Oroboros Virtual O2k-Workshop

Virtual O2k-Workshop
Basic O2k and NextGen-O2k PhotoBiology 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**. 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 guide you step-by-step on **O2k instrumental setup** and service of the polarographic oxygen sensors (**OroboPOS**) for instrumental quality control, an essential component of HRR. The **virtual coaching sessions include individual training hours**. This offers the opportunity to analyse and discuss your first experimental DatLab files obtained with your NextGen-O2k with the bioenergetics experts of Oroboros. Instrumental and biological experiments demonstrate the unique advantages and limitations of monitoring of oxygen concentration consumption and production, simultaneously with monitoring 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. In this especially adapted workshop for the algae Key Opinion Leaders (KOLs), the focus will be also on providing specific training on the **NextGen-O2k PB-Module** and the hands-on demo experiments will be specific for conducting measurements of microalgae dark respiration and photosynthesis.

At our workshops, IOC participants invariably ask for a detailed discussion about protocol design. The **Blue Book** (5th edition in prep.) and the MitoEAGLE Bioenergetics Communication **Mitochondrial physiology** 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 (Open 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. Additional videos will be available specifically for Virtual O2k-Workshop participants.

O2k-Procedures (Open Access) explain various applications of the O2k: 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. Find the best SUIT protocol for your research questions with the Oroboros **SUITbrowser** and **library of SUIT protocols** (Open Access).

Instrumental and SUIT **DL-Protocols** are included in **DatLab 7.4**, guiding step-by-step through instrumental and biological experiments.

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.

Face-to-face **virtual coaching** sessions are mutually arranged for individual tutoring, guidance, and discussions.

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 getting familiar with the "**Materials for self-study**" for instrumental set-up and high-resolution respirometry HRR. Each KOL will receive **virtual coaching** sessions individually and in groups. For the group sessions, the possibility of signing an NDA first can be discussed. After receipt of the registration form, the date of the live sessions will be arranged with the participants. Information from the form will be used to plan the joint session "Discussion about biological samples and experimental design". Although this virtual workshop is specific for the PB-Module, training on algal dark respiration will be available upon request.

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

Program

For the virtual coaching sessions, the approximate durations of Start-up sessions (live face-to-face) and 'Do-it-yourself' sessions are indicated for planning the time schedule.

O2k-Basic

Session	Duration
Part 1.1: OroboPOS service and O2k instrumental setup	
Hands-on: OroboPOS service 1. OroboPOS 2. Cathode cleaning 3. Anode cleaning 4. Membrane mounting	Start-up 2 h
Hands-on: O2k instrumental setup 5. O2k FluoRespirometer 6. Insert OroboPOS 7. Insert O2k Chamber 8. Chamber volume calibration	Start-up 2 h
Part 1.2: DatLab	
DatLab overview	Start-up 1 h
Part 1.3: Oxygen calibration and instrumental background	
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: Quality control 2: Oxygen background 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
Quality Control and DatLab 7.4 analysis and discussion	Start-up 1 h

NextGen-O2k
Dark respiration measurements and PB-Module

Part 2.1: PB-Module, biological samples, and experimental design

Introduction to the design and functioning of the PB-Module. Discussion about biological samples, experimental design, SUIT protocols. All together session Get prepared with "Materials for self-study" Section 2.1	Start-up 2 h Virtual Coaching

Part 2.2: Biological experiment and data analysis. Dark respiration (Optional)

Hands-on: Quality control 1: Oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	Do-it-yourself 1.5 h DatLab
Hands-on: Biological experiment: cell or mitochondrial respiration. DL-Protocol: SUIT protocol will be selected/discussed individually	Do-it-yourself 1 to 3 h DatLab
Hands-on: O2k-cleaning after use DatLab Select one DL-Protocol according to your needs: O2k-cleaning AfterUse O2k-cleaning AfterUse inhibitors O2k-cleaning AfterUse stirrers	Do-it-yourself 1 h

Part 2.3: Biological experiment and data analysis. Photosynthesis-Irradiance curves

Hands-on: Quality control 1: Oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	Do-it-yourself 1.5 h DatLab
Hands-on: light intensity dependence of inorganic carbon-saturated maximal photosynthesis rate. PI curve in microalgal living cells SUIT protocol: SUIT-030_PB_ce_D070	Do-it-yourself 1 to 3 h DatLab
Hands-on: O2k-cleaning after use DatLab Select one DL-Protocol according to your needs: O2k-cleaning AfterUse O2k-cleaning AfterUse inhibitors O2k-cleaning AfterUse stirrers	Do-it-yourself 1 h

Part 2.4: Biological experiment and data analysis. Inorganic carbon dependence of photosynthesis

Hands-on: Quality control 1: Oxygen calibration DL-Protocol: O2k-cleaning BeforeUse DL-Protocol: O2 calibration air	Do-it-yourself 1.5 h DatLab
Hands-on: inorganic carbon dependence of light-saturated maximal photosynthesis rate. SUIT protocol: SUIT-030_PB_ce_D069	Do-it-yourself 1 to 3 h DatLab

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
DatLab 7.4 analysis and DatLab performance evaluation. Discussion. This could be a group session		

Tutor

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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 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 Living Communications

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

Acknowledgements

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 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.
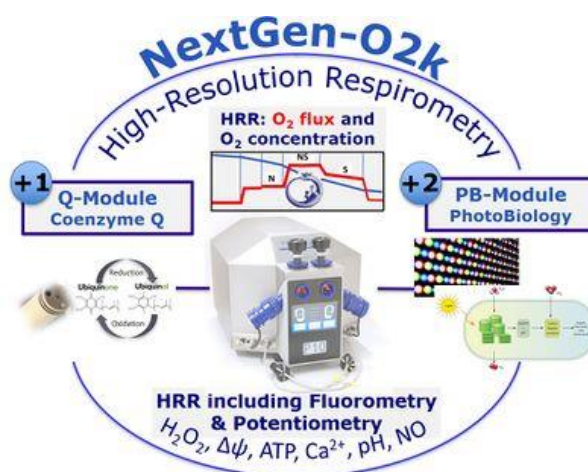
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Mitochondria and cell research

Virtual O2k-Workshops are listed as MitoGlobal Events