

uSense Solutions

Software Manual

uSense Solutions Manual

UNISENSE A/S

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WARRANTY AND LIABILITY

The uSense Solutions software is checked and validated on the operating systems as given in the specification, running English language settings.

Software must be installed under administrator rights. Customer must ensure PC is fully updated and no conflicting third party software is installed. Unisense do not warrant compliance with any other operating systems, language settings or third party software.

For instrumentation and sensors, please refer to our warranty conditions as given in the document "General Terms of Sale and Delivery of Unisense A/S" found on www.unisense.com.

License agreement

The following terms shall apply to the software provided by Unisense A/S ("Unisense") in connection with the simultaneous sale to you ("Customer") of a Unisense "uSense Solutions" Software.

All rights, title and interest in the software belong to Unisense. Unisense grants the Customer a royalty-free, non-exclusive and non-transferable license to use the software solely in connection with the Unisense Product purchased from Unisense simultaneously with the purchase of the software. The Customer undertakes not to copy, modify, reverse engineer, disassemble or de-compile all or any part of the software or rent, lease, distribute or sell the software. The Customer shall, however, be entitled to make one copy of the software for back-up and recovery purposes for use solely in connection with the Unisense Products supplied by Unisense together with the software.

Nothing in this License Agreement or any other agreement between Unisense and the Customer shall be construed as an obligation for Unisense to provide to the Customer updates of the software. This License Agreement shall automatically terminate if the Customer violates the terms of the license. In case of termination of the license the Customer shall immediately destroy the software and any copy thereof. Unisense reserves the right to register the license activation and use of the software license via the internet. The software may automatically upload bug reports from time to time. These uploads are only designed to improve, enhance and further develop the uSense Solutions software.

THE CUSTOMER TAKES THE SOFTWARE "AS IS." UNISENSE MAKES NO WARRANTY OR REPRESENTATION CONCERNING THE SOFTWARE, AND EXPRESSLY DISCLAIMS ALL OTHER WARRANTIES AND CONDITIONS, EXPRESS OR IMPLIED, STATUTORY OR OTHERWISE, OF WHATEVER KIND OR NATURE, INCLUDING BUT NOT LIMITED TO ANY AND ALL IMPLIED WARRANTIES, INCLUDING IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. UNISENSE SHALL NOT BE LIABLE FOR ANY DAMAGES OF ANY KIND, INCLUDING INCIDENTAL, SPECIAL, PUNITIVE, CONSEQUENTIAL, AND SIMILAR DAMAGES, INCLUDING, WITHOUT LIMITATION, LOSS OF PRODUCTION, LOSS OF PROFIT, LOSS OF DATA, LOSS OF GOODWILL, LOSS OF CONTRACTS, OR BUSINESS INTERRUPTION

This License Agreement and any dispute arising out of or in relation to this License Agreement shall be governed by and construed in accordance with the laws of Denmark exclusive of its choice of law provisions. The venue for any such dispute shall be the Danish courts provided however that Unisense shall be entitled to instigate legal proceedings against the Customer before the courts with jurisdiction over the matter located in a country where the Customer has a place of business or is incorporated or organized.

CONGRATULATIONS ON YOUR NEW PRODUCT

Support, ordering, and contact information

If you wish to order additional products or if you encounter any problems and need scientific/technical assistance, please do not hesitate to contact our sales and support team. We will respond to your inquiry within one working day.

E-mail: sales@unisense.com

Unisense A/S

Tel: +45 8944 9500

Further documentation and support is available on our website www.unisense.com.

OVERVIEW

uSense Solutions is a software package including uSense Log, Profile, Rate, and Photo. All uSense Solutions programs have the same overall frame and functions but with application specific differences. In this manual, we first describe all the general parts of the software followed by a software specific description for the different programs.

The specific programs offer the following features:

Log (freeware): For sensor calibration and time series datalogging.

Log+: For basic data acquisition. It offers sensor calibration, time series data-logging and motor control.

Profile: For data logging along transects, visualization of these measurements and calculations of activity rates. The software supports motor controlled, automated measurements, but also manual positioning of individual sensors.

Rate: For microrespiration experiments, to measure the metabolic rates including respiration rates of small aquatic animals, bacteria or oxygen production of phytoplankton.

Photo: For photosynthetic experiments using the light-dark switch technique.

uSense Solutions is compatible with all Unisense instruments designed for use in laboratories and some of the field/in situ instruments. All data can be exported into Excel or csv formatted files for subsequent data analysis.

Minimum PC requirements

- Windows 10 (1809) or newer
- 8 GB RAM
- 500 MB free harddisk space
- .NET 8/9 Runtime installed

INSTALLING THE SOFTWARE

Make sure that you are installing using full administrator rights. The software must be installed locally, not on a network drive.

Make sure that you always run the latest version of uSense Solutions.

The latest version is always available on www.unisense.com.

License activation

uSense Log can be used without a license (freeware). To activate any of the other applications in uSense Solutions (Log+, Photo, Rate and Profile) a license key is necessary.

Contact sales@unisense.com to purchase a license key. It is possible to try out the full uSense Solutions free of charge for 14 days by selecting **Activate 14 days trial**.

Register your License

License activation

uSense Log

uSense Log
Software for data logging

Free

uSense Profile (Includes uSense Photo and uSense Motor)

uSense Profile
Software for MicroProfiling System

uSense Photo
Software for photosynthesis studies using light-dark shift

uSense Motor
Software for motor controller

Enter license key

uSense Rate

uSense Rate
Software for MicroRespiration System

Enter license key

uSense Field

uSense Field
Software for programming field systems

Enter license key

The Unisense software uSense Solutions is available as:

Freeware version

Logging data: Log data as a function of time.

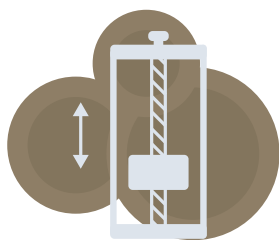
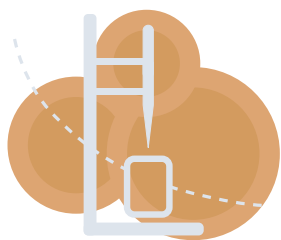
Contains: uSense Log



Licensed versions

Profiling: Use MicroProfiling System to measure microprofiles and photosynthesis.

Contains: uSense Profile, uSense Photo, uSense Motor



MicroRespiration: Use MicroRespiration System to measure changes over time.

Contains: uSense Rate



In Situ Systems: For programming MiniChamber Lander, MiniProfiler, Eddy Covariance System

Contains: uSense Field. Contact sales@unisense.com for further information.

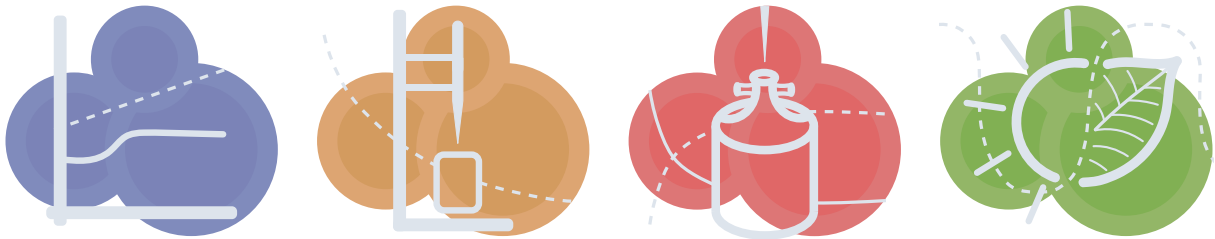


GETTING STARTED

1. Connect the Unisense amplifier(s) and motor(s) to the computer.
2. Set the PC power management to **Always on** and make sure that your PC does not enter sleep mode or standby during measurements, as this will interrupt the connection to the instruments and it will be necessary to restart the program. See "Troubleshooting" below.

IMPORTANT! Please make sure that your PC does not enter sleep mode or standby during measurements as this will interrupt the connection to the instruments and it will be necessary to restart the program

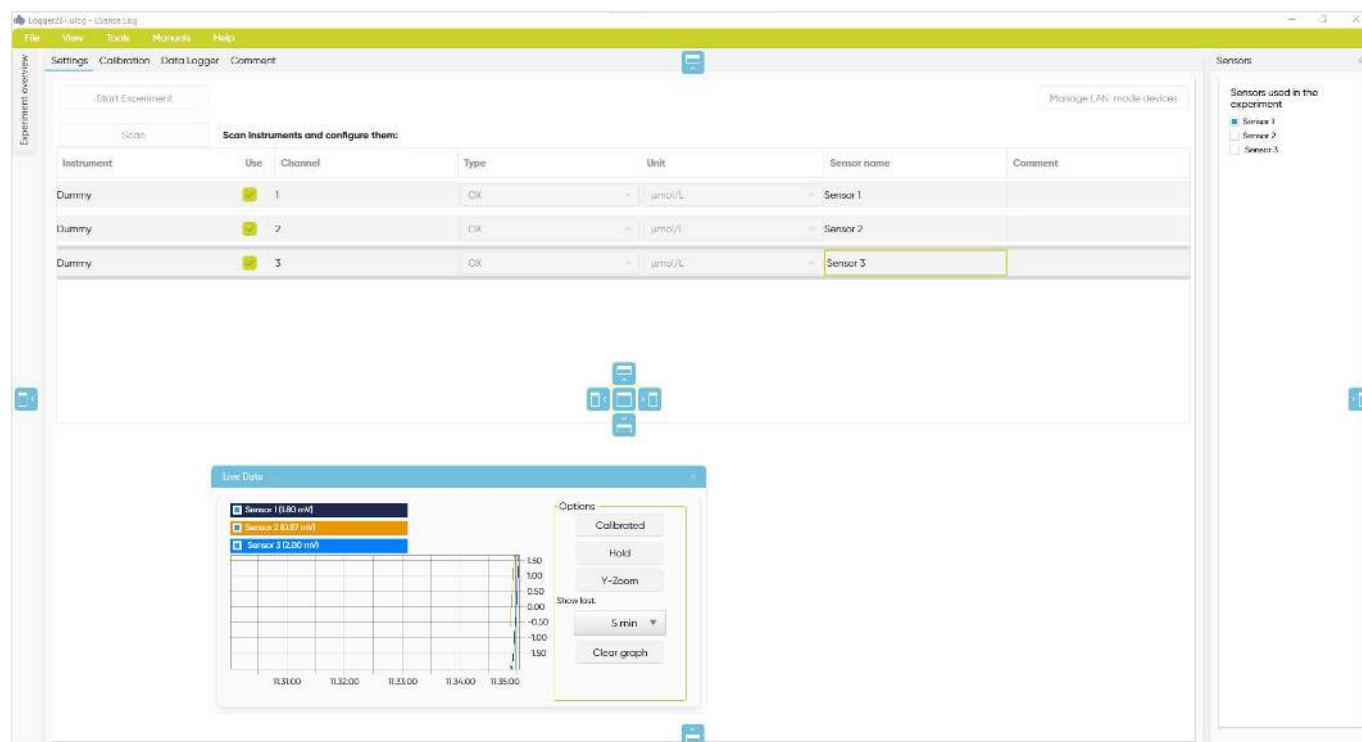
3. Start the free uSense Log software or one of the programs in the uSense Solutions package: Profile, Rate or Photo.



4. In the settings window you have to ensure that all the instruments, sensors, and motors connected to the computer have been recognized. For starting an experiment select **Start Experiment**. You will be asked to name your experiment and save the file on your computer. By default, the files will be saved in Unisense Data under Documents.
5. Load an experiment: You can load an old experiment using the File menu. Alternatively, you can double click the experiment file and the file will open in the program it was made in.
Working with old data; settings and parameters cannot be changed and new measurements cannot be started.

Re-arrange windows on the screen

The different frames on the screen can be moved around and can either be docked to one of the four sides or be floating.



To move a frame, click and hold on the top bar of the frame and drag it. The frame is now floating. To dock the frame again, drag it onto one of the blue icons and let go.

The frames can be moved around between and function on multiple screens. For example, you can view Live Data on one screen and follow the profiling on another.

The size of the frames can be changed by moving the mouse to the light grey area between two frames. When the mouse symbol changes to a double arrow, click and drag to change the size of the frames.

The frames can be shown or hidden by toggling the pin symbol in the upper right corner of a frame.

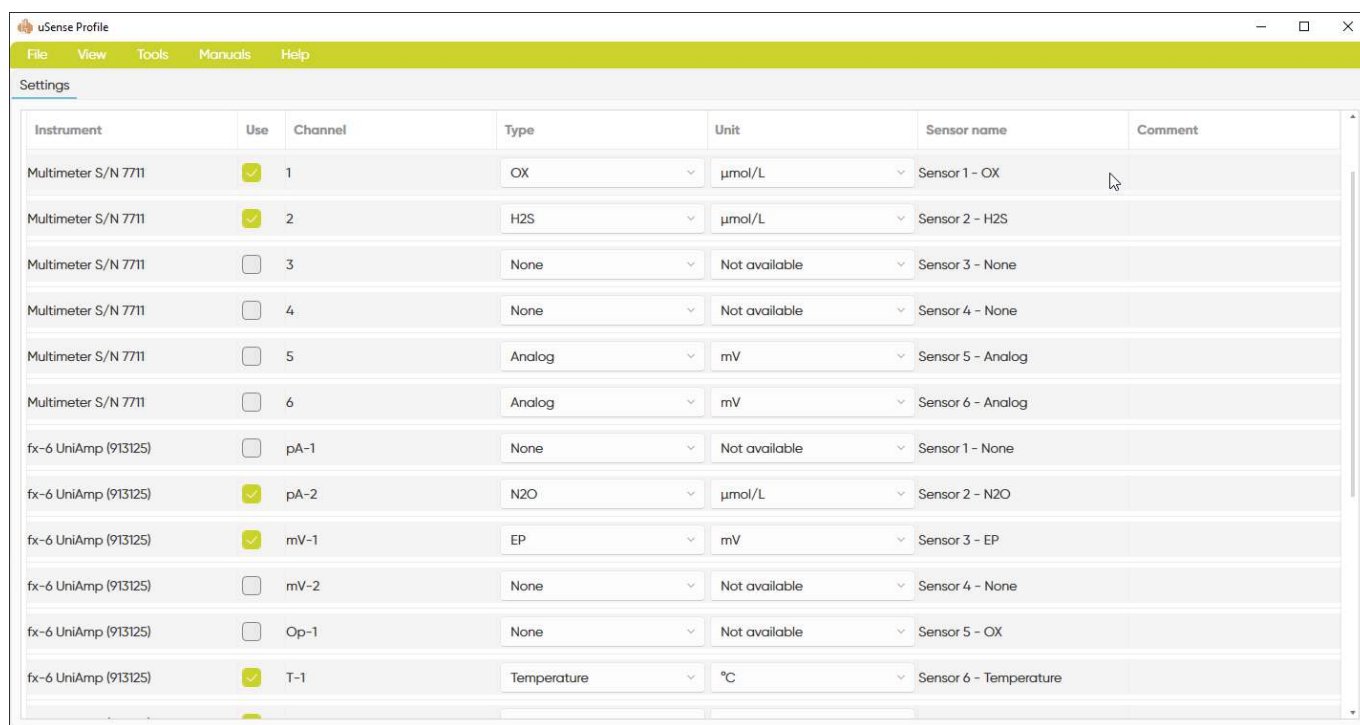
The arrangement of frames can be reset to the default view by clicking View – Reset to default.

SETTINGS TAB

The first tab to appear is the **Settings tab**. This tab will display the detected hardware, motors, and sensors and you can change the settings for the sensors and motors here.

Sensor Settings

In the Settings window, all identified sensors are marked. For each sensor, there are several setting options. From left to right, you can find the following information: channel number on the instrument where the sensor is connected, sensor type, sensor measuring unit, and sensor name. Furthermore, it is possible to add a short comment for each sensor.



The screenshot shows the 'uSense Profile' application window with the 'Settings' tab selected. The window has a menu bar with 'File', 'View', 'Tools', 'Manuals', and 'Help'. Below the menu bar is a table with the following columns: 'Instrument', 'Use', 'Channel', 'Type', 'Unit', 'Sensor name', and 'Comment'. The table lists 15 sensors, grouped by instrument type: 'Multimeter S/N 7711' (6 sensors) and 'fx-6 UniAmp (913125)' (9 sensors). Each row has a 'Use' checkbox, a 'Channel' value, a 'Type' dropdown menu, a 'Unit' dropdown menu, a 'Sensor name' dropdown menu, and a 'Comment' text field. The 'Use' checkboxes for the first two sensors of each instrument type are checked.

Instrument	Use	Channel	Type	Unit	Sensor name	Comment
Multimeter S/N 7711	☒	1	OX	$\mu\text{mol/L}$	Sensor 1 - OX	
Multimeter S/N 7711	☒	2	H2S	$\mu\text{mol/L}$	Sensor 2 - H2S	
Multimeter S/N 7711	☐	3	None	Not available	Sensor 3 - None	
Multimeter S/N 7711	☐	4	None	Not available	Sensor 4 - None	
Multimeter S/N 7711	☐	5	Analog	mV	Sensor 5 - Analog	
Multimeter S/N 7711	☐	6	Analog	mV	Sensor 6 - Analog	
fx-6 UniAmp (913125)	☐	pA-1	None	Not available	Sensor 1 - None	
fx-6 UniAmp (913125)	☒	pA-2	N2O	$\mu\text{mol/L}$	Sensor 2 - N2O	
fx-6 UniAmp (913125)	☒	mV-1	EP	mV	Sensor 3 - EP	
fx-6 UniAmp (913125)	☐	mV-2	None	Not available	Sensor 4 - None	
fx-6 UniAmp (913125)	☐	Op-1	None	Not available	Sensor 5 - OX	
fx-6 UniAmp (913125)	☒	T-1	Temperature	$^{\circ}\text{C}$	Sensor 6 - Temperature	

Sensor: Mark the checkboxes for the channels/sensors you want to view and record signals from.

Type: Choose sensor type from the drop-down menu if the default type is not correct.

Unit: Select an appropriate concentration unit for the sensor signal when calibrated.

NOTE: In uSense Profile, Rate and Photo only $\mu\text{mol/L}$ will give access to subsequent data analysis features.

Name: Write a name describing your sensor (optional).

Comment: Write a comment about your sensor (optional).

Name: Write a name describing your sensor (optional).

Comment: Write a comment about your sensor (optional).

Motor Settings

Available in Log+, Profile and Photo.

NOTE: If a Field Microsensor Multimeter is connected, the two motor channels will be shown even if no motor is connected.

Motors: The connected motors are detected by the software. Click the Scan button if motor connections have been changed.

Motor Axis: In this software Z denotes the vertical axis, X and Y the horizontal axes. For a 1D setup, only the Z-axis is available, in a 2D setup Z- and X-axes are available, and in a 3D setup Z-, X- and Y-axes are available.

For motors connected via the lab motor controller (MOTCON), the axis designation may be modified using the dropdown menu.

To identify the motor controller connected to a given row in the motor list, click anywhere in that row and the green light on the connected motor controller will blink 10 times. The configuration of axes will be saved and used for subsequent experiments until a new axis configuration is made.

Device	Motor Axis	Velocity	Acceleration	Port connected
Lab Motor (UniMOTCON)	Z	1000	1000	COM50
Lab Motor (UniMOTCON)	X	1000	1000	COM50
Lab Motor (UniMOTCON)	Y	1000	10000	COM50

Manual motor: Z Add Remove

For Field Motors, the motor connected to the Motor 1 connector on the Field Multimeter is per default configured as the Z-axis and the motor connected to the Motor 2 connector is configured as the X-axis.

This may be modified using the dropdown menu as shown above, however, this configuration is only for the current experiment and the axis configuration will revert to default in the next experiment.

For motors connected via the MC-232 motor controller, the axis designation is coded in each motor controller. Contact Unisense if you need to modify this.

NOTE: For a manual microprofiling setup with only a Micromanipulator (MM33 or MM33-2) and no Motor Stages, it is not necessary to add an axis. A manual Z-axis will always be available in the experiment file.

Velocity and acceleration: For lab motor stages controlled by MOTCON or MC-232, the velocity and acceleration can be specified. The default velocity is 1000 $\mu\text{m/s}$, and the default acceleration is 1000 $\mu\text{m/s}^2$. Maximum values are 20000 $\mu\text{m/s}$ for velocity and 300000 $\mu\text{m/s}^2$ for acceleration.

Manual Motor: It is possible to add extra axes to the setup here. If an axis is defined as a Manual motor (e.g. Micromanipulator), the user will be prompted to manually move the sensor each time this is required. Once the setup has been completed, click **Start experiment**. A 'save file' dialog will appear. Select where the experiment file should be saved. The software will subsequently make several new windows available depending on which program of uSense Solutions you use.

The sensor and motor configuration will be saved.

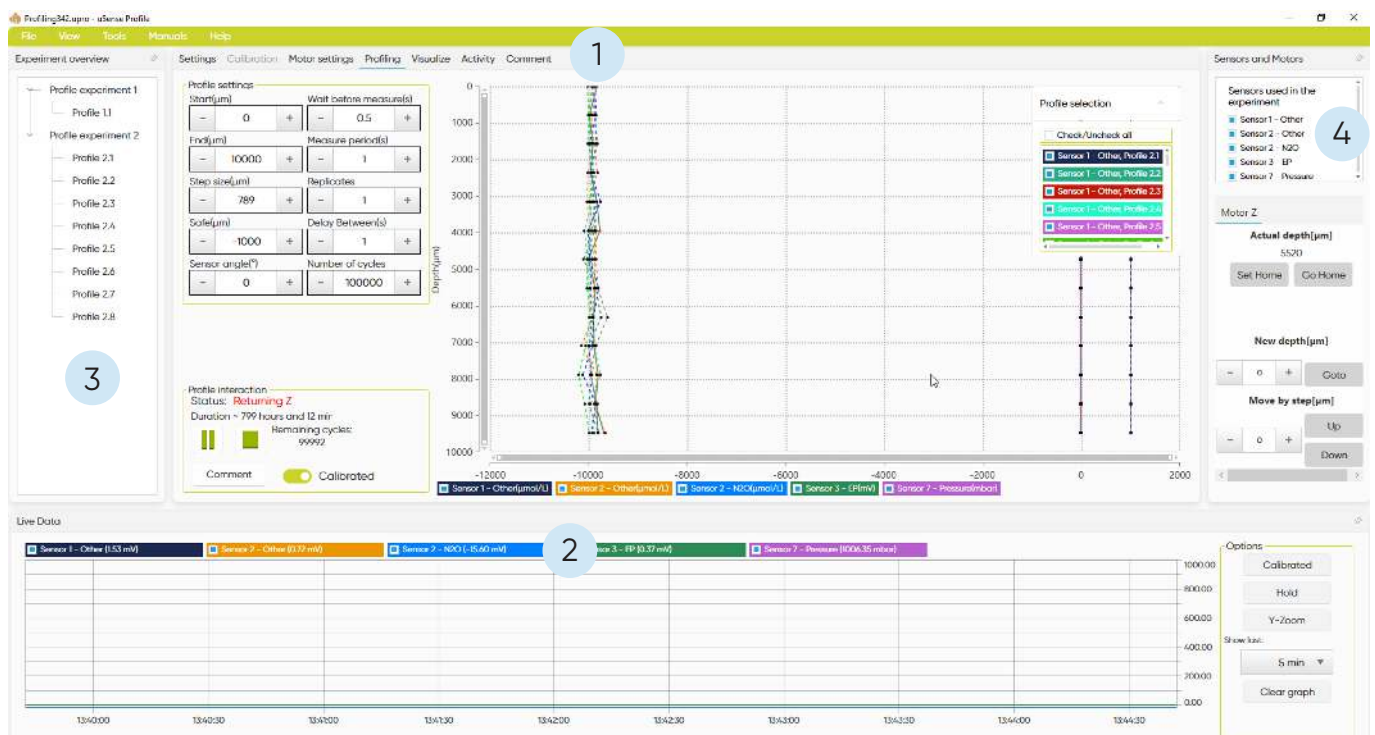
NOTE: Data can be added to a uSense Experiment file until it is closed. After that, it can be opened for working with data but new data cannot be saved to a re-opened file.

Any event (recording of data, calibration, add a comment, etc.) will be saved to the open file immediately. The File - Save as menu is only available if a file has been opened as read only or from a location where it cannot be saved (e.g. from an email).

GENERAL FEATURES

All uSense Solutions programs have windows that by default are divided into four main areas: 1. Window selection, 2. Live Data, 3. Experiment overview, and 4. Sensors and Motors. The windows can be pinned or unpinned depending whether you wish to have a permanent view of the window or not. The height and width of the windows can be changed by dragging its borders

NOTE: You can navigate between the windows at any time when the software is running a program.



Window selection (1): The upper area is divided into windows which allow you to access different functions of the program. The number of windows and the specification of the windows will vary depending on the specific program. See the description of the specific windows in the respective sections for uSense Log, Profile, Rate, and Photo. The Settings window, Calibration window and the Comment window are the same for all programs.

Live data (2): The lower area, the Live Data, shows the live sensor signals of all sensors.

Experiment overview window (3): In the left side area, Experiment overview lists the finished experiments.

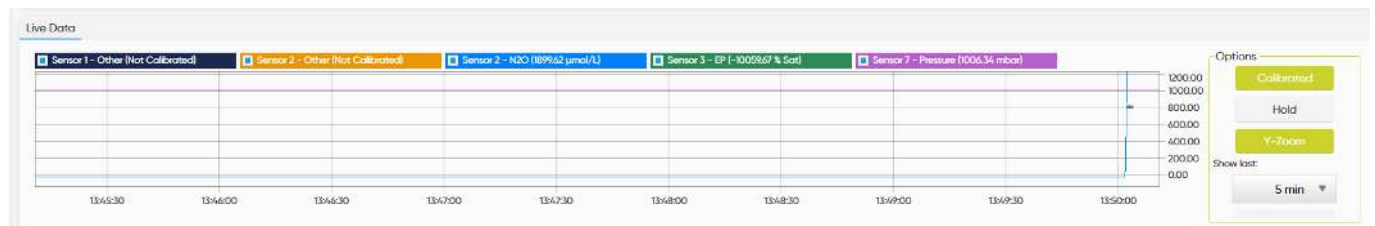
Sensor and Motors (4): In the right side area, Sensors and Motors show the sensor selection and, if available, the motor control. A motor control is available in uSense Log+, Photo, and Profile.

All components will be described in detail in the following pages.

LIVE DATA GRAPH

The Live Data graph is permanently visible in the lower part of the interface. It allows you to view sensor signals continuously. By default, uncalibrated raw sensor signals are shown but if the Calibrated box is checked, calibrated values are plotted. You can always see the Live Data from the last 24 hours.

Comments, calibrations points, and other events generated by the user or the program can be seen as colored marks in the Live Data window. For further information on comments and events, see the Comment window section.



X-axis scale: The time scale (x-axis) is controlled in the **Show Last** drop down list, where a number of time intervals can be chosen. To have a look at a certain time span, scroll to zoom in and out, or drag a rectangle with the mouse from the upper left corner to the lower right corner of the area of interest. To zoom out, right-click and select **Reset Zoom-Level** or double-click directly on the graph.

Y-axis scale: By default, the y-axis auto scales to accommodate the maximum and minimum signals that are shown in the Live Data. A certain time span can be zoomed in and out as described above.

Graph: To have a look at a certain part of the graph, zoom in on this area as described above.

Calibrated/uncalibrated: Here you can control whether the graphs show calibrated signals or raw signals. If no calibration has been applied and the checkbox is checked, no signals will be plotted.

Hold: Here the auto updating is paused and zooming and scrolling through the data is easier. Data are still collected and unchecking **Hold** will update the graph.

Y-zoom: By selecting **Y-zoom** the graph will be zoomed only on the y-axis and not on the x-axis.

***ZOOM FUNCTION:** It is possible to zoom in and out on the graph by using the mouse wheel. Point the cursor on part of the graph of interest and use the mouse wheel to zoom in and out. A certain time span: drag a rectangle with the mouse from the upper left corner to the lower right corner. Fast zoom out: double-click on the graph*

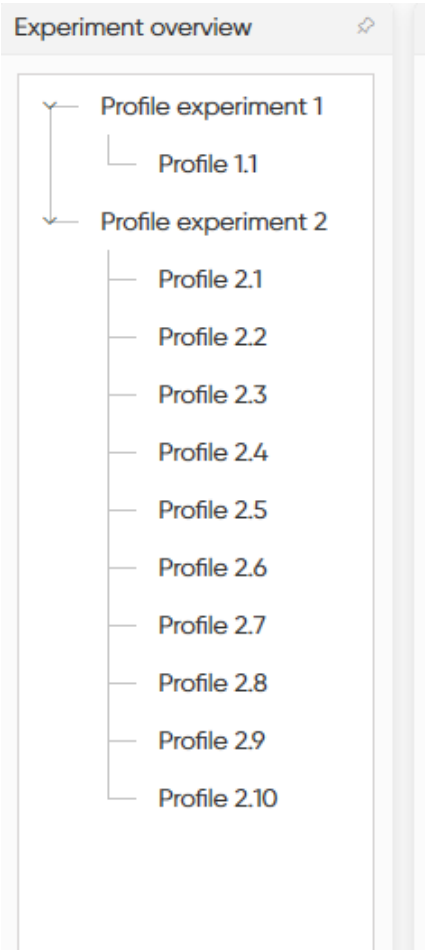
Chart legend: At the top of the Live Data there is a sensor legend showing the graph color for the associated sensors and their current signal/concentration value. Data for all channels is shown at startup. Click the check box in the legend of a channel to show or hide data.

Data points: The data points in the Live Data window can be cleared by selecting **Clear graph**. This will NOT affect the stored data.

Save data from Live Data: Right-click in the graph and select Export Data. This saves all the available data in the graph to an Excel or csv file. Data that has been deleted using the Clear graph (above) are not available for export.

EXPERIMENT OVERVIEW

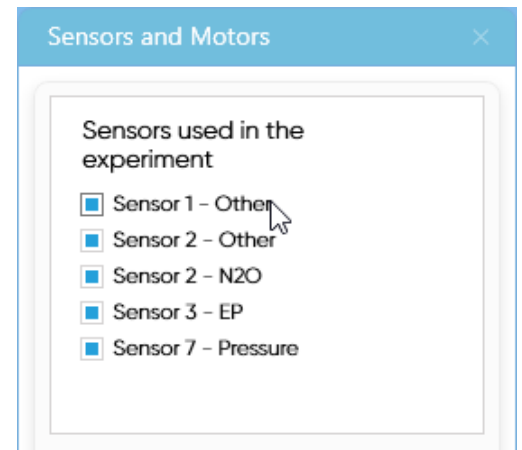
The Experiment overview frame shows all the sub experiments and analysis made in an experiment. They can be loaded or deleted and a note can be added. All sub experiments are stored in one experiment file.



SENSORS AND MOTORS

Sensors

Select the sensors you wish to use here.



Motors

The motor control box is found in uSense Log+ (licensed version), Photo, and Profile and allows you to read, adjust, and redefine your sensor position.

The motors detected by the program can be controlled from here. For a 1D system, the motor z is used, for a 2D motorized system, motor z and x are used, and for a 3D system, motors z, x, and y are available. For the x-axis and y-axis motor, the up and down movement refers to horizontal movement.

Going **Down** means the direction of movement will be away from the motor head.

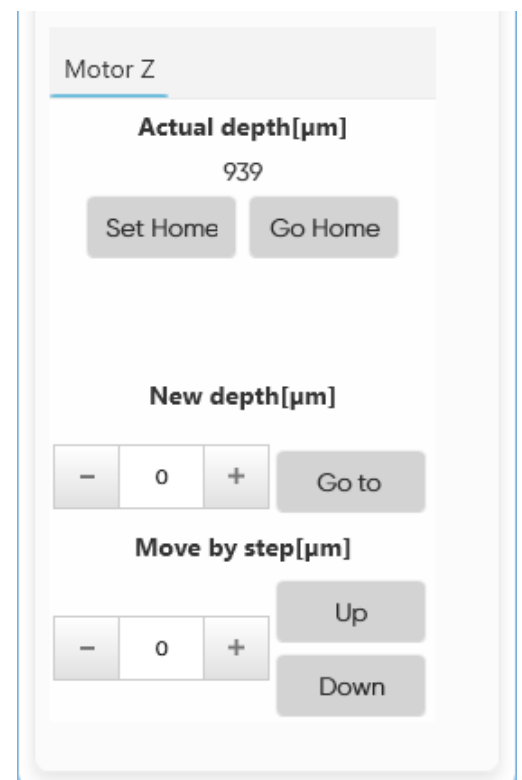
Actual depth (μm): Indicates the current vertical position of the sensor tip.

NOTE: The Actual depth is arbitrary until the user has related the position of the sensor tip to the position of the study sample – see example on the next page.

Set Home: Sets the current position to zero, i.e. defines it as the Home position.

Go Home: By pressing **Go Home**, the motor will move the sensor tip to the Home position.

New depth: Enter a position that the motor should move to and click the **Go To** button. Example: Enter "2000", click **Go To** and the motor will move the sensor tip to a position 2,000 μm deeper than the Home position.



IMPORTANT! The depth scale is positive downwards. If the zero depth is set at the sample surface, positive values in the boxes below (Actual depth, and New depth) will indicate positions in the sample. All depths and step sizes are given in μm unit.

Go To: Click this button to move the sensor to the position entered in the New depth field.

Move by step [μm]: Enter the step size that the motor should move the sensor up or down.

Example: If you want to define the surface of the study sample as the zero position: Move the sensor tip to the sample surface using Move by step or by manually moving the sensor using the micromanipulator. When the sensor approaches the surface or if the sensor is hard to see, it is a good idea to use small increments. Verify the position, e.g. with a microscope. With the sensor tip on the surface of the sample, click **Set Home**. This position will now be 0 μm .

Sometimes, if it is not possible to see the sensor, you might be able to identify the surface from the data. Make the first profile and identify the sample surface from there. Place the sensor at the sample surface and click **Set Home** to define this position as zero. If subsequently you want to move the sensor to a specified position, e.g. 1,000 μm above the sample, type -1000 in the New Depth (μm) and click **Go To**.

NOTE: It is not necessary to define the sample surface as zero. It is just as fine to use an arbitrary depth scale. The definition of the depth scale when recording a profile is not important for analysing the profile in the Activity tab (however, here it is necessary to define the position of the sample surface.).

CALIBRATION

Calibrations are performed in the Calibration window. A list of connected sensors will be shown in the green Sensor column to the left. A red cross indicates that the sensor has not been calibrated, and a grey tick mark indicates that the sensor has been calibrated.

NOTE: Calculation of specific activities from a microprofile is only possible if units are $\mu\text{mol/L}$

The source of the calibration may be:

- Direct recording of calibration values in the Calibration tab
- An already recorded calibration in the current experiment
- Import of the most recent calibration from a previous experiment
- Import of calibration from a stored experiment file
- Calibration stored on the sensor (only for UniAmp sensors, optical sensors of the Opto-series)

UNITS: The concentration unit used for calibration is defined for each sensor in the "Settings" tab before start of experiment and cannot be changed in a running experiment.

For information on calibration of a specific sensor consult the sensor manuals.

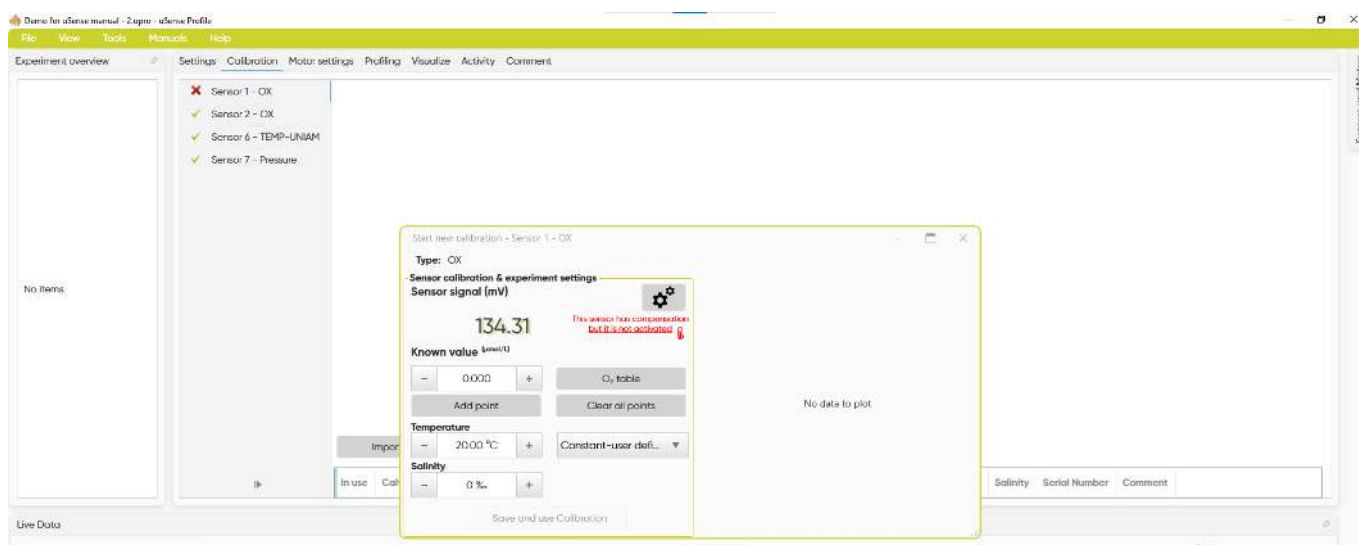
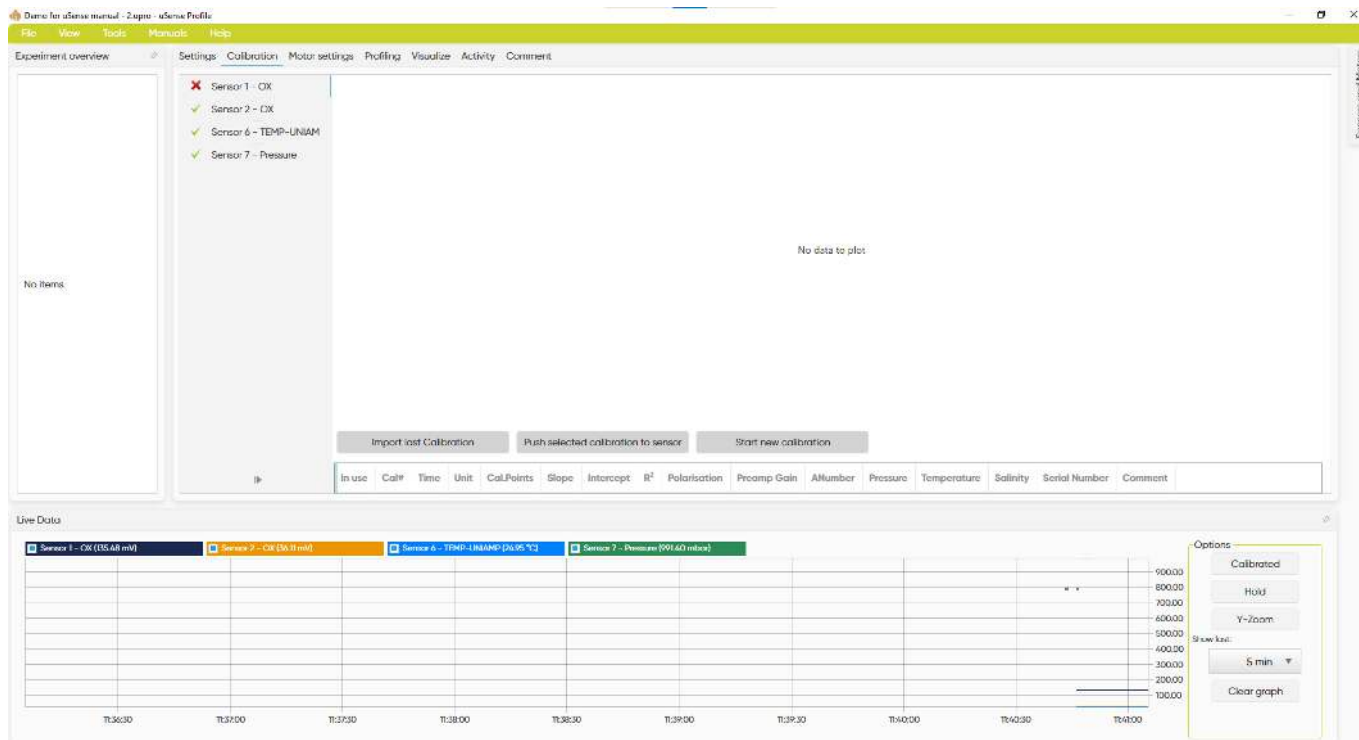
All raw sensor signals can be seen in the **Live Data** section at the bottom of the screen. After calibration, the calibrated signals can also be seen here by clicking the **Calibrated** button in the **Live Data** section.

Calibration procedure for Clark Type Sensors

When a new sensor without calibrations is connected, the Calibration window will be shown. The grey sensor column shows the available sensors. Note that if the instrument connected has a built-in pressure sensor, this is always shown as the last one, and the current atmospheric pressure can be seen in the Live data section.

Principles for calibration: Unisense amperometric sensors for gases (O_2 , H_2S , H_2 , N_2O , NO) can be calibrated with two points: Zero and one known concentration. pH electrodes also require two points, whereas redox potential electrodes can be calibrated with one or two points, please see the specific manuals.

Start a new calibration: Click the **Start new calibration** button to open the Calibration pop-up window.



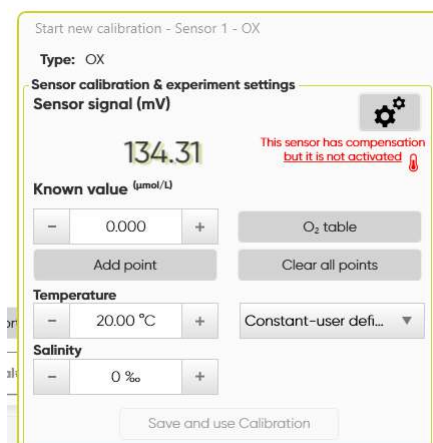
Simple calibration

Sensor signal:

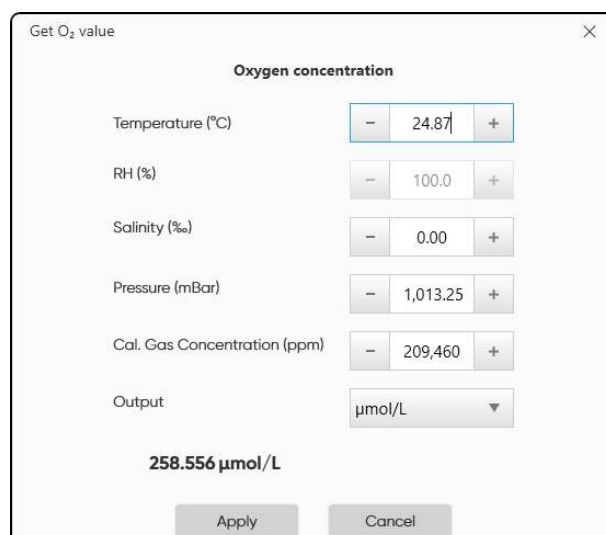
Shows the current sensor raw signal

Known value:

Enter the concentration of the current calibration standard. There is a built-in calculator for the concentration of O₂, H₂, H₂S and N₂O, see the window Get O₂ value. When calibrating a sensor for one of these gases, click the button O₂ table (see screenshot above) to open a calculator for that specific gas. In the screenshot, an O₂ sensor is connected and the calculator for O₂ concentration is shown when clicking the button.



In the calculator, the concentration of dissolved gas at a given temperature, gas composition, salinity, and pressure is calculated. It is assumed that there is equilibrium between water and gas. Note that for oxygen, the value for Calibration gas concentration (ppm) corresponds to the standard atmospheric composition, and this should be left unchanged if atmospheric air is used for calibration. The current atmospheric pressure can be seen in the Live Data window for UniAmp electrochemical instruments and on the display of the Multimeter and Monometer. To get the actual concentration of the specific gas in the calibration standard, enter the actual temperature, salinity, pressure, and gas composition. Click **Apply** to transfer the calculated concentration to the Known value box.



*NOTE: The values for Temperature, Salinity, Pressure, and Calibrated Gas Concentration (ppm) must be entered manually. The calculated concentration is transferred to the **Known value box** when clicking **Apply**.*

Temperature: Temperature may be entered manually or be continuously read from a temperature sensor. In the dropdown menu to the right, any connected temperature sensor can be selected as input.

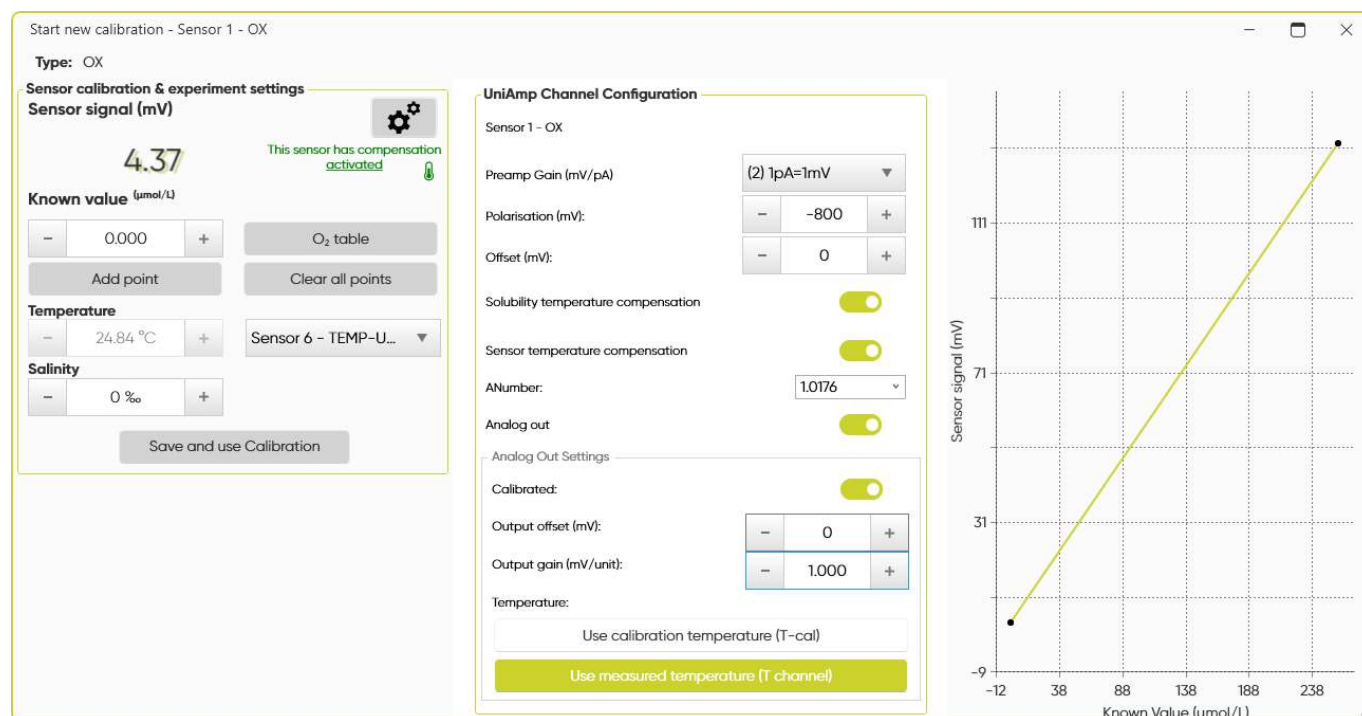
The value in the temperature box in **Sensor calibration** and **experiment settings** is used for:

- The current value will be saved as the calibration temperature when **Save and use Calibration** is clicked. This is the reference temperature for temperature compensation. It is shown in the "Temperature" column in the list of calibrations just above the **Live data** section.
- During measurement, the current value will be saved with each data point. If the temperature compensation is selected, the difference between the measured temperature and the calibration temperature is used for temperature compensation.

NOTE: The temperature in the "Get O₂ value" window must be entered manually and is not connected to this temperature box.

Advanced calibration

Click the gear icon  to expand the Calibration pop-up window. The **UniAmp Channel Configuration frame** is now visible.



This frame is only visible when a UniAmp instrument for electrochemical sensors is connected.

Some of these settings are also available through the UniAmp Service in the Windows Notification Area (see the UniAmp manual for details).

Preamplifier Gain: The Pre-Amp Range determines how the sensor signal in pico ampere (pA) is converted into a signal in millivolt (mV). Default setting is 1 pA = 1 mV

Polarization: Displays the current polarization of the sensor. This may be changed by entering a new value in the box.
NOTE: Wrong polarization may destroy the sensor. See Sensor Manual for correct polarization.

Offset: The Offset may be used to displace the signal in a positive or negative direction.

Solubility temperature compensation: If this is activated, the calculated concentration is corrected for the effect of temperature on the relation between solubility and partial pressure. It is enabled by default.

Sensor temperature compensation: If this is activated, the sensor signal is corrected for the temperature induced change in sensor response that will occur at constant partial pressure. The compensation will work within $\pm 3^\circ\text{C}$ from the calibration temperature. See the UniAmp series manual for details about the temperature compensation. It is enabled by default.

ANumber:

The ANumber is used for the sensor temperature compensation and is loaded from the E²PROM. A custom value may be entered. The factory value is always available in the drop-down field. It is enabled by default.

Analog out: Check this checkbox to get an analog signal out on the Analog channel on the back of the UniAmp instrument (adaptor needed). If Calibrated is selected, the calibrated sensor signal is scaled using the values entered in the Output offset and Output gain boxes. The analog out calibrated data are calculated from the calibration present on the E²PROM of the sensor. Therefore, the **Push selected calibration to sensor button** must be clicked to make a calibration active for the analog out signal. See Appendix 1 for details.

In use	Cal#	Time	Unit	Cal.Points	Slope	Intercept	R ²	Polarisation	Preamp Gain	ANumber	Pressure	Temperature	Salinity	Serial Number	Comment	
☒	3	23-06-2025 11:48:58	µmol/L	4	0.508	4.540	1.000	-800	2	1.01760005950928	991	24.84379	0	506017		
☐	4	23-06-2025 11:54:13	µmol/L	2	0.495	4.405	1.000	-800	2	1.01760005950928	991	24.864565	0	506017		
☐	5	23-06-2025 11:55:30	µmol/L	2	0.495	4.405	1.000	-800	2	1.01760005950928	991	24.858075	0	506017		

Calibration procedure

1. Prepare the calibration samples (see the specific sensor manual).
2. Choose the sensor you want to calibrate in the grey sensor column to the left in the main **Calibration** window.
3. Click the **Start new calibration** button and the Calibration pop-up window opens.
4. Enter the concentration of the current calibration standard in the "Known value (µmol/L)" box. See "Known value" above for an explanation of how to use the built-in gas concentration calculators.
5. After entering the current concentration, record the calibration point by clicking **Add point**. Several points can be added for each concentration and the average of these will be used.
6. Move the sensor to another calibration standard and repeat steps 3 – 4. It is possible to use several different standards and make a multi-point calibration to verify linearity.
7. If a calibration point is not valid (e.g. due to typing errors), a single point can be cleared by clicking on it and choosing **Delete point**. If you wish to remove all calibration points, select **Clear all points**.
8. When you are satisfied with your calibration click **Save and use calibration**. A linear regression will be performed based on the recorded calibration points which will be used for converting raw signals to concentrations. The active calibration will be marked with a blue circle in the **In use** column.

Re-calibration procedure

It is possible to modify an existing calibration and to make a new one. The calibration marked with the blue ring in the **In use** column in the main **Calibration** window will be shown in the **Calibration pop-up** window, when clicking the **Start new calibration** button.

- Make a new calibration: Click the **Clear all points** button, and follow the **Calibration procedure** above.
- Modify an existing calibration:
 - Click the calibration points in the graph that you want to remove and click the **Delete** button that pops up.
 - Record a new calibration point by exposing the sensor to the appropriate calibration standard and enter the appropriate value in the **Known value box**.
 - Click **Add point**.
 - Repeat this procedure for all the calibration points that should be changed.
 - Click the **Save and use Calibration** button and the modified calibration will be saved as a new calibration.

Saving and applying calibrations

In use	Cal#	Time	Unit	Cal.Points	Slope	Intercept	R ²	Polarisation	Preamp Gain	ANumber	Pressure	Temperature	Salinity	Serial Number	Comment
	3	23-06-2025 11:48:58	µmol/L	4	0.508	4.540	1.000	-800	2	1.01760005950928	991	24.84379	0	506017	
	4	23-06-2025 11:54:13	µmol/L	2	0.495	4.405	1.000	-800	2	1.01760005950928	991	24.864565	0	506017	

All calibrations are automatically saved in the current file. An existing calibration may be activated by clicking in the circle in the **In use** column in the list of calibrations.

When making a new calibration, or activating an existing calibration, all data recorded with this sensor will be calibrated according to this calibration. This applies to data already recorded in the current file, as well as to data recorded after the calibration.

When starting up a new experiment, it is possible to import the most recent calibration from the previous experiment by clicking **Import Last Calibration**.

For UniAmp instruments, it is possible to save a single calibration on the E²PROM on the sensor by clicking **Push selected calibration to sensor**. This calibration will be available in the calibration table whenever the sensor is plugged in. It can be activated by clicking the corresponding blue button in the **In use** column. Only calibration parameters are saved to the E²PROM, not the calibration points. Therefore, the calibration curve is not shown in the graph.

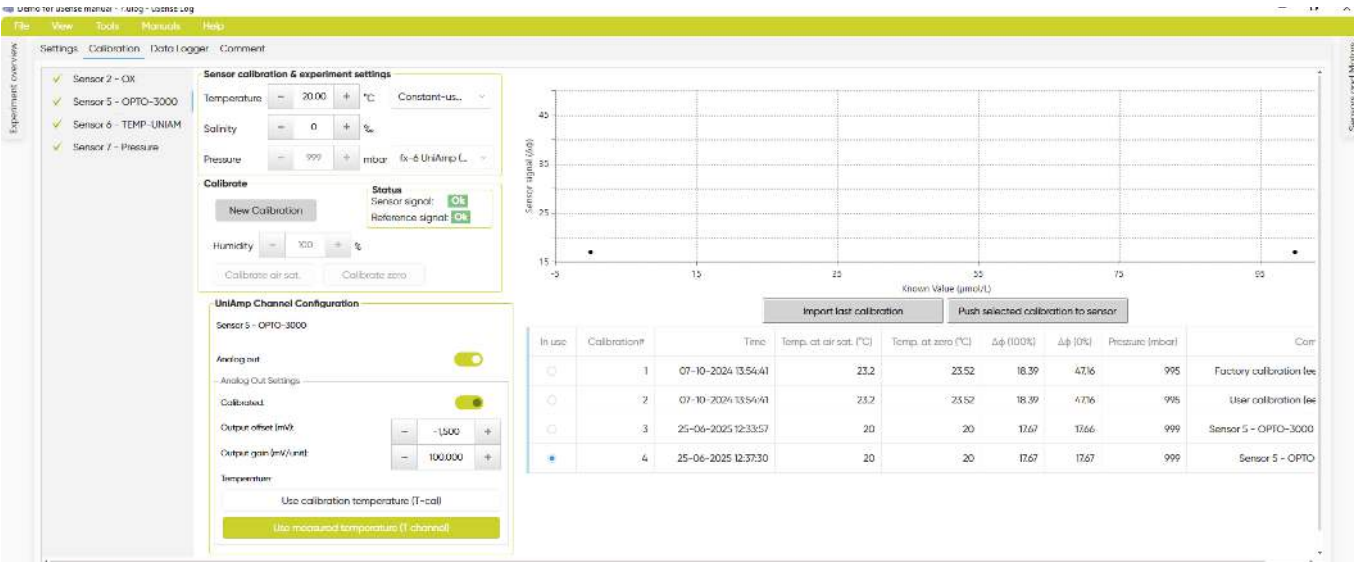
Sensors can be recalibrated at any time during an experiment and another calibration can be selected from the list of calibrations. The new calibration will be applied both to data already recorded and data recorded after the calibration with that sensor.

All calibration data are stored in the experiment file.

Calibration procedure for oxygen optodes

Sensor column

In the grey column to the left, you select the sensor you want to calibrate. The sensors can be renamed (in the Settings tab) before a Logger experiment is started. A red cross indicates that the sensor has not been calibrated or that a sensor is not connected to a given channel. A green checkmark indicates that the sensor has been calibrated. The Opto series microoptodes will always appear calibrated. If you want to make a new calibration, use **New Calibration** as described below.



Sensor calibration and experiment settings

Temperature: Select the appropriate temperature sensor for automated temperature compensation of the oxygen signal. Select Constant – user defined and enter the appropriate temperature if the optode is to be used at a constant known temperature.

NOTE: Temperature compensation is always enabled. It compensates automatically if a temperature sensor is connected and selected as input in the drop-down field next to the Temperature field. If measuring at a temperature different from that of the calibrations, this temperature should be entered here.

Salinity: Enter the salinity of the calibration solution. The salinity should match the salinity of your sample.

Pressure: The UniAmp instruments have a built in pressure sensor that measures the ambient air pressure. To show the current atmospheric pressure in the Pressure field, select the UniAmp in the drop-down field next to the Pressure field. It is also possible to enter pressure manually. The pressure displayed in the Pressure field is used for calculation of the partial pressure of O₂ which is used for calibration.

Calibrate

New calibration: Click this button to record a new calibration. **Calibrate air sat.** and **Calibrate zero** now become active, one after the other. Once the **New Calibration** button is clicked, the measuring frequency changes from idle mode (flash every minute) to active mode (flash every 1 sec.). The optode signal is thus shown in real time and can be used to estimate when the signal is stable and a calibration reading can be recorded.

NOTE: When a new calibration is recorded this will be applied to data already measured with this sensor as well as new data measured with this sensor. You can shift between calibrations easily. Just click the button in the **In use** column for the calibration you wish to apply and sensor as well as already recorded data will be calibrated accordingly.

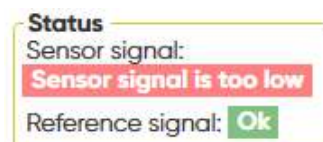
Humidity: 100% humidity is used for measurements in water. When calibrating the Optode in a gas you must enter the relative humidity of this gas. The software then automatically corrects the O₂ concentration for relative humidity.

Calibrate air sat.: Calibrate the MicroOptode by exposing the optode and the temperature sensor to air or an air saturated solution as described in the MicroOptode manual. The oxygen concentration will be calculated based on the temperature and salinity specified in the **Sensor calibration & experiment settings** frame. When a stable signal is obtained click **Calibrate air sat.** The software now records 5 measurements over 2.5 seconds and stores the average.

Calibrate zero: Expose the MicroOptode and temperature sensor to a solution or gas without oxygen and click Calibrate zero when the signal is stable. The software meter now records 5 measurements over 2.5 seconds and stores the average.

Status

Information about the sensor quality is given here. The Sensor signal and Reference signal in the Status box must show a green OK. When the sensor signal is low e.g. because no sensor is connected, the tip of the fiber is broken off or the fluorophore at the tip has been worn off or bleached, the status bar will turn red and read "Sensor signal is too low". The Reference signal status is independent of the optode signal status and should always show a green OK to be able to measure a signal.



List of calibrations and associated buttons

Import last calibration: Clicking this button will import and apply the most recent calibration for the selected sensor from the latest experiment.

Push selected calibration to device: Will save the active calibration, marked with a blue dot in the **In use** column, to the E²PROM. This calibration will then be available here until it is actively replaced by clicking this button again. This is also the case if the E²PROM is disconnected and later reconnected to the same or another meter.

Calibration table: Shows which calibration is active (indicated by a blue dot in the In use column), the time stamp for the calibration, the temperatures at which the air saturation and zero calibration were obtained and the optode signal at air saturation and zero oxygen. The raw optode signal is a phase shift measured in degrees. Under Comment the name of the calibration is shown. Click in the Comment field to edit this.

In use	Calibration#	Time	Temp. at air sat. (°C)	Temp. at zero (°C)	$\Delta\phi$ (100%)	$\Delta\phi$ (0%)	Pressure (mbar)	Comment
☐	1	02-06-2025 09:30:02	23.04	23.69	18.22	51.25	1002	Factory calibration (eeprom)
☐	2	23-06-2025 12:55:51	24.9	24.93	18.61	50.89	991	Sensor 5 - OPTO-50
☒	3	23-06-2025 12:59:14	24.94	24.96	18.63	50.86	991	Sensor 5 - OPTO-50

Switch between calibrations: If there is more than one calibration for a given sensor, it is possible to switch between the calibrations. To apply a calibration, click the button in the **In use** column for the calibration to be applied. This calibration will be applied to all data in the experiment recorded with this sensor and, if a sensor is connected, the sensor itself.

UniAmp Channel Configuration

This frame is only displayed if an UniAmp with electrochemical channels is connected and not if the dedicated optode meter are connected (Opto-F1 or Opto-F4 UniAmp).

The analog output can be activated by clicking the **Analog out** button (adaptor needed). For optodes only the calibrated signal is available as analog output. The analog output is temperature compensated and click the buttons **Use calibration temperature (T-cal)** or **Use measured temperature (T-channel)** to select which temperature that should be used for this.

The analog out calibrated data are calculated from the calibration present on the E²PROM of the sensor. Therefore, the **Push selected calibration to sensor** button must be clicked to make a calibration active for the analog out signal. See Appendix 1 for details.

Importing calibrations from file

Calibrations stored in uSense files may be retrieved and used to calibrate or recalibrate data in another uSense file and to calibrate a sensor in an active experiment.

To import calibrations:

1. Open the file to which you wish to import calibrations.
2. Click **File** -> **Import calibration**.
3. Select the file with the wanted calibrations and click **Open**.

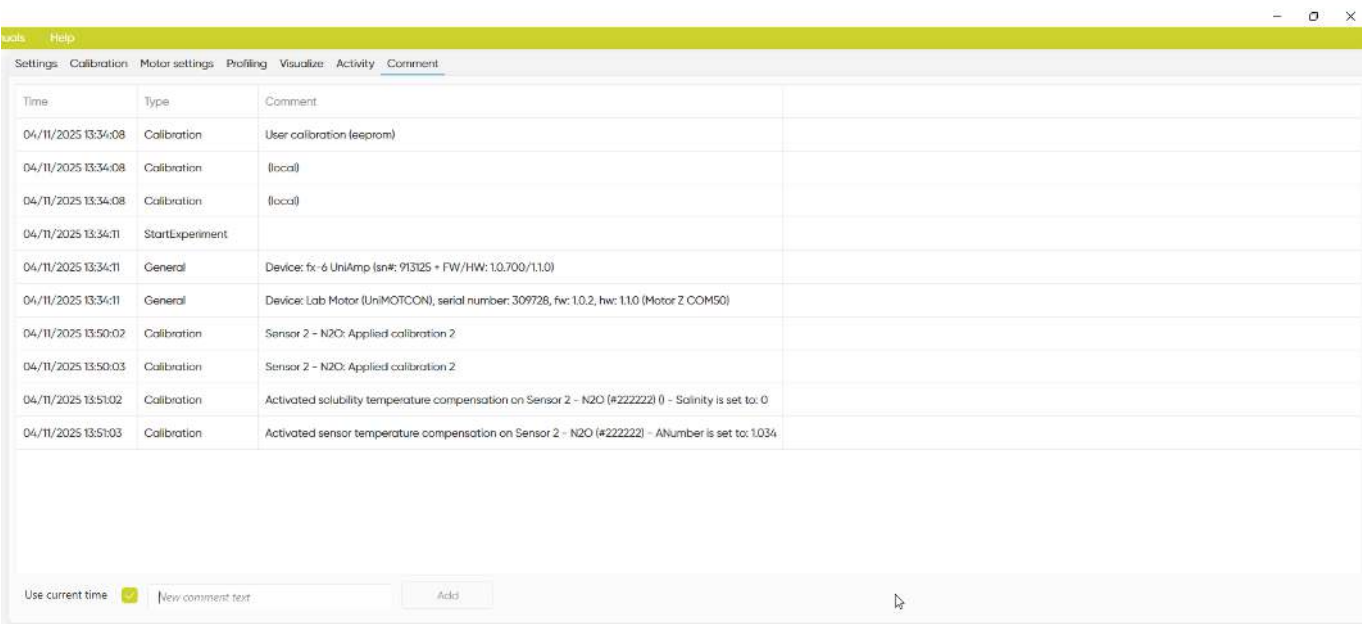
The imported calibrations will now be available in the list of calibrations in the calibration tab for each sensor. Click the button in the **In use** column for the calibration you want to apply. This calibration will be applied to all data in the experiment recorded with this sensor and, if a sensor is connected, the sensor itself. Any of the available calibrations may be applied just by clicking the associated button in the **In use** column.

COMMENT WINDOW, OUTPUT AND DOWNLOAD FILES

Comment window

Comments, calibrations and other events generated by the user or the program are recorded in the Comment window. The window allows you to enter notes and comments. Any text that you want to save with your data (e.g. a general description of your experiment) can be entered. Text entered in the Calibration window and the other program specific windows are also listed in the Comment window. All activities made during an experiment can also be seen as colored marks in the Live Data window.

A comment can be added using 1) the current time or 2) by writing the text first, wait for an expected event, and then update the time.



Time	Type	Comment
04/11/2025 13:34:08	Calibration	User calibration (eeprom)
04/11/2025 13:34:08	Calibration	(local)
04/11/2025 13:34:08	Calibration	(local)
04/11/2025 13:34:11	StartExperiment	
04/11/2025 13:34:11	General	Device: fx-6 UniAmp (sn#: 913125 + FW/HW: 1.0.700/1.1.0)
04/11/2025 13:34:11	General	Device: Lab Motor (UniMOTCON), serial number: 309728, fw: 1.0.2, hw: 1.1.0 (Motor 2 COM50)
04/11/2025 13:50:02	Calibration	Sensor 2 - N2O: Applied calibration 2
04/11/2025 13:50:03	Calibration	Sensor 2 - N2O: Applied calibration 2
04/11/2025 13:51:02	Calibration	Activated solubility temperature compensation on Sensor 2 - N2O (#222222) () - Salinity is set to: 0
04/11/2025 13:51:03	Calibration	Activated sensor temperature compensation on Sensor 2 - N2O (#222222) - ANumber is set to: 1.034

☒ Use current time

Output file

All logged data, calculations, and comments entered are stored in the data file. The file can be exported as Excel file or as a text file (CSV) to facilitate processing and graphic presentation of the data. Note that CSV file imported into e.g. Excel should have the decimal separator as '.' and delimiter ','.

Choose how to export data

Export as Excel (.xlsx) file (Recommended).

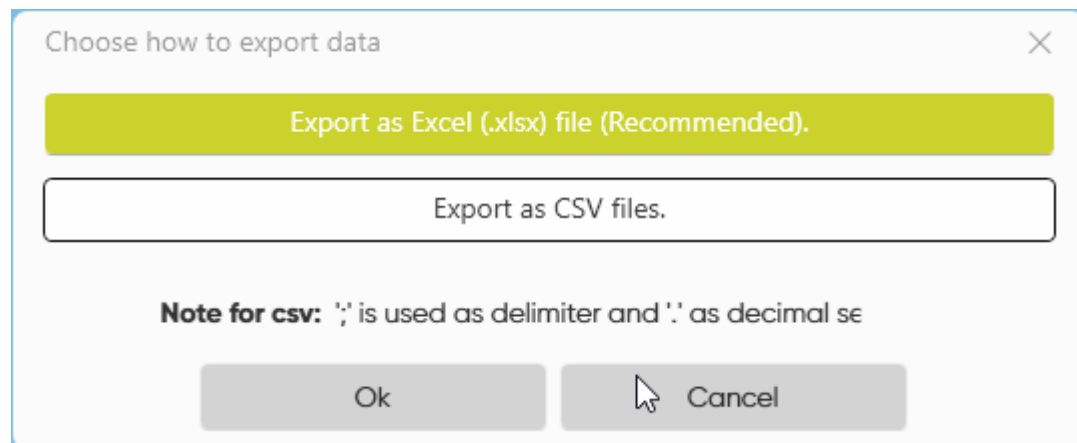
Export as CSV files.

Note for csv: ';' is used as delimiter and '.' as decimal se

Ok

Cancel

There will be an Excel file for each experiment, containing separate sheets for 1. Data points (raw and calibrated), 2. Calibration, 3. Comments and Miscellaneous. When exporting CSV files, separate files will be created for the different information's.



*NOTE: All figures in uSense Solutions can be exported into separate files by right-clicking in the graph and selecting **Export as image**.*

Download data from Field Datalogger, Field Datalogger Mini and Field Microsensor Multimeter

Data stored on the Field Microsensor Multimeter, Field Datalogger, and Field Datalogger Mini can be downloaded into uSense Solutions. Connect the instrument to the computer and open uSense Log or uSense Profile. Use Profile for downloading profile data and use Log for downloading other types of data. Start the relevant application and click File > Import from instrument. The data file can now be opened.



uSENSE LOG

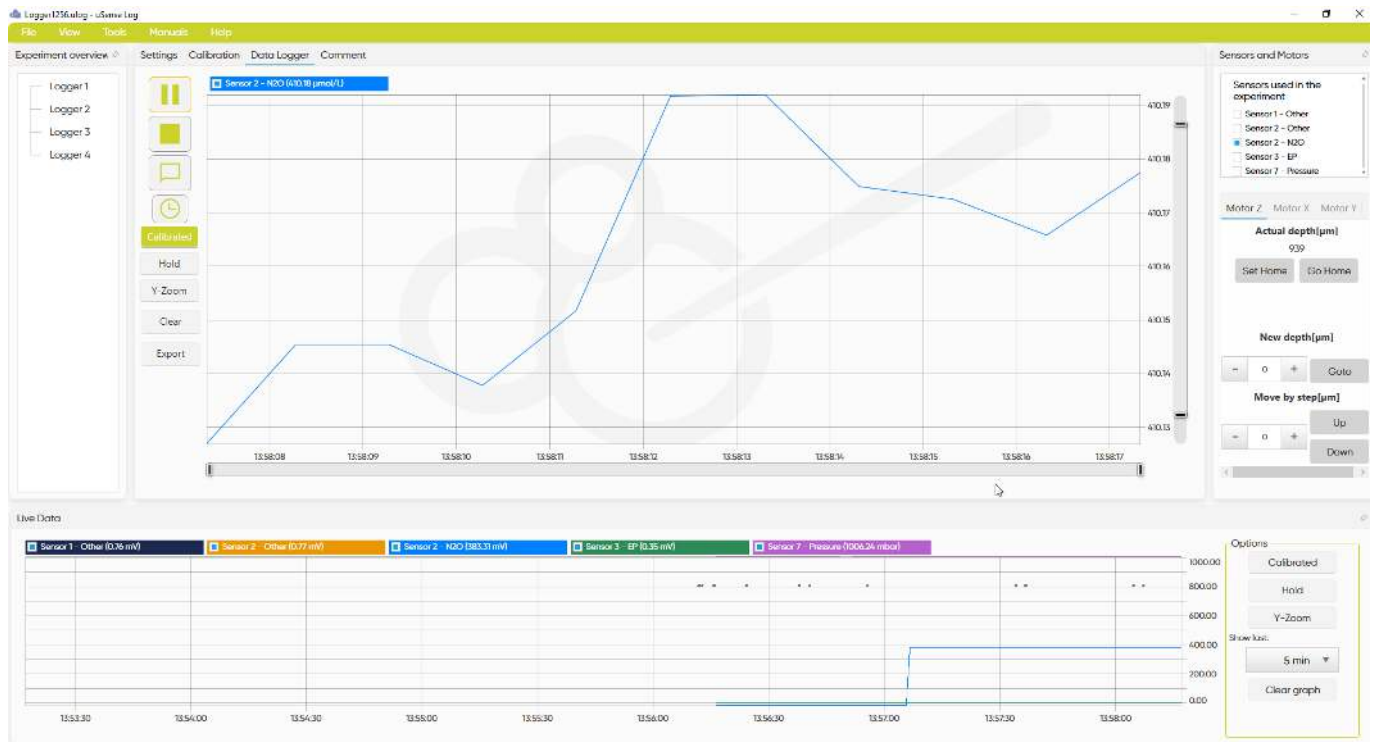
uSense Log is for basic data application and comes as freeware (without motor control) or as part of the licensed uSense Solutions software package (with motor control). It offers time series datalogging, calibration features and motor control. It supports simultaneous logging from multiple channels with a frequency of up to 50 samples per second depending on the amplifier connected. The program automatically saves all data in a uSense Log file (*.ulog).

The general description of the software and how to use the software to calibrate microsensors can be found at the beginning of this manual. In the following, the specific features of uSense Log will be described.

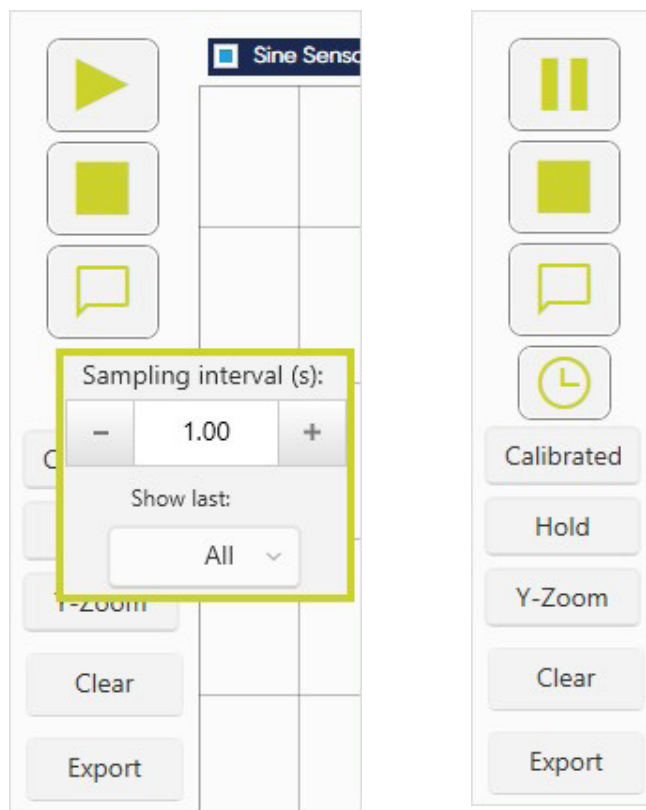


DATA LOGGER WINDOW

The Logger window is where data recording is done.



Sampling interval (s): The sampling interval denotes how often the program will make a data point (for the log file and in the graph). A single data point consists of an average of measurements sampled with a high speed background frequency in the sampling interval preceding the data point (up to 1,000 ms). In other words, with a sampling interval of 10 s, the data points are logged every 10 s as an average of samples over the last second just before the next sampling point. The background frequency will be, depending on the amplifier, between 1 and 10 Hz.





It is important to know the system you are working with, in order to choose the right sampling interval and not missing the fluctuation that you are expecting. For a relatively active system for example, it is preferable to choose a short sampling interval. For a long, perhaps linear, monitoring situation, a longer sampling interval will save computer memory.

After starting the recording, all data are stored continuously in the uSense Log file (*.ulog). No data are, therefore, lost during logging an experiment. The logging is stopped when selecting **Stop**.

The sampling interval cannot be changed while logging. If you need to change your sampling interval, sampling must be interrupted (**Pause** or **Stop** see below) before the interval can be changed.

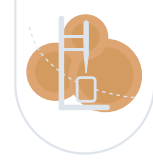
Show last: Select the scaling of the horizontal axis in the logging graph from the drop-down list.

The buttons for: Calibrated values, Hold, Y-zoom, and Show Last works in the same way as described in the Live Data Graph section.

Zoom: You can zoom the Logger graph using the scroller bars both on the x- and y-axis. When zooming on the x-axis or when zooming in and out of the graph, logging will automatically go on Hold. Data are still logged. When you deselect Hold the graph will be updated. More information on how to zoom in and out on the graph is found in the Live Data Graph section.

Clear graph: Clear graph will clear all the logging data points shown in the Data Logger graph. Clearing the graph will not delete any logged measurements as all data are saved in the uSense Log file. New logged data will start to show on the graph immediately after the old graph has been cleared.

Export: Logger data are exported as described in **Output file** (see page 27).



uSENSE PROFILE

uSense Profile is the profiling program from uSense Solutions. It offers datalogging along a transect, it controls the motor that positions the sensor, it visualizes measurements, and calculates consumption and production rates from the measured profiles.

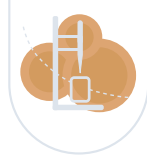
The general description of the software and how to use the software to calibrate microsensors can be found above in this manual. In the following the uSense Profile specific features of the software will be described.

System features

uSense Profile is the software used for the MicroProfiling System or the Field Microprofiling System. An overview of the equipment needed for a profiling measurement setup can be found in our MicroProfiling System Manual or the Field MicroProfiling System manual.

Basic features of uSense Profile are:

- Control of settings for profile start-ups, motor run, and datalogging
- It keeps track of measurements collected in different profiles and can show them simultaneously for comparison
- Multiple sensors can be used simultaneously
- Raw and calibrated data are continuously saved for later data interpretation
- Graph view of measurements obtained by one or more sensors during and after profiling
- It models the distribution of production and consumption based on measured concentration profiles
- All saved data are exported into an Excel or CSV file for further data handling and analysis



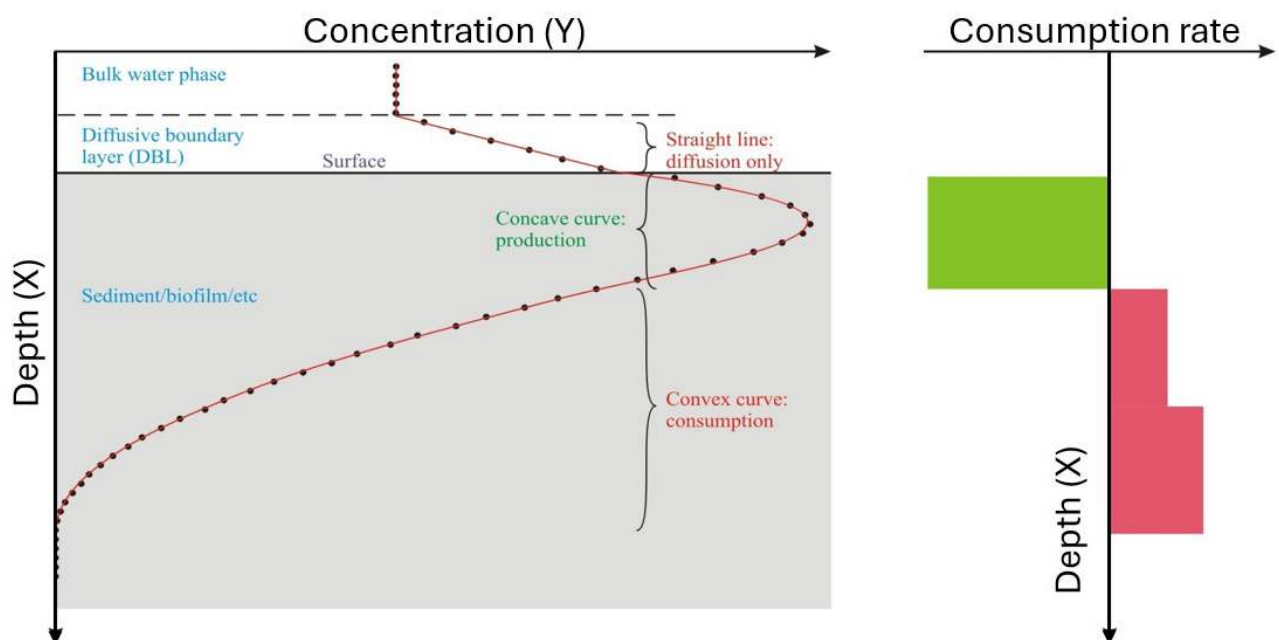
RATE CALCULATIONS FROM CONCENTRATION PROFILES

High resolution concentration profiles measured with microsensors can be used to identify the location of micro size zones of activity (production and consumption), the sizes of these zones, and the diffusive exchange rate across interfaces e.g. sediment water interface and biofilm water interface. The uSense Profile program gives you an interactive platform to experiment with activity calculations from these concentration profiles. For the interpretation, the program uses the shape of a measured concentration profile together with modeling techniques. The model implemented in uSense Profile is partly based on the method published by Peter Berg and co-workers in Berg et al., 1998, optimized for biogeochemical interpretations of solutes in sediment pore water.

The microprofiles may be analyzed in the "Activity" window of the software. See the "Profiling application note" that can be downloaded from: https://www.unisense.com/Sediment_profiling_analysis

Background

A large amount of information and understanding can be achieved from analyzing the shape of the concentration curve (see rules of thumb). However, going beyond the crude qualitative statement, the model used in the Activity window describes the measured concentration profile by the transport phenomena and processes occurring at different layers within the investigated system, and how the concentration in each layer is affected by the transport phenomena and processes in neighboring layers. The model assumes steady-state conditions where transport of solutes only occurs by diffusion. In many samples, e.g. sediments and biofilms, the assumption is valid, however, if your system is heavily affected by pore water movements due to burial, groundwater flow, wave action, and similar movements, the uSense Profile activity model should be used with care.



RULES OF THUMB:

If the diffusion coefficient and porosity is constant in a layer, the following rules of thumb can be used for a qualitative interpretation of the processes in that layer:

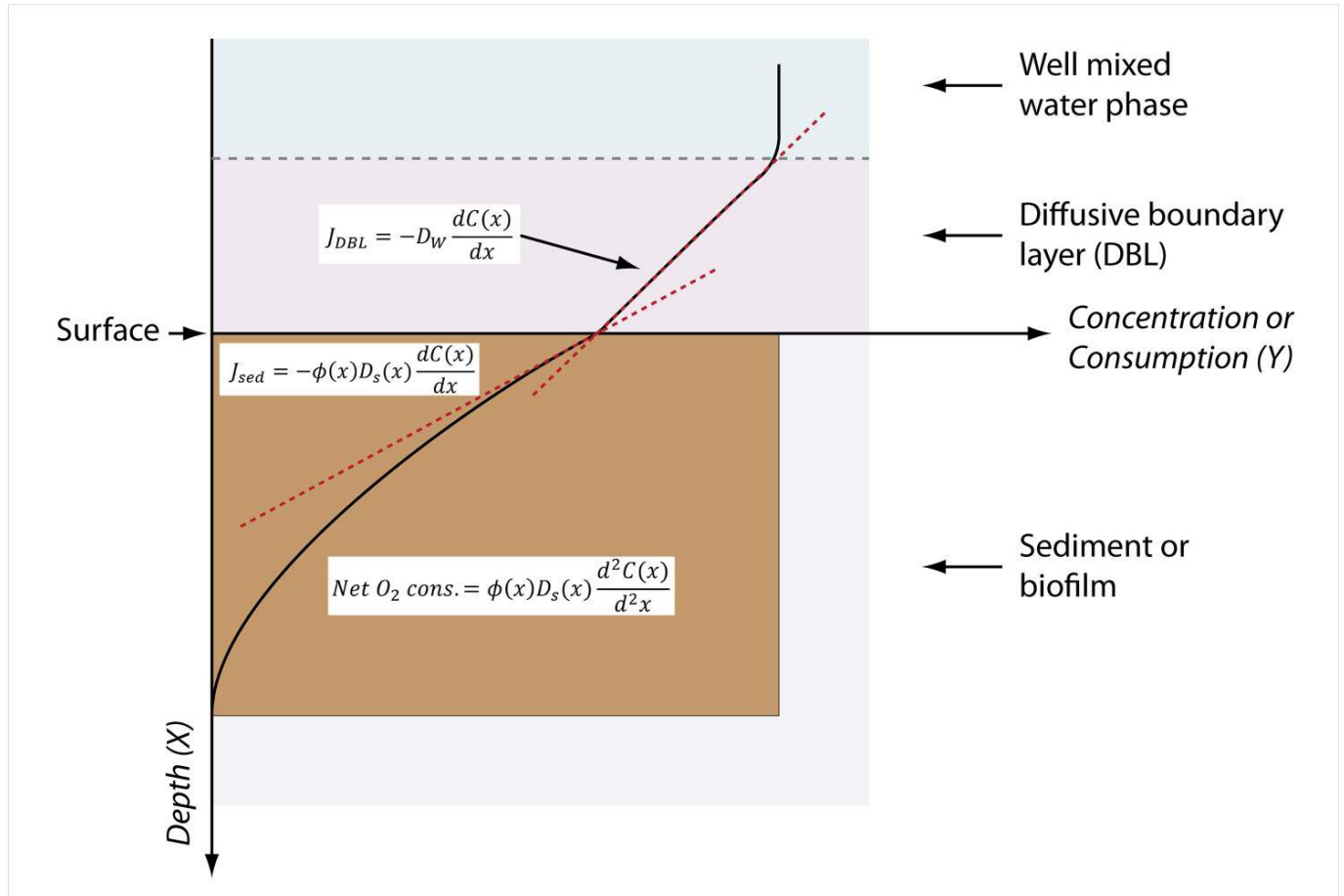
- If the concentration curve is convex, there is net consumption (e.g. respiration, oxidation of reduced compounds).
- If the concentration curve is concave, there is net production (e.g. photosynthetic production of oxygen, sulfide production by sulfate reduction).
- If the concentration curve is linear, there is no net consumption or production, only diffusional transport.



Theory

Under steady-state conditions, the activity can be calculated from microprofiles by different approaches (see e.g. Glud, 2008).

NOTE: In this section, 'X' refers to the depth axis and 'Y' to the concentration axis.



1. The mass flux, e.g. the diffusive O_2 uptake, can be calculated from the linear approximation to the concentration profile in the diffusive boundary layer (DBL see figure) using the one-dimensional mass conservation equation, Ficks 1st law:

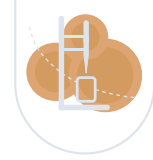
$$J = D_s \times \frac{dC}{dz}$$

where J is the mass flux ($\text{mol cm}^{-2} \text{s}^{-1}$), $\frac{dC(x)}{dx}$ is the slope of the concentration gradient in the DBL and D_w is the molecular diffusion coefficient in water.

2. Just below the samples surface, the mass flux can be calculated from the straight concentration profile, Ficks 1st law, using following equation:

$$J = -\phi(x) D_s(x) \frac{dC(x)}{dx}$$

where ϕ is sediment porosity and D_s is diffusivity (x denotes that both these parameters may vary with depth). Diffusivity may be calculated in different ways depending on the sample.



The equation $D_s = D_w \times \phi$ is often for samples with high porosity such as biofilms or fine sand or mud. In samples with low porosity, such as more compact sediment, the equation $D_s = D_w \times \phi^2$ is often used. Finally, the equation $D_s = D_w / (1 + 3 \times (1 - \phi))$ can be used for all kinds of material (e.g. Iversen and Jørgensen, 1993; Ullman and Aller, 1982).

3. The volume specific consumption and production can be determined from the shape of the concentration profile using Ficks 2nd law of diffusion:

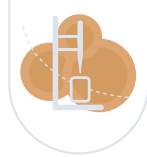
$$Net\ O_2\ cons. = \phi(x) D_s(x) \frac{d^2 C(x)}{dx^2}$$

where Net O₂ cons. is the net rate (nmol cm⁻³ s⁻¹) of O₂ consumption or production.

uSense Profile activity model uses the steady-state concentration profile to:

1. Calculate the diffusive mass flux.
2. The volume specific activity rate for different depth intervals and the integrated production or consumption.

Before starting the analysis model, you must provide estimates for the diffusion coefficient and the porosity in all zones, and some boundary conditions (see Activity tab). The model makes an initial guess of the activity distribution and compares the calculated profile with the actual measured profile. Using a stepwise optimization method, the activity distribution is refined until the calculated profile does not deviate from the measured profile within some statistical margin. Statistical values like the sum of squared error and the P-value together with the modeled graph will help you to estimate the best fit for the activity calculations.



EXPERIMENTAL OVERVIEW IN PROFILING

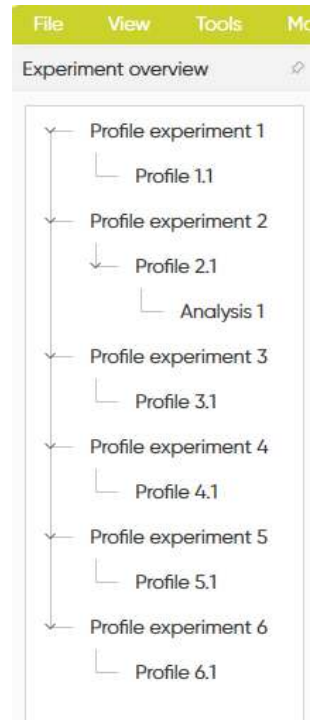
The experimental overview shows all the profile experiments, profiles, and analyses made in an experiment.

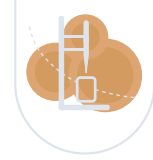
Profile experiment

The program automatically saves all the profiles made in an experiment. One profile experiment can contain one profile or multiple profiles repeated in one run defined in the Profiling tab. These are stored as Profile 1, Profile 2, and so on.

Analysis

Analysis of a profile made in the Activity window is stored as an Analysis under the studied profile. One or more are stored under one profile as Analysis 1, Analysis 2, and so on. To open an Analysis double click it.





MOTOR SETTINGS

The manual or motorized movements of x, y, and z-axis are indicated here. The program will automatically set **Motor** when it recognizes a motor. If no z-motor is connected, it will automatically set a manual movement. If no x or y motor is connected (or found by the software), it will automatically set **None**.

The screenshot shows the 'Motor settings' tab in a software interface. It contains three panels for Z-axis, X-axis, and Y-axis. Each panel has a dropdown menu for movement type (Manual or Motor) and three input fields for Start(μm), End(μm), and Step size(μm) with increment/decrement buttons. The Z-axis is set to 'Motor'. The X-axis and Y-axis are set to 'None'. The Start, End, and Step size fields are all set to 0, 100, and 100 respectively.

Axis	Movement Type	Start(μm)	End(μm)	Step size(μm)
Z-axis	Motor	0	100	100
X-axis	None	0	100	100
Y-axis	None	0	100	100

Z-axis: The z-axis is the vertical movement of the sensor. The movement of this motor is controlled in this window and in the **Sensors and Motors** frame in the Profiling window.

X- and Y-axis: The x and y horizontal movements are controlled from this tab and from the motor control window. The start position of the sensor in the x- and y-movement is defined in **Start**. The start position of the sensor is defined compared to its current position. Define also **End position** and **Step size** moving in the x- or y-direction.

2D option: For the 2D option, the program will make one z-profile defined in the Profiling tab, then move in the x-direction and make another z-profile and so on. The y-axis is set on **None**. In a 2D setting, one cycle includes all the profiles measured along the x-axis.

3D option: In a 3D setting, one cycle includes all the profiles measured along both the x-axis and y-axis. The program will make one z-profile, then move in the x-direction, make another profile, and so on. When all profiles are performed in the x-direction, it will move one step in the y-direction and repeat. When all profiles are made in the x/y-grid, it will return to the safe position. In a 3D setup, one cycle includes a number of profiles measured along the x- and y-axis.

If you want more than one cycle executed automatically, specify the number in the "Number of cycles" box in the "Profile settings" frame (see below). The delay between each cycle is specified in the "Delay between" box.

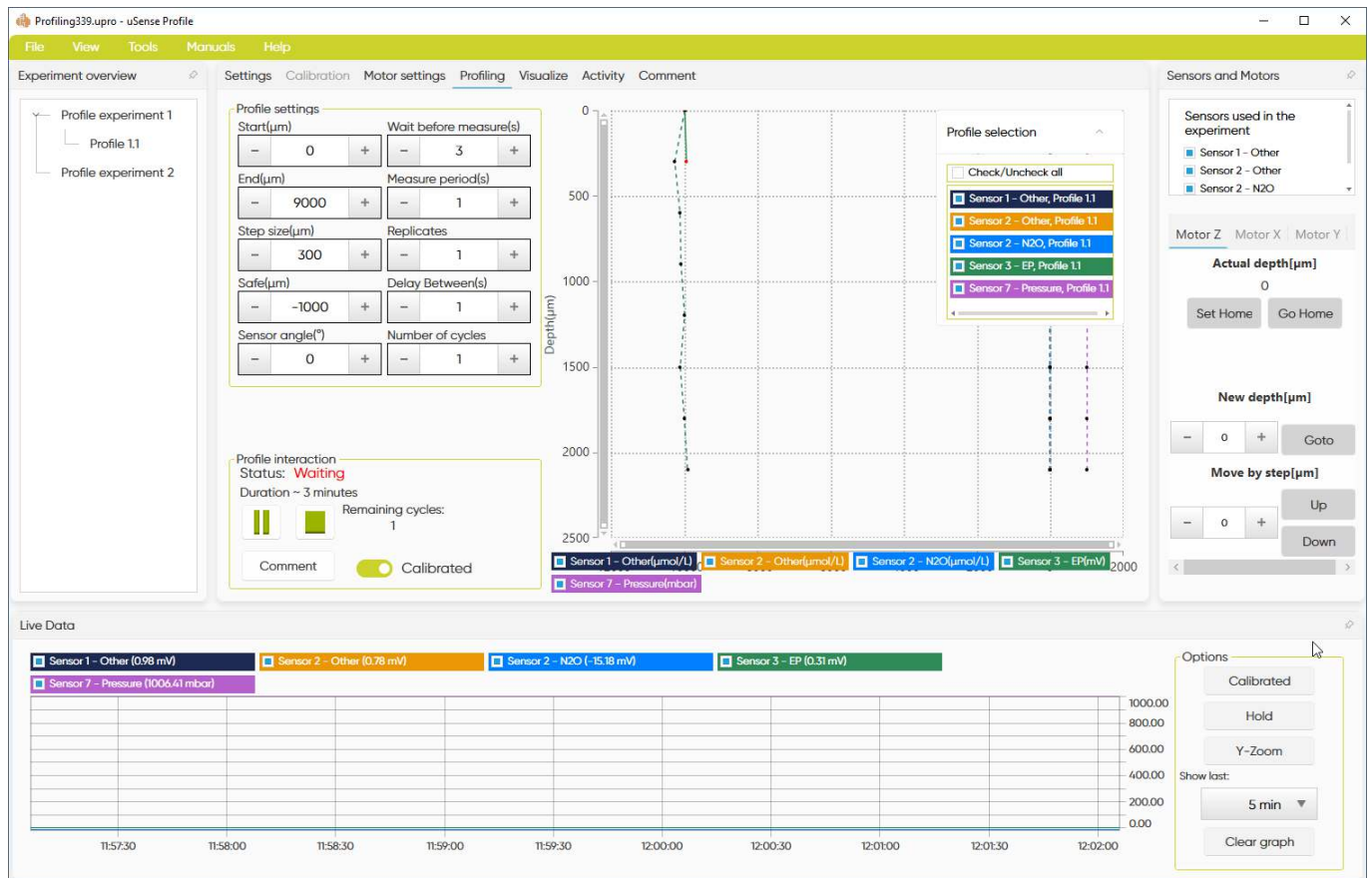


PROFILING WINDOW

The Profiling window controls the z-axis (depth) of your profile. For x- and y-settings, go to Motor setting and Sensors and Motors.

IMPORTANT! The depth scale is positive downwards.

Profiling with depth can be done with or without a motor unit (motorized or manual). The functions of this window are the same whether you do your profiles manually or motorized.



Profile settings

Start (μm): Is the depth position relative to the Home depth from where the profile is started. Negative values are above the surface and thus normally the start position should be 0 or negative.

End (μm): Is the depth position relative to the Home depth where the profile is stopped.

Step size (μm): Is the vertical distance between each measurement. The step size should not be smaller than the size of the sensor tip, e.g. if the sensor has a tip size of 50 μm, the step sizes should not be smaller than 50 μm.

Safe (μm): The Safe position is the position where the motor will rest between cycles. The safe position is to ensure that the sensor is resting outside the sample between replica profiles. In 2D and 3D

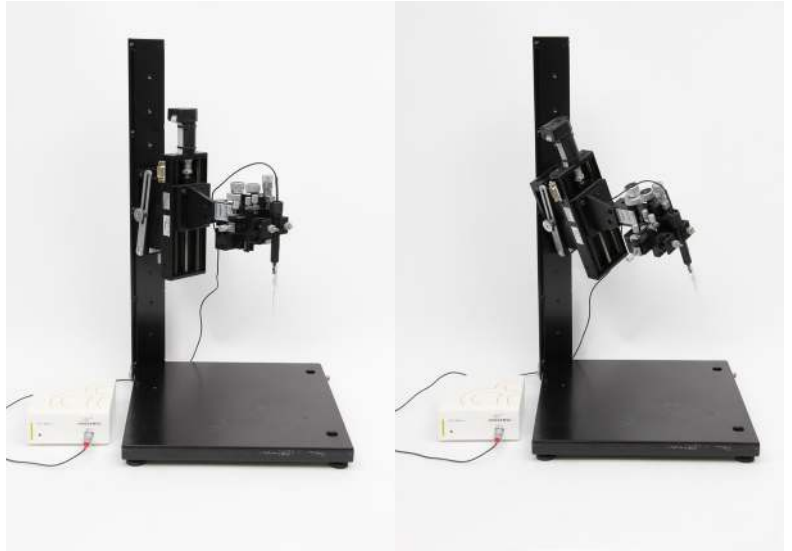
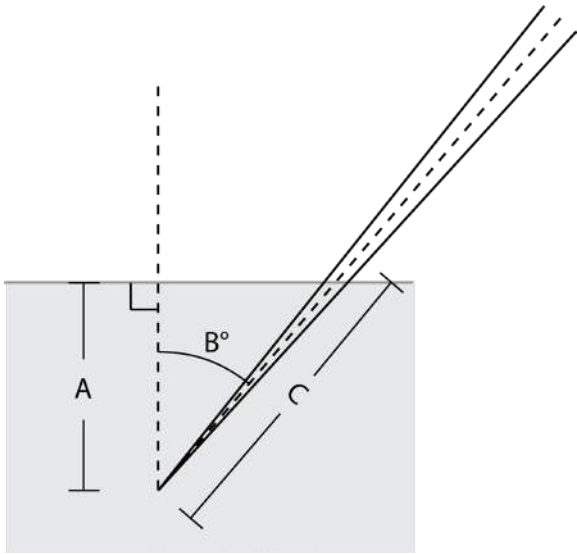
Profile settings	
Start(μm)	Wait before measure(s)
- 0 +	- 3 +
End(μm)	Measure period(s)
- 9000 +	- 1 +
Step size(μm)	Replicates
- 300 +	- 1 +
Safe(μm)	Delay Between(s)
- -1000 +	- 1 +
Sensor angle(°)	Number of cycles
- 0 +	- 1 +

Depth(μm)



profiles, this is also the height above the sample where the sensor tip will be while the sensor is moved sideways (x- and y-direction). Therefore, be sure that the Safe position is well above the sample surface.

Sensor angle (°): When the sensor enters the sample at an angle B (see Figure below), the software can calculate the actual distance that the sensor should move (C) to reach a certain distance perpendicular to the sample surface (A). The distance A is indicated in the Step size box. Enter the angle, B, in the Sensor angle (°) box and the software will calculate and move the sensor the distance C. For example, to move the sensor 100 μm into the sample along the axis perpendicular to the surface and the sensor is inserted at an angle of 30° , the sensor must be moved $100 \mu\text{m} / \cos(30^\circ) = 115 \mu\text{m}$.



Wait before measure (s): When the position during a profile is reached, the system will wait for a period of seconds before it starts measuring. This is to ensure that the sensor signal is stable before the measurements begins. The default setting is 3 seconds, however, most sensors require longer waiting time to respond fully to changes.

Measure period (s): Sets the duration of the measurement in each position. Each measurement will be an average value over this period of time. When making profiles in a noisy environment, such as on a ship or in a cold room, it can be helpful to average over a longer period, i.e. increase the measure period. The default setting is 1 second but should be set to match the measuring conditions. The standard deviation for the values are shown in the visualize tab when tagged.

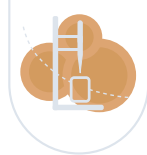
Delay Between (s): Is used when starting a profile and during repeated profiles. Each time a cycle is started, the sensor is placed in the Safe position, and the profile is started after a delay period given here.

Replicates: Enter the number of measurements that should be performed at each depth.

Number of cycles: Set the desired number of cycles here. In a 1D setting, one profile is identical to one cycle. In a 2D setting, one cycle includes all the profiles measured along the x-axis, whereas in a 3D setting one cycle includes all the profiles measured along both the x-axis and y-axis.

EXAMPLE: SETTINGS FOR PROFILING WITH A 50 μm SENSOR

Start: -1500 μm	Wait before measure: 5 s
End: 4000 μm	Measure period: 1 s
Step size: 50 μm	Replicates: 1
Safe: -4000 μm	Delay between: 1 s
Sensor angle: 0°	Number of cycles: 1



Profile interaction

Here the profile can be started and stopped at any time during profiling.

Start Profiling: When all parameters are set, select **Start** and the data will be logged continuously to a uSense Profile file (x.upro).

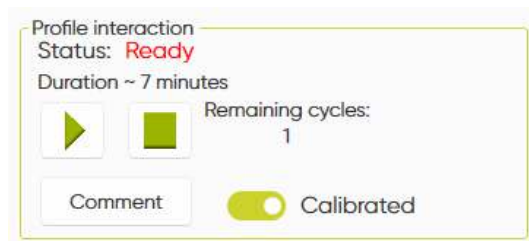
Pause profiling: The same button is used to **Start** and **Pause** the profiling. Simply select **Pause** to pause the profiling and continue again by pressing **Start**.

Stop profiling: **Stop** will stop the profiling program immediately and ask the user if the sensor shall be moved to the Safe position and the x/y profiling to its start position, or stay in its current position.

Comments: At all times during the experiment, it is possible to enter a comment. All comments are listed in the Comment window

Remaining cycles: Is a status reading on how many cycles the program still needs to run.

Calibrated: Selecting **Calibrated** will show the calibrated values in the profile graph.

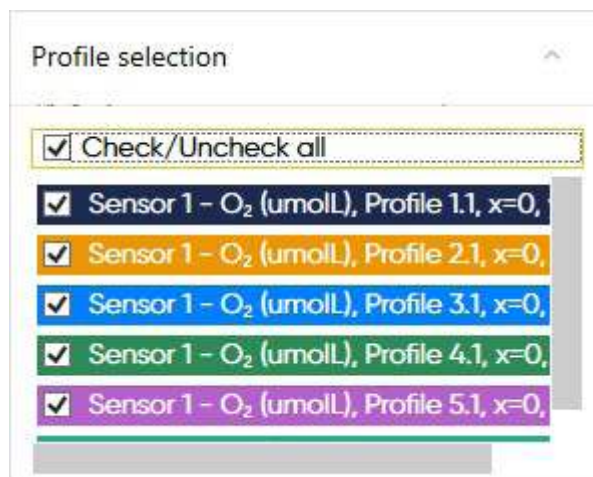


Profile selection

A profile plot is shown while profiling. During profiling, it is possible to show previous measured profiles. Mark the profiles you want to view during the profile run.

Manual profiling

If you do not have a motor unit, you have to move the sensors manually with a micromanipulator. In this case, a dialog box will appear after each measurement that tells you which depth to go to. When you have reached the depth appearing in the dialog box, select OK. The program will use the time indicated in Wait before measure, Measure period, and Replicates before a new dialog box will appear.





VISUALIZE WINDOW

This tab is for advanced visualization of the measured profiles. You can plot one or more profiles and you can choose to see the profiles of one or multiple sensors. The zero depth can be adjusted and the new adjusted profile can be saved and used in the Activity window.

*NOTE: Update a new filter setting by choosing **Profile**. This will plot the new graph.*

Filters

Sensor: Here you choose the sensor or sensors you want to see the profiles from.

Cycle: It is possible to choose how many cycles you want to see on the graph. In a 1D setting using only z-depths, one profile is identical to one cycle. For 2D and 3D cycle, the profile of one or more profiles in one cycle or multiple cycles will be plotted in a 1D plot. In a 2D setting, one cycle includes all the profiles measured along the x-axis or y-axis, whereas in a 3D setting one cycle includes all the profiles measured along both the x-axis and y-axis.

Z-depth: This setting currently has no function because uSense Profile cannot make contour plot. It will be available in a future update.

X- and Y-depth: Profile plots from a defined x- and y-position can be chosen here.

Filters

Sensor

All Se... ▾

Cycle

All ▾

Z Depth

All ▾

X Position

All ▾

Y Position

All ▾

Plot

Profile

☒ Calibrated

☒ Standard deviation

Adjust interface position (μm)

Current

0

Adjust by

-

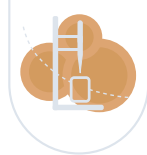
0

+

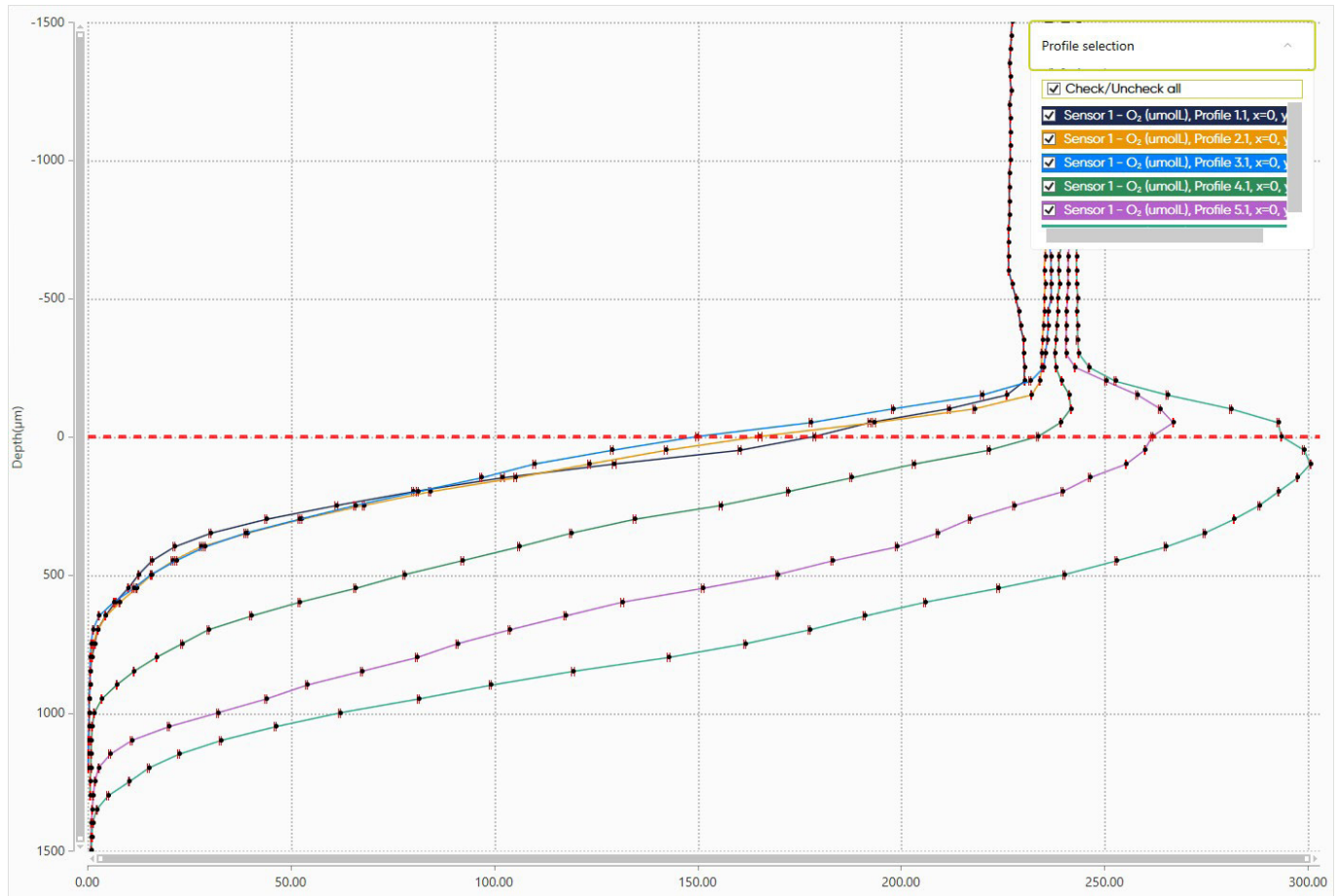
Apply

Reset

Export profiles



Plot



Profile: Click here to replot all the graphs chosen according to your filter setting.

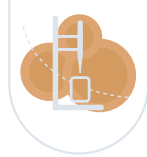
All profiles including profiles in 2D and 3D dimensions will be plotted in a 1D graph. uSense Profile can currently not make contour plot.

Adjust zero depth: On the graph, it is possible to change the zero depth position by dragging the red horizontal line to the new zero depth position. **Adjust zero** will adjust only the profiles shown on the graph. The profiles are re-scaled to their original depth when selecting **Reset depth**. The zero depth adjustments will be saved into the Activity window. Analysis can be made in the Activity window with the adjusted zero depths and be saved as an analysis. However, only the original depths are saved for future data handling.

Calibrated: When checking **Calibrated**, the calibrated values are visible on the graph.

Standard deviation: The standard deviation is based on internal measurements in the time-period given under **Measure period** in the profile settings. The average value is shown as a dot and the standard deviation as red bars.

Export profiles: How to export profiling data is described in Output File in the general section. If you have adjusted the zero depth, then the profile with the zero depth adjustment will be exported but not permanently saved. If you want to export the original depth values select **Reset depth** before you Export profiles.



ACTIVITY TAB

In the Activity tab, you calculate consumption and production rates based on the model calculation of the measured profiles. Before starting the activity model, you need to set different parameters and boundaries that are defined in this tab.

For background information and the theory behind the Activity calculation, see the Rate calculation from concentration profile section.

Settings and Analysis

Sensor 1 - O₂ Profile 6.1 Select analysis...

Boundary Conditions

Bottom conc + bottom flux

Bottom conc: - 1.00 + Bottom flux: - 0.00 +

Intervals and zones

Start depth(μm): - 150 + End depth(μm): - 1500 +

Max zones: - 7 + Min width (μm): - 200 +

DS & Theta Analyze

Settings and analysis

NOTE: The analysis can only be performed when the concentration is measured in μmol/L and if the sensor has been calibrated before measuring the profile.

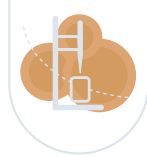
Sensor and profile: In the **Select sensor** and **Select profile** dropdown, you choose the sensor and profile you wish to analyse. This will display the selected profile in the graph window.

Boundary Conditions: When running the analysis the model needs two boundary conditions as target values. It is possible to choose between several different boundary conditions (see Berg et al., 1998). To choose the right set of conditions, it is important to consider the characteristics of the profile (see examples in Boundary condition examples box). Concentration and flux values for the boundary condition are set automatically or added manually.

BOUNDARY CONDITION EXAMPLES BOX

Oxygen profiles in many cases end with a constant concentration of zero as all the oxygen is used up at the bottom of the profile. This implies that there is a zero concentration and also a zero concentration gradient at the bottom of the profile, and the boundary concentration **Bottom conc. + bottom flux** is appropriate with zero as the parameters entered in the boxes below. For sulfide profiles on the other hand, the concentration is typically zero over an interval at the top of the profile, and consequently the flux is also zero, so in this case **Top conc. + top flux** is appropriate.

For flux calculations in the DBL **Top conc. + bottom conc.** is selected and the concentrations at the top of the DBL at the sediment surface are entered.



Intervals and zones: Choose the **Start** – and **End depth** of the profile where the calculation should be performed. The start depth will typically be the top of the sediment and the end depth where oxygen is depleted. Then choose the maximum number of zones in **Max zones** to be used in the calculation (1-10 zones can be selected). Note that the maximum number of zones multiplied with the minimum zone width has to be smaller than the distance between the start and end points.

INTERVALS AND ZONES EXAMPLE BOX

1. Diffusive mass flux in the DBL (Equation 1 in the Theory section): **Start depth** is the top depth of the boundary layer, **End depth** is the depth of the sample surface, **Max zones** is one. D_0 is used for this calculation. Notice that a mass flux calculated from the DBL can only be recommended when sufficient data points are available from the DBL, e.g. by using sensors with tip size $< 50 \mu\text{m}$.
2. For volume specific and integrated consumption and production rate calculations (Equation 2 and 3 in the Theory section): **Start depth** is the depth of the sediment surface, **End depth** is the depth where the profile ends and **Max zones** must be between 1 and 10. D_s values are used for these calculations.

The min width (μm) is the smallest zone width that the method allows. It should not be smaller than twice the step size in the profile. The number of zones giving the best solution can vary between profiles. First, you start choosing a high number of zones. The statistical numbers shown in the Statistic table can, after the first analysis, guide you to find the most appropriate number of zones for the final result.

D_s and Phi (ϕ)

Before the activity model can run, it is necessary for the program to have values for diffusion coefficients and porosity. These values can either be measured values obtained by different methods (see e.g. Ullman and Aller 1982, Iversen and Jørgensen 1993, Revsbech et al 1998), or they can be estimates based on literature values.

Settings (D_s and Phi)

Zones with different diffusion parameters: Add the number and borders of the zones with different diffusion coefficient and porosity. First, select the number of zones in the entry box. Then adjust the start depth of each zone.

D_s and Phi

Settings

Zones with different diffusion parameters: 1

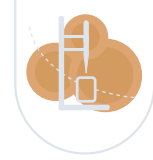
Calculate

D₀ coefficient free water (10⁻⁵ cm² s⁻¹): O₂ Table 2.104

Sediment diffusion coefficient equation: D_s = D₀ / (1 + 3 * ...

Start	End	Porosity (φ)	D _s
-1500	1500	1	0

Confirm Cancel ☐ Enter D_s manually



Default Porosity: To specify a uniform porosity in all depths, enter a measured or estimated value and select **Porosity**. To enter variable porosity, enter measured or estimated porosity values by typing directly in the table. If only a subset of the porosity values need to be different, set a uniform porosity and modify the subset by typing in the relevant cells afterwards.

Calculate (D_s and Φ)

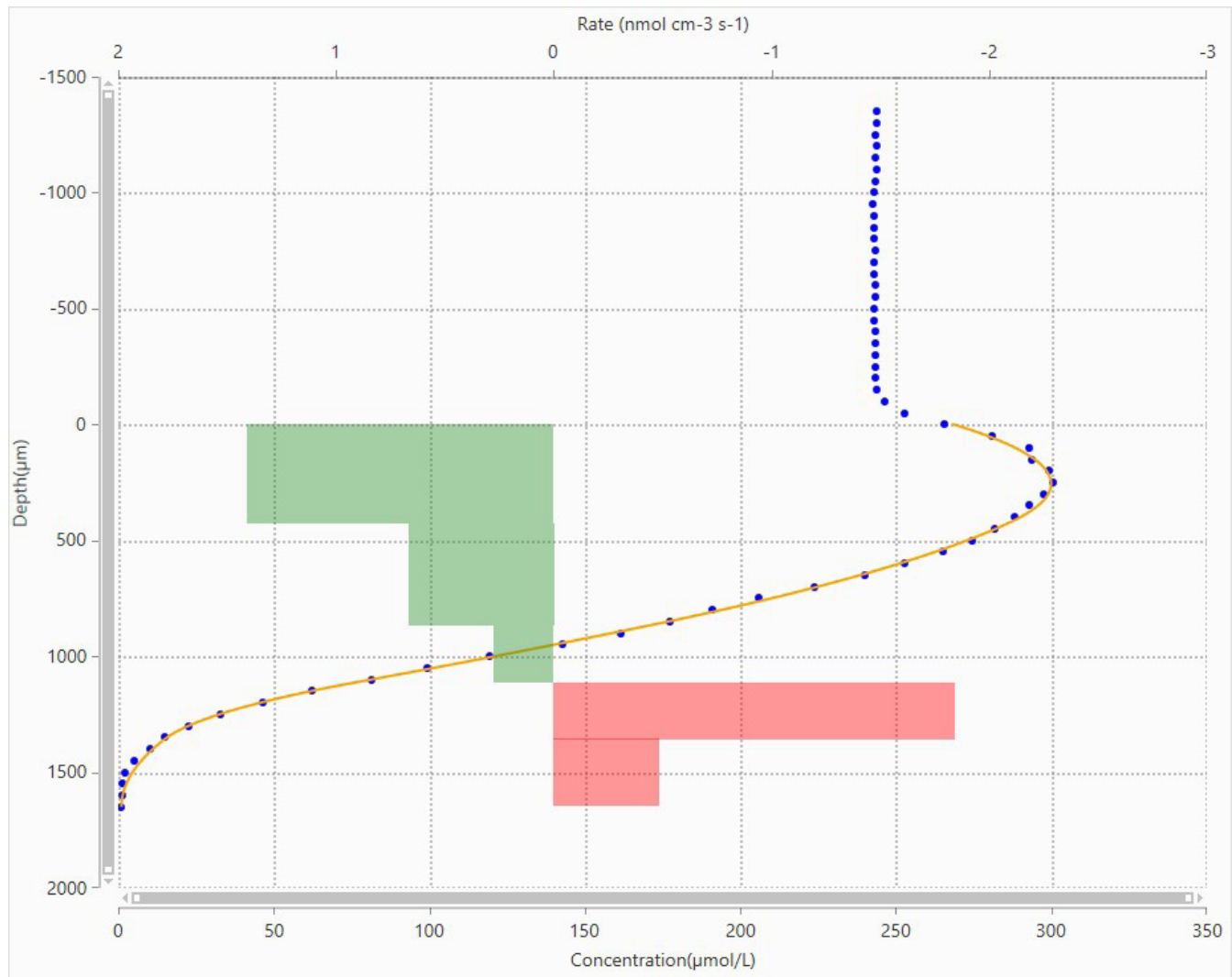
D_0 in free water: Enter the diffusion coefficient in free water at the actual salinity and temperature. For oxygen, D_0 is found by using **O₂-Table**. The Unisense oxygen diffusion coefficient table is also available in the **Knowledge - Technical Information** section of our website www.unisense.com. In the table, you also find information on how to calculate D_0 for other solutes. Alternatively, the D_0 value for the other solutes can be found in the literature.

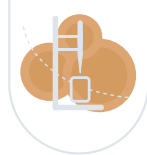
Sediment diffusion coefficient equation (D_s): Select an equation to calculate the sediment diffusion coefficient. $D_s = D_0 \times \phi$, where ϕ is the porosity of the sample, is often used for samples containing a high porosity. $D_s = D_0 \times \phi^2$ is often used in samples with low porosity and $D_s = D_0 / (1 + 3 \times (1 - \phi))$ is used for all kinds of material. See also the Theory section.

Table (D_s and Φ)

In the table, you can manually set the start and end depths, porosity, and calculate the diffusion coefficient (D_s).

Sediment diffusion coefficient (D_s): D_s is calculated using the porosity set in the D_s table, the diffusion coefficient in free water (D_0), and the diffusion coefficient equation. To use independent diffusion coefficient values: Type diffusion coefficient values – measured or estimated – directly in the table.





Analyze

Select **Analyze** to start the activity analysis. The calculation starts automatically by first performing the analysis with one zone and then makes the calculation for increasingly high zone numbers until the specified maximum zone number.

Statistics							
Save solution		Export selected analysis					
No. of Zones	SSE	P-Value	Top Cor (μmol/L)	Bottom Cor (μmol/L)	Top Flux (nmol cm ⁻² s ⁻¹)	Bottom Flux (nmol cm ⁻² s ⁻¹)	Integrated prod (nmol cm ⁻² s ⁻¹)
1		0.000	243.69	1.00	-2737.50669	7591.28254	10328.78922
2		0.017	243.69	1.00	805.27249	8904.55390	8099.28141
3		0.000	243.69	1.00	1426.04292	-3939.24446	-5365.28738
4		0.004	243.69	1.00	-641.20556	-4289.35979	-3648.15424
5		0.009	243.69	1.00	-737.89997	322.47378	1060.37375

Statistics

For each zone number a row in the table shows the statistical values, concentrations, and rates. The program highlights the zone number that has the best statistical values. Following parameters are listed:

Zones: The number of zones in the calculation

SSE: Sum of Squared Errors is the difference between the simulated and observed profile. The smaller this number, the better.

P-Value: The probability of the hypothesis that including an extra zone significantly improves the prediction. The hypothesis is calculated based on the F value (see Berg et al., 1998). The smaller this number, the better.

Top conc.: Calculated top concentration in μmol/L

Bottom conc.: Calculated bottom concentration in μmol/L

Top flux: Calculated top flux in nmol cm⁻² s⁻¹

Bottom conc.: Calculated bottom flux nmol cm⁻² s⁻¹

Integrated prod.: Calculated integrated production in nmol cm⁻² s⁻¹

Volume specific rate: The volume specific consumption or production rates in nmol cm⁻³ s⁻¹ are shown when moving the cursor over the green or red rate bars in the activity plot. The values are also shown in the exported file under Zones.

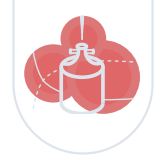
When all calculations are done, the solution with the highest number of zones that is significantly better than the previous solution (P<0.05) will be the best choice. However, it is advisable to visually check the other estimated solutions by selecting and thus highlighting these in the table. Furthermore, the effect of changing boundary conditions and/or minimum zone width can also be tested.



Save solution: This will save the analysis in the Experimental overview tab under the analyzed profile. If the statistics indicate that the number of zones you have chosen in the **Max zones** are too high or too low, you can either reduce or increase zone number in the **Max zones** setting. Repeat the profile analysis and save the best solution. An analysis can be deleted in the Experiment overview.

Export data: The following information will be exported to an Excel or a CSV file:

1. Statistics table including all data shown in the statistics table
2. Zones with the volume specific rate
3. Settings. Here you find the settings of the profiles
4. Observed profile, including the calibrated data of the profile
5. Simulated profile, including the values of the modeled profile
6. Devices shows which amplifier(s) have been active during profiling
7. Calibration, including the calibrated values
8. Comments, including all comments and actions
9. Miscellaneous



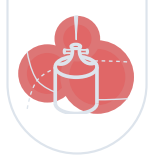
uSENSE RATE

uSense Rate is a program for measuring consumption and production rates of analytes, typically oxygen using the MicroRespiration System.

Basic features of the program are:

- Control of settings for experimental start-ups
- It keeps track of measurements obtained in different chambers/samples
- Multiple sensors can be used simultaneously
- Calculations based on linear interpolation and regression results in respiration rate data from several chambers using only one sensor
- Oxygen or other analytes consumption and/or production are followed in real time
- Data is continuously saved for later data interpretation
- After the experiment, all measurements can be exported into an Excel or a CSV file for easy data handling and analysis

The general description of the software and how to use the software to calibrate a microsensor can be found at the beginning of this manual. In the following the uSense Rate specific features of the software will be described. General information about the MicroRespiration System is found in the MicroRespiration System user manual.



SETTINGS TAB FOR μ SENSE RATE

When μ Sense Rate is started, the Settings window for μ Sense Rate appears. This window allows you to define the settings of the experimental set-up. The amplifier and sensors as well as the number of chambers should be selected. The volume in μL and a description of the contents in the different chambers can be entered directly into the table. As all microrespiration chambers are handmade, the exact volume of a chamber can vary. It is therefore important that you weigh the exact chamber volume with water.

IMPORTANT! You can choose a maximum of 24 chambers in the software

When the settings for the experimental setup are defined, click **Start experiment**.

μ Sense Rate File View Tools Manuals Help

Settings

Start Experiment Manage LAN-mode devices

Scan **Scan instruments and configure them:**

Instrument	Use	Channel	Type	Unit	Sensor name	Comment
fx-6 UniAmp (401561)	☒	pA-1	OX	$\mu\text{mol/L}$	Sensor 1 - OX	
fx-6 UniAmp (401561)	☐	pA-2	None	Not a...	Sensor 2 - None	
fx-6 UniAmp (401561)	☐	mV-1	None	Not a...	Sensor 3 - None	
fx-6 UniAmp (401561)	☐	mV-2	None	Not a...	Sensor 4 - None	
fx-6 UniAmp (401561)	☐	Op-1	None	Not a...	Sensor 5 - OX	
fx-6 UniAmp (401561)	☐	T-1	None	Not a...	Sensor 6 - Temperature	
fx-6 UniAmp (401561)	☒	Pressure	Press...	mbar	Sensor 7 - Pressure	
fx-6 UniAmp (401561)	☐	Analog In 1 (0 - 4000 mV)	Analog	mV	Sensor 8 - Analog	
fx-6 UniAmp (401561)	☐	Analog In 2 (0 - 4000 mV)	Analog	mV	Sensor 9 - Analog	

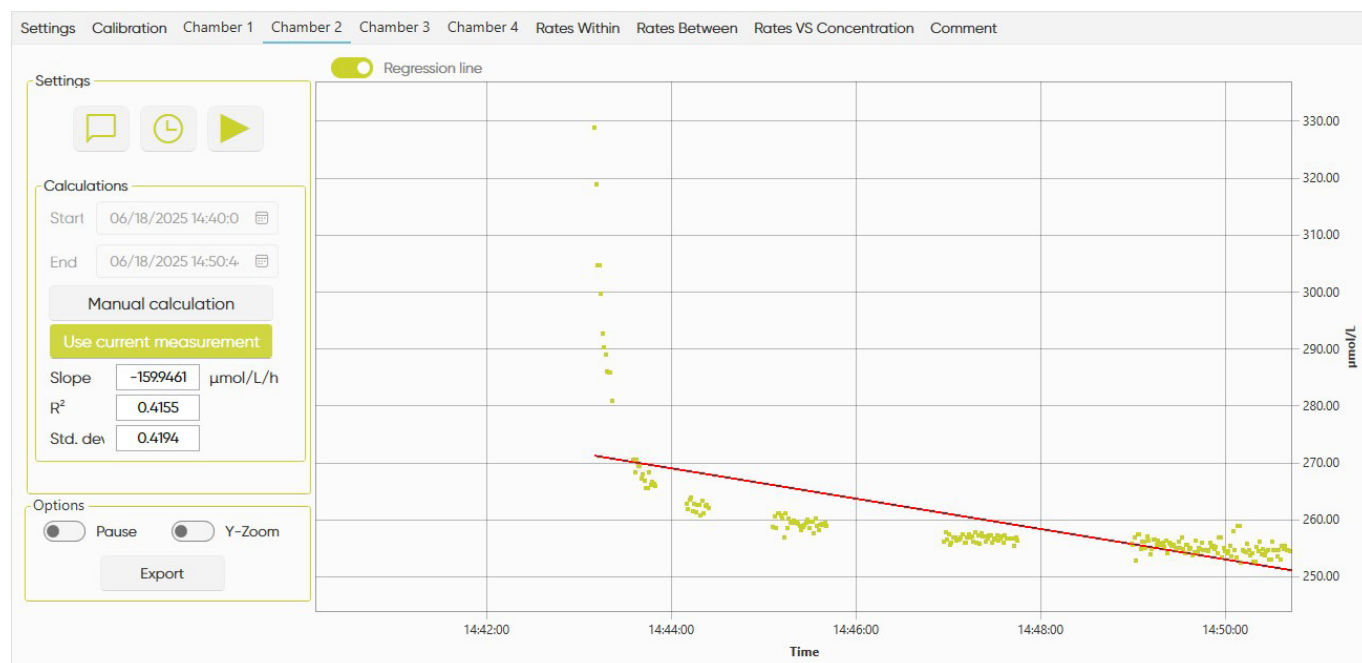
Total chambers used in the experiment: - 4 +

Number	Volume, μL	Description	Double chambers
1	4025		☐
2	4123		☐
3	4105		☐
4	3985		☐



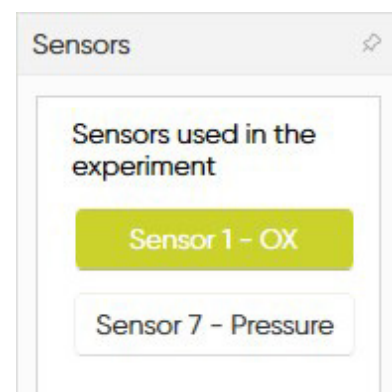
CHAMBER TAB

The Chamber window shows the microsensor measurements that have been done in a chamber. There is a Chamber tab for each chamber added in the Settings tab. Under each Chamber tab, the concentration of the substance can be continuously followed and when **Regression line** has been selected, the linear regression line is continuously calculated and shown as a line on the graph.



To start a rate measurement in a selected chamber, select the correct sensor in the right sensor panel.

Once a sensor is lowered into a sample, it will take a few seconds before the correct signal is shown (sensor response time). In order to avoid these measurements being included in the interpolations and regressions performed by the software, the signal should be allowed to stabilize before measurements are started. After lowering the sensor, wait for a stable signal before selecting **Start measuring**. When **Start measuring** has been selected, data are plotted in the logging window and a regression line is continuously calculated based on all the measurements made until **Stop measuring** is selected.





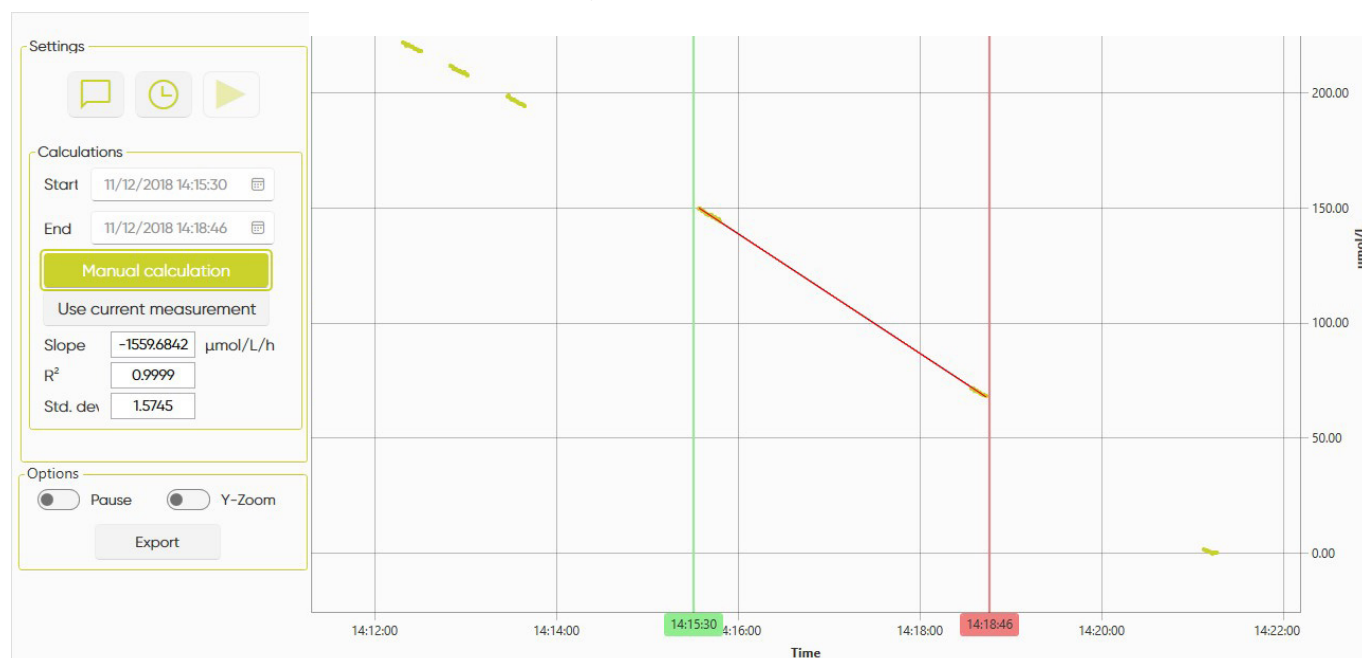
Sampling interval(s): Open the **Sampling Interval** dialogue by clicking the clock icon. This specifies how often the program will make a data point (for the log file and in the graph). A single data point consists of an average of measurements sampled with a high speed background frequency in the sampling interval preceding the data point (up to 1,000 ms). In other words with a sampling interval of 10 s, the data points are logged every 10 s as an average of samples over the last 1,000 ms just before the next sampling point. The background frequency is dependent on the amplifier type. It is typically preset to 1 to 10 kHz.

It is important to know the system you are measuring in, to make sure that you choose the right sampling interval and do not miss the fluctuation that you are expecting. For a relatively active system for example, it is preferable to choose a short sampling interval. For a long, perhaps linear, monitoring situation, a longer sampling interval will save computer memory.

Start and stop measuring: Here you start and stop the data logging from a sensor placed in the selected chamber. The Regression line is automatically shown for the latest logged data points. Several chambers can be in use simultaneously with their individual sensors and log data, while another Chamber tab is inspected.

Comment: Here you can place a comment to the experiment. The comment is saved in the Comment tab.

Manual calculation: During a measurement, it is possible to do a fast analysis of the rate by using the manual rate calculation. This can be useful if you have included unstable data or if the rate is changing over time during the experiment. When selecting **Manual calculation**, a start and end time bar is shown on the figure. Move them to the timeframe you want a manual rate calculation from. The Slope ($\mu\text{mol L}^{-1} \text{h}^{-1}$), R^2 , and standard deviation (StDev) for the data measured within the timeframe will be calculated and shown in the Settings window.



Use current measurement: It is possible to start and stop data logging in a chamber several times e.g. when returning the microsensor to the chamber after measuring in other chambers. The regression line will by default be calculated from all logged data. By selecting **Use current measurements** the regression line will be calculated only from the last logged data series.

Options: How to use **Pause**, **Y-Zoom**, **Show last**, **Clear** and **Export** please see the uSense Log section and Live Data Graph.



RATES WITHIN WINDOW

Rates Within window shows all rates calculated from the concentration measurements in a chamber during a time interval. The green dots show the concentration measurements in the individual periods. The line is the regression line through these measurements. The time interval from where the rates are calculated are marked as red bars for a consumption and green bars for a production. The height of the bars marks the rate. The rate values (nmol/h) are found on the right y-axis.



Options: Here you can select what data should be posted in the result table.

Table: The table shows all chambers and the rates measured in the separated periods marked as M1, M2, and so on. The results are shown per sensor across the chambers for easy comparison.

Chamber: Indicates the chamber number from where the rates have been calculated.

Rate: The rate is expressed as nmol/h. The data are based on the concentration change over the indicated time interval (slope). The volume of the chamber entered in Setting for uSense Rate has been used to convert the rate from µM/h unit to nmol/h.

Slope: The slope of the regression line is expressed in µM per seconds.

95% confidence level: The 95% confidence level is calculated for the regression line from which the rate is calculated.

Average concentration: The average concentration is calculated from the concentration values measured in the indicated time interval.

Start and End: The Start and End time shows the time interval for the rate calculation of the separated periods marked as M1, M2, and so on.

Export table: All values calculated in Rate Within are logged in the experiment file and can be exported by selecting **Export table**.

	A	B	C	D	E	F	G	H
1	Chamber	Interval	Rate (nmol/h)	Slope (uM/s)	95% C.L (nmol/h)	Start (YYYY-MM-DD hh:mm:ss)	End (YYYY-MM-DD hh:mm:ss)	Avg.Conc (nmol/L)
2	1	M 1	-4076.8902	-0.2831	0.53	2018-11-12 14.11.22	2018-11-12 14.11.40	236.6691
3	1	M 2	-4631.1207	-0.3216	0.7046	2018-11-12 14.12.19	2018-11-12 14.12.30	220.044
4	1	M 3	-4686.7457	-0.3255	0.7046	2018-11-12 14.12.50	2018-11-12 14.13.01	209.7206
5	1	M 4	-5353.0059	-0.3717	0.7046	2018-11-12 14.13.28	2018-11-12 14.13.39	196.5153
6	1	M 5	-5440.3674	-0.3778	0.5992	2018-11-12 14.15.34	2018-11-12 14.15.48	147.0656



RATES BETWEEN WINDOW

In this tab the rates are calculated as the slope of the interpolation line between the concentration measurements of two following measuring periods, M1-M2, M2-M3, and so on. It calculates the rate between the last measurement points from the previous measurement period and the first measurement points from the following measurement period. The rates (nmol/h) are shown on the right y-axis and marked as red blocks if the concentrations are decreasing (consumption), and as green blocks when the concentrations are increasing (production).



Samples before and after measurement: Here you can indicate how many sample points should be included from the last and previous measuring periods.

Mid-Time Conc.: Mid-Time Concentration is the concentration half way through the period between measurements, M1-M2, M2-M3, and so on. The purpose of this value is to give the user an impression of the concentration of analyte during this period, analogous to the average concentration from Rate within.

Options and Table: For further information on Options and Table see Rate within section

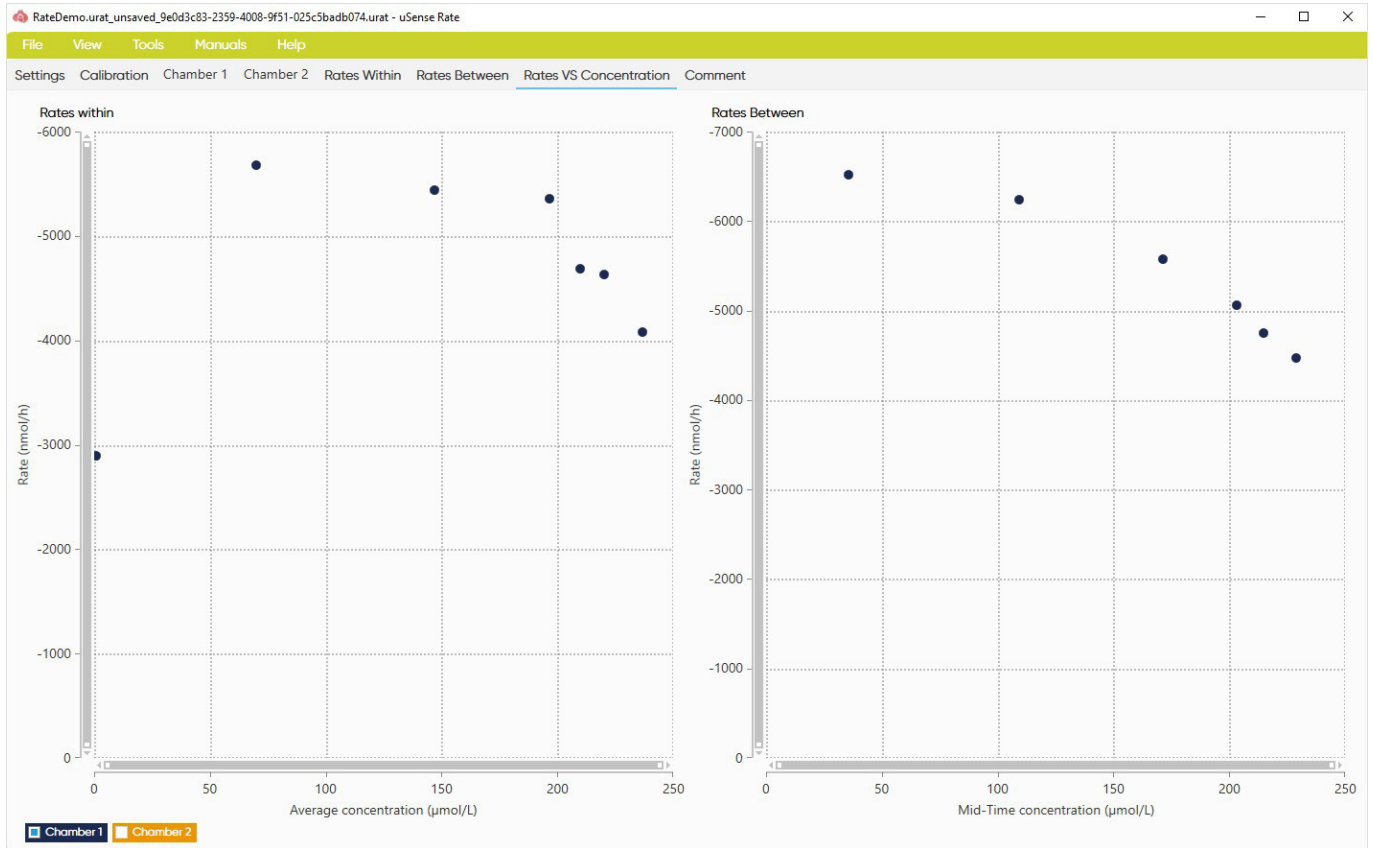
Export table: All values calculated in Rates Between are logged in the experiment file and can be exported by selecting **Export table**.

	A	B	C	D	E	F	G	H
1	Chamber	Interval	Rate (nmol/h)	Slope (µM/s)	95% C.L (nmol/h)	Start (YYYY-MM-DD hh:mm:ss)	End (YYYY-MM-DD hh:mm:ss)	Mid-Time Conc. (nmol/L)
2	1	M1-M2	-4467.8917	-0.3103	0.3871	2018-11-12 14.11.22	2018-11-12 14.12.30	228.8253
3	1	M2-M3	-4753.0534	-0.3301	0.4422	2018-11-12 14.12.19	2018-11-12 14.13.01	214.8713
4	1	M3-M4	-5061.9906	-0.3515	0.4422	2018-11-12 14.12.50	2018-11-12 14.13.39	203.1064
5	1	M4-M5	-5573.2459	-0.387	0.4119	2018-11-12 14.13.28	2018-11-12 14.15.48	171.5026
6	1	M5-M6	-6238.737	-0.4332	0.4314	2018-11-12 14.15.34	2018-11-12 14.18.44	109.0831
7	1	M6-M7	-6522.6477	-0.453	0.5117	2018-11-12 14.18.34	2018-11-12 14.21.16	35.4245
8	2	M1-M2	-4824.6905	-0.335	0.3949	2018-11-12 14.11.56	2018-11-12 14.12.46	221.3581



RATES VS. CONCENTRATION WINDOWS

The Rates vs. Concentration produces a view of the respiration/production rate versus the average concentration. This gives a good picture of how the metabolism of the invested organism varies with the concentration level in the environment.





uSENSE PHOTO

The uSense Photo software is made for photosynthetic experiments using the light-dark switch technique for gross and net photosynthetic rate measurements. In the following, the uSense Photo specific features will be described.



MEASURING SETUP FOR μ SENSE PHOTO

In order to carry out a light-dark measurement, the following components are required for the experimental set-up:

- Fast responding O_2 micro-sensor, 90% response time <400 ms
- Light sensor including amplifier box
- Micromanipulator
- Microsensor amplifier
- Photosynthetic active sample (i.e. algae mat, coral, microbial mat)
- μ Sense Photo software with valid license key

The microsensor is connected to a pA channel on the front of the meter, while the Light Sensor box is connected to an analogue input channel at the rear. The appropriate amplifier is connected to the computer via USB. The microsensor is placed in a micromanipulator that is used to position the sensor tip at the intended measuring site of the studied sample. The tip of the light sensor is positioned, i.e. with a lab stand, within the light field, but placed so that it is not interfering with the path of light towards the measuring site. Thus, the light sensor signal acts as an exact indicator for the time of light to dark transition.



THE LIGHT-DARK SHIFT METHOD

This paragraph gives a short description of the theory on which the light-dark shift method is based. The tip of an oxygen microsensor is placed at a certain position r in a photosynthetic system. It measures at time t the oxygen concentration $C(r,t)$. The time dependency (i.e. the derivative in time) of the oxygen concentration can be described by:

Where P is the volumetric gross photosynthesis rate of oxygen ($\text{mol m}^{-3} \text{s}^{-1}$) and R the volumetric oxygen respiration rate ($\text{mol m}^{-3} \text{s}^{-1}$), respectively. The volumetric transport rate T represents the changes in oxygen concentration due to transport phenomena ($\text{mol m}^{-3} \text{s}^{-1}$). The type of transport is dependent on the specific sample under investigation. It can be due to advection, convection, or diffusion. The light-dark shift method works for all types of transport.

For the light-dark shift method, it is important that the oxygen concentration reaches a measurable steady state value under a constant illumination setting. At steady state the above equation is equal to zero:

$$\frac{\partial C}{\partial t} = 0 \Rightarrow -T - R + P = 0$$

If the illumination is now switched off the steady state situation is disturbed. The photosynthesis rate P is now zero, whereas transport and respiration rates are assumed to remain unchanged within the next short time period. Thus, the time dependency of the oxygen concentration C immediately after the onset of darkness is given by:

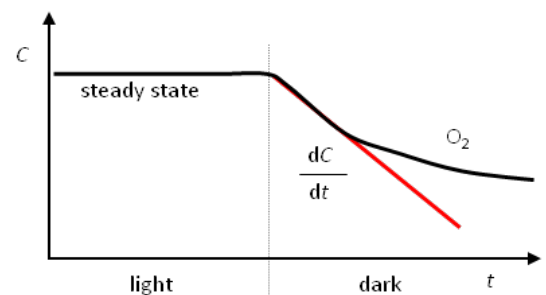
$$\frac{\partial C}{\partial t} = -T - R$$

The latter two equations can be subtracted from each other, which yield:

$$\frac{\partial C}{\partial t} = -T - R + P$$

Thus, the volumetric gross photosynthesis rate P can be determined by the slope of the oxygen concentration C immediately after the light-dark shift. This is indicated in the following figure.

Practically, the measurements are done by moving the oxygen microsensor tip to the measuring site with the help of a micromanipulator. The sample of interest is illuminated with photosynthetically active light of a constant intensity. Steady state is achieved when the microsensor reading is stable over time. At this point the illumination is switched off. It is essential that the switching event is fast ($\ll 100$ ms). Hence, just switching off the light source will usually not work, as most light sources exhibit an afterglow. Normally, sample shading will be the best method. This can be done by either blocking the light path manually (e.g. using a piece of cardboard or aluminium foil) or by applying an electronic shutter.



The computer samples the voltage readings of the microsensor at a frequency of 10–50 Hz. The typical sampling interval starts 1–3 seconds before the switching event and stops 2–4 seconds after it. The time interval before the switching event is used to calculate an average value of the steady state oxygen concentration.

The volumetric gross photosynthesis rate is obtained by fitting a linear slope to the data sampled immediately after darkening. The user has to define the start and end time of the fitting interval relative to the switching event. Typical values for the fitting interval are 0–0.5 and 0.5–2 seconds for the start and end times respectively.

As the uSense Photo software has to know exactly when the switching took place, a light sensor is placed within the light field near the measuring site. Its signal is used by the computer as a trigger for the switching event.

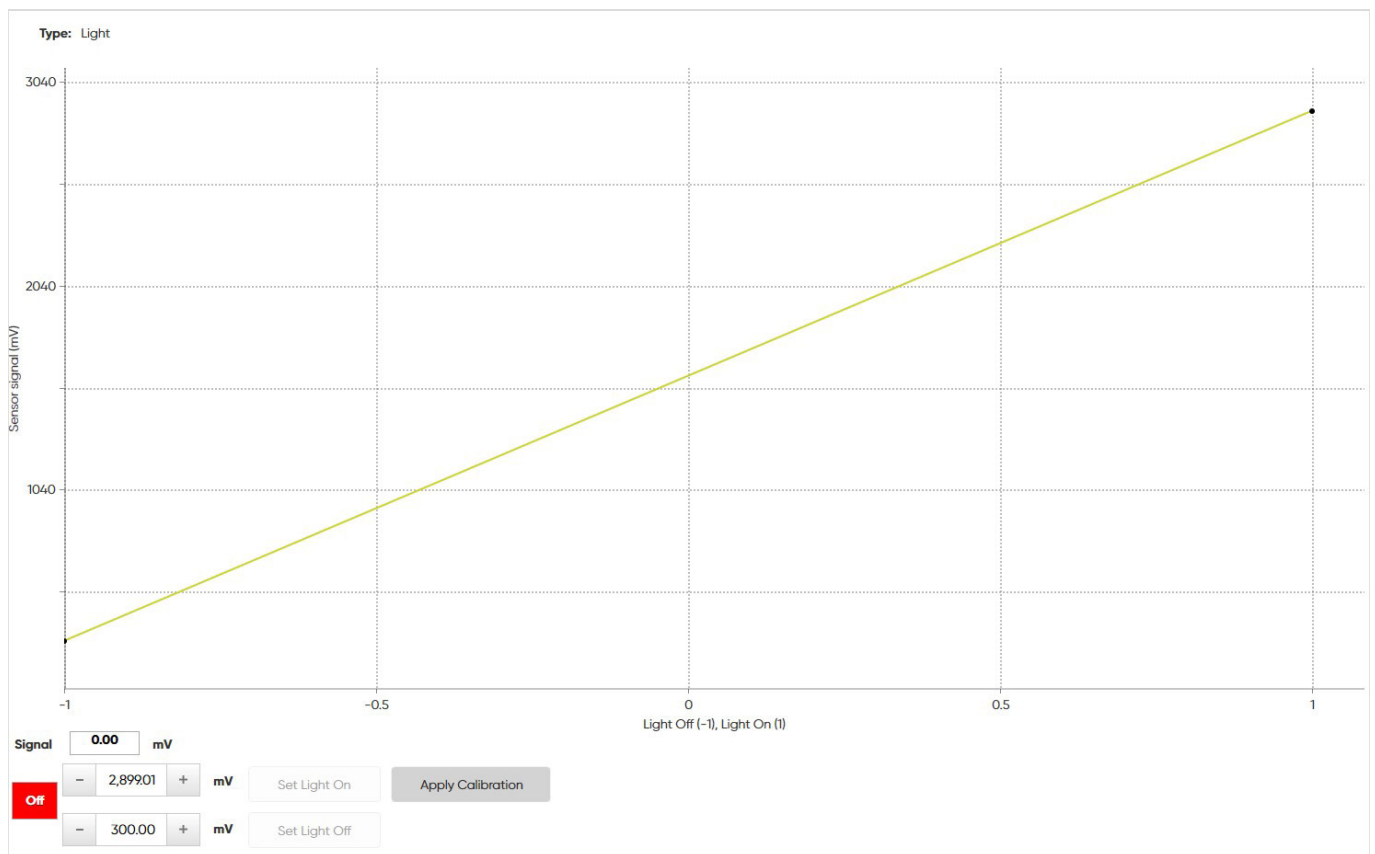


CALIBRATION OF LIGHT SENSOR

For the light-dark shift method you will need data from a calibrated oxygen microsensor and from the light sensor. Information on how to use uSense Photo for calibration of an oxygen microsensor can be found under Calibration window in the General section. See how to calibrate an oxygen microsensor in the sensor specific manual.

Calibration of the light sensor

During calibration of the light sensor, it is recommended that the sensor is physically placed in the same position that is used for the actual measurements. **Set light On** during illumination. Then switch the illumination off and select **Set light Off**. The actual voltage readings of the light sensor are shown in the boxes next to the buttons. The calibration of the light sensor is now activated by pressing the **Apply calibration** button. It is recommended to test that the light trigger will actually capture a change in light regime during light on-off transitions, by turning the light source repeatedly on and off a couple of times while observing the status of the sensor.

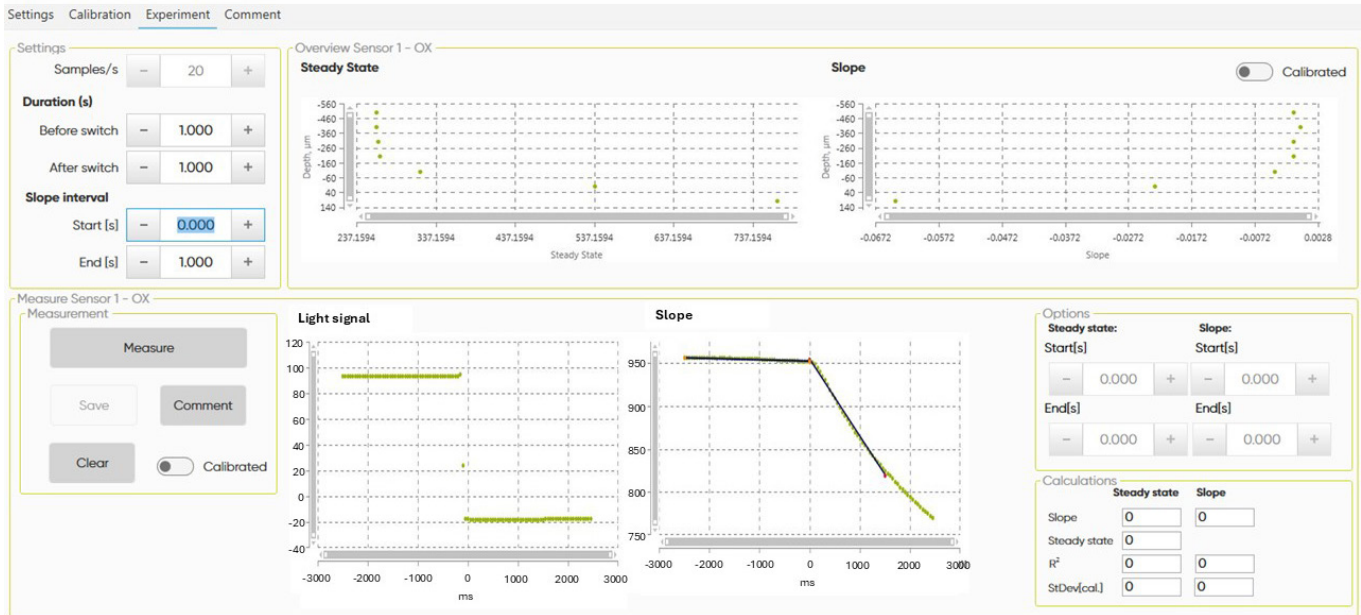


The software is preprogrammed to respond to light threshold values that are somewhat lower than the recorded calibration values for light on and somewhat higher when light off. It is further possible to adjust these readings, i.e. the light on value downwards and the light off value upwards, to further ensure that the light transition is captured during experiments. The calibrated light signal is shown as RAW and the value represents the current signal in units of % compared with the set signal value for light on.



EXPERIMENT WINDOW

The main experimental window is used during photosynthesis rate measurements and is composed of a number of different elements.



The main display features are:

Settings: In this window the experimental settings are preadjusted for the photosynthetic rate measurements.

Overview: This window, contains two graphs that summarize the results of the performed experiments by showing the measured steady state concentrations and slopes as a function of sample depth.

Measurement: Displays the sensor signals during the last performed light-dark measurement. The values for steady state and slope are shown and the time interval for calculations can be manually adjusted.



PERFORMING AN EXPERIMENT

Settings: Before the actual measurement can be started, some parameters have to be adjusted in the experimental window. See also the Light-Dark Shift Method.

Samples (seconds): The sampling rate (or sampling frequency) is set here.

Duration (seconds): The total sampling duration is set in **Before switch** and **After switch**. These parameters define the recorded time interval in seconds before the switching event and after it respectively.

Slope interval (seconds): The time interval used for the slope fitting is defined in **Start** and **End**. The values are given in seconds relative to the switching event.

Settings

Samples/s	-	20	+
Duration (s)			
Before switch	-	1.000	+
After switch	-	1.000	+
Slope interval			
Start [s]	-	0.000	+
End [s]	-	1.000	+

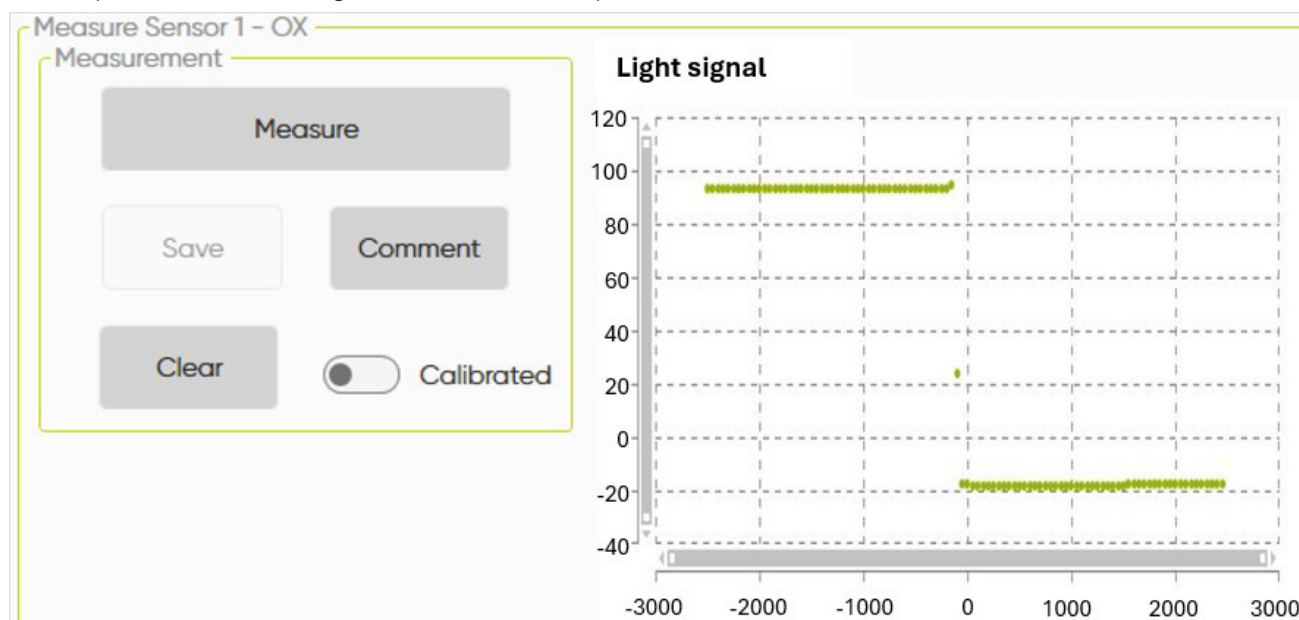
NOTE: The fitting interval can be interactively changed for each experiment after the measurement has been made

Photosynthesis rate measurements

For the light-dark measurement, the oxygen microsensor tip is positioned at the measuring site of the illuminated sample. The oxygen sensor reading should reach a stable value, which thus indicates that the system is in a steady state.

Now click **Measure**. On the screen, the message Measuring will be shown, indicating that the measurements have started. After a short time period (as set in **Before switch**) this message will change to Turn off the light, signaling that it is time to darken the sample. It is important to wait with the switch event for at least as many seconds as set under **Before switch**, because the trigger signal of the light sensor can otherwise not be detected.

Keep the sample in the dark until the Measurement window is opened, but not unnecessarily long as an extended darkening period will prolong the time period before a new steady state is reached and the next rate measurement can be made. The Measurement window displays two charts with recorded signal data for the light sensor and the oxygen sensor during the light-dark transition. The captured data are shown either as calibrated values or as raw signals. The graph that shows the oxygen data includes the fitted lines for both steady state and slope intervals. Both intervals can be manually adjusted in the Option window following the measurement to optimize the curve fit.





The calculated values for steady state ($\mu\text{M O}_2$) and slope ($\mu\text{M O}_2/\text{ms}$), including statistical analysis of the data, are shown in 'Calculations'. These values will automatically be updated when the interval is adjusted. The R^2 of the line fittings is a value between 0 and 1, and the closer the slopline is to 1, the better the fit.

When a measurement has been completed the data is being saved to the file by clicking **Save** in the Measurement window. Alternatively, if the signal data from the current measurement for some reason should not be saved, e.g. if uSense Photo did not detect the trigger signal or if the measured data was of poor quality, the **Clear** button will erase the recorded data, and the measurement can be repeated.

After the measurement has been saved, the sensor can be moved from the current to the next position using the motor control or micromanipulator, and a new light-dark experiment can be performed. Depending on the selected step size (i.e. $100\mu\text{m}$) this will generate a data set that at high spatial resolution describes the vertical distribution of the photosynthetic activity within the analyzed sample.

The profile windows

All measurements that are saved will be displayed in two separate graphs in the Overview window and these graphs will be updated gradually as the experiment progresses.

The two graph plots show steady state values and calculated slopes respectively as a function of depth. The y-axis refers to the depth position (μm) where the data points have been acquired. The x-axis refers either to the voltage value or to the calibrated value.



The two plots give the user an overview of the measured photosynthetic activity in the sample. From the combined data, it is possible to clearly identify where the photosynthetic activity zone is found.



Export file: In the output file you will find the following information:

1. Slope calculations
2. Data, including raw and calibrated values from the oxygen and light sensor
3. Devices, including amplifiers that have been used during the experiments
4. Calibration, including the calibrated values
5. Comments, including all comments and actions
6. Miscellaneous

For more information see the Output file section.

REFERENCES

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TROUBLESHOOTING

NOTE! Always ensure that you are using the latest version of uSense Solutions. You can always download the latest version from www.unisense.com

Problem Instruments not found

Possible cause 1 You have not connected your digital sensor instrument.

Possible cause 2 An amplifier with different bit-resolution has just been connected.

Possible cause 3 There is something wrong with the cable/s

Solution Disconnect and reconnect the instruments and select **Scan**.
Use a different cable.

Problem "Noisy" measurements

Possible cause 1 Physical vibrations from other appliances on the table are causing movements of the sensor resulting in instability of experimental setup and disturbances in the measurements.

Solution Remove all unrelated appliances from surfaces in contact with the experimental setup and ensure completely stable conditions for the sample.

Possible cause 2 Electric noise in the system.

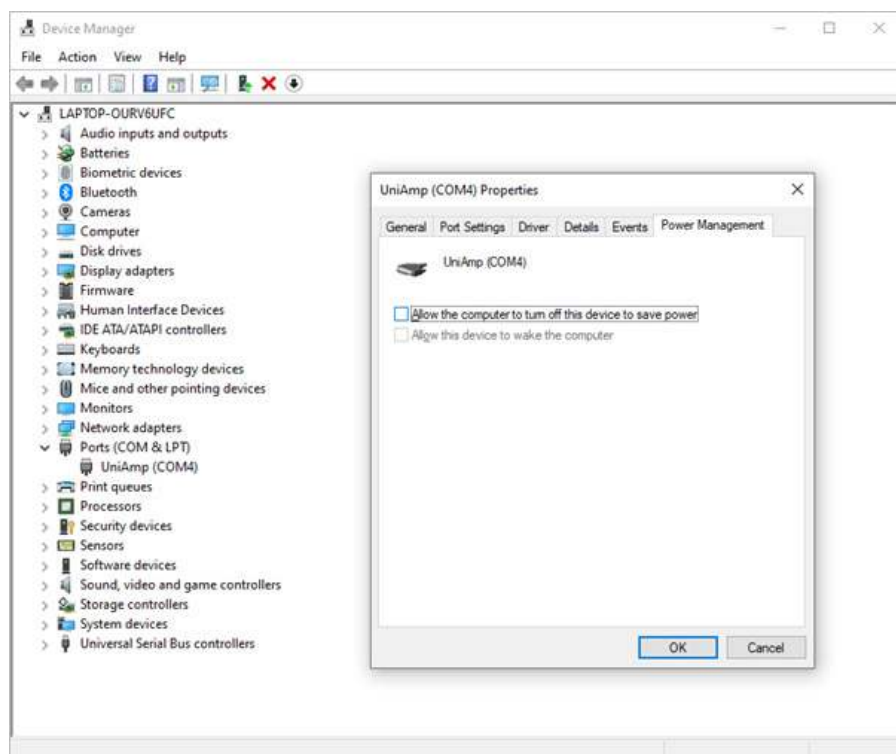
Solution Check that the system is properly grounded. Connect the Ground connection on your sensor instrument to a ground source (a waterpipe or similar). Alternatively, you can ground the meter by connecting the ground cable to the Ground outlet of the sensor amplifier and place the other end of the ground cable into the liquid you are measuring in.

Loss of connection to the Unisense instruments because of PC power saving

Windows may turn USB connections off to save power. To avoid this, open Device manager, double click the Ports (COM & LPT) to unfold it, double click the connected amplifier, UniAmp in the figure below, to open the Properties window (see figure below). Make sure that the checkbox Allow the computer to turn off this device to save power is unchecked.

Disable power saving on the PC

Make sure that the PC is powered from a stable mains outlet or, if running on battery, that this has sufficient power for the duration of your experiment. In the power saving settings on your PC, disable all power saving options. Please note that it may be necessary to disable power saving options both in Windows settings as well as in any proprietary power management software on the PC.



APPENDIX 1

Analogue output for Laboratory UniAmp instruments

Analogue output is available on the following laboratory UniAmp instruments: fx-6 UniAmp, fx-3 pA UniAmp, fx-3 mV UniAmp, x5 UniAmp, O₂ UniAmp, H₂ UniAmp, N₂O UniAmp, H₂S UniAmp, and pH/Redox UniAmp. It is not available for the Opto-F1 and Opto-F4.

The calibrated analogue output is based on the calibration on the E²PROM of the sensor. To save a calibration on the E²PROM, select the calibration in the Calibration window you want to use and then click the Push selected calibration to sensor button. There can only be one calibration on the sensor E²PROM at a time, so if a calibration is pushed to the sensor, this will automatically replace the previous and become the active calibration.

IMPORTANT: Calibrated analogue output is based on the calibration on the E²PROM of the sensor. To save a calibration on the E²PROM, click Push calibration to sensor in the calibration window.

Temperature compensation of the analogue out signal is possible. For electrochemical sensors, activate or deactivate this in the Calibration pop-up window before clicking the Save and use calibration button. For O₂ optodes, the temperature calibration is always active. For both types of sensors, the temperature to use for this must be selected in the Analogue Out Settings in the Calibration pop-up window. This can either be the input from a temperature sensor or it can be the calibration temperature.

Uncalibrated analogue output

The uncalibrated analogue output is only available for electrochemical sensors and not for optodes.

The software calculates the uncalibrated analogue output as:

$$\text{Analogue output (mV)} = \text{Sensor signal (mV)} \times \text{Gain} + \text{Offset}$$

For uncalibrated analogue out Gain and Offset are constant and cannot be changed:

$$\text{Gain} = 0.667 \text{ (mV/mV)}$$

$$\text{Offset} = 667.7 \text{ (mV)}$$

Uncalibrated analogue out is, therefore, always calculated as:

$$\text{Analogue output (mV)} = \text{Sensor signal (mV)} \times 0.667 + 667.7$$

Calibrated analogue output

The software calculates the analogue output as:

$$\text{Analogue output (mV)} = \text{Concentration (unit)} \times \text{Gain (mV/unit)} + \text{Offset (mV)}$$

The unit can be any of the available units in uSense (e.g. $\mu\text{mol/l}$, mg/l etc.).

Example with $\mu\text{mol/l}$ as the unit:

$$\text{Gain} = 5 \text{ mV}/(\mu\text{mol/l})$$

$$\text{Offset} = 100 \text{ mV}$$

$$\text{Conc.} = 75 \mu\text{mol/l}$$

$$\text{Analogue output (mV)} = 75 (\mu\text{mol/l}) \times 5 (\text{mV}/(\mu\text{mol/l})) + 100 (\text{mV}) = 475 \text{ mV}$$

Note: For the Optode channels on fx-6 UniAmp and fx-3 UniAmp, analogue out is only available as Calibrated analogue out.

Setting the Analogue Out

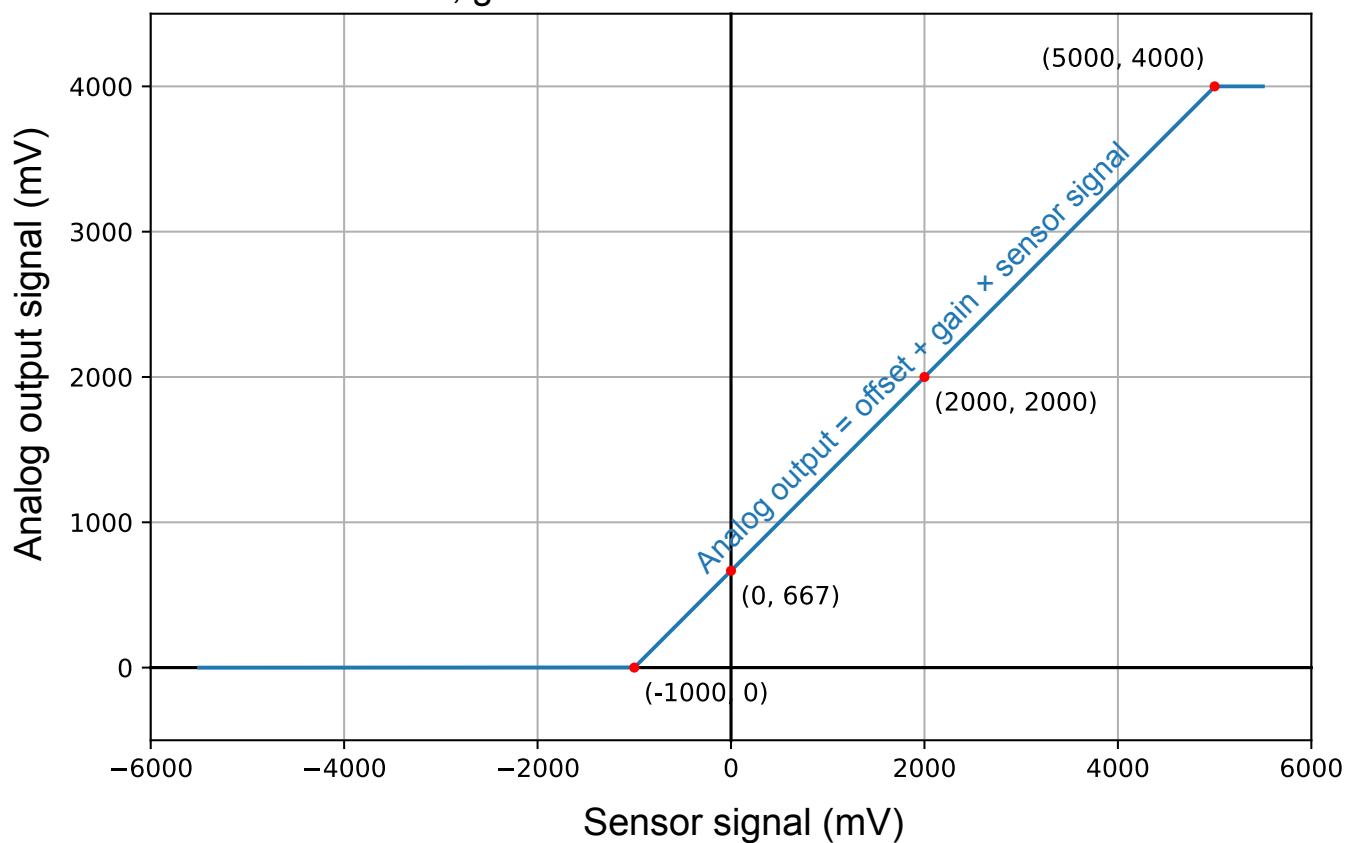
The Analogue Out signal is configured in the UniAmp Channel Configuration window in the calibration window for each channel.

The screenshot shows the 'UniAmp Channel Configuration' window for 'Sensor 1 - OX'. The settings are as follows:

- Preamp Gain (mV/pA):** (2) 1pA=1mV
- Polarisation (mV):** -800
- Offset (mV):** 0
- Solubility temperature compensation:** Enabled (toggle)
- Sensor temperature compensation:** Enabled (toggle)
- ANumber:** 1.0176
- Analogue out:** Enabled (toggle)
- Analogue Out Settings:**
 - Calibrated:** Enabled (toggle)
 - Output offset (mV):** 0
 - Output gain (mV/unit):** 1.000
 - Temperature:** Use calibration temperature (T-cal) [Selected]
 - Use measured temperature (T channel) [Button]

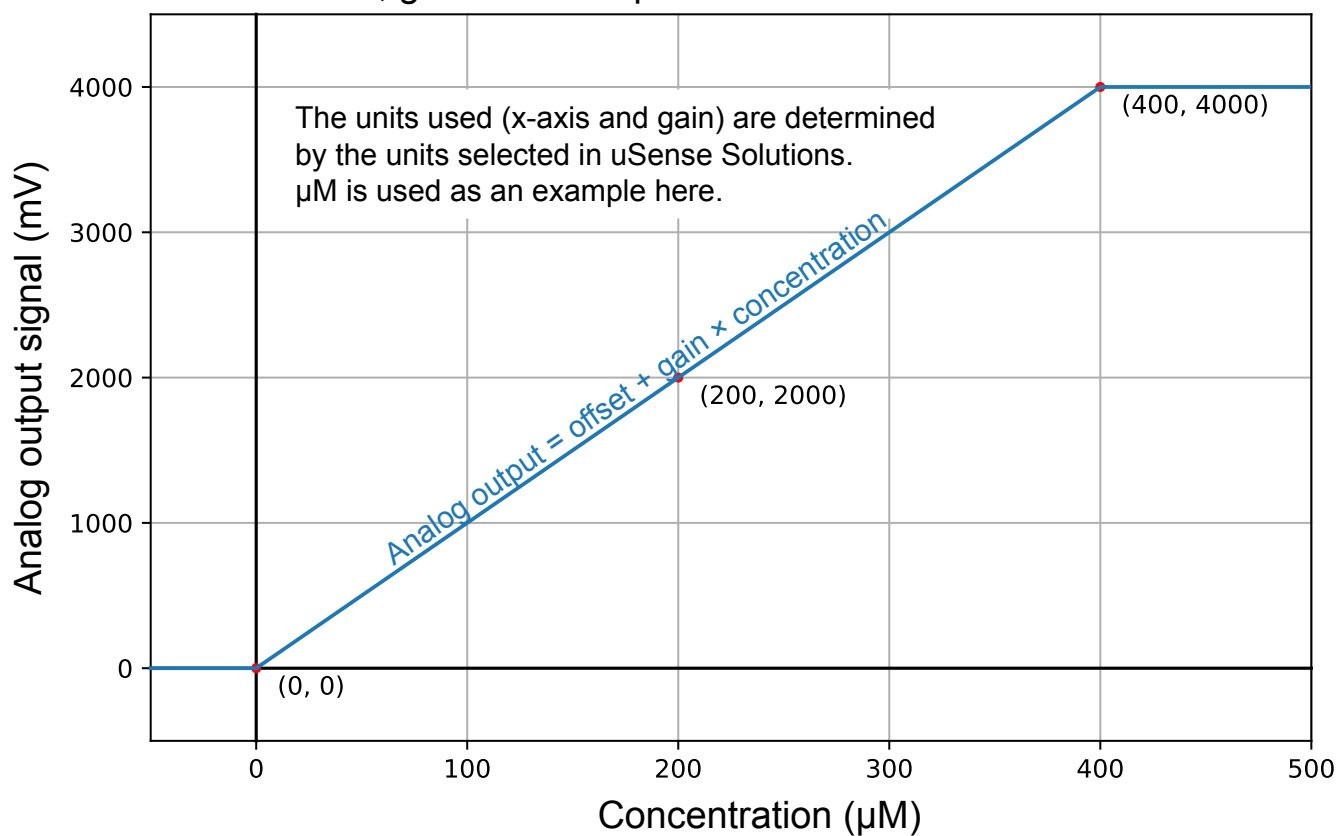
Uncalibrated analog output:

Offset = 667.7 mV, gain = 0.667 mV/mV



Example for Calibrated analog output:

Offset = 0 mV, gain = 10 mV/ μ M



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