



Course on High-Resolution Respirometry

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

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72nd International Workshop on HRR

2012 December 05-10
Schröcken, Vorarlberg, Austria



The **72nd Workshop on High-Resolution Respirometry (HRR)** is the **28th** 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 Burtcher

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	-
Arrival		
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	<i>Welcome reception at Hotel Körbersee.</i>	
19:00	<i>Dinner</i>	
20:30-21:00	Get-together: Introduction of participants and their research interests	

2	Thursday, December 06	
Workshop 1		Weblink
07:30-08:30	<i>Breakfast</i>	
08:00	<i>Organize loan of skiing equipment</i>	
O2k-Core	Principles of high-resolution respirometry - from switching on the Oxygraph-2k to the experimental result	Gnaiger 2008 POS
08:30-09:00	Erich Gnaiger: Get O2k-Connected with your OROBOROS-USB: a guided tour to the O2k	get O2k-Connected
09:00-10:00	Instrumental quality control 1: The oxygen sensor OroboPOS - calibration, stability testing, and evaluation of sensitivity to measure oxygen flux.	O2k-Calibration
10:00	<i>Organize special lunch or bad weather task groups (fibre and homogenate prep, fluorometry and spectrophotometry, NO, pH and Ca, Bioblast wiki login) and loan of skiing equipment</i>	
11:00	<i>Practice - skiing, walk & talk, or individual O2k-tasks / Lunch</i>	
14:00	<i>Coffee / Tea</i>	
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 H₂O₂ production (add-on O2k-Fluorescence LED2-Module)	O2k-Fluorometry Workshop
16:00	<i>Coffee / Tea</i>	
16:30-17:15	DatLab Guide through the menus: DL-Demo files and DL-Excel templates	DatLab Guide
17:15-18:00	Experimental design 1: Coupling control of mitochondrial respiration: LEAK, OXPHOS, ETS, ROX	DatLab Flux Analysis
18:30	<i>Dinner</i>	

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

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

4 Saturday, December 08

Workshop 3		Weblink
07:30	<i>Breakfast</i>	
08:30-09:30	<p>MiPNet-Lectures by guest tutors Chuck Hoppel (US): OXPHOS protocols for diagnosis of mitochondrial diseases with isolated mitochondria. Steve Hand (US): OXPHOS protocols for the study of mitochondrial function in active and dormant states.</p>	
09:30-10:00	DatLab O₂ flux analysis: Flux per volume, flux per mass, flow per cell, flux control ratio	Glossary: Respiratory states
10:00	<i>Practice - skiing, walk & talk, or individual O2k-tasks / Lunch</i>	
14:00	<i>Coffee / Tea</i>	
14:30-16:00	O2k-Demo experiment 2: SUIT protocol with mouse permeabilized fibres and on-line DatLab analysis	Pesta 2012 Methods Mol Biol

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

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

6 Monday, December 10	
Departure	
<i>Breakfast</i>	
Early morning: Departure	

IOC72 List of Participants

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

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

MiPNet Abstracts IOC72: 10+5 min

Hot topics in Mitochondrial Physiology

Online: www.bioblast.at/index.php/IOC72_Abstracts_MiPNet17.13

Continue the discussion:

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 Ca²⁺-induced mitochondrial permeability transition but presence of bongkrekate-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

T +43 5519 265; hotel@koerbersee.at



Further information

O2k-Manual – www.oroboros.at/?O2k-Manual

O2k-Protocols – www.oroboros.at/?O2k-Protocols

O2k-Publications – 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, Hofer 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* (Dykens 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, MiRO5.*

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www.oroboros.at/?MitoCom-Tyrol



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