

O2k-Manual: NextGen-O2k Series XB



Mitochondrial Physiology Network 28.07(02):1-23 (2023)

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Updates: https://wiki.orooboros.at/index.php/MiPNet28.07_NextGen-O2k_Series_XB_manual

NextGen-O2k Series XB manual

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1. Overview



NextGen-O2k All-in-one



NextGen-O2k Redox



NextGen-O2k Fluo



NextGen-O2k Startup

Fluo



O2k-Fluo Smart-Module

PB



PB-Module

NADH



NADH-Module

Q



Q-Module

The **Oroboros NextGen-O2k** provides a unique high-resolution approach to the monitoring of cellular and mitochondrial respiratory function and supports an innovative combination of add-on modules for measurement of NAD and Coenzyme Q redox state, as well as photosynthesis together with dark respiration (PhotoBiology-Module) and fluorescence (O2k-Fluo Smart-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 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 pediatric 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. In addition, 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.

Oroboros

NextGen-O2k



TIP2k MiR05-Kit

Titration Set

sV-Module

ISS

Software:

DatLab 8

O2k-Modules:

NADH redox

Q redox

PhotoBiology

Smart Fluo (ROS, ATP, mtMP, Ca^{2+})TPP⁺, Ca^{2+}

pH

NO

+ auxiliary O2k-Tools:

Oxia

Dissection Tools

Microbalance

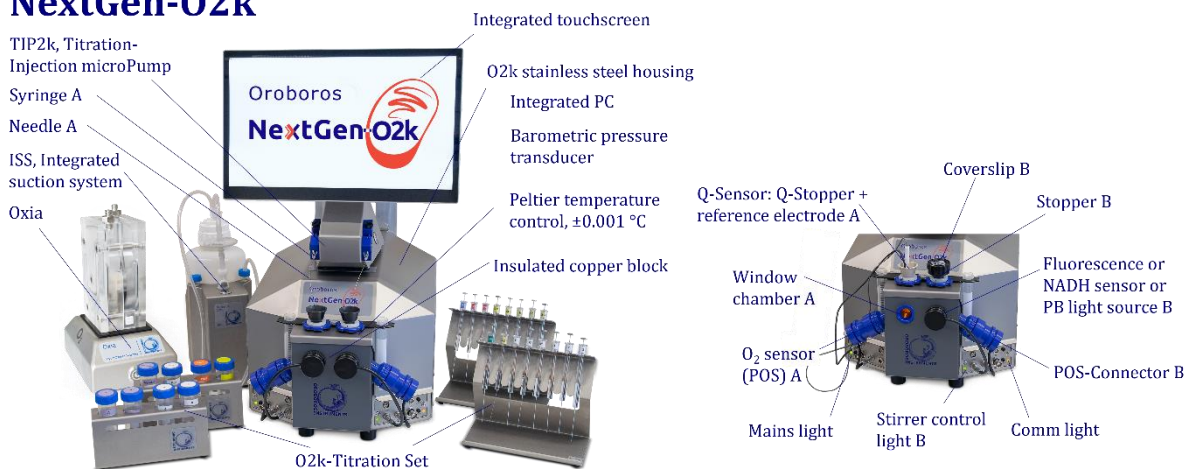
The Oroboros O2k is the modular 2-chamber system for high-resolution respirometry (HRR). NextGen-O2k:

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, Integrated Suction System (ISS). **Add-on O2k-Modules:** high flexibility to expand the spectrum in HRR.

HRR with the Oroboros NextGen-O2k 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 Smart polarographic oxygen sensor ([SmartPOS](#)), [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](#).

NextGen-O2k



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, webcam, 2 [SmartPOS](#), [O2k-Accessory box](#), 2 [PVDF Stirrer-Bars](#), 2 [PEEK O2k-Stoppers](#), [DatLab 8 software](#), [ISS-Integrated Suction System](#), and [O2k-Titration Set](#).

The following NextGen-O2k packages are available:

- The [NextGen-O2k Startup](#), including the items mentioned above.
- The [NextGen-O2k all-in-one](#), also including the [NADH-Module](#), [Q-Module](#), [O2k-Fluo Smart-Module](#) with 4 Smart Fluo-Sensors (2 blue and 2 green), the [PB-Module](#) with 6 PhotoBiology light sources – PB-Light Sources (2 blue, 2 red and 2 white).
- The [NextGen-O2k Redox](#), also including the [NADH-Module](#) and [Q-Module](#).
- The [NextGen-O2k Fluo](#), also including [O2k-Fluo Smart-Module](#) with 4 Smart Fluo-Sensors (2 blue and 2 green).

All packages, excluding NextGen-O2k Startup, also include [O2k-sV-Module](#), [Titration-Injection microPump \(TIP2k\)](#) and [Oxia](#).



O2k-Manual user information

- » [MiPNet07.08 User information](#) - PLEASE STUDY THIS MANUAL
- » [O2k-Manual](#)



2. Unpack the Oroboros-O2k

[Box 1](#) (or [Peli Case](#)) contains the O2k-Main Unit. After unpacking, store the original Box 1 (or Peli Case) and packing material for any future shipment (return for repair) of the O2k-Main Unit.

For other modules and accessories, please refer to your dispatch note.

3. Integrated PC and DatLab 8

DatLab 8 comes installed in the NextGen-O2k integrated PC and is ready to use for protocol design, O2k control/data acquisition and data analysis. The integrated PC is operated by Linux and has the following specifications: Intel® Pentium® Silver Processor, J5005 (QC @1.5 GHz/2.8 GHz), SSD, 256GB, 2280 M.2 SATA, 8GB DDR4, Integrated Intel® UHD Graphics 600/605.

The NextGen-O2k comes with a US 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.

If installing and running DatLab 8 on another PC or laptop for data acquisition or analysis, a full HD screen is recommended. PC or laptop minimum configuration: 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. To complete the installation, download DatLab 8 from our website ([here](#)) and follow the instructions. 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) and save them on an external device. Please note that Oroboros Instruments cannot be held responsible for securing data generated with the O2k.

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 comes with 'Sleep' mode already disabled.

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:

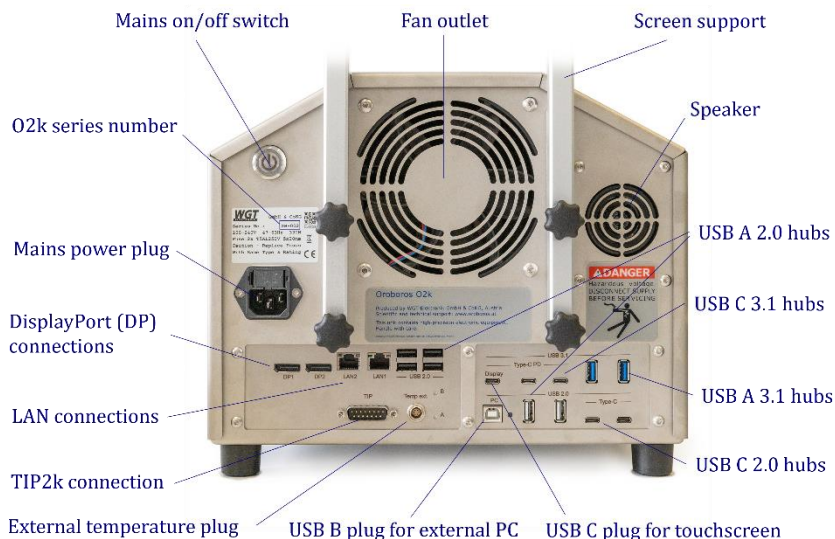
- EU:** O2k-Main Power Cable/230 V/Europe, 21111-01
Note: Use O2k-Main Power Cable/230 V/Europe (Hybrid German/French plug CEE7/7, Type E+F) only for a compatible socket.
- DK:** Some Danish sockets will also accept the CEE7/7 plugs, but there is no grounding connection with these plugs without male 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 (Series XB): The mains on/off button, O2k series number, , mains power plug, two DisplayPort connections (DP1, DP2), two LAN connections (LAN1, LAN2), TIP2k connection, external temperature (temp ext.) plug, 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 two USB C 2.0 hubs (5 V, 1.5 A, max. 7.5 W), two USB A 3.1 hubs (5 V, 1.5 A, max. 7.5 W), two USB C 3.1 hubs (5 V, 3 A, max. 60 W), and six USB A 2.0 hubs (5 V, 0.5 A, max. 2.5 W).

If an external PC is connected to the O2k via the USB 2.0 Type B “PC” port, then the four lower USB ports will connect to the external PC, while all other USB ports will still connect to the integrated 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 left 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 susceptible to viruses and ransomware like any other PC. Ensure that no contaminated USB is connected to the NextGen-O2k. Do not click on suspicious links or install untrustworthy software. 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 Limited 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 intended for persistent data storage.



It is the responsibility of the user to copy all important data to dedicated permanent storage outside the O2k. Oroboros is not liable for any data losses. 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. To prevent accidental deletion of non-backed-up data by an unauthorized user, the unsecure initial password must be changed to a more secure one upon first login.

For previous series of the NextGen-O2k please see this Manual:

» [MiPNet26.13 NextGen-O2k manual](#).

6. Starting the NextGen-O2k

6.1. Using the integrated PC

1. Fix the screen support to the main unit using the 4 long screws/bolts. Holding the screen with two hands, insert the touchscreen into 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 power source.
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 with either the main power button at the rear or the button on the top of the screen. The four front control lights of the O2k-Main Unit should now be illuminated: 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**) is yellow. The power button at the back of the O2k illuminates blue and the button on the screen illuminates green if PC and screen are on or blue if PC is on and the screen is off.
5. Start DatLab 8 and connect to the O2k. The outer right diode (**COMM**) changes from yellow to green when data is being recorded with DatLab 8.
6. To switch off the screen only, press the button on the screen top left, the light on screen turns blue. To switch off the O2k, disconnect the O2k from DatLab 8 and shut down 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 power 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 with the main power button at the rear. The four front control lights of the O2k-Main Unit should now be illuminated: 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**) is yellow if a USB connection is available. In this case, the power button at the back of the O2k does **not** illuminate and the screen button remains red.
7. Start DatLab 8 using the external PC and connect to the O2k. The outer right diode (**COMM**) changes from yellow 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 shut down the computer.

For daily use repeat steps 1, and 5-8.

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 to the electronics. Touch a grounded object, such as 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, there are Amp and Fluo/PB 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-Accessory box](#). The O2k-Main Unit comes mounted with the blue [O2k-Chamber Holders](#) and [POS-Holders](#). Two 0.5-mL O2k-Chambers with black O2k-Chamber Holders and POS-Holders are in the [O2k-sV-Module](#) box, if included in the NextGen-O2k package. To use the Smart Fluo-Sensors or the NADH-, PB- or Q-Modules, assemble the NextGen-O2k with the 2-mL O2k-Chambers.

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



[ClipA1](#)

7.3. SmartPOS assembly

The [SmartPOS](#) are shipped dry without electrolyte, in a Perspex housing, in the a storage box. Before assembling the O2k, apply electrolyte and membrane, following the SmartPOS service instructions.



Go to: SmartPOS assembly
» [MiPNet28.10 SmartPOS service](#)



- Push a *wetted* [POS-Seal Tip](#) (black gasket) over the SmartPOS head ([arrow 1](#)). Position the pore centrally and do not stretch the POS-Seal Tip. Each SmartPOS can be used on either O2k-chamber A or B.
- Connect the SmartPOS cable to the O2k-Main Unit. Insert the cable plug ([2](#)) into the O2s port. The red dot on the cable plug must face straight up when inserting it.



Only the SmartPOS, and not the OroboPOS-Connector, can be connected to the O2s port.

- After inserting the plug of the SmartPOS cable into the O2k-Main Unit, the SmartPOS cable ([3](#)) must be in a relaxed, undistorted position. While pressing the sleeve of the SmartPOS downwards ([arrow 4](#)), insert the SmartPOS with the POS-Seal Tip into the POS-Holder ([5](#)) and release the sleeve to fix the SmartPOS in its final position.



Do not rotate the SmartPOS ([6](#)) after it is attached to the POS-Holder to prevent any potential damage of the POS-Seal Tips against the sharp edges of the glass O2k-Chamber, ensuring the integrity of the tight seal provided by them.

After the SmartPOS service, the SmartPOS needs time to stabilize. It is recommended to leave the O2k switched on and running, from a few hours to overnight, until stabilization.

7.4. O2k-chamber disassembly

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

7.5. Checking the O2k-chamber and SmartPOS 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 entering the O2k-chambers. To test for proper assembly, follow these steps:

1. 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.
2. Decrease the oxygen concentration down to ~ 50 μM (using [dithionite](#) or N₂ or H₂ injection); see: [Setting the oxygen concentration, Oxia](#). When using H₂O do not use dithionite!
3. Leave the O2k recording overnight with the [O2k-chamber closed](#) and illumination switched off, at 25 °C.
4. The O₂ concentration should decrease over time due to the O₂ consumed by the SmartPOS. 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 power source (Section 4), connect the O2k with DatLab and familiarize yourself with the O2k control keys (stirrer; Section 8). 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; if needed, rinse the chamber with 100 % EtOH and let it dry. Place a dry stirrer bar into each chamber.
2. Add exactly 2.07 mL (0.54 mL) of H₂O into the O2k-chamber for calibration of an 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 8.5).
4. Loosen the [Volume-Calibration Rings](#) (A) and (B) with the screwdriver Allen wrench (O2k-Accessory box). 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. Additional modules assembly

For instructions on additional modules for simultaneous measurements in combination with oxygen, please follow the instructions for the assembly of the specific module. All listed modules are compatible with the NextGen-O2k (series XB).

7.7.1. Smart Fluo-Sensor

The [Smart Fluo-Sensors](#) are shipped in a separate box and are pre-calibrated with sensor specific memory and direct input into DatLab 8. For more information on its assembly, please see:

» [MiPNet28.09 O2k-Fluo Smart-Module manual](#)

7.7.2. NADH-Sensor assembly

The [NADH-Sensors](#) are shipped in a separate box and present sensor specific memory and direct input into DatLab 8. For more information on its assembly, please see:

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

7.7.3. PB-Light Source assembly

The [PB-Light Sources](#) are shipped in a separate box and present sensor specific memory and direct input into DatLab 8. For more information on its assembly, please see:

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

7.7.4. Q-Sensor assembly

The [Q-Sensors](#) are shipped in a separate box and present sensor specific memory and direct input into DatLab 8. For more information on its assembly, please see:

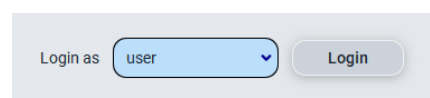
» [MiPNet24.12 NextGen-O2k: Q-Module.](#)

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 and start measurement** on the main screen or select on the top menu **O2k** and **Connect** (Ctrl+Shift+C) or **Connect and start measurement** (Ctrl+Shift+M). On the O2k, the **COMM** LED still appears yellow.

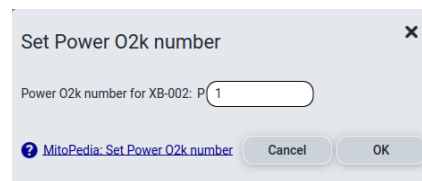


O2k	TIP2k	Protocols	Experiment
O2k control (active)			F7
O2k info (view)			Ctrl+F7
Connect			Ctrl+Shift+C
Disconnect			Ctrl+Shift+D
Connect and start measurement			Ctrl+Shift+M
Stop measurement			
Illumination on/off			F10
Stirrer A on/off			F11
Stirrer B on/off			F12
Stirrer test			
Set Power O2k number			Ctrl+P
Data labels and units			Ctrl+D
Reboot O2k			

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 **Set Power O2k number** in the top menu **O2k**, 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 (P1 as default) is visible during the measurement in the left lower corner and is automatically saved in each DatLab file, which have the format:



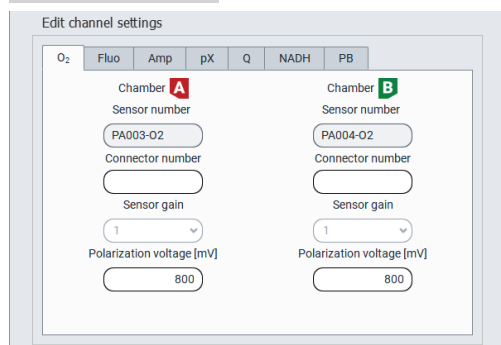
YYYY-MM-DD_P#_##.dld8: date as Year-Month-Day, P#=**P**ower-O2k;
 ## =sequential number of the experiment on the same day;
 extension **dld8** for **D**at**L**ab **D**ata, DatLab 8, e.g.: **2021-10-28 P1-01.dld8**

8.3. Settings

Select on the top menu **O2k** and then **O2k Control (active)** (F7). In **Edit channel settings** 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. Enter the following settings:

Sensor number: The SmartPOS serial number can be found on the bottom side of the



sensor. When the Smart POS is connected to the O2k, the sensor number is automatically detected by DatLab 8 and set in **O₂** “Sensor number”. If the serial numbers are not automatically recognized, unplug the sensors and plug them into the O2k again. 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 recognized, for traceability purposes.

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.

Fluorometric, 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 **Fluo** “Sensor number” if the Smart Fluo-Sensor is connected to the O2k. If the serial numbers are not automatically recognized, unplug the sensors and plug them in again.

For more information on the settings please see:

» [MiPNet28.09 O2k-Fluo Smart-Module manual](#)

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.

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.

For more information on the settings please see:

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

Q redox, Q is the label for the [Q-Module](#).

Sensor number and **reference electrode number**: The Q-Sensor serial number can be found on the side of the sensor. The serial number is automatically detected by the NextGen-O2k and set in **Q** “Sensor number” if the Q-Sensor is connected to the O2k. If the serial numbers are not automatically recognized, unplug the sensors from the O2k and plug them in again. The reference electrode numbers are not automatically detected by the NextGen-O2k and must be typed manually.

For more information on the settings please see:

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

NADH redox, NADH is the label for the [NADH-Module](#).

Sensor number: The NADH-Sensor serial number can be found on the inside of the sensor. The serial number is automatically detected by the NextGen-O2k and set in **NADH** “Sensor number” if the NADH-Sensor is connected to the O2k. If the serial numbers are not automatically recognized, unplug the sensors from the O2k and plug them in again.

For more information on the settings please see:

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

PhotoBiology, PB is the label for the [PB-Module](#).

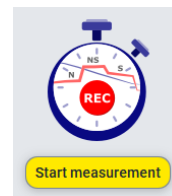
PhotoBiology light source (PBLs) number: The serial number can be found on the inside of the PBLs. The serial number is automatically detected by the NextGen-O2k and set in **PB** “PBLs number” if the PBLs is connected to the O2k. If the serial numbers are not automatically recognized, unplug the PBLs from the O2k and plug them in again.

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 **Connect and start measurement** or **Start measurement** (if already connected). The “Start recording” window pops up and includes the 3 following steps:




1. Select where to save the file:

The file is saved by default under the “Users” folder with a default name as explained in section 8.2. To select a different folder to save the file, or to change the file name, click on the folder button.





2. Select the protocol:

- a. DatLab protocol: Select this option to run an experiment using a [DatLab protocol \(dlp8\)](#). To choose the protocol, click on the folder button  below “Chamber A” and “Chamber B” and select a protocol for each chamber or the same for both (by ticking “Apply to both chambers”).

The screenshot shows the 'Start recording' dialog box. The 'Proceed with' section has 'DatLab protocol' selected. Under 'Chamber A' and 'Chamber B', there are folder icons for selecting a protocol. The 'Apply to both chambers' checkbox is checked. Below, there are settings for Temperature [°C] (37.00), Data recording interval [s] (2.0), Stirrer on/speed [rpm] (750), and Volume [mL] (2.0).

- b. Free protocol: If this option is checked, a list of channels will appear below “Chamber A” and “Chamber B”. Select all the channels that should be recorded besides O_2 , which is always included.

The screenshot shows the 'Start recording' dialog box with 'free protocol' selected. Under 'Chamber A' and 'Chamber B', there are lists of channels to be recorded. The 'Apply to both chambers' checkbox is checked. Below, there are settings for Temperature [°C] (37.00), Data recording interval [s] (2.0), Stirrer on/speed [rpm] (750), and Volume [mL] (2.0).



3. Adjust the settings of your measurement:

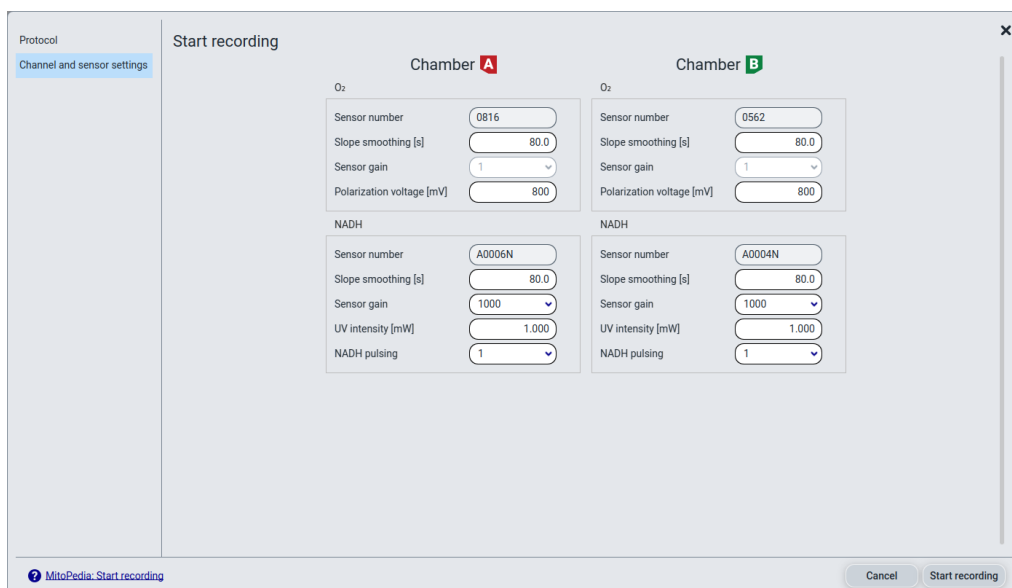
Temperature [°C]: Left click on the input window to enter the block (=experimental) temperature (range 4.0 to 47.0 °C at room temperature; 2 °C at lower ambient temperature). The default temperature is 37 °C.

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.

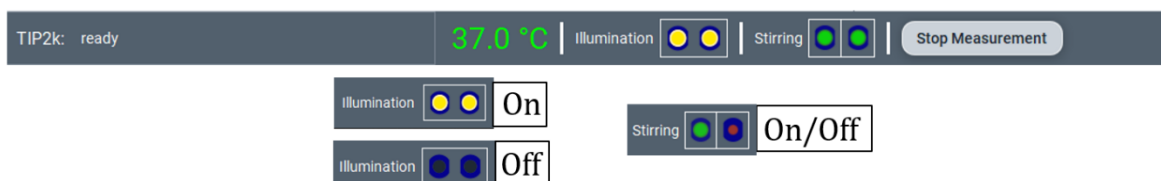
Stirrer on: Select or unselect the box to set the stirrer of each chamber on or off when connecting. If the box is selected, the corresponding stirrer speed can be chosen (see “Stirrer speed” below).

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 at lower rpm but uncoupling of the magnet occurs at very high stirring speeds causing high signal noise.

To check the settings of the specific channels and sensors, select the tab **Channel and sensor settings** on the left bar.



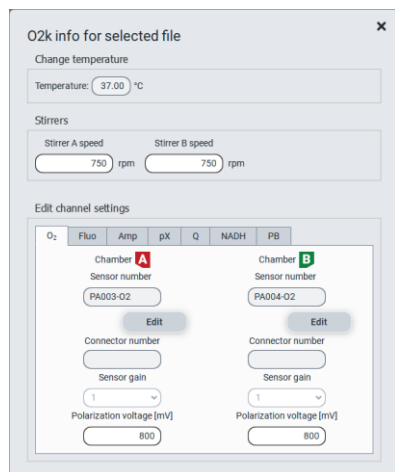
Click on **Start recording** at the bottom right of the “Start recording” window. On the control bar, the set temperature, the chamber illumination and the stirring status are shown and can be modified by clicking on the respective icon.



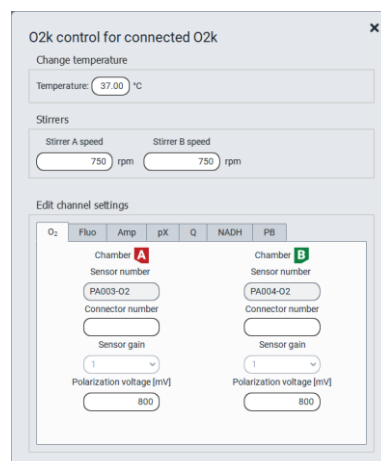
On the O2k, the **COMM** LED which was yellow while DatLab was not yet recording, becomes green.

8.5. While recording a file

Anytime during the experiment, settings can be changed by clicking on the top menu **O2k** and **O2k control (active)** or by clicking on the temperature in the control bar. The following fields can be edited: **Change temperature**, **Stirrers**, or **Edit channel settings**. The chamber illumination and stirrers can be turned on/off through the control bar as



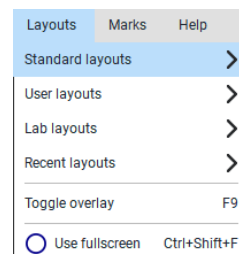
mentioned above or the top menu **O2k** and **Illumination on/off (F10)**, **Stirrer A on/off (F11)**, and **Stirrer B on/off (F12)**. When settings are changed during the experimental run, automatic instrumental events are set on the graphs.



After a file is recorded the experimental settings can be found on the top menu **O2k** and **O2k info (view)** or by pressing Ctrl+F7. This window displays the settings but does not allow modifying them. While recording, **O2k info (view)** and **O2k control (active)** display the same information for the recording file. If more saved files are open while recording, **O2k control (active)** will always show the settings of the active recording, while **O2k info (view)** will show the settings of the recorded selected file.

8.5.1 Layouts

To display different combinations of plots with standardized layouts, select on the top menu **Layouts**, then **Standard layouts**, and choose one of the pre-set layouts according to the combination of channels used in the measurement. When using DatLab 8 protocols the appropriate default layout is automatically loaded by the selected DL8-protocol.



8.5.2 Sample and medium

Fill-in the window "Sample and medium," which can be found under the top 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. Data are normalized using the information entered in this window.

Sample and medium

Chamber A Chamber B

Experimental code: MIR05_Kit QC

Protocol: SU1F001 O2 ce-pce D003

Sample type: HEK cryo

Sample preparation: ce to pce

Cohort: 22J01040

Sample code: 2022-09-20

Sample number: 1

Subsample number: 17

Medium: MIR05-ki Lot22J

Chamber volume [mL]: 2.00

Normalization: Type: cell count, Unit: Mx, Concentration per mL: 1.0000, Amount per chamber: 2.0000

Comments: MIR05-kit prepared on 2022-04-05

MitoPedia_Sample Cancel OK

8.5.3 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**. It can only be changed when connected to the O2k.

Set lower O₂ limit

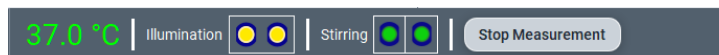
Chamber A Chamber B

Lower O₂ limit [µM]: 0.00 0.00

MitoPedia: Lower O₂ limit Cancel OK

8.6. Stopping a measurement

After the experiment is finished, click on **Stop measurement** on the control bar 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 (active)** and **O2k info (view)** windows show the last values used during operation before disconnecting. If the Experimental log is opened (**Experiment** - Experimental log on the top menu or Ctrl+F3), the settings 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 control bar or on **O2k** in the top menu, then **Start measurement (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.

O2k	TIP2k	Protocols	Ex
O2k control (active)			F7
O2k info (view)			Ctrl+F7
Connect			Ctrl+Shift+C
Disconnect			Ctrl+Shift+D
Start measurement			Ctrl+Shift+M
Stop measurement			
Illumination on/off			F10
Stirrer A on/off			F11
Stirrer B on/off			F12
Stirrer test			
Set Power O2k number			Ctrl+P
Data labels and units			Ctrl+D
Reboot O2k			

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 **Calibration** in the top 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 provides information on the calibrations:

- For **O₂** channel: Orange indicates that the channel has not been calibrated during the current run or in the current day with the same temperature and sensor number. Green indicates that the channel has been calibrated during the current run or a calibration was applied in the current day with the same conditions mentioned above.
- For other channels: the indicator appears green only when the channel has been calibrated during the current run. The calibration from the previous run is maintained, although the indicator will appear in orange.

Calibrations A:	O ₂	Fluo
Calibrations B:	O ₂	Fluo
Calibrations A:	Fluo	Fluo
Calibrations B:	Fluo	Fluo

For further instructions on calibration, see:

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

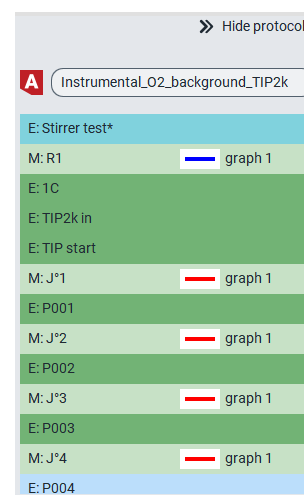
9.2. DL-Protocols

The sequence of events and marks of the selected DL-Protocol is shown on the right side (if it is not visible, click on **Show protocols** on the top). 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 top menu **Protocols** and **Instrumental: Browse DL-Protocols** or **SUIT: Browse 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:

» [MiPNet28.07 NextGen-O2k Series XB manual](#)

Links to O2k-catalogue and support

» [Products](#)

» [O2k-Open Support](#)

11. References

[MiPNet07.08 User information](#)

[MiPNet26.13 NextGen-O2k manual](#)

[MiPNet14.01 ESD-damage](#)

[MiPNet28.10 SmartPOS-Service](#)

[MiPNet28.09 O2k-Fluo Smart-Module manual](#)

[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

Grings M and Baglivo E prepared the MiPNet. Gnaiger E is responsible for the project and instrumental development of the NextGen-O2k.

13. Acknowledgements

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