



OOSensors Fiber Optic Sensors System

Installation and Operation Manual

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About This Manual

Document Purpose and Intended Audience

This document provides you with an installation and configuration instructions to get your system up and running. In addition, user interface for SpectraSuite is provided.

What's New in this Document

This version adds information concerning the MFPP unit, and FOSPOR and HIOXY probes.

Document Summary

Chapter	Description
Chapter 1: Sensors System Overview	Contains descriptions of what is included in a sensor system.
Chapter 2: OOISensors Software	Contains instructions for installing and configuring the OOISensors software.
Chapter 3: Oxygen Sensors	Provides information on how the sensors work, as well as instructions for installing and calibrating the sensors.
Chapter 4: pH Sensors	Provides a description of the pH sensors and a procedure for calibration.
Chapter 5: Compatible Products and Accessories	Contains descriptions of compatible accessories and their specifications.
Chapter 6: Hardware Datasheets and Instructions	Contains descriptions of the functions on the Spectrometer Menu.
Chapter 7: Troubleshooting	Contains a table of troubleshooting suggestions and
Appendix A: External Triggering	Provides information on external triggering as it relates to sensors.
Appendix B: Chemical Effects on Oxygen Probes	Lists chemicals known to be harmful and chemicals that have no effect on oxygen probes.

Chapter	Description
Appendix C: pH Sensor Theory of Operation and Calculations	Contains equations that express the theory of pH sensor operation and calculations.

Product-Related Documentation

- *MultiFrequency Phase Fluorometer Installation and Operation Manual*
- Various USB spectrometer documents such as the *USB4000 Spectrometer Installation and Operation Manual*.
- *SpectraSuite Spectrometer Operating Software Installation and Operation Manual*
- *OOIBase32 Spectrometer Operating Software Installation and Operation Manual*

You can access documentation for Ocean Optics products by visiting our website at <http://www.oceanoptics.com>. Select *Technical* → *Operating Instructions*, then choose the appropriate document from the available drop-down lists. Or, use the **Search by Model Number** field at the bottom of the web page.

You can also access operating instructions for Ocean Optics products on the *Software and Technical Resources* CD included with the system.

Engineering-level documentation is located on our website at *Technical* → *Engineering Docs*.

Upgrades

Occasionally, you may find that you need Ocean Optics to make a change or an upgrade to your system. To facilitate these changes, you must first contact Customer Support and obtain a Return Merchandise Authorization (RMA) number. Please contact Ocean Optics for specific instructions when returning a product.

Sensors System Overview

Overview

The following sections provide a general overview of the Oxygen Sensors System and the pH Sensors System.

Oxygen Sensors System Overview

Fiber Optic Oxygen Sensors are spectrometer and/or phase fluorometer-coupled chemical sensors for monitoring oxygen partial pressure in gases and liquids. A fluorescence method measures the partial pressure of dissolved or gaseous oxygen. Optical fiber carries excitation light produced by the blue LED to the thin-film coating at the probe tip. The probe collects fluorescence generated at the tip and carries it via the optical fiber to the high-sensitivity spectrometer. When oxygen in the gas or liquid sample diffuses into the thin film coating, it quenches the fluorescence. The degree of quenching correlates to the level of oxygen pressure.

Fiber Optic Oxygen Sensors are low-power, portable devices that offer high sensitivity, reversibility, and stability. Their small size is useful for remote monitoring. What's more, the thin film used in the probe tips consumes no oxygen, allowing for continuous contact with the sample. FOXY Sensors offer other key advantages: They're ideal for viscous samples and are immune to interference caused by pH change or from changes in ionic strength, salinity, and biofouling.

pH Sensors System Overview

Fiber optic probes for pH monitoring couple to Ocean Optics spectrometers to measure pH by monitoring color changes in indicator dyes. These dyes are immobilized in polymer films. You then place the films in the optical path of a transfection dip probe and exposed to the sample solution. These pH probe sensors are especially useful for monitoring low conductivity samples such as boiler water, where potentiometric devices fail; or for turbid, fouling environments, where particulate matter, slurries, and other media can coat or destroy electrodes.

Ocean Optics' fully integrated pH systems provide full spectral analysis to help eliminate errors from dye leaching or from changes in turbidity, temperature, and ionic strength. Inherent calibration based on the physical properties of the immobilized indicator dye eliminates the need for frequent calibration.

FOXY Probes

FOXY oxygen sensors are designed for monitoring oxygen partial pressure in gas and aqueous solutions. Standard FOXY comes with different probe sizes and configurations depending on the application.

FOSPOR Probes

FOSPOR is a new generation of highly sensitive probes for monitoring traces of oxygen in gas and liquids. This sensor is capable of monitoring low ppm levels of oxygen in gas and ppb levels of dissolved oxygen in liquids. This sensor can be used in environments where oxygen traces need to be monitored. Some applications include vacuum systems, and food and pharmaceutical packaging. The sensor material consists of a sol gel thin film immobilized with Pt-porphyrin.

HIOXY Probes

HIOXY (FOXY-hydrocarbon) is designed for monitoring oxygen partial pressure in nonaqueous vapors and solutions. The sensor coating chemistry is designed for compatibility with oils, alcohols and hydrocarbon-based vapors and liquids. HIOXY has been tested successfully in commercial and military aviation fuels, gasoline, diesel, some alcohols, glycol, military hydraulic fluids, and wine.

MultiFrequency Phase Fluorometer (MFPF)

The MultiFrequency Phase Fluorometer (MFPF) is a flexible platform for measuring luminescence lifetime, phase and intensity. The MFPF is a compact, self-contained, frequency-domain luminescence monitor that is invariant to fiber bending and stray light, and has a wide dynamic range of optical intensity as well as low optical and electronic crosstalk, and low drift and phase noise. Therefore, the MFPF is especially useful for oxygen sensing applications where sensitivity to drift is important and where sample set-ups must be left undisturbed for long periods of time. The MFPF is available for use with FOXY, HIOXY, and FOSPOR custom probes. See the *MultiFrequency Phase Fluorometer Installation and Operation Manual* for more information on the MFPF.

The MFPF software works in conjunction with OOISensors software to manage the MFPF unit. If your system uses a spectrometer instead, you will not install or use the MFPF software. See [Installing OOISensors](#) for more information on installing software.

The application for measuring oxygen concentrations is essentially the same for spectrometers and MFPF. The main difference is that the spectrometers' application is with raw data of spectrum intensity of fluorescence at a particular wavelength, while the MFPF's application raw data is Tau, which is an indication of the decay of fluorescence over time as the result of a light pulse on sensor material.

Hardware

The following hardware is included with the Fiber Optic Sensors System or is compatible with the Fiber Optic Sensors System.

Included Hardware (Oxygen Sensor System)

- ❑ Fiber optic fluorescence probe with proprietary oxygen-sensing thin-film coating on the tip
- ❑ Miniature fiber optic spectrometer
- ❑ LS-450 Blue LED Light Source
- ❑ Bifurcated optical fiber assembly with splice bushing that connects the fluorescence probe to the spectrometer and the LED
- ❑ A/D Converter (if needed)

Included Hardware (pH Sensor System)

- ❑ pH probe & film
- ❑ Light Source (such as LS-1 or Mini-D2T)
- ❑ Miniature fiber optic spectrometer (see [Spectrometers](#) for options)
- ❑ A/D Converter (if needed)

See [Compatible Products and Accessories](#) for information on these and other Ocean Optics products designed for use with the Fiber Optic Sensor System.

Caution

Do not connect the spectrometer included with the Fiber Optics Sensors System to the PC prior to installing the OOISensors software. Connecting this equipment prior to software installation will result in incorrect driver configuration and may require Technical Support to correct. See [Troubleshooting](#) for more information.

Software

OOISensors software is Ocean Optics' next generation of software for use with the Fiber Optics Sensor System. OOISensors is an advanced data acquisition and display program that provides a real-time interface to a variety of signal processing functions for users of Windows 95/98/ME/NT/2000/XP.

MFPP software is used with OOISensors when you have an MFPP unit. See [MultiFrequency Phase Fluorometer \(MFPP\)](#) for more information on the MFPP.

Documentation

The Fiber Optic Sensors System contains various documentation components on paper, floppy diskette and CD. These components include:

Packing List

The packing list is located inside a plastic bag attached to the outside of the shipment box (the invoice is mailed separately). The items listed on the packing slip include all of the components in the order, including customized items installed in the spectrometer, such as the grating, detector collection lens, and slit. The packing list also includes important information, such as the shipping and billing addresses, as well as components on back order.

Wavelength Calibration Data

The spectrometer included with the Fiber Optic Sensors System is shipped in a silver-gray anti-static bag. This bag also contains wavelength calibration information that is unique to your individual spectrometer. Our spectrometer operating software simply reads these values from the spectrometer; however, this information is provided in case, for some reason, you need to manually enter these values into the software.

Software and Technical Resources CD

Each spectrometer order comes with Ocean Optics' *Software and Technical Resources* CD. This disc contains all Ocean Optics software and manuals for software operation, spectrometers, and spectroscopic accessories. (Your OOISensors software and documentation might be included on a separate CD labeled Custom Software.) Documentation is provided in Portable Document Format (PDF). You need Adobe Acrobat Reader version 6.0 or higher to view these files. Adobe Acrobat Reader is included on the CD.

With the exception of OOIBase32 Spectrometer Operating Software, all Ocean Optics software is password protected. OOISensors Software must be purchased with your Fiber Optic Sensors System, and the installation program is password protected. The password for the software can be found on the back of the *Software and Technical Resources* CD package.

Temperature Calibration Diskette (if requested)

The Temperature Calibration Diskette can be used during temperature calibration setup. Insert the diskette in the disk drive, and then load the data via the Multiple Temperature Calibration Screen.

OOISensors Software

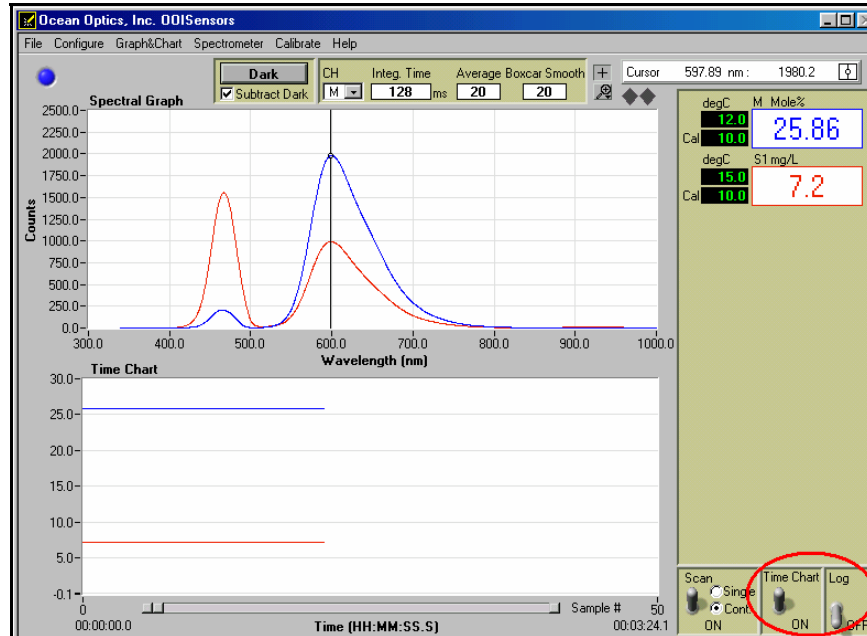
OOISensors Overview

OOISensors Software is Ocean Optics' next generation of operating software for the Fiber Optic Sensors System. OOISensors is an advanced acquisition and display program that provides a real-time interface to a variety of signal-processing functions for all Windows platforms. With OOISensors, you have the ability to obtain oxygen partial pressure and concentration values, control all system parameters, collect data from up to 8 spectrometer channels simultaneously and display the results in a single spectral window, perform time acquisition experiments, and display and correct for temperature and/or ambient pressure fluctuations in the sample.

OOISensors allows you to use the Second Order Polynomial algorithm in the calibration procedure. This algorithm often provides more accurate data than the linear Stern-Volmer algorithm. Furthermore, you can now monitor temperature with the OOISensors application. OOISensors corrects the data for any fluctuations in temperature.

OOISensors can also display up to eight spectrometer channels in one spectral window, while providing unique data acquisition parameters to each spectrometer channel.

Additionally, the Time Chart feature in OOISensors can display O₂ values, pH values, and the data from all active channels at a specific wavelength over a period. During a timed data acquisition procedure, you can enter text for an event into the log file. Both the Time Chart and Data Logging features are enabled by a simple switch next to the graph.



Installing OOISensors

Before installing OOISensors Software, make sure that no other applications are running.

► Procedure

Perform the steps below to install the OOISensors software on your PC:

1. Close all other applications running on the PC.
2. Start the software installation process.

Installing from CD:

- a. Insert the CD containing the OOISensors and software.
- b. Select the drive on your computer with the software CD.
- c. Double-click on the Setup.exe. The installation wizard appears.

Installing from the Web:

- a. Go to <http://www.oceanoptics.com/technical/softwaredownloads.asp>.
 - b. Right-click on OOISensors Oxygen Measurement Software and select Save Target As... to download the executable to your machine.
 - c. Double-click on the downloaded file. The installation wizard appears.
3. Click the **Next** button at the **Welcome** screen. The **Choose Destination Location** screen appears.

4. Select a location for your software files. Click **Next**. The **Backup Replaced Files** screen appears.
5. Select **Yes** or **No**, depending on whether you want to create backup replacement files. If you select **Yes**, choose a location for these files. Then click **Next**. The **Select Program Manager Group** screen appears.
6. Select the name of the Program Manager group to which you want to add the OOISensor icons. Then click **Next**. The **Start Installation** dialog box appears.
7. Click **Next**. The **Installation Password** dialog box appears. Enter the password for your OOISensors software. Passwords are located on the back of the software CD jacket. The software begins installing.
8. The MFPP dialog box appears asking you if you have MFPP hardware.



Note

If you have an MFPP unit, refer to the *MultiFrequency Phase Fluorometer Installation and Operation Manual* for more information. These instructions assume that you are installing OOISensors to use with a spectrometer.

9. Click **No**. The installation begins. A message then appears informing you that your installation is complete.
10. Click **OK**, then click **Finish**. You have now installed both OOISensors software. You must restart your computer to use the software.

Configuring OOISensors Software

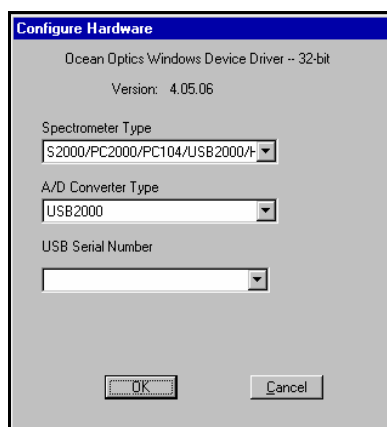
Once OOISensors is installed and the system has been rebooted, connect the spectrometer to the PC and start the OOISensors program.

The first time that you run OOISensors, you will be prompted to configure the software for use with your hardware.

► Procedure

Follow the steps below to configure OOISensors:

1. Run OOISensors. If this is the first time you have opened the software, or if the software is not configured yet, the **Configure Hardware** screen appears. You can also access this screen manually by selecting **Configure | Hardware** from the OOISensors menu bar.



2. Click on the **Spectrometer Type** drop-down menu and select the type of spectrometer you received with your Fiber Optic Sensors System.

Note

The S2000-FL, SF2000, and USB series are S2000-series spectrometers.

3. Click on the **A/D Converter Type** drop-down menu and select the type of A/D converter you are using to interface the spectrometer to the PC.
4. Enter the parameters for the A/D Converter you selected in Step 3, if applicable.

For ADC1000 and DAQ700 A/D Converters

Enter the **Base Address** and **IRQ** for the A/D Converter. Consult your A/D Converter documentation for more information.

For ADC1000-USB A/D Converters

Specify the serial number of the ADC1000-USB, or select the First Available ADC1000-USB option. Consult your A/D Converter documentation for more information.

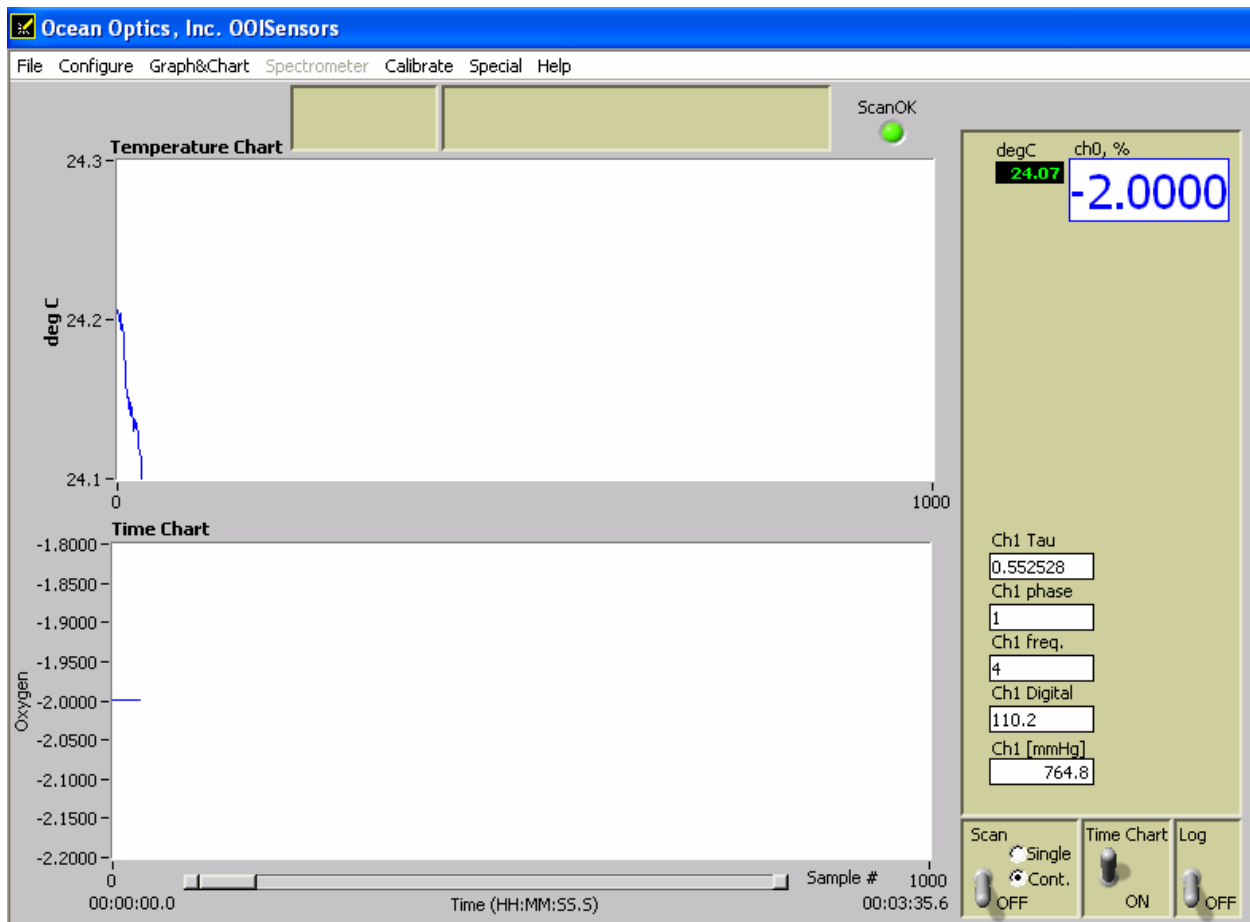
For SAD500 and Serial USB2000 A/D Converters

- a. Enter the **COM port** (serial port) to which the A/D Converter is connected.
- b. Enter the **Baud Rate** (speed) that the A/D Converter will operate.
- c. Enter the **SAD500 Pixel Resolution**, which specifies that the spectrometer will transfer every Nth pixel to the PC. This option does not appear for Serial USB2000 users.
- d. Enable the **Compressed Data** function to minimize the amount of data transferred over the serial connection.

For USB2000 A/D Converters

Click on the **USB Serial Number** drop-down menu and select the serial number of the USB2000 spectrometer you will be using.

The main OOISensors screen is shown below (the following example is for the MFPP configuration):



Configure Menu Functions

Select **Configure** from the menu bar to configure the hardware and software parameters for your system.

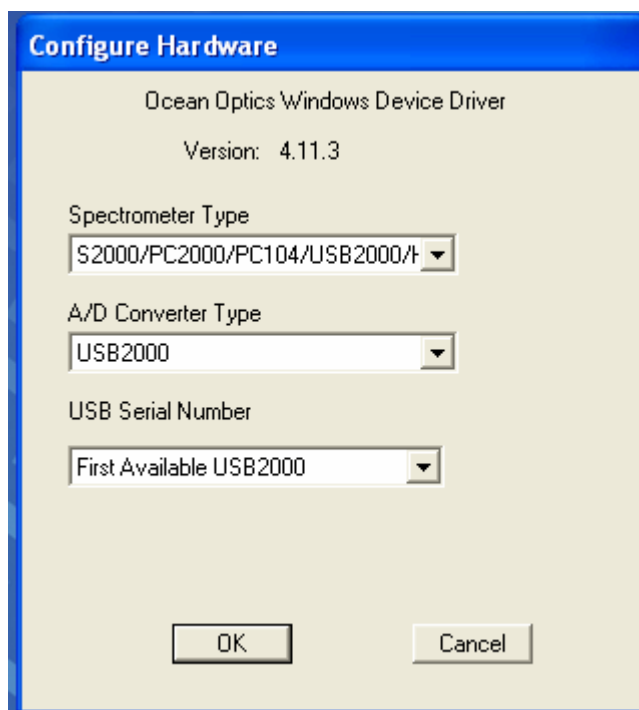
Hardware

The **Configure | Hardware** dialog box allows you to specify the hardware parameters for the spectrometer. Typically, you set these parameters only once, when you first install OOISensors. Upon running OOISensors for the first time after installation, you will need to specify hardware settings in the **Configure Hardware** dialog box. However, you can reconfigure these settings at any time by selecting **Configure | Hardware** from the menu bar.

► Procedure

Follow the steps below to reconfigure hardware parameters:

1. Select **Configure | Hardware** from the OOISensors menu bar. The **Configure Hardware** screen appears.



2. Specify a spectrometer type in the **Spectrometer Type** drop-down menu (the S2000-FL, SF2000 and USB series are S2000-series spectrometers).
3. Specify the A/D converter you are using to interface your spectrometer to your computer in the **A/D Converter Type** drop-down menu.

If you choose the USB2000 A/D Converter:

Select the USB2000 A/D Converter from the drop-down menu. Then, specify the serial number of the USB2000 in the **Serial Number** drop-down menu. Consult the USB2000 Operating Instructions for more information.

If you choose the ADC1000 or DAQ700 A/D Converter:

Select an available Base Address (or I/O Range) and IRQ (Interrupt Request) from the drop down menus. Consult the operating instructions for the specific A/D converter more information.

If you choose the ADC1000-USB A/D Converter:

Specify the serial number of the ADC1000-USB, or select the First Available ADC1000-USB option. Consult your A/D Converter documentation for more information.

If you choose the SAD500 or Serial USB2000 Spectrometer:

4. Specify the PC **Serial Port** (or COM Port) number to which the device is connected.
5. Select the **Baud Rate** at which the device will operate.
6. Enter a **SAD500 Pixel Resolution**, which specifies that the spectrometer transmit every nth pixel from the SAD500 to the PC. Valid pixel resolutions are 1-500, and resolution requirements vary by experiment.

Note

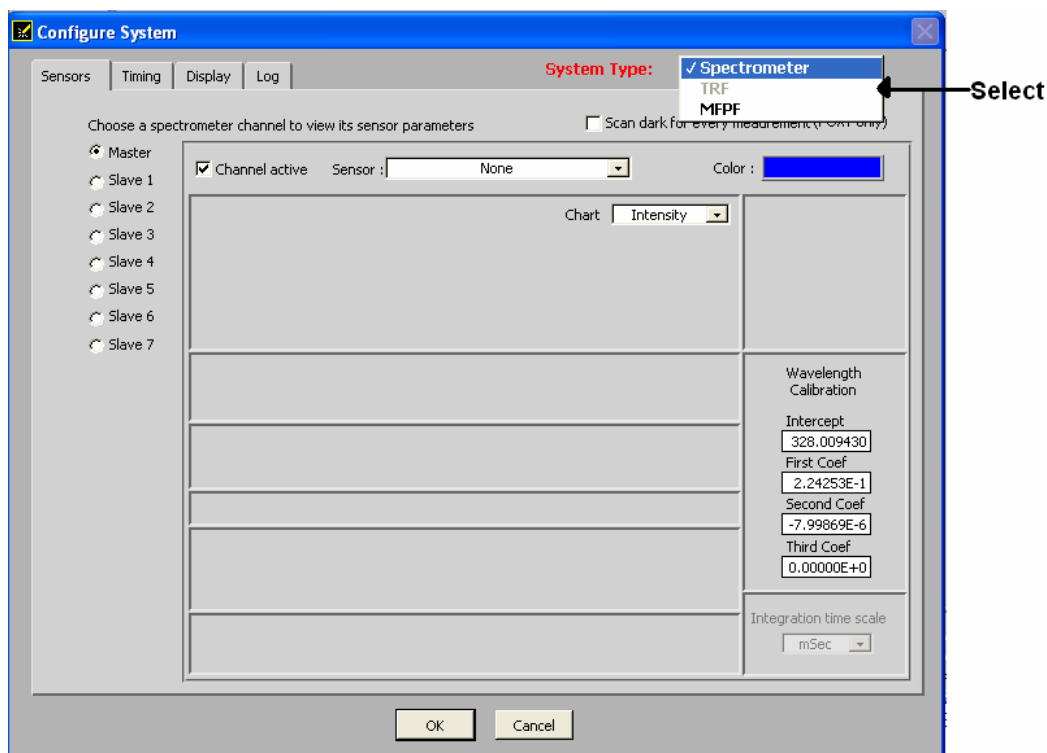
Lower pixel resolutions result in increased speed. The transfer of one complete spectra requires ~0.4 seconds when communicating at 115,200 baud rate. If you need your information in <0.4 seconds, increase the resolution or enable data compression. (This option does not appear for Serial USB2000 users.)

7. Enable the Compress Data function to minimize the amount of data transferred over the RS-232 connection. Transmission of spectral data over the serial port is a relatively slow process. Enabling this function insures that every scan transmitted will be compressed, which greatly increases the data transfer speed.
8. Select the USB2000 Serial Number for the USB2000 you wish to use (Serial USB2000 users only).

Software

Select **Configure | Software** to specify the spectrometer parameters in the OOISensors software.

To access the **Configure System** screen, choose **Configure | Software** from the OOISensors menu bar. The **Configure System** screen contains tabs for specifying Sensor, Timing, Display, and Log options.



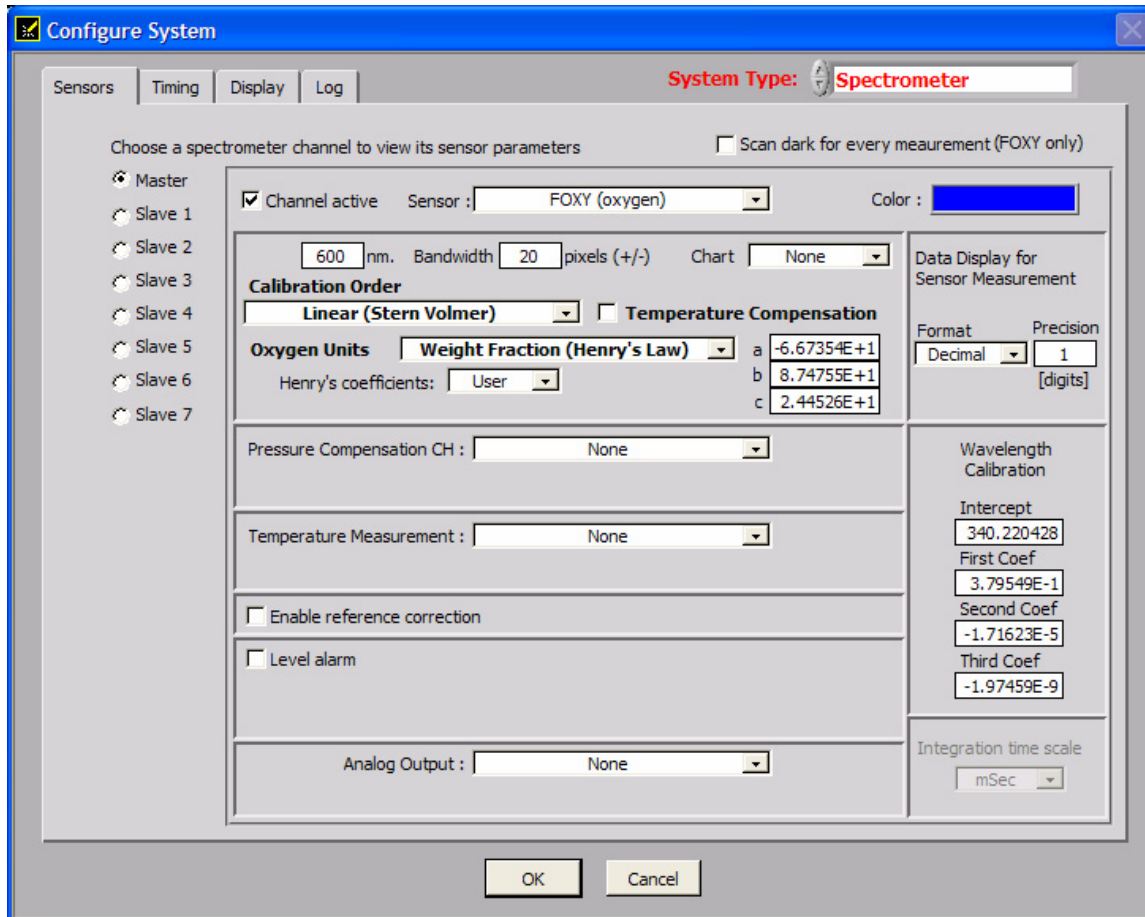
The **System Type** field in the upper, right corner allows you to select either Spectrometer or MFPF for the attached hardware. The differences between the two system types when using OOISensors are noted where applicable throughout this manual. Depending on this selection, some tabs and other user interface objects will be visible (or not) in main application window and in this configuration window.


Sensors Tab for Spectrometer Systems

Click on the Sensors tab in the **Configure System** screen to access Sensor controls. The Sensors tab contains the following options:


- None
- [FOXY \(oxygen\)](#)
- [pH](#)
- [Pressure](#)
- [CO2](#)

FOXY (oxygen)



Option Name	Description
Choose a spectrometer channel to view its sensor parameters	Selects the spectrometer channel for which all sensor options will be applied. Each spectrometer channel has its own parameters.
Scan dark for every measurement (oxygen sensor only)	Automatically stores a dark spectrum each time a simple scan is taken. Only for use with the SF2000 in pulsed mode, and the USB-LS-450 light source.
Channel active	Activates the currently selected spectrometer channel (see above). For some multichannel systems such as the ADC1000-USB, the software sets the active channels according to what is stored in hardware and will not allow you to activate or deactivate channels.
Sensor	Selects the type of sensor used for each spectrometer channel.
Color 	Changes the color of the spectral trace in the display graph. Click directly on the color box to customize the color.

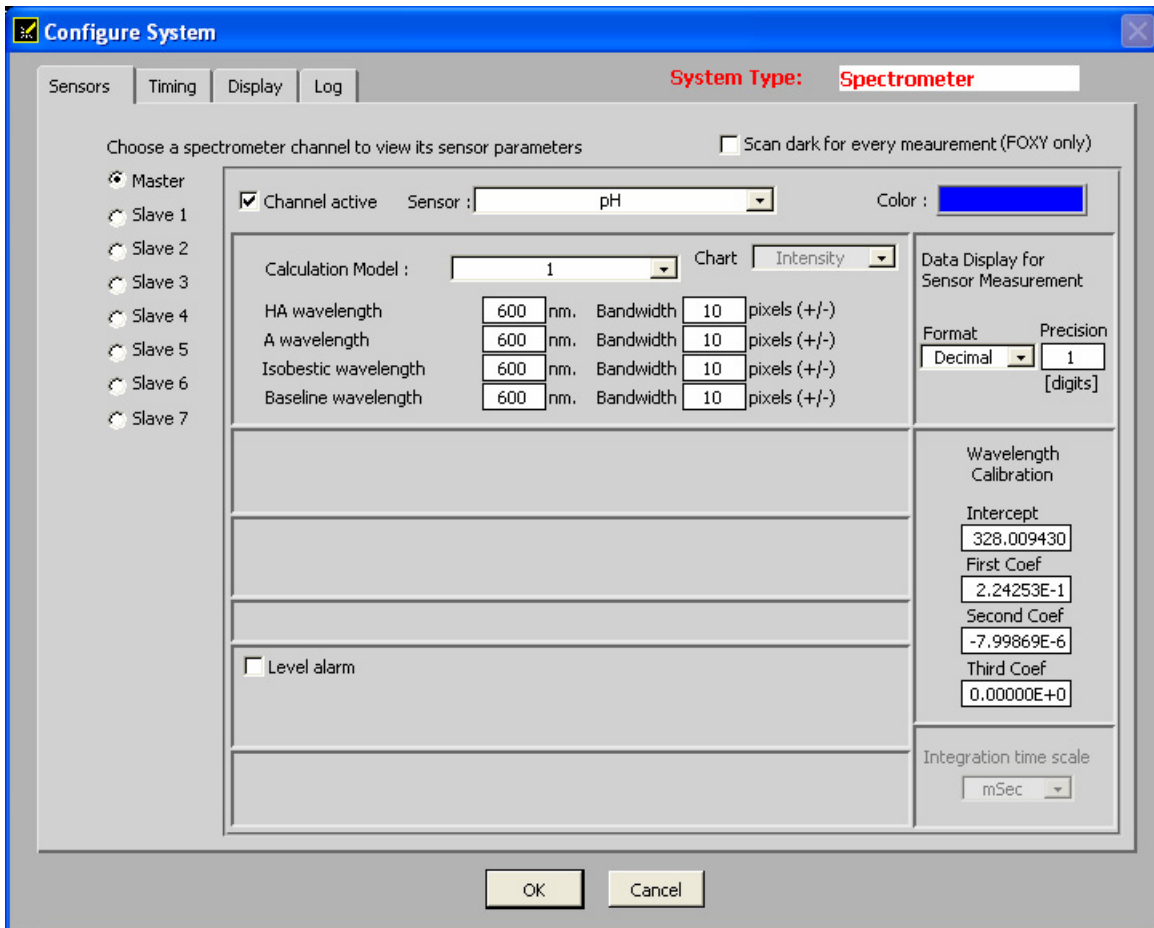
Option Name	Description
Analysis Wavelength	Enter the analysis wavelength. This number should be close to 600 nm, since the ruthenium complex at the tip of the oxygen probe emits energy at ~600 nm when it fluoresces.
Bandwidth Pixels	Enter the number of pixels to average on either side of the analysis wavelength, typically 25. This value is in addition to the boxcar smoothing on the main window. For best results, keep the boxcar value low.
Chart	Specifies the type of information charted in the spectral graph.
Data Display for Sensor Measurement	Specify the data display format and precision of the data. Select Decimal or Scientific from the Format drop-down menu, and select a value (max. 5) to specify the precision of the oxygen data displayed on the screen and saved in log files.
Calibration Order	Select Linear (Stern Volmer) or Second Order Polynomial.
Temperature Compensation	Select this checkbox to enable temperature compensation.
Oxygen Units	Select to use % oxygen (based on factory calibration for oxygen in air), or Weight Fraction (Henry's Law) for water
Henry's coefficients	Enter Henry's coefficients if you are using a liquid other than water (water is the default).
Wavelength Calibration Coefficients	Compare these values with the Wavelength Calibration Data Sheet that accompanied your system to ensure that the values are identical. If necessary, enter the correct values for each channel. Software retrieves coefficients from hardware when possible.
Pressure Compensation CH	<p>Monitors and corrects for pressure fluctuations in the sample (if you have your own pressure transducer). You can use the pressure transducer separately, or interface it with the sensor system.</p> <p>If interfacing to the sensor system, you must have an available spectrometer channel that is NOT connected to an oxygen sensor or have the OMEGA D5251 4-20mA general purpose module. Specify the pressure-monitoring method with the drop-down menu.</p> <p>If an unused spectrometer channel is used as an analog-to-digital converter (A/D) for your pressure transducer, typically Slave1 to Slave7, activate it and assign that spectrometer channel as a pressure channel and enter its calibration coefficients there. If a D5251 module is used, select the serial COM port used and enter its calibration coefficients.</p> <p>Additionally, you can enter the pressure manually.</p>

Option Name	Description
	<p>The Pressure applies to all channels which activated manual pressure compensation and is displayed in the upper left corner of the OOISensors screen as follows:</p> 
<p>Enable reference correction</p> <p>CAUTION: This option is not recommended. Speak to an Ocean Optics Application Scientist before setting.</p>	<p>Monitors and corrects for any drift or change in the LS-450 Blue LED light source. Click to enable this function, and then enter the reference wavelength in the nm box.</p> <p>To use this option correctly, be sure the peak at 470 nm is not saturated.</p>
<p>Bandwidth Pixels</p>	<p>Only available if “Enable reference correction” is enabled. Specifies the number of pixels around the reference wavelength to average.</p>
<p>Level alarm</p>	<p>Configures alarm properties. Click this box to enable the alarm feature, and then enter alarm-monitoring parameters. When the value is within the specified value range, the alarm indicator is green. If the value falls below the specified alarm parameters, the on-screen indicator will turn red. If the value rises above the specified alarm parameters, the indicator turns yellow.</p>
<p>Temperature Measurement</p>	<p>Specifies the method used to monitor and correct for temperature fluctuations, if desired:</p> <p>None: No monitoring</p> <p>Manual: Temperature is monitored, but OOISensors will not read and display temperature values. You must manually enter temperature values in the display window.</p> <p>Select Omega D5xx1 RS232 monitor temperature and have OOISensors automatically read and display temperature values from thermistor or thermocouple.</p> <p>Ocean Optics offers the Omega Thermistor and the Omega Thermocouple for monitoring temperature. The thermistor and thermocouple should already be connected to your PC via an RS-232 module. Select the COM Port number (next to Serial Port) on your PC to which the thermistor or thermocouple is connected. Because the RS-232 module can support up to four thermistors or thermocouples, there are four labeled ports. Next to D5xx1 CH, select the port to which the thermistor or thermocouple connects to the RS-232 module. (If you only have one thermistor or thermocouple, select 0.)</p>

Option Name	Description
	<p>Select USB2000 LS-450 to instruct the USB-LS-450 to monitor and correct for temperature fluctuations. The A/D converter in the USB-LS-450 front end is configured for a 100 ohm platinum RTD (resistance temperature device).</p> <p>A similar method is used with the OMEGA D5251 general purpose 4-20mA module, which requires your own temperature sensor with current output. You must calibrate the input and create its calibration coefficients.</p> <p>Enable the Compensate function if you want the software to correct for temperature fluctuations. Enabling the Chart function allows you to view a chart of the temperature values.</p>

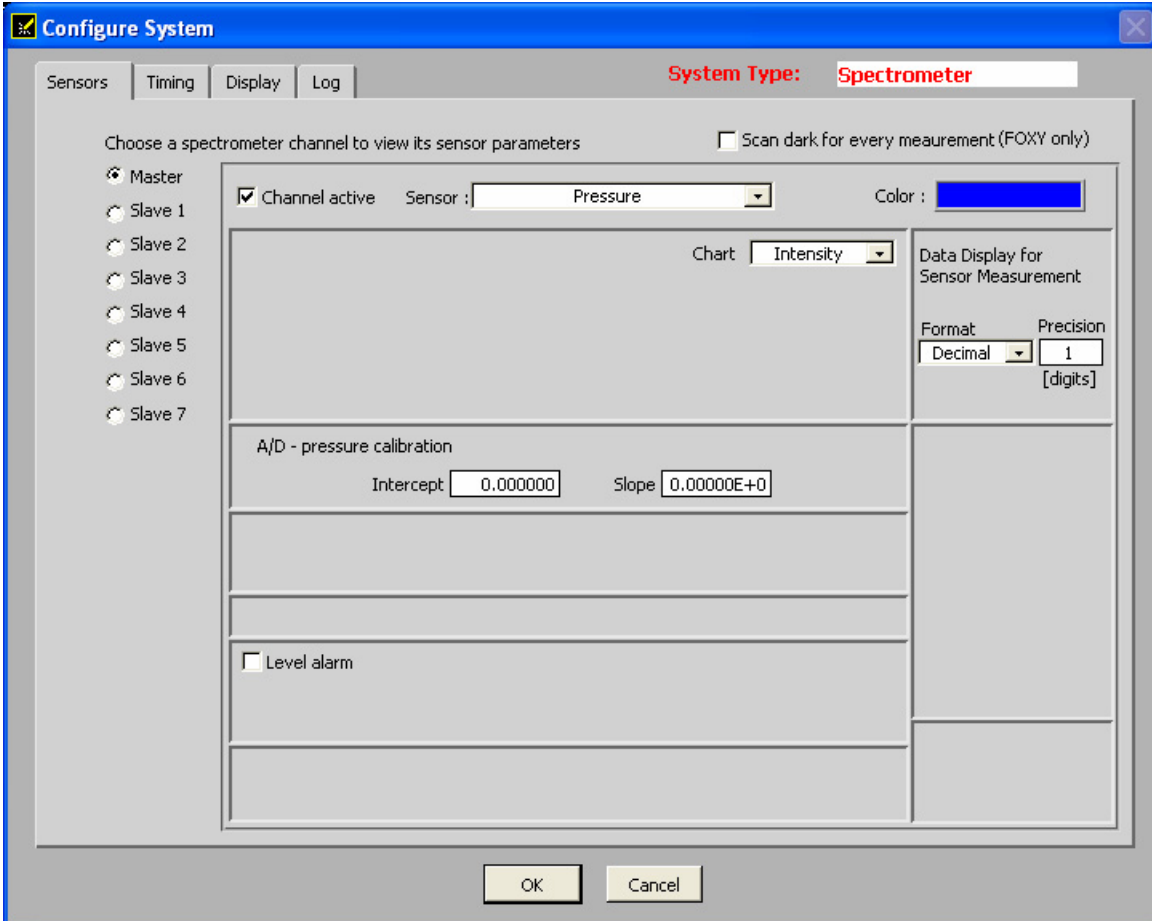
pH

Use this screen to set parameters for a pH sensor.



Pressure

Use this screen to set parameters for a pressure sensor.



Configure System

Sensors | Timing | Display | Log

System Type: Spectrometer

Choose a spectrometer channel to view its sensor parameters Scan dark for every measurement (FOXY only)

Master
 Slave 1
 Slave 2
 Slave 3
 Slave 4
 Slave 5
 Slave 6
 Slave 7

Channel active Sensor : Pressure Color :

Chart: Intensity

Data Display for Sensor Measurement

Format: Decimal Precision: 1 [digits]

A/D - pressure calibration

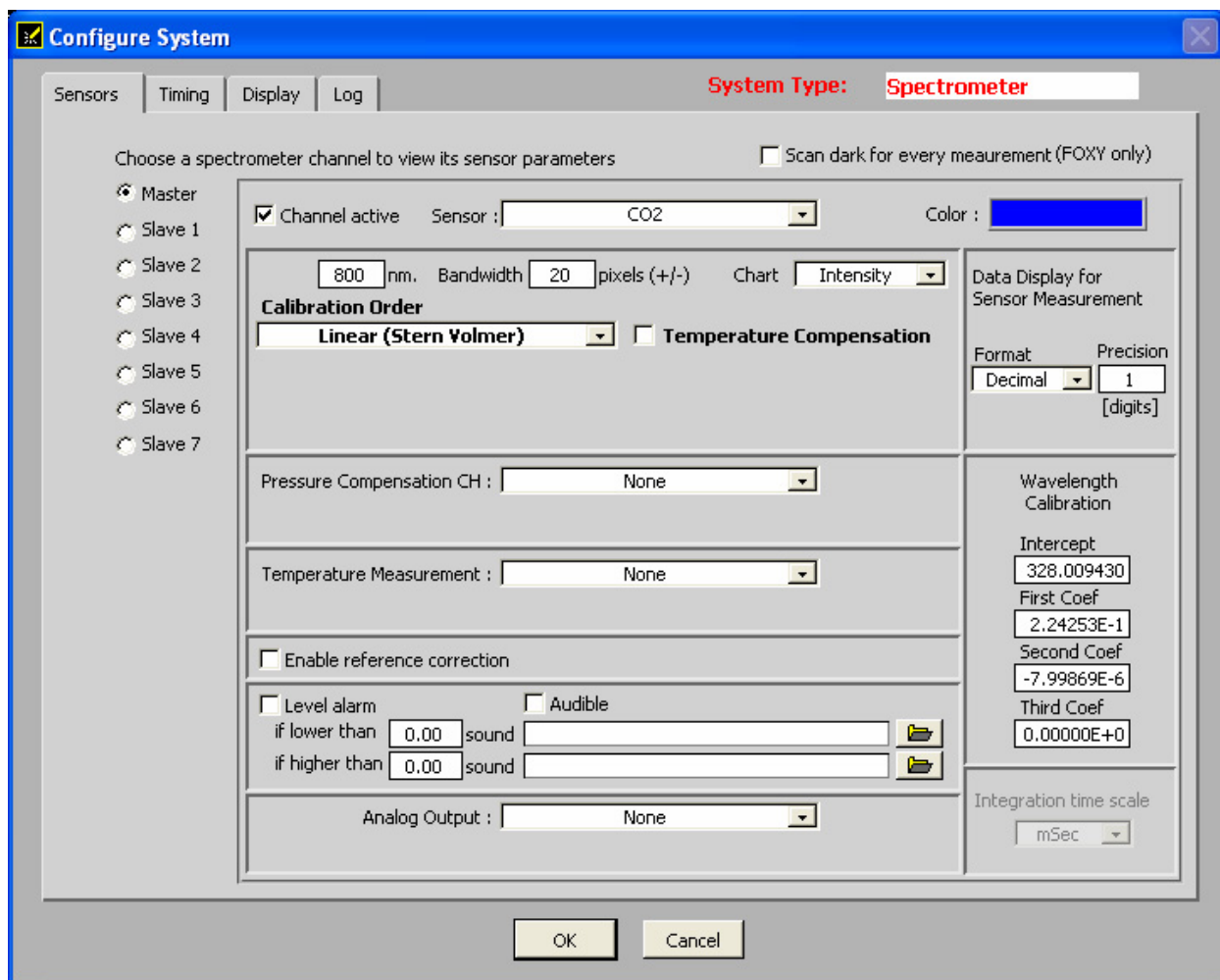
Intercept: 0.000000 Slope: 0.00000E+0

Level alarm

OK Cancel

CO2

Use this screen to enter parameters for a CO2 sensor.



Configure System

Sensors | Timing | Display | Log

System Type: **Spectrometer**

Choose a spectrometer channel to view its sensor parameters Scan dark for every measurement (FOXY only)

Master
 Slave 1
 Slave 2
 Slave 3
 Slave 4
 Slave 5
 Slave 6
 Slave 7

Channel active Sensor : Color :

nm. Bandwidth pixels (+/-) Chart

Calibration Order
 Temperature Compensation

Pressure Compensation CH :

Temperature Measurement :

Enable reference correction

Level alarm Audible
 if lower than sound

if higher than sound

Analog Output :

Data Display for Sensor Measurement

Format: Precision: [digits]

Wavelength Calibration

Intercept:

First Coef:

Second Coef:

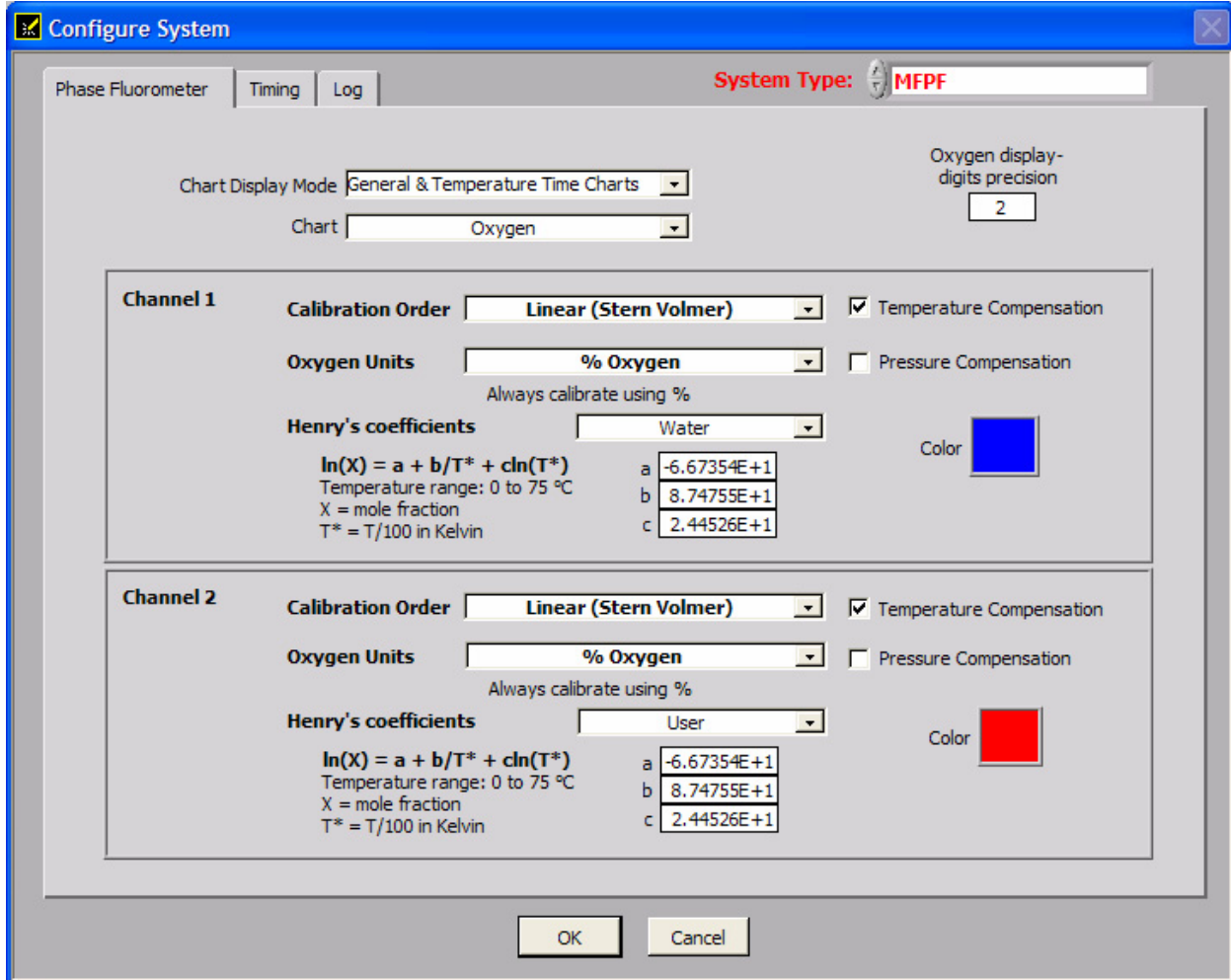
Third Coef:

Integration time scale:

OK Cancel

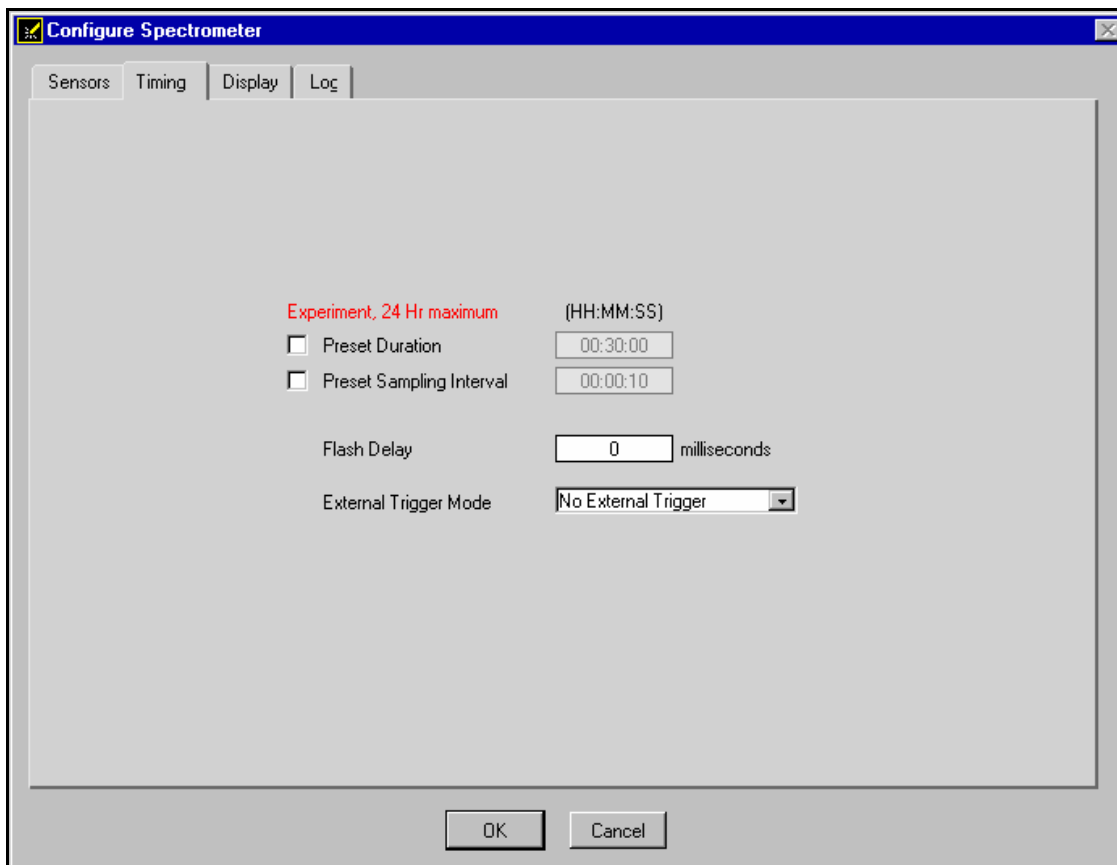
Phase Fluorometer Tab for MFPF Systems

Use this screen to set the parameters for systems using an MFPF unit.



Timing Tab

Click on the Timing tab in the **Configure System** screen to access Timing controls. This screen allows you to configure timed data acquisition parameters.



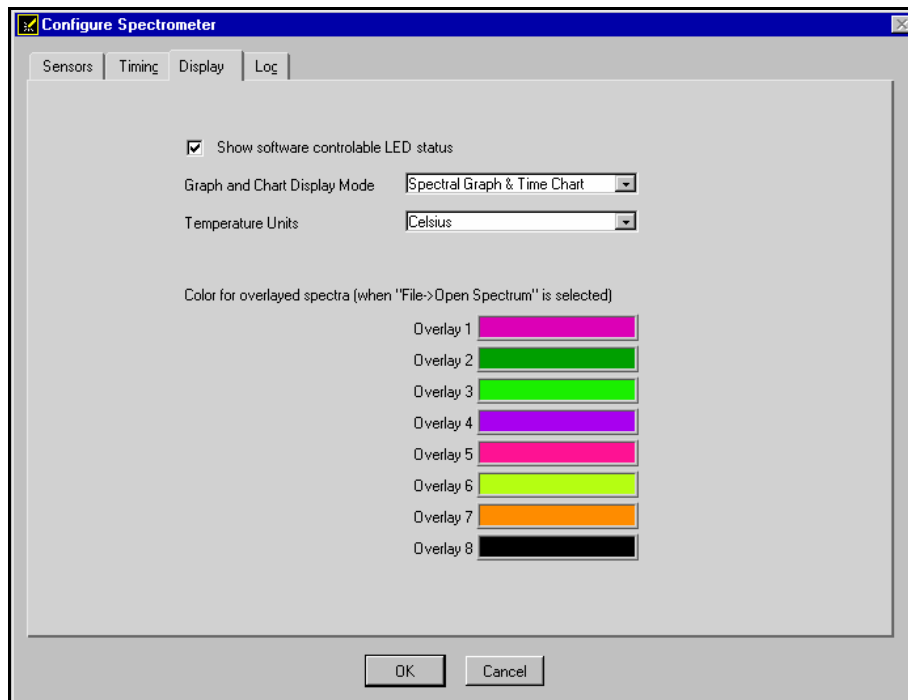
The Timing tab contains the following options:

Option Name	Description
Preset Duration	Specifies the length of the entire timed data acquisition process. Enter the duration in HH:MM:SS format.
Preset Sampling Interval	Specifies the frequency of the data collected in the timed acquisition. Enter the frequency interval in HH:MM:SS format.
Flash Delay (For spectrometer systems only)	Controls the flash delay time of the lamp, in milliseconds. This feature is only applicable when using an ADC1000-PCI or ADC2000-PCI A/D Converter and the LS-450 or R-LS-450 light sources.
External Trigger Mode (For spectrometer systems only)	Specifies the external trigger mode used when acquiring data: No External Trigger: The spectrometer continuously scans, acquires, and transmits data to the PC according to previously configured acquisition parameters. This mode offers no way to synchronize data acquisition with an external event.

Option Name	Description
	<p>External Software Trigger: This mode allows data acquisition to be synchronized with external events. In this level-triggered mode, the spectrometer is free running, only while level is high, just as in normal mode. However, upon each trigger, the data collected up to the trigger event is transferred to the software. For more information, consult the External Triggering document on the Ocean Optics web site at: http://www.oceanoptics.com/technical/externaltriggering.pdf</p>

Display Tab

Click on the Display Tab in the **Configure Spectrometer** screen to access Display controls. This screen allows you to configure display parameters for spectrometers in OOISensors. This tab does not exist for systems with MFPP units.



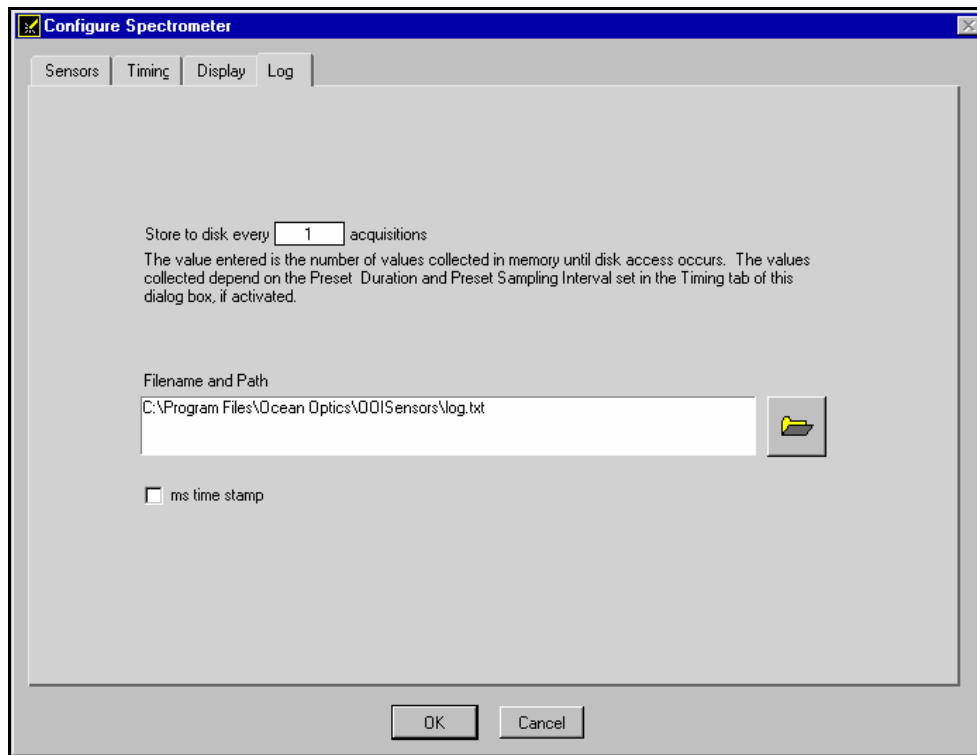
The Display Tab contains the following options:

Option Name	Description
Show software controllable LED status	Displays the status of the LS-450's blue LED.
Graph and Chart Display Mode	<p>Specifies the information that appears in the display window.</p> <p>Spectral Graph Only: The Spectral Graph appears in the display window.</p>

Option Name	Description
	<p>Spectral Graph & Time Chart: The Spectral Graph appears in the top of the display window and the Time Chart appears in the bottom of the display window.</p> <p>To view a Temperature Chart, select Graph&Chart View Temperature Chart from the menu bar. The Temperature Chart will replace the Spectral Graph until View Temperature Chart is unchecked.</p>
Temperature Units	<p>Specifies the format for temperature display. Select Celsius or Fahrenheit from the drop-down menu.</p> <p>Note: OOISensors measures all temperatures in Kelvin and converts to Celsius or Fahrenheit.</p>
Color for overlaid spectra	<p>Specifies colors for static spectra opened with the File Open Spectrum command from the menu bar. Stored static spectra are displayed as overlays, and you should distinguish overlays from real-time spectra by modifying the color of the traces.</p>

Log Tab

Click on the Log Tab in the Configure Spectrometer screen to access data logging controls. These controls allow you to configure the data logging parameters available in OOISensors.



The Log Tab contains the following options:

Option Name	Description
Store to disk every X acquisitions	Specifies the number of scans stored in RAM before the data is saved to a log file. Smaller numbers cause saves that are more frequent. Larger numbers cause saves that are less frequent, but increase application performance by reducing save time.
Filename and Path	Specifies the log file name for the timed data acquisition. Click on the folder icon to browse to a specific folder. Note: The Insert Event in Log File option enables you to enter text in the log file. Select Spectrometer Insert Event in Log File from the menu bar. A dialog box will prompt you to enter text, which will be displayed in the log file next to the data that was acquired at the time of text entry.
Insert Event in Log File	Enables you to enter text in the log file. Select Spectrometer Insert Event in Log File from the menu bar. A dialog box will prompt you to enter text, which will be displayed in the log file next to the data that was acquired at the time of text entry.
MS time stamp	Stamps the data in the log file with the system time.

Display Functions

The following sections provide information on controls that are available directly from the OOISensors main display panel. From the display window, you can choose a mode to acquire data, take scans of your sample, store a dark spectrum, configure the cursor, configure the graph, enter data acquisition parameters and analyze data.

Scan Control

This switch controls the mode of the OOISensors scan function. The **Single** mode option instructs the Scan function to take one scan. The **Cont.** (continuous) mode option instructs the Scan function to take continuous scans until scans are manually stopped.

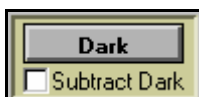


► Procedure

1. Select the **Single** or **Cont.** button from the Scan section of the OOISensors screen in the lower right hand corner.
2. Click on the switch graphic to turn the Scan function on and acquire data. In **Single** mode, the switch will automatically revert to the off position after one scan is completed. In the **Cont.** mode, the switch will remain on and OOISensors will perform multiple scans.
3. Click on the switch graphic to turn the Scan function off and cease data acquisition (if in **Cont.** mode).

Store Dark

This function stores the current spectrum as the dark spectrum for all active channels. You should store the dark after you set the data acquisition parameters in OOISensors.



The dark spectrum is a sample spectrum taken with the light path to the sample blocked. Storing a dark spectrum is required before the computer can make accurate measurements. This button stores the dark values at the fluorescence and reference wavelengths.

If you are using an O₂ sensor without an overcoat, you must physically place the sensor in a dark location before storing a dark spectrum. Ambient light can interfere with dark spectrum measurements on probes with no overcoat.

You should see a flat line in the display screen prior to storing a dark spectrum.

Note

This selection is not recommended for the USB-LS-450 light source.

If you have configured the spectrometer to control the LS-450, the software can take automatic dark scans if you select **Configure | Software** from the menu, click on the Sensors tab, and select Scan Dark for every measurement. When this function is enabled, the LS-450 automatically turns off, and a dark scan is stored, each time you take a sample scan.

Subtract Dark

This feature affects only the display of data on the screen.

Selecting this box under the Store Dark button subtracts the current dark spectrum from the spectra displayed in OOISensors. This command is useful if you are trying to eliminate fixed pattern noise (caused by a very long integration time) from the spectra.

OOISensors always subtracts the dark measurement from the sample measurement in the OOISensors algorithm.

Data Acquisition Parameters

This control, located at the top of the OOISensors screen, allows you to specify the integration time, averaging and boxcar smoothing values. This control provides you with immediate access to the data acquisition settings.

CH	Integ. Time	Average	Boxcar Smooth
M	64 ms	1	3

Channel [CH]

To set the data acquisition parameters (such as integration time, averaging and boxcar smoothing) **for a specific spectrometer channel**, first select the spectrometer channel from the **CH** pull down menu.

Note

This pull down menu is not for selecting the spectrometer channels that are active in the display graph. It is only used to specify the data acquisition parameter for a specific channel.

To activate and display specific spectrometer channels, select **Configure | Spectrometer** from the menu bar and click on the **Sensors** tab. Then, enable each spectrometer in the system.

Integration Time

This parameter specifies the length of the spectral acquisition in milliseconds for the specified spectrometer channel (in the CH pull down menu). The integration time of the spectrometer is analogous to the shutter speed of a camera. The higher the value specified for the integration time, the longer the detector "looks" at the incoming photons.

For Oxygen Sensors:

If your signal intensity is too low, increase this value. If the signal intensity is too high, decrease the value. Adjust the integration time until the fluorescence peak (~600 nm) is about 2000 counts in air or saturated water. The fluorescence peak should not exceed 3500 counts. The intensity of the LED peak (~475 nm) does not affect your measurements. You only need to adjust the integration time if the fluorescence peak is saturating the detector.

Note

If you are using any light source and the SF2000 with the switch on **Pulse**, only set the integration time to powers of two (2, 4, 8, 16, 32, 64... etc.). This ensures a constant number of LED pulses during each spectral acquisition.

Average

This parameter specifies the number of spectra to average for the specified spectrometer channel. A higher value for this parameter provides a better signal-to-noise (S:N) ratio.

Note

The S:N improves by the square root of the number of scans averaged.

Boxcar Smooth

This parameter specifies the number of pixels on either side of a particular pixel to average across an entire spectral acquisition. This method averages a group of adjacent detector elements. A value of five, for example, averages each data point with 5 points (or bins) to its left and 5 points to its right.

The greater this value, the smoother the data and the higher the signal-to-noise ratio. However, if the value entered is too high, a loss in spectral resolution results.

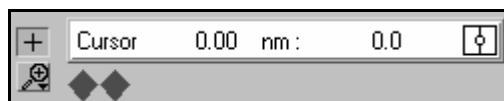
Note

The S:N improves by the square root of the number of pixels averaged.

For best performance when using oxygen sensors, set the boxcar smoothing value to no more than 25 pixels.

Cursor Functions

The cursor functions section of the OOISensors screen allows you to configure specific cursor parameters. You can label the cursor, monitor cursor X and Y values, magnify the cursor, and move the cursor in small increments. Additionally, there is a cursor selection button (located to the right of the XY values) used for choosing cursor and pointer style.

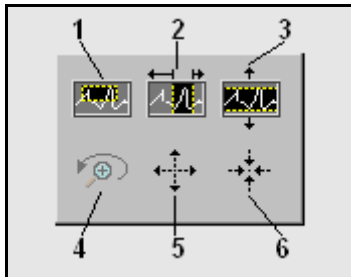


+ Button

When the + button is depressed, the pointer becomes a crosshair symbol, enabling you to drag the cursor around the graph.

Magnification Options

There are several magnification options from which to choose. The function chosen remains in use until another magnification icon or the crosshair symbol is selected. Clockwise, beginning with the top left symbol, the magnify icons perform the following functions:



1. Magnifies a specific area by clicking and dragging a box around the area
2. Zooms in on the horizontal scale, but the vertical scale remains the same
3. Zooms in on the vertical scale, but the horizontal scale remains the same
4. Reverts to the last zoom function
5. Zooms out approximately one point vertical and horizontal, click once or press continuously
6. Zooms in approximately one point vertical and horizontal, click once or press continuously

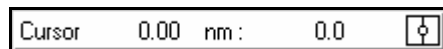
Cursor Diamonds

To move the cursor left or right in small increments in the graph area, click on the left and right cursor diamonds.



Cursor Bar

The functions listed in the following sections are located in the Cursor Taskbar, inside the Cursor Function section of OOISensors.



To move the cursor with the mouse, click directly on the cursor in the display panel and drag.

Cursor Label

The first box in the configure cursor taskbar allows you to label the cursor.

X and Y Values

The cursor taskbar displays the X value and Y value of the cursor point.

Cursor Properties

To the right of the X and Y values of the cursor is a cursor properties button that allows you to utilize many cursor features such as choosing a cursor style, selecting a point style and finding a color for the cursor trace.

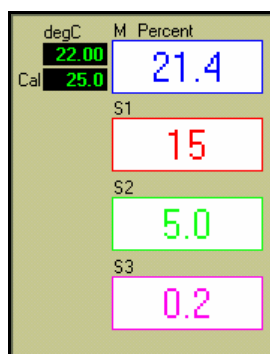


Data Values

The data displayed to the right of the graphs and chart areas provides you with the oxygen or pH values for each spectrometer channel and probe combination. If you are monitoring and correcting for temperature, these values appear in this area as well.

The upper temperature represents the current temperature reading. Additionally, this is where you would manually enter the temperature, depending on your configuration.

The lower temperature represents the single point calibration temperature.



Spectral Graph

The Spectral Graph section is located in the upper half of the OOISensors main screen.

The Spectral Graph area of the display window provides you with real-time spectral scans of your sample. You can change the vertical and/or horizontal scales of the graph by clicking on an X and Y endpoint and manually typing in a value. The graph will then resize itself.

Temperature Chart

The Temperature Chart, when enabled, displays in the upper half of the OOISensors main screen. When this option is enabled, the Spectral Graph screen is replaced by the Temperature Chart.

Display the Temperature Chart

► *Procedure*

Perform the steps below to display the Temperature Chart:

1. Select Graph & Chart from the OOISensors menu bar.
2. Select the View Temperature Chart option from the pop up menu.

The Temperature Chart then takes the place of the Spectral Graph.

Save and Display the Temperature Chart

► Procedure

Perform the steps below to save the Temperature Chart and display it on the screen:

1. Select **File** from the OOISensors menu bar.
2. Select **Save Time Chart** from the pop up menu.
3. Specify the save information in the **Save** prompt that appears.

Note

The saved file is in ASCII format. The first column is the Time column, the second column is the sensor time chart values, and the third column is the temperature readings.

4. Select **Graph & Chart | View Temperature Chart** again to de-select the View Temperature Chart option. Once this option is disabled, the Spectral Graph reappears.

Save the Temperature Without Displaying It

You can also save Temperature Chart data without displaying the chart.

► Procedure

To save the Temperature Chart without displaying the chart:

1. Select **Configure** from the OOISensors menu bar.
2. Select **Spectrometer** from the pop-up menu.
3. Click on the **Sensors** tab and enable the **Chart** function, located under the Temperature Measurement option. Temperature data will be collected, whether or not the Temperature Chart is displayed.
4. Save the chart using the **Save** function.

Time Chart

The time chart displays the data from all active channels at a specific wavelength over time.

► Procedure

Perform the following steps to view the Time Chart:

1. Select **Configure | Spectrometer** from the menu bar.
2. Click on the **Display** tab.
3. Ensure that **Spectral Graph & Time** is selected next to Graph and Chart Display mode.

To configure a timed data acquisition procedure, select **Configure | Spectrometer** from the menu bar and click on the **Timing** tab.

Time Chart Switch

The **Time Chart** switch allows you to start and stop data acquisition. Click on the switch to enable or disable data acquisition.

► Procedure

To set timed data acquisition parameters:

1. Select **Configure | Spectrometer** from the menu bar.
2. Click on the **Timing** tab.
3. Enter the data acquisition parameters.

Log On/Off Switch

You can enable or disable the saving of data to a log file with the Log switch. Click on the switch to enable or disable data logging. Only the last 10,000 scans of a timed data acquisition can be saved in the log file.



► Procedure

To set data logging parameters for timed data acquisition:

1. Select **Configure | Spectrometer** from the menu bar.
2. Click on the **Log** tab.
3. Specify the frequency in between data saves.
4. Specify a file name for the saved log.

File Menu Functions

The following sections detail commands available from the OOISensors File menu.

Save Spectrum	Ctrl+S
Save Time Chart	Ctrl+K
Open Spectrum	Ctrl+O
Open Time Chart	Ctrl+I
Page Setup...	
Print Spectrum	Ctrl+P
Print Time Chart	Ctrl+B
Exit	Ctrl+Q

Save Spectrum

This menu option saves the current spectrum as a tab-delimited ASCII file. You can then open these files as overlays in the spectral graph or import them into other software programs, such as Microsoft Excel.

Save Time Chart

This menu option saves the current time chart as a tab-delimited ASCII file. You can then open these files as a static chart or import them into other software programs, such as Microsoft Excel.

Open Spectrum

This menu option opens a dialog box that allows you to open a previously saved spectrum and to open it as an overlay (a static spectrum) while still acquiring live data.

Open Time Chart

This menu option opens a dialog box that allows you to choose a previously saved time chart and open it as a static chart.

Page Setup

This menu option allows you to select printing parameters.

Print Spectrum and Time Chart

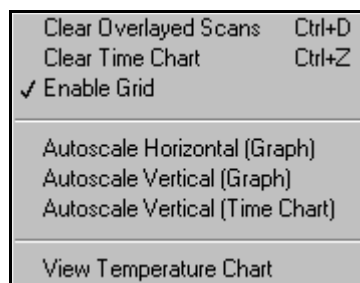
This menu option allows you to print the current display in the Spectral Graph, or select **File | Print Time Chart** from the menu to print the time chart.

Exit

This menu option closes the OOISensors software. You will be prompted to confirm before OOISensors closes.

Graph&Chart Menu Functions

The following section provides information on options available from the Graph&Chart menu.



Clear Spectrum Graph

This option removes static spectra overlays from the graph.

Clear Time Chart

This option clears the time chart traces. A message box confirms this action.

Enable Grid

This option generates a grid in the spectral graph. If you also have the time chart displayed, this function generates a grid in the time chart as well. De-select **Enable Grid** from the menu to turn this option off.

Autoscale Horizontal (Spectral Graph)

This option automatically adjusts the horizontal scale of a current graph so the entire horizontal spectrum fills the display area.

Autoscale Vertical (Spectral Graph)

This option automatically adjusts the vertical scale of a current graph so the entire vertical spectrum fills the display area.

Autoscale Vertical (Time Chart)

This option automatically adjusts the vertical scale of a current time chart so the entire vertical chart fills the display area. You can also adjust the vertical scale of a temperature time chart (if you first select the **View Temperature Chart** option).

View Temperature Chart

This option displays temperature data. The temperature chart replaces the spectral chart. To view the spectral graph again, select **Graph&Chart | View Temperature Chart** from the menu.

Spectrometer Menu Functions

The following section provides information on options available from the Spectrometer menu.



Scan

This option takes a scan of your sample.

When in **Single** mode, this function acts as a snapshot. When the button is depressed, the Stop function replaces the Scan function. The Scan switch enables and the scan begins. The switch remains on until the scan completes (according to configured integration time).

When in **Continuous** mode, this function continuously takes multiple scans of the sample. After each integration cycle, another scan begins immediately. The Scan switch enables and the system begins data acquisition. Select **Spectrometer | Scan** from the menu bar or click on the Scan switch again to halt the scanning process and discontinue acquiring data.

Insert Event in Log File

This option allows you to enter text into the log file during a logged, timed data acquisition.

When this option is selected, a dialog box appears prompting you to enter text. In the log file, this text appears next to the data that was acquired at the time of text entry.

To use this feature, both the Time Chart and Log switches in the display window should be in the **On** position.

Calculate Sensor Values with Scan

The values displayed in the Data Values boxes to the right of the Spectral Graph will appear illogical the first time you run OOISensors. System calibration will correct this condition. However, if you don't want to see these illogical values displayed, deselect **Spectrometer | Calculate Sensor Values with Scan**. Once the system is calibrated, this function should always be selected to display sensor values.

Pulse LED

When the LED is computer-controlled, you have the option of pulsing the LED. This cuts the exposure time in half, thus decreasing the photodegradation of the probe.

This option is not recommended for use with the USB-LS-450.

Calibrate Menu Functions

The following section provides information on options available from the Spectrometer menu.

Oxygen, Single Temperature Oxygen, Multiple Temperature pH Pressure
--

Option Name	Description
Oxygen, Single Temperature	Allows for oxygen probe calibration at a single temperature. Used most frequently.
Oxygen, Multiple Temperature	Allows for oxygen probe calibration at multiple temperatures.
pH	Allows for pH probe calibration.
Pressure	Allows for pressure probe calibration.

Configuring a Spectrometer in OOISensors

Once you install OOISensors, you will need to configure the spectrometer in OOISensors using the Wavelength Calibration data included with the spectrometer shipment.

► Procedure

Perform the steps below to configure the spectrometer in OOISensors:

1. Select **Configure | Spectrometer** from the menu bar. The Configure Spectrometer screen appears.
2. Click on the Sensors tab, if necessary.
3. Ensure that the values in the **Wavelength Calibration Coefficients** section of this screen are the same as the values on the Wavelength Calibration Data you received with the spectrometer.
4. Repeat Step 3 for each channel in the spectrometer. In the left pane of the screen, select the slave channel number, then verify and/or enter the values in the **Wavelength Calibration Coefficients** section of the screen.

Notes

Be sure to retain the Wavelength Calibration Data. You will need this information if you reinstall OOISensors.

If you have a USB-series spectrometer, you do not need to manually enter the Wavelength Calibration Coefficients. The software retrieves these values directly from a memory chip on the spectrometer.

This option does not work with the ADC1000-USB A/D Converter.

Chapter 3

Oxygen Sensors

The following section provides information on operation of the oxygen sensors, as well as the theory behind sensor operation.

How the Oxygen Sensors Work

Oxygen sensors use the fluorescence of a ruthenium complex in a sol-gel to measure the partial pressure of oxygen.

► *Procedure*

This measurement is taken as follows:

1. The pulsed blue LED sends light, at ~475 nm, to an optical fiber.
2. The optical fiber carries the light to the oxygen probe. The distal end of the probe tip consists of a thin layer of a hydrophobic sol-gel material. A ruthenium complex is trapped in the sol-gel matrix. This immobilizes the ruthenium complex and protects it from water.
3. The light from the LED excites the ruthenium complex at the probe tip.
4. The excited ruthenium complex fluoresces, emitting energy at ~600 nm.
5. If the excited ruthenium complex encounters an oxygen molecule, the excess energy is transferred to the oxygen molecule in a non-radiative transfer, decreasing or quenching the fluorescence signal (see [Fluorescence Quenching](#)).
6. The energy is collected by the probe and carried through the optical fiber to the spectrometer. The A/D converter converts this analog data to digital data, which is displayed in the OOISensors software.

Fluorescence Quenching

The following paragraph explains the principle of fluorescence quenching as it relates to oxygen sensors.

Oxygen, as a triplet molecule, is able to quench efficiently the fluorescence and phosphorescence of certain luminophores. This effect (first described by Kautsky in 1939) is called "dynamic fluorescence quenching." Collision of an oxygen molecule with a fluorophore (in the case of oxygen sensors, a ruthenium complex) in its excited state leads to a non-radiative transfer of energy. The degree of fluorescence quenching relates to the frequency of collisions, and therefore to the concentration, pressure and temperature of the oxygen-containing media.

Connecting the Fiber Optic Sensors System

Once you have configured system hardware and installed OOISensors, you will need to connect the sampling optics (probes, optical fiber assemblies) to the Fiber Optic Sensors System.

► **Procedure**

Follow the steps below to properly connect the sampling optics:

1. Connect the probe to the bifurcated optical fiber assembly that came with the Fiber Optic Sensors System. To obtain operating instructions for specific probes, consult the Ocean Optics website or the documentation that accompanied the probe.
2. Locate the 21-02 SMA Splice Bushing that came with the system. This item is a 0.75" screw with two female ends.
3. Screw one end of the splice bushing into the SMA 905 connector on the proximal end of the probe.
4. Locate the bifurcated fiber that came with the system. This optical fiber assembly has a "Y" shaped design.
5. Connect the common end of the bifurcated fiber to the splice bushing/probe.
6. Connect one arm of the bifurcated fiber to the SMA connector on the spectrometer, and then connect the other arm to the SMA connector on the LS-450 Blue LED light source.

Note

It does not matter which arm of the fiber is connected to the light source or spectrometer.

Calibration Requirements for Oxygen Sensors

The following section explains the principles of calibration for oxygen sensors, as well as the algorithms required in the calibration procedures.

For accurate oxygen measurements of a sample, you must first calibrate the oxygen system. There are two major considerations when calibrating the Fiber Optic Sensors System:

- **Temperature:** Determine if you need to compensate for changes in temperature in the sample. If so, you must calibrate the Fiber Optic Sensors System accordingly. (Sample Temperature Duration $\leq \pm 3$ °C)
- **Algorithm:** Determine which algorithm you will use for the calibration. The Linear (Stern-Volmer) algorithm requires at least two standards of known oxygen concentration while the Second Order Polynomial algorithm requires at least three standard of known oxygen concentration.

Calibration curves are generated from your standards and the algorithms to calculate concentration values for unknown samples. The **Second Order Polynomial** algorithm provides a better curve fit. Thus, this algorithm returns accurate data during oxygen measurements, particularly when working in a broad oxygen concentration range.

Note

A linear calibration is good from 0 to 30% O₂. Higher values of O₂ require a second order fit.

Linear (Stern-Volmer) Algorithm

The Stern-Volmer algorithm requires at least two standards of known oxygen concentration. The first standard must have 0% oxygen concentration and the last standard must have a concentration in the high end of the concentration range in which you will be working. The fluorescence intensity can be expressed in terms of the Stern-Volmer equation where the fluorescence is related quantitatively to the partial pressure of oxygen:

$$\frac{I_0}{I} = 1 + k p_{O_2}$$

I_0 is the intensity of fluorescence at zero pressure of oxygen

I is the intensity of fluorescence at a pressure p of oxygen

k is the Stern-Volmer constant

For a given media, and at a constant total pressure and temperature, the partial pressure of oxygen is proportional to oxygen mole fraction.

The Stern-Volmer constant (k) is primarily dependent on the chemical composition of the ruthenium complex. Ocean Optics probes have shown excellent stability over time, and this value should be largely independent of the other parts of the measurement system. However, the Stern-Volmer constant (k) does vary among probes, and it is temperature dependent. All measurements should be made at the same

temperature as the calibration experiments. If this is not possible, temperature-monitoring devices should be used.

Compensating for Temperature with the Linear (Stern-Volmer) Algorithm

If you decide to compensate for temperature, the relationship between the Stern-Volmer values and temperature are defined as:

$$I_0 = a_0 + b_0 * T + c_0 * T^2$$

$$k = a + b * T + c * T^2$$

The *intensity of fluorescence at zero pressure of oxygen* (I_0) depends on various factors in the optical setup: the power of the LED, the quality and type of optical fibers, light loss at the probe due to fiber coupling, and backscattering from the sample. To account for these inconsistencies, you must measure the *intensity of fluorescence at zero pressure of oxygen* (I_0) for each experimental setup.

It is evident from the equation that the sensor will be most sensitive to low levels of oxygen. Deviations from the Stern-Volmer relationship occur primarily at higher oxygen concentration levels. To correct these deviations, use the **Second Order Polynomial** algorithm.

Second Order Polynomial Algorithm

The Second Order Polynomial algorithm requires at least three standards of known oxygen concentration. The first standard must have 0% oxygen concentration and the last standard must have a concentration in the high end of the concentration range in which you will be working. The Second Order Polynomial algorithm is considered to provide more accurate data because it requires at least three known concentration standards while the Linear (Stern-Volmer) algorithm requires a minimum of two known concentration standards. The Second Order Polynomial algorithm is defined as:

$$\frac{I_0}{I} = 1 + K_1 * [O] + K_2 * [O]^2$$

I_0 is the fluorescence intensity at zero concentration

I is the intensity of fluorescence at a pressure p of oxygen,

K_1 is the first coefficient

K_2 is the second coefficient

Compensating for Temperature with the Second Order Polynomial Algorithm

If you decide to compensate for temperature, the relationship between the Second Order Polynomial algorithm and temperature are defined as:

$$I_0 = a_0 + b_0 * T + c_0 * T^2$$

$$K_1 = a_1 + b_1 * T + c_1 * T^2$$

$$K_2 = a_2 + b_2 * T + c_2 * T^2$$

Theory - Henry's Law

It is possible to calibrate the Fiber Optic Sensors System in gas and then use the system in liquid or vice versa. In theory, the Fiber Optic Sensors System detects the partial pressure of oxygen. In order to convert partial pressure to concentration, you can use Henry's Law. When the temperature is constant, the weight of a gas that dissolves in a liquid is proportional to the pressure exerted by the gas on the liquid. Therefore, the pressure of the gas above a solution is proportional to the concentration of the gas in the solution. The concentration (mole %) can be calculated if the absolute pressure is known:

$$\text{Oxygen mole fraction} = \text{oxygen partial pressure} / \text{absolute pressure}$$

Since the Fiber Optic Sensors System detects partial pressure of oxygen, the response in a gas environment is similar to a liquid environment in equilibrium with gas. Therefore, it is possible to calibrate the Fiber Optic Sensors System in gas and then use the system with liquid samples and vice versa if you utilize Henry's Law.

However, Henry's Law does not apply to gases that are extremely soluble in water. The solubility of oxygen in water at different temperatures is shown below:

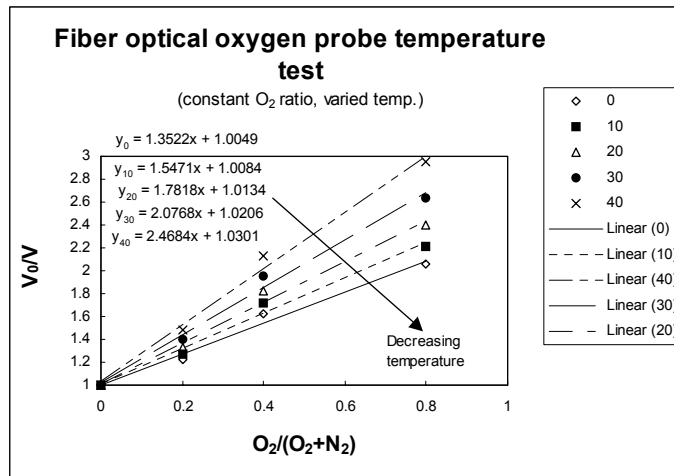
$\ln(X) = a + b/T^* + c \ln(T^*)$	
Temperature Range	0° C - 75° C
X	mole fraction
T^*	T/100 in Kelvin
a	-66.7345
b	87.4755
c	24.4526

Oxygen Solubility in Water at Differing Temperatures

T (°C)	T* (T/100K)	Mole Fraction of oxygen in water at 1 atmosphere pO2	Weight Fraction (ppm) at 1 atmosphere pO2 (pure O2)	Weight Fraction (ppm) at 0.209476 atmospheres pO2 (Air)
5	2.7815	3.46024E-05	61.46203583	12.87482142
10	2.8315	3.06991E-05	54.52891411	11.42249881
15	2.8815	2.75552E-05	48.94460474	10.25272002
20	2.9315	2.50049E-05	44.41468119	9.303809756
25	2.9815	2.29245E-05	40.71933198	8.529722785
30	3.0315	2.12205E-05	37.69265242	7.895706058
35	3.0815	1.98218E-05	35.20817214	7.375267068
40	3.1315	1.86735E-05	33.16861329	6.948028438

Temperature

Temperature affects the fluorescence decay time, the fluorescence intensity, and the collisional frequency of the oxygen molecules with the fluorophore. This, therefore, affects the diffusion coefficient of oxygen. Temperature also affects the solubility of oxygen in samples. The net effect of temperature fluctuations is seen as a change in the calibration slope:



Calibration Slope with Temperature Fluctuations

Samples should be maintained at a constant ($\pm 3^{\circ}\text{C}$) temperature. If this is not practical, calibrate the Fiber Optic Sensors System by using the temperature compensation features and measuring temperature and oxygen concurrently. To monitor the temperature of the sensing environment and compensate for temperature fluctuations, temperature electrodes can now be used in conjunction with the oxygen probe. (Optional thermistor and K-type thermocouple accessories are available.) OOISensors Software corrects for changes in data due to temperature fluctuations.

Note

Although Ocean Optics' miniature spectrometers are extremely sensitive, the spectrometer cannot detect temperature fluctuations $\pm 3^{\circ}\text{C}$.

Scattering Media

Note

This section is relevant only if there is no overcoat applied to the oxygen probe.

Fluorescence emissions from the ruthenium complex propagate in all directions. In clear media, only those emissions propagating toward the fiber within the acceptance angle of the probe are detected. If the probe tip is held near a reflecting surface, or immersed in a highly scattering media, the fluorescence signal will increase. The increase will be proportional for both the intensity of the fluorescence at a pressure of oxygen and the intensity of fluorescence at zero pressure of oxygen, but will not affect the Stern-Volmer constant. For this reason, it is necessary to measure the intensity of fluorescence at zero pressure of oxygen in the sample. Furthermore, if you are measuring oxygen in highly scattering media, then the standards you use for your calibration procedure should be in the same media as your sample for the most accurate results.

Samples to Use

If you are using the probe in gases, N_2 can be used for the low value (0%) and either air (20.9%) or O_2 (100%) can be used for the high value.

If you are using the probe in liquid media, it may be difficult to prepare standards. Sodium hydrosulfite dissolved in aqueous media consumes O_2 rapidly. You can use this to prepare a 0% concentration. Air-saturated values for various solvents and salt solutions can be found in textbooks.

Note

Bubbling standardized gasses through the sample is an option but, depending on your setup, may take more than 24 hours to reach equilibrium

Calibrating Oxygen Sensors

The following sections provide instructions on calibrating your oxygen sensors both with and without temperature compensation.

Calibrating Oxygen Sensors without Temperature Compensation

Perform the steps below to calibrate your oxygen sensors without using temperature compensation:

Preparing for Calibration

► Procedure

1. Set the data acquisition parameters for the calibration procedure (integration time, averaging, and boxcar smoothing).
2. Set the integration time for the entire calibration procedure when the probe is measuring the standard with zero concentration. The fluorescence peak (~600 nm) will be at its maximum at zero concentration.
3. Adjust the integration time so that the fluorescence peak does not exceed 3500 counts.

Note

If you are using the LS-450 in **Pulse** mode, set the integration time to powers of 2 (2, 4, 8, 16, 32, 64 etc.) to ensure a constant number of LED pulses during the integration time.

The intensity of the LED peak (~475 nm) does not affect your measurements.

4. Take a Dark measurement.
5. Select **Calibrate | Oxygen, Single Temperature** from the menu bar. The **Single Temperature Calibration** screen appears.
6. Enter the serial number of the probe in the **S/N** box. The **Date** box should display the current date. The file name and path will appear under **Calibration File Path** once you select **File | Save Calibration Chart** and save the chart. At the bottom of the screen is a box to assign a text label to the **Oxygen Concentration Units**.
7. Select **Multi Point** from the drop-down menu located next to **Calibration Type**.
8. Locate the **Channel** section and select the spectrometer channel associated with the sensor you are calibrating.

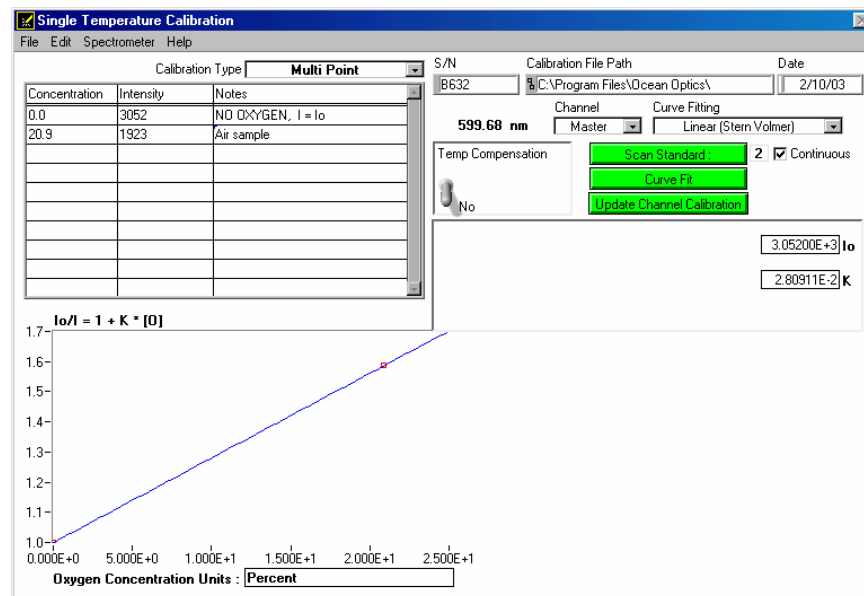
9. Select the algorithm type from the **Curve Fitting** drop-down menu. This algorithm will be used to calibrate your sensor system. See the [Calibration Requirements for Oxygen Sensors](#) section for more information on algorithms

You have configured the system for calibration. Proceed to the next section to calibrate your standards.

Calibrating Standards

► Procedure

1. Enter information for the first standard in the Calibration Table. The first standard should have 0% oxygen concentration, such as in a nitrogen flow or in a solution of sodium hydrosulfite. Enter **0** in the **Concentration** column of the Calibration Table.
2. Leave the oxygen probe in the standard for at least 5 minutes. This guarantees equilibrium.



3. Move your cursor to the **Intensity** column.
4. Click the green **Scan Standard** button or select **Spectrometer | Scan Standard** from the menu.

Note

Enabling the optional **Continuous** function, located to the right of the **Scan Standard** button, will allow you to receive continuous intensity values of a standard. To use this function, check the **Continuous** box.

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5. Once you click the green **Scan Standard** button, a red **Scanning** button appears. Watch the values in the **Intensity** column. When there appears to be no changes in this value, select the red **Scanning** button to accept the intensity value.

Note

If the probe has an overcoat, then the standards used in the calibration can be of a state of matter different from the sample that you are using. You could switch between gas and liquid and your calibration data will be valid. If the probe does not have an overcoat, standards used in the calibration not only must be of the same state of matter as your sample, but also must have the same refractive index as your sample.

6. Enter the known oxygen concentration of the standard in the **Concentration** column and leave the oxygen probe in the standard for 5 minutes.

7. Move the cursor to the **Intensity** column.
8. Click the green **Scan Standard** button or select **Spectrometer | Scan Standard** from the menu bar. The intensity of the standard should then appear in the **Intensity** column.
9. Repeat the steps above for each standard you wish to measure.

You have now calibrated all standards. Proceed to [Finalizing Calibration](#).

Finalizing Calibration

► Procedure

1. Click the green **Curve Fit** button. A graph displaying the results of your calibration procedure appears in the bottom of the screen. Verify the results of the calibration, and then proceed to Step 2.
2. Click the green **Update Channel Calibration** button to save information from this calibration procedure for the specified spectrometer channel into data files.
3. Save the Calibration Table for future use. Select **File | Save Calibration Table** from the menu bar.
4. To print the entire screen, select **File | Print** from the menu bar.
5. Select **File | Close** from the menu bar to return to the main display window.

Calibrating Oxygen Sensors with Temperature Compensation

Perform the steps below to calibrate your oxygen sensors using temperature compensation.

About Factory Calibration

If you want to compensate for temperature changes, you can purchase a Factory Calibration to be performed at Ocean Optics prior to shipping the Fiber Optic Sensors System. Consult an Ocean Optics catalog, contact an Ocean Optics Applications Scientist, or visit our website at www.oceanoptics.com for a price for this service.

Note

Factory Calibration can only be performed if you specify the oxygen concentration range and temperature in which you will be working when you purchase the Fiber Optic Sensors System.

Calibrating With Factory Calibration

Perform the steps below to calibrate the oxygen sensors for temperature compensation using the factory calibration.

Note

If you are not using (or do not have) a factory calibration file, proceed to [Calibrating Without Factory Calibration](#) later in this chapter.

Preparing for Calibration Using the Factory Calibration

If you ordered the **Factory Calibration**, you are provided with an additional file that includes data for the Calibration Table. You will need this file in Step 5.

► Procedure

1. Set the data acquisition parameters for the calibration procedure (integration time, averaging, and boxcar smoothing).
2. Set the integration time for the entire calibration procedure when the probe is measuring the standard with zero concentration at the lowest sample temperature you will be using. The fluorescence peak (~600 nm) will be at its maximum at zero concentration.
3. Adjust the integration time so that the fluorescence peak does not exceed 3500 counts. Set the integration time to powers of two (2, 4, 8, 16, 32, 64 etc.) to ensure a constant number of LED pulses during the integration time. (The intensity of the LED peak [~475 nm] does not affect your measurements.)
4. Select **Calibrate | Oxygen, Multiple Temperature** from the menu bar. The **Multiple Temperature Calibration** screen appears.
5. Select **File | Open Calibration Table** from the menu bar. The name of the file corresponds to the serial number of the probe that you are calibrating. Once this file is opened, the Calibration Table should be populated with oxygen concentration amounts and temperatures.

Note

The serial number is etched on the SMA connector, located at the top of the probe.

6. Select the green **Curve Fit** button. Graphs displaying the curves appear in the bottom of the dialog box. Verify that the graphs are satisfactory, and then proceed to Step 7.

7. Click the green **Update Channel Calibration** button to save information from this calibration procedure.
8. Select **File | Close** from the menu bar to return to the main display window.

You have now configured the system for calibration using the factory calibration file. Proceed to the next section to calibrate your standards.

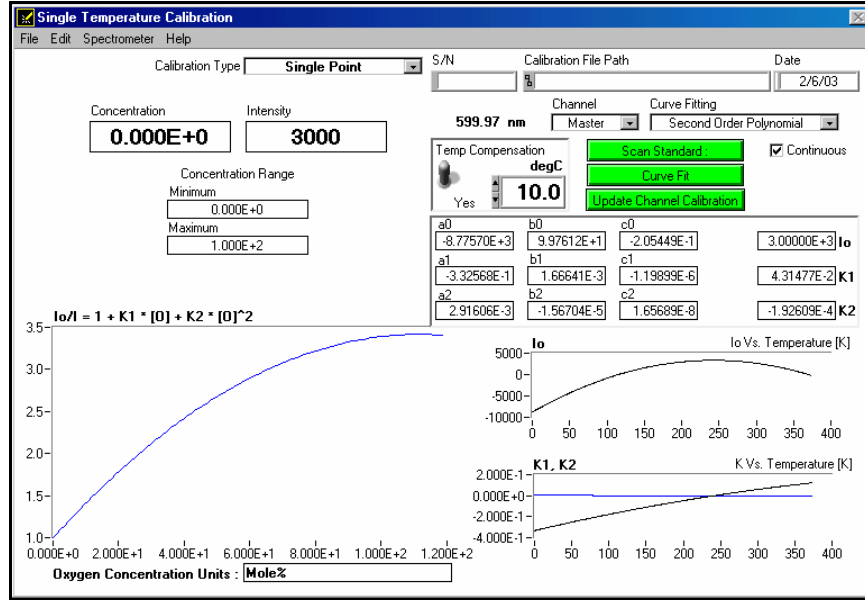
Note

Second order is strongly recommended for all temperature compensation.

Calibrating Standards Using Factory Calibration

► Procedure

1. Select **Calibrate | Oxygen, Single Temperature** from the OOISensors menu bar.
2. Click on the **Calibration Type** drop-down menu and select Single Point.
3. Click on the **Curve Fitting** drop-down menu and select **Second Order Polynomial**.
4. Enter the known oxygen concentration of your standard under **Concentration**.
5. Change the switch in the **Temp Compensation** section of the **Single Temperature Calibration** screen to **Yes**.
6. Get the proper temperature reading. Either:
 - Select Spectrometer | Sample Temperature from the menu bar in the Single Temperature Calibration screen, or
 - Enter the temperature manually.



7. Leave the oxygen probe in the standard for at least 5 minutes. This guarantees equilibrium.
8. Place the cursor in the **Intensity** box.
9. Click the green **Scan Standard** button or select **Spectrometer | Scan Standard** from the menu bar.

Note

Enabling the optional **Continuous** function, located to the right of the **Scan Standard** button, will allow you to receive continuous intensity values of a standard. To use this function, check the **Continuous** box.

Once you click the green Scan Standard button, a red Scanning button appears. Watch the values in the Intensity column (If this value is < 1.0, then the Enable reference correction option is checked on the Configure Software screen.). When there appears to be no changes in this value, select the red Scanning button to accept the intensity value.

Note

If the probe has an overcoat, then the standards used in the calibration can be of a state of matter different from the sample that you are using. You could switch between gas and liquid and your calibration data will be valid. If the probe does not have an overcoat, standards used in the calibration not only must be of the same state of matter as your sample, but also must have the same refractive index as your sample.

Finalizing Calibration Using Factory Calibration

► Procedure

1. Click the green **Curve Fit** button. A graph displaying the results of your calibration procedure appears in the bottom of the screen. Verify the results of the calibration, and then proceed to Step 2.
2. Click the green **Update Channel Calibration** button to save information from this calibration procedure for the specified spectrometer channel into data files.
3. Save the calibration table for future use. Select **File | Save Calibration Table** from the menu bar.
To print the entire screen, select **File | Print** from the menu bar.
4. Select **File | Close** from the menu bar to return to the main display window.

Calibrating Without Factory Calibration

Perform the steps below to calibrate the oxygen sensors for temperature compensation without using the factory calibration.

Note

If you are using a factory calibration file, refer to [Calibrating With Factory Calibration](#).

When calibrating for temperature compensation without using factory calibration, calibration becomes a two-part task.

Preparing for Calibration without Using Factory Calibration – Part 1

► Procedure

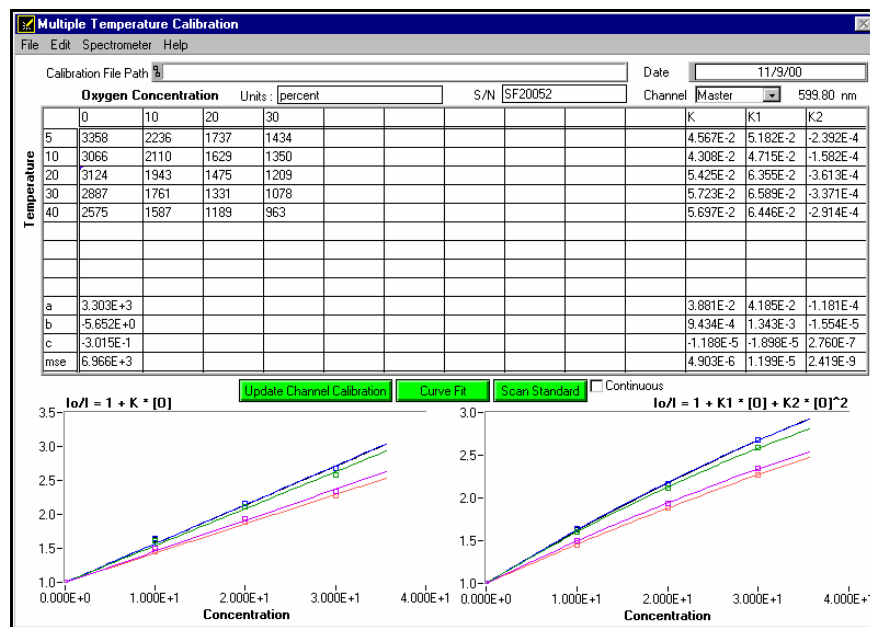
1. Set the data acquisition parameters for the calibration procedure (integration time, averaging, and boxcar smoothing).
2. Set the integration time for the entire calibration procedure when the probe is measuring the standard with zero concentration at the lowest sample temperature you will be using. The fluorescence peak (~600 nm) will be at its maximum at zero concentration.
3. Adjust the integration time so that the fluorescence peak does not exceed 3500 counts. Set the integration time to powers of two (2, 4, 8, 16, 32, 64 etc.) to ensure a constant number of LED pulses during the integration time. (The intensity of the LED peak [~475 nm] does not affect your measurements.)
4. Click on the **Dark Measurement** icon to take a Dark measurement.

5. Select **Calibrate | Oxygen, Multiple Temperature** from the menu bar. The **Multiple Temperature Calibration** screen appears.
6. Enter the serial number of the probe in the **S/N** box. The **Date** box should display the current date. The file name and path will appear under **Calibration File Path** once you select **File | Save Calibration Chart** and save the chart. At the bottom of the screen is a box to assign a text label to the **Oxygen Concentration Units**.

Calibrating Standards without Using Factory Calibration – Part 1

► Procedure

1. Enter the concentration values for each standard with known oxygen concentrations in the top row of the table. The first standard should have 0% oxygen concentration, such as in a nitrogen flow or in a solution of sodium hydrosulfite
2. Leave the oxygen probe in the standard for at least 5 minutes. This guarantees equilibrium.
3. Manually enter the temperature of this standard in the second cell of the left column or select **Spectrometer | Sample Temperature** from the menu.



4. Place your cursor under the oxygen concentration of the first standard.
5. Click the green **Scan Standard** button or select **Spectrometer | Scan Standard** from the menu bar.

Note

Enabling the optional **Continuous** function, located to the right of the **Scan Standard** button, will allow you to receive continuous intensity values of a standard. To use this function, check the **Continuous** box.

Once you click the green **Scan Standard** button, a red **Scanning** button appears. Watch the values in the **Intensity** column. When there appears to be no changes in this value, select the red **Scanning** button to accept the intensity value.

Note

If the probe has an overcoat, then the standards used in the calibration can be of a state of matter different from the sample that you are using. You could switch between gas and liquid and your calibration data will be valid. If the probe does not have an overcoat, standards used in the calibration not only must be of the same state of matter as your sample, but also must have the same refractive index as your sample.

6. Repeat steps 1 through 5 in this section until you have a minimum 3 X 3 grid of calibration data.

Finalizing Calibration without Using Factory Calibration – Part 1

► Procedure

1. Click the green **Curve Fit** button. A graph displaying the results of your calibration procedure appears in the bottom of the screen. Verify the results of the calibration, and then proceed to Step 2.
2. Click the green **Update Channel Calibration** button to save information from this calibration procedure for the specified spectrometer channel into data files.
3. Save the Calibration Table for future use. Select **File | Save Calibration Table** from the menu bar.

To print the entire screen, select **File | Print** from the menu bar.

4. Select **File | Close** from the menu bar to return to the main display window.

Preparing for Calibration without Using Factory Calibration – Part 2

► Procedure

1. Select **Calibrate | Oxygen, Single Temperature** from the menu bar. The **Single Temperature Calibration** screen appears.

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2. Enter the serial number of the probe in the **S/N** box. The **Date** box should display the current date. The file name and path will appear under **Calibration File Path** once you select **File | Save Calibration Chart** and save the chart. At the bottom of the screen is a box to assign a text label to the Oxygen Concentration Units.
3. Select Single **Point** from the drop-down menu located next to **Calibration Type**.

Note

Single Point calibration is based on the procedure you just completed in the Oxygen, Multiple Temperature dialog box. Because you are using the data from the Oxygen, Multiple Temperature dialog box, you only need one standard of known oxygen concentration to complete your calibration.

4. Locate the **Channel** section and select the spectrometer channel associated with the sensor you are calibrating.
5. Select the algorithm type from the **Curve Fitting** drop-down menu. This algorithm will be used to calibrate your sensor system. See [Calibration Requirements for Oxygen Sensors](#) for more information on algorithms.

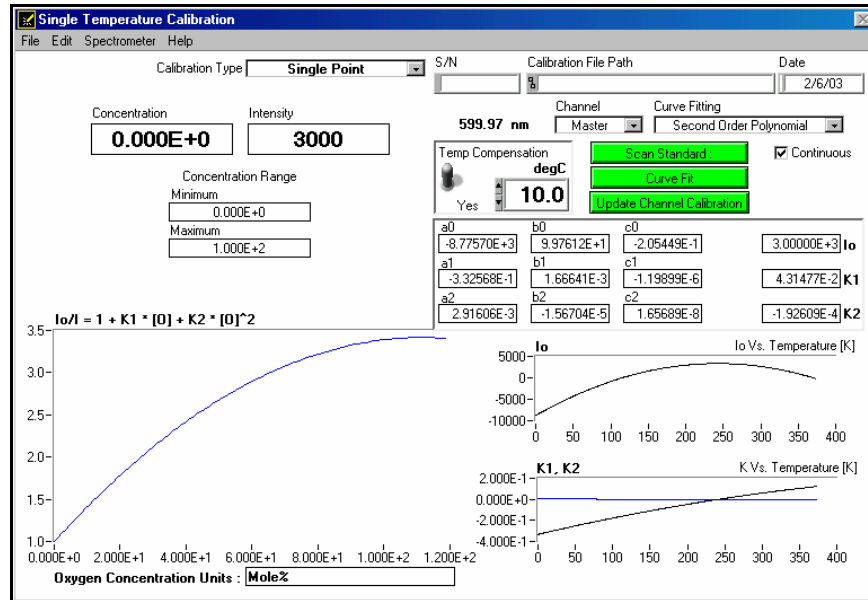
Note

In order to use the Second Order Polynomial algorithm, you must have had at least three standards of known oxygen concentration when scanning standards in the **Oxygen, Multiple Temperature** dialog box, discussed in Part 1.

Calibrating Standards without Using Factory Calibration – Part 2

► Procedure

1. Enter the known oxygen concentration of your standard under **Concentration**.
2. Change the switch in the **Temp Compensation** section of the **Single Temperature Calibration** screen to **Yes**.
3. Get the proper temperature reading. Either:
 - Select **Spectrometer | Sample Temperature** from the menu bar in the **Single Temperature Calibration** screen, or
 - Enter the temperature manually.



4. Leave the oxygen probe in the standard for at least 5 minutes. This guarantees equilibrium.
5. Place the cursor in the **Intensity** box.
6. Click the green **Scan Standard** button or select **Spectrometer | Scan Standard** from the menu bar.

Note

Enabling the optional **Continuous** function, located to the right of the **Scan Standard** button, will allow you to receive continuous intensity values of a standard. To use this function, check the **Continuous** box.

Once you click the green **Scan Standard** button, a red **Scanning** button appears. Watch the values in the **Intensity** column. When there appears to be no changes in this value, select the red **Scanning** button to accept the intensity value.

Note

If the probe has an overcoat, then the standards used in the calibration can be of a state of matter different from the sample that you are using. You could switch between gas and liquid and your calibration data will be valid. If the probe does not have an overcoat, standards used in the calibration not only must be of the same state of matter as your sample, but also must have the same refractive index as your sample.

Finalizing Calibration without Using Factory Calibration – Part 2

► Procedure

1. Click the green **Curve Fit** button. A graph displaying the results of your calibration procedure appears in the bottom of the screen. Verify the results of the calibration, and then proceed to Step 2.
2. Click the green **Update Channel Calibration** button to save information from this calibration procedure for the specified spectrometer channel into data files.
3. Save the calibration table for future use. Select **File | Save Calibration Table** from the menu bar.
To print the entire screen, select **File | Print** from the menu bar.
4. Select **File | Close** from the menu bar to return to the main display window.

Calibration Data

Once you have calibrated your sensor system, the calibration data is stored in two files:

- OOISensors.cfg
- chXFoxy.cal

The **OOISensors.cfg** file is the application configuration file. Calibration data is called from this binary file each time you use the sensor system and software.

The **chXFoxy.cal** file is an ASCII (text) data file that can be read and is portable to Microsoft Word or Excel. In this file name, X represents the spectrometer channel (0 for master, 1 for channel 1, etc.). This file is not used by the OOISensors application. It is strictly for analyzing calibration data. If you have temperature data in this file, the temperature will be displayed in Kelvin.

If you ordered the Factory Calibration, you are provided with an additional file that includes data for the Calibration Table in the **Multiple Temperature Calibration** dialog box. The name of this file corresponds to the serial number of the probe.

Recalibration

oxygen probes need recalibration from time to time. You may need to recalibrate the oxygen probe if any of the following conditions occur:

- You use a oxygen probe in a harsh environment that degrades the probe coating.
- You expose a oxygen probe to the LED source for long periods, causing faster photobleaching of the ruthenium compound.
- You recondition the oxygen probe.

- You sterilize the oxygen probe with processes such as autoclave or gamma radiation.

Note

After calibrating or recalibrating the probe, shut down OOISensors and restart the software. This procedure saves all new information to the default files, which load upon startup.

Chapter 4

pH Sensors

Overview

The Fiber Optic pH Sensor system consists of a fiber optic probe designed to hold immobilized indicator dye materials, a light source, a spectrometer, and OOISensors software. Calibration requires recording spectra in high and low pH samples, as well as in at least one pH standard solution (such as a NIST-traceable buffer).

pH Films

The Fiber Optic pH Sensor System uses two different types of films: Transmissive and Reflective. Transmissive indicator dye films are used for clean, transparent samples. Reflective indicator dye films are used for turbid or absorbing media.

Each type of film consists of a cellulose mechanical matrix surrounded by a hydrophilic polymer that entraps the indicator molecule. The films are stable when stored dry, and are suitable for nearly any aqueous sample environment.

When immersed in water, the film dyes may leach very slowly over time and will have to be replaced. The film response rate is slow (on the order of minutes), being limited by diffusion of ions into the material. Increasing stirring speed, ionic strength, and temperature all tend to increase the response rate.

Probe Options

The following sections detail options available for the Fiber Optic pH Sensor probes.

TP300-UV-VIS Probe

The TP300-UV-VIS probe is a chemically inert PEEK polymer transfection probe that can be equipped with a special tip (RT-PH) for mounting transmissive films in the optical path. Light is directed via one fiber through the mounted film to a mirror. The light is then redirected back through the film to a receive fiber that returns the light to the spectrometer. The sample is free to flow over the sides of the film.

Note

You can also use an RT-2-10 or RT-10-20 transflection tip, which enables the TP300-UV-VIS to be used for routine transmission measurements.

RFP200-UV-VIS Reflective Film Probe

The RFP200-UV-VIS Reflective Film probe consists of a 6-around-1-fiber bundle in a chemically inert 6.35 mm OD Torlon body. The open tip of the probe screws onto the body to hold 3.17 mm to 4.76 mm discs of reflective indicator material. The 6-fiber leg attaches to the light source, while the central fiber leg connects to the spectrometer. The sample has access to the sensing material from one side only.

More Information

For more information on the Fiber Optic pH Sensor system, visit the following page:
<http://www.oceanoptics.com/products/phsensor.asp>.

Start Up

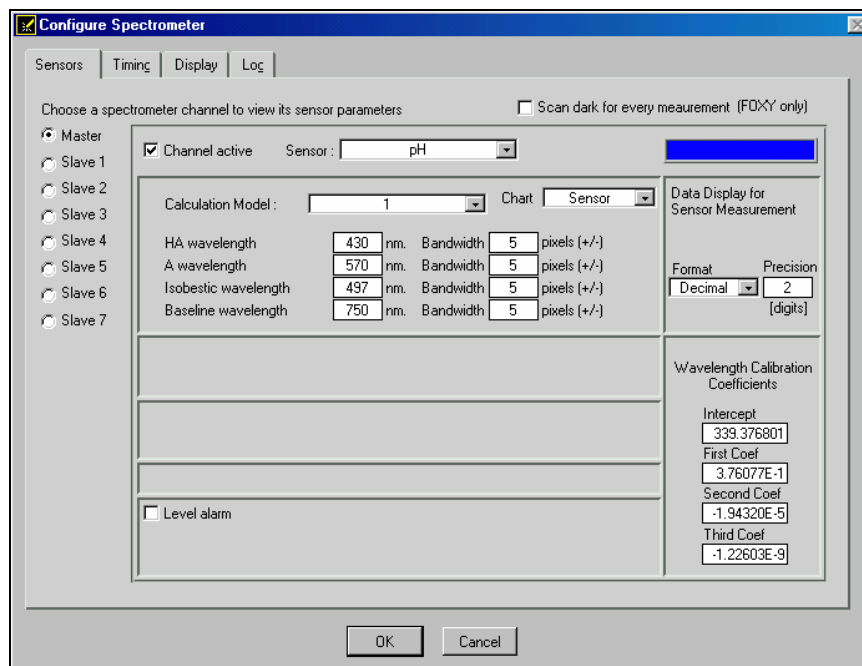
The following sections provide information on starting all components of the Fiber Optic pH Sensor system.

pH Sensor Set Up

► Procedure

Perform the steps below to set up the pH Sensor:

1. Open OOISensors and go to **Configure > Spectrometer**.



2. Activate channels with pH sensors and enter their analysis wavelengths (table on next page):

- HA (acid peak)
- A (base peak)
- Baseline

The isobestic point is not currently used in the OOISensors pH module.

Note

If you aren't sure what these wavelengths are, measure the absorbance in OOIBase32 or SpectraSuite to determine the peaks and where an appropriate baseline is. The baseline should be beyond the last peak (no absorbance should be present). These wavelength values are used in all of the computations and must be entered correctly. See the table on the following page for more information.

3. Click the **OK** button to close the configuration panel.
4. Unscrew the tip of the probe (both the transmission and reflection probe have a screw tip) and place a single piece of the film inside the probe.
5. Screw the tip back onto the probe.

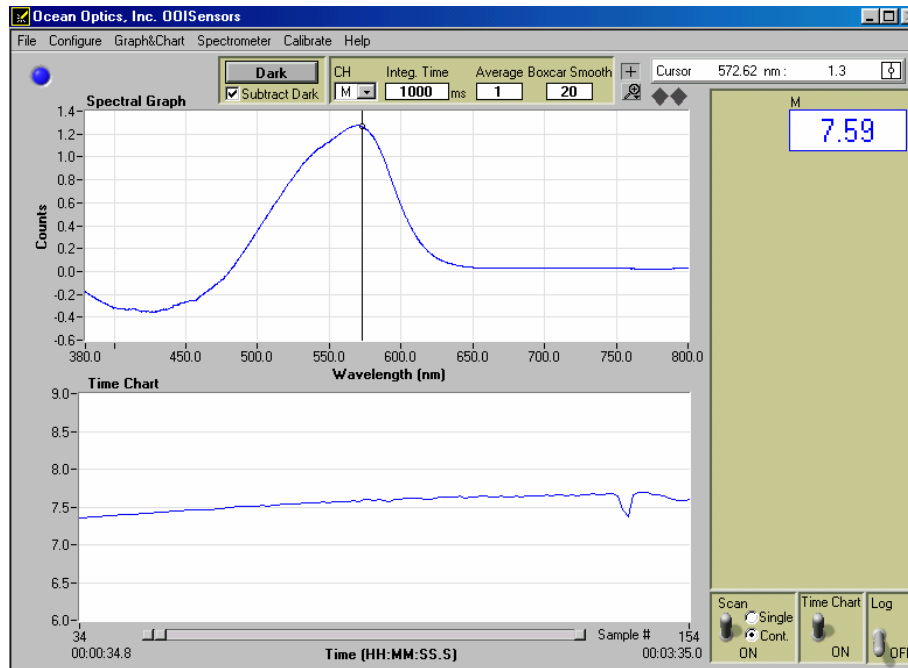
Note

Be careful not to over-tighten the tip. Over-tightening the tip can break the mirror in the transmission probe or break the film in the reflection probe.

- Place the probe in the first pH sample, ensuring that there are no bubbles in the light path of the probe (only applicable to transfectance probes).

pH Sensors Analysis Wavelengths

Indicator Film	Valid pH Range	Isobestic Wavelength (nm)	HA Peak Wavelength (nm)	A Peak Wavelength (nm)	Baseline Wavelength (nm)
Phenol Red	6.5 – 8.5	470	435	575	750
Cresol Red	8.0 – 10.0	490	424	587	750
m-Cresol Purple	8.5 – 10.5	487	435	593	800
Thymol Blue	9.0 – 12.0	495	440	605	800
Brilliant Yellow	7.0 – 9.0	465	412	523	700



Calibration

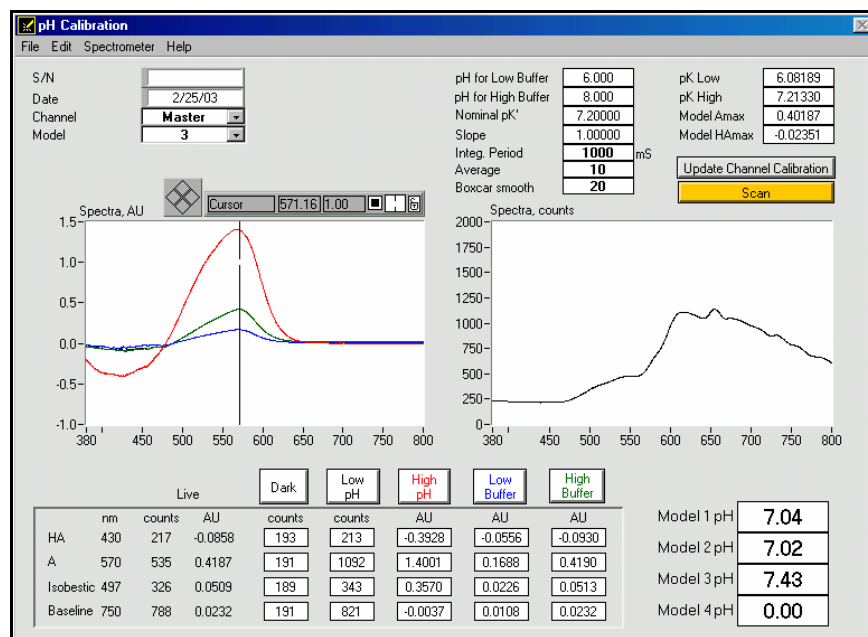
The following sections contain information on calibrating your Fiber Optic pH Sensor system. Calibration requires recording spectra in high and low pH samples, as well as in at least one pH standard solution (such as a NIST-traceable buffer).

pH Sensor Calibration

► Procedure

Perform the steps below to calibrate the pH sensor:

1. Click on the **Calibrate** option in the menu and select the **pH** option from the drop down menu. The **pH Calibration** screen appears.



2. Click on the **Channel** list-box in the upper left corner of the screen and select a spectrometer channel to calibrate.
3. Enter values for **pH for Low Buffer**, **pH for High Buffer**, and **Nominal pK**. Set the **Slope** value to 1.

The pH for the low buffer and high buffer should be within the linear range of the indicator being used. For example, for phenol red the low and high buffer should be pH 6.5 and 8.5 or pH 7 and 8. These numbers are used in the calculations and should be entered correctly. Each indicator has a different linear range. Ensure that you use the correct indicator in the appropriate range for measurements.

Integration Period, **Average**, and **Boxcar Smoothing** are displayed for the active channel. These values can be modified and will be used in the rest of the application if accepted.

Tip

Click on the scan button and optimize the integration period based on the spectra on the right side of the screen. Monitor the spectra and adjust the maximum to peak to ~ 2500 counts. Average should definitely be greater than 20; Boxcar should be around 10 to 20.

4. Block the light path and click the **Dark** button. OOISensors stores intensity (counts) values for all wavelengths of interest.
5. Place the optrode in a low pH sample and allow the optrode to equilibrate for 10 minutes. Then, click on the **Low pH** button to take its measurement.

The Low pH measurement is the reference point for your absorbance measurements and should be less than the lowest value in the linear range of the indicator. For example, for phenol red a good reference pH would be 5. OOISensors stores intensity (counts) values for all wavelengths of interest. These values serve as the reference values in the absorbance calculations.

6. Place the optrode in a high pH sample and allow the optrode to equilibrate for 10 minutes. Then, click on the **High pH** button to take its measurement.

The High pH value should also be outside the linear range of the indicator. For phenol red, pH 10 would be appropriate. An absorbance (AU) curve will be stored in the graph (red line). OOISensors also stores values for all wavelengths of interest.

7. Place the optrode in a Low Buffer sample and allow the optrode to equilibrate for 10 minutes. The low buffer should be the same number entered earlier. Then, click on the **Low Buffer** button to take its measurement.

An absorbance (AU) curve will be stored in the graph (blue line). OOISensors also stores values for all wavelengths of interest.

8. Place the optrode in a High Buffer sample and allow the optrode to equilibrate for 10 minutes. Then, click on the **High Buffer** button to take its measurement.

An absorbance (AU) curve will be stored in the graph (green line). OOISensors also stores values for all wavelengths of interest.

Tip

The absorbance graphs for each measurement appear in the graph on the left side of the pH Calibration screen. The intensity measurements appear in the graph on the right side. Pay attention to the intensity measurements. A sudden overall drop in intensity may be attributed to a bubble, particularly when using the transmission probe. If an increase is noticed where there is a saturation of the detector, you must drop the integration time and restart the calibration from Step 4.

9. Click on the **Update Channel Calibration** button.

From this point on, when you click the **Scan** button, OOISensors displays intensity and absorbance curves for the samples (black line) along with the values for wavelengths of interest. All pH values (for the different models) will be calculated and displayed in the lower right-hand corner. The Pk Low, Pk High, Model Amax, and Model HAmx values in the upper right corner are also calculated by the software and displayed.

10. Click on the **Model** list-box in the upper left corner of the screen and choose a calculation model from the drop down menu. This model will be displayed on the main OOISensors screen. See **Appendix 3: pH Sensor Theory of Operation and Calculations** for an explanation of models.

Tip

Models 2 and 3 normally work best for all of the indicators.

11. Close the calibration screen.
12. Go to **Configure >Spectrometer** and select the spectrometer model that you want to display in the upper right-hand corner on the main screen (the same model you chose at the pH Calibration screen). Then, click the **OK** button.
13. Click on the **Scan On** switch in the lower right corner of the screen. The pH value of your sample will be reported in the upper left corner of the screen. The absorbance curve will appear in the upper graph. Be sure to rescale to see the spectra fully.

Note

Shut down the software correctly when finished. In the event of improper software shutdown or system crash, OOISensors will not retain the calibration values, forcing you to recalibrate the optrode. Shutting down the software ensures that the calibration values are properly stored.

Chapter 5

Compatible Products and Accessories

Overview

The following sections provide ordering information for products and accessories compatible with OOISensors and the Fiber Optic Sensors System.

For each category of accessory, you can visit the associated link for specific information on each product, including specifications and pricing.

Probes

<http://www.oceanoptics.com/products/foxyprobes.asp>

Part Number	Description
FOXY-R	1000- μ m core diameter stainless steel optical fiber, 1/16" outer diameter stainless steel tube beveled at 45°, approximately 6" in length, designed to couple to a 600 μ m bifurcated fiber and splice bushing.
FOXY-AL300	300- μ m aluminum jacketed fiber optic probe, 1 m in length, designed to couple to a 200 μ m bifurcated fiber and splice bushing.
FOXY-PI600	600- μ m polyimide coated fiber optic probe, 2 m in length, designed to couple to a 400 μ m bifurcated fiber and splice bushing
FOXY-OR125	1000- μ m core diameter stainless steel fiber optic probe, 1/8" outer diameter, 2.5" in length, designed to couple to a 600- μ m bifurcated fiber and splice bushing (direct replacement for 1/8" diameter oxygen electrodes)
FOXY-OR125G	1000- μ m core diameter stainless steel fiber optic probe, 1/8" outer diameter, O-ring groove at tip, 2.5" in length, designed to couple to a 600- μ m bifurcated fiber and splice bushing (direct replacement for 1/8" diameter oxygen electrodes)

5: Compatible Products and Accessories



Part Number	Description
FOXY-OR125-GT	1000- μ m core diameter titanium fiber optic probe, 1/8" outer diameter, O-ring groove at tip, 2.5" in length, designed to couple to a 600- μ m bifurcated fiber and splice bushing (direct replacement for 1/8" diameter oxygen electrodes)
FOXY-T1000	1000- μ m core diameter stainless steel fiber optic probe with screw-on light shield, 1/4" outer diameter, approximately 7" in length, designed to couple to a 600- μ m bifurcated fiber and splice bushing
FOXY-T1000-RTD	1000- μ m core diameter stainless steel fiber optic probe with screw-on light shield, 1/4" outer diameter with an imbedded platinum RTD; Only for use with the USB-LS-450 light source; Temperature range of -60 °C to 600 °C
FOXY-18G	300- μ m aluminum jacketed fiber optic probe with 18-gauge needle tip for penetrating vial septa, designed to couple to a 200- μ m bifurcated fiber and splice bushing
FOXY-21G	300- μ m aluminum jacketed fiber optic probe with 21-gauge needle tip for penetrating vial septa, designed to couple to a 200- μ m bifurcated fiber and splice bushing
FOXY-RESP	200- μ m optical fiber housed in a 1 mm OD plastic ferrule for applications that require fast response times.
FOSPOR-18G	300- μ m fiber probe with 18-gauge needle tip
FOSPOR-21G	300- μ m fiber probe with 21-gauge needle tip
FOSPOR-AL300	300- μ m fiber probe in aluminum jacketing, 1m
FOSPOR-OR125	1000- μ m fiber probe housed in a 1/8 th OD stainless steel ferrule
FOSPOR-OR125-G	1000- μ m fiber probe housed in a 1/8 th OD stainless steel grooved ferrule
FOSPOR-OR125-GT	1000- μ m fiber probe housed in a 1/8 th OD titanium O-ring
FOSPOR-R	1000- μ m fiber probe in 1/16 th OD stainless steel ferrule
FOSPOR-R-FLAT	1000- μ m fiber probe in 1/16 th OD, flat polish
FOSPOR-T1000	1000- μ m fiber probe in 1/4" OD stainless steel ferrule
FOSPOR-T1000-RT	1000- μ m fiber probe in 1/4" ferrule with RTD
HIOXY-18G	300- μ m fiber probe in 18-gauge needle tip
HIOXY-21G	300- μ m fiber probe in 21-gauge needle tip

Part Number	Description
HIOXY-AL300	300- μ m fiber probe in aluminum jacketing, 1m
HIOXY-OR125	1000- μ m fiber probe in 1/8 th OD stainless steel ferrule
HIOXY-OR125-G	1000- μ m fiber probe in 1/8 th OD stainless steel grooved ferrule
HIOXY-OR125-GT	1000- μ m fiber probe in 1/8 th OD titanium O-ring
HIOXY-R	1000- μ m fiber probe in 1/16 th OD stainless steel ferrule
HIOXY-T1000	1000- μ m fiber probe in 1/4" OD stainless steel ferrule
HIOXY-T1000-RTD	1000- μ m fiber probe in 1/4" ferrule with RTD

Factory Calibration Service

<http://www.oceanoptics.com/products/foxyprobes.asp>

Part Number	Description
FOXY-CAL	Factory calibration service for FOXY probes, effective in the 0 °C to 80 °C temperature range, 0% to 100% oxygen range
FOXY-CAL-EXT	Factory calibration service for FOXY probes, effective in the -20 °C to 80 °C temperature range, 0% to 100% oxygen range
FOSPOR-CAL	In-house calibration for Fiber Optic Sensors Systems
FOSPOR-CAL-EXT	In-house calibration for Fiber Optic Sensors Systems, extended range
HIOXY-CAL	In-house calibration for HIOXY systems
HIOXY-CAL-EXT	In-house calibration for HIOXY systems, extended range

Overcoats

<http://www.oceanoptics.com/products/foxysystem.asp>

The following silicone overcoats exclude ambient light, improve chemical resistance, and eliminate refractive index effects. A silicone overcoat is required for applications involving liquids or gas-to-liquid activity.

Part Number	Description
FOXY-AF	RTV healthcare-grade silicone overcoat for oxygen probes
FOXY-AF-MG	RTV high-strength medical implant-grade silicone overcoat for oxygen probes (provides a thicker and stronger coating than the FOXY-AF)
FOSPOR-AF	Overcoat for FOSPOR probes, RTV silicone
FOSPOR-AF-MG	Overcoat for FOSPOR probes, medical-grade RTV silicone
FOSPOR-RECOV	Oxygen sensor reconditioning/recoating service
HIOXY-RECOV	Oxygen sensor reconditioning/recoating service

Temperature Compensating Components

<http://www.oceanoptics.com/products/foxytempcomp.asp>

Since oxygen probes are temperature dependent, temperature electrodes can be used in conjunction with oxygen probes to compensate for temperature effects. The following table details the temperature probes offered by Ocean Optics:

Part Number	Description
FOXY-TK1	Omega K-type Thermocouple has 1/8" outer diameter, temperature range of -150° C to 220° C with accuracy of 2.2° C or 0.75% of reading with precision of 1° C (requires FOXY-T-MOD-K module for data acquisition and 10-foot wire)
FOXY-TK-1-W	20-gauge K-type Wire Thermocouple with exposed end has temperature range of -58°F to +392°F (requires FOXY-T-MOD-K module for data acquisition)
USB-LS-450-TP	16-gauge needle type RTD only for use with the USB-LS-450; Temperature range of -60 °C to 600 °C

Sol-Gel Coated Membranes, Microscope Coverslips, and Slides

Ocean Optics' sol-gel oxygen-sensitive film can be applied to any glass substrate, including membranes, microscope coverslips, and microscope slides. Ocean Optics can also apply the oxygen-sensitive film to other products, such as cuvettes and micro-well plates. The table below contains a list of off the shelf products coated with the sol-gel:

Part Number	Description
FOXY-GF	Sol-gel coated glass fiber membranes (pack of 5) for testing viability of reactive material; useful in evaluating membrane-holding probes
FOXY-SGS	1" x 1" sol-gel coated, microscope glass coverslips (pack of 5) for qualitative feasibility testing
FOXY-SGS-M	1" x 3" sol-gel coated microscope slide for testing viability of reactive material when used with microscopes

Spectrometers

<http://www.oceanoptics.com/products/spectrometers.asp>

Spectrometers for use with the Fiber Optic Sensors System are optimized for low light level applications. The following spectrometers are optimized for use with the Fiber Optic Sensors System:

Part Number	Description
MFPPF100-1	Multifrequency Phase Fluorometer, single channel
MFPPF100-2	Multifrequency Phase Fluorometer, dual channel
MC2000 Series	Multichannel S2000 Series Spectrometers
USB4000-FL	USB4000 spectrometer preconfigured for fluorescence without light source
USB4000-FL-395	USB4000 spectrometer preconfigured for fluorescence with USB-LS-395 light source
USB4000-FL-450	USB4000 spectrometer preconfigured for fluorescence with USB-LS-450 light source

Light Sources

<http://www.oceanoptics.com/products/ls450.asp>

The LS-450 Blue LED Light Sources are compact, low-cost light-emitting diodes that produce either pulsed or continuous spectral output at 470 nm – the blue region – for fluorescence measurements. The LS-395 is a pulsed blue LED excitation source with a 395 nm center wavelength. These light sources are specially designed for use with the Fiber Optic Sensor System:

Part Number	Description
LS-450	Blue LED Pulsed Light Source with cable for external connection to spectrometer

Part Number	Description
LS-395	Blue LED Pulsed Excitation Source, 395 nm center wavelength

Bifurcated Optical Fiber Assemblies and Splice Bushing

<http://www.oceanoptics.com/products/premgradebif.asp>

Ocean Optics' bifurcated fiber optic assemblies connect easily to sensors, light sources, and miniature fiber optic spectrometers via SMA terminations and an additional splice bushing. These single-strand optical fibers are silica-core and silica-clad. Standard assemblies are two meters in length.

Part Number	Description
QBIF200-VIS/NIR	200- μ m bifurcated optical fiber assembly (for use with FOXY-AL300 and FOXY-24G probes)
QBIF400-VIS/NIR	400- μ m bifurcated optical fiber assembly (for use with FOXY-PI600 probe)
FOXY600-VIS/NIR	600- μ m bifurcated optical fiber assembly (for use with the FOXY-R, FOXY-OR125, FOXY-OR125G and the FOXY-T1000 probes)
21-02	SMA splice bushing for connecting an oxygen probe to a bifurcated optical fiber assembly

Chapter 6

Hardware Datasheets and Instructions

The following section contains product data and operating instructions for hardware that is specific to Ocean Optics' Fiber Optic Sensor System.

Overview

The following products are detailed in this chapter:

- Fiber Optic Sensing Probes – General Information
- FOXY-R Stainless-steel 1/16" OD Fiber Optic Probe
- FOXY-AL300 Aluminum-jacketed Fiber Optic Probe
- FOXY-PI600 Polyimide coated Fiber Optic Probe
- FOXY-18G/21G Needle-tipped 18/21-gauge Fiber Optic Probes
- FOXY-OR125 and FOXY-OR125G O-ring Fiber Optic Probes
- FOXY-T1000 Stainless-steel Fiber Optic Probe with Light Shield
- FOXY-T1000-RTD Stainless-steel Fiber Optic Probe with Light Shield
- FOXY-RESP Respiration Probe

Oxygen Sensing Probes – General Information

The following general information applies to all of oxygen probes detailed in the following sections.

**FOXY Probes**

Probe Construction

The standard oxygen probe has its distal tip polished and coated with the oxygen-sensing material. The active material used for the oxygen probe coating is a sub-micron thin-glass film with an immobilized ruthenium organic complex. The material is applied as a thin film to glass substrates such as optical fibers. The proximal end of the oxygen probe has an SMA 905 termination for coupling to a bifurcated fiber optic assembly.

Accuracy

The accuracy of the probe is based on the algorithm used during the calibration process and the range in which you are working:

- 1.0% of full range for 0-20% when calibrating with the Linear (Stern-Volmer) algorithm
- 0.1% of full range for 0-20% when calibrating with the Second Order Polynomial algorithm
- 1.0% of full range for 0-100% when calibrating with the Second Order Polynomial algorithm

Measurement Range

Each oxygen probe will respond to gaseous samples from 0-100% at 1 atmosphere pressure. In water, the probe will respond from 0-40.7 ppm, which is the saturation level for oxygen in water. In other liquids, the probe responds from 0 to the saturation level for oxygen in that particular liquid.

Sampling Chambers

The oxygen probe should be installed in a suitable sampling compartment. If you are not using an overcoat on the probe, it is critical that ambient light be excluded from the field of view of the probe tip. For gases, a flow chamber with connections to N₂, dry air, and/or O₂ is suggested. For fluids, these gases can be used with glass frits (airstones) and a stirrer to bring the liquid into equilibrium with the calibration gas. As an option, sodium hydrosulfite can be used to strip O₂ from aqueous solutions. It is critical that standards with known oxygen concentration levels are available for use.

Ocean Optics has both clear and black/opaque sampling chambers available for sale in various sizes. Consult an Applications Scientist for more information.

Response Time

The response time of a oxygen probe is limited by the speed of diffusion of oxygen into the sensor. Our standard thin films are very fast (<1 second) in gases and liquids. In viscous samples, the diffusion through the sample will determine the response rate. The probe will respond quickly in samples such as water, and more slowly in viscous samples such as oils, emulsions, and creams. Unlike an electrode, the optical sensor will not consume oxygen. This means that stirring the sample will increase the response rate, but will not affect the final equilibrium reading. Also, optional probe overcoats, applied to exclude ambient light and improve chemical resistance, will slow response time from 20 seconds to as long as 90 seconds, depending on the overcoat.

Environmental Conditions

Environmental changes, such as changes in pH, salinity, and ionic strength, do not affect oxygen probes.

Chemical Compatibility

oxygen probes are not affected by pH change or salinity. They can be used safely with 50% methanol, acids, sodium sulfite, NF₃, hexane, CH₄, acetone vapor, moisture, CO₂ and CO. We recommended not using the probes with strong bases (pH>10), styrene, ethanol, liquid acetone, acetonitrile, and HF and BTEX solvents.

For a complete list of disruptive and benign chemicals for use with oxygen probes, see [Chemical Effects on Oxygen Probes](#).

Temperature and the Oxygen Probe

Temperature affects the fluorescence decay time, the fluorescence intensity and the collisional frequency of the oxygen molecules within the fluorophore – and therefore, the diffusion coefficient of oxygen. Temperature also affects the solubility of oxygen in samples. The net effect of temperature fluctuations is seen as a change in the calibration slope. It is best to maintain the sample at a constant ($\pm 3^{\circ}\text{C}$) temperature. If this is not practical, then you should calibrate your Fiber Optic Sensors System by using the temperature compensation features and measuring temperature and oxygen concurrently. To monitor the temperature of the sensing environment and compensate for temperature fluctuations, temperature electrodes can now be used in conjunction with the oxygen probe. OOISensors Software corrects for changes in data due to temperature fluctuations.

Photodegradation

Because the ruthenium may be subject to photodegradation, the total exposure to the excitation source should be minimized. OOISensors Software can control the strobe of the LED so that it illuminates the probe only during a measurement cycle, as long as the measurement cycle is greater than three minutes.

Ambient Light

Our miniature fiber optic spectrometers are very sensitive, and the oxygen probe will readily detect ambient light. The probe must be shielded from ambient light to obtain reliable data. Install it in a closed vessel, or shield the probe with a dark housing. An overcoat of silicone can be applied to the probe (at no charge) to help reduce ambient light effects. However, the overcoat can slow the response time of the probe from 20 to 90 seconds.

You can determine if ambient light is entering the probe by turning off the LED. The spectra in OOISensors should appear flat, near the bottom of the scale.

Lifetime

Each oxygen probe is warranted for 1 year. Probes used in benign environments may last for a very long time. However, severe biofouling, physical abrasion, and chemical etching of the glass may erode the probes' sensing surface. Cleaning the probe and protecting it from damaging environments will extend its life. After 1 year (or, if necessary, before 1 year), you can send your oxygen probe back to Ocean Optics for re-conditioning and re-coating services (item code FOXY-RECOV or FOXY-RECOV-N for needle probes).

Cleaning Oxygen Probes

You can use detergents to clean the probe. Using detergents to clean the probe does not necessitate calibration.

Avoid cleaning oxygen probes with ketones (acetone and alcohols included) or organic solvents.

Use Warnings

- Handle with care. Dropping the probe may cause permanent damage.
- Gently remove the plastic cover from the SMA connector before use.
- Pulling the SMA connector away from the probe when removing the plastic cover could damage the probe.

Oxygen Probe Specifications

The following specifications pertain to all oxygen probes:

Specification	Dissolved Oxygen in H ₂ O	Oxygen Gas (at 1 atmosphere)
Dynamic range	0-40.7 ppm 0-760 mm Hg partial pressure	0-100% (mole percent) 0-760 mm Hg partial pressure
Response time (without overcoat)	< 1 second	< 1 second
Response time (with overcoat)	30-50 seconds	20-30 seconds
Resolution	0.003 ppm at 0 ppm O ₂	0.01% at 0% O ₂
	0.02 ppm at 8.5 ppm O ₂	0.04% at 20.9% O ₂
	0.2 ppm at 40 ppm O ₂	0.40% at 100% O ₂
Stability:	Drift <0.02 ppm per day	Drift <0.05% per day
Minimum Detection Limit	0.01 ppm	0.025%

Sterilization Methods Tested on Oxygen Probes

The following sterilization methods have been tested for use with oxygen probes. The results of each sterilization method are listed to in the right column of the table:

Method	Results
Autoclaving (steam sterilization 30 minutes or more at 121 °C)	Each cycle decreases signal by more than 50%. Recalibration is necessary; lifetime of probe is 6- 8 cycles.
Gamma Radiation	No effect.

Method	Results
ETO (at room temperature)	Unknown.
ETO (at temperatures above 100 °C)	Unknown.
Hydrogen Peroxide	Destroys probe signal with extended exposure, even with overcoat applied to probe.
Hydrogen Peroxide Plasma (Plazlyte)	Degrades probe signal with each cycle. Each cycle degrades probe by ~ 15%.
Methanol and Ethanol Wash	Destroys probe signal with extended exposure, even with overcoat applied to probe.
Ozone	Preliminary results indicate ozone may not be a viable method for sterilization.
Sodium Hypochlorite (bleach or Clorox)	No effect.
UV	Unknown.

FOXY-R Stainless-steel 1/16" OD Fiber Optic Probe

The FOXY-R is a 1000- μm silica-core, silica-clad fiber encased in a stainless-steel ferrule. The 7" long semi-rugged probe has an outer diameter of 1/16". The FOXY-R was the first probe designed for the Fiber Optic Sensors System. The hypodermic needle-sized probe is designed for use with 600- μm bifurcated optical fiber. It has an SMA 905 connector at the proximal end for easy coupling to the bifurcated fiber. The gap between the stainless steel and the silica cladding is filled with epoxy.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-R Probe

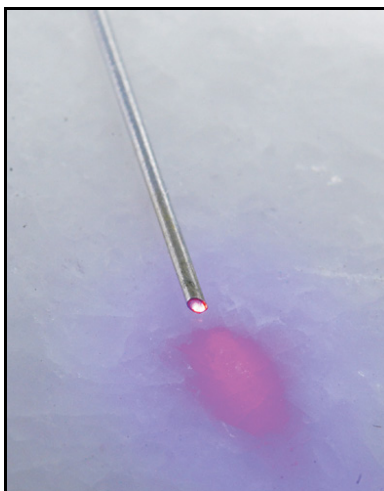
FOXY-R Specifications

Specification	Value
Fiber core	1000 μm core silica
Fiber cladding	Silica
Fiber jacketing	Stainless steel
Outer diameter	1/16"
Length	7"
Maximum applied pressure	300 psi
Probe temperature range	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector	SMA 905
Response time (without overcoat)	<1 second
Compensation	Temperature compensation only
Probe reconditioning	Yes (FOXY-RECOV @ \$100)
Re-calibration	Required when probe is replaced or sterilized by autoclave
Probe lifetime	1 year

FOXY-AL300 Aluminum-jacketed Fiber Optic Probe

The FOXY-AL300 is a 300- μm aluminum jacketed fiber optic probe. The aluminum jacketing, which is FDA approved, is designed for applications where extraordinary environmental performance is required. Aluminum jacketed fibers do not weaken under stress (as ordinary fibers do) and have an extremely high break strength. This extremely flexible and thin probe is 1 meter in length and is designed for use with the BIF200-VIS/NIR 200- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-AL300 Aluminum-Jacketed Fiber Optic Probe

FOXY-AL300 Specifications

Specification	Value
Fiber core	300 μm silica
Fiber cladding	Silica
Fiber jacketing	Aluminum
Outer diameter	410 to 420 μm
Length	1 meter
Bending radius:	2 cm

Specification	Value
Maximum applied pressure:	300 psi
Probe temperature range:	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector:	SMA 905
Response time (without overcoat):	<1 second
Compensation:	Temperature compensation only
Probe reconditioning:	Yes (FOXY-RECOV @ \$100)
Re-calibration:	Required when probe is replaced or cleaned by autoclave
Probe lifetime:	1 year

FOXY-PI600 Polyimide coated Fiber Optic Probe

The FOXY-PI600 is a 600- μm polyimide-coated fiber optic probe. The polyimide is a coating designed to operate at temperatures up to 300°C. The polyimide coating allows the sensor to operate in many harsh chemicals and solvents. The FOXY-PI600 is 2 meters in length and is designed for use with the BIF400-VIS/NIR 400- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-PI600 Polyimide Coated Fiber Optic Probe

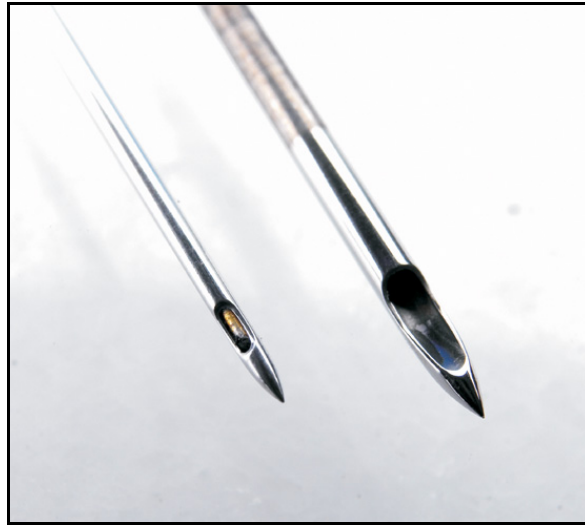
FOXY-PI600 Specifications

Specification	Value
Fiber core	600 μm silica
Fiber coating	25 μm polyimide
Outer diameter	~1 mm
Length	2 meters
Amount of pressure that can be applied	300 psi
Probe temperature range	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector	SMA 905
Response time (without overcoat)	<1 second
Compensation	For temperature only
Probe reconditioning	Yes (FOXY-RECOV @ \$100)
Re-calibration	Required when probe is replaced or cleaned by autoclave
Probe lifetime	1 year

FOXY-18G/21G Needle-tipped 18/21-gauge Fiber Optic Probes

The FOXY-18G/21G are silica-core, aluminum-jacketed, 1-meter probes with 18/21-gauge needle tip at the ends for penetrating vial septa. The length of the needle tip is 4", while the entire length of the probe is 1 meter. The FOXY-18G/21G is designed for use with the BIF200-VIS/NIR 200- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



Foxy 21G (left) and 18G (right) Fiber Optic Probe

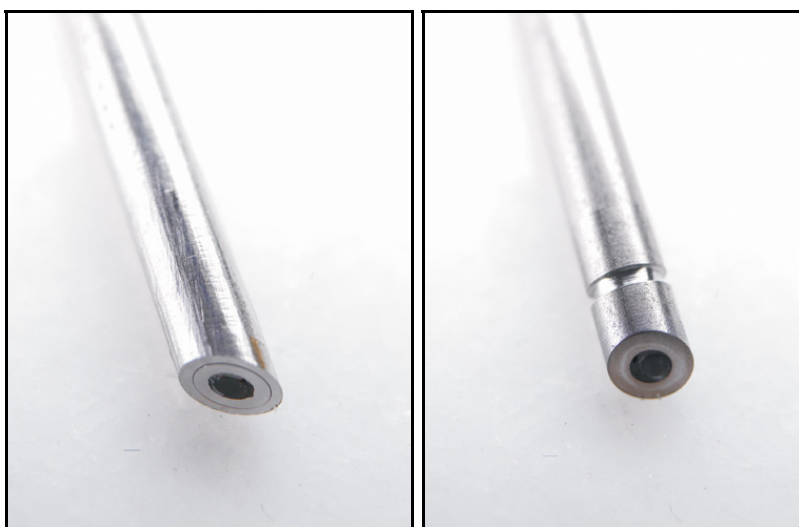
FOXY-18G/21G Specifications

Specification	Value
Fiber core	300 μ m silica
Fiber jacketing	Aluminum
Needle tip	18 or 21-gauge, 0.022" stainless steel wire
Length	4" for needle tip, 1 meter for entire probe
Amount of pressure that can be applied	300 psi
Probe temperature range	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector	SMA 905
Response time (without overcoat)	<1 second
Compensation	Temperature compensation only
Probe reconditioning	Yes (FOXY-RECOV-N @ \$125)
Re-calibration	Required when probe is replaced or cleaned by autoclave
Probe lifetime:	1 year

FOXY-OR125 and FOXY-OR125G O-ring Fiber Optic Probes

The FOXY-OR125 is a silica-core, 1000- μm stainless steel fiber optic probe with a 1/8" outer diameter and an O-ring seal. The FOXY-OR125G is a silica-core, 1000- μm stainless steel fiber optic probe with a 1/8" outer diameter and an O-ring groove at the tip. Both probes are 2.5" in length and are designed for use with a 600- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-OR125 (left) and FOXY-OR125G (right) O-ring Probe

FOXY-OR125 and FOXY-OR125G Specifications

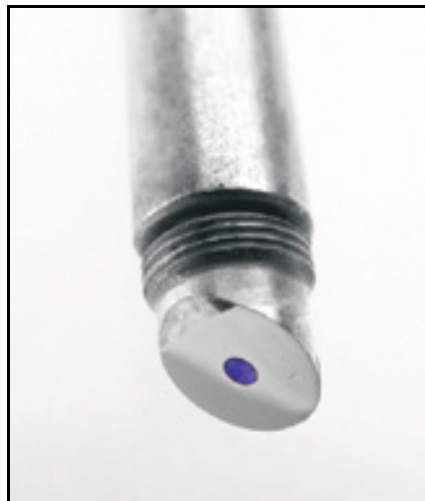
Specification	Value
Fiber core	1000 μm silica
Fiber cladding	Silica
Fiber jacketing	Stainless steel
Outer diameter	1/8"
Length	2.5"
Amount of pressure that can be applied	300 psi

Specification	Value
Probe temperature range	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector	SMA 905
Response time (without overcoat)	<1 second
Compensation	Temperature compensation only
Probe reconditioning	Yes (FOXY-RECOV @ \$100)
Re-calibration	Required when probe is replaced or cleaned by autoclave
Probe lifetime	1 year

FOXY-T1000 Stainless-steel Fiber Optic Probe with Light Shield

The FOXY-T1000 is a 1000- μm silica core, silica-clad fiber encased in a stainless-steel ferrule with a screw-on tip. The 7" long probe has an outer diameter of 1/4" and is extremely rugged. The probe can withstand pressure up to 3000 psi and temperature up to 80° C. It is designed for use with the 600- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-T1000 Stainless Steel Probe with Light Shield

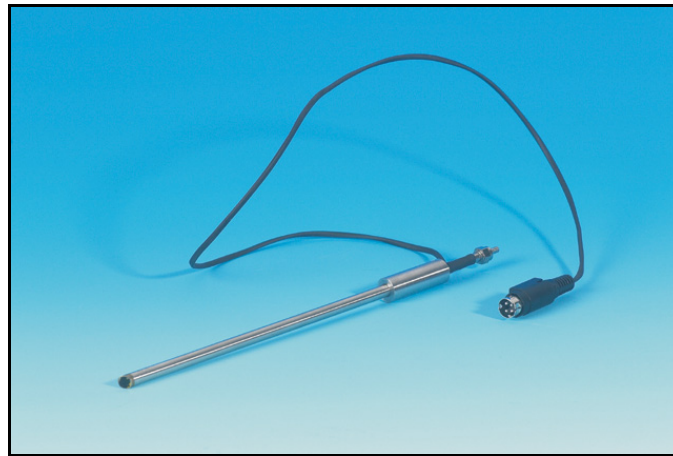
FOXY-T1000 Specifications

Specification	Value
Fiber core:	1000 μm silica
Fiber cladding:	Silica
Fiber jacketing:	Stainless steel
Outer diameter:	1/4"
Length:	7"
Amount of pressure that can be applied:	3000 psi
Probe temperature range:	-80° C to 80° C (reflects range of ruthenium complex in the sol-gel matrix)
Connector:	SMA 905
Response time (without overcoat):	<1 second
Compensation:	Temperature compensation only
Probe reconditioning:	Yes (FOXY-RECOV @ \$100)
Re-calibration:	Required when probe is replaced or cleaned by autoclave
Probe lifetime:	1 year

FOXY-T1000-RTD Stainless-steel Fiber Optic Probe with Light Shield

The FOXY-T1000-RTD is a 1000- μm silica core, silica-clad fiber encased in a stainless-steel ferrule with a screw-on light shield. The light shield prevents ambient light from entering the probe. The 7" long probe has an outer diameter of 1/4" and is extremely rugged. The probe can withstand pressure up to 3000 psi and temperature up to 80° C. It is designed for use with the 600- μm bifurcated optical fiber assembly.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY-T1000-RTD Stainless Steel Probe with Light Shield

FOXY-T1000-RTD Specifications

Specification	Value
Fiber core	1000 μm silica
Fiber cladding	Silica
Fiber jacketing	Stainless steel
Outer diameter	1/4 "
Length	7 "
Amount of pressure that can be applied	3000 PSI
Probe temperature range	-80 °C to 80 °C
Connector	SMA 905
Response time (without overcoat)	< 1 second
Compensation	Temperature compensation only
Probe reconditioning	Yes (FOXY-RECOV @ \$100)
Recalibration	Required when probe is replaced or cleaned by autoclave
Probe lifetime	1 year

FOXY-RESP Respiration Probe

The FOXY-RESP Respiration Probe is typically used for measuring oxygen tension in respiratory gasses. It is composed of a 2-meter cluster of seven 200- μm fibers in a Torlon jacket. The probe can withstand pressure up to 300 psi and temperature up to 80° C.

See [Oxygen Sensing Probes – General Information](#) at the beginning of this chapter for information on cleaning and sterilizing oxygen probes.



FOXY Respiration Probe

FOXY-RESP Specifications

Specification	Value
Fiber bundle	(7) 200 μm core diameter fibers (6 illumination, 1 read)
Fiber assembly length	2 meters
Fiber jacketing	Torlon
Outer diameter:	1/4"
Amount of pressure that can be applied	300 PSI
Probe temperature range	-80 °C to 80 °C
Connector	SMA 905
Response time (without overcoat)	< 50 msec (no overcoat)

Specification	Value
Compensation	Temperature
Probe reconditioning	N/A – disposable sensor films
Re-calibration	Required with each new film
Probe lifetime	>1 year

Factory Calibration Services

When measuring oxygen, you normally must keep the sample at a constant temperature. However, if you cannot maintain the sample at a constant temperature (+/-3 °C), you can perform a temperature calibration using the calibration function in the OOISensors Software.

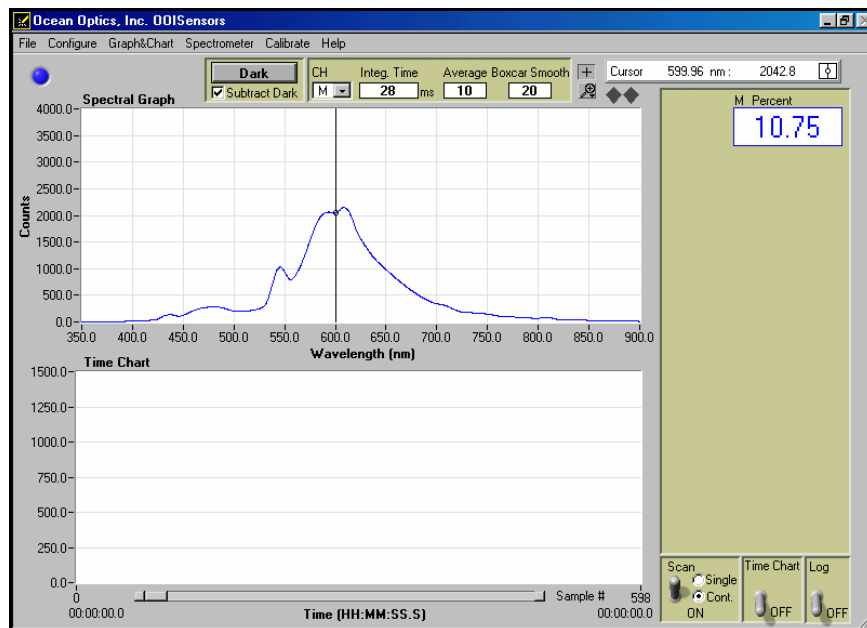
Ocean Optics can also perform the temperature calibration for your. The FOXY-CAL is an in-house factory-calibration service for environments from 0 °C to 80 °C. The FOXY-CAL-EXT is a factory-calibration service for extended temperature ranges below 0 °C or above 80 °C. You'll need to determine the temperature and the oxygen concentration range of your sample before ordering an in-house calibration service.

Calibration Option	Temperature Range	O2 % Range	Price
FOXY-CAL	0 °C to 80 °C	0 to 100%	\$199.00
FOXY-CAL-EXT	-20 °C to 80 °C	0 to 100%	\$299.00

FOXY-RECOV Probe Reconditioning

Occasionally, your probe overcoats may become compromised and require reconditioning. Ocean Optics offers the FOXY-RECOV and FOXY-RECOV-N (for needle probes) probe reconditioning services.

When a probe overcoat becomes compromised, you will observe spectra similar to what is displayed below:



Sample spectra indicative of a compromised probe overcoat

FOXY-AF and FOXY-AF-MG Silicone Overcoats

The FOXY-AF Silicone Overcoat and the FOXY-AF-MG Silicone Medical-grade Overcoat are room temperature vulcanized (RTV) silicone adhesives used as additional coatings for the oxygen probes. Overcoats protect the oxygen probes from ambient light, improve chemical resistance, and eliminate refractive index effects of the sensing environment. A silicone overcoat is required for applications involving liquids or gas-to-liquid activity.

FOXY-AF is a multi-surface healthcare-grade silicone while the FOXY-AF-MG is a high-strength acetoxy, implant-grade silicone. The FOXY-AF-MG provides a thicker and stronger coating than the FOXY-AF. Both overcoats increase the response time of the sensor.

The overcoats are made from a silicone adhesive designed for use in fabricating elastic bonds to silicone, polyester, and other substrates. Following cure, the overcoat is of the same chemical composition as other implant-grade silicone elastomers and it contains no solvents or plasticizers. Both overcoats are designed and tested to meet ISO 10993 biocompatibility test requirements.

Cleaning

Use a 10% hypochlorite detergent solution to clean probes with an overcoat. Using detergents to clean the probe does not necessitate calibration.

Sterilizing

See the Sterilization table in the [Oxygen Sensing Probes – General Information](#) section of this chapter for information on sterilizing probes with overcoats. It is the user's responsibility to validate any sterilization method chosen.

Overcoat Specifications

Specification	FOXY-AF Overcoat	FOXY-AF-MG Overcoat
Response time in oxygen gas (at 1 atmosphere):	20 to 30 seconds	20 to 45 seconds
Response time in dissolved oxygen in H ₂ O:	30 to 50 seconds	60 to 90 seconds
Coating stability:	Poor in long term	Very good
Resistance to chemicals (organic solvents):	Very good	Excellent
Coating hardness:	Soft-hard	Hard
Tensile strength:	780	
Tear strength:	70 ppi	
Cohesive/adhesive strength:	18 ppi	
Cytotoxicity:	Non-toxic	

Oxygen Process Temperature Control Accessories

Temperature affects the fluorescence decay time, the fluorescence intensity and the collisional frequency of the oxygen molecules with the fluorophore -- and therefore, the diffusion coefficient of oxygen. Temperature also affects the solubility of oxygen in samples. The net effect of temperature fluctuations is seen as a change in the calibration slope. If you cannot maintain the sample at a constant ($\pm 3^{\circ}\text{C}$) temperature, then you should calibrate your Fiber Optic Sensors System by using the temperature compensation features and measuring temperature and oxygen concurrently. The FOXY-TS1 Thermistor and the FOXY-TK1 Thermocouple monitor the temperature of the sensing environment and OOISensors Software corrects for changes in data due to temperature fluctuations.

FOXY-TS1 Omega Thermistor

A thermistor, a type of temperature-to-resistance transducer, is able to transduce temperature into a continuous electrical signal. A circuit measures the resistance of the thermistor and provides an output voltage proportional to the temperature. The FOXY-TS1 Thermistor from Omega is a stainless-steel tubular electrode probe most often used for liquid immersion. The FOXY-TS1 can monitor temperatures from 0°C to 100°C . It connects to the FOXY-T-MOD-1, an RS-232 module that interfaces the thermistor to your PC.

Application Tips

- Immerse the FOXY-TS1 to its cap.
- Use detergents to clean the FOXY-TS1 after use.

Operation

► Procedure

1. Connect the FOXY-TS1 to the FOXY-T-MOD-1 RS-232 module. (Rack-mounted systems have the modules installed into the rack box.)
2. Connect the FOXY-T-MOD-1 RS-232 module to the PC. You must have an available serial port on your PC. Note the serial port number (also called COM Port) on the PC to which you are interfacing.
3. Plug the +12VDC wall transformer into an outlet and connect it to the FOXY-TS1.
4. Open OOISensors software and select **Configure | Spectrometer** from the menu. Select the **Sensors** tab. Next to the **Temperature Measurement** option, select **Omega D5xx1 RS232** for automatic temperature monitoring. Finally, enable the **Compensate** function.
5. Select the COM Port number (next to **Serial Port**) on your PC to which the thermistor is connected.

6. Locate the port on the module that is connected to the thermistor in use.

Note

The RS-232 module can support up to four thermistors, and each port on the module is labeled.

7. Next to **D5xx1**, select between 0 and 3. (If you only have one thermistor, select 0.)
8. Enable the **Chart** function to view a chart of the temperature values.

FOXY-TS1 Specifications

Specification	Value
Length	5.375"
Outer diameter	1/8"
Temperature range	0° C to 100° C
Accuracy	+/-0.1° C
Data acquisition	FOXY-T-MOD-1 RS-232 module
Probe material	Stainless steel
Type	2252 Ohm at 25° C

FOXY-TK1 K-type Omega Thermocouple

A thermocouple consists of two dissimilar metals bonded to each other, typically by welding. The bimetallic junction develops a small voltage that varies with temperature. A thermocouple also provides accurate and consistent measurements and operates over a wide temperature range. The FOXY-TK1 Thermocouple from Omega is an electrode that can monitor temperatures from -150° C to 220° C. It is smaller and more flexible than the thermistor. The thermocouple must be connected to the FOXY-T-MOD-K, which is an RS-232 module that interfaces the thermocouple to your PC. The FOXY-T-MOD-K can support up to four thermocouples. The software can then apply algorithms to the readings to correct for temperature fluctuations.

Application Tips

- Immerse the FOXY-TK1 to its cap.
- Use detergents to clean the FOXY-TK1.

Operation

► Procedure

1. Connect the FOXY-TK1 to the 10-foot wire (model number FOXY-T-WIRE) that comes with the thermocouple.
2. Connect the FOXY-TK1 and the wire to the FOXY-T-MOD-K RS-232 module. (Rack-mounted systems have the modules installed into the rack box.)
3. Connect the FOXY-T-MOD-K RS-232 module to your PC. You must have an available serial port on your PC. Note the serial port number (also called COM Port) on the PC to which you are interfacing.
4. Plug the +12VDC wall transformer into an outlet and connect it to the FOXY-TK1.
5. Open OOISensors and select **Configure | Spectrometer** from the menu. Select the **Sensors** tab. Next to **Temperature Measurement**, select **Omega D5xx1 RS232** for automatic temperature monitoring. Finally, enable the **Compensate** function.
6. Select the COM Port number (next to **Serial Port**) on your PC to which the thermocouple is connected.
7. Locate the port on the module that is connected to the thermistor in use.

Note

The RS-232 module can support up to four thermocouples, and each port on the module is labeled.

8. Next to **D5xx1**, select between 0 and 3. (If you only have one thermocouple, select 0.)
9. Enable the **Chart** function to view a chart of the temperature values.

FOXY-TK1 Specifications

Specification	Value
Length	6"
Outer diameter	1/8"
Temperature range	-150° C to 220° C
Accuracy	2.2° C or 0.75% of reading with precision of 1° C

Specification	Value
Data acquisition	FOXY-T-MOD-K RS-232 module
Probe material	Chomega-Alomega 304 stainless steel
Connector body	Glass-filled Nylon

FOXY-TK1-W K-type Omega Thermocouple

The FOXY-TK1-W operates in a similar manner as the FOXY-TK1. However, the FOXY-TK1-W is a wire thermocouple, as opposed to a K-type thermocouple.

FOXY-TK1-W Specifications

Specification	Value
Length	1 meter
Outer diameter	3 mm"
Temperature range	-50° C to 220° C
Accuracy	2.2° C or 0.75% of reading with precision of 1° C
Data acquisition	FOXY-T-MOD-K RS-232 module

USB-LS-450-TP Temperature Probe

This is a 16-gauge needle type RTD only for use with the USB-LS-450 Blue LED Pulsed Light Source. The probe has a temperature range of -50 °C to 200 °C. The USB-LS-450-TP connects to the USB-LS-450 Light Source via a circular 4-pin connector located on the side of the USB-LS-450.

USB-LS-450-TP Specifications

Specification	Value
Length	6"
Outer diameter	1/8"
Temperature range	-50° C to 200° C

Specification	Value
Element type	Platinum 100 ohm Single 3 Wire
Sheath material	316 SS

Light Sources

The LS-450 Blue LED is specially designed for use with the oxygen sensor. A linear voltage regulator provides constant power to the LED. External power is supplied by a 12 Volt DC power transformer. The LS-450 Blue LED pulsed source has a cable for external connection to the spectrometer. The R-LS-450 Blue LED is a card-mounted pulsed source for mounting in a spectrometer enclosure or 19" rack system.

Other light sources available include the USB-LS-395 Blue LED (395 nm) for direct attachment to the USB4000 Spectrometer, the rack-mounted R-LS-395, and the rack-mounted Dual Blue 395 nm LED light source.

LS-450 Blue LED Pulsed Light Source

The LS-450 Blue LED Pulsed Light Source is a compact, low-cost light-emitting diode that produces pulsed or continuous spectral output at 470 nm -- the blue region -- for high-sensitivity emission fluorescence measurements. The LS-450 excitation source can be combined with other sampling optics for fluorescence applications.



LS-450 Blue LED Pulsed Light Source

Using the Continuous Mode

► Procedure

1. Turn the switch on the back of the LS-450 to **Contin.** This means that the light coming from the LS-450 is continuous.
2. Change the position switch to **Off** to turn off the lamp.

Using the Pulsed Mode

► Procedure

1. Plug one end of the DB-15 accessory connector into the back of the LS-450 and the other end into the back of the S2000.
2. Turn the switch on the back of the LS-450 to "pulsed" for pulsed mode of operation. In this mode, the spectrometer controls the pulsing of the LS-450.
3. Remove the spectrometer from its housing. Do not tamper with the optical bench.

Note

If you have more than one channel in your system, you may have to disconnect the channels from one another. The master spectrometer channel is always on the bottom of a multiple channel system.

4. Locate Jumper Block 3 (JP3). This jumper block consists of 10 pins, which are labeled by rows: /16, /14, /12, /10 and 2.

Consult the table below for information on which pins you should jumper to create the required pulses per second for the A/D converter interfaced to the S2000. (The default setting from the factory is /16.) For example, if you have an ADC1000 A/D converter, you have four choices for how many pulses per second can come out of your LS-450: 976, 244, 60 and 15. If you select 244 pulses per second, place a jumper over the pins next to the /14 label.

S2000 JP3 Post #	DAQ700 Frequency (Hz)	SAD500 Frequency (Hz)	ADC1000 Frequency (Hz)
/10	98.0	488.0	976.0
/12	24.0	122.0	244.0
/14	6.1	30.0	60.0
/16	1.5	7.6	15.2

When using the pulsed flash mode, you need to ensure that a constant number of flashes occur for every integration cycle. To achieve a constant number of flashes per integration cycle, the integration time must be a multiple of that shown in the table below. Integration times are set in the software.

S2000 JP3 setting	Integration time for DAQ700 (multiple of)	Integration time for SAD500 (multiple of)	Integration time for ADC1000 (multiple of)
/10	8 (with a min. value of 24 ms)	4	4
/12	32	8	4
/14	128	32	16
/16	512	128	64

LS-450 Specifications

Specification	Value
Physical dimensions:	9.0 cm x 5.0 cm x 3.2 cm (LWH) 3.5" x 2.0" x 1.25" (LHW)
Spectral output:	20 mA Total output power = 500 μ W Typical power into 600 μ m fiber = 75 μ W (+/-25%) Typical power into 200 μ m fiber = 6 μ W (+/-25%)
Stability (after 2 minutes):	+/-2 counts

R-LS-450 Rack-mount Blue LED Pulsed Light Source

The R-LS-450 Blue LED Pulsed Light Source is a compact, low-cost light-emitting diode that produces pulsed or continuous spectral output at 470 nm - the blue region - for high-sensitivity emission fluorescence measurements. The R-LS-450 is the rack mount version of the LS-450 and can be configured to operate in continuous wave mode or pulsed mode through both manual operation and software.



R-LS-450 Rack Mount Blue LED Pulsed Light Source

The R-LS-450 is shipped with the following jumper pin configuration:

- Jumper over pins in JP1
- Jumper over the Manual pins in JP3
- Jumper over the 2¹⁰ pins in JP2 (for the fastest available pulse rate)

Configuring the R-LS-450 Board

You can configure the lamp's performance through the use of a switch and three jumper blocks on the circuit board of the R-LS-450 and, if desired, through one jumper block on the circuit board of the S2000 spectrometer. The following sections detail the different configuration options for the R-LS-450. Determine the best mode of operation for your application, and configure your system accordingly.

S1 Switch

The S1 Switch on the R-LS-450 can be positioned in three modes: continuous wave operation, no operation, and pulsed operation.

Jumper Block 1 (JP1)

There is one set of pins in JP1. If other jumper blocks are configured correctly, a jumper over JP1 allows you to turn the R-LS-450 on and off via the Enable Strobe feature in OOISensors Software, and even allows you to control the pulse rate through OOISensors' Flash Delay feature.

Note

This feature is only available with an ADC1000 A/D converter and with a J-series or later version of the S2000. If you have a J-series S2000, the third letter in your S2000 serial number will be J.

Jumper Block 2 (JP2)

There are nine sets of pins in JP2. The number of pulses per second emitted from the R-LS-450 depends on the pins you jumper on JP2. However, pulses per second are also dependent upon the master frequency of your A/D converter.

- A jumper over the CW pins causes the R-LS-450 to continually operate. This means there is no pulsing of the light source. However, other jumper blocks must be configured correctly. *This configuration is not recommended for use with the Fiber Optic Sensors System.*
- A jumper over the CS pins controls the pulse rate via the OOISensors Software. (See Using JP3 on the S2000 on the following pages for more information.)
- A jumper over the 2^{16} , 2^{15} , 2^{14} , 2^{13} , 2^{12} , 2^{11} and 2^{10} pins controls the pulse rate per second of the R-LS-450 (depending on the A/D converter interfaced to the S2000). See Table 6-3 for pulse rates.

Jumper Configuration and Pulse Rates for A/D Converters

Pins on the JP2	Function	DAQ700 Frequency (Hz)	SAD500 Frequency (Hz)	ADC1000 Frequency (Hz)
CW	Continuous Mode	0	0	0
2^{16}	Divide by 2^{16}	1.5	7.6	15.2
2^{15}	Divide by 2^{15}	3.1	15.2	30.4
2^{14}	Divide by 2^{14}	6.1	30.0	60.8
2^{13}	Divide by 2^{13}	12.2	60.8	122.0
2^{12}	Divide by 2^{12}	24.0	122.0	244.0
2^{11}	Divide by 2^{11}	48.0	244.0	488.0
2^{10}	Divide by 2^{10}	98.0	488.0	976.0
CS*	Continuous Strobe	N/A	N/A	Software Controlled

Jumper Block 3 (JP3)

There are two sets of pins in JP3. The jumper position determines the source of control for the R-LS-450: manual or remote (software) control. However, other jumper blocks must be configured correctly.

R-LS-450 Operating Matrix

The following table provides information on configuring the jumper blocks on the R-LS-450.

S1 Switch	JP1	JP3	LED Status
Off	No jumper	No jumper	Off
CW	No jumper	No jumper	Continuously on
CW	Jumpered	Jumper Remote pins	Continuous wave mode controlled by software (see Continuous Wave Mode with the S2000's JP3 for more information)
CW	Jumpered	Jumper Manual pins	Continuously on
Pulsed	No jumper	No jumper	Pulse rate determined by JP2 on the R-LS-450 board (see the JP2 table for pulse rates)
Pulsed	Jumpered	Jumper Remote pins	Pulsed mode controlled by software (see Pulsed Mode with the S2000's JP3 for more information)
Pulsed	Jumpered	Jumper Manual pins	Pulse rate determined by JP2 on the R-LS-450 board (see the JP2 table for pulse rates)

Using JP3 on the S2000

You can also control the R-LS-450 with the S2000 Spectrometer and OOISensors by configuring Jumper Block 3 (JP3) on the S2000 circuit board. JP3 allows you to control the R-LS-450 through the OOISensors Software.

Continuous Wave Mode with the S2000's JP3

The Continuous Wave Mode is **not** recommended for use with the Fiber Optic Sensors System. However, if you want to enable or disable the lamp on the R-LS-450 while it is in continuous wave mode through the Enable Strobe function in the OOISensors Software, only do so if the following conditions are met:

- The switch is turned to continuous wave mode
- There is a jumper over the pins in JP1 of the R-LS-450
- There is a jumper over the CW pins in JP2 of the R-LS-450
- There is a jumper over the Remote pins in JP3 of the R-LS-450
- There is a jumper over the 2 pins in JP3 of the S2000 board

Pulsed Mode with the S2000's JP3

You can control the R-LS-450's pulses per second if the following conditions are met:

- The switch is turned to pulsed mode
- There is a jumper over the pins in JP1 of the R-LS-450
- There is a jumper over the CS pins in JP2 of the R-LS-450
- There is a jumper over the over the Remote pins in JP3 of the R-LS-450
- There is a jumper over pins labeled /16, /14, /12, or /10 in JP3 of the S2000, depending on the pulse rate you need. The pulses per second are also dependent upon the frequency of your A/D converter.

See the table below for configuration choices:

Pulse Mode Configuration Options for A/D Converters

Pins on the S2000's JP3	DAQ700	SAD500	ADC1000
/16	1.5	7.6	15.2
/14	6.1	30.4	60.8
/12	24.0	122.0	244.0
/10	98.0	488.0	976.0

OOISensors Flash Delay

You can control the pulses per second of the R-LS-450 through the **Flash Delay** function in the OOISensors Software if the following conditions are met:

- You are using an ADC1000 A/D converter
- The switch is turned to pulsed mode
- There is a jumper over the pins in JP1 on the R-LS-450 board
- There is a jumper over the pins labeled CS in JP2 on the R-LS-450 board
- There is a jumper over the pins labeled Remote in JP3 on the R-LS-450 board
- There is a jumper over the pins labeled 2 in JP3 on the S2000 board

Setting the Integration Time

When using any of the pulsed modes for the R-LS-450, you need to ensure that a constant number of flashes occurs for every integration cycle. This achieves a continuous and stable signal. Set the appropriate integration time in the OOISensors Software.

To achieve a constant number of flashes per integration cycle, the integration time must be a multiple of those shown in the table below, according to the A/D converter being used:

Pins on the JP3 (of the S2000)	Integration time for DAQ700 (multiple of)	Integration time for SAD500 (multiple of)	Integration time for ADC1000 (multiple of)
/16	512	128	64
/14	128	32	16
/12	32	8	4
/10	8 (with a min. value of 24 ms)	4	4
2	N/A	N/A	N/A

USB-LS-450

The USB-LS-450 module connects to the USB2000-FL Spectrometer via a 10-pin connector on the front of the spectrometer. The spectrometer provides power to the USB-LS-450, and enables synchronization functions and I2C communications.

In addition, the USB-LS-450 features a built-in, 24-bit analog-to-digital converter with an analog front end that is configured for a 100 ohm platinum RTD (resistance temperature device). This is particularly useful when configured as part of the Fiber Optic Oxygen Sensor. As an added benefit for oxygen probe users, the USB-LS-450 has on-board memory to store temperature calibration coefficients and oxygen calibration coefficients.

The USB-LS-450 board allows for software-controlled switching between pulsed and continuous operation of the LED.

USB-LS-450 LED -- Specifications

Specification	Value
Power output	No less than 60 μ W into a 600 μ m optical fiber
LED drive current	20 mA +/-150 μ A
Maximum modulation frequency	1 kHz
0.5% stability time	Less than 1 minute
Temperature-dependent drift	+0.1%/degree C

USB-LS-450 Temperature Measurement Specifications

Specification	Value
Precision:	Better than 0.1° C
Accuracy:	Better than 0.5° C
Maximum data rate:	15 samples/second

USB-LS-450 Power Requirements

Specification	Value
Input voltage range	3.0-8.0 volts
Quiescent range	~20 mA
Current with light on	~20 mA

Bifurcated Optical Fiber Assemblies

Ocean Optics' bifurcated fiber optic assemblies easily connect to oxygen sensors, light sources, and miniature fiber optic spectrometers via SMA terminations and an additional splice bushing. These single-strand, multi-mode optical fibers are silica-core and silica-clad. Standard assemblies are 2 meters in length, although custom fibers can be greater than 2 meters. The ends of the fibers are cleaved, epoxied into the connectors, and polished.

Bifurcated assemblies are shaped like a "Y" with a stainless steel breakout located midway from the ends of the fibers. The common end (the tail of the "Y") of a bifurcated assembly has two fibers side by side. The common end of a bifurcated fiber can be coupled to a larger diameter probe with a splice bushing. Bifurcated fibers used in Fiber Optic Sensors System include the following:

Bifurcated Fiber	Description
BIF200	2 separate 200- μ m optical fibers in a "Y" configuration. For use with the FOXY-AL300 and FOXY-24G probes
BIF400	2 separate 400- μ m optical fibers in a "Y" configuration. For use with the FOXY-PI600 probe
BIF600	2 separate 600- μ m optical fibers in a "Y" configuration. For use with the FOXY-R, FOXY-OR125, FOXY-OR125G and the FOXY-T1000 probes

Use Notes

- Gently remove the plastic cover from the SMA connector before use. Pulling the SMA connector away from the fiber when removing the plastic cover will permanently damage the fiber.
- When fibers break, they stop transmitting light. Inspect fibers by eye to determine if light is being transmitted.
- Do not coil the fiber too tightly, or bend it at a sharp angle. The maximum sustained bend radius of a 400 μm fiber is 10 cm. Bending the fiber can cause attenuation. To minimize this effect, add extra strain relief to both ends of the fiber.
- Do not exceed the temperature specifications for the materials involved: 200°C for the fiber, 100°C for PVC cabling, and 100°C for standard epoxy.
- Keep connectors and probe tips covered when the fibers are not being used.
- Clean ends of the fibers with lens paper and distilled water, alcohol, or acetone. Avoid scratching the surface.
- Do not immerse fiber ends in caustic materials or other solutions that can damage quartz or aluminum.

Bifurcated Fiber Assembly Specifications

Specification	Value
Connector/termination:	SMA 905
Fiber core:	Pure fused silica
Fiber cladding:	Doped fused silica
Fiber buffer:	Polyimide (temperature range -40° C to +300° C)
Sheathing/cabling:	PVC with Kevlar reinforcement (standard) Silicone Monocoil (optional – autoclaveable) Stainless steel BX flexible jacketing (optional)
Mode structure:	Step-index multi-mode
Numerical aperture:	0.22 \pm .02
Recommended minimum bend radius:	Momentary = 200x the fiber radius; long term = 400x the fiber radius

FOXY/HOXY/FOSPOR General Specifications

Specification	FOXY	HIOXY	FOSPOR
Principle	Photoluminescence-quenching using Ru compound. Measures O ₂ partial pressure.	Photoluminescence-quenching using Ru compound. Measures O ₂ partial pressure.	Photoluminescence-quenching using Pt compound. Measures O ₂ partial pressure.
Applications	Benign environment Aqueous liquids and vapors	Hydrocarbon-based liquids and vapors Fuels Alcoholic beverages Vegetable oil	Low O ₂ (0–5%) Vacuum systems packaging
Time constant (μsec) dynamic range	Air: 2.50 N ₂ : 5.00 Low: 1.00 High: 7.00	Air: 0.50 N ₂ : 2.50 Low: 0.30 High: 6.00	Air: 10.00 N ₂ : 50.00 Low: 3.00 High: 70.00
Temperature range	-50°C to +80°C	-50°C to +80°C	0°C to +60°C
Response time	~1 sec – liquid/gas no overcoating 15–20 sec in gas-overcoated 30–45 sec in liquid-overcoated	~1 sec – liquid and gas	~1 sec – liquid/gas no overcoating 15–20 sec in gas-overcoated 30–45 sec in liquid-overcoated
Overcoating	With or without overcoating	No overcoating	With or without overcoating

FOSPOR Specifications

Parameter	Dissolved Oxygen in Liquid	Oxygen Gas (at 1 atmosphere)
Sensor mechanism	Phase shift due to change in partial pressure of O ₂	
Dynamic range	0–40.7 ppm, 0–760 mmHg partial pressure	0–100 mole%, 0–760 mmHg partial pressure
Response time	Standard: ~1 sec 20–50 seconds with silicone overcoating	Standard: ~1 sec 20–30 seconds with silicone overcoating
Resolution	0.002 ppm at room temperature	0.005% at room temperature
Stability	Drift < 0.00008 ppm O ₂ per hour at low DO Drift ~ 0.006 ppm O ₂ per hour in air-saturated water	Drift ~ 0.0002% O ₂ drift per hour at low O ₂ Drift ~ 0.015% O ₂ per hour air
Minimum detectable limit	0.002 ppm at room temperature	0.005 mole% (0.04 mm Hg)
Accuracy (0-20% O ₂ , 0-50°C)	5% of reading (using 2 nd order polynomial fit to multipoint calibration)	
Temperature	Temperature sensor and software-supported compensation	
Temperature range	0–60°C	
Probe reconditioning	HIOXY-RECOV: \$150 HIOXY-RECOV-N (for HIOXY needle probes: \$175)	
Calibration	Standard 2-point calibration (linear fit) Multipoint calibration (2 nd order polynomial fit) for improved accuracy and broad dynamic range applications Single-temperature, single point calibration using factory-calibrated oxygen probes	
Temperature recalibration	Only when probe is replaced/refurbished	
Storage conditions	No specific requirements	
Cleaning	10% hypochlorite detergent	
Sterilization	Gamma radiation or sodium hypochloride (bleach or Clorox)	

FOXY/MFPF Specifications

Specification	Dissolved Oxygen in H ₂ O	Oxygen Gas (at 1 atmosphere)
Sensor mechanism	Phase shift due to change in partial pressure of O ₂	
Dynamic range	0–40.7 ppm 0–760 mm Hg partial pressure	0–100% (mole percent) 0–760 mm Hg partial pressure
Response time (standard)	~ 1 second	
Response time (w/overcoat)	30–50 seconds	20–30 seconds
Temperature	Temperature sensor and software-supported compensation	
Temperature range	-50° C to 80° C	
Chemical compatibility of coating	Unaffected by pH change or salinity Safe with 50% methanol, acids, sodium sulfite, moisture, CO ₂ , and CO Not recommended with strong bases (pH>10), styrene, ethanol, liquid acetone, acetonitrile, HF and BTEX solvents	
Resolution	0.02 ppm at room temperature	0.05% (0.04 mmHg) at room temperature
Calibration	Standard 2-point calibration (linear fit) Multipoint calibration (2 nd order polynomial fit) for improved accuracy and broad dynamic range applications Single-temperature, single-point calibrations using factory-calibrated oxygen probes	
Accuracy: (0–20% O ₂ , 0–50°C)	5% of reading (using 2 nd order polynomial fit to multipoint calibration)	
Lowest detectable limit	0.02 ppm	0.05% (0.04 mm Hg)
Stability	Drift<0.01 ppm per hour	Drift ~0.03% O ₂ per hour
Probe reconditioning	FOXY-RECOV \$100 FOXY-RECOV-N (for FOXY needle probes) \$125	
Temperature recalibration	Only when probe is replaced/refurbished	
Storage conditions	No specific requirements	
Cleaning	10% hypochlorite detergent for overcoated probes	
Sterilization	Gamma radiation or sodium hypochlorite (bleach or Clorox)	
Probe lifetime	Refurbishing recommended once per year	

HIOXY/MFPF Specifications

Specification	Dissolved Oxygen in Liquid	Oxygen Gas (at 1 atmosphere)
Sensor mechanism	Phase shift due to change in partial pressure of O ₂	
Dynamic range	0–40.7 ppm 0–760 mm Hg partial pressure	0–100% (mole percent) 0–760 mm Hg partial pressure
Response time (standard)	~ 1 second	
Response time (w/overcoat)	Not applicable	
Temperature	Temperature sensor and software-supported compensation	
Temperature range	-50° C to 80° C	
Chemical compatibility of coating	Compatible with variety of hydrocarbon-based fluids and vapors such as solvents, fuels and oils Tested with jet fuel, diesel, gasoline, hydraulic fluids, ethanol, and wine	
Resolution	0.02 ppm at room temperature	0.05% (0.4 mm Hg) at room temperature
Calibration	Standard 2-point calibration (linear fit) Multipoint calibration (2 nd order polynomial fit) for improved accuracy and broad dynamic range applications Single-temperature, single-point calibrations using factory-calibrated FOXY probes	
Accuracy: (0–20% O ₂ , 0–50°C)	5% of reading (using 2 nd order polynomial fit to multipoint calibration)	
Lowest detectable limit	0.02 ppm at room temperature	0.05% (0.4 mm Hg)
Stability	Drift < 0.002 ppm per hour	Drift ~ 0.01% O ₂ per hour
Probe reconditioning	HIOXY-RECOV \$150 HIOXY-RECOV-N (for HIOXY needle probes) \$175	
Temperature recalibration	Only when probe is replaced/refurbished	
Storage conditions	No specific requirements	
Cleaning	10% hypochlorite detergent	
Sterilization	Gamma radiation or sodium hypochlorite (bleach or Clorox)	
Probe lifetime	Refurbishing recommended once per year	

Troubleshooting

Overview

The following sections contain information that may assist you in troubleshooting some of the more common problems encountered when using the Fiber Optic Sensor System.

General Troubleshooting Procedures

Issue	Possible Cause	Resolution
OOISensors not performing properly.	Various, including incorrect hardware installation, incorrect constants, or calibration issues.	Perform the following steps to troubleshoot the OOISensors system: 1. Load OOISensors. 2. Ensure that no other Ocean Optics software application is running in conjunction with OOISensors. 3. Unplug the USB cable from the PC and restart OOISensors. 4. Ensure that the entered constants are correct, then shut down OOISensors. This guarantees that the correct constants are in place each time OOISensors starts up.
Receiving Incorrect O2 readings	Various	1. Check the units provided by the Factory Calibration 2. Ensure that the temperature probe reading is accurate (if applicable). 3. Zero Percent Calibration information – Ensure you are using a container that is impervious to oxygen in order to maintain a consistent level in your sample.

Issue	Possible Cause	Resolution
Peaks displayed in the graph screen are in incorrect places.	Wavelength coefficients may be incorrect, or probe overcoat may not be in place or may be damaged.	<p>Reload or re-enter the wavelength coefficients correctly.</p> <p>Confirm that the probe overcoat is in place.</p>
Incorrect counts obtained on Calibration screen	Incorrect fluorescence peak entered or dark measurement taken incorrectly.	<p>Ensure that the fluorescence peak is entered correctly on the Configuration Spectrometer screen. Verify that the LED was not on while the dark measurement was obtained, and retake the dark measurement if required.</p> <p>Note: If Enable Reference is selected on the Sensors tab of the Configure Spectrometer screen, you will not see counts. Rather, you will see the ratio of the fluorescence peak to the LED peak.</p>
Large peak at 900 nm with little fluorescence signal	There is a broken fiber inside probe and/or loose splice bushings.	Verify the condition of the fiber inside the probe and replace the probe if necessary. Tighten all splice bushings to ensure maximum performance.
Signal decay is excessively quick	There may be solvents or harmful chemicals in the sample, or you may have autoclaved or washed the probe with solvents.	Remove any solvents or harmful chemicals from the sample you are measuring. Furthermore, you should never autoclave or wash the probe with solvents. For information on chemicals harmful to Ocean Optics probes, see Chemical Effects on Oxygen Probes .
Unstable readings	A variety of possible causes, including loose fittings, low averaging, and probes or coupling fibers moving during measurements.	<p>Double-check and tighten all fittings.</p> <p>Increase signal averaging to at least 20.</p> <p>Immobilize probe during sample measurements.</p> <p>If these suggestions do not correct the unstable readings, contact Ocean Optics technical support for assistance.</p>
Calibration settings not retained	You may have disconnected the probe between sample measurements. Additionally, overcoat condition and sample temperature can affect calibration.	<p>Ensure that the probe overcoat is intact when moving the probe from gas to liquids.</p> <p>Confirm that the sample temperature is constant when taking sample measurements.</p> <p>Keep the probe connected to the spectrometer in between sample measurements.</p>

Issue	Possible Cause	Resolution
High response times when measuring Samples	Sample condition may be adversely affecting response time, or sampling port (on needle probes) is obstructed.	<p>Confirm that the sample is not viscous slurry. If this is the case, response times may range from 5 to 15 minutes.</p> <p>Ensure that the sampling port of the needle probe is unobstructed, if applicable.</p>
oxygen measurements are drifting	Unstable sample temperatures can cause drift. Additionally, drift can occur in either the LED or the probe (or both).	<p>Ensure that the sample temperature remains stable during measurement.</p> <p>To determine if the LED or probe is responsible for the drift, open OOIBase32 or SpectraSuite and run a time acquisition. Monitor the LED peak (if possible) and the fluorescence peak.</p> <p>For more information on using OOIBase32 or SpectraSuite, consult the appropriate instructions on the Ocean Optics website at http://www.oceanoptics.com.</p>
When calibrating the Fiber Optic Sensors System, the intensity counts do not change.	The fluorescence peak is saturated, or the software has stopped responding.	Verify that the fluorescence peak is not saturated and that OOISensors is still responding. If necessary, reboot the system and restart OOISensors.
LED is saturating the entire spectrum.	Hardware failure or configuration error.	<p>Verify that the bifurcated fiber is not broken, and replace the fiber if necessary.</p> <p>Verify that you properly connected the bifurcated fiber to the sensors system, and correct if necessary.</p>

Troubleshooting Device Installation

If you connect your Ocean Optics USB or PCI device to the computer prior to installing your Ocean Optics software application, you may encounter installation issues that must be corrected before your Ocean Optics device will operate properly.

Follow the applicable steps in this document to remove the incorrectly installed device, device driver, and installation files.

Note

If these procedures do not correct your device driver problem, you will need to obtain the *Correcting Device Driver Issues* document from the <http://www.oceanoptics.com>.

Remove the Unknown Device from Windows Device Manager

► Procedure

1. Open Windows Device Manager. Consult the Windows operating instructions for your computer for directions, if needed.
2. Locate the **Other Devices** option and expand the **Other Devices** selection by clicking on the "+" sign to the immediate left.

Note

Improperly installed USB devices can also appear under the Universal Serial Bus Controller option. Be sure to check this location if you cannot locate the unknown device.

3. Locate the unknown device (marked with a large question mark). Right-click on the **Unknown Device** listing and select the **Uninstall** or **Remove** option.
4. Click the **OK** button to continue. A warning box appears confirming the removal of the Unknown Device. Click the **OK** button to confirm the device removal.
5. Disconnect the USB device from your computer.
6. Locate the section in this chapter that is appropriate to your operating system and perform the steps in the following [Remove Improperly Installed Files](#) section.

Remove Improperly Installed Files

► Procedure

Open Windows Explorer.

1. Navigate to the **Windows | INF** directory.

Note

If the INF directory is not visible, you must disable the Hide System Files and Folders and Hide File Extensions for Known File Types options in Windows Folder Options. Access Windows Folder Options from Windows Explorer, under the **Tools | Folder Options** menu selection.

2. Delete the **OOI_USB.INF** in the INF directory. If your computer is running either the Windows 2000 or XP operating system, you must also delete the **OOI_USB.PNF** file in the INF directory.
3. Navigate to the **Windows | System32 | Drivers** directory.
4. Delete the **EZUSB.SYS** file.
5. Reinstall your Ocean Optics application and reboot the system when prompted.
6. Plug in the USB device.

The system is now able to locate and install the correct drivers for the USB device.

External Triggering

Ocean Optics S2000-series spectrometers, when used with OOISensors Software, provide two methods of acquiring data: no triggering for free running acquisitions controlled only from application's front window or external software trigger which limits scanning to when a high level signal is detected. See the Timing Tab section of Chapter 2: [OOISensors Software](#) for more information. OOISensors does not support external triggering.

External Software Trigger Mode allows you to use an external event to initiate the scan process in OOISensors. In the External Software Trigger Mode, you set the integration time (as well as all other acquisition parameters) in the software. The source for the integration clock comes from the A/D converter.

Note

In order for you to use the External Software Trigger option, it is imperative that you know the specifications and limitations of your triggering device. The design of your triggering device may prevent you from using the External Software Trigger mode as it is described here.

Consult the External Triggering Options document on the Ocean Optics website for more information: <http://www.oceanoptics.com/technical/externaltriggering.pdf>.

Chemical Effects on Oxygen Probes

Summary of Chemical Effects on Oxygen Probes

(Data Available upon Request)

Chemicals Known to be Harmful to Oxygen Probes

Chemical	Supporting Data Available
Strong Bases pH >10	Yes
Styrene	Yes
Ethanol	Yes
Toluene/Ethyl Acetate	No
Acetone	No
Acetonitrile	Yes
HF	No
Xylene (dissolves silicone and quenches fluorescence)	No
Benzene (Removes Silicone Overcoat)	No
Isopropyl Acetate	No
Heptane (Removes Silicone Overcoat)	Yes
Hexane (Removes Silicone Overcoat)	No

Chemical	Supporting Data Available
Gasoline (even in headspace)	Yes
N-Vinyl-2-Pyrrolidinone (even at 2.5%)	Yes
DMSO (>60 hours test slides)	No
Acrylonitrile	No
Hydrogen Peroxide (30%) irreversible quencher (smaller concentrations may be fine)	No

**Chemicals Known to Have No Effect on Oxygen Probes
(SOME REQUIRE PROTECTIVE OVERCOAT)**

Benign Chemicals	Supporting Data Available
50% Methanol (WITH OVERCOAT)	Yes
Ammonia	No
Acids	No
Sodium Sulfide	Yes
SF ₆ (test slide results)	Yes
NF ₃ (test slide results)	Yes
Perfluorohexane	Yes
Perfluorodecalin (C ₁₀ F ₁₈)	Yes
1 M NaOH	No
Benzene (short term measurements without silicone overcoat)	Yes
Sodium Hypochlorite	No
60% Isopropyl alcohol (w/ overcoat)	No

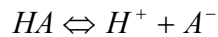
B: Chemical Effects on Oxygen Probes

pH Sensor Theory of Operation and Calculations

Overview

The following sections contain the equations that express the theory of pH sensor operation and calculations.

Theory of Operation and Calculations



$$pH = -\log[H^+]$$

$$K = \frac{[A^-][H^+]}{[HA]}$$

$$pK = -\log K = \log[HA] - \log[A^-] - \log[H^+]$$

↓

$$pH = pK + \log[A^-] - \log[HA]$$

or

$$pH = pK + \log \frac{[A^-]}{[HA]}$$

Model # 1

Looks at base peak only. No baseline correction. Uses nominal pK and slope.

$$[A^-] = \frac{[A^-]_{Abs}}{[A^-]_{HighpHAbs}}$$

$$[HA] = 1 - [A^-]$$

If

$$\frac{[A^-]}{[HA]} \leq 0 \text{ then } pH = 0$$

else

$$pH = pK + \log \frac{[A^-]}{[HA]}$$

Model # 2

Looks at base peak only. Corrects for baseline.

$$[A^-] = \frac{[A^-]_{Abs} - BaselineAbs}{[A^-]_{HighpHAbs} - HighpHBAbs}$$

$$[HA] = 1 - [A^-]$$

If

$$\frac{[A^-]}{[HA]} \leq 0 \text{ then } pH = 0$$

else

$$pH = pK + \log \frac{[A^-]}{[HA]}$$

Model # 3

Looks at base peak only. Uses the values of two buffers to predict absorbance of [A] High pH and [HA] High pH.

$$LowH = 10^{-pH_{LowBuffer}}$$

$$HighH = 10^{-pH_{HighBuffer}}$$

correct for baseline

$$[A^-]_{LowBufferAbs} = [A^-]_{LowBufferAbs} - LowBufferBaselineAbs$$

$$[A^-]_{HighBufferAbs} = [A^-]_{HighBufferAbs} - HighBufferBaselineAbs$$

calculate theoretical maximum absorbance at peak

$$[A^-]_{HighpHAbs} = \frac{LowH * [A^-]_{LowBufferAbs} * [A^-]_{HighBufferAbs} - HighH * [A^-]_{LowBufferAbs} * [A^-]_{HighBufferAbs}}{LowH * [A^-]_{LowBufferAbs} - HighH * [A^-]_{HighBufferAbs}}$$

calculate pK from the LowBuffer

$$[A^-] = \frac{[A^-]_{LowBufferAbs}}{[A^-]_{HighpHAbs}}, \quad [HA] = 1 - [A^-]$$

$$\text{if } \frac{[A^-]}{[HA]} > 0 \text{ then } pK_{low} = pH_{lowBuffer} - \log \frac{[A^-]}{[HA]} \text{ else } pK_{low} = 0$$

calculate pK from the HighBuffer

$$[A^-] = \frac{[A^-]_{HighBufferAbs}}{[A^-]_{HighpHAbs}}, \quad [HA] = 1 - [A^-]$$

$$\text{if } \frac{[A^-]}{[HA]} > 0 \text{ then } pK_{high} = pH_{HighBuffer} - \log \frac{[A^-]}{[HA]} \text{ else } pK_{high} = 0$$

use the average

$$\text{if } pK_{high} > 0 \text{ and } pK_{low} > 0 \text{ then } pK = \frac{pK_{high} + pK_{low}}{2}$$

$$[A^-] = \frac{[A^-]_{abs} - BaselineAbs}{[A^-]_{HighpHAbs}}, \quad [HA] = 1 - [A^-]$$

$$\text{If } \frac{[A^-]}{[HA]} \leq 0 \text{ then } pH = 0 \text{ else } pH = pK + \log \frac{[A^-]}{[HA]}$$

Model # 4

In addition to previous, it uses both acid and base peaks

$$\begin{aligned} LowH &= 10^{-pH_{LowBuffer}} \\ HighH &= 10^{-pH_{HighBuffer}} \end{aligned}$$

correct for baseline

$$\begin{aligned} [A^-]_{LowBufferAbs} &= [A^-]_{LowBufferAbs} - LowBufferBaselineAbs \\ [A^-]_{HighBufferAbs} &= [A^-]_{HighBufferAbs} - HighBufferBaselineAbs \\ [HA]_{LowBufferAbs} &= [HA]_{LowBufferAbs} - LowBufferBaselineAbs \\ [HA]_{HighBufferAbs} &= [HA]_{HighBufferAbs} - HighBufferBaselineAbs \end{aligned}$$

(Continued)

calculate theoretical maximum absorbance at peak

$$\begin{aligned} [A^-]_{HighpHAbs} &= \frac{LowH * [A^-]_{LowBufferAbs} * [A^-]_{HighBufferAbs} - HighH * [A^-]_{LowBufferAbs} * [A^-]_{HighBufferAbs}}{LowH * [A^-]_{LowBufferAbs} - HighH * [A^-]_{HighBufferAbs}} \\ [HA]_{HighpHAbs} &= \frac{LowH * [HA]_{LowBufferAbs} * [HA]_{HighBufferAbs} - HighH * [HA]_{LowBufferAbs} * [HA]_{HighBufferAbs}}{LowH * [HA]_{LowBufferAbs} - HighH * [HA]_{HighBufferAbs}} \\ DyeConstant &= \frac{-[HA]_{HighpHAbs}}{[A^-]_{HighpHAbs}} \end{aligned}$$

if $[HA]_{HighpHAbs} > [HA]_{Abs} - BaselineAbs$ then $Ratio = 5000$ else

$$Ratio = \frac{DyeConstant}{[HA]_{Abs} - BaselineAbs - [HA]_{HighpHAbs}}$$

If ($Ratio \leq 0.0005$) or ($Ratio \geq 5000$) then $pH = 0$

else

$$\begin{aligned} Ratio &= \log(Ratio) \\ pH &= pK + Ratio \end{aligned}$$

Revised Model #4

Looks at base peak only. Corrects for baseline.

$$[A^-] = \frac{[A^-]_{Abs} - BaselineAbs}{[A^-]_{HighpHAbs} - HighpHBaselineAbs}$$

$$[HA] = 1 - [A^-]$$

Then calculate the pK and slope values from...

$$pH = pK + Slope * \log \frac{[A^-]}{[HA]}$$

...where the two points you are using are the low buffer and high buffer

Then proceed to calculation

If

$$\frac{[A^-]}{[HA]} \leq 0 \text{ then } pH = 0$$

else

$$pH = pK + Slope * \log \frac{[A^-]}{[HA]}$$

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