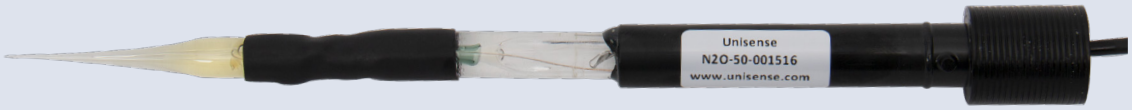


N_2O Sensor

User Manual

UNISENSE
SCIENTIFIC

Nitrous Oxide Sensor User Manual

UNISENSE A/S

Version: May 2026

TABLE OF CONTENTS

1. WARRANTY AND LIABILITY

1.1 Notice to Purchaser

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

1.2 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.

1.3 Warranty and Liability

The Nitrous Oxide sensor is covered by a 60 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 in 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.

1.4 Repair and 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.

2. CONGRATULATIONS WITH YOUR NEW PRODUCT!

2.1 Support, ordering, and contact information

The Unisense nitrous oxide microsensor is a miniaturized Clark-type nitrous oxide sensor with a guard cathode designed for research applications within environmental sciences.

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
Langdyssen 5
DK-8200 Aarhus N, Denmark
Tel: +45 8944 9500

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

3. 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 delivery note and the manual.
- The seal is still intact.
- the sensors are returned to Unisense for inspection within two weeks (contact the sales team before returning).
- the sensors are correctly packed for return to Unisense, in accordance with the note included in the sensor shipping box.

4. OVERVIEW

This manual covers all the Unisense N_2O sensors. With its minute tip size, excellent response time, and insignificant stirring sensitivity the Unisense nitrous oxide sensor makes it possible to make reliable and fast measurements with a high spatial resolution.

WARNING: Unisense MIcroOptodes are neither intended nor approved for use in humans

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

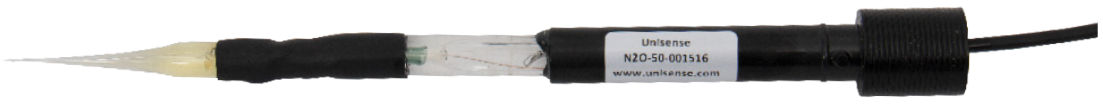
N_2O Sensor Characteristics:

Type	Tip diameter	Measuring range	Detection limit	Response tim (90%)
N_2O-50	40-60 µm	0-200 µm	0.3 µM	< 30 sec
N_2O-100	90-110 µm	0-200 µm	0.1 µM	< 30 sec
N_2O-500	400-600 µm	0-200 µm	0.1 µM	< 35 sec
N_2O-R-LR	400-600 µm with cap*	0-10 µm	25 µM	< 65 sec
N_2O-R-SR	400-600 µm with cap*	0-50 µm	0.1 µM	< 65 sec
N_2O-R-IR	400-600 µm with cap*	0-300 µm	0.1 µM	< 65 sec
N_2O-R-HR	400-600 µm with cap*	0-4 µm	1 µM	< 65 sec
N_2O-MR	500 µm for Microrespiration system	0-50 µm	0.1 µM	< 65 sec
N_2O-NP	1.6 x 40 mm needle for piercing	0-50 µm	0.1 µM	< 65 sec
N_2O-FT	With glass flow cell, 6 or 8 mm OD	0-50 µm	0.1 µM	< 65 sec
N_2O-SL	With Swagelok flow cell, 1/8" or 1/4"	0-50 µm	0.1 µM	< 65 sec
N_2O-ST-12	With 12 mm x 20 cm steel tube	0-50 µm	0.1 µM	< 65 sec
N_2O-PEEK	For 1/8" or 1/6" PEEK tube	0-50 µm	0.1 µM	< 65 sec

Table 1: N_2O Sensor Characteristics

**Stainless steel cap, 10 mm outer diameter*
Stirring sensitivity for all types < 2%
Sensors must never be exposed to N_2O concentrations above their working range.
LR: Low Range; SR: Standard Range; IR:Intermediate Range; HR: High Range.

The Unisense nitrous oxide microsensor is a miniaturized Clark-type sensor with an internal reference and a guard cathode. In addition, the sensor is equipped with an oxygen front guard, which prevents oxygen from interfering with the nitrous oxide measurements. The sensor is connected to a high-sensitivity picoammeter and the cathode is polarized against the internal reference. Driven by the external partial pressure, nitrous oxide from the environment will penetrate through the sensor tip membrane and be reduced at the metal 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 stabilization time.



5. GETTING STARTED

5.1 Unpacking a new sensor

When receiving a new sensor, first remove the shock-absorbing grey plastic net. Then secure the sensor in a safe position (e.g. micromanipulator or calibration chamber) before connecting it to the measuring meter.

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

NOTE: Sensors for the Microrespiration System are shipped unassembled and the glass sensor and the blue aluminium guide must be assembled as described in the delivery note and Appendix 2 of the sensor manual. Test the sensor before assembley.

The signal from the nitrous oxide sensor is generated in picoamperes. Therefore the nitrous oxide sensor must be connected to a picoammeter amplifier unit during measurements.

5.2 Pre-activation and Polarization

Please see procedure below.

WARNING: Incorrect polarization may destroy the sensor.

Pre-activation period: Nitrous oxide sensors have a metal cathode which during normal exposure to oxygen over time will acquire an oxidized surface layer. If the sensor is new or has not been used recently, this layer must be "pre-activated" in order for the sensor to work. This is done by applying a voltage of -1.3 V for 30 minutes prior to the period of polarization which is required for most electrochemical sensors.

Stabilization period: The electrolyte inside the sensor can contain large amounts of oxygen which must always be removed before the sensor is stable. This is done by the guard cathode inside the sensor whenever the sensor is polarized, i.e., connected to an amplifier. This process requires some time, depending on the dimensions and exact proportions of the individual sensor.

IMPORTANT: If the sensor is new or has been disconnedcted for > 4 hours, follow the PRE-ACTIVATION PROCEDURE below.
For recently used N_2O sensors, follow the POLARIZATION PROCEDURE below.
Please read both sections before starting the relevant procedure.

For directions on adjusting the polarization, please consult the relevant instrument manual.

Pre-activation procedure

1. Secure the nitrous oxide sensor with its tip immersed in nitrous oxide free water. (It does not harm the sensor to be in air instead, but pre-activation, polarization, and calibration might as well be made with the same set-up).

2. **Using an fx-6, fx-3 pA, x-5 or N_2O UniAmp:**
   Change the polarization to -1.3 V in the Calibration tab of uSense Solutions.

> *NOTE: The software converts the pA signal into mV. Standard setting is 1 mV = 1 pA. This may be modified by changing the Pre-Amp setting (mV/pA) in the software (UniAmp) or on the instrument (Multimeter, Monometer, and Field Multimeter).*

Using a Microsensor Multimeter or Monometer:
Connect the sensor to the amplifier.
Turn the polarization to -1.3V.

Using a PA2000 amplifier:
Connect ONLY the BNC connector of the adaptor cable, not the yellow banana plug and turn the polarization voltage to -1.3 V.

Leave the polarization at -1.3 V for approx 30 min. You will see a very high signal in the beginning but this will decrease after 5 - 10 minutes.

Polarization procedure

1. **Using a fx-6, fx-3 pA, x-5, or N_2O UniAmp**
   Change the polarization back to -0.8 V.

   Using a Microsensor Multimeter or Monometer:
   Turn the polarization voltage to -0.8 V.

   Using a PA2000 amplifier:
   Turn the polarization voltage to -0.8 V.
   Connect the yellow banana plug to the yellow connection on the meter.

2. You will see a sharp decrease followed by a rapid increase in the signal. After this, the signal will stabilize with an initial rapid and then a more slow decrease. Wait until the signal is below 20 mV and stable. This may take many hours, e.g. 12 hours (see below). If the sensor signal does not reach this value, please go to the Troubleshooting section.

In general the sensor should be polarized and allowed to stabilize for as long as possible before calibration and measurements to get maximum stability.
Unisense recommends that you perform the pre-activation the day before measurements and then leave the sensor polarized overnight. If the signal does not stabilize or is too high or too low, see 'Trouble-shooting'.

> *IMPORTANT: Calibration must first be performed after the sensor signal has stabilized.*

5.3 Calibration principles

Calibration must be performed after the sensor signal has stabilized. The N_2O sensors respond linearly to the N_2O concentration within their measuring range (see Table 1) and signals can thus be linearly converted to partial pressure. Check and repeat calibration at appropriate intervals. Shortly after taking a sensor into use, the appropriate interval may be 2 hours; when the sensor has been used for some time, it may be 24 hours. To minimize the need for calibrations, keep the sensor polarized between measurements, unless the time between measurements exceeds several days.

NOTE: The lifetime of the N_2O sensor is the same whether the sensor is continuosly polarized or store un-polarized.

The membrane permeability of nitrous oxide microsensors changes with time, so a change in signal of up to 50% may occur over months.

5.4 Calibration

It is recommended to use the Unisense N_2O Sensor Calibration Kit for calibrating the N_2O sensors (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-subscript Manual (www.unisense.com/manuals)

5.6 Alternative calibration

Zero calibration point: Place/keep the sensor tip in nitrous oxide free water and record the signal. This signal is your calibration value for zero nitrous oxide. The temperature in the solutions for zero calibration and for known N_2O concentration must be the same.

Known concentration calibration point: The nitrous oxide sensor responds linearly to N_2O within its measuring range (see Table 1 for the measuring range for your type of sensor). Therefore, a two-point calibration is sufficient. Prepare water with a defined nitrous oxide concentration (partial pressure), which is slightly above the maximum expected nitrous oxide concentration (partial pressure) in the samples. Never expose the sensor to concentrations more than the specified measuring range - it may cause irreversible damage to the sensor (see Table 1 for the measuring range for your sensor).
A defined nitrous oxide concentration can be obtained by two different procedures:

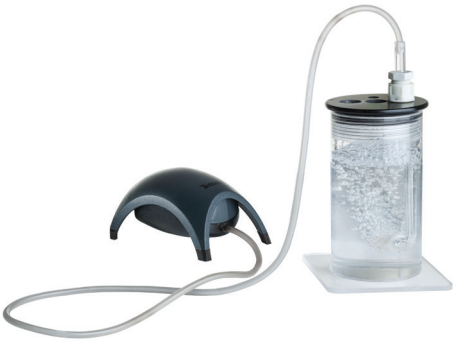
WARNING: Never expose the N_2O sensors to concentrations above their measuring range. This will destroy the senor!
See Table 1 for the measuring range for each type of N_2O sensor.

A. Use a gas mixture controller to obtain a defined mixture of nitrous oxide and nitrous oxide-free inert bulk carrier gas from a gas tank (e.g. N_2) . For instance, to obtain a nitrous oxide concentration of 100 μM in the calibration chamber at 22°C, bubble the water in the calibration chamber with a gas mixture containing 1 atm N2 and 0,003679 atm N_2O (nitrous oxide partial pressure 0.003679 atm × solubility 27,05 × 10-3 mol/liter/atm. = 100 μmol/liter, see Table 2). For a Unisense 300 ml calibration chamber CAL300, 5 minutes of bubbling at a rate of 5 L per minute is sufficient time to achieve 99% of the concentration. If the equipment (gas mixture controller) is available, this method can be 13 convenient, because it is possible to switch between different nitrous oxide levels without changing the water.
   Calculate the appropriate mixture from the solubilities given in Table 2. Alternatively, use the N_2O calculator in the

WARNING: Bubbling of water with any gas may cause the water to cool considerably. Monitor the temperature to find a suitable bubbling rate, which does not cool the water significantly.

Unisense software (Tools - N_2O Calculator) or the Unit Converter on the Unisense website: Click **Knowledge** - **Unit Converter**.

For obtaining correct concentrations it is important that the headspace above the water in the calibration chamber is closed except for one hole, which should be only slightly larger than the microsensor shaft. This effectively prevents ambient air from entering the vessel. We recommend the Unisense Calibration chamber, CAL300, and rubber stoppers for calibrations.



Calibration chamber CAL300

B. Add a defined volume of nitrous oxide saturated water to a defined volume of water in a calibration chamber. For instance adding 3,679 ml of nitrous oxide saturated to 996,32 ml water gives a concentration of 100 μM at 22°C (calculated from Table 2), as 3,679 ml of nitrous oxide saturated water contains 100 μmol nitrous oxide. Using a calibration chamber containing 200 ml, reduce the volume of added nitrous oxide saturated water by a factor of 5 for obtaining 100 μM, and so on. After adding nitrous oxide-saturated water to the calibration chamber, mix it thoroughly for a few seconds and read the signal when it is stable.

   Do not stir bubbles into the water and do not mix by bubbling, as this will flush nitrous oxide from the water. A magnetic stirrer is also not recommended as a mixing tool because it can introduce electrical noise to the signal. The nitrous oxide in the water will slowly escape to the atmosphere and the concentration can only be considered constant for a few minutes. concentration can only be considered constant for a few minutes.

5.7 Approval of new sensor

If the sensor functions according to the criteria given in the delivery note, carefully remove the seal and the protective tube before making measurements.

6. MEASUREMENTS

Nitrous oxide sensors can be used for a wide variety of measurements (please see our web page www.unisense.com for further information). They are for example used for making profiles in sediments or tissues where a high spatial resolution is required or for nitrous oxide measurements in water samples.

TEMPERATURE: Closely monitor the temperature. The temperature coefficient varies from sensor to sensor but is approximately 2-3% per °C. UniAmp instruments has automatic temperature compensation within ±3°C from the calibration temperature, if a temperature sensor is connected.

6.1 Sensor orientation

The N_2O sensor cannot be used with the tip pointing upwards. The sensor can be horizontal or the tip can point downwards. The sensor has a gas bubble inside and if the sensor points upwards, it will float into the narrow part of the sensor and block electrical connectivity in the electrolyte. This will result in no or very little response to N_2O. See **Troubleshooting** below for how to resolve this problem.

6.2 Use of glass-tip microsensors

Although the Unisense microsensors are made of glass, the tip is flexible and can adjust slightly around physical obstacles. The sensor is thus rather sturdy in the longitudinal direction. However, large obstacles like stones or lateral movements of the sensor when the tip is in contact with a solid substrate may cause the tip to break.

Furthermore, 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 only a few microns may change its environment.

Therefore, when working with glass-tip microsensors, we recommend that measurements are performed only in a stable set-up free of moving or vibrating devices. We recommend the Unisense laboratory stand LS and the Unisense micromanipulator MM33 (double MM33-2) for laboratory use. For in situ use we recommend our in situ stand (IS19) and a micromanipulator.

6.3 Use of sensors without visible glass tip

Sensors with stainless steel protection caps, stainless steel tubes, needles, flow through cells, etc. do not have an exposed glass tip and are, therefore, less fragile. However, these sensors still contain a glass sensor inside which can be damaged by physical shock. To protect the sensor, do not let the sensor drop onto the table or floor. If the sensor has a needle, make sure that the needle does not bend or flex. This will break the glass sensor inside.

6.4 Electrical noise

The signal of the microsensor is in the 10^{-13} to 10^{-10} ampere range. Although both the Unisense picoammeter and the Unisense nitrous oxide microsensors are quite 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 and the sensor or wires are not touched during measurements.

6.5 Interference

Exposure to high concentrations of sulfide should be avoided as it can affect the sensitivity of the nitrous oxide microsensor.

Nitric Oxide (NO) is interfering the N_2O sensor signal.

CO_2 in mM concentrations may affect the zero signal of the sensor.

On suspicion of sensor damage, repeat calibration and consult 'Trouble-shooting'.

7. ADVANCED USE OF THE N_2O SENSOR

Unisense can construct nitrous oxide sensors for customer requested applications at an additional cost. Unisense provides several options for customizations and adaptions (e.g. tip size, response time, pressure tolerance, and stirring sensitivity) making accurate measurements possible for even more applications.

Please visit our website for more information (unisense.com/products/customizations)

8. STORAGE AND MAINTENANCE

7.1 Storage

Always store the sensor in the protective plastic tube used for shipping. The nitrous oxide microsensor can be stored with the tip exposed to water or air. The sensor can be stored dry at room temperature (15-30°C) or in the fridge (approx. 4°C) which may give a longer lifetime. If the sensor is used regularly it can be stored polarized.

7.2 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 other detergent.

REFERENCES

- Andersen, K., T. Kjaer, and N. P. Revsbech. 2001. An oxygen insensitive microsensor for nitrous oxide. Sensors and Actuators B-Chemical. 81(1):42-48.

- Elberling, B., Christiansen, H.H. and Hansen, B.U. 2010. High nitrous oxide production from thawing permafrost. Nature geoscience 3:332-335. Abstract

- Horn, M., A. Schramm, and H.L. Drake. 2003. The earthworm gut: an ideal habitat for ingested N_2O-producing microorganisms. Appl. Environ. Microbiol. 69, 1662-1669.

- 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.

TROUBLESHOOTING

Problem	Sensor has a good zero signal but has a very low response to N_2O
Possible cause	Air bubble in the narrow parts of the sensor
Solution	Shake the sensor as you would shake an old school mercury thermometer. See video guide here: unisense.com/video-guides/#troubleshooting
Problem	A slow response
Possible cause	Insoluble compounds deposited at the sensor tip
Solution	Rinse with 96% ethanol, rinse with 0.01M HCl and rinse with water
Problem	An unstable signal or the signal fluctates if the setup is touched or equipmen 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
Problem	High and drifting signal
Possible cause	The sensor tip is broken
Solution	Replace the microsensor
Problem	A high signal
Possible cause 1	Possible oxygen interference if the oxygen front guard is damaged
Solution 1	Replace the microsensor
Possible cause 2	Air is trapped in the tip
Solution 2	Degas water by boiling and subsequent cooling or by 10 min of vacuum treatment. Immerse the sensor tip for 20 min in the degassed water. Repeated or prolonged treatment may be necessary.
Problem	The signal is very low
Possible cause	Contamination of the cathode surface (e.g. by sulfide) or loss of the cathode material due to excessive vibration.
Solution	Replace the microsensor

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 1: EQUILIBRIUM N_2O CONCENTRATIONS

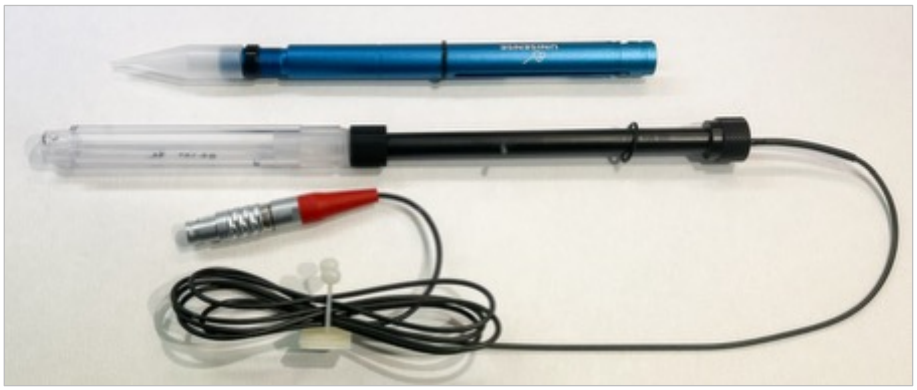
		Temperature (°C)								
Salinity (parts per thousand)		**0**	**5**	**10**	**16**	**22**	**26**	**30**	**36**	**40**
	0	59.35	48.46	40.16	32.66	27.05	24.09	21.61	18.61	16.98
	10	55.85	45.73	37.99	30.96	25.69	22.91	20.57	17.73	16.18
	20	52.58	43.15	35.93	29.35	24.40	21.78	19.58	16.89	15.42
	30	49.50	40.73	33.98	27.82	23.18	20.71	18.63	16.09	14.70
	35	48.03	39.56	33.05	27.09	22.59	20.19	18.18	15.70	14.35
	38	47.17	38.88	32.50	26.66	22.24	19.89	17.91	15.48	14.15
	40	46.60	38.43	32.14	26.37	22.01	19.69	17.73	15.33	14.01

Table 2. Nitrous oxide (mmol N_2O/liter) at ambient partial pressure of 1 atm. in water as a function of temperature and salinity.
Source: Weiss, R.F; Price, B. A.: Marine, Chemistry, 1980, 8, 347-359

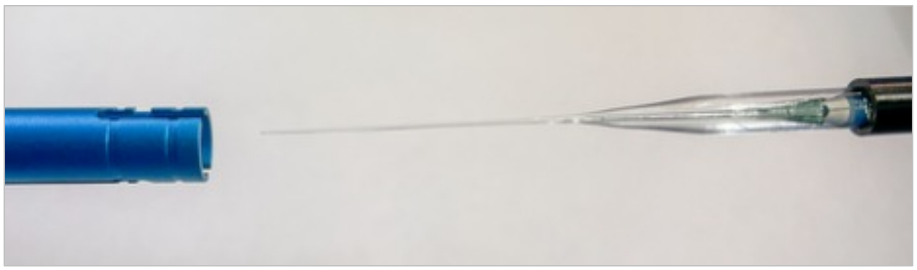
For conversion between units go to the Unit Converter on Unisense website: unisense.com/unit-converter

The Unisense software has a built-in calculator, giving the solubility of N_2O at a given combination of temperature and salinity ("Tools - N_2O Calculator" or the "N_2O table" button in the calibration dialog).

APPENDIX 2: ASSEMBLY OF MICRORESPIRATION SENSOR



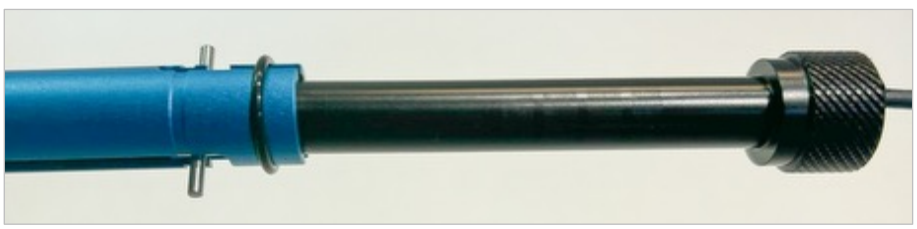
The sensor and the blue aluminium guide before assembly.



Hold the sensor and blue aluminium guide by hand and align them longitudinally.

Insert the sensor gently into the blue aluminium guide.
The sensor tip should not touch the inside of the guide.

Push the sensor in so that the steel pins engage with the slits in the guide.



Mount the O-ring in the groove. This ensures that the sensor stays in place.

UNISENSE
SCIENTIFIC