

## Course on High-Resolution Respirometry

**IOC-39.** *Mitochondrial Physiology Network* 12.14: 1-8 (2007)

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# International Course on High-Resolution Respirometry 13-17 April 2007



The 39<sup>th</sup> Course on High-Resolution Respirometry starts with a demo experiment with permeabilized muscle fibers, providing a practical overview of the **Oxygraph-2k**, with a simple multiple substrate/inhibitor titration protocol and integrated on-line analysis by **DatLab 4**. Emphasis will be placed on hands-on sessions to introduce O2k-high-resolution respirometry and application of the **TIP-2k**.



Experienced tutors guide small working groups step-by-step through the approach of high-resolution respirometry. Five Oxygraph-2k, two TIP-2k and several PCs are available for a do-it-yourself application of both hardware and software.

During lunch breaks, sufficient time is available for skiing, relaxing walks and talks, to enjoy the refreshing scenery of the alpine environment, or use the spare time for specific tutorials.



New features of DatLab 4.2 will be presented, and data analysis is accomplished on-line during the experiment, providing final results and their graphical presentation by the end of an experimental run. A special interest group will see the Titration-Injection microPump TIP-2k with new feedback-control in action and practice its simple and automatic operation.



## Tutors and Invited Guest Lecturers



Erich Gnaiger, Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - erich.gnaiger@oroboros.at

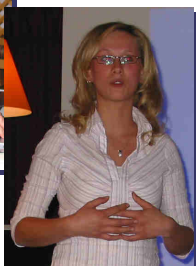
- Francesca Scandurra, Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - francesca.scandurra@oroboros.at



H el ene Lemieux, Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - helene.lemieux@oroboros.at



Ellen Lenaers, Department of Movement Sciences, Postbus 616, Universiteitssingel 50, 6200 MD Maastricht, The Netherlands. - ellen.lenaers@bw.unimaas.nl



Esther Phielix, Department of Human Biology, Maastricht University, Universiteitssingel 50, P.O. Box 616, NL-6229 ER Maastricht, Netherlands. - esther.phielix@HB.unimaas.nl

- Haas Richard, Neurosciences and Pediatrics, UCSD Mitochondrial Disease Laboratory, San Diego, California, USA. - rhaas@ucsd.edu
- Hoppel Charles, Case Western Reserve University, Department of Pharmacology, Center for Mitochondrial Diseases, Cleveland, Ohio, USA. - charles.hoppel@case.edu

## Programme

The final time schedule will depend on weather conditions and corresponding timing for breaks.

### Friday, 13. April

**Participants arriving in Innsbruck:** Departure from Innsbruck airport to Schr ocken. Two hour drive along the Inn valley, across the Arlberg Pass (St. Anton, St. Christoph), Flexenpass (Z urs, Lech), from Warth across the Tannberg Pass (Salober, lake Kalbeleseee), to Schr ocken. Usually the road from Lech to Warth is closed in winter, but now open due to exceptionally low snowfall.

**Participants arriving in Bregenz:** Meeting point at Bregenz train station, 1 hour drive to Schr ocken through the Bregenzerwald.

**Afternoon/Evening** Check in at Hotel Mohnenfluh, 19:30 dinner.

After dinner: Welcome; setting up the O2k-instruments with a glass of wine.

### Saturday, 14. April

**08:45 – 11:45 From switching on the Oxygraph-2k to the experimental result.**

See Protocol for the O2k Demo Experiment (below).

See also: **OROBOROS Protocols 2.1.A.** An experiment with high-resolution respirometry: Phosphorylation control in cell respiration. *MiPNet*. 10.4.

- Oxygraph-2k demo experiment with DatLab 4;



Salober, April 2007

- Oxygen calibration;
- Permeabilized fiber preparation;
- Filling and closing the chambers;
- MultiSubstrate/inhibitor titration protocol (see below):  $PMG_{+D+c} + S_{+u[F-titration]} + (Rot) + (Azd)$ ;
- Re-oxygenation.

12:00 – 16:00 Lunch break, skiing (bus leaves at 12:22 from Hotel Mohnenfluh); walk&talk, tutorials.

**16:10 - 19:00 Working Group Session 1:**  
Hands-on with the Oxygraph-2k (four instruments - eight parallel chambers): Respiration experiment with permeabilized fibers.



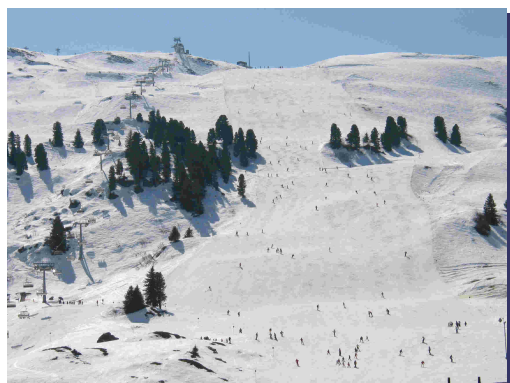
19:15 Dinner

**22:00 Short presentations of projects/topics of interest by participants**

Questions and discussion

**Sunday, 15. April**

**08:45 - 11:45 Introduction to Instrumental Background and Working Group Session 2:** Hands-on experiments with the Oxygraph-2k - O2k-instrumental background; chemical background.



12:00 – 16:00 Ski break (bus leaves at 12:22 from Hotel Mohnenfluh); alpine walks and talks; tutorials. Optional lunch at Hotel Körbersee - [www.koerbersee.at](http://www.koerbersee.at).

**16:15 -19:00 Working Group Session 3:** Hands-on experiments with the Oxygraph-2k, oxygen sensor service, O2k-assembly; DatLab 4.

19:30 Dinner

**22:00 Short presentations of topics of interest by participants**  
Questions and discussion

**Monday, 16. April**

**08:45 – 09:45 Demo DatLab Analysis.**  
**10:00 – 12:30 Working Group Session 4:** Hands-on experiments with the Oxygraph-2k, oxygen sensor service, O2k-assembly; DatLab 4; special tasks (TIP-2k; MultiSensor).



Alpmuseum uf m Tannberg, Batzen [www.alpmuseum.at](http://www.alpmuseum.at)

13:00 – 17:00	<b>Snowshoe walk</b> from Schröcken to a welcome at the <b>Alpmuseum uf m Tannberg</b> - <a href="http://www.alpmuseum.at">www.alpmuseum.at</a> . Snowshoes can be rented, water-proof hiking boots and skiing sticks are recommended.
<b>17:30 - 19:30</b>	<b>Working Group Session 5:</b> Hands-on experiments with the Oxygraph-2k, oxygen sensor service, O2k-assembly; DatLab 4.
20:00	Dinner
<b>22:00</b>	Discussion - Summary – Conclusions

## Tuesday, 17. April

- Departure to Innsbruck and Bregenz / MitoPathways Workshop (17-20 April)

## CONTENTS: OVERVIEW ON HIGH-RESOLUTION RESPIROMETRY

**Introduction: Mitochondrial and cellular respiratory physiology – new challenges for high instrumental performance.**

**High-resolution respirometry – what makes the difference? Presentation of the OROBOROS Oxygraph-2k**

- Low oxygen and measurement of cellular oxygen consumption – pushing the limits of detection.
- Optimum system design - the OROBOROS Oxygraph-2k.
- DatLab 4: on-line recording of oxygen concentration and flux; linear slope versus oxygen flux as a function of time.
- DatLab 4: the specialized software for high-resolution respirometry; high-resolution calibrations.

**OROBOROS Oxygraph-2k and TIP-2k: On-line instrumental performance**

- Instrumental background: measurement and correction as a function of  $pO_2$ .
- High resolution of respiratory flux at various steady-states.
- The Titration-Injection microPump TIP-2k: automatic titrations.
- Conceptual and methodological advantages of measurement at physiological low levels of oxygen.
- High time resolution for kinetic analyses: Determination of the time constant, dynamic corrections.

**Polarographic oxygen sensor (O2S) and O2k service**

- Cleaning of anode and cathode.
- Electrolyte and membrane application.
- Oxygraph-2k and TIP-2k: instrumental maintenance.

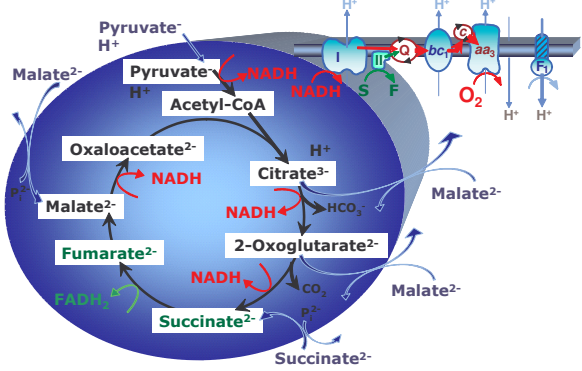
## The Protocol for the O2k Demo Experiment

Permeabilized Mouse Heart Fibers: After preparation of permeabilized muscle fibers, the air-calibrated Oxygraph-2k chambers (2 ml, MiR05, 37 °C) are oxygenated to c. 500  $\mu M$   $O_2$ . After closing the chambers, identical titration protocols are applied to both chambers.

**Protocol** **PMG<sub>+D+c</sub>+S<sub>+u</sub>[F-titration]+(Rot)+(Azd)**

State PMG Pyruvate, malate and glutamate (PMG) are added to induce State 2 (no adenylates) with substrates feeding NADH into Complex I.

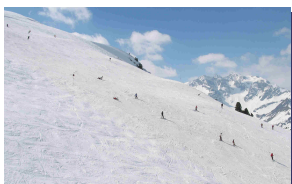
State PMG<sub>D</sub> After titration of 2 mM ADP (D), flux increases steeply to State 3, and does not return to State 4 since ADP is added at high concentration (required when working with permeabilized fibers) and ATPases maintain a high ADP concentrations due to hydrolysis of the ATP (T) formed in OXPHOS.

State PMG <sub>CD</sub>	Addition of 10 μM cytochrome c (c) provides a test for the intactness of the outer mitochondrial membrane, as indicated by the lack of stimulation or respiration by external cytochrome c.
State PMGS <sub>CD</sub>	Further addition of succinate (S) activates respiration due to convergent electron flow through Complexes I+II.
State PMGS <sub>cDu</sub>	Subsequently, FCCP is titrated in steps of 0.05 μM (manually or using the TIP-2k), to test for a stimulation of flux due uncoupling compared to State 3 (coupled), which is expected in cases when the phosphorylation system is limiting (ANT, ATP synthase, phosphate transporter).
State PMGS <sup>Rot</sup> <sub>cDu</sub>	<div style="display: flex; align-items: flex-start;"> <div style="flex: 1;"> <p>Rotenone is added to inhibit Complex I, now providing an estimate of linear electron transport through Complex II. If flux in this State S<sup>Rot</sup><sub>cu</sub> and the previous State State PMG<sub>CD</sub> are lower than flux driven by combined Complex I+II electron input,</p> </div> <div style="flex: 1;">  </div> </div> <p>then an auxiliary effect of convergent electron flow is demonstrated.</p>
Complex IV	It would be possible to continue this protocol after inhibition of Complex III with Antimycin A, and addition of ascorbate and TMPD, for evaluation of the activity of Complex IV (cytochrome c oxidase).
State PMGS <sup>RotAzd</sup> <sub>cDu</sub>	Sodium azide inhibits Complex IV and reduces respiration to a minimum. In the presence of ascorbate, TMPD and cytochrome c, this oxygen-dependent minimum flux would correspond to the chemical background.
Further information:	<b>OROBOROS Protocols</b>
#2.2.A.	Mitochondrial pathways to Complex I: Respiration with pyruvate, malate and glutamate. <i>Mitochondr. Physiol. Network</i> 11.4: 1-11.
#2.2.B.	Mitochondrial pathways to Complex II, glicerophosphate dehydrogenase and electron transferring flavoprotein. <i>Mitochondr. Physiol. Network</i> 11.9: 1-4.
#2.2.C.	Mitochondrial pathways through Complexes I+II: Convergent electron transport at the Q-junction and auxiliary effect of multiple substrates. <i>Mitochondr. Physiol. Network</i> 12.12: 1-10.
#4.2.1.	MitoPathways: Compilation on the auxiliary effect of succinate and flux ratio with substrates for Complexes I+II/Complex I. 12.13: 1-4.
#2.2.D.	Isolated Mitochondria and Permeabilized Tissues or Cells. <i>Mitochondr. Physiol. Network</i> 11.4: 1-4.
#2.3.A.	Mitochondrial respiration medium - MiR05. <i>Mitochondr. Physiol. Network</i> 8.5: 1-4.
#2.3.B.	Oxygraph-2k titrations: Mitochondria, permeabilized cells and biopsies. <i>Mitochondr. Physiol. Network</i> 9.12: 1.

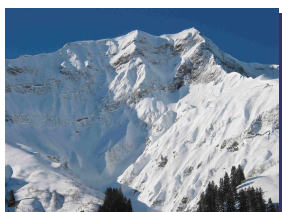
## Accommodation and Location

**Hotel Mohnenfluh** [www.mohnenfluh.at](http://www.mohnenfluh.at); Tel.: +43 5519 203; [hotel@mohnenfluh.at](mailto:hotel@mohnenfluh.at). The course takes place at Hotel Mohnenfluh (Sylvia Schramm-Strolz, right). Accommodation for all participants is arranged at Hotel Mohnenfluh and Hotel Tannberg. Breakfast and all meals will be served jointly at Hotel Mohnenfluh.

**Skiing:** Warth-Schröcken - <http://www.snowworld.at/>.



Bus trips are free from Schröcken to the skiing area of Salober, leaving at 12:20/12:22 at Hotel Tannberg / Hotel Mohnenfluh (or 11:04/11:06). For the afternoon after 12:30, the skiing pass is € 22.50 for the skiing lifts of Salober and Warth. There is also excellent crosscountry skiing around lakes Kalbelesee and Körbersee, as well as easy walking in magnificent winter scenery. Ski rental is available in Schröcken and at the skiing lift Salober. Top ski (+boots) is € 16.- (+7.-; 1 day), 30.- (+12.-; 2 days), 42.- (+17.-; 3 days) or 52.- (+22.-; 4 days). You can return to Schröcken on skies (depending on snow conditions) or by the free bus (leaving 15:30 at Salober). *Details may have changed and must be checked individually.*



**Weather:** Bright sunshine and warm temperatures are expected in April. Sunshine will be strong – bring sunglasses and sunscreen, even if you do not plan to go skiing. Protect yourself against wind and alpine weather (gloves, jacket, etc.).

**Further information** Introductory course material is available on our homepage [www.orooboros.at](http://www.orooboros.at).

## Contact

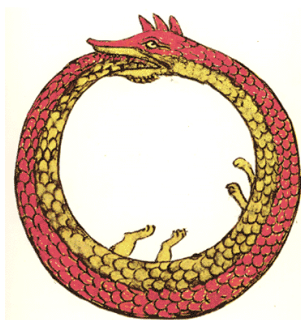
Erich Gnaiger, PhD  
 Medical University of Innsbruck  
 Department of Transplant Surgery  
 D. Swarovski Research Laboratory  
 Innrain 66/6  
 A-6020 Innsbruck, Austria  
 T +43 512 504 24623 (24626)  
 F +43 512 504 24625  
 Email [erich.gnaiger@i-med.ac.at](mailto:erich.gnaiger@i-med.ac.at)  
<http://www.mitophysiology.org>

OROBOROS INSTRUMENTS  
 high-resolution respirometry



Oxygraph-2k

OROBOROS INSTRUMENTS GmbH  
 Schöpfstrasse 18  
 A-6020 INNSBRUCK, Austria  
 T/F +43 512 566796  
 Email [instruments@orooboros.at](mailto:instruments@orooboros.at)  
 Homepage: <http://www.orooboros.at>



## Participants

Aiyar Raeka, EMBL Heidelberg, Gene Expression Unit, Heidelberg, Germany. - [aiyar@embl.de](mailto:aiyar@embl.de)

Chiesi Michele, Novartis Pharma AG, Basel, Switzerland. - [michele.chiesi@novartis.com](mailto:michele.chiesi@novartis.com)

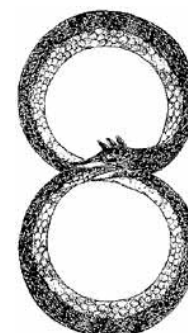
Elmer Eskil, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - [eskil.elmer@expbr.lu.se](mailto:eskil.elmer@expbr.lu.se)

Galina Antonio, Institute of Medical Biochemistry, Federal University of Rio de Janeiro, Brazil. - [galina@bioqmed.ufrj.br](mailto:galina@bioqmed.ufrj.br)

Gnaiger Erich, Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - [erich.gnaiger@orooboros.at](mailto:erich.gnaiger@orooboros.at)

Gomez Ludovic, INSERM U 886, Laboratoire de Physiologie Lyon-Nord, Lyon, France. - [lgomez@rockefeller.univ-lyon1.fr](mailto:lgomez@rockefeller.univ-lyon1.fr)

- Grüninger Stephan, Pharma NOVARTIS, Basel, Switzerland. - stephan.grueninger@novartis.com
- Haas Richard, Neurosciences and Pediatrics, UCSD Mitochondrial Disease Laboratory, San Diego, California, USA. - rhaas@ucsd.edu
- Hagland Hanne, University of Bergen, Haukeland University Hospital, Department of Clinical Biology, Division of Biochemistry, Bergen, Norway. - hanne.hagland@biomed.uib.no
- Hansson Magnus, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - magnus.hansson@expbr.lu.se
- Hoppel Charles, Case Western Reserve University, Department of Pharmacology, Center for Mitochondrial Diseases, Cleveland, Ohio, USA. - charles.hoppel@case.edu
- Khalid Murtaz Ahmed, Department of Medical Physiology, Institute of Medical Biology, Faculty of Medicine, University of Tromsø, Norway. - ahmed.khalid@fagmed.uit.no
- Larsen Steen, Copenhagen Muscle Research Centre, Dept. Medical Physiology, The Panum Institute, Copenhagen, Denmark. - slarsen@mfi.ku.dk
- Lemieux Hélène, Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - helene.lemieux@oroboros.at
- Lenaers Ellen, Department of Movement Sciences, Postbus 616, Universiteitssingel 50, 6200 MD Maastricht, The Netherlands. - ellen.lenaers@bw.unimaas.nl
- Phielix Esther, Department of Human Biology, Maastricht University, Universiteitssingel 50, P.O. Box 616, NL-6229 ER Maastricht, Netherlands. - esther.phielix@HB.unimaas.nl
- Riebel Virginie, Novartis Pharma AG, Biochem.+Molecular Toxicology, Muttenz, Switzerland. - virginie.riebel@novartis.com
- Rosca Mariana, Case Western Reserve University, Department of Pharmacology, Center for Mitochondrial Diseases, Cleveland, Ohio, USA. - mxr93@cwru.edu
- Sabatini Massimo, Pathologie Cardiovasculaire, Institut de Recherches Servier, Suresnes, France. - massimo.sabatini@fr.netgrs.com
- Scandurra Francesca M., Medical University of Innsbruck, Dept. Transplant Surgery, D. Swarovski Research Laboratory, Innsbruck, Austria; and OROBOROS INSTRUMENTS, Innsbruck, Austria. - francesca.scandurra@oroboros.at
- Sjövall Fredrik, Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, Lund, Sweden. - figge@flamman.org
- Smolkova Katarina, INSERM, Université Victor Segalen-Bordeaux 2, Bordeaux, France. - smolkova@biomed.cas.cz
- Vazquez Edwin, Case Western Reserve University, Department of Pharmacology, Center for Mitochondrial Diseases, Cleveland, Ohio, USA. - edwin.vazquez@case.edu
- Wan Kah Fei, Institute of Molecular and Cell Biology, Proteos, Singapore. - kfwan@imcb.a-star.edu.sg
- Wergedahl Hege, Institute of Medicine, Section of Medical Biochemistry, Haukeland University Hospital, Bergen, Norway. - hege.wergedahl@med.uib.no



# MiPNet Notes

