



## Course on High-Resolution Respirometry

**IOC49.** *Mitochondrial Physiology Network* 14.2: 1-9 (2009)

©2009 OROBOROS®

Last update: 2009-02-19

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

ICBR, Interdisciplinary Center for Biotechnology  
Research  
1376 Mowry Road, Gainesville, Florida, USA



**23-25 February 2009** Gainesville, Florida, USA

Local organizer:

Christiaan Leeuwenburgh, PhD, Associate Prof.  
Dept Aging and Geriatric Research, University of Florida  
1329 SW 16th St., Rm. 5277  
Gainesville FL 32610-0107, USA



O2k-Course (IOC41, July 2007) - POS service (from left to right: Hélène Lemieux, Steffi Wohlgemuth, Danina Munteaux, David Julian, foreground Francesca Scandurra and Zuzana Jilkova).



The OROBOROS Oxygraph-2k for high-resolution respirometry, the Titration-Injection microPump (TIP-2k) on top of the O2k, and the Integrated Suction System (ISS) on the right.

The 49<sup>th</sup> O2k-Course provides a special opportunity for an expert introduction into high-resolution respirometry in the USA. This O2k-Course presents a practical overview of the **Oxygraph-2k**, with integrated on-line analysis by **DatLab 4.3** (new upgrade), and application of the **TIP-2k**. 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. Four Oxygraph-2k instruments are available for a do-it-yourself application of both hardware and software.

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.



## Tutors

**Erich Gnaiger**, Medical University of Innsbruck, and OROBOROS INSTRUMENTS, Innsbruck, Austria

**Mario Fasching**, OROBOROS INSTRUMENTS, Innsbruck, Austria

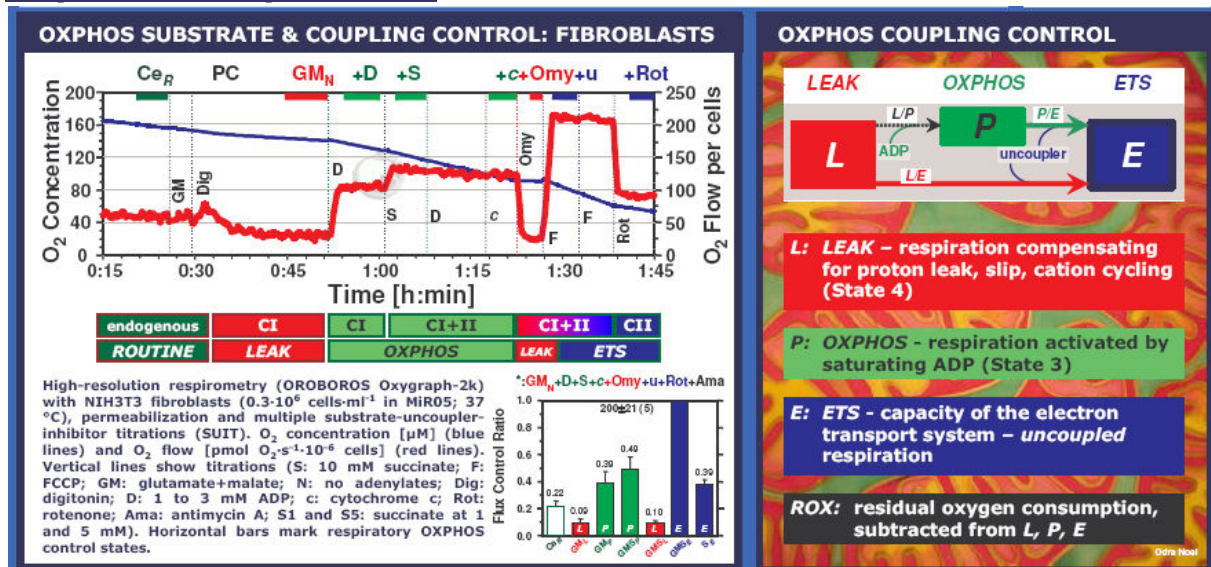
**Stephanie Wohlgemuth**, University of Florida, FL-Gainesville, USA

## Programme IOC49

### Sunday, Feb 22: O2k set-up

13:00 – 16:00 **Participants meet with their Oxygraph-2k** at: ICBR (Interdisciplinary Center for Biotechnology Research) 1376 Mowry Road, Gainesville, FL 32610 to set up their instrument together with the tutors (directions & map page 7).

### Day 1: Monday, Feb. 23



8:00 – 10:00 Session 1: Erich Gnaiger: Introduction to high-resolution respirometry and mitochondrial respiratory physiology

10:00 – 10:30 Coffee break (coffee stand located in the building)

10:30 – 12:15 Session 2: Mario Fasching: Introduction to O2k-design and function.

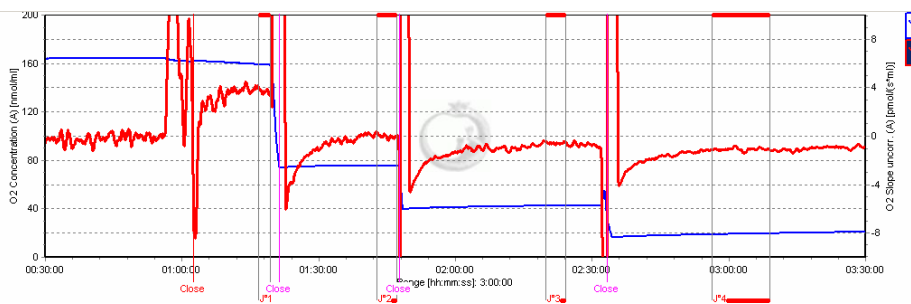
12:15 – 14:00 Lunch break (food court in the Health Science building, ca. 15 min walk)

14:00 – 17:30 Session 3: Hands on - O2k setup and service

17:30 – 18:30 Session 4: Summary of instrumental setup – Questions (p. 4)

**Day 2: Tuesday, Feb. 24**

8:00 – 09:00 Session 5: Erich Gnaiger and Mario Fasching: O2k calibration and instrumental background oxygen flux



09:00 – 10:00 Session 6: Hands on - O2k calibration and instrumental background oxygen flux

10:00-10:30 Coffee break

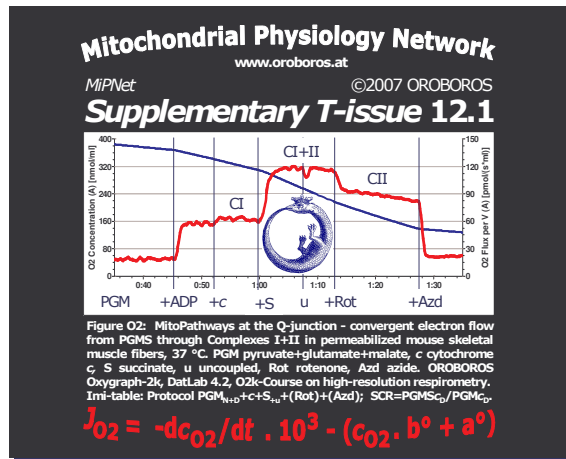
10:30 – 12:15 Session 6 (continued)

12:15 – 14:00 Lunch break (food court in the Health Science building, ca. 15 min walk)

14:00 – 15:30 Session 6: Introduction to DatLab and analysis of instrumental background

15:30 – 16:30 Seminar in the Zoology/Biology Department in Bartram Hall (15 min walk minimum from Cancer Genetics Building)

**Erich Gnaiger: Mitochondrial respiratory control in heart and skeletal muscle: Evaluation of the mouse model from the perspective of comparative mitochondrial physiology.**



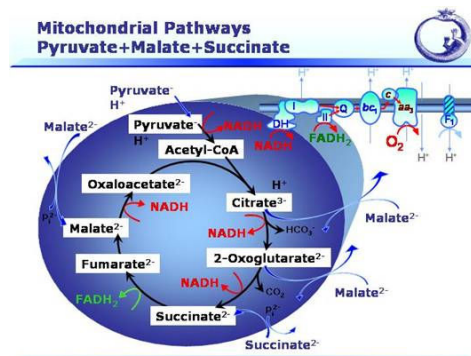
17:00 – 18:30 Session 7: Hands on – DatLab analysis (Questions – page 4)

**Day 3: Wednesday, Feb. 25**

8:00 – 9:00 Session 7: Hands on session - the O2k: calibration, DatLab on-line

9:00 – 10:00 Session 8: Erich Gnaiger: Substrate-uncoupler-inhibitor titration protocols. High-resolution respirometry with mitochondrial preparations.

10:00 – 10:30 Coffee break



10:30 – 12:00	Session 8 (continued)
12:00 – 13:30	Lunch break (food court in the Health Science building, ca. 15 min walk)
13:30 – 14:30	Session 9: Hands on - DatLab analysis of experimental oxygen flux
14:30 – 15:30	Session 10: Special topics; , troubleshooting (Questions – page 4)
15:30 – 17:00	Conclusions, summary, discussion

**Thursday, Feb. 26**

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.

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

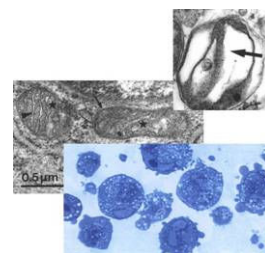
- Instrumental background: measurement and correction as a function of  $pO_2$ .
- High resolution of respiratory flux at various steady-states.
- The Titration-Injection microPump TIP-2k: automatic titrations.
- Conceptual and methodological advantages of measurement at physiological low levels of oxygen.

**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> edition: online [www.orooboros.at](http://www.orooboros.at)

**Questions for the O2k-Course**

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

**Oxygraph-2k assembly** (O2k-Manual 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 (O2k-Manual #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 (O2k-Manual #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 (O2k-Manual #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 (O2k-Manual #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?

## Literature

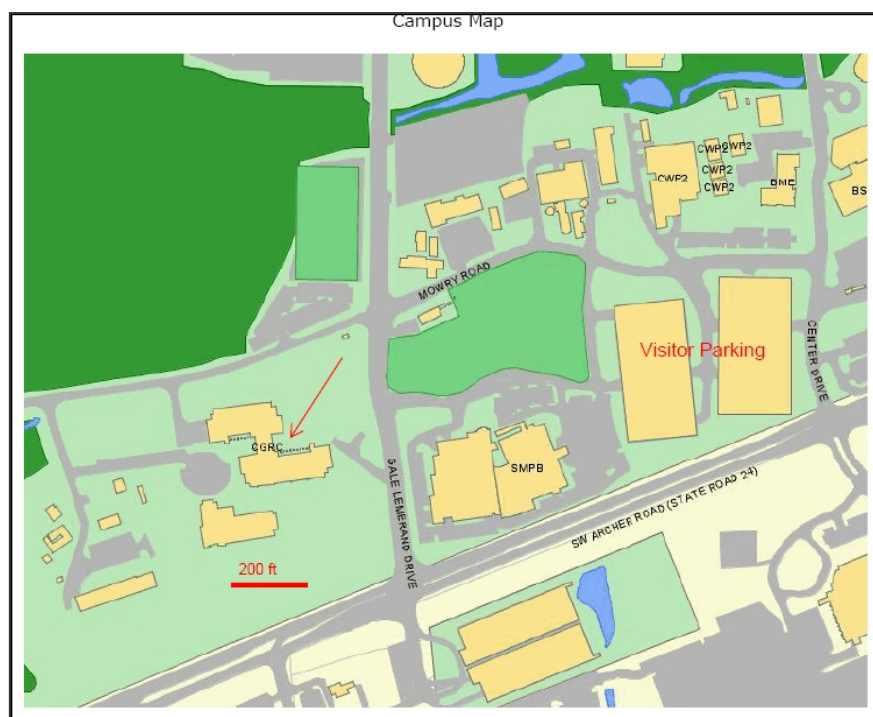
- Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial Dysfunction in Drug-Induced Toxicity (Dykens JA, Will Y, eds) John Wiley: 327-352. – *A methodological introduction into high-resolution respirometry, with 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 (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:*
- 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/O<sub>2</sub> 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*
- Gnaiger E (2009) Mitochondrial Pathways to Complex I: Respiration with Pyruvate, Glutamate and Malate. In: Mitochondrial Pathways and Respiratory Control. OROBOROS MiPNet Publications 2009, 2<sup>nd</sup> ed.  
www.orooboros.at/index.php?id=o2k-protocols

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

1. Oxygraph-2k and Manual
2. MiPNet Protocols
3. O2k-Publications
4. WorldWide Mitochondrial Physiology Network

## Directions & Contacts

ICBR, Interdisciplinary Center for Biotechnology Research 1376 Mowry Road, Gainesville



**The ICBR is located in the South Wing of the first floor of the Cancer and Genetics Research Complex (CGRC).**

At the intersection of Gale Lemerand Dr (was North-South Dr) and 1900 SW Archer Rd:

**Turn North** onto Gale Lemerand Dr (was North-South Dr) from SW Archer Rd.

**Turn West** (left) onto Mowry Rd.

**Turn South** (left) at the CGRC/ICBR sign. South Wing of the first floor of the Cancer and Genetics Research Complex

Sunday: O2k set-up between 1-4pm (Stephanie Wohlgemuth; Mobile 352-213-2111)

Monday: 8 am (Brian Bouverat; Mobile 352-283-2983)

## Participants and Areas of Interest

**Bossy Blaise**, Burnett School of Biomedical Sciences, College of Medicine University of Central Florida, FL-Orlando, USA. - [bbossy@mail.ucf.edu](mailto:bbossy@mail.ucf.edu)

**Chen Jing**, PhD, University of Florida, FL-Gainesville, USA. - [jic24@ufl.edu](mailto:jic24@ufl.edu) [*Role of mitochondrial DNA mutations in mouse and human diabetes.*]

**Crane Justin**, Department of Pediatrics and Medicine, McMaster University Medical Center, Hamilton, Canada. - [jcrane19@gmail.com](mailto:jcrane19@gmail.com) [*Human and animal skeletal muscle, mitochondrial respiration with regards to aging, oxidative stress and mitochondrial myopathies.*]

- Darmyn Ritchie**, Faculty of Medicine, University of Calgary, Calgary, Canada. - [ddritchi@ucalgary.ca](mailto:ddritchi@ucalgary.ca) [*Mitochondrial dysfunction in aging skeletal muscle.*]
- Dutta Debapriya (Priya)** Biochemistry of Aging Laboratory, Dept. of Aging and Geriatric Research, University of Florida, FL-Gainesville, USA. - [ddutta@aging.ufl.edu](mailto:ddutta@aging.ufl.edu)
- Fasching Mario**, PhD, OROBOROS INSTRUMENTS, Innsbruck, Austria. - [mario.fasching@oroboros.at](mailto:mario.fasching@oroboros.at)
- Gnaiger Erich**, PhD, Prof., Dept. General and Transplant Surgery, Medical University of Innsbruck, D. Swarovski Research Laboratory Innsbruck, Austria; and OROBOROS INSTRUMENTS; Austria. - [erich.gnaiger@oroboros.at](mailto:erich.gnaiger@oroboros.at)
- Hwang Judy**, Biochemistry of Aging Laboratory, Dept. of Aging and Geriatric Research, University of Florida, FL-Gainesville, USA. - [jhwang@aging.ufl.edu](mailto:jhwang@aging.ufl.edu)
- Johnson John**, Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, FL-Orlando, USA. - [johnajoh@mail.ucf.edu](mailto:johnajoh@mail.ucf.edu)
- Joseph Anna-Maria**, Biochemistry of Aging Laboratory, Dept. of Aging and Geriatric Research, University of Florida, FL-Gainesville, USA. - [annamj@yorku.ca](mailto:annamj@yorku.ca)
- Julian David**, PhD, Dept. of Zoology, University of Florida, FL-Gainesville, USA. - [djulian@ufl.edu](mailto:djulian@ufl.edu)
- King Adrienne**, Nutrition Sciences, Department of Environmental Health Sciences University of Alabama at Birmingham. - [AKing@ms.soph.uab.edu](mailto:AKing@ms.soph.uab.edu) [*Alcoholic liver disease and mitochondrial dysfunction. In particular how calcium may effect mitochondrial respiration.*]
- Koves Timothy R**, PhD, Sarah W. Stedman Nutrition & Metabolism Center, Duke University Medical Center, NC-Durham, USA. - [koves001@mc.duke.edu](mailto:koves001@mc.duke.edu) [*Mitochondrial phenotypes underlying the development or protection against skeletal muscle insulin resistance.*]
- Leeuwenburgh Christiaan**, PhD, Prof., Dept Aging and Geriatric Research University of Florida, FL-Gainesville, USA. - [cleeuwen@aging.ufl.edu](mailto:cleeuwen@aging.ufl.edu)
- Lucioni Jumbo Patricia P**, PhD Student, Nutrition Sciences, Department of Environmental Health Sciences University of Alabama at Birmingham, AL-Birmingham, USA. - [patjumbo@uab.edu](mailto:patjumbo@uab.edu) [*Mitochondrial mechanisms of hydrogen sulphide induced suspended animation. Natural variations in mitochondrial bioenergetic traits in Drosophila melanogaster.*]
- Manani Todd**, Department of Aging and Geriatrics, and Rehabilitation Research Center (ARRC), University of Florida, Aging FL-Gainesville, USA. - [tmanani@aging.ufl.edu](mailto:tmanani@aging.ufl.edu)
- Martello Ralph D**, PhD, University of Florida, FL-Gainesville, USA. - [martelr@ufl.edu](mailto:martelr@ufl.edu)
- Muoio Debbie**, Sarah W. Stedman Nutrition & Metabolism Center, Duke University Medical Center, NC-Durham, USA.



**Niedziela Genevieve**, PhD, Medical Neurogenetics, GA-Atlanta, USA. - [GNiedziela@MedicalNeurogenetics.com](mailto:GNiedziela@MedicalNeurogenetics.com)

**Song Huang**, PhD Student, Department of Biochemistry, UT Southwestern Medical Center at Dallas, TX-Dallas, USA. - [Song.Huang@UTSouthwestern.edu](mailto:Song.Huang@UTSouthwestern.edu) [*Warburg effect.*]

**Wahl Dan**, University of Michigan, MI-Ann Arbor, USA. - [dwahl@umich.edu](mailto:dwahl@umich.edu) [*Bioenergetics of pathologic lymphocytes.*]

**Wohlgemuth Stephanie**, PhD, Dept Aging and Geriatric Research, University of Florida, FL-Gainesville, USA. - [swohlgemuth@aging.ufl.edu](mailto:swohlgemuth@aging.ufl.edu)

## 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  
 Email [erich.gnaiger@i-med.ac.at](mailto:erich.gnaiger@i-med.ac.at)  
<http://www.mitophysiology.org>

**OROBOROS INSTRUMENTS**  
 high-resolution respirometry

**Oxygraph-2k**



Schöpfstrasse 18  
 A-6020 INNSBRUCK, Austria  
 T/F +43 512 566796  
 Email [instruments@oroboros.at](mailto:instruments@oroboros.at)  
 Homepage: [www.oroboros.at](http://www.oroboros.at)  
 Cooperation and Feedback in Science

**MiPart - 'Mitochondria - the Organism in our Cells'**  
 Mitochondria meet Art – [www.mipart.at](http://www.mipart.at)

