

## 132<sup>nd</sup> International Workshop on High- Resolution FluoRespirometry

2018 August 24-25  
Budapest, Hungary

**Venue:**

Department of Medical Biochemistry  
Semmelweis University  
Budapest, Hungary  
37-47 Tuzolto Str, HU-1088

**Host:**

Prof. László Tretter  
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The **132<sup>nd</sup> O2k-Workshop on High-Resolution FluoRespirometry (HRFR)** is held in cooperation with our O2k-Network Lab in Hungary. This O2k-Workshop presents a basic introduction to the **Oroboros O2k** with integrated real-time analysis by **DatLab**. We introduce the new **DatLab 7** software with innovative **DatLab-Protocols** and the concept of a quality control system including the MitoFit interlaboratory Proficiency Test.

HRFR provides information on cell respiration with basic coupling control protocols. State-of-the-art OXPHOS analysis is extended using mt-preparations (permeabilized cells, permeabilized muscle fibers, tissue homogenate, isolated mitochondria), to evaluate coupling efficiencies and OXPHOS capacities with electron transfer into the Q-junction converging from NADH, FADH<sub>2</sub>, succinate and  $\alpha$ -glycerophosphate (N,F,S,Gp), to diagnose defects in respiratory electron transfer system pathways and the phosphorylation system. Novel developments are presented on **substrate-uncoupler-inhibitor titration (SUIT) protocols** in HRFR using the **O2k-FluoRespirometer** for simultaneous measurement of respiration and hydrogen peroxide production (Amplex UltraRed®). Discussions are extended on comparison of measurement of mt-membrane potential using Safranin (fluorometric) versus TMRM (fluorometric) and on perspectives of HRFR in mitochondrial physiology.

## Lecturers and tutors

<a href="#">Gnaiger Erich</a>	CEO, Oroboros Instruments (AT)
<a href="#">Doerrier-Velasco Carolina</a>	CSO, Oroboros Instruments (AT)
<a href="#">Komlodi Timea</a>	Research assistant, Oroboros Instruments (AT)
<a href="#">Meszaros Andras</a>	CRO, Oroboros Instruments (AT)

## Programme

### 1 Friday, August 24

\*printed in workshop materials

Workshop 1		Weblink
<b>08:30</b>	<b>Registration, welcome</b> <i>Venue:</i> Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary 37-47 Tuzolto Str, HU-1088	<a href="#">IOC132</a>
<b>09:00-09:30</b>	<b>Get-together: Introduction of participants and their research interests</b>	
<b>09:30-10:00</b>	<b>Application of the O2k Series H</b>	
<b>10:00-11:00</b>	<b>Real-time experiment: Oxygen calibration (instrumental quality control 1) and DatLab 7</b> DL-Protocol for air calibration	<a href="#">Gnaiger 2008 POS SOP: O2-calibration</a>
11:00-11:30	<i>Coffee / Tea</i>	
<b>11:30-13:00</b>	<b>Introduction and real-time experiment: High-Resolution FluoRespirometry (mt-respiration and H<sub>2</sub>O<sub>2</sub> production) with isolated mitochondria:</b> studying the effect of oxygen concentration on H <sub>2</sub> O <sub>2</sub> production	<a href="#">Amplex UltraRed</a>
<b>13:00-13:30</b>	<b>DatLab analysis</b>	<a href="#">Amplex UltraRed</a>
13:30-14:30	<i>Lunch</i>	
<b>14:30-15:30</b>	<b>Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay.</b>	<a href="#">The Blue Book*</a> <a href="#">SUIT reference protocol</a>
<b>15:30-16:30</b>	<b>DatLab data analysis and normalization of results</b> Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	<a href="#">MitoPedia: Respiratory control ratios</a> <a href="#">MitoPedia: SUIT</a>
<b>16:30-17:00</b>	<i>Coffee / Tea</i>	
<b>17:00-18:00</b>	<b>Introduction to analysis of mitochondrial oxygen kinetics and O2kinetics software</b>	
<b>18:00</b>	<b>Questions and answers</b>	

### 2 Saturday, August 25

Workshop 2		Weblink
<b>08:30-10:30</b>	<b>Determination of mitochondrial (mt) membrane potential via Safranin and TMRM with isolated mitochondria: Introduction and Real-time experiment</b>	<a href="#">Safranin,TMRM</a>
10:30-11:00	<i>Coffee / Tea</i>	
<b>11:00-12:00</b>	<b>Data analysis and interpretation: TMRM/safranin</b>	
<b>12:00-13:00</b>	<b>Final discussion</b>	

## O2k-Workshop: OUR COMMON AIMS

- **Mitochondrial physiology:**  
Study mitochondrial function in the **context** of cell physiology and pathology
- **Instrumental performance – the O2k:**
  - Learn **High**-Resolution FluoRespirometry
  - Gain **hands-on** experience
  - Extend to O2k-**Multi**Sensor applications
- **Excellence in research:**
  - Instrumental **quality** control
  - Experimental design for **innovation**
  - Data analysis meeting superior **standards**

OROBOROS INSTRUMENTS

O2k

Mitochondria and cell research

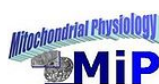


## Oroboros: O2k in numbers



- **25 years** - since 1992
- **>950** instruments world-wide
- **>576** O2k-Network Labs in 49 countries
- **>2,600** O2k-Publications: [www.orooboros.at](http://www.orooboros.at)
- **Oroboros-Team: 20**
- **131** O2k-Workshops

2018 Aug

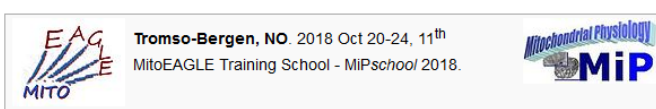


MiP2018/MitoEAGLE  
Jurmala LV

MiPschool  
Tromso-  
Bergen  
2018



## MiPschool Tromso-Bergen 2018



## More detail?

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. » [Full text in Bioblast](#)

Doerrier C, Garcia-Souza LF, Krumschnabel G, Wohlfarter Y, Mészáros AT, Gnaiger E (2018) High-Resolution FluoRespirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. Methods Mol Biol 1782:31-70. » [Full text in Bioblast](#)

Komlodi T, Sobotka O, Krumschnabel G, Bezuidenhout N, Hiller E, Doerrier C, Gnaiger E (2018) Comparison of mitochondrial incubation media for measurement of respiration and hydrogen peroxide production. Methods Mol Biol 1782:137-55. » [Full text in Bioblast](#)

**O2k-Manual** – <http://wiki.oroboros.at/index.php/O2k-Manual>

**O2k-Protocols** – <http://wiki.oroboros.at/index.php/O2k-Protocols>

**>2,200 O2k-Publications** – <http://wiki.oroboros.at/index.php/O2k-Publications: Topics>

## COST Action CA15203 MitoEAGLE



### MitoEAGLE preprint publication

[Mitochondrial respiratory states and rates: Building blocks of mitochondrial physiology](#)

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K-Regio of Standortagentur Tirol. [www.mitofit.org](http://www.mitofit.org)



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**Mitochondria and cell research**

O2k-Workshops are listed as [MitoGlobal Events](#)

