## **Oroboros Virtual O2k-Workshop**



Mitochondrial Physiology Network 26.02(01):1-6 (2021) Version 01: 2021-02-01 NextGen-O2k DatLab 7 ©2021 Oroboros Updates: https://wiki.oroboros.at/index.php/MiPNet26.02\_Virtual\_O2k-Workshop:Q-Module

# Virtual O2k-Workshop Basic and Advanced NextGen-O2k: Q-Module





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. 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 guide you step-bystep on **O2k instrumental setup** and service of the sensors polarographic oxygen (OroboPOS) for 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-uncouplerinhibitor-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. In this adapted workshop for NextGen-O2K Key Opinion Leaders (KOLs), the focus will be also on providing specific training on theNextGen-O2k Q-Module and the hands-on demo experiments will be specific for conducting simultaneous measurement of O<sub>2</sub> flux and the redox changes of the Q-pool.

At our workshops, IOC participants invariably ask for a detailed discussion about protocol design. The <u>Blue Book</u> (5<sup>th</sup> edition) 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-Analyisis, 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</u> **protocols** 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 (<u>O2k-Videosupport: DatLab 7 installation</u>).

## Program

For the 10 hours of individual virtual coaching sessions, we recommend that new users follow the O2k-Basic sessions denoted by as Start-up \*. 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	2 h
1. OroboPOS	
2. Cathode cleaning	(have)
3. Anode cleaning	OZK
4. Membrane mounting	Virtual Coaching
Hands-on: O2k instrumental setup	
	2 h
<ol> <li>O2k FluoRespirometer</li> <li>Insert OroboPOS</li> </ol>	
7. Insert O2k Chamber	
8. Chamber volume calibration – including Q-Stopper with	
reference electrode	Virtual Coaching
DatLab overview	1 h
Part 1.3: Oxygen calibration and instrumental backgrou Hands-on: Quality control 1: Oxygen calibration	Ind Do-it-yourself 1.5 h
DL-Protocol: O2k-cleaning BeforeUse	
DL-Protocol: O2 calibration air	DatLab
DL-Protocol: O2 calibration air Hands-on: Quality control 2: Oxygen background	DatLab
Hands-on: Quality control 2: Oxygen background	Do-it-yourself 2 h
Hands-on: Quality control 2: Oxygen background Select one DL-Protocol according to your needs:	Do-it-yourself 2 h
Hands-on: Quality control 2: Oxygen background Select one DL-Protocol according to your needs: Instrumental O2 background TiP2k	Do-it-yourself 2 h
Hands-on: Quality control 2: Oxygen background Select one DL-Protocol according to your needs:	Do-it-yourself 2 h

Hands-on: Quality control 1: Oxygen calibration with the Q-Stopper and reference electrode	Do-it-yourself 1.5 h
DL-Protocol: O2k-cleaning BeforeUse	
DL-Protocol: O2 calibration air	DatLab
Hands-on: Quality control 2: Oxygen background with the	
Q-Stopper and reference electrode	Do-it-yourself 2 h
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	DatLab
	1 h
Quality Control and DatLab 7.4 analysis and discussion	02K Virtual Coaching

### NextGen-O2k Simultaneous determination of O<sub>2</sub> flux and the redox state of the Q-pool

Session	Duration	
Part 2.1: Q-Module, CV, biological samples, and experimental design		
Introduction to the design and functioning of the Q-Module and cyclic voltammetry. Discussion about biological samples, experimental design, SUIT protocols.	1-2 h	
Get prepared with "Materials for self-study" Section 2.1 and NextGen-O2k KOL Training – Q-Module	Virtual Coaching	
Part 2.2: Instrumental quality control and Q-Service		
Hands-on: Assembly and Polishing of the Q-Stopper and chamber volume calibration with Q-Sensor	0.25-0.5 h	
Part 2.3: Cyclic voltammetry		
Hands-on: Cyclic voltammetry with and without CoQ2	0.25 h	
Hands-on: O2k-cleaning after CV	0.25 h	

Part 2.4: Biological experiment and analysis	
Hands-on: Biological experiment: simultaneous measurement of O2 flux and the redox changes of the Q- pool	Do-it-yourself 2 h
DL-Protocols: SUIT-006 Q mt D071 SUIT-031 Q mt D072	DatLab
Hands-on: O2k-cleaning after use Cleaning after Q-experiments	Do-it-yourself 1 h
Select one DL-Protocol according to your needs: O2k-cleaning AfterUse O2k-cleaning AfterUse inhibitors O2k-cleaning AfterUse stirrer	DatLab
Hands-on: DatLab 7.4 Q redox ratio analysis and DatLab performance evaluation. Discussion	1 h Virtual Coaching

## **Tutors**

Cardoso Luiza	Mitochondrial Wizard, PostDoc, Oroboros Instruments
Komlodi Timea	Mitochondrial Explorer, PostDoc, Oroboros Instruments

### **COST Action CA15203 MitoEAGLE**



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**



**MitoFit** Preprints The Open Access preprint server for mitochondrial physiology and bioenergetics

» <u>https://www.mitofit.org/index.php/MitoFit\_Preprints</u>

## **Bioenergetics Communications**



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

» <u>https://www.bioenergetics-communications.org</u>

#### Acknowledgements

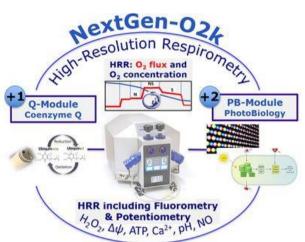
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Oroboros - as a driving force in mitochondrial physiology - extends the analytical and diagnostic power of high-resolution respirometry by integration of NADH- and Q-redox monitoring in the **NextGen-O2k**. We aim at establishing the Oroboros quality 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 resolve the *inflation crisis* and 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.

### Contact

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Virtual O2k-Workshops are listed as MitoGlobal Events

