

122nd International Workshop on HRR and O2k-Fluorometry and TRACT training course

2017 June 26 - July 01
Schröcken, Vorarlberg, Austria



TRACT

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.

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
Dikova Valentina	Marie Sklodowska-Curie PhD student in TRACT, University of Valencia
Velika Beata	Pavol Jozef Šafárik University in Kosice, Republic Slovakia

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 <i>Welcome reception at Hotel Körbersee & get-together:</i> Introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	Schroecken
19:30 <i>Dinner</i>	

2 Tuesday, Jun 27

Workshop 1	Weblink
07:30-08:30 <i>Breakfast</i>	
08:30-09:30 O2k instrumental setup – overview with video clips	O2k-Manual
09:30-11:30 Hands-on (10 groups) O2k instrumental setup	
09:30-10:15 Groups 1-5	OroboPOS service
10:15 <i>Coffee / Tea</i>	
10:45-11:30 Groups 6-10	O2k-Start
11:30-12:30 Applications of the O2k: DatLab guide through the menus	POS Service
12:30 <i>Lunch packages/ Walk & Talk alternative: individual O2k-tasks</i>	Gnaiger 2008 POS O2k-Calibration
14:30-15:30 Oxygen calibration (instrumental quality control 1) and cell respiration (Demo-Experiment)	O₂-Flux Analysis
15:30 <i>Coffee / Tea</i>	

16:00-18:00	Hands-on (7 groups): Oxygen calibration and cell respiration Advanced groups: Cell respiration and simultaneous measurement of H ₂ O ₂ production.	Yeast reference assay
18:30	<i>Dinner</i>	
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	<i>Breakfast</i>	
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	<i>Coffee / Tea</i>	
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	<i>Lunch packages / Walk & Talk</i> <i>alternative: individual O2k-tasks</i>	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	<i>Coffee / Tea</i>	
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	<i>Dinner</i>	
20:30-21:00	O2k perspectives: 10+5 min presentations of abstracts	
21:00	<i>Registration for the walk to the Alpmuseum</i>	

4 Thursday, Jun 29

Workshop 3		Weblink
07:30-08:30	<i>Breakfast</i>	
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	<i>Coffee / Tea</i>	
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	<i>Lunch packages / walk & talk</i> <i>alternative: individual O2k-tasks</i>	
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	<i>Coffee / Tea</i>	
16:30-17:15	DatLab analysis: summary discussion	
17:15-18:00	OXPHOS analysis: diagnosis of respiratory defects	
18:30	<i>Dinner</i>	
20:00	<i>Feedback discussion: Next steps in the individual projects</i>	

5 Friday, Jun 30

Workshop 4		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-10:00	Coupling control protocol for intact cells in 7 O2ks Advanced groups: CCP for intact cells with measurement of H ₂ O ₂	
10:00	<i>Coffee / Tea</i>	MiPNet18.10 O2kvsMultiwell*
10:30-12:00	Data analysis	The Blue Book* pp 43-57
12:00	<i>Lunch packages</i>	
12:30-15:30	<i>Walk to the Alpmuseum - guided tour and reception: € 15.-</i>	Alpmuseum*
15:30	<i>Coffee / Tea</i>	
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	<i>Dinner</i>	

6 Saturday, Jul 01

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

Participants – in preparation

Participant	Institution

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

**MiPNet22.01 Abstracts IOC121: 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.orooboros.at/index.php/O2k-Manual>

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

>2,124 O2k-Publications – <http://wiki.orooboros.at/index.php/O2k-Publications: Topics>

Acknowledgements

Programme prepared for printing by M Beno, 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



This event is part of training in the Marie Skłodowska-Curie project TRACT 721906. H2020-MCSA-ITN 2016.



Contact

Erich Gnaiger, PhD
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria
www.mitofit.org

OROBOROS INSTRUMENTS
Schöpfstrasse 18
A-6020 Innsbruck, Austria
T +43 512 566796 F +43 512 566796 20
instruments@orooboros.at | www.orooboros.at
Mitochondria and cell research

O2k-Workshops are listed as [MitoGlobal Events](#)



COST Action CA15203 Mitochondrial fitness mapping

MITOEAGLE: Evolution - Age - Gender - Lifestyle - Environment

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 performance 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 immensely, permitting routine screening and repeated monitoring of the MitoFit score.

More detail? » www.mitofit.org