



## Course on High-Resolution Respirometry

IOC47. Mitochondrial Physiology Network 13.4: 1-8 (2008)

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# 47<sup>th</sup> International Course on High-Resolution Respirometry



12-16 July 2008

Schröcken, Vorarlberg, Austria

The 47<sup>th</sup> O2k-Course is the 16<sup>th</sup> presentation of high-resolution respirometry in Schröcken since 1988. This O2k-Course includes experiments with permeabilized muscle fibers and cells, providing a practical overview of the **Oxygraph-2k**, with integrated on-

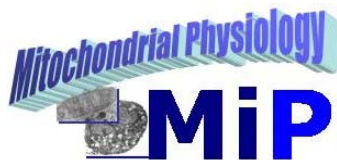
line analysis by **DatLab 4.3** (new upgrade), application of the **TIP-2k** and demonstration of **MultiSensor modules** for high-resolution respirometry. The O2k-system is introduced with specific perspectives of mitochondrial physiology. Emphasis is placed on hands-on applications by all participants.



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

During lunch breaks, sufficient time is available for relaxing walks and talks, to enjoy the refreshing scenery of the alpine environment, or use the spare time for specific tutorials. With DatLab 4.3 we accomplish data analysis on-line during the experiment, providing final results and their graphical presentation by the end of an experimental run. Thus we gain sufficient time to see the Titration-Injection microPump TIP-2k with new feedback-control in action and practice its simple and automatic operation.





Organized in parallel to the 2<sup>nd</sup> MiPsummer School on Mitochondrial Respiratory Physiology, 12-18 July 2008, Schröcken, Austria - [www.mitophysiology.org](http://www.mitophysiology.org).

**Support**

**MITOFOOD** COST Action Number FA0602 (Coordinator: Dr. Jaap Keijer, RIKILT-Institute of Food Safety, Wageningen University, The Netherlands.

**Tutors**

Med. Univ. Innsbruck, Dept. General Transplant Surgery, D. Swarovski Res: Lab., Innsbruck; and OROBOROS INST., Austria

- **Mario Fasching**, PhD, [mario.fasching@oroboros.at](mailto:mario.fasching@oroboros.at)
- **Erich Gnaiger**, PhD, [erich.gnaiger@i-med.ac.at](mailto:erich.gnaiger@i-med.ac.at)
- **Simone Köfler**, Mag, [simone.koefler@oroboros.at](mailto:simone.koefler@oroboros.at) (admin.)
- **Hélène Lemieux**, PhD, [helene.lemieux@oroboros.at](mailto:helene.lemieux@oroboros.at)
- **Francesca Scandurra**, PhD, [francesca.scandurra@oroboros.at](mailto:francesca.scandurra@oroboros.at)
- **Patrick Subarsky**, MSc, [patrick.subarsky@oroboros.at](mailto:patrick.subarsky@oroboros.at)

**Programme IOC47**

**Saturday, 12. July**

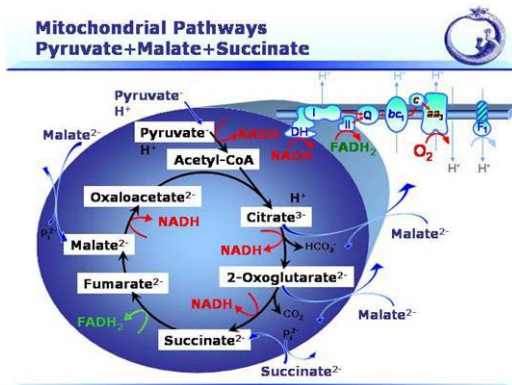
**16:15 Participants arriving in Bregenz:**  
Meeting point at 4:15 pm in Bregenz train station; 1.1 hour drive to Schröcken. Check in at Hotel Mohnenfluh.



**18:30 Welcome reception**  
**19:00 Dinner**

**Day 1: Sunday, 13. July**

**09:00 - 12:45** **Joint Session with MiPsummer school (lecture hall) Mitochondrial respiratory capacity and respiratory control: Erich Gnaiger (Innsbruck, AT) and Charles Hoppel (Cleavland, US)**



**13:00** Lunch break

**14:30 - 15:00** **Introduction to O2k-Design (seminar room, hotel Mohnenfluh)**

**15:00 - 17:45** **Parallel group sessions 1: Hands-on experiments with the Oxygraph-2k - instrumental performance: O2k-calibration; Instrumental setup and service.**



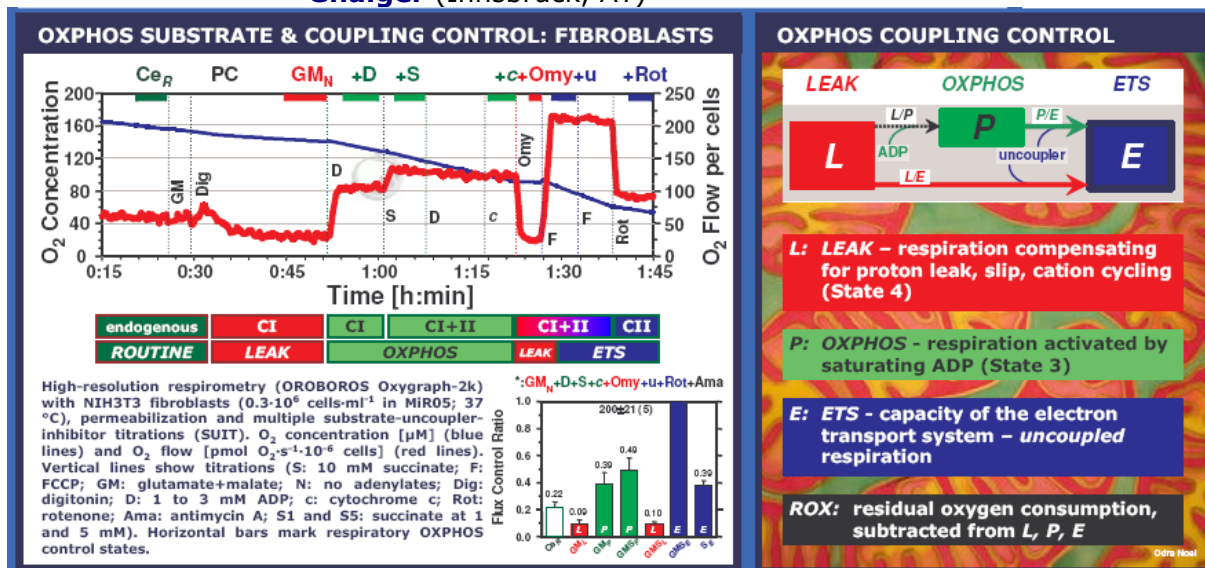
**17:45 - 18:45** **Summary of instrumental setup, trouble shooting.**

**19:00** Dinner

**Day 2: Monday, 14. July**

09:00 - 12:45

**Joint Session wit MiPsummer School (lecture hall)**  
**Membrane potential, coupling, substrates and respiratory control: Vilma Borutaite (Kaunas, LT), Patrick Subarsky (Innsbruck, AT), Dominique-Marie Votion (Liège, BE), Erich Gnaiger (Innsbruck, AT)**



13:00

Lunch break

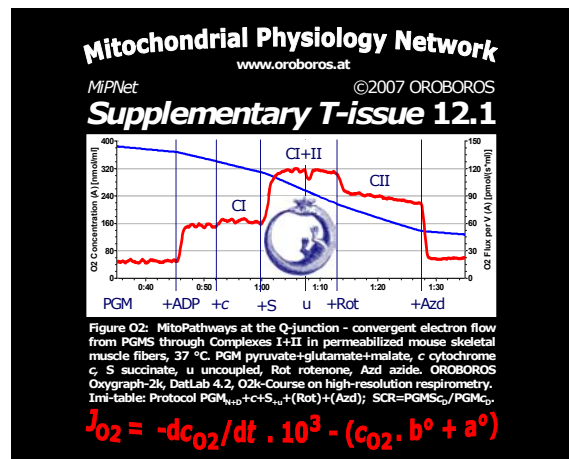
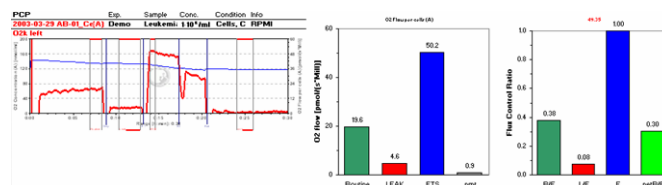
14:30 - 15:00

**Introduction to Datlab**

15:00 - 19:00

**Parallel group sessions: Hands-on with the Oxygraph-2k (five instruments - ten parallel chambers):**

Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result (demo experiment).



- Oxygraph-2k demo experiment with DatLab 4.3.
- Oxygen calibration of the polarographic oxygen sensors (POS).
- Preparation of permeabilized muscle fibers.
- Determination of fiber wet weight with [METTLER TOLEDO](http://www.mettler.com) microbalance [XS205DU](http://www.mettler.com) (1 to 3 mg per chamber).
- Addition of muscle fibers, closing the chamber.

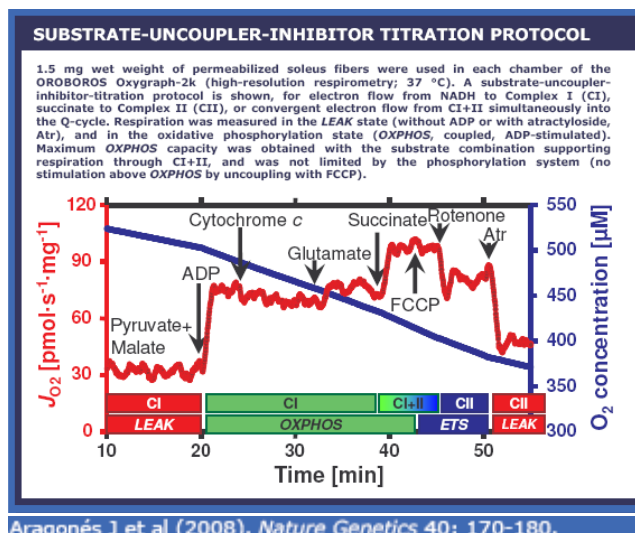


- **Demo experiment: Multiple substrate-uncoupler-inhibitor titration (OXPHOS titration protocols) and on-line DatLab analysis.**

17:30 -18:45

Special Interest Group: TPP+ / MultiSensor Application with the O2k (parallel to a 2<sup>nd</sup> run of the Demo experiment)

19:00 Dinner



**Day 3 (Tuesday, 15. July)**

08:30 – 09:00

**Introduction: Instrumental Background**

09:00 - 12:00

**Parallel group sessions:**

Hands-on experiments with the Oxygraph-2k - instrumental performance: O2k-calibration; instrumental background competition, DatLab analysis, O2k-manual.



12:00

**MiPsummer Walk** to the cheese and wine reception at the *Alpmuseum uf m Tannberg* ([www.alpmuseum.at](http://www.alpmuseum.at)); refreshment in the lake Körebersee or at Hotel Körbersee.

17:00-17:45

**Parallel group sessions:** Hands-on with Datlab: DatLab analysis of Demo Experiment and Background.

18:00–19:00

**Demo Experiment MultiSensor (lecture hall)**

Oxygen and pH: Calibration and rationale of application.

19:00 Dinner

21:15

*Discussion - Summary – Conclusions*

**Wednesday, 16. July**

Departure

**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 4.3: on-line recording of oxygen concentration and flux; linear slope versus oxygen flux as a function of time.
- DatLab 4.3: the specialized software for high-resolution respirometry; high-resolution calibrations.

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

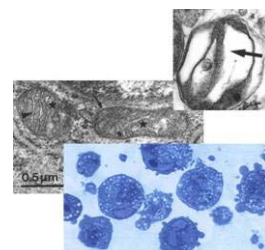
- Instrumental background: measurement and correction as a function of  $pO_2$ .
- High resolution of respiratory flux at various steady-states.
- The Titration-Injection microPump TIP-2k: automatic titrations.
- Conceptual and methodological advantages of measurement at physiological low levels of oxygen.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

### Polarographic oxygen sensor (POS) and O2k service

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

## Protocols for the O2k Demo Experiment

Gnaiger E, ed (2007) *Mitochondrial Pathways and Respiratory Control*. OROBOROS MiPNet Publications, Innsbruck: 96 pp.  
Electronic 1<sup>st</sup> ed ISBN 978-3-9502399-0-4  
2<sup>nd</sup> addition: online [www.orooboros.at](http://www.orooboros.at)



## Questions for the O2k-Course

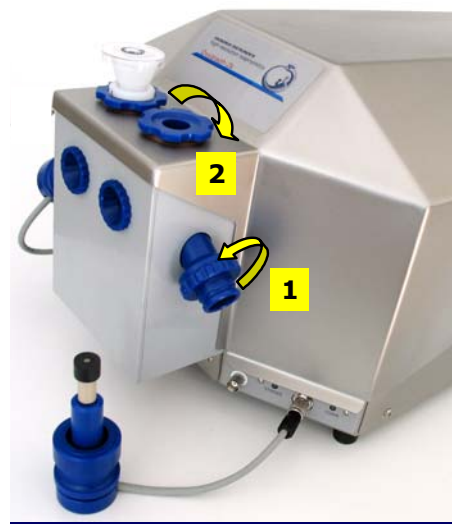
The O2k-Manual (# refers to Chapter numbers) provides the answers to many of these questions – and you find more information on [www.orooboros.at](http://www.orooboros.at) ...

### Oxygraph-2k assembly (#1.O2k.A)

- What is the most important consideration for positioning the glass chamber during assembly of the O2k?
- How do I detect a leak in the chamber?

### Polarographic oxygen sensor (POS)

- Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the polarographic oxygen sensor?
- Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
- Does the POS respond to oxygen concentration,  $c_{O_2}$  [ $\mu\text{mol}\cdot\text{dm}^{-3} = \mu\text{M}$ ], or partial oxygen pressure  $p_{O_2}$  [kPa]? (#1.4.A)



### POS calibration (#1.O2k.D)

- How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
- During POS calibration, should the chamber be open or closed?
- What is an acceptable voltage (raw signal) of the POS at (a) air calibration, and (b) zero oxygen calibration, and how are these raw signals affected by the gain setting?
- Why should you check the raw voltage during calibration?
- The sensor voltage is above 9.9 V. What should I do?
- What does the stirrer test tell me?
- How do I perform a zero oxygen calibration?

- The oxygen solubility,  $S_{O_2}$  [ $\mu\text{M}\cdot\text{kPa}^{-1}$ ], relates oxygen concentration to partial pressure. Which variables need to be considered for estimation of the oxygen solubility of an aqueous solution, for example of a respiration medium? (#1.4.A)
- When is the oxygen calibration of a POS preferentially performed?
- How long does it take approximately (5, 15, 30 or 45 min) to perform an oxygen calibration at air saturation, after the O2k is switched on (at experimental temperature in the range of 20 to 37 °C)?
- Do you need to consider the instrumental background when performing an oxygen calibration of the POS at zero oxygen concentration?
- Do you need to consider the instrumental background when performing an oxygen calibration of the POS at air saturation?
- Does the oxygen signal have to be stable for an oxygen calibration of the POS?
- How do you define POS signal stability? (#1.1.D)
- Do you have to perform a zero oxygen calibration of the POS before air calibration?
- Can you perform an oxygen calibration of the POS with biological sample and respiratory activity in the aqueous solution, when equilibration is performed with a gas phase in the chamber and stability of the signal is observed?
- What is the difference between static calibration (#1.O2k.D) and dynamic sensor calibration (#1.O2k.G; time constant – for advanced users)? How can I use a dynamic calibration (stirrer test) as a quick sensor test? (#1.O2k.G)

### POS Service (#1.O2k.B)

- Can I wash the sensor with 70 % or 99 % EtOH?
- What should I do if the sensor connector threads appear dark and dirty?
- The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?
- How can I avoid creating bubbles when filling the electrolyte reservoir of the POS?
- Can I repeat the ammonia treatment?
- How can I check sensor performance?
- What precautions should be taken when handling the sensor connector?

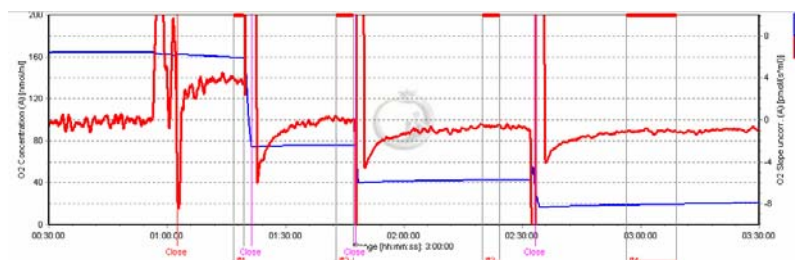


### Cleaning of the Chamber (#1.5.C)

- What solution should be placed in the chamber when the O2k is not in use (i.e. overnight, for a few days)?
- Can detergents be used to clean the chamber and the PVDF stoppers?
- What is the recommended cleaning procedure between experimental runs?
- The glass chambers appear to have surface residue. Can this be removed, what is the procedure?
- The stirring bar gets stuck. What can I do?

### Instrumental background calibration (#1.O2k.E)

- Does the oxygen signal have to be stable for an instrumental background calibration?
- Does the oxygen flux have to be stable for an instrumental background calibration?
- How do we define flux stability? Is a flat red line always an indication of a stable flux?
- Do I need to calibrate instrumental background flux at air saturation and zero oxygen concentration?
- Do I need to calibrate the POS before performing an instrumental background calibration?



- We use the symbol  $a^{\circ}$  for the intercept at zero oxygen concentration, and the symbol  $b^{\circ}$  for the slope of background oxygen flux as a function of oxygen concentration. In the analysis of instrumental background, we have obtained 0.022 and -1.7. Which value is  $a^{\circ}$  and  $b^{\circ}$ ?
- Does the background-corrected flux have to be zero when the oxygen signal is stable?
- How often do I have to check the instrumental background?

## Accommodation and Location

**Hotel Mohnenfluh** [www.mohnenfluh.at](http://www.mohnenfluh.at);  
Tel.: +43 5519 2031;  
[hotel@mohnenfluh.at](mailto:hotel@mohnenfluh.at). The course takes place at Hotel Mohnenfluh, including accommodation for all participants with breakfast, meals and coffee breaks.



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

## Participants and Areas of Interest

Comelli Marina, Dr., Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Udine, Udine, Italy. - [mcomelli@mail.dstb.uniud.it](mailto:mcomelli@mail.dstb.uniud.it)

The role of mitochondria in energy metabolism during differentiation.

Hawke Emma, PhD Student, Verona University, Verona, Italy. - [emma.hawke@crimper.co.nz](mailto:emma.hawke@crimper.co.nz)

Optimizing training induced improvements in PGC-1 $\alpha$  and mitochondrial biogenesis (exercise, training adaptations, mitochondria respiration, mitochondria biogenesis).

Hughey Curtis, PhD Student, Department of Biochemistry & Molecular Biology, University of Calgary, Canada. - [cchughey@ucalgary.ca](mailto:cchughey@ucalgary.ca)

Ischemia, myocardial infarction, mitochondrial respiration, substrate utilization.

Latini Alexandra, Prof.Dr., Center of Biological Sciences UFSC, Universidade Federal de Santa Catarina Campus Universitario Trindade, Florianopolis, Brazil. - [alagini@ccb.ufsc.br](mailto:alagini@ccb.ufsc.br)

Involvement of mitochondrial function in the physiopathology of brain disorders.

Lucchinetti Eliana, Dr., Institute of Anesthesiology, E-HOF University Hospital Zurich, Switzerland. - [eliana.lucchinetti@usz.ch](mailto:eliana.lucchinetti@usz.ch)

Effects of anesthetics on mitochondrial function (anesthetics, mitochondrial respiration, ROS, hypoxia/ischemia, Ca<sup>2+</sup> overload, mitochondrial K<sub>ADP</sub> channels).

Milenkovic Dusanka, Institute for Biochemistry and Molecular Biology, Freiburg, Germany. - [dusanka.milenkovic@biochemie.uni-freiburg.de](mailto:dusanka.milenkovic@biochemie.uni-freiburg.de)  
Coupling of respiratory chain to the ATP Synthese.

Miinalainen Ilkka, PhD, Centre for Bioanalytical Sciences (CBAS), Dublin City University, Dublin, Ireland. - [ilkka.miinalainen@dcu.ie](mailto:ilkka.miinalainen@dcu.ie)

Bioenergetics and mitochondrial function in podocytes under diabetic stress (podocytes, ATP production and distribution, ROS, mitochondria).

Polyak Erzsebet, Ph.D., Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, USA. - [polyake@email.chop.edu](mailto:polyake@email.chop.edu)

(C.elegans mitochondria, mouse liver mitochondria, complex I mutant).



Praturi Gopala Krishna, PhD Student, Centre for cellular and molecular Biology, Hyderabad, India. - [praturi@ccmb.res.in](mailto:praturi@ccmb.res.in)

We want to look into the oxygen levels in normal and diseased persons particularly neuromuscular, cardiomyopathy, periodontitis (mutations, deletions, cardiomyopathy, periodontitis).

Pustovidko Antonina, Dr., A.N. Belozersky Institute of Physiko-Chemical Biology, MSU, Leninskie, Moscow, Russia. - [nell\\_a@mail.ru](mailto:nell_a@mail.ru)

Oxidative stress and expiration of mitochondria from normal and cancer cells (mitochondria, reactive oxygen species, oxidative stress, antioxidants).

Rennison Julie, PhD Student, Rennison Julie, Dept. of Molecular Cardiology, Cleveland Clinic, Cleveland, USA. - [julie.rennison@case.edu](mailto:julie.rennison@case.edu)

Assesing oxidative phosphorylation in mitochondria and permeabilized cardiac muscle fibers.

Sarna Neha, PhD Student, Faculty of Kinesiology, University of Calgary, Canada. - [neha\\_sarna@hotmail.com](mailto:neha_sarna@hotmail.com)

The mitochondrial function in cultured muscle cells in response to nutritional overload (myotube, obesity, mitochondria).

Stride Nis, Copenhagen Muscle Research Centre, Copenhagen, Denmark. - [nis.stride@gmail.com](mailto:nis.stride@gmail.com)

Mitochondrial function in the failing heart, mitochondrial function with varying degrees of heart failure (mitochondrial function, high resolution respirometry, heart failure, metabolic switch, elective cardiac surgery, coronary artery bypass graft (CABG), cardiac assist device (HeartMate), heart transplantation).

Vögtle Nora, Institute for Biochemistry and Molecular Biology, Freiburg, Germany. [nora.voegtle@biochemie.uni-freiburg.de](mailto:nora.voegtle@biochemie.uni-freiburg.de)

Oxygen consumption during catalysis of disulfide bond formation by mitochondrial IMS protein.

## Contact

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**OROBOROS INSTRUMENTS**  
high-resolution respirometry

**Oxygraph-2k**



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Cooperation and Feedback in Science

