# Oroboros FAT4BRAIN Virtual O2k-Workshop

Mitochondrial Physiology Network 26.09(01):1-6 (2021) Version 01: 2021-08-15 NextGen-O2k DatLab 7 ©2021 Oroboros Updates: https://wiki.oroboros.at/index.php/FAT4BRAIN Advanced O2k-Workshop IOC150 Virtual



FAT4BRAIN Virtual O2k-Workshop

Advanced - TMRM and Calcium Green





The **Oroboros O2k-Workshop on high-resolution respirometry (HRR) – Advanced** provides an overview of the **O2k- FluoRespirometer**, including data analysis with **DatLab 7.4**. This provides a unique opportunity to receive advanced training in simultaneous O<sub>2</sub> flux and mitochondrial membrane potential (mtMP) or Ca<sup>2+</sup> measurements.



Via a live video link, Oroboros experts will guide you step-by-step on **O2k-Multisensor applications**, with hands-on training on mtMP using TMRM and Ca<sup>2+</sup> uptake and release using Calcium Green.



During the **FAT4BRAIN School IOC147 Virtual Event** topics covered in the **Blue Book** (5<sup>th</sup> edition) and the MitoEAGLE Bioenergetics Communication **Mitochondrial physiology** were presented and discussed, providing a basic introduction to mitochondrial physiology and protocol design, and during **FAT4BRAIN Virtual O2k-Workshop** – **Basic** the participants received hands-on training on the quality controls for high-resolution respirometry and SUIT protocols performance and

analysis. The **FAT4BRAIN Virtual O2k-Workshop – Advanced – Amplex UltraRed** gave the participants the first contact with fluorespirometry. Now the **FAT4BRAIN Virtual O2k-Workshop – Advanced – TMRM and Calcium Green** will introduce further O2k-Multisensor applications.



### 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.



**Virtual coaching** sessions includes tutoring, guidance, questions and discussions.

### Materials for self-study

» https://wiki.oroboros.at/index.php/FAT4BRAIN Advanced O2k-Workshop IOC150 Virtual#Virtual O2k-Workshop self-study material

It is recommended that participants prepare for the live sessions by going through the self-study material found at the link above.

### **Program**

### August 24th:

### **02k-Advanced**

## Simultaneous determination of O<sub>2</sub> flux and mitochondrial membrane potential

### Session

08:00-08:30 (CEST), 09:00-09:30 (EEST): Hands-on: Quality control 1: Oxygen calibration

Do-it-yourself

DL-Protocol: 02k-cleaning BeforeUse DL-Protocol: 02 calibration air



 $08:\!30\text{-}09:\!00$  (CEST),  $09:\!30\text{-}10:\!00$  (EEST): Introduction to mitochondrial membrane potential measurements and discussion



Get prepared with "Materials for self-study"

09:00-09:30 (CEST), 10:00-10:30 (EEST): Hands-on: TMRM calibration

DL-Protocol: TMRM\_calibration



\* One O2k will be selected to show the traces real time for those who are present only virtually – Afterwards the DLD files will be shared among all participants

09:30-11:00 (CEST), 10:30-12:00 (EEST): Hands-on: Biological experiment: simultaneous measurement of  $O_2$  flux and mt membrane potential

SUIT protocol: SUIT-006



\* One O2k will be selected to show the traces real time for those who are present only virtually – Afterwards the DLD files will be shared among all participants

11:00-12:00 (CEST), 12:00-13:00 (EEST): Lunch break

12:00-13:00 (CEST), 13:00-14:00 (EEST): Hands-on: O2k-cleaning after use





DL-Protocol: O2k-cleaning AfterUse

13:00-13:30 (CEST), 14:00-14:30 (EEST): Hands-on: Chemical background with TMRM

SUIT protocol: TMRM\_calibration, SUIT-006 chemical background



\* One O2k will be selected to show the traces real time for those who are present virtually – Afterwards the DLD files will be shared among all participants

13:30-15:00 (CEST), 14:30-16:00 (EEST): Hands-on: DatLab 7.4 mitochondrial membrane potential and O<sub>2</sub> flux analysis and performance evaluation (and hands-on: O2k-cleaning after use)



DL-Protocol: 02k-cleaning AfterUse

15:00-16:00 (CEST), 16:00-17:00 (EEST): Discussion: Applications of mitochondrial membrane potential measurements



### August 25th:

#### **O2k-Advanced**

## Simultaneous determination of O<sub>2</sub> flux and mitochondrial Ca<sup>2+</sup> uptake and release

### Session

08:00-08:30 (CEST), 09:00-09:30 (EEST): Hands-on: Quality control 1: Oxygen calibration

Do-it-yourself

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

08:30-09:00 (CEST), 09:30-10:00 (EEST): Introduction to Ca<sup>2+</sup> measurements in mitochondria and discussion



Get prepared with "Materials for self-study"

09:00-10:30 (CEST), 10:00-11:30 (EEST): Hands-on:  $Ca^{2+}$  uptake – experiment with biological sample.

DL-Protocol:  $Ca^{2+}$  uptake and release



\* One O2k will be selected to show the traces real time for those who are present o virtually – Afterwards the DLD files will be shared among all participants

10:30-11:00 (CEST), 11:30-12:00 (EEST): Hands-on: O2k-cleaning

Do-it-yourself



DL-Protocol: 02k-cleaning BeforeUse

 $11:\!00\text{-}12:\!00$  (CEST),  $12:\!00\text{-}13:\!00$  (EEST): Hands-on: CaG calibration with biol sample

Protocol: Ca<sup>2+</sup> calibration



\* One O2k will be selected to show the traces real time for those who are present o virtually – Afterwards the DLD files will be shared among all participants

12:00-13:00 (CEST), 13:00-14:00 (EEST): Lunch break

13:00-14:00 (CEST), 14:00-15:00 (EEST): Hands-on: O2k-cleaning after use

Do-it-yourself

DL-Protocol: O2k-cleaning AfterUse



14:00-15:00 (CEST), 15:00-16:00 (EEST): Hands-on:  $Ca^{2+}$  analysis and DatLab performance evaluation



15:00-16:00 (CEST), 16:00-17:00 (EEST): Discussion on  $Ca^{2+}$  in mitochondria and applications



### **Tutors**

<u>Cardoso Luiza</u>	Mitochondrial Wizard, PostDoc, Oroboros Instruments
Cecatto Cristiane	Mitochondrial Phoenix, PostDoc, Oroboros Instruments

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### **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 Preprints**



The Open Access preprint server for mitochondrial physiology and bioenergetics

» https://www.mitofit.org/index.php/MitoFit Preprints

### **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

### NextGen O2k

Oroboros - as a driving force in mitochondrial physiology - extends the analytical and high-resolution diagnostic power of respirometry by integration of NADH- and Qredox monitoring in the NextGen-O2k. We aim at establishing the Oroboros quality control dissemination management for 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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Mitochondria and cell research



Virtual O2k-Workshops are listed as MitoGlobal Events