IOC91 Mitochondrial Physiology Network 19.06: 1-4 (2014)
Updates: www.bioblast.at/index.php/MiPNet19.06

Version 4: 2014-05-30

91st Workshop on High-Resolution Respirometry & O2k-Fluorometry

2014 June 2-3
Philadelphia, PA, USA

Pre-conference workshop: Mitochondrial Medicine, Pittsburgh, USA. UMDF 2014 June 4-7

Venue:
Center for Mitochondrial and Epigenomic Medicine (CMEM)
The Children's Hospital of Philadelphia - Research Institute
Colket Translational Research Building

Host:
Douglas C Wallace, MD, PhD, Prof.
Alessia Angelin, PhD, Sherri Ghee
Center for Mitochondrial and Epigenomic Medicine (CMEM)
The Children's Hospital of Philadelphia - Research Institute
Colket Translational Research Building
AngelinA@email.chop.edu; ghees@email.chop.edu
www.bioblast.at/index.php/US_PA_Philadelphia_Wallace_DC

Lecturers and tutors:
Erich Gnaiger, Ao.Univ.-Prof. PhD
Verena Laner, Mag.
OROBOROS INSTRUMENTS Corp
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The 91st O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is an **Oxygraph-2k Workshop** held in cooperation with one of our prominent MiPNet Labs in Philadelphia. The O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS Oxygraph-2k** with integrated on-line analysis, introducing new features of **DatLab 5.2**.

The workshop will include a discussion on optimization of OXPHOS analysis in various mitochondrial (mt) preparations (permeabilized muscle fibres, tissue homogenate, isolated mitochondria). HRR provides information on cell respiration with simple phosphorylation control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations, to evaluate coupling efficiencies and OXPHOS capacities with carbohydrate versus fatty acid substrates, and to diagnose defects in respiratory complexes of the electron transfer system and phosphorylation system. Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols** in HRR using the **O2k-Fluorescence LED2-Module** for simultaneous measurement of hydrogen peroxide production (Amplex red®). Discussions are extended on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TPP^+ or $TPMP^+$ (potentiometric), and on perspectives of HRR in mitochondrial physiology.

Dr. DC Wallace will open the workshop. Several OROBOROS MiPNet Reference Labs will be represented. In particular, Drs. MJ Falk, BA Irving and C Sims will provide their views on 'Sharing our experience as a MiPNet Lab'.

Program IOC91

Monday, June 2:

08:45 Registration

09:00 – 09:15 **Douglas C Wallace (CMEM)** Welcome – perspectives of mt-function and mt-medicine.

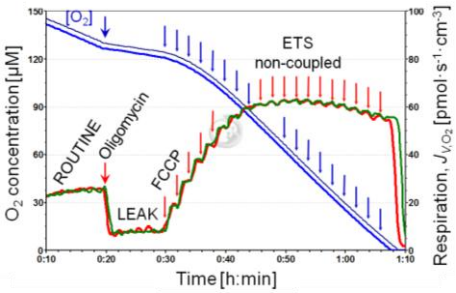
09:15 – 09:30 **Introduction of participants:** who is who?

09:30 – 10:30 **Erich Gnaiger: Get started with the Oxygraph-2k.**

10:30 *Coffee break – Registration ctn.*

11:00 – 12:15 **Pro's and con's of mt-preparations:** Coupling and substrate control of O_2 consumption and H_2O_2 production in homogenate, permeabilized fibres – or isolated mitochondria?

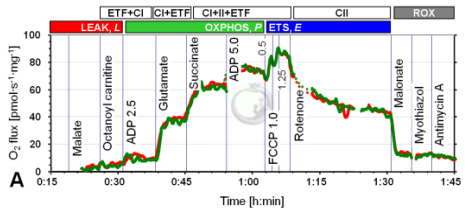
12:15 – 12:30 **Brian A Irving** (Geisinger Health System, Danville, PA) Mitochondrial homogenate preparation: PBI-Shredder.



12:30 *Lunch*

13:15 – 14:00 **Phosphorylation protocol for intact cells.**

14:00 – 15:00 **Comprehensive OXPHOS analysis:** A challenge for simultaneous measurements of respiration and mt-membrane potential: solving a puzzle.



15:00 – 15:30 **Experimental setup 1:** OroboPOS - sensor quality control, calibration.

15:30 *Coffee Break*

16:00 – 17:00 **Experimental setup 2:** Calibration of O2k-Fluo Sensors

17:00 – 17:30 **Marni J Falk** (CHOP) / **Carrie Sims** (Div Traumatol Surgical Crit Care, Univ Penn): Sharing our experience as a MiPNet Lab.

17:30 – 18:00 **Q&A session on HRR and OXPHOS analysis:** Design of experimental protocol - day 2.

18:30 *O2k-Workshop dinner*

Tuesday, June 3:

09:00 – 10:30 **Experiment:** HRR and O2k-Fluorometry with intact cells – respiration and extracellular H_2O_2 production.

10:30 *Coffee break*

11:00 – 12:30 **Experiment** continued

12:30 *Lunch*

13:15 – 15:30 **Data analysis**

15:30 *Coffee break*

16:00 – 16:40 **Trouble shooting**

16:40 – 18:00 **Feedback – conclusions – stay connected** as a MiPNet Lab.

IOC91 Participants

Lab US PA Philadelphia Wallace DC

www.bioblast.at/index.php/US_PA_Philadelphia_Wallace_DC

Douglas Wallace, host
Angelin Alessia, co-organizer
Colas Carman, Haroon Suraiya, Holczbauer Agnes, Kopinski Piotr, Lvova Masha, McCormack Shana, Morrow Ryan, Ortiz-Gonzales Xilma, Peng Min, Polyak Zsoka, Potluri Prasanth, Tintos Alonso, Vermulst Mark, Wang Ting

Other labs

Barsotti Robert, US_PA Philadelphia_Barsotti R: Department of Neuroscience, Physiology & Pharmacology, Philadelphia College of Osteopathic Medicine.

Breton Sophie, CA_Montreal_Breton S: Department of Biological Sciences, University of Montréal. - Mitochondrial DNA divergence and aerobic capacity in invertebrates.

Curtis Jessica, US_MD Baltimore_Curtis J: Translational Gerontology Branch, National Institute on Aging, Baltimore. - Respiration, ROS, fluorometry, ADP titration.

DeBoer Erik, US_PA Philadelphia_DeBoer E: The Children's Hospital of Philadelphia.

Dometita Crystal, US_PA Danville_Irving BA: Obesity Institute, Center for Nutrition and Weight Management, Geisinger Health System, Danville.

Falk Marni J, US PA Philadelphia Falk MJ: Sharing our experience as a MiPNet Lab.

Gnaiger Erich, AT Innsbruck Gnaiger E: Lecturer, OROBOROS INSTRUMENTS.

Gómez Luis A, CO_Medellin_Gomez LA: Department of Basic Sciences, EAFIT University, Medellin. - Electron transfer system, cardiolipin, supercomplexes.

Guan Yuxia, US_PA Philadelphia_Sims C: University of Pennsylvania, Philadelphia.

Hao Ling-Yang, US_MI Ann Arbor_Glick GD: Lycera Corp., Ann Arbor.

Hsiao Chao-Pin, US_OH Cleveland_Hsiao CP: School of Nursing, Case Western Reserve University, Cleveland. - Mitochondrial bioenergetic profiles, fatigue, prostate cancer, radiotherapy.

Irving Brian A, US_PA Danville_Irving BA: Obesity Institute, Center for Nutrition and Weight Management, Geisinger Health System, Danville. - Sharing our experience as a MiPNet Lab

Kavanagh Robert P, US_PA Philadelphia_Kavanagh RP: The Children's Hospital of Philadelphia. - TBI, cardiac arrest, neurologic injury.

Kienesberger Petra, CA_Saint John_Pulinilkunnil T: Department of Biochemistry and Molecular Biology, Dalhousie University, Saint John. - Autotaxin, lysophospholipids, heart, triacylglycerol, obesity.

Laner Verena, AT Innsbruck OROBOROS: Tutor, OROBOROS INSTRUMENTS.

Moellering Douglas R, US_AL Birmingham_Moellering DR: Department of Nutrition Sciences, University of Alabama at Birmingham. - Mitochondria, Bioenergetics, ROS, Diabetes, Obesity, CMD, Aging.

Morgan Rodney, US_MI Ann Arbor_Glick GD: Lycera Corp., Ann Arbor.

Previte Dana, US_PA Pittsburgh_Previte D: Immunology Program, University of Pittsburgh. - Immunology, CD4+ T cells.

Pulinilkunnil Thomas, CA_Saint John_Pulinilkunnil T: Department of Biochemistry and Molecular Biology, Dalhousie University, Saint John. - Autophagy, mitophagy, branched chain amino acid, leucine, heart, muscle.

Sims Carrie, US PA Philadelphia Sims C: Sharing our experience as a MiPNet Lab

Wade Mark, US_IN Indianapolis_Brozinick JT: Eli Lilly and Co., Indianapolis.

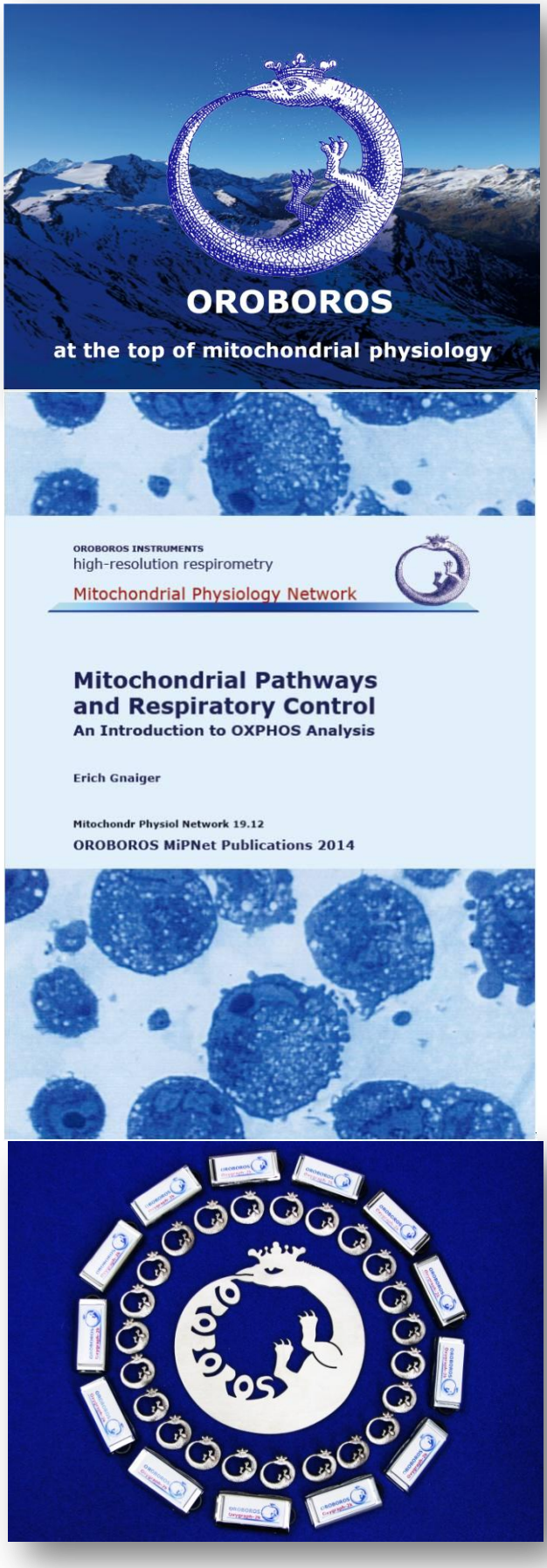
Weiss Scott, US_PA Philadelphia_Becker LB: The Children's Hospital of Philadelphia. - Sepsis, pediatrics, peripheral blood mononuclear cells.

Zhang Donglan, US_PA Philadelphia_Zhang D: The Children's Hospital of Philadelphia. - Intact cells.

www.oroboros.at **www.bioblast.at** - the *information synthase* for Mitochondrial Physiology and high-resolution respirometry

Contribution to K-Regio *MitoCom Tyrol*:

This project is funded by the Tyrolean Government and the European Regional Development Fund (ERDF) and is subject to the regulations of EU law as well as to the Directive of the Tyrolean Government on the Funding of Science, Research and Development.



Recommended reading

O2k-Core Manual

New: **»O2k-Core Manual.pdf**

SUIT protocols for high-resolution respirometry

Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopisies of human muscle. Methods Mol Biol 810: 25-58. »Bioblast Access

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: 327-52. »Bioblast Access

HRR and O2k-Fluorometry

» Manual: O2k-Fluorescence LED2-Module
Eigentler A, Fontana-Ayoub M, Gnaiger E (2013) O2k-Fluorometry: HRR and H_2O_2 production in mouse cardiac tissue homogenate. Mitochondr Physiol Network 18.05(01): 1-6.

» O2k-Fluorometry

Mitochondrial pathways

Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. OROBOROS MiPNet Publications, Innsbruck: 80 pp. »Open Access - ***handout to O2k-Workshop participants***