

139th O2k-Workshop on high-resolution respirometry

2019 Jun 17 – 22

Schröcken, Vorarlberg, Austria



The **139th O2k-Workshop on high-resolution respirometry (HRR)** is the **41st** International Oxygraph Course held in Schroecken since 1988. We provide an overview of the **O2k-FluoRespirometer**, with real-time analysis by **DatLab 7 (new)** and applications of the **Titration-Injection microPump TIP2k**. O2k-Demo experiments demonstrate the unique advantages and limitations of simultaneous monitoring of oxygen concentration, respiration, and hydrogen peroxide production. HEK 293T cells are used as a biological reference sample, which can be stored and shipped on dry-ice – introducing the MitoFit Proficiency Test. **Instrumental setup** and service of the polarographic oxygen sensor (**OroboPOS**) are demonstrated, followed by hands-on practice in 10 teams. A wide range of mitochondrial topics is covered; abstracts and experimental experiences are presented by participants. IOC participants invariably asked for a detailed discussion of protocol design. The **Blue Book** and the **Mitochondrial respiratory states and rates** provide a basic introduction to mitochondrial physiology and is complemented by overview presentations with examples, including **DatLab Analysis** of demo files. **Instrumental quality control** is a fundamental component of HRR and will be put to the practical test in teams using eight O2k (16 chambers). The **O2k-FluoRespirometer**, fully supporting **O2k-MultiSensor** applications, particularly fluorescence measurements, has become an integral part of the O2k-Workshop. Optimization of protocol design for various O2k-MultiSensor applications helps to critically evaluate basic principles of mitochondrial physiology. You

will also see the **TIP2k** with feedback-control in action and practice its simple and automatic operation.

Lunch breaks provide an opportunity for relaxing Walks & Talks, enjoying the refreshing scenery of the secluded alpine environment or using spare time for individual practice. Join for a visit to the *Alpmuseum*.

Lecturers and tutors

Bastos Sant'Anna Silva Ana Carolina	PhD student TRANSMIT , Oroboros Instruments (AT)
Di Marcello Marco	Scientific Assistant, Oroboros Instruments (AT)
Doerrier Carolina	Chief scientific officer, Oroboros Instruments (AT)
Gnager Erich	CEO, Oroboros Instruments (AT)
Laner Verena	Chief operating officer, Oroboros Instruments (AT)
Passrigger Manuela	Biomedical assistant, Oroboros Instruments (AT)
Zdrzilova Lucie	Scientific researcher, Charles University (CZ)



Programme

1 Monday, Jun 17

*printed in workshop materials

Arrival	Weblink
15:00 Arrival in Bregenz: Meeting point Bregenz train station at 3:00 pm; approx. 1 h bus drive to Schröcken and Hochtannberg (Salober); walk to Hotel Körbersee (approx. 40 min)	IOC-travel
18:30-19:30 <i>Welcome reception at Hotel Körbersee & get-together:</i> Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:30 <i>Dinner</i>	

2 Tuesday, Jun 18

Workshop 1	Weblink
07:30-08:30 <i>Breakfast</i>	
08:30-09:30 Challenges of innovation and continuation: transition to O2k-Series H and DatLab 7 O2k instrumental setup – overview with video clips	O2k-FluoRespirometer MitoPedia: DatLab DL-Protocols O2k-Videosupport O2k-Start
09:30-11:30 Hands-on (10 groups) DatLab 7	OroboPOS service
09:30-10:15 Groups 1-5	Groups 6-10
10:15 <i>Coffee / Tea</i>	
DatLab 7	OroboPOS service
10:45-11:30 Groups 6-10	Groups 1-5
11:30-12:30 Oxygen calibration (instrumental quality control 1) DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	POS Service O2k-Start Gnager 2008 POS SOP: O2-calibration
12:30 <i>Lunch packages/ Walk & Talk</i> <i>Alternative: individual O2k-tasks</i>	

14:30-15:30	Cell respiration and simultaneous measurement of H₂O₂ production (Demo-Experiment) DL-Protocol (O2&AmR): SUIT-013 AmR ce D023	O₂-Flux Analysis SUIT-013 AmR ce D023
15:30	<i>Coffee / Tea</i>	
16:00-18:00	Hands-on (7 groups): Oxygen calibration and cell respiration Cell respiration and simultaneous measurement of H ₂ O ₂ production in intact cryopreserved HEK cells DL-Protocol: O2 calibration air DL-Protocol (O2&AmR): O2k-cleaning AfterUse	Coupling control protocol SUIT-013 AmR ce D023
18:30	<i>Dinner</i>	
20:00-21:00	DatLab analysis: Reproducibility of technical repeats	DatLab-Analysis

3 Wednesday, Jun 19

Workshop 2	Weblink
07:30-08:30 <i>Breakfast</i>	
08:30-10:00 Experimental design: Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
10:00 <i>Coffee / Tea</i>	
10:30-11:00 Substrate-uncoupler-inhibitor titration (SUIT) protocols – fundamental principles	MitoPedia: SUIT
11:00-11:30 O2k-Demo experiment: Respiration of permeabilized cells: Measurement of oxygen consumption with Reference protocols RP1 (SUIT-001) and RP2 (SUIT-002) DL-Protocol (O2): SUIT-001 O2 ce-pce D003 and SUIT-002 O2 ce-pce D007	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
11:30-12:30 Hands-on (7 groups) - getting started with an O2k experiment: washing, stirrer test, air calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	SOP: O2k-cleaning and ISS SOP: O2-calibration
12:30 <i>Lunch packages / Walk & Talk alternative: individual O2k-tasks</i>	The Blue Book p 56*
14:00-16:30 Hands-on (7 groups) - O2k-experiment Respiration with permeabilized cells: SUIT protocols (RP1 and RP2) with 7 Power-O2k DL-Protocol (O2): SUIT-001 O2 ce-pce D003 and SUIT-002 O2 ce-pce D007 DL-Protocol: O2k-cleaning AfterUse	SUIT reference protocol SUIT-001 O2 ce-pce D003 SUIT-002 O2 ce-pce D007
16:00 <i>Coffee / Tea - split team, continue with experiment</i>	
16:30-17:45 DatLab analysis and SUIT protocols Flux per volume, flux per mass, flow per cell, flux control ratio, flux control factor	MitoPedia: Respiratory control ratios MitoPedia: SUIT
17:45-18:45 DatLab analysis: hands-on in teams Analysis of the hands-on experiment with permeabilized cells.	O₂-Flux Analysis MitoPedia: DatLab
19:00 <i>Dinner + registration for the walk to the Alpmuseum</i>	
20:30-21:30 O2k perspectives: 10+5 min presentations of abstracts 1-4	

4 Thursday, Jun 20

Workshop 3	Weblink
07:30-08:30 <i>Breakfast</i>	
08:30-10:30 Hands-on (7 groups): Standard H₂O₂ protocol for permeabilized cells in 7 O2ks DL-Protocol (O2&AmR): SUIT-009 AmR ce-pce D019 DL-Protocol: O2k-cleaning AfterUse	Standard H2O2 protocol: SUIT-009 AmR ce-pce D019
10:00 <i>Coffee/Tea - split team, continue with experiment</i>	

10:30-12:30	H₂O₂ data analysis: introduction and hands-on in teams	
12:30	<i>Lunch packages / walk & talk</i> <i>alternative: individual O2k-tasks</i>	
14:30-15:30	DatLab analysis: summary discussion	O₂-Flux Analysis
15:30-16:30	From isolated mitochondria to tissue fibres and tissue homogenate preparation: The PBI-Shredder (overview with video clips)	MiPNet17.03 Shredder vs Fibres O2k-Videosupport
16:30	<i>Coffee / Tea</i>	
17:00-18:00	Data interpretation using SUIIT protocols. OXPHOS analysis: diagnosis of respiratory defects	MitoPedia: SUIIT
18:00-19:00	Introduction to analysis of mitochondrial oxygen kinetics and O2kinetics software	
19:00	<i>Dinner</i>	
20:30-21:30	O2k perspectives: 10+5 min presentations of abstracts 5-9	

5 Friday, Jun 21

	Workshop 4	Weblink
07:30-08:30	<i>Breakfast</i>	
08:30-09:00	Introduction to instrumental O₂ background (Demo-Experiment), using the TIP2k DL-Protocol: Instrumental O ₂ background TIP2k	SOP: O₂ background TIP2k manual
09:00-11:00	Hands-on (7 groups): Instrumental O₂ background (instrumental quality control 2) O ₂ background test with the TIP2k; analysis of oxygen flux; O ₂ background from air saturation to zero oxygen concentration; or for permeabilized muscle fibres in the high-oxygen range of 500 – 200 µM DL-Protocol: Instrumental O ₂ background TIP2k	SOP: O₂ background
10:30	<i>Coffee / Tea - split team, continue with experiment</i>	MiPNet18.10 O2kvsMultiwell*
11:00-12:00	Data analysis	The Blue Book* pp 43-57
12:00	<i>Lunch packages</i>	
12:30-15:30	<i>Walk to the Alpmuseum - guided tour and reception: € 15.-</i>	Alpmuseum*
15:30	<i>Coffee / Tea</i>	
16:00-17:30	Data interpretation using O2k publications	O2k-Publications
17:30-18:15	Tutorial on the Bioblast wiki www.bioblast.at/	O2k-Network www.bioblast.at
18:30	<i>Dinner</i>	
20:00	<i>Feedback discussion: Next steps in the individual projects</i>	

6 Saturday, Jun 22

Departure	
06:30-7:30	<i>Breakfast</i>
Early morning: departure from Hotel Körbersee at 08:15 am, bus departure 9.00 am at Salober.	

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



Preliminary list of participants

Participant	Institution
Bach de Courtade Sandra Monica *	NO Oslo Eide L - Oslo University Hospital, Oslo (NO)
Bardal Tora **	NO As Egelandsdal B - Norwegian University of Science and Technology, Trondheim (NO)
Chabi Beatrice	FR Montpellier Wrutniak-Cabello C - INRA/University of Montpellier, Montpellier Cedex 1 (FR)
Davis Michael ****	US OK Stillwater Davis MS - Oklahoma State University, Stillwater (US)
Decker Stephen *	US_MA Amherst Busa MA - University of Massachusetts Amherst, Amherst (US)
Fulton Montana ****	US OK Stillwater Davis MS - Oklahoma State University, Stillwater (US)
Grams Bente *	DE Kronshagen Tillmans F - Kiel University, Kronshagen (DE)
Leffler Märta *****	SE Lund Elmer E - Lund University, Lund (SE)
Liang Liping *	US CO Denver Patel M - University of Colorado, Aurora Denver (US)
Mauil Felicia *	DE Mainz Methner A - Universitätsmedizin Mainz, Mainz (DE)
Mihanovic Ivan *	HR Split Ljubkovic M - University of Split School of Medicine, Split (HR)
Mila Guasch Maria *	ES_Barcelona Claret M - Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona (ES)
Moore Christy *	US TN Nashville Fessel JP - Vanderbilt University Medical Center, Nashville (US)
O'Brien Kristin *	US_AK Fairbanks O'Brien K - University of Alaska, Fairbanks (US)
Olsen Rolf Erik **	NO As Egelandsdal B - Norwegian University of Science and Technology, Trondheim (NO)
Scaife Paula *	UK Nottingham Chakrabarti L - University of Nottingham, Nottingham (UK)
Glombik Katarzyna *	PL_Krakow Budziszewska B - Institute of Pharmacology PAS, Cracow (PL)

*Asteriks indicate the number of O2k instruments in the participant's lab.

Oroboros: O2k in numbers



- **26 years** - since 1992



>1,100 instruments world-wide



610 O2k-Network Labs in 49 countries



>3,200 O2k-Publications: www.orooboros.at



Oroboros-Team: 21



138 O2k-Workshops



OROBOROS INSTRUMENTS

O2k

Mitochondria and cell research

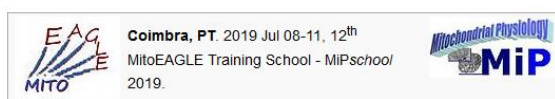


MiPNet24.01 Abstracts IOC139: 10+5 min O2k perspectives

in progress



MiPschool Coimbra 2019



Accommodation and location

Hotel Körbersee www.koerbersee.at
T +43 5519 265 hotel@koerbersee.at



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](#)

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

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

>3,200 O2k-Publications – <http://wiki.oroboros.at/index.php/O2k-Publications: Topics>

COST Action CA15203 MitoEAGLE



MitoEAGLE Mitochondrial respiratory states and rates.
doi:10.26124/mitofit:190001

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

Acknowledgements

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

Contribution to K-Regio project MitoFit.

The project MitoFit is funded by the Land Tirol within the program

K-Regio of Standortagentur Tirol. www.mitofit.org



Contact

Erich Gnaiger, PhD
Medical University of Innsbruck
D. Swarovski Research Laboratory
A-6020 Innsbruck, Austria
www.mitofit.org

Oroboros Instruments
Schöpfstrasse 18
A-6020 Innsbruck, Austria
T +43 512 566796 F +43 512 566796 20
instruments@oroboros.at | www.oroboros.at
Mitochondria and cell research

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

