Oroboros FAT4BRAIN Virtual O2k-Workshop

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Updates:https://wiki.oroboros.at/index.php/FAT4BRAIN Advanced O2k-Workshop IOC149 Virtual



FAT4BRAIN Virtual O2k-Workshop

Advanced - Amplex® UltraRed





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_2 and H_2O_2 production measurements.



Via a live video link, Oroboros experts guide you step-bystep on **O2k-Multisensor applications**, particularly fluorescence measurements, with hands-on training on ROS production measurement using Amplex® UltraRed. Instrumental and biological experiments demonstrate the unique advantages and limitations of simultaneously monitoring respiration and H_2O_2 production.



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.

During the **FAT4BRAIN School IOC147 Virtual Event** topics covered in the <u>Blue Book</u> (5th edition) and the MitoEAGLE Bioenergetics Communication <u>Mitochondrial physiology</u> 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. Now the **FAT4BRAIN Virtual O2k-Workshop – Advanced – Amplex UltraRed will introduce 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/Virtual O2k-Workshop self-study material#O2k-Advanced

It is recommended that participants prepare for the live sessions by going through the self-study material found at the "Materials for self-study – 02k-Advanced" page. The content will lead participants through simultaneous determination of O_2 and H_2O_2 fluxes.

Program

June 14th:

02k-Advanced

Simultaneous determination of O2 and H2O2 fluxes

Session

08:00-08:30: Hands-on: Quality control 1: Oxygen calibration

Do-it-yourself

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



08:30-09:30: Introduction to H₂O₂ measurements and discussion

Get prepared with "Materials for self-study: 02k-Advanced, 02k-Applications: Simultaneous determination of 0_2 and H_2O_2 fluxes"



09:30-10:00: Hands-on: Amplex UltraRed calibration:

Amplex UltraRed calibration in the absence of biological sample.

DL-Protocol: AmR calibration

 $\ensuremath{^{*}}$ One 02k should be selected to show the traces for those who are present only virtually



(Collect the DLD files to send for those who are only online)

10:00-11:30: Hands-on: Biological experiment: simultaneous measurement of O_2 and H_2O_2 production

Suggested SUIT protocol: <u>SUIT-026</u>



* One O2k should be selected to show the traces for those who are present only virtually

(Collect the DLD files to send for those who are only online)

11:30-13:00: Lunch break

13:00-14:00: Hands-on: O2k-cleaning after use

Do-it-yourself

DL-Protocol: 02k-cleaning AfterUse



 $14{:}00{-}15{:}00{:}$ Hands-on: DatLab $7.4~H_2O_2$ flux analysis and DatLab performa evaluation. Discussion



15:00-16:00: Timea Komlodi: Facts and artifacts on oxygen dependence of H_2O_2 production using the Amplex UltraRed assay



Tutors

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

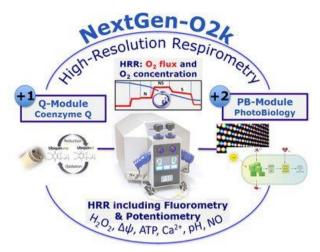


The Open Access journal for publishing scientific and technical advances in bioenergetics and mitochondrial physiology as <u>Living Communications</u>

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

NextGen O2k

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



 $Virtual\ O2k\text{-}Workshops\ are\ listed\ as\ \underline{MitoGlobal\ Events}$