pyroscience
sensor technology

FireSting O$_2$

FIBEROPTIC OXYGEN METER

USER MANUAL

Document Version 2.04
Refers to *FireSting Logger Software* version >2.35

The *FireSting O$_2$* is manufactured by

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1 Overview

The compact PC-based fiber-optic oxygen meter *FireSting O2* with 1, 2 or 4 channels unifies several innovative technological improvements making it the new standard of high precision oxygen sensing with fiber-optical oxygen sensors (optodes). The *FireSting O2* utilizes a new unique measuring principle based on red light excitation and lifetime detection in the near infrared using novel luminescent oxygen indicators (*REDFLASH technology*, see Appendix 13.2 for more details). It is a multipurpose oxygen meter

- working with oxygen needle-type (retractable, fixed), bare fiber and contactless sensors (sensor spots, sensor cuvettes and flow-through cells), as well as robust miniprobes and
- working with full range and trace oxygen sensors.

Along with the *FireSting O2* we offer a variety of fiber-optical oxygen sensors, differing in tip diameter, tip geometry and measuring concentration range, which can be adjusted at your needs. Furthermore, we also offer turnkey motorized microprofiling setups enabling the measurement of depth-profiles of oxygen concentration in environmental samples at high temporal and spatial resolution.

The *FireSting O2* enables a diverse range of applications for measuring oxygen at different concentration ranges, sample types and spatial scales. This multipurpose oxygen meter allows measurements with microsensors in small samples and detection of depth-resolved oxygen microgradients in semi-solid samples like sediments, biofilms, or tissue. With the same oxygen meter it is also possible to monitor oxygen concentrations in air or aquatic environments using minisensors, robust miniprobes or sensor spots for long-term measurements.

Our consulting service is available to inform you about the

- new and outstanding *FireSting O2*,
- different sophisticated sensor types,
- microprofiling setups from *PyroScience*,
- their applicability and
- integration into existent devices as OEM solution.

More information concerning our products can be found at
www.pyro-science.com

or contact us under info@pyro-science.com

We would be pleased to serve you concerning all needs for sensing oxygen at high precision and resolution with state-of-the-art technology.

Profit from our innovation, scientific expert knowledge and flexibility to develop customized solutions for high performance sensing technology.

Your *PyroScience* Team

2 Safety Guidelines

The *FireSting O2* is a laboratory instrument to be used with fiber-optic oxygen sensors (optodes) from *PyroScience* for measuring oxygen at high precision and resolution. In order to guarantee an optimal performance of the *FireSting O2* please follow these operation instructions and safety guidelines. Before starting a measurement with the *FireSting O2* please allow for an appropriate

- installation of the *FireSting Logger* software before plugging the *FireSting O2* for the first time into a USB-port of your PC
- ambient temperature, cleanness and humidity,
- connection of sensors and products (extension module) supplied from *PyroScience*,
- calibration of the sensors,
- precaution during handling of the needle-type sensors to avoid injuries or breaking of the sensor and
- functioning of the *FireSting O2*.

If any problems or damage evolve, please disconnect the instrument immediately, mark it to prevent any further use and consult *PyroScience* for repair or maintenance service. The *FireSting O2* 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 *FireSting O2* 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 is

not intended for medical, military or other safety relevant areas. It 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 manufacturer of chemicals used during measurements.

Calibration 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 *FireSting O2*, which is on the user's authority.
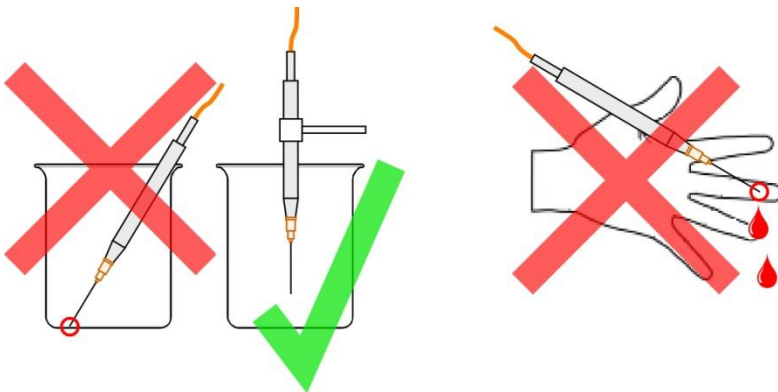
Before using the *Firesting O2* and its sensors, read carefully the instructions and user manuals for the oxygen meter *Firesting O2*.

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

The *Firesting O2* 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).

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 *Firesting O2* are not intended for medical or military purposes or any other safety-critical applications.

The sensors should be used in the laboratory only by qualified personal 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 *Firesting O2* outside the reach of children!

3   Introduction to the *FireSting O2*

The *FireSting O2* is a multipurpose oxygen meter that is compatible with all available oxygen sensors from *PyroScience* (needle-type, bare fiber and contactless sensors, miniprobes). Most sensors are available for the full range (0-100% O2) and for the trace range (0-21% O2). The optical detection technology is based on the unique oxygen-sensitive *REDFLASH indicators* which use red light excitation and lifetime detection in the near infrared (see Appendix 13.2 for more details). The oxygen-sensitive *REDFLASH indicators* show excellent brightness and their red light excitation significantly reduces stress in biological systems and interferences caused by autofluorescence.

The *FireSting O2* is a high precision, compact PC-based fiber-optic oxygen meter with 1, 2 or 4 channels for measurements in the laboratory. Additionally, one temperature sensor can be connected allowing for automatic temperature compensation of the oxygen measurement. The *FireSting O2* is operated via an USB connection to a PC with a Windows operation system. The included logging software *FireSting Logger* provides comfortable calibration and logging functionality.

The *FireSting O2* can be used in various environments, but is designed as a laboratory instrument. If used in the field, please protect the *FireSting O2* from heating, moisture and corrosion.

The *FireSting O2* comes with 1, 2, or 4 channels (connectors **S1** to **S4**) on the right side panel for up to 4 fiber-optic oxygen sensors and one connector (**T**) for a temperature sensor. The USB-connector on the left side panel provides the energy supply and the data exchange with the PC.
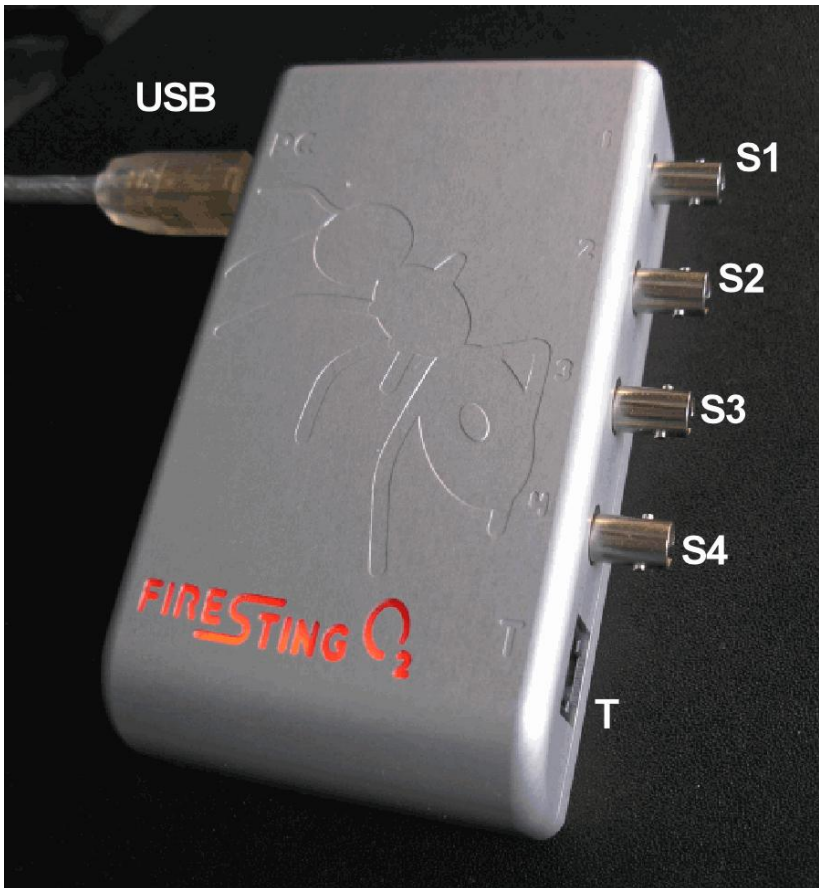
4 Software Installation

System requirements: PC with Windows XP/Vista/7 (but not "Windows 7 Starter Edition") and min. 200 MB free disk space.

> IMPORTANT: Do not connect the *FireSting O2* to your PC before the *FireSting Logger* software has been installed. The software will install automatically the appropriate USB-drivers.

Installation steps:

- download the installer package for the newest version of the *FireSting Logger* software from the *PyroScience* homepage: www.pyro-science.com/downloads.html

- unzip and start the installer and follow the instructions

- connect the *FireSting O2* with the USB-cable to the computer. The red logo will flash shortly indicating the correct startup of the oxygen meter.

After the successful installation a new program group „Pyro FireSting O2" is added to the start menu, and a short-cut named "FireSting" can be found on the desktop.

> Remark to "Windows 7 Starter Edition": Some laptops or netbooks are delivered only with a limited Windows version called "Windows 7 Starter Edition", which does not support the *FireSting O2*. In that case you should look in the Windows system settings for "Windows Anytime Upgrade". Here you can purchase and download online an upgrade to a full Windows version.

5 Oxygen Sensor Types

5.1 Needle-Type Sensors

The needle-type oxygen sensors are composed of a fiber-optical cable (**C**; 2.5 m standard length) with a plug (**P**) for connection to the *FireSting O2* meter, a metal housing (**H**, fixed or retractable), and a syringe needle (**N**; 40 mm standard length) including the fiber with a fragile sensing tip. On each sensor, a specific Sensor Code (**SC**) is attached to the cable.
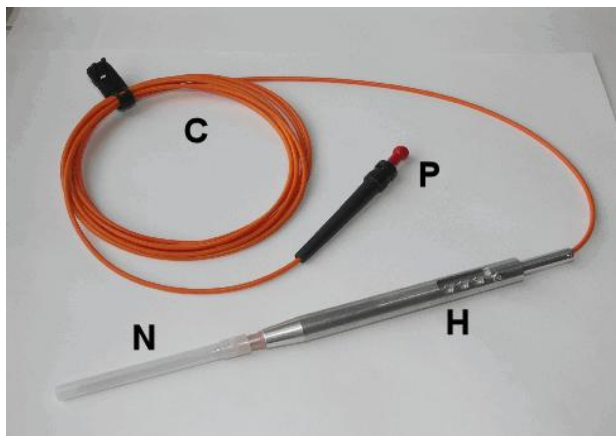
At the tip of the sensors (**T**), the oxygen-sensitive *REDFLASH indicator* is immobilized in a raisin. The needle-type oxygen sensors are offered with different tip diameters, including microsensors with a tapered sensor tip of ca. 40-60 µm in diameter (**A**) and minisensors with a flat sensor tip (**B**) of 230 µm, 430 µm, 0.5 mm and 1.1 mm in diameter.
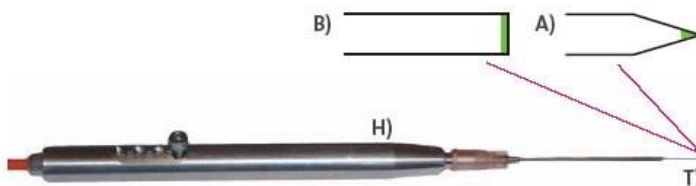
The sensor tip can be retractable (**R**) or fixed (**F**) in the housing.

Microsensor (retractable)

Minisensor (fixed)

The retractable sensor tip allows sensor insertion through septa, package material or tissue and subsequent extension of the sensor tip into the sample for oxygen measurements. The retractable sensors are available as microsensors (40-60 µm) and as minisensors (230 µm, 430 µm).

The sensors are shipped in **position 0** (retracted position) to protect the tip from breaking. The needle is covered with a protective cap (**PC**), which has to be removed carefully before using the sensor. After removal of the protective cap from the needle, the sensor should be fixed in a stable set-up like a solid laboratory stand or a micromanipulator mounted on a heavy stand from *PyroScience*. In case of retractable sensors, move gently the push-button (**PB**) from the retracted position 0 to the extended **positions 1** (tip flush with needle tip), **2** or **3**, thereby moving the sensor tip ca. 6 mm (**position 2**) or 12 mm (**position 3**) out of the needle into the water or gas phase of the sample.

Please take care when handling the needle-type sensors to prevent injuries and breaking of the sensor. Ensure enough space in front of the fragile sensor tip, especially when pushing it out!

Retracted Position (position 0)	tip inside needle
Extended position 1	tip flush with needle
Extended position 2	tip ca. 6 mm extended
Fully extended position 3	tip ca. 12 mm extended
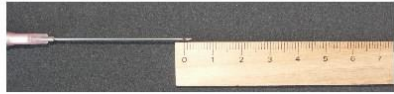
The needle-type **microsensors** are appropriate for high resolution measurements in semi-solid samples, like sediments, biofilms or tissues with *PyroScience* turnkey motorized microprofiling setups. They allow measurements in small sample volumes, along steep gradients and of depth-resolved oxygen profiles at high spatial resolution.

Please always use a stable stand and a micromanipulator to move the sensor into a semisolid sample like sediment, biofilm, tissue.

The **minisensors** are standard sensors for robust long-term oxygen measurements in gaseous or aquatic environments. Fixed minisensors with a mechanically protected tip (0.5 mm diameter) can be used for insertion through a rubber septum without addition of air to gas samples. Here the very sensor tip is flush with the tip of a beveled needle and glued into it.

In case of fixed minisensors with a tip diameter of 1.1 mm, the fixed sensor tip extends 1 mm from the needle tip and is hence unprotected after removal of the protective cap. **Avoid any contact to harsh surfaces!**

The needle-type sensors can be sterilized with ethylene oxide (EtO) and can be cleaned with peroxide (3% H_2O_2), soap solution or ethanol. They can be applied in gas phases, aqueous solutions, ethanol, methanol and isopropanol. Other organic solvents and gaseous chlorine (Cl_2) induce interferences with the sensor reading. No cross-sensitivity was found for pH 1-14, CO_2, CH_4, H_2S and any ionic species.

After finalization of the measurements, the sensor tip of the oxygen sensors should be rinsed carefully with demineralized water. In case of retractable sensors, retract the sensor tip and put on the protective cap (PC) onto the needle to protect the sensor. Store the sensor at a dry, dark and secure place.

A signal drift of the sensor can indicate photobleaching of the oxygen-sensitive *REDFLASH indicator*, depending on the intensity of the excitation light and frequency of measurements.

The needle-type oxygen sensors are also optionally available with **optical isolation** for measurements in samples with auto-fluorescence. With this optical isolation, interferences between the *REDFLASH indicator* luminescence and autofluorescence, e.g. from photosynthetic pigments, are reduced. However, the optical isolation causes an increase in the response time t_{90}, which is the time for 90% of the total sensor signal change (see chapter 12).

5.2   Bare Fiber Sensors

The bare fiber sensors are identical to the retractable needle-type oxygen sensors, except that they do not contain a housing. They comprise only the bare optical fiber with the *REDFLASH indicator* immobilized on the fragile sensor

tip. The bare fiber sensors are available both as microsensors (40-60 μm tip diameter) and as minisensors (230 μm, 430 μm tip diameter). They can be used with customized housing with complex geometries or they might be inserted directly into samples ("implantable sensors").

5.3   Sensor Spots

PyroScience offers a range of **contactless oxygen sensors**, comprising sensor spots, sensor cuvettes and flow-through cells. The sensor spots are coated with the novel *REDFLASH indicator* with PET foil or glass as carrier material. The sensor spots allow oxygen measurements in closed vessels in combination with the *FireSting O2*. They have standard diameters of 5 mm or 8 mm and can also be obtained with an optical isolation (black coating). The sensor spots have a rough sensing surface (**SF**) which is whitish-green and facing towards the paper side of the packaging. The backside (**BS**) is smooth, shiny and dark-green.
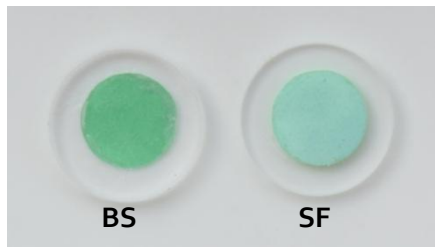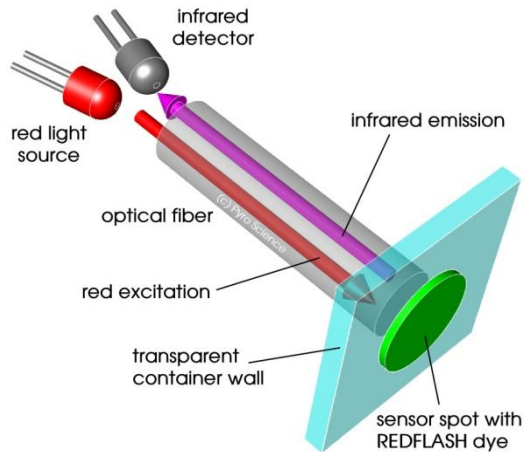
The sensor spots can be glued with their **backside** on transparent, clean and dry **inner** container walls (plastic or glass, wall thickness 0-6 mm) using an appropriate adhesive (e.g. transparent silicone based on acetic acid, item no. SPGLUE). After the glue has dried,

the gas or liquid sample has to be filled into the container so that the whitish-green sensing surface of the spot is completely covered and in contact with the sample.

For containers with **wall thicknesses of 0-2 mm**, the *Basic Spot Adapter* (a 10x10x10 mm plastic cube, item no. SPADBAS) needs to be fixed tightly or glued to the outer container wall at the spot position. Then the sensor signal can be read out using the *Spot Fiber* (item no. SPFIB) connecting the basic spot adapter with the *FireSting O2*. For measurements

through containers with **wall thicknesses of 2-6 mm**, the metallic *Lens Spot Adapter* (item no. SPADLNS) with an integrated collimating lens has to be used instead. It comes together with a flexible re-adjustable cable binder for quick positioning of spot adapters e.g. on laboratory flasks with a maximal diameter of ~10 cm. The spot adapter is then connected with the *Spot Fiber* to a channel of the *FireSting O2*.

It is important to fix or glue the spot adapter firmly to the outer container wall. **The position of the spot adapter should not be changed after calibration of the sensor spot (otherwise the spot has to be re-calibrated).**

The general characteristics, like cross-sensitivity, optimal and maximal range of measurements, limit of detection, accuracy and resolution, are the same as for the needle-type sensors (see chapter 5.1 and 12).

The sensor spots offer a versatile field of application for multi-sampling measurements and online-monitoring of oxygen at greater scales. This includes the application in e.g. respiration and photosynthesis chambers, bioreactors, growth experiments, studies of enzyme kinetics, cell biological approaches and industrial process water-monitoring, including aqua-farming, sewage treatment and cooling water cycling.

5.4 Sensor Cuvettes

The oxygen sensor cuvettes are standard plastic cuvettes (10x10x45 cm) with an oxygen sensor spot glued to one side wall of the cuvette. They allow precise contactless oxygen measurements within the liquid content of the cuvette. A *Basic Spot Adapter* (item no. SPADBAS) has to be fixed/glued to the outside of the cuvette at the spot position. The spot adapter is then connected with the *Spot Fiber* (item no. SPFIB) to a channel of the *FireSting O2*.

5.5 Flow-Through Cells

The flow-through cell has an integrated oxygen sensor spot, allowing online-monitoring of the oxygen concentration in a gas or

liquid sample pumped through the cell. The flow-through cell can be directly connected with the *Spot Fiber* (item no. SPFIB) to the *FireSting O2*. The tubing has an outer/inner diameter of ca. 2/1.5 mm.

5.6 Robust Miniprobe

The sensor tips of the robust oxygen miniprobes consist of a stainless steel tubing 3 mm in diameter, and 3 or 10 cm in length (item no. OXROB3, OXROB10). It can be used for long-term measurements in gases and liquids. The whole sensor including its cabling is completely submersible in water and specified for longtime submersion in liquid media. In contrast to the needle-type sensors, the sensitive sensor tip of the miniprobe is protected by the steel tubing and is more robust for standard laboratory applications. Due to the significantly bigger dimensions of this miniprobe compared to our micro- and minisensors, diffusion limitation in liquid samples of oxygen towards the 3 mm tip has a measurable effect on the response time. For optimum operation conditions it is therefore recommended to utilize these miniprobes in permanently stirred liquids (this diffusion limitation is neglectable in gaseous samples).

5.7 Connecting the Sensors

The fiber-optic oxygen sensors, including needle-type and bare fiber micro- and minisensors, miniprobes, as well as spot fibers

needed for contactless sensors (spots, cuvettes, flow-through cells) are connected to the ST-connectors of the *FireSting O2* (**S1** to **S4**) with a male fiber plug. First, remove the protective caps from both the plug of the sensor (see picture) and the receptacles at the *FireSting O2*.
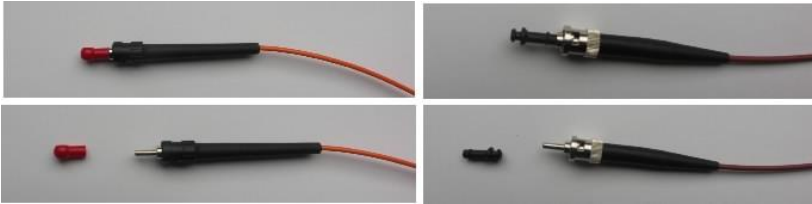
Then insert the male fiber plug (**M**) of the sensor cable into the ST-receptacle (female fiber connector) of the *FireSting O2* and turn the bayonet coupling gently clockwise until the plug is locked firmly.

Note: Detailed sensor specifications are listed in chapter 12.

6   The Software "*FireSting Logger*"

This chapter describes all functions of the *FireSting Logger* software excluding the calibration. Please refer to the chapters 7, 8, and 9 for a detailed description of the calibration procedure.

6.1   Main Window

After start of the software *FireSting Logger* the following main window is shown:
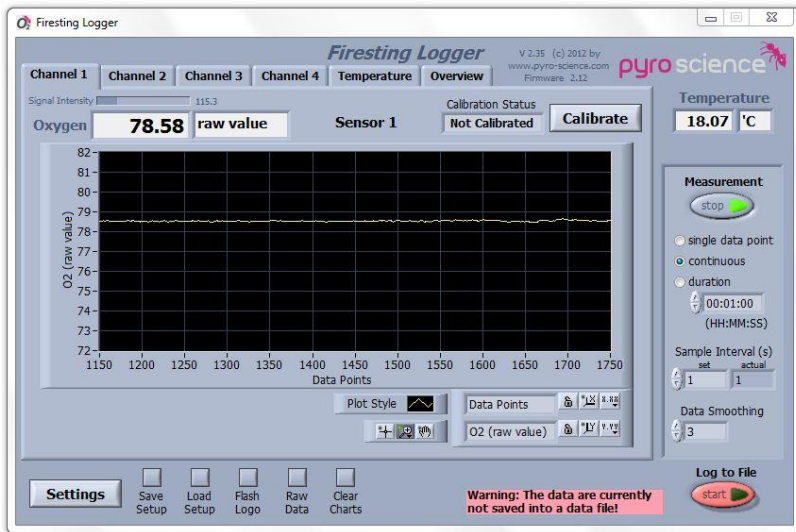
The four panels **Channel 1-4** correspond to the channels 1-4 of the fiber-optic oxygen sensors connected to the *FireSting O2*. For the 1- or 2-channel version of the *FireSting O2*, only the respective panels will be visible.

The default sensor readings are shown as **raw value** (i.e. the uncalibrated sensor readings), which give **no** quantitative information of the actual oxygen concentration.

After the activation of the respective channels in the **Settings** (see chapter 6.2), 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 oxygen unit (**UD**). The color and appearance of each graph can be changed by clicking on the color-control (**CC**). The description of the sensor (as defined in the **Settings**) is shown in the description display (**DD**). The **Signal Intensity** (**SI**) of the oxygen sensor is shown as a horizontal indicator bar just above the numeric display (**D**). A reasonable oxygen sensor shows signal intensities well above 50 (typically 100-1000)[1]. If the signal intensity drops below 50, this indicator bar turns gradually from grey to red indicating that the sensor might get degraded soon. Most sensors will still work even at a signal intensity of ca. 10 (yet with increased noise). But at even lower signal intensity the warning **Low signal** will appear in the warning display (**WD**) (see below and chapter 13.1).

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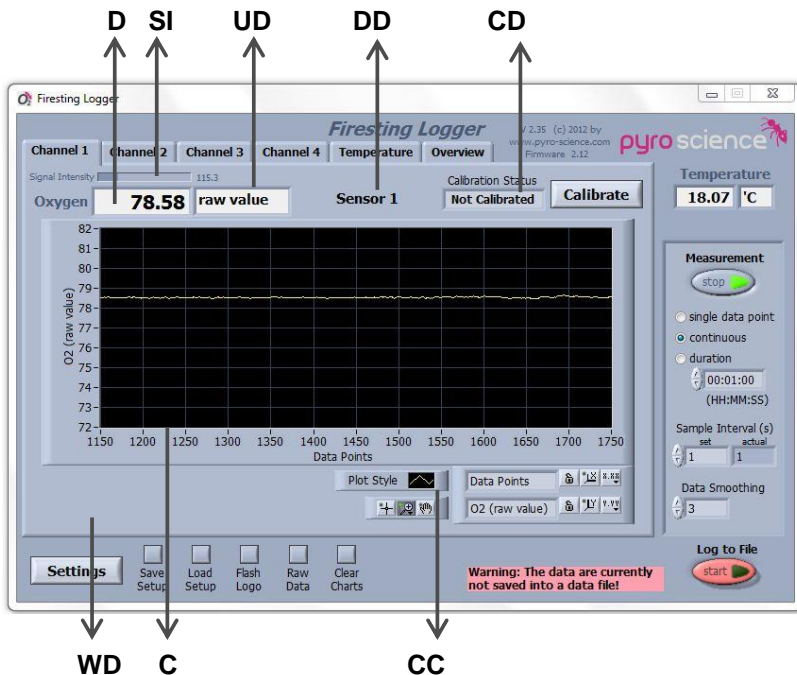
[1] Note: Exceptions are trace oxygen sensors. During the 100%-calibration at 21% O2, these sensors show naturally a very low signal intensity (as low as 10). But the signal intensity will strongly increase when a trace oxygen sensor is applied within its specified range of 0-10% O2.

Note that the signal intensity can be influenced by varying the **LED Intensity** or the **Amplification** in the advanced settings (see chapter 6.2.2).

The calibration status is shown in a separate calibration display (**CD**). As long as the sensors are not calibrated, it shows **Not Calibrated** and the data can only be displayed as uncalibrated "raw value" [2] or "dphi". The "raw value" reflects only qualitatively the measured oxygen concentration, i.e. increasing oxygen concentrations will result in increasing "raw values". However, this dependency is not linear and especially 0 % O_2 does not correspond to a "raw value" of zero. In order to switch to quantitative oxygen units like hPa or %O_2, a calibration has to be performed by clicking on the button **Calibrate.** The calibration procedure is explained in chapter 7.

The plot style of the data in the charts can be changed by different chart tools.
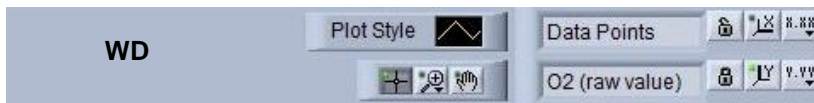
The **Plot Style** of the chart displayed can be changed by clicking with the right mouse button onto the black field of the color control ⬜, opening a pop-up menu. With **Common Plots**, **Color**, **Line Style and Width**, **Interpolation**, and **Point Style** the chart appearance can be changed.

The button with the magnifying glass offers different zoom options. After clicking the button with the hand, the user has the possibility to click onto the chart and move the whole area while keeping the mouse button pressed.

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[2] For advanced users: raw value=100-dphi, whereby dphi denotes the phase shift of the luminescence life time measurement

There are several possibilities to change the display range of the chart. The upper and lower limits of both axes can be changed by clicking with the mouse onto the limit tags so that a new value can be entered (if autoscaling is off!). The buttons ⬚ and ⬚ provide autoscaling for the x- or y-axis, respectively. This feature can be permanently activated by clicking on the respective lock buttons ⬚. By default only the y-axis is in autoscale mode. The buttons ⬚ and ⬚ can be used for changing the format, precision or the mapping mode (linear, logarithmic).

The warning display (**WD**) can show the following warnings:

Low Signal
Signal too high
Bad reference

Low signal indicates that 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 spot fiber and the sensor spot is too large. (For advanced users: increase the LED intensity and/or the amplification in the Advanced Settings)

Signal too high requires a change of the environmental conditions during measurements, avoiding direct sun light exposure or illumination with a lamp. (For advanced users: decrease the LED intensity and/or the amplification in the Advanced Settings)

Bad reference indicates internal problems of the *FireSting O2*. Please contact *PyroScience* for support.

Refer also to Troubleshooting in chapter 13.1.

On the ride hand side of the main window, the actual temperature as measured by the connected and activated temperature sensor is shown in the temperature display (**TD**) in the chosen unit (**TUD**). The temperature can be set in the **Settings** to the unit degree

Celsius (°C), degree Fahrenheit (°F) or Kelvin (K). **NaN** denotes **N**ot **a N**umber when no temperature sensor is connected or if it is not activated in the **Settings** (see chapter 6.2).
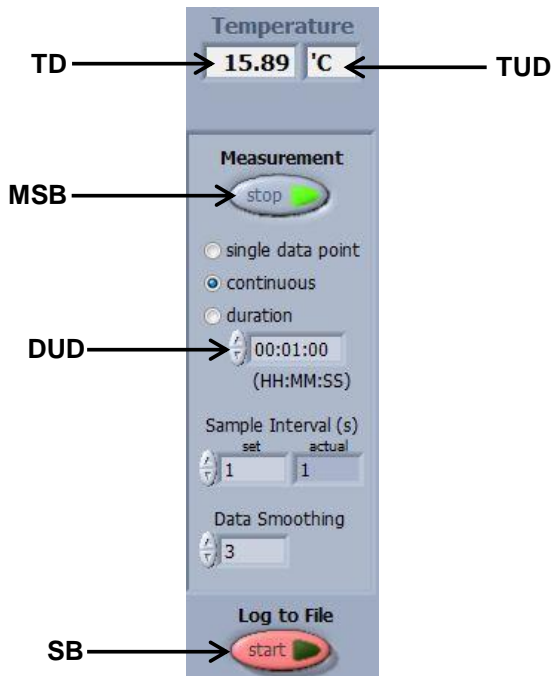
A **Measurement** is started by clicking on the measurement start button (**MSB**). The arrow in the button turns from dark green to light green indicating that a measurement is in progress. Clicking again on it will stop the measurement.
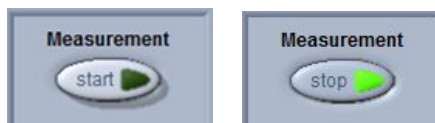
The mode of **Measurement** can be chosen as **single data point** acquisition, **continuous** sampling (default setting) or as continuous

sampling limited to a defined time interval. The **duration** of the time interval can be adjusted in the duration display (**DUD**) shown as hour (HH): minutes (MM): seconds (SS).

The **Sample Interval** 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.**

The 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.

To activate data saving, 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 indicators **Comment** and **File Path** are additionally shown in the main window. In the field **Comment**, 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 **Show File**. The data saving is indicated by the light green arrow in the grey **Log to File** button and be can be stopped by clicking this button again.

NOTE: During data logging, the buttons **Settings** and **Calibrate** (see chapter 6.2 and 7) are not active and cannot be used before **Log to File** is stopped again.

The button **Save Setup** can be used to the save the current settings and calibration data. They can be reloaded anytime by pressing the button **Load Setup**. This allows e.g. to switch between different laboratory setups with a single *FireSting O2*. 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 *Firesting O2* to a second computer, you can load there again this configuration with **Load Setup** giving you calibrated sensors immediately ready for the measurement.

Note, that all current settings and calibration data are automatically saved when closing the *FireSting Logger* software (by clicking on the cross in the upper right corner of the main window after finalization of the measurements), and they are automatically loaded again at the next startup.

The button **Flash Logo** induces a flashing of the logo *FireSting O2* on the corresponding *FireSting O2* meter. Several *FireSting O2* meters can be connected to the PC in parallel and multiple measurements can be performed by opening the *FireSting Logger*

software a number of times corresponding to the number of connected *FireSting O2*. The different windows operate completely independent of each other and are assigned to exactly one *FireSting O2*. This allows measurements in different setups at the same time. The flashing of the logo (for ca. 1 sec after pressing the button) can help to assign a specific logger window to the corresponding *FireSting O2* meter (more details see chapter 13.3).

Clicking on **Raw Data** opens a pop-up window **FireSting Raw Data** which is described in chapter6.5.

Clicking on **Clear Charts** will clear the charts in all panels. This will not affect the saved data in the data file.

6.2 Settings

In the settings the user has to define for each channel (1) the measuring parameters and (2) also the environmental conditions of the sample under investigation.

Settings can only be adjusted if data logging is **not** active.

To open the dialog window **FireSting Settings** click on **Settings** in the Main Window:

Each oxygen channel of the *FireSting O2* has its own tab in the settings window. By clicking on **Copy these Settings,** all settings adjusted in the active channel tab can be pasted to all other channels.

The channels can be activated independently by clicking on the button **Active.** Activation is indicated by changing from dark to light green of the arrow in the button. A text for describing the channel's application during the measurements can be entered in the **Description** display. This description will be shown above the charts in the main window (**DD**) and also in the data file.
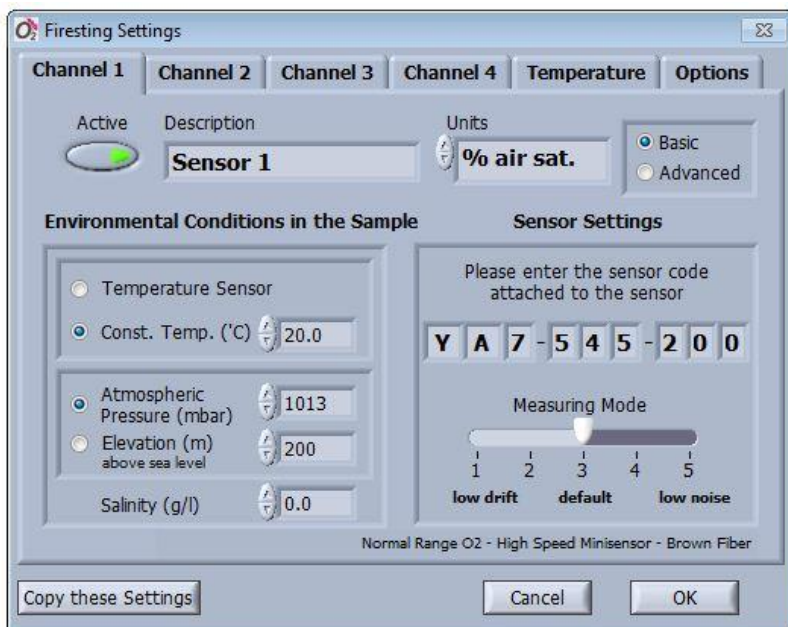
The units of oxygen concentration can be selected for each channel by the selector **Units**. The selectable units include raw value[3] (default), % air saturation, % O_2 (gas), ml L^{-1}, µmol L^{-1}, mg L^{-1} (ppm), hPa, Torr, dphi and µg L^{-1} (ppb). For detailed information refer to the chapters 13.2 and 13.4).

> **NOTE**: The Charts are automatically cleared in the panels of the **Main Window** after the **Settings** have been modified. Readjustments in the **Settings** might require also a recalibration of the sensor(s).

---

[3] for advanced users: raw value=100-dphi, whereby dphi denotes the phase shift of the luminescence life time measurement

6.2.1 Basic Settings

The settings mode has to be chosen as **Basic** or **Advanced**. The first time user is advised to work with the **Basic Settings**. If **Basic** is chosen, the sensor code attached to the sensor has to be entered into the field **Sensor Settings**. A detailed explanation of the sensor code is given in chapter 13.6.

Please take care that the sensor code of the sensor connected to a specific channel of the *FireSting O2* meter is entered in the same channel panel in the window **Settings**. It includes information for optimal sensor settings and for calibration data needed for the factory and the 1-point calibration. A sensor description is then displayed below the **Sensor Settings**.
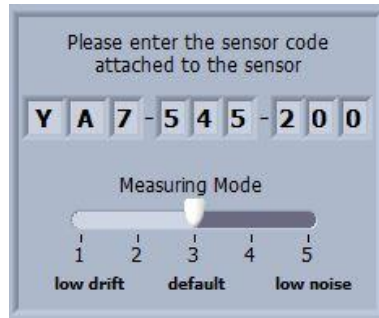
The **Measuring Mode** can be adjusted gradually between low drift (1) and low noise (5) of the sensor signal by moving the arrow with the mouse along the scale, thereby changing the oxygen measuring time. An intermediate mode (3) is default.

NOTE: The chosen oxygen units are only displayed in the **Units Display** of a channel panel of the **Main Window** after a successful calibration of the respective sensor. Ensure that the correct sensor code has been entered. If the sensor is not yet calibrated, either the units "raw value" or "dphi" are shown.

6.2.2 Advanced Settings

If **Advanced Settings** are chosen, more complex settings controls get visible. In order to get a good starting point for the advanced settings, you should first go to the **Basic Settings** and enter there the **Sensor Code** and the preferred **Measuring Mode.** If you now change to the **Advanced Settings**, the **Advanced Settings** will

reflect exactly the measuring conditions as defined in the **Basic Settings**.

The first letter of the sensor code attached to the sensor cable defines the **Sensor Type** (e.g. **R** stands for Normal range O2-Minisensor -Brown Fiber) and has to be adjusted accordingly.

For contactless sensors (e.g. sensor spots, flow-through cells with the **Sensor Type** S or W), a **Background Compensation** can be activated optionally by checking the little square. The background compensation is described in detail in chapters 7.5 and 9.2.

The **Advanced Measuring Parameters** comprise the **LED Intensity** for excitation of the *REDFLASH indicator* (in %) and the **Amplification** of the sensor signal (default: 200x). As a rule of thumb, the **LED Intensity** should be 10-30% for microsensors, minisensors and miniprobes, but can be increased up to 100% for contactless sensors. The **Amplification** should be typically chosen as 80x, 200x or 400x. Note, that varying the LED Intensity and the Amplification has direct influence on the signal intensity and therefore on the signal-to-noise-ratio (see chapter 6.1).
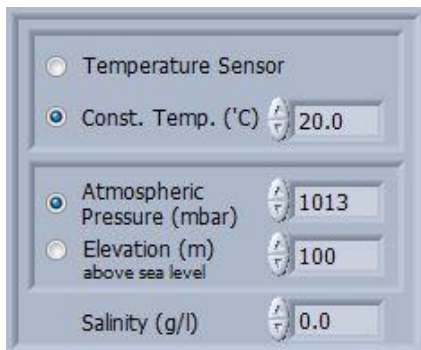
The **Oxygen Measuring Time** (default 10 ms) defines the integration time for the acquisition of a single data point. Shorter measuring times provide low long-term drift, whereby longer

measuring times assure less noise. The maximal possible value is 200 ms.

> **NOTE:** The use of the **Advanced Settings** necessitates a **2-point calibration** of the oxygen sensor. Later readjustments in the **Advanced Settings** might require also a recalibration of the sensor.

6.2.3   Environmental Conditions

The next step is the determination of the **Environmental Conditions in the Sample**, in which the oxygen measurements will be performed. It needs to be selected if the temperature will be determined by the **Temperature Sensor** connected to the *FireSting O2* or if measurements are performed under a **Constant Temperature** (see also chapter 10).

If **Temperature Sensor** is selected, automatic temperature compensation of the respective oxygen sensor readings is activated (see chapter 10.2). Please note that if **Temperature Sensor** is chosen in one of the 4 oxygen **Channel** panels of **FireSting Settings**, the temperature sensor connected to the *FireSting O2* is automatically activated in the **Temperature Panel** (see chapter 6.2.4).

> Please ensure that the temperature sensor is fixed in the container containing the sample in which the oxygen measurements with automatic temperature compensation will be performed.

If **Fixed Temperature** is chosen, the temperature of the environmental sample has to be determined with an external

thermometer or with a temperature sensor from *PyroScience* connected to the *FireSting O2* and has to be adjusted manually.

The **Atmospheric Pressure** (mbar) should be determined with a barometer and changed in the software under conditions of increased/decreased pressures, e.g. if measurements are performed in locations above or below sea level. Normal conditions refer to 1013 mbar (default setting).

If the actual atmospheric pressure cannot be determined on site, it is also possible to enter the **Elevation** in meters (m) **above sea level**. For this click on **Elevation** and enter the actual elevation. This procedure will only calculate the average atmospheric pressure for this elevation; therefore this option is less precise than measuring the actual atmospheric pressure.

The **Salinity** of the environmental sample (in g L^{-1}), in which the oxygen measurements will be performed, needs to be adjusted e.g. in case of saline water. For measurements in gaseous samples this value has no relevance.

6.2.4 Temperature Settings

The temperature sensor connected to the *FireSting O2* can be independently made **Active** in the panel **Temperature,** even if the oxygen measurements of all sensors are performed with **Constant Temperature** (and hence are not affected by the temperature sensor measurements). After activation, the measured temperature is displayed in the **Temperature** panel (see chapter 6.3) and **Overview** panel (see chapter 6.4) of the main window and saved also into the data file.

The temperature units can be adjusted in **Units** of degree Celsius (°C; default setting), degree Fahrenheit (°F) or Kelvin (K).

The **Temperature Measuring Time** (default 300 ms; only multiples of 100ms possible) 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.
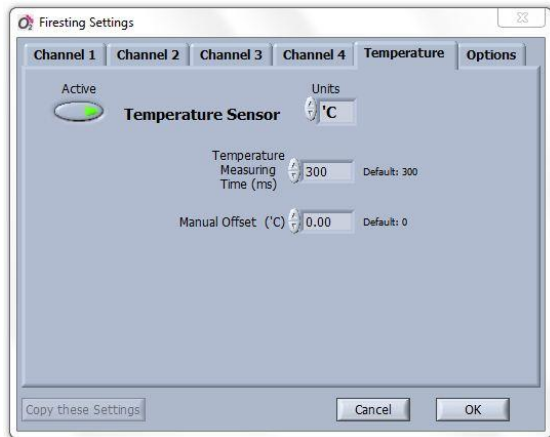
A **Manual Offset** might be entered for possible recalibration of the temperature sensor (default: 0).

6.2.5   Optional Settings

The panel **Options** is **only for advanced users!** Here the **USB communication speed** can be adjusted e.g. for improving the maximum sampling rate (default: 19200). Activation of the button **Enable High-Speed Sampling** will enable the adjustment of a Sample Interval <0.25 s in the main window. Clicking on the **Flash** button of **Flash Settings and Calibration** will save the current Settings and Calibration data into the flash memory of the *FireSting O2*. This is **only relevant for OEM modules** or **third-party software**.
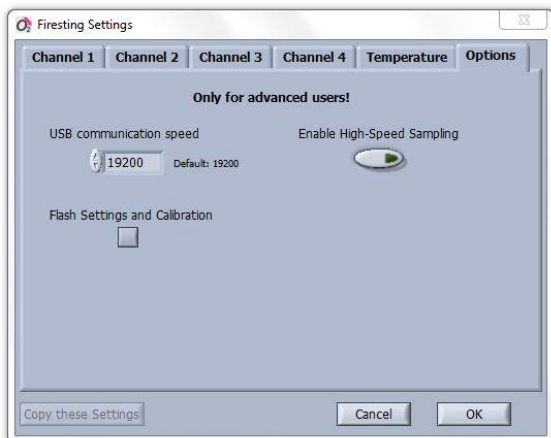
6.3   Temperature Panel

In the **Temperature** panel of the **Main Window** the measured temperature of the connected and activated temperature sensor can be displayed. The temperature unit can be chosen in the **Settings** in degree Celsius (°C; default setting), degree Fahrenheit (°F) or Kelvin (K). **NaN** denotes **N**ot **a** **N**umber when no temperature sensor is connected or the sensor was not activated in the **Settings**.
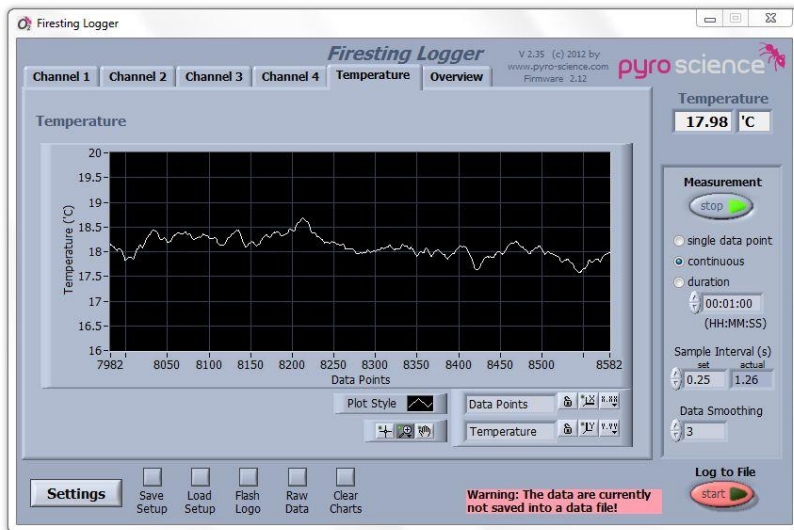
Please note that each *FireSting O2* meter provides only a single port for a temperature sensor. For **Automatic Temperature Compensation** (see chapter 6.2) of >1 oxygen sensor, all sensors connected to the selected channels with automatic temperature compensation have to measure at the same temperature condition, as determined with the connected and activated temperature sensor (see also chapter 10).

6.4 Overview Panel

The sensor readings of all activated oxygen sensors and the temperature sensor are graphically displayed in the panel **Overview.** Each channel reading is shown also as a numerical value in the chosen unit. The plot style of each channel in the chart can be changed by clicking on ◾. With Common Plots, Color, Line Style and Width, Interpolation and Point each plot in the chart can be changed. The items Bar Plot, Fill BaseLine, and Y-Scale are not appropriate for this application.
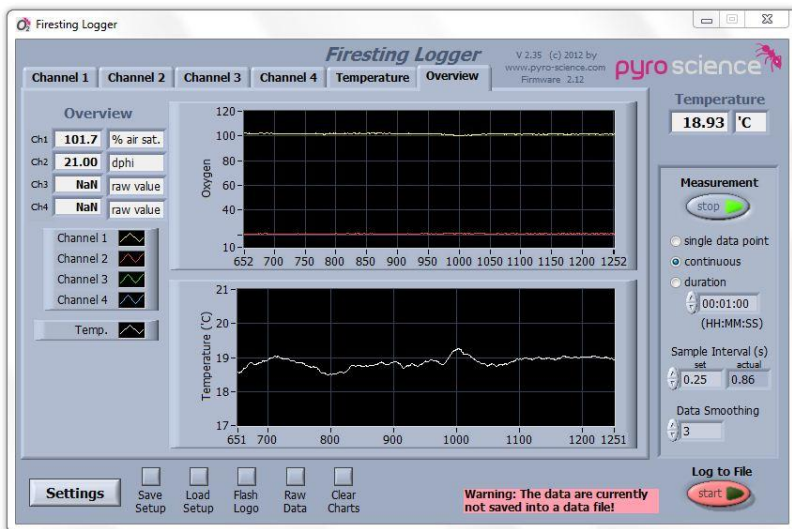
6.5 The Raw Data Window

The Raw Data Window is only intended for trouble shooting, not for use during normal measurements. After clicking on the **Raw Data** button in the **Main Window** (see chapter 6.1) the following **FireSting Raw Data** window opens:

NOTE: While the Raw Data window is opened, all raw values are additionally saved into the data file in additional columns behind the standard data columns.

The panels of each 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 **O2 (µM),** oxygen partial pressure **O2 (hPa)** and % air saturation **O2 (% air sat)** (see also chapter 13.2).

The temperature measured by the temperature sensor and the internal temperature in the *FireSting O2* meter are also displayed.
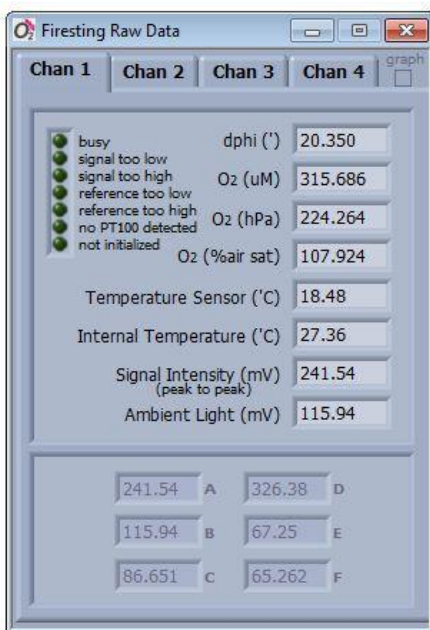
The **Signal Intensity** (in mV) gives a measure of the

O₂ Firesting Raw Data			graph
Chan 1	Chan 2	Chan 3	Chan 4

● busy	dphi (') 20.350
● signal too low	
● signal too high	O2 (uM) 315.686
● reference too low	
● reference too high	O2 (hPa) 224.264
● no PT100 detected	
● not initialized	O2 (%air sat) 107.924

Temperature Sensor ('C) 18.48
Internal Temperature ('C) 27.36
Signal Intensity (mV) (peak to peak) 241.54
Ambient Light (mV) 115.94

241.54	A	326.38	D
115.94	B	67.25	E
86.651	C	65.262	F

quality of the oxygen measurement, which is also displayed in the horizontal bar indicator in the main window (see chapter 6.1).

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 *FireSting O2* might get saturated giving the warning **Signal too high** in this window and in the warning display of the main window (see chapter 6.1).

On the left side, 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.

7   Calibration Procedure

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**. A dialog window opens in which the **Calibration Mode** for the corresponding channel can be selected:
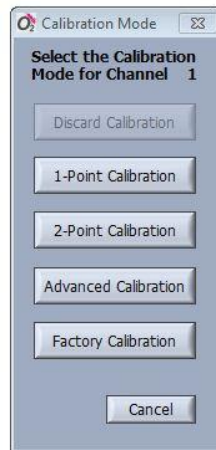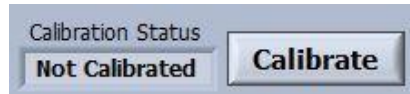
Three *main modes* of calibration can be chosen:

1-Point Calibration: taking the 0% value from the sensor code and the 100% value from a manual calibration

2-Point Calibration: taking the 0% and the 100% value from a manual calibration

Factory Calibration (for a quick, rough calibration): taking the 0% and the 100% values from the sensor code.

During the calibration of a sensor connected to a specific channel, the **Sample Interval** is automatically set to 0.25 s and the **Data Smoothing** to 10. Furthermore, all other oxygen sensor channels

are temporarily de-activated. This allows a fast determination of a precise mean value during the sensor calibration. After finalization of the calibration, the program returns automatically to all former settings.

> **NOTE**: The correct sensor code must have been entered before in the **Settings** (see chapter 6.2). If "**Advanced"** sensor settings were chosen in the **Settings**, the dialog window **Calibration Mode** enables only a **2-Point Calibration** or **Advanced Calibration**.

7.1 Factory Calibration

> **NOTE**: **Factory Calibration** (only for rough measurements and testing purposes) is only possible if "**Basic"** is chosen in the **Settings** and the **correct Sensor Code** has been entered in the **Settings** (see chapter 6.2).

If **Factory Calibration** is chosen, the dialog window **Calibration Mode** closes and a new dialog window opens to ensure that the correct sensor code has been entered in the **Settings**. After clicking on **OK** and thereby returning to the main window of the corresponding channel, the **Calibration Status** displays "**Factory"**.
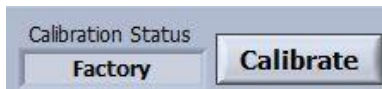
7.2 The 1-Point Calibration

> **NOTE**: The **1-Point Calibration** is only possible if **"Basic"** is chosen in the **Settings** and the **correct Sensor Code** has been entered in the **Settings** (see chapter 6.2).

If **1-Point Calibration** is selected, the dialog window **1-Point Calibration** for the corresponding channel opens to enter the **Calibration Conditions** and to determine the calibration point at 100% air saturation (**Set 100% value**). The preparation of appropriate 100% calibration standards is described in chapter 8.

In the **Calibration Conditions** the **Temperature** of the 100% calibration standard has to be adjusted.

> **NOTE**: The calibration temperature is *not* read automatically from the temperature sensor, but it needs to be determined and entered *manually*. Ensure constant calibration conditions!
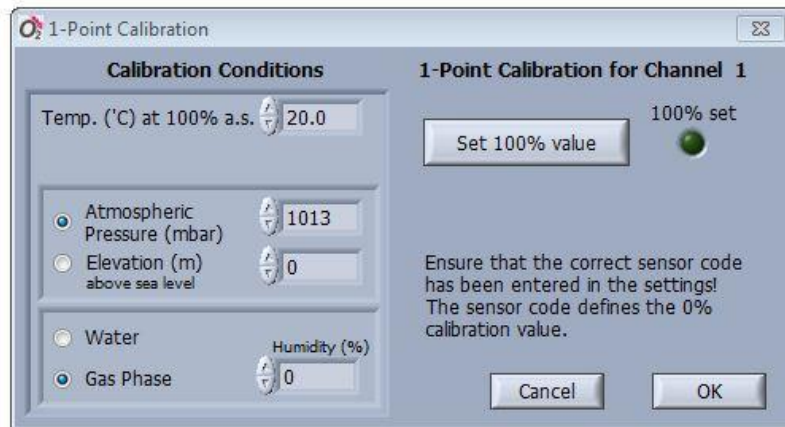
Also the actual **Atmospheric Pressure** determined with a barometer during the calibration must be entered. Normal conditions refer to 1013 hPa/mbar (default setting).

If the actual atmospheric pressure cannot be determined on site, it is alternatively possible to enter the actual **Elevation** in meters (m) **above sea level**. For this click on **Elevation** and enter the actual elevation. Please note, that this option takes only the elevation-dependent pressure change into account, but not the variations due to the actual weather conditions. Therefore, determining the actual atmospheric pressure with a barometer gives more precise results.

Depending on the 100% calibration standard used, select **Water** or **Gas Phase** (see chapter 8). In the latter case the % of **Humidity** of the gas phase (e.g. air) needs to be determined with a hygrometer and entered. If the current **Humidity** cannot be determined, a

medium value of 50% is a good estimate (under normal conditions around 20°C, an incorrectly entered humidity will cause a max. calibration error of ca. 1% deviation).

After insertion of the sensor into the 100% calibration standard and after reaching stable readings (observe the chart in the main window!) click on **Set 100% value** for a manual 1-Point Calibration. The actual sensor reading shown in the main window is then taken as 100% calibration value and the green indicator **100% set** lights up. A re-calibration is possible at any time, simply by clicking on **Set 100% value** again.

Finalize the calibration of the sensor by clicking on **OK**, closing the calibration window and returning to the main window, where now the **Calibration Status** shows **"1-Point"**.
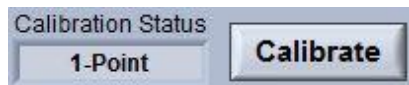
7.3 The 2-Point Calibration

If in the window **Calibration Mode** the **2-Point Calibration** is selected, the dialog window **2-Point Calibration** for the corresponding channel opens to enter the **Calibration Conditions** and to determine the calibration points at 100% and 0% air saturation (**Set 100% value** and **Set 0% value**). The preparation of appropriate 0% and 100% calibration standards is explained in chapter 8.
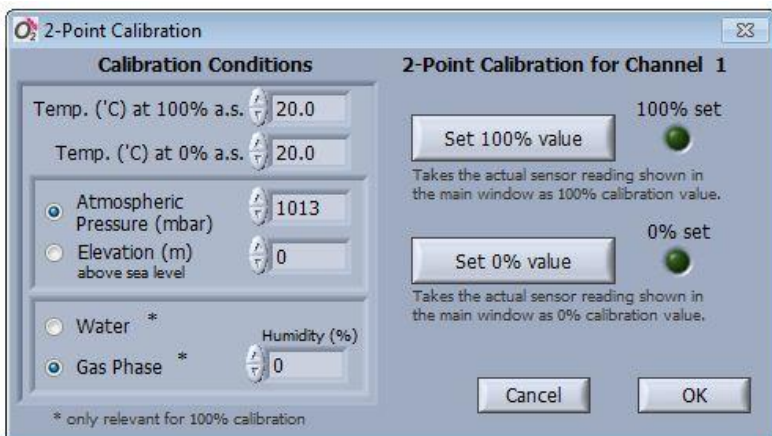
The **Calibration Conditions** need to be defined and entered as described for the 1-point-calibration (see chapter 7.2). The **Temperature** for both the 0% and the 100% calibration standard needs to be determined and entered manually.

After insertion of the sensor into the defined 100% calibration standard and after reaching stable readings (observe the chart in the main window!) click on **Set 100% value**. The actual sensor reading is then taken as 100% calibration and the green indicator **100% set** lights up.

Then insert the sensor subsequently into the 0% calibration standard and after reaching stable readings click on **Set 0% value**. Now the actual sensor reading shown in the main window is taken as 0% calibration value and the green indicator **0% set** lights up. Both calibration values can be re-calibrated any time, simply by clicking again on **Set 100% value** or **Set 0% value**.
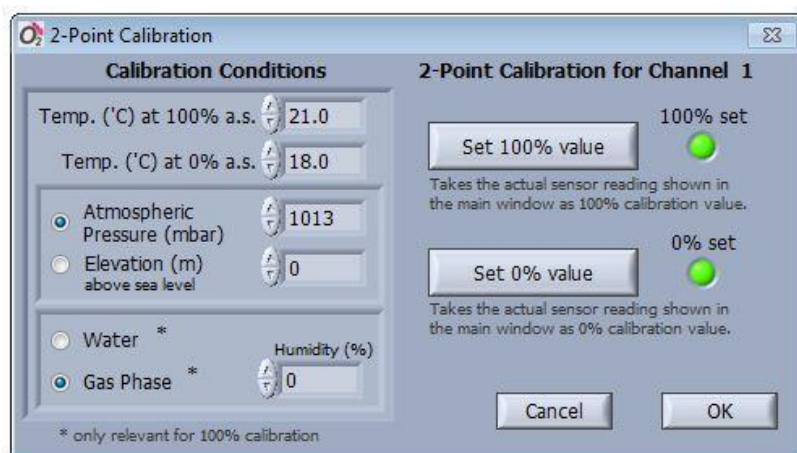
If both indicators **100% set** and **0% set** are bright green, the **2-Point Calibration** can be finalized by clicking on **OK**. In the main window the **Calibration Status** shows then "**2-Point**".
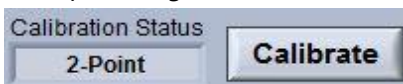
7.4 Advanced Calibration

NOTE: The **Advanced Calibration** is only for **advanced users**!

Besides the three main modes of calibration (factory, 1-point, 2-point) it is also possible to select **Advanced Calibration** in the window **Calibration Mode**.

In the dialog window **Advanced Calibration** the **Calibration Conditions** have to be defined and entered for the corresponding channel as described for the 1-point-calibration (see chapter 7.2).

In the **Advanced Calibration** the phase shift "dphi" (**dphi**, see chapter 6.5 and 13.2) for the 0% calibration standard (**dphi 0%** in °) and for the 100% calibration standard (**dphi 100%** in °) can be adjusted manually.

The parameters **f**, **m**, **F**, **kt**, **mt** 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. *If not otherwise communicated by PyroScience, it is strongly advised to leave these parameters at their default values.*

Further, the **Background Amplitude** (in mV) and the **Background dphi** (phase shift in °) can be adjusted (refer to chapter 9 for more details). These two values are only relevant, if **Background Compensation** for measurements with contactless sensors has been activated in the Advanced Settings of the respective channel.

The **Advanced Calibration** is finalized by clicking **OK**, closing the **Advanced**

Calibration window and returning to the main window, where **Calibration Status** shows now "**Advanced**".

7.5 Background Compensation of Contactless Sensors

The calibration of contactless sensors (i.e. sensor spots, sensor cuvettes and flow-through cells) offers additionally a background compensation (i.e. compensation of potential background fluorescence from the setup). So if in the settings a contactless **Sensor Type** (i.e. S or W) has been selected, then a separate **Background Compensation** will open automatically during the calibration procedure just before the main calibration window opens. Further details about the background compensation and the calibration of contactless sensors are provided in chapter 9.

NOTE: The **Background Compensation** is only relevant for the calibration of contactless sensors (i.e. sensor spots, sensor cuvettes and flow-through cells) and is described in detail in chapter 9.

8 Calibration Standards

8.1 The 100% Standard

The 100% calibration standard can be

- water-vapor saturated air (20.95 vol.-%)
- ambient air with known humidity
- 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 e.g. the bottom of the flask or any other hard object. Always use a proper lab stand for mounting the oxygen sensor! Extend the tip of retractable sensors out of the protecting needle using the push button (extended position 2 or 3).

8.1.1 Water-Vapor Saturated Air

Enclose wet cotton wool in a flask (e.g. DURAN flask) with a lid prepared with holes for the oxygen sensor and a temperature sensor. Select in the **Calibration Conditions "Gas Phase"** and enter **100%** for the **Humidity**. Adjust also the temperature (**Temp. (°C) at 100% a.s.**) and the **Atmospheric Pressure** of your calibration standard. Insert the oxygen sensors into the flask, let it equilibrate for about 15 min and perform the calibration as described in chapter 7.

8.1.2 Ambient Air

If the normal ambient air should be used as the 100% a.s. standard, it is important to measure the humidity with a hygrometer. Select in the **Calibration Conditions "Gas Phase"** and enter the measured **Humidity**. Adjust also the temperature (**Temp. (°C) at 100% a.s.**) and the **Atmospheric Pressure** of your calibration standard. Expose the oxygen sensors to the air and perform the calibration as described in chapter 7.

8.1.3 Air-Saturated Water

Fill water into a flask and stream for 10 min. air through it 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 a flask leaving about 50% air in the head space and shake it strongly for about 3 min. Select in the **Calibration Conditions "Water"** and adjust also the temperature (**Temp. (°C) at 100% a.s.**) and the **Atmospheric Pressure** of your calibration standard. Insert the sensor into the water and perform the calibration as described in chapter 7.

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

8.2 The 0% Standard

The 0% calibration standard can be

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

8.2.1 Water Flushed with Nitrogen Gas

Stream nitrogen gas for about 10 min through water enclosed in a glass flask (e.g. Duran flask) with a lid prepared with holes for inserting the oxygen sensor and a temperature sensor. 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. Adjust in the **Calibration Conditions** the temperature of the calibration standard (**Temp. (°C) at 0% a.s.**) and the current **Atmospheric Pressure**. Insert the oxygen sensor into the flask and perform the calibration as described in chapter 7.

8.2.2 Water Mixed with a Strong Reductant

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}, in water enclosed in a flask, 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 and let the solution stand for about 3 minutes. Adjust in the **Calibration Conditions** the temperature of the calibration standard (**Temp. (°C) at 0% a.s.**) and the actual **Atmospheric Pressure**. Insert the oxygen sensor into the flask and perform the calibration as described in chapter 7.

> Do not store the sensors in this solution and rinse them very carefully after the calibration with demineralized water to avoid salt crystallization which can damage the sensor.

8.2.3 Nitrogen Gas

Flush 100% nitrogen gas through a 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. Adjust in the **Calibration Conditions** the temperature (**Temp. (°C) at 0% a.s.**) and the **Atmospheric Pressure** of your calibration standard. Insert the oxygen sensor into the flask 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

For preparing a setup with contactless oxygen sensors, please refer to the chapters 5.3-5.5.

9.1 Calibration Procedure

In general, the calibration procedure for contactless sensors is the same as for the needle-type/bare fiber oxygen sensors. However, if a 1-point or a 2-point calibration should be performed, the calibration standards have to be filled directly into the vessel intended for the later measurements (e.g. the chamber/flask/ reactor where a sensor spot is glued into), or into the tubing of the flow-through-cell.

It is important that the sensor spot is completely submersed in the specific calibration standard.

9.2 Background Compensation

Further, for an optimal measuring precision of contactless sensors, a background compensation should be performed. This compensates for potential background fluorescence of the fiber-optic setup.

In order to perform a background compensation, ensure in the **Settings**

- either that in the **Basic** settings the correct **Sensor Code** for the contactless sensor is entered (starting with S or W)
- or that in the **Advanced** settings **Sensor Type** S or W is selected and **Background Compensation** is activated.

These settings ensure that the dialog window **Background Compensation** will automatically open during the calibration process before the actual calibration window is shown:
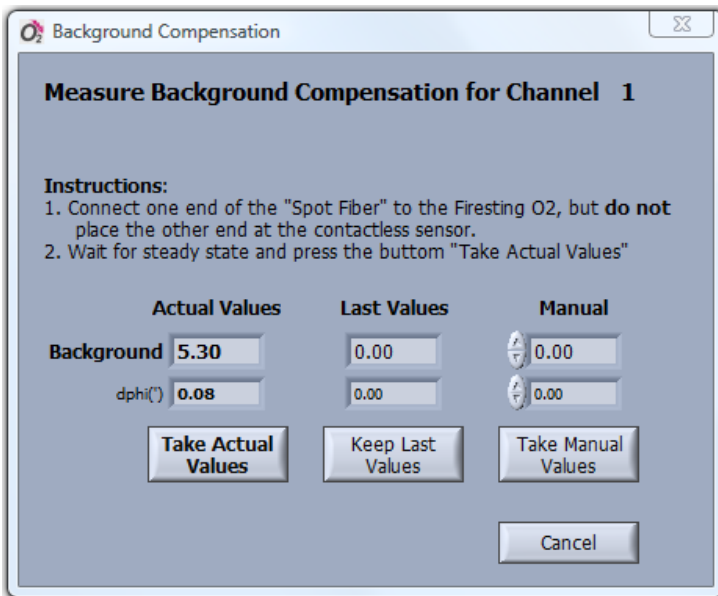
Here the background fluorescence of the connected **Spot Fiber** can be compensated. For this it is *important* that

- one end of the *Spot Fiber* is connected to the corresponding channel of the *FireSting O2* and

- the other end of the *Spot Fiber* is *not* placed underneath the sensor spot (i.e. disconnect this end from the spot adapter 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 (re-)calibrated with the *same* spot 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 both for **Background** and for **dphi,** no background compensation is performed.

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

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

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

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

10 Temperature Measurement

10.1 Available Temperature Sensors

The *FireSting O2* provides one port for a temperature sensor. *PyroScience* offers two standard temperature sensors: the dipping-probe temperature sensor *TDIP15* (with 1.5 mm tip diameter) and the submersible temperature sensor *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. The measuring range of the temperature sensors is -70°C to 200°C (-95°F to 390°F). Please note, that the oxygen sensors have a different temperature range (typ. 0-50°C specified, -20°C to 70°C not specified). The resolution is typically 0.02°C and the absolute accuracy is typically +-0.2-0.3°C.

10.2 Automatic Temperature Compensation

The temperature sensor can be simply used for recording the temperature in the measuring setup. However, the temperature sensor is 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 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, the option **Temperature Sensor** has to be selected in the

Environmental Conditions in the **Settings** of the respective channel. The temperature readings are also saved into the data file.

Please ensure that the oxygen and the temperature sensor are both inserted into the same experimental setup, if an automatic temperature compensation for the oxygen measurement is activated!

Each *FireSting O2* provides only a single port for a temperature sensor. For an automatic temperature compensation of >1 oxygen sensor, all oxygen sensors have to measure under identical temperature conditions.

However, it is also possible to use the automatic temperature compensation e.g. only for a single channel, whereas the other channels can be used for measurements at a **Constant Temperature**. For the channels running at constant temperature, this temperature must be entered in the **Environmental Conditions** in the **Settings.** It is on the users` authority to ensure that the sample is kept under this constant temperature during the measurements.

Even if measurements with all activated oxygen sensors are performed under **Constant Temperature**, the temperature sensor can be used for independent temperature measurements. This requires a separate **activation** of the temperature sensor in the **Temperature** panel of the **Settings** (see chapter 6.2).

11 Specifications of the *FireSting O2*

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 2000, XP, VISTA, 7 (but not "Windows 7 Starter Edition")
Operating temperature	0 to 50ºC
Max. relative humidity	Non-condensing conditions
Oxygen channels	1, 2, or 4 (dependent on model)
Oxygen measuring principle	lifetime detection of REDFLASH indicator luminescence
Oxygen channel connector	fiber-optic ST-plug
Excitation wavelength	620 nm (orange-red)
Detection wavelength	760 nm (near infrared)
Temperature channel	1
Temp. measuring principle	4-wire PT100
Max. sample rate (standard)	4 samples per second
Max. sample rate (with enabled "high speed sampling" in Settings->Options)	20 samples per second (contact us for details)
Extension port	UART interface (contact us for details)

12 Sensor Specifications

12.1 Measuring Range

All "full range" oxygen sensors show an optimal and a maximal possible range of measurements in the gas phase and in water (*dissolved oxygen*):

Full Range Sensors		Units
Optimal Range	*Maximal Range*	
0-50%	0-100%	% O_2 (gas)
0-500	0-1000	hPa
0-500	0-1000	mbar
0-250%	0-500%	*% air saturation*
0-750	0-1500	*µM*
0-23	0-45	*mg L^{-1} (ppm)*

Trace Sensors		Units
Optimal Range	*Maximal Range*	
0-10%	0-21%	% O_2 (gas)
0-100	0-200	hPa
0-100	0-200	mbar
0-50%	0-100%	*% air saturation*
0-150	0-300	*µM*
0-4.5	0-9	*mg L^{-1} (ppm)*

12.2 Available Sensor Dimensions

The following standard sensor dimensions are available:

Type of Sensor	Tip or Spot Diameter
Microsensor	40-60 μm (retractable)
Minisensor	230 μm, 430 μm (retractable) 0.5 mm, 1.1 mm (fixed)
Miniprobe	3 mm
Sensor Spot	5 mm, 8 mm

Please refer to the homepage www.pyro-science.com, whether in the meantime additional standard sensor types are available! Other sizes on request.

12.3 Possible Calibration Modes

Full Range Sensors	Type of Sensor			
Calibration Mode	*Micro-sensor*	*Mini-sensor*	*Mini-probe*	*Sensor Spots*
Rough factory calibration (sensor code attached to the sensor)	yes	yes	yes	
1-point calibration (100% air saturation)	yes	yes	yes	yes
2-point calibration (0% + 100% air saturation)	yes	yes	yes	yes

Trace Sensors: 2-point calibration

12.4 Detection Limit and Resolution

Typical detection limit and resolution for 2-point-calibrated sensors for **Measuring Mode** = *default* (see settings in chapter 6.2.1):

Full Range Sensors (for Measuring Mode = *default*)			
Unit	Limit of Detection	Resolution (low levels)	Resolution (high levels)
% O_2(gas)	0.02% O_2	±0.01% O_2 at 1% O_2	±0.05% O_2 at 20% O_2
hPa / mbar	0.2 hPa	±0.1 hPa at 10 hPa	±0.5 hPa at 200 hPa
mg L^{-1} / ppm	0.01 mg L^{-1}	±0.005 mg L^{-1} at 0.5 mg L^{-1}	±0.025 mg L^{-1} at 8 mg L^{-1}
% air saturation	0.1% a.s.	±0.05% a.s. at 5% a.s.	±0.25% a.s. at 100% a.s.

Trace Sensors (for Measuring Mode = *default*)		
Unit	Limit of Detection	Resolution
% O_2(gas)	0.004% O_2	±0.002% O_2 at 0.2% O_2
hPa / mbar	0.04 hPa	±0.02 hPa at 2 hPa
mg L^{-1} / ppm	0.002 mg L^{-1}	±0.001 mg L^{-1} at 0.1 mg L^{-1}
% air saturation	0.02% a.s.	±0.01% a.s. at 1% a.s.

For **Measuring Mode** = *low drift* the values in the tables must be typically multiplied by a factor of 2, for **Measuring Mode** = *low noise* the values must be divided by 2.

12.5 Accuracy

The absolute accuracy of the full range sensors depends on the calibration mode:

Full Range Sensors			
	Unit	Accuracy (low level)	Accuracy (high level)
Factory Calibration	% O_2 (gas)	±0.3% O_2 at 1% O_2	±1% O_2 at 20% O_2
	hPa / mbar	±3 hPa at 10 hPa	±10 hPa at 200 hPa
	mg L^{-1} / ppm	±0.15 mg L^{-1} at 0.5 mg L^{-1}	±0.5 mg L^{-1} at 8 mg L^{-1}
	% air saturation	±1.5 % a.s. at 5% a.s.	±5% a.s. at 100% a.s.
1-point Calibration	% O_2 (gas)	±0.04% O_2 at 1% O_2	±0.2% O_2 at 20% O_2
	hPa / mbar	±0.4 hPa at 10 hPa	±2 hPa at 200 hPa
	mg L^{-1} / ppm	±0.02 mg L^{-1} at 0.5 mg L^{-1}	±0.1 mg L^{-1} at 8 mg L^{-1}
	% air saturation	±0.2% a.s. at 5% a.s.	±1% a.s. at 100% a.s.
2-point Calibration	% O_2 (gas)	±0.02% O_2 at 1% O_2	±0.2% O_2 at 20% O_2
	hPa / mbar	0.2 hPa at 10 hPa	±2 hPa at 200 hPa
	mg L^{-1} (ppm)	±0.01 mg L^{-1} at 0.5 mg L^{-1}	±0.1 mg L^{-1} at 8 mg L^{-1}
	% air saturation	±0.1% a.s. at 5% a.s.	±1% a.s. at 100% a.s.

Trace Sensors: typically 2% of reading, but not better than 0.01% O_2

12.6 Response Time

The response time t_{90} is defined as the time for 90% of the total sensor signal change in stirred media. Beside the standard sensor types, *PyroScience* offers on request also selected high speed sensor types. Note that the response time increases slightly if the sensor is provided with an optional optical isolation.

	Response Time (t90), no optical isolation			
	Microsensor	*Minisensor*	*Miniprobe*	*Sensor Spots* *Flow-through-cells*
Gas phase	<2 sec (standard)	<3 sec (standard)	<3 sec (standard)	<3 sec (standard)
	<0.5 sec (high speed)	<1 sec (high speed)		<0.5 sec (high speed)
in water	<2 sec (standard)	<10 sec (standard)	<15 sec (standard)	<15 sec (standard)
	<0.5 sec (high speed)	<5 sec (high speed)		<5 sec (high speed)

	Response Time (t90), with optical isolation			
	Microsensor	*Minisensor*	*Miniprobe*	*Sensor Spots* *Flow-touch-cells*
Gas phase	<3 sec (standard)	<3 sec (standard)	<3 sec (standard)	<3 sec (standard)
	<1 sec (high speed)	<1 sec (high speed)		<0.5 sec (high speed)
in water	<4 sec (standard)	<15 sec (standard)	<20 sec (standard)	<20 sec (standard)
	<1 sec (high speed)	<7 sec (high speed)		<5 sec (high speed)

12.7 Long-term Drift

Optical oxygen sensors show a slight long-term drift due to photobleaching. The drift is proportional to the number of sampled data points (i.e. decreasing the sample rate will decrease the drift!), and it is dependent on the chosen **Measuring Mode** in the settings (see chapter 6.2.1).

	Typical absolute drift for full range sensors after 100,000 data points in aerated solution			
Measuring Mode	*Micro- sensor*	*Mini- sensor*	*Mini- probe*	*Sensor Spots*
low drift	0.03% O_2	0.015% O_2	0.005% O_2	0.01% O_2
default	0.1% O_2	0.05% O_2	0.02% O_2	0.04% O_2
low noise	0.4% O_2	0.2% O_2	0.08% O_2	0.16% O_2

12.8 Typical Values for Advanced Settings

Full Range Sensors	**Type of Sensor**			
Setting	*Microsensor*	*Minisensor*	*Miniprobe*	*Sensor Spots*
LED Intensity	10%	10%	10%	30-100%
Amplification	400x	400x	400x	400x
Oxygen Measuring Time	10 ms	10 ms	10 ms	10 ms

Trace Sensors	**Type of Sensor**			
Setting		*Minisensor*	*Miniprobe*	*Sensor Spots*
LED Intensity		10%	10%	30-100%
Amplification		400x	400x	400x
Oxygen Measuring Time		60 ms	60 ms	60 ms

Refer to chapter 6.2.2 for more details.

13 Appendix

13.1 Troubleshooting

How to respond to the warnings shown in the *FireSting 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 **Amplification** in the **Advanced** settings
→ and/or increase **LED Intensity** in the **Advanced** settings
→ replace sensor, the tip might be broken/bleached

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

13.2 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).
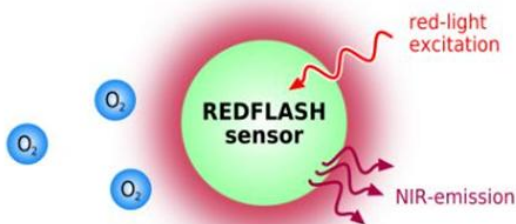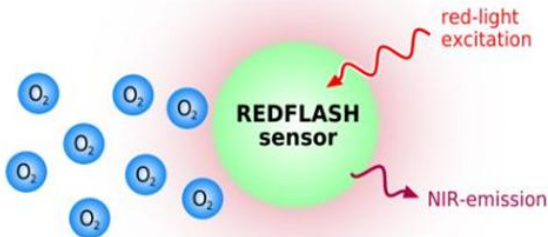
Principle: red light excited REDFLASH indicators show luminescence in the near infrared (NIR), which decreases with increasing oxygen (quenching effect). A) high NIR emission at low oxygen and B) low NIR at high oxygen

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.
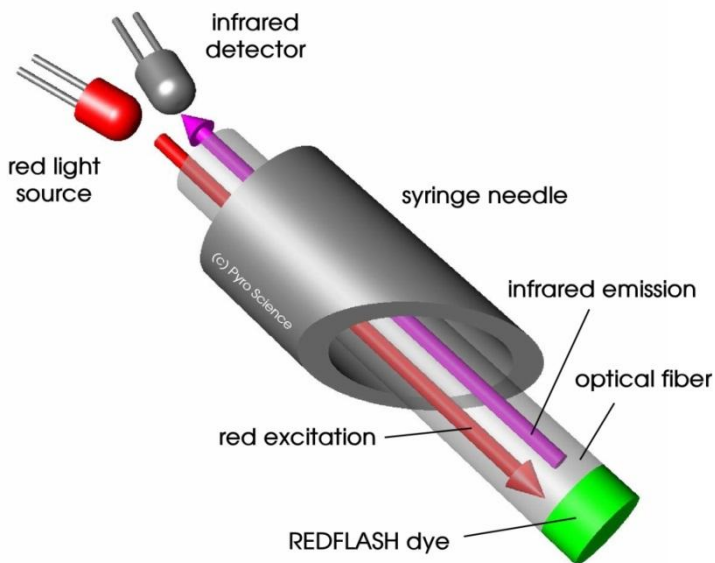
The measuring principle is based on a sinusoidally modulated red excitation light. This results in a phase-shifted sinusoidally modulated emission in the NIR. The *FireSting O2* measures this phase shift (termed "dphi" in the software). The phase shift is then converted into oxygen units based on the Stern-Vollmer-Theory.

13.3 Operating several *FireSting O2* in parallel

The fiber-optic oxygen meter *FireSting O2* 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 *FireStings* can be easily operated at a single PC as described in the following:

Connect each *FireSting* 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 *FireSting* needs max. 70 mA); an external power supply for the USB-hub might be advisable.

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

When closing the *FireSting 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 *FireSting* serial number, i.e. each *FireSting* in the above described setup keeps its own settings and calibration data.

13.4 Definition of Oxygen Units

phase shift   *dphi*

The phase shift *dphi* is the fundamental unit measured by the optoelectronics in the *Firesting O2* (see chapter 13.2). 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* = 100 – *dphi*

The unit *raw value* has the sole purpose to avoid confusion about increasing or decreasing oxygen conditions. In contrast to *dphi*, the *raw value* increases with increasing oxygen levels and vice versa. Therefore, the unit *raw value* is the default unit for uncalibrated sensors.

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 *Firesting O2*.

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(gas)*

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 $p100_{O_2} = 0.2095 \, (p_{atm} - p_{H_2O}(T))$
    $p_{H_2O}(T) = 6.112mbar \times \exp (17.62 \, T[°C] / (243.12 + T[°C]))$
    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 13.5).

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

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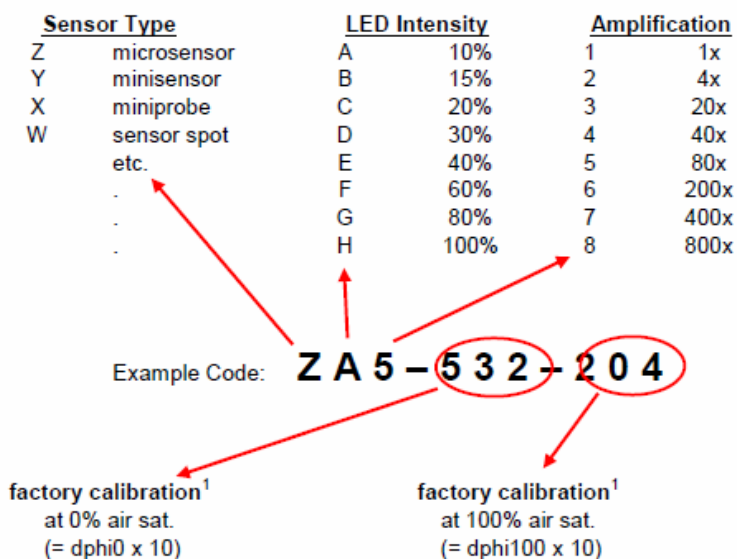
                 Millero, FJ and Poisson, A (1981)
                 International one-atmosphere equation of state of seawater.
                 Deep Sea Res. 28A: 625-629

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

13.6 Explanation of the Sensor Code

The oxygen sensors are delivered with an attached sensor code which can be entered in the basic settings (refer to chapter 6.2.1). The following figure gives a short explanation about the information given in the sensor code.

Sensor Type		LED Intensity		Amplification	
Z	microsensor	A	10%	1	1x
Y	minisensor	B	15%	2	4x
X	miniprobe	C	20%	3	20x
W	sensor spot	D	30%	4	40x
	etc.	E	40%	5	80x
	.	F	60%	6	200x
	.	G	80%	7	400x
	.	H	100%	8	800x

Example Code: **Z A 5 – 5 3 2 – 2 0 4**

factory calibration[1]
at 0% air sat.
(= dphi0 x 10)

factory calibration[1]
at 100% air sat.
(= dphi100 x 10)

The shown example code refers to a full range oxygen microsensor measured at 10% LED intensity at 80x amplification. The factory calibration is given as dphi0 = 53.2 and dphi100 = 20.4

[1] the factory calibration is not given for trace oxygen sensors