Oroboros Virtual O2k-Workshop

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Version 01: 2020-10-20 NextGen-O2k DatLab 7 ©2020 Oroboros Website: https://wiki.oroboros.at/index.php/MiPNet25.16_Virtual_O2k-Workshop



Virtual O2k-Workshop Basic and Advanced





The Oroboros O2k-Workshop on high-resolution respirometry (HRR) provides an overview of the O2k-FluoRespirometer, including applications of the Titration-Injection microPump TIP2k and data analysis by DatLab 7.4. The Virtual O2k-Workshop offers flexibility to the participants, allowing you to choose the virtual coaching sessions that most fit your personal needs. This provides a unique opportunity to receive a start-up introduction and learn about new developments in HRR.



Via a live video link, Oroboros experts quide you step-bystep on O2k instrumental setup and service of the polarographic (OroboPOS) oxygen sensors instrumental quality control, an essential component of HRR. The virtual coaching sessions include 10 **individual training hours**. This offers the opportunity to analyse and discuss your first experimental DatLab files obtained with your O2k-FluoRespirometer with the bioenergetics experts of Oroboros. Instrumental and biological experiments demonstrate the unique advantages and limitations of monitoring of oxygen concentration and respiration, simultaneously with monitoring hydrogen peroxide production and several other MultiSensor options.



A wide range of standardized substrate-uncoupler-inhibitor-titration (**SUIT**) protocols is available to address

your specific research questions, which can be further customized for application to your biological samples. **Online supporting material** is provided to make it easy for you to use the many features of the DatLab software from instrumental control to the analysis of results.

At our workshops, IOC participants invariably ask for a detailed discussion about protocol design. The <u>Blue Book</u> (5th edition in prep.) and the MitoEAGLE Bioenergetics Communication <u>Mitochondrial physiology</u> provide a basic introduction to mitochondrial physiology, as an introduction to get prepared for the training course.

The Virtual O2k-Workshop is composed of:



O2k-Manual: Repository of online manuals (unlimited access) which guide beginners and experienced users from the instrumental set-up to data analysis.



The **O2k-Videosupport** provides valuable assistance, complementary to the O2k-Manual. These video clips are Open Access. Exclusive videos will also be available for Virtual O2k-Workshop participants.



O2k-Procedures (unlimited access) explain various applications of the O2k (i.e. mitochondrial pathways, O2k-Demo experiments, O2k-Analysis, chemicals and media, O2k-mitochondrial preparations and mitochondrial and marker-enzymes).



Substrate-uncoupler-inhibitor titration (SUIT) protocols are applied to living cells and mitochondrial preparations. Oroboros <u>library of SUIT protocols</u> and the <u>SUITbrowser</u> offer help to find the best SUIT protocol for your research questions. Instrumental and SUIT **DL-Protocols** (DatLab 7.4 software) provide a guide through the sequence of steps for instrumental and biological experiments. The library of SUIT protocols and the SUITbrowser are available online with unlimited access. DL-Protocols are included in **DatLab 7.4**.



MitoPedia includes a continuous development of a consistent nomenclature, terms, abbreviations and concepts in mitochondrial physiology and nonequilibrium thermodynamics, in the spirit of Gentle Science.



Bioenergetics Communications is the Open Access journal for publishing scientific and technical advances in bioenergetics and mitochondrial physiology as Living Communications.



O2k-Publications include relevant information of high-resolution respirometry.



Individual face-to-face **virtual coaching** sessions (this takes place on the dates to be confirmed). The virtual coaching includes tutoring, guidance, questions and discussions. **10 hours** of virtual coaching are included in the Virtual O2k-Workshop.

Materials for self-study

» https://wiki.oroboros.at/index.php/Virtual O2k-Workshop study material

It is recommended that participants prepare for their first live sessions by going through the self-study material found at the "Materials for self-study" file. The content will lead participants through the set-up of the instrument and introduce the field of HRR. The date of the live sessions will be communicated to the participants once a registration form is received. Each participant will receive 10 h to be used on these virtual coaching sessions.

DatLab 7.4 has to be installed on the computer to which the O2k is connected (O2k-Videosupport: DatLab 7 installation).

Program

For the 10 hours of individual virtual coaching sessions, we recommend that new users follow the O2k-Bassic sessions denoted by an *. Advanced users may choose to select sessions from both the O2k-Basic and Advanced programs.

O2k-Basic		
Session	Duration	
Part 1.1: OroboPOS service and O2k instrumental setup		
Hands-on: OroboPOS service	Start-up 2 h *	
1. OroboPOS		
2. Cathode cleaning		
3. Anode cleaning		
4. Membrane mounting	Virtual Coaching	
Hands-on: 02k instrumental setup Start-up 2 h *		
5. O2k FluoRespirometer	Start-up 2 II	
6. Insert OroboPOS		
7. Insert O2k Chamber		
8. Chamber volume calibration	Virtual Coaching	
Part 1.2: DatLab		
	Start-up 1 h *	
DatLab overview	Virtual Coaching	
Part 1.3: O ₂ calibration and instrumental background		
Hands-on: Quality control 1: Oxygen calibration	Do-it-yourself 1.5 h	
DL-Protocol: O2k-cleaning BeforeUse		
DL-Protocol: O2 calibration air	DatLab	
Hands-on: Quality control 2: Oxygen background	Do it vermels 2 b	
Select one DL-Protocol according to your needs: Instrumental O2 background TiP2k Instrumental O2 background manual injections Instrumental high O2 background TiP2k Instrumental high O2 background manual injections	Do-it-yourself 2 h	
DatLab 7.4 analysis and discussion	1 h * Virtual Coaching	

Part 2.1: Biological samples and experimental design

Discussion about biological samples, experimental design, SUIT protocols

Get prepared by "Materials for self-study" Section 2.1

1 to 2 h *

02K

Part 2.2: Biological experiment and data analysis

Hands-on: Quality control 1: Oxygen calibration

DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air

Do-it-yourself 1.5 h



Hands-on: Biological experiment: cell or mitochondrial respiration

DL-Protocol: SUIT protocol will be selected/discussed individually

Do-it-yourself 1 to 3 h



Hands-on: O2k-cleaning after use



Select one DL-Protocol according to your needs:

O2k-cleaning AfterUse

O2k-cleaning AfterUse inhibitors O2k-cleaning AfterUse stirrers Do-it-yourself 1 h

Hands-on: DatLab 7.4 analysis and DatLab performance

evaluation. Discussion



O2k-Advanced

Simultaneous determination of O2 and H2O2 fluxes

Simultaneous determination of O ₂ and H ₂ O ₂ fluxes		
Session	Option to select hours for virtual coaching	
Introduction to H ₂ O ₂ measurements		
and discussion		
	1 h	
Get prepared with "Materials for self-study", "O2k-Advanced, O2k-Applications: Simultaneous determination of O2 and H2O2 fluxes"		
Hands-on: Quality control 1: Oxygen calibration	1.5 h	
DL-Protocol: O2k-cleaning BeforeUse		
DL-Protocol: O2 calibration air		DatLab
Hands-on: Amplex UltraRed calibration: Amplex UltraRed calibration in the absence of biological sample.	1 h	
DL-Protocol: AmR calibration		DatLab
Hands-on: Biological experiment: simultaneous	2 h	
measurement of O ₂ and H ₂ O ₂ production		
Suggested SUIT protocol: <u>SUIT-026</u>		DatLab
DL-Protocol: SUIT-026 AmR mt D064		Duttus
Hands-on: O2k-cleaning after use	1 h	
DL-Protocol: O2k-cleaning AfterUse		DatLab
Hands-on: DatLab 7.4 H ₂ O ₂ flux analysis and DatLab		
performance evaluation. Discussion	1.5 h §	

Tutors

Cardoso Luiza	Mitochondrial Wizard, PostDoc, Oroboros Instruments
Cecatto Cristiane	Mitochondrial Phoenix, PostDoc, Oroboros Instruments
<u>Di Marcello Marco</u>	Research Magician, Oroboros Instruments
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Mitochondrial physiology. Gnaiger Erich et al — MitoEAGLE Task Group (2020) Mitochondrial physiology. Bioenerg Commun 2020.1. doi:10.26124/bec:2020-0001.v1.

Mitochondrial physiology

MitoFit Preprint Archives



The Open Access preprint server for mitochondrial physiology and bioenergetics

» https://www.mitofit.org/index.php/MitoFit Preprint Archives

Bioenergetics Communications



The Open Access journal for publishing scientific and technical advances in bioenergetics and mitochondrial physiology as <u>Living Communications</u>

» https://www.bioenergetics-communications.org

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NextGen O2k

Oroboros - as a driving force in mitochondrial physiology - extends the analytical and diagnostic power of high-resolution respirometry by integration of NADH- and O-redox monitoring in the **NextGen-O2k**. We aim at establishing the Oroboros quality control management for dissemination to our worldwide O2k-Network laboratories. This will become an effective contribution to address the acute reproducibility crisis of scientific investigation. In the spirit of Open Science and global networking, we will enable data sharing across projects and institutions in an Open Access database on mitochondrial physiology and pathology, to the *inflation* crisis and resolve ultimately



the *value-impact crisis* of present academic publication. This will support key developments in mitochondrial medicine. In addition, we expand our business to algal biotechnology and ecology with the photobiology module of the NextGen-O2k, widening our focus from medicine to environment and climate.

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Mitochondria and cell research

Virtual O2k-Workshops are listed as MitoGlobal Events

