



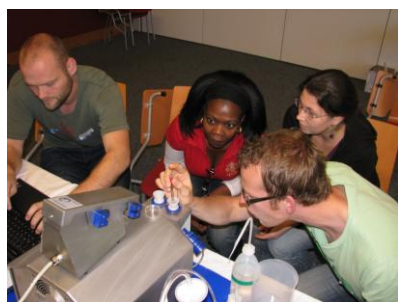
Course on High-Resolution Respirometry

IOC65. Mitochondrial Physiology Network 16.03: 1-8 (2011)

©2011 OROBOROS®
Version 3: 2013-10-08

65th International Workshop on HRR

2011 December 10 - 15 Schröcken, Vorarlberg, Austria

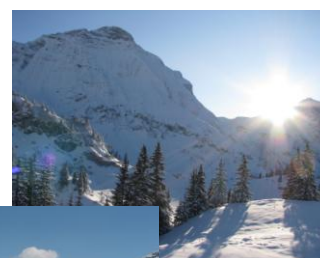


The **65th Workshop on High-Resolution Respirometry (HRR)** is the 26th international Oxygraph Course held in Schröcken since 1988. The workshop includes experiments with biological samples, providing a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.3**, applications of the **TIP2k**, and perspectives of HRR in mitochondrial physiology. Parallel to an introductory O2k-Basic workshop, a TPP-Basic workshop will focus on **O2k-MultiSensor** applications for measurement of mt-membrane potential, Ca^{2+} , and acidification rate (pH).

An international team of experienced tutors guides small working groups step-by-step through the approach of HRR. Five Oxygraph-2k (10 chambers) are available for do-it-yourself applications of both hardware and software. Combined with an introduction and demo experiment, it is best to put the O2k into action yourself.

Lunch breaks provide an opportunity 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 feedback-control in action and practice its simple and automatic operation.

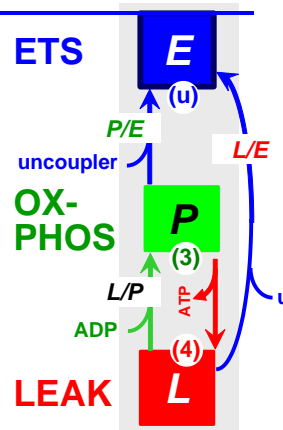
Tutors Erich Gnaiger, AT
Mario Fasching, AT
Dominik Pesta, AT
Anna Draxl, AT
Andrea Eigentler, AT
Anita Wiethuechter, AT
Carolina Doerrier, ES



Programme IOC65

Day 1 Saturday, December 10

- 15:00** **Arrival in Bregenz:** Meeting point Bregenz train station at 3:00 pm; 1.1 hour bus drive to Schröcken and Hochtannberg (Salober). Transfer/walk to Hotel Körbersee.
- 18:30** Welcome reception at Hotel Körbersee.
- 19:00** *Dinner*
- 21:00** **Introductions of participants and their research interests.**



Day 2 Sunday, December 11

- 08:30 - 9:00** **Dominik Pesta:** General introduction.
- 09:00 - 09:45** **Mario Fasching:** O2k-MultiSensor overview.
- 09:45** *Coffee break*
- 10:15 - 10:45** **Anita Wiethüchter:** DatLab 4.3 – An overview. ☺MiPNet12.07
- 10:45 - 12:30**

O2k-Basic	TPP-Basic
<p>The O2k-System: Introduction and oxygen calibration of the polarographic oxygen sensors (OROBOPoS).</p> <p>Hands-on: Oxygen calibration with DatLab. ☺MiPNet12.08</p>	<p>Mario Fasching: Planning and performing a TPP Experiment: Introduction.</p> <p style="text-align: right;">☺MiPNet14.05</p>

12:30 - 14:00 *Lunch break*

14:00 - 16:30

<p>14:00 - 14:30 Dominik Pesta: Introduction: Instrumental background ☺MiPNet14.06</p> <p>14:30 - 16:30 Hands-on (3 groups): Instrumental background test for experiments with cells and isolated mitochondria, from air saturation to zero oxygen concentration or for permeabilized muscle fibres, in the high-oxygen range of 500 - 200 µM.</p> <p>A. Instrumental background test, with automatic TIP2k titration protocol.</p> <p>B. Instrumental background test, with manual titrations.</p>	<p>Hands-on: Assembly and maintenance of TPP and reference electrodes; Set up of the instrument with TPP and reference electrodes. ☺MiPNet15.03</p> <p>Hands-on: Instrumental background oxygen flux in the presence of the TPP electrode.</p>
--	---

16:30 Coffee break

17:00 – 19:00


O2k-Basic	TPP-Basic
Hands-on: <i>continued</i> Background analysis and summary.	Hands-on: <i>continued</i> Background analysis and summary.

19:00 Dinner

21:00 – 21:45 Hot topics: MiPNet Session 1 (3 x 10+5 min)

Day 3 Monday, December 12

08:30 – 10:30

<p>Parallel group sessions: Hands-on with the Oxygraph-2k.</p> <table border="1"> <thead> <tr> <th></th> <th>Setup</th> <th>POS Service</th> <th>Dat Lab Analysis</th> </tr> </thead> <tbody> <tr> <td>08:30 – 09:30</td> <td style="color: red;">Gr.1</td> <td>Gr.2</td> <td style="color: blue;">Gr.3</td> </tr> <tr> <td>09:30 – 10:30</td> <td style="color: blue;">Gr.3</td> <td style="color: red;">Gr.1</td> <td>Gr.2</td> </tr> </tbody> </table>		Setup	POS Service	Dat Lab Analysis	08:30 – 09:30	Gr.1	Gr.2	Gr.3	09:30 – 10:30	Gr.3	Gr.1	Gr.2	<p>Hands-on: TPP calibration and TPP chemical background.</p> 
		Setup	POS Service	Dat Lab Analysis									
	08:30 – 09:30	Gr.1	Gr.2	Gr.3									
09:30 – 10:30	Gr.3	Gr.1	Gr.2										

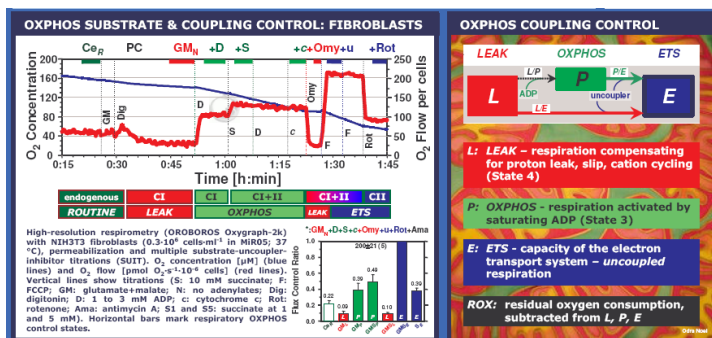
10:30 Coffee break

11:00 – 12:00

<p>Parallel group sessions: <i>continued</i></p> <table border="1"> <thead> <tr> <th></th> <th>Setup</th> <th>POS Service</th> <th>Dat Lab Analysis</th> </tr> </thead> <tbody> <tr> <td>11:00 – 12:00</td> <td>Gr.2</td> <td style="color: blue;">Gr.3</td> <td style="color: red;">Gr.1</td> </tr> </tbody> </table>		Setup	POS Service	Dat Lab Analysis	11:00 – 12:00	Gr.2	Gr.3	Gr.1	<p>Hands-on: <i>continued</i></p>
		Setup	POS Service	Dat Lab Analysis					
11:00 – 12:00	Gr.2	Gr.3	Gr.1						

12:00 - 16:00 Lunch break - exercise

16:00 Coffee, tea



16:30 – 17:30 Erich Gnaiger: MitoPathways: Respiratory states and coupling control ratios. [MiPNet12.15](#)

17:30 – 18:30 Erich Gnaiger: MitoPathways: through Complexes I+II: Convergent electron transfer at the Q-junction and additive effect of substrate combinations. [MiPNet12.12](#)

19:00 Dinner

21:00 - 22:15 Announcement of special interest groups and Hot topics: MiPNet Session 2 (4 x 10+5 min)

Day 4 Tuesday, December 13

08:30 - 10:00

<p>Erich Gnaiger: Experimental protocols Talk and parallel hands-on with DatLab demo files: a) Basic protocol with isolated mitochondria: LEAK, OXPHOS, ETS, ROX. 📍 MiPNet12.11 b) Coupling Control Protocol with intact cells: ROUTINE, LEAK, ETS, ROX. 📍 MiPNet08.09</p> <p>Hands-on: Start air calibration</p>	<p>Hands-on: TPP calibration and extending the TPP method for the determination of mitochondrial membrane potential beyond isolated mitochondria: TPP calibration and experiment with biological sample.</p>
---	---

10:00 *Coffee break*

10:30 - 12:30

<p>Hands-on: Experiment with biological sample in the Oxygraph-2k: SUIT protocol and on-line DatLab analysis.</p>	<p>Hands-on: <i>continued</i></p>
--	--

12:30 - 16:00 *Lunch break*

16:00 *Coffee, tea*

16:30 - 17:15 **Dominik Pesta:** Preparation of permeabilized muscle fibres in the context of an exercise training study.



17:15 - 19:00

O2k-Basic	TPP-Basic
<p>Hands on: <i>continued</i> Data analysis and guide through the Excel templates.</p>	<p>Mario Fasching: From the TPP⁺ signal to mitochondrial membrane potential - Guide through the Excel templates. Hands-on: Data evaluation.</p>

19:00 *Dinner*

21:00 - 22:00

<p>Design of SUIT-protocols: Questions from participants and discussion.</p>	<p>Hands-on: <i>continued</i> and discussion.</p>
---	--

Day 5 Wednesday, December 14

08:30 - 10:00 **Special interest groups - Parallel sessions**

- A. Hands-on permeabilized fibres with TPP-electrodes:** Fibre preparation and permeabilization; Respiration of permeabilized fibres and membrane potential of permeabilized fibres.
- B. Hands-on TIP2k: Oxygen kinetics**
- C. Hands-on Repeats:** Biological experiment or Instrumental background test (in the high oxygen range), with/without TIP2k.

10:00 *Coffee break*

10:30 – 12:00 **Special interest groups:**
continued

12:00 - 16:30 *Lunch at Hotel Körbersee*

Snowshoe walk (rental of snowshoes) **to the Alpmuseum:**
Guided tour and reception: € 15



Alpmuseum uf m Tannberg, Batzen www.alpmuseum.at

16:30 – 17:30 **Mario Fasching:** Introduction to trouble shooting.

17:30 – 19:30

O2k-Basic	TPP-Basic
<p>Working groups: Elaborate answers to the 'Questions for the O2k-Course'</p> <p>Discussion of 'Answers'.</p>	<p>Analysis of TPP experiment from Day 4.</p> <p>Discussion of results and final discussion and conclusions for the TPP special interest group.</p>

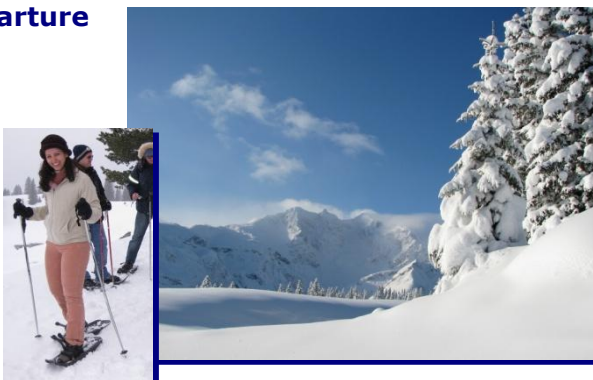
19:30 *Dinner*

21:00 **Panel Discussion – Feedback IOC65**
Lundby Carsten
Goncalves Renata de Lima Sales
Carolina Doerrier
Mc Donald Allison

Farewell party of IOC65

Day 6 **Thursday, December 15**

Early morning: Departure



MiPNet Abstracts–

Hot topics in Mitochondrial Physiology

Online on: http://wiki.oroboros.at/index.php/IOC_Abstracts_MiPNet16.03

Literature

- Boushel R, Gnaiger E, Calbet JA, Gonzalez-Alonso J, Wright-Paradis C, Sondergaard H, Ara I, Helge JW, Saltin B (2011) Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion* 11: 303-307.
- 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, 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.
- 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 (2009) Capacity of oxidative phosphorylation in human skeletal muscle. New perspectives of mitochondrial physiology. *Int J Biochem Cell Biol* 41: 1837-1845.
- 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, MiR06.*
- 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:

O2k-Manual - www.oroboros.at/index.php?o2k-manual

Protocols - www.oroboros.at/index.php?mipnet-protocols

wiki.oroboros.at - the *information synthase* for Mitochondrial Physiology and high-resolution respirometry:

Publications - <http://www.oroboros.at/index.php?publications>

Continue the discussion - <http://wiki.oroboros.at/index.php/Talk:IOC65>

Accommodation and Location

Hotel Körbersee www.koerbersee.at;

Tel +43 5519 265; hotel@koerbersee.at



Participants and Areas of Interest

- Bechara-Hirvonen Nada**, Institute of Biomedicine, University of Helsinki, FI. nada.bechara-hirvonen@helsinki.fi (*mitochondrial dysfunction in experimental heart failure*).
- Bienholz Anja**, Universitätsklinikum Essen, DE. anja.bienholz@uk-essen.de (*role of non-esterified fatty acids in respiratory impairment and mitochondrial de-energization of proximal tubules secondary to hypoxia/reoxygenation*).
- Bosch Marta**, Cell Biology Department, Hospital Clínic, Barcelona, ES. martabosch@ub.edu.
- Cannon Megan**, Experimental Cardiology, Groningen, NL. m.v.cannon@umcg.nl (*metabolic remodeling in cardiac hypertrophy and heart failure*).
- Cesnekova Jana**, Laboratory for Study of Mitochondrial Disorders, Charles University and General University, Prague, CZ. tesarova.jana@post.cz (*oxidative phosphorylation system*).
- Cotter Matthew**, University of Texas Medical Branch, Shriners Burn Hospital, Galveston, TX, USA. macotter@utmb.edu (*changes in skeletal muscle respiration in burned children*).
- Draxl Anna**, OROBOROS INSTRUMENTS, Innsbruck, AT. anna.draxl@oroboros.at (*tutor*).
- Eigentler Andrea**, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck, AT. andrea.eigentler@i-med.ac.at (*tutor*).
- Farsijani Navid**, Vanderbilt University School of Medicine, Nashville, TN, USA. navid.farsijani@gmail.com (*high-resolution respirometry of mouse kidney tissue*).
- Fasching Mario**, OROBOROS INSTRUMENTS, Innsbruck, AT. mario.fasching@oroboros.at (*lecturer, tutor*).
- Ghosh Sangeeta A.**, The University of Texas Health Science Center San Antonio, TX, USA. ghoshs4@uthscsa.edu (*effect of aging and exercise training on mitochondrial function: relationship with insulin sensitivity and inflammation*).
- Gnaiger Erich**, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; OROBOROS INSTRUMENTS; AT. erich.gnaiger@oroboros.at (*organizer, tutor*).
- Goncalves Renata de Lima Sales**, Federal University of Rio de Janeiro, Institute of Biomedical Medicine, Botafogo, BR. rlsales@bioqmed.ufrj.br (*mitochondrial membrane potential, metabolic states*).
- Gonzalez Franquesa Alba**, IDIBAPS, Barcelona, ES. agonzalf@clinic.ub.es (*mitochondrial dysfunction in insulin-dependent tissues such as liver, skeletal muscle and adipose tissue*).
- Hinkeldein Tanja**, Universitätsklinikum Essen, DE. tanja.hinkeldein@uni-due.de (*respiration in isolated proximate tubules (kidney) after hypoxia/ reoxygenation supported by different cac metabolites*).
- Hoffman David L.**, Lycera Corporation, Plymouth, USA. hoffman@lycera.com (*mitochondrial ROS production; open flow respirometry mitochondrial therapeutics*).
- Keipert Susanne**, German Institute of Human Nutrition, Dept. of Pharmacology/ Group of Energy Metabolism, Nuthetal, DE. Susanne.Keipert@dife.de (*Transgenic mice with ectopic expression of UCP1 in skeletal muscle with beneficial effects on energy metabolism, diet-induced obesity and aging*).
- Kerätär Juha**, Department of Biochemistry, University of Oulu, FI. juha.keratar@oulu.fi (*effect of mitochondrial fatty acid synthesis defects on mitochondrial respiration*).
- Knaub Leslie**, University of Colorado Denver, Aurora, Colorado, USA. Leslie.Knaub@ucdenver.edu (*mitochondrial dysfunction in the vasculature and skeletal muscle in disease states*).
- Lau Gigi**, Dept. of Zoology, The University of British Columbia, Vancouver, CA. glau@zoology.ubc.ca (*physiological adaptations of mitochondria to hypoxia in fishes*).

- Lundby Carsten**, Center for Integrative Human Physiology (ZIHP), University of Zurich, CH. carsten.lundby@access.uzh.ch
- McDonald Allison**, Dept. of Biology, Wilfrid Laurier University, Waterloo, ON, CA. amcdonald@wlu.ca (*functional and regulatory characterization of alternative oxidases in various kingdoms of life*).
- Meissner Barbara**, OROBOROS INSTRUMENTS, Innsbruck, AT. barbara.meissner@oroboros.at (*administrative assistant*)
- Nielsen Morten Hostrup**, Research Unit of Respiratory Medicine, Bispebjerg Hospital, Copenhagen, DK. mortenhostrup@gmail.com (*effects of beta2-agonists on human skeletal muscle mitochondrial enzyme activity*).
- Oliveira Marcus F.**, Federal University of Rio de Janeiro, Institute of Medical Biochemistry, BR. maroli@bioqmed.ufrj.br (*studies on the mitochondrial metabolism in the blood flow Schistosoma mansoni*).
- Orynbayeva Zulfiya**, Dept. of Surgery, Drexel University College of Medicine, Philadelphia, USA. Zulfiya.Orynbayeva@DrexelMed.edu (*the bioenergetic signature of cancer; the case of prostate adenocarcinoma*).
- Ost Mario**, German Institute of Human Nutrition, Dept. of Pharmacology/ Group of Energy Metabolism, Nuthetal, DE. Mario.Ost@dife.de (*transgenic mice with ectopic expression of UCP1 in skeletal muscle with beneficial effects on energy metabolism, diet-induced obesity and aging*).
- Pesta Dominik**, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck; and OROBOROS INSTRUMENTS, AT. dominik.pesta@student.uibk.ac.at (*lecturer and tutor*)
- Pinho Joao Paulo**, Federal University of Rio de Janeiro, Institute of Medical Biochemistry, BR. xjpinho@gmail.com (*studies on the dynamic changes of mitochondrial function following a blood meal in the kissing bug Rhodnius prolixus*).
- Plenge Ulla**, Bispebjerg Hospital, University of Copenhagen, DK. ullaplenge@gmail.com (*effects of rhEPO in human mitochondria in muscle cells and blood cells*).
- Sondergard Nielsen Hans**, Bispebjerg Hospital, University of Copenhagen, DK. hans@cmrc.dk (*regulation of mitochondria in different bioethnic groups; impact of diet and exercise/ impact of klima and light, circadian rythm on mitochondria*).
- Spacilova Jana**, Laboratory for Study of Mitochondrial Disorders, Charles University and General University, Hospital in Prague, CZ. spacilova.jana@gmail.com (*oxidative metabolism in human skin fibroblast from patients with mitochondrial disorders*).
- Strand Harald**, University Hospital of North Norway, NO. harald.strand@unno.no (*diagnosis of mitochondrial diseases*).
- Wiethuechter Anita**, D. Swarovski Research Laboratory, Dept. General Transplant Surgery, Medical University Innsbruck, AT. anita.wiethuechter@i-med.ac.at (*tutor*).

Contact

Erich Gnaiger, PhD
 Medical University of Innsbruck
 Department of Transplant Surgery
 D. Swarovski Research Laboratory
 Anichstrasse 35
 A-6020 Innsbruck, Austria
 Email erich.gnaiger@i-med.ac.at

<http://wiki.oroboros.at>
www.mipart.at



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

