# DatLab 7.4

Innovations: DL-Protocols

For all O2k users

Oroboros developed the advanced DatLab 7 software, featuring new dimensions in:

- DL-Protocols (DLP): fusion with MitoPedia SUIT
  - » http://www.bioblast.at/index.php/MitoPedia:_SUIT

- DL-Protocol User (DLPU): flexibility in DL-Protocols
  - » https://www.bioblast.at/index.php/Export_DL-Protocol_User_(*.DLPU)

- Data acquisition & analysis for HRR: context-sensitive help
  - » http://wiki.oroboros.at/index.php/MitoPedia:_DatLab

- Quality control, traceability

Two-in-one

- DatLab 7 is optimized for high-resolution respirometry (HRR) – two techniques combined in each of the two O2k-chambers

- Matching O2k hardware and software

- DatLab 7 supports all O2k configurations and files generated in previous DatLab versions.
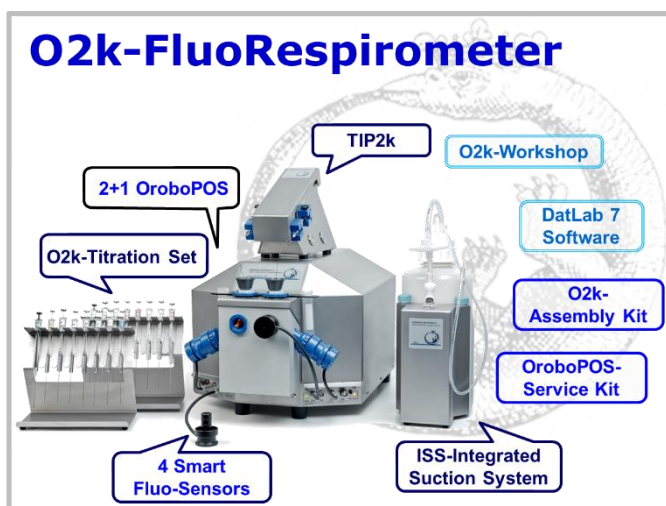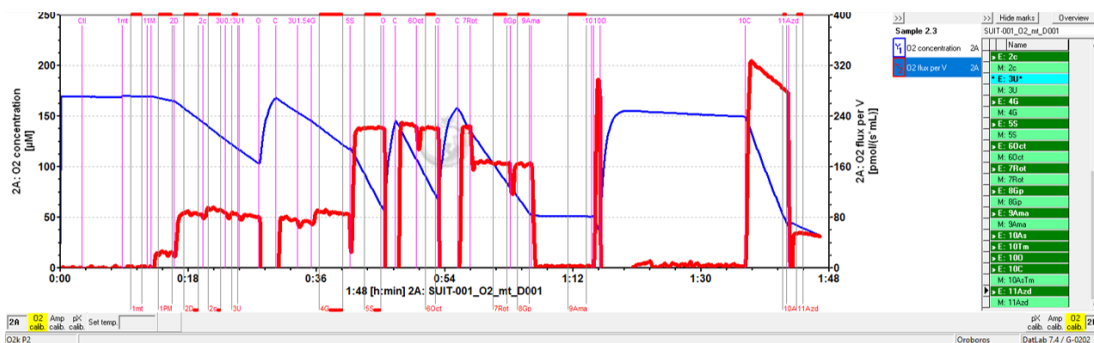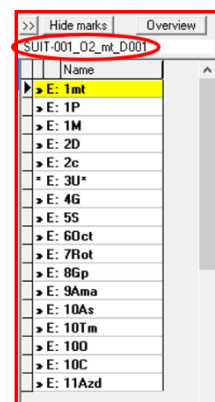

DL-Protocols

DL-Protocol applications define the sequence of **Events** and **Marks**, instructions on titrations in instrumental and substrate-uncoupler-inhibitor titration (SUIT) protocols or instrumental quality control.
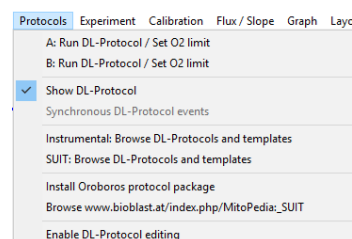
Top O2k-Applications: Standardized DL-Protocols are installed with the DatLab software. Titration steps in these SUIT protocols are fixed. However, events, marks, titration volumes and final concentrations can be personalized and exported as a customized DL-Protocol by the user (*.DLPU).

Why standardized DL-Protocols? For generating a database on mitochondrial respiratory physiology, standardized protocols are a requirement. In view of the variety of mitochondrial respiratory control patterns between species, tissues and cell types, and the diversity of specific questions addressed, a large library of standardized SUIT protocols will be required. Yet, user-specific variations of SUIT-protocols do not always follow the strict rationale required for optimum protocol design. Even a minor variation of titration steps may obscure the meaning of a respiratory state, and the nomenclature requires standardization. For instance, titration of oligomycin in the presence of ADP and a substrate combination supporting the NADH- and succinate-pathway (NS), makes sense *after* titration of rotenone (succinate-pathway, S), inducing a well-defined S-LEAK state with two coupling sites (CIII and CIV). In contrast, titration of oligomycin without rotenone yields NS-LEAK respiration, which cannot be interpreted with respect to coupling, if the relative contributions of NADH- and succinate pathway fluxes are not known.

Using a DL-Protocol: The user is guided stepwise through the titration sequence of a DL-Protocol, with standard names of **Events** (E) for titrations of chemicals and **Marks** (M) for respiratory states and statistical analysis. This minimizes handling errors and greatly improves performance during O2k measurements: The <u>**yellow**</u> line indicates the next titration step during the experiment. Titration steps that are completed correctly are shown in **green**. **Cyan** indicates multiple titrations. Any titration event and mark that is not properly set in sequence is indicated in **red**. The events and marks in gray can be skipped. An automatic warning at low oxygen levels can be set to remind the user to re-oxygenate the chamber in due time.

User-specific DL-Protocols: The flexibility to perform user-defined experiments is maintained. Personalized DL-Protocols, however, require programming which can be done by the user or by Oroboros scientific support team – a contribution to Cost Action MitoEAGLE.

a) Create a user-specific DL-Protocol: Fixed sequence of events and marks can be changed (Skip/Added) in a SUIT protocol by the user. Moreover, text, instructions, concentrations and titration volumes of injections in a specific DL-Protocol can be edited ("Enable DL-Protocol editing" by clicking on "Overview" to edit) and
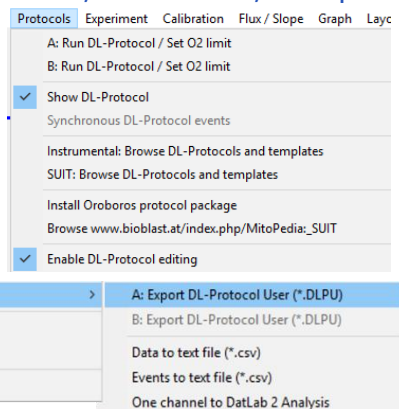
saved as user-specific DL-Protocol [File]\Export\DL-Protocol User (*.DLPU).

b) In the spirit of Open Innovation and COST Action CA15203 MitoEAGLE: To obtain your user-specific DL-Protocol, send us your DatLab file (*.DLD) with a detailed description of your O2k-application. A discussion forum on the MitoEAGLE website (or MitoPedia: SUIT) may extend the evaluation of the suitability of the SUIT protocol. You will be the author of the SUIT protocol (if it has not been published before), which you make available for the scientific community on the website. The Oroboros team will edit the protocol according to a standardized nomenclature [1] and provide you the DL-Protocol free of charge. The rights of the DLP remain with Oroboros Instruments.

c) If you do not agree on Open Access, Oroboros will ask for a service charge to generate a DL-Protocol for your exclusive use, without publishing it on the website. Oroboros would consider such a protocol as your intellectual property, without warranty of priority rights. Any other O2k-laboratory may submit an identical SUIT protocol for Open Access, which then would become available for the scientific community.

[1]   MitoEAGLE preprint 2019-05-20 Mitochondrial respiratory states and rates.
        https://www.mitoeagle.org/index.php/Gnaiger_2019_MitoFit_Preprint_Arch