



Course on High-Resolution Respirometry

IOC88. Mitochondrial Physiology Network 19.02(02): 1-8 (2014)
http://www.bioblast.at/index.php/MiPNet19.02_IOC_2014-04_Schroecken

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88th International Workshop on HRR and O2k-Fluorometry

2014 April 07-12
Schröcken, Vorarlberg, Austria



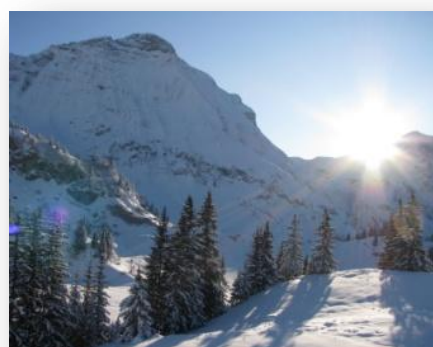
The **88th Workshop on High-Resolution Respirometry (HRR)** is the **31st** International Oxygraph Course held in Schroecken since 1988. A practical overview is provided of the **Oxygraph-2k and O2k-Fluo LED2-Module**, with real-time analysis by **DatLab** and applications of the **TIP2k**. Demo experiments 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 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.

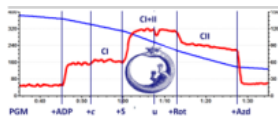
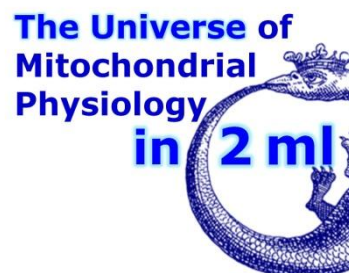
Lunch breaks provide an opportunity for relaxing skiing or walks and talks, enjoying the refreshing scenery of the secluded alpine environment, offer a visit to the Alpmuseum, or give sufficient spare time for individual practice.



Lecturers and tutors

[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 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	IOC80*

2 Tuesday, Apr 08

	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
09:15-10:00	Introduction to an O2k-Demo experiment	MiPNet18.05 Amplex-Mouse-heart
10:00	<i>Practice: skiing / walk & talk / individual O2k-tasks / lunch / or bad weather task groups</i>	
14:00	<i>Coffee / Tea</i>	
14:30-16:00	O2k-Demo experiment 1: Respiration of intact cells and real-time DatLab Analysis: Simultaneous measurement of oxygen flux and H ₂ O ₂ production (O2k-Fluo LED2-Module)	MiPNet18.06_Amplex-Yeast*
16:00	<i>Coffee / Tea</i>	
16:30-17:15	DatLab guide: DL-Demo files and DL-Excel templates	DatLab Guide
17:15-18:30	DatLab Analysis 1: hands-on in 10 teams - with your laptops	DatLab Flux Analysis
18:30	<i>Dinner</i>	
20:00-21:00	O2k instrumental setup and sensor service - overview	O2k-Start

3 Wednesday, Apr 09

Workshop 2		Weblink
07:30-08:30	Breakfast	
08:30-09:15	O2k instrumental setup	OroboPOS service
	Groups 1-5	Groups 6-10
09:15-10:00	Groups 6-10	Groups 1-5
	10:00	Practice: skiing / walk & talk / individual O2k-tasks / lunch
14:00	Coffee / Tea	
14:30-16:00	O2k-Demo experiment 2: SUIT protocol with mouse permeabilized fibres and real-time DatLab analysis	Pesta 2012 Methods Mol Biol
	16:00	Coffee / Tea
16:30-17:45	Hands-on: SUIT experiment continued with DatLab Analysis and Excel templates	MiPNet18.10 O2kvsMultiwell*
	17:45-18:30	Experimental design 1: Substrate and coupling control of mitochondrial respiration - MitoPathways through CI and CII
18:30	Dinner	
20:00-21:15	Hot MiPNet-Topics 1 (10+5) Chair: Krumschnabel Gerhard Speakers: Agrimi Gennaro, Pereira de Almeida Nogueira Natalia, Paes Marcia Cristina, Laner Verena and Davis Mike, Mendonça Ana Paula	DatLab Flux Analysis The Blue Book* IOC88 Abstracts

4 Thursday, Apr 10

Workshop 3		Weblink
07:30-08:30	Breakfast	
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 2: Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	Glossary: Respiratory states
	10:00	Practice: skiing / walk & talk / individual O2k-tasks / lunch
14:00	Coffee / Tea	
14:30-15:15	Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.	-
	15:15-16:00	Instrumental quality control 2: O2k-Background test with TIP2k; analysis of oxygen flux.
16:00	Coffee / Tea	
16:30-17:45	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-Calibration O2k-Background
	17:45-18:30	OXPHOS analysis: A challenge for the simultaneous measurement of respiration and mt-membrane potential
18:30	Dinner	

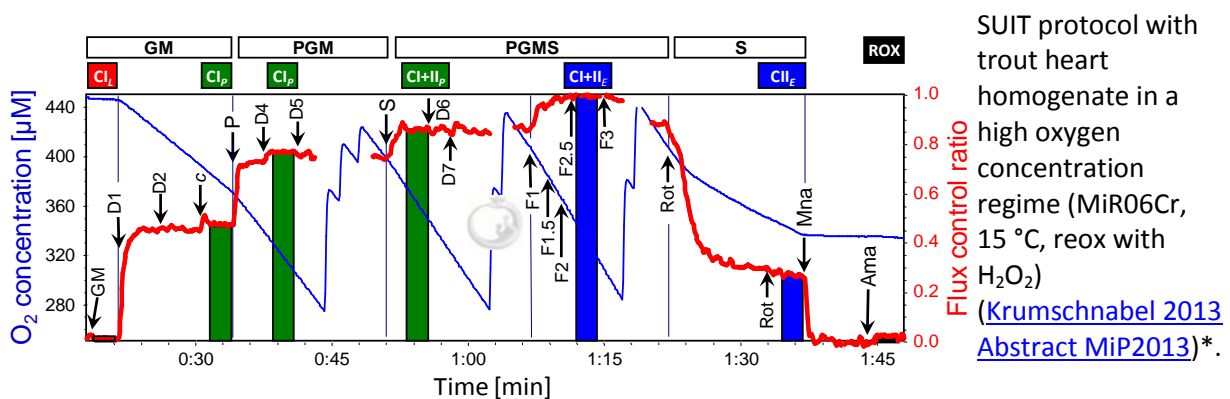
20:00-21:15	Hot MiPNet-Topics 2 (10+5) Chair: Bauer Christiane Maria Speakers: Andersen Marianne, Dhandapani Praveen Kumar, Legkun German, Mendonça Ana Paula, Wagenaars Jori, Maedo Kati	IOC88 Abstracts
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5 Friday, Apr 11

Workshop 4		Weblink
07:30-08:30	Breakfast	
08:30-09:45	Experimental design 3: MitoPathways through Complexes I+II - perspectives of comparative mitochondrial physiology	Gnaiger 2009 Int J Biochem Cell Biol
09:45-10:15	Tissue homogenate preparation: the PBI-Shredder	MiPNet17.03 Shredder vs Fibres*
10:15	Coffee / Tea	-
10:45-12:00	O2k-Demo experiment 3: Respiration and steady-state feedback control of oxygen levels with the TIP2k.	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 IOC88	O2k-Feedback
	Farewell party	-

6 Saturday, Apr 12

Departure	
	Breakfast
	Early morning: Departure



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: Assistant
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: Assistant
Fontana-Ayoub Mona	AT_Innsbruck_OROBOROS INSTRUMENTS: Tutor
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	AT_Innsbruck_OROBOROS INSTRUMENTS: D. Swarovski Research Laboratory, Medical University Innsbruck: Lecturer
Hoppel Florian	AT_Innsbruck_OROBOROS INSTRUMENTS: Assistant
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.
Krumtschnabel Gerhard	AT_Innsbruck_OROBOROS INSTRUMENTS: Tutor
Laner Verena	AT_Innsbruck_OROBOROS INSTRUMENTS: Tutor
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: Administrator

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.

MiPNet19.02 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

>1,100 O2k-Publications – www.bioblast.at/index.php/O2k-Publications

Recommended reading – general introduction

- Gnaiger E (2012) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 3rd ed. Mitochondr Physiol Network 17.18. OROBOROS MiPNet Publications, Innsbruck: 64 pp. » 📄
- 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.* » 📄
- 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.* » 📄
- 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. » 📄

Recommended reading – specific topics

- 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, 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.* » 📄
- 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. » 📄
- 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. » 📄
- 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. » 📄



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www.orooboros.at/?MitoCom-Tyrol



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