



## Course on high-resolution respirometry

**IOC104** Mitochondrial Physiology Network 20.05(01): 1-3 (2015)  
Updates: [http://wiki.orooboros.at/index.php/MiPNet20.05\\_IOC104](http://wiki.orooboros.at/index.php/MiPNet20.05_IOC104)

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# 104<sup>th</sup> Workshop on O2k high-resolution respirometry & O2k-Fluorometry

**2015 August 09-10**  
**Greenville, NC, USA**

*Pre-conference workshop:*  
*[MiPschool Greenville](#) 2015, USA.*  
*August 09-10*

### Venue:

East Carolina Diabetes & Obesity Institute  
East Carolina University  
East Carolina Heart Institute Building at ECU

### Host:

P. Darrell Neuffer, Ph.D., Professor, Director ECDIO  
David A. Brown, Associate Professor  
Ashley Busada  
East Carolina University, Brody School of Medicine, NC, USA  
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[http://bioblast.at/index.php/US\\_NC\\_Greenville\\_Neuffer\\_PD](http://bioblast.at/index.php/US_NC_Greenville_Neuffer_PD)

### Lecturers and tutors:

Erich Gnaiger, Ao.Univ.-Prof. PhD  
Verena Laner, Mag.

### OROBOROS INSTRUMENTS

O2k high-resolution respirometry  
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This O2k-Workshop on high-resolution respirometry and O2k-Fluorometry is held in cooperation with one of our prominent O2k-Network Labs in Greenville. The O2k-Workshop includes a basic introduction to quality control of instrumental performance of the **OROBOROS O2k** with integrated on-line analysis, introducing new features of **DatLab 6**.

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<sup>+</sup> or TPMP<sup>+</sup> (potentiometric), and on perspectives of HRR in mitochondrial physiology.



# Program IOC

## Sunday, August 09:

08:45 Registration  
 09:00 – 09:15 Welcome  
 09:15 – 09:30 Introduction of participants: who is who?  
 09:30 – 10:30 Erich Gnaiger: Get started with the O2k.

10:30 Coffee break – Registration ctn.

11:00 – 12:15 **Pro’s and con’s of mt-preparations:** Coupling and substrate control of O<sub>2</sub> consumption and H<sub>2</sub>O<sub>2</sub> production in homogenate, permeabilized fibres – or isolated mitochondria?  
 12:15 – 12:30 Permeabilized fibre preparation – what to take care of?

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 **Neufer P. Darrel:** Sharing our experience as an O2k-Network Lab.

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

18:30 O2k-Workshop dinner

## Monday, August 10:

09:00 – 10:30 **Experiment:** HRR and O2k-Fluorometry with intact cells – respiration and extracellular H<sub>2</sub>O<sub>2</sub> production.

10:30 Coffee break

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

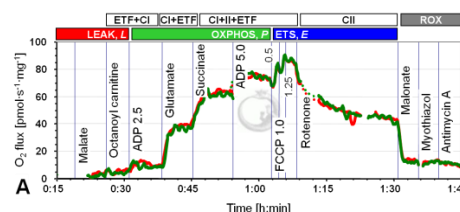
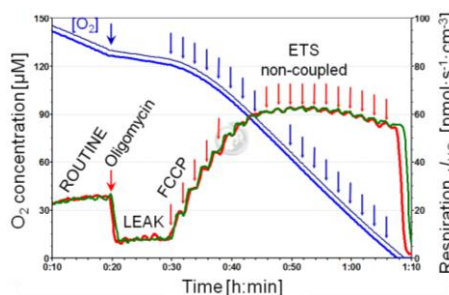
12:30 Lunch

13:15 – 15:30 **Data analysis**

15:30 Coffee break

16:00 – 16:40 **Technical support**

16:40 – 18:00 **Feedback – conclusions – stay connected** as an O2k-Network Lab



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the *information* *synthase* for  
Mitochondrial Physiology and O2k high-  
resolution respirometry

## Recommended reading

### O2k-Core Manual

[»O2k-Core Manual«](#)

### SUIT protocols for O2k high-resolution respirometry

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.

[»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<sub>2</sub>O<sub>2</sub> 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.

[»Bioblast Access«](#)

