# FireStingO2

*FIBER-OPTIC OXYGEN & TEMPERATURE METER*

*USER MANUAL*

Document Version 3.081
Refers to *Pyro Oxygen Logger Software* version >3.3

The *FireStingO2* is manufactured by

*PyroScience* GmbH
Hubertusstr. 35
52064 Aachen
Germany

Phone          +49 (0)241 5183 2210
Fax            +49 (0)241 5183 2299
Email          info@pyroscience.com
Internet       www.pyroscience.com

Registered: Aachen HRB 17329, Germany

# 1 Overview

The compact USB-powered fiber-optic meter *FireStingO2* with 1, 2, or 4 channels unifies several innovative technological improvements making it the new standard of oxygen sensing with fiber-optical oxygen sensors (optodes). The *FireStingO2* utilizes a measuring principle based on red light excitation and lifetime detection in the near infrared using luminescent oxygen indicators (*REDFLASH technology*, see Appendix 12.4). It is a multifunctional oxygen meter working

- for oxygen measurements in water as well as in gas phases,
- with fiber-based sensors comprising several size classes like microsensors, minisensors, or robust probes,
- with contactless sensors for oxygen and temperature like sensor spots, flow-through cells or respiration vials, and
- with full range and trace oxygen sensors,
- with optical temperature minisensors.

The *FireStingO2* has integrated atmospheric pressure and humidity sensors for a precise and easy sensor calibration, but also for automatic pressure compensation of the oxygen measurements. Furthermore, the *FireStingO2* offers 4 analog outputs and a built-in temperature port for an external PT100 temperature probe for automatic temperature compensation of the oxygen measurement. If independent temperature compensation for all oxygen channels is needed, a USB-powered temperature extension module *TeX4* can be easily fixed below the *FireStingO2* meter with a smart docking mechanism. Or the new optical temperature sensors can be connected to any channel of the *FireStingO2* meter. By placing the same sensor type for oxygen and temperature into the sample, true temperature compensation of the oxygen measurements can be achieved.

The user-friendly *Pyro Oxygen Logger* software allows operation of several *FireStingO2* meters in parallel as a scalable multi-channel system.

Along with the *FireStingO2* we offer turnkey motorized setups for measurements of depth-profiles in semi-solid environmental samples and along micro-gradients at high temporal and spatial resolution. And several customized OEM solutions are available for application in industry and underwater.

More information concerning our products can be found at www.pyroscience.com

or contact us at info@pyroscience.com.

Your *PyroScience* Team

# 2 Safety Guidelines

The *FireStingO2* is a laboratory instrument to be used with fiber-optic oxygen and temperature sensors (optodes) from *PyroScience*. In order to guarantee an optimal performance of the *FireStingO2*, please follow these operation instructions and safety guidelines.

If any problems or damage evolve, disconnect the instrument immediately, mark it to prevent any further use and consult *PyroScience* for repair or maintenance service. The *FireStingO2* should not be manipulated or opened by unauthorized persons, only by *PyroScience* or persons advised directly from *PyroScience*.

Please note that opening the housing will void the warranty. There are no serviceable parts inside the device.

The *FireStingO2* and sensors should be kept and stored outside the reach of children in a secure place under dry and clean conditions at room temperature, avoiding moisture, dust, corrosive conditions and heating of the instrument. This device and the sensors are not intended for medical, military or other safety-relevant areas. 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 *FireStingO2* should be used in the laboratory by qualified personal only, following the operation instructions and safety guidelines of this manual. Please follow the appropriate laws and guidelines for safety, like EEC directives for protective labor legislation, national protective labor legislation, safety regulations for accident prevention and safety data-sheets from manufacturers of chemicals used during the measurements.

Calibration and application of the sensors, data acquisition, data processing and data publication is on the user's authority.

When used in the field, the environmental conditions (like high humidity, dust, exposure to direct solar radiation) may cause damage or interference of the *FireStingO2*, which is on the user's authority.
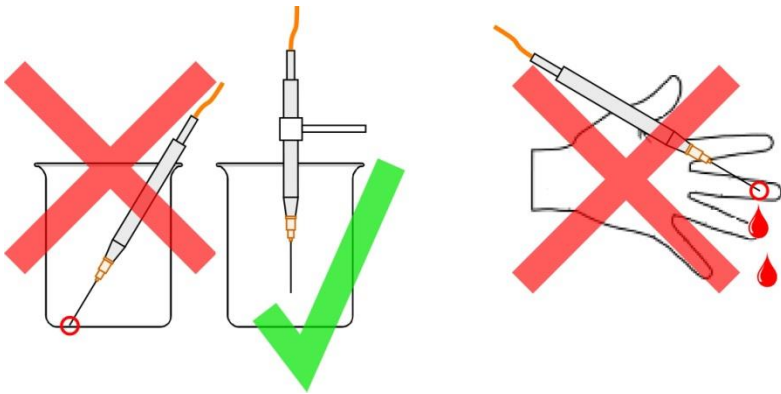
Before using the *FireStingO2* and its sensors, read carefully the instructions and user manual for the oxygen meter *FireStingO2*.

In case of problems or damage, disconnect the instrument and mark it to prevent any further use! Consult *PyroScience* for advice! There are no serviceable parts inside the device. Please note that opening the housing will void the warranty!

The *FireStingO2* is not watertight, is sensitive to corrosive conditions and to changes in temperature causing condensation. Avoid any condition (e.g. direct sun light) causing a heating of the device above 50°C (122°F) or below 0°C (32°F). Avoid any elevated humidity causing condensing conditions.

Handle the sensors with care especially after removal of the protective cap! Prevent mechanical stress to the fragile sensing tip! Avoid strong bending of the fiber cable! Prevent injuries with needle-type sensors!

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

The sensors and the oxygen meter *FireStingO2* are not intended for medical, diagnostic, therapeutic, or military purposes or any other safety-critical applications. The sensors must not be used for applications in humans and must not be brought in direct contact with foods intended for consumption by humans.

The *FireStingO2* and sensors should 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 sensors and the oxygen meter *FireStingO2* out of reach of children!

# 3 Introduction to the FireSting Meter

The *FireStingO2* is an optical oxygen meter that is compatible with a broad range of optical oxygen and temperature sensors from *PyroScience*:

- microsensors and minisensors (retractable needle-type, fixed needle-type, or bare fiber),
- robust probes and
- contactless sensors (sensor spots, respiration vials, flow-through cells)

Most oxygen sensors are available in versions for the full range (0-50% $O_2$, max. range 0-100% $O_2$) and for the trace range (0-10% $O_2$). The optical detection technology is based on the oxygen-sensitive *REDFLASH* indicators which use red light excitation and lifetime detection in the near infrared (see Appendix 12.4 for more details).

The *FireStingO2* is a compact USB-powered fiber-optic oxygen meter with 1, 2 or 4 channels for oxygen and temperature measurements in the laboratory. The optical temperature sensors can be used for true automatic temperature compensation of the oxygen measurements. Additionally, one external PT100 temperature probe can be connected for calibration of the optical temperature sensors and for automatic temperature compensation of the oxygen measurements. The integrated sensors for atmospheric pressure (mbar) and relative humidity (% RH) of the ambient air enable a precise and easy sensor calibration, as well as automatic pressure compensation of the oxygen measurements.

The *FireStingO2* is operated via a Micro-USB connection to a PC / tablet with a Windows operation system. The included logging software *Pyro Oxygen Logger* provides comfortable calibration and logging functionality.

The *FireStingO2* is a laboratory instrument. Use in the field is on the user´s authority and then it is recommended to protect the *FireStingO2* from heating, moisture and corrosion.

The *FireStingO2* comes with 1, 2, or 4 channels (connectors **1** to **4**) for up to 4 fiber-optic sensors and one connector (**T**) for an external PT100 temperature probe.



optical sensors
connector **1 - 4**

PT100 temperature probe
connector **T**

The Micro-USB connector on the left side panel provides the energy supply and the data exchange with the **PC**. Right-hand side of it, a connector X1 for power and digital interface (7-pins) and a connector X2 for analog output (5 pins) is located. The holes function as air inlet for the internal temperature, pressure and humidity sensors. Avoid covering these holes to ensure free air circulation towards the internal sensors.



*air inlet*

Extension Port
connector **X1** & **X2**

Micro-USB
connector **PC**

# 4 Software Installation

IMPORTANT: Do not connect the *FireStingO2* to your PC before the *Pyro Oxygen Logger* software has been installed. The software will install automatically the appropriate USB-drivers.

**System requirements:**

- PC with Windows 7 / 8 / 10 (but not Windows RT) and min. 700 MB free disk space

**Installation steps:**

- download the installer package for the newest version of the *Pyro Oxygen Logger* software from the *PyroScience* homepage: www.pyro-science.com/downloads.html
- unzip and start the installer and follow the instructions
- connect the *FireStingO2* with the Micro-USB cable to the computer. The red logo will flash shortly indicating the correct startup of the *FireStingO2* meter.

After successful installation, a new program group "Pyro Oxygen Logger" is added to the start menu, and a short-cut named "Oxygen Logger" can be found on the desktop.

# 5 Optical Oxygen & Temperature Sensors

For an overview of available optical oxygen and temperature sensor types, please see http://www.pyro-science.com/products.html.

## 5.1 Overview of Sensor Types

| Sensor Type Fiber-Based | Available Versions | Analyte | Application |
|---|---|---|---|
| Robust Probes | OXROB3 | $O_2$ | stirred water, gas & semi-solid samples |
| | OXROB10 | O2 | |
| Retractable Needle-Type | OXR50 | $O_2$ | water, gas & semi-solid samples - for profiling & small volumes, puncturing septa |
| | OXR230 | $O_2$ | |
| | OXR430 | $O_2$ | |
| | TPR430 | Temp. | |
| Fixed Needle-Type | OXF50 | $O_2$ | gas & water - gas-tight housing, sensor insertion through a port |
| | OXF1100 | $O_2$ | |
| | TPF1100 | Temp. | |
| | OXF500-PT | $O_2$ | gas - gas-tight housing, puncturing septa, packing material |
| | OXF900-PT | $O_2$ | |
| Bare Fiber Sensors | OXB50 | $O_2$ | water, gas & semi-solid samples - integration into custom housings |
| | OXB230 | $O_2$ | |
| | OXB430 | $O_2$ | |
| Solvent-Resistant Probes | OXSOLV | $O_2$ | approved polar and non-polar solvents |
| | OXSOLV-PTS | $O_2$ | |

| Sensor Type Contactless | Available Versions | Analyte | Application |
|---|---|---|---|
| **Nanoprobes** | OXNANO | O2 | **aqueous solutions** - microfluidics, cultures, enzymatic react. |
| **Sensor Spots** | OXSP5 | O2 | **water & gas*** - measurements in closed containers with transparent window |
| | TPSP5* | Temp. | |
| **Respiration Vials** | OXVIAL4 | O2 | **water & gas** - measurements in closed vials, of respiration / net photosynthesis rates |
| | OXVIAL20 | | |
| | TOVIAL4 | Temp. & O2 | |
| | TOVIAL20 | | |
| **Flow-Through Cells** | OXFTC | O2 | **water & gas*** pumped through the cell |
| | OXFTC2 | | |
| | TPFTC* | Temp. | |
| | TPFTC2* | | |
| | TOFTC2 | Temp. & O2 | |

**\*** Gas measurements only in combination with corresponding contactless O2 sensor for true temperature compensation

For details on the different sensor types, please see their respective websites.

## 5.2 Connecting the Sensors

The fiber-optic oxygen and temperature sensors, including needle-type and bare fiber micro- and minisensors, robust probes, as well as optical fibers needed for read-out of contactless sensors (sensor

spots, flow-through cells, respiration vials) are connected to the ST-connectors of the *FireStingO2* (**1** to **4**) with a male fiber plug.



First, remove the black caps from the plug of the sensor / fiber. Then remove the red caps from the sensor ports at the *FireStingO2* (the red caps should be put on again if the *FireStingO2* is not in use anymore to protect the optics). Insert the male fiber plug of the sensor cable into the ST-port (female fiber connector) of the *FireStingO2* and turn the bayonet coupling gently clockwise until the plug is locked firmly.

## 5.3   Cleaning and Maintenance of the Sensors

The oxygen sensors can be sterilized with ethylene oxide (EtO) and can be cleaned with peroxide (3% $H_2O_2$), soap solution or ethanol (do **not** use bleach!). They can be applied in gas phases, aqueous solutions and in ethanol, methanol and isopropanol (robust probes: only short-term application in diluted ethanol, methanol or isopropanol). Other organic solvents and gaseous chlorine ($Cl_2$) induce interferences with the sensor reading. 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.

The optical temperature sensors can be applied in water / aqueous samples and gas. If required, soap solution or 3% $H_2O_2$ can be used for cleaning, as specified on the sensor website.

A signal drift of the sensor can indicate photo-bleaching of the oxygen-sensitive *REDFLASH indicator* or the temperature sensitive *THERMOGREEN*/*THERMOBLUE* indicator depending on the ambient light intensity, as well as the intensity of the excitation light and the sample frequency. This can necessitate a new calibration of the sensor and possibly also a re-adjustment of the **Sensor Settings** (LED intensity; see also chapter 6.1). 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 too low, as indicated by the horizontal indicator bar in the *Pyro Oxygen Logger* software and by the respective warning (see chapter 6.2), the sensor needs to be replaced.

A reasonable optical sensor shows signal intensities well above 50.

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, 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 plug of the fiber 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.**

# 6  The Software "Pyro Oxygen Logger"

This chapter describes all functions of the *Pyro Oxygen Logger* software excluding the calibration. Refer to the chapters 7-9 for a detailed description of the calibration procedure of optical oxygen and temperature sensors.

If the Pyro Oxygen Logger software is opened for the first time, the **FireSting Settings** window opens automatically:
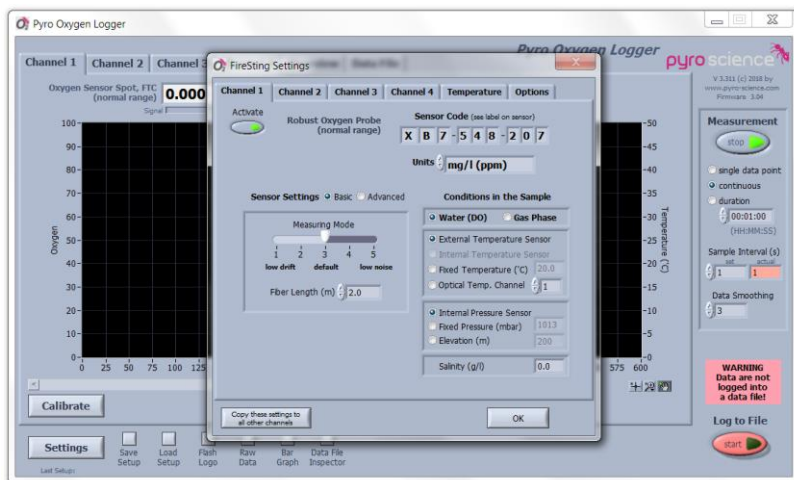


Activate each connected sensor in the respective channel tab of the **Settings** window, corresponding to the channel number at the *FireStingO2* meter (see chapter 3). Enter the **Sensor Code** and all relevant parameters (**Units, Measuring Mode, Conditions in the Sample**) into **each** channel tab before calibration and measurements, as described in detail in chapter 6.1.

## 6.1  FireSting Settings

Each channel (1-4) of the connected *FireStingO2* meter has its own tab (channel 1-4) in the **FireSting Settings** window.

For all optical sensors, enter first the sensor code of each connected optical sensor into the field **Sensor Code** in the corresponding channel panel of the **Settings** window. It includes information for optimal sensor settings and for calibration. The first letter of the sensor code defines the sensor type. A detailed explanation of the sensor code is given in chapter 12.8.

The channels can be activated independently by clicking on the button **Activate**. Activation is indicated by a change from dark to light green of the arrow in the button.

A text describing the connected sensor type appears on the right-hand side of this button after the **Sensor Code** (see label on sensor) has been entered. This description will be shown in the description display of the main window (see chapter 6.2) and also in the data file (see chapter 6.4).

Depending on the optical sensor type and analyte, there are different parameters which need to be adjusted in the respective channel tab of the Settings window (see chapter 6.1.1 for oxygen and 6.1.2 for optical temperature sensors).

If the same sensor type (with the same Sensor Code) is connected to all channels (e.g. 4 sensor spots from the same packaging unit), the settings adjusted in the active channel tab can be pasted to all other channels by clicking on **Copy these Settings to all other channels**.

After the first start, the dialog window **FireSting Settings** opens automatically. For later adjustments, it can be opened by clicking on the **Settings** button in the Main Window.

> **Settings** can only be adjusted if data logging is **not** active (see chapter 6.2.3).

### 6.1.1  Channel Tab: Optical Oxygen Sensors

Enter the **Sensor Code** of each connected oxygen sensor (see label on sensor) and define for each channel (1) the **Sensor Settings** (Measuring Mode), (2) the environmental **Conditions in the Sample** under investigation and (3) the oxygen **Units** for the measurements.

**(1) Sensor Settings**

The **Sensor Settings** can be adjusted in a **Basic** or an **Advanced** mode. Ensure that the correct sensor code attached to the sensor has been entered in the field **Sensor Code**.
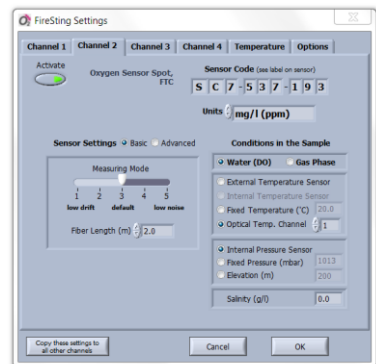


> The first-time user is advised to work with the **Basic Sensor Settings**.

Basic Settings

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 interested users: the entered fiber length is used for automatic background compensation; refer to chapter 9.2 for more details).

The **Measuring Mode** can be adjusted gradually between low drift (1) and low noise (5) of the sensor signal by moving the arrow with

19

the mouse along the scale, thereby changing the measuring time. An intermediate mode (3) is default.

## Advanced Settings (for advanced users/applications only)

If **Advanced Sensor Settings** are chosen, more complex setting controls get visible. Ensure that the correct **Sensor Code** has been entered.
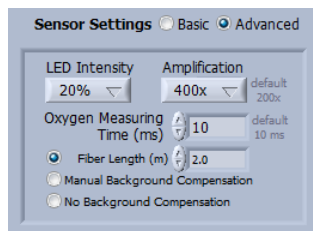
The **Advanced Sensor Settings** comprise the **LED Intensity** for excitation of the *REDFLASH* indicator (in %) and the **Amplification** of the sensor signal. As a rule of thumb, the **LED Intensity** should be 10-30% for microsensors, minisensors and robust probes, but can be increased up to 100% for contactless sensors (sensor spots, flow-through cells, respiration vials). The **Amplification** is typically 200x or 400x and should not be changed. Note, that varying the LED Intensity and the Amplification has direct influence on the signal intensity and therefore on the signal-to-noise-ratio!

The **Oxygen Measuring Time** (default: 10 ms) defines the integration time for the acquisition of a single data point. Shorter measuring times provide low long-term drift, whereby longer measuring times assure less noise. The maximum value is 250 ms.

For background compensation of robust probes (sensor type: X), the **Fiber Length (m)** of the connected robust probe must be entered.

For background compensation of contactless sensors (sensor spots, flow-through cells, respiration vials, nanoprobes; sensor type: S, W, T, P), the **Fiber Length (m)** of the connected optical fiber must be entered. Alternatively, it is possible to select **Manual Background Compensation**, which is described in detail in chapter

9.2. Finally, the background compensation can be completely de-activated by selecting **No Background Compensation** (not recommended in general).

---

**NOTE:** Recommended is to enter the **Fiber Length (m)** for background compensation. The alternative options **Manual Background Compensation** or **No Background Compensation** are only intended for advanced users/applications.

If using **Advanced Sensor Settings**, a **2-Point** calibration of the oxygen sensor must be performed. Later re-adjustments in the **Advanced Settings** require new sensor calibration.

---

## (2) Conditions in the Sample

The next step is the determination of the **Conditions in the Sample** during the measurements. For this, select the

SAMPLE: **Water (D**issolved **O**xygen**)** or **Gas Phase**.

TEMPERATURE compensation: by
- an **External Temperature Sensor** (PT100) connected to the temperature port of the *FireStingO2*,
- a **Fixed Temperature** (needs to be determined, adjusted manually and kept constant), or
- an **Optical Temperature** sensor connected to a **Channel** at the *FireStingO2* meter (its respective channel number needs to be entered here).

For **Gas** measurements, temperature could be measured in principal also by the **Internal Temperature Sensor** of the *FireStingO2*, but this is **NOT** recommended**!**

If **External Temperature Sensor**, **Internal Temperature Sensor** (in a Gas Phase) or **Optical Temperature Channel** is selected, automatic compensation of temperature changes on the respective oxygen sensor readings is activated (see chapter 10.3). The **Compensation Temperature** will be displayed in the corresponding channel panel of the main window (see chapter 6.2).

---

**NOTE**:

If an **External** or **Optical Temperature Sensor** was selected, it has to be fixed in the sample container/calibration standard in which oxygen measurements/calibration with automatic temperature compensation will be performed.

If internal temperature sensor was selected (not recommended), ensure the **same** temperature conditions for the gas sample and the *FireStingO2*.

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!

---

PRESSURE compensation: by
- the **Internal Pressure Sensor** (recommended as default) or
- at **Fixed Pressure (mbar)** (recommended for applications with different pressure conditions experienced by the oxygen sensor and *FireStingO2* meter). The actual pressure at the sensor position needs to be determined with e.g. a barometer and adjusted manually (default: 1013 mbar).

If the actual atmospheric pressure cannot be determined on site, it is also possible to enter the

- **Elevation (m)** above sea level. For this click on **Elevation** and enter the actual elevation in meters. This procedure will only calculate the average atmospheric pressure for this elevation; therefore this option is less precise than measuring the actual atmospheric pressure.

If **Internal Pressure Sensor** is selected, the oxygen measurements are automatically compensated for pressure changes e.g. caused by weather changes.

SALINITY: The **Salinity (g/L)** of the environmental sample 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. For measurements in gas samples this value has no relevance (and is not active).

**(3) Units**

The oxygen unit can be selected for each channel by the selector **Units**. The selectable units include raw value (default), % air saturation, % $O_2$, mL/L, µmol/L, mg/L (ppm), hPa (mbar), mmHg (Torr), dphi and µg/L (ppb).

For measurements in a **Gas Phase** only the units raw value, % $O_2$, hPa (mbar), mmHg (Torr) and dphi can be selected, whereas for measurements of dissolved oxygen in a **Water** sample (**DO**) all units except % $O_2$ can be selected. For detailed information please refer to chapter 12.6.

### 6.1.2   Channel Tab: Optical Temperature Sensors

To enter the **Settings** for an optical temperature sensor connected to a *FireStingO2* channel, open the respective channel tab in the **FireSting Settings** window of the *Oxygen Logger* software.

Enter the **Sensor Code** of the connected optical temperature sensor (see label on sensor).
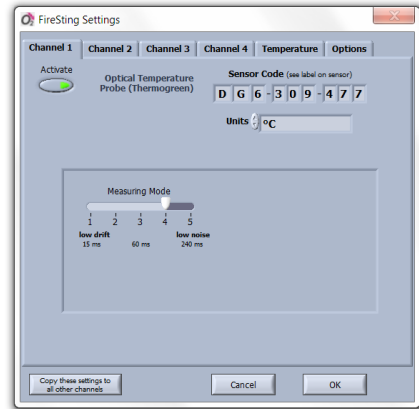
The **Unit** for the temperature readings is **°C**.

The **Measuring Mode** for the optical temperature sensor can be adjusted gradually between low drift (1) and low noise (5) of the sensor signal by moving the arrow with the mouse along the scale, thereby changing the measuring time. An intermediate mode (4) is default.

> Ensure that the sensor code of a sensor connected to a specific channel (e.g. 2) of the *FireStingO2* meter is entered in the same channel panel (e.g. 2) in the window **FireSting Settings**.

### 6.1.3   Temperature Tab

An **External Temperature Sensor (PT100)** connected to the temperature port **T** of the *FireStingO2* meter (see chapter 3) and the **Internal Temperature Sensor (within instrument)** can be activated in the FireSting Settings tab **Temperature**.
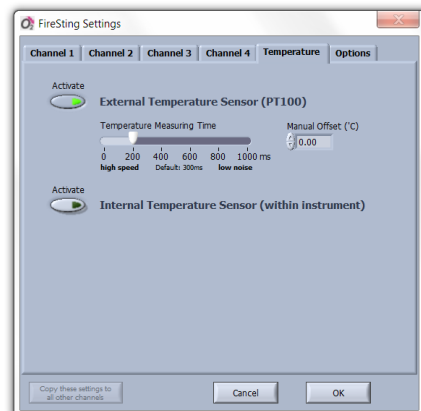
Both temperature sensors can be activated independently even if **Fixed Temperature** or **Optical Temperature Sensor** was selected for temperature compensation of all connected oxygen sensors (in this case the oxygen measurements are not affected by these temperature sensor measurements). The measured temperature is then displayed in the **Overview** panel (see chapter 6.3) of the main window and saved into the data file (see chapter 6.4).

After activation of an external PT100 / internal temperature sensor in an oxygen sensor channel tab of the Settings (see 6.1.1), the temperature measured by these temperature sensors is then displayed in the respective channel tab and the **Overview** tab of the Main Window in units of degree Celsius (y-axis on the right-hand side) and is saved into the data file (see chapter 6.4).

The **Temperature Measuring Time** (only multiples of 100 ms possible) of the **External Temperature Sensor (PT100)** can be optionally (a) increased in order to reduce the noise of the temperature measurement or (b) decreased in order to achieve higher sampling rates.

A **Manual Offset** of the External Temperature Sensor (PT100) might be entered for a 1-point calibration of the temperature sensor (default: 0). It is recommended to check the reading of the external temperature PT100 probe periodically in stirred water/ water bath/incubator of known temperature at steady state. This is especially important if a concentration unit (like µmol/L or mg/L) was selected for the oxygen measurements in the Settings.

Changing the temperature offset will lead to a loss of the oxygen sensor calibration.

To check for a temperature offset 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. Wait for steady state, read the measured temperature, and enter it as a **negative**

Temperature Offset. Then perform a new calibration of the connected optical sensor.

Please note that each *FireStingO2* meter provides only a **single** port for an external PT100 temperature probe. For **Automatic Temperature Compensation** (see chapter 6.1.1) of >1 oxygen sensor with an external PT100 temperature probe, all oxygen sensors with automatic temperature compensation have to measure at the same temperature condition. Alternatively, optical temperature sensors can be connected to optical sensor channels or the temperature extension module *TeX4* can be coupled to the *FireStingO2*, offering 4 ports for PT100 temperature probes.

### 6.1.4   Options Tab

In the Settings tab **Options**, several internal sensors and an **Analog In** can be activated. The **Internal Pressure Sensor** and the **Internal Humidity Sensor** inside the *FireStingO2* meter can be independently activated by clicking on the respective buttons and are displayed in the **Overview** panel of the main window and saved into the data file. If the **Internal Pressure Sensor** of the *FireStingO2* was activated in one of the oxygen channel tabs (see 6.1.1), it is automatically activated in the **Options** panel.

It is possible to designate a specific name to the connected *FireStingO2* in **Device Name** e.g. "Water Container Nr.42". This device name is then indicated in the top line of the main window. This option is especially useful if several *FireStingO2* devices are operated in parallel in order to distinguish the opened logger windows.
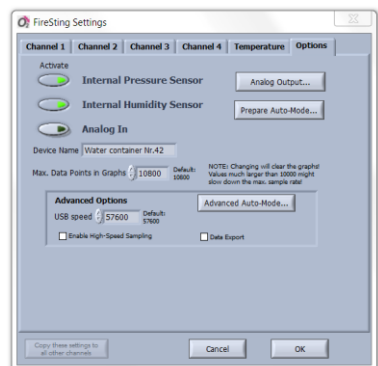


The activation of the **Analog In** button allows to read in a voltage signal at the extension port, e.g. from third-party sensors. The

measuring range is 0-2.5V and the display and output of the signal is in mV. For details refer to chapter 11.

The maximum number of data points kept in the graphs can be changed by the selector **Max. Data Points in Graphs** (default: 10800). A change of the number will clear the graphs and high values (>>10000) might decrease the maximum sample rate.

The *FireStingO2* offers four analog outputs (0-2.5V) at the extension port which can be configured by pressing the **Analog Output** button. For details refer to chapter 11.

## Only for advanced users:

The **USB** communication **speed** can be adjusted e.g. for improving the maximum sampling rate (default: 57600).
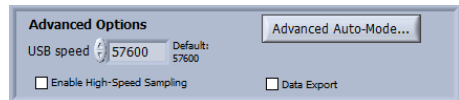


Activation of the button **Enable High-Speed Sampling** will enable the adjustment of a Sample Interval <0.25 s in the main window (and disable **Max. Data Points in Graph**). Details on request!

The **Advanced Auto-Mode** button allows advanced configuration options for the Auto-Mode. For details refer to chapter 11.

## 6.2   Main Window

After adjusting the Settings for all connected sensors after start of the Oxygen Logger software, the following main window is shown:



The four panels **Channel 1-4** correspond to the fiber-optic sensors connected to the ports **1-4** at the *FireStingO2* (see chapter 3). For the 1- or 2-channel version of the *FireStingO2*, only the respective panels will be visible.
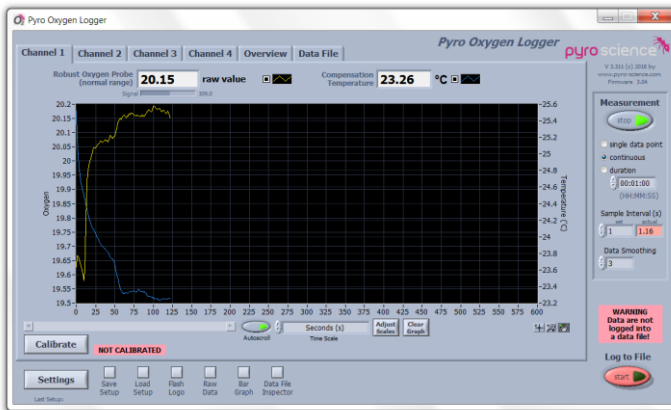
The default sensor readings show uncalibrated sensor readings (in raw value). For oxygen sensors this gives only qualitative information of the actual oxygen level.

After activation of the respective channels in the **Settings** (see chapter 6.1), the sensor readings of each channel are displayed in its corresponding panel in a numeric display (**D**) and in a chart recorder (**C**) in the chosen unit (**UD**).

The description of the sensor type, as defined in the **Settings** by the **Sensor Code**, is shown in the description display (**DD**). The **Signal** intensity (**SI**) of each oxygen sensor is shown in its channel tab as horizontal indicator bar underneath the numeric display (**D**).

 If a sensor was not yet calibrated or its Settings were changed, a warning **Not Calibrated** is displayed in the **W**arning **D**isplay.
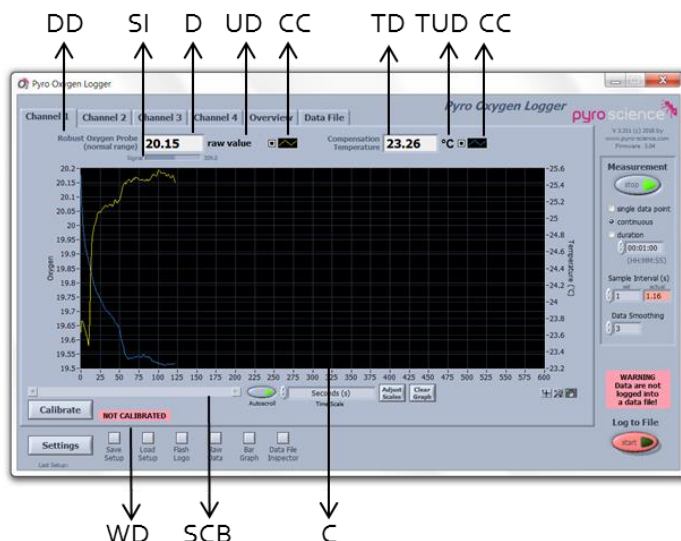
### 6.2.1 Chart Recorder

The color and appearance of each graph can be changed by clicking on the color-control (**CC**), opening a pop-up menu. With **Common Plots**, **Color**, **Line Style**, **Line Width**, **Interpolation**, and **Point Style** the chart appearance can be changed. Clicking on the small rectangular button allows hiding / showing the respective graph.

The visible time frame of the chart recorder (**C**) can be changed by moving the bar along the scroll bar (**SCB**). Switching off the **Autoscroll** button will allow inspection of older data which are not visible anymore in the time frame, e.g. during longtime measurements.

The display of the data in the charts can be changed by different chart tools arranged underneath the chart recorder. The button with the magnifying glass offers different zoom options. After clicking the button with the hand, the user can click onto the chart and move the whole area while keeping the mouse button pressed.
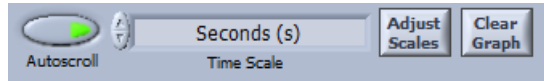
## Clear Graph

The button **Clear Graph** offers the options to clear only the graph of one channel (**Clear Single Graph**), or to **Clear Graphs of all Channels & Zero Time Scale** (i.e. the graphs in all other panels will be also cleared). Note, that this will not affect the saved data in the data file.
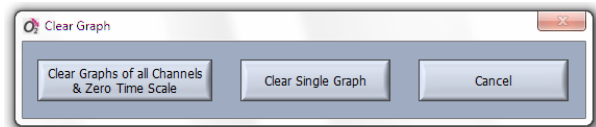


## Adjust Scales

The unit of the x-axis (Time) can be changed with the selector **Time Scale** (using the arrows or clicking onto the field). The time scale can be displayed in Seconds (s), Minutes (min), Hours (h), Relative Time (HH:MM:SS), Absolute Time (HH:MM:SS), Absolute Time & Date and in Data Points.

For a channel tab with connected optical oxygen sensor and external temperature PT100 sensor, the y-axes can be **Oxygen** (left) and **Temperature** (right). For a channel tab with connected optical temperature sensor the y-axis is optical Temperature (left; **opt. Temp.**).

The scales of the x-axis (Time) and of the y-axes (depending on connected sensor type, see above) can be adjusted by clicking on **Adjust Scales**, opening the respective pop-up window:
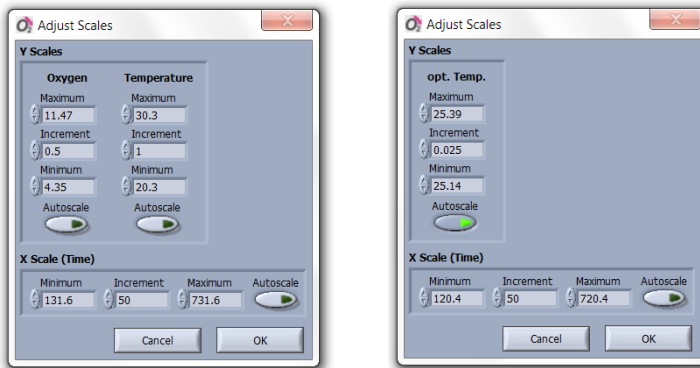


The upper (**Maximum**) and lower limits (**Minimum**) and the **Increment** of the **Y Scales** and **X Scale (Time)** can be changed by clicking on the respective selector or by double-clicking directly onto the field and entering the values manually (changing these parameters will automatically deactivate the auto-scaling). Autoscaling for all axes can be activated with the **Autoscale** button. The arrow in the button turns from dark green to light green indicating that Autoscale is activated. By default only the y-axis **Oxygen** is in Autoscale mode.

**NOTE**: The Charts are automatically cleared in the panels of the main window after the **Settings** have been modified, which might require also new sensor calibration.

If changes of the Settings require new sensor calibration, a warning **Not Calibrated** appears right-hand side of the **Calibrate** button in the corresponding channel panel of the Main Window.
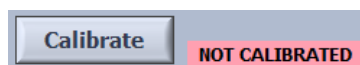
## 6.2.2  Warnings

A reasonable oxygen sensor shows signal intensities well above 50 (typically 50-500)[1]. If the **Signal** intensity drops below 50, the horizontal indicator bar underneath the numeric display turns gradually from grey to red, indicating that the sensor gets degraded soon. 
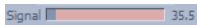
At signal intensities of <10, the warning  will appear in the warning display (**WD**). At a signal intensity of <5, the display changes to **NaN** (**N**ot **a N**umber), indicating that the signal is too low and the sensor needs to be replaced (or moved to another position on the sensor spot).
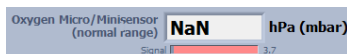


Besides the warning **Not calibrated** for an uncalibrated sensor, the warning display (**WD**) can show the following warnings:



**Low signal** ⎯⎯ The sensor is either not connected or needs to be replaced by a new one. In case of contactless sensors it might indicate that the distance between the optical fiber and the sensor spot is too large. For advanced users: increase the LED intensity in the Advanced Settings (see chapter 6.1.1).

**Signal too high** ⎯⎯ There might be too much ambient light on the sensor tip or the sensor spot. Avoid direct sun light exposure or strong direct illumination with a lamp. For advanced users: decrease the LED intensity and / or the amplification in the Advanced Settings (see chapter 6.1.1)

**Bad reference** ⎯⎯ This indicates internal problems of the *FireStingO2*. Please contact *PyroScience* for support.

**NOTE**: Do not continue with measurement if a warning is shown!

---

[1] Note: Exceptions are trace oxygen sensors. During the air calibration at 21% O2, these sensors naturally show a very low signal intensity (as low as 10). But the signal intensity will strongly increase when a trace oxygen sensor is applied within its specified range of 0-10% O2.

Refer also to the Troubleshooting in chapter 12.3.

The actual **Compensation Temperature** (see chapter 6.1.1), i.e. the temperature

Compensation Temperature **20.00** °C

used for calculating the oxygen values, is shown in the temperature display (**TD**) in units of **degree Celsius** (**°C**). When no external PT100 temperature sensor is connected, but activated in the **Settings** (see chapter 6.1), **NaN** is displayed and a warning **Bad Temperature** appears below the chart recorder (also for broken or nonfunctional temperature sensors).
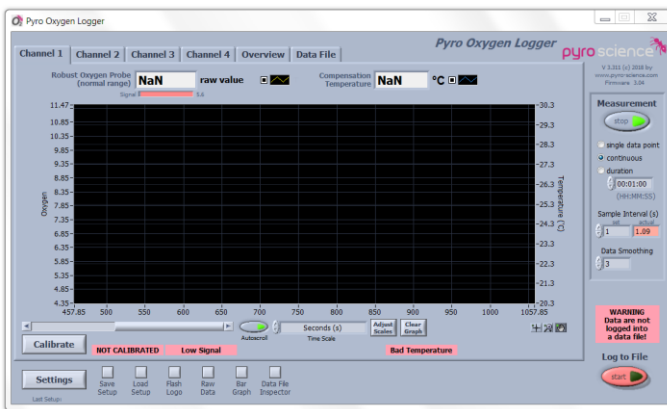


If a sensor is not yet calibrated, the warning **Not Calibrated** is shown on the right-hand side of the **Calibrate** button and the data are shown in units of "raw value", reflecting the measured oxygen levels only qualitatively. In order to switch to **quantitative** oxygen units, a sensor calibration has to be performed by clicking on **Calibrate.** The calibration procedure is explained in detail in chapter 7.

### 6.2.3   Measurement and Logging
Measurement

A measurement start button (**MSB**) is activated as default. The light green arrow in the button indicates that a **Measurement** is in

progress. Clicking again on it will stop the measurement (dark green arrow).

The mode of **Measurement** can be chosen as **single data point** acquisition, **continuous** sampling (default setting) or as continuous sampling limited to a defined time interval. The **duration** of this time interval can be adjusted in the duration display (**DUD**) shown as hour (HH): minutes (MM): seconds (SS).

MSB ←

DUD ←

The **Sample Interval (s)** for continuous sampling can be defined in the field designed with **set.** Setting the sample interval to 0.25 will give the maximal possible scan rate. The exact maximal rate depends on the settings and the number of activated channels. The actual sample interval is shown in the display **actual** and is displayed in red if the **actual** ≠ the **set** sample interval.

SB ←
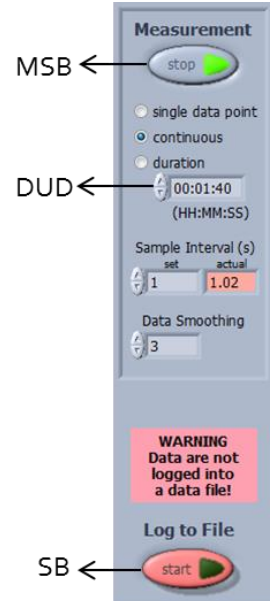
Acquired data can be smoothed by a **Data Smoothing** (range 1..10, **default**: 3, a value of 1 means no data smoothing). For **continuous** or **duration** measurements with a sample interval <10 s, data smoothing is done by a simple running average (e.g. with **Data Smoothing**=5 always the last 5 sampled data points are averaged). However, for **single data point** measurements and for **continuous** or **duration** measurements with sample intervals >10 s, the data smoothing is done by averaging repetitive measurements (e.g. with **Data Smoothing**=3 for each data point 3 oxygen measurements are performed as fast as possible sequentially, and the average of these 3 measurements is displayed as the new data point).

**IMPORTANT**: By default the displayed data are not automatically saved to a file.

## Data Logging

To activate data logging, click on the red **start** button (**SB**) of **Log to File**. Select a file name in the appearing file dialog. The saved data files are simple text-files with the file extension ".txt", which can be easily imported into common spreadsheet programs.
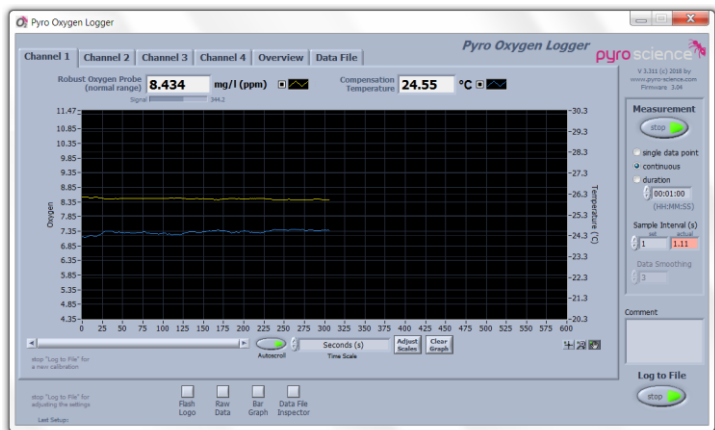


Thereafter, the indicator **Comment** is shown additionally. Here, the measurements can be commented and this comment is then saved together with the next data point into the data file.

During data logging, the data file can be displayed and opened by clicking on the button **Data File Inspector**. The data logging is indicated by the light green arrow in the grey **Log to File** button and can be stopped by clicking this button again.

**NOTE**: During data logging, the buttons **Settings** and **Calibrate** are not active and cannot be used before **Log to File** is stopped.

All current settings and calibration data are automatically saved when closing the *Pyro Oxygen Logger* software (by clicking on the cross in the upper right corner of the main window), and are automatically loaded again at the next startup. The **Last Setup** loaded is shown underneath the **Settings** button.

## Save Setup & Load Setup

The button **Save Setup** can be used to save the current settings and calibration data of all channels. They can be reloaded anytime by pressing the button **Load Setup**. This allows e.g. to switch between different laboratory setups with a single *FireStingO2*. This function might be also useful if different computers are used for the calibration and for the actual measurements. You might calibrate the sensors with the first computer, save the configuration with **Save Setup**. By transferring this file and also the oxygen meter *FireStingO2* to a second computer, you can load there again this configuration with **Load Setup** giving you calibrated sensors ready for the measurement. Note that for this procedure identical software version of the *Pyro Oxygen Logger* must be installed on both computers!
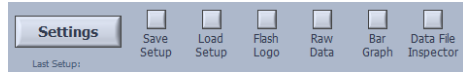
**Save Setup** and **Load Setup** might be useful also if e.g. a 1-channel *FireStingO2* should be used repeatedly with several oxygen sensors. Initially each sensor needs to be calibrated only once and the configuration of each sensor is saved with **Save Setup**. If later on a measurement should be performed with a specific sensor, it is only necessary to load the configuration for this sensor with **Load Setup** (but it must be checked if the calibration is still valid or the sensor needs to be calibrated again).

## Flash Logo

The button **Flash Logo** causes a short flashing of the illuminated logo of the *FireStingO2* meter. Several *FireStingO2* meters can be connected to the PC in parallel and multi-channel measurements can be performed by opening the *Pyro Oxygen Logger* software a number of times corresponding to the number of connected *FireStingO2* meters. The different windows operate completely independent of each other and are assigned to exactly one

*FireStingO2*. This allows measurements in different setups at the same time. The flashing of the logo (for ca. 1 sec after pressing the **Flash Logo** button) can help to assign a specific logger window to the corresponding *FireStingO2* meter (more details in chapter 12.5).

## Bar Graph

Clicking on the button **Bar Graph** opens the **Bar Graph Window**. Here, different parameters can be chosen to be displayed in the bar graphs by clicking next to "Select data type", like the **Signal Intensity (mV)** of the connected optical sensors.
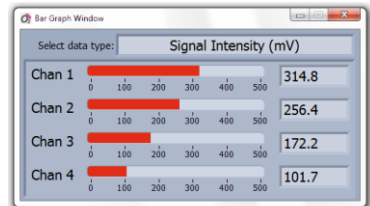


## Data File Inspector

By clicking on the button **Data File Inspector,** a saved data file can be opened with **Open File** and exported to Excel® with the button **Export to Excel**. Data saved currently can be exported during logging, as well as when the logging is terminated. For details see also chapter 6.4.

## Raw Data

Clicking on **Raw Data** opens a pop-up window **Oxygen Sensor Raw Data** which is described in chapter 6.2.4.

### 6.2.4   Raw Data Window

The Raw Data Window is mostly intended for trouble shooting and advanced users. During standard measurements it is in general not needed. After clicking on the **Raw Data** button in the **Main Window** (see chapter 6.2) the following **Oxygen Sensor Raw Data** window opens:
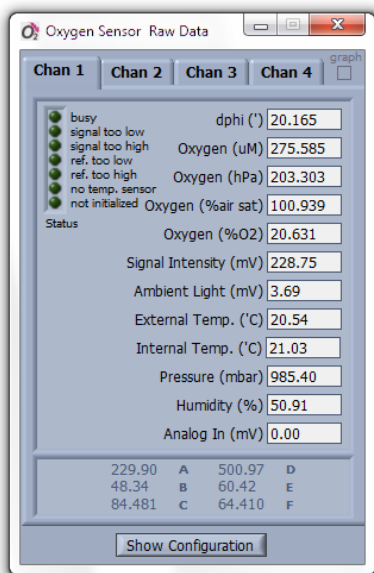
The panels of each oxygen channel (**Chan 1-4**) show the phase shift as "delta phi" (**dphi, in °**). **dphi** is the actual measured raw value which is used for the internal calculation of the oxygen concentration **Oxygen (µM)**, **Oxygen** partial pressure **(hPa)**, **Oxygen** in % air saturation **(% air sat)** and **Oxygen** (**% O2**) (see also chapter 12.4).

The **Signal Intensity** (in mV) gives a measure of the quality of the oxygen measurement, which is also displayed in the horizontal bar indicator in the main window (see chapter 6.2).

**Ambient Light** (in mV) gives a measure of the ambient light entering the sensor from outside. At too high ambient light levels the detector of the *FireStingO2* might get saturated giving the warning **Signal too high** in this window and in the warning display of the main window (see chapter 6.2.2).

The temperature measured by the connected external PT100 temperature sensor (**External Temp. (°C)**) and the internal temperature sensor (**Internal Temp. (°C)**) in the *FireStingO2* meter, the **Pressure (mbar)** and **Humidity (%)** measured by the internal sensors inside the *FireStingO2*, as well as the **Analog In (mV)** are also displayed.

On the left side, the **Status** and different warnings can be indicated by the software concerning the signal and reference intensity (too low, too high) and the detection of the temperature sensor.

On the right-hand side of the channel tabs, a **graph** can be activated, showing the dphi (°) and Signal Intensity (mV) in the graph (default setting). Plotting of additional parameters can be activated by clicking on the small rectangular button next to the color control of the respective parameter.
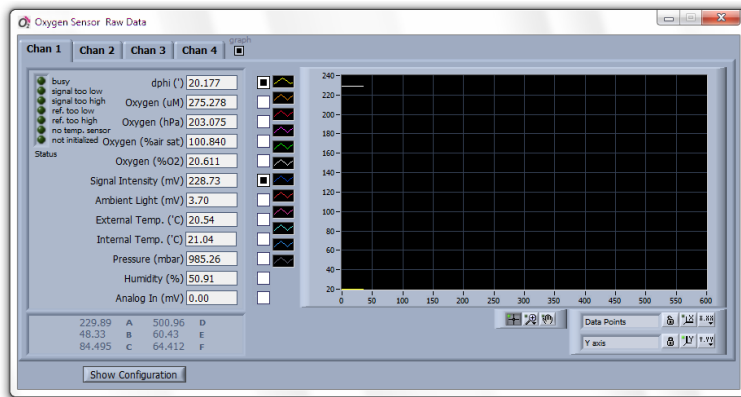
## 6.3  Overview Panel

In the panel **Overview,** the sensor readings of all activated oxygen sensors and the signals from the Analog In are displayed on the left y-axis. The readings of the optical, external and internal temperature sensors and of the internal pressure and humidity sensors are displayed on the right y-axis.

Each sensor reading is shown also as a numerical value in the chosen unit on top of the overview graph. The plot style of each channel in the chart can be changed by clicking on the color control:

By clicking on the small rectangular button of the color control, it is possible to show or hide the respective graph. This show / hide functionality is especially useful, if e.g. a single graph should be inspected in detail while all other graphs are hidden.
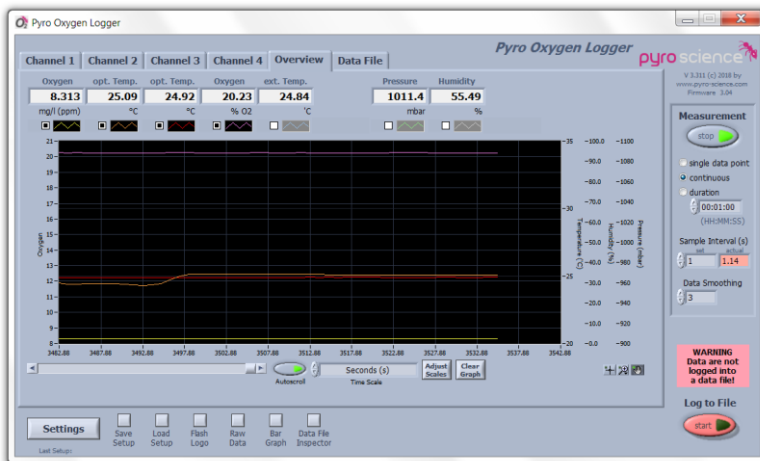


With Common Plots, Color, Line Style and Width, Interpolation and Point Style each plot in the chart can be changed. The items Bar Plots, Fill BaseLine, and Y-Scale are not appropriate for this application.

## 6.4 Data File Panel

In the Data File Panel, the current log file is shown and can be exported to Excel. By clicking on the **Update Table** button, the latest data are displayed in the table. By clicking on **Export to Excel**, the data file is exported to Excel®. Current data can be exported during logging, as well as when the logging is terminated.

> **NOTE:** Data are only displayed in the data file panel when they are logged to a file.
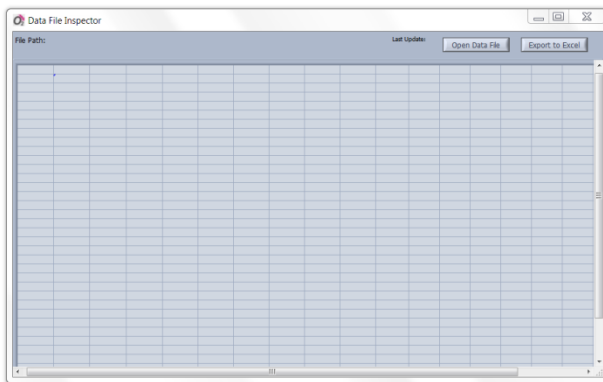
# 7   Sensor Calibration

This chapter describes the possible calibration modes for optical oxygen and temperature sensors from *PyroScience* using the logger software "*Pyro Oxygen Logger*".

Please note that the calibration modes described in this chapter can be only performed with a *FireStingO2* device with firmware version >3.0 (having integrated humidity and pressure sensors) and a software version >3.3 of the *Oxygen Logger*.

This chapter covers only the necessary steps during the calibration procedure. For details regarding the preparation of oxygen calibration standards refer to chapter 8.

**Note:** It is strongly recommended to perform a manual calibration at conditions close to the environmental conditions during measurements.

## 7.1   Optical Oxygen Sensor Calibration

Before starting the calibration, ensure that the correct **Sensor Code** has been entered in the settings (refer to chapter 6.1.1) and prepare appropriate calibration standards (refer to chapter 8). For calibration of contactless sensors, please refer also to chapter 9.

To calibrate a sensor click on the button **Calibrate** in the corresponding channel panel. Note that during data logging this button cannot be used until **Log to File** was **stopped**.
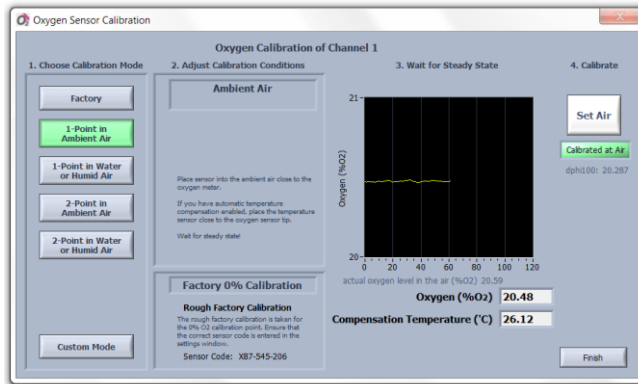


Five *standard modes* can be chosen for the calibration:

**Factory** calibration (for a quick, rough calibration): taking the 0% and the air calibration values from the sensor code, **advised only for rough measurements or testing purposes (not recommended for measurements)**.

**1-Point in Ambient Air**: taking the 0% calibration value from the sensor code and the air calibration value from a manual calibration in ambient air (see chapter 8.1.1) for precise measurements around 21% O2.

**1-Point in Water or Humid Air**: taking the 0% calibration value from the sensor code and the air calibration value from a manual calibration in air saturated water (see chapter 8.1.3) or water-vapor

saturated air (100% RH, see chapter 8.1.2) for precise measurements around 100% air saturation.

**2-Point in Ambient Air**: taking the 0% and the air calibration value from a manual calibration for precise measurements over the full range (0-21% O2). This mode uses the ambient air (see chapter 8.1.1) for determining the air calibration value. For determining the 0% value, specially prepared 0% calibration standards can be used (see chapter 8.2).

**2-Point in Water or Humid Air**: taking the 0% and the air calibration value from a manual calibration for precise measurements over the full range (0-100% dissolved O2 (DO)). This mode uses air saturated water (see chapter 8.1.3) or water-vapor saturated air (100% RH, see chapter 8.1.2) for determining the upper (air) calibration value. For determining the 0% value, e.g. water mixed with a strong reductant can be used (see chapter 8.2.1).

**NOTE**: It is recommended to determine the air calibration value in air saturated water (see chapter 8.1.3) if the measurements will be performed in water samples (aqueous liquids).

During the calibration of a sensor connected to a specific channel, the **Sample Interval** is automatically set to 0.5 s and the **Data Smoothing** to 5, ensuring a fast determination of a precise mean value during sensor calibration. After finalization of the calibration, the program returns automatically to the former settings.

Please note that the adjustment of the **Settings** concerning temperature and pressure compensation (see chapter 6.1.1) are taken automatically also during the calibration. Ensure appropriate Settings and calibration standards.

For advanced users and applications, a **Custom Mode** can be selected, allowing the user to combine freely the possible calibration types for the air and the 0% calibration.

Possible Air Calibration Types in the Custom Mode:

- **Air with 100% Humidity or Air Saturated Water**,
- **Ambient Air**,
- **Custom Calibration** (allowing also calibrations at freely chosen oxygen levels)
- **Factory Air Calibration**.

Possible 0% Calibration Types in the Custom Mode:

- manual **0% Calibration**
- **Factory 0% Calibration**

In the following the different calibration modes are described.

### 7.1.1   Calibration Mode: Factory

**NOTE**: The **Factory Calibration is intended only for rough measurements and testing purposes**. It is only possible if the **correct Sensor Code** has been entered in the **Settings** (see chapter 6.1.1).

If the calibration mode **Factory Calibration** is chosen, ensure that the correct sensor code has been entered in the **Settings** of the corresponding channel (as displayed in 2. **Adjust Calibration Conditions** of the **Oxygen Sensor Calibration** window).

If the sensor code displayed is not correct, click on **Finish**, go to the **Settings**, enter the correct **Sensor Code** and repeat the **Factory Calibration**.

If the Sensor code is correct, the factory calibration is completed by clicking on **Finish**, thereby returning to the main window of the corresponding channel.

### 7.1.2   Calibration Mode: 1-Point in Ambient Air

**NOTE**: The calibration **1-Point in Ambient Air** is only possible with precautions (see chapters 8.1 and 8.1.1) and with the **correct Sensor Code** entered in the **Settings** (see chapter 6.1.1).

The calibration mode 1-Point in Ambient Air is selected by clicking on the button **1-Point in Ambient Air**. This mode uses the ambient air (see chapter 8.1.1) for determining the air calibration value. The 0% calibration value is taken from the **Sensor Code**.
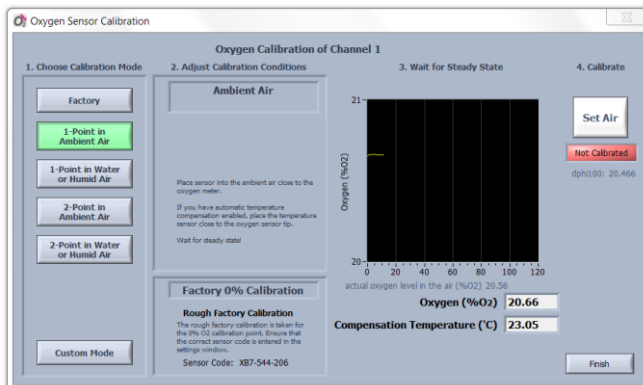
## Temperature, Pressure and Humidity

Please note that the adjustments of the **Settings** concerning temperature, pressure and humidity (see chapter 6.1.1) are taken automatically also during the calibration. Before calibrating the sensor, please ensure that the appropriate Settings are chosen.

If the atmospheric pressure and the relative humidity of the ambient air are read from the internal **Pressure** and **Humidity sensors**, it is important that both the *FireStingO2* device and the connected oxygen sensor are exposed to **identical environmental conditions**.

For compensation of the air **Temperature** either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from the

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

## Factory 0% Calibration

Ensure that the correct **Sensor Code** has been entered in the **Settings** of the corresponding channel. If the sensor code displayed in **Factory 0% Calibration** is not correct, click on **Finish**, go to the **Settings**, enter the correct **Sensor Code** and repeat the calibration.

## Set Air

For the air calibration value (in **Ambient Air,** see chapter 8.1.1), place the oxygen and external/optical temperature sensor **close to the air holes** at the backside of the *FireStingO2* meter.

> Ensure that the **oxygen sensor and the temperature sensor (if used) are completely dry**; otherwise the relative humidity around the sensor will differ from the measured humidity inside the *FireStingO2*. And even worse, the evaporation of water drops would cool down the sensor tips causing undefined temperatures.
>
> It is recommended that the device and the sensor are **placed for >30 min. under constant environmental conditions** before the calibration is performed.

Wait now until the sensor reading is stable by observing the graph and the numerical display of the oxygen sensor reading. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**.

Note that the button **Set Air** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type. If all readings have reached their steady-state, click on **Set Air,** and the actual oxygen sensor reading is taken for air calibration.

**4. Calibrate**

**Set Air**

Calibrated at Air

dphi100: 21.650

If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the calibration (Note: it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

A completed calibration is indicated by the green indicator **Calibrated at Air**.

Finish

Finally, click on **Finish** for returning to the main window.

### 7.1.3    Calibration Mode: 1-Point in Water or Humid Air

The calibration mode **1-Point in Water or Humid Air** uses air saturated water or water-vapor saturated air for determining the air calibration value. The preparation of appropriate air calibration standards is explained in chapters 8.1.3 and 8.1.2. The 0% calibration value is taken from the **Sensor Code**.
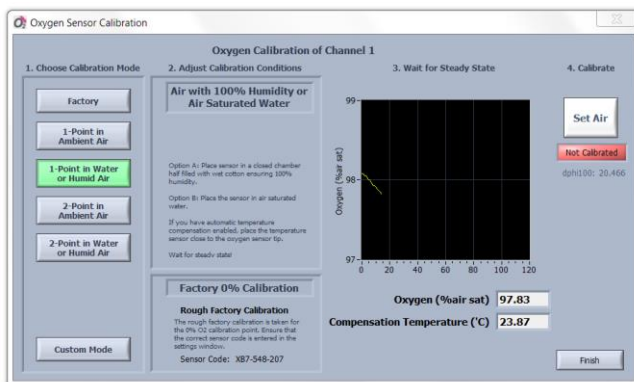


Temperature and Pressure Compensation

Please note that the adjustments of the **Settings** concerning temperature and pressure compensation (see chapter 6.1.1) are taken automatically also during the calibration. Before calibrating the sensor, ensure that appropriate Settings are chosen.

If the atmospheric pressure is read from the internal **Pressure Sensor**, it is important that the oxygen sensor in the calibration standard (air saturated water or water-vapor saturated air) is exposed to the same atmospheric pressure as the *FireStingO2* device (which is given in typical applications[2]).

For compensation of the **Temperature** in the calibration standards during oxygen sensor calibration either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from the

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from the

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

## Factory 0% Calibration

Ensure that the correct sensor code has been entered in the **Settings** of the corresponding channel. If the sensor code displayed in **Factory 0% Calibration** is not correct, click on **Finish**, go to the respective channel tab in the **Settings**, enter the correct **Sensor Code** and repeat the calibration.

## Set Air

Insert the oxygen sensor and, if used, the temperature sensor into the flask containing the air-saturated water or water-vapor saturated air, prepared as described in chapters 8.1.3 and 8.1.2.



4. Calibrate

Set Air

Calibrated at Air

dphi100: 21.650

---

[2] In rare cases and for special applications the calibration standard might be exposed to different pressures than the ambient air where the *FireStingO2* is positioned. In this case you have to choose the Custom Mode (chapter 7.1.6) where the calibration pressure can be entered manually.

Wait now until the sensor reading is stable by observing the graph and the numerical display of the oxygen sensor reading. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**. Note that the button **Set Air** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type.

> **NOTE**: Ensure constant calibration conditions! If a temperature sensor is selected, ensure that the temperature sensor is placed close to the oxygen sensor in the calibration standard.

If all readings have reached their steady-state, click on **Set Air**, and the actual oxygen sensor reading is taken for the air calibration.

If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the calibration (Note: it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

A completed calibration is indicated by the green indicator **Calibrated at Air**.

Finally, click on **Finish** for returning to the main window.

> **NOTE**: If using retractable needle-type sensors (e.g. item *R50*, *OXR230*, (*TR*)*OXR430* or *TPR430*), it is important that the sensor tip is extended out of the needle when the calibration value is taken.

### 7.1.4   Calibration Mode: 2-Point in Ambient Air

> **NOTE**: The calibration **2-Point in Ambient Air** is only possible with precautions (see chapters 8.1 and 8.1.1) and with the **correct Sensor Code** entered in the **Settings** (see chapter 6.1.1).

In the calibration mode **2-Point in Ambient Air** both the air calibration value and the 0% calibration value are determined from a manual calibration. Ambient air (see chapter 8.1.1) is used for determining the air calibration value and a specially prepared 0% calibration standard for determining the 0% calibration value. The preparation of 0% calibration standards is explained in chapter 8.2.
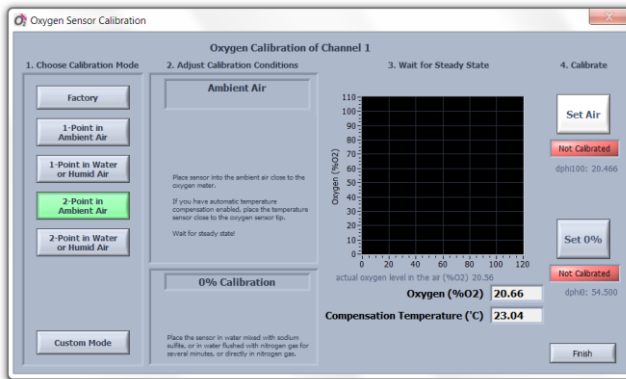


## Temperature, Pressure and Humidity

Please note that the adjustments of the **Settings** concerning temperature, pressure and humidity (see chapter 6.1.1) are taken automatically also during the calibration. Before calibrating the sensor, please ensure that appropriate Settings are chosen.

If the atmospheric pressure and the relative humidity of the ambient air are read from the internal **Pressure** and **Humidity Sensors**, it is important that both the *FireStingO2* device and the connected oxygen sensor are exposed to **identical environmental conditions**.

For **Temperature** compensation during oxygen sensor calibration in the air and 0% calibration standards either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from the

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

## Set Air

For the air calibration value (**Ambient Air**, see chapter 8.1.1), place the oxygen sensor and the external/optical temperature sensor (if used) **close to the air holes** at the backside of the *FireStingO2*.

Ensure that the **oxygen sensor and the temperature sensor (if used) are completely dry**; otherwise the relative humidity around the sensor will differ from the measured humidity inside the *FireStingO2*. And even worse, the evaporation of water drops would cool down the sensor tips causing undefined temperatures.

It is recommended that the device and the sensor are **placed for >30 min. under constant environmental conditions** before the calibration is performed.

Wait now until the sensor reading is stable by observing the graph and the numerical display of the oxygen sensor reading. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**.

Note that the button **Set Air** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type. If all readings have reached their steady-state, click on **Set Air**, and the actual oxygen sensor reading is taken for the air calibration.

If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the

calibration (Note: it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

A completed calibration is indicated by the green indicator **Calibrated at Air**.

<span style="color:red">Set 0%</span>

Subsequently, insert the oxygen and external/optical temperature sensor (if used) into an appropriate 0% calibration standard, prepared as described in chapter 8.2.

Also for the 0% calibration standard either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from an

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

If the internal **Pressure sensor** was selected for pressure compensation, it is important that both the *FireStingO2* device and the connected oxygen sensor in the 0% calibration standard are exposed to **identical pressure conditions**.

Wait until the oxygen sensor reading is stable by observing the graph. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**.



4. Calibrate

Set Air

Calibrated at Air

dphi100: 20.000

Set 0%

Calibrated at 0%

dphi0: 52.000

Finish

Note that the button **Set 0%** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type.

If all readings have reached their steady-state, click on **Set 0%**, and the actual oxygen sensor reading is taken for the 0% calibration. If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the calibration (it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

A completed calibration is indicated by the green indicator **Calibrated at 0%**.

Finally, click on **Finish** for returning to the main window.

> **NOTE**: If using retractable needle-type sensors (e.g. *OXR50*, *OXR230*, (*TR*)*OXR430* or *TPR430*), it is important that the sensor tip is extended out of the needle when the calibration value is taken.

### 7.1.5   Calibration Mode: 2-Point in Water or Humid Air

> **NOTE**: The calibration **2-Point in Water or Humid Air** is only possible with preparation of appropriate calibration standards (see chapter 8) and with the **correct Sensor Code** entered in the **Settings** (see chapter 6.1.1).

In the calibration mode **2-Point in Water or Humid Air** both the air calibration value and the 0% calibration value are determined from a manual calibration. Air saturated water (see chapter 8.1.3) or water-vapor saturated air (see chapter 8.1.2) is used for determining the air calibration value and a specially prepared 0% calibration standard (see chapter 8.2.1) for determining the 0% calibration value. The preparation of appropriate calibration standards is explained in chapter 8.

## Temperature and Pressure Compensation

Please note that the adjustments of the **Settings** concerning temperature and pressure compensation (see chapter 6.1.1) are taken automatically also during the calibration. Before calibrating the sensor, please ensure that appropriate Settings are chosen.

If the atmospheric pressure is read from the internal **Pressure Sensor,** it is important that the calibration standards (air saturated water or water-vapor saturated air, de-oxygenated water) are exposed to the same atmospheric pressure as the *FireStingO2* device (which is given in typical applications[3]).

For **Temperature** compensation during oxygen sensor calibration in the air and 0% calibration standards either a
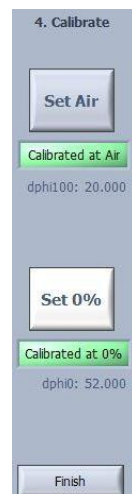
**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from an

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

---

[3] In rare cases and for special applications the calibration standard might be exposed to different pressures than the ambient air where the *FireStingO2* is positioned. In this case you have to choose the Custom Mode (chapter 7.1.6) where the calibration pressure can be entered manually.

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

## Set Air

For the air calibration point (**Air with 100% Humidity or Air Saturated Water**), insert the oxygen and temperature sensor into the flask containing either water-vapor saturated air (see 8.1.2) or air-saturated water (100% air saturation, see 8.1.3).

Wait now until the sensor reading is stable by observing the graph and the numerical display of the oxygen sensor reading. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**. Note that the button **Set Air** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type.

If all readings have reached their steady-state, click on **Set Air**, and the actual oxygen sensor reading is taken for the air calibration. If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the calibration (Note: it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

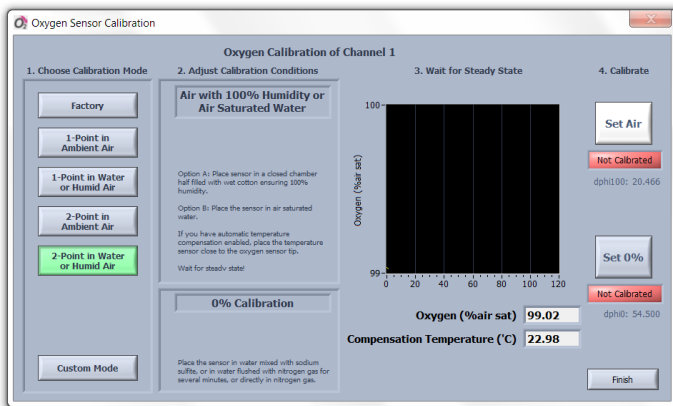A completed calibration is indicated by the green indicator **Calibrated at Air**.

## Set 0%

Subsequently, insert the oxygen and external/optical temperature sensor (if used) into the 0% calibration standard (de-oxygenated water, see 8.2.1).

Also for the 0% calibration standard either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from an

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

If the internal **Pressure Sensor** was selected for pressure compensation, it is important that both the *FireStingO2* device and the connected oxygen sensor in the 0% calibration standard are exposed to **identical pressure conditions**.

Wait until the oxygen sensor reading is stable by observing the graph. If **External** or **Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**.

Note that the button **Set 0%** will be highlighted as soon as the oxygen readings are within the expected range for the connected sensor type.

If all readings have reached their steady-state, click on **Set 0%**, and the actual oxygen sensor reading is taken for the 0% calibration. If the oxygen reading seems to be out of the expected range, a warning will be shown offering the possibility to repeat the calibration (Note: it is **not** recommended to continue **without** checking the calibration standard and the sensor!).

A completed calibration is indicated by the green indicator **Calibrated at 0%**.

Finally, click on **Finish** for returning to the main window.

### 7.1.6   Calibration Mode: Custom Mode

The custom calibration mode is selected by clicking on the button **Custom Mode**. This mode allows the user to combine freely all possible calibration types for the air calibration and the 0% calibration. The air calibration type can be selected by clicking on the "Air Calibration Selector". The 0% calibration type can be selected by clicking on the "0% Calibration Selector".
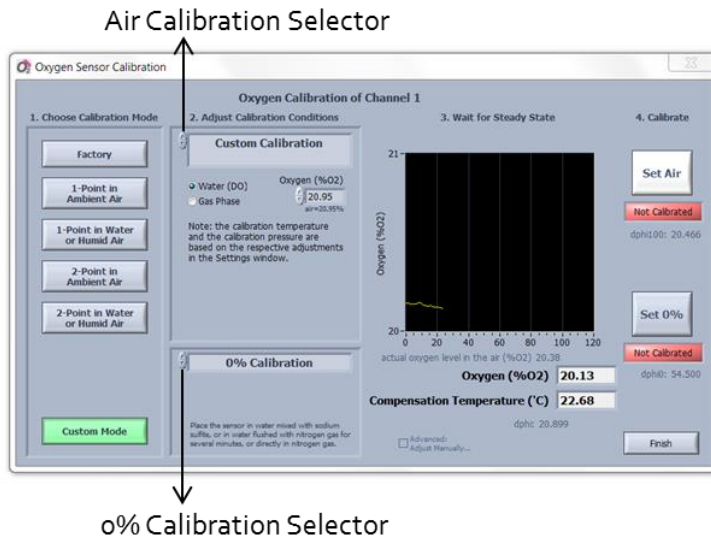


The following air calibration types can be chosen:

- **Factory Air Calibration** (refer to chapter 7.1.1)
- **Air with 100% Humidity or Air Saturated Water** (refer to chapter 7.1.3)
- **Ambient Air** (refer to chapter 7.1.2)
- **Custom Calibration** (more details below)

And for the 0% calibration the following types can be selected:

- **Factory 0% Calibration** (refer to chapter 7.1.1)
- **0% Calibration** (refer to chapter 7.1.4)

Most calibration types have been explained already in the preceding chapters (see references in the above list). Only the air calibration type **Custom Calibration** is a unique feature only available in the **Custom Mode**. The **Custom Calibration** offers the most flexible options for performing an air calibration as explained in the following.

The oxygen level in the calibration standard can be freely chosen in **Oxygen (%O2)**. If the air calibration standard is based on ambient air or air saturated water, then this value should be kept at 20.95% O2, representing the standard oxygen volume fraction in ambient air. However, other values can be adjusted if custom calibration gases are used, of e.g. 5% O2, which is useful when using trace oxygen sensors.

Depending on the calibration standard used, select either **Water (DO)** for dissolved oxygen or **Gas Phase**.

## Temperature, Pressure and Humidity

Please note that the adjustments of the **Settings** concerning temperature, pressure and humidity (see chapter 6.1.1) are taken automatically also during the calibration. Before calibrating the sensor, please ensure that appropriate Settings are chosen.

If the atmospheric pressure and the relative humidity of the ambient air are read from the internal **Pressure** and **Humidity Sensors**, it is important that both the *FireStingO2* device and the connected oxygen sensor are exposed to **identical environmental conditions**. Especially the use of the internal humidity sensor

requires several precautions; please refer to chapter 7.1.2 for more details.

For precision calibrations, it is generally advised to prepare calibration standards with 100% **R**elative **H**umidity (refer to chapter 8), which eliminates any possible error source by the usage of the internal humidity sensor.

For **Temperature** compensation during oxygen sensor calibration in the air and 0% calibration standards either a

**(1) Fixed Temperature** can be adjusted manually (needs to be determined and kept constant) or the temperature is read from an

**(2) External** (PT100) **Temperature Sensor** connected to the temperature port of the *FireStingO2* or from an

**(3) Optical Temperature Sensor** connected to a channel at the *FireStingO2* meter (its respective channel number needs to be entered at **Optical Temp. Channel**).

## Set Air

Place now the oxygen sensor and the external/optical temperature sensor (if used) into the calibration standard.

Wait until the oxygen sensor reading is stable by observing the graph. If **External or Optical Temperature Sensor** was selected, ensure also stable temperature readings indicated at **Compensation Temperature (°C)**.

If all readings have reached their steady-state, click on **Set Air**, and the actual oxygen sensor reading is taken for the air calibration. A completed calibration is indicated by the green indicator **Calibrated at Air**.
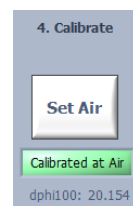
Consequently, the 0% calibration should be performed. If the calibration type **0% Factory Calibration** was selected no further steps are necessary (refer also to chapter 7.1.1).

If **0% calibration** was selected, follow the instructions given in chapter 7.1.4. Click on **Finish** for reverting to the main window.

---

**NOTE**: If using retractable needle-type sensors (e.g. *OXR50*, *OXR230*, (*TR*)*OXR430* or *TPR430*), it is important that the sensor tip is extended out of the needle when the calibration values are taken.

# Advanced Adjustments

For very advanced applications it is possible to manipulate all internal calibration parameters of the *FireStingO2* manually. This option is accessible by selecting **Custom Mode** and subsequently clicking on **Advanced: Adjust Manually,** which opens a separate window showing all internal calibration parameters.
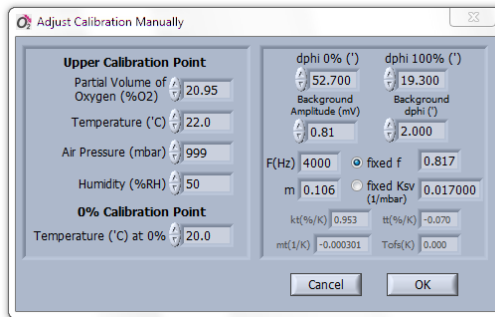


Here the **Upper Calibration Point** (default: **Partial Volume of Oxygen** 20.95**% O2**) can be defined, as well as the calibration conditions (Temperature, Air Pressure, Humidity) as described for the **Custom Calibration** (see chapter 7.1.6). The **Temperature (°C)** needs also to be determined and entered for the **0% Calibration Point**.

Further, the phase shift "dphi" (**dphi,** see chapter 12.4) for the 0% calibration standard (**dphi 0%** in °) and for the 100% calibration standard (**dphi 100%** in °) can be adjusted manually.

The **Background Amplitude** (in mV) and the **Background dphi** (phase shift in °) can be adjusted (refer to chapter 9.2 for more details). These two values are only relevant, if a background compensation for measurements with contactless sensors has been activated in the **Advanced Settings** of the respective channel.

The parameters **f, m, F, kt, mt, Tofs,** and **tt** are needed for the internal calculation of the oxygen concentration data. These parameters are specific constants for the *REDFLASH indicators*, and are automatically adjusted for the selected **Sensor Type** in the settings. *Unless otherwise communicated by PyroScience, it is strongly advised to leave these parameters at their default values.*

## 7.2 Optical Temperature Sensor Calibration

**NOTE**: The calibration of the optical temperature sensor (recommended every day) is only possible if the **correct Sensor Code** has been entered in the **Settings** (see chapter 6.1.2).

To calibrate the optical temperature sensor, click on the button **Calibrate** in the corresponding channel tab of the main window. Note that during data logging this button cannot be used until **Log to File** was **stopped**.

In the dialog window **Optical Temperature Calibration** two calibration modes can be selected for 1-point calibration of the optical temperature sensor connected to a channel at the *FireStingO2* device:
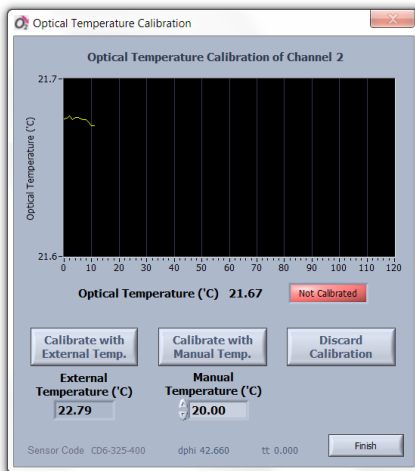


- **Calibrate with External Temp.**
- **Calibrate with Manual Temp.**

By clicking on **Calibrate with External Temp.,** the optical temperature sensor connected to a channel at the *FireStingO2* device can be calibrated against an **External Temperature Sensor** (PT100) connected to the temperature port of the *FireStingO2* (see chapter 3).

For a calibration with the external temperature sensor (item no. *TDIP15* or *TSUB21*), first check for a **temperature offset** of the connected PT100 sensor (see chapter 6.1.3). After adjusting this manual offset, the 1-point calibration of the optical temperature sensor can be performed subsequently in its calibration window. Ensure that the external temperature sensor is placed close to and exactly to the same temperature conditions as the optical temperature sensor.

For precise absolute temperature measurements, it is strongly recommended to determine manually if the external (PT100) temperature has an offset before calibrating the optical temperature sensor against the external temperature sensor.

All temperature readings are displayed in °C.

Alternatively, the optical temperature sensor can be calibrated manually against a **Manual Temperature (°C)**, which has to be determined and entered here. For this it is recommended to use a calibrated incubator (for measurements in the gas phase) or water bath of constant and known temperature (for measurements in water/aqueous samples).

By clicking on **Discard Calibration** the latest calibration is deleted and the optical temperature sensor is uncalibrated and needs to be calibrated again.

In the lower line of the **Optical Temperature Calibration** window the actual **Sensor Code** and the calibration value ("dphi") of the last calibration are displayed. If the displayed sensor code of the connected optical temperature sensor is not correct, return to the **Setting**s, enter there the correct **Sensor Code** and repeat the optical temperature sensor calibration.

Wait until the temperature sensor readings are stable by observing the graph and the numerical display of the **Optical Temperature (°C)** sensor. If all readings have reached their steady-state, calibrate the optical temperature sensor accordingly.

A completed calibration is indicated by the green indicator **Calibrated**.

Finally, click on **Finish** for returning to the main window.
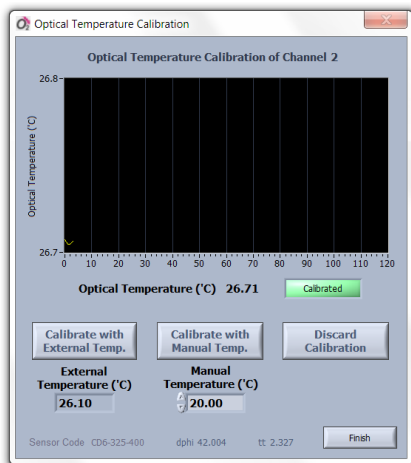
# 8 Oxygen Calibration Standards

## 8.1 The Air Calibration Standard

The Air Calibration standard can be

- ambient air
- water-vapor saturated air
- air saturated water (100% air saturation)

> When inserting fragile needle-type oxygen sensors into the calibration standards, ensure that the sensor tips are not hitting against e.g. the bottom of the flask or any hard object. Always use a proper lab stand for mounting the oxygen sensor!

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.

Furthermore, 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:

(1) The relative humidity and the temperature of the ambient air must be determined during calibration. The *Pyro Oxygen Logger* software calculates then automatically the real oxygen level under these conditions.

(2) 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.

Option (1) is utilized in the calibration standard "Ambient Air" (see section 8.1.1), whereby option (2) is utilized for the calibration standards "Water-Vapor Saturated Air" (see section 8.1.2) and "Air Saturated Water" (see section 8.1.3).

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 12.6). 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 *Pyro Oxygen Logger* 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 12.6):

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

giving a partial oxygen pressure of e.g. 205 mbar. This is the essential calibration value used internally by the *Pyro Oxygen Logger* software. The atmospheric pressure can be influenced 1) by weather changes (e.g. varying between ca. 990 and 1030 at sea

level) and 2) 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).

Thus in summary, there are three important parameters to be known for the air calibration standard:

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

The *FireSting* device and the *Pyro Oxygen Logger* software will guide the user through all calibration steps, not requiring the theoretical knowledge given above. And in case of a second generation *FireStingO2* (with micro USB connector), the built-in humidity and pressure sensors together with the internal or external temperature sensor will measure these parameters automatically for most calibration types (see also chapter 7).

### 8.1.1   Ambient Air

If ambient air is used as air calibration standard, there is no need for preparation. The dry oxygen sensor, optionally together with the dry external or optical temperature sensor, is simply exposed to the ambient air. Otherwise, follow the calibration procedures given in chapter 7.

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.

### 8.1.2  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 the wet cotton wool, while the other volume fraction is left free for inserting the tip of the oxygen sensor, and optionally also the temperature sensor. Otherwise, follow the calibration procedures given in chapter 7.

### 8.1.3  Air Saturated Water

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 for about 10 minutes air through the water with an air stone connected to an air pump (available as commercial equipment for fish aquaria). 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. Insert the oxygen sensor and temperature sensor into the flask and ensure that the tips of the sensors are immersed in the water and free of air bubbles. Otherwise, follow the calibration procedures given in chapter 7.

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

## 8.2 The 0% Oxygen Standard

The 0% calibration standard can be

- water mixed with a strong reductant
- water flushed with nitrogen gas ($N_2$)
- nitrogen gas ($N_2$)

### 8.2.1 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 $L^{-1}$, creating oxygen-free water by chemical reaction. It is not recommended to use saline water (e.g. sea water) for this, because the high salinity of the water might prevent a proper dissolution of the reductant. Stir the solution until the salt is completely dissolved, then stop the stirring and let the solution stand 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. Otherwise, follow the calibration procedures given in chapter 7.

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.

### 8.2.2   Water Flushed with Nitrogen Gas

Fill water into a glass flask (e.g. Duran flask) with a lid prepared with holes for inserting the oxygen sensor and a temperature sensor. Close it and stream for about 10 minutes nitrogen gas through the water. You might speed up this process by first boiling the water (and thereby removing all dissolved gases) and then stream the nitrogen gas during cooling through it. Insert the oxygen and temperature sensor into the flask, let it equilibrate and perform the calibration as described in chapter 7.

Please consider that streaming $N_2$ gas through water causes cooling of the water. Ensure a **correct temperature determination** of the 0% calibration standard!

### 8.2.3   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 as described in chapter 7.

Ensure that no ambient air enters the flask again during the calibration process. Convectional gas transport is a very fast process! It is therefore advised 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!

# 9 Calibration of Contactless Sensors

First prepare a setup with contactless oxygen sensors.

## 9.1 Calibration Procedure

In general, the calibration procedure for contactless sensors (e.g. sensor spots, flow-through cells, respiration vials) is the same as for fiber-based oxygen sensors as described in chapters 7 and 8. However, if a 1-point or a 2-point calibration should be performed, the calibration standards have to be filled directly into the vessel in which a sensor spot is glued, into the tubing of the flow-through cell, or into the respiration vial.

If "Ambient Air" is used for the air calibration standard (see chapter 8.1.1), a good air circulation of ambient air into the **dry** setup is important, ensuring that the relative humidity within the setup is identical to the relative humidity outside the setup (experienced by the *FireStingO2*). A falsely determined relative humidity gives a maximum relative error of 1% for the air calibration (refer also to chapter 8.1).

For precision applications, where it is not possible to ensure a dry setup for the calibration procedure, the alternative air calibration standards "Water-Vapor Saturated Air" (see also chapter 8.1.2) or "Air Saturated Water" (see also chapter 8.1.3) should be preferred. In the first case some part of the inner volume of the setup can be filled with e.g. wet cotton wool ensuring 100% RH around the oxygen sensor position. In the latter case, the inner volume of the setup is simply filled with air saturated water prepared as described in chapter 8.1.3. Ensure that the oxygen sensor is **completely** covered with the air saturated water and that **no air bubbles** adhere to the sensor surface!

## 9.2 Manual Background Compensation

The calibration of contactless sensors (i.e. sensor spots, flow-through cells and respiration vials) includes a compensation of potential background fluorescence from the fiber-optic cable connecting the *FireStingO2* with the contactless oxygen sensor. Based on the **Fiber Length (m)** entered in the **Settings** (see chapter 6.1.1), a background signal for compensation is estimated automatically by the *Pyro Oxygen Logger* software. So the user usually does not notice the background compensation at all. For standard applications this should be the preferred procedure.

But for precision applications and especially for low signal intensities or for application of nanoprobes in microfluidics, a manual background compensation should be performed by the user. For this, **Manual Background Compensation** must be selected in the **Advanced Settings** (see chapter 6.1.1). After opening the calibration window by clicking on **Calibrate** in the main window, a separate **Background Compensation** window will open automatically:
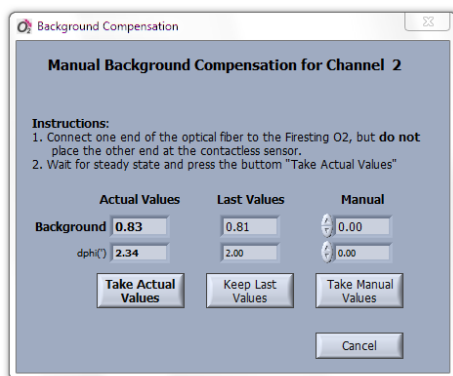


Here the background fluorescence of the connected *Optical Fiber* can be compensated. For this it is *important* that

- one end of the *Optical Fiber* is connected to the corresponding channel of the *FireStingO2* and

- the other end of the *Optical Fiber* is *not* attached to the sensor spot (i.e. disconnect this end from the spot adapter, adapter ring or from the flow-through cell)

Then wait for steady-state and press the button **Take Actual Values**.

Alternatively, the button **Keep Last Values** can be used if the sensor spots are again calibrated with the *same* optical fiber, which was background compensated before. Then the last values for the background compensation are kept.

It is also possible to enter values for the **Background** and **dphi** (°) manually into the field **Manual** and subsequently clicking on **Take Manual Values.** If you enter manually zero for **Background**, no background compensation is performed (not recommended).

After the background compensation is finished, the window closes and the program proceeds with the main calibration window (see chapter 7). It is important that for the subsequent calibration process the *Optical Fiber* is again attached to the sensor spot position, e.g. by connecting this optical fiber end again to the spot adapter, adapter ring or to the flow-through cell.

Please ensure that *during the background compensation* the *Optical Fiber is not connected* to the contactless sensor.

Please ensure that *during the subsequent calibration process* the *Optical Fiber is again attached to* the contactless sensor.

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.

# 10 Temperature Measurement

For the measuring range, precision and accuracy of the internal and external PT100 temperature sensors refer to chapter 12.1 and for the optical temperature sensors refer to their website: https://www.pyro-science.com/products.html

## 10.1 External (PT100) Temperature Sensors

The *FireStingO2* provides one port for an external temperature sensor. *PyroScience* offers e.g. the dipping-probe temperature sensor *TDIP15* (with 1.5 mm tip diameter) and the submersible temperature sensors *TSUB21* (with 2.1 mm tip diameter, completely Teflon coated). The *TSUB21* is fully specified for long-term submersion into aquatic samples, i.e. the complete sensor including the cable can be submersed. In contrast, the *TDIP15* is only specified for long-term immersion of the 100 mm long probe tip into aquatic samples. However, the complete *TDIP15* including the cable is splash-proof and withstands easily short-term submersion into water.

## 10.2 Internal Temperature Sensor

**It is not advised to use the internal temperature sensor for precision oxygen measurements.**

The *FireStingO2* meter has also a built-in internal temperature sensor for temperature measurements of the ambient air. If used during calibration or during temperature compensation of the oxygen readings in a gas sample or in air, ensure that the internal temperature in the *FireStingO2* equals the ambient temperature in air or in the gas sample (a certain degree of **warming** by the instrument cannot be excluded!). It is advised to compare the Internal Temperature Sensor reading with an External

Temperature Sensor connected to the *FireStingO2* or with an external thermometer.

## 10.3 Optical Temperature Sensors

For precise temperature measurements and true automatic temperature compensation of the oxygen measurements, it is recommended to use optical temperature sensors for automatic temperature compensation. The optical temperature sensors are connected to the same ports as the optical oxygen sensors. For details on the different optical temperature sensor types, please refer to chapter 5.1. For precise absolute temperature readings and true automatic temperature compensation, the optical temperature sensors need to be calibrated on a daily basis (see chapter 7.2).

## 10.4 Automatic Temperature Compensation

The external or optical temperature sensors can be simply used for recording the temperature in the measuring setup. However, the temperature sensors are especially useful in order to compensate automatically the oxygen sensor signals for temperature variations in the setup. The temperature compensation is needed due to two reasons:

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

In order to activate automatic temperature compensation for measurements in a sample, the type of temperature sensor needs to be selected at **Conditions in the Samples** in the **Settings** of the respective channel (see chapter 6.1.1). For measurements in water there are two options:

1) Select **External Temperature Sensor** and connect a PT100 temperature sensor (item: *TSUB21* or *TDIP15*) to the temperature port of the *FireStingO2* or

2) Select **Optical Temp. Channel** and enter the respective channel number of an optical temperature sensor connected to a channel (1-4) of the *FireStingO2*.

The temperature readings are also saved into the data file.

For measurements in a gas sample, a third option is to select the **Internal Temperature Sensor** of the *FireStingO2* meter (**not** recommended for precision measurements). In the latter case, please ensure free circulation of the ambient air around the *FireStingO2* meter and exclude internal warming.

Please ensure that the oxygen and the temperature sensor are both inserted into the same experimental setup/sample! Each *FireStingO2* meter provides only a single port for an external PT100 temperature sensor. For automatic temperature compensation with an external temperature sensor of >1 oxygen sensor, all oxygen sensors have to measure under identical temperature conditions or a temperature extension module with 4 temperature channels *TeX4* has to be coupled to the *FireStingO2*. Then the automatic temperature compensation is performed individually using the temperature sensor connected to the corresponding port of the *TeX4*. For true temperature compensation it is recommended to choose an optical temperature sensor connected to a channel of the *FireStingO2*.

However, it is also possible to use the automatic temperature compensation e.g. only for a single oxygen channel, whereas the other oxygen channels can be used for measurements at a **Fixed Temperature**. For the oxygen channels running at a fixed temperature, this temperature must be entered in the **Conditions in the Sample** in the **Settings** (see chapter 6.1.1). It is on the users`

authority to ensure that the sample is kept under this fixed temperature during the measurements.

Even if measurements with all activated oxygen sensors are performed under a **Fixed Temperature**, the external and optical temperature sensor can be used for independent temperature measurements. This requires a separate **activation** of the external temperature sensor in the **Temperature** panel of the **Settings** (see chapter 6.1.3) or a channel tab for an optical temperature sensor (see chapter 6.1.2).

# 11 Analog Output and Auto-Mode

The standard operation mode of the *FireStingO2* is based on a PC running the *Pyro Oxygen Logger* software operating the *FireStingO2* via the USB interface (as described in this manual). This user-friendly operation mode is generally recommended, as it offers easy control over the full functionality of the *FireStingO2*. However, several advanced features are available in addition for integrating the *FireStingO2* in customized setups.

An integrated 4-channel **Analog Output** at the extension port can be used for transferring measurement results (e.g. oxygen concentration, temperature, pressure, humidity, signal intensity) as voltage signals to other electronic equipment (e.g. loggers, chart recorders, data acquisition systems).

Further, the *FireStingO2* can be operated in a so-called **Auto-Mode**, in which the *FireStingO2* performs measurements autonomously without any PC connected to it. The auto-mode *does not* possess any integrated logging functionality, but the measured values must be read out via the analog output e.g. by an external data logger.

And finally, the extension port offers also a complete digital interface (UART) for advanced integration possibilities into custom electronics equipment (see appendix 12.2). This UART interface might be also utilized during auto-mode operation for a digital read-out of the measured values.

## 11.1 Analog Output

The analog output is provided at the connector X2 of the extension port. It offers 4 channels with an output range of 0-2500 mV. The pin configuration is given in appendix 12.2. The analog output is always active and automatically updated each time the

*FireStingO2* performs a new measurement (independent whether the *FireStingO2* is operated via a PC with the *Pyro Oxygen Logger* software, or if it is operated autonomously in the auto-mode).

The analog output can be configured by opening the **Settings** window and clicking there on the **Analog Output** button in the **Options** tab. A separate window will open.
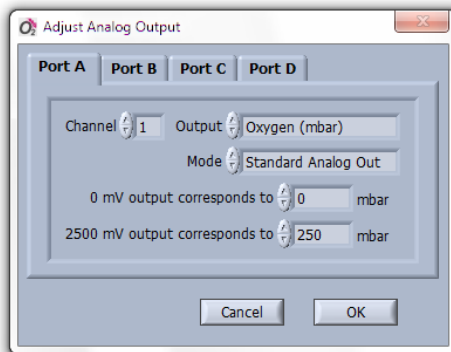


The settings of the 4 analog outputs can be adjusted in the respective tabs designated as **Port A-D**.

**NOTE:** The 4 analog outputs are deliberately designated with A, B, C, and D for distinguishing them clearly from the numbering 1, 2, 3, and 4 of the oxygen channels. The background is that the analog outputs are not fixed to specific oxygen channels ensuring highest flexibility.

Each analog output can be freely mapped to the oxygen **Channel** 1-4. The output parameter can be chosen with the selector **Output**. The following parameters can be selected: dphi (deg), Oxygen (µM), Oxygen (mbar), Oxygen (% air sat), Oxygen (% O2), Signal Intensity (mV), Ambient Light (mV), External Temperature (°C), Internal Temperature (°C), Pressure (mbar), Humidity (%) or Analog In (mV). Note that the selected **Channel** number is only significant if an oxygen related parameter is selected (first 7 options in the **Output** selector).

The operation **Mode** of the analog output can be chosen as **Standard Analog Out** or as **Alarm if out of range**. The **Standard Analog Out** mode represents the standard mode resulting in

voltage signals proportional to the measured value. The linear scaling of the voltage signal can be freely adjusted at **0 mV output corresponds to** and **2500 mV output corresponds to**.

<div style="background-color: yellow;">

**NOTE:** Due to hardware restrictions, the very lower range of the analog output around 0-3 mV shows slight non-linearities. For precision measurements e.g. around 0% O2 it is therefore recommended to map the zero oxygen level e.g. to 100 mV.

Example:     **0 mV output corresponds to**       -1   % O2

             **2500 mV output corresponds to**       24   % O2

This will give 100 mV for 0% O2, and 2200 mV for 21% O2.

</div>

The second operation mode of the analog output is **Alarm if out of range**. In this mode, the analog output can output only two possible voltages: either 0 mV or 2500 mV (so actually it is now a digital signal). The 0 mV are given if the measured parameter falls within a specific range, which can be freely adjusted at **lower alarm limit** and **upper alarm limit**. If the measured parameter gets out of this range, the analog output will switch to 2500 mV. This feature can be e.g. utilized for monitoring oxygen levels in a fish tank. If the oxygen levels might get dangerous for the fish, the 2500 mV of the alarm output could trigger some external electronic equipment which e.g. rings a bell.
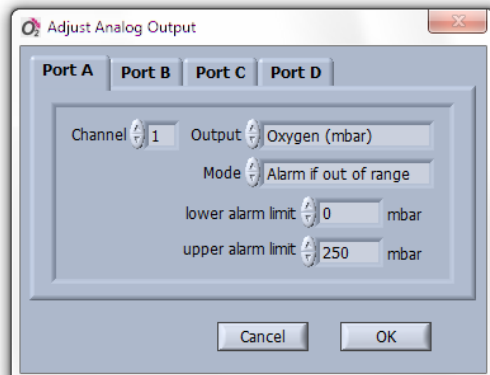
## 11.2 Auto-Mode

The auto-mode is simply activated (1) by connecting the USB port of the *FireStingO2* with a standard micro-USB-charger as used for many mobile phones or (2) by connecting two pins at the extension port (more details in appendix 12.2). So there is no connection to a PC anymore. The correct initiation of the auto-mode can be recognized by a significantly slower flashing frequency (ca. 4 flashes with a total duration of ca. 2-3 s) of the *FireSting* logo after power-up, compared to the faster flashing frequency when connecting it to a PC (see chapter 0). During auto-mode operation, the measurement results can be read out e.g. by an analog data logger from the analog output (see chapter 11.1).

**NOTE:** Some older micro-USB-charger do not fulfill the new common standard for micro-USB-chargers. Such an old micro-USB-charger might not be recognized by the *FireStingO2*, and thus the auto-mode is not started. This can be checked by observing the flashing frequency of the logo.

**NOTE:** Some micro-USB-charger have built in safety timers which shut down the power supply after e.g. 4 hours.

The basic idea behind the auto-mode is that all operations related to sensor settings and sensor calibrations are still performed during the normal operation with a PC. When this is done, the auto-mode can be configured by opening the **Settings** window and clicking there on the **Prepare Auto-Mode** button in the **Options** tab. A separate window will open.
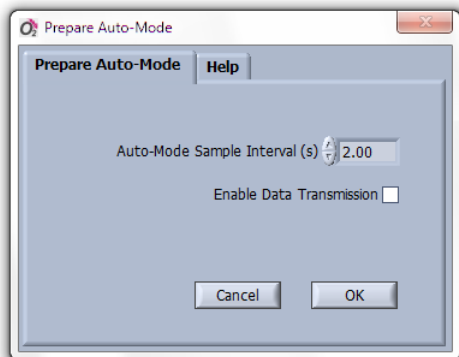
Here the **Auto-Mode Sample Interval (s)** can be adjusted, defining the time interval between consecutive measurements in the auto-mode. As an advanced feature, the option **Enable Data Transmission** activates digital data transmission via the UART interface of the extension port (details on request).

**NOTE:** Only sensors (e.g. oxygen channel 1-4, temperature, pressure, humidity) which are activated in the *Pyro Oxygen Logger* software will be also measured during the auto-mode. If e.g. the humidity sensor should measure during the auto-mode, the respective **Activate** button in the **Settings** window must be enabled.

## 11.3 Advanced Auto-Mode

**NOTE:** This section is only for advanced users!

For advanced applications, the auto-mode can be configured even more flexible as described in the preceding chapter. First, it is possible to define an independent sample interval for each oxygen channel; e.g. channel 1 is measured every 10 s, while channel 2 is measured only every 10 min. Second, optionally the measurements of each channel can be triggered externally by using the trigger input of the extension port (see appendix 12.2).

The advanced auto-mode can be configured by opening the **Settings** window and clicking there on the **Advanced Auto-Mode** button in the **Options** tab. A separate window will open.
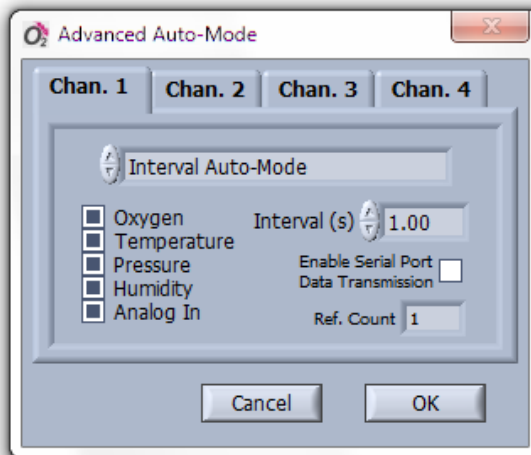


Three options can be selected. The first option **Disable Auto-Mode** disables any measurement in the auto-mode for the respective channel (but it does not disable the auto-mode in general!). For the second option **Interval Auto-Mode**, the sample **Interval (s)** can be adjusted for each channel independently. If the last option **Triggered Auto-Mode** is chosen, the measurement of the

respective channel is only performed during the auto-mode if a trigger signal was detected at the trigger input of the extension port (see appendix 12.2).

By checking **Enable Serial Port Data Transmission,** the results for the respective channel are additionally transmitted via the UART interface of the extension port. **Ref. Count** is an advanced feature only relevant for high speed applications (details on request).

**IMPORTANT: Leave Ref. Count=1 for standard applications!**

# 12 Appendix

## 12.1 Specifications of the *FireStingO2*

| | |
|---|---|
| **Dimensions** | 68 x 120 x 30 mm |
| **Weight** | 350 g |
| **Interface** | USB 2.0 |
| **Power Supply** | USB-powered (max 70mA at 5V) |
| **Supported operating systems** | Windows 7,8, 10<br>(but not Windows RT) |
| **Operating temperature** | 0 to 50ºC |
| **Max. relative humidity** | Non-condensing conditions |
| **Optical sensor port** | 1, 2, or 4 (dependent on model) |
| **Oxygen measuring principle** | lifetime detection of REDFLASH indicator luminescence |
| **Optical sensor connector** | fiber-optic ST-plug |
| **Temperature port** | 1 channel for 4-wire PT100 External Temperature Sensor |
| **Max. sample rate** | 4 samples per second |
| **Max. sample rate** with enabled "high speed sampling" in Settings->Options | 20 samples per second<br>(ext. temperature sensor not activated) |
| **External Temperature Sensors\***<br>Range, Resolution, Accuracy | PT100<br>-30°C to 150°C, 0.02°C, ±0.3°C |
| **Internal Temperature Sensor\***<br>Range, Resolution, Accuracy | -40 to 125°C, 0.01°C, ±0.3°C |
| **Internal Pressure Sensor**<br>Range, Resolution, Accuracy | 300 to 1100 mbar, 0.06 mbar, typ. ±3 mbar |
| **Internal Humidity Sensor**<br>Range, Resolution, Accuracy | 0 to 100% rel. humidity (RH), 0.04% RH, typ. ±0.2% RH |
| **Analog Input (1 channel)**<br>at extension port X1 | 0 to 2.5 VDC, 12 bit resolution<br>Note: slight non-linearities around 0..5mV |
| | |

| | |
|---|---|
| **Analog Output (4 channels)** at extension port X2 | 0 to 2.5 VDC, 14 bit resolution<br>Note: slight non-linearities around 0..5mV |
| **Connector plug** for extension port X1 | Phoenix Contact<br>item no. 1778887 |
| **Connector plug** for extension port X2 | Phoenix Contact<br>item no. 1778861 |
| **Digital interface** at extension port X1 | UART with 3.3V levels<br>(5V tolerant)<br>19200 baud, 8 data bit, 1 stop bit, no parity, no handshake |

*Please note, that the oxygen sensors have a different temperature range (typ. 0-50°C specified, -20°C to 70°C not specified).

## 12.2 Extension Port X1

The extension port of the *FireStingO2* consists of the two connectors X1 and X2 (**fitting connector plugs** can be obtained from *Phoenix Contact* item **no. 1778887** and **1778861**).
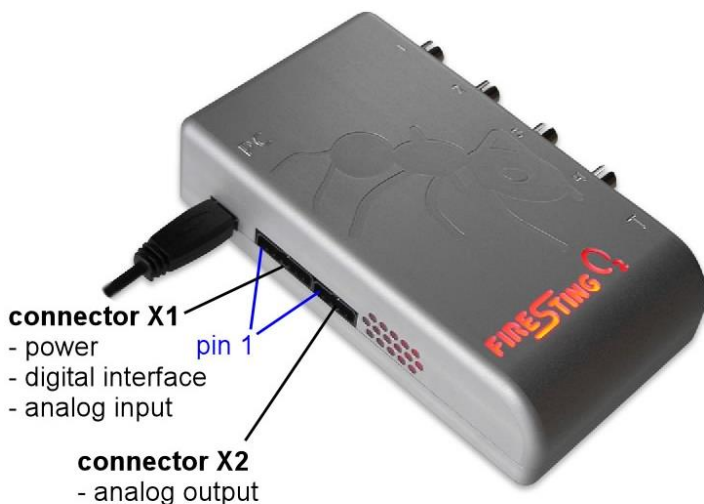


### 12.2.1 Connector X1 (Power, Digital Interface, Analog In)

The pin configuration of the connector X1 is given in the table below. Pins 1-2 (GND and VCC) can be used for providing an external power supply (3.5...5.0 VDC), if the *FireStingO2* should not be powered via the USB port. Pin 3 (/USB_DISABLE) should be tied to pin 1 (GND), if the *FireStingO2* should be operated in full-control mode (see below). The transmit and receive pins of the UART-interface are given at pins 4 (TXD) and 5 (RXD). If pin 6 (/AUTO) is tied to pin 1 (GND), then the auto-mode of the *FireStingO2* is activated (see chapter 11.2).

**NOTE**: While the auto-mode is activated by connecting pin 6 to pin 1, the USB interface and the receive pin of the UART interface are disabled. The *FireStingO2* will not respond to the *Pyro Oxygen Logger* software or any command sent to it via the UART interface!

| Pin | Name | Function | Description |
|-----|------|----------|-------------|
| 1 | GND | Power | Ground |
| 2 | VCC | Power | Power supply, 3.5V to 5.0V DC max. 70 mA (typ 40 mA) |
| 3 | /USB_DISABLE | Disables USB interface | Ground |
| 4 | TXD | Digital Output (0V or 3.3V) | Data transmission pin of the UART interface |
| 5 | RXD | Digital Input (0V or 3.3V) (5V tolerant) | Data receive pin of the UART interface |
| 6 | /AUTO | Digital Input (0V or 3.3V, internally pulled-up to 3.3V) | Leave the /AUTO pin unconnected for normal operation. Connect to GND for auto-mode operation. |
| 7 | /TRIG_AIN | Digital Trigger Input or Analog Input (0...2.5VDC) If used as trigger input, a 10kOhm pull-up resistor must be connected between pin 7 and pin 2 (VCC).) | The trigger input is used for triggering a measurement in the "triggered auto-mode". The trigger is activated at the moment, when the pin is tied to GND. Alternatively this pin can be used to read in analog voltage signal. |

Pin 7 (/TRIG_AIN) can be used for two alternative functions. Either it is used as a trigger input for triggering measurements in "triggered auto mode" (see chapter 11.3). In this case, the pin 7 must be permanently connected via a 10kOhm resistor ("pull-up resistor") to VCC at pin 2. The trigger is then activated by connecting pin 7 shortly to GND at pin 1 ("falling edge sensitive trigger").

Alternatively, pin 7 can be used as an analog input for reading in voltage signals (0..2.5 VDC), e.g. from an external sensor. If **Analog In** is activated in the **Settings** of the *Pyro Oxygen Logger* software,

then this voltage signal is logged along with the normal oxygen measurements (see chapter 6.1.1).

The following illustrations show typical use cases for the extension port X1:

### USB-Powered Auto-Mode
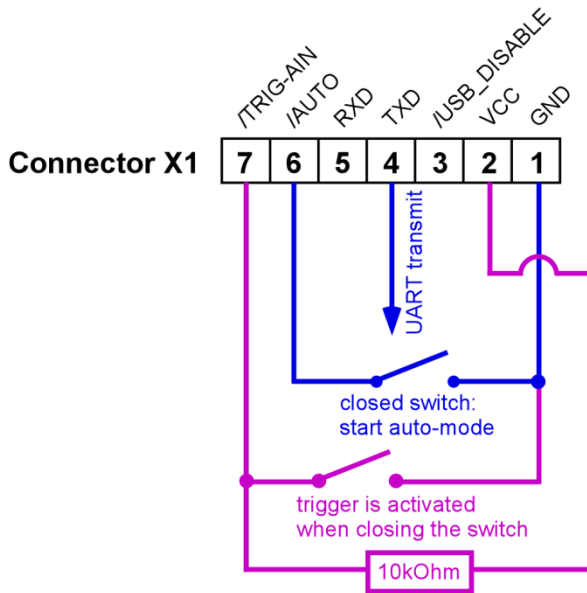


The *FireStingO2* is powered e.g. by a USB connection to a PC. By closing the blue switch between pin 6 and pin 1, the auto-mode is activated. Note, if the *FireStingO2* is powered by a micro-USB-charger, then the auto-mode is automatically activated (see chapter 11) and the blue switch has no function. If the advanced functionality "triggered auto-mode" should be utilized (see chapter 11.3), the pink circuit including a 10kOhm resistor has to be added. Closing the pink switch can then trigger the measurement in the auto-mode.

### *Auto-Mode with External Power Supply*
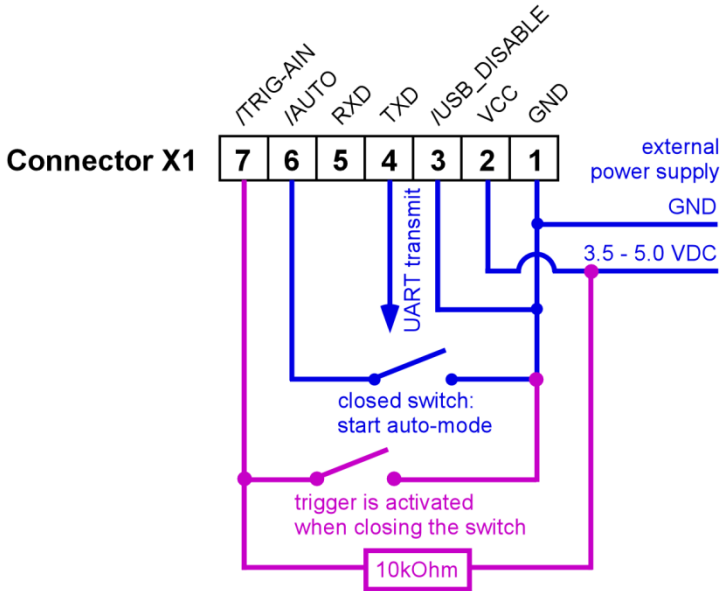


This configuration is almost identical to the preceding case, only the power supply is now given at pins 1 and 2. The USB connector of the *FireStingO2* is left unconnected. Note the additional connection between pin 1 and 3 powering down the internal USB interface of the *FireStingO2*.

### *Full Control Mode via the UART Interface*
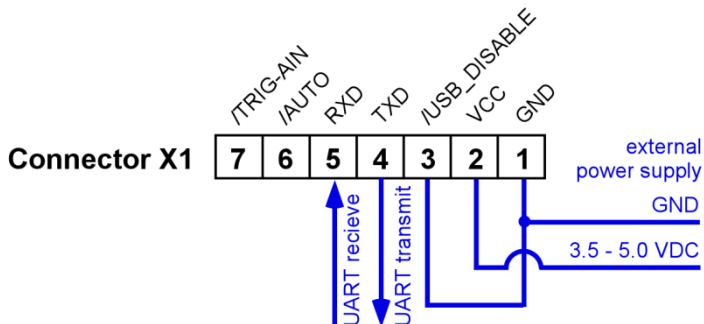
This configuration is used if the *FireStingO2* should be controlled completely via the UART interface by external custom electronic equipment (OEM applications). Note the additional connection between pin 1 and 3 powering down the internal USB interface of the *FireStingO2*. Otherwise unintended data communication via a potentially still connected PC might disrupt the UART communication. More information is available on request.

### *Connecting an external sensor voltage to the analog input:*



DC voltage $U_{in}$ from e.g. external sensor
max. range 0..2500mV
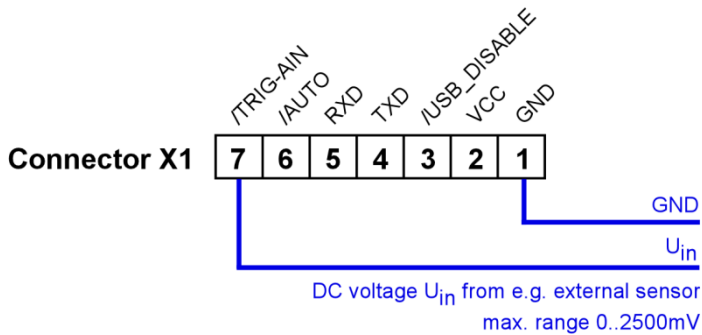
For reading in voltage signals between 0 and 2.5 VDC of e.g. an external sensor, simply connect the voltage to pin 1 and pin 7. Ensure a correct polarity. And ensure that the voltage does not exceed 2.5 VDC. Voltages above 3.3 VDC might damage the device.

### 12.2.2 Connector X2 (Analog Output)

The connector X2 provides 4 independent analog outputs with a range of 0-2.5V DC at a resolution of 14 bits (see table below). Refer to chapter 11.1 how to configure the analog outputs.

| Pin | Name | Function | Description |
|-----|------|----------|-------------|
| 1 | GND | | Ground |
| 2 | AO_A | Analog Output (0 – 2.5 V DC) (14 bit resolution) | Analog Output Port A (alternatively digital alarm output) |
| 3 | AO_B | Analog Output (0 – 2.5 V DC) (14 bit resolution) | Analog Output Port B (alternatively digital alarm output) |
| 4 | AO_C | Analog Output (0 – 2.5 V DC) (14 bit resolution) | Analog Output Port C (alternatively digital alarm output) |
| 5 | AO_D | Analog Output (0 – 2.5 V DC) (14 bit resolution) | Analog Output Port D (alternatively digital alarm output) |

## 12.3 Troubleshooting

How to respond to the warnings shown in the *Pyro Oxygen Logger*:

**Signal Too High**
Too much ambient light exposed to the sensor, or amplification is too high, or LED intensity is too high:
→ darken the surrounding
→ and / or decrease **Amplification** in the **Advanced** settings
→ and / or decrease **LED Intensity** in the **Advanced** settings

**Low Signal**
Sensor signal is too low:
→ check whether the sensor cable is connected
→ increase **LED Intensity** in the  **Advanced** settings
→ replace sensor, the tip might be broken / bleached

**Bad Reference**
Internal problem of the electronics
→ contact *PyroScience*

## 12.4 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).
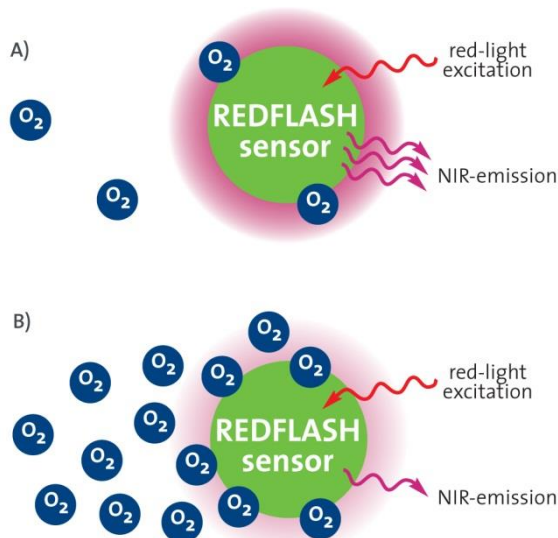


**Principle:** red light excited REDFLASH indicators show luminescence in the near infrared (NIR), which decreases with increasing oxygen (quenching effect).
**A)** high NIR emission at low oxygen and **B)** low NIR at high oxygen

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.
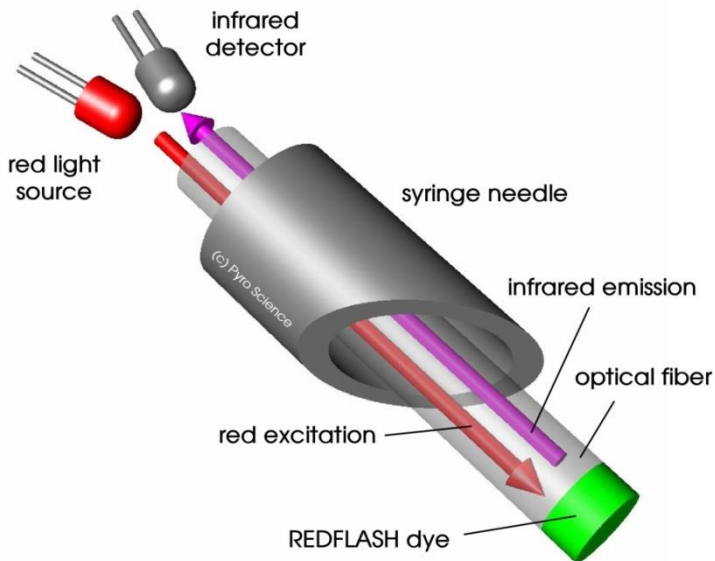


The measuring principle is based on a sinusoidally modulated red excitation light. This results in a phase-shifted sinusoidally modulated emission in the NIR. The *FireStingO2* measures this phase shift (termed "dphi" in the software). The phase shift is then converted into oxygen units based on the Stern-Vollmer-Theory.

## 12.5 Operating several *FireStingO2* in parallel

The fiber-optic oxygen meter *FireStingO2* is available as 1-, 2-, or 4-channel version. However, in order to realize extendable multichannel systems with higher channel numbers (e.g. 8, 16, 32, or 64), several *FireStingO2* meters can be easily operated at a single PC as described in the following:

Connect each *FireStingO2* to a free USB port of your PC. If the PC does not provide a sufficient number of USB ports, you can use an external USB-hub. Ensure that the USB-hub provides sufficient power (each *FireStingO2* needs max. 70 mA); an external power supply for the USB-hub might be advisable.

The *Pyro Oxygen Logger* software has now to be started separately for each connected *FireStingO2*. So, if you want to operate e.g. 6 different *FireStingO2* meters, you have to start the *Pyro Oxygen Logger* software 6 times, which will open 6 *Pyro Oxygen Logger* windows on your desktop. The different windows operate completely independent from each other, and are associated to exactly one of the *FireStingO2* meters. In order to check which window is associated to a specific *FireStingO2*, simply press the **Flash Logo** button in the main window of the *Pyro Oxygen Logger* software, which induces a flashing of the red *FireSting* logo on the associated device for about 1 sec.

When closing the *Pyro Oxygen Logger* software, all settings and all current calibration data are saved in a "setup file", which is automatically loaded at the next startup. This setup file is saved specifically for each *FireStingO2* serial number, i.e. each *FireStingO2* in the above described setup keeps its own settings and calibration data.

## 12.6 Definition of Oxygen Units

### phase shift                                    *dphi*

The phase shift *dphi* is the fundamental unit measured by the optoelectronics in the *FireStingO2* (see chapter 12.4). 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 *FirestingO2*.

### 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 12.7).

## 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

## 12.7 Table of Oxygen Solubility

The following Table shows the equilibrium oxygen concentration $C_{100}$(T, P=1013mbar, S) in units of µmol/L 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 values for the actual atmospheric pressure $p_{atm}$, the following formula has to be applied:

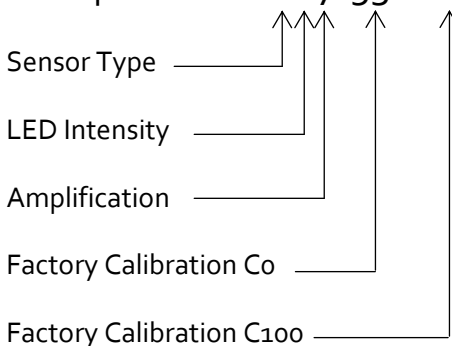$$C_{100}(T,P,S) = C_{100}(T,P=1013mbar,S) \times p_{atm} / 1013mbar$$

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

| Sal (PSU) | Temp (°C) 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 456.6 | 398.9 | 352.6 | 314.9 | 283.9 | 257.9 | 235.9 | 217.0 | 200.4 |
| 2 | 450.4 | 393.6 | 348.1 | 311.1 | 280.6 | 255.0 | 233.3 | 214.7 | 198.3 |
| 4 | 444.2 | 388.5 | 343.7 | 307.3 | 277.3 | 252.1 | 230.8 | 212.4 | 196.3 |
| 6 | 438.1 | 383.3 | 339.4 | 303.6 | 274.0 | 249.3 | 228.3 | 210.2 | 194.3 |
| 8 | 432.1 | 378.3 | 335.1 | 299.9 | 270.8 | 246.5 | 225.8 | 207.9 | 192.3 |
| 10 | 426.1 | 373.3 | 330.8 | 296.2 | 267.6 | 243.7 | 223.3 | 205.7 | 190.3 |
| 12 | 420.3 | 368.4 | 326.7 | 292.6 | 264.5 | 240.9 | 220.9 | 203.6 | 188.4 |
| 14 | 414.5 | 363.5 | 322.5 | 289.1 | 261.4 | 238.2 | 218.5 | 201.4 | 186.5 |
| 16 | 408.8 | 358.7 | 318.4 | 285.5 | 258.3 | 235.5 | 216.1 | 199.3 | 184.6 |
| 18 | 403.2 | 354.0 | 314.4 | 282.1 | 255.3 | 232.8 | 213.7 | 197.2 | 182.7 |
| 20 | 397.7 | 349.3 | 310.4 | 278.6 | 252.3 | 230.2 | 211.4 | 195.1 | 180.8 |
| 22 | 392.2 | 344.7 | 306.5 | 275.2 | 249.3 | 227.6 | 209.1 | 193.0 | 179.0 |
| 24 | 386.8 | 340.2 | 302.6 | 271.9 | 246.4 | 225.0 | 206.8 | 191.0 | 177.1 |
| 26 | 381.5 | 335.7 | 298.7 | 268.5 | 243.5 | 222.5 | 204.5 | 189.0 | 175.3 |
| 28 | 376.2 | 331.2 | 294.9 | 265.3 | 240.6 | 219.9 | 202.3 | 187.0 | 173.5 |
| 30 | 371.0 | 326.9 | 291.2 | 262.0 | 237.8 | 217.4 | 200.1 | 185.0 | 171.7 |
| 32 | 365.9 | 322.5 | 287.5 | 258.8 | 235.0 | 215.0 | 197.9 | 183.0 | 170.0 |
| 34 | 360.9 | 318.3 | 283.9 | 255.7 | 232.2 | 212.5 | 195.7 | 181.1 | 168.2 |
| 36 | 355.9 | 314.1 | 280.3 | 252.5 | 229.5 | 210.1 | 193.6 | 179.2 | 166.5 |
| 38 | 351.0 | 309.9 | 276.7 | 249.5 | 226.8 | 207.7 | 191.4 | 177.3 | 164.8 |
| 40 | 346.2 | 305.8 | 273.2 | 246.4 | 224.1 | 205.4 | 189.3 | 175.4 | 163.1 |

## 12.8 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 6.1). The following figure gives a short explanation about the information given in the sensor code.

Example Code:  XB7-532-205

Sensor Type ————

LED Intensity ————

Amplification ————

Factory Calibration C0 ————

Factory Calibration C100 ————

### Sensor Type

| | |
|---|---|
| Z | Oxygen Micro / Minisensor (normal range) |
| Y | Oxygen Minisensor (normal range) |
| X | Robust Oxygen Probe (normal range) |
| V | Oxygen Minisensor (trace range) |
| U | Robust Oxygen Probe (trace range) |
| T | Oxygen Sensor Spot / FTC (trace range) |
| S | Oxygen Sensor Spot / FTC (normal range) |
| Q | Solvent-Resistant Oxygen Probe |
| P | Oxygen Nanoprobes |
| C | Optical Temperature Minisensor (Thermoblue) |
| D | Optical Temperature Sensor Spot/FTC (Thermogreen) |

## 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

### C0 (Factory Calibration at 0% O2)

dphi0 = C0 / 10

### C100 (Factory Calibration at 100% O2)

dphi100 = C100 / 10

The values of the factory 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 |

## Optical Temperature Sensors

**C0** and **C100**: values needed for calibration.