAGLE	O2k-Network www.bioblast.at
19:00	*Dinner & Farewell party*	

6 Saturday, Oct 08

	Departure
06:30-7:30	*Breakfast*
08:15	**Departure from Hotel Körbersee**
09:00	**Bus departure at Hochtannberg (Salober)**



Participants

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

MiPNet21.10 Abstracts IOC115: 10+5 min

O2k perspectives

1. Wasilewski M, Mohanraj K, Chacinska A (2016) MitoRUSH, microscopy approach to mitochondrial protein import in mammalian cells. Mitochondr Physiol Network 21.10.

The majority of mitochondrial proteins is encoded by nuclear DNA and synthesized in the cytosol, thus import machineries are required to allow proper localization of proteins into multiple subcompartments of mitochondria. There are five major import pathways, which specialize in the import of different classes of proteins. Proteins of the mitochondrial matrix as well as many proteins of the inner mitochondrial membrane, which are characterized by an N terminal signal known as a presequence, are imported on the presequence translocase of the inner membrane (TIM23) pathway. Most of our knowledge about TIM23 pathway stems from yeast were the main components and mechanisms governing TIM23 were characterized. Despite a high level of conservation of import machineries between yeast and Metazoa our understanding of TIM23 pathway in mammals is still rudimental. For example mammalian genomes encode for additional

isoforms of some key subunits of TIM23 complex like TIM17A and B, which are both expressed ubiquitously but form separate sub-populations of the TIM23.

The progress in our understanding of mitochondrial protein import in mammalian cells is hindered by the lack of proper tools, which could substitute for in *organello* import strategy successful in yeast studies where large scale mitochondria isolation is feasible. In contrast studying mammalian cells often allows only limited amount of material. We propose a novel, microscopy-based approach named mitoRUSH (retention using selective hooks), which allows observation of mitochondrial import in living mammalian cells. MitoRUSH is based on expression of two fusion proteins called a "hook" and a "reporter". The hook contains a transmembrane domain targeted to the ER membrane and a streptavidin domain exposed to the cytoplasm. The reporter consists of streptavidin binding peptide, a presequence and a fluorescent tag. Expressed together these proteins interact through streptavidin and a streptavidin binding peptide, which leads to accumulation of the reporter on the ER membrane. The reporter can be synchronously released from the hook by biotin applied to the cells and its translocation to mitochondria can be followed in a confocal microscope. We propose that this method can be applied to study mitochondrial protein import along the TIM23 pathway *in vivo*.

2. Bundgård AG, James AM, MP, Fago A (2016) Does nitrite protect the turtle heart from oxidative damage in the spring? Mitochondr Physiol Network 21.10.

Nitrite protects the heart from toxic oxygen radicals when oxygen returns after a period of oxygen deprivation, such as after heart attack or infarct. During anoxia, nitrite can inhibit inactive proteins, such as complex I in the electron transport chain, by a post-translational modification termed S-nitrosation, where an NO-moiety binds protein cysteines. Inhibition of complex I have been shown to limit the production of oxygen radicals, thereby protecting the heart from oxidative damage.

Some extreme animals, such as the red-eared slider turtle, survive the winter at the bottom of frozen ponds, and remain completely deprived of oxygen for several months. Unlike mammals, these turtles are not maimed by reoxygenation, but wake up in the spring with healthy hearts. Nitrite is naturally accumulated in the hearts of these animals during anoxia, which might protect them from reperfusion damage.

In this study, we investigate the protective effects of nitrite on the turtle heart. *In vitro* studies on isolated mitochondria have shown that the artificial S-nitrosating agent MitoSNO S-nitrosates turtle complex I which decreases activity of the enzyme and reduces ROS production upon reoxygenation, but does not affect respiration rate. Further, we have shown that succinate is accumulated in the anoxic turtle heart, which has been shown in mice to fuel the ROS production that occurs upon reoxygenation. This would corroborate the need for inhibition of complex I in the turtle.

We further wish to investigate whether the accumulated nitrite in the anoxic turtle mimics the protective effect of S-nitrosation *in vitro* and whether this is involved in keeping the turtle heart healthy upon awakening in the spring after a long, oxygen-deprived winter. Using extreme animals such as the turtle as models for coping with extreme situations like oxygen deprivation might teach us how to protect the more sensitive human heart.

3. Bird M (Team at UZ Leuven/KU Leuven, Belgium) (2016) Test of High Resolution Respirometry for the diagnosis of mitochondrial disease.

Using fresh (and frozen?) patient: muscle, liver and fibroblasts, we aim to.

- Establish reference ranges
- Validate the technique in biopsies and cultured fibroblasts from patients with a confirmed inherited mitochondrial disorder and patients with non alcoholic liver steatosis AND
- Prospectively validate the assay in 150 patients suspected of a mitochondrial disorder

In parallel, we will test these samples for mitochondrial function using established protocols to measure ATP production, and respiratory chain enzyme activities, such that we can compare the diagnostic capabilities of these parallel diagnostic tools.

Accommodation and location

Hotel Körbersee www.koerbersee.at
T +43 5519 265 hotel@koerbersee.at

More detail?

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck: 80 pp. » Full text in Bioblast

O2k-Manual – http://wiki.oroboros.at/index.php/O2k-Manual
O2k-Protocols – http://wiki.oroboros.at/index.php/O2k-Protocols
>1,700 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

Acknowledgements

Programme prepared for printing by E Gnaiger, C Doerrier, V Laner and V Erhart, OROBOROS INSTRUMENTS.

Contribution to K-Regio project MitoFit.
The project MitoFit is funded by the Land Tirol within the program K-Regio of Standortagentur Tirol.
www.mitofit.org

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Mitochondria and cell research

NextGen-O2k
A project supported by the "Technologieförderungsprogramm Tiroler Innovationsförderung" of the Tyrolean Government.

O2k-Workshops are listed as MitoGlobal Events

COST Action CA15203 Mitochondrial fitness mapping

MITOEAGLE: Evolution - **A**ge - **G**ender - **L**ifestyle - **E**nvironment

The MITOEAGLE Network aims at:

- Improving our knowledge on mitochondrial function in health and disease with regard to **E**volution, **A**ge, **G**ender, **L**ifestyle and **E**nvironment
- Interrelating results of studies performed world-wide with the help of a MITOEAGLE data management system
- Providing standardized measures to link mitochondrial and physiological per-formance to understand the myriad of factors that play a role in mitochondrial physiology

Join the COST Action MITOEAGLE and contribute to the quality management network: http://www.mitoglobal.org/index.php/MITOEAGLE

MitoFit in health and protective medicine

MitoFit develops novel laboratory standards and diagnostic monitoring of a mitochondrial fitness score. MitoFit provides a signature for high-end health tourism, introducing a scientific perspective on the benefits of mitochondrial fitness.

The O2k-Core and O2k-Fluorometer represent the gold standard for generating reliable quantitative respirometric data to develop the MitoFit Knowledge Management Platform (KMP) and MitoFit database.

- **Reference sample of cryopreserved mitochondria:** The availability of a reference sample for respirometry will provide enormous benefits for scientific research and open up new perspectives on clinical applications. Its use enables a new level of quality control in respiratory studies to be attained.
- **MitoFit proficiency test:** A ring test allows evaluation of the proficiency of a laboratory by measuring respiration of reference samples at pre-defined times and following standard experimental protocols. Reporting the reproducibility of measurements is a quality control for the evaluation of compliance with defined standard requirements.
- **MitoFit test on human blood cells:** Tissue biopsy for the study of mitochondrial function is a practical but invasive approach. Measurement of mitochondrial performance in human blood cells allows a non-invasive sampling procedure, enabling collection and cryopreservation of samples for later measurement and analysis. This will widen the applicability of respirometry for the study of human physiology immensly, permitting routine screening and repeated monitoring of the MitoFit score.

More details? » www.mitofit.org