

136th International Workshop on High- Resolution FluoRespirometry

2018 November 27-28
Melbourne, Australia

Venue:

Building P, P138
Institute of Sport, Exercise and Active Living (ISEAL)
Victoria University
Ballarat Road
Melbourne, Australia

Host:

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The 136th 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, FADH₂, succinate and α -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_o/O₂ ratio.

Programme

1 Tuesday, November 27

*printed in workshop materials

	Workshop 1	Weblink
08:30	Registration, welcome Venue: Building P, P138 Institute of Sport, Exercise and Active Living (ISEAL) Victoria University Ballarat Road Melbourne, Australia	IOC136*
09:00-09:30	Get-together: Introduction of participants and their research interests	
09:30-10:00	Applications of the O2k	
10:00-11:00	Real-time experiment: Oxygen calibration (instrumental quality control 1) and DatLab 7 Instrumental DL-Protocol: O2k-cleaning before use Instrumental DL-Protocol: O2 calibration air	Gnaiger 2008 POS SOP: O2-calibration
11:00-11:30	<i>Coffee / Tea</i>	
11:30-12:30	Experimental design: Pathway and coupling control of mitochondrial respiration	
12:30-13:00	Comprehensive OXPHOS analysis: substrate-uncoupler-inhibitor titration (SUIT) protocols for respiratory control by coupling and mitochondrial pathways, SUIT reference assay.	MitoPedia: Respiratory control ratios MitoPedia: SUIT
13:00-14:00	<i>Lunch</i>	
14:00-15:30	O2k-Demo experiment: Measurement of oxygen consumption with a SUIT protocol	SUIT reference protocol
15:30-16:30	DatLab analysis and normalization Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	O₂-Flux Analysis MitoPedia: DatLab MitoPedia: Respiratory control ratios MitoPedia: SUIT
16:30-17:00	<i>Coffee / Tea</i>	
17:00-18:00	Instrumental quality control 2: Instrumental O₂ background	Instrumental background

2 Wednesday, November 28

	Workshop 2	Weblink
08:30-10:00	H₂O₂ production in tissue normoxia O2k-Demo experiment: Simultaneous measurement of respiration and H ₂ O ₂ production	Amplex UltraRed
10:00-10:30	<i>Coffee / Tea</i>	
10:30-12:30	P_i / O₂ ratio: effects of ADP and hypoxia	
12:30-13:30	<i>Lunch</i>	
13:30-15:00	SUIT protocols: diagnostic approaches with mitochondrial preparations	SUIT protocols
15:00-16:00	SUIT protocols: diagnostic approaches with intact cells	SUIT protocols
16:00-16:30	<i>Coffee / Tea</i>	
16:30-17:00	Tutorial on the Bioblast wiki www.bioblast.at/	O2k-Network www.bioblast.at MitoPedia: SUIT
17:00-18:00	Data interpretation using SUIT protocols OXPHOS analysis: diagnosis of respiratory defects	
18:00-18:30	Feedback discussion	

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



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.orooboros.at/index.php/O2k-Manual>

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

COST Action CA15203 MitoEAGLE



MitoEAGLE preprint publication

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



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Mitochondria and cell research

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

