

# OXYGEN MICROOPTODE USER MANUAL



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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 is covered by a 1 year limited warranty or 1 million data points (estimated 300 hours of measurements).

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.

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Further documentation and support is available at our website [www.unisense.com](http://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
- The MicroOptodes are correctly packed for return to Unisense, in accordance with the note included in the MicroOptode shipping box.

## RECOMMENDED METERS

Unisense MicroOptode Meter

## OVERVIEW

This manual covers all the Unisense MicroOptodes. 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 MicroOptodes are designed for research applications within physiology, biotechnology, environmental sciences and related areas.

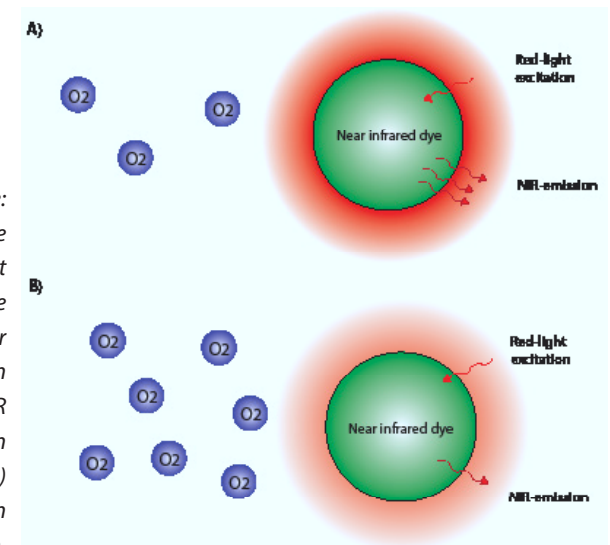
### MEASURING PRINCIPLE

The very tip of the optical fiber is coated with a specialized dye that is excitable with red light (610-630 nm). When the MicroOptode is connected to a Unisense MicroOptode Meter the dye is excited with sinusoidal modulated red excitation light. The dye shows an oxygen-dependent luminescence in the near infrared (NIR, 760-790 nm), and by detecting quenching of the luminescence the oxygen concentration can be determined in the sample.

### WARNING

Unisense MicroOptodes are neither intended nor approved for use on humans

*Measuring principle:  
The near infrared dye shows oxygen dependent luminescence 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

### UNPACKING A NEW MICROOPTODE

The MicroOptode sensor is shipped in a protective tube with the optic fiber positioned half exposed in the needle. Please do not remove the white seal or move the fiber further out of the needle before the calibration procedure has been successfully completed.

### CONNECTING THE MICROOPTODE

Remove the black cap from the MicroOptode connector and remove the red cap from the MicroOptode Meter. Insert the male fiber plug of the MicroOptode cable into the ST-receptacle of the MicroOptode Meter and turn the bayonet coupling gently clockwise until the plug is locked firmly.



*The MicroOptode and the connecting male fiber plug.*

### CALIBRATION

Please consult the software manual for instructions on how to calibrate the MicroOptode in the software.

Calibration of the oxygen MicroOptode sensor is performed as a two-point-calibration by measuring the MicroOptode response in a solution saturated with oxygen and an anoxic solution.

Remember that a temperature sensor is required for correct oxygen measurements unless the measurement and calibration is completed at stable temperature.

#### OXYGEN SATURATED SOLUTION

Place the MicroOptode in a well-aerated calibration solution (e.g. by bubbling with air in the Unisense calibration chamber).

### WARNING

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

After 5 min. of vigorous bubbling turn off the air, follow the MicroOptode signal and when the signal is stable add the point to the calibration in the software. This signal is your calibration value for 100% oxygen saturation at atmospheric partial pressure. Zero reading

#### ZERO READING

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

1. Prepare a solution of sodium ascorbate and NaOH, both to final concentrations of 0.1M (~2 g sodium ascorbate in 100 ml of 0.1M NaOH). This zero calibration solution can be stored in a closed container for 1-2 weeks. Keep the MicroOptode in its protective tube and expose only the fiber tip and the needle part of the sensor to the ascorbic solution, place the MicroOptode in the anoxic solution, read the signal and add the point to the software calibration. Clean the MicroOptode thoroughly in either tap water or demineralized water to avoid any carry-over of ascorbic solution to your sample.
2. Vigorous bubbling with oxygen-free inert gas (e.g. N<sub>2</sub>). It is important to apply vigorous bubbling over a time period (> 5 min.) sufficient to ensure that all oxygen has been removed. Furthermore, 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 the headspace above the water must be closed except for a hole slightly larger than the MicroOptode shaft. This effectively prevents

### IMPORTANT

*Calibration must be performed after pre-polarization when the sensor signal has stabilized. Always use a calibration solution with the same temperature and salinity as the sample solution.*

### IMPORTANT

*The O<sub>2</sub> sensor signal is sensitive to temperature, and the O<sub>2</sub> solubility depends on both salinity and temperature.*

ambient air from entering the vessel. We recommend the Unisense calibration chamber CAL300. Place the MicroOptode in the anoxic solution, read the signal and add the point to the software calibration.

Place the MicroOptode in the anoxic solution. The signal reading is the calibration value for zero oxygen partial pressure ( $S_0$ ). The value should be less than 10% of the signal for atmospheric saturation (otherwise see 'Troubleshooting').

**When the calibration procedure has been successfully complete, test that the MicroOptode responds as expected by inserting it in oxygen saturated solution and anoxic solution. If the MicroOptode meets these requirements the white seal can be removed.**

### CALIBRATION

*As oxygen MicroOptodes respond linearly to changes in oxygen concentrations a two-point calibration is sufficient.*

## MEASUREMENTS

### HANDLING OF MICROOPTODES

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.

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



*Adjustment of the fiber position.*

### 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 (LS18) and micromanipulator (MM33, MM33-2) to avoid damages on 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.

Please avoid bending of the fiber 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 ( $\text{Cl}_2$ ) induce interferences with the sensor reading. No cross-sensitivity is found for pH 1-14,  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{H}_2\text{S}$  and any ionic species.

## 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 peroxide (3%  $\text{H}_2\text{O}_2$ ), soap solution or ethanol and can furthermore be sterilized with ethylene oxide (EtO).



## 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<sub>2</sub> 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<sub>2</sub>/l = 0.032 mg O<sub>2</sub>/l = 0.024 ml O<sub>2</sub>

Table 1. Equilibrium concentrations of oxygen (µmol O<sub>2</sub>/litre) at ambient partial pressure of 0.21 atm. in water as a function of temperature and salinity.

%o / °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	360.9	318.3	283.9	255.7	232.2	212.5	195.7	181.1	168.2
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





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