



## Course on High-Resolution Respirometry

**IOC72.** *Mitochondrial Physiology Network* 17.14: 1-8 (2012)

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## 72<sup>nd</sup> International Workshop on HRR

**2012 December 05-10**  
**Schröcken, Vorarlberg, Austria**



The **72<sup>nd</sup> Workshop on High-Resolution Respirometry (HRR)** is the **29<sup>th</sup>** 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**, applications of the **TIP2k**, the new **O2k-Fluorescence LED2-Module**, and perspectives of HRR in mitochondrial physiology. Hands-on training will be done in six parallel groups.



An international team of experienced tutors guides small working groups step-by-step through the approach of HRR. Six O2k (12 chambers) are available for do-it-yourself applications of both hardware and software. Combined with an introduction and demo experiments, it is best to put the O2k into action yourself.



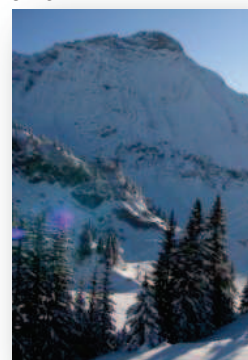
Lunch breaks provide an opportunity for skiing, relaxing walks and talks, to enjoy the refreshing scenery of the secluded alpine environment, or use the spare time for specific tutorials. With DatLab 5 (new) we accomplish data analysis on-line, providing final results and their graphical presentation 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.

### Lecturers and Tutors

**Erich Gnaiger**  
**Mona Fontana-Ayoub**  
**Johannes Bartscher**

### Guest Tutors

**Hand Steven C, Prof., PhD (LSU, Baton Rouge, US)**  
**Hoppel Charles L, Prof., MD (CWRU, Cleveland, Ohio, US)**  
**Iyer Shilpa, Ass.Prof., PhD (VCU, Richmond, US)**  
**Le Catherine (CSU, Fort Collins, US)**  
**Votion Dominique-Marie, PhD (University of Liège, BE)**  
**Konrad Csaba, (Semmelweis University, Budapest, HU)**  
**Kiss Gergely, (Semmelweis University, Budapest, HU)**



## Programme IOC72



1 Wednesday, December 05		
<b>Arrival</b>		
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	
18:30	Welcome reception at Hotel Körbersee.	
19:00	Dinner	
20:30-21:00	Get-together: Introduction of participants and their research interests	
2 Thursday, December 06		
	Workshop 1	Weblink
07:30-08:30	Breakfast	
08:00	Organize loan of skiing equipment	
O2k-Core	Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result	<a href="#">Gnaiger 2008 POS</a>
08:30-09:00	Erich Gnaiger: Get O2k-Connected with your OROBOROS-USB: a guided tour to the O2k	<a href="#">get O2k-Connected</a>
09:00-10:00	Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.	<a href="#">O2k-Calibration</a>
10:00	Organize special lunch or <i>bad weather</i> task groups (fibre and homogenate prep, fluorometry and spectrophotometry, NO, pH and Ca, Bioblast wiki login) and <i>loan of skiing equipment</i>	
11:00	Practice - skiing, walk & talk, or individual O2k-tasks / Lunch	
14:00	Coffee / Tea	
14:30-16:00	O2k-Demo experiment 1: Respiration of intact cells and on-line DatLab Analysis: Simultaneous measurement of oxygen consumption (O2k-Core) and H <sub>2</sub> O <sub>2</sub> production (add-on O2k-Fluorescence LED2-Module)	<a href="#">O2k-Fluorometry Workshop</a>
16:00	Coffee / Tea	
16:30-17:15	DatLab Guide through the menus: DL-Demo files and DL-Excel templates	<a href="#">DatLab Guide</a>
17:15-18:00	Experimental design 1: Coupling control of mitochondrial respiration: LEAK, OXPHOS, ETS, ROX	<a href="#">DatLab Flux Analysis</a>
18:30	Dinner	

<b>20:00-21:00</b>	<b>MiPNet-Lectures by guest tutors</b> <b>Dominique-Marie Votion (BE):</b> OXPHOS protocols for the study of training and rare myopathies in horse skeletal muscle fibres. <b>Shilpa Iyer (US):</b> Respiration in intact cells.
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### 3 Friday, December 07

Workshop 2		Weblink
07:30	Breakfast	
<b>O2k-Basic</b>	<b>O2k instrumental setup</b>	<b>OroboPOS service</b>
<b>08:30-09:15</b>	Groups A and B	Groups C and D
<b>09:15-10:00</b>	Groups C and D	Groups A and B
10:00	Practice - skiing, walk & talk, or individual O2k-tasks / Lunch	
14:00	Coffee / Tea	
<b>14:30-16:00</b>	<b>Instrumental quality control 2: O2k-Background test and on-line analysis of oxygen flux.</b>	<a href="#">O2k-Background</a>
16:00	Coffee / Tea	
<b>16:30-18:30</b>	<b>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 <math>\mu</math>M. O2k-Background with automatic TIP2k or manual titrations.</b>	<a href="#">O2k-Background</a>
18:30	Dinner	
<b>20:00-21:00</b>	<b>Hot MiP topics 10 + 5 min</b> Rodrigues MF (BR), Towheed MA (US), Konrad C (HU), Kiss G (HU)	<a href="#">IOC72 Abstracts</a> <a href="#">MiPNet17.13</a>

### 4 Saturday, December 08

Workshop 3		Weblink
07:30	Breakfast	
<b>08:30-09:30</b>	<b>MiPNet-Lectures by guest tutors</b> <b>Chuck Hoppel (US):</b> OXPHOS protocols for diagnosis of mitochondrial diseases with isolated mitochondria. <b>Steve Hand (US):</b> OXPHOS protocols for the study of mitochondrial function in active and dormant states.	
<b>09:30-10:00</b>	<b>DatLab O<sub>2</sub> flux analysis: Flux per volume, flux per mass, flow per cell, flux control ratio</b>	<a href="#">Glossary: Respiratory states</a>
10:00	Practice - skiing, walk & talk, or individual O2k-tasks / Lunch	
14:00	Coffee / Tea	
<b>14:30-16:00</b>	<b>O2k-Demo experiment 2: SUIT protocol with mouse permeabilized fibres and on-line DatLab analysis</b>	<a href="#">Pesta 2012 Methods Mol Biol</a>

16:00	Coffee / Tea	
16:30-17:30	<b>Hands-on: SUIT experiment continued with DatLab Analysis and guide through Excel templates</b>	<a href="#">DatLab Flux Analysis</a>
17:30-18:00	<b>Experimental design 2: Coupling Control Protocol with intact cells: ROUTINE, LEAK, ETS, ROX</b>	<a href="#">Cells: PCP</a>
18:00-18:30	<b>Experimental design 3: Substrate and coupling control of mitochondrial respiration.</b>	<a href="#">CI+II</a>
18:30	Dinner	
20:00-21:00	<b>Hot MiP topics 10 + 5 min</b> Dzialowski EM (US), Horscroft J (UK), Irving BA (US), Kamath VG (US)	<a href="#">IOC72 Abstracts</a> <a href="#">MiPNet17.13</a>

## 5 Sunday, December 09

Workshop 4		Weblink
07:30	Breakfast	
8:30-09:15	<b>Working groups: Elaborate answers to the 'Questions for the O2k-Workshop'</b>	<a href="#">IOC-Questions</a>
9:15-09:45	<b>IOC-Questions - discussion of 'Answers'</b>	
09:45-10:15	<b>The O2k-Workshop continues with the Bioblast wiki</b>	<a href="http://www.bioblast.at">www.bioblast.at</a>
10:15	Coffee / Tea	
10:45-12:00	<b>O2k-Demo experiment 3: Respiration and steady-state feedback control of oxygen levels with the TIP2k.</b>	<a href="#">TIP2k User Manual</a>
12:00	Lunch	
13:00-13:45	<b>Mona Fontana-Ayoub: Introduction into trouble shooting</b>	
14:00	<i>Walk (snowshoe rental) to the Alpmuseum: Guided tour and reception: 15 €</i>	<a href="http://www.alpmuseum.at">www.alpmuseum.at</a>
17:30	Coffee / Tea	
18:00-19:00	<b>Erich Gnaiger: MitoPathways through Complexes I+II - perspectives of comparative mitochondrial physiology</b>	<a href="#">Gnaiger 2009 Int J Biochem Cell Biol</a>
19:00	Dinner	
20:30-21:00	<b>Panel Discussion - Feedback IOC72</b>	<a href="#">O2k-Feedback - Bioblast</a>
<b>Farewell party</b>		

## 6 Monday, December 10

<b>Departure</b>	
Breakfast	
Early morning: Departure	

## IOC72 List of Participants

Name	Lab
<b>Ali Sameh S</b>	Center for Aging and Associated Diseases, Helmy Institute of Medical Sciences, IBBE, Mitochondria, synaptosomes, cardiomyocytes, oxidative stress – <b>ET_Giza_Ali SS</b>
<b>Amoedo Nivea Dias</b>	Federal University of Rio de Janeiro, Medical Biochemistry Institute, Mitochondria, cells, oxygen consumption, membrane potential, proton efflux – <b>BR_Rio De Janeiro_Rumjanek FD</b>
<b>Atlante Anna</b>	Consiglio Nazionale delle Ricerche, IBBE, Mitochondria, cells, oxygen consumption, membrane potential, proton efflux – <b>IT_Bari_Atlante A</b>
<b>Bikman Benjamin T</b>	Brigham Young University, Sphingolipids, Insulin resistance – <b>US_UT Provo_Bikman BT</b>
<b>Bresciani Martins de Andrade Paula</b>	Institute of Physical Activity and Sport Sciences, Cruzeiro do Sul University, White adipose tissue, fatty acids, mitochondria – <b>BR_Sao Paulo_BM de Andrade P</b>
<b>Burtscher Johannes (team tutor)</b>	Dep. of Pharmacology, Medical Uni Innsbruck – <b>AT_Innsbruck_Gnaiger E</b>
<b>Colleoni Francesca</b>	Department of Physiology, Development & Neuroscience, University of Cambridge, Placenta, hypoxia, bioenergetics, metabolism, pregnancy – <b>UK_Cambridge_Murray AJ</b>
<b>Dudzinska Dominika</b>	Department of Haemostasis and Haemostatic Disorders, Medical University of Lodz - Polyphenols, neutrophils, respiratory burst – <b>PL_Lodz_Watala C</b>
<b>Dzialowski Edward M</b>	Department of Biological Sciences, University of North Texas - Endothermy, avian, reptile, cardiac, skeletal, liver, hypoxia – <b>US_TX Denton_Dzialowski EM</b>
<b>Figueiredo Rodrigues Mariana</b>	Federal University of Rio de Janeiro, Medical Biochemistry Institute, Sodium butyrate, mitochondrial function, glycolysis – <b>BR_Rio De Janeiro_Rumjanek FD</b>
<b>Fontana-Ayoub Mona (team tutor)</b>	<b>AT_Innsbruck_OROBOROS INSTRUMENTS</b>
<b>Friederich Marisa</b>	University of Colorado, Respiratory chain enzyme analysis, mitochondrial diseases – <b>US_CO Denver_Van Hove J</b>
<b>Gnaiger Erich (organizer, tutor)</b>	DSL, Dept Visceral, Transpl Thoracic Surgery, Medical Univ Innsbruck – <b>AT_Innsbruck_OROBOROS INSTRUMENTS</b>
<b>Goy Sarah</b>	Department of Biological Sciences, University of North Texas, Endothermy, avian, reptile, cardiac, skeletal, liver, hypoxia – <b>US_TX Denton_Dzialowski EM</b>
<b>Hand Steven (guest tutor)</b>	Louisiana State University – <b>US_LA Baton Rouge_Hand SC</b>
<b>Hoppel Charles (guest tutor)</b>	Case Western Reserve University School of Medicine – <b>US_OH Cleveland_Hoppel CL</b>
<b>Horscroft James</b>	Department of Physiology, Development and Neuroscience, University of Cambridge, Heart, cardiac metabolism, Angiotensin II, mitochondria – <b>UK_Cambridge_Murray AJ</b>
<b>Irving Brain</b>	Obesity Institute Center for Nutrition and Weight Management, Aging, diabetes, NASH, NAFLD, exercise – <b>US_PA Danville_Irving BA</b>
<b>Iyer Shilpa (guest tutor)</b>	Virginia Commonwealth University – <b>US_VA Richmond_Iyer S</b>
<b>Kamath Vasudeva Gurupur</b>	College of Medicine, Central Michigan University, Nucleoside, Nrtl – <b>US_MI Mt Pleasant_McKee E</b>



<b>Karlsson Michael</b>	Mitochondrial Pathophysiology Unit, University Lund, Malaria, sepsis, multiple organ failure – <b>SE_Lund_Elmer E</b>
<b>Kiss Gergely</b> (guest tutor)	Semmelweis University – <b>HU_Budapest_Chinopoulos C</b>
<b>Konrad Csaba</b> (guest tutor)	Semmelweis University – <b>HU_Budapest_Chinopoulos C</b>
<b>Lai Nicola</b>	Case Western Reserve University School of Medicine, Department of Pharmacology, Center for Mitochondrial Diseases, Oxygen transport and energy metabolism, skeletal muscle mitochondrial function in Type 2 Diabetes – <b>US_OH Cleveland_Hoppel CL</b>
<b>Le Catherine</b> (guest tutor)	Colorado State University Fort Collins - <b>US_CO Fort Collins_Chicco AJ</b>
<b>Leo Elettra</b>	Alfred Wegener Institute for Polar and Marine Research, Mitochondrial complexes, temperature, pH, membrane potential, ATP turnover – <b>DE_Bremerhaven_Mark FC</b>
<b>Leonhardt Andrea</b>	Sanofi-Aventis Deutschland GmbH, Respiriometry, ATP production, skeletal muscle function, mitochondrial biogenesis, sarcopenia and muscle atrophy – <b>DE_Frankfurt_Leeuw T</b>
<b>Lundby Stine</b>	Institute of Physiology, University Zuerich, Skeletal muscle mitochondrial function – <b>CH_Zurich_Lundby C</b>
<b>Petrosillo Guiseppe</b>	Department of Biochemistry and Molecular Biology, University Bari, Mitochondria, cells, oxygen consumption, membrane potential, proton efflux – <b>IT_Bari_Atlante A</b>
<b>Pidcoke Heather</b>	US Army Institute of Surgical Research, Mitochondrial impact on platelet biology and function, <b>US_TX Houston_Cap A</b>
<b>Romestaing Caroline</b>	Laboratoire de Physiologie Intégrative, Cellulaire et Moléculaire, Mitochondria in ecophysiology, energy allocation, oxidative stress, ROS, ATP, fitness – <b>FR_Villeurbanne_Romestaing C</b>
<b>Senyilmaz Deniz</b>	German Cancer Research Center (DKFZ), Signal transduction in cancer and metabolism – <b>DE_Heidelberg_Teleman A</b>
<b>Shahini Albana</b>	NeuroVive, Pharmaceuticals AB, Mitochondrial pathophysiology – <b>SE_Lund_Elmer E</b>
<b>Sirsat Tushar</b>	Department of Biological Sciences, University of North Texas, Endothermy, avian, reptile, cardiac, skeletal, liver, hypoxia – <b>US_TX Denton_Dzialowski EM</b>
<b>Sparks Lauren</b>	Burnham Institute for Medical Research, Muscle mitochondria, fatty acids, human cells – <b>US_FL Orlando_Smith SR</b>
<b>Surampudi Vasudha</b>	Center for the Study of Biological Complexity, Virginia Commonwealth University, Respiriometric studies in stem cells – <b>US_VA Richmond_Iyer S</b>
<b>Towheed Mohammad Atif</b>	Pittsburgh Institute for Neurodegenerative Diseases (PIND), University of Pittsburgh, Mitochondrial encephalomyopathies, Drosophila, ATP6, ATP synthase, OXPHOS – <b>US_PA Pittsburgh_Palladino MJ</b>
<b>Tsygankova Polina</b>	Lab metab. Disorders, Research centre for medical genetics, Pdh-deficiency, Alpers syndrome, Leigh syndrome, mitochondrial DNA depletion syndromes – <b>RU_Moscow_Tsygankova P</b>
<b>Votion Dominique-Marie</b> (guest tutor)	University of Liège – <b>BE_Liege_Votion DM</b>
<b>Wohlwend Martin</b>	K.G. Jebsen Centre of Exercise in Medicine, Norwegian University of Science and Technology (NTNU), Exercise, mitochondria, aging – <b>NO_Trondheim_Rognmo O</b>

## MiPNet Abstracts IOC72: 10+5 min

### Hot topics in Mitochondrial Physiology

**Online:** [www.bioblast.at/index.php/IOC72\\_Abstracts\\_MiPNet17.13](http://www.bioblast.at/index.php/IOC72_Abstracts_MiPNet17.13)

**Continue the discussion:**

[www.bioblast.at/index.php/Talk:IOC72\\_Abstracts\\_MiPNet17.13](http://www.bioblast.at/index.php/Talk:IOC72_Abstracts_MiPNet17.13)

Dzialowski EM, Sirsat T, Goy S (2012) **T3: Does it really make the alligator go "Tick, Tock"?**

Horscroft J, Kotwica AO, Ashmore T, Colleoni F, Morash AJ, Gilbert E, Gnaiger E, Martin DS, Murray AJ (2012) **Mitochondrial function at altitude in lowland Europeans and highland Sherpas.**

Irving BA (2012) **AgRP deficient female mice have elevated body weight and hypothalamic mitochondrial oxidative capacity.**

Kamath VG, Lizenby ZJ, Adusumilli VRKR, Hsiung CH, McKee EE (2012) **Measuring mitochondrial OXPHOS as a nucleoside reverse transcriptase inhibitor induced cardiac toxicity endpoint.**

Rodrigues MF, Amoedo ND, Rumjanek FD (2012) **Studies of bioenergetics alterations in breast cancer lines induced by sodium butyrate.**

Towheed MA (2012) **Elucidating pathogenesis of ATP synthase dysfunction in a *Drosophila* model of mitochondrial encephalomyopathy.**

Konrad C, Kiss G, Torocsik B, Adam-Vizi V, Chinopoulos C (2012) **Absence of  $\text{Ca}^{2+}$ -induced mitochondrial permeability transition but presence of bongrekate-sensitive nucleotide exchange in *C. crangon* and *P. serratus*.**

Kiss G, Konrad C, Doczi J, Starkov AA, Kawamata H, Manfredi G, Zhang SF, Gibson GE, Beal MF, Adam-Vizi V, Chinopoulos C (2012) **The negative impact of alpha-ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation.**

## Accommodation and Location

**Hotel Körbersee** [www.koerbersee.at](http://www.koerbersee.at)  
T +43 5519 265; [hotel@koerbersee.at](mailto:hotel@koerbersee.at)



## Further information

**O2k-Manual** – [www.oroboros.at/?O2k-Manual](http://www.oroboros.at/?O2k-Manual)

**O2k-Protocols** – [www.oroboros.at/?O2k-Protocols](http://www.oroboros.at/?O2k-Protocols)

**O2k-Publications** – [www.oroboros.at/?O2k-Publications](http://www.oroboros.at/?O2k-Publications)

## Recommended Reading

- 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.
- Lemieux H, Semsroth S, Antretter H, Hoefer 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, 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.
- 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 (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: *Mitochondrial Dysfunction in Drug-Induced Toxicity* (Dyken JA, Will Y, eds) John Wiley: 327-352. – *A methodological introduction into high-resolution respirometry*.
- 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.
- 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, MiR05*.

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[www.orooboros.at/?MitoCom-Tyrol](http://www.orooboros.at/?MitoCom-Tyrol)



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[www.mipart.at](http://www.mipart.at)

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