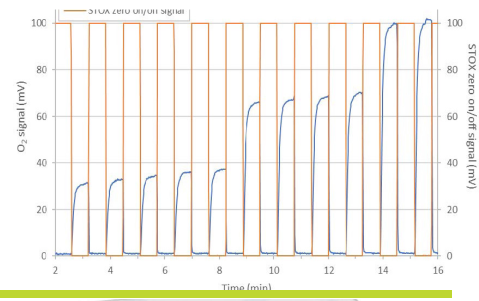
STOX SENSOR USER MANUAL

FOR STOX SENSORS USED WITH UNIAMP SERIES INSTRUMENTS



STOX SENSOR USER MANUAL

Version March 2020

STOX 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 STOX 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 Switchable Trace Oxygen microsensor is a specialized product. It is handmade and characteristics vary between individual sensors. This means that pre-polarization time (see section on Pre-polarization), the optimal zeroing on/off intervals (see section with same title), the response time, and the sensor signal for a given oxygen concentration will vary between sensors.

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 sensors

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

- The sensors were tested immediately upon receipt in accordance with the manual.
- The seal is still intact.
- The sensors are returned to Unisense for inspection within two weeks.

- The sensors are correctly packed for return to Unisense, in accordance with the packing guide included in the sensor box.

STOX SENSOR PRINCIPLE

The Switch-able Trace Oxygen Sensor (STOX) is designed for measuring trace amounts of oxygen.
All amperometric oxygen sensors give a small residual current in absence of oxygen due to unspecific reactions at the cathode. This fact makes it difficult to establish whether small signals are due to exposure to trace amounts of oxygen or due to the residual current.

The design of the STOX sensor enables detailed measurements of sub-micromolar oxygen concentrations. The Unisense STOX sensor is based on a Clark-type oxygen sensor, but it is modified to have two cathodes; a very porous front guard cathode at the very tip of the sensor and a measuring cathode placed behind it. Polarization of the front cathode (front guard) is switchable. When the front guard is switched on (zeroing period), it consumes all oxygen entering the sensor through the silicone rubber membrane, and the measuring cathode signal is solely due to residual current. When it is switched off (measuring period), the measuring cathode signal is the result of both oxygen in the sample, that passes through the two silicone membranes and the porous front guard cathode, and the residual current. The signal difference between the measuring period and the zeroing period is proportional to the oxygen concentration at the sensor tip. This signal difference is continuously measured during experiments and hereby measurements are continuously compensated for any baseline drift.

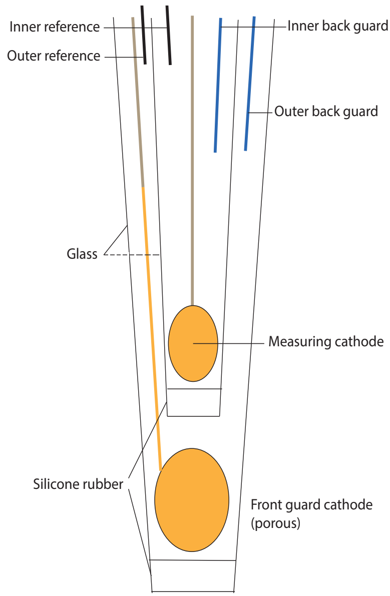


Figure 1:Schematic drawing of STOX microsensor tip.

The detection limit of a sensor is the lowest concentration at which there is a statistically significant difference between the signals for measuring and zeroing.

SETTING UP THE UNIAMP AND STOX SENSOR

The signal from the measuring cathode of the STOX sensor is generated in picoampere. Therefore, the oxygen sensor must be connected to a Unisense picoampere amplifier during measurements. STOX sensors should be polarized with -800 mV. This happens automatically when connected to a UniAmp series instrument.

Connecting and polarizing the sensor

Connect the STOX sensor to a pA channel on the fx-6, x-5 and fx-3 UniAmp or the O_2 channel on an O_2 UniAmp. The UniAmp instrument will recognize the STOX sensor and the Enable STOX-mode will be available in the UniAmp Channel Configuration window (Figure 4).

Figure 2: The fx-6 UniAmp

Figure 3: The O2 UniAmp

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 back 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 several hours.

CALIBRATION

If possible, polarize the STOX sensor one to several days before the intenden measurements to get a baseline as stable as possible. The pre-polarization period required for a sensor to become stable depends on the individual sensor and the above is only a guideline.

SETTING THE STOX TIMING

The front guard of the STOX sensor must be polarized in a cyclic manner, switching between STOX zero on and STOX zero off. This creates the periodic signals as shown in Figure 5. The optimal duration of the STOX zero on and off periods depends on the response time of the individual sensor (see 7.1 Finding of the optimal zeroing on/off intervals). In the example in Figure 4, both STOX zero on and off times are set for 40 seconds. For the example in Figure 5, a STOX zero on and off time of 36 seconds was used. The signal didn't quite stabilize at the end of the measuring period, but it was still possible calculate a meaningful average of the last few datapoints before the shifting to zeroing mode.

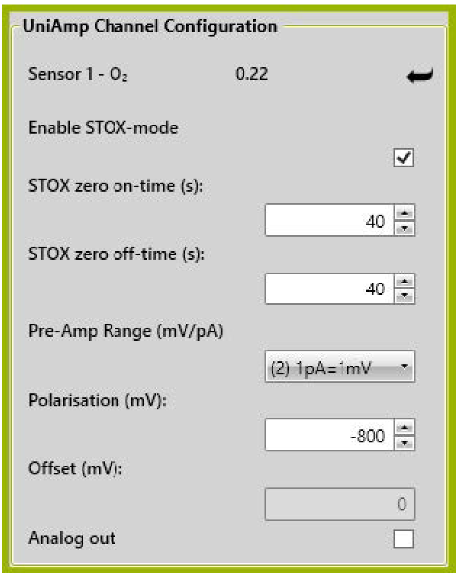


Figure 4: UniAmp Channel Configuration for STOX sensors. When the "Enable STOX-mode" box is checked, the "STOX zero on-time" and "STOX zero-off time" boxes appear.

CALIBRATION AND MEASURING

Calibration of the STOX sensor should not be performed using the calibration function in SensorTrace Logger as this is not designed for this type of signals. Instead it is recommended to record un-calibrated data and transform data to calibrated signal using spread sheet or other software after data have been collected. The principles of calibration are explained below.

Calibration must be performed after the sensor signal has stabilized after a period of pre-polarization (see Pre-polarization). As oxygen sensors are sensitive to temperature, it is necessary to perform calibration and measurements at the same temperature. Temperature compensation in the UniAmp instrument cannot be used for STOX data.

When the front guard is switched off (measuring mode), the signal will increase if there is oxygen present at the tip of the sensor. If no oxygen is present the measuring signal will be identical to the signal obtained during the front guard on period (zeroing mode). Due to the complex construction of the sensor tip, the response time of a STOX sensor is much longer than a standard Unisense microsensor. The signal will not reach a completely stable plateau (if oxygen is present) but will continue to have a slow upwards drift until next period of zeroing, e.g. Figure 5. It is not necessary to reach a completely stable O_2 signal as long as the STOX zero on/off timing is the same during calibration and measuring.

Finding of the optimal zeroing on/off intervals

The STOX zero on and zero off timing should be optimized for the individual sensor. In principle, these intervals should be so long that the sensor responds fully, i.e. the signal becomes constant. However, this will give a very slow response to changes in oxygen concentration and is not necessary. In practice it is sufficient to obtain 80 - 95% of the full response. This is possible because the on/off timing is identical for calibration and measurements and because the switching between front guard on and off is precisely repeated. Therefore, the fraction of the response obtained is identical for all the intervals, i.e. if a certain fraction of the full response is reached in one interval, the exact same fraction of full response will be reached in all other intervals.

To find the optimal STOX zero on and zero off intervals:

1. Set both the *STOX zero on-time* and *STOX zero off-time* to a minimum of 10 minutes and place the sensor in water with an O_2 concentration close to that expected in the experiment (recommended) or water equilibrated with atmospheric air. Note the signals at the end of these long periods.
2. Reduce the *STOX zero on-time* to the shortest possible period required for obtaining a stable zero signal (typically 30 to 60 seconds).
3. Reduce the *STOX zero off-time* to the time is takes to achieve approximately 80-95% of the 10 minute signal (typically 30 to 60 seconds).

The *STOX zero off-time* is often a bit longer than the *STOX zero on-time* in order to obtain a stable zero signal.

It is important that the pre-polarization is complete, i.e. that the zero signal is constant, before the process of finding the optimal *STOX zero on-time* and *STOX zero off-time* is started. If the zero signal is not constant, the sensor needs longer polarization before it can be used. Checking the stability of the zero signal can be done in one of two ways:

1. Place the STOX sensor in an oxygen free solution for half an hour and check that the signal is constant. See the O_2 Microsensor

manual at https://www.unisense.com/manuals/ for how to prepare this solution.

2. Place the STOX sensor at constant temperature in air or water. Set the *STOX zero on-time* and *STOX zero off-time* to approx 50 seconds each and record data for at least half an hour. Check that the O_2 signal reaches the same low value at the last few seconds of each *STOX zero on* interval for these periods.

Calibration using oxygen amendments

The net O_2 signal from the STOX sensor, which is the difference between the signals with front guard polarization off and on, is proportional to the O_2 concentration. This is the case because the net O_2 signal for zero O_2 is zero mV and calibration can be done solely by amendment of known amounts of O_2. It may be described as a traditional linear calibration curve with an intercept of zero and it is, therefore, only necessary to determine the slope of this - the calibration factor below.

The calibration factor A is determined by amendment of O_2 as:

$$A\,(mV/\mu M) = \frac{\Delta O_2\ signal\ cal\ (mV)}{\Delta O_2\ conc\ (\mu M)} \qquad \text{(Equation 1)}$$

Where *ΔO_2 signal cal (mV)* is the difference between the O_2 signal before and after O_2 amendment and *ΔO_2 conc. (µM)* is the increase in concentration by the amendment. During measurement, the O_2 concentration is then calculated as:

$$O_2\ conc.\,(\mu M) = \frac{\Delta O_2\ signal\ net\ (mV)}{A\,(mV/\mu M)} \qquad \text{(Equation 2)}$$

Where *ΔO_2 signal net (mV)* is the difference between the O_2 signal with the front guard off and on. Please see the example below (Figure 5).

Calibration may be done as shown in the example in Figure 5. In this experiment the STOX sensor was placed in a glass bottle with a rubber stopper. The stopper slowly released oxygen to the water giving the increasing difference between the O_2 signal with front guard on and off. O_2 was added in the form of 5 ml air saturated water. This resulted in an increase in O_2 concentration by 1.37 µM which gave an increase in the O_2 signal of 29.1 mV for the first and 29.3 mV for the second amendment (average = 29.2 mV).
The calibration factor is thus:

$$A\ (mV/\mu M) = \frac{29.2\ (mV)}{1.37\ (\mu M)} = 21.314\ mV/\mu M$$

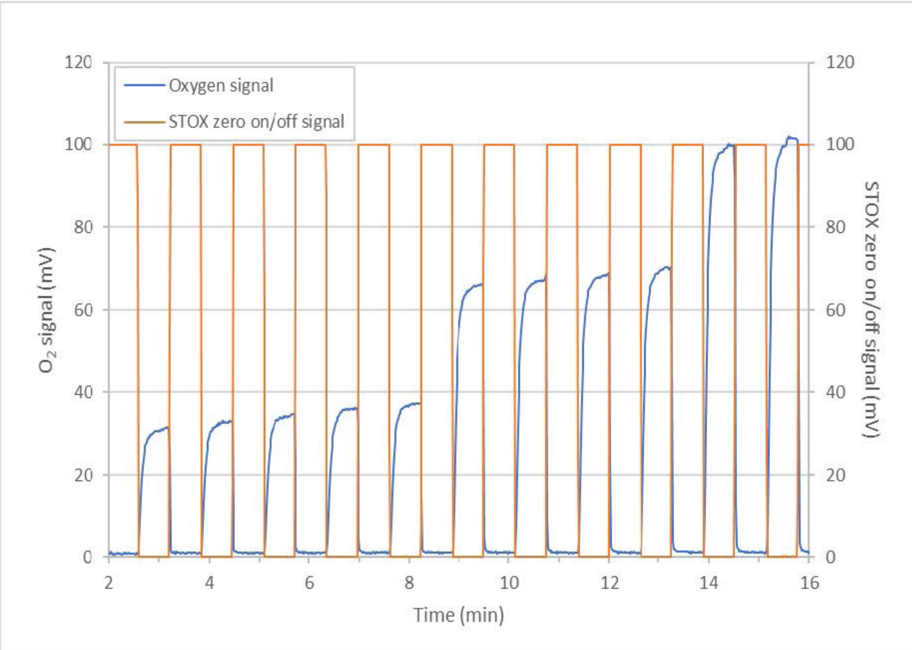


Figure 5: Example of raw STOX data with both the O_2 signal and the STOX zero on/off signal plotted. The STOX zero on-time and off-time was 36 seconds each. A STOX on/off signal of 100 mV indicates that the front guard is on, 0 mV indicates that it is off. The green arrows indicate amendment of 1.37 µM of oxygen (5 ml air saturated water (273 µM) in 1 litre).

The oxygen concentration before the first amendment can now be calculated from equation 2. The O_2 signal was 1.00 mV with the front guard on and 37.16 mV with the front guard off.

$$O_2\,(\mu M) = \frac{37.16 - 1.00\ (mV)}{21.314\ (mV/\mu M)} = 1.697\ \mu M$$

Calibration of the sensor and measuring should be done with the same STOX zero on/off timing.

The STOX sensors are designed to give a very high signal in order to optimize the signal to noise ratio and give a very low detection limit. The millivolt signal for a given oxygen concentration will depend on the *Pre-Amp Range* (Figure 4). This could cause the signal to be out of range with the sensor at atmospheric O_2 concentrations. If this is a problem, you must decrease the *Pre-Amp Range*.

NOTE
The millivolt signal for a given oxygen concentration will vary between individual sensors (see Support, ordering, and contact information)

It is recommended to calibrate within the same range of concentrations as you will measure. It is possible to calibrate directly in water equilibrated with atmospheric air. However, with such a large difference in signal with the front guard on and off, it might take longer time to get a stable signal.

Calibration using fixed oxygen concentration

Place the sensor in water with a known oxygen concentration at the same temperature as that of the measurements. Adjust the STOX zero on/off times to fit the specific sensor (See section 'Finding of the optimal zeroing on/off intervals'). Record data with these STOX zero on/off settings for at least 5 cycles in order to achieve a precise estimate of the difference between the O_2 signal with the front guard on and off for a known concentration of oxygen. Hereafter the sensor can be transferred to the experimental setup.

IMPORTANT
Keep the same timing settings for the STOX zero on and off times during both calibration and measurements.

Calculate the calibration factor A for fixed oxygen concentration:

$$A\,(mV/\mu M) = \frac{\Delta O_2\ signal\ cal\ \ (mV)}{O_2\ conc\ (\mu M)} \qquad \text{(Equation 3)}$$

Where *ΔO_2 signal cal (mV)* is the difference in O_2 signal with the STOX front guard off and on (average for the 5 periods), and *O_2 conc (µM)* is the oxygen concentration in the calibration water. The oxygen concentrations during measurements can now be calculated as:

$$O_2\ conc.\ (\mu M) = \frac{\Delta O_2\ signal\ net\ \ (mV)}{A\ (mV/\mu M)} \qquad \text{(Equation 4)}$$

where *ΔO2 signal net (mV)* is the difference between the O_2 signal with the front guard off and on. Please see the example in Figure 5.

REFERENCES

N. P. Revsbech, L. H. Larsen, J. K. Gundersen, T. Dalsgaard, O. Ulloa, and B. Thamdrup. Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. Limnol Oceanography: Methods 7:371-381, 2009.

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