



Course on High-Resolution Respirometry

IOC88. *Mitochondrial Physiology Network* 19.02: 1-8 (2014)
http://www.bioblast.at/index.php/IOC_2014-04_Schroecken

©2014 OROBOROS®
Version 1: 2014-03-28

88th International Workshop on HRR and O2k- Fluorometry

2014 April 07-12
Schroëcken, Vorarlberg, Austria



The **88th Workshop on High-Resolution Respirometry (HRR)** is the **31st** International Oxygraph Course held in Schroëcken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluorescence LED2-Module**, with real-time analysis by **DatLab** and applications of the **TIP2k**. A demo experiment illustrates the principle and shows the unique advantage of simultaneous monitoring of oxygen concentration, respiration and hydrogen peroxide production. Yeast cells are used as a biological reference material which can be obtained world-wide 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.

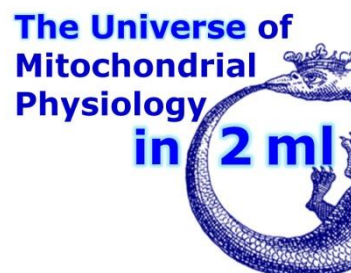
Lunch breaks provide an opportunity for relaxing walks and talks, enjoying the refreshing scenery of the secluded alpine environment, offer a visit to the Alpmuseum, or give sufficient spare time for individual practice.



Lecturers and tutors

[Gnaiger Erich](#)
[Laner Verena](#)

[Fontana-Ayoub Mona](#)
[Krumnschnabel Gerhard](#)



Programme

1 Monday, Apr 07

*printed in workshop materials

	Arrival	Weblink
15:00	Arrival in Bregenz: Meeting point Bregenz train station at 3pm; 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>	
20:30-21:00	Get-together: introduction of participants and their research interests - a welcome by OROBOROS INSTRUMENTS	IOC 2014-04 Schroecken

2 Tuesday, Apr 08

	Workshop 1	Weblink
07:30-08:30	<i>Breakfast</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
09:15-10:00	Introduction to a Demo experiment with the O2k	Pesta 2012 Methods Mol Biol*
10:00	<i>Coffee / Tea</i>	-
10:30-12:00	O2k-Demo experiment 1: Respiration of intact cells and on-line DatLab Analysis: Simultaneous measurement of oxygen consumption (O2k-Core) and H ₂ O ₂ production (O2k-Fluorescence LED2-Module)	MiPNet18.06 Amplex-Yeast*
12:00	<i>Lunch - Walk & Talk</i>	-
15:00-16:00	O2k instrumental setup and sensor service - overview	O2k-Manual
16:00	<i>Coffee / Tea</i>	
	O2k instrumental setup	OroboPOS service
16:30-17:15	Groups 1-5	Groups 6-10
17:15-18:00	Groups 6-10	Groups 1-5
18:30	<i>Dinner</i>	
20:00-21:00	Protocol design: mt-prep - tissues, limitations imposed by fluorometry - controls parallel to fluorometry	MiPNet18.05 Amplex-Mouse-heart

3 Wednesday, Apr 09

Workshop 2		Weblink
07:30-08:30	Breakfast	
08:30-09:15	Experimental design 1: Coupling control protocol with intact cells: ROUTINE, LEAK, ETS, ROX	Cells: PCP
09:15-10:00	Experimental design 2: Substrate and coupling control of mitochondrial respiration - MitoPathways through CI+II	The Blue Book* pp 29-43
10:00	Coffee / Tea	
10:30-12:00	SUIT protocol with DatLab Analysis and guide through Excel templates	DatLab Flux Analysis
12:00	Lunch	The Blue Book p 56*
15:00-16:00	Tissue homogenate preparation: The PBI-Shredder	MiPNet17.03 Shredder vs Fibres*
16:00	Coffee / Tea	
16:30-18:00	O2k-Demo experiment 2: Respiration with tissue homogenate: SUIT protocol, oxygen regime, washing	Krumshnabel 2013 Abstract MiP2013: 26-27*
18:30	Dinner	
20:00-21:00	Hot MiP-Topics: 10+5 min presentations of abstracts by participants	IOC 2014-04 Schroecken Abstracts MiPNet 19.02

4 Thursday, Apr 10

Workshop 3		Weblink
07:30-08:30	Breakfast	
08:30-09:15	DatLab O₂ flux analysis: Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
09:15-10:00	DatLab Guide through the menus: DL-Demo files and DL-Excel templates	DatLab Guide
10:00	Coffee / Tea	
10:30-12:00	DatLab Analysis: hands-on in 10 teams	DatLab Flux Analysis
12:00	Lunch - Walk & Talk	-
15:00-16:00	Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.	O2k-Calibration
16:00	Coffee / Tea	MiPNet18.10 O2kvsMultiwell*
16:30-17:15	Instrumental quality control 2: O2k-Background test and on-line analysis of oxygen flux.	O2k-Background*

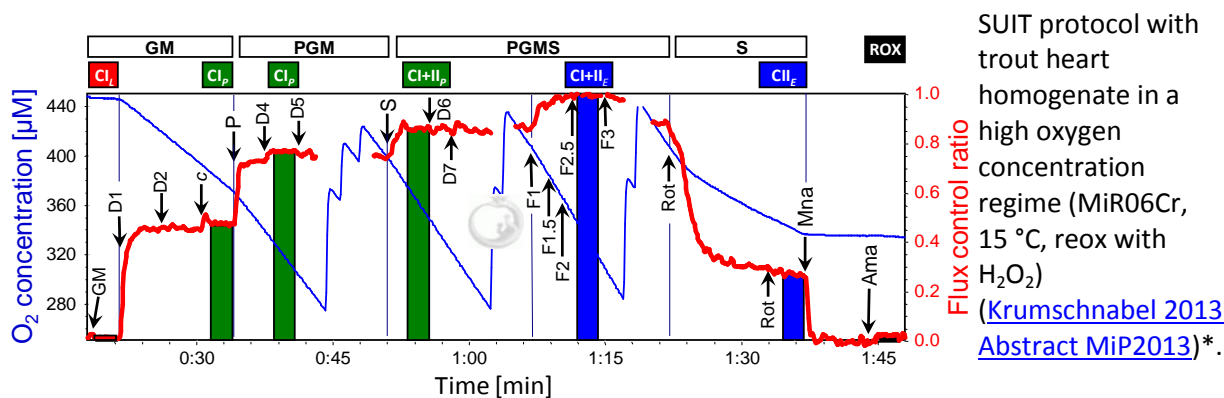
17:15-18:00	Hands-on (6 groups): O2k-Background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 - 200 μ M. O2k-Background with automatic TIP2k or manual titrations.	O2k-Background*
18:30	<i>Dinner</i>	
20:00-21:00	10x5-min team presentations: DatLab Analysis: results, what did we learn, what was difficult, open questions?	

5 Friday, Apr 11

	Workshop 4	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:15	O2k-MultiSensor overview and O2k-Fluorometry applications: Amplex™ red and safranin	MiPNet17.17 Amplex-Mouse-brain*
09:15-10:00	OXPPOS analysis: A challenge for the simultaneous measurement of respiration and mt-membrane potential	
10:00	<i>Coffee / Tea</i>	-
10:30-12:00	O2k-Demo experiment 3: Respiration and steady-state feedback control of oxygen levels with the TIP2k.	TIP2k User Manual
12:00	<i>Lunch packages</i>	-
12:30-15:30	<i>Walk to the Alpmuseum: Guided tour and reception (15 €)</i>	http://www.alpmuseum.at
16:00	<i>Coffee / Tea</i>	-
16:00-16:45	Working groups: Elaborate answers to the 'Questions for the O2k-Workshop'	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	<i>Dinner</i>	-
20:30-21:00	Panel Discussion - Feedback IOC	O2k-Feedback
	Farewell party	-

6 Saturday, Apr 12

Departure	
	<i>Breakfast</i>
	Early morning: Departure



SUIT protocol with trout heart homogenate in a high oxygen concentration regime (MiR06Cr, 15 °C, reox with H₂O₂) (Krumshabel 2013 Abstract MiP2013)*.

Participants

Name	Lab
Agrimi Gennaro	IT_Bari_Agrimi G: Department of Biosciences, Biotechnology and Biopharmacology, University of Bari. - Carriers, metabolism, organic acids
Andersen Marianne	DK_Copenhagen_Treebak JT: Section on Integrative Physiology, Center for Basic Metabolic Research, University of Copenhagen
Bauer Christiane	AT_Innsbruck_OROBOROS INSTRUMENTS
Boeck Christina	DE_Ulm_Karabatsiakis A: Clinical and Biological Psychology, University of Ulm. - Psychopathological stress
Byro Melissa	US_PA Philadelphia_Margulies S: Department of Bioengineering, University of Pennsylvania, Philadelphia.
Calloway Jones Jessica	US_MA Cambridge_Vernochet C: Cardiovascular and Metabolic Diseases Research Unit, Pfizer Research Technology Center (RTC), Cambridge. - Obesity, Diabetes, Mitochondria, Metabolism
Cejvanovic Vanja	DK_Copenhagen_Poulsen HE: Laboratory of Clinical Pharmacology, Rigshospitalet, Copenhagen. - Oxidative stress, Hemochromatosis
Dhandapani Praveen Kumar	FI_Helsinki_Szibor M: Biomedicum, University of Helsinki. - alternative oxidase, ROS, respiratory chain complex
Davis Michael	US_OK Stillwater_Davis M: Center for Veterinary Health Sciences, Oklahoma State University, Stillwater.
Fischer Sylvia	DE_Frankfurt_Schmoll D: R&D TD Metabolism FFM, Sanofi-Aventis Deutschland GmbH, Frankfurt.
Fleischmann Sandra	AT_Innsbruck_OROBOROS INSTRUMENTS
Fontana-Ayoub Mona (tutor)	AT_Innsbruck_OROBOROS INSTRUMENTS
Gaustad Svein Erik	NO_Tromsoe_Gaustad SE: Institute of Clinical Medicine, Faculty of Medical Sciences, University of Tromso. - Hypothermia, Rewarming
Giordano Luca	IT_Bari_Cantatore P: Department of Biosciences, Biotechnology and Biopharmacology, University of Bari. - Mitochondrial dysfunctions, mtDNA mutations, LHON, DNMT1, ROS production, cross-talk nucleus-mitochondrion
Gnaiger Erich (Lecturer)	AT_Innsbruck_OROBOROS INSTRUMENTS: DSL, Dept Visceral, Transpl Thoracic Surgery, Medical University Innsbruck.
Hoppel Florian	AT_Innsbruck_OROBOROS INSTRUMENTS
Kachappilly Nicole	CH_Zurich_Gassmann M: Institute of Veterinary Physiology, Faculty of Medicine, University of Zurich.

Kjaer Hansen Lis	DK_Copenhagen_Poulsen HE: Laboratory of Clinical Pharmacology, Rigshospitalet, Copenhagen.
Krumschnabel Gerhard (tutor)	AT_Innsbruck_OROBOROS INSTRUMENTS
Laner Verena (tutor)	AT_Innsbruck_OROBOROS INSTRUMENTS
Legkun German	RU_Dolgoprudny_Motovilov KA: Department of General and Applied Physics, Moscow Institute of Physics and Technology, Dolgoprudny. - Membrane protons
Lehti Maarit	FI_Jyväskylä_Kainulainen H: Department of Biology of Physical Activity, University of Jyväskylä. - Exercise, mitochondria, skeletal muscle
Maedo Kati	EE_Tallinn_Kaambre T: National Institute of Chemical Physics and Biophysics, Tallinn. - Permeabilized cancer tissue
Marte Verena	AT_Innsbruck_OROBOROS INSTRUMENTS
Mendonça Ana Paula	BR_Rio de Janeiro_Oliveira MF: Institute of Medical Biochemistry, Federal University of Rio de Janeiro. - Oxidative stress, tumors, hemorrhage, brain
Menna Barreto Rubem	BR_Rio de Janeiro_Menna Barreto R: Laboratório de Biologia Celular, Instituto Oswaldo Cruz, Rio de Janeiro. - Parasitology, Parasitic diseases
Nair Syam	SE_Gothenburg_Hagberg H: Institute for Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg.
Nunes Patricia	UK_London_Anastasiou D: MRC National Institute for Medical Research, London.
Olsen Rolf Erik	NO_Matredal_Olsen RE: Institute of Marine Research, Matre Research Station, Matredal.
Pereira de Almeida Nogueira Natalia	BR_Rio de Janeiro_Paes MC: Department of Biochemistry, Institute of Biology, Rio de Janeiro State University. - ROS, Parasites, Mitochondria, Bioenergetics
Paes Marcia Cristina	BR_Rio de Janeiro_Paes MC: Department of Biochemistry, Institute of Biology, Rio de Janeiro State University. - ROS, Parasites, Mitochondria, Bioenergetics
Reynolds Merrick	US_UT Provo_Hancock C: Department of Nutrition, Dietetics & Food Science, Brigham Young University, Provo.
Wagenaars Jori	NL_Nijmegen_Koopman WJ: Department of Biochemistry, Radboud University Medical Centre, Nijmegen. - Mouse model, Skeletal muscle, Complex 1, mPTP
Weidinger Adelheid	AT_Vienna_Kozlov A: Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna.

MiPNet18.07 Abstracts IOC88: 10+5 min

Hot topics in Mitochondrial Physiology

Agrimi G, Mena MC, Izumi K, Pisano I, Germinario L, Fukuzaki H, Palmieri L, Blank LM, Kitagaki H (2014) **Increased mitochondrial pyruvate dissimilation in sake yeast.**

Andersen M, Vienberg SG, Novod JB, Brandauer J, Holst B, Treebak JT (2014) **Nicotinamide phosphoribosyltransferase knockdown impairs mitochondrial function in mouse myoblasts.**

- Dhandapani PK, Dufour E, Jacobs HT, Szibor M (2014) **Alternative oxidase-based therapy for heart failure.**
- Legkun G, Motovilov K (2014) **Investigation on alternative mechanism of electron transport in the respiratory system.**
- Maedo K, Kaldma A, Planken A, Klepinin A, Chekulayev V, Varikmaa M, Tepp K, Chevchuk I, Kaambre T (2014) **Regulation of mitochondrial respiration in human colorectal cancer cells in situ: the possible role of beta-tubulins.**
- Mendonca AP, Rodrigues MF, Amoedo ND, Oliveira MF (2014) **Effects of iron on mitochondrial physiology of central nervous system cell lines.**
- Nogueira NP, Baldow QCS, Costa DSS, Domingos J, Laranja GAT, Costa PRR, Dias AG, Paes MC (2014) **The effect of nitrones upon clinically relevant forms of *Trypanosoma cruzi*.**
- Paes MC, Saraiva FMS, Nogueira NP, Laranja GAT, Coelho MGP, Oliveira MF (2014) **Heme increases mitochondrial membrane potential and ROS production in *Trypanosoma cruzi* epimastigotes.**
- Wagenaars J, Willems PHGM, Koopman WJ (2014) **Oxygen consumption in OXPHOS-deficient cells.**

Accommodation and Location

Hotel Körbersee www.koerbersee.at

T +43 5519 265; hotel@koerbersee.at

More detail?

O2k-Manual – www.oroboros.at/?O2k-Manual

O2k-Protocols – www.oroboros.at/?O2k-Protocols

>1,000 O2k-Publications – www.bioblast.at/index.php/O2k-Publications



Recommended Reading

- Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58.
- Lemieux H, Semsroth S, Antretter H, Hoefler D, Gnaiger E (2011) Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart. *Int J Biochem Cell Biol* 43: 1729-1738.
- Pesta D, Hoppel F, Macek C, Messner H, Faulhaber M, Kobel C, Parson W, Burtscher M, Schocke M, Gnaiger E (2011) Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. *Am J Physiol Regul Integr Comp Physiol* 301: R1078-R1087.
- Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int J Biochem Cell Biol* 41: 1837-1845.
- Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dykens JA, Will Y, eds) John Wiley: 327-352. – A *methodological introduction into high-resolution respirometry*.

- Hütter E, Renner K, Pfister G, Stöckl P, Jansen-Dürr P, Gnaiger E (2004) Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. *Biochem J* 380: 919-928.
- Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir Physiol* 128: 277-297. – *A detailed introduction into high-resolution respirometry with particular emphasis on kinetics and measurements at low oxygen.*
- Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold* (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: 431-442. – *Isolated mitochondria and permeabilized muscle fibres, MiR05.*

Acknowledgements

Programme prepared for printing by V Marte, OROBOROS INSTRUMENTS Corp.



Contribution to K-Regio project *MitoCom Tyrol*, funded in part by the Tyrolian Government and the European Regional Development Fund (ERDF).

www.orooboros.at/?MitoCom-Tyrol



Contact

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

OROBOROS INSTRUMENTS Corp
 Schöpfstrasse 18
 A-6020 INNSBRUCK, Austria
 T +43 512 566796 F +43 512 566796 20
 Email instruments@orooboros.at
 Homepage: www.orooboros.at
 Cooperation and Feedback in Science

