

SENSORTRACE SUITE USER MANUAL



SENSORTRACE SUITE V3.3.000 USER MANUAL

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SENSORTRACE SUITE USER MANUAL

UNISENSE A/S

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WARRANTY AND LIABILITY

SensorTrace Suite software is checked and validated on the operating systems as given in the specification, running English language settings. It comes with lifetime updates.

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

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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.

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Further documentation and support is available at our website
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OVERVIEW

SensorTrace Suite is a software package including SensorTrace Logger, Profiling, Rate, and Photo. All SensorTrace Suite 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 following features:

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

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

Profiling: 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.

SensorTrace Suite 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 SYSTEM REQUIREMENTS

- 2 GHz PC
- Windows 7 or newer
- 200 MB free harddisk space
- Ports to connect Unisense instruments
- 4 GB RAM
- Screen resolution 1280 x 800
- Unisense amplifier (UniAmp series, Microsensor Multimeter, Microsensor Monometer, OXY-Meter, MicroOptode Meter, pH/mV-Meter, Field Microsensor Multimeter, Field DataLogger or Field DataLogger Mini (Firmware version 2 or later))

INSTALLING THE SOFTWARE

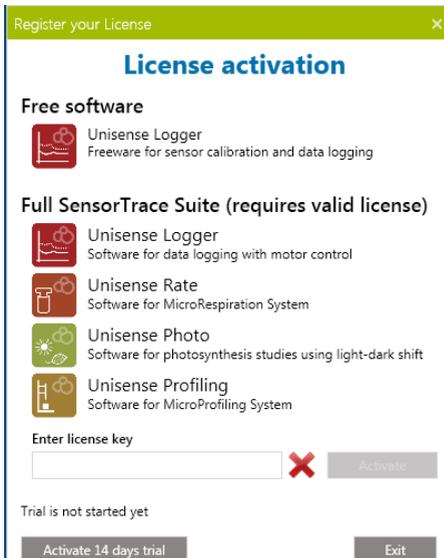
Make sure that you are installing using full administrator rights. The software must be installed locally, not on a network drive.

Make sure that you always run the latest version of SensorTrace Suite. The latest version is always available on www.unisense.com

LICENSE ACTIVATION

SensorTrace Logger and Programming Tool can be used without a license (freeware). To activate the rest of SensorTrace Suite (Logger+, Photo, Rate and Profiling) a license key is necessary.

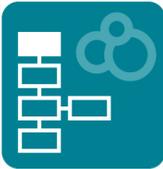
Contact sales@unisense.com to purchase a license key. It is possible to try out the full SensorTrace Suite free of charge for 14 days by selecting **ACTIVATE 14 DAYS TRIAL**.



After installation of SensorTrace Suite the four programs are shown as following icons on your desktop.



Programming Tool is used in combination with the Field Microprofiling Multimeter and the Field Data Logger (Firmware version 2 or later). Information on how to use Programming Tool can be found in the Programming Tool manual.



Motor Tool is used to operate the motors manually.



UniAmp service is installed in the Windows System Tray and is started automatically when Windows starts. If this is turned off, the UniAmp service can be started manually. This service is need for the UniAmp series of amplifiers.



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.
3. Start the free SensorTrace Logger software or one of the programs in the SensorTrace

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.



Suite package: Profiling, 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 Documents under Unisense Data
5. Load an experiment: You load an old experiment in the File Tab. Alternatively, you can double click the experiment file

and the file will open in the program it was made in.

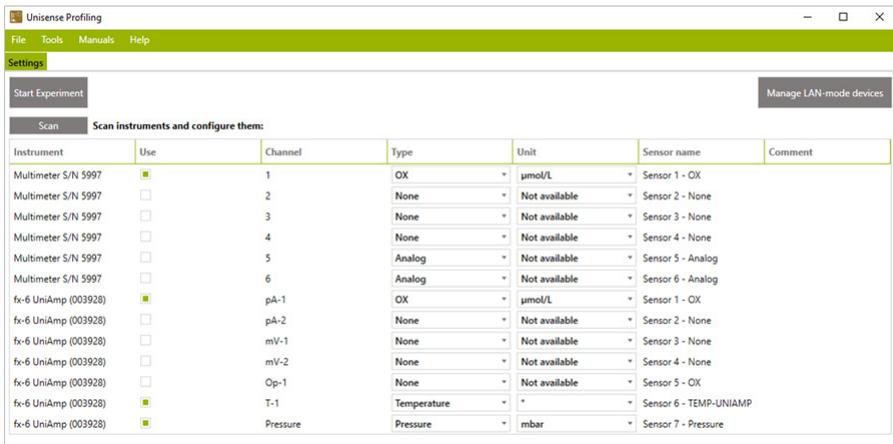
Working with old data; settings and parameters cannot be changed and new measurements cannot be started.

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.



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 SensorTrace Profiling, 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).

MOTOR SETTINGS

Available in Logger+, Profiling and Photo

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.

NOTE:

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

Scan Scan motors and configure them:					
Device	Motor Axis	Velocity	Acceleration	Port connected	
Lab Motor (MOTCON)	Z	1000	1000	COM7	
Lab Motor (MOTCON)	X	1000	1000	COM4	
Lab Motor (MOTCON)	Y	1000	1000	COM15	
	Z				
	X				
	Y				
	Disabled				

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.

Velocity and acceleration

For lab motor stages (MMS-type) 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, 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 tabs available depending on which program of SensorTrace Suite you use.

The sensor and motor configuration will be saved.

NOTE:

Data can be added to a SensorTrace 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).

NOTE:

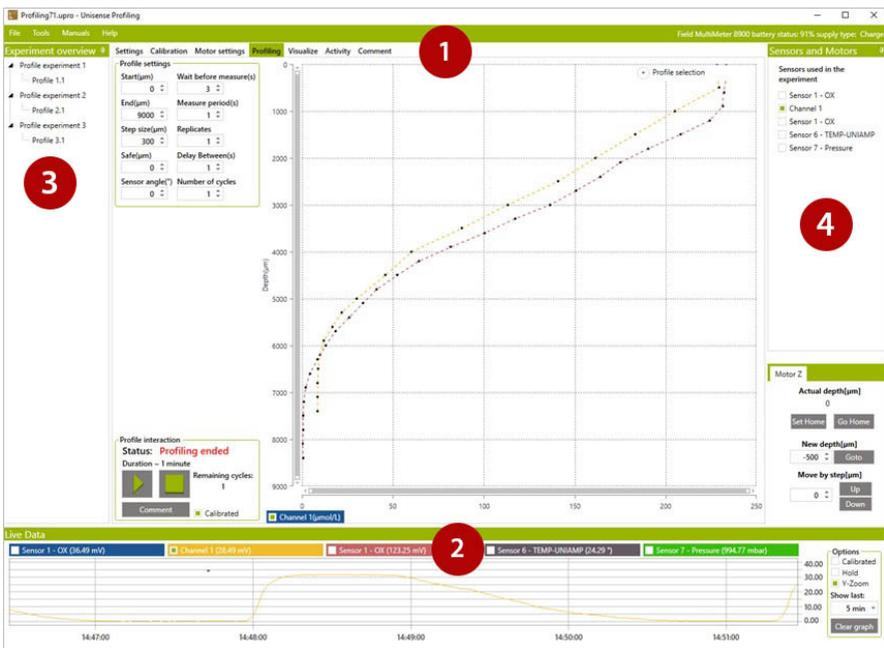
For a manual microprofiling setup with only a Micromanipulator (MM33 or MM33-2) and no Motor Stages, it is not necessary to add an axis. A manual Z-axis will always be available in the experiment file.

GENERAL FEATURES

All SensorTrace Suite programs have windows that by default are divided into four main areas: 1. Tab 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 tabs at any time when the software is running a program



Tab selection (1)

The upper area is divided into tabs which allow you to access different functions of the program. The number of tabs and the specification of the tabs will vary depending on the specific program. See the description of the specific tabs in the respective sections for SensorTrace Logger, Profiling, Rate and Photo. The Settings tab, Calibration tab and the Comment tab 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 tab (3)

In the left side area Experiment overview lists the finished experiments.

Sensor and Motors (4)

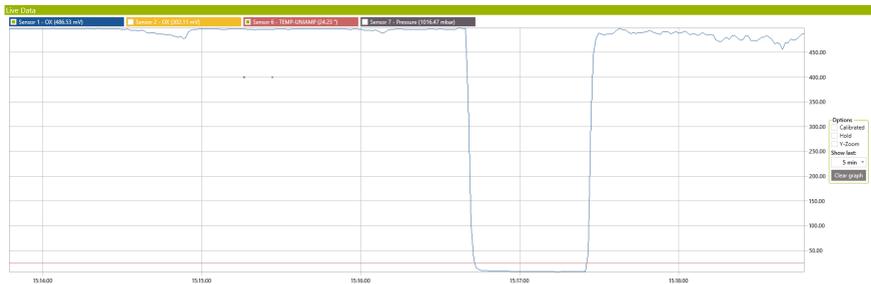
In the right side area Sensors and Motors show the sensor selection and, if available, the motor control. A motor control is available in SensorTrace Logger+, Photo and Profiling.

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 also the Comments tab 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: Selecting **Y-ZOOM** the graph will be zoomed only on the y-axis and not on the x-axis.

Show last: By default the time scale (x-axis) is controlled in the Show Last drop down list, where a number of preset time intervals can be chosen.

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.

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

ZOOM FUNCTION

It is possible to zoom in and out on the graph by using the mouse wheel.

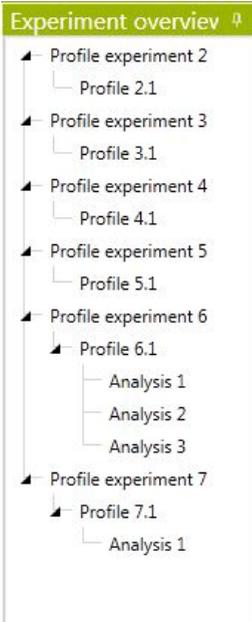
Point the cursor on part of the graph of interest and use the mouse wheel to zoom in and out. A certain time span: drag a rectangle with the mouse

from the upper left corner to the lower right corner.

Fast zoom out: double-click on the graph

EXPERIMENT OVERVIEW

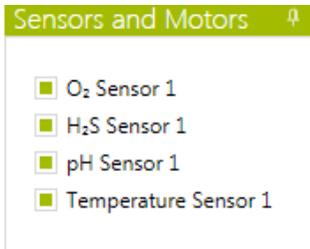
The Experiment overview window shows all the sub experiments and analysis made in an experiment. They can be loaded or deleted and a note can be added. All sub experiments are stored in one experiment file.



SENSORS AND MOTORS

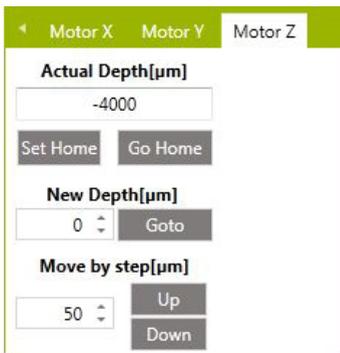
SENSORS

Select the sensors you wish to use here.



MOTORS

The motor control box is found in SensorTrace Logger+ (licensed version), Photo and Profiling 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 **GOTO** button. Example: enter “2000”, click **GOTO** and the motor will move the sensor tip to a position 2000 μm deeper than the Home position.

GoTo: Click this button to move the sensor to the position entered in the New Depth field.

Move by step: [μm]. Enter the step size the motor should move the sensor up or down. Click the **UP** or **DOWN** button to move the sensor one step in that direction.

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.

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. 1000 μm above the sample, type -1000 in the New Depth (μm) and click **GOTO**.

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 tab. 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 green tick mark indicates that the sensor has been calibrated.

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 (only for Logger files)
- Calibration stored on the sensor (only for UniAmp sensors, optical sensors of the Opto-series and temperature sensors)

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

The Sensor signal in the Calibration frame displays the actual raw sensor signal in mV for the chosen sensor. The sensor signal can also be followed continuously in the Live Data graph at the bottom of the screen.

NOTE:

Calculation of specific activities from a micro-profile is only possible if units are $\mu\text{mol/L}$

UNITS:

The concentration unit used for calibration is defined for each sensor in the "Settings" tab before start of experiment

CALIBRATION PROCEDURE FOR CLARK TYPE SENSORS



The settings within the two dashed boxes are only available for UniAmp instruments

Details for the calibration

Sensor calibration & experiment settings frame

Sensor signal:

Shows the current sensor 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" below. 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 above, an O₂ sensor is connected and the calculator for O₂ concentration is shown when clicking the button.

In the calculator, the dissolved gas concentration is calculated. It is assumed that water at the specified temperature and salinity is in equilibrium with a gas at the specified pressure and composition.

Note that for oxygen the value for “Calibration gas concentration (ppm)” corresponds to the standard atmospheric composition and if this should be left unchanged it is assumed that atmospheric air is used for calibration. The current atmospheric pressure can be seen in the “Live Data” window for UniAmp 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 must be entered manually and the calculated concentration is transferred to the “Known value” box when clicking **APPLY**.

Get O₂ value

Oxygen concentration

Temperature (°C) 20.00

Salinity (‰) 0.00

Pressure (mBar) 1,013.25

Cal. Gas Concentration (ppm) 209,460

Concentration 283.895 μmol/L

Apply Cancel

Temp.:

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 is used for:

- The current value will be saved the calibration temperature when **SAVE AND USE CALIBRATION** is clicked. This is the reference temperature for temperature compensation, and it is shown in the "Temperature" column in the list of calibrations just above the "Live data" window.
- During measurement, the current value will be saved with each data point. If the temperature compensation is selected, the difference between the current 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.

Pres.:

The air pressure may be entered manually or be continuously read from the pressure sensor built into the UniAmp instruments. In the dropdown menu to the right, select whether to use the pressure from this or to enter it manually. The current value will be saved with the calibration when **SAVE AND USE CALIBRATION** is clicked and it is shown in the "Pressure" column in the list of calibrations just above the "Live data" window.

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

UNIAMP CHANNEL CONFIGURATION FRAME

UniAmp Channel Configuration

Sensor 1 - OX

Preamp Gain (mV/pA) (2) 1pA=1mV ▾

Polarisation (mV): -800 ▾

Offset (mV): 0 ▾

Solubility temperature compensation

Sensor temperature compensation

Analog out

Analog Out Settings

Calibrated:

Output offset (mV): 0 ▾

Output slope (mV/unit): 1.000 ▾

Temperature:

Use calibration temperature (T-cal)

Use measured temperature (T channel)

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).

Preamp 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.

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.

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.

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.

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 slope boxes. See Appendix 1 for details.

Calibration procedure

1. Prepare the calibration samples (see the specific sensor manual).
2. Choose the sensor you want to calibrate in the green sensor column to the left
3. Enter the concentration of the current calibration standard in the "Known value ($\mu\text{mol/L}$)" box. See "Known value" above for an explanation of how to use the built in gas concentration calculators.

NOTE:

The Calibrated Analog out signal is based on the "User Calibration" on the E2PROM. Click the "Push selected calibration to sensor" for the selected calibration to be used with the Calibrated Analog out signal.

4. 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.
5. 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.
6. 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**.
7. 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 green dot in the In use column.
8. Repeat steps 1-7 for other sensors.

Saving and applying calibrations

All calibrations are automatically saved in the current file. An existing calibration may be activated by clicking the green radio button in the In use column in the calibration table below the graph window.

Import last Calibration		Push selected calibration to sensor													
In use	Cal#	Time	Unit	Cal.Points	Slope	Intercept	R ²	Polarisation	Preamp Gain	A#Number (temp. compensati	Pressure	Temperature	Salinity	Serial Num	Comment
<input checked="" type="radio"/>	1	15-10-2020 15:04:57	µmol/	6	1.826	7.530	1.000	-800	2	1.01760005955928	1016	20	0	10464	(local)
<input type="radio"/>	2	15-10-2020 15:05:00	µmol/	0	1.826	7.531	0.000	-800	2	1.01760005955928	1016	20	0	10464	User calibration (eeprom)

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 one user calibration on the E²PROM on the sensor by clicking the '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 green 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.

Recalibration procedure

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

To recalibrate a sensor:

9. Press **CLEAR ALL POINTS**
10. Follow steps 1-7 in the above calibration procedure.

All calibration data are stored in the experiment file. One calibration may be stored on the E²PROM on the sensor by clicking the Push selected calibration to sensor.

CALIBRATION PROCEDURE FOR OXYGEN MICROOPTODES

The oxygen microoptodes are calibrated using the Calibration tab. The E²prom of a new optode comes with a Factory calibration (Cal. # 1 in the screenshot below) and has room for one user calibration, the user calibration (E²prom) (Cal. # 2 in the screenshot below). In addition, it is possible to store an unlimited number of calibrations in the experiment files. The individual parts of the Calibration tab are explained below.

In use	Cal#	Time	Temperature at air sat. (°C)	Temperature at zero (°C)	Δφ (100%)	Δφ (0%)	Pressure (mbar)	Comment
<input checked="" type="radio"/>	1	10-09-2019 13:12:03	25	25	18	48	1013	Factory calibration (eeprom)
<input type="radio"/>	2	13-11-2019 20:21:39	20000	20000	17574	16424	0	User calibration (eeprom)

Sensor column

In the green column to the left you select the sensor you want to calibrate. The sensors can be renamed before a Logger experiment is started in the Settings tab. A red cross indicates that the sensor has not been calibrated or that a sensor is not connected to a given channel, green tag 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 parameters

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

FLASH RATES

The flash rate of optodes connected to a MicroOptode UniAmp depends on its use. When idle, this is 1 flash/minute. At start of an Experiment file and when connecting an optode this is 1 flash/second for 30 seconds, then idle.

temperature.

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

Calibrate

New calibration: Click this button to record a new calibration. The Calibrate air sat., Calibrate zero and the Humidity field are now active. Clicking this button will change the measuring frequency from the 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 that sensor and to the sensor itself. You can shift between calibrations easily. Just click the button in the In use column for the calibration you wish to apply and sensor and already recorded data will be calibrated accordingly.

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

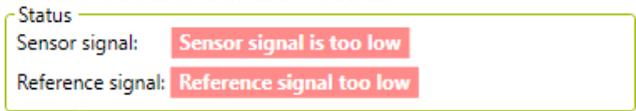
Calibrate air sat.: Calibrate the MicroOptode by exposing the optode and the temperature sensor to air or an air saturated solution as described in the MicroOptode manual. The oxygen concentration will be calculated based on the temperature and salinity specified above. When a stable signal is obtained click **CALIBRATE AIR SAT**. The Opto-F1/F4 UniAmp 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 MicroOptode 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 from the previous experiment.

Push selected calibration to device: Will save the active calibration, marked with a green dot in the "In use" column, to the eeprom. 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: Here you find which calibration is active (indicated by a green 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 its content.

Switch between calibrations: When you have 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

recorded with this sensor

itself.

Import last Calibration				
In use	Cal#	Time	Unit	Cal.Points
<input type="radio"/>	1	20-06-2018 10:44:31	umolL	2
<input checked="" type="radio"/>	2	20-06-2018 10:45:12	umolL	4

IMPORTING CALIBRATIONS FROM FILE

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

To import calibrations in Logger:

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. Only calibrations compatible with a give sensor will be available in the calibration table. 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.

COMMENTS TAB, OUTPUT AND DOWNLOAD FILES

COMMENT TAB

Comments, calibrations and other events generated by the user or the program are recorded in the Comment tab. The tab 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 tab and the other program specific tabs are also listed in the Comment tab. 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.

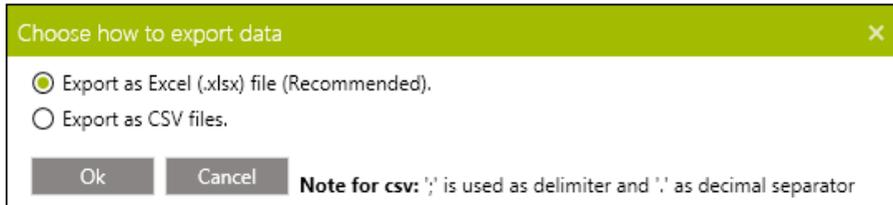
Settings Calibration Motor settings Profiling Visualize Activity **Comment**

Time	Type	Comment
05/07/2014 10:09:31	StartExperiment	
05/07/2014 10:10:13	Calibration	Calibrate O ₂ Sensor 2
05/07/2014 10:11:31	Calibration	Calibrate O ₂ Sensor 2
05/07/2014 10:35:58	Calibration	Calibrate O ₂ Sensor 2
05/07/2014 10:39:28	Calibration	Calibrate O ₂ Sensor 2
05/07/2014 10:41:13	Calibration	Calibrate O ₂ Sensor 2
05/07/2014 11:43:10	Calibration	Calibrated O ₂ Sensor 1
05/07/2014 12:27:28	Profiling	The zero depth has been reset
05/07/2014 12:57:43	Calibration	Water

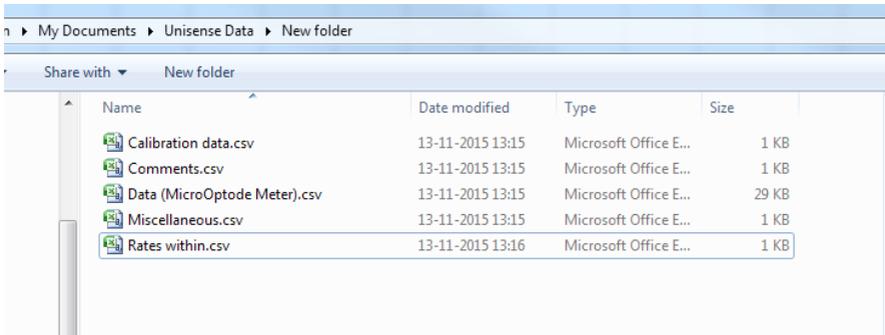
Use current time

OUTPUT FILE

All logged data, calculations and comments entered are stored in the data file. The file can be exported as Excel file (MS Office 2007 or later and LibreOffice) 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 “.



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 SensorTrace Suite can be exported into separated files by selecting **EXPORT FIGURE**.*

DOWNLOAD DATA FROM FIELD DATALOGGER , FIELD DATALOGGER MINI AND FIELD MICRO-SENSOR MULTIMETER

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



SENSORTRACE LOGGER

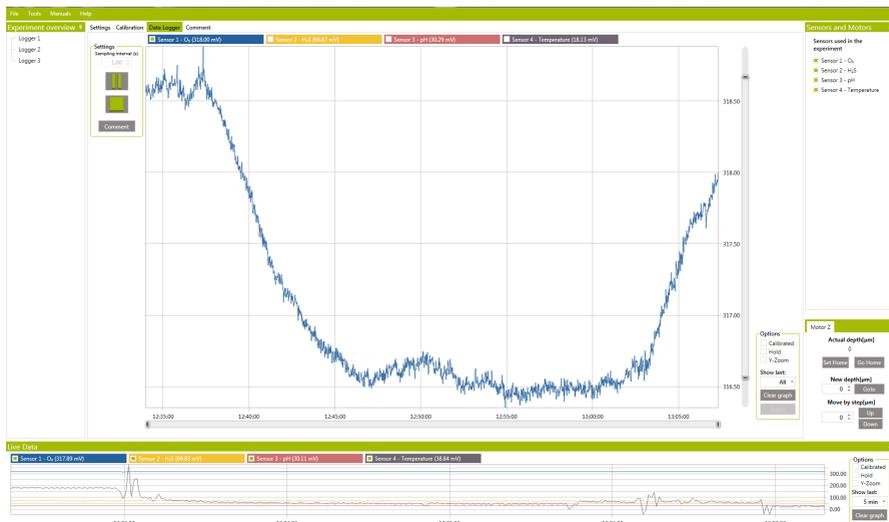
SensorTrace Logger is for basic data application and comes as freeware (without motor control) or as part of the licensed SensorTrace Suite software package (with motor control). It offers time series datalogging, calibration features and motor control. It supports simultaneous logging from multiple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

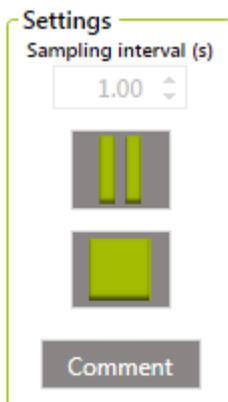
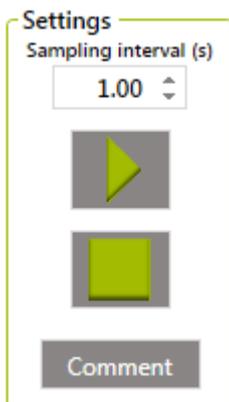


LOGGER TAB

The Logger tab is where data recording is done.

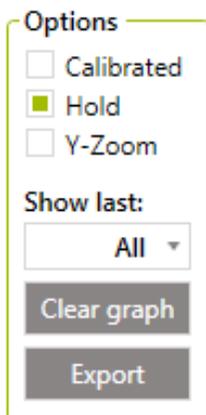


Sampling interval (s): The sampling interval denotes how often the program will make a data point (for the log file and in the graph). A single data point consists of an average of measurements sampled with a high speed background frequency in the sampling interval preceding the data point (up to 1000 ms). In other words, with a sampling interval of 10 s, the data point 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 relative 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 SensorTrace Logger 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.





Options: 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 SensorTrace Logger 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.



SENSORTRACE PROFILING

SensorTrace Profiling is the profiling program from SensorTrace Suite. It offers time series datalogging along a transect, it controls the motor that runs this path, it can visualize these 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 at the beginning of this manual. In the following the SensorTrace Profiling specific features of the software will be described.

SYSTEM FEATURES

SensorTrace Profiling is the software used for the MicroProfiling System or the Field Microprofiling System. An overview of the equipment needed for a profiling measurement set-up can be found in our MicroProfiling System User Manual or the Field MicroProfiling System manual.

Basic features of SensorTrace Profiling 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



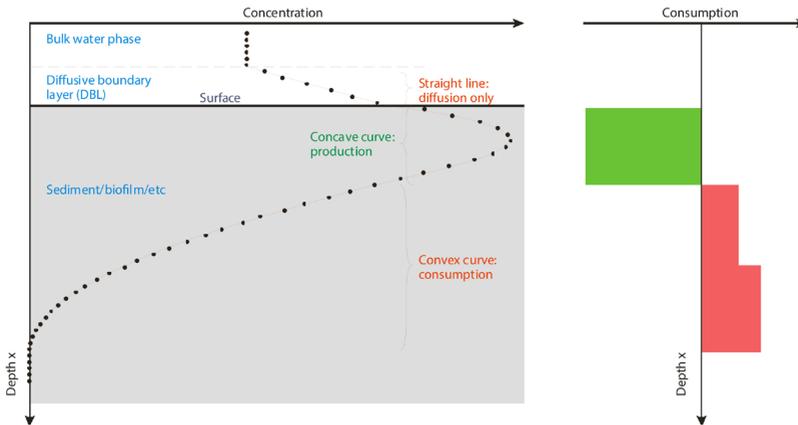
RATE CALCULATIONS FROM CONCENTRATION PROFILES

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 SensorTrace Profiling 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 SensorTrace Profiling is partly based on the method published by Peter Berg and co-workers in 1998, optimized for biogeochemical interpretations of solutes in sediment pore water.

The microprofiles may be analysed in the “Activity” tab of the software. See the “Profiling application note.pdf” 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 tab 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 biobilms, 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 SensorTrace Profiling 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:

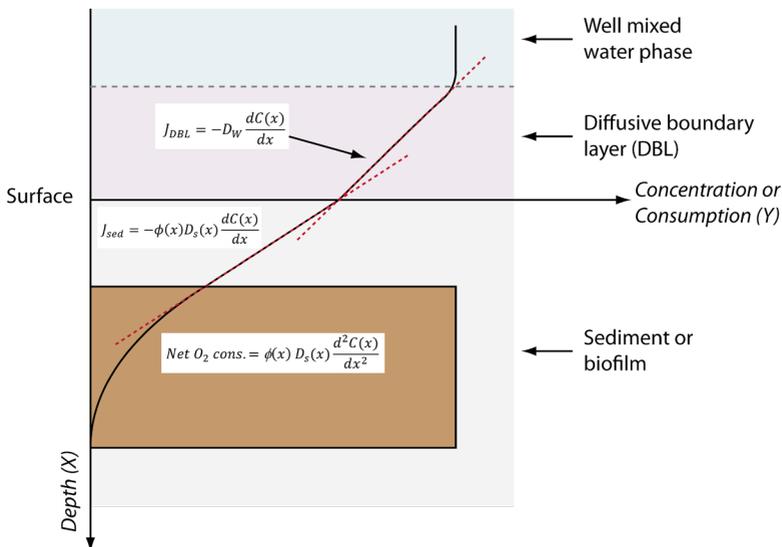
- If the concentration curve is convex there is net consumption (e.g. respiration, oxidation of reduced compounds).
- If the concentration curve is concave there is net production (e.g. photosynthetic production of oxygen, sulfide production by sulfate reduction).
- If the concentration curve is linear, 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:

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

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:

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

where Net O_2 cons. is the net rate (nmol cm⁻³ s⁻¹) of O_2 consumption or production.

SensorTrace Profiling 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.

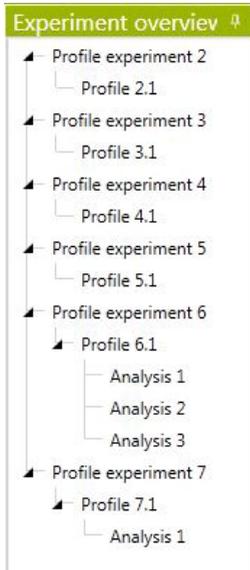


Before starting the analysis model you must provide estimates for the diffusion coefficient and the porosity in all zones, and some boundary conditions (see Activity tab). The model makes an initial guess of the activity distribution and compares the calculated profile with the actual measured profile. 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 analysis 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 and these are stored as Profile 1, Profile 2 and so on.

Analysis

Analysis of a profile made in the Activity tab 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 SETTING

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**. Pin the Sensors and Motors tab and control the sensor position from here (see how under the Sensors and Motors tab).

Z-axis	X-axis	Y-axis
<input type="radio"/> Manual <input checked="" type="radio"/> Motor	<input checked="" type="radio"/> None <input type="radio"/> Manual <input type="radio"/> Motor	<input checked="" type="radio"/> None <input type="radio"/> Manual <input type="radio"/> Motor
	Start(μm) <input type="text" value="0"/>	Start(μm) <input type="text" value="0"/>
	End(μm) <input type="text" value="1,000"/>	End(μm) <input type="text" value="100"/>
	Step size(μm) <input type="text" value="100"/>	Step size(μm) <input type="text" value="100"/>

Z-axis: The z-axis is the vertical movement of the sensor. The movement of this motor is controlled in the Profiling tab and in the motor control 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.

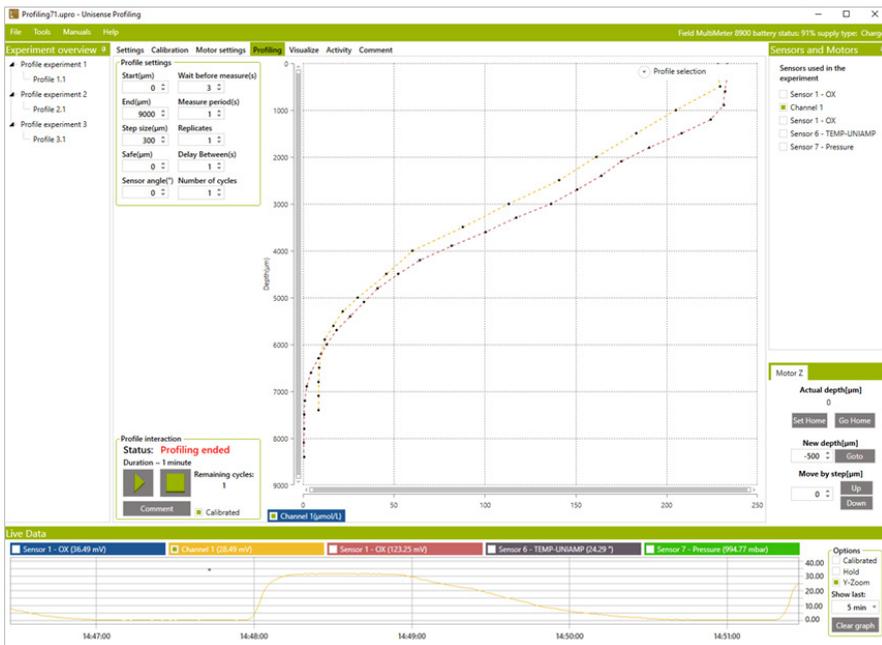


PROFILING TAB

The Profiling tab 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 sheet are the same whether you do your profiles manually or motorized.



PROFILE SETTINGS

Profile settings	
Start(μm)	Wait before measure(s)
-2,000	3.00
End(μm)	Measure period(s)
2,000	1.00
Step size(μm)	Replicates
50	1
Safe(μm)	Delay Between(s)
-4,000	1.00
Sensor angle($^{\circ}$)	Number of cycles
0	1

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.

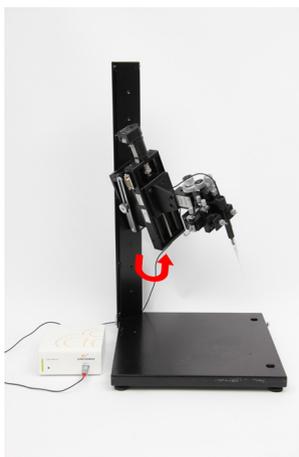
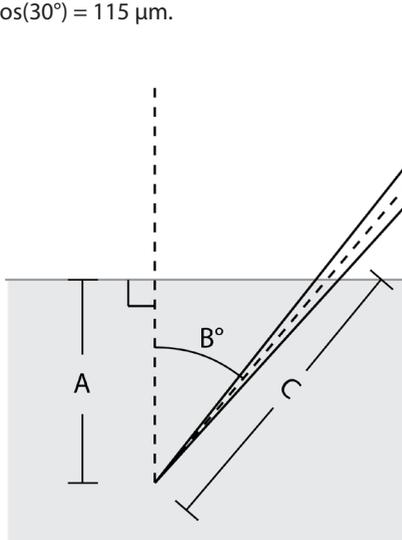
End (μm): Is the depth position relative to the Home depth where the profile is stopped.

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 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 ($^{\circ}$): 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 ($^{\circ}$) box and the software will calculate and move the sensor the distance C. For example, to move the sensor $100\ \mu\text{m}$ 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 $100\ \mu\text{m} / \text{Cos}(30^{\circ}) = 115\ \mu\text{m}$.



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.



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 a 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: 3 s

END: 4000 μm

MEASURE PERIOD: 1 s

STEP SIZE: 50 μm

REPLICATES: 1

SAFE: -4000 μm

DELAY BETWEEN: 1 s

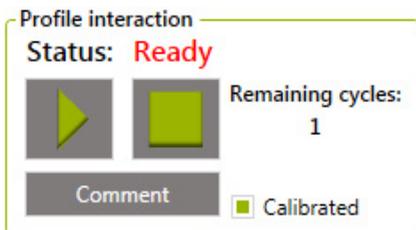
SENSOR ANGLE: 0°

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 SensorTrace Profiling 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 tab

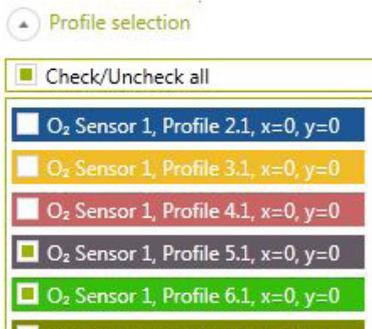
Remaining cycles: Is a status reading on how many cycles the program still needs to run.

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 TAB

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 tab.

FILTERS

The screenshot shows the 'Filters' and 'Plot' sections of the software interface. The 'Filters' section contains five dropdown menus: 'Sensor' (set to 'All Sensors'), 'Cycle' (set to 'All'), 'Z Depth' (set to 'All'), 'X Position' (set to 'All'), and 'Y Position' (set to 'All'). Below this is the 'Plot' section, which includes a 'Profile' button, two checkboxes for 'Calibrated' and 'Standard deviation', and an 'Adjust interface position (µm)' section. The 'Adjust interface position' section has a 'Current' value of 0 and an 'Adjust by' value of 0, with 'Apply' and 'Reset' buttons. At the bottom is an 'Export profiles' button.

NOTE

Update a new filter setting by choosing **PROFILE**. This will plot the new graph.

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

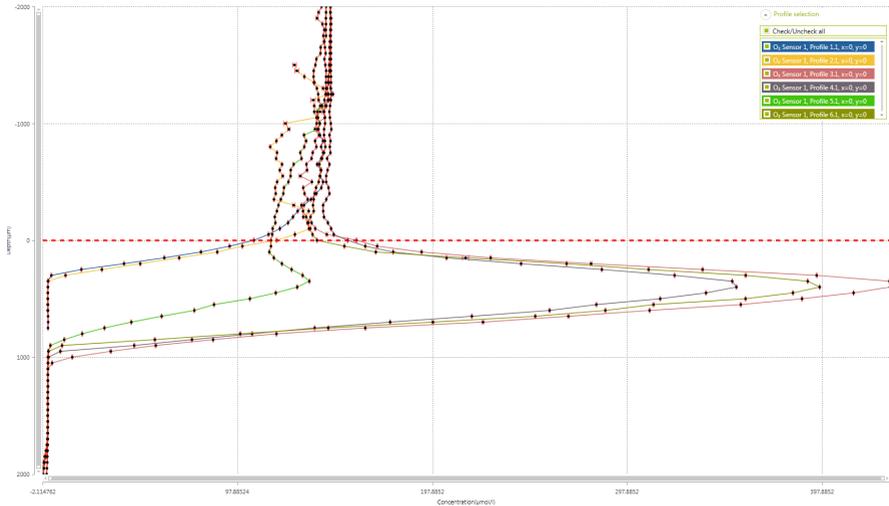
Cycle: It is possible to choose how many cycles you want to see on the graph. In a 1D setting using only z-depths one profile is identical to one cycle. For 2D and 3D cycle the profile of one or more profiles in one cycle or multiple cycles will be plotted in a 1D plot. In a 2D setting one cycle includes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

PLOT



Profile: Here you will see the graphs chosen according to your filter setting.

All profiles including profiles in 2D and 3D dimensions will be plotted in a 1D graph. SensorTrace Profiling 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 original depth position is re-found when selecting **RESET DEPTH**. The zero depth adjustments will be saved into the Activity tab. Analysis can be made in the Activity tab 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

Settings and Analysis

O₂ Sensor 1

Profile 7.1

Select analysis...

Boundary Conditions

Bottom conc + bottom flux

Bottom conc: -11.30

Bottom flux: 0.00

Intervals and zones

Start depth(µm): -1.500

End depth(µm): 1.500

Max zones: 1

Min width (µm): 100

Production

Consumption

Observed

Simulated

DS & Theta

Analyse

Sensor and profile: In the **SENSOR** and **PROFILE** fields 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.

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.



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 $\text{BOTTOM CONC.} + \text{BOTTOM FLUX}$ 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 also the flux is zero, so in this case $\text{TOP CONC.} + \text{TOP FLUX}$ is appropriate. For flux calculations in the DBL $\text{TOP CONC.} + \text{BOTTOM CONC.}$ is selected and the concentration at the top of the DBL and the conc. at the sediment surface are added.

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. The min width (μm) is the smallest zone width that the method allows and 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.



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 surfaces, **END DEPTH** is the depth where the profile ends and **MAX ZONES** between 1 and 10. D_s values are used for these calculations.

D_s AND THETA

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 THETA)

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.

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.

DS and Theta

Settings
Zones with different diffusion parameter:

Calculate
D0 coefficient free water ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$) O2 Table
Sediment diffusion coefficient equation

Start	End	Porosity	DS
-1500	0	1	2.325E-05
0	3000	0.8	1.86E-05

Enter DS manually

CALCULATE (D_s AND THETA)

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 Documentation and Downloads-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 kind of material. See also the Theory section.

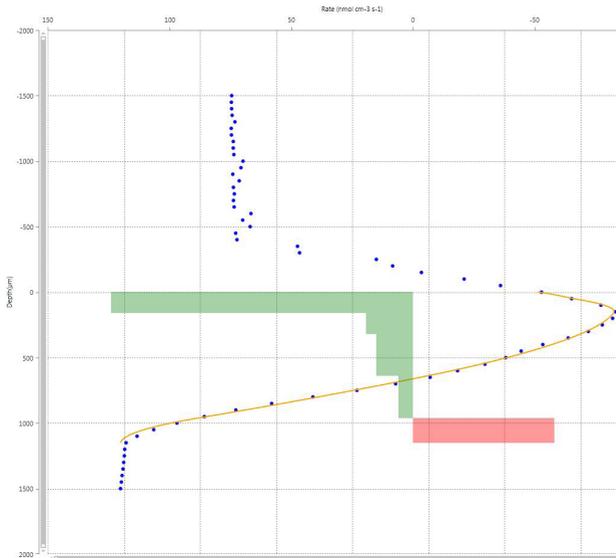
TABLE (D_s AND THETA)

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

Statistics							
Save solution		Export data					
No. of Zones	SSE	P-Value	Top Conc (µmol/L)	Bottom Conc (µmol/L)	Top Flux (nmol cm ⁻² s ⁻¹)	Bottom Flux (nmol cm ⁻² s ⁻¹)	Integrated prod (nmol cm ⁻² s ⁻¹)
1	992.53	0.000	234.85	1.89	0.03	0.00	-0.03
2	44.80	0.000	248.24	1.89	0.04	0.00	-0.04
3	30.65	0.063	247.51	1.89	0.04	0.00	-0.04
4	22.28	0.108	247.29	1.89	0.04	0.00	-0.04
5	14.08	0.064	248.53	1.89	0.04	0.00	-0.04

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 $\text{nmol cm}^{-3} \text{ s}^{-1}$ 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: **SAVE SOLUTION** 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



SENSORTRACE RATE

SensorTrace 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 and online
- 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 SensorTrace Rate specific features of the software will be described. General information about the MicroRespiration System is found the MicroRespiration System user manual.



SETTINGS TAB FOR SENSORTRACE RATE

When SensorTrace Rate is started, the Settings tab for SensorTrace 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 set-up are defined **START EXPERIMENT**.

File Tools Manuals Help

Settings

Start Experiment

Scan Scan instruments and configure them:

Instrument	Use	Channel	Type	Unit	Range	Sensor name
MicroOptode Meter	<input checked="" type="checkbox"/>	1	O ₂	$\mu\text{mol/L}$	± 5	Sensor 1 - O ₂
MicroOptode Meter	<input checked="" type="checkbox"/>	2	O ₂	$\mu\text{mol/L}$	± 5	Sensor 2 - O ₂
MicroOptode Meter	<input checked="" type="checkbox"/>	3	Temperature	$^{\circ}\text{C}$	± 5	Sensor 3 - Temperature

Total chambers used in the experiment:

Number	Volume	Description	Double chambers
1	4000		<input type="checkbox"/>
2	4000		<input type="checkbox"/>
3	4000		<input type="checkbox"/>
4	<input type="text" value="4000"/>		<input type="checkbox"/>



CHAMBER TAB

The Chamber tab shows the microsensors 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, first select the correct sensor in the right sensor panel.

Sensors

Sensors used in the experiment

Sensor 1 - O₂

Sensor 2 - H₂S

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.



Settings

Sampling interval(s):

Calculations

Start:

End:

Manual calculation

Use current measurement

Slope: $\mu\text{mol/L/h}$

R²:

StDev:

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 1000 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 1000 ms just before the next sampling point. The background frequency is dependent on the amplifier type but 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.

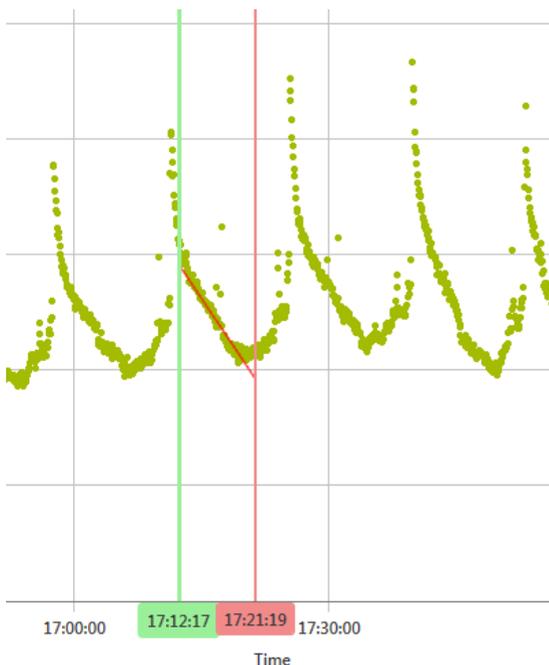
Start and stop measuring: Here you start and stop the data logging from a sensor placed in the selected chamber. The Regression line is automatically shown for the latest logged data points. Several chambers can be in use simultaneously with their



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 derivation (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 micorsensor 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 SensorTrace Logger section and Live Data Graph.



RATE WITHIN TAB

Rate Within tab 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.



Option: 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 SensorTrace Rate has been used to convert the rate from $\mu\text{M/h}$ 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.

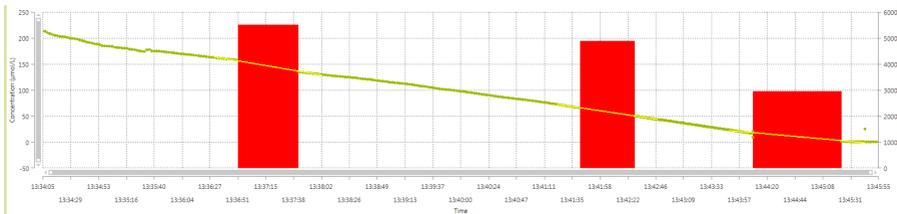
Export table: All values calculated in Rate Within are logged in the experiment file and can be exported by selecting [EXPORT TABLE](#).

	A	B	C	D	E	F	G	H
1	Chamber	Period	Rate (nmol/h)	Slope ($\mu\text{M}/\text{s}$)	95% C.L (nmol/h)	Start (hh:mm:ss)	End (hh:mm:ss)	Avg.Conc (nmol/L)
2	1	M 1	-4405,5225	-0,3059	0,1588	2015-09-29 13:34:06	2015-09-29 13:36:52	180,0749
3	1	M 2	-4180,067	-0,2903	0,1314	2015-09-29 13:37:43	2015-09-29 13:41:42	102,2875
4	1	M 3	-4793,5739	-0,3329	0,2037	2015-09-29 13:42:29	2015-09-29 13:44:09	33,6108
5	1	M 4	-472,3353	-0,0328	0,3798	2015-09-29 13:45:24	2015-09-29 13:45:55	0,5511



RATE BETWEEN TAB

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).



Sensor 1 - O₂ ▼

Samples before and after measurement:

Export table

Chamber	Parameter	Unit	M1-M2	M2-M3	M3-M4
1	Rate	nmol/h	-5508.2441	-4886.1851	-2945.9428
1	Slope	µM/s	-0.3825	-0.3393	-0.2046
1	95% C.L	nmol/h	0.3284	0.3284	0.3284
1	Start	hh:mm:ss	13:36:31	13:41:22	13:43:48
1	End	hh:mm:ss	13:38:02	13:42:48	13:45:43
1	Mid-Time Conc.	nmol/L	146.382	58.0727	10.4559

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.

Option and Table: For further information on Options and Table see Rate Within section

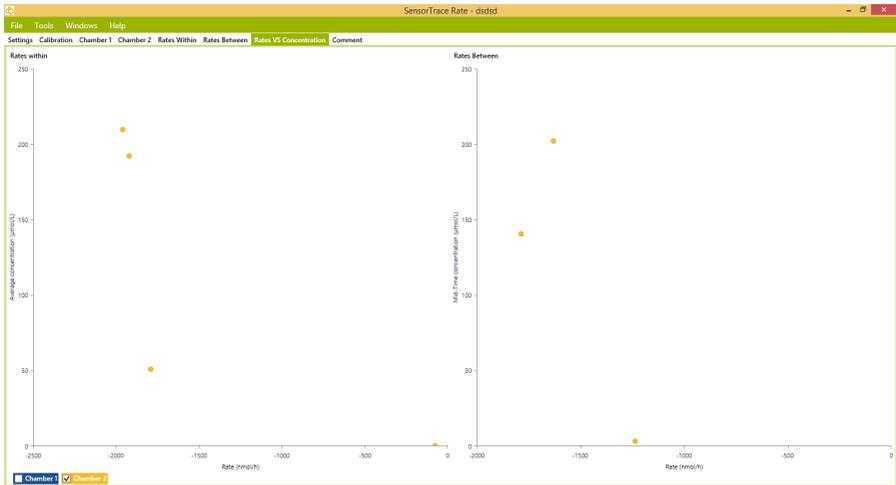
Export table: All values calculated in Rates Between are logged in the experiment file and can be exported by selecting **EXPORT TABLE**.

	A	B	C	D	E	F	G	H
1	Chamber	Period	Rate (nmol/h)	Slope ($\mu\text{M/s}$)	95% C.L. (nmol/h)	Start (hh:mm:ss)	End (hh:mm:ss)	Mid-Time Conc. (nmol/L)
2	1	M1-M2	-5508,2441	-0,3825	0,3284	2015-09-29 13:36:31	2015-09-29 13:38:03	146,382
3	1	M2-M3	-4886,1851	-0,3393	0,3284	2015-09-29 13:41:22	2015-09-29 13:42:48	58,0727
4	1	M3-M4	-2945,9428	-0,2046	0,3284	2015-09-29 13:43:49	2015-09-29 13:45:44	10,4559



RATE VS. CONCENTRATION TAB

The Rate 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.





SENSORTRACE PHOTO

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



MEASURING SET-UP FOR SENSORTRACE PHOTO

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

- Fast responding O₂ micro-sensor
- Light sensor including amplifier box
- Micromanipulator
- Microsensor amplifier
- Photosynthetic active sample (i.e. algae mat, coral, microbial mat)
- SensorTrace Photo software with valid license key

The microsensor is connected via the LEMO connector 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:

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

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:

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

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:

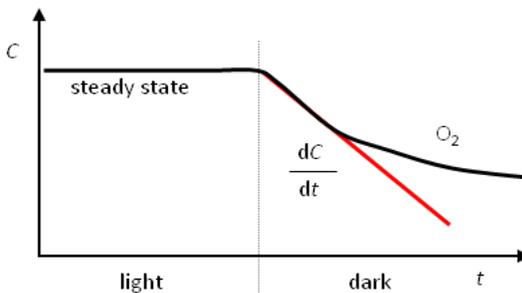


$$\frac{\partial C}{\partial t} = -T - R$$

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.



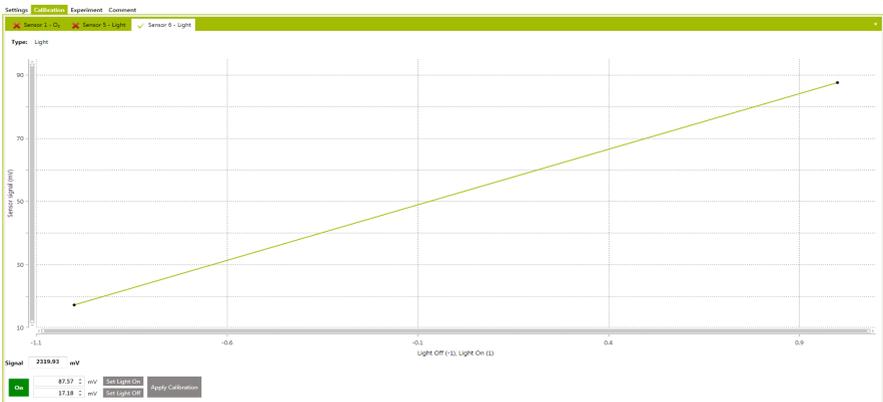
The computer samples the voltage readings of the microsensor at a frequency of 10- 50 Hz. The typical sampling interval starts 1-3 seconds before the switching event and stops 2-4 seconds after it. The time interval before the switching event is used to calculate an average value of the steady state 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 SensorTrace 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 SensorTrace Photo for calibration of an oxygen microsensor can be found under Calibration tab in the General section. See how to calibrate an oxygen microsensor in the sensor specific manual.

Below we will describe the 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



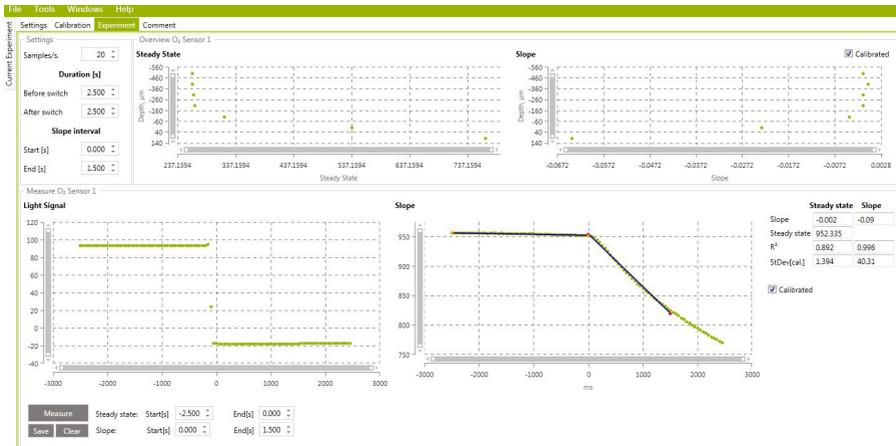
values for light on and somewhat higher when light off. It is further possible to adjust these readings, i.e. the light on value downwards and the light off value upwards, to further ensure that the light transition is captured during experiments.

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 TAB

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.

Measuring: 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.

The screenshot shows a 'Settings' window with the following parameters and values:

- Samples/s.**: 20
- Duration [s]**
 - Before switch: 2,000
 - After switch: 3,500
- Slope interval**
 - Start [s]: 0,500
 - End [s]: 1,500

NOTE:

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

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.

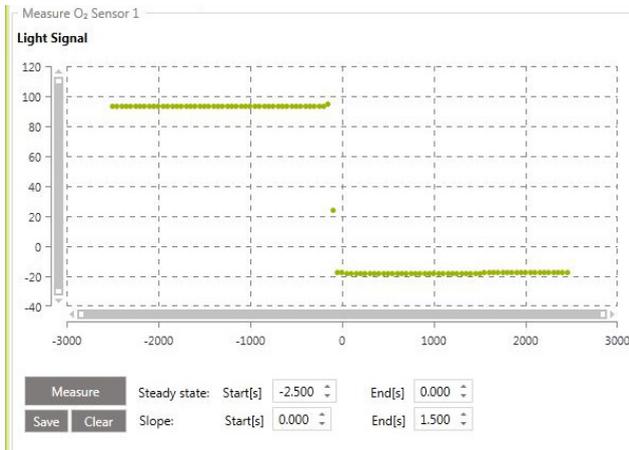


PHOTOSYNTHESIS RATE MEASUREMENTS

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.

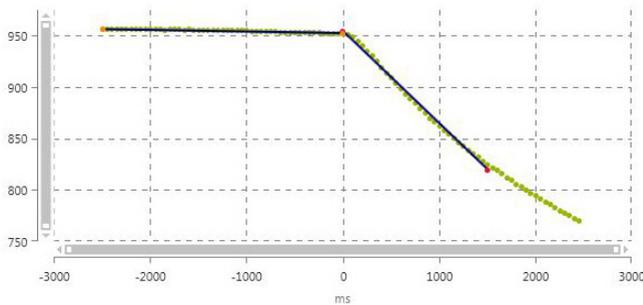
Now select **MEASURE**. On the screen the message Measuring will be shown, indicating that the measurements have started. After a short time period (as set in **BEFORE SWITCH**) this message will change to Turn of the light, signaling that it is time to darken the sample. It is important to wait with 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.





Slope



	Steady state	Slope
Slope	-0.002	-0.09
Steady state	952.335	
R ²	0.892	0.996
StDev[cal.]	1.394	40.31

Calibrated

Options

Steady state:		Slope:	
Start[s]	-2.000	Start[s]	0.500
End[s]	0.000	End[s]	1.500

Calculations

	Steady state	Slope
Slope	-1.437	-3.706
Steady state	-560.105	
R ²	0.221	0.383
StDev[cal.]	1809.335	1813.225

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 R² of the line fittings is a value between 0 and 1, and the closer the sloped line is to 1, the better the fit. When a measurement has been completed the data is being



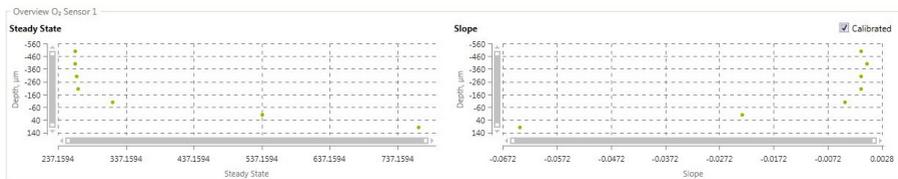
saved to the file by clicking **SAVE** in the Measurement window. Alternatively, if the signal data from the current measurement for some reason should not be saved, e.g. if SensorTrace Photo did not detect the trigger signal or if the measured data was of poor quality, the **CLEAR** button will erase 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 μm) 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 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:

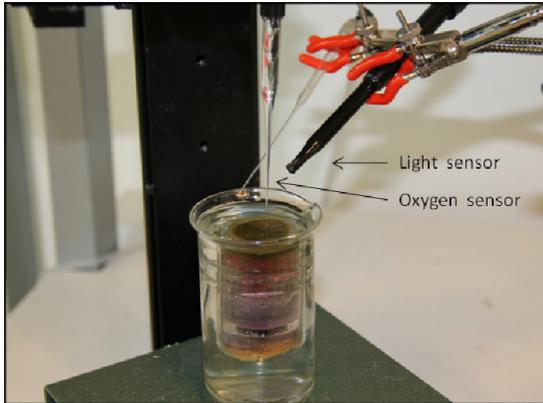
1. Slope calculations
2. Data, including raw and calibrated values from the oxygen and light sensor
3. Devices, including amplifiers that have been used during the experiments
4. Calibration, including the calibrated values
5. Comments, including all comments and actions
6. Miscellaneous

For more information see the Output file section.

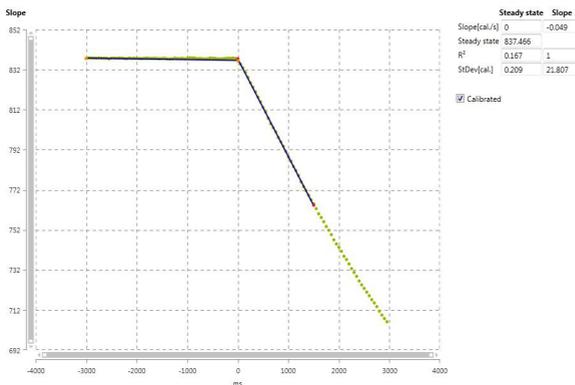


EXPERIMENTAL DATA EXAMPLE

This section gives an example of how to obtain photosynthesis rate measurements using the light-dark-shift method. The measurements were performed in a highly diverse microbial sediment community with high photosynthetic activity.



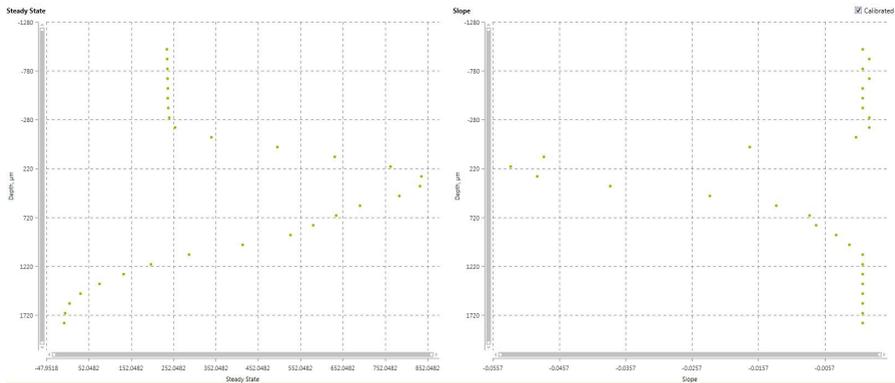
In the exemplified study the photosynthetic rates were measured over a vertical micro-gradient from 1000 μm above the sediment surface to a depth of 2000 μm . Measurements were performed at a spatial resolution of 200 μm . A fast responding oxygen sensor with a tip diameter of 50 μm and a response time <0.5 seconds was used.



Example of rate measurement performed at 300 μm sample depth within the zone with very high photosynthetic activity.



In the sample, the photosynthetic active zone spanned a layer of about 1 mm from the sample surface. The highest rate of photosynthesis was measured at 200-400 μm depth, and the highest rates of activity coincided with the depth region where the highest oxygen levels were found.



Summary of the results of the performed light – dark shift measurements in the photosynthetic mat environment.

REFERENCES

REFERENCES FOR SENSOR TRACE PROFILING

- Berg, P., Risgaard-Petersen, N., and Rysgaard, S. 1998. Interpretation of measured concentration profiles in sediment pore water. *Limnology and Oceanography*. 43(7):1500-1510.
- Glud, N. R. 2008. Oxygen dynamics of marine sediments. *Marine Biology Research* 4: 243-289.
- Iversen, N. and Jorgensen, B.B. 1993. Diffusion Coefficients of Sulfate and Methane in Marine Sediments: Influence of Porosity. *Geochimica et Cosmochimica Acta* 57(3): 571-578
- Ullman, W.J and Aller, R.C. 1982. Diffusion Coefficients in Nearshore Marine Sediments. *Limnology and Oceanography* 27(3): 552-556.
- Revsbech, N.P., Nielsen, L.P., and Ramsing, N.B. 1998. A Novel Microsensor for Determination of Apparent Diffusivity in Sediments. *Limnology and Oceanography* 43(5): 986-992.

REFERENCES FOR SENSOR TRACE RATE

- Alonzo, F., Gilbin, R., Bourrachot, S., Floriani, M., Morello, M., Garnier-Laplace, J. 2006. *Aquatic Toxicology* 80:228–236
- Alonzo, F., Gilbin, R., Zeman, F.A., Garnier-Laplace, J. 2008. *Aquatic Toxicology* 87:146–156
- Bang, A., Frederiksen, T.M., Bruhn, A., Meilvang, A.S, and Grønkjær, P. (2004). A. Bang Ph.D. Thesis
- Brodersen, K.P., Pedersen, O., Walker, I.R. and Jensen, M.T. 2007. *Freshwater Biology* doi:10.1111/j.1365-2427.2007.01922.x
- Jensen, T.C., Anderson, T.R., Daufresne, M, Hessen, D.O. 2006. *Freshwater Biology* 24-Oct-2006.
- Marshallon, D. and Pinckney, J.L. 2007. *Journal of Plancton Research* 29(12):1031- 1040.
- Martens-Habbena, W., Berube, P.M., Urakawa, H., Torre, J.R. d. I. Stahl, D.A. 2009. *Nature* 461(7266):976-979. Abstract
- Sommer, S., Linke, P., Pfannkuche, O., Niemann, H., Trude, T. 2010. *Marine Geology* 272:223-232 Abstract
- Warming T.P., Mulderij G.M., Christoffersen K.S. 2008. *Environ Toxicol Chem.* 22:1
- Zeman, F.A., Gilbin, R., Alonzo, F., Lecomte- Pradines, C., Garnier-Laplace, J., Aliaume, C. 2008. *Aquatic Toxicology* 86:370–378

TROUBLESHOOTING

NOTE! Always ensure that you are using the latest version of SensorTrace Suite. 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 set-up and disturbances in the measurements.

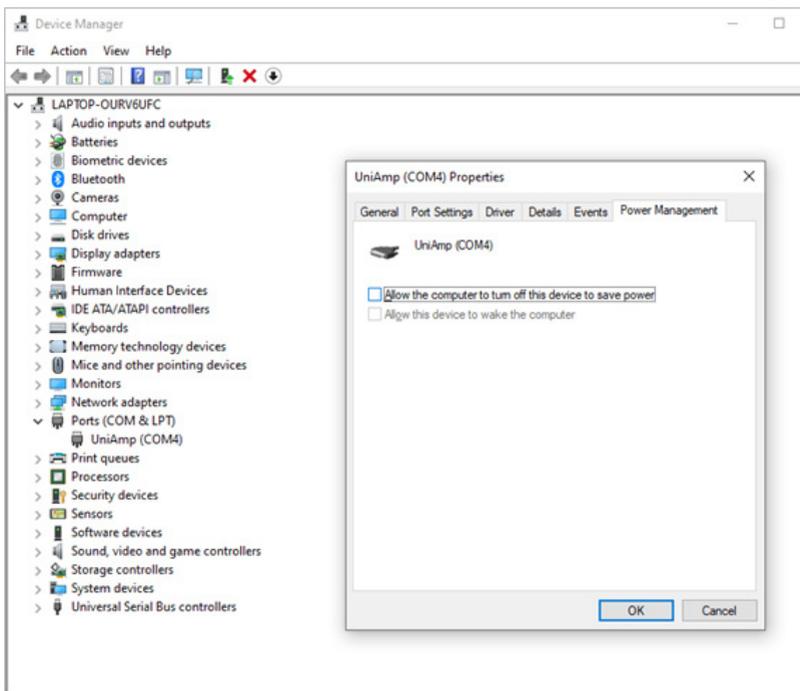
Solution Remove all unrelated appliances from surfaces in contact with the experimental set-up and ensure completely stable conditions for the sample.

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

ANALOG OUTPUT FOR LABORATORY UniAMP INSTRUMENTS

Analog 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.

UNCALIBRATED ANALOG OUTPUT

The uncalibrated analog output is only available for electrochemical sensors and not for optodes. The software calculates the uncalibrated analog output as:

$$\text{Analog output (mV)} = \text{Sensor signal (mV)} \times \text{Slope} + \text{Offset}$$

For uncalibrated analog out Slope and Offset are constant and cannot be changed:

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

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

Uncalibrated analog out is, therefore, always calculated as:

CALIBRATED ANALOG OUTPUT

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

The software calculates the analog output as:

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

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

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

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

Offset = 100 mV

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

Analog 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, analog out is only available as Calibrated analog out.

SETTING THE ANALOG OUT

The Analog Out signal is configured in the UniAmp Channel

Configuration window in the calibration window for each channel:

UniAmp Channel Configuration

Sensor 1 - OX

Preamp Gain (mV/pA) (2) IpA=1mV

Polarisation (mV): -800

Offset (mV): 0

Solubility temperature compensation

Sensor temperature compensation

Analog out

Analog Out Settings

Calibrated:

Output offset (mV): 0

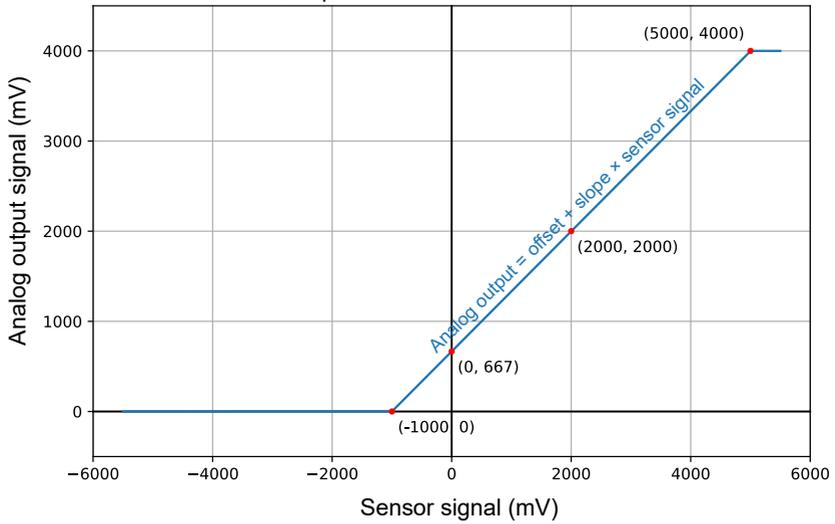
Output slope (mV/unit): 1.000

Temperature:

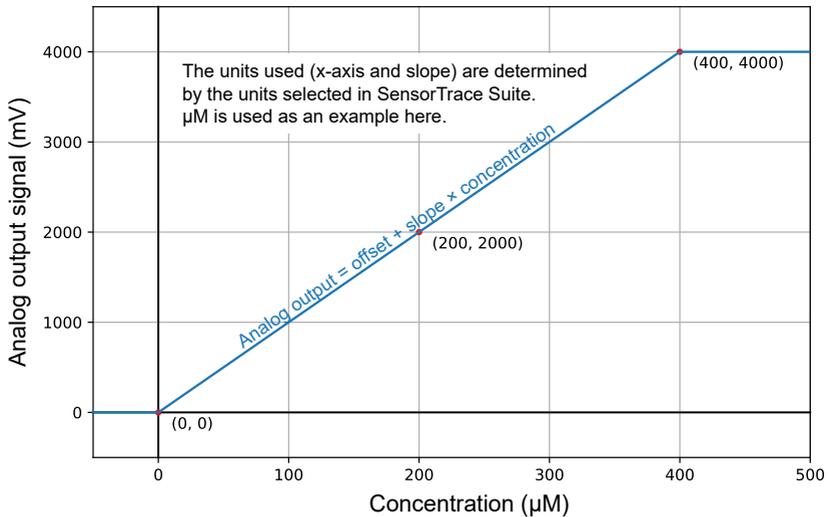
Use calibration temperature (T-cal)

Use measured temperature (1 channel)

Uncalibrated analog output:
 Offset = 667.7 mV, slope = 0.667 mV/mV



Example for Calibrated analog output:
 Offset = 0 mV, slope = 10 mV/ μM





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