STOX-sensor and Controller
**STOX sensor principle**

The Switch-able Trace Oxygen Sensor (STOX) is a unit 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 front guard at the very tip of the sensor and a measuring cathode placed behind it. Polarization of the front cathode (front guard) is switch-able. When the front guard is switched on (zeroing), it consumes all oxygen entering the sensor tip and the signal from the measuring cathode is solely due to residual current. When it is switched off (measuring), the signal on the measuring cathode is due to both oxygen in the sample and the residual current. Thus the signal difference (amplitude) between periods of zeroing and measuring linearly reflects the oxygen concentration at the sensor tip. This amplitude is continuously measured during experiments and hereby measurements are continuously compensated for any baseline drift.

**STOX-Sensor Controller**

The STOX-Sensor Controller works by turning the polarization of the front guard of a STOX sensor on and off in a sequential manner while measuring the signal of an internal oxygen sensor. The signal with the front guard turned on (zeroing) gives the baseline signal, and the increase in signal (amplitude) with front guard off (measuring) indicates the oxygen concentration (see Revsbech et al. 2009 for more details).

**Connecting a STOX sensor**

The STOX sensor is delivered from Unisense with two sensor connection cables, one with a black labeled LEMO connector and one with a red labeled LEMO connector. See fig. 3.

The red labeled LEMO connector contains the internal oxygen sensors signal and should be connected to the ‘Measuring Input’ socket on the STOX-Sensor Controller.

The black labeled LEMO connects the front guard oxygen sensor and should be connected to the...
‘Zero Input’ socket on the STOX-Sensor Controller, see fig. 2. Connecting controller unit to a microsensor amplifier

The following list of Unisense amplifiers can be used: PA2000, Microsensor Multimeter, Microsensor Monometer or OXY-Meter.

From the STOX-Sensor Controller two connections must be made; one connecting the controller ‘Measure out’ to an amplifier, and one with a set of banana-plug connectors for exporting the timing data.

For connecting ‘Measure out’ to a Microsensor Multimeter, Monometer or OXY-Meter, use the supplied Lemo to Lemo cable. For users with a PA2000, this cable will be a 2-wired Lemo to BNC/banana plug cable.

When connecting the STOX sensor to the Microsensor Multimeter, Monometer and OXY-Meter the amplifier will automatically recognize the sensor as an oxygen sensor and polarize the sensor. The PA2000 must be manually adjusted for oxygen sensor (see the oxygen sensor manual).

**Recording Timing**

The timing signal can be logged in Unisense data acquisition software, SensorTrace Basic using a separate A/D converter (PA2000 and OXY-Meter users) or by connecting the timer signal to one of the analog inputs on the Microsensor Multimeter or Monometer. The “timing output” is found on the backside of the STOX-Sensor Controller, see fig. 4. Recording the timing signal along with the STOX sensor signal, will enable the user to distinguish the measurement period from the zeroing period.

**Setting timing intervals**

Once the sensor, amplifier, and optionally the A/D converter connections have been made, the measurement timing interval should be set.

There are two times to be set, the zeroing and the measuring times. Each of these is set using a second’s selection and a multiplication factor selection.

As example, setting the zeroing time to 10 seconds and the measuring time to 1 minute set the following:

Nub 1: set to 1
Nub 2: set to x10
Nub 3: set to 1
Nub 4: set to x60

The red and green LEDs will go on when the respective time frame is active.
Calibration and measuring:

Calibration of the STOX sensor should not be performed using the calibration function in SensorTrace BASIC, instead it is recommended to record un-calibrated data and transform data to calibrated signal in a spreadsheet after experiments are terminated. Calibration values are obtained as described in the following section.

Calibration must be performed after the sensor signal has stabilized during pre-polarization (see oxygen sensor manual). As oxygen sensors are sensitive to temperature, it is necessary to perform calibration and measurements at the same temperature. Ex. sample temperature is 4 °C the calibration should be completed in solutions of 4 °C.

The auto zeroing function by the front guard remove any uncertainty regarding baseline drift from the data set, so detailed measurements in the nanomolar range is possible.

When the zeroing is switched off (green light on) 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 zeroing period. 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, see fig. 6.

Figur 6 Data from 2 cycles with different cycle length. Note the slow upward drift in the two min. measuring period.

The net signal increase between 2 periods of zeroing (amplitude) reflects the oxygen concentration, and by keeping the periods constant in water with known concentration and for experiments, the experiment data can calibrated. 1 cycle reflects 1 oxygen concentration.
Calibration of the sensor and measuring should thus be done with the same cycle periods e.g. if the calibration value is obtained after one minute, the oxygen concentration should also be measured after one minute. Below see a typical data set obtained with a STOX sensor in water containing 2 micromolar of oxygen where cycle length is kept constant.

![Figur 7. Raw STOX sensor data obtained in sample with 2 µM oxygen.](image)

The signal from a STOX sensor is made to give the largest possible signal for a given oxygen concentration to optimize the signal to noise ratio. The millivolt signal from a given oxygen concentration will depend on the amplification range chosen for the amplifier in request. At highest microsensor amplification range on the amplifier the signal could be out of range in atmospheric readings.

It is recommended to do calibration in water with a low concentration corresponding to the maximum expected concentration in the experiments. It is possible to use atmospheric saturated water, but then it might take longer time to get a stable baseline signal with zeroing is switch on.

**Calibration procedure:**

- Set both the zeroing and measuring time to minimum 10 minutes and place the sensor in either low concentrated water (recommended) or atmospheric oxygen saturated water.
- Observe the time it takes for the baseline to stabilize after zeroing is switch on and adjust the zeroing time to the shortest zeroing time for obtaining a stable baseline
- Adjust the measuring period to the time is takes to achieve approximately 80-90% of the 10 minute signal.
- Record data with these STOX-Sensor Controller settings in at least 5 cycles in order to achieve a precise estimate of the signal in a known concentration of oxygen.
Hereafter the sensor can be transferred to the experiment setup. IMPORTANT: Keep the same timing settings on STOX-Sensor Controller and amplifier used for the calibration cycles.

References: