UNISENSE

OXYGEN MICROOPTODE - OPTO SERIES
USER MANUAL

Oxygen MicroOptode - Opto Series user manual

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OXYGEN MICROOPTODE AND MINIOPTODE - OPTO SERIES USER MANUAL

UNISENSE A/S

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WARRANTY AND LIABILITY

NOTICE TO PURCHASER

This product is for research use only. Not for use in human diagnostic or therapeutic procedures.

WARNING

Microsensors have very pointed tips and must be handled with care to avoid personal injury and only by trained personnel. Unisense A/S recommends users to attend instruction courses to ensure proper use of the products.

WARRANTY AND LIABILITY

The oxygen MicroOptode Sensor Opto series is covered by a 1 year limited warranty or a maximum of datapoints, whichever comes first. The warranty covers a maximum of 1.0 million datapoints for Opto-50 and Opto-430 Fast, 1.5 million datapoints for Opto-430 and 3.0 million datapoints for Opto-3000. Wear-off of dye resulting in reduced signal quality, e.g. from profiling into biological matrices like sediments,and bend and breakage of the fiber optic cable is not covered by the warranty.

MicroOptodes are consumables. Unisense will only replace dysfunctional sensors if they have been tested in accordance with the instructions in the manual within 14 days of receipt of the sensor(s). The warranty does not include repair or replacement necessitated by accident, neglect, misuse, unauthorized repair, or modification of the product. In no event will Unisense A/S be liable for any direct, indirect, consequential or incidental damages, including lost profits, or for any claim by any third party, arising out of the use, the results of use, or the inability to use this product.

Unisense mechanical and electronic laboratory instruments must only be used under normal laboratory conditions and a dry and clean environment. Unisense assumes no liability for damages on laboratory instruments due to unintended field use or exposure to dust, humidity or corrosive environments.

REPAIR OR ADJUSTMENT

Sensors and electrodes cannot be repaired. Equipment that is not covered by the warranty will, if possible, be repaired by Unisense A/S with appropriate charges paid by the customer. In case of return of equipment please contact us for return authorization.

For further information please see the document General Terms of Sale and Delivery of Unisense A/S as well as the manuals for the respective products.

CONGRATULATIONS WITH YOUR NEW PRODUCT!

Support, ordering, and contact information

The Unisense MicroOptode is an oxygen measuring system based on the latest development within optical fiber technology. Use the MicroOptode for fast and accurate oxygen measurement in a broad variety of research applications.

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

E-mail: sales@unisense.com

Unisense A/S
Tueager 1
DK-8200 Aarhus N, Denmark
Tel: +45 8944 9500
Fax: +45 8944 9549

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

REPLACEMENT OF MICROOPTODES

Unisense will replace MicroOptodes that have been damaged during shipment provided that:

- *The MicroOptodes were tested immediately upon receipt in accordance with the delivery note and the manual.*
- *The white seal is still intact*
- *The MicroOptodes are returned to Unisense for inspection within two weeks (contact the sales team before returning)*
- *The MicroOptodes are correctly packed for return to Unisense, in accordance with the note included in the MicroOptode shipping box.*

RECOMMENDED METERS

Opto-F1 UniAmp
Opto-F4 UniAmp
fx-6 UniAmp
fx-3 UniAmp

OVERVIEW

WARNING

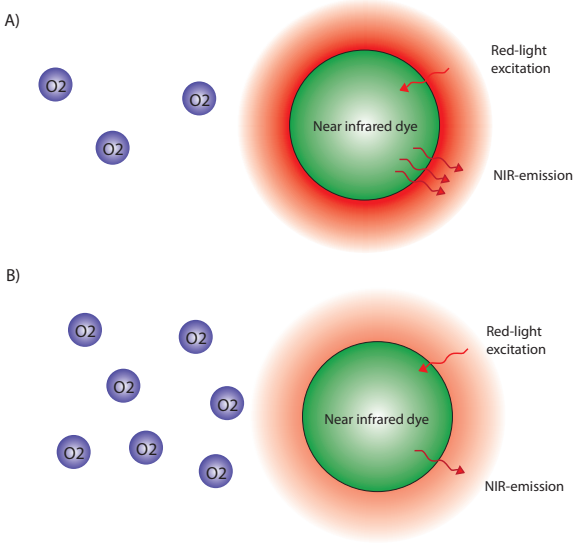
Unisense MicroOptodes are neither intended nor approved for use on humans

This manual covers all the Unisense MicroOptodes and MiniOptodes in the Opto series. The MicroOptode relies on the latest developments within optical fiber technology utilizing near infrared dyes for improved performance, high precision, high reliability, low cross-interference and fast response times.

The Unisense Optodes are designed for research applications within physiology, biotechnology, environmental sciences and related areas.

Measuring principle

The very tip of the optical fiber is coated with a specialized dye that is excitable with red light (610-630 nm). The Opto-F1 and Opto-F4 UniAmp exites this dye with modulated red excitation light. The dye shows an oxygen-dependent fluorescence in the near infrared (NIR, 760-790 nm) and the oxygen concentration is determined from the quenching of the fluorescence signal.



Measuring principle: The near infrared dye shows oxygen dependent fluorescence in the near infrared (NIR) after exposure to excitation light. A) High NIR emission at low oxygen concentration and B) low NIR at high oxygen concentration.

GETTING STARTED

WARNING

FOR THE OPTO-50 AND OPTO-430 VERSIONS:

Do not remove the seal and protective plastic tube before these steps and calibration are successfully completed.

Unpacking a new MicroOptode (Opto-50, Opto-430)

The MicroOptode sensor is shipped in a protective tube with the optic fiber positioned half exposed in the needle. Do not remove the white seal before the calibration procedure has been successfully completed (see Warranty and liability above).

Unpacking a new MiniOptode (Opto-3000)

The MiniOptode is ready for use out of the box.

Connecting the Optodes

Remove the cap from the Optode connector and the red cap from the Optode Meter. Insert the male fiber plug of the Optode cable into the ST-receptable of the Optode Meter and turn the bayonet coupling gently clockwise until the plug is locked firmly. Insert the E^2PROM connector on the sensor into the E^2PROM connector on the Optode Meter.

The MicroOptode cable with optical and E^2PROM Connectors

The MicroOptode and temperature sensor connected to the Opto-F4 UniAmp

Calibration principles and temperature compensation

Please consult the software manual for instructions on how to calibrate the MicroOptode or MiniOptode using the software. Calibration of the Unisense oxygen Optodes is performed as a two-point-calibration by measuring the Optode response in an air saturated solution and an anoxic solution. Remember that a temperature sensor is required for correct oxygen measurements unless the measurement and calibration are performed at identical temperatures.

Automatic temperature compensation

For automatic temperature compensation the appropriate temperature channel must be selected in the "Sensor calibration & experiment settings" box in the Calibration Tab in SensorTrace Suite. If you don't have a temperature sensor connected to the Optode Meter you should record the temperature with another thermometer and enter this manually in the calibration tab in SensorTrace Suite.

IMPORTANT

If possible use a calibration solution with the same temperature and salinity as the sample solution. Use temperature sensor to correct the oxygen signal if measuring at temperatures different from calibration temperature

Calibration

It is recommended to use the Unisense O_2 Sensor Calibration Kit for calibrating the O_2 optodes (https://www.unisense.com/calibration_kits/). This kit ensures accurate and simple calibration both in the lab and in the field. The calibration kit can be shipped as normal cargo and does not require dangerous goods shipping. Therefore, it is ideal also for shipping to field work, research cruises etc. The detailed calibration procedure can be found in the Calkit-O2 Manual (https://www.unisense.com/manuals/)

Alternative calibration

Air saturated solution

Place the Optode in a well-aerated calibration solution (e .g . by bubbling with air in the Unisense calibration chamber). The temperature sensor should also be placed in the calibration solution. Please note that bubbling may lower the temperature due to evaporation induced by the bubbling.

IMPORTANT

The O_2 sensor signal is sensitive to temperature, and the O_2 solubility depends on both salinity and temperature.

The response of the optodes to oxygen is well described mathematically and a two-point calibration is sufficient

After 5 min. of vigorous bubbling the water in the calibration chamber is close to atmospheric saturation. Then follow the Optode signal and when the signal is constant, turn off the bubbling to minimize noise and add the point to the calibration in the software. This signal is your calibration value for 100% air saturation corresponding to atmospheric oxygen partial pressure.

Zero reading

An anoxic solution can be prepared in one of several ways; below we describe two methods recommended by Unisense:

1. Chemical removal of oxygen. Prepare a solution of sodium ascorbate and NaOH, both to final concentrations of 0.1 M (2 g sodium ascorbate and 0.4 g NaOH 100 ml of water). This zero-calibration solution can be stored in a closed container for 1-2 weeks.

   For MicroOptodes
   Keep the MicroOptode in its protective tube and submerge only the fiber tip in the ascorbate solution.

   For MiniOptodes
   Dip the MiniOptode 1-2 cm into the ascorbate solution.

   With the MicroOptode in the anoxic solution, allow the signal to stabilize and add the point to the software calibration. Clean the Optode thoroughly in either tap water or demineralized water to avoid any carry-over of ascorbate solution to your sample.

   NOTE for MicroOptodes: To remove any

ascorbate solution that has entered the space between needle and fiber, dip the needle into clean water and move the fiber in and out several times, until the ascorbate has been washed away.

2. Vigorous bubbling with oxygen-free gas (e.g. N2). Apply vigorous bubbling with N_2, He or Ar gas until all oxygen is removed (typically > 5 min). If you place the sensor in the calibration solution while bubbling you can follow the decrease in signal as oxygen is removed. You can assume that all oxygen has been removed when the signal reaches a low level that is constant over time. However, it is important to prevent any contact of oxygen with the water during bubbling, as oxygen will otherwise be continuously reintroduced to the water. In practice this means that contact between the atmosphere and the headspace above the water must be minimized, i.e. by using a calibration vessel with a narrow opening that is almost blocked when the sensor, mounted in the protection tube, is inserted. This effectively prevents ambient air from entering the vessel. We recommend the Unisense calibration chamber CAL300. Closing the holes in the top that are not in use, with a stopper or a piece of tape, will minimize re-oxygenation of the water. Place the MicroOptode in the anoxic solution, read the signal and add the point to the software calibration.

Approval of new sensor

If the sensor cfunctions according to the criteria given in the delivery note, the seal may now be removed, and the sensor can be used (see Warranty and Liability above).

MEASUREMENTS

Handling of MicroOptodes

NOTE:
Avoid bending of the cable as this might break the MicroOptode.

The MicroOptode fiber can be either retracted into the needle or exposed; the flexible design of the MicroOptodes enables optimal positioning of the optic fiber tip in various samples.

MicroOptode with its components

Adjusting the exposure of the optic fiber

Positioning of the optical fiber is adjusted by loosening the locking nut on the MicroOptode and pushing the optical fiber cable towards the plastic shaft. The optical fiber can be pushed maximally 1 cm out from the needle protection. Tighten the locking nut after positioning the optical fiber.

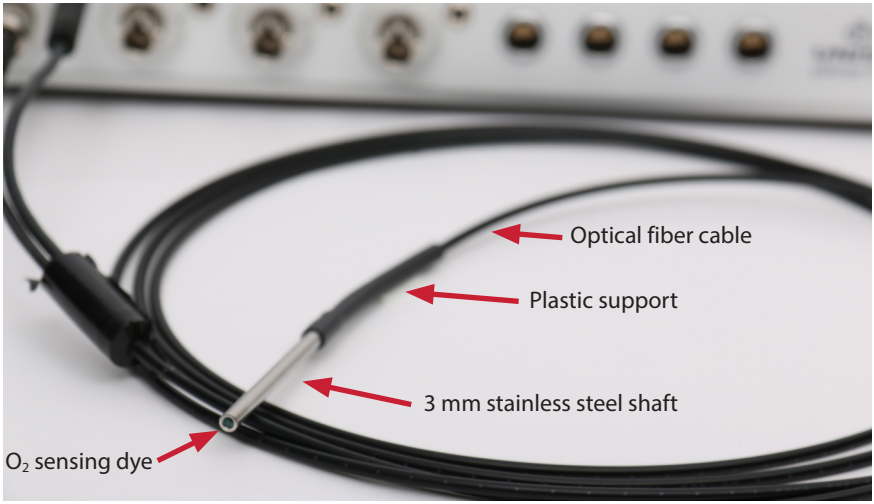
Mounting of the MicroOptode

When the optical fiber is exposed Unisense recommends mounting the MicroOptode in a stabilized setup using the Unisense lab stand (LS) and micromanipulator (MM33, MM33-2) to avoid breaking the MicroOptode.

When the optical fiber is retracted into the needle in protected position, the MicroOptode can be pushed through a relatively hard surface such as a rubber septum. The optical fiber can then subsequently be adjusted to the exposed position, when the needle tip is positioned in the environment.
Note that the space between the fiber and the shaft and needle is open and gases may pass freely along the fiber.

Avoid bending the fiber cable sharply as this may break the optical fiber inside the cable.

Handling of MiniOptodes



MiniOptode with its components

The MiniOptode will measure the O_2 that reaches the O_2 sensing dye in the tip. Avoid bending the fiber optical cable as this may break the optical fiber inside the cable.

Interference

The oxygen sensitive dye on the optic fiber shows excellent brightness and their red light excitation significantly reduces stress in biological systems and interferences caused by autofluorescence. The MicroOptodes can be applied in gas phases, aqueous solutions, ethanol, methanol and isopropanol. Other organic solvents and gaseous chlorine (Cl_2) induce interferences with the sensor reading. No cross-sensitivity is found for pH 1-14, CO_2, CH_4, H_2S and any ionic species.
Extremely bright and/or extremely flickering ambient light may interfere with the signal for oxygen.

Note: Strong organic solvents such as acetone or toluene will destroy the sensor.

STORAGE AND MAINTENANCE

Storage

Store the sensor in the protective plastic tube used for shipping, and store it in a dry, dark and secure place

Cleaning the sensor

All oxygen MicroOptodes can be cleaned with hydrogen peroxide (3% H_2O_2), soap solution or ethanol and can furthermore be sterilized with ethylene oxide.
Note: Strong organic solvents such as acetone or toluene will destroy the sensor.

TROUBLE SHOOTING

Problem	Drifting signal
Possible cause 1	Can indicate photobleaching of the dye
Solution	Recalibration of the MicroOptode
Problem	High signal
Possible cause	Can occur from too much ambient light exposed to the MicroOptode
Solution	Darken the surroundings
Problem	Low signal
Possible cause	Broken tip
Solution	Replace the MicroOptode

If you encounter other problems and need scientific/technical assistance, please contact sales@unisense.com for online support (we will answer you within one workday)

APPENDIX: EQUILIBRIUM O_2 CONCENTRATIONS

Detailed tables are available at our web page http://www.unisense.com/support/tables.html
At 20 °C and 1 atm.: 1 µmol O_2/l = 0.032 mg O_2/l = 0.024 ml O_2
Table 1. Equilibrium concentrations of oxygen (µmol O_2/litre) at ambient partial pressure of 0.21 atm. in water as a function of temperature and salinity.

‰ /°C	0.0	5.0	10.0	15.0	20.0	25.0	30.0	35.0	40.0
0.0	456.6	398.9	352.6	314.9	283.9	257.9	235.9	217.0	200.4
2.0	450.4	393.6	348.1	311.1	280.6	255.0	233.3	214.7	198.3
4.0	444.2	388.5	343.7	307.3	277.3	252.1	230.8	212.4	196.3
6.0	438.1	383.3	339.4	303.6	274.0	249.3	228.3	210.2	194.3
8.0	432.1	378.3	335.1	299.9	270.8	246.5	225.8	207.9	192.3
10.0	426.1	373.3	330.8	296.2	267.6	243.7	223.3	205.7	190.3
12.0	420.3	368.4	326.7	292.6	264.5	240.9	220.9	203.6	188.4
14.0	414.5	363.5	322.5	289.1	261.4	238.2	218.5	201.4	186.5
16.0	408.8	358.7	318.4	285.5	258.3	235.5	216.1	199.3	184.6
18.0	403.2	354.0	314.4	282.1	255.3	232.8	213.7	197.2	182.7
20.0	397.7	349.3	310.4	278.6	252.3	230.2	211.4	195.1	180.8
22.0	392.2	344.7	306.5	275.2	249.3	227.6	209.1	193.0	179.0
24.0	386.8	340.2	302.6	271.9	246.4	225.0	206.8	191.0	177.1
26.0	381.5	335.7	298.7	268.5	243.5	222.5	204.5	189.0	175.3
28.0	376.2	331.2	294.9	265.3	240.6	219.9	202.3	187.0	173.5
30.0	371.0	326.9	291.2	262.0	237.8	217.4	200.1	185.0	171.7
32.0	365.9	322.5	287.5	258.8	235.0	215.0	197.9	183.0	170.0
34.0	3609	3183	2839	2557	2322	2125	1957	1811	1682
36.0	355.9	314.1	280.3	252.5	229.5	210.1	193.6	179.2	166.5
38.0	351.0	309.9	276.7	249.5	226.8	207.7	191.4	177.3	164.8
40.0	346.2	305.8	273.2	246.4	224.1	205.4	189.3	175.4	163.1
42.0	341.4	301.8	269.4	243.4	221.5	203.1	187.3	173.6	161.5

Sources:
Garcia, H.E. and Gordon, L.I. 1992. Limnol. Oceanogr. 37:1307-1312
Millero, F.J. and Poisson A. 1981. Deep Sea Res. 28A:625-629)

UNISENSE, DENMARK
www.unisense.com · info@unisense.com