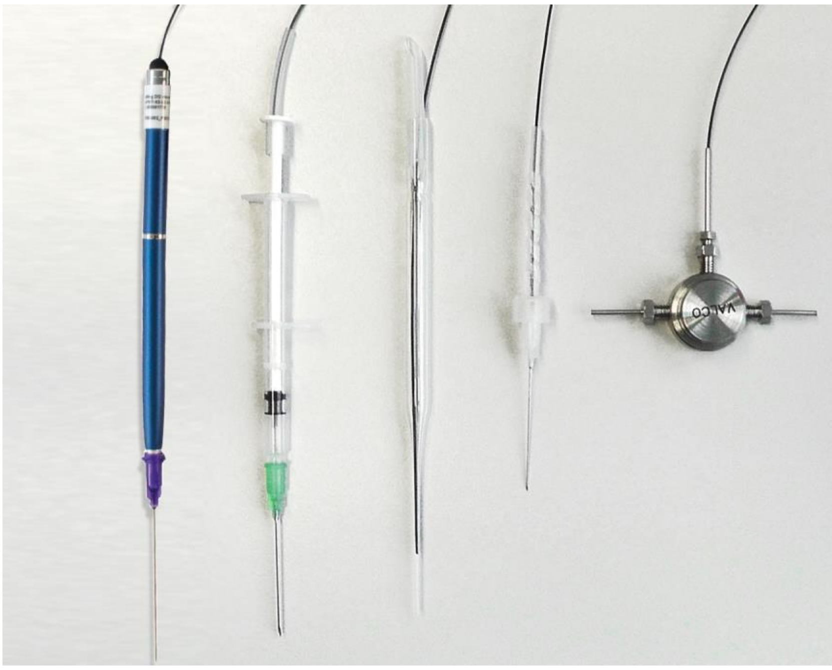
SENSOR PROBES

Oxygen Microsensors

Instruction Manual



PreSens
PRECISION SENSING

Oxygen Microsensors

Specification:

Chemical optical oxygen microsensors for non-invasive or minimal invasive oxygen measurements

Document filename: IM_OxMicro_dv3

Specifications may change without prior notice.

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1 Preface

You have chosen a new, innovative technology for measuring oxygen.

Chemical optical oxygen microsensors (also called optrodes) have several important features:

- They are small.
- Their signal does not depend on the flow rate of the sample.
- They allow measurements with high spatial resolution whenever this is required.

Therefore, they are ideally suited for the examination of small sample volumes.
A set of different oxygen microsensors is available to make sure you have the sensor which matches your application.

Please feel free to contact our service team to find the best solution for your application.

Your PreSens Team

PLEASE READ THE FOLLOWING INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS DEVICE.

2 Sensors and Housings

Oxygen microsensors are designed for all research and packaging applications where a small tip size (< 50 µm) and fast response time (t90 < 1 s) are necessary. They are based on 200 / 230 µm silica fiber and are available with two different sensor tip diameters, a < 50 µm tapered tip and a 230 µm flat-broken tip. Therefore, they are ideally suited for the examination of very small sample volumes and for measuring oxygen gradients in heterogeneous systems with high spatial resolution. Their small dimensions even allow measurements in living systems.
The oxygen microsensors are mounted in different housings.

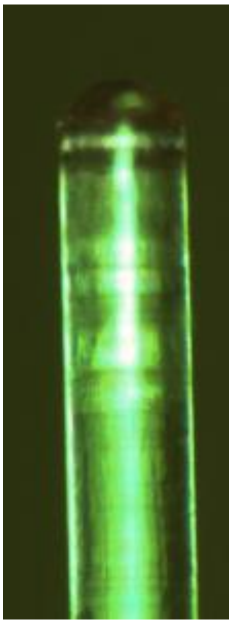


Fig. 1 Tapered sensor tip Flat broken sensor tip

2.1 Needle-Type Oxygen Microsensor (NTH)

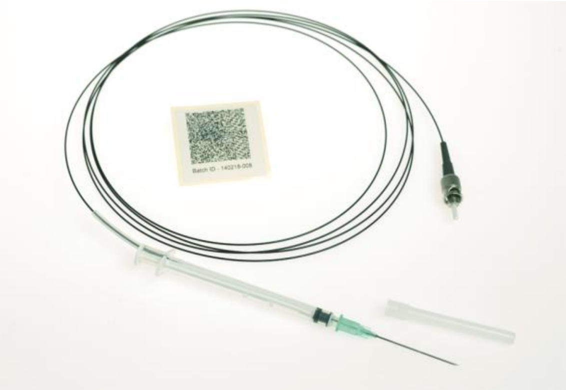


Fig. 2 Needle-type oxygen microsensor NTH

The glass fiber with its oxygen-sensitive tip is protected inside a stainless steel needle and can be extended for measurement. As long as the sensor tip is sheltered inside this needle, it can be easily penetrated through septum rubber or any other harsh material. You can also watch our video on oxygen microsensors on www.presens.de/video-micro.

Fig. 3 Needle-type oxygen microsensor pierced through septum rubber of a pharmaceutical vial

A 1 mL syringe tube made from polypropylene is used as the probe housing.
Fiber cables with a length of up to 10 m and an outer diameter of 0.9 mm are available.

Features

- Measuring range for sensor type PSt7 is 0 - 100 % O_2, 0 - 45 mg/L dissolved oxygen; for sensor type PSt8 it is 0 – 10 % O_2, 0 – 5 mg/L dissolved oxygen.
- Limit of detection for sensor type PSt7 is 0.04 % O_2, 20 ppb dissolved oxygen; for a sensor typePSt8 it is 0.01 % O_2, 4 ppb dissolved oxygen.
- High spatial resolution
- Penetration probe for insertion into semisolids like sediments or biofilms

- Sterilization possible (H_2O_2, EtO, EtOH)
- **Not** autoclavable since the syringe is made out of polypropylene
- Easy to handle and robust

2.2 Needle-Type Housing with Fixed Oxygen Microsensor (NFSG)

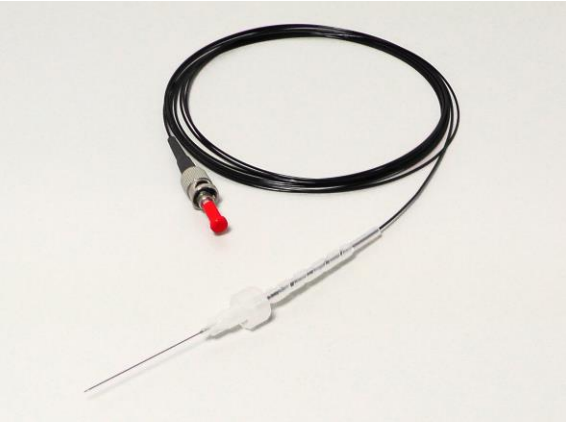


Fig. 4 Needle-type housing with fixed oxygen microsensor NFSG

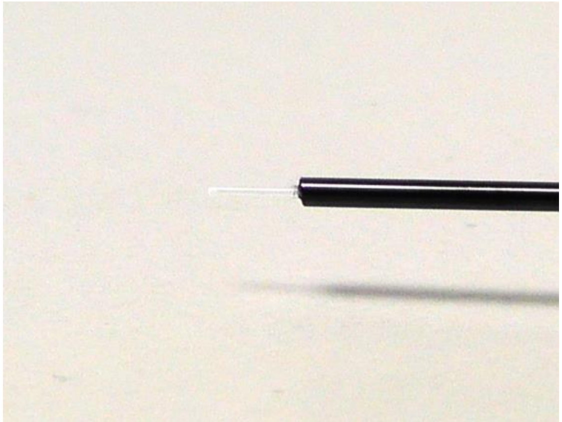
This type offers an oxygen microsensor for all applications where a fixed sensor tip is necessary. As the sensor tip is fixed inside the stainless steel needle, this sensor is advantageous for measurements in packages. It is specially designed for measurement in the gas phase (NFS**G**).
Fiber cables with a standard length of 2.5 m and an outer diameter of 0.9 mm are available.

Features

- Measuring range for sensor type PSt7 is 0 - 100 % O_2, 0 - 45 mg/L dissolved oxygen; for sensor type PSt8 it is 0 – 10 % O_2, 0 – 5 mg/L dissolved oxygen.
- Limit of detection for sensor type PSt7 is 0.04 % O_2, 20 ppb dissolved oxygen; for a sensor type PSt8 it is 0.01 % O_2, 4 ppb dissolved oxygen.
- High spatial resolution
- Penetration probe for insertion into semisolids like sediments or biofilms
- Sterilization possible (H_2O_2, EtO, EtOH)
- **Not** autoclavable since the syringe is made out of polypropylene
- Easy to handle and robust

2.3 Implantable Oxygen Microsensor (IMP)

Fig. 5 Implantable oxygen microsensor IMP



IMP: Bare fiber sensor tip

The microsensor tip is not housed in any additional housing. The bare glass fiber tip can be mounted to custom made housings, home-made steel tubes, or custom-made respirometer chambers. It can be deployed in soil or implanted into animal or plant tissue to measure oxygen online and in real-time.
Small outer diameters of 900 or 500 µm allow insertion into implantable Venflon-tubes. Fiber cables with a length of up to 10 m and an outer diameter of 900 or 500 µm are available.

Features

- Measuring range for sensor type PSt7 is 0 - 100 % O_2, 0 - 45 mg/L dissolved oxygen; for sensor type PSt8 it is 0 – 10 % O_2, 0 – 5 mg/L dissolved oxygen.
- Limit of detection for sensor type PSt7 is 0.04 % O_2, 20 ppb dissolved oxygen; for a sensor type PSt8 it is 0.01 % O_2, 4 ppb dissolved oxygen.
- High spatial resolution
- High flexibility
- Without any housings (the microsensor is protected with a glass housing during transport)
- Sterilization possible (H_2O_2, EtO, EtOH)
- Autoclavable (130 °C / 266 °F, 1.5 atm)
- Implantation into animal blood circuits
- Soil implantation
- Implantation in custom-made housings

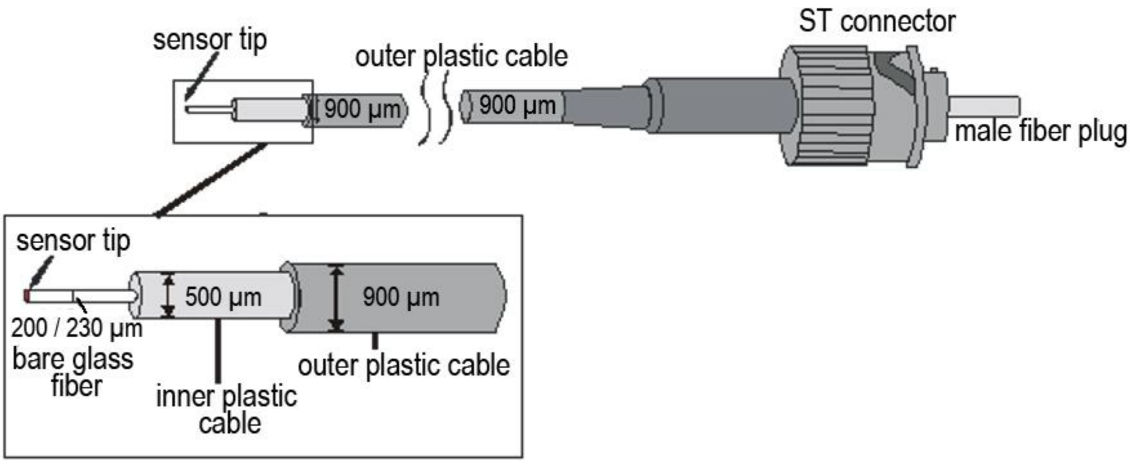


Fig. 6 Schematic illustration of an implantable microsensor's dimensions

! The implantable microsensor can also be delivered without the 900 µm cladding, if the sensor needs to be integrated e. g. in Venflon tubes or catheters.

2.4 Profiling Oxygen Microsensor (PM)

Fig. 7 Profiling O_2 Microsensor

Profiling Microsensors (PM) are the sturdiest microsensor version PreSens offers – with a firmer fiber construction and a splash proof metal housing. They are specifically designed for profiling applications and should be used whenever measurements in semi-solid substrates are performed, such as e. g. sediments, microbial mats or biofilms. Easy handling and their robust design makes them also applicable in many other fields of research and development. The PMs have a close fitting fiber guidance and mechanical interlock for precise vertical localization of the measurement tip. The sensor tip is protected by a blunt steel cannula and can be extended with a turning mechanism.

Features

- Measuring range for sensor type PSt7 is 0 - 100 % O_2, 0 - 45 mg/L dissolved oxygen; for sensor type PSt8 it is 0 – 10 % O_2, 0 – 5 mg/L dissolved oxygen.
- High spatial resolution
- Penetration probe for insertion into semisolids like sediments or biofilms
- Close fitting fiber guidance & mechanical interlock
- Precise vertical localization
- Splash proof metal housing
- No electrical interference due to optical measurement
- Easy to handle

2.5 Metal Flow-Through Cell Housed Oxygen Microsensor (FTCM)

Fig. 8 Metal Flow-through cell housed oxygen microsensor FTCM

The metal flow-through cell housed oxygen microsensor is a miniaturized fiber optic oxygen sensor optimized for fast response time (t_{90} < 1 sec. in gases, < 5 sec. in liquids). The microsensor is integrated in a stainless steel tee with connectors for 1/16” steel tubing. The inner volume of 2.1 µL is extraordinarily small.

Features

- Measuring range for sensor type PSt7 is 0 - 100 % O_2, 0 - 45 mg/L dissolved oxygen; for sensor type PSt8 it is 0 – 10 % O_2, 0 – 5 mg/L dissolved oxygen.
- Limit of detection for sensor type PSt7 is 0.04 % O_2, 20 ppb dissolved oxygen; for a sensor type PSt8 it is 0.01 % O_2, 4 ppb dissolved oxygen.
- Easy to handle and robust
- Online monitoring
- Very fast response time
- Sterilization possible (autoclave (130 °C, 1.5 atm), EtOH, H2O2)

3 Use of NTH and NFSG

3.1 Mounting the Needle-Type Oxygen Microsensor

You can also watch our video about handling oxygen microsensors on http://www.presens.de/support/presens-tv.html.

Carefully remove the microsensor from the protective cover. The needle-type oxygen microsensor is housed in a e. g. 0.8 x 40 mm syringe needle (many other dimensions available) mounted to a 1 mL plastic syringe housing with integrated Push & Pull mechanism. The syringe needle is covered by a protective plastic cap. Carefully remove the protective cap from the syringe needle.

! Make sure to hold the plastic base of the needle so it does not come off when removing the cap.

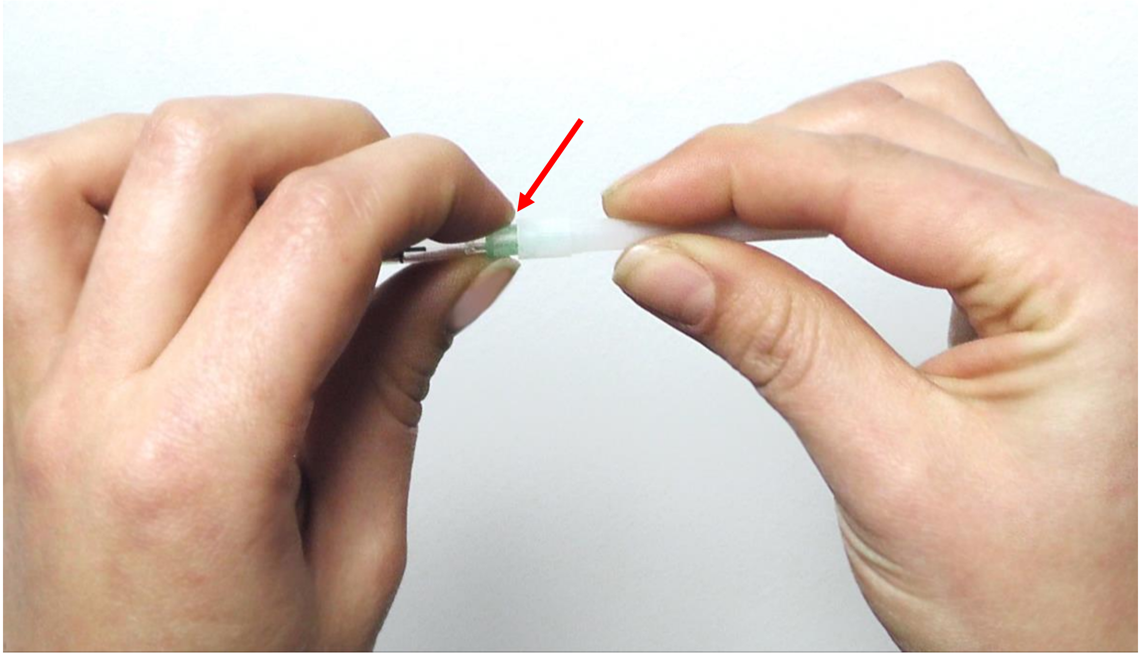


Fig. 9 Hold the plastic needle base when removing the protective cap

Fix the microsensor to the Manual Micromanipulator (www.presens.de/MM) or a similar stable construction.

! Do not handle the microsensor without the support especially when the sensor tip is extended.

Then remove the protective cap at the end of the optical fiber and attach it to the connector on the front panel of your fiber optic transmitter. The ST plug has to be inserted and slightly turned clockwise to fasten it. Be careful not to snap off the optical fiber.

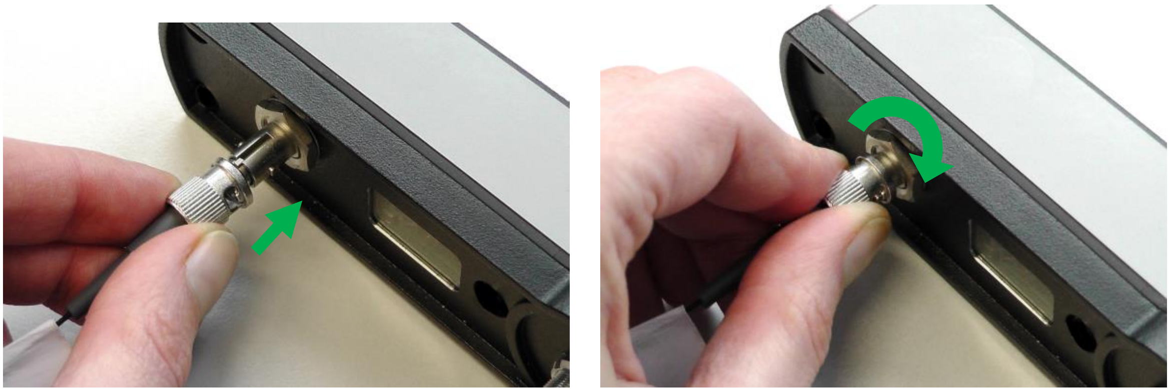


Fig. 10 Attaching the oxygen microsensor to the connector on the transmitter

The glass fiber with its sensing tip is prevented from sliding by a transport block. Remove the transport block from the syringe housing. Now it is possible to extend or retract the optical fiber with its sensor tip by pushing or pulling the plunger.

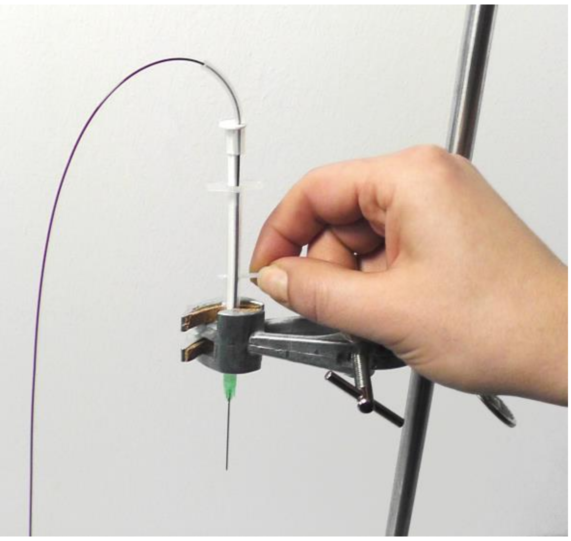


Fig. 11 Removing the transport block from the NTH

! Make sure you have removed the protective plastic cap from the syringe needle and that there is enough space in front of the needle tip before you extend the sensor tip.

3.2 Calibration of a Needle-Type Oxygen Microsensor

It is recommended to calibrate the oxygen microsensor prior to each measurement. Especially after longer measurements (more than 18,000 measuring points or 8 h continuous sensor illumination) the sensor should be re-calibrated. For using calibration values of a prior calibration see the instruction manual of the respective transmitter!

3.2.1 Calibration of an NTH / NFSG Type PSt7

3.2.1.1 Preparation of the Calibration Standards

Calibration of needle-type oxygen microsensors type PSt7 is performed using a conventional two-point calibration in oxygen-free water (**cal 0**) and air-saturated water (**cal 100 / cal 2nd**) or in the gas phase with nitrogen-saturated atmosphere (**cal 0**) and air-saturated water / 20.9 % O_2 (**cal 100 / cal 2nd**).

Cal 0:

- Oxygen-free water:
  To prepare oxygen-free water dissolve 1 g of sodium sulfite (Na_2SO_3) and 50 µL cobalt nitrate ($Co(NO_3)_2$) standard solution (ρ(Co) = 1000 mg/L; in nitric acid 0.5 mol/L) in 100 mL water. Use a suitable vessel with a tightly fitting screw top and label it **cal 0**. Make sure there is only little headspace in your vessel. Due to a chemical reaction of oxygen with the Na_2SO_3 the water becomes oxygen-free. Additional oxygen, diffusing from air into the water, is removed by surplus Na_2SO_3. Close the vessel with the screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free. Keep the vessel closed after calibration with a screw top to minimize oxygen contamination.
  To prepare oxygen-free water you also can use sodium dithionite ($Na_2S_2O_4$). The shelf life of **cal 0** is about 24 hours provided that the vessel has been closed with the screw top.

For calibration in the gas phase (NFSG):

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- Air-saturated water:
  Add 100 mL water to a suitable vessel and label it **cal 100 / cal 2nd**. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (air stone), creating a multitude of small air bubbles, while stirring the solution. After 20 minutes, switch of the air-pump and stir the solution for another 10 minutes to ensure that the water is not supersaturated.

For calibration in the gas phase (NFSG):

- Water vapor-saturated air or 20.9 % O_2
  Use a certified test gas of 20.9 % O2 (suppliers are e. g. Air Liquide, Linde, Westfalen AG) or water-vapor saturated air as calibration standard **cal 100 / cal 2nd**. Place wet cotton wool in the vessel you have mounted the sensor in. Close the vessel with a fitting screw top or lid. (For inserting the microsensor and temperature sensor in the vessel you might have to drill holes in the lid.) Wait about 2 minutes to ensure that the air is water vapor-saturated.

3.2.1.2 Calibration Procedure for NTH in Liquid Phase

For instructions and further information on software settings and handling see the respective transmitter manual!

First record the second calibration value, air-saturated water. Place **cal 100 / cal 2nd** below the microsensor fixed to the laboratory support. (If you want to use automatic temperature compensation connect the temperature sensor to the respective connector on the oxygen meter, and put it into **cal 100 / cal 2nd**. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.)

! Please ensure that the NTH glass fiber with its sensor tip is not yet extended.

Carefully locate the syringe needle of the NTH above the water surface and extend the sensor tip. Ensure that only the sensor tip is dipped into **cal 100 / cal 2nd** and not the protective syringe needle.

Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the values are recorded, pull back the NTH sensor tip into the protective syringe needle and remove **cal 100 / cal 2nd**.

Then place the vessel with the label **cal 0** underneath the microsensor. (Put the temperature sensor into **cal 0**. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.)

Fig. 12 NTH and temperature sensor placed in calibration standard 0 (Cal 0)

! Please ensure that the NTH glass fiber with its sensor tip is not extended.

Carefully locate the NTH syringe needle above the water surface. Slowly press the syringe plunger and extend the glass fiber with its sensor tip from the protective syringe needle. Ensure that only the sensor tip is dipped into **cal 0** and not the protective syringe needle.

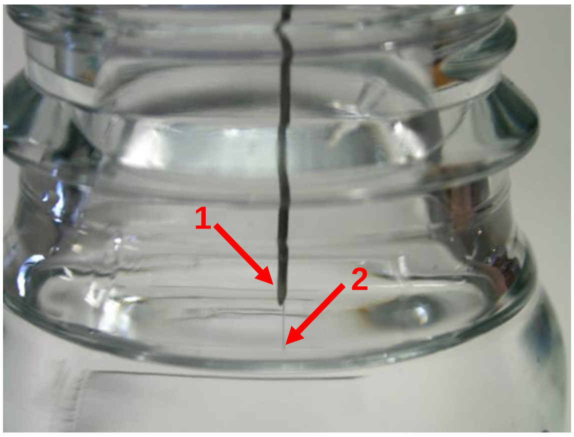


Fig. 13 The syringe needle has to be located over the water surface (1) – then extend the microsensor fiber into the liquid (2)

Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively).

Fig. 14 Wash the NTH with distilled water if the needle had been dipped into water with sodium sulfite

Whenever the glass fiber and temperature sensor have been dipped into **cal 0** sodium sulfite solution wash them with distilled water to clean them from sodium sulfite. If the syringe needle has been dipped into **cal 0** by mistake, wash the glass fiber and the syringe needle thoroughly with distilled water to avoid salt crystallization within the syringe needle. Salt crystallization may seal the needle and the glass fiber with its sensor tip will break when extended.

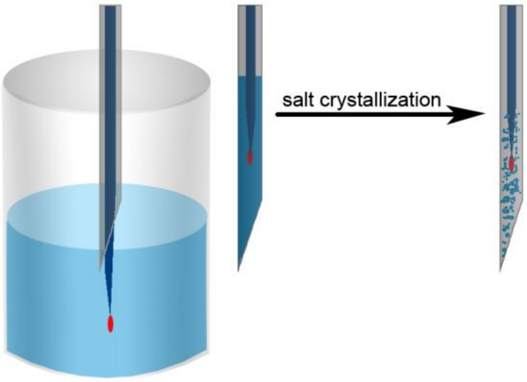


Fig. 15 Schematic illustration of salt crystallization inside the syringe needle

Pull the sensor tip back into its protective syringe needle before removing the microsensor from the calibration vessel. Protect the syringe plunger against slipping out by inserting the transport block back into the syringe housing and do not remove it again until just before measurement.

3.2.1.3 Calibration Procedure for NTH and NFSG in the Gas Phase

! Please use nitrogen-saturated atmosphere and water-vapor saturated air / 20.9 % O_2 for calibrating the NFSG, as this sensor is not designed for use in the liquid phase.

Place the oxygen microsensor fixed to a laboratory support inside the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the oxygen microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.) In case you are working with an NTH you can then carefully extend the sensor tip.

Lead the first gas (N_2 5.0) in the vessel with the oxygen microsensor (Dry mode). In case you want to perform the calibration in Humid mode lead the gas into a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 16 for schematic illustration of set-up). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (20.9 % O_2) into in the vessel with the oxygen microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel, or place the microsensor in a vessel with water-vapor saturated atmosphere (as described in 3.2.1.1). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply or remove the sensor from water-vapor saturated atmosphere.

! Please ensure that the NTH glass fiber with its sensor tip is retracted in the steel needle before removing it from the calibration vessel.

3.2.2 Calibration of an NTH / NFSG Type PSt8

3.2.2.1 Preparation of Calibration Standards

Calibration of an oxygen microsensor type PSt8 is performed with certified gases (suppliers are e. g. Air Liquide, Linde, Westfalen AG).

Cal 0:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- 2 – 4 % O_2
  The second calibration value **cal 100 / cal 2nd** for a PSt8 sensor is ideally in the range between 10 and 20 % air sat. (ca. 2 - 4 % O_2 → % air sat. = % O_2 x 100/20.95). Use a commercially available test gas of 2 – 4 % O_2 as a second calibration standard **cal 100 / cal 2nd**.

3.2.2.2 Calibration Procedure

Place the oxygen microsensor fixed to a laboratory support inside the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the oxygen microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.) In case you are working with an NTH you can then carefully extend the sensor tip.

Lead the first gas (N_2 5.0) into the vessel with the oxygen microsensor (Dry mode). If you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 16). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (2 – 4 % O_2) into the vessel with the oxygen microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 16). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply.

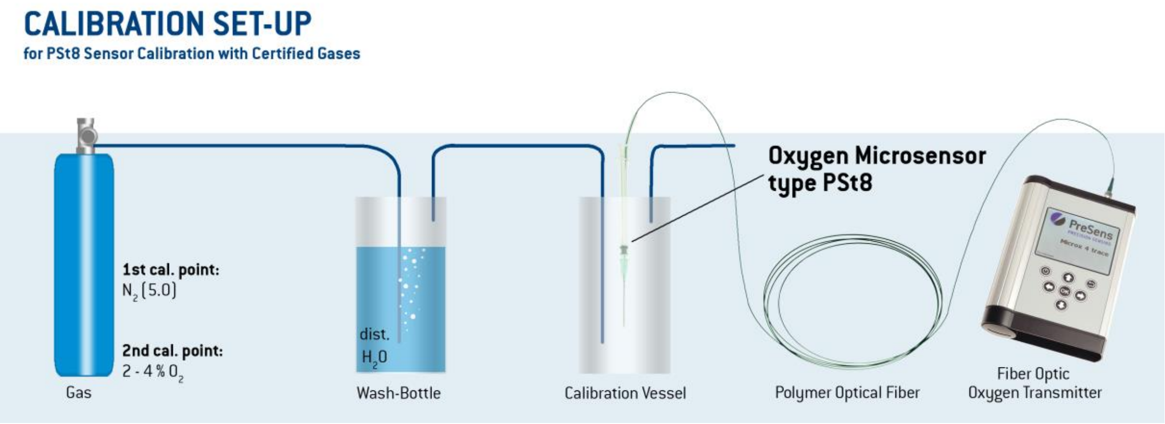


Fig. 16 Schematic illustration: Calibration in Humid mode of an oxygen microsensor type PSt8 with certified gases

If it is not possible to use gases or to build a suitable calibration chamber, a manual calibration or calibration via barcode scan can be performed, using calibration values obtained from the final inspection protocol or the barcode delivered with the sensor. You can find more information about manual calibration in the instruction manual of the respective transmitter.

3.2.3 Manual Calibration

Manual calibration is performed using the respective transmitter or PreSens Measurement Studio software. Please refer to the respective transmitter or PreSens Measurement Studio instruction manual for further information!

3.3 Measurement with a Needle-Type Oxygen Microsensor

Carefully read chapter 3.1 on mounting a needle-type microsensor before starting your measurements.

Position the microsensor right above your sample. The syringe needle - with retracted glass fiber – can be pierced through a septum or immersed into a tissue. Then remove the transport block.

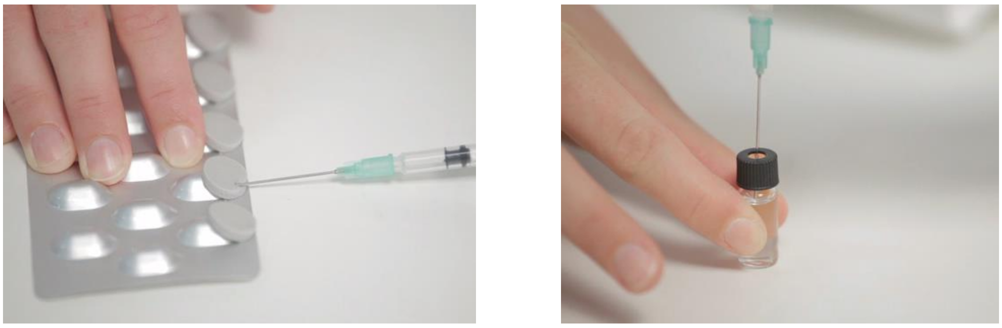


Fig. 17 Measurement with a needle-type oxygen microsensor in a blister and a pharmaceutical vial – the syringe needle is pierced through a septum, then the sensor tip is extended.

Extend the glass fiber with its sensor tip from the syringe needle by carefully pressing the syringe plunger.

! Please take into account that the fine glass fiber with its sensor tip is very delicate! Avoid mechanical stress as far as possible.

! For measurements inside tissue or semisolids it is recommended to use the Manual Micromanipulator (www.presens.de/MM), so the sensor can be inserted in the sample without any vibrations. The micromanipulator has a safe-insert function, which prevents breaking of the microsensor tip, and it also allows exact positioning in a certain sample depth.

Fig. 18 Needle-type oxygen microsensor mounted on the Manual Micromanipulator.

The sensor tip of an NTH will only measure accurately if the glass fiber with its sensor tip has been completely extended from the syringe needle. Inside the needle there is an air reservoir, in which the oxygen content is different from your sample!

Rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Retract the sensor tip into the protective housing and insert the transport block to prevent the syringe plunger from slipping.

4 Use of IMP

4.1 Mounting the Implantable Oxygen Microsensor

Carefully remove the microsensor from the protective cover. The implantable oxygen microsensor is protected by a glass housing during transport.
Fix the microsensor with the glass housing to a laboratory support or a similar stable construction.

! Do not handle the microsensor without the support especially when the sensor tip is unprotected.

Then remove the protective cap from the ST plug of the optical fiber and attach it to the connector on the front panel of your fiber optic transmitter. The ST plug has to be inserted and slightly turned clockwise to fasten it. Be careful not to snap off the optical fiber.

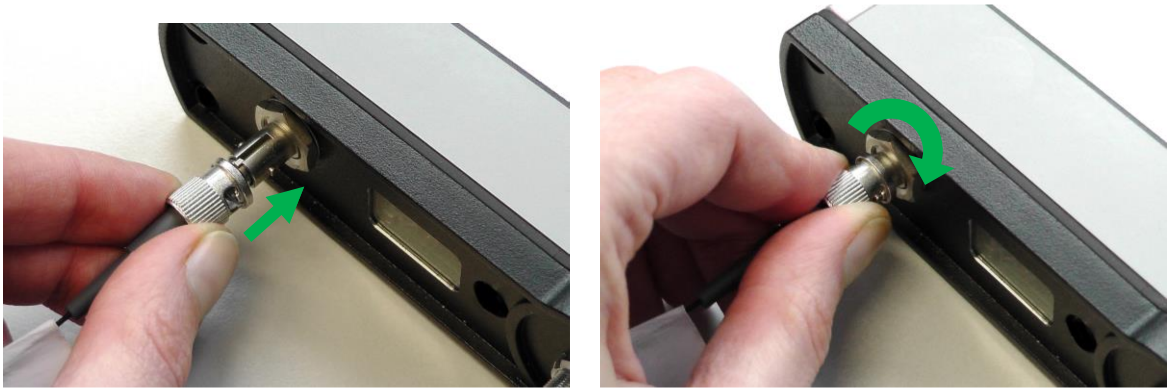


Fig. 19 Attaching the oxygen microsensor to the connector on the transmitter

The glass fiber with its sensing tip is prevented from slipping by a protection tubing. Loosen the protection tubing from the glass housing, extend the sensor tip about 1 cm and fix the glass fiber again with the protection tubing.

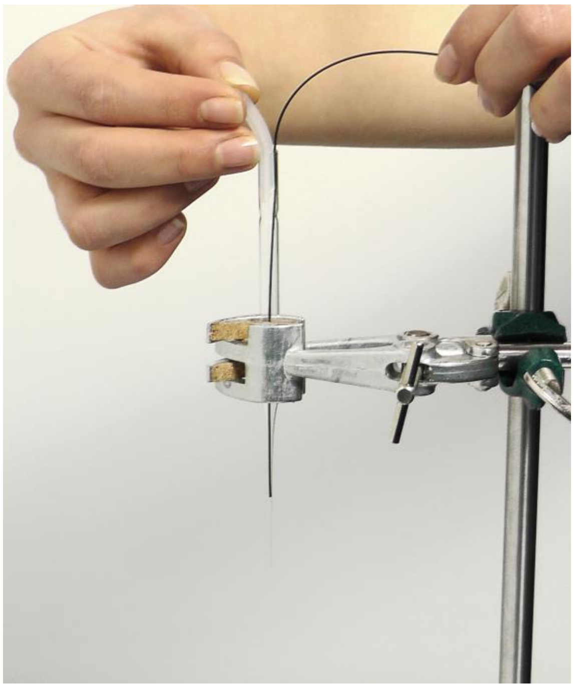


Fig. 20 Loosening the protection tubing to extend the sensor tip from the glass protection tubing

! Handle the microsensor with care when the glass fiber with its sensor tip is extended. The glass fiber is unprotected and might break!

4.2 Calibration of an Implantable Oxygen Microsensor

It is recommended to calibrate the oxygen microsensor prior to each measurement. Especially after longer measurements (more than 18,000 measuring points or 8 h continuous sensor illumination) the sensor should be re-calibrated.
For using calibration values of prior calibration see the instruction manual of the respective transmitter!

4.2.1 Calibration of an IMP Type PSt7

4.2.1.1 Preparation of the Calibration Standards

Calibration of implantable oxygen microsensors type PSt7 is performed using a conventional two-point calibration in oxygen-free water (**cal 0**) and air-saturated water (**cal 100 / cal 2nd**) or in the gas phase with nitrogen-saturated atmosphere (**cal 0**) and water vapor-saturated air or 20.9 % O_2 (**cal 100 / cal 2nd**).

Cal 0:

- Oxygen-free water:
  To prepare oxygen-free water dissolve 1 g of sodium sulfite (Na_2SO_3) and 50 µL cobalt nitrate ($Co(NO_3)_2$) standard solution (ρ(Co) = 1000 mg/L; in nitric acid 0.5 mol/L) in 100 mL water. Use a suitable vessel with a tightly fitting screw top and label it **cal 0**. Make sure there is only little headspace in your vessel. Due to a chemical reaction of oxygen with the Na_2SO_3 the water becomes oxygen-free. Additional oxygen, diffusing from air into the water, is removed by surplus Na_2SO_3. Close the vessel with the screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free. Keep the vessel closed after calibration with a screw top to minimize oxygen contamination.
  To prepare oxygen-free water you also can use sodium dithionite ($Na_2S_2O_4$). The shelf life of **cal 0** is about 24 hours provided that the vessel has been closed with the screw top.

For calibration in the gas phase:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- Air-saturated water:
  Add 100 mL water to a suitable vessel and label it **cal 100 / cal 2nd**. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (air stone), creating a multitude of small air bubbles, while stirring the solution. After 20 minutes, switch of the air-pump and stir the solution for another 10 minutes to ensure that the water is not supersaturated.

For calibration in the gas phase:

- Water vapor-saturated air or 20.9 % O_2
  Use a certified test gas of 20.9 % O2 (suppliers are e. g. Air Liquide, Linde, Westfalen AG) or water-vapor saturated air as calibration standard **cal 100 / cal 2nd**. Place wet cotton wool in the vessel you have mounted the sensor in. Close the vessel with a fitting screw top or lid. (For inserting the microsensor and temperature sensor in the vessel you might have to drill holes in the lid.) Wait about 2 minutes to ensure that the air is water vapor-saturated.

4.2.1.2 Calibration Procedure for IMP in the Liquid Phase

For instructions and further information on software settings and handling see the respective transmitter manual!

First record the second calibration value, air saturated water. Place **cal 100 / cal 2nd** below the microsensor fixed to the laboratory support. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the oxygen microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.)

! Please ensure that the glass fiber with its sensor tip is not extended.

Carefully locate the glass housing about 5 mm above the water surface and loosen the protection tubing. Then extend the sensor tip about 1 cm and fix it again with the protection tubing. Ensure that the sensor tip is dipped about 4 mm into **cal 100 / cal 2nd** but not the protective glass housing.
Wait for about 3 minutes until the phase angle and temperature value displayed in the software are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the values are recorded, pull back the sensor tip into the protective syringe needle and remove **cal 100 / cal 2nd**.

Place the vessel with the label **cal 0** underneath the microsensor fixed to the laboratory support. Put it into **cal 0**. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.

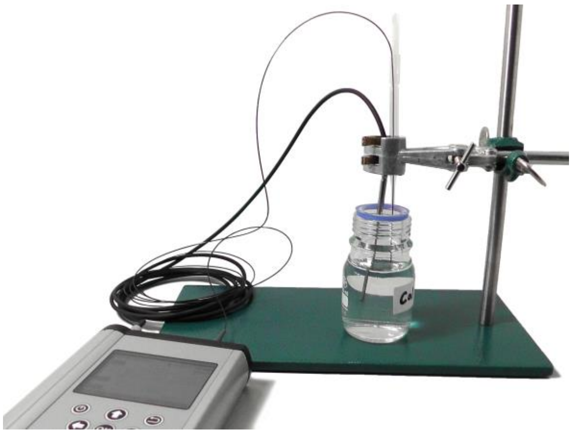


Fig. 21 IMP and temperature sensor placed in calibration standard 0 (Cal 0)

Ensure that the sensor tip is inside the protective glass housing. Carefully locate the glass housing about 5 mm above the water surface. Loosen the protection tubing from the glass housing, extend the sensor tip about 1 cm and fix the glass fiber again with the protection tubing.

Ensure that the sensor tip is dipped about 4 mm into **cal 0** but not the protective glass housing.

Wait for about 3 minutes until the phase angle and temperature value displayed in the software are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively).

Whenever the glass fiber and temperature sensor have been dipped into **cal 0** wash them with distilled water to clean them from sodium sulfite. If the glass housing has been dipped into **cal 0** by mistake, wash the glass fiber and the glass housing thoroughly with distilled water to avoid salt crystallization. Salt crystallization may seal the glass housing and the glass fiber with its sensor tip will break when extended.

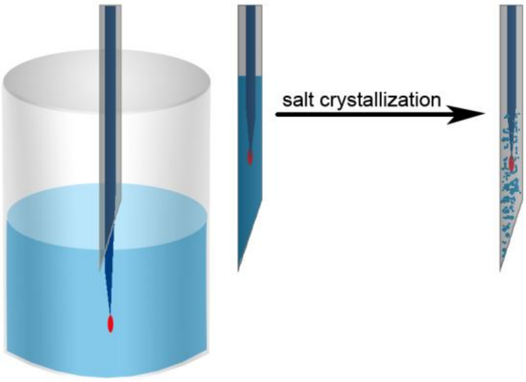


Fig. 22 Schematic illustration of salt crystallization inside the syringe needle

Pull the sensor tip back into its protective glass housing before removing the microsensor from the calibration vessel. Use the protection tubing to fix the glass fiber and do not remove it again until just before measurement.

4.2.1.3 Calibration Procedure for IMP in the Gas Phase

Place the oxygen microsensor fixed to a laboratory support inside the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the oxygen microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.)

Lead the first gas (N_2 5.0) in the vessel with the oxygen microsensor (Dry mode). In case you want to perform the calibration in Humid mode lead the gas into a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 23 for schematic illustration of set-up). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (20.9 % O_2) into in the vessel with the oxygen microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel, or place the microsensor in a vessel with water-vapor saturated atmosphere (as described in 4.2.1.1). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply or remove the sensor from water-vapor saturated atmosphere.

4.2.2 Calibration of an IMP Type PSt8

4.2.2.1 Preparation of Calibration Standards

Calibration of an implantable oxygen microsensor type PSt8 is performed with certified gases (suppliers are e. g. Air Liquide, Linde, Westfalen AG).

Cal 0:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- 2 – 4 % O_2
  The second calibration value **cal 100 / cal 2nd** for a PSt8 sensor is ideally in the range between 10 and 20 % air sat. (ca. 2 – 4 % O_2 → % air sat. = % O_2 x 100/20.95). Use a commercially available test gas of 2 – 4 % O_2 as a second calibration standard **cal 100 / cal 2nd**.

4.2.2.2 Calibration Procedure

For instructions and further information on software settings and handling see the respective transmitter manual!

Place the oxygen microsensor fixed to a laboratory support inside the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the oxygen microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.) Then carefully extend the sensor tip.

Lead the first gas (N_2 5.0) into the vessel with the oxygen microsensor (Dry mode). If you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 23). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (2 – 4 % O_2) into the vessel with the oxygen microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 23). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply.

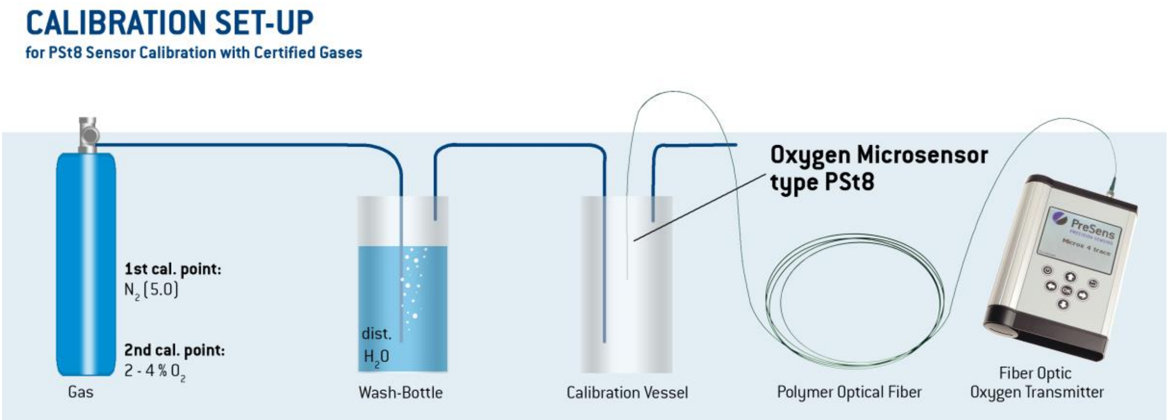


Fig. 23 Schematic illustration: Calibration in Humid mode of an implantable oxygen microsensor type PSt8 with certified gases

If it is not possible to use gases or to build a suitable calibration chamber, a manual calibration or calibration via barcode can be performed, using calibration values obtained from the final inspection protocol or the barcode delivered with the sensor. You can find more information about manual calibration in the instruction manual of the respective transmitter.

4.2.3 Manual Calibration

Manual calibration is performed using the respective transmitter or PreSens Measurement Studio software. Please refer to the respective transmitter or PreSens Measurement Studio instruction manual for further information!

4.3 Measurement with an Implantable Oxygen Microsensor

Carefully read chapter 4.1 on mounting an implantable microsensor before starting your measurements.

The glass fiber with its sensing tip is prevented from slipping from the glass housing by a protection tubing. Remove the fiber cable from the glass housing for implantation. Loosen the protection tubing from the glass housing and carefully extract the fiber. Be careful not to touch the glass housing with the sensor tip!

! When the glass fiber with its sensor tip is extracted from the protective housing, handle it with care. The glass fiber is unprotected and might break.

Be careful while implanting the microsensor into your specially designed system. Please contact our service team when using the microsensor with custom-designed systems.

Rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Place it back in the protective housing and insert the protection tubing when you are done with your measurements.

5 Use of PM

5.1 Mounting the Profiling Oxygen Microsensor

! We recommend the use of a Manual Micromanipulator (MM) or Automated Micromanipulator (AM) when applying the Profiling Microsensor.

A PM microsensor can simply be placed in the sensor holder of the MM or AM. Fasten the holder with the screw on top so the microsensor is attached below the turning mechanism of the PM (silver ring). Tighten the holder firmly so the microsensor cannot move back or forth, or even wedge in the holder. In case you are not using a micromanipulator, at least fix the microsensor with a clip to a laboratory support, or a similar stable construction.
Remove the protective plastic cap that covers the needle.

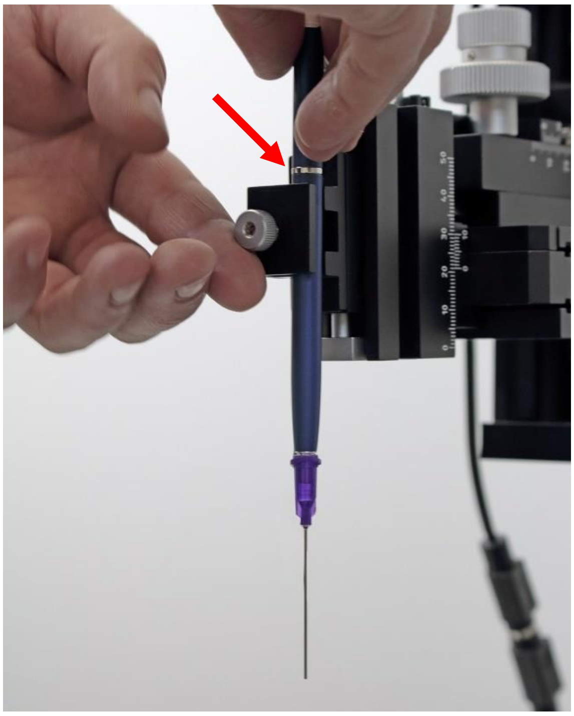


Fig. 24 Mounting a PM to the sensor holder; the turning mechanism (silver ring) has to be placed above the holder.

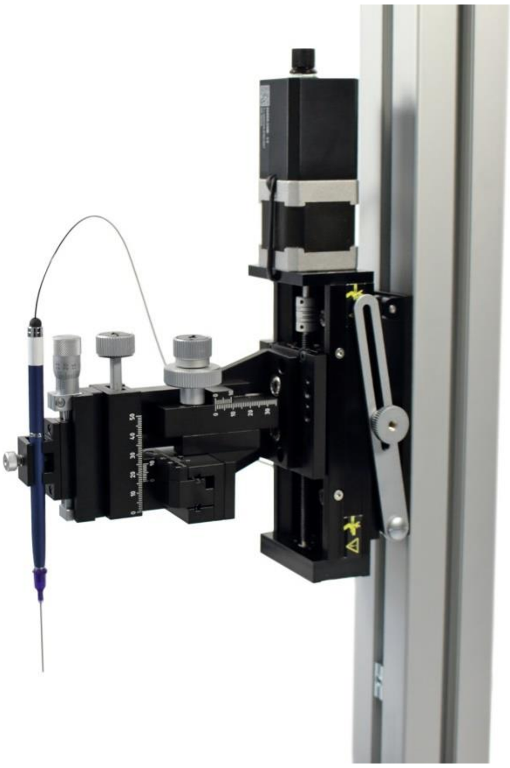


Fig. 25 PM fixed in an Automated Micromanipulator

Then remove the protective cap at the end of the optical fiber and attach it to the sensor connector on your fiber optic oxygen meter. The ST plug has to be inserted and slightly turned clockwise to fasten it. Be careful not to snap off the optical fiber.

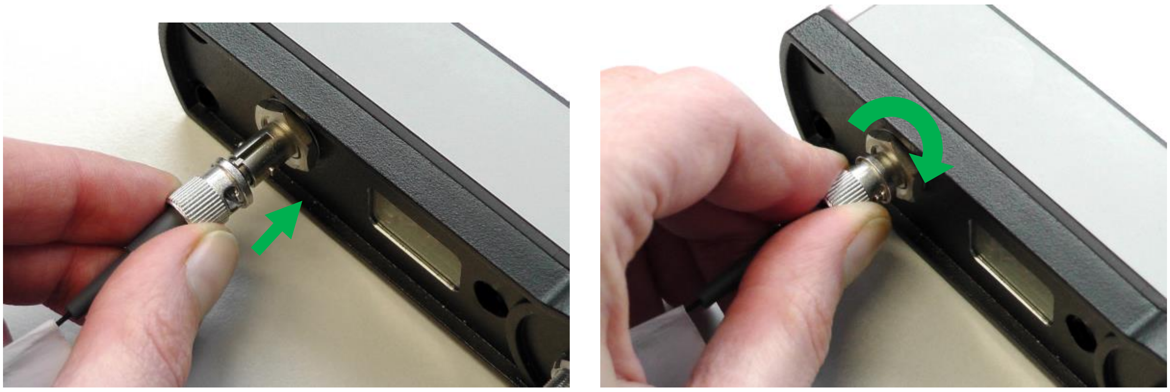


Fig. 26 Attaching a PM to the sensor connector on the meter (here PM-PSt7 and Microx 4).

5.2 Calibration of the Profiling Oxygen Microsensor

It is recommended to calibrate the oxygen Profiling Microsensor prior to each measurement. Especially after longer measurements (more than 18,000 measuring points or 8 h continuous sensor illumination) the sensor should be re-calibrated. For using calibration values of a prior calibration see the instruction manual of the respective oxygen meter!

5.2.1 Calibration of PM Type PSt7

5.2.1.1 Preparation of the Calibration Standards

Calibration of a Profiling Microsensor type PSt7 is performed using a conventional two-point calibration in oxygen-free water (**cal 0**) and air-saturated water (**cal 100 / cal 2nd**) or in the gas phase with nitrogen-saturated atmosphere (**cal 0**) and water vapor-saturated air / 20.9 % O_2 (**cal 100 / cal 2nd**).

Cal 0:

- Oxygen-free water:
  To prepare oxygen-free water dissolve 1 g of sodium sulfite (Na_2SO_3) and 50 µL cobalt nitrate ($Co(NO_3)_2$) standard solution (ρ(Co) = 1000 mg/L; in nitric acid 0.5 mol/L) in 100 mL water. Use a suitable vessel with a tightly fitting screw top and label it **cal 0**. Make sure there is only little headspace in your vessel. Due to a chemical reaction of oxygen with the Na_2SO_3 the water becomes oxygen-free. Additional oxygen, diffusing from air into the water, is removed by surplus Na_2SO_3. Close the vessel with the screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free. Keep the vessel closed after calibration with a screw top to minimize oxygen contamination.

For calibration in the gas phase:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- Air-saturated water:
  Add 100 mL water to a suitable vessel and label it **cal 100 / cal 2nd**. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (air stone), creating a multitude of small air bubbles, while stirring the solution. After 20 minutes, switch of the air-pump and stir the solution for another 10 minutes to ensure that the water is not supersaturated. (Please consider that this procedure might lead to a temperature drop in the liquid. Best use automatic temperature compensation during calibration, or make sure to enter the correct calibration temperature.)

For calibration in the gas phase:

- Water vapor-saturated air or 20.9 % O_2
  Use a certified test gas of 20.9 % O2 (suppliers are e. g. Air Liquide, Linde, Westfalen AG) or water-vapor saturated air as calibration standard **cal 100 / cal 2nd**. Place wet cotton wool in the vessel you have mounted the sensor in. Close the vessel with a fitting screw top or lid. (For inserting the microsensor and temperature sensor in the vessel you might have to drill holes in the lid.) Wait about 2 minutes to ensure that the air is water vapor-saturated.

5.2.1.2 Calibration Procedure for PM-PSt7 in Liquid Phase

For instructions and further information on software settings and handling see the respective transmitter manual!

First record the second calibration value, air-saturated water. Place **cal 100 / cal 2nd** below the microsensor fixed to a laboratory support or micromanipulator. (If you want to use automatic temperature compensation connect the temperature sensor to the respective connector on the oxygen meter, and put it into **cal 100 / cal 2nd**. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.)

! Please ensure that the PM glass fiber with its sensor tip is not extended.

Carefully locate the cannula of the PM above the water surface and extend the sensor tip. Ensure that the sensor tip is dipped into **cal 100 / cal 2nd**.

Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the values are recorded, pull back the sensor tip into the protective cannula and remove **cal 100 / cal 2nd**.

Then place the vessel with **cal 0** underneath the microsensor. (Put the temperature sensor into **cal 0**. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.)

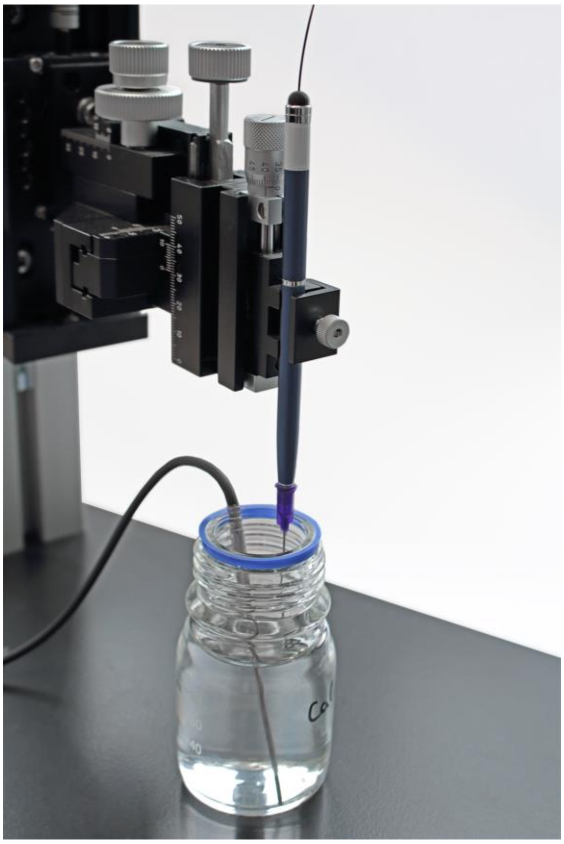


Fig. 27 PM and temperature sensor placed in calibration standard 0 (Cal 0)

! Please ensure that the PM glass fiber with its sensor tip is not extended.

Carefully locate the PM cannula above the water surface and extend the glass fiber with its sensor tip. Ensure that the sensor tip is dipped into **cal 0**.

Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively).

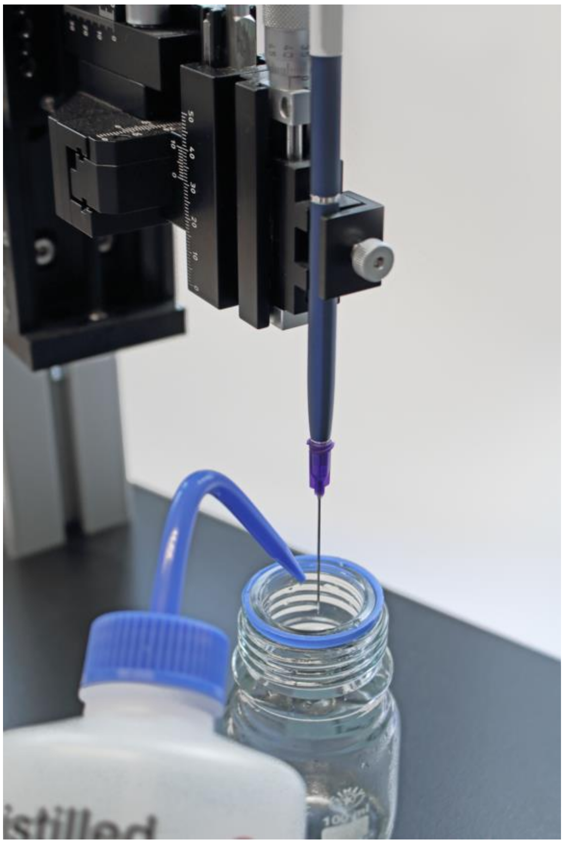


Fig. 28 Wash the PM with distilled water if the needle has been dipped into water with sodium sulfite

Whenever the glass fiber has been dipped into **cal 0** containing sodium sulfite, wash the glass fiber and the syringe needle thoroughly with distilled water to avoid salt crystallization within the cannula. Salt crystallization may seal the cannula and the glass fiber with its sensor tip might break when extended.

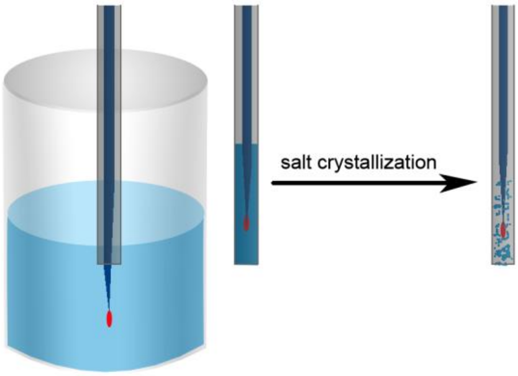


Fig. 29 Schematic illustration of salt crystallization inside the cannula

Pull the sensor tip back into its protective cannula before removing the microsensor from the calibration vessel.

5.2.1.3 Calibration Procedure for PM-PSt7 in the Gas Phase

Place the oxygen Profiling Microsensor fixed to a laboratory support or micromanipulator inside the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the microsensor. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.) Then carefully extend the sensor tip.

Lead the first gas (N_2 5.0) in the vessel with the oxygen Profiling Microsensor (Dry mode for Microx 4 & Microx 4 trace). In case you want to perform the calibration in Humid mode lead the gas into a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 30Fig. 16 for schematic illustration of set-up). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (20.9 % O_2) in the vessel with the oxygen microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel, or place the microsensor in a vessel with water-vapor saturated atmosphere (as described in 5.2.1.1). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply or remove the sensor from water-vapor saturated atmosphere.

! Please ensure that the PM glass fiber is retracted in the steel cannula before removing it from the calibration vessel.

5.2.2 Calibration of a PM Type PSt8

5.2.2.1 Preparation of Calibration Standards

Calibration of Profiling Microsensors type PSt8 is performed with certified gases (suppliers are e. g. Air Liquide, Linde, Westfalen AG).

Cal 0:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- 2 – 4 % O_2
  The second calibration value **cal 100 / cal 2nd** for a PSt8 sensor is ideally in the range between 10 and 20 % air sat. (ca. 2 - 4 % O_2 → % air sat. = % O_2 x 100/20.95). Use a commercially available test gas of 2 – 4 % O_2 as a second calibration standard **cal 100 / cal 2nd**.

5.2.2.2 Calibration Procedure for PM-PSt8

Place the oxygen Profiling Microsensor fixed to a laboratory support or micromanipulator in the calibration vessel / calibration chamber. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it into the calibration vessel, together with the PM. If necessary fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor tip.) You can then carefully extend the sensor tip.

Lead the first gas (N_2 5.0) into the vessel with the oxygen Profiling Microsensor (Dry mode for Microx 4 & Microx 4 trace). If you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 30). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (2 – 4 % O_2) into the vessel with the oxygen Profiling Microsensor. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the calibration vessel (see Fig. 30). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply.

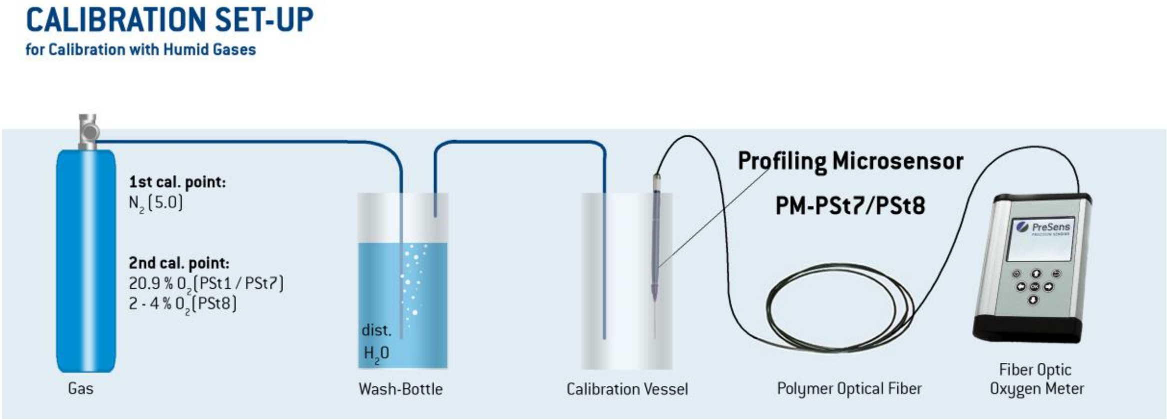


Fig. 30 Schematic illustration: Set-up for calibration with humid gases

If it is not possible to use gases or to build a suitable calibration chamber, a manual calibration or calibration via barcode scan can be performed, using calibration values obtained from the final inspection protocol or the barcode delivered with the sensor. You can find more information about manual calibration in the instruction manual of the respective oxygen meter.

5.2.3 Manual Calibration

The Profiling Microsensors are pre-calibrated. The enclosed Final Inspection Protocol contains the calibration values that have to be entered into the PreSens Profiling Studio or Measurement Studio software, or the oxygen meter you are using. Please refer to the oxygen meter's or the respective software instruction manual for further information on "Manual Calibration".

5.3 Measurement with a Profiling Oxygen Microsensor

! The sensor tip will only measure accurately if the glass fiber with its sensor tip has been completely extended from the cannula.

Carefully read chapter 5.1 on mounting a Profiling Microsensor before starting your measurements. The sensor cannula - with retracted glass fiber – can be pierced through a septum or immersed into tissue.

1. Position the PM with retracted sensor tip above your sample and move it manually with the micromanipulators coarse and fine drive into starting position for profiling.

2. Then turn the upper part of the PM clockwise until the sensor tip is extended.

3. In case you are working with an Automated Micromanipulator you will have to perform **Zero Level Adjustment** via the software now (read the AM instruction manual for more details).

4. Start you profiling measurements via the PreSens Profiling Studio or the oxygen meter's software.

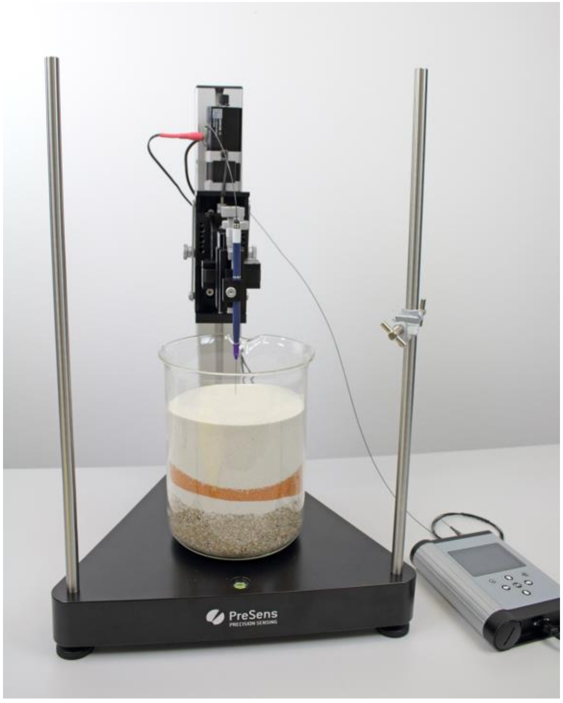


Fig. 31 Sediment profiling with a PM-PSt7 mounted in an AM

5. When you have finished your measurements, remove the sensor from the sample and rinse the glass fiber and sensor tip with distilled water to remove any sample residues. Finally retract the sensor tip into the protective steel cannula.

6 Use of FTCM

6.1 Mounting the Flow-Through Cell Metal Housing Oxygen Microsensor (FTCM)

Fig. 32 Flow-through cell metal housing oxygen microsensor (FTCM)

Carefully remove the metal flow-through cell with its integrated oxygen microsensor from the protective cover.
Connect the flow-through cell with the tubing of your flow-through system. Make sure the optical fiber is not exceedingly bent, else it might break. You can use a laboratory support or similar stable construction to hold the FTCM in place.
Then remove the protective cap at the end of the optical fiber and attach it to the connector on the front panel of your fiber optic transmitter. The ST plug has to be inserted and slightly turned clockwise to fasten it. Be careful not to snap off the optical fiber.

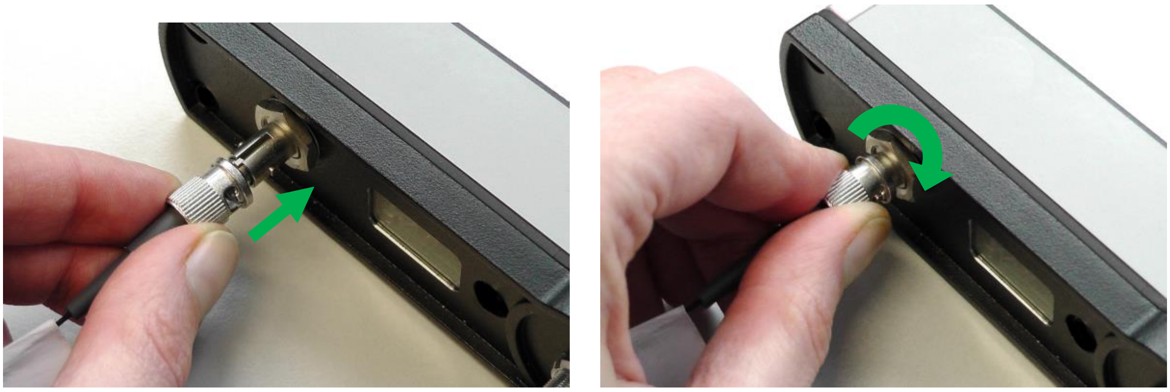


Fig. 33 Attaching the oxygen microsensor to the connector on the transmitter

6.2 Calibration of a Flow-Through Cell Metal Housing Oxygen Microsensor

It is recommended to calibrate the oxygen microsensor prior to each measurement.
Especially after longer measurements (more than 18,000 measuring points or 8 h continuous sensor illumination) the sensor should be re-calibrated.
For using calibration values of prior calibrations see the instruction manual of the respective transmitter!

6.2.1 Calibration of FTCM Type PSt7

6.2.1.1 Preparation of Calibration Standards

Calibration of FTCM type PSt7 is performed using a conventional two-point calibration in oxygen-free water (**cal 0**) and air-saturated water (**cal 100 / cal 2nd**) or in the gas phase with nitrogen-saturated atmosphere (**cal 0**) and 20.9 % O_2 (**cal 100 / cal 2nd**).

Cal 0:

- Oxygen-free water:
  To prepare oxygen-free water dissolve 1 g of sodium sulfite (Na_2SO_3) and 50 µL cobalt nitrate ($Co(NO_3)_2$) standard solution (ρ(Co) = 1000 mg/L; in nitric acid 0.5 mol/L) in 100 mL water. Use a suitable vessel with a tightly fitting screw top and label it **cal 0**. Make sure there is only little headspace in your vessel. Due to a chemical reaction of oxygen with the Na_2SO_3 the water becomes oxygen-free. Additional oxygen, diffusing from air into the water, is removed by surplus Na_2SO_3. Close the vessel with the screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that the water is oxygen-free. Keep the vessel closed after calibration with a screw top to minimize oxygen contamination.
  To prepare oxygen-free water you also can use sodium dithionite ($Na_2S_2O_4$). The shelf life of **cal 0** is about 24 hours provided that the vessel has been closed with the screw top.

For calibration in the gas phase:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- Air-saturated water:
  Add 100 mL water to a suitable vessel and label it **cal 100 / cal 2nd**. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (air stone), creating a multitude of small air bubbles, while stirring the solution. After 20 minutes,

switch of the air-pump and stir the solution for another 10 minutes to ensure that the water is not supersaturated.

For calibration in the gas phase:

- 20.9 % O_2
  Use a certified test gas of 20.9 % O2 (suppliers are e. g. Air Liquide, Linde, Westfalen AG) as calibration standard **cal 100 / cal 2nd**.

6.2.1.2 Calibration Procedure for FTCM Type PSt7 in the Liquid Phase

For instructions and further information on software settings and handling see the respective transmitter manual!

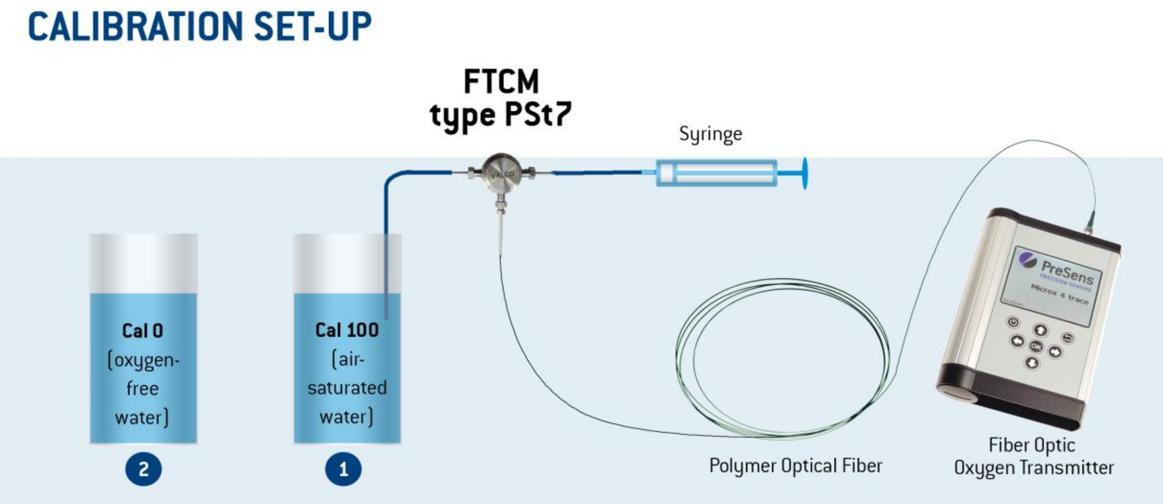


Fig. 34 Schematic illustration of calibration set-up: The FTCM O_2 is connected with tubing to a container with Cal. solution and to a syringe.

Connect one of the connectors of the FTCM with a plastic tube. The tube is dipped into the vessel containing the calibration standard **cal 100 / cal 2nd** (air saturated water). Then connect a syringe – using a small piece of tube – to the opposite FTCM connector (see Fig. 34). Fill the flow-through cell with **cal 100 / cal 2nd** by slowly pulling the syringe plunger. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, put it into the calibration vessel and ensure that there is no difference in temperature between the calibration vessel and the flow-through cell.)

Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and ± 0.2°C, respectively). When the first calibration value **cal 100 / cal 2nd** is recorded press the syringe plunger and empty the calibration solution form the flow-through cell into a waste container.

To record the second calibration value, oxygen-free water, dip the plastic tube into the vessel containing the calibration solution **cal 0**. Again pull the syringe plunger and slowly fill the flow-through cell with **cal 0**. (If you want to use automatic temperature compensations, transfer the temperature sensor to the calibration vessel with **cal 0**).
Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and ± 0.2°C, respectively). When the second calibration value **cal 0** is recorded press the syringe plunger and empty the calibration solution form the flow-through cell into a waste container.

After calibration the FTCM has to be cleaned from sodium sulfite. Dip the plastic tube in a vessel containing distilled water and fill the flow-through cell using the syringe. Then press the syringe plunger and empty the washing solution into a waste vessel. Repeat this cleaning procedure 3 times. (Also clean the temperature sensor with distilled water, if it has been dipped into **cal 0**).

6.2.1.3 Calibration Procedure for FTCM in the Gas Phase

For instructions and further information on software settings and handling see the respective transmitter manual!

Connect the FTCM to your calibration set-up. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it in an environment of the same temperature as in your perfusion system.)

Lead the first gas (N_2 5.0) into the FTCM (Dry mode). In case you want to perform the calibration in Humid mode lead the gas into a vessel filled with distilled water before introducing it in the FTCM (see Fig. 35 for schematic illustration of set-up). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (20.9 % O_2) into the FTCM. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the FTCM. Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply.

6.2.2 Calibration of an FTCM Type PSt8

6.2.2.1 Preparation of Calibration Standards

Calibration of an FTCM type PSt8 is performed with certified gases (suppliers are e. g. Air Liquide, Linde, Westfalen AG).

Cal 0:

- Nitrogen-saturated atmosphere
  Use nitrogen (N_2 5.0) as a first calibration standard **cal 0**.

Cal 100 / Cal 2nd:

- 2 – 4 % O_2
  The second calibration value **cal 100 / cal 2nd** for a PSt8 sensor is ideally in the range between 10 and 20 % air sat. (ca. 2 – 4 % O_2 → % air sat. = % O_2 x 100/20.95). Use a commercially available test gas of 2 – 4 % O_2 as a second calibration standard **cal 100 / cal 2nd**.

6.2.2.2 Calibration Procedure

For instructions and further information on software settings and handling see the respective transmitter manual!

Connect the FTCM to your calibration set-up. (If you want to use automatic temperature compensation, connect the temperature sensor to the respective connector on the oxygen meter, and put it in an environment of the same temperature as in your perfusion system.)

Lead the first gas (N_2 5.0) into the FTCM (Dry mode). In case you want to perform the calibration in Humid mode lead the gas into a vessel filled with distilled water before introducing it in the FTCM (see Fig. 35 for schematic illustration of set-up). Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2 °C, respectively). When the first calibration value **cal 0** is recorded cut the gas supply.

Then lead the second gas (2 – 4 % O_2) into the FTCM. Again, if you want to perform the calibration in Humid mode lead the gas in a vessel filled with distilled water before introducing it in the FTCM. Wait for about 3 minutes until the phase angle and temperature value displayed in the software or on your oxygen meter are constant (the variation of the phase angle and temperature should be smaller than ± 0.2 ° and 0.2°C, respectively). When the second calibration value **cal 100 / cal 2nd** is recorded cut the gas supply.

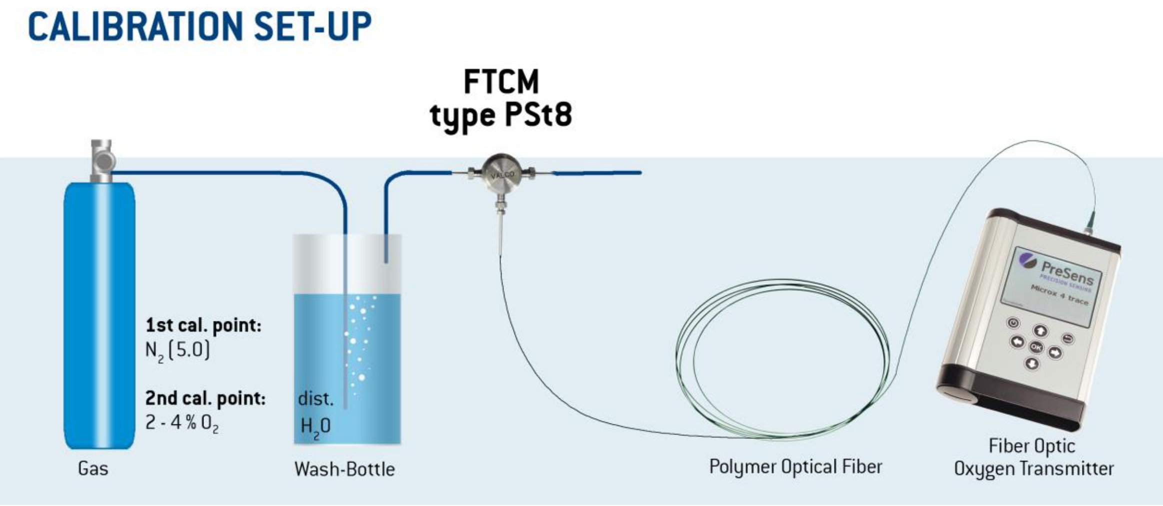


Fig. 35 Schematic illustration: Calibration in Humid mode of an FTCM type PSt8 with certified gases.

If it is not possible to use gases or to build a suitable calibration chamber, a manual calibration or calibration via barcode can be performed, using calibration values obtained from the final inspection protocol or the barcode delivered with the sensor. You can find more information about manual calibration in the instruction manual of the respective transmitter.

6.2.3 Manual Calibration

Manual calibration is performed using the respective transmitter or PreSens Measurement Studio software. Please refer to the respective transmitter or PreSens Measurement Studio instruction manual for further information!

6.3 Measurement with a Flow-Through Cell Metal Housing Oxygen Microsensor

Carefully read chapter 6.1 on mounting the FTCM before starting your measurements.

Connect the end pieces of the T-connector with your tubing and pump your sample through the flow-through cell.

In case liquid samples are used, rinse the flow-through cell with distilled water after your sample had been pumped through to remove any residues, and repeat this procedure until it is clean.

7 Some Advice for Correct Measurement

7.1 Signal drifts due to oxygen gradients

It has to be taken into account that the sensor has a high spatial resolution. Commonly oxygen gradients occur in unstirred solutions which are in contact with ambient air.

Whenever unexpected drifts, gradients or unstable measurement values occur while using a needle-type sensor, please check if the tip is completely extended from the needle or if air bubbles are on the sensor tip.
Critical conditions for bubble formation are, for example, purging with air or other gases and increasing temperature during measurements.
The formation of a biofilm during long-term measurements or the accumulation of other sample components like oil or solid substances may also induce oxygen gradients.

7.2 Signal drifts due to temperature gradients

Imprecise measurement might also be caused by insufficient temperature compensation. If you are using temperature compensation, make sure that there are no temperature gradients between the microsensor and the temperature sensor. If you perform measurements without temperature compensation, make sure that the temperature in the sample is constant during measurements and is the same as you have typed in the software at the beginning of the measurement. If the temperature is measured with a precision of ± 0.2 °C, there is a variation of 100 ± 0.3 % air saturation in the measurement value at 100 % air saturation. Please choose the measurement with temperature compensation to minimize temperature gradients.

7.3 Performance Proof

A performance proof is usually done in any pharmaceutical application. To prove the performance of the microsensor during measurement, the sensor tip is inserted in the cal 0 and cal 100 standards before and after the measurement. If the device shows 0 % air saturation when inserting the sensor tip into cal 0 solution and 100 % air saturation when measuring cal 100, the sensor works perfectly during the whole measurement.

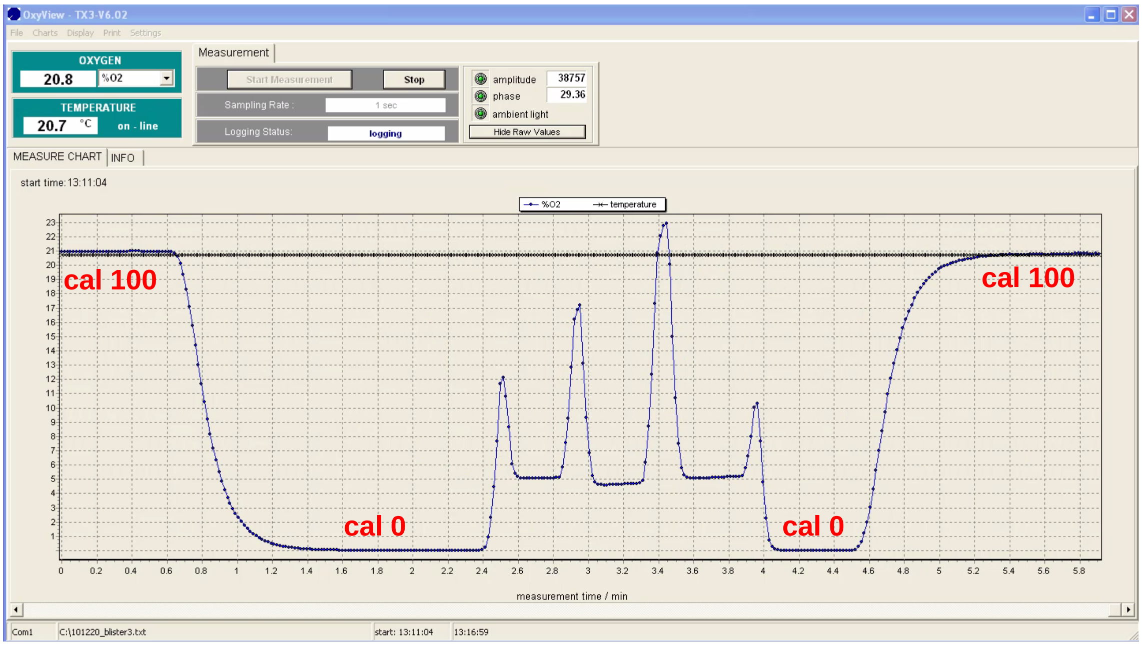


Fig. 36 Performance proof before and after measurement

8 Technical Data

	Sensor Type PSt7		Sensor Type PSt8	
Specifications	**Gaseous & Dissolved O_2**	**Dissolved O_2**	**Gaseous & Dissolved O_2**	**Dissolved O_2**
Measurement range	optimal: 0 – 50 % O_2 0 – 500 hPa max.: 0 – 100 % O_2 0 – 1000 hPa	optimal: 0 – 22.5 mg/L 0 – 700 µmol/L max.: 0 – 45 mg/L 0 – 1400 µmol/L	optimal: 0 – 10 % O_2 0 – 100 hPa max.: 0 – 20.9 % O_2 0 – 200 hPa	optimal: 0 – 4.5 mg/L 0 – 140 µmol/L max.: 0 – 9 mg/L 0 – 200 µmol/L
Limit of detection	0.03 % oxygen	15 ppb	0.007 % oxygen	3 ppb
Resolution	± 0.01 % O_2 at 1 % O_2 ± 0.05 % O_2 at 20.9 % O_2	± 0.005 mg/L at 0.4 mg/L ± 0.025 mg/L at 9.06 mg/L	± 0.002 % O_2 at 0.008 % O_2 ± 0.06 % O_2 at 2.5 % O_2	± 0.7 ppb at 3 ppb ± 2.5 ppb at 1000 ppb
Accuracy*	optimal: ± 0.05 % O_2 or ± 3 % rel.		optimal: ± 3 ppb or ± 3 % rel.	
Measurement temperature range	0 – 50 °C		0 – 50 °C	
Response time (t_{90})	TF**: < 3 sec. (gas), < 10 sec. (liquid) TS***: < 0.3 sec.		TF**:< 3 sec. (gas), < 10 sec. (liquid)	
Properties				
Compatibility	Aqueous solutions, ethanol, methanol			
No cross-sensitivity with	pH 1 – 14 CO2, H_2S, SO_2 Ionic species			
Cross-sensitivity to	Organic solvents, such as acetone, toluene, chloroform or methylene chloride Chlorine gas			
Sterilization procedure	Ethylene oxide (EtO)			
Cleaning procedures	3 % H_2O_2, ethanol, soap solution			
Calibration	Two-point calibration in oxygen-free environment (nitrogen, sodium sulfite) and air sat. environment		Two-point calibration in oxygen free-environment (nitrogen) and a second calibration value optimally between 2 and 4 % oxygen	

*at 20 °C, after two-point calibration
**TF = flat-broken sensor tip with 230 µm diameter
***TS = tapered sensor tip with < 50 µm diameter

9 Concluding Remarks

Dear Customer,

With this manual, we hope to provide you with an introduction to work with the oxygen microsensors.

This manual does not claim to be complete. We are endeavored to improve and supplement this version.

We are looking forward to your critical review and to any suggestions you may have.

You can find the latest version at www.PreSens.de.

With best regards,

Your PreSens Team

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