

## 136<sup>th</sup> International Workshop on High- Resolution FluoRespirometry

2018 November 27-28  
Melbourne, Australia

**Venue:**

Building P  
Institute of Sport, Exercise and Active Living (ISEAL)  
Victoria University  
Ballarat Road  
Melbourne, Australia

**Host:**

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The 136<sup>th</sup> O2k-Workshop on High-Resolution FluoRespirometry (HRFR) is held in cooperation with our O2k-Network Lab and **Oroboros O2k-Training Centre** in Melbourne, Australia. This O2k-Workshop presents a basic introduction to the **Oroboros O2k** with integrated real-time analysis by **DatLab**. We introduce the **DatLab 7** software with innovative **DatLab-Protocols** and the quality control management 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 determination and application of the P<sub>o</sub>/O<sub>2</sub> ratio.

## Programme

### 1 Tuesday, November 27

\*printed in workshop materials

	Workshop 1	Weblink
08:30	<b>Registration, welcome</b> <i>Venue:</i> Building P Institute of Sport, Exercise and Active Living (ISEAL) Victoria University Ballarat Road Melbourne, Australia	<a href="#">IOC136</a>
09:00-09:30	<b>Get-together: Introduction of participants and their research interests</b>	
09:30-10:00	<b>Applications of the O2k</b>	
10:00-11:00	<b>Real-time experiment: Oxygen calibration (instrumental quality control 1) and DatLab 7</b> Instrumental DL-Protocol: O2k-cleaning before use Instrumental DL-Protocol: O2 calibration air	<a href="#">Gnaiger 2008 POS SOP: O2-calibration</a>
11:00-11:30	<i>Coffee / Tea</i>	
11:30-12:30	<b>Experimental design: Pathway and coupling control of mitochondrial respiration</b>	
12:30-13:00	<b>Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay.</b>	<a href="#">MitoPedia: Respiratory control ratios</a> <a href="#">MitoPedia: SUIT</a>
13:00-14:00	<i>Lunch</i>	
14:00-15:30	<b>O2k-Demo experiment:</b> Measurement of oxygen consumption with a SUIT protocol	<a href="#">SUIT reference protocol</a>
15:30-16:30	<b>DatLab analysis and normalization</b> Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	<a href="#">O<sub>2</sub>-Flux Analysis</a> <a href="#">MitoPedia: DatLab</a> <a href="#">MitoPedia: Respiratory control ratios</a> <a href="#">MitoPedia: SUIT</a>
16:30-17:00	<i>Coffee / Tea</i>	
17:00-18:00	<b>Instrumental quality control 2: Instrumental O<sub>2</sub> background</b>	<a href="#">Instrumental background</a>

### 2 Wednesday, November 28

	Workshop 2	Weblink
08:30-10:00	<b>Introduction: H<sub>2</sub>O<sub>2</sub> production in tissue normoxia</b> <b>O2k-Demo experiment:</b> Simultaneous measurement of respiration and H <sub>2</sub> O <sub>2</sub> production	<a href="#">Amplex UltraRed</a>
10:00-10:30	<i>Coffee / Tea</i>	
10:30-12:30	<b>P»/O<sub>2</sub> ratio: effects of ADP and hypoxia</b>	
12:30-13:30	<i>Lunch</i>	
13:30-15:00	<b>SUIT protocols: diagnostic approaches with mitochondrial preparations</b>	<a href="#">SUIT protocols</a>
15:00-16:00	<b>SUIT protocols: diagnostic approaches with intact cells</b>	<a href="#">SUIT protocols</a>
16:00-16:30	<i>Coffee / Tea</i>	
16:30-17:00	<b>Tutorial on the Bioblast wiki</b> <a href="http://www.bioblast.at/">www.bioblast.at/</a>	<a href="#">O2k-Network</a> <a href="http://www.bioblast.at">www.bioblast.at</a> <a href="#">MitoPedia: SUIT</a>
17:00-18:00	<b>Data interpretation using SUIT protocols</b> <b>OXPHOS analysis: diagnosis of respiratory defects</b>	
18:00-18:30	<b>Feedback discussion</b>	

## Lecturer

Gnaiger Erich, CEO, Oroboros Instruments (AT)

## 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

- **26 years** - since 1992



**>1,000** instruments world-wide



**596** O2k-Network Labs in 49 countries



**>3,000** O2k-Publications: [www.orooboros.at](http://www.orooboros.at)



**Oroboros-Team: 20**



**137** O2k-Workshops

## More details?

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

## COST Action CA15203 MitoEAGLE



### MitoEAGLE preprint publication

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



## Acknowledgements

Programme prepared for printing by T Komlodi, M Beno, C Doerrier, E Gnaiger, Oroboros Instruments.



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**Mitochondria and cell research**

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

