OROBOROS O2k-Workshops

Mitochondrial Physiology Network 22.01(01):1-8 (2017)
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122nd International Workshop on HRR and O2k-Fluorometry and TRACT training course

2017 June 26 - July 01
Schröcken, Vorarlberg, Austria

The **122nd Workshop on High-Resolution Respirometry (HRR)** is the **37th** 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 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 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.

TRACT

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
Doerrier-Velasco Carolina	CSO, OROBOROS INSTRUMENTS
Bufe Anja	Marie Sklodowska-Curie PhD student in TRACT, OROBOROS INSTRUMENTS
Velika Beata	Pavol Jozef Šafárik University in Kosice, Republic Slovakia
Wohlfarter Yvonne	Trainee

Programme

1 Monday, Jun 26

*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
19:30	*Dinner*	

2 Tuesday, Jun 27

	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, Jun 28

	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*	
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*	
20:30-21:00	**O2k perspectives:** 10+5 min presentations of abstracts	
21:00	*Registration for the walk to the Alpmuseum*	

4 Thursday, Jun 29

	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	O2 background TIP2k User Manual
10:00	*Coffee / Tea*	
10:30-12:00	**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.	
12:00	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
14:30-15:00	**Bufe Anja: a tutorial for using the wiki-based website** www.bioblast.at	
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 the individual projects*

5 Friday, Jun 30

	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', Introduction to 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*	

6 Saturday, Jul 01

	Departure
06:30-7:30	*Breakfast*
	Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9.00 am at Salober.

Participants – *in preparation*

Participant	Institution
Abdul Karim Norwahidah*	**MY Kuala Lumpur Abdul Karim N:** National University of Malaysia, Kuala Lumpur (MY)
Corbet Cyril	**BE Brussels Feron O:** University of Louvain, Brussels (BE)
Doleželová Eva*	**CZ Ceske Budejovice Zikova A:** Biology Center, Ceske Budejovice (CZ)
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Han Woo Hyun**	**CA Edmonton Lemieux H:** University of Alberta, Edmonton (CA)
Jönsson Sofia***	**SE Uppsala Liss P:** Uppsala University (SE)
Karavyraki Marilena*	**IE Dublin Porter RK:** Trinity College Dublin (IE)
Lerfall Jørgen *	**NO Trondheim Barstad T:** Norwegian University of Science and Technology, Trondheim (NO)
Lopes de Carvalho Carla***	**SE Uppsala Liss P:** Uppsala University (SE)
Magnano Stephania*	**IE Dublin Porter RK:** Trinity College Dublin (IE)
Mukai Kazutaka	**JP Shimotsuke Mukai K:** Japan Racing Association, Shimotsuke (JP)
Newsom Sean**	**US OR Corvallis Robinson MM:** Oregon State University, Corvallis (US)
Robinson Matt**	**US OR Corvallis Robinson MM:** Oregon State University, Corvallis (US)
Skolik Robert**	**US KY Louisville Menze MA:** University of Louisville (US)
Truu Laura****	**EE Tallinn Kaambre T:** National Institute of Chemical Physics and Biophysics, Tallinn (EE)
Wack Gesine	Goethe University Frankfurt (DE)
Zakaria Fazaine*	**MY Kuala Lumpur Abdul Karim N:** National University of Malaysia, Kuala Lumpur (MY)

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

MiPNet22.01 Abstracts IOC122: 10+5 min

O2k perspectives

In preparation

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
>2,124 O2k-Publications – http://wiki.oroboros.at/index.php/O2k-Publications:_Topics

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www.mitofit.org

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Mitochondria and cell research

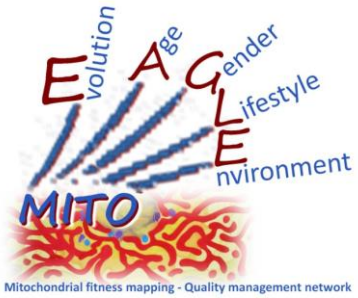
O2k-Workshops are listed as MitoGlobal Events

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 detail? » www.mitofit.org