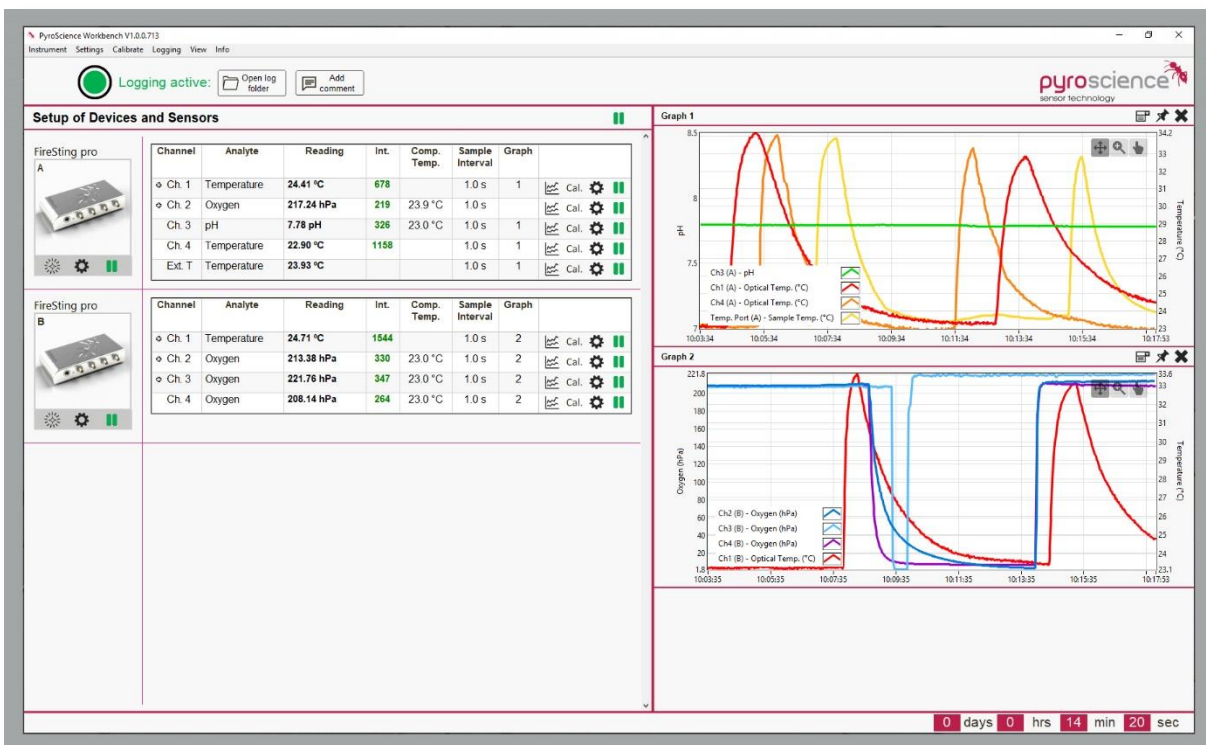# Pyro Workbench &
Data Inspector
PyroScience Logger Software

QUICKSTART MANUAL

O₂ pH T

Pyro Workbench &
Data Inspector
PyroScience Logger Software

Document Version 3.04

Refers to Pyro Workbench & Data Inspector Version V1.4.7.2305

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1   INTRODUCTION

The **Pyro Workbench** is the new customer-friendly logger software with advanced functionality for operation of the newest generation of fiber-optic measuring devices in combination with the complete optical sensor portfolio from **PyroScience**. The intuitive design and new features meet the user requirements to obtain, visualize and store data from multi-analyte measurements (pH, O2, Temperature) with multiple **FireSting-PRO**, **FireSting-O$_2$**, **AquapHOx** or **Pico devices**.

1.1   Technical Requirements

|	**Minimal requirements**	**Recommended requirements**
Operating system	Windows 7 / 8 / 10 / 11 (but not Windows RT)	Windows 10
Processor	Intel i3 Gen 3 (or equivalent) or later	Intel i5 Gen 6 (or equivalent) or later
Graphic	1366 x 768 pixel (Windows scaling: 100%)	1920 x 1080 pixel (Full HD)
Disk space	1 GB	3 GB
RAM	4 GB	8 GB

1.1 Installation

> **Important:** Do not connect the **PyroScience** device to your PC before the **Pyro Workbench** software has been installed. The software will install automatically the appropriate USB-driver. After software installation, first connect a supported device and then open the **Pyro Workbench** software.

Installation steps:

- Please find the correct software in the downloads tab of your purchased device on www.pyroscience.com

- Unzip and start the installer and follow the instructions

- Restart your PC after software installation

- Connect the supported device(s) with the micro-USB cable to the computer **before** opening the **Pyro Workbench** software. The port LEDs of the device will flash shortly indicating correct startup

- After successful installation, a new program short-cut "Pyro Workbench" and "Pyro Data Inspector" are added to the start menu and can be found on the desktop:

- Additionally, a registry entry in Windows is made that links the **Workbench** unique data file format *.pyr to the "Pyro Data Inspector":
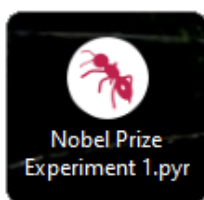
By double clicking on a *.pyr file in the windows explorer, the respective experiment gets opened automatically in the **Pyro Data Inspector** for further data processing or data export to a e.g. *.txt or *.xls file format.

1.2   Supported Devices

Multi-analyte meter FireSting-PRO with

- 4 optical channels (item no.: **FSPRO-4**)

- 2 optical channels (item no.: **FSPRO-2**)

- 1 optical channel (item no.: **FSPRO-1**)

Oxygen meter FireSting-O$_2$ with

- 4 optical channels (item no.: **FSO2-C4**)

- 2 optical channels (item no.: **FSO2-C2**)
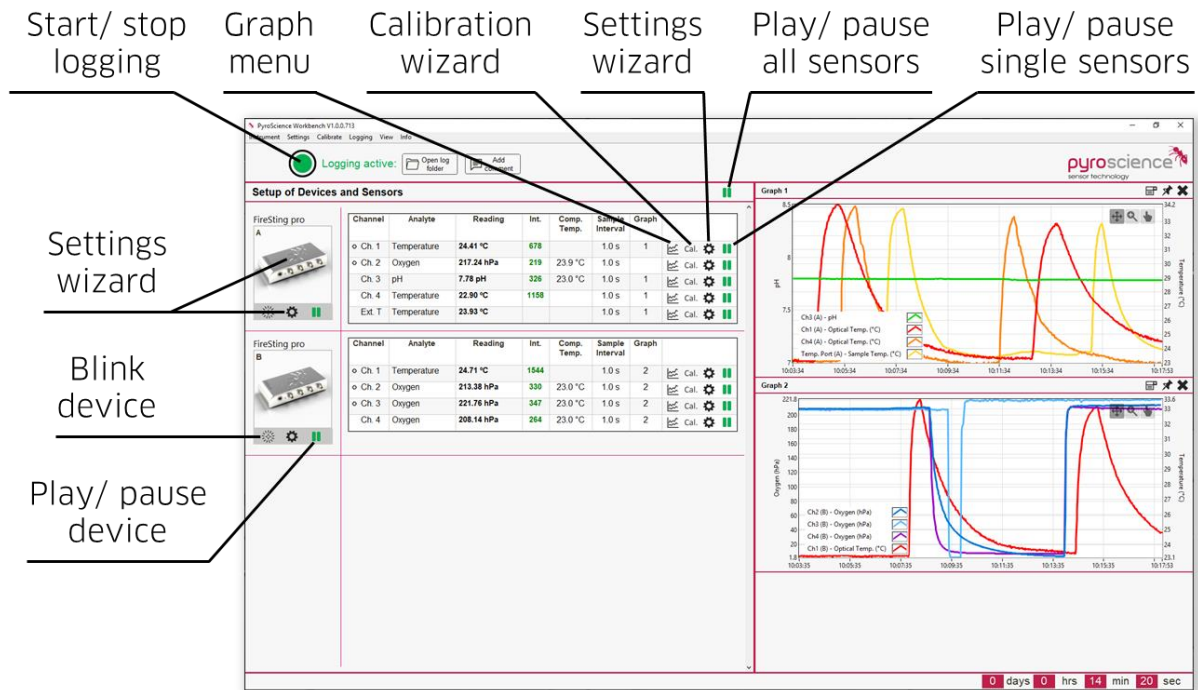
- 1 optical channel (item no.: **FSO2-C1**)

OEM meters

- Oxygen OEM module (item no.: **PICO-O$_2$, PICO-O$_2$-SUB**)

- pH OEM module (item no.: **PICO-PH, PICO-PH-SUB**)

- Temperature OEM module (item no.: **PICO-T, PICO-T-SUB**)

Underwater AquapHOx meters

- Logger (item no.: **APHOX-LX, APHOX-L-O$_2$, APHOX-L-PH**)

- Transmitter (item no.: **APHOX-TX, APHOX-T-O$_2$, APHOX-T-PH**)

2 OVERVIEW MAIN WINDOW

Start/ stop logging · Graph menu · Calibration wizard · Settings wizard · Play/ pause all sensors · Play/ pause single sensors

Settings wizard

Blink device

Play/ pause device

The main window of the **Pyro Workbench** can be separated in 4 parts: Menu bar (top), device overview (left), configuration overview and numerical data (middle) and graphs (right). In the top part you find the top menu bar, important global buttons and enough space for possible warning massages. On the left side all connected and compatible **PyroScience** devices are visible and listed. In the middle, there is an overview of the respective configurations of the connected devices and current sensor data. The right side is reserved for the graphical presentation of the measured sensor data.

Please see the overview above for a short explanation of all buttons of the main Window. All options are also accessible via the top menu bar. The two buttons **Open log folder** and **Add comment** are only visible when data are logged.

2.1 Graph Tools

The measurements of all sensors can be displayed in graphs by clicking on the **Graph** menu symbol. Please note that the left y-axis is available for a single optical sensor analyte (e.g. pH or O_2), whereas the right y-axis is ascribed to temperature only (e.g. compensation temperature for optical sensors). However, all sensor analytes can be displayed in several graphs, each graph can be unpinned and the graph image can be exported (see tools in the upper right corner).
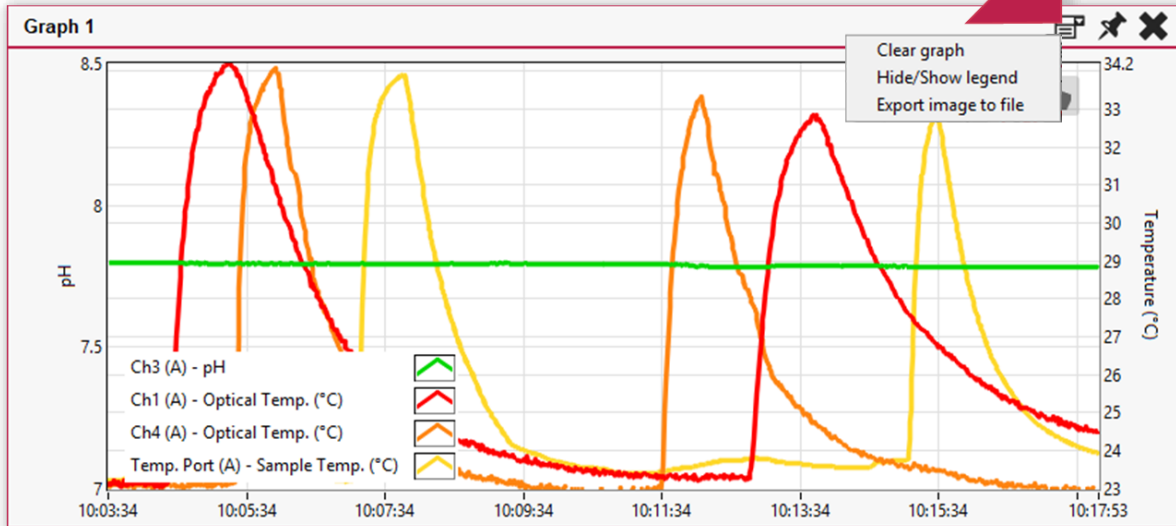
There are two ways to change the scale of a graph. The easiest way would be to change the minimal and maximal value of the y-axes by clicking on the value and entering the desired minimum/ maximum.
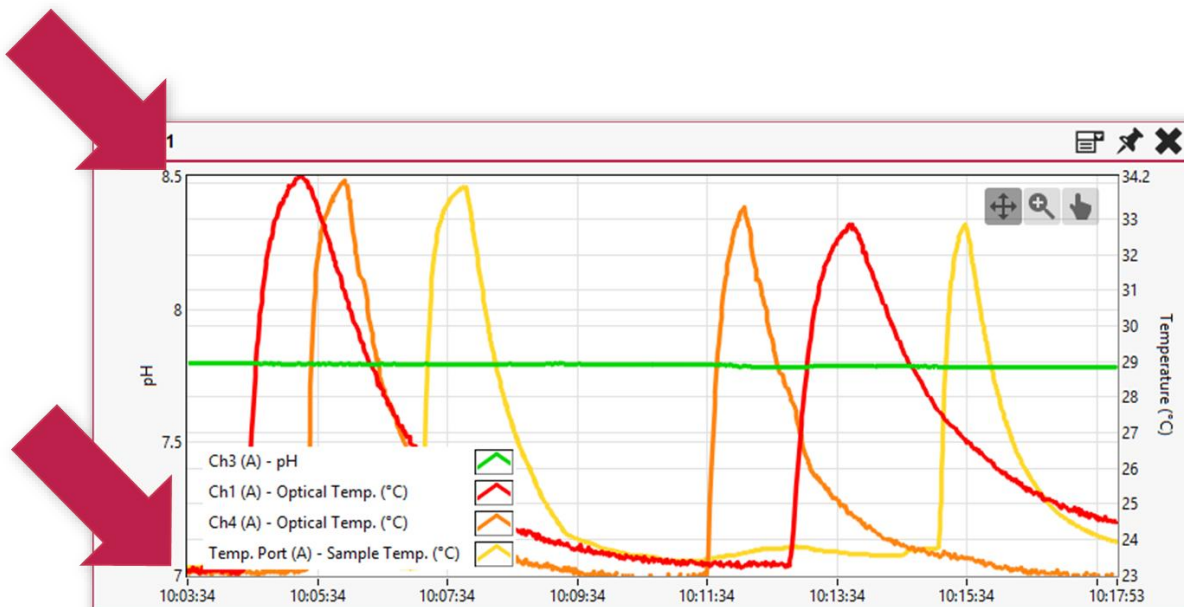
Additionally, there are zoom tools accessible in the upper right corner of every graph. With the help of these tools and by selecting the desired part of the graph with the mouse, a graph cutout can be performed.
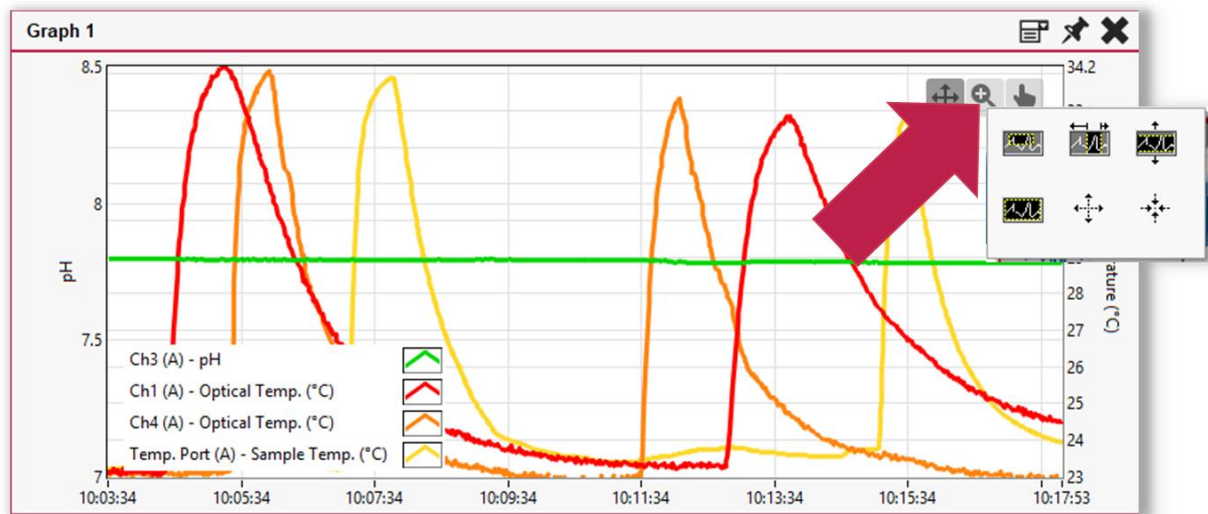
The appearance of every single curve can be modified in various ways. To enter the respective options, do a **right click** on the graph legend. If the graph legend is not visible, use the "Hide/show legend" option in the upper right corner.

3   SENSOR SETTINGS

To setup and configure a device, a guided and user-friendly **Settings Wizard** is implemented.

3.1   Settings Wizard

- Open the **Settings Wizard** by clicking on the appearing device picture, the little gear symbol underneath or use the menu bar at the top of the main window:

- Enter the respective settings, click **Next** until the required settings for all connected sensors are entered and apply with **Save**.
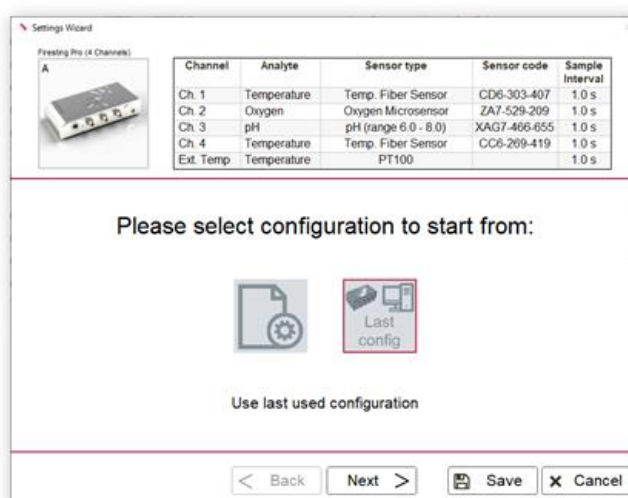
> **Important:** Enter the correct **Sensor Code** for the connected sensor. The sensor code can be found on the label attached to the cable (fiber-based sensors) or on the bag of contactless sensors (see example below).

- In some cases (e.g. OXNANO), it can be beneficial/ necessary to change the recommended default LED intensity. For this, switch the **Settings** from "Recommended" to "Advanced" and increase the **LED intensity** on the shown panel below:

3.2   Environmental Parameters

Depending on the analyte and desired unit of the optical sensor reading, there are different important environmental parameters, which need to be considered and determined (as requested in a respective window of the settings wizard).

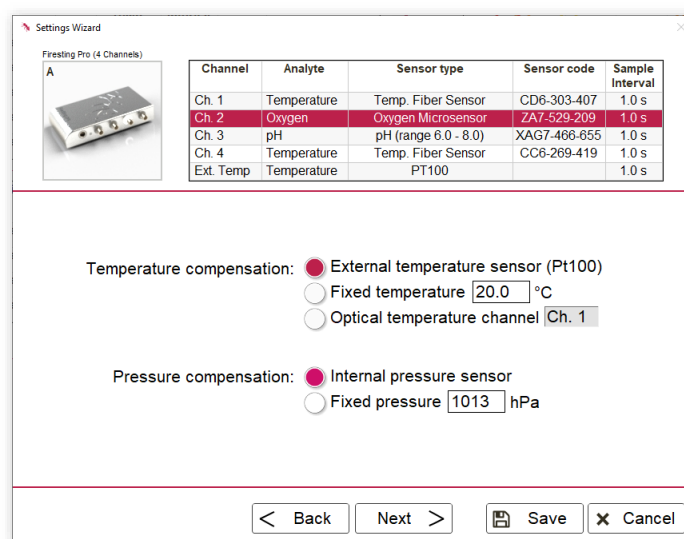Please note that you can use several options for **Temperature compensation** of optical analyte sensors (pH, O_2):

- External Temperature Sensor (Pt100, temperature port)

- Fixed Temperature (must be entered, kept constant and controlled!)

- Optical Temperature sensor connected to an optical channel (its channel number must be selected)

Another parameter, which needs to be defined in the settings (for O_2 sensors) is the atmospheric pressure, which can be compensated by:

- the **Internal pressure sensor** for automatic compensation of pressure changes e.g. caused by weather changes or

- by entering a **Fixed pressure (hPa)**. The actual pressure at the sensor position needs to be determined with e.g. a barometer and adjusted manually (default: 1013 mbar).

For more details on compensation of important parameters, please see the manuals for the optical oxygen and/or optical pH sensors.

4   SENSOR CALIBRATION

Similar to the **Settings Wizard**, the user is guided through a **Calibration Wizard** for performing the required optical sensor calibration.

4.1   Calibration of Oxygen Sensors

- For gas measurements: the sensor needs to be calibrated (temperature-controlled) in ambient air (21% O_2) and in some cases also in nitrogen gas N2 (0% O_2).

- For measurements in aqueous/water samples: the sensor needs to be calibrated (temperature-controlled) in air-saturated water (upper calibration, 100% air saturation) and in some cases also in de-oxygenated water (0% DO).

The oxygen sensor calibration can be performed in two ways:

- 1-point calibration (required): upper **or** 0% calibration (e.g. for TRACE oxygen sensors)

- 2-point calibration (optional): upper **and** 0% calibration; recommended for measurements at low O_2 and high accuracy measurements over the full range.

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

- After completing the Settings Wizard for all channels, open the **Calibration Wizard** by clicking the "Cal." button in the respective row ("Ch. 1" to "Ch. 4") of the table in the main window or use the menu bar at the top of the main window:

Channel	Analyte	Reading	Int.	Comp. Temp.	Sample Interval	Graph	
✛ Ch. 1	Temperature	23.85 °C	680		1.0 s	1	⬚ Cal
✛ Ch. 2	Oxygen	Not calibrated	218	23.5 °C	1.0 s		⬚ Cal. ⚙ ▶

> **Important:** For precise absolute measurements and optical sensor calibration using **External Temperature Sensor**, it needs to be determined manually if the external (Pt100) temperature has an offset before calibrating the optical sensor. Then, the Pt100 temperature sensor needs to be calibrated first (see sensor manual).

- Click on **Calibrate** and follow the calibration instructions.
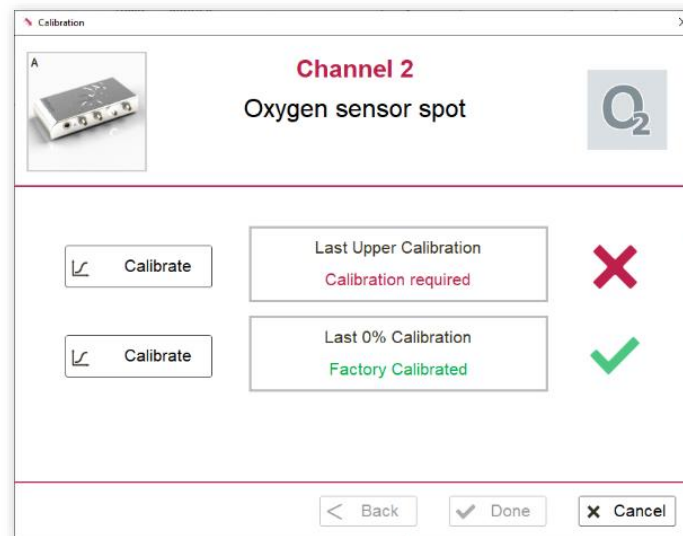
- Enter all required parameters, click **Next** and perform a sensor calibration. Click **Take Value** when the readings of the optical sensor and temperature probe are stable in the respective calibration standard and the **Take Value** button is enabled. With increasing stability of the sensor signal the color will change from red to orange to yellow to green. Although a calibration is possible in the orange state, we strongly recommend to wait until **Take Value** and the frame of the graph have turned to **green**!
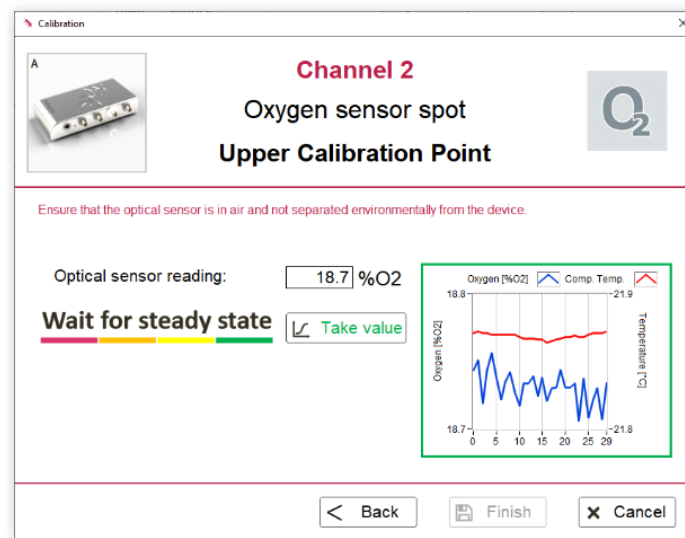
- Click on **Save Calibration** to complete the calibration. A successful calibration is indicated in green, showing the (latest) calibration date. Confirm with **Finish** and later **Done** to return to the main window.
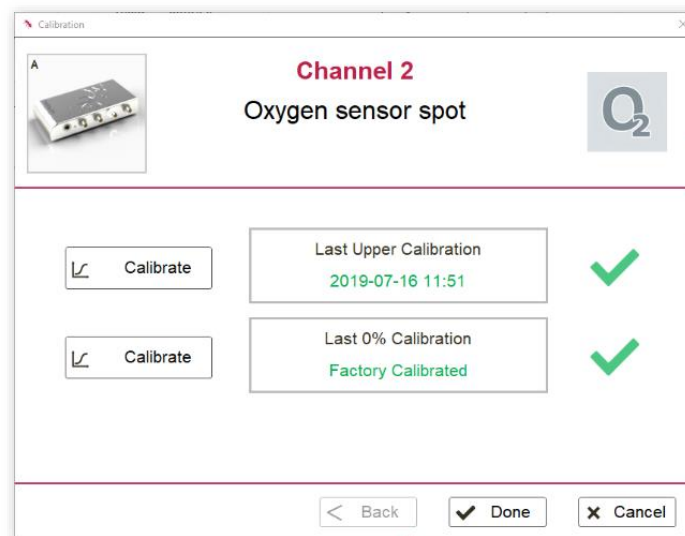

4.2 Calibration of pH Sensors

Depending on the applied equipment and requirements, the following calibration modes are possible:

- **Calibration free measurements** are possible with new pH sensors (SN>231450494) in combination with pre-calibration ready **FireSting-PRO** devices (SN>23360000 and labeled devices)

- **A one-point calibration** is obligatory for re-used pre-calibrated pH sensors or readout-devices that are not pre-calibration ready. A manual calibration is generally recommended for higher accuracy.
- **A two-point calibration** before every measurement is highly recommended for precision measurements
- **pH offset adjustment** is recommended for measurements in complex media (only advanced applications)
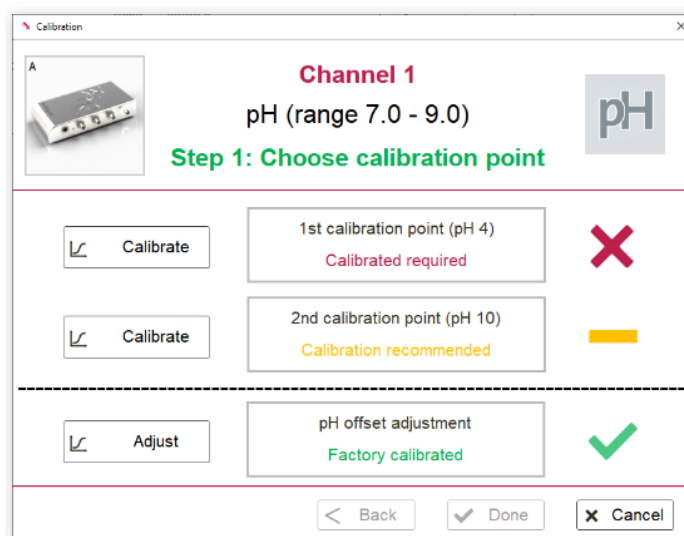
> **Do not use commercially available buffer solutions used for pH electrodes.**
> Theses buffers (colored and uncolored) will change the sensor performance irreversibly. It is important to only use **PyroScience** buffer capsules or self-made buffers with known pH and ionic strength for calibration (more details on request).
>
> It is strongly recommended to perform a calibration at temperatures close to the temperature of the subsequent measurements. Ensure constant conditions during the whole calibration.

1-point calibration

- After completing the Settings Wizard for all channels, open the **Calibration Wizard** by clicking the "Cal." button in the respective row ("Ch. 1" to "Ch. 4") of the table in the main window or use the menu bar at the top of the main window:

Channel	Analyte	Reading	Int.	Comp. Temp.	Sample Interval	Graph	
⟳ Ch. 1	Temperature	23.86 ºC	679		1.0 s	1	⎿ Cal.
⟳ Ch. 2	Oxygen	20.81 %O2	172	23.5 °C	1.0 s		⎿ Cal.
Ch. 3	pH	Not calibrated	348	23.5 °C	1.0 s	1	⎿ Cal. ⚙ ⏸

> **Important:** For precise absolute measurements and optical sensor calibration using **External Temperature Sensor**, it needs to be determined manually if the external (Pt100) temperature has an offset before calibrating the optical sensor. Then, the Pt100 temperature sensor needs to be calibrated first.

- Click on **Calibrate** and follow the calibration instructions.

- Immerse the pH sensor and the temperature sensor into the **stirred** pH 2 buffer solution (freshly prepared using item no. **PHCAL2**). For more information see the pH sensor manual.

- Click on **Take Value** when the readings of the pH and temperature sensor are stable. With increasing stability of the sensor signal the color will change from red to orange to yellow to green. Although a calibration is possible in the orange state, we strongly recommend to wait until **Take Value** and the frame of the graph have turned to **green**!

2-point calibration

A 2-point calibration is highly recommended before every measurement.

- Immerse the pH sensor and the temperature sensor into the **stirred** pH 11 buffer solution (freshly prepared using item no. **PHCAL11**). For more information see the pH sensor manual.

- Click on **Take Value** when the readings of the pH and temperature sensor are stable. With increasing stability of the sensor signal the color will change from red to orange to yellow to green. Although a calibration is possible in the orange state, we strongly recommend to wait until **Take Value** and the frame of the graph have turned to **green**!

- Click **Finish** to apply the 2-point calibration.

- Finish the calibration and start measurements with **Done**.

pH offset adjustment (only for advanced applications)

This will perform a pH-offset adjustment to a buffer with exactly known pH value. This can be used for measurements in highly complex media (e.g. cell culture media). Please refer to the pH sensor manual for more information.

To undo the pH offset, please click on **Delete calibrations** in the calibration wizard. This will delete all current calibration values and set the sensor back to factory calibration. A 2-point calibration has to be performed afterwards to start a measurement.

4.3   Calibration of Optical Temperature Sensors

> **Note:** The calibration of the optical temperature sensor is recommended on a weekly basis.

- After completing the Settings Wizard for all channels, open the **Calibration Wizard** by clicking the "Cal." button in the respective row ("Ch. 1" to "Ch. 4") of the table in the main window or use the menu bar at the top of the main window:

Channel	Analyte	Reading	Int.	Comp. Temp.	Sample Interval	Graph	
⊕ Ch. 1	Temperature	24.13 ºC	679		1.0 s	1	⬚ Cal. ⚙
⊕ Ch. 2	Oxygen	20.81 %O2	172	23.5 °C	1.0 s		⬚ Cal
Ch. 3	pH	pH < 5.5	883	23.5 °C	1.0 s	1	⬚ Cal
Ch. 4	Temperature	Not calibrated	860		1.0 s	1	⬚ Cal. ⚙ ⏸

> **Important:** For precise absolute measurements and optical sensor calibration using **External Temperature Sensor**, it needs to be determined manually if the external (Pt100) temperature has an offset before calibrating the optical sensor. Then, the Pt100 temperature sensor needs to be calibrated first.

- Click on **Calibrate** and follow the calibration instructions.

- Select the desired calibration against a **Pt100 Temperature Sensor** (external temperature sensor) connected to the temperature port of the device or against a **Fixed Reference Temperature**.

- Click on **Take Value** when the readings of the optical temperature sensor and the Pt100 temperature probe are stable in the respective calibration standard. With increasing stability of the sensor signal the color will change from red to orange to yellow to green. Although a calibration is possible in the orange state, we strongly recommend to wait until **Take Value** and the frame of the graph have turned to **green**!

Important: Ensure that the external Pt100 temperature probe is placed close to the optical temperature sensor and exposed to the exact same temperature conditions.

5   MEASUREMENT AND LOGGING

After a successful sensor calibration, **Measurements** and **Logging** can be started individually for each sensor.

5.1   Measurements

- The **Measurement** of the respective sensor will start automatically (indicated by "II") after sensor calibration has been performed successfully.

> **Note:** A measurement can be paused by clicking on "II", then changing to ▶.
>
> **Note:** The sampling interval can be selected individually for each optical channel.

- Configure the **Graphs** according to your preferences by clicking on the graph symbol in the respective row of the table (see chapter 2.1).

Channel	Analyte	Reading	Int.	Comp. Temp.	Sample Interval	Graph	
⊕ Ch. 1	Temperature	23.76 ºC	679		1.0 s	1	〰 Cal. ⚙ ❚❚
⊕ Ch. 2	Oxygen	20.48 %O2	172	22.4 °C	1.0 s		〰 Cal. ⚙ ❚❚
Ch. 3	pH	pH < 5.5	878	22.4 °C	1.0 s	1	〰 Cal. ⚙ ❚❚
Ch. 4	Temperature	22.37 ºC	861		1.0 s	1	〰 Cal. ⚙ ❚❚
Ext. T	Temperature	22.44 ºC			1.0 s	1	〰 Cal. ⚙ ❚❚

5.2   Data Logging

5.2.1   PC Logging

- Start **Data Logging** into a file by clicking on the **Record** button in the upper left corner:

- If you have a device connected, which features data logging, the following window will show up:

- Select the "PC Logging" option

- Enter all Record Settings and **Start logging**:
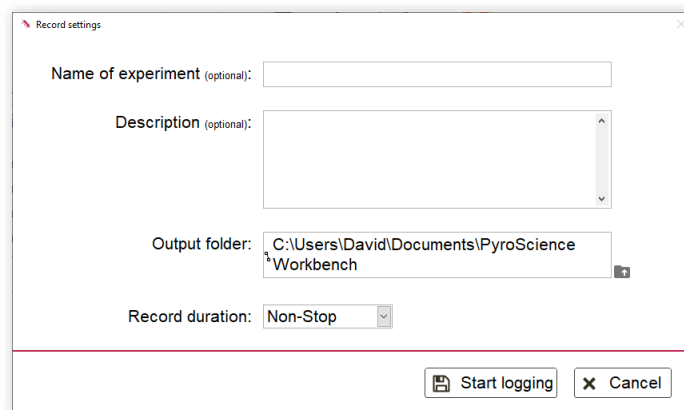
- After your experiments, the saved data files are available in the entered data folder as simple text-files with the file extension ".txt". These files can be easily imported into common spreadsheet programs. It is not recommended to open and/ or copy the data files during an experiment. This could lead to a forced stop of the data recording.

5.2.2   Device Logging/ Stand-Alone Logging

Some devices (e.g. **AquapHOx Logger**) offer the option to log data without the connection to a PC.

- Select the "Stand-alone Logging" option instead of the PC Logging option after clicking in the **Record** button on the front panel or select "**Stand-alone logging**" of the respective device via "**Settings**" in the top menu
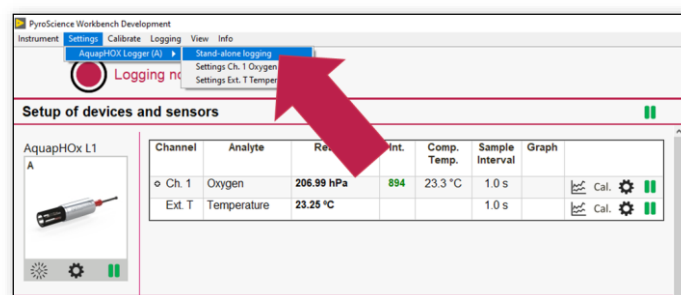
- Enter a unique **Log Name** with 1 to 6 characters.
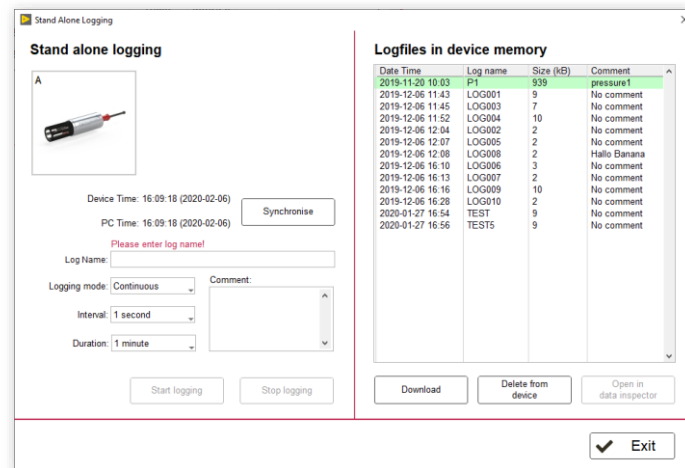
- Start the logging by clicking on "**Start logging**". The device can now be disconnected from the PC and will continue logging the data.

- The obtained data can be downloaded after the experiment on the right side of this window by selecting the correct logfile and clicking on "Download". These ".txt" files can be easily imported into common spreadsheet programs.

> **Note:** PC logging (see 5.2.1) and device logging can be active simultaneously at different or identically set sampling intervals. The obtained data via PC Logging will not show up in the device logging file and vice versa.
> But, if the combined measurement rate supersedes the capacity of the device, measurement values will be skipped automatically. Therefore, it is recommended to pause the PC measurements while setting up and starting the device logging.

5.2.3   Analog Output/ Broadcast Mode

The standard operation mode is based on a Windows PC running the **Pyro Workbench** operating firmware 4 devices via the USB interface. This user-friendly operation mode is generally recommended, as it offers easy control over the full functionality. However, several advanced features are available in addition for integrating the devices in customized setups.

Some devices (e.g. **FireSting-PRO**) offer an integrated 4-channel **Analog Output** at the extension port. It can be used for transferring measurement results (e.g. oxygen, pH, temperature, pressure, humidity, signal intensity) as voltage/ current (depending on the device) signals to other electronic equipment (e.g. loggers, chart recorders, data acquisition systems).

Further, some devices can be operated in a so-called **Broadcast Mode**, in which the device performs measurements autonomously without any PC connected. 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. The basic idea behind the auto-mode is that all operations related to sensor settings and sensor calibrations are still performed during the general operation with a PC. When this is done, the broadcast modus can be configured and the device will trigger a Measurement autonomously as long as a power supply is given via the USB or extension port.

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

To enter the Analog Output Settings, use the top menu bar of the **Pyro Workbench** via settings -> respective device -> Analog output/ broadcast mode
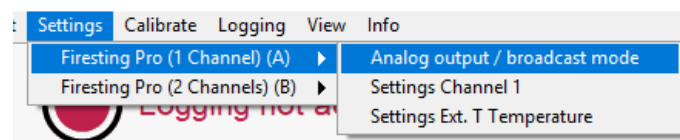
The respective settings window for the analog output options will open:
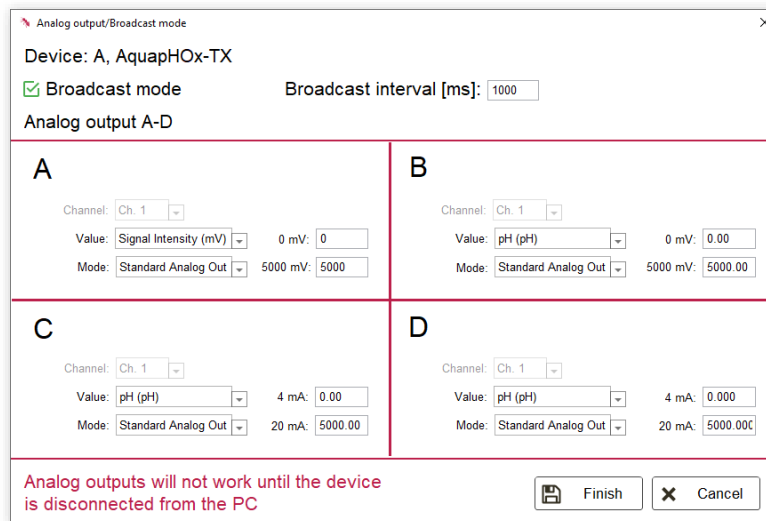
Broadcast mode: The device can trigger a measurement on its own, without a connection to a PC. Please refer to the respective device manual on how to connect a necessary supply voltage to the device. During broadcast modus operation, the measurement results can be read out e.g. by an analog data logger from the analog outputs. For this, enable **Broadcast mode** and set a respective **Broadcast interval**.

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 optical channels. The background is that the analog outputs are not fixed to specific channels ensuring highest flexibility.

Please select which value of which optical channel you would like to map on the respective analog output A-D. The output of the analog output is device dependent. In the example above all analog outputs offer a voltage output between 0 and 2500 mV. Please note that only values can be selected which are in line with the settings of this particular optical channel. Meaning you cannot select an oxygen unit, when this channel was configured as a pH channel in the workbench settings.

Note: The corresponding values of the minimum and maximum outputs are always in the unit of the selected value. Meaning in the example above, 0 mV corresponds to 0° dphi and 2500mV corresponds to 250° dphi.

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 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: In this case either 0 mV or 2500 mV (so actually it is now a digital signal). The 0 mV are given if the measured parameter falls below the entered value. Equally the 2500mV are given, if the measured parameter is above the entered value. If the measured parameter gets out of this range or there is a conflict, the analog output will switch to the maximum voltage/ current. 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 maximum voltage/ current of the alarm output could trigger some external electronic equipment which e.g. rings a bell.

Note: For devices with a MODBUS interface (e.g. APHOX-TX) the analog outputs will not work until the device is disconnected from the PC.

The **Finish** button will apply all settings (Analog output + Broadcast modus).

6   PYRO DATA INSPECTOR

During an experiment, the data and configuration are always stored in a database *.pyr file. Data from logging devices can be downloaded and converted to *.pyr files via clicking on "Download and open in data inspector" in the stand-alone logging window (See section 5.2.2).

Obtained live data of a *.txt file (enabled "Live export" option in the PC-logging window) cannot be loaded in the data inspector, only *.pyr files.

The **Pyro Data Inspector** does not require a connection(s) of the respective **PyroScience** device(s), which was/were used during the experiment.

Main functions and benefits of the **Pyro Data Inspector**:

- Experiment information such as start time, duration, name, made comments during the experiment, occurred errors and so on are stored and can be evaluated.

- The complete device setup including all settings and calibration data of the experiment can be revisited.

- Powerful data processing steps, such as smoothing, drift compensation, calculation of respiration rates, can be user friendly performed. (see section 6.2)

- Obtained data as well as processed data can easily be compared and visualized in graphs.

- All or only targeted data can be exported into a variety of standard data file formats such as *.txt or *.xls for further data analyses. (see section 6.3)

6.1   Overview Main Window

The main window of the **Pyro Data Inspector** can be separated in 4 parts: Menu bar (top), device overview (upper left), data processing steps (down left) and graphs (right).
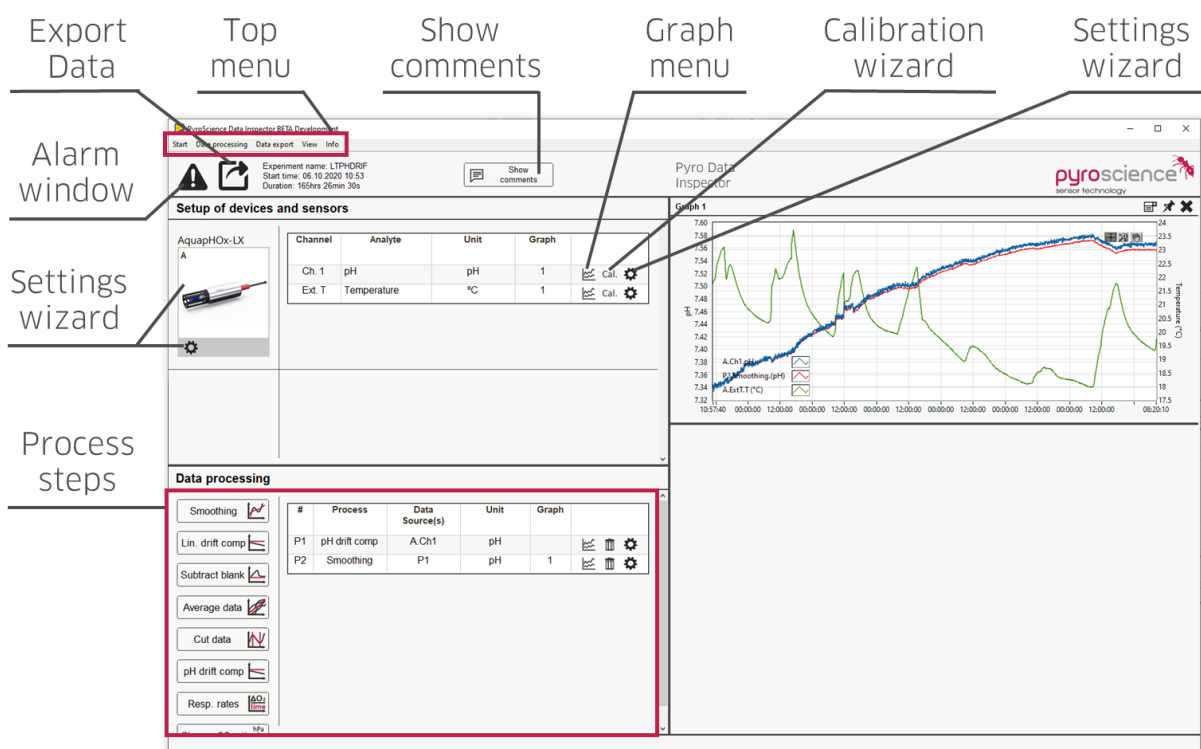
Please see the overview above for a short explanation of most buttons of the main Window. All options are accessible via the top menu bar as well.

Besides the identical "look and feel", the button functions are mostly identical to the respective buttons of the **Pyro Workbench**. To revisit the settings and calibration, the respective wizards of the **Pyro Workbench** are shown with greyed out and not configurable options.

6.2 Data Processing Steps

Every process step consists of 3 panels. The data source for the respective process step has to be selected on the first panel (see screenshot below). Depending on the process step, this could be either measured or already processed data. Additionally, certain requirements need to be matched. For the detailed constraints for each specific process step, refer to the respective chapter.

On the second panel, process specific settings can be entered. This panel also contains a down sampled graphical quick view of the expected results of the process step.

> The data is down sampled to 2000 values. The quick view results can therefore differ slightly compared to the later proper calculation, which contains all data points.

This panel varies from process step to process step and a screenshot can be found in the respective chapter.

By clicking on "Start" of the third and last panel, the calculation of the process step for all data of the data source is triggered. The progress can be monitored by the progress bars (see screenshot below). A setting change of a process step will trigger the recalculation of depending process steps automatically. For example: Measured oxygen values get smoothed. These values are used as data source for the calculation of respiration rates afterwards. When changing the settings of the smoothing process step, the depending calculation of the respiration rates is automatically triggered and performed. A list of depending and therefore to be recalculated process steps is always visible in the down left part of this panel.

6.2.1   Smoothing

Requirement(s):

- Only one source can be selected
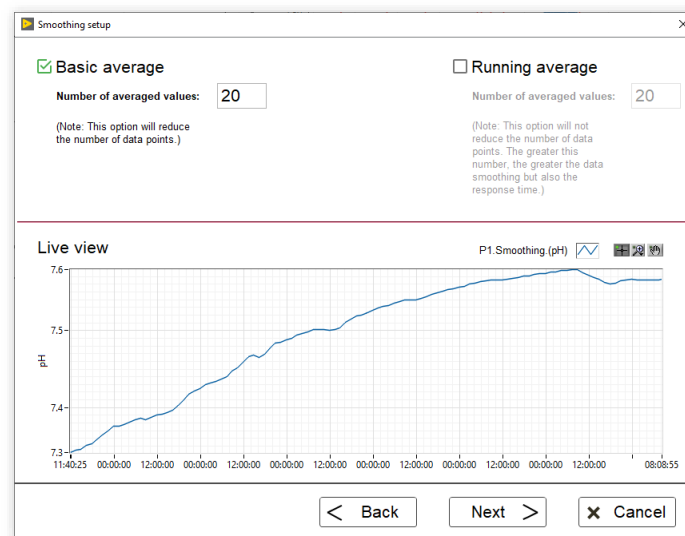
There are two smoothing options available. "Basic average" averages n values to one. Consequently, this option reduces the number of data points.
"Running average" calculates the average of the last n data points for every data point. This option does not reduce the number of data points, but leads to an increased response time. Keep in mind that for the first n-1 values less then n values are

considered and averaged and consequently the smoothed curve can be nosier at the beginning compared to the rest of the curve.

6.2.2   Linear Drift Compensation

Requirement(s):

• Only one source can be selected
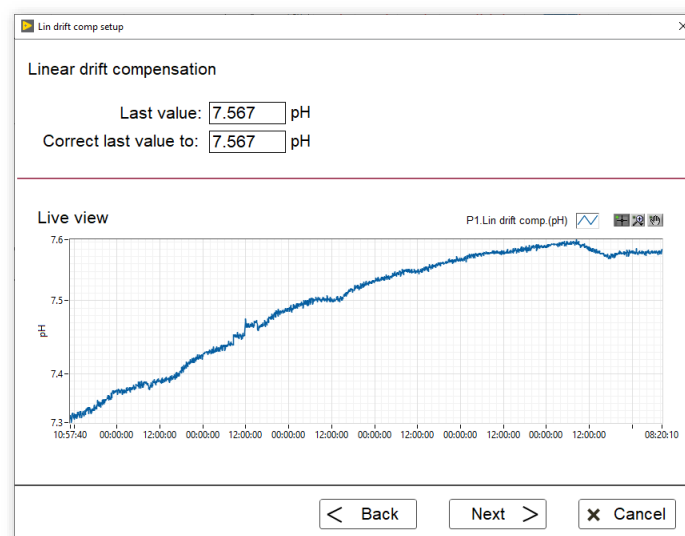
If an external reference value is known at the end of the experiment, this external value can be entered in "correct last value to" in the panel above. I linear increasing drift compensation, ranging from 0 at the beginning to the difference of measured and entered value at the end, will be performed.

6.2.3   Subtract Blank

Requirement(s):

• Two sources have to be selected

• The units of both data sources have to match

For some applications it might be beneficial to subtract a blank/ reference from a data source. On the panel above the blank/ reference has to be selected from the two chosen data sources from the panel before. The blank/reference will be subtracted from the other data source. To be more exact: Since both sources have unique timestamps and potentially different sample intervals, the blank/ reference data source is linearly interpolated. For every time stamp of the non-blank/ reference data source a value for the blank/ reference is calculated. This interpolated and calculated value is subtracted from the value of the non-blank/ reference data source.

6.2.4 Average Data

Requirement(s):

- At least two sources have to be selected

- The units of all data sources have to match
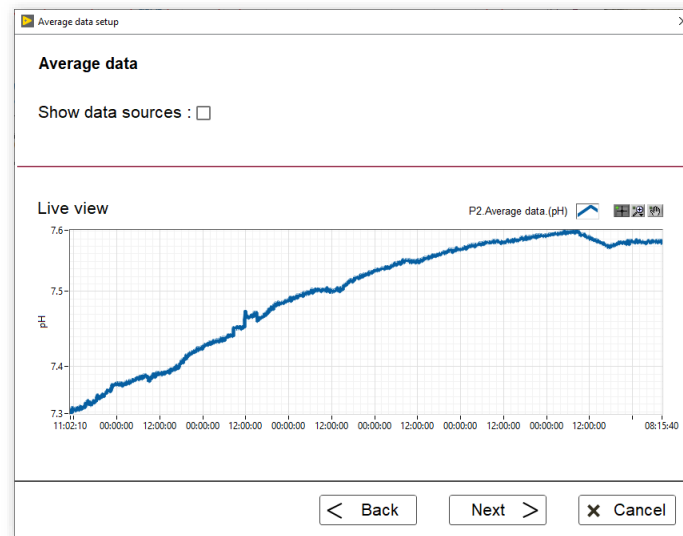
With this process step, n data sources can be averaged. The "show data source" option inserts plots of the previously selected data sources to the live view graph for better visualization. Information about the standard deviation is not accessible and lost after averaging.

6.2.5   Cut Data

Requirement(s):

• Only one source can be selected
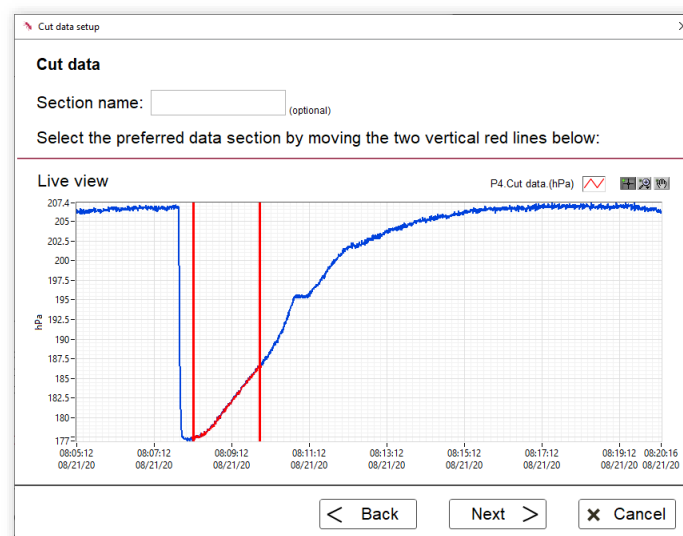
Certain parts of data sources can be selected and "cut" from the original data source and processed individually afterwards. The section can be named. The name will be displayed later in the header of exported data files.

6.2.6   pH Drift Compensation

Requirement(s):

•   Only one source can be selected

•   The data source has to be measured (and not already processed) pH data
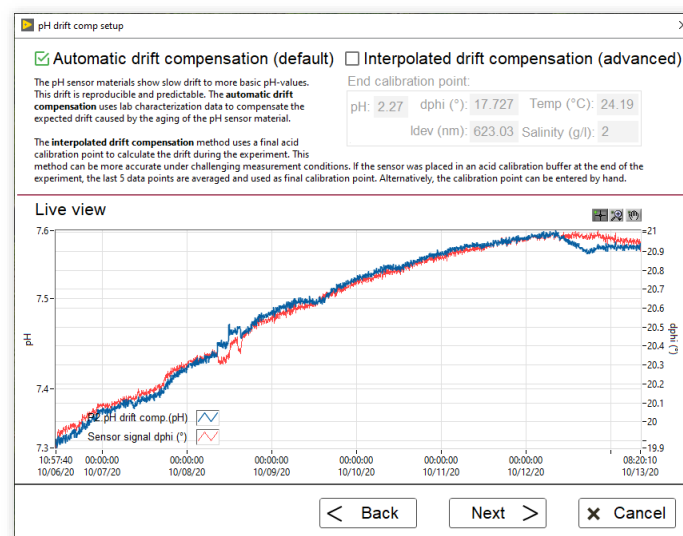
The pH sensor materials show slow drift to more basic pH-values over time. This drift is reproducible and predictable. The "Automatic drift compensation (default)" uses lab characterization data to compensate the expected drift caused by the aging of the pH sensor material. This option is recommended and requires no recalibration. In the example above a pH sensor drift of roughly 1/100th pH occurred at room temperature in one week.
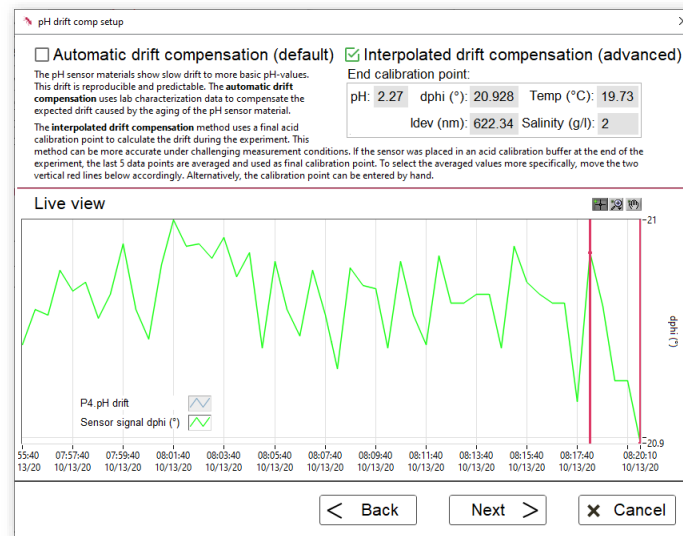
The "Interpolated drift compensation (advanced)" method uses a final acid calibration point to calculate the drift during the experiment. Meaning at the end of the experiment, the sensor had to be placed in the acid calibration buffer **WITHOUT** stopping the data logging.

Caution: If the sensor was not placed in the acid calibration buffer at the end of the experiment, this option will lead to meaningless pH data!

However, this method can be more accurate under challenging measurement conditions. The last 5 data points are automatically averaged and used as final calibration point. To select the averaged values more specifically and more meaningfully, move the two vertical red lines in the live view graph accordingly. Alternatively, the "End calibration point" can be entered by hand. For this put the sensor in acid calibration buffer and log data into a file. All values (except the pH) can be seen from the logger data file. For pH, enter the pH value of the buffer found on the label.

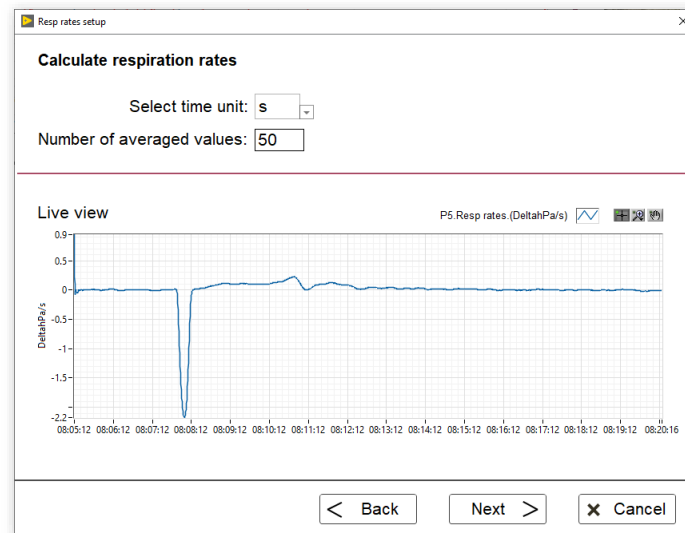### 6.2.7   Respiration Rates

Requirement(s):

- Only one source can be selected

- The data source can be measured or processed oxygen data

With this process step continues respiration rates can be calculated. In detail: For every time stamp of the fundamental oxygen data, a linear regression is calculated on the

bases of the prior n oxygen values. The number of used values can be modified by the user (see screenshot below). The greater the number, the smoother the respiration rate curve, but also the greater the response time. Therefore, for timely fast changing oxygen values, a small number of averaged values is recommended. For slowly changing oxygen data a greater number can be beneficial, since it leads to smoother respiration curves.

The slope of this linear regression of the prior n oxygen values is shown as delta oxygen over time in the live view graph. The user can select the preferred time unit for the respiration rate.

6.2.8 Linear Respiration Rates

Requirement(s):

- Only one source can be selected

- The data source can be measured or processed oxygen data

In contrast to the prior continuous respiration rates, single linear respiration rates can be calculated with this process step. For this, select your preferred data section of your oxygen data section by moving the two vertical lines in the live view graph. This selected section can be named and the preferred time unit can be selected. After releasing the curser, a linear regression is performed on the bases of the oxygen data between the two vertical red lines and is highlighted in the live view graph as third red line. The slope represents the shown "preliminary respiration rate".
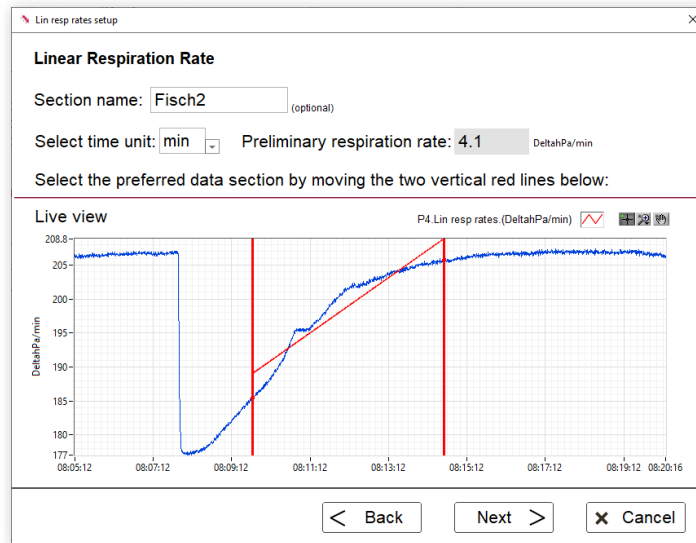
Since the live view graph may be down sampled to 2000 values, due to performance reasons, the final respiration rate is calculated on the later panel.

6.2.9 Change Oxygen Unit

Requirement(s):

- Only one source can be selected

- The data source has to be measured (and not already processed) oxygen data

It is possible to change the oxygen unit after an experiment with this process step. There are different sets of units available, depending on the medium the experiment was performed in. For example, you cannot choose a unit, which is only valid in liquids (e.g. %air saturation), if the measurement was performed i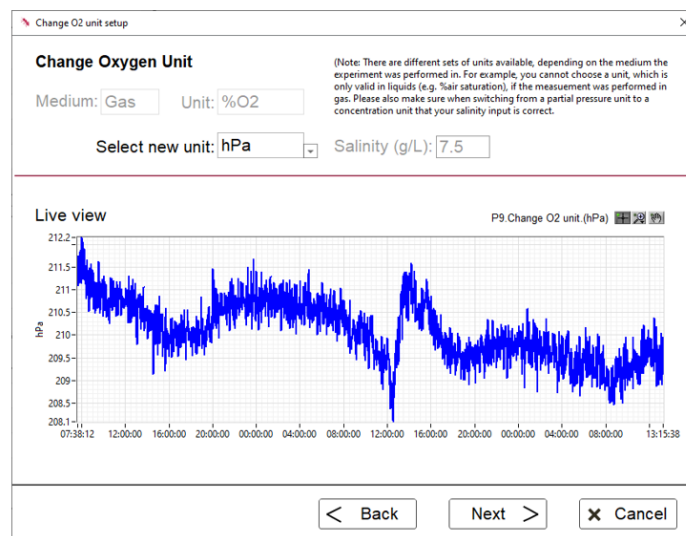n gas. Please also make sure when switching from a partial pressure unit to a concentration unit that you put in the correct salinity and your liquid medium was water and not e.g. solvent-based.

6.2.10   Recalculate oxygen

Requirement(s):

• Only one source can be selected

• The data source has to be measured (and not already processed) oxygen data
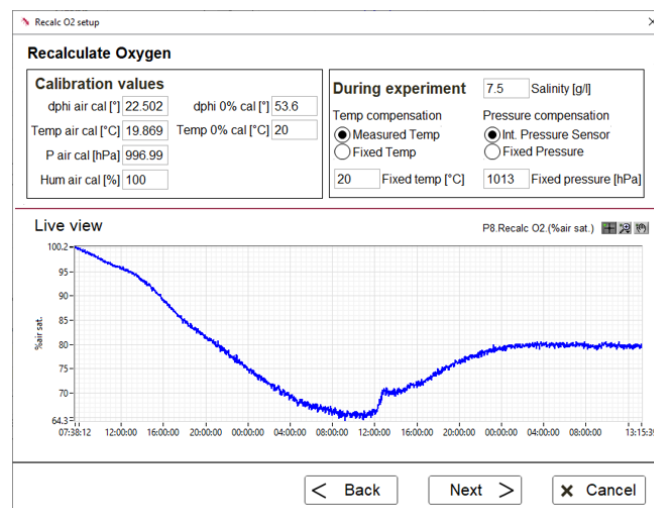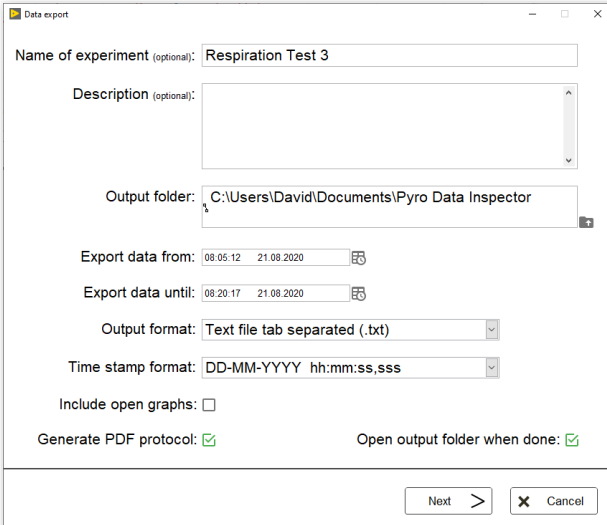
The measured oxygen units can be recalculated after the experiment with this process step. The calibration values, the salinity, the temperature and pressure compensation can be adjusted. Subsequently, the raw data is used again to calculate the selected oxygen unit using the adjusted parameters. For example, the calibration values of a recalibration of the oxygen sensor after the performed experiment can be used for a recalculation. Only the oxygen unit which was selected in the settings prior to the experiment can be recalculated.

6.3   Data export

There are two panels for exporting data into standard file formats. On the first panel of the data export, the experiment name and description can be entered. Since the data export will contain multiple files, a respective output folder can be chosen. To select

only a preferred data section, the start and end time can be modified. The default values are always the first and last time stamp of all data sources. There are several standard file formats as well as time stamp formats available to choose from. With the option "Include open graphs" the currently opened graphs will be exported as single *.png files. To add an automatically generated PDF protocol of the experiment to the output folder, select the respective option. The protocol contains information about all basic information of this experiment, such as start, end time, name, utilized devices, performed process steps, currently open graphs und the added comments during the experiment.

After confirming the options by clicking "next", the data selection panel will be visible (see screenshot below). To save disc space, only the desired data sources for export can be selected. For this, uncheck data which should not be exported. Additionally, the data can be discreetly down sampled to facilitate the later data processing in the case of a great data amount. Per default (100%), all data points will be exported. By choosing for example 50% only every second value will be exported.
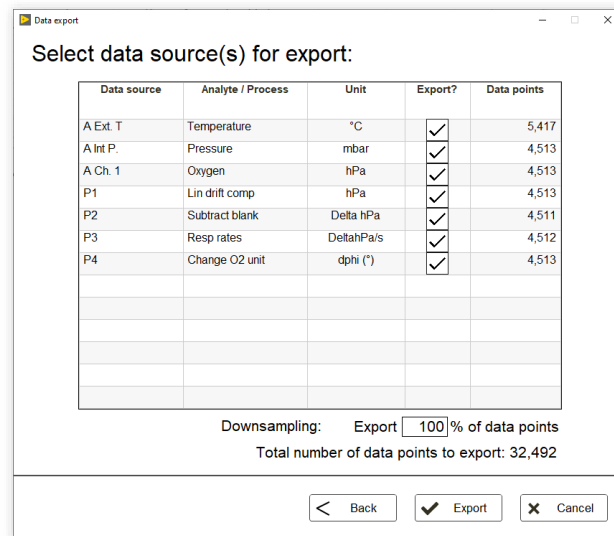
The output folder will contain single subfolder for every selected data source and a combined data file, which contains all selected data.

Note: For a great amount of data points, the export can take up to several minutes. Please do not interrupt the export by closing the software.

7   SPECIAL APPLICATIONS

7.1   Luminescence Background Compensation

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

When using fiber robs with a length <1m, please insert 0.1m as fiber length in the settings.

For precision applications and especially for low signal intensities or for applications utilizing oxygen nanoprobes (item no.: **OXNANO**), a **manual background compensation** should be performed by the user.

For this, instead of clicking **Next** when asked to select the calibration temperature in the calibration wizard, click on **Background compensation** in the lower left corner. If that button is not visible, a background compensation is not supported for the applied sensor type.
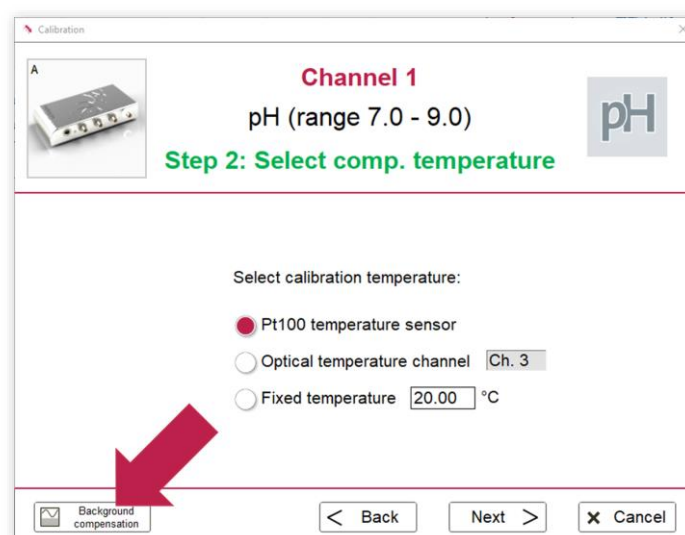
For **sensor spots**, **flow-through cells** and **respiration vials**, please select **Manual** if the luminescence background compensation method is required.
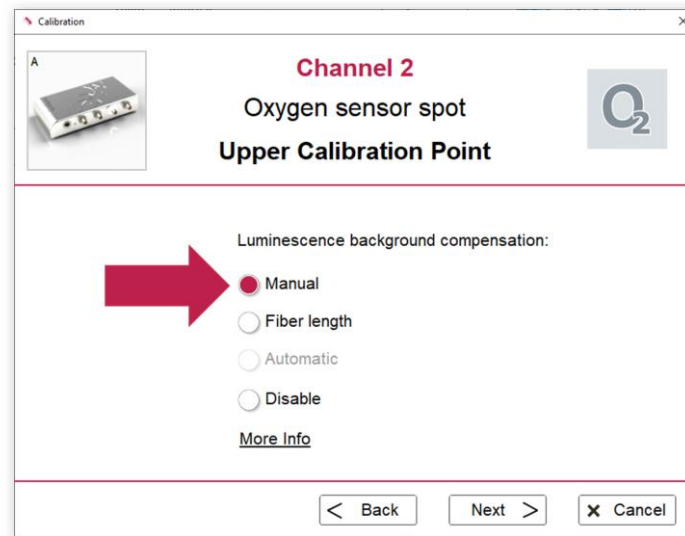
After selecting **Manual** and clicking **Next**, a manual background compensation can be performed:
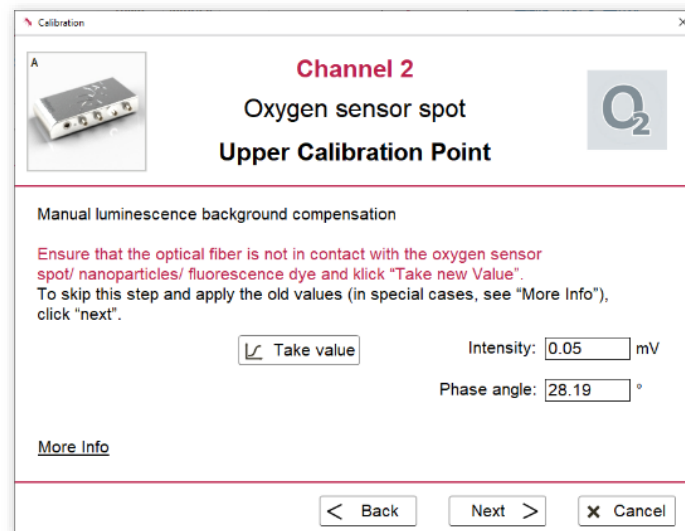
Here, the background fluorescence of the connected optical fiber or of the medium used for the measurements (in case of oxygen nanoprobes), can be compensated. For this it is important that:

- one end of the optical fiber is connected to the corresponding channel of the device and

- the other end of the optical fiber is not attached to the sensor spot, flow-through cell, respiration vial or oxygen nanoprobes (i.e. disconnect this end from the spot adapter, adapter ring or from the flow-through cell)
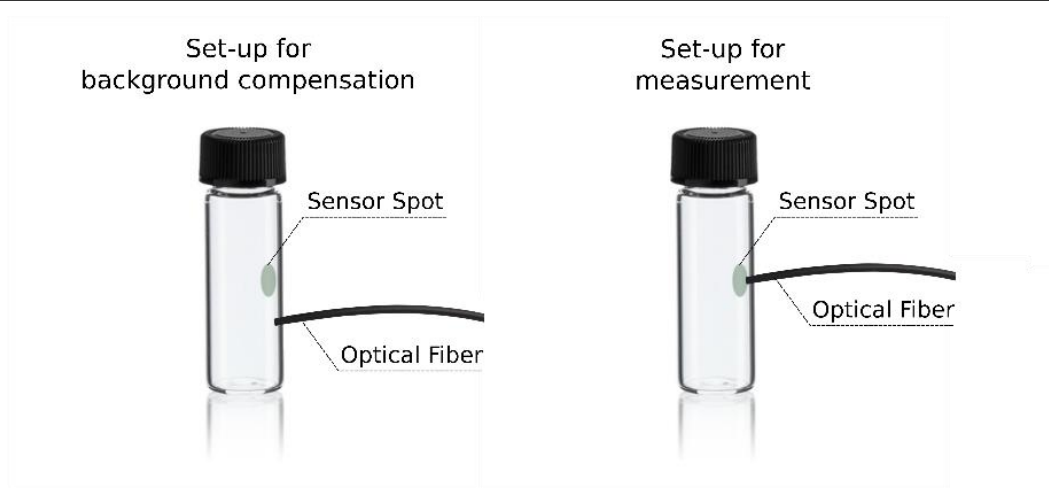
For the **manual luminescence background compensation**, wait for steady-state and press the button **Take value**.

Alternatively, click **Next** without taking a new value. Then the last values for the background compensation are kept.

After that the program proceeds with the main calibration window. For the subsequent calibration process, it is important that the optical fiber is reattached to the sensor spot, flow-through cell, respiration vial or oxygen nanoprobes, e.g. by reconnecting this optical fiber end to the spot adapter, adapter ring or to the flow-through cell.

> Please ensure that during background compensation the optical fiber is not connected to the contactless sensor.
>
> Please ensure that during the subsequent calibration process the optical fiber is reattached to the contactless sensor.
>
> Remind that the position of the spot adapter or adapter ring must not be changed after calibration of the sensor spot; otherwise, the sensor has to be calibrated again.

7.2   Save & Load Setup

It is possible to switch between different sensor setups with a single device without entering new settings and performing new calibrations each time. For this, use **Load/Save instrument configuration** for the respective device in the **Instrument** drop-down menu of the top menu.
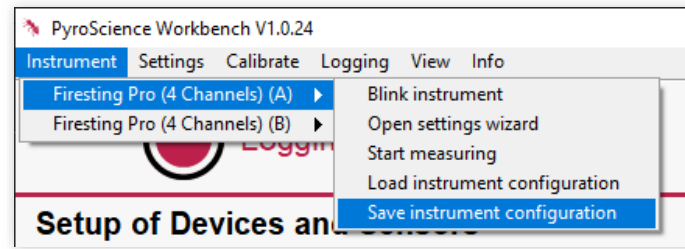
This tool can be used to save the current settings and calibration data of all channels. They can be reloaded anytime by pressing the button **Load Instrument Configuration**. This allows e.g. to switch between different laboratory setups with a single **FireSting-PRO** or **Pico** device.

This function might also be 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 instrument configuration**. By transferring this file and also the measurement device to a second computer, you can reload this configuration with **Load instrument configuration**, giving you calibrated sensors ready for the measurement.

> **Note:** For this procedure the identical software version of the **Pyro Workbench** must be installed on both computers.

7.3   Copy Settings
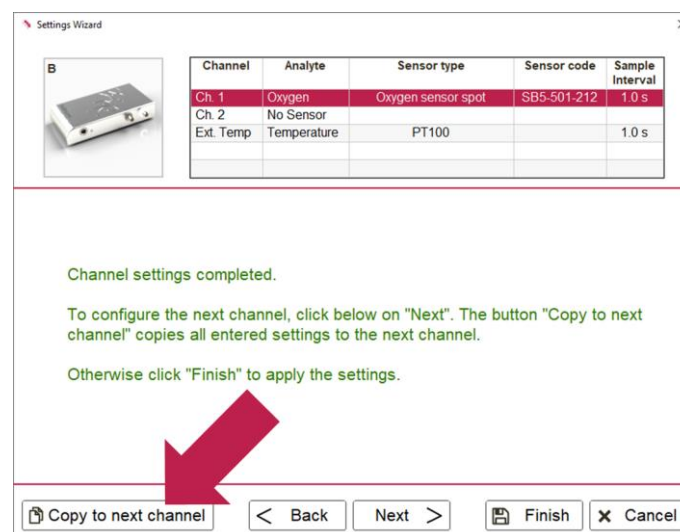
If the same sensor type (with the **same Sensor Code**) is connected to all channels (e.g. 4 sensor spots from the same packaging unit), the entered settings can be copied to all other channels by clicking repeatedly on **Copy to next channel**. The button **Copy to next channel** appears not until the settings of all channels have been completed.

7.4   High-Speed Sampling

For some advanced applications, it is crucial to measure with a high sample rate, e.g. using ultra-high speed oxygen sensors. In order to achieve this:
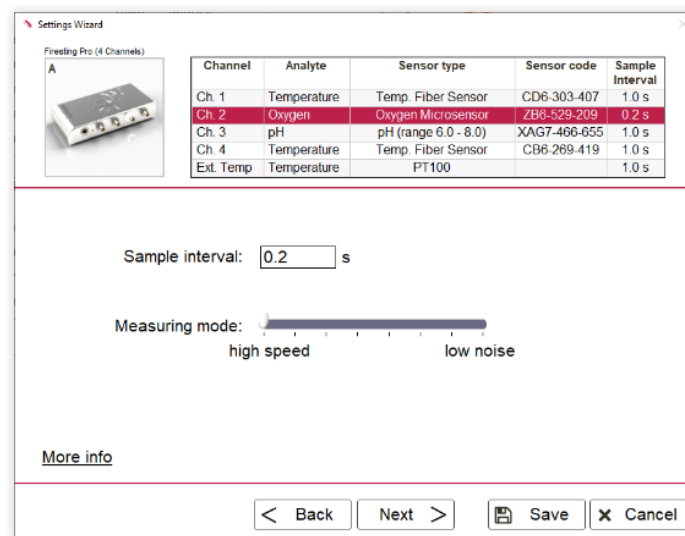
Set the **sample interval** to 0.1 seconds.

Set the **measuring mode** all the way to the left to "**low drift**".

All other optical channels of this device must be set to "No sensor" in their respective sensor settings.

8   WARNINGS & ALARM WINDOW

There are several warnings and errors that can occur. They can strongly affect the quality of the measurement or prohibit a reasonable measurement at all. The overview of all current errors and warnings is accessible via the top menu: **View → Alarm Window** or by clicking on the blinking warning triangle in the upper left corner of the main window. If a new error appears, the alarm window will also open automatically.

> **Important:** Do not continue with the measurements if an error is shown. Please check the sensor and the setup.
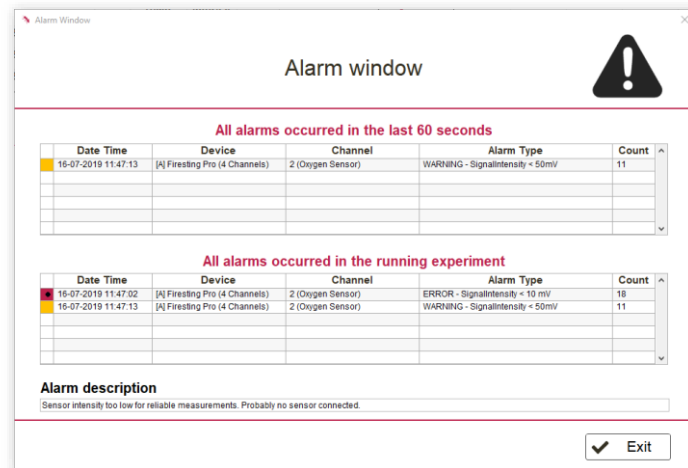
Entries are divided into warnings and errors. If a warning occurs, the effect on the measurement is typically minor. Consequently, the measurement continues and measurements are still possible. Errors have a strong impact on the data and hence measurements are not possible anymore.

> The presence of a **warning** or an **error** is indicated by a blinking warning triangle in the upper left corner of the **Pyro Workbench** main window. If a new **error** occurs, the Alarm Window will pop up additionally.

Please refer to the following two tables for an overview of all possible warnings and errors:

Warning message	Description	What to do?
Reference too low (Bit 3)	Reference signal intensity low (<20mV). Increased noise in optical sensor reading.	Contact info@pyroscience.com for support.
High humidity (Bit 7)	High humidity (>90%RH). The device can get permanently damaged in condensing conditions.	Reduce humidity in the environment of the device.
Signal intensity <50 mV	Sensor intensity low. Elevated noise in sensor readings.	For contactless sensors: check the connection between fiber and sensor. Alternatively, change the LED intensity under advanced settings. **Important:** this requires a new sensor calibration.

Error message	Description	What to do?
Optical detector saturated (Bit 2)	Detector of the device is saturated due to too much ambient light.	Reduction of ambient light (e.g. lamp, sunlight) recommended. Alternatively, change the second letter of the sensor code to the prior letter of the alphabet. This decreases the LED intensity. **Important:** this requires a new sensor calibration!
Reference too high (Bit 4)	Reference signal too high (>2400mV). This can have a strong negative effect on the accuracy of the sensor reading.	Contact info@pyroscience.com for support.
Failure of sample temp. sensor (Bit 5)	Failure of sample temperature sensor (Pt100).	Connect a Pt100 temperature sensor to the Pt100 connector. If a sensor is already connected, the sensor might be broken and needs to be replaced.
Case temp. sensor (Bit 8)	Failure of case temperature sensor.	Contact info@pyroscience.com for support.
Pressure sensor (Bit 9)	Failure of pressure sensor.	Contact info@pyroscience.com for support.
Humidity sensor (Bit 10)	Failure of humidity sensor.	Contact info@pyroscience.com for support.
Signal Intensity <10 mV	Sensor intensity too low for reliable measurements. Probably no sensor connected.	Check whether a sensor is connected. If yes: check the connection between fiber and sensor. Alternatively, change the second letter of the sensor code to the next letter of the alphabet. This increases the LED intensity. **Important:** this requires a new sensor calibration!

9   WARNINGS & SAFETY GUIDELINES

In case of problems or damage, disconnect the device 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!**

Follow appropriate laws and guidelines for safety in the laboratory, 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 and of **PyroScience** buffer capsules.

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!

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

The device and the sensors must be used in the laboratory by qualified personnel only, following the user instructions and the safety guidelines of the manual.

Keep the sensors and the device out of reach of children!

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