Oroboros O2k-Workshop

Mitochondrial Physiology Network 22.07(04):1-12 (2017)
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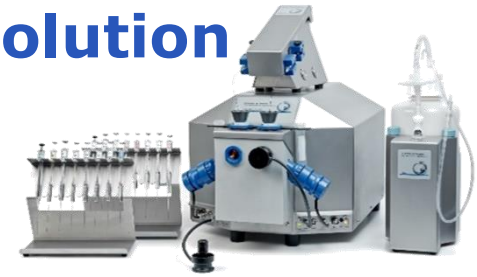
124th International Workshop on High-Resolution FluoRespirometry



2017 October 03-08
Schröcken, Vorarlberg, Austria

The **124th Workshop on High-Resolution FluoRespirometry (HRFR)** is the **38th** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **O2k-FluorRespirometer**, 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 and shipped 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 HRFR and will be put to the practical test in teams using seven O2k (14 chambers). The **O2k-MultiSensor** and particularly O2k-Fluorometry have 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

Chang Shao-Chiang	PhD student Marie-Curie Project 'TRACT', Oroboros Instruments
Gnaiger Erich	CEO, Oroboros Instruments and Medical University of Innsbruck
Komlódi Tímea	Scientific Researcher, Oroboros Instruments
Laner Verena	Chief Operating Officer, Oroboros Instruments
Meszaros Andras	Postdoctoral researcher, Oroboros Instruments
Passrugger Manuela	Biomedical Assistant, Oroboros Instruments
Velika Beata	Pavol Jozef Šafárik University in Kosice, RS

Programme

1 Tuesday, Oct 03

*printed in workshop materials

	Arrival	Weblink
15:00	**Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; 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 Wednesday, Oct 04

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
08:30-09:30	**Challenges of innovation and continuation: transition to O2k-Series H and DatLab 7** O2k instrumental setup – overview with video clips		O2k-Videosupport
09:30-11:30	**Hands-on (10 groups)**		
	O2k instrumental setup	**OroboPOS service**	O2k-Start
09:30-10:15	Groups 1-5	Groups 6-10	POS Service
10:15	*Coffee / Tea*		
	O2k instrumental setup	**OroboPOS service**	POS Service
10:45-11:30	Groups 6-10	Groups 1-5	O2k-Start
11:30-12:30	**Oxygen calibration (instrumental quality control 1)** DL-Protocol O2-calibration air		Gnaiger 2008 POS SOP: O2-calibration
12:30	*Lunch packages/ Walk & Talk* *Alternative:* individual O2k-tasks		
14:30-15:30	**Cell respiration and simultaneous measurement of H_2O_2 production** (Demo-Experiment)		O_2-Flux Analysis
15:30	*Coffee / Tea*		
16:00-18:00	**Hands-on (7 groups): Oxygen calibration and cell respiration** Cell respiration and simultaneous measurement of H_2O_2 production.		MiPNet15.09 Yeast reference assay
18:30	*Dinner*		
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats *Bring your laptop for DL7 installation.*		POS-Calibration-SOP O2 background

3 Thursday, Oct 05

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Experimental design:** Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
10:00	*Coffee / Tea*	
10:30-11:30	**O2k-Demo experiment**: Respiration of permeabilized cells: Measurement of oxygen consumption (Oroboros O2k) 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-Proficiency test** Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 7 Power-O2k	SUIT Reference Protocols
16:00	*Coffee / Tea*	
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	**DatLab analysis: hands-on in teams** Analysis of the hands-on experiment with permeabilized cells.	DatLab Flux Analysis MitoPedia: DatLab
19:00	*Dinner + registration for the walk to the Alpmuseum*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts 1-3	

4 Friday, 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	**Introduction to instrumental O2 background** (Demo-Experiment), using the TIP2k DL-Protocol: Instrumental O2 background_TIP2k.	SOP: O2 background TIP2k manual
10:00	*Coffee / Tea*	
10:30-12:00	**Hands-on (7 groups) - Instrumental quality control 2** O2 background test with the TIP2k; analysis of oxygen flux.	SOP: QC2
12:00	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:00	**Tutorial on the Bioblast wiki** www.bioblast.at	O2k-Network www.bioblast.at
15:00-16:00	**DatLab analysis: hands-on** *You can practice DL7 on your own laptop – install before* Collect results of background tests and RP1/RP2 for database and publication	DatLab Flux Analysis
16:00	*Coffee / Tea*	
16:30-17:15	**DatLab analysis: summary discussion**	
17:15-18:00	**SUIT protocols**	MitoPedia: SUIT
18:30	*Dinner*	
20:00	**Option for discussing specific topics of your projects**	

5 Saturday, Oct 07

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Hands-on (7 groups): Coupling control protocol for intact cells in 7 O2ks** CCP for intact cells with measurement of H_2O_2.	Coupling control protocol
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', introduction to O2k-technical support**	O2k-technical support
17:50-18:45	**OXPHOS analysis: diagnosis of respiratory defects**	
19:00	*Dinner*	
20:20	*Summary of experiments for database and manuscript; feedback discussion; IOC party*	

6 Sunday, 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.

Participants

Participant	Institution
Bailey Stephen*	**UK Loughborough Bailey S:** Loughborough University
Bastos Sant'Anna Silva Ana Carolina	**AT Innsbruck Oroboros:** Oroboros Instruments, Innsbruck
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Fernandez-Oriz Marisol*	**ES Granada Acuna-Castroviejo D:** University of Granada
Gumpp Anja****	**DE Ulm Karabatsiakis A:** Clinical & Biological Psychology Ulm University
Hugh Guan	**CN Shanghai Oroboros Scientific China:** Oroboros Instruments, Shanghai
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Martins Luis*	**UK London Carling D:** MRC London Institute of Medical Sciences
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Petit Michele*	**AT Innsbruck Jansen-Duerr P:** University of Innsbruck
Pranger Florian	**AT Innsbruck Oroboros**: Oroboros Instruments, Innsbruck
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Touron Julianne*	**FR Clermont-Ferrand Costes F:** CRNH Auvergne
Virding Susanne*	**SE Stockholm Freyer C:** Karolinska Institute, Stockholm
Woyda-Ploszczyca Andrzej*	**PL Poznan Jarmuszkiewicz W:** Adam Mickiewicz University, Poznan

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

MiPNet22.01 Abstracts IOC124: 10+5 min

O2k perspectives

1. **Agnieszka Koziel, Karolina Ogrodna, Wieslawa Jarmuszkiewicz (2017) The influence of chronic exposure to hypoxia on mitochondrial oxidative metabolism in human endothelial EA.hy926 cell. Mitochondr Physiol Network 22.07.**

   Endothelium is a monolayer of cells lining each blood vessel. Disturbances of endothelial functions are implicated in the development of many cardiovascular diseases. In endothelial cells, the ATP synthesis occurs in a major part via a glycolytic pathway. The slight dependence of endothelial cells on mitochondrial oxidative phosphorylation could suggest that mitochondria play no significant role in these cells. The general goal of our research is to study the aerobic metabolism of endothelial cells under physiological and pathophysiological conditions. The goal of this study was to determine mitochondrial respiratory function in endothelial cells and isolated mitochondria and assess the influence of chronic hypoxia on the aerobic metabolism of these cells. Human umbilical vein endothelial cells (EA.hy926 cell line) were cultured for 6 days at 20% and 1% O2 concentrations.

   In endothelial cells, chronic hypoxia provoked a shift from aerobic toward anaerobic catabolic metabolism. Growth of endothelial cells at 1% O2 tension did not change endothelial cells viability and mitochondrial biogenesis. However, elevated fermentation was observed. Under hypoxic conditions, mitochondrial respiration during carbohydrate, fatty acid and amino acid oxidation were declined, but ketogenic amino acid oxidation were increased. Hypoxia led to increased reactive oxygen species (ROS) generation (intracellular and mitochondrial), although antioxidant defence (superoxide dismutases SOD1 and SOD2, and uncoupling proteins, UCPs) were not escalated. Moreover, decreased UCP2 activity and expression were observed. Furthermore, in

mitochondria from hypoxic cells, increased expression and activity of complex II, and decreased expression and activity of complex I were observed. The increased activity of complex II resulted in an elevation in succinate-sustained mitochondrial ROS generation, mainly through increased reverse electron transport.
Chronic exposure to hypoxia lead to numerous changes in aerobic metabolism of endothelial cells. Endothelial mitochondria play a significant role in response to metabolic adaptation related to hypoxia. Our results point out an important role of succinate, complex II, and reverse electron transport in hypoxia adaptation of endothelial cells. Mitochondrial ROS could play significant signaling role in endothelial cells under hypoxic conditions.

2. **Woyda-Ploszczyca A.M. and Jarmuszkiewicz W (2017) GDP-induced oxidative phosphorylation alleviates the GDP-dependent inhibitory effect on mitochondrial proton leak. Mitochondr Physiol Network 22.07.**
The purine nucleotide GDP is commonly used as a diagnostic inhibitor of mitochondrial uncoupling proteins (UCPs). UCPs belong to the mitochondrial carrier family and play the role of main catalysts of futile proton leak of the proton electrochemical gradient generated by the respiratory chain. However, we found that a high concentration of GDP (1 mM), but not GTP, stimulates respiratory rate and decreases membrane potential of mammalian (rat kidney and human endothelial cells) [Woyda-Ploszczyca and Jarmuszkiewicz, 2014], plant (potato tubers), as well as unicellular eukaryote (amoeba) isolated mitochondria [Woyda-Ploszczyca and Jarmuszkiewicz, 2017]. Therefore, we have observed exactly an opposite effect than that expected for the UCP-mediated H+ leak inhibition where an oxygen consumption rate inhibition is accompanied by an increase in mitochondrial membrane potential. We obtained the GDP stimulatory effect both in the absence and presence of exogenous free fatty acids (linoleic acid) considered strong positive effectors of UCP homologues. Interestingly, under conditions excluding oxidative phosphorylation, i.e. in the presence of carboxyatractyloside and/or oligomycin, the GDP stimulatory effect was completely abolished. We propose that the GDP-induced stimulatory effect depends on nucleoside diphosphate kinase (NDPK) activity. This enzyme transphosphorylates GDP in the presence of ATP and generates ADP pool (ATP + GDP → ADP + GTP) what subsequently induces oxidative phosphorylation. Our results indicate that the GDP-dependent inhibition of UCPs could have a minor physiological significance and consequently GDP should not be used as a diagnostic inhibitor of UCPs.

3. **Miljenko Panajatovic, François Singh, Stephan Krähenbühl, and Jamal Bouitbir (2017) Role of PGC-1α associated mitochondrial biogenesis in statin-induced myotoxicity. Mitochondr Physiol Network 22.07.**
At the request of the authors, this abstract is not made available online.

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
>2,200 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications: Topics

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Mitochondria and cell research

O2k-Workshops are listed as MitoGlobal Events