

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*.

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| 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> | |

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|--------------------|--|--|
| 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 O2 background TIP2k | SOP: O2 background TIP2k manual |
| 09:00-11:00 | Hands-on (7 groups): Instrumental O2 background (instrumental quality control 2) O2 background test with the TIP2k; analysis of oxygen flux; O2 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 O2 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) |
| Joseph Vincent * | CA Quebec Soliz J - Laval University, Quebec (CA) |
| Lefler 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) |
| Paez Hector * | US TN Memphis Alway S - University of Tennessee Health Science Center, Memphis (US) |
| Roshanravan Baback * | US CA Davis Roshanravan B - University of California, Davis (US) |

| | |
|------------------------------------|--|
| Scaife Paula * | UK Nottingham Phillips BE - University of Nottingham, Nottingham (UK) |
| Crossland Hannah * | UK Exeter Blackwell JR - University of Nottingham, Nottingham (UK) |

*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



MitoEAGLE Matrei a Brenner 2018-03-11 - WG4



MiPschool Coimbra 2019



Coimbra, PT. 2019 Jul 08-11, 12th
MitoEAGLE Training School - MiPschool
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](#)

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



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Mitochondria and cell research

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

