# O2k-Manual: NextGen-O2k

# NextGen-O2k manual

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## Contents

## 1. Overview



NextGen-O2k RedoxBiology        NextGen-O2k PhotoBiology



NADH-Module              Q-Module              PB-Module

The **Oroboros NextGen-O2k** provides a unique high-resolution approach to the monitoring of cellular and mitochondrial respiratory function and brings an innovative combination of add-on modules for measurement of NAD and Coenzyme Q redox state, as well as a PhotoBiology Module. High-resolution respirometry (HRR) is indispensable for application of SUIT protocols at controlled oxygen levels and when measuring low respiratory activity or kinetic transitions. High-resolution is required for analysis of pathological effects causing reduced respiration (mitochondrial and metabolic diseases, ischemia-reperfusion injury, oxidative stress, apoptosis, aging). The NextGen-O2k, like the O2k-FluoRespirometer, enables simultaneous measurement of respiration and

fluorometric signals (H$_2$O$_2$ flux, mt-membrane potential, Ca$^{2+}$, ADP-ATP phosphorylation) for analysis of biopsies with limited amounts of sample (diagnosis of genetic and acquired mitochondrial defects in paediatric patients, diabetes); cell cultures with limited cell numbers or low activity; mutants with diminished respiratory capacity; chemical oxidation rates and antioxidant capacities; and oxygen kinetic measurements at low, physiological intracellular oxygen levels. Besides this, the NextGen-O2k breaks new ground by complementing the abovementioned features with modules to detect NAD or Coenzyme Q redox state in real time, concomitantly with high-resolution respirometry. The novel PhotoBiology (PB) module extends the high-resolution of the O2k oxygen measurement to photosynthesis studies and more, opening new horizons in photobiology research.
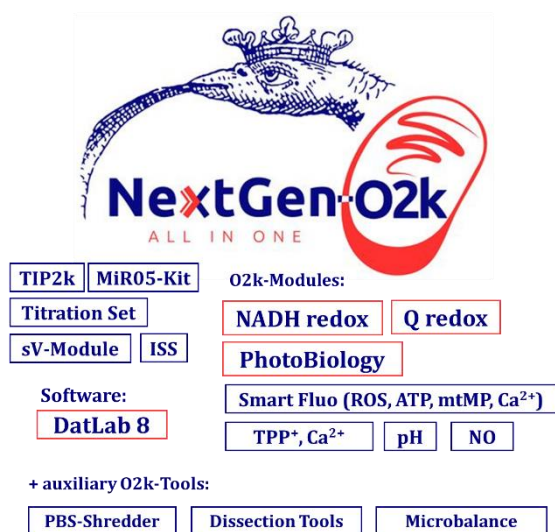


**The Oroboros O2k is the modular 2-chamber system for high-resolution respirometry (HRR).**
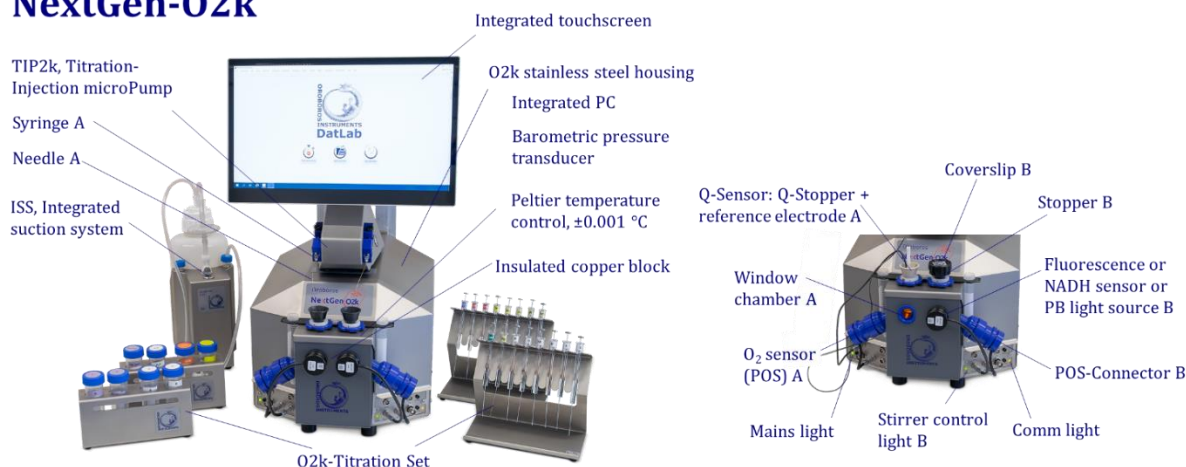**NextGen-O2k**:
Simultaneous monitoring of oxygen consumption, NAD or Coenzyme Q redox state, fluorescence or potentiometry measurement with small amounts of sample (isolated mitochondria to permeabilized tissues and cells). Measurement of photosynthesis and dark respiration. **Included**: O2k-Titration Set, Titration-Injection microPump (TIP2k), Integrated Suction System (ISS). **Add-on O2k-Modules:** high flexibility to expand the spectrum in HRR.

HRR with the Oroboros O2k-FluoRespirometer is based on a radically different systems approach to traditional polarographic measurement of oxygen dynamics. HRR rests on tightly connected instrumental features and concepts integrated by the DatLab 8 software, providing real-time display of oxygen concentration and respiration (oxygen flux), and a fluorometric, amperometric, or potentiometric signal and its time derivative (slope). The mechanics, critical selection of materials, and state-of-the-art electronics provide the basis for signal stability of an optimized polarographic oxygen sensor (**OroboPOS**), **Smart Fluo-Sensors**, **NADH-Sensors**, **PB-Light Sources**, **Q-Sensors** in an electronically controlled thermal environment with high temperature stability. Calibration and quality control follow standardized operating procedures (**O2k-SOP**) with automatic real-time analysis supported by DatLab. Scientific support complements HRR. Detailed laboratory **O2k-Procedures** provide step-by-step instructions, integrated as DL-Protocols into DatLab 8. Numerous new **O2k-Publications** on applications of the Oroboros O2k focus on critical issues of mitochondrial physiology.

While the Oroboros NextGen-O2k provides the instrumental basis for high-resolution respirometry and other measurements, successful operation at high accuracy up to the limit of detection depends on a professional application by the technician, scientist, or student. Participation in a virtual **Installation and startup support session**, **O2k-Workshop** or **O2k On-site Training** is recommended to save time and gain from the experience obtained over many years of application in various settings. All NextGen-O2k instruments come with a free remote **Installation and startup support session**.

**The NextGen-O2k is the complete system for high-resolution respirometry (HRR),** including the O2k-Main Unit with stainless steel housing, integrated PC and touchscreen, keyboard, 3 OroboPOS (2+1 spare oxygen sensor), OroboPOS-Service Kit, 2 OroboPOS-Connectors, 2 PVDF Stirrer-Bars, 2 PEEK O2k-Stoppers, DatLab 8 software, ISS-Integrated Suction System, Titration-Injection microPump (TIP2k) and O2k-Titration Set.
The NextGen O2k-FluoRespirometry and Redox package also includes NADH-Sensors, Q-Sensors  and its accessories, O2k-Fluo Smart-Module with 4 Smart Fluo-Sensors (2 blue and 2 green), and the O2k-sV-Module.
The NextGen O2k-Respirometry and PhotoBiology package also includes 6 PhotoBiology light sources - PB-Light Sources (2 blue, 2 red and 2 white).

**O2k-Manual user information**
» MiPNet07.08 User information  - PLEASE STUDY THIS MANUAL
» O2k-Manual

## 2.    Unpack the NextGen-O2k

Box 1 (or Peli Case) contains the O2k-Main Unit, the OroboPOS-Connectors without polarographic oxygen sensors (OroboPOS) and the TIP2k syringes. After unpacking, **store the original Box 1 (or Peli Case) and packing material for any future shipment of the** O2k-Main Unit.

Box 2 contains the touchscreen, keyboard and standard accessories [SA], the Oroboros USB-flash drive and any additional accessories.

## 3.    Integrated PC and DatLab 8

DatLab 8 comes installed in the NextGen-O2k integrated PC and is ready for use with protocol design, O2k control/data acquisition and data analysis.

The NextGen-O2k comes with an US American keyboard. If you wish to use a different keyboard, change the keyboard layout/settings in the operating system.

The integrated PC default user password is **OroborosNextGen**, and we recommend you change it in Settings/Users.

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To install DatLab 8 on another PC or laptop, for data acquisition or analysis, it is recommended to use a full HD screen. The PC or laptop minimum configuration is: Intel-Core-2 or equivalent CPU, 2GB RAM, USB port, Windows XP or later Windows versions. Recommended: Intel i5 or equivalent CPU, 4GB RAM, Windows 10, SSD.

It is recommended that you exit all computer applications before running the DatLab installation program. Read the short instruction windows and proceed by left click on Next. By default, DatLab is installed to "C:\DatLab". Installation is quick and easy and generates this icon on your desktop: **DatLab 8** for control of the O2k, TIP2k, data acquisition & analysis.

For data safety reasons and optimal DatLab performance, it is strongly recommended to operate your DatLab files, DL-Protocols and DatLab templates on the PC's local hard drive (HDD).

If using an external PC, make sure the ´Sleep´ mode of your computer is disabled during data acquisition to avoid errors of data transmission. The built-in PC already comes with ´Sleep´ mode disabled and ready to use.

Updates of the O2k-Manual and O2k-Procedures are posted on our website.

**MitoPedia: O2k-Open-Support** is integral to the O2k-Manual, where specifically advanced sections on high-resolution respirometry are included:

» MitoPedia: O2k-Respirometry

» MitoPedia: O2k_hardware

» MitoPedia: DatLab

» MitoPedia: SUIT

» MitoPedia: NextGen-O2k

# 4.   Electric specifications and electric/space requirements

Max. power input: 330 W
Current at 100-120 V: max. 3.0 A
Current at 220-240 V: max. 1.5 A
Power consumption, with screen switched on, during standard operation (when block temperature is stable at 37 °C and ambient temperature of 22 °C): approx. 40–50 W

Check the suitability of the delivered cable for the mains power (230/120 V) and socket. The O2k is delivered with one of three different cable types:

**EU:**     O2k-Main Power Cable/230 V/Europe, 21111-01
Note: Use O2k-Main Power Cable/230 V/Europe (Hybrid German/French plug CEE7/7, Type E+F) only for a compatible socket.

DK:     Some Danish sockets will also accept the CEE7/7 plugs, but there is no grounding connection with these plugs without male ground pin. Exchange for an appropriate Danish power cable.

CH:     A different main power cable or adapter is required.

**US:**     O2k-Main Power Cable/120 V/US-CA, 21112-01

**AU/NZ:**     O2k-Main Power Cable/230 V/AU-NZ, 21113-01

The NextGen-O2k requires a minimum of lab space      (0.5 m bench space), with sufficient space for the ISS, O2k-Titration Set and handling. The built-in PC and touchscreen allow the NextGen-O2k to be used in a compact space, however it is also possible to connect to an external PC or laptop, or another screen. For maximum signal stability and optimal temperature control, the NextGen-O2k should be placed in a position in which it is not exposed to direct sunlight and sudden changes in room temperature. Keep the space on the table underneath the O2k clear to prevent any interference with the ventilation beneath the O2k-Main Unit.

Avoid damage due to condensation. When moving the O2k from a cold to warm environment, let it equilibrate for at least 4 h in the warm room before connecting the power.
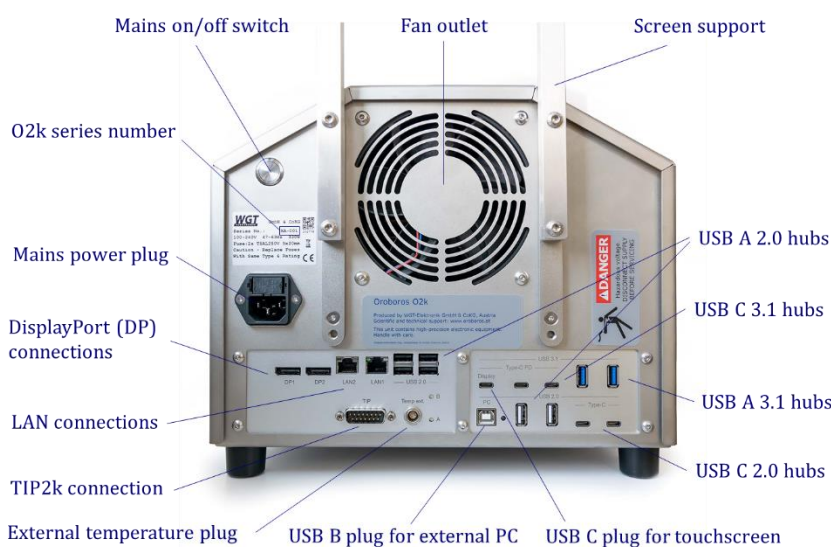


**Connections and information at the rear of the NextGen-O2k:** The O2k series number, mains on/off button, mains power plug, TIP2k connection, external temperature (temp ext.) plug, two DisplayPort connections (DP1, DP2), two LAN connections (LAN1, LAN2), one USB B 2.0 for connection to a PC, one USB C 3.1 hub for the touchscreen (display, 5 V, 3 A, max. 60 W) plus six USB A 2.0 hubs (5 V, 0.5 A, max. 2.5 W), two USB A 3.1 hubs (5 V, 1.5 A, max. 7.5 W), two USB C 2.0 hubs (5 V, 1.5 A, max. 7.5 W), and two USB C 3.1 hubs (5 V, 3 A, max. 60 W) are on the rear of the NextGen-O2k (Series XA).

If an external PC is connected to USB 2.0 Type B "PC" port, then the four lower USB will be connected to the external PC, while all other USB´s will remain connected to the internal PC. Use one of the four lower USB ports to connect a USB stick with the NextGen-O2k calibration data.

Do not manipulate the USB ports "A" and "B" unless advised and instructed by the Oroboros Open Support team.

Please connect or disconnect the TIP2k cable only with the O2k switched off.

**DANGER:** Hazardous voltage. Disconnect from power supply before servicing.

**WGT quality control seal:** Do not open. An intact quality control seal is required for warranty obligations related to the electronics of the O2k.
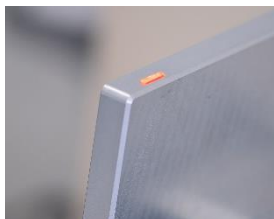
**The NextGen O2k comes with an integrated touchscreen (15,6"; FHD 1920×1080).** A power button is on the top right of the touchscreen (picture) and a USB A 3.1 port (5 V, 0.9 A, max. 4.5 W) on the screen's left side. On the back of the screen, the plug for connection to the O2k can be found.

## 5. Operating system and software

The NextGen O2k is delivered with a pre-installed operating system (Ubuntu 22.04 LTS by Canonical Ltd.). Ubuntu is a trademark of Canonical Limited and is used with the permission of Canonical Limited. We are not endorsed by or affiliated with Canonical Limited or the Ubuntu project.

⚠ The installation of alternative operating systems is neither supported by Oroboros nor covered by any warranty.

If the NextGen O2k is connected to a network:

⚠ The internet connection must be configured by qualified personnel of your institution to avoid possible risks. Beware that the integrated PC is suceptible to viruses and ransomware like any other PC. Ensure that no contaminated USB is connected to the NextGen-O2k. Do not click on links or install software that are suspicious. It is the responsibility of the user to install all Ubuntu security updates and patches in a timely manner. These updates are provided by Canonical Ltd. and not subject to prior testing by Oroboros.

In the unlikely event that such updates may result in a malfunction of DatLab 8 or later versions, Oroboros shall deliver a DL update in a timely manner. In the meantime, an external PC with DatLab 8 or later could be connected to the NextGen O2k. Oroboros is not liable for any issues caused by connecting the O2k to a network. Canonical releases new long-term-support (LTS) versions every two years, each supported for five years. The upgrade to a later LTS release shall only be performed upon prior notification by Oroboros as it requires a new DatLab version.

The NextGen O2k comes with all necessary software required for its operation. It constitutes a measuring device and shall not be used as a personal computer.

⚠ The installation of any other software may harm the functionality of, but not only, DatLab 8 or later and is therefore strongly discouraged and not covered by any warranty by Oroboros.

As a measurement device, the NextGen O2k is not meant to be a persistent data storage.

⚠ It is the responsibility of the user to copy all important data to dedicated permanent storages outside the O2k. Oroboros is not liable for any data losses. In order to prevent data loss and damage to the software and hardware, the O2k must be switched off via the operating system.

The NextGen O2k is equipped with an internal reset mechanism that restores the initial state at the time of delivery of the device, thereby deleting ALL user data and user settings. The user must change the unsafe initial passwords to more secure ones upon first login.

For previous series of the O2k-FluoRespirometer please see this Manual: MiPNet22.11 O2k-FluoRespirometer manual.

## 6.     Start the NextGen-O2k

### 6.1.   Using the integrated PC

1. Fix the screen support to the main unit using 4 long screws/bolts. Holding the screen with two hands, insert the touchscreen to the support, adjusting the angle in relation to the support. When the desired position is reached, use the two short screws/bolts to fix the position.
2. Connect the O2k to the electricity supply.
3. Connect the screen to the NextGen-O2k with the USB cable (on the O2k: "Display" USB C port; on the screen: port on the back).
4. Switch on the O2k at the rear (main power button) or using the button on top of the screen. Now the four front control lights of the O2k main unit illuminate: The outer left diode (POWER) is green, and temperature is regulated at 25 °C. The inner left and right diodes (STIRRER) are green and stirrer rotation is on. The outer right diode (COMM) changes to yellow. The power buttons at the back of the O2k and on the screen illuminate (green if PC and screen are on, blue if PC is on and the screen is off).
5. Start DatLab 8 and connect to the O2k. The outer right diode (COMM) is yellow and changes to green when data is being recorded with DatLab 8.
6. To switch off the screen only, press again the button on the screen top right, the light on screen turns blue. To switch off the O2k, disconnect the O2k from DatLab 8 and switch off the computer (the light on the screen turns red).

For daily use repeat steps 4 – 6.

### 6.2.   Using an external PC

1. Switch on the laptop or PC and wait for it to finish starting up.
2. Install DatLab 8.
3. Connect the O2k to the electricity supply.
4. Connect the O2k with the USB cable to the laptop or PC ("PC" USB B port on the back of the O2k).
5. Switch on the O2k at the rear (main power button). Now the four front control lights of the O2k main unit illuminate: The outer left diode (POWER) is green, and temperature is regulated at 25 °C. The inner left and right diodes (STIRRER) are green and stirrer rotation is on. The outer right diode (COMM) changes to yellow if a USB connection is available. In this case, the Mains on/off switch at the back of the O2k and the screen button do **not** illuminate.

7. Start DatLab 8 using the external PC and connect to the O2k. The outer right diode (COMM) is yellow and changes to green when data is being recorded with DatLab 8.
8. Disconnect at DatLab 8, switch off the O2k using the button at the rear and switch off the computer.

For daily use repeat steps 1, 5, 6 , 7, and 8.

In case of any problems (with the connection or temperature control) switch off the O2k and wait ~ 8–10 s before switching on the instrument again.

# 7.   Assembly of the NextGen-O2k

⚠ Dispel yourself of static charge to avoid possible damage of the electronics. Shortly connect to electrical ground or simply touch the O2k steel housing. For details, see: MiPNet14.01 ESD-damage

## 7.1.  Instrument overview

The NextGen-O2k is electronically equipped with all required ports to connect sensors: in addition to the O2 channel, the Amp channel is available with separate Fluo ports. The Fluo ports are used for Fluo-Sensors, PB Light Sources and NADH-Sensors. There are ports for the Q and pX channels, each with separate Ref ports for the reference electrode.

## 7.2.  O2k-Chamber assembly

Two 2-mL O2k-Chambers are supplied in the O2k-Service box. The O2k-Main Unit comes mounted with the blue O2k-Chamber Holders and OroboPOS-Holders. Two 0.5-mL O2k-Chambers with black O2k-Chamber Holders and OroboPOS-Holders are in the O2k-sV-Module box, provided in the NextGen-O2k Redox package. To use the Smart Fluo-Sensors or the NextGen NADH-, PB- or Q-Modules, assemble the NextGen-O2k with the 2-mL O2k-Chambers.



- Unscrew and remove the O2k-Chamber Holders. Remove the OroboPOS-Connectors.
- Insert the glass O2k-Chamber into the copper block of the NextGen-O2k, with the angular cut aligned towards the OroboPOS-holder. Add the PVDF stirrer-bar.
- Screw the OroboPOS-Holder (arrow 1) into a position where the glass O2k-Chamber is slightly lifted upwards.
- Screw the O2k-Chamber Holder loosely to the copper block (arrow 2). Loosen the OroboPOS-Holder stepwise (counterclockwise, slow successive quarter turns; arrow 1) and follow any downwards movement of the glass O2k-Chamber by screwing down the O2k-ChamberHolder (arrow 2). When a slight backwards movement of the OroboPOS-Holder (1) cannot be followed by any further downwards movement of the O2k-Chamber-Holder (2), then screw the
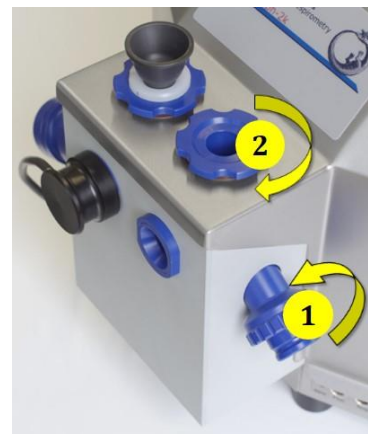
OroboPOS-Holder (1) clockwise (tight), thus securing the glass O2k-Chamber in a fixed position.

## 7.3.  OroboPOS assembly

The OroboPOS are shipped dry without electrolyte, in a Perspex housing, in the O2k-Service box. Before assembling the O2k, apply electrolyte and membrane, following the OroboPOS service instructions.
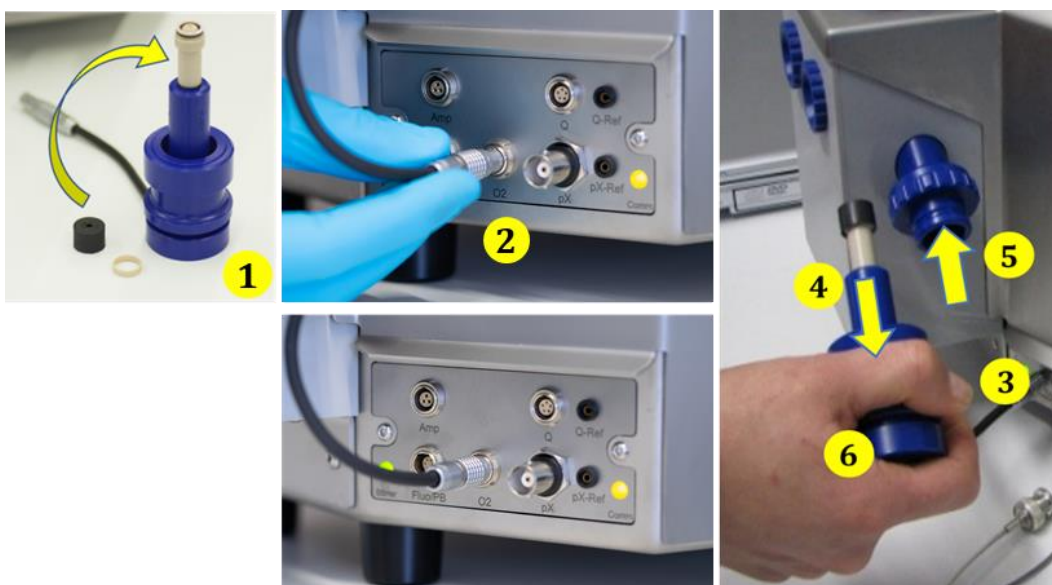
**Go to: OroboPOS assembly**
» MiPNet19.18B_POS-service

⮑Clip A3

- Screw an OroboPOS head onto each OroboPOS-Connector (finger-tight). Push a *wetted* OroboPOS-Seal Tip (black gasket) over the OroboPOS head (arrow 1). Position the pore centrally and do not stretch the OroboPOS-Seal Tip. Each OroboPOS head can be used on either O2k-chamber A or B. Note down the OroboPOS number (marked on the cylindrical body of each OroboPOS) for each O2k-chamber to input later into DatLab (see Section 6).
- Connect the OroboPOS-Connector cable to the O2k-Main Unit. Insert the male plug of the cable (2) into the female O2 plug. The red dot on the male plug must accurately face straight upwards when inserting the plug.
- After inserting the plug of the OroboPOS-Connector cable into the O2k-Main Unit, the OroboPOS-Connector cable (3) must be in a relaxed, undistorted position. While pressing the sleeve of the OroboPOS downwards (arrow 4), insert the OroboPOS with the OroboPOS-Seal Tip into the OroboPOS-Holder (5) and release the sleeve to fix the OroboPOS in its final position.

⚠ **Do not rotate the OroboPOS-Connector** (6) after it is attached to the OroboPOS-Holder in order to prevent damage of the OroboPOS-Seal Tips, which provide a tight seal against the glass O2k-Chamber that has sharp edges.

After the OroboPOS service, the OroboPOS needs some time to stabilize. It is recommended to leave the O2k switched on, from a few hours up to a run overnight, until stabilization.

### 7.4. O2k-chamber disassembly

1.  Remove the OroboPOS-Connectors from the OroboPOS-Holders by pressing the sleeve downwards against the bottom plate of the OroboPOS-Connector. Unplug the cable.
2.  Unscrew the blue O2k-Chamber Holders (and the OroboPOS-Holder) from the O2k-Main Unit.
3.  Carefully insert your finger (with gloves on) into the O2k-Chamber and then gently take it out from the copper block of the NextGen-O2k.

### 7.5. Checking the O2k-chamber and OroboPOS assembly

The O2k assembly can be tested during the Instrumental $O_2$ background test, which requires full attention during performance. An overnight test can be done to rule out media leakage out of the O2k-chambers or gas getting into the O2k-chambers. To test if the assembly is properly done, follow the steps:

1.  Add MiR05 (or $H_2O$) to the O2k-chamber and close it by gently pushing the stopper downwards. Siphon off excess medium from the top of the stopper using the ISS. Check that any air bubbles are removed from the O2k-chamber.
2.  Decrease the oxygen concentration down to ~ 50 µM (using dithionite or $N_2$ or $H_2$ injection); see: Setting the oxygen concentration. When using $H_2O$ do not use dithionite!
3.  Leave the O2k recording overnight with the O2k-chamber closed and illumination switched off, at 25 °C.
4.  The $O_2$ concentration should decrease over time due to the $O_2$ consumed by the OroboPOS. In this case, the chamber is assembled properly.
5.  If the oxygen concentration increases over time and/or air bubbles are observed in the chamber, a reassembly of the O2k-chamber is needed.

### 7.6. O2k-chamber volume calibration

The following instructions are for the black PEEK stoppers. For calibration of Q-Module stoppers, please see MiPNet24.12 NextGen-O2k: Q-Module, topic 4.1.

DatLab must be installed before calibrating the chamber volume. Connect the O2k to the electricity supply (Section 4), connect the O2k with DatLab (Section 6), and familiarize yourself with the O2k control keys (stirrer; Section 7.1). The standard O2k-chamber volume is 2.0 mL (0.5 mL for the O2k-sV-Module; corresponding values below in parentheses).

1.  Dry the O2k-chamber, removing all liquid droplets from the glass O2k-Chamber. Place a dry PVDF stirrer bar into each chamber.
2.  Add exactly 2.07 mL (0.54 mL) of $H_2O$ into the O2k-chamber for calibration of a O2k-chamber volume of 2.0 mL (0.5 mL).
    The volume of the capillary of the stopper (black PEEK) is not part of the effective O2k-chamber volume. The volume of the stopper capillary is 0.065 mL or 65 µL (0.04 mL or 40
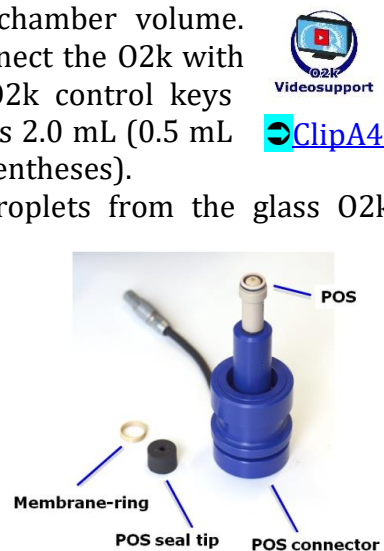
µL). When titrating 2.07 mL (0.54 mL), insert the tip of the pipette to the wall of the glass O2k-Chamber to add the entire volume. Liquid must not be lost between the top of the glass cylinder and the blue (black) O2k-Chamber Holder.

3. Switch on the stirrers (Section 6.4).
4. Loosen the Volume-Calibration Rings (A) and (B) with the screwdriver Allen wrench (OroboPOS-Service Kit). Push them slightly downwards in the direction of the O-rings.
5. Insert the stopper with dry capillary (the O-rings should be moistened) and push it downwards until the gas phase fully extrudes through the capillary of the stopper, while the fixation ring is fully pushed onto the O2k-Chamber Holder.
6. Gently push the stopper further downwards until the capillary fills up and a small droplet appears on the top of the capillary. This marks the volume calibration position.
7. Tighten the screws of the calibration rings (A) and (B) gently.

## 7.7. Smart Fluo-Sensor assembly



Green LED, ex. 525 nm

Blue LED, ex. 465 nm

The Smart Fluo-Sensors are shipped in a separate box as part of the NextGen-O2k. Smart Fluo-Sensors are precalibrated with sensor-specific memory and direct input into DatLab 8.

⚠️  **The Smart Fluo-Sensors cannot be used with O2k-Series G and earlier.**

1. Connect the Smart Fluo-Sensor cable to the Fluo/PB port. (7). The red dot on the male plug should be pointing straight up. Each Smart Fluo-Sensor can be used on either O2k-chamber A or B.
2. The blue frame of the chamber window and the Smart Fluo-Sensor are specially designed to only connect at a specific orientation, when the flat edges align (8). With these aligned, the Smart Fluo-Sensor is carefully inserted in the window opening until the sensor fully covers the chamber window and is flush against the O2k-Main Unit (9a,b). In this position, the cable routing is horizontal (10).
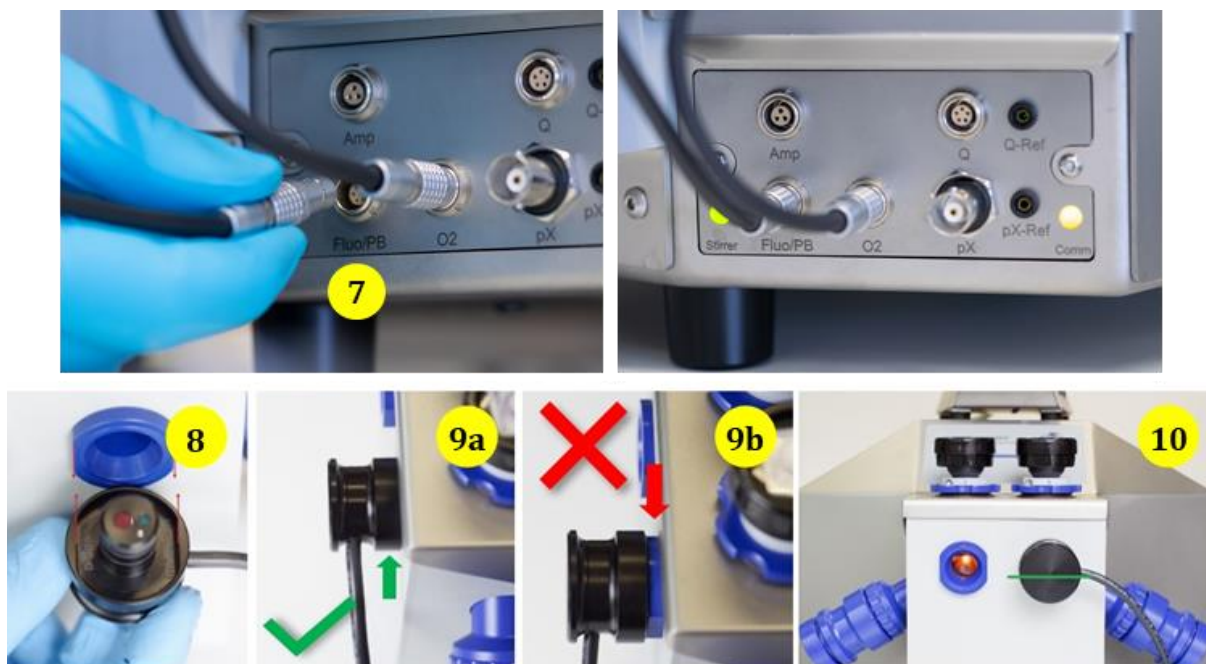
### 7.8.  NADH-Sensor assembly

The NADH-Sensors are shipped in a separate box as part of the NextGen-O2k Redox package and can only be used with the NextGen-O2k (series XA). For more information on its assembly, please see:

» MiPNet26.12 NextGen-O2k: NADH-Module.

### 7.9.  PB-Light Source assembly

The PB-Light Sources are shipped in a separate box as part of the NextGen-O2k PhotoBiology package and can only be used with the NextGen-O2k (series XA). For more information on its assembly, please see:

» MiPNet26.11 NextGen-O2k: PB-Module.

### 7.10.  Q-Sensor assembly

The Q-Sensors are shipped in a separate box as part of the NextGen-O2k Redox package and can only be used with the NextGen-O2k (series XA). For more information on its assembly, please see:

» MiPNet24.12 NextGen-O2k: Q-Module.

## 8.  Start DatLab 8
### 8.1.  Login

On your PC desktop, left click on the icon "DatLab 8" to start the program.

Enter the username and click Login. Click on User at the top right to open the "Manage users" window.
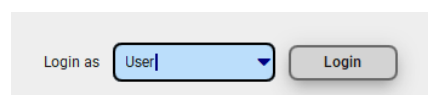


In this window it is possible to manage the information of the user who is currently logged in, add new users or delete users. The information entered on this window will be saved with the DatLab files saved by the user.

Select "Always ask" so that DatLab always requires the username to be entered (or selected in a dropdown menu, after the user is saved) before starting to record or open a file. This option is recommended for computers used by multiple users. Select previous user to skip this step and always enter as the previous user. This option is recommended only for personal computers.

## 8.2. Connect

Click on Connect to O2k on the right, lower side. The serial number is automatically recognized, e.g., Connected to XA-### and an indicator becomes green . On the O2k, the COMM LED still appears yellow.
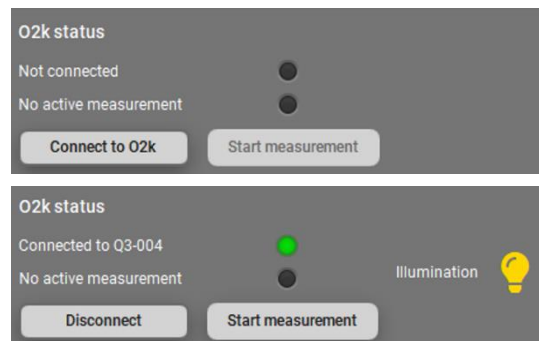
**COMM LED of the O2k-Main Unit**

● Yellow   USB connection is available, but DatLab connection (measurement) is not started.

● Green   USB connection to a PC is established and a Datlab measurement is in progress.

● Red flashing (1 s on/1 s off): USB connection was lost during a Datlab measurement (USB communication error or USB unplugged). If Datlab reconnects automatically, the COMM LED remains green without flashing.

Under O2k information, it is possible to assign a label (**Power-O2k** or **P**-number) to your O2k. This is especially recommended if you have more than one O2k (to differentiate them, e.g., P1 for the first O2k, P2 for the second, and so on). The P-number is automatically saved in each DatLab file, which have the format:
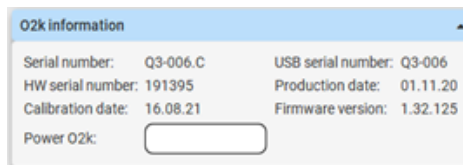
> **YYYY-MM-DD_P#_##.dld8**: date as Year-Month-Day, P#=Power-O2k; **##** =sequential number of the experiment on the same day; extension **dld8** for **D**at**L**ab **D**ata, DatLab 8, e.g.: **2021-10-28 P1-01.dld8**

## 8.3. Settings

Click on the arrow ▼ in Edit channel settings and fill the required information for each channel by clicking on the respective tabs.
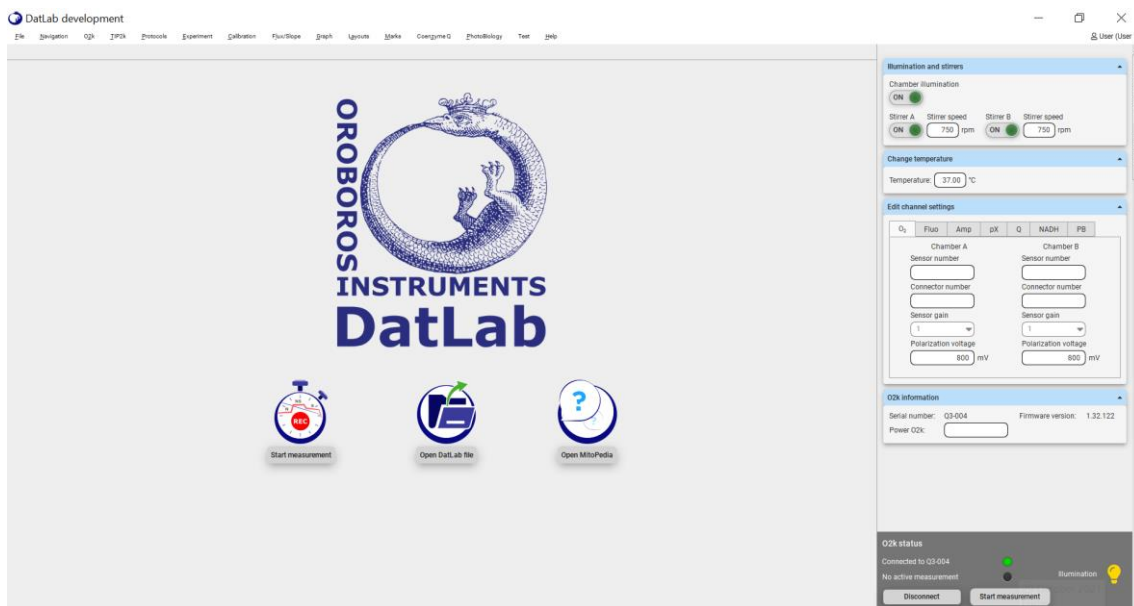
**Oxygen, O₂** is the label for the oxygen channel, which is always active and cannot be deselected. Enter the following settings:



Sensor number: Enter the oxygen sensor number, which can be found on the side of the OroboPOS, for O2k-chambers A and B. Usually, sensors should not be switched between chambers, except for troubleshooting. The individual sensor number is included in the protocol for generating a database of calibration values (MiPNet06.03 POS-calibration-SOP). DatLab 8 will not proceed to recording a file if this number is not entered, for traceability purposes.

Connector number:    Enter the POS-Connector number, which can be found on the side of each POS-Connector, for chambers A and B.

Sensor gain: The gain for the POS is always set to 1 V/μA.

Polarization voltage [mV]: The default for the POS is 800 mV, which is maintained in all routine experiments. Any changes affect the calibration. The voltage can be modified in the range of −2,000 to +2,000 mV for scanning the plateau region of the polarographic oxygen sensor, or for different types of sensors.

**Fluorimetric, Fluo** is the label for the fluorescence channel.

Sensor number: The Smart Fluo-Sensor serial number and LED excitation can be found on the inside of the sensor. The serial number is automatically detected by the NextGen-O2k and set in "Amp sensor #" if the Smart Fluo-Sensor is connected to the O2k. If the serial numbers are not automatically recognized, unplug and plug again the sensors to the O2k.

Enter the following settings:

Sensor gain: Select a gain of 1, 10, 100 or 1000.

Polarization voltage: Select a polarization voltage [mV].

**Amperometric, Amp** is the label for an Amp signal. For applications such as the O2k-NO_Amp-Module, NO, $H_2O_2$ and $H_2S$ sensitive electrodes. Enter the following settings:

Sensor number:   The sensor numbers are not automatically detected by the NextGen-O2k and must be typed manually.

Sensor gain: Select a gain of 1, 10, 100 or 1000.

Polarization voltage: Select a polarization voltage [mV].

For more information on the settings please see:

> » MiPNet15.05 O2k-NO Amp-Module

**Potentiometric, pX** is the label for a potentiometric channel which is used for the ion selective electrodes (TPP$^+$, pH, Ca$^{+2}$). Enter the following settings:

Sensor number and reference electrode number: The sensor and reference electrode numbers are not automatically detected by the NextGen-O2k and must be typed manually.

Sensor gain: Select a gain of 10, 20, 40 or 80.

Polarization voltage: Select a polarization voltage [mV].

For more information on the settings please see:

> » MiPNet15.03 O2k-MultiSensor-ISE

**Q redox, Q** is the label for the Q-Module. For more information on the settings please see:
> » MiPNet24.12 NextGen-O2k: Q-Module

**NADH redox, NADH** is the label for the NADH-Module. For more information on the settings please see:
> » MiPNet26.12 NextGen-O2k: NADH-Module

**PhotoBiology, PB** is the label for the PB-Module. For more information on the settings please see:
> » MiPNet26.11 NextGen-O2k: PB-Module

### 8.4.   Starting a measurement

With the O2k switched on, click on Start measurement. The `Start recording´ window pops up. Two options to proceed with are available:
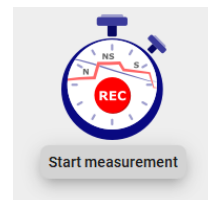


   a. DatLab protocol: Use this option to run an experiment using a DatLab protocol (DLP). If this box is checked, select the protocol clicking on the folder button [ 📁 ] below "Chamber A" and "Chamber B" and select a protocol for each chamber or the same for both. All the settings will be automatically set for the protocol selected.

b. Free protocol: If this box is checked, a list of channels will appear below "Chamber A" and "Chamber B". Select the boxes ☑ for each channel that should be recorded besides O₂, which is always selected. Adjust the other parameters (temperature, data recording interval, illumination in the chamber, stirrer speed) if needed.



**Temperature [°C]:**   Left click on the input window to enter the block (=experimental) temperature (range 4.0 to 47.0 °C at room temperature; 2 °C at lower ambient temperature).

**Data recording interval [s]** can be set between 0.1 s and 10 s, identical for both chambers. The default of 2.0 s is optimal for most applications. Do not decrease the data recording interval unless necessary, since at low data recording intervals, the apparent noise of flux is increased, and real-time information is reduced.

**Illumination in chamber:** Toggle ☑ between On/Off to switch the illumination on or off when connecting. It can be also switched on and off during the experiment by pressing F10. Illumination is different from the light intensity of the Smart Fluo-Sensors, NADH-module or PB light sources.

**Stirrer on:** Toggle ☑ between On/Off to set the stirrer of each chamber on or off when connecting. Independent of this On/Off setting, the left and right stirrers are switched on and off during the experiment by pressing F11 (left) and F12 (right) or F7 (left and right). For a stirrer test: Start a stirrer test by pressing F9: stirring is stopped in both O2k-chambers and restarted after a selected period (default: 30 s).

» [MiPNet06.03 - POS Calibration SOP](#)

**Stirrer speed [rpm]:** The default is 750 rotations per minute (12.5 Hz), which may be varied between 100 and 900 rpm for each chamber independently. The signal level and signal stability increase with stirrer speed in the region of low rpm but uncoupling of the magnet occurs at very high stirring speeds causing high signal noise.

Click on the folder button after 🗀 "Save as" to select a different folder to save the file, or to change the file name. If this is not done, the files will be saved under the "Users" folder.

Click on Start recording at the bottom right. The O2k status window will show that a measurement is active with a green indicator. On the right, the set temperature and the chamber illumination status is shown.
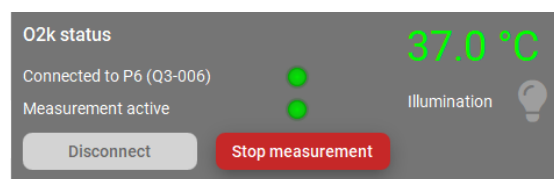
On the O2k, the COMM LED which was yellow while DatLab was not yet recording, becomes green.

### 8.5. *While recording a file*

Anytime during the experiment, settings can be changed by clicking on the right side menus from O2k control: Illumination and stirrers, Change temperature, or Edit channel settings and then selecting each channel and editing. When settings are changed during the experimental run, automatic events appear on the plots to indicate the change.
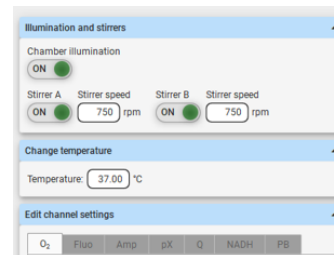
The O2 sensor numbers cannot be changed during the run.

### 7.5.1 *Layouts*

To display different combinations of plots with standardized layouts, select in the upper menu **Layouts**, select "Standard layouts", then the combination of channels used in the measurement, and select one of the pre-set layouts according to needs.
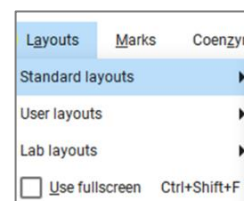
### 7.5.2 *Sample and medium*

Fill-in the window "Sample and medium", which can be found under the upper menu Experiment, then Sample and medium, or by pressing F3. Edit experimental code, sample information, medium, and additional information. Entries can be edited at any time during

real-time or post-experiment analysis. All related results are recalculated instantaneously with the new parameters.



### 7.5.3 Set the O2 limit

Set the $O_2$ limit by clicking on the upper menu Protocols , then Set lower O2 limit or Set upper O2 limit . Can only be changed when connected to the O2k.
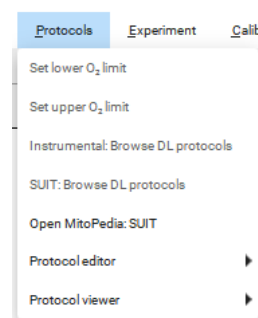




### 8.6.  Stopping a measurement

After the experiment is finished, click on Stop measurement to stop recording data. The dld8. file will be automatically saved.
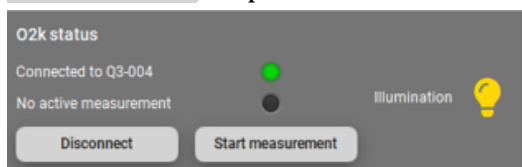


Upon stopping the measurement, the COMM light on the O2k main unit switches from green to yellow. The O2k control window shows the last values used during operation before disconnecting. If the Experimental log is opened (Experiment – Experimental log on the upper menu or Ctrl + F3 ), the settings at the beginning of the experimental run can be seen.

Importantly, after the measurement is stopped, the O2k continues to operate at constant experimental temperature, stirring speed and illumination in the chamber. No data are recorded until another measurement is started to be recorded with DatLab.

### 8.7.  Starting a next measurement

To start another measurement, click on Start measurement in the lower right menu or on O2k in the upper menu, then Start measurement, or press Ctrl+Shift+M.



Again, a default file name is given, as described above, with the next sequential number. After re-connecting, the O2k continues operation uninterrupted, the experimental time is reset to zero, and data recording is restarted, automatically saving to file.

After disconnecting the communication between the O2k and DatLab, the O2k can be switched off.

# 9.    O2k calibration, DL-Protocols and data analysis

## 9.1.  Calibration

**Instrumental DL-Protocols** provide real-time guides to calibrations.

**O2** » O₂ calibration - DatLab
**Amp** » Amp calibration - DatLab
**pX** » pX calibration - DatLab

In DatLab 8, click on Calibration and then Open in the upper menu, or press F5, to open the calibration window. Select the channels and chambers to be calibrated.

The status bar at the bottom of the screen/window provides information on the calibrations. Yellow indicates that the channel has not been calibrated during the current run. Green indicates that the channel has been calibrated during the current run. The calibration from the previous run is maintained, although the indicator will appear in yellow.

For further instructions on calibration, see:
    » MiPNet06.03 POS-calibration-SOP

## 9.2.  DL-Protocols

The sequence of events and marks of the selected DL-Protocol is shown after selecting the Protocol window on the right side. During an experiment, events are set by clicking on the next event shown in yellow. Mark information is added automatically to marks set after the corresponding event. Events and marks are highlighted in green when properly set according to the DL-Protocol (events with sequential steps appear in blue). An erroneous sequence of events or marks is indicated by highlighting the respective events and marks in red.
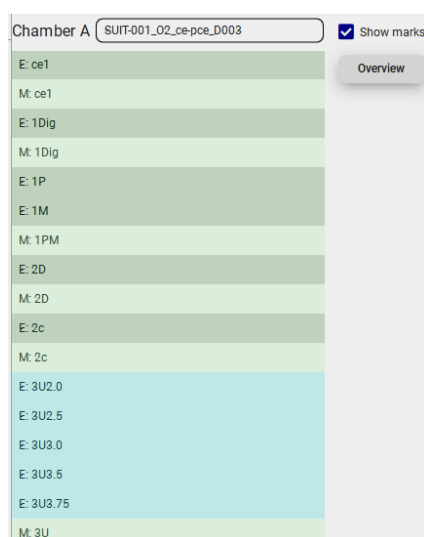
A set of current DL-Protocols can be found on the **Oroboros USB-flash drive** (…\Oroboros O2k-Course on HRR\O2k\DL-Protocols) and can be accessed via the 'Oroboros-FileFinder'. The section 'O2k Manual' lists Instrumental DL-Protocols; the section 'O2k Protocols' lists SUIT DL-Protocols).

    Further details:
    » Library of substrate-uncoupler-inhibitor-titration (SUIT) protocols

Use the **SUITbrowser** to find the best SUIT protocol for your research questions.
Further details:
» SUITbrowser

## 9.3.  Flux analysis

The calculations used for data analysis in DatLab 8 are provided complying with Oroboros transparency policy:
    » Flux / Slope.

## 10.  Further information and links

Further information and updated versions:
»[MiPNet26.13_NextGen-O2k_manual](#)

Links to O2k-catalogue and support
»[Products](#)
»[O2k-Open Support](#)


## 11.  References

[MiPNet07.08 User information](#)

[MiPNet22.11 O2k-FluoRespirometer manual](#)

[MiPNet14.01 ESD-damage](#)

[MiPNet19.18B_POS-service](#)

[MiPNet26.12 NextGen-O2k: NADH-Module](#)

[MiPNet26.11 NextGen-O2k: PB-Module](#)

[MiPNet24.12 NextGen-O2k: Q-Module](#)

[MiPNet06.03 POS-calibration-SOP](#)

[MiPNet15.05 O2k-NO Amp-Module](#)

[MiPNet15.03 O2k-MultiSensor-ISE](#)


## 12.  Author contributions

Cardoso LHD and Cecatto C prepared the MiPNet. Haider M and Niedenzu W developed the software DatLab 8. Gollner M, Merth A, Schwaninger H, Walter-Vracevic M participated in the electronic and mechanical development of the NextGenO2k. Gradl P is responsible for the logistics of electronic and mechanical development of the O2k. Gnaiger E is responsible for the project and instrumental development of the NextGen-O2k.


## 13.  Acknowledgements

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