



Course on High-Resolution Respirometry

IOC100. Mitochondrial Physiology Network 20.01(01): 1-8 (2015)
Updates: http://wiki.orooboros.at/index.php/MiPNet20.01_IOC100

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100th International Workshop on HRR and O2k- Fluorometry

2015 April 09-14
Schröcken, Vorarlberg, Austria



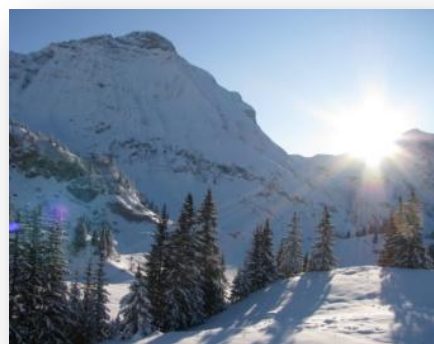
The **100th Workshop on High-Resolution Respirometry (HRR)** is the **33th** International Oxygraph Course held in Schroecken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluo LED2-Module**, with real-time analysis by **DatLab** and applications of the **TIP2k**. Demo experiments illustrate the principle and show the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, hydrogen peroxide production or mt-membrane potential. Yeast cells are used as a biological reference material which can be obtained worldwide as freeze dried samples.

Instrumental setup and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. In the evenings, general mitochondrial topics are 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 six O2k (12 chambers). **O2k-MultiSensor** and particularly O2k-Fluorometry has become an integral part of the O2k-Workshop. Optimization of protocol design for

various 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 skiing or walks and talks, enjoying the refreshing scenery of the secluded alpine environment, offer a visit to the Alpmuseum, or give sufficient spare time for individual practice.



Lecturers and tutors

Doerrier Carolina	Post-doctoral scientist, OROBOROS INSTRUMENTS
Garcia-Roves Pablo M	MiPNet Lab: ES Barcelona Garcia-Roves PM
Gnaiger Erich	CEO, OROBOROS INSTRUMENTS
Hickey Anthony J	MiPNet Lab: NZ Auckland Hickey AJ
Laner Verena	Chief Operating Officer (COO), OROBOROS INSTRUMENTS
Sumbalova Zuzana	MiPNet Lab: SK Bratislava Sumbalova Z

Programme

1 Thursday, Apr 09

*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). Transfer/walk to Hotel Körbersee	IOC-travel
18:30	<i>Welcome reception at Hotel Körbersee</i>	Schroecken
19:00	<i>Dinner</i>	
20:30-21:15	Get-together: introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	IOC100*

2 Friday, Apr 10

	Workshop 1	Weblink
07:30-08:30	<i>Breakfast</i>	
08:00	<i>Organize loan of skiing equipment</i>	
	Principles of high-resolution respirometry and O2k-Fluorometry - from switching on the Oxygraph-2k to the experimental result	Gnaiger 2008 POS*
08:30-09:15	Get O2k-Connected with OROBOROS: a guided tour to the Oxygraph-2k	get O2k-Connected
09:15-12:00	Hands-on (6 groups) introduction to DatLab: DL Installation, DL-Demo files and DL-Excel templates	DatLab Guide DatLab Flux Analysis
12:00	<i>Practice: skiing / walk & talk / lunch packages/ or bad weather task groups; alternative: individual O2k-tasks</i>	
15:00-16:00	Hands-on (10 groups) O2k instrumental setup: OroboPOS service, chamber assembly	O2k-Start POS Service
16:00	<i>Coffee / Tea</i>	
16:30-18:00	Hands-on (6 groups) Getting started with an O2k experiment 1: washing procedures, stirrer test, air calibration	DatLab Guide
18:30	<i>Dinner</i>	
20:00-21:00	Hot MiP-Topics 1: 10+5 min presentations of abstracts 1-X	IOC100 Abstracts

3 Saturday, Apr 11

Workshop 2		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:15	Tissue homogenate preparation: the PBI-Shredder and general discussion of sample preparation	MiPNet17.03 Shredder vs Fibres*
09:15-10:00	Hands-on (6 groups) getting started with an O2k experiment 2: washing, stirrer test, air calibration	O2k-Start
10:00	<i>Practice: skiing / walk & talk / lunch packages / or bad weather task groups; alternative: individual O2k-tasks</i>	
14:00	<i>Coffee / Tea</i>	
14:30-16:00	Hands-on (6 groups) O2k-experiment with cell lines: SUIT protocol with HEK 293T cells and real-time DatLab analysis	Pesta 2012 Methods Mol Biol
16:00	<i>Coffee / Tea</i>	MiPNet18.10 O2kvsMultiwell*
16:30-17:45	Hands-on: SUIT experiment continued with DatLab Analysis and Excel templates	DatLab Flux Analysis
17:45-18:30	Experimental design 1: Substrate and coupling control of mitochondrial respiration - MitoPathways through CI and CII	The Blue Book*
18:30	<i>Dinner</i>	
20:00-21:15	Hot MiPNet-Topics 2: 10+5 min presentations of abstracts X-Y	IOC100 Abstracts

4 Sunday, Apr 12

Workshop 3		Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:15	Experimental design 2: Coupling control protocol with intact cells vs. mt-preparations: ROUTINE, OXPHOS, LEAK, ETS	Cells: PCP
09:15-10:00	DatLab Analysis: Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
10:00	<i>Practice: skiing / walk & talk / lunch packages; alternative: individual O2k-tasks</i>	
14:00	<i>Coffee / Tea</i>	
14:30-16:00	Hands-on (6 groups) Multisensor O2k-experiment 1: combined respirometry and fluorometric detection of mitochondrial membrane potential with TMRM and safranin using permeabilized HEK 293T cells	Krumnschnabel 2014 Methods Enzymol MiPNet19.19 Safranin Data Acquisition and Analysis
16:00	<i>Coffee / Tea</i>	
16:30-18:30	Hands-on (6 groups) Multisensor O2k-experiment 2: combined respirometry and fluorometric detection of H ₂ O ₂ production with Amplex Red using permeabilized HEK 293T cells	MiPNet19.20 Amplex Red Data Acquisition and Analysis
18:30	<i>Dinner</i>	
20:00-21:15	DatLab Analysis: diagnosis of respiratory defects	

5 Monday, Apr 13

Workshop 4		Weblink
07:30-08:30	Breakfast	
08:30-09:15	Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux	O2k-Calibration
09:15-10:00	Instrumental quality control 2: O2k-Background test with TIP2k; analysis of oxygen flux	O2k-Background
10:00	Coffee / Tea	-
10:30-12:00	Hands-on (6 groups): O2k-Background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 - 200 μM. O2k-Background with automatic TIP2k or manual titrations.	O2k-Background TIP2k User Manual
12:00	Lunch packages	-
12:30-15:30	Walk to the Alpmuseum: Guided tour and reception (15 €)	http://www.alpmuseum.at
16:00	Coffee / Tea	-
16:00-16:45	Working groups: Elaborate answers to the 'Questions for the O2k-Workshop' - come prepared*	IOC-Questions*
16:45-17:15	IOC-Questions - discussion of 'Answers'	-
17:15-18:00	Introduction to trouble shooting	O2k-Troubleshooting
18:00-18:45	The O2k-Workshop continues with the Bioblast wiki - in the spirit of Gentle Science	www.bioblast.at
19:00	Dinner	-
20:30-21:00	Panel Discussion - Feedback IOC100	O2k-Feedback
	Farewell party	-

6 Tuesday, Apr 14

Departure	
	Breakfast
	Early morning: Departure

Participants

Name	Lab

MiPNet20.01 Abstracts IOC100: 10+5 min

Hot topics in Mitochondrial Physiology

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. [»Open Access](#)

O2k-Manual – <http://wiki.oroboros.at/index.php/O2k-Manual>

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

>1,300 O2k-Publications – <http://wiki.oroboros.at/index.php/O2k-Publications: Topics>



Acknowledgements



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 Cooperation and Feedback in Science