UNISENSE

# OXYGEN SENSOR USER MANUAL

**OXYGEN SENSOR USER MANUAL**



Version March 2020

# OXYGEN SENSOR USER MANUAL

UNISENSE A/S

# TABLE OF CONTENTS

# 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 sensor is covered by a 180 days limited warranty.
Microsensors are a consumable. Unisense will only replace dysfunctional sensors if they have been tested according 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 oxygen microsensor is a miniaturized Clark-type oxygen sensor that facilitates reliable and fast measurements with a high spatial resolution designed for 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.

## *RECOMMENDED AMPLIFIERS*

*One-channel amplifier: OXY-Meter or Microsensor Monometer*
*Multi-channel amplifiers for oxygen: Microsensor Multimeter or*
*Microsensor Multimeter - 4-channel Oxymeter*

# OVERVIEW

**WARNING**
*Unisense sensors are neither intended nor approved for use on humans*

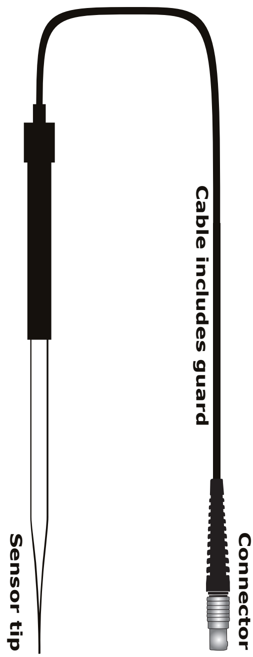
This manual covers all the Unisense oxygen sensors.

The Unisense oxygen microsensor is a miniaturized Clark-type oxygen sensor with a guard cathode designed for research applications within physiology, biotechnology, environmental sciences, and related areas.

With the minute tip size, excellent response time, and insignificant stirring sensitivity, the Unisense oxygen sensor facilitates reliable and fast measurements with a high spatial resolution.

## Measuring principle

The sensor should be connected to a high-sensitivity picoampere amplifier, e.g. the UniAmp multichannel series or the $O_2$ UniAmp. The cathode is polarized against the internal reference. Driven by the external partial pressure, oxygen from the environment penetrates through the sensor tip membrane and is reduced at the gold cathode surface. The picoammeter converts the resulting reduction current to a signal. The internal guard cathode is also polarized and scavenges oxygen in the electrolyte, thus minimizing zero-current and pre-polarization time.




*The connector contains connections for both reference, guard, and sensing cathode.*

# GETTING STARTED

## Unpacking a new sensor

When receiving a new microsensor, remove the shock-absorbing grey plastic net. **Please do not remove the seal and protective tube before the following steps are successfully completed.**

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

## Polarization

The signal from the oxygen sensor is generated in picoampere. Therefore the oxygen sensor must be connected to a Unisense picoampere amplifier during measurements. Oxygen sensors should be polarized with -0.80 V. This happens automatically on the UniAmp instruments, on the Microsensor Multimeter, Monometer, OXY-Meter, Field Multimeter, and Unisense In Situ Systems. If you are using any other amplifier, polarization must be set manually.

***NOTE:***
*The conversion of sensor signal in pA to amplifier signal in mV is controlled by the Pre-Amp Range (mV/pA) setting on the amplifer (not PA-2000)*

**Note that incorrect polarization may destroy the sensor.**
For details on how to set the polarization, consult the user manual of the amplifier that you are using.

## Connecting the microsensor

Connect the sensor to the amplifier. The LEMO connector contains connections for both reference, guard, and sensing cathode. For fast responding sensors it is strongly recommended to ground the system as the ultra thin silicone membrane could be damaged by static electricity.

## Pre-polarization

When the sensor is not in use, oxygen will build up inside the electrolyte. This oxygen must be removed by the sensing cathode and the guard cathode before stable operation of the sensor is possible. Therefore, a period of polarization is necessary before you can use the sensor. This is called the pre-polarization period. If the sensor is new or has not been operated for several days,

it must be pre-polarized for at least 2 hours before it can be calibrated and used. After shorter periods without polarization, the sensor should be pre-polarized until it has shown a stable signal for at least ten minutes.
When pre-polarization is initiated, the signal will be very high and then drop rapidly over the first few minutes. After that, the signal will drop slowly for up to two hours (for needle- and some custom made sensors slightly longer).
The sensor signal depends on the specific sensor (see the delivery note which came with the sensor). If the signal does not stabilize or is too high or too low, refer to the 'Troubleshooting' section.

## Calibration

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

It is recommended to use the Unisense $O_2$ Sensor Calibration Kit for calibrating the $O_2$ sensors (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/)

**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.*

## Alternative Calibration

### Atmospheric reading

Place/keep the sensor tip in a well aerated calibration solution (e.g. by bubbling with air in the Unisense calibration chamber). After complete aeration of the water (5 minutes of vigorous bubbling), turn off any bubbling. After the water movement has stopped, note the signal reading on the display and/or your data acquisition device or add the point to the calibration in the software. This signal is your calibration value for atmospheric partial pressure (Sat) conditions.

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

## ZERO READING

An anoxic solution can be prepared in ONE OF several ways:

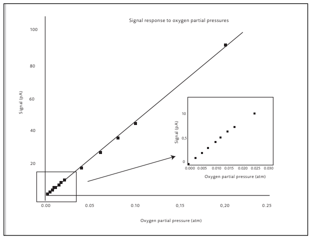
1. Prepare a solution of sodium ascorbate and NaOH, both to final concentrations of 0.1M (~2 g sodium ascorbate in 100 ml of 0.1M NaOH). Stir briefly, and allow the water to stagnate. This zero calibration solution can be stored in a closed container for 1-2 weeks. Place the sensor (in its protective tube) in the solution and wait for the signal to stabilize. Rinse the sensor by flushing it in tap water. Make sure that all the anoxic solution is rinsed off the sensor before you insert it in to your sample.

2. Vigorous bubbling with oxygen-free inert gas (e.g. $N_2$). It is important to ensure vigorous bubbling over a time period sufficient to flush all oxygen out. 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 microsensor shaft. This effectively prevents ambient

*Calibration chamber CAL300*

**CALIBRATION**

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




*2 examples of calibration curves*

**WARNING**

*Bubbling of water with gas may cause the water to change temperature due to the gas temperature or evaporative cooling. Monitor the temperature to find a suitable bubbling rate, which does not change the water temperature significantly.*

air from entering the vessel. Please see warning below right.
We recommend the Unisense calibration chamber CAL300 in which 5 minutes of bubbling at a rate of 0.5 L gas per minute is sufficient to drive 99% of the oxygen out.

3. A zero reading can be obtained with many other methods. A solution of yeast will use all oxygen within ½ hour or a small container with active sediment/sludge will also become anaerobic.
   When measuring in sediment you can also use the oxygen-free sediment to obtain your zero-reading.

Place the sensor in the anoxic solution. The signal reading is the calibration value for zero oxygen partial pressure (S0). The value should be less than 10% of the signal for atmospheric saturation (otherwise see 'Troubleshooting').
Read the value on the display or add it to the software calibration.

Oxygen sensors respond linearly in the range of 0 to 100% oxygen and signals can be linearly converted to partial pressure. To convert a signal S from partial pressure to the equivalent concentrations of oxygen (C), perform a linear conversion and multiply with the atmospheric level solubility (a) of oxygen in the relevant liquid at the relevant temperature (see Table 1 in this manual):

$$C = a \times (S - S0) / (Sat - S0)$$

If you use any of our programs in the SensorTrace series this is done by the software. Consult your software manual for details.

Check and repeat calibration frequently to ensure that all measurements are calibrated to correct concentrations. When the sensor is new, you may need to calibrate more often, while

an older and extensively used sensor may require calibration only every 24 hours or less. To minimize the need for calibration, it is recommended to keep the sensor polarized between measurements.
The membrane permeability of oxygen microsensors changes with time, so a change in signal of up to 50% may occur over months. This does not affect the quality of the measurements as long as the sensor is regularly calibrated.

## Approval of new sensor

If the sensor functions according to the above procedure, the seal and protective plastic tube can be carefully removed and measurements can be started.

# MEASUREMENTS

## Mounting the sensors

Although the Unisense microsensors are made of glass, the tip is flexible and can bend slightly. The sensor is thus surprisingly sturdy even in coarse sediments or tough tissues. However, larger obstacles or lateral movements of the sensor while the tip is in contact with a solid substrate may cause the tip to break.
Also, due to the small size of the microsensor tip and to the steepness of gradients in many environments, a displacement of the sensor tip of even a few microns may change its environment. **We therefore recommend that measurements are performed only in a stabilized set-up free of moving or vibrating devices**. We recommend the Unisense lab stand (LS18) and micromanipulator (MM-33, MM33-2) for laboratory use. For in-situ use we recommend our in situ stand (IS19) and a micromanipulator.

**TEMPERATURE**

*Closely monitor the temperature, because the sensor signal is very dependent on temperature. The temperature coefficient varies from sensor to sensor but is approximately 2-3 % per °C.*

## Electrical noise

The signal of the microsensor is very small ($10^{-13}$ to $10^{-10}$ ampere). Although both the Unisense amplifiers and oxygen microsensors are very resistant to electrical noise from the environment, electrical fields may interfere with the sensor signal. Therefore we recommend that unnecessary electrical/mechanical equipment is switched off. Also, be careful not to touch the sensor or wires unnecessarily during measurements.

## Interference

Sulfide can affect the sensitivity of the oxygen microsensor and the calibration of the oxygen sensor can thus change when exposed to sulfide. Exposure to high concentrations of sulfide should be avoided. If measuring in sulfide rich environments the oxygen sensors can be "pre-contaminated" with sulfide to avoid large changes in calibration values for the sensor. Contact Unisense for more information.

# ADVANCED USE OF THE OXYGEN SENSORS

Unisense can construct oxygen sensors for customer requested applications providing several options for customizations (e.g. tip size, response time, pressure tolerance, and stirring sensitivity) and adaptations (e.g. mounting in needle or flow-through cell), making accurate measurements possible for even more applications.

## Examples of advanced applications

- Gross photosynthesis measurements by the light-dark shift method
- Respiration/production rates in small samples in Unisense micro-respiration chambers
- Measurements of oxygen under high external pressure e.g. in closed pressurized systems, underwater, and deep sea applications
- Long-term oxygen monitoring
- Oxygen in flow-through cells

Please visit our website for more information.

# STORAGE AND MAINTENANCE

## Storage

Store the sensor in the protective plastic tube used for shipping. The oxygen microsensor can be stored with the tip exposed to water or air. The room in which the oxygen microsensor is stored should be dry and not too hot (10-30°C). If the sensor is used regularly it can be stored polarized, connected to a Unisense amplifier.

## Cleaning the sensor

Depending on which substance is present on the sensor tip or membrane, the sensor can be cleaned with different solutes.
The standard method is to rinse with 96% ethanol, then rinse with 0.01 M HCl and rinse with water. This will remove most substances. Alternatively it is possible to rinse with 0.1M NaOH, isopropanol, or different detergent.

# REFERENCES


- Revsbech, N. P. 1989. An oxygen microsensor with a guard cathode. Limnology and Oceanography. 34(2):474-478.
- Gundersen, J.K., Ramsing, N.B., and Glud, R.N. 1998. Predicting the signal of $O_2$ microsensors from physical dimensions, temperature, salinity, and $O_2$ concentration. Limnology and Oceanography 43(8): 1932-1937.
- Revsbech, N. P., and B. B. Jørgensen. 1986. Microelectrodes: Their Use in Microbial Ecology, p. 293-352. In K. C. Marshall (ed.), Advances in Microbial Ecology, vol. 9. Plenum, New York.


Please see our website for more references

# TROUBLE SHOOTING

| | |
|---|---|
| **Problem** | High and drifting signal |
| **Possible cause 1** | Gas bubbles present inside the sensor tip due to short circuit or electrical shock. |
| **Solution** | Degas water by boiling and **subsequent cooling** or by 10 minutes of vacuum treatment. Immerse the sensor tip for 20 min in the degassed water. Repeated or prolonged treatment may be necessary. |
| **Possible cause 2** | The sensor tip is broken. |
| **Solution** | Replace the oxygen microsensor. |
| **Problem** | Signal very low |
| **Possible cause** | Contamination of the cathode surface (e.g. by sulfide) or loss of gold tip due to excessive vibrations. |
| **Solution** | Replace the oxygen microsensor. |
| **Problem** | Signal constantly very low and no response to oxygen |
| **Possible cause** | Gas bubbles present inside the tip of the sensor causes a disruption in the electrolyte. |
| **Solution** | Degas water by boiling and subsequent cooling or by 10 minutes of vacuum treatment. Immerse the sensor tip for 20 min in the degassed water. Repeated or prolonged treatment may be necessary. |
| **Problem** | Slow response. |

# TROUBLE SHOOTING

| | |
|---|---|
| **Possible cause** | Insoluble compounds deposited at the sensor tip. |
| **Solution** | Rinse with 96% ethanol, rinse with 0.01 M HCl and rinse with water. |

| | |
|---|---|
| **Problem** | Slow response - OX-N sensors |
| **Possible cause** | A gas bubble trapped in the needle tip. |
| **Solution** | Remove the air bubble gently by repositioning the sensor |

| | |
|---|---|
| **Problem** | Unstable signal or the signal fluctuates if the setup is touched or equipment is introduced in the medium you are measuring in. |
| **Possible cause** | Electrical disturbance of the sensor through the tip membrane |
| **Solution** | Ground the set-up using the blue grounding cable supplied with the picoammeter. Connect the reference plug on the picoammeter (blue plug) with the medium you are measuring in. |

*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/technical_information/*
*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