IOC59. *Mitochondrial Physiology Network* 15.07: 1-12 (2010)

59th International Course on High-Resolution Respirometry

2010 Oct 1 – 6
Obergurgl, Tyrol, Austria

The **59th O2k-Course** includes experiments with biological samples, providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.3** (new upgrade), applications of the **TIP2k**, and perspectives of high-resolution respirometry in mitochondrial physiology. Emphasis is placed on hands-on applications by all participants.

An international team of experienced tutors guide small working groups step-by-step through the approach of HRR. Four Oxygraph-2k (8 chambers) are available for a do-it-yourself application of both hardware and software. Combined with an introduction and demo experiment, it is best to put the O2k into action yourself.

During lunch breaks, sufficient time is available for relaxing walks and talks, to enjoy the refreshing scenery of the secluded 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 TIP2k with new feedback-control in action and practice its simple and automatic operation.

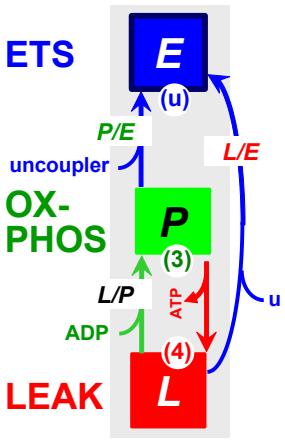
Tutors

Erich Gnaiger, PhD (Innsbruck, AT)
Mario Fasching, PhD (Innsbruck, AT)
Kathrin Renner-Sattler, Mag.,PhD (Regensburg, DE)
Anita Wiethüchter, Mag. (AT)

MacDonald Julia, NZ *(guest tutor)*

Programme IOC59

Day 1: Friday, October 1



Participants arriving in Innsbruck: Meeting point at 2:20 pm at Innsbruck main railway station, at 2:45 pm at the OROBOROS Office and at 3:15 pm at the airport; 1h45min drive to the University Center Obergurgl.

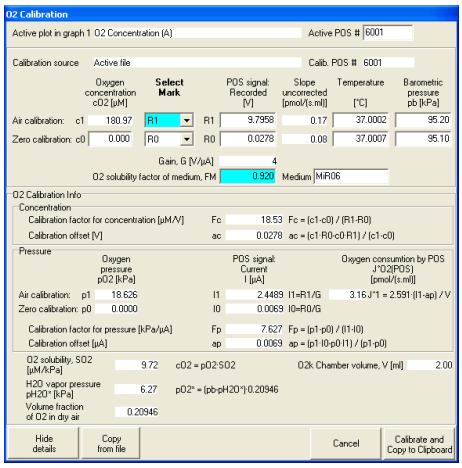
18:30 Welcome Reception University Center Obergurgl

19:00 Dinner

21:00 **Erich Gnaiger: Beyond respiratory states 3 and 4: Electron transport system (ETS), OXPHOS capacity and LEAK respiration - Experimental advances with high-resolution respirometry (HRR).**

Day 2: Saturday, October 2

Principles of HRR - from switching on the Oxygraph-2k to the experimental result - with a little help from a friend: the O2k-Manual.

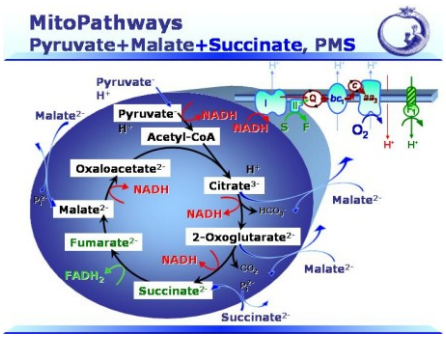


08:30 – 09:30 **The O2k system: Introduction and oxygen calibration of the polarographic oxygen sensors (OROBoPOS).**

09:30 – 10:30 **Hands-on: Oxygen sensor calibration with DatLab 4.3**

10:30 Coffee break

11:00 – 12:00 **Erich Gnaiger:**



Phosphorylation Control Protocol (PCP): An introduction.

12:00 Lunch break - exercise

MiPNet12.12

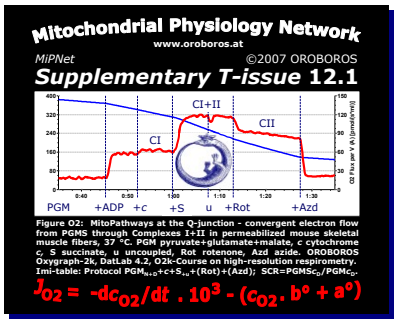
15:00 -16:30 **Demo experiment – the Oxygraph-2k and on-line DatLab analysis.** Yeast as a HRR-model.

16:30 Coffee break

17:15 – 18:45 **Hands-on: Experiment with the Oxygraph-2k (four O2k - 8 chambers) and on-line DatLab analysis.**

19:00 Dinner

21:00 **Discussion of results, protocol, DatLab analysis.**



Day 3: Sunday, October 3

08:30 – 09:00 **Erich Gnaiger: Instrumental background - Introduction.**

09:00 – 12:00 **Hands-on (four groups): Oxygen calibration and instrumental background test with the Oxygraph-2k** - Washing and filling the O2k chambers with experimental media; air calibration; instrumental background *competition*, DatLab background analysis (see Protocols: MiPNet14.06_Instrumental background correction and accuracy of oxygen flux).

A. Instrumental background test for experiments with cells and isolated mitochondria, from air saturation to zero oxygen concentration, with automatic TIP2k titration protocol.

B. Instrumental background test for experiments with permeabilized muscle fibres, in the high-oxygen range of 500 to 200 µM. Manual titration of hydrogen peroxide into MiR06 (MiR05 with catalase).

12:00 Lunch break - sports

16:00 Coffee break

MiPNet08.09/10.04

MiPNet12.07

16:30 – 17:15 **Background analysis – summary.**

17:15 – 17:45 **DatLab 4.3 – An overview.**

17:45 – 18:45 **Hands-on (four groups): Instrumental background analysis**

19:00 Dinner

21:00 - 21:30 **Design of HRR protocols: Questions from participants and discussion**

Day 4 Monday, October 4

08:15 **Parallel group sessions - Introduction**

	Setup	POS Service	DatLab Analysis
08:30 – 09:15	**Gr. 1**	**Gr. 2**	**Gr. 3**
09:15 – 10:00	**Gr. 3**	**Gr. 1**	**Gr. 2**
10:00	Coffee break		
10:30 - 11:15	**Gr. 2**	**Gr. 3**	**Gr.1**

11:15 – 12:00 Working groups: Elaborate answers to the „Questions for the O2k-Course"

12:00 Lunch break - sports

15:00 – 15:30 **Introduction to trouble shooting**

15:00 – 16:00 **Mitochondrial membrane potential and how to measure it**

16:00 -16:30 Coffee break

IOC 59

16:30- 17:00	**Introduction to MultiSensor methodologies. The TPP^+ electrode – an example for ion selective electrodes.**
17:00 -18:30	**Demo experiment: Calibration of the TPP^+ electrode, and instrumental oxygen background in the presence of additional sensors**
19:00	Dinner
21:00 - 21:45	**Presentation of 'Answers for the O2k-Course' – Trouble shooting; Participant Questions for Troubleshooting and Standard O2k Operation**

Day 5: Tuesday, October 5

08:30 - 10:30	**Paralell group sessions:** Hands on: Set up of the instrument with TPP^+ and reference electrodes; Electrode assembly and maintenance
10:30 -11:00	Coffee break
11:00 – 12:45	**Paralell group sessions:** continued

Alternative program (in parallel):

08:30 - 12:45	**Special interest group: High-Resolution Respirometry (HRR) and mitochondrial physiology**
12:00	Lunch break
14:00 - 15:15	**From the TPP^+ signal to mitochondrial membrane potential –** Introduction
15:15 – 15:45	Coffee break
15:45 – 17:00	**Parallel group sessions:** From the TPP^+ signal to mitochondrial membrane potential – exercise
17:00 – 18:00	***Discussion - Summary – Conclusions***

University Center Obergurgl **Farewell party of IOC59**

Day 6: Wednesday, October 6

Early morning: Departure

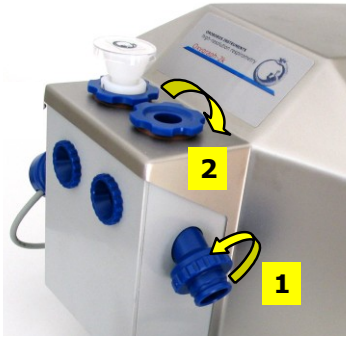
Questions for the O2k-Course

The **O2k-Manual** and **Protocols** provides answers to many of these questions ([] MiPNet numbers in the O2k-Compendium on the CD) – and you find more information on www.oroboros.at …

1. Oxygraph-2k assembly [MiPNet12.06]

1.1. What is the most important consideration for positioning the glass chamber during assembly of the O2k?

1.2. How do you detect an oxygen leak in the chamber?



2. Polarographic oxygen sensor (POS)

2.1. Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the polarographic oxygen sensor, and how can you quickly see the raw signal on-line?

2.2. The sensor voltage is above 9.9 V. What should you do?

2.3. Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
2.4. Does the POS respond to oxygen concentration, c_{O2} [µmol·dm^{-3} = µM], or partial oxygen pressure p_{O2} [kPa]?

3. POS calibration [MiPNet12.08]

3.1. How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
3.2. Should the chamber be open or closed during POS calibration?
3.3. 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?
3.4. Why should you check the raw voltage during calibration?
3.5. How do you perform a zero oxygen calibration?
3.6. The oxygen solubility, S_{O2} [µM·kPa^{-1}], relates oxygen concentration to partial pressure. How is S_{O2} related to the solubility factor, F_M? Which variables need to be considered for estimation of the oxygen solubility of an aqeous solution, for example of mitochondrial respiration medium MiR06? [MiPNet06.03]
3.7. When is the oxygen calibration of a POS preferentially performed?
3.8. 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)?
3.9. Do you have to consider the instrumental background when performing an oxygen calibration of the POS at zero oxygen concentration?
3.10. Do you need to consider the instrumental background when performing an oxygen calibration of the POS at air saturation?
3.11. Does the oxygen signal have to be stable for an oxygen calibration of the POS?
3.12. How do you define POS signal stability? [MiPNet06.05]
3.13. Do you have to perform a zero oxygen calibration of the POS before air calibration?
3.14. Can you calibrate 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?
3.15. What is the difference between static calibration [MiPNet12.08] and dynamic sensor calibration (time constant – for advanced users)? How can you use a dynamic calibration (stirrer test) as a quick sensor test? [MiPNet02.04]

4. POS Service [MiPNet08.04]

4.1. What should be done if the sensor connector threads appear dark and dirty?
4.2. The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?
4.3. How can you avoid creating bubbles when filling the electrolyte reservoir of the POS?
4.4. Can the ammonia treatment be applied repeatedly?
4.5. How can you check sensor performance?
4.6. What precautions should be taken when handling the sensor connector?

5. Cleaning of the Chamber [MiPNet06.03]

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

6. Instrumental background test [MiPNet12.09; MiPNet14.06]

6.1. Does the oxygen signal have to be stable for setting a mark in an instrumental background test?

6.2. Does the oxygen flux have to be constant for setting a mark in an instrumental background test?
6.3. How do you define flux stability? Is a flat horizontal red line always an indication of a stable flux?
6.4. Do you need to determine instrumental background flux at air saturation and zero oxygen concentration?
6.5. Do you need to calibrate the POS before performing an instrumental background calibration?
6.6. We use the symbol *a*° for the intercept at zero oxygen concentration, and the symbol *b*° 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*° and *b*°, respectively?
6.7. Does the background-corrected flux have to be zero when the oxygen signal is stable?
6.8. How often do you have to check the instrumental background?

Literature

Pesta D, Gnaiger E (2010) High-Resolution Respirometry. OXPHOS protocols for human cell cultures and permeabilized fibres from small biopisies of human muscle. In: Mitochondrial bioenergetics: methods and protocols (Series Editor: Sir John Walker), edited by Carlos Palmeira and António Moreno. In press.

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, with focus on*

- Polarographic oxygen sensor and traditional oxygraphy
- High-resolution respirometry: The Oxygraph-2k
- Calibration of Polarographic Oxygen Sensors and Oxygen Concentration in Respiration Media at Air Saturation
- From Oxygraph Slopes to Respiratory Flux Corrected for Background Effects
- Phosphorylation control protocol with intact cells
- Titration Steps of the PC Protocol
- Experimental Example for the PC Protocol
- Flux Control Ratios from the PC Protocol
- Intact cells, permeabilized cells and tissue, or isolated mitochondria?

Gnaiger E (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int. J. Biochem. Cell Biol.* 41: 1837–1845.

- Respirometry with permeabilized fibres and isolated mitochondria
- Convergent CI+II electron input and OXPHOS capacity
- Tissue-OXPHOS capacity in human permeabilized muscle fibres and isolated mitochondria
- Tissue-OXPHOS capacity and functional diversity

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 respirrometry with particular emphasis on kinetics and measurements at low oxygen:*

- Mitochondrial kinetics measured by high-resolution respirometry
- Calibrations and corrections for response time and instrumental background
- Steady-state injection respirometry
- Mitochondrial respiratory control at low oxygen
- Apparent oxygen affinity and catalytic efficiency of mitochondrial respiration
- Effect of ADP and oxygen limitation on ADP/O2 flux ratios
- The low-oxygen environment of the cell: Mitochondria between hypoxic and oxidative stress

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 fibers, MiR05.*

- Optimization of mitochondrial cold storage
- Mitochondrial respiration medium, MiR05
- Mitochondrial cold ischemia-reperfusion injury

Renner K, Amberger A, Konwalinka G, Kofler R, Gnaiger E (2003) Changes of mitochondrial respiration, mitochondrial content and cell size after induction of apoptosis in leukemia cells. *Biochim. Biophys. Acta* 1642: 115-123. – *Intact cells, cytochrome c oxidase, cytochrome c test, respiration per million cells, per citrate synthase, per mg protein, or per cytochrome c oxidase activity.*

Further information: Introductory course material is available on our homepage www.oroboros.at, within the following sections:

- **Oxygraph-2k**
- **Protocols** - www.oroboros.at/index.php?id=mipnet-protocols
- **Publications**
- **WorldWide**
- **O2k-Manual** - http://www.oroboros.at/index.php?id=o2k-manual

Please also visit: www.bioblast.at

Accomodation and Location

University Center Obergurgl

www.uz-obergurgl.at/;
Tel.: +43 512 507-37201 obergurgl@uibk.ac.at

Participants and Areas of Interest

Berthelson Per, GIH – The Swedish School of Sport and Health Sciences, Åstrandlaboratoriet Lidingövägen 1, Box 5626, SE-114 86 Stockholm, Sweden. – per.perthelson@gih.se (*exercise, high fat load, ROS*)

Deepika Verma, PhD Student, 7S-06, Kadoorie Biological Sciences Building, Division of Plant Biology, Faculty of Science, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R.China. - h0892104@hku.hk *(Cysteine, plant mitochondria, Cyanide)*

Duicu Oana Maria , MD, Assistant-Professor, PhD student - University of Medicine and Pharmacy 2, Eftimie Murgu Sq., 300041 Timisoara, Romania - oanaduicu@umft.ro

Hagen Christian, Cand.scient. - Department of Clinical Biochemistry and Immunology, Statens Serum Institut, 5 Artillerivej, 2300 Copenhagen S, Denmark - UNC@ssi.dk

Fasching Mario, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. – mario.fasching@oroboros.at (*tutor*)

Forkink Marleen, Dept. of Biochemistry (286), Nijmegen Centre for Molecular Life Sciences Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. – m.forkink@ncmls.ru.nl (*life cell fluorescence microscopy; ROS*)

Garlid Anders O., Portland State University, Dept. Biol., Portland OR, USA. - aogarlid@gmail.com

Gnaiger Erich, A.Univ.-Prof. PhD, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; OROBOROS INSTRUMENTS; Austria. – erich.gnaiger@oroboros.at (*organizer, tutor*)

Ho Qin Shi, Kate, National University of Singapore, Yong Loo Lin School of Medicine, MD7, 8 Medical Drive, #05-05, Singapore 117597, Republic of Singapore - bchhqs@nus.edu.sg (*Oxphos, Warburg effect*)

Hroudová Jana, MPharm, Department of Psychiatry, First Faculty of Medicine, Charles University, Ke Karlovu 11121 08 Praha 2, Czech Republic. – jana.hroudova@centrum.cz (*mitochondria, mood stabilizers, antidepressants, respirometry*)

Jensen Martin Gram, Faculty of Health Sciences, Dept Biomedical Sciences, The Panum Institute, Blgn. 12.4.7, Blegdamsvej 3, DK 2200 Copenhagen N, Denmark. – martingram@gmail.com (*mitochondrial membrane potential, ROS production, respirometry*)

Larsen Anna Karina, Dept. of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark. – akl@farma.ku.dk *(mitochondrial response to the presence of lipophilic polycations, apoptosis – necrosis and factors controlling mitochondrial reorganization)*

MacDonald Julia, University of Auckland, School of Biological Sciences, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand. – j.macdonald@auckland.ac.nz *(permeabilized muscle fibres, vastus lateralis, obesity, peripheral blood, mononuclear cells; guest tutor)*

Page Melissa, University of Abderdeen, University of Aberdeen, Dept. of Zoology, Aberdeen, Scotland AB242TZ, U.K. - melissa.page@brocku.ca *(mitochondrial ex-phos, permeabilized skelet muscle fibres, lifespan and stress restistance)*

Renner-Sattler Kathrin, Mag.Dr., MiPNet OROBOROS INSTRUMENTS, St.-Georgen-Platz 6, D-93047 Regensburg, Germany. – kathrin.renner@oroboros.at (*tutor*)

Sims Carrie, MD, MS, FACS, Assistant Professor in Surgery, Division of Traumatology and Surgical Critical Care, University of Pennsylvania, 3451 Walnut St., Philadelphia, PA 19104-6205, United States. – carrie.sims@uphs.upenn.edu (*exercise, high fat load, ROS*)

Wiethüchter Anita, MSc., OROBOROS INSTRUMENTS, Innsbruck, Austria. – anita.wiethuechter@oroboros.at (*tutor*)

William Ashley S., Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, 2200 Pierce Ave., Nashville, TN 37232-0615, USA. – ashley.s.williams@vanderbilt.edu (*hepatic insulin resistance)*

Zungu Makhosazane, Dr. (PhD (Medicine),UCT), Cardiac Scientist and Biochemist, Discipline of Human Physiology, University of KwaZulu Natal, P/BagX54001, Durban, 4000, South Africa - zungumak@ukzn.ac.za (*mitochondria, electron transport chain activity, substrates, ADP/O ratio, uncoupling, Hansatech, oxidative phosphorylation*)

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 20
Email erich.gnaiger@i-med.ac.at
www.mitophysiology.org

www.mipart.at

OROBOROS INSTRUMENTS
high-resolution respirometry
Oxygraph-2k

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