

O2k-Manual: NextGen-O2k

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NextGen-O2k manual

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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 NADH 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, as the O2k-FluoRespirometer, enables simultaneous measurement of respiration and fluorometric signals (H_2O_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 NADH or Coenzyme Q redox state 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.



NextGen-O2k
ALL IN ONE

TIP2k	MiR05-Kit	O2k-Modules:	
Titration Set		NADH redox	Q redox
sV-Module	ISS	PhotoBiology	
Software:		Smart Fluo (ROS, ATP, mtMP, Ca ²⁺)	
DatLab 8		TPP ⁺ , Ca ²⁺	pH NO
+ auxiliary O2k-Tools:			
PBS-Shredder	Dissection Tools	Microbalance	

The Oroboros O2k is the modular 2-chamber system for high-resolution respirometry (HRR).

NextGen-O2k:

Simultaneous monitoring of oxygen consumption, NADH and Coenzyme Q redox state, photosynthesis or photobiology, fluorescence or potentiometry measurement with small amounts of sample (isolated mitochondria to permeabilized tissues and cells). **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, or 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](#) or [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 instrument come with a free remote [Installation and startup support session](#).



Copy the folder “**Oroboros O2k-Course on HRR**” from the **Oroboros USB-flash drive** to your computer. The ‘Oroboros-FileFinder’ is the tool to find topics and open the files. Updates of the [O2k-Manual](#) and [O2k-Procedures](#) are posted on our website.

It is recommended that you exit all computer applications before running the DatLab installation programme. In the ‘Oroboros-FileFinder’, left click the Oroboros symbol or ‘O2k-DatLab install’. 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: **Oroboros DatLab** 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.

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](#)
- » [NextGen-O2k](#)

3. Electric and lab 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

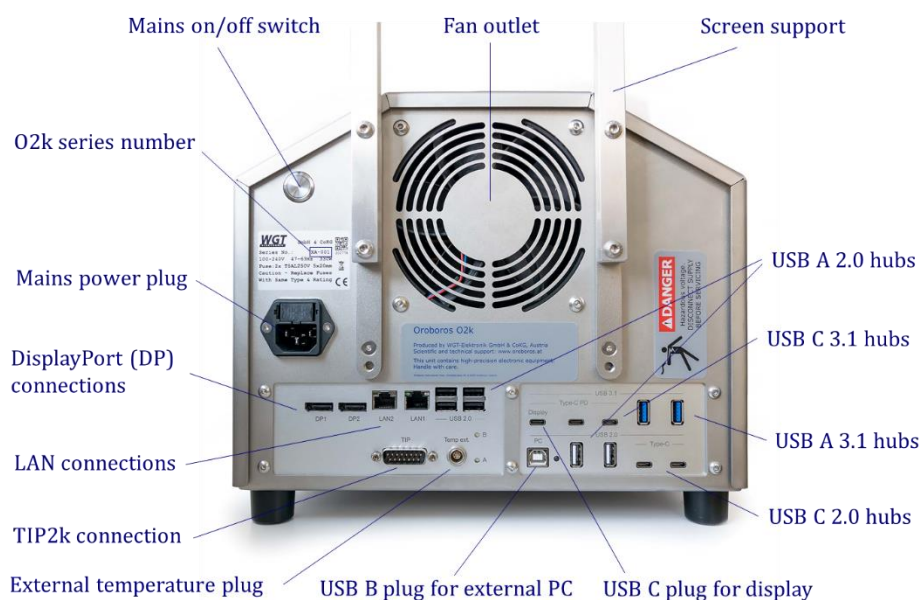
AU/NZ: O2k-Main Power Cable/230 V/AU-NZ, 21113-01



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 with a small 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 of room temperature. Keep the space on the table below the O2k bottom clear to prevent any interference of 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 to the mains.



Connections and information at the rear of the NextGen-O2k: The O2k series number, mains on/off switch, 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 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).

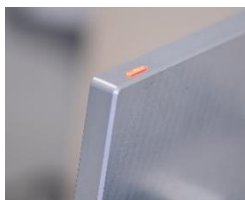
If an external PC is connected to USB 2.0 Type B “PC” socket, then the four lower USB are connected to the external PC, while all other USB’s are still connected to the internal PC. Use one of the four lower USB ports to connect a USB stick with the NextGen-O2k calibration data.

Do not manipulate the plugs “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.

WGT quality control seal: Do not open. An intact quality control seal is required for warranty obligations related to the electronics of the O2k.

For previous series of the O2k FluoRespirometer please see this Manual: [MiPNet22.11 O2k-FluoRespirometer manual](#).



The NextGen O2k comes with an integrated touchscreen (15,6"; FHD 1920x1080). A switch button can be found on the top right of the touchscreen (picture). An USB A 3.1 plug (5 V, 0.9 A, max. 4.5 W) can be found on the screen's left side. On the back of the screen, the plug for connection to the O2k can be found.

4. Start the NextGen-O2k

Using the integrated PC:

1. Connect the O2k to the electricity supply.
2. Connect the screen to the NextGen-O2k with the USB cable (on the O2k: "Display" USB C plug; on the screen: plug on the back).
3. Switch on the O2k at the rear (Mains on/off switch: I/O) **or** at the button at the screen top right. Now the four front control lights illuminate: The outer left diode (**MAINS**) 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. The Mains on/off switch at the back of the O2k and the screen button illuminate.
4. Start DatLab 8 and connect to the O2k. The outer right diode (**COMM**) is yellow and changes to green when data is being recorded.
5. To switch off the screen only, press again the button at the screen top right. To switch off the O2k, disconnect the O2k of DatLab 8, switch off the computer, and switch off the mains at the rear.

For daily use repeat steps 3 – 5.

Using another PC - Installation:

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 plug on the back of the O2k).
5. Switch on the O2k at the rear (Mains on/off switch: I/O). Now the four front control lights illuminate: The outer left diode (**MAINS**) 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.
6. Start DatLab 8 and connect to the O2k. The outer right diode (**COMM**) changes to green.
7. Disconnect at DatLab 8, switch off the O2k at the button at the rear and switch off the computer.

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

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.

5. 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](#)

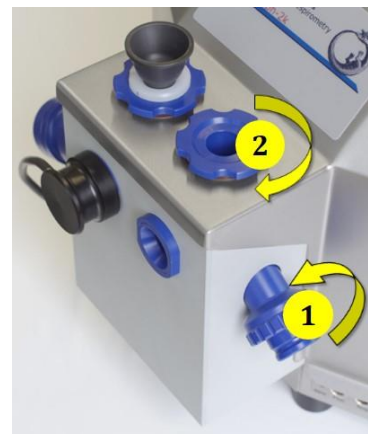
5.1. Instrument overview

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

5.2. 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.

- Remove the O2k-Chamber Holders, unscrewing them. Remove the [OroboPOS-Connectors](#).
- Insert the glass O2k-Chamber into the copper block of the NextGen-O2k, with the angular cut 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.



5.3. 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.



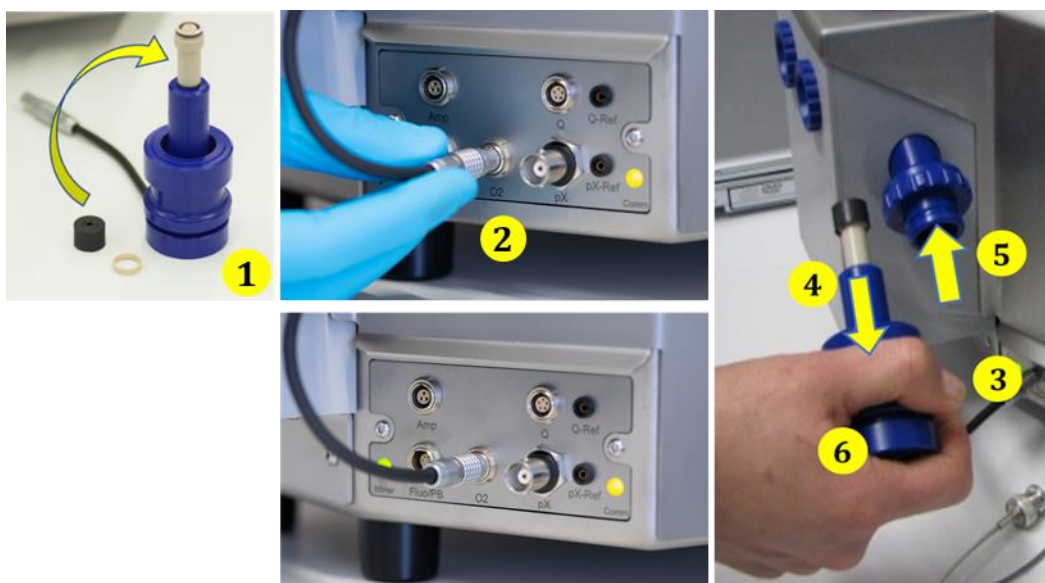
Go to: OroboPOS assembly
» [MiPNet19.18B POS-service](#)



[Clip A3](#)

1. Screw a 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 the OroboPOS number (marked on the cylindrical body of each OroboPOS) for each O2k-chamber (see Section 6).
2. 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.
3. After inserting the plug of the OroboPOS-Connector cable to the O2k-Main Unit, the OroboPOS-Connector cable **(3)** must be in a relaxed, undistorted position. Press the sleeve downwards (arrow **4**) insert the OroboPOS with the OroboPOS-Seal Tip straight into the OroboPOS-Holder **(5)** and release the sleeve for fixing the OroboPOS in its final position.

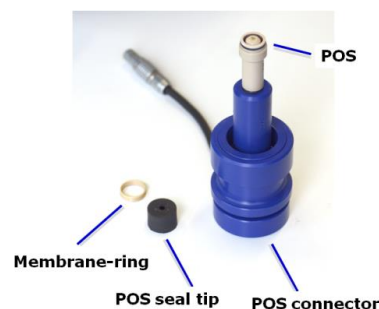
! Do not rotate the OroboPOS-Connector **(6)** after it is attached to the OroboPOS-Holder in order to prevent damage of the OroboPOS-Seal Tips, which provide a tight seal against the glass O2k-Chamber that has sharp edges.



After 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.

5.4. 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.



- 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.

5.5. Checking the O2k-chamber and OroboPOS assembly

The O2k assembly can be tested during the Instrumental O₂ 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:

- Add MiR05 (or H₂O) 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.
- Decrease the oxygen concentration down to ~ 50 μM (using [dithionite](#) or N₂ or H₂ injection); see: [Setting the oxygen concentration](#). When using H₂O do not use dithionite!
- Let the O2k recording overnight with the [O2k-chamber closed](#) and illumination switched off, at 25 °C.
- The O₂ concentration should decrease over time due to the O₂ consumed by the [OroboPOS](#). In this case, the chamber is assembled properly.
- If the oxygen concentration increases over time and/or air bubbles are observed in the chamber, a reassembly of the O2k-chamber is needed.

5.6. O2k-chamber volume calibration

The following instructions are for the [black PEEK stoppers](#), for use of the [Q-Module](#), please follow the instructions in [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 make yourself familiar with the O2k control keys (stirrer; Section 7.1). The standard O2k-chamber volume is 2.0 mL (0.5 mL with the [O2k-sV-Module](#); corresponding values below in parentheses).



[ClipA4](#)

- Dry the O2k-chamber. Remove all liquid droplets from the glass O2k-Chamber. Place a dry PVDF stirrer bar into each chamber.
- Add accurately 2.07 mL (0.54 mL) of H₂O 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.
- Switch on the stirrers (Section **Error! Reference source not found.**).
- 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](#).
- 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.

5.7. Smart Fluo-Sensor assembly



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 up to Series G.

1. Connect the Smart Fluo-Sensor cable to the **Fluo/PB** plug. Similar to the OroboPOS (2), insert the male plug of the cable into the female **Fluo/PB** plug (7). The red dot on the male plug faces straight upwards. 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 unbowed notches align (8). In this optimal position, the Smart Fluo-Sensor is carefully inserted in the window opening until the sensor covers the chamber window without gap (9a,b). In this position, the cable routing is horizontal (10).



5.8. NADH-Sensor assembly

The [NADH-Sensors](#) are shipped in a separate box as part of the NextGen-O2k Redox package and cannot be used with other O2k than the NextGen-O2k (series XA). For more information on its assembly, please see:

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

5.9. PB-Light Source assembly

The [PB-Light Sources](#) are shipped in a separate box as part of the NextGen-O2k PhotoBiology package and cannot be used with other O2k than the NextGen-O2k (series XA). For more information on its assembly, please see:

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

5.10. Q-Sensor assembly

The [Q-Sensors](#) are shipped in a separate box as part of the NextGen-O2k Redox package and cannot be used with other O2k than the NextGen-O2k (series XA). For more information on its assembly, please see:

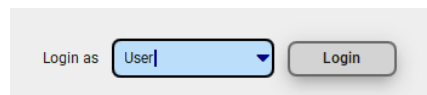
» [MiPNet24.12 NextGen-O2k: Q-Module.](#)

6. Start DatLab 8

6.1. 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.

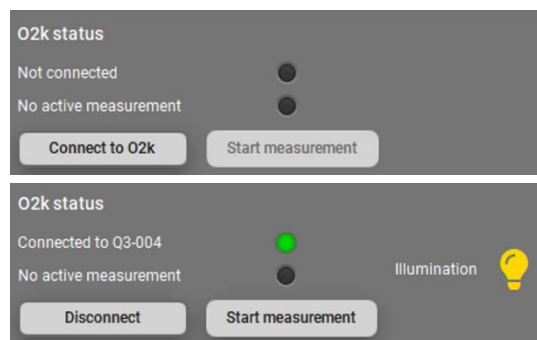


In this window it is possible to manage the information of the [user](#) who is currently logged in, add new users or delete users. The information entered on this window will be saved with the DatLab files saved by the user.

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.

6.2. Connect

Click on **Connect to O2k** at the right, lower side. The serial number is automatically recognized, e.g., **Connected to XA-###** and an indicator becomes **green** at its right side. 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:

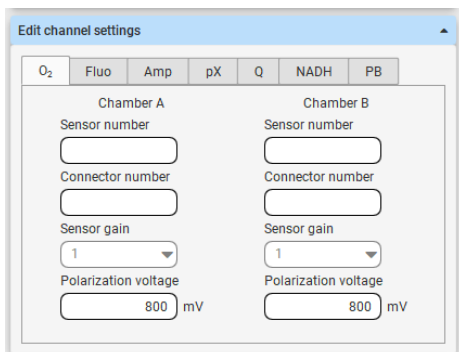
YYYY-MM-DD_P#_##.dld8: date as Year-Month-Day, P#=**P**ower-O2k; ## =sequential number of the experiment on the same day; extension **dld8** for **DatLab Data**, DatLab 8, e.g.: **2021-10-28 P1-01.dld8**

O2k information	
Serial number:	Q3-006.C
USB serial number:	Q3-006
HW serial number:	191395
Production date:	01.11.20
Calibration date:	16.08.21
Firmware version:	1.32.125
Power O2k:	<input type="text"/>

6.3. Settings

Click on the arrow ▼ in **Edit channel settings** and fill the required information for each channel by clicking on the 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](#)). 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.



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, H₂O₂ and H₂S 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, Ca²⁺). 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 1, 10, 100 or 1000.

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:

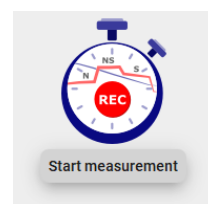
» [MiPNet26.12 NextGen-O2k: NADH-Module](#)


PhotoBiology, **PB** is the label for the [PB-Module](#). For more information on the settings please see:

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



6.4. Starting a measurement

1. 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.

The 'Start recording' dialog box contains the following elements:

- Proceed with:** DatLab protocol, free protocol
- Save as:** C:\Users\Cardoso\2021-10-29_Q3-004_01.dld8
- Chamber A:** 
- Chamber B:** 
- Apply to both chambers:**
- Temperature [°C]:** 37.00
- Data recording interval [s]:** 2.0
- Illumination in chamber:**
- Stirrer on:**
- Stirrer speed [rpm]:** 750
- Volume [mL]:** 2.0, 0.5, 0.00

- 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.

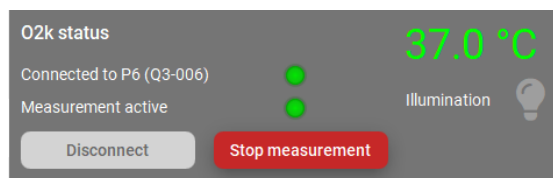
Stirrer on: Toggle between On/Off to set the stirrer of each chamber on or off when connecting. Independent of this On/Off setting, the left and right stirrers are switched on and off during the experiment by pressing **F11** (left) and **F12** (right) or **F7** (left and right). For a stirrer test: 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.

2. 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.

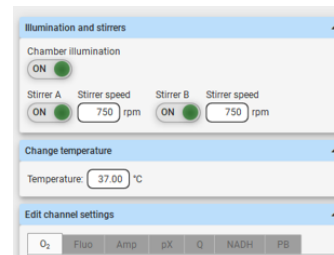
3. Click on **Start recording** at the bottom right. The O2k status window will show that a measurement is active with a **green** indicator. On the right side, there will be indicators of the temperature which was set, and whether the chamber illumination is switched **on** or off. On the O2k, the **COMM** LED which was yellow while DatLab was not yet recording, becomes green.



6.5. While recording a file

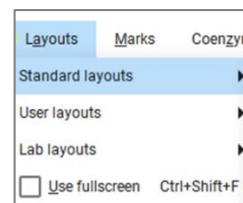
Anytime during the experiment, settings can be changed by clicking on the right side menus from **O2k control**: **Illumination and stirrers**, **Change temperature**, or **Edit channel settings** and then selecting each channel and editing. When settings are changed during the experimental run, automatic events appear on the plots to indicate the change.

The O2 sensor numbers cannot be changed during the run.



6.5.1 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 the needs.

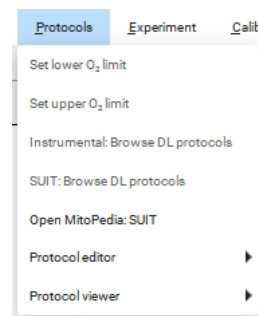
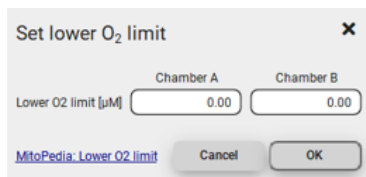


6.5.2 Sample and medium

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.

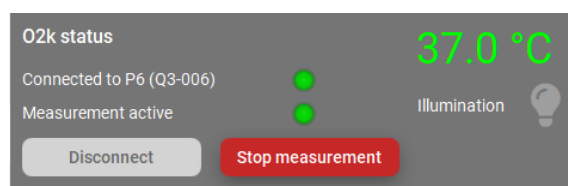
6.5.3 Set the O₂ limit

Set the O₂ 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.



6.6. 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.

6.7. Starting a next measurement

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



O2k	TIP2k	Protocols
Connect		Ctrl+Shift+C
Disconnect		Ctrl+Shift+D
Start measurement		Ctrl+Shift+M
Illumination on/off		F10
Stirrer A on/off		F11
Stirrer B on/off		F12
Stirrers A+B on/off		F7
Stirrer test		F9
Channel and data vector labels		

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 with automatic saving to file.

After disconnecting the communication between the O2k and DatLab, the O2k can be switched off on the rear of the O2k-Main Unit.

7. O2k calibration, DL-Protocols and data analysis

7.1. 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 the **Open** on the upper menu, or press **F5**, to open the calibration window. Select the channels and chambers to be calibrated.

The bottom menu shows the calibration status. 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.

Calibrations A: O₂ Amp pX

Calibrations A: O₂ Amp pX

For further instructions on calibration, see:

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

7.2. DL-Protocols

The sequence of events and marks of the selected DL-Protocol is shown after selecting the **Protocol** window at 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](#)

7.3. Flux analysis

The calculations used for data analysis in DatLab 8 are provided complying with Oroboros transparency policy:

» [Flux / Slope](#).



Further information and links

Further information and updated versions:

» [MiPNet26.13 NextGen-O2k manual](#)

Links to O2k-catalogue and support

» [Products](#)

» [O2k-Open Support](#)

8. Author contributions

Gnaiger E is responsible for the project and instrumental development and preparation of the MiPNet. 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.



9. Acknowledgement

Supported by the NextGen-O2k and MitoFit project. Paolo Cocco, member of Oroboros Instruments, took and edited the photos and images specific to the NextGen-O2k and its modules. Andras Mészáros, as a former member of the Oroboros Instruments, took and edited part of the photos and images.



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