pyroscience
sensor technology

Optical Oxygen Sensors

USER MANUAL

OPERATING INSTRUCTIONS

Document Version 1.013

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1 Introduction

PyroScience offers a variety of fiber-based and contactless oxygen sensors. For an overview see our homepage www.pyroscience.com.

These sensors can be read-out with different fiber-optic meters from *PyroScience*, including

- the multi-channel PC-operated *FireStingO2* (with *Pyro Oxygen Logger* software)
- the single channel *Piccolo2* (with *Pyro Oxygen Logger* software)
- the multi-analyte & multi-channel PC-operated *FireSting pro* (with *PyroWorkbench*) and
- the (single channel) pocket meter *FireStingGO2* for stand-alone operation (with *FireStingGO2 Manager* software for data management or lab applications).

All software versions are available as free downloads from the *PyroScience* website and must be installed on the Windows PC/laptop before connecting the respective oxygen meter for the first time. For details on the read-out devices, their software and user interface, please see their respective manuals and handling guidelines.

This manual is intended to provide all necessary information on standard application of optical oxygen sensors from *PyroScience*.

For more information concerning advanced applications, please contact us at info@pyroscience.com.

Your *PyroScience* Team

2 Quick Start

Step 1: For PC operation, download the respective software from our homepage. The software can be found in the download tabs of the respective read-out device. Download, unzip and start the installer, and follow the instructions.

Step 2: For PC operation, connect the ***PyroScience*** read-out device to the Windows PC/laptop with the micro-USB cable.

Step 3: Carefully remove the protective caps from the sensor tip, fiber plug and from the optical connector(s) at the read-out device.

Step 4: Connect the ***PyroScience*** oxygen sensor(s) to the optical connector(s) of the device.

Step 5: **For automatic temperature compensation,** connect an appropriate Pt100 temperature sensor to the temperature port or, alternatively, an optical temperature sensor to one of the remaining channel connectors (multi-channel devices only).

Step 6: Prepare appropriate oxygen calibration standards (see chapter 4.2).

Step 7: Start the respective ***PyroScience*** software by clicking on the short-cut on your desktop or the LCD user interface of the ***FireStingGO2*** (stand-alone operation).

Step 8: Enter all required **Sensor Settings,** including the **Sensor Code**, the **Fiber Length (m)** (sensor type: S, W, T, P, X, U), **Medium** and **Oxygen Unit** for each sensor, as well as the **compensation of environmental parameters** (temperature, pressure, salinity, where indicated/applicable).

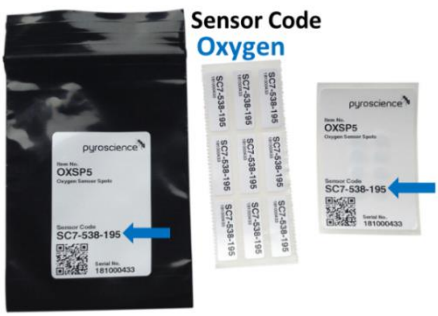
Step 9: Perform a 1- or 2-point sensor **Calibration**.

Step 10: Start measurements and activate **Data Logging**.

3 Sensor Settings

Each optical oxygen sensor comes with an individual **Sensor Code**, containing important information for optimal sensor settings and for calibration. The first letter of the sensor code defines the sensor type. Therefore, it is important to enter the Sensor Code of the connected sensor into the **Sensor Settings** of the respective software. For multi-channel devices, the number of the channel tab must correspond with the channel number at the *PyroScience* read-out device.

IMPORTANT: Enter the correct **Sensor Code** for sensors connected to a channel at a *PyroScience* read-out device. The sensor code can be found on the label attached to the cable (fiber-based sensors) or on the bag of contactless sensors (see example below).



For contactless sensors (sensor spots, flow-through cells, respiration vials, nanoprobes; sensor type: S, W, T, P) and for robust probes (sensor type: X, U), the **Fiber Length (m)** of the connected optical fiber (e.g. *SPFIB*) or of the connected robust probe (e.g. *OXROB10*) must be entered additionally (for automatic background compensation).

The **Measuring Mode** can be adjusted gradually between low drift and low noise of the sensor signal by moving the arrow with the mouse along the scale. Typically, an intermediate mode is default.

3.1 Conditions in the sample

When entering the sensor settings, the **Conditions in the Sample** during the measurements have to be determined. There are three important parameters to be taken into account, which can be automatically compensated:

- Temperature
- Atmospheric Pressure
- Salinity

3.1.1 Temperature

Several options for **Temperature Compensation** of optical oxygen sensors are available:

- External Temperature Sensor (Pt100, temperature port)
- Fixed Temperature (must be entered, kept constant and controlled!)
- Optical Temperature Sensor connected to a channel connector (its channel number must be selected) of a multi-channel read-out device (**not** for ***FSGO2***, ***Piccolo2***)

If **External Temperature Sensor** or **Optical Temperature Channel** is selected, **automatic compensation** of **temperature changes** on the respective oxygen sensor readings is activated. The **Compensation Temperature** will be displayed in the corresponding channel panel of the main window.

NOTE: If an External or Optical Temperature Sensor was selected, the sensor has to be fixed in the sample/calibration standard in which oxygen measurements/calibration will be performed.

IMPORTANT: For precise absolute oxygen measurements and optical temperature sensor calibration using an External Temperature Sensor, please determine manually if the external (Pt100) temperature has an offset. In case of an offset, the Pt100 temperature sensors needs to be calibrated first (see Appendix 8.6) before calibrating the optical sensor.

If a Fixed Temperature was selected, the temperature in the sample/calibration standard must be measured, adjusted and kept constant (needs to be controlled)! Ensure constant and defined conditions!

3.1.2 Atmospheric Pressure

Another parameter, which has to be defined in the settings is the atmospheric pressure (for details please see chapter 8.1). Atmospheric pressure can be compensated by:

- the **Internal Pressure Sensor** for automatic compensation of pressure changes, e.g. caused by weather changes. Possible with all ***FireSting***-based devices if oxygen sensor and device experience the same pressure conditions,

or

- by entering a **Fixed Pressure (mbar)**: for applications with ***Piccolo2*** and for set-ups with different pressure conditions experienced by the oxygen sensor and the ***FireSting***-based devices. The actual pressure at the sensor position needs to be determined with e.g. a barometer and adjusted manually (default: 1013 mbar).

For older software versions it is also possible to enter the **Elevation (m) above sea level**. Note that this option takes only the elevation-dependent pressure change into account, but not the variations due to actual weather conditions. Thus, determining the actual atmospheric pressure with a barometer gives more precise results (more information in the respective read-out device manual).

3.1.3 Salinity

The **Salinity (g/L)** of the environmental sample (based on seawater salinity) is only relevant if a concentration unit for dissolved oxygen **DO** measurements was selected (e.g. mg/L or µmol/L). The sample salinity needs to be measured and entered, e.g. in case of saline water/seawater. For measurements in gas samples this value has no relevance (and is not active).

4 Sensor Calibration

Ensure that the correct **Sensor Code** has been entered in the settings (refer to chapter 3) and prepare appropriate calibration standards (see chapter 4.2). For calibration of contactless sensors, refer also to chapter 4.4.

Oxygen sensor calibration can be performed in two different ways:

- 1-point calibration (required): upper (standard) OR in special applications 0% calibration (only for measurements exclusively at very low O2, e.g. with trace range oxygen sensors; only possible with the ***FSPRO***)
- 2-point calibration (optional): upper AND 0% calibration; recommended for measurements from air saturation/21% to low O2 and for accuracy measurements

NOTE: It is strongly recommended to perform a manual calibration at conditions close to the environmental conditions during measurements. Ensure constant conditions during calibration!

- Gas measurements: the sensor needs to be calibrated (temperature-controlled) in ambient air (upper calibration) and in some cases also in nitrogen gas N2 (0% calibration).
- Measurements in aqueous/water samples: the sensor needs to be calibrated (temperature-controlled) in air-saturated water (upper calibration) and in some cases also in de-oxygenated water (0% calibration).

Please note: In most cases the upper calibration point is defined as the air calibration point, which can be ambient air, air saturated water or water-vapor saturated air (with 100% RH).

Depending on the application (only for advanced users), the upper calibration point can also be user-defined via a **Custom Calibration**.

4.1 Important parameters

All air calibration standards described in the following rely on the virtually constant oxygen content in the earth's atmosphere of about 20.95% O_2 in dry air. Slight deviations might be given in closed rooms occupied by many people (or e.g. candles, combustion engines) consuming the oxygen. So if in doubt, ensure a good ventilation of the room with fresh air, e.g. by opening a window for some minutes.

HUMIDITY

The relative humidity of the air causes deviations from the ideal value of 20.95% O_2. Simply speaking, the water vapor in humid air replaces a fraction of the oxygen, resulting in a diminished oxygen level of e.g. 20.7% O_2. For temperatures around and below 20°C, this effect causes fortunately only a maximum deviation of about 0.5% O_2. However, for higher temperatures at 30°C or even 40-50°C, the humidity of the air gets a significant influence on the actual oxygen level. For example, ambient air at body temperature (37°C) with 100% relative humidity contains only 19.6% O_2 compared to dry air with 20.95% O_2.

During the calibration of oxygen sensors, there are two possibilities to take the humidity into account:

- The relative humidity and the temperature of the ambient air must be determined during calibration. The respective software then calculates automatically the real oxygen level under these conditions.
- The calibration standard is prepared in a closed vessel either filled with water or partly filled with e.g. wet cotton wool or a wet sponge. This ensures a constant humidity of 100% RH and there is no need to measure the humidity.

ATMOSPHERIC PRESSURE

Another parameter even more important for the air calibration standard is the atmospheric pressure. The principle parameter measured by oxygen sensors is not the partial volume (i.e. "% O2"), but the partial oxygen pressure (i.e. "mbar") (see also appendix 8.1). So an oxygen level of e.g. 20.7% O2 (determined as described above by a given humidity and temperature) is converted internally by the respective software into a partial pressure of oxygen essentially by multiplying the relative oxygen level with the atmospheric pressure of e.g. 990 mbar (see chapter 6):

$$0.207 \quad \times \quad 990 \text{ mbar} \quad = \quad 205 \text{ mbar}$$

giving a partial oxygen pressure of e.g. 205 mbar. This is the essential calibration value used internally by the software. The atmospheric pressure can be influenced

- by weather changes (e.g. varying between ca. 990 and 1030 at sea level) and
- by the elevation above sea level (e.g. at 1000 m elevation the typical atmospheric pressure is about 900 mbar compared to 1013 mbar at sea level).

TEMPERATURE

Precise temperature compensation of the oxygen sensor readings during calibration and measurements is needed due to two reasons:

- the luminescence of the ***REDFLASH indicators*** is temperature dependent and
- the conversion of some oxygen units needs to be compensated for temperature.

SUMMARY

There are three important parameters to be known for the air calibration standard:

- Temperature (°C)
- Relative Humidity (% RH)
- Atmospheric Pressure (mbar)

For the ***FireSting***-based read-out devices, the built-in humidity and pressure sensors together with the external temperature sensor will measure these parameters automatically for most calibration types.

For the ***Piccolo2***, these parameters need to be determined, entered and kept constant.

4.1.1 Temperature

It is crucial to determine exactly the temperature in the upper and 0% calibration standards during the oxygen sensor calibration process via one of the following possibilities:

- Manual adjustment of a **Fixed Temperature** (needs to be determined and kept constant)
- Temperature Compensation with an **External** (Pt100) **Temperature Sensor** connected to the temperature port of a ***FireSting***-based device, or
- Temperature Compensation with an **Optical Temperature Sensor** connected to a channel at a multi-channel ***FireSting*** device (its respective channel number needs to be entered at **Optical Temp. Channel**).

4.1.2 Atmospheric Pressure

As for the oxygen measurements, the actual atmospheric pressure is an important parameter for the calibration and calibration standards and needs to be compensated.

If the atmospheric pressure is read from the internal **Pressure Sensor** of a ***FireSting*** device, it is important that the calibration standards are exposed to the same atmospheric pressure as the ***FireSting*** device.

For **Pressure** compensation with a ***Piccolo2*** read-out device,

- the actual atmospheric pressure in the calibration standard must be measured and entered manually. Normal conditions refer to 1013 mbar (default setting).
- **Elevation (m)** in meters above sea level can be entered (see above).

4.1.3 Relative Humidity

During the calibration of oxygen sensors, there are two possibilities to take the humidity into account:

- The relative humidity of the ambient air must be determined during calibration. The software then automatically calculates the actual oxygen level under these conditions.
- The calibration standard is prepared in a closed vessel either filled with water or partly filled with e.g. wet cotton wool or a wet sponge. This ensures a constant humidity of 100% RH and there is no need to measure the humidity.

For precision calibrations, it is generally recommended to prepare calibration standards with 100% **R**elative **H**umidity, which eliminates any possible error source by the usage of the internal humidity sensor.

4.2 Preparation of Calibration Standards

4.2.1 Gas measurements: upper calibration

AMBIENT AIR

The **dry** oxygen sensor, optionally together with the **dry** external or optical temperature sensor, is simply exposed to ambient air.

For precise calibrations in ambient air, it is important that the measuring tips of the oxygen and temperature sensor are **completely dry**. Wet sensor tips will cause undefined humidity levels around the sensor tips. And even worse, the evaporation of water drops would cool down the sensor tips causing undefined temperatures.

WATER-VAPOR SATURATED AIR

Enclose wet cotton wool into a flask (e.g. DURAN flask) with a lid prepared with holes for the oxygen sensor and a temperature sensor from ***PyroScience***. Typically, about 1/3 to 1/2 of the flask volume is filled with wet cotton wool, while the other volume fraction is left free for inserting the tip of the oxygen and temperature sensor.

After insertion of the sensors and equilibration, follow the calibration procedure given by the software.

4.2.2 Gas measurements: 0% calibration

NITROGEN GAS

Flush 100% nitrogen gas through a glass flask (e.g. Duran flask) with a lid prepared with holes for inserting the oxygen sensor and a temperature sensor. Ensure that all air has been replaced by the nitrogen gas before performing the calibration. Insert the **dry** oxygen and temperature sensor into the flask, let it equilibrate and perform the calibration.

Important: Ensure that no ambient air enters the flask again during the calibration process. Convectional gas transport is a very fast process! It is therefore recommended to keep flushing the flask with nitrogen gas during the complete calibration process!

Please consider that nitrogen gas from gas bottles might be significantly cooled down by the decompression process. Ensure a correct temperature determination of the calibration standard!

4.2.3 Measurements in water: upper calibration

AIR SATURATED WATER

For calibration in air saturated water, it is very important that the water is indeed 100% saturated with air. Please follow one of the two options below to prepare an accurate calibration standard:

- Fill an appropriate amount of water into a flask (e.g. Duran flask) with a lid prepared with holes for inserting the oxygen sensor and a temperature sensor. Stream air through the water with an air stone connected to an air pump (available as commercial equipment for fish aquaria) for about 10 minutes.
- Alternatively, if no air pump is available, fill water into the flask leaving **>50% air in the headspace**, close it with a lid and shake the flask strongly for about 1 minute. Open the lid shortly for ventilating the headspace with fresh air. Close it again and shake the flask for 1 more minute.

In both cases insert the oxygen and temperature sensor into the flask and ensure that the sensor tips are immersed in the water and are free of air bubbles. Afterwards follow the calibration procedures given by the software.

Please consider that streaming air through water may cause cooling of the water. Ensure a **correct temperature determination**!

4.2.4 Measurements in water: 0% calibration

WATER MIXED WITH A STRONG REDUCTANT

Fill an appropriate amount of water into a glass flask (e.g. Duran flask) with a lid prepared with holes for inserting the oxygen sensor and a temperature sensor.

Add a strong reductant, like sodium dithionite ($Na_2S_2O_4$) or sodium sulfite (Na_2SO_3) at a concentration of 30 g/L, creating oxygen-free water by chemical reaction. Please note that 0% calibration capsules are available from ***PyroScience***, giving 50mL 0% calibration standard (item no.: ***OXCAL***).

DO NOT use saline water (e.g. seawater) for this, but demineralized water. Saline water prevents proper dissolution of the reductant and can lead to false 0% sensor calibration.

Stir the solution until the salt is completely dissolved, then stop the stirring and leave the solution for about 15 minutes. Ensure that there is **no headspace and no air bubbles in the closed flask**.

Then insert the oxygen and temperature sensor into the flask, and ensure that the sensor tips are completely immersed into the water and free of air bubbles. Let equilibrate and perform the calibration.

Important: Do not store the sensors in this solution and rinse them carefully after the calibration with demineralized water. Especially the retractable needle-type sensors (item no. ***OXR50***, ***OXR230***, (***TR***)***OXR430*** and ***TPR430***) need to be rinsed very thoroughly, because salt crystallization within the needle might damage them irreversibly.

4.2.5 Custom Calibration: upper custom calibration

Instead of air (ambient air, air saturated water, water-vapor saturated air) for the upper calibration point, a custom calibration can be performed if custom calibration gases are used. There are two applications, where custom calibration mode is recommended:

- using trace range sensors in the range of 0-10% O2
- measurements at high oxygen levels (>21% O2)

For a custom calibration, the oxygen level in the calibration standard can be freely chosen in **Oxygen (%O2)**. Here, the correct value has to be adjusted if custom calibration gases are used, of e.g. 5% O2, which is useful when using trace range oxygen sensors.

Important: Custom calibration is only recommended for advanced applications/users! The relevant parameters (%O2, humidity, pressure, temperature) must be entered correctly (and need to be controlled)!

4.3 Calibration procedure

Calibration should be performed following the instructions of the software (***Pyro Workbench***) or read-out device manual. We generally recommend to perform a two-point calibration in gas (*water*) for gas (*water*) measurements. A one-point calibration close to environmental conditions is obligatory.

Important: The device and sensors must be placed **for >30 min. under constant environmental conditions** before the calibration is performed.

Each time the sensor is placed into a new calibration standard, wait until the sensor reading is stable by observing the graph and the numerical display of the oxygen sensor reading. Ensure also stable temperature readings of the External or Optical Temperature Sensor indicated at Compensation Temperature (°C).

For calibration of optical oxygen sensors from ***PyroScience***, it is important to follow these steps:

Step 1: Connect the sensor to the respective read-out device and remove the protective caps from the sensor tip, from the fiber plug and from the optical connector(s) at the read-out device

Step 2: Connect an appropriate Pt100 temperature sensor to the temperature port or, alternatively, an optical temperature sensor to one of the remaining channel connectors (multi-channel devices only) **for automatic temperature compensation** of the oxygen measurements.

Step 3: Enter the correct **Sensor Code** for sensors connected to a channel at a ***PyroScience*** read-out device and their **Fiber Length (m)** (only for sensor type: S, W, T, P, X, U).

Step 4: Prepare appropriate oxygen calibration standards:

- For measurements in GAS: ambient air (upper calibration); nitrogen gas N_2 (0% calibration)
- For measurements in WATER/AQUEOUS samples: air-saturated de-mineralized water (upper calibration); de-oxygenated water (0% calibration) using sodium dithionite ($Na_2S_2O_4$) or sodium sulfite (Na_2SO_3).
- For measurements in SEAWATER/SALINE WATER: **DO NOT** use saline water for preparation of 0% calibration standards, but de-mineralized water.

Step 5: Insert the oxygen and temperature sensor into the flask, and ensure that the sensor tips are completely immersed into the water and free of air bubbles. Let equilibrate and perform a 1- or 2-point oxygen sensor calibration:

NOTE: It is strongly recommended to perform a manual calibration at conditions close to the environmental conditions during measurements. Ensure constant conditions during calibration! Rinse the sensors carefully after calibration with demineralized water.

Step 6: After successful 1- or 2-point calibration at constant and comparable temperature conditions of the successive measurements, perform the measurements in your samples. Ensure a sufficiently high signal intensity of the sensor (>50), regular cleaning, re-calibration and careful handling of the sensors.

4.4 Background Compensation

A background compensation is recommended for optical fibers used for read-out of contactless sensors and for robust probes.

- For **robust probes, respiration vials, flow-through cells** and **sensor spots** with a black optical fiber (sensor type: S, W, T, P, X, U), the FIBER LENGTH needs to be entered into the software for an automatic background compensation (recommended for most applications).
- For precision applications, for applications with low signal intensities and for application of **nanoprobes**, the option MANUAL background compensation must be used.

FIBER LENGTH

Based on the **Fiber Length (m)** entered into the **Settings,** a background signal for compensation is estimated automatically by the software. For standard applications, this is the preferred procedure.

MANUAL

For precision applications, for measurements at low signal intensities and for application of oxygen nanoprobes in microfluidic applications, a **Manual** background compensation must be performed to determine the individual luminescence background of the applied optical fibre.

Especially in the case of oxygen nanoparticles (item no. ***OXNANO***) the luminescence background compensation is important.

Please ensure that during manual background compensation the ***Optical Fiber*** is connected to the medium WITHOUT oxygen nanoprobes.

For other contactless sensors, it is important that the fiber is not attached to the sensor spot (i.e. disconnect this end from the adapter, adapter ring or from the flow-through cell).

Please ensure that during the subsequent calibration process the ***Optical Fiber*** is again attached to the medium WITH oxygen nanoprobes or to the position with sensor spots.

Remind that the position of the spot adapter or adapter ring should not be changed after calibration of the sensor spot; otherwise it has to be calibrated again.

DISABLE

This option disables the background compensation and is only recommended for expert users.

5 Sensor application

PyroScience oxygen sensors can be applied in gas phases, water, aqueous solutions and in ethanol, methanol and isopropanol (robust probes: only short-term application in diluted ethanol, methanol or isopropanol). Other organic solvents (like e.g. acetone), bleach and gaseous chlorine (Cl_2) induce interferences with the sensor reading and potentially destruction of the sensor. No cross-sensitivity is found for pH 1-14, CO_2, CH_4, H_2S and any ionic species.

For application in organic solvents, a special solvent-resistant oxygen probe (item no. ***OXSOLV*** or ***OXSOLV-PTS***) is available.

Specific application instructions are listed for different sensors in the table below.

5.1 Oxygen Sensors

5.1.1 Fiber-based sensors

Sensor item	Sensor-specific application instructions
OXROB…	**Application**: water & gas **Calibration**: 1- or 2-point calibration* **Features**: optical isolation **Sterilization**: short term treatment with ethylene oxide (EtO), 70% ethanol, 70% isopropanol **Note**: Remove air bubbles from sensor surface, stirring is obligatory for application in water/aqueous samples
OXR… *OXF…*	**Application**: water & gas and semi-solid samples **Calibration**: 1- or 2-point calibration* in same application medium obligatory: gas (*water*) calibration for gas (*water*) measurements **Sterilization**: ethylene oxide (EtO), 70% ethanol (not for option -OI), 70% isopropanol (not for option -OI) **Note**: Handle with care! Unprotected fragile sensor tip. Extend sensor tip for calibration and measurements.
OXF…-PT	**Application**: gas **Calibration**: 1- or 2-point calibration* in gas **Sterilization**: ethylene oxide (EtO), 70% ethanol (not for option -OI), 70% isopropanol (not for option -OI) **Note**: Handle with care! Piercing of packaging materials/septa.
OXB…	**Application**: water & gas, semi-solid & custom samples **Calibration**: 1- or 2-point calibration* in same application medium obligatory: gas (*water*) calibration for gas (*water*) measurements **Sterilization**: ethylene oxide (EtO), 70% ethanol (not for option -OI), 70% isopropanol (not for option -OI) **Note**: Handle with care, especially during custom integration! Unprotected fragile sensor tip. Avoid breakage!
TROX….	**Application**: water & gas at low oxygen concentration around 0% O_2 (max. 10% O_2) **Calibration**: 1- or 2-point calibration** in application medium, manual 0% calibration obligatory **Note**: Low signal intensity/signal-to-noise at air-saturated conditions during upper calibration!

OXSOLV...	**Application**: approved polar and non-polar solvents **Calibration**: 2-point calibration in air-saturated water (*air*) and de-oxygenated water for measurements in approved solvents (*solvent vapour*) **Note**: Only measurement in hPa or mmHg for max. 1 h. Handle with care and mind air bubbles!

* depending on application: 1-point for measurements around 21%/air saturation, 2-point for complete range between 0% and 21%/air saturation
** 0% calibration obligatory. For measurements around 0%, 1-point calibration at 0% O2 or custom calibration at custom <21% O2 upper and at 0% O2 recommended.

5.1.2 Contactless sensors

Sensor item	Sensor-specific application instructions
OXSP5	**Application**: water & gas **Calibration**: 1- or 2-point calibration* **Features**: optical isolation **Sterilization**: ethylene oxide (EtO), 70% ethanol, 70% isopropanol, can be autoclaved few cycles at 121°C for 15 min with special precautions (details on request) **Note**: Mind air bubbles! Glue carefully with silicone glue and let dry for 24h.
OXVIAL...	**Application**: water & gas **Calibration**: 1- or 2-point calibration* **Features**: optical isolation **Sterilization**: ethylene oxide (EtO), 70% ethanol, 70% isopropanol **Note**: Remove air bubbles! Determine specific volume before measurements. Ensure stable temperature conditions.
OXFTC...	**Application**: water & gas **Calibration**: 1- or 2-point calibration* **Sterilization**: ethylene oxide (EtO), 70% ethanol, 70% isopropanol **Note**: Flow rate 10-100/20-500 mL/min. Remove air bubbles! Clean regularly.

OXNANO	**Application**: water/aqueous samples **Calibration**: 2-point calibration in application medium **Sterilization**: can be autoclaved few cycles at 121°C for 15 min with special precautions (details on request) **Note**: Manual background compensation necessary in microfluidic applications. Not in coloured, illuminated or fluorescing samples.
TROX...	**Application**: water & gas at low oxygen concentration around 0% O_2 (max. 10% O_2) **Calibration**: 1- or 2-point calibration** in application medium, manual 0% calibration obligatory **Note**: Low signal intensity/signal-to-noise at air-saturated conditions during upper calibration!

* depending on application: 1-point for measurements around 21%/air saturation, 2-point for complete range between 0% and 21%/air saturation
** For measurements around 0%, 1-point calibration at 0%O_2 or custom calibration at custom <21% O_2 upper and at 0% O_2 recommended.

5.2 Combined Sensors

Sensor item	Sensor-specific application instructions	
TOVIAL...	O_2 & Temp	**Application**: water & gas **Calibration**: 1- or 2-point calibration for each sensor necessary* **Features**: optical isolation **Note**: Remove air bubbles! Determine specific volume before measurements. Ensure stable temperature conditions.
PHTOVIAL ...	pH & O_2 & Temp	**Application**: water **Calibration**: 1- or 2-point calibration in specified buffers/calibration standards for each sensor necessary** **Features**: optical isolation **Note**: Remove air bubbles! Ensure stable conditions.
TOFTC2	O_2 & Temp	**Application**: water & gas **Calibration**: 1- or 2-point calibration in application medium for each sensor necessary* **Note**: Flow rate 20-500 mL/min Remove air bubbles! Clean regularly.

* depending on application: 1-point for temperature sensors, 1-point for oxygen measurements around 21%/air saturation, 2-point for complete range between 0% and 21%/air saturation
** 1-point for temperature sensors, 1-point for oxygen measurements around 21%/air saturation, 2-point for complete range between 0% and 21%/air saturation, 2-point calibration for pH sensors at pH 4 and pH 10, using ***PyroScience*** buffer capsules

6 Sterilization, Cleaning and Storage

6.1 Sterilization

Most oxygen sensors can be sterilized with ethylene oxide (EtO) and cleaned with peroxide (3% H_2O_2), soap solution or ethanol.

Please refer to the specifications on the respective ***PyroScience*** website.

The oxygen sensor spots (item no. ***OXSP5***) and nanoprobes (item no. ***OXNANO***) can be autoclaved (few cycles at 121°C for 15 min) with special precautions. More details on request.

IMPORTANT: do **not** use bleach, acetone or any solvent/agent not approved by ***PyroScience***!

6.2 Cleaning and Storage

After finalization of the measurements, the sensor tip of the needle-type and bare fiber sensors, as well as the robust probes should be rinsed carefully with demineralized water. After cleaning, let dry and put on the protective cap / tubing for storage in a dry, dark and secure place at room temperature. For all sensors and fibers, put the black caps on the fiber plug to prevent that light is entering the fiber possibly causing photo-bleaching of the indicator.

In case of retractable sensors and application in seawater / aqueous samples with dissolved salts, the sensor has to be cleaned thoroughly with demineralized water to prevent salt crystallization in the needle which can cause breaking of the sensor tip. **After drying**, retract the sensor tip into the needle and put on the protective cap onto the needle to protect the sensor tip and to avoid injuries.

Store the sensor in a dry, dark and secure place at room temperature.

A signal drift of the sensor can indicate photo-bleaching of the oxygen-sensitive ***REDFLASH indicator*** depending on the ambient light intensity, as well as the intensity of the excitation light and the sample frequency. This can necessitate new calibration of the sensor and possibly also a re-adjustment of the **Sensor Settings**. In case of sensor spots, this could require a re-positioning of the optical fiber on the sensor spot and a subsequent new calibration.

If the signal intensity is getting below 50 mV, the sensor needs to be replaced, as indicated by the respective warning.

7 Related documents

Related documents for more detailed instructions on fiber-optic read-out devices, software and optical sensors are available:

- manual for logger software "***Pyro Workbench***" (Windows)
- manual for multi-analyte meter ***FireSting pro***
- manual for oxygen meter ***FireStingO2*** (with ***Oxygen Logger*** software)
- manual for portable oxygen meter ***FireStingGO2*** (with ***FireStingGO2 Manager*** software)
- manual for oxygen meter ***PICO2*** (with ***Oxygen Logger*** software)
- manual for optical pH sensors
- manual for optical temperature sensors

8 Appendix

8.1 Definition of Oxygen Units

phase shift *dphi*

The phase shift *dphi* is the fundamental unit measured by the optoelectronics in the ***PyroScience*** read-out device (see chapter 8.3). Please note, that *dphi* is not at all linearly dependent on the oxygen units, and **increasing** oxygen levels correspond to **decreasing** *dphi* values, and vice versa! As a thumb of rule, anoxic conditions will give about *dphi*=53, whereby ambient air will give about *dphi*=20.

raw value *raw value*

Definition: *raw value* = %O2 (uncalibrated)

The unit *raw value* is the default unit for uncalibrated sensors and shows only qualitative oxygen sensor readings.

partial pressure p_{O_2} *hPa = mbar*

Used in: gas and water phase

For a calibrated sensor, the partial oxygen pressure p_{O_2} in units of *hPa* (equivalent to *mbar*) is the fundamental oxygen unit measured by the ***PyroScience*** read-out device.

partial pressure p_{O_2} *Torr*

Definition: $p_{O_2}[Torr] = p_{O_2}[hPa] \times 759.96 / 1013.25$

Used in: gas or water phase

volume percent p_V *%O2*

Definition: $p_V = p_{O_2}[hPa] / p_{atm} \times 100\%$

Used in: gas

with p_{atm}: actual barometric pressure

% air saturation A *% a.s.*

Definition: $A[\%a.s.] = 100\% \times p_{O_2} / p100_{O_2}$

Used in: water phase

with $p100_{O_2} = 0.2095 \; (p_{atm} - p_{H_2O}(T))$

$p_{H_2O}(T) = 6.112\text{mbar} \times \exp (17.62 \; T[°C] / (243.12 + T[°C]))$

p_{O_2}: actual partial pressure

p_{atm}: actual barometric pressure

T: actual temperature

$p_{H_2O}(T)$: saturated water vapor pressure at temperature T

Dissolved O2 concentration C *µmol/L*

Definition: $C\,[\mu mol/L] = A[\%a.s.] / 100\% \times C_{100}(T,P,S)$

Used in: water phase

with $C_{100}(T,P,S)$: interpolation formula for dissolved oxygen concentration in units of µmol/L at temperature T, atmospheric pressure P and Salinity S (see chapter 0).

Dissolved O2 concentration C *mg/L = ppm*

Definition: $C\,[mg/L] = C\,[\mu mol/L] \times 32 / 1000$

Used in: water phase

Dissolved O2 concentration C *mL/L*

Definition: $C\,[mL/L] = C\,[\mu mol/L] \times 0.02241$

Used in: water phase

8.2 Oxygen Solubility

The calculation of the equilibrium oxygen concentration C_{100}(T, P=1013mbar, S) in units of µmol/L is done at standard atmospheric pressure of 1013 mbar as a function of water temperature in units of °C and salinity in units of PSU ("practical salinity unit" ≈ g/L). In order to correct these for the actual atmospheric pressure p_{atm}, the following formula has to be applied:

$$C_{100}(T,P,S) = C_{100}(T,P=1013mbar,S) \times p_{atm} / 1013mbar$$

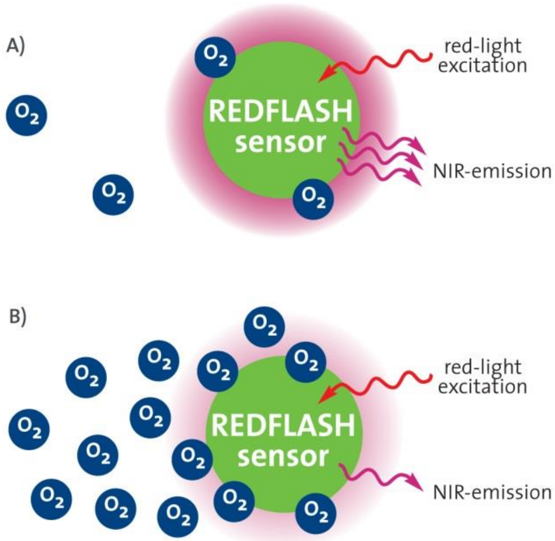
References:

Garcia, HE and Gordon, LI (1992)
Oxygen solubility in seawater: Better fitting equations.
Limnol. Oceanogr. 37: 1307-1312

Millero, FJ and Poisson, A (1981)
International one-atmosphere equation of state of seawater.
Deep Sea Res. 28A: 625-629

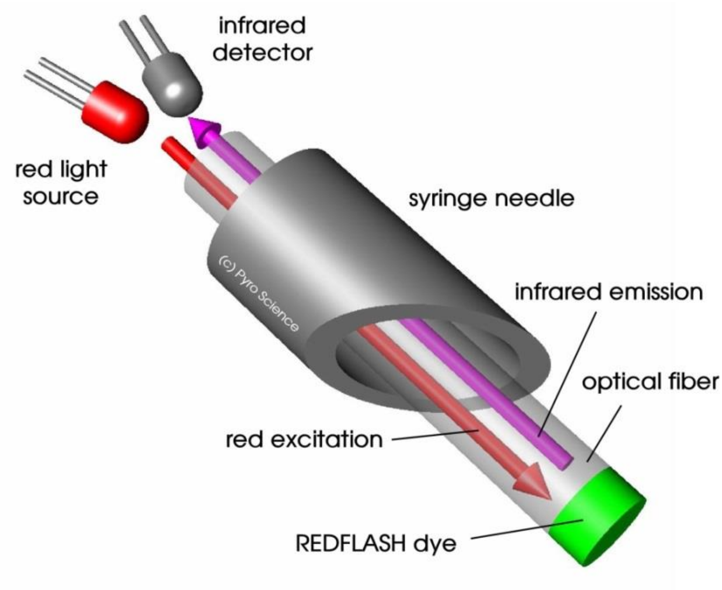
8.3 Oxygen Measuring Principle

The new ***REDFLASH technology*** is based on the unique oxygen-sensitive ***REDFLASH indicator*** showing excellent brightness. The measuring principle is based on the quenching of the ***REDFLASH indicator*** luminescence caused by collision between oxygen molecules and the ***REDFLASH indicator*** immobilized on the sensor tip or surface. The ***REDFLASH indicators*** are excitable with red light (more precisely: orange-red at a wavelength of 610-630 nm) and show an oxygen-dependent luminescence in the near infrared (NIR, 760-790 nm).



Principle: red light excited REDFLASH indicators show luminescence in the near infrared (NIR), which decreases with increasing oxygen (quenching effect).
A) high NIR emission at low oxygen and **B)** low NIR at high oxygen

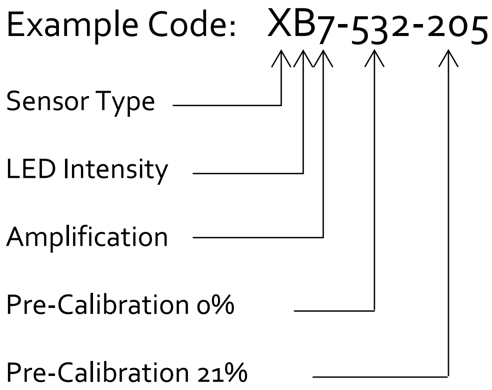
The ***REDFLASH technology*** impresses by its high precision, high reliability, low power consumption, low cross-sensitivity, and fast response times. The red light excitation significantly reduces interferences caused by autofluorescence and reduces stress in biological systems. The ***REDFLASH indicators*** show much higher luminescence brightness than competing products working with blue light excitation. Therefore, the duration of the red flash for a single oxygen measurement could be decreased from typically 100 ms to now typically 10 ms, significantly decreasing the light dose exposed to the measuring setup. Further, due to the excellent luminescence brightness of the ***REDFLASH indicator***, the actual sensor matrix can be now prepared much thinner, leading to fast response times of the ***PyroScience*** oxygen sensors.



The measuring principle is based on a sinusoidally modulated red excitation light. This results in a phase-shifted sinusoidally modulated emission in the NIR. The ***PyroScience*** read-out device measures this phase shift (termed "dphi" in the software). The phase shift is then converted into oxygen units based on the Stern-Vollmer-Theory.

8.4 Explanation of the Sensor Code

The oxygen sensors are delivered with an attached sensor code which must be entered in the Settings (refer to chapter 3). The following figure gives a short explanation about the information given in the sensor code.



Sensor Type

Z	Oxygen Micro / Minisensor
Y	Oxygen Minisensor
X	Robust Oxygen Probe
V	Oxygen Minisensor (trace range)
U	Robust Oxygen Probe (trace range)
T	Oxygen Sensor Spot / FTC (trace range)
S	Oxygen Sensor Spot / FTC
Q	Solvent-Resistant Oxygen Probe
P	Oxygen Nanoprobes

LED Intensity

A	10%	E	40%
B	15%	F	60%
C	20%	G	80%
D	30%	H	100%

Amplification

4	40x
5	80x
6	200x
7	400x

Oxygen Sensors

Co (Pre-Calibration at 0% O2)

dphi0 = C0 / 10

C100 (Pre-Calibration at 100% O2)

dphi100 = C100 / 10

The values of the pre-calibration are valid for the following calibration conditions:

Partial Volume of Oxygen (% O2)	20.95
Temperature at both calibration points (°C)	20.0
Air Pressure (mbar)	1013
Humidity (% RH)	0

8.5 Available sensors and read-out devices

Sensor Type	Compatible read-out devices				
	FSO2-X	*FSGO2*	*FSO2-SUBPORT**	*Piccolo2*	*FS pro*
OXR...	✓	✓	✓	✗	✓
OXF...	✓	✓	✓	✗	✓
OXF...PT	✓	✓	on request	✗	✓
OXB...	✓	✓	✓	✗	✓
OXROB...	✓	✓	✓	✗	✓
OPROB3	✗	✗	✗	✓	✗
OPDIP20	✗	✗	✗	✓	✗
OXSOLV...	✓	✓	✗	✗	✓
OXSP5	✓	✓	✓	✓	✓
OXFTC...	✓	✓	✗	✓	✓
TOFTC2	✓	✗	✗	✗	✓
OXVIAL....	✓	✓	✗	✓	✓
TOVIAL4 *TOVIAL20*	✓	✗	✗	✗	✓
OXNANO	✓	✗	✗	✓	✓

*only in combination with UNDERWATER CONNECTOR (option -SUB)

8.6 Pt100 Temperature Sensor Calibration

For precise absolute temperature readings, an optional **1-point calibration of the external temperature sensor** is recommended.

For this, check the reading of the external temperature Pt100 probe periodically in stirred water / water bath / incubator of known temperature at steady state. It is also possible to prepare a water-ice-mixture giving 0°C, where at least 50 mm of the Pt100 temperature probe tip is submerged. After calibration of the Pt100, a new optical sensor calibration must be performed.

9 Warnings and Safety Guidelines

Before using *PyroScience* oxygen sensors, carefully read the instructions and user manuals for the respective *PyroScience* read-out device. The manuals are available for download on www.pyroscience.com

Prevent mechanical stress (e.g. scratching) to the sensing surface at the tip of the oxygen sensor! Avoid strong bending of the fiber-optic cables. They might break!

Ensure that the complete sensing surface at the tip is always covered by the sample and is free of air bubbles, and that liquid samples are stirred.

Calibration and application of the oxygen sensors is on the user's authority, as well as data acquisition, treatment and publication!

***PyroScience* oxygen sensors and read-out devices are not intended for medical or military purposes or any other safety-critical applications. They must not be used for applications in humans; not for in vivo examination on humans, not for human-diagnostic or therapeutic purposes. The sensors must not be brought in direct contact with foods intended for consumption by humans.**

The sensors must be used in the laboratory by qualified personnel only, following the user instructions and the safety guidelines of the manual, as well as the appropriate laws and guidelines for safety in the laboratory!

Keep the *PyroScience* oxygen sensors and read-out devices out of reach of children! Store the oxygen sensors in a secure, dry and dark place at room temperature.