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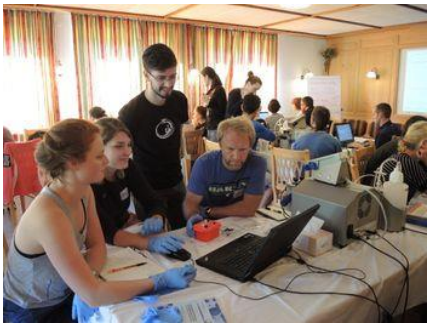
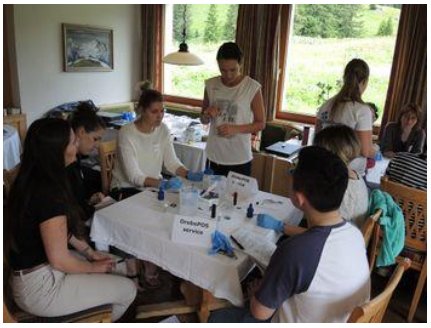
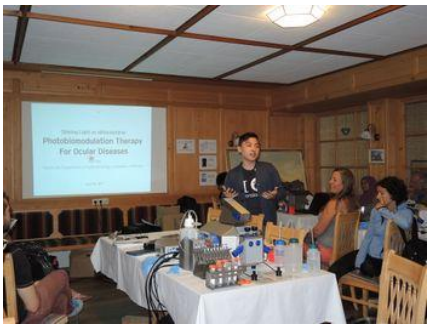
134th International Workshop on High-Resolution FluoRespirometry

2018 Oct 1 – Oct 6
Schröcken, Vorarlberg, Austria







The **134th Workshop on High-Resolution FluoRespirometry (HRFR)** is the **40th** 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** provides 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 HRFR 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

Aasander Frostner Eleonor	Invited guest tutor, Mitochondrial Medicine, Lund University (Lund, SE) and NeuroVive (SE)
Gnaiger Erich	CEO, Oroboros Instruments (AT)
Javier Iglesias-Gonzalez	Principal investigator, Medical University of Innsbruck (AT)
Komlodi Timea	Research assistant, Oroboros Instruments (AT)
Passrugger Manuela	Biomedical assistant, Oroboros Instruments (AT)

Programme

1 Monday, Oct 1

*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	*Welcome reception at Hotel Körbersee* & ***get-together:*** Introduction of participants and their research interests - a welcome by Oroboros Instruments	Schroecken
19:30	*Dinner*	

2 Tuesday, Oct 2

	Workshop 1		Weblink
07:30-08:30	*Breakfast*		
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	POS Service
10:15	*Coffee / Tea*		
	DatLab 7	**OroboPOS service**	POS Service
10:45-11:30	Groups 6-10	Groups 1-5	O2k-Start
11:30-12:30	**Oxygen calibration (instrumental quality control 1)** DL-Protocol: O2k-cleaning before use DL-Protocol: O2 calibration air		Gnaiger 2008 POS SOP: O2-calibration
12:30	*Lunch packages/ Walk & Talk* *Alternative:* individual O2k-tasks		
14:30-15:30	**Cell respiration and simultaneous measurement of H_2O_2 production** (Demo-Experiment)		O_2-Flux Analysis SUIT-6_AmR_ce_D17

	DL-Protocol (O2&AmR): SUIT-6_AmR_ce_D17	
15:30	*Coffee / Tea*	
16:00-18:00	**Hands-on (7 groups): Oxygen calibration and cell respiration** Cell respiration and simultaneous measurement of H_2O_2 production in intact cryopreserved HEK cells DL-Protocol: O2 calibration air DL-Protocol (O2&AmR): SUIT-6_AmR_ce_D17 DL-Protocol: O2k-cleaning after use	Coupling control protocol SUIT-6_AmR_ce_D17
18:30	*Dinner*	
20:00-21:00	**DatLab analysis:** Reproducibility of technical repeats	DatLab-Analysis

3 Wednesday, Oct 3

	Workshop 2	Weblink
07:30-08:30	*Breakfast*	
08:30-10:00	**Experimental design:** Pathway and coupling control of mitochondrial respiration	MitoPedia: Respiratory states
10:00	*Coffee / Tea*	
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 1) and RP2 (SUIT 2) DL-Protocol (O2): SUIT-1_O2_pce_D03 and SUIT-2_O2_pce_D07	SUIT reference protocol SUIT-1_O2_pce_D03 SUIT-2_O2_pce_D07
11:30-12:30	**Hands-on (7 groups) - getting started with an O2k experiment:** washing, stirrer test, air calibration DL-Protocol: O2k-cleaning before use DL-Protocol: O2 calibration air	SOP: O2k-cleaning_and_ISS SOP: O2-calibration
12:30	*Lunch packages / Walk & Talk* *alternative:* individual O2k-tasks	*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-1_O2_pce_D03 and SUIT-2_O2_pce_D07 DL-Protocol: O2k-cleaning after use	SUIT reference protocol SUIT-1_O2_pce_D03 SUIT-2_O2_pce_D07
16:00	*Coffee / Tea - split team, continue with experiment*	
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.	O_2-Flux Analysis MitoPedia: DatLab
19:00	*Dinner + registration for the walk to the Alpmuseum*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts 1-4	

4 Thursday, Oct 4

	Workshop 3	Weblink
07:30-08:30	*Breakfast*	
08:30-10:30	**Hands-on (7 groups): Standard H_2O_2 protocol for permeabilized cells in 7 O2ks** DL-Protocol (O2&AmR): SUIT-9_AmR_pce_D19 DL-Protocol: O2k-cleaning after use	Standard H2O2 protocol: SUIT-9_AmR_pce_D19
10:00	*Coffee/Tea - split team, continue with experiment*	
10:30-12:30	**H_2O_2 data analysis: introduction and hands-on in teams**	

12:30	*Lunch packages / walk & talk* *alternative:* individual O2k-tasks	
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	*Coffee / Tea*	
17:00-18:00	**Data interpretation using SUIT protocols.** **OXPHOS analysis: diagnosis of respiratory defects**	MitoPedia: SUIT
18:00-19:00	**Introduction to analysis of mitochondrial oxygen kinetics and O2k**inetics **software**	
19:00	*Dinner*	
20:30-21:30	**O2k perspectives:** 10+5 min presentations of abstracts 5-9	

5 Friday, Oct 5

	Workshop 4	Weblink
07:30-08:30	*Breakfast*	
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	*Coffee / Tea - split team, continue with experiment*	MiPNet18.10 O2kvsMultiwell*
11:00-12:00	**Data analysis**	The Blue Book* pp 43-57
12:00	*Lunch packages*	
12:30-15:30	*Walk to the Alpmuseum - guided tour and reception: € 15.-*	Alpmuseum*
15:30	*Coffee / Tea*	
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	*Dinner*	
20:00	*Feedback discussion: Next steps in the individual projects*	

6 Saturday, Oct 6

	Departure
06:30-7:30	*Breakfast*
	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**

Participants

Participant	Institution
Ganetzky Rebecca*****	**US_PA Philadelphia_Falk MJ:** Abramson Research Center
Wickert Anita*	**DE_Greifswald_Fielitz J:** Universitätsmedizin Greifswald
Dörmann Niklas*	**DE_Greifswald_Fielitz J:** Universitätsmedizin Greifswald
Espino Guarch Mertixell**	**QA_Doha_Espino M:** Sidra Medical & Research Center
Jensen Brigitte**	**DK_Aarhus_Fago A:** Aarhus University
Jansone Baiba*	**LV_Riga_Jansone B:** Universtiy of Latvia
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Starr Jonathan	**US_PA Philadelphia_Kilbaugh T:** Children's Hospital of Philadelphia
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Davidova Eliska**	**CZ_Prague_Neuzil J:** Institute of Biotechnology CAS
Bovard Josh**	**CA_Vancouver_Boushel RC:** Chan Gunn Pavilion
Clever Sheila*****	**US_PA Philadelphia_Falk MJ:** Abramson Research Center
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Donelly Chris*	**CH_Lausanne_Kayser B:** Institute of Sport Sciences (ISSUL)
Tausan Daniel**	**CA_Vancouver_Boushel RC:** Chann Gunn Pavillion
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Hattori Naoya	**JP_Tokyo_Berthold:** Berthold Japan KK
Winwood-Smith Hugh*	**AU_Melbourne_White C:** School of Biological Sciences
Cano Sanchez Maria Consolacion*	**FR_Lille_Neviere R:** Maison de la Recherche
Blindheim Dan Filip*	**NO_Bergen_Berge RK:** University of Bergen
Krajcova Adela*	**CZ_Prague_Krajcova A:** Charles University in Prague
Alton Lesley	**AU_Clayton Victoria_Alton L:** Monash University
Ford Ellen*	**US_CO Denver_Patel M:** University of Colorado, Denver Anschutz

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

Oroboros: O2k in numbers

2018 Aug

- **25 years** - since 1992
- **>1000** instruments world-wide
- **>592** O2k-Network Labs in 49 countries
- **>2,900** O2k-Publications: www.oroboros.at
- **Oroboros-Team: 20**
- **133** O2k-Workshops

MiPNet23.06 Abstracts IOC134: 10+5 min

O2k perspectives

1. **Blindheim DF, Giil LM, Tzoulis C, Berge RK, Bjoerndal B (2018) Bioactive lipophilic substances and their effect on neuronal cells. Mitochondr Physiol Network 23.08.**

Neurodegenerative diseases, including Alzheimer´s Disease (AD) and Parkinson´s Disease (PD), lack efficient medications to modify pathogenetic mechanisms. Affecting millions of people worldwide every year, the need for disease-modifying therapies is pressing.

There is strong evidence for mitochondrial dysfunction playing a critical role in the development of AD and PD, implicated by the accumulation of amyloid-β and α-synuclein respectively. Synaptic failure and neuronal death are also consequences of impaired mitochondrial biogenesis, bioenergetics and transport [1,2].

Studies have shown that synthetic heteroatom-substituted fatty acids in β-position such as tetradecylthioacetic acid (TTA) have favorable effects on mitochondrial function. This includes stimulation of mitochondrial and peroxisomal fatty acid oxidation [3], antioxidant capacity and mild uncoupling by UCP2 and UCP3. Induction of mitochondrial biogenesis and respiration by TTA have the potential to repopulate neurites with mitochondria, possibly preventing neurodegeneration, synaptic failure and neuronal death.

During the work with my master´s thesis, I wish to investigate the effects of TTA along with other novel modified fatty acids on neuronal cells. These include triple-TTA with a triple bond at the methyl end, possibly slowing the catabolism, and N-TTA which has a nitrogen atom in β-position instead of sulphur.

Starting procedures have included viability tests on the cell lines used in the project, and determination of cell toxicity of the fatty acids using WST-1-assay and spectrophotometric detection. When appropriate concentrations of the fatty acids are known, the plan is to perform in vitro respiration assays to determine mitochondrial activity in the cell lines after treatment with the selected compounds. Oxygen utilization in response to the treatment will be quantified by polarographic respirometry (OROBOROS® Oxygraph), after permeabilization of the cells. By

employing various metabolic substrates and molecular manipulators we can differentiate functional and regulatory aspects of single components of the respiratory chain. Specifically, we will examine if the fatty acids alter the capacity or coupling state of the mitochondria.

2. Bovard J, Boushel R (2018) Integrative determinants of oxygen uptake and biomolecular markers of exercise training. Mitochondr Physiol Network 23.08.

Physical activity is a necessity for healthy living. Essential to this is the assessment of cardiorespiratory fitness by measuring maximal oxygen uptake (VO2max), which is the one of the strongest predictors of morbidity and mortality. While classically thought to be determined by oxygen delivery to working muscle, the adaptive responses of muscle oxidative capacity and therefore mitochondrial contributions are not fully understood. Moreover, changes in VO2max with standardized training programs vary substantially. A greater understanding of this variation may be achieved by a systems biology approach characterizing the biomolecular response to exercise ("the exercise responsome"), including differences in arterial and venous concentrations of proteins and metabolites (i.e., fluxomics). Given the "drug-like" effects of molecules secreted by muscle during exercise, characterizing the exercise responsome can highlight exercise dosages that optimize circulating biomolecule levels, adaptations to training, and therefore health benefits of exercise. Thus, the purposes of this study are three-fold: (1) To understand the relative and integrated contributions of the circulatory and muscle oxidative components to oxygen uptake with exercise training; (2) to assess the "exercise responsome"; and (3) to associate determinants of oxygen uptake with biomolecular markers of health.

Trained and untrained individuals will be recruited. At Visit 1, maximal oxygen uptake and critical power will be assessed. At Visit 2, blood samples will be drawn in the morning (fasted), prior to an exhaustive bout of exercise, and at multiple post-exercise time points to assess the proteomic and metabolomic responses to exercise. Body composition will be assessed, and muscle biopsies will be taken prior to and after exercise to assess mitochondrial function and oxidative stress. Specifically, a substrate and inhibitor protocol will be applied to assess OXPHOS, substrate and coupling control, LEAK respiration, mitochondrial p50, and COX excess capacity. At Visit 3, subjects will be instrumented with femoral arterial and venous catheters, as well as antecubital venous catheterization, and complete multiple incremental exercise tests on 2-legged cycling and 1-leg knee extension ergometers. During each exercise stage, blood samples will be drawn to measure fluxomics and circulatory responses to exercise will be determined. Integrative determinants of oxygen uptake will be modeled to include muscle mass-normalized O2 delivery, mitochondrial excess capacity, relative activation of mitochondria, and the role of p50 in O2 extraction. Bioinformatic analysis of omic responses alongside integrative determinants will investigate molecular-to-organ signaling networks. Trained vs. untrained groups and males vs. females will be compared. Untrained subjects will then complete a 12-16-week exercise training program, including aerobic intervals and resistance exercise, before repeating the 3 visits. Pre- and post-training will be compared.

3. Ganetzky RB, Falk MJ (2018) SUIT protocol development for zebrafish embryos. Mitochondr Physiol Network 23.08.

At the request of the author, this abstract is not made available online.

4. Janowska J, Piel S, Ehinger JK, Karlsson M, Kilbaugh T (2018) Mitochondrial targeted biofuels as countermeasures against chemical threats. Mitochondr Physiol Network 23.08.

At the request of the author, this abstract is not made available online.

5. **Krajčová A, Tomáš Urban, Petr Waldauf, Barbora Blahutová, Jan Gojda, František Duška (2018) Skeletal muscle bioenergetics in critically ill patients: effect of early rehabilitation on mitochondrial functions and insulin resistance during and 6 months after critical illness. Mitochondr Physiol Network 23.08.**

The hallmark of metabolic changes in skeletal muscle during critical illness is impaired aerobic phosphorylation in mitochondria [1] and reduced insulin-stimulated glucose disposal [2]. We asked whether these parameters can be influenced by very early (started <48 hours) rehabilitation using functional-electrical stimulation assisted supine cycling (FESCE).

In tested subgroup of patients in a prospective randomized clinical trial of early rehabilitation (NCT 02864745) we performed serial vastus lateralis muscle biopsies and euglycemic hyperinsulinaemic (120 mIU.m-2 BSA.min-1) clamps at days 0, 7 and 180. Mitochondrial functions were assessed by high resolution respirometry (Oroboros O2k) using native skeletal muscle homogenates, as previously described [3], with a cohort (n=8) of metabolically healthy patients undergoing hip replacement surgery as the control group. Electron flux through mitochondrial respiratory complexes was measured by addition of specific substrates and inhibitors [3].

In the control group, the mean rehabilitation dose was 22 min a day, whilst interventional group was receiving 77 min/day ($p<0.01$). Insulin resistance: Glucose disposal was lowest in the acute phase of critical illness (1.53±0.99 vs. 1.21±0.92 mmol/min) and improved a little after 7 days in both groups (to 2.23±1.01 vs. 2.05±0.82 mmol/min) and after 6 months (3.32±0.59 vs. 2.72±0.90 mmol/min). Bioenergetic functions: Critical illness led to a mild impairment of aerobic phosphorylation, with major defect being in respiratory complex I and II, whilst fatty acid oxidation was upregulated (see Table 1). In a standard rehabilitation group, this pattern persisted up until 6 months after the critical illness, whilst in the early rehabilitation group it seems to normalize or even achieve the supra-normal values. The major limitation indeed is the low number of subjects accumulated so far in this ongoing study. This is the reason why these data are to be considered preliminary and have not been formally statistically processed.

In conclusion, our preliminary data show that critical illness leads to profound changes in skeletal muscle bioenergetics, which seem to persist in survivors at least 6 months, but could be influenced by early rehabilitation.

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
>2,900 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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Mitochondria and cell research

O2k-Workshops are listed as MitoGlobal Events