

INSTECH

Fiber Optic Oxygen Monitor

Models 110 and 210

Operating Manual

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Most current version is available online at
<http://www.instechlabs.com/manuals.html>

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Software installation precautions-Order is very important

See page 11

With no USB connected—Install software but do not run

Attach USB

Run software.

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System Overview

The Model 110 is a single channel monitor containing 1 Serial A/D card, 1 light source card, 1 spectrometer card, 1 bifurcated fiber optic cable and a power supply. The Model 210 adds an extra light source, spectrometer and fiber optic cable.

These systems incorporate the latest in fiber optic probe technology to detect and record the concentration of oxygen, either in gaseous form or dissolved in liquids. Oxygen is sensed by the quenching of fluorescence of an indicator dye trapped in a matrix at the tip of the probe. Since this is an equilibrium measurement, there will be no "motion artifact" as is seen with polarographic electrodes. The fluorophore is excited by a pulsed blue LED light source and the resulting fluorescence is detected using a miniature spectrometer. The OOISensors software controls the spectrometer, light sources, display and data logging.

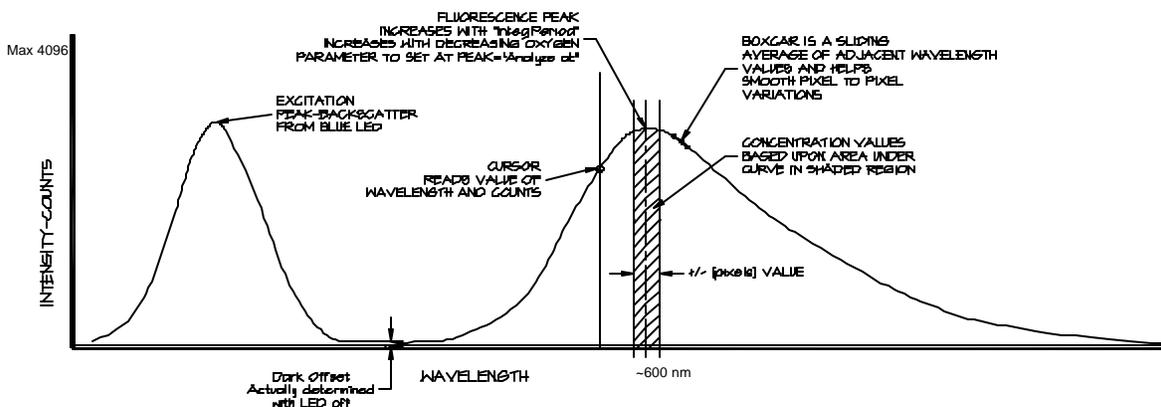
Probes can be provided in several configurations. The 125/FO version has the same external dimensions as our 125 series polarographic electrodes and is physically interchangeable, except for some flow cells. The tip of the probe is coated with an opaque layer of black silicone. The coating permits use in ambient light. No electrolytes or replaceable membranes are required. Other probes as small as 500 micron OD are available.

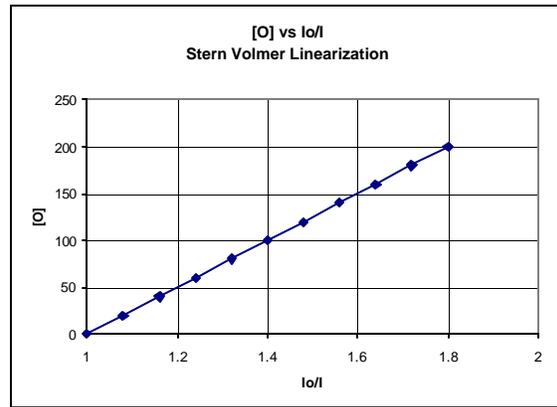
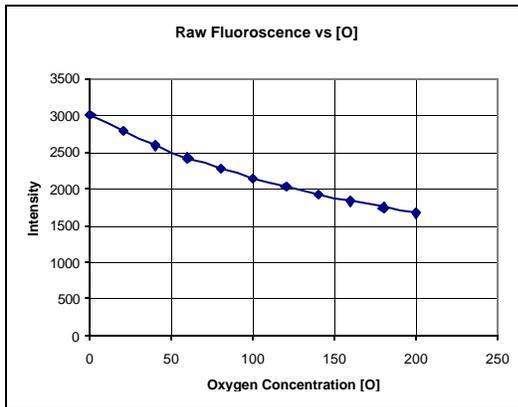
How it Works

Light from the blue LED travels from the "out" port, down the fiber to the probe where it excites the fluorescent dye. Some blue light is returned along with the fluorescence and travels back up the fiber to the "in" port where it enters a miniature spectrometer. The diagram below shows a typical spectral trace and some explanation of the parameters that you will be using in setting up the monitor. The dye fluoresces most brightly when no oxygen is present and decreases with increasing oxygen concentration.

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 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.

The graphs below illustrate how the raw intensity signal is converted within the software to a linear output by use of the Stern-Volmer linearization. Appendix 1 covers the equations governing the linearization and temperature corrections.





Summary of Setup Procedure

- Setup up Monitor with power supply, serial cable, and bifurcated fibers and attach probes. For USB A/D converter version, do not attach USB cable to computer yet.
- Install software (first time)
- Attach USB cable.
- Turn on Oxygen Monitor unit.
- Configure hardware (first time).
- Enter spectrometer wavelength calibration coefficients for each channel (first time).
- Prepare calibration setup for one temperature and 2 oxygen concentrations.
- Do zero oxygen first and establish acquisition parameters for each probe.
- Calibrate at ambient oxygen level for each probe.
- Ready to run.

System Assembly

Monitor Assembly



For the SAD500 version the monitor requires that the DC power adapter be plugged into the rear as well as the RS-232 cable. Use the DIN to 9-pin adapter cable to attach the oxygen monitor to an available COM port on your PC.

For USB version, just attach USB cable to computer after software has been installed.

Attach the bifurcated ends of the fiber optic cable to the "out" and "in" SMA optical connectors on the front panel of the monitor. Use the tubular wrench to secure the fittings. The single end will attach to the probe via the 3/4" sleeve coupler. If possible, keep the fiber optic cable positions the same once calibrated to avoid small errors due to cable differences. If using a dual system, keep the probes associated with a given channel once calibrations have been performed. Positions are labeled A, B..., on the Monitor front panel to facilitate this. Switches on the rear are disabled.

125 Series Probe Assembly



The 125/FO is a silica-core, 1000- μm stainless steel fiber optic probe with a 1/8" outer diameter and an O-ring seal and 2.5" in length. It is designed for use with a 600- μm bifurcated optical fiber assembly.

Caution!

- ◆ Avoid using ketones (acetone and alcohols included) with the 125/FO.
- ◆ Handle with care. Dropping the probe may cause permanent damage.
- ◆ Gently remove the plastic cover from the SMA connector before use. Cleaning
- ◆ Sterilize the 125/FO by gamma radiation or ETO. If you sterilize the probe, you must recalibrate.
- ◆ You can use detergents to clean the probe. Using detergents to clean the probe does not necessitate calibration.
- ◆ Avoid cleaning the 125/FO with ketones (acetone and alcohols included) or organic solvents.

Assembly

The tip of the probe is covered with a thin layer of black silicone. Care must be exercised to prevent puncturing or peeling of this layer. To prepare the 125/FO for use in Instech compatible systems, slide the threaded sleeve over the electrode first. Load O-ring onto the installing tool and place the tool over the end of the probe. Push the O-ring off and into the groove. The probe can now be installed into the chamber and the sleeve tightened to form a seal and hold it in place.



Installing the o-ring on the 125/FO probe.



Removing the o-ring from 125/FO probe. **WARNING:** cut it rather than trying to push it off over the tip. It is better to sacrifice the O-ring rather than to damage the tip coating.

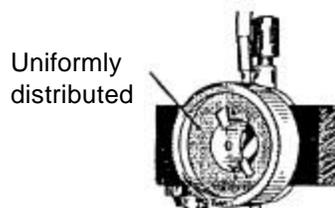
Instech Series 600 Chambers

Setting Up the Batch Cell Chamber

The batch cell mode uses the chamber cup with a magnetic stirring motor mounted behind it. The chamber cup is sealed with the window valve that is held in place by a thin layer of silicone grease.

1. *Plug in the speed controller.* Plug the AC adapter into the DC IN jack of the speed controller first, then plug the AC adapter into a wall outlet.
2. *Plug the motor into MOTOR OUT jack of the speed controller.* The motor should run when the speed controller is turned on.
3. Temporarily insert the chamber cup into the chamber block. *Push in the red leak protection divider from the rear of the chamber block* using a pencil until it hits the chamber cup. The opening should face away from the chamber cup.
4. *Insert the motor/magnet assembly into the chamber block* until it hits the red spacer, then pull it back about 1 mm.
5. *Gently tighten the set screw* to hold the motor using the provided allen wrench.

6. *Insert the chamber cup into the front of the chamber block.*
7. *Place the stir bar into the chamber cup.* It should couple to the magnet and rotate freely against the back of the cup when the speed controller is turned on.
8. *Apply a small amount of silicone grease to the flat front surface of the chamber cup.* Avoid the small fill and overflow port holes.
9. *Press the window against the cup and rotate it to distribute the grease uniformly across the face.*
10. *Pull off the window valve and clean off excess grease from the inside of cup using a toothpick.* Check the port holes as well. Do not clean the layer of grease from the face of the cup – this will form the seal for the window.



11. *Clean off all grease from the window valve.* An acetone dampened tissue works well.
12. *Press the window valve back on to the chamber cup and rotate to distribute the grease across the face.*
13. *Install the appropriate fill port plug into the top of the chamber.* If you plan to use a plastic-tipped micropipette to add fluid to the chamber during your experiment, use the single-piece pipette plug. If you plan to use a microliter syringe, use the two-piece syringe plug.

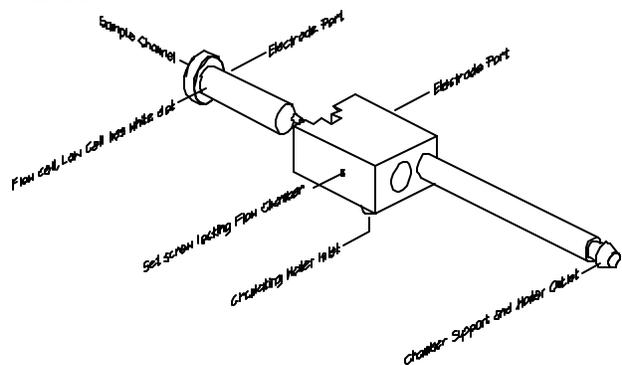


Pipette Fill Port Plug

Hamilton Syringe Fill Port Plug

The chamber is now ready for the electrode. When inserted, the electrode will hold the cup firmly in place.

Setting up the Flow Cell Chamber



Flow cell chamber assembly

The various pieces of equipment designed for the batch cell mode (i.e., speed controller, motor, chamber cup, window valve, and red spacer) are not needed and should be set aside.

Most in-line experiments involve measuring the difference in oxygen level at two points in a system, and thus will require that you set up two flow cells.

To set up the flow cell chamber:

1. *Insert the flow cell into the chamber block from the front.* Be sure that batch cell parts have been removed

from the chamber block and that the setscrew does not protrude into the hole.

2. *Attach inlet and outlet tubes of your experiment to the flow cell.* The system is designed for 1/16" ID tubing, such as Tygon. The flow cell is now ready for the probe.



Titanium Micro Chamber Assembly

Introduction

This chamber is constructed of non-reactive titanium with volumes of 250 microliters to 1 ml. It is top loading for easy sample loading and clean out. A beveled, transparent, sample-sealing plug serves also as a valve by closing off the angled fill port when rotated. The angle on the plug allows air to be easily purged. An alternative acrylic plug with a central fill hole may also be provided. Miniature stirring system and a nickel/Teflon coated aluminum block are standard. Stirring is not required to achieve a stable reading with this type of probe but faster thermal equilibrium will be achieved and any particulates will be kept in suspension.



Water Jacket Plumbing

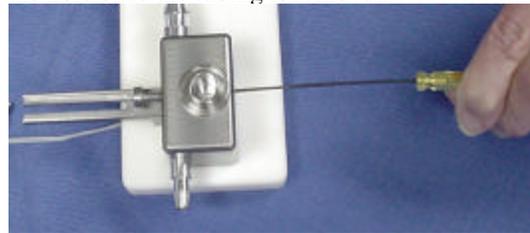
1. Use 5/16" ID Tygon tubing on the two outboard fittings.
2. Secure in place using clamps or cable ties.
3. If the center coupling should require changing, remove the stirring motor from one block, remove the block mounting screws and attach short length of tubing between the inner block fittings. Again secure tubing. **DO NOT ATTEMPT THIS WITH THE PROBE IN PLACE.**



Stirring Motor Installation

1. Loosen the motor holding setscrew (lower front center of block) until it does not protrude into the bore.
2. Back off the chamber holding setscrew and temporarily place the titanium chamber into the top of the aluminum block.
3. From the bottom, insert the motor/magnet assembly and push it until it contacts the bottom of the titanium chamber.
4. Pull the motor back about 1 mm and gently tighten the motor holding setscrew. Excessive force will damage the motor.

4. When the seal screw is seated, tighten the front chamber holding setscrew. This will assure that the tip of the fiber will not be damaged when inserted subsequently. The seal will remain in the hole once the tubing is removed.



Titanium Chamber Insertion and Removal

1. Drop chamber into opening at the top of the block with the fiber entry hole in the rear and roughly aligned with the threaded hole that will accept the fiber sealing screw.
2. Use the fiber sealing screw (without the fiber and seal) to set the alignment by screwing it into the block until seated. Some rotation of the chamber may be necessary.
3. The length of 22 ga.. stainless steel hypodermic tubing should be inserted prior to installing the probe in check the alignment once the seal is in place. Place the seal over the end of the tubing.

Installing the Probe

Note: At all times take care not to damage the fiber probe.



1. Attach the probe to the unmounted L-bracket by screwing it into the 3/4" hollow sleeve coupler. Fiber will be on the side nearest the L-bracket tightening screw. Do not attach the bifurcated bundle yet. Before tightening the probe, rotate it until the black angled face is upward to minimize light leakage.
2. Place the bracket with probe installed on the groove in the back supporting rail.
3. Loosen the seal tightening screw so that the probe will pass through the seal without friction.

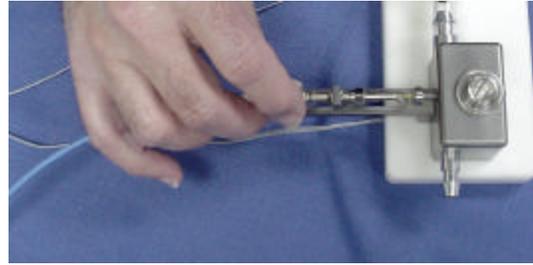
- Loosen the bracket thumbscrew and place the bracket in the track.



- Very carefully slide the probe through the hole in the seal tightening screw and advance it until the tip just protrudes into the chamber. *Do not allow the tip to touch the far side of the well, it could jam and crack the tip.*
- The final position of the probe tip should have just the angled portion of the tip exposed with the bevel facing upward. As the seal is tightened, it may advance into the chamber. Tighten seal and then reposition as necessary until the seal no longer can be moved.



- Repeat for the second chamber and then attach the single end of each bifurcated fiber bundle.



Chamber Plugs

Depending upon the chamber size, different style plugs may be use, either beveled tip style or O-ring style with single vertical fill hole.

The beveled type can be rotated to seal off the side fill hole.

For large volume chambers, e.g. 1000 microliters, the central hole can be plugged to reduce oxygen leakage although little reoxygenation actually occurs.

Larger chambers have no side fill hole.

Setting the Optional Beveled Plug Depth



- Slide a fine tip micro pipette tip into the fill hole until it touches the far side of the chamber well.
- Drop the beveled transparent plug into the well with the bevel matching the angle of the pipette tip.
- Place the collar over the plug and tighten the holding screw facing forward. This will be the position indicator as well.
- This process will position the depth of the plug so that air will clear properly when the chamber is initially filled

Using the Titanium Micro Chamber with Beveled Plug

- Fill the chamber with buffer solution that has been equilibrated at your operating temperature with the oxygen tension to be used in the experiment. Use slightly more than the specified

volume of the chamber. A small overflow will occur.

2. If working at ambient pO_2 , you may allow some time to allow thermal and oxygen equilibration to occur before sealing off the sample.
3. Add cells or organelles and slide in the central transparent core plug. Orient the top collar so that the air rises to the top of the bevel and exits through the fill port.
4. The setscrew should be facing you when the plug has been correctly installed.
5. Turn off stirring, if on.
6. Slowly continue to slide the plug down while watching from above to ensure that all air is expelled from the chamber.
7. When no air bubbles are visible, rotate the top collar until the setscrew faces toward the rear which will seal the chamber.
8. Additions can be made through the fill port in the right front of the chamber. First return the plug/valve to the forward position to allow entry.
9. This should be done with a loosely fitting needle or pipette so that the overflow will flow by the needle. No separate vent port is needed. Inject near the bottom and the overflow will flow out from the top part of the chamber and into the circular trough. Turning the stirrer off during this procedure is recommended to reduce mixing.

Using the Titanium Chamber with O-ring fitted plugs with central fill hole.

This type does not depend on the use of the side fill hole and may not even exist with larger chambers.

1. Fill chamber with plug out to slightly more than the stated chamber volume.
2. Gently push plug into chamber. Air should be expelled and a small amount of solution will be expelled insuring that no bubbles remain.
3. Continue as in step 9 above.

Software Overview

This software is designed to operate Instech Laboratories Model 110 or 210 Fiber Optic Oxygen Monitoring systems. These systems incorporate Ocean Optics' spectrometers, light sources and A/D boards. It is a native 32 bit application for Windows 95, 98, NT and 2000. USB unit requires Windows 98, 2000 or NT This version includes an optional second order Oxygen calibration to the linear regression (Stern-Volmer). Temperature calibration data is valid for gaseous measurements but not for dissolved oxygen. See Appendix 1 for procedures when measuring gaseous oxygen.

The operating screen or “front panel” displayed on the PC monitor, is a virtual instrument with graphs, charts, controls and indicators. Depending on selection, it is possible to view the full wavelength spectrum, intensity or concentration time chart, current concentration values. This information can be viewed simultaneously for up to eight channels, although only Master and Slave1 are used in the Model 210 and Master only in the Model 110.

Users have to do a single temperature Multipoint calibration - Linear/Polynomial, without temperature compensation for dissolved oxygen measurements. System settings are saved upon exiting the system. Once all settings have been established, exit and reenter the OOISensors program. They will then become the defaults next time the program is started. Calibration routines will save to their files during the operation of the program.

Nominal Setup Parameters-Adjust as needed

Spectrometer type*	S2000/PC2000...
A/D converter type*	ADC1000USB
USB Serial Number*	Select detected
Channel Active	Check for each
Scan dark for every measurement	check
Sensor	Foxy
Chart	sensor
Nm	Peak wavelength
Bandwidth	25
Pressure compensation	None
Temperature measurement	None
Enable Reference correction	unchecked
Wavelength coefficients*	Fill from sheet
Subtract dark box	check
Integ. time	64-512
Average	2
Boxcar smooth	10
Scan	Continuous
Calculate sensor values with scan	Pull down-checked
Calibrate	Oxygen, single temperature
Temp compensation	no
Calibration type	Multi Point

* Required first time

Software Installation

The following files are included on the CD provided:

110-210 Manual v6.pdf	Operating manual
Websetup.exe	Sets up and installs software
Intake Demo	Used as oxygen partial pressure or content calculator-old DOS program but front end is useful

SAD500 Serial Port Interface Version

You have this version if the unit shipped with a round DIN to DB9 RS-232 cable.

The SAD500 Serial Port Interface is a microprocessor-controlled A/D converter for serial port connection or stand-alone operation. The SAD500 can be used to interface to desktop or portable PCs, PLCs and other devices that support the RS-232 communication protocol. The following are directions for setting up your SAD500. Because A/D converter installation goes hand-in-hand with software installation, you will find directions for installing OOISensors Software in this section as well.

Interface the SAD500 to your PC

Interfacing the SAD500 to a desktop or portable PC is simple.

1. If your 110 or 210 came equipped with a SAD500 mounted onto your spectrometer, simply connect the 6-pin DIN end of the serial cable to the SAD500 and the DB9 end to your PC.
2. For either configuration, note the serial port number (also called COM Port) on the PC to which you are interfacing. (Older PCs may not have numbered ports.)
3. Plug the +12VDC wall transformer into an outlet and connect it to the power jack on the rear of the monitor unit.

ADC1000-USB Interface Version

Overview

Do not attach USB cable until software has been loaded.

Load the software using the Password provided.

Attach USB cable to computer and monitor. Windows should recognize USB connection.

Only now should you run the software.

This is the newer of the two versions and will ship with units after Jan. 1, 2002. This version requires a USB port on your computer and is shipped with the appropriate USB cable.

1. Plug 12VDC power supply to rear of monitor unit. You may leave this turned off at this time.
2. Plug the flat end of the USB cable into the computer and leave the monitor end of the cable *unconnected* for now.

Install OOISensors

Before installing OOISensors, make sure that no other applications are running. Also, make sure you have the OOISensors password, which can be found on the CD cover. During installation, you will have to enter this password.

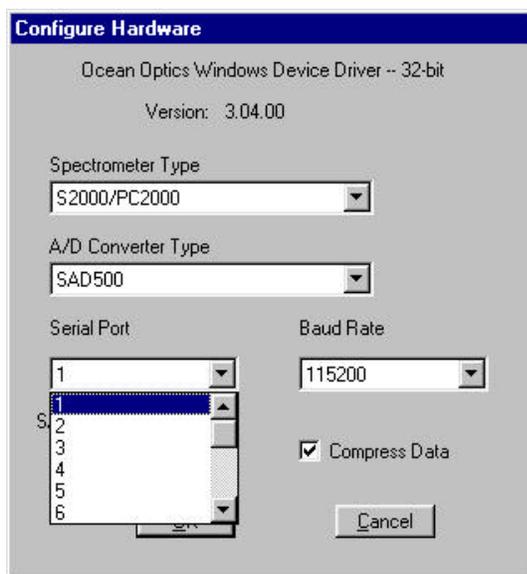
1. Execute **Websetup.exe**. At the "Welcome" dialog box, click **Next**>
2. At the "Destination Location" dialog box, accept the default or choose **Browse** to pick a directory. Click **Next**>
3. At the "Backup Replaced Files" dialog box, select either **Yes** or **No**. We recommend choosing Yes. If you select Yes, you can choose **Browse** to pick a destination directory. Click **Next**>.
4. Select a Program Manager Group. Click **Next**>. At the "Start Installation" dialog box, click **Next**>.
5. At the "Installation Complete" dialog box, choose **Finish**>. Restart your computer after installation is complete.
6. You may wish to create a shortcut and drag it to the desktop.

Configuring for SAD500

After restarting your computer, *turn the monitor unit on* and then start OOISensors.

The first time you run OOISensors after installation, you will need to initialize some parameters.

Configure Hardware Dialog Box



Select **Configure | Hardware** from the menu. The parameters in this dialog box are usually set only once -- when OOISensors is first installed and the software first runs.

1. Under **Spectrometer Type**, select S2000/PC2000 (the SF2000, S2000-FL and USB2000-FL are S2000-series spectrometers).
2. Under **A/D Converter Type**, choose SAD500.
3. Under **Serial Port**, choose the COM port number your computer is using to interface to your SAD500. See the **Troubleshooting** section to determine the COM Port.
4. Under **Baud Rate**, select the speed at which the SAD500 will operate. (We recommend 115,200 baud to start with. If no spectrum is seen, try a lower value).
5. Under **SAD Pixel Resolution**, enter resolution values from 1 to 500. This value specifies that every nth pixel of the spectrometer is transmitted from the SAD500 to the PC. By sacrificing resolution, you gain 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.

6. Enable the **Compress SAD500 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 ensures that every scan transmitted by the SAD500 will be compressed, greatly increasing the data transfer speed of the SAD500.

For your setup, only these parameters apply to your system. Click OK.

Troubleshooting Serial Ports

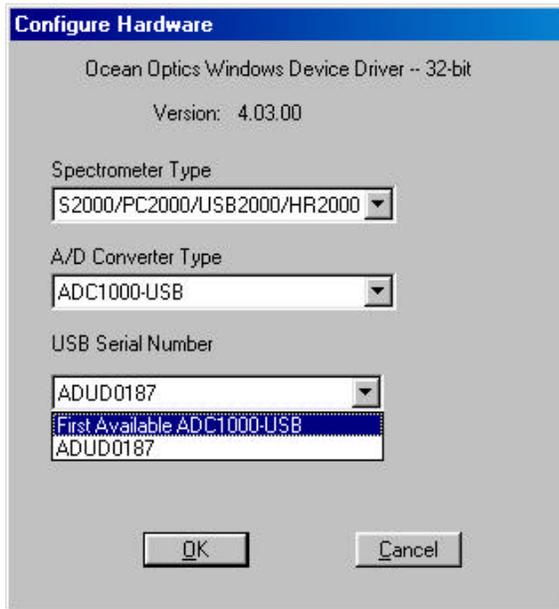
Occasionally, there will be problems associated with your PC configuration and the software. Here are a few tips to assist you.

- To ensure that the software and hardware are in-synch, exit OOISensors, cycle power on the SAD500, and restart OOISensors.
- In Windows 95/98, you can find out your Serial Port (or COM Port) number by selecting **Start | Settings | Control Panel**. Then double-click on the System icon and select the **Device Manager** tab. Double-click on **Ports (COM & LPT)** to display the COM port numbers. Ensure that there is no yellow or red warning sign next to the COM Port you are attempting to use.
- If the ports on your PC are not labeled and you don't know which COM port you are using for your SAD500, you may have to resort to trial and error. If you choose the wrong serial port number, you will not see a dynamic trace responding to light near the bottom of the displayed graph. Instead, you will see a straight line at 0 counts.
- On some computers, users may have to disable any virus protection software to ensure timely and complete transfer of the data.
- If spectral peaks do not align with expected values make sure wavelength coefficients have been correctly entered.

Configuring ADC1000-USB

Note: ORDER IS IMPORTANT. After restarting your computer, *first turn the monitor unit on and attach USB cable to the rear of the 110 or 210 monitor*. Initiate OOISensors.

The first time you run OOISensors after installation, select the Configure Hardware dialog box.



1. Select **Configure | Hardware** from the menu. The parameters in this dialog box are usually set only once -- when OOISensors is first installed and the software first runs.
2. Under **Spectrometer Type** S2000,PC2000,HR2000
3. Under **A/D Converter Type**, choose ADC1000-USB.
4. Under **USB Serial Number**, select the spectrometer serial number, this should match the calibration sheet number.
5. Select **Configure|Spectrometer**, activate the channel/s you will be using and check that the spectrometer coefficients appear as on the calibration sheet provided. If they do not match, enter each value and exit the program to save these values to non-volatile ram on the ADC1000.

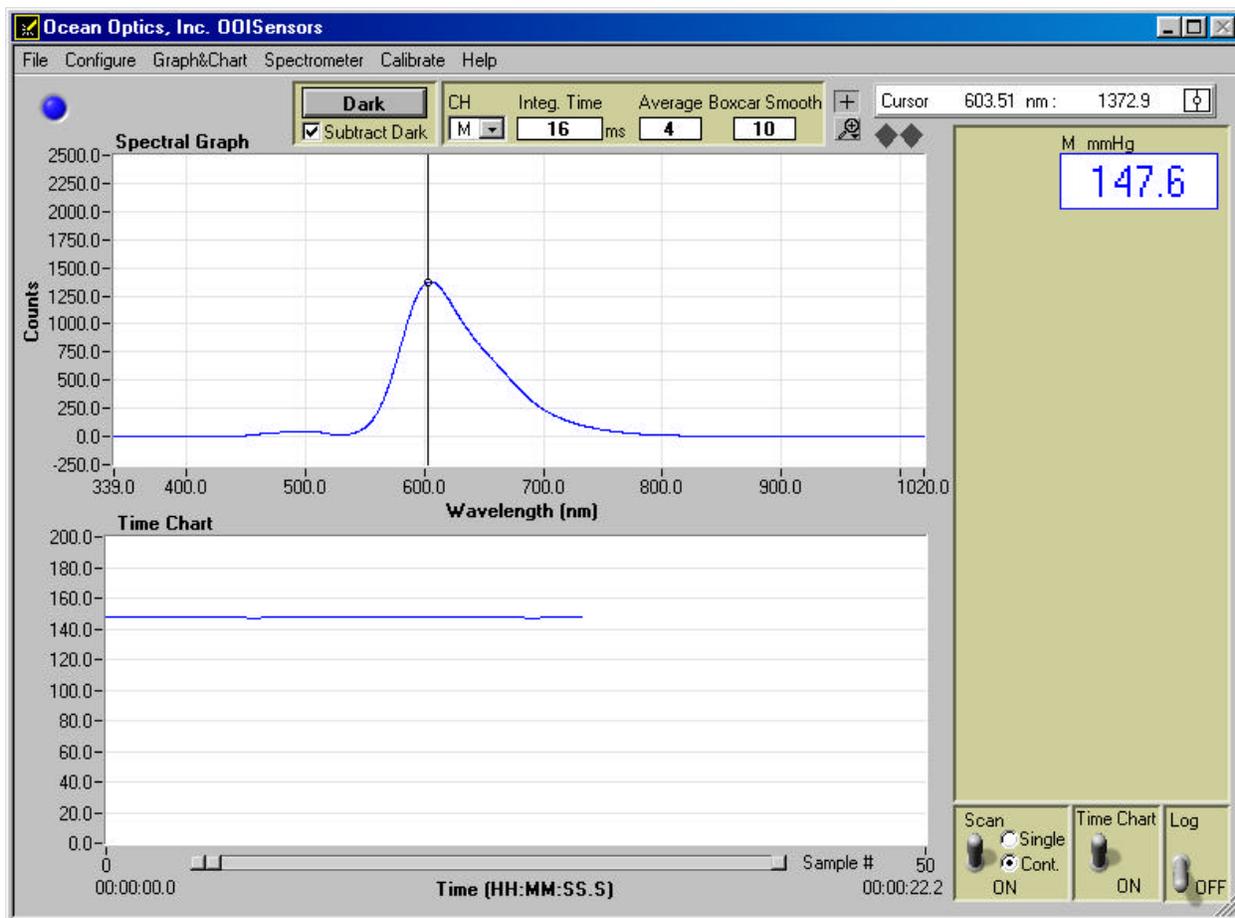
Software Operation

OOISensors Software is our next generation of operating software for our FOXY Fiber Optic Oxygen Sensing systems. OOISensors is a 32-bit, advanced acquisition and display program that provides a real-time interface to a variety of signal-processing functions for Windows 95/98/2000/NT users. With OOISensors, users 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 fluctuations in the sample.

The most important change from the previous oxygen sensing software, the 16-bit OOIFOXY, is the ability to use the Second Order Polynomial algorithm in the

calibration procedure. This algorithm often provides more accurate data than the linear Stern-Volmer algorithm. Also, with OOISensors, you can now monitor temperature. The software corrects the data for any fluctuations in temperature. Another improvement over OOIFOXY is that OOISensors can display up to 8 spectrometer channels in one spectral window, and yet each spectrometer channel can have its own data acquisition parameters.

What's more, a time chart displays the data from all active channels at a specific wavelength over time. During a timed data acquisition procedure, you can enter text for an event into the log file. Enabling the time chart and the data logging function are as easy as clicking on switches next to the graph.



Display Functions

Several functions are accessed not through the menu but through buttons and taskbars directly on the display window, on the top and to the right of the spectral graph and time chart areas. 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 Single and Continuous

When in **Single** mode, the Scan function acts as a snapshot. After selecting the Single mode, click the Scan switch to **ON** to take a scan of the sample. The switch stays in the **ON** position until the scan has been completed (the time set in the Integration Time box). The switch then moves to the **OFF** position.



When in **Cont.** (continuous) mode, the recommended setting, the Scan function continuously takes as many scans of the sample as needed. After each integration cycle, another scan

will immediately begin. Click the switch to **OFF** to discontinue acquiring data.

Store Dark



This function stores the current spectrum as the dark spectrum for all active channels

It is not necessary to use this function as long as the Subtract Dark box is checked **Configure | Spectrometer** from the menu, click on the **Sensors** tab and select **Scan dark for every measurement**. When this function is enabled, while the LS-450 automatically turns off, and a dark scan is stored, each time you take a sample scan.

Subtract Dark

Selecting this box subtracts the current dark spectrum from the spectra being displayed. This command is useful if you are trying to eliminate from the spectra fixed pattern noise caused by a very long integration time. This function is only for display purposes. It should be checked along with **Scan dark for every measurement**.

Data Acquisition Parameters

When altering parameters, turn scan to OFF to speed up program response to your actions.

Functions at the top of the display window such as choosing the integration time, averaging and boxcar smoothing values provide you with immediate access to important data acquisition settings.



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. This pull down menu is not for selecting the spectrometer channels that are active in the display graph; it's only for setting data acquisition values for each channel. (To activate your spectrometer channels and have them displayed, select **Configure | Spectrometer** from the menu and click on the **Sensors** tab. Enable each spectrometer in your system.)

Integration Time

Enter a value to set the integration time in milliseconds for the chosen spectrometer channel. 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. 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 1500-2000 counts in air or saturated water. The fluorescence peak should not exceed 3500 counts when oxygen is absent. 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.

Average

Enter a value to implement a sample averaging function that averages the specified number of spectra for the chosen spectrometer channel. The higher the value entered the better the signal-to-

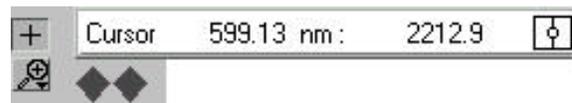
noise ratio (S:N). The S:N improves by the square root of the number of scans averaged.

Boxcar Smooth

Enter a value to implement a boxcar smoothing technique that averages across spectral data for the spectrometer channel chosen. This method averages a group of adjacent detector elements. A value of 5, 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. The S:N improves by the square root of the number of pixels averaged. When using the oxygen sensors, we recommend setting the boxcar smoothing value to no more than 25 pixels.

Cursor Functions

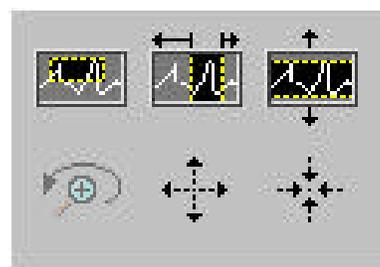
In this bar, you can label the cursor, monitor its X and Y values and move the cursor. To the right of the X and Y values of the cursor is a cursor selection button that allows you to choose a cursor style and a point style.



+ Sign

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

Magnify Symbols



There are several magnify functions from which to choose. The function chosen remains in use until another magnify 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

- zooms in on the horizontal scale, but the vertical scale remains the same
- zooms in on the vertical scale, but the horizontal scale remains the same
- zooms in approximately one point vertical and horizontal, click once or press continuously
- zooms out approximately one point vertical and horizontal, click once or press continuously
- reverts to the last zoom function

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 Label

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

X and Y Values

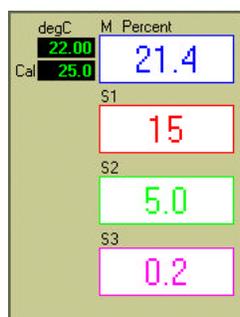
The cursor taskbar displays the X value and Y value of the cursor point.

Cursor Properties



To the right of the X and Y values of the cursor is a cursor selection 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 values for each spectrometer channel and probe combination. If you are monitoring and correcting for temperature, these values appear in this area as well.

Spectral Graph

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 simply clicking on an X and Y endpoint and manually typing in a value. The graph will then resize itself.

Temperature Chart

To display the temperature chart, select **Graph&Chart | View Temperature Chart** from the menu. The Temperature Chart will then take the place of the Spectral Graph. To save the Temperature Chart, select **File | Save Time Chart** from the menu. You will receive two Save prompts, one for the Time Chart and one for the Temperature Chart. By selecting **Graph&Chart | View Temperature Chart** again (deselecting the function), the Spectral Graph will return.

You can also save Temperature Chart data without displaying the chart. By selecting **Configure | Spectrometer** from the menu, clicking on the **Sensors** tab, and enabling the **Chart** function under Temperature Measurement, temperature data is collected, whether or not the Temperature Chart is displayed. Then you can use the save function.

Time Chart

The time chart displays the data from all active channels at a specific wavelength over time. To view the Time Chart, select **Configure | Spectrometer** from the menu and click on the **Display** tab. Make sure that **Spectral Graph & Time Chart** is selected next to **Graph and Chart Display Mode**. To configure a timed data acquisition procedure, select **Configure | Spectrometer** from the menu and click on the **Timing** tab. (For details on configuring a timed acquisition procedure, see page 53.)

Time Chart and Log On/Off Switches



Once you have configured a timed data acquisition procedure, you can start and stop the acquisition by clicking on the **Time Chart** switch.

(To set the parameters for a timed data acquisition procedure, select **Configure | Spectrometer** from the menu, click on the **Timing** tab and enter your settings.) Turn on and off saving this data to a log file by clicking on the **Log** switch. (To set parameters for saving timed acquisition data, select **Configure | Spectrometer** from the menu, click on the **Log** tab, select how frequently you want to save data and choose the file name for the log.) Only the last 10,000 scans of a timed data acquisition can be saved in the log file.

File Menu Functions

Save Spectrum

Select **File | Save Spectrum** from the menu to save 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

Select **File | Save Time Chart** from the menu to save 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

Select **File | Open Spectrum** from the menu to open 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

Select **File | Open Time Chart** from the menu to open a dialog box that allows you to choose a previously saved time chart and open it as a static chart.

Page Setup

Select **File | Page Setup** to select printing parameters.

Print Spectrum and Time Chart

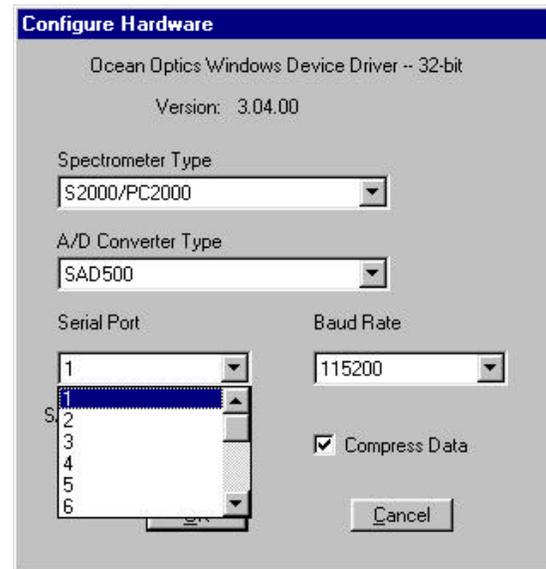
Select **File | Print Spectrum** from the menu to print the current display in the Spectral Graph, or select **File | Print Time Chart** from the menu to print the time chart.

Exit

Select **File | Exit** from the menu to quit OOISensors. A message box appears asking you if you are sure you want to exit the software.

Configure Menu Functions

Hardware



The Configure Hardware dialog box sets the hardware parameters for the spectrometer. The parameters in this dialog box are usually set only once -- when OOISensors is first installed. The first time you run OOISensors after installation, you need to select settings in the Configure Hardware dialog box. Select **Configure | Hardware** from the menu. First, select a **Spectrometer Type** (the S2000-FL, SF2000 and USB2000 are S2000-series spectrometers). Next, select an **A/D Converter Type**. Select the A/D converter you are using to interface your spectrometer to your computer. Your choices are the ADC500/PC1000, ADC1000/PC2000, DAQ700, SAD500, Serial USB2000 or USB2000.

Depending on the Spectrometer Type and the A/D Converter Type you chose, other choices must be made:

◆ For SAD500 users:

- Enter your computer's **Serial Port** (or COM Port) number to which the device is connected.
- Select the **Baud Rate** or speed at which the device will operate.
- Enter a **SAD500 Pixel Resolution**, which specifies that every nth pixel of the spectrometer is transmitted from the SAD500 to the PC. Enter resolution values from 1 to 500. Your resolution value depends on your experiment. By sacrificing

pixel resolution, you gain 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.)

- 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, greatly increasing the data transfer speed.

- ◆ **For USB2000 users:** Select the **USB2000 Serial Number** for the USB2000 you wish to use.

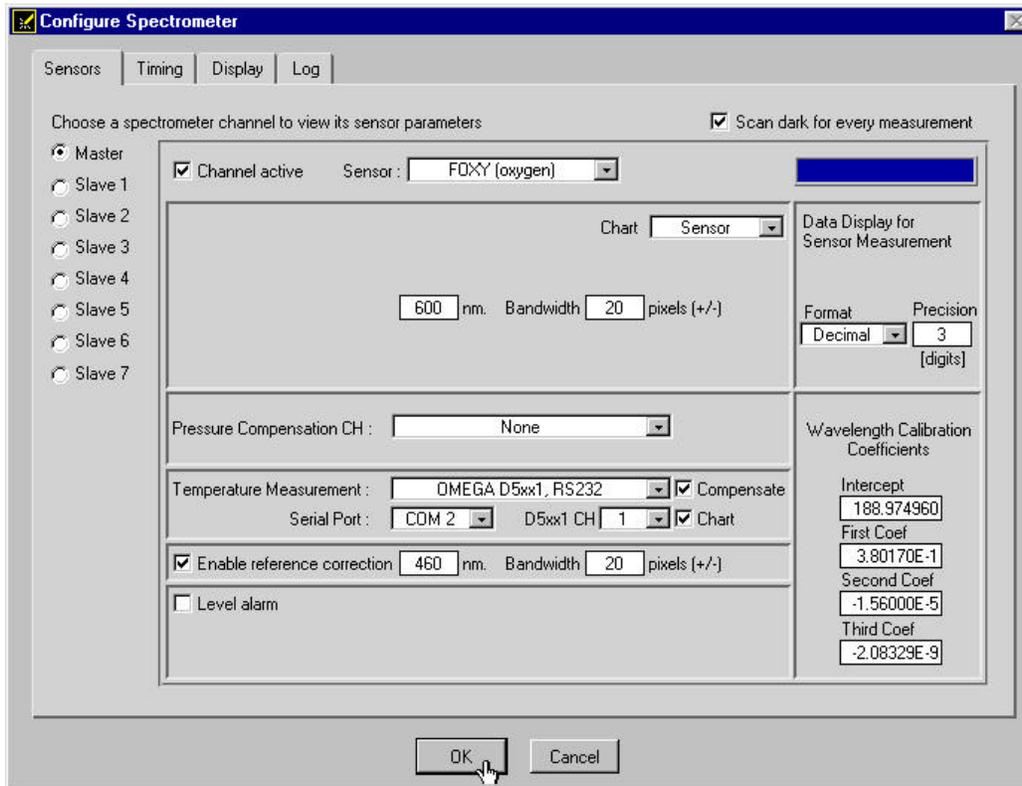
Spectrometer

Choose **Configure | Spectrometer** from the menu. Go through each page of this dialog box to select options for sensing, timing, displaying and logging options.

Sensor Tab

To set the parameters for your sensors, select **Configure | Spectrometer** from the menu and then click on the **Sensors** tab.

- ◆ **Choose a spectrometer channel to view its sensor parameters.** Set parameters for each spectrometer channel in your system by first selecting a channel. Each spectrometer channel has its own parameters.
- ◆ **Scan dark for every measurement.** Storing a dark spectrum is requisite before the computer can make accurate measurements. If you have configured the spectrometer to control the LS-450, the software can take *automatic* dark scans if you 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.
- ◆ **Channel active.** Select this box to activate the spectrometer channel.
- ◆ **Sensor.** Use the pull down menu to select the type of sensor you are using for each spectrometer channel. FOXY (oxygen)
- ◆ Click on the solid colored box to change the color of the spectral trace that will appear in the display graph.
- ◆ **Chart.** Select the type of information you want charted in the Spectral Graph. You can choose to view the spectral graph of the sensor or the intensity at the analysis wavelength. Set to Sensor.



- ◆ **Analysis Wavelength Box.** Enter the analysis wavelength. The analysis wavelength should be very close to 600 nm. (When the excited ruthenium complex at the tip of the oxygen probe fluoresces, it typically emits energy at ~600 nm.) In the **Bandwidth [box] pixels** area, select the number of pixels around the analysis wavelength to average.
- ◆ **Data Display for Sensor Measurement.** Choose the data display format and precision of the data. Under **Format** select Decimal or Scientific. Under **Precision**, select a value to specify the precision of the oxygen data displayed and saved in files. The maximum is 5.
- ◆ **Wavelength Calibration Coefficients**
- ◆ **Do not forget to enter these the first time using the system.**

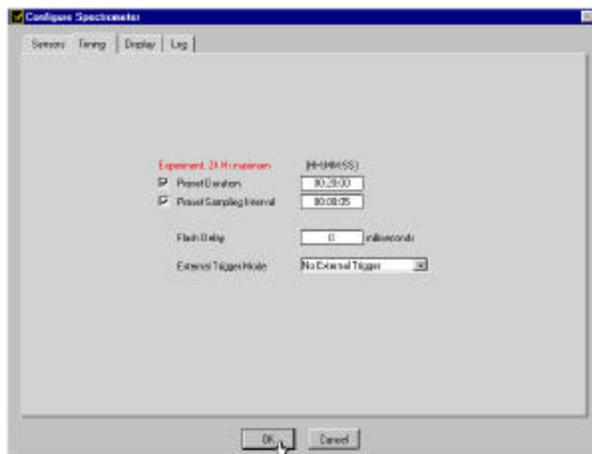
Check the Wavelength Calibration Data Sheet that came with your system to make sure the values on the Data Sheet and in this dialog box are the same. If not, enter the coefficients otherwise spectral data may be skewed.

- ◆ **Pressure Compensation CH.** If you have your own external pressure measuring system, you can use this feature to monitor and correct for pressure fluctuations in your sample. You can either use a pressure transducer separately from the system or interface it to your sensor system. If you interface a pressure transducer to your sensor system, you must have an available spectrometer channel that is not connected to an oxygen sensor. Use the pull down menu to select how you want to monitor pressure. See Spectrometer DB-25 connector details for attaching the analog signal to an unused A/D input channel.
- ◆ **Temperature Measurement.** If you want to monitor and correct for temperature fluctuations in your sample, select a method from the pull down menu.
 - Select **None** if you are not monitoring temperature. For now, this is the recommended setting.
 - Select **Manual** if you are monitoring temperature, but you do not want OOISensors Software to read and display the temperature values. The Manual selection means that you must

manually type temperature values in the display window. Enable the **Compensate** function if you want the software to correct for temperature fluctuations *only if you calculated the temperature coefficients*. Enabling the **Chart** function allows you to view a chart of the temperature values.

- Select **Omega D5xx1 RS232** if you are monitoring temperature and if you want the software to automatically read and display the temperature values. 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. Next to **Serial Port**, select the COM Port number on your PC to which the thermistor or thermocouple is connected. Because the RS-232 module can support up to four thermistors or thermocouples, it has these four ports labeled. 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.) 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.
- ◆ **Enable reference correction.** Disable this function.
- ◆ **Level alarm.** Enable this feature to set alarm properties. A green indicator appears in the display window if this feature is activated. If the values fall below the alarm parameters, the green indicator turns red. If the values rise above the alarm parameters, the green indicator turns yellow.

Timing Tab



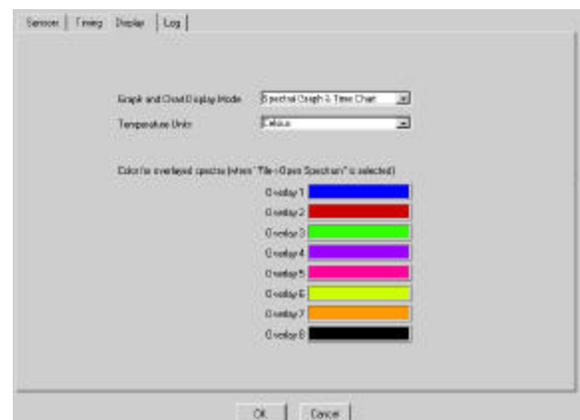
To configure a timed data acquisition procedure, select **Configure | Spectrometer** from the menu and select the **Timing** tab. In this dialog box, you can set the parameters for a timed data acquisition procedure.

- ◆ **Preset Duration.** Enable this box and enter values to set the length for the entire timed acquisition process. Be sure to enter hours (HH), minutes (MM) and seconds (SS).
- ◆ **Preset Sampling Interval.** This is frequently used to reduce the number of data points saved to disk by inserting a delay between samples. Enable the Preset Sampling Interval box and enter a value to set the frequency of the data collected in a timed acquisition process. Be sure to select hours (HH), minutes (MM) and seconds (SS).
- ◆ **Flash Delay.** (Normally not selected) Enter a value to set the delay, in milliseconds, between external strobe signals of the LS-450 Blue LED light source. *You can only use this feature if you have an ADC1000 A/D converter.*
- ◆ **External Trigger Mode.** (Not normally used). You have two methods of acquiring data. Choose a triggering mode from the pull down menu:
 - In the normal mode (called **No External Trigger**), the spectrometer is continuously scanning, acquiring, and transferring data to your computer, according to parameters set in the software. In this mode, however, there is no way to synchronize the acquisition of data with an external event.

-- To synchronize data acquisition with external events, choose **External Software Trigger**. In this level-triggered mode, the spectrometer is "free running," just as it is in the normal mode. With each trigger, the data collected up to the trigger event is transferred to the software. (See **Appendix D** for details.)

- ◆ Once you have configured a timed data acquisition procedure, you can start and stop the acquisition by clicking on the **Time Chart** switch on the main display window.

Display Tab

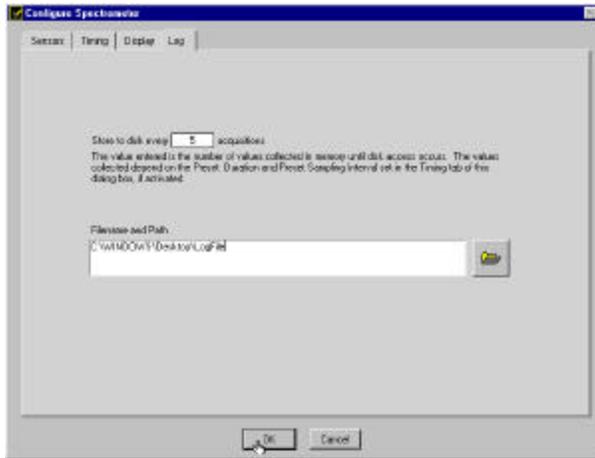


To configure your display window, select **Configure | Spectrometer** from the menu and click on the **Display** tab. In this dialog box, select the graphs and charts to appear in the display window.

- ◆ **Graph and Chart Display Mode.** Choose the information that appears in the display window. If you choose **Spectral Graph Only**, a spectral graph appears in the display window. If you choose **Spectral Graph & Time Chart**, the spectral graph appears in the top of the display window and the time chart appears in the bottom. (To view a temperature chart, select **Graph&Chart | View Temperature Chart** from the main menu. The temperature chart then replaces the spectral graph.)
- ◆ **Temperature Units.** Select either **Celsius** or **Fahrenheit** as the temperature units. (The application works in Kelvin, and converts to Celsius or Fahrenheit.)
- ◆ **Color for overlays.** Select colors for static spectra that open when selecting **File | Open Spectrum** from the menu. These static

spectra are called overlays and you may wish to distinguish overlays from real-time spectra by changing the colors of their traces.

Log Tab



To configure the data logging feature for a timed data acquisition procedure, select **Configure | Spectrometer** from the menu and select the **Log** tab.

- ◆ **Store to disk every x acquisitions.** Enter a value to set how many scans are stored in RAM before they are saved permanently into a file. The smaller this number, the more frequently data is saved permanently to a file. The larger this number, the less frequently data is saved permanently to a file, but entering a large number enhances the performance of the process.
- ◆ **Filename and Path.** Name the log file for the timed data acquisition process. Click on the file folder icon to navigate to a designated folder.
- ◆ **Insert Event in Log File.** If you want to enter text into the log file, you can select **Spectrometer | Insert Event in Log File** from the menu. A dialog box then appears allowing you to enter text. In the log file, this text appears next to the data that was acquired at the time you entered the text.
- ◆ To turn the logging function on for the time chart, you must select the **Log On/Off Switch** on the main display window.

Graph & Chart Menu Functions

Clear Spectrum Graph

Select **Graph&Chart | Clear Spectrum Overlays** from the menu to remove static spectra from the graph.

Clear Time Chart

Select **Graph&Chart | Clear Time Chart** from the menu to clear the time chart traces. A message box then appears, asking if you are sure you want to clear the time chart.

Enable Grid

Select **Graph&Chart | Enable Grid** from the menu to generate a grid in the spectral graph. If you also have the time chart displayed, this function will create a grid in the time chart as well. De-selecting **Enable Grid** from the menu makes the grid disappear.

Autoscale Horizontal (Spectral Graph)

Select **Graph&Chart | Autoscale Horizontal (Spectral Graph)** from the menu to automatically adjust the horizontal scale of a current graph so the entire horizontal spectrum fills the display area.

Autoscale Vertical (Spectral Graph)

Select **Graph&Chart | Autoscale Vertical (Spectral Graph)** from the menu to automatically adjust the vertical scale of a current graph so the entire vertical spectrum fills the display area.

Autoscale Vertical (Time Chart)

Select **Graph&Chart | Autoscale Vertical (Time Chart)** from the menu to automatically adjust the vertical scale of a current time chart so the entire vertical chart fills the display area.

View Temperature Chart

Select **Graph&Chart | View Temperature Chart** from the menu to view temperature data. The temperature chart replaces the spectral chart. To view the spectral graph again, select **Graph&Chart | View Temperature Chart** from the menu and the spectral graph replaces the temperature chart.

Spectrometer Menu Functions

Scan-usually Continuous

Select **Spectrometer | Scan** from the menu to take a scan of your sample. When in **Single** mode, (seldom used) the Scan function acts as a snapshot. The button depresses and Stop replaces Scan. The button will stay depressed until the scan has been completed (the time set in the Integration Time box).

When in **Continuous** mode, the Scan button continuously takes as many scans of the sample as needed. After each integration cycle, another scan immediately begins. The button depresses and Stop replaces Scan. Select **Spectrometer | Scan** from the menu or click on the Stop button to halt the scanning process and discontinue acquiring data.

Insert Event in Log File

During a timed data acquisition procedure, you can enter text into the log file by selecting **Spectrometer | Insert Event in Log File**. A dialog box then appears allowing you to enter text. In the log file, this text appears next to the data that was acquired at the time you entered the text. Both the Time Chart and Log switches in the display window should be turned to the On position to use this feature.

Enable Strobe Not normally used

If you have configured the spectrometer to control the LS-450 selecting this function in the software allows you to enable or disable the triggering of the LS-450 Blue LED light source. The value entered in **Flash Delay** of the **Timing** tab in the **Configure Spectrometer** dialog box sets the delay, in milliseconds, between strobe signals of the LS-450 Blue LED light source.

Calculate Sensor Values with Scan

When you first start OOISensors, the values displayed in the Data Values boxes to the right of the Spectral Graph will appear illogical. These values will continue to appear this way until you have calibrated your system. If you don't want to see these illogical values displayed, deselect **Spectrometer | Calculate Sensor Values with Scan**.

Once you have calibrated your system, this function should always be enabled (or have a check mark in front of it) if you want the oxygen values displayed.

Probe Calibration

Physical Calibration Setup

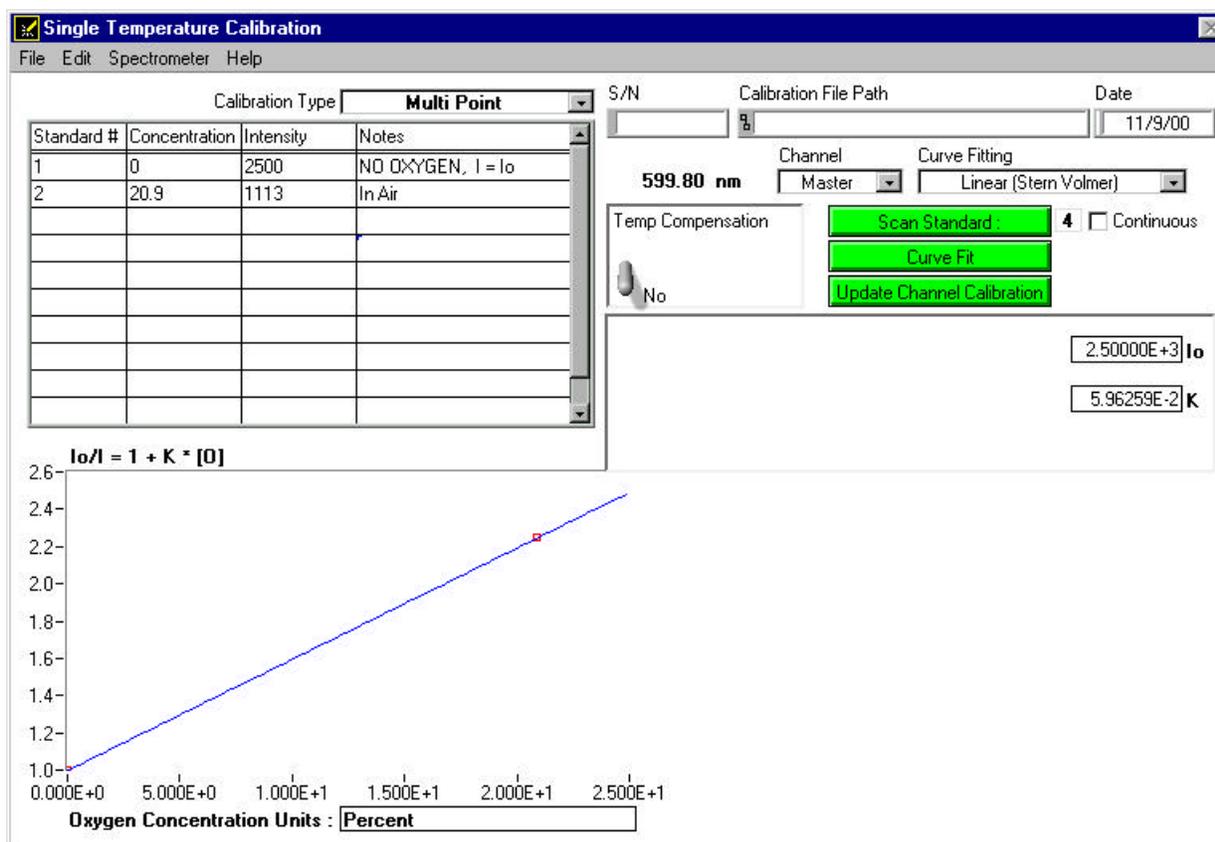
Assemble a system that will maintain constant temperature of the medium and probe and a means of presenting known oxygen concentration to the probe. The easiest concentrations will be atmospheric oxygen levels (20.9%) and zero. Zero oxygen is best attained by adding sodium dithionite to the solution. This will chemically remove the oxygen. Bubbling with nitrogen or an inert gas is more difficult and fraught with pitfalls. Decide on the units of oxygen concentration you will be using.

Included with the unit is equipment to facilitate the calibration procedure for dissolved oxygen levels if the OR125 series probes are being used. A black holder attached to a long ¼” handle has two holes for probes and one small hole for a thermocouple temperature monitoring device (user supplied).

Calibration Procedure

This procedure does not include temperature compensation as this not yet possible with dissolved oxygen measurements.

1. Set data acquisition parameters for your calibration procedure, such as 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. Adjust the integration time so that the fluorescence peak does not exceed 3500 counts. If your signal intensity is too low, increase the integration time. If the signal intensity is too high, decrease the integration time. Set the integration time to powers of 2 (2, 4, 8, 16, 32, 64, 128, 256, 512, etc.) to ensure a constant number of LED pulses during the integration time. The intensity of the LED peak [~475 nm] will not affect your measurements providing that compensation has



not been enabled. It will affect readings if the LED peak becomes saturated and compensation is enabled.

3. Select **Calibrate | Oxygen, Single Temperature** from the menu.
4. Enter the serial number of the probe in the **S/N** box. Today's date should enter automatically in the **Date** box. The file name and path appears under **Calibration File Path** once you select **File | Save Calibration Chart** and save the chart. At the
5. This area is for typing in a label; it does not affect data in any way.
6. Next to **Calibration Type**, select **Multi Point** from the pull down menu.
7. Under **Channel**, select the spectrometer channel to which the sensor you are calibrating is connected.
8. Under **Curve Fitting**, select the kind of algorithm you want to use to calibrate your sensor system: **Linear (Stern-Volmer)** or **Second Order Polynomial**. 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 and therefore more accurate data during oxygen measurements, especially if you are working in a broad oxygen concentration range.
 - ◆ If you choose **Linear (Stern-Volmer)**, you must have 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.
 - ◆ If you choose **Second Order Polynomial**, you must have 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. *Since achieving 3 known dissolved oxygen levels is usually difficult, this mode is not recommended.*
9. In the Calibration Table, in the **Standard #** column, enter **1** for your first standard of known oxygen concentration. The first standard should have 0% oxygen concentration, such as can be

found in a nitrogen flow or in a solution of sodium hydrosulfite or sodium dithionite.

10. Under the **Concentration** column, enter **0**.
11. Leave your FOXY probe in the standard for at least 5 minutes to guarantee equilibrium. Checking the continuous box will allow watching of Intensity values to ensure that they are stable.

Calibration Data

Once you have calibrated your sensor system, the calibration data is stored in two files. It is stored in the **OOISensors.cfg** file, which is the application configuration file. The calibration data is called from this binary file each time you use your sensor system and software.

Calibration data is also stored in an ASCII file (or text file) so that you use read the data and even import it into other application programs such as Microsoft Word and Excel. This ASCII file is called **chXFoxy.cal**, where "**X**" stands for the spectrometer channel ("0" for master spectrometer, "1" for spectrometer channel 1, "2" for spectrometer channel 2 and so on). The **chXFoxy.cal** file is not used by the OOISensors application; it is strictly for analyzing calibration data. (If you have temperature data in this file, temperature will be displayed as 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 the file corresponds to the serial number of the probe. Unless otherwise specified, these coefficients are applicable to *gaseous measurements only*.

INTAKE Utility Software

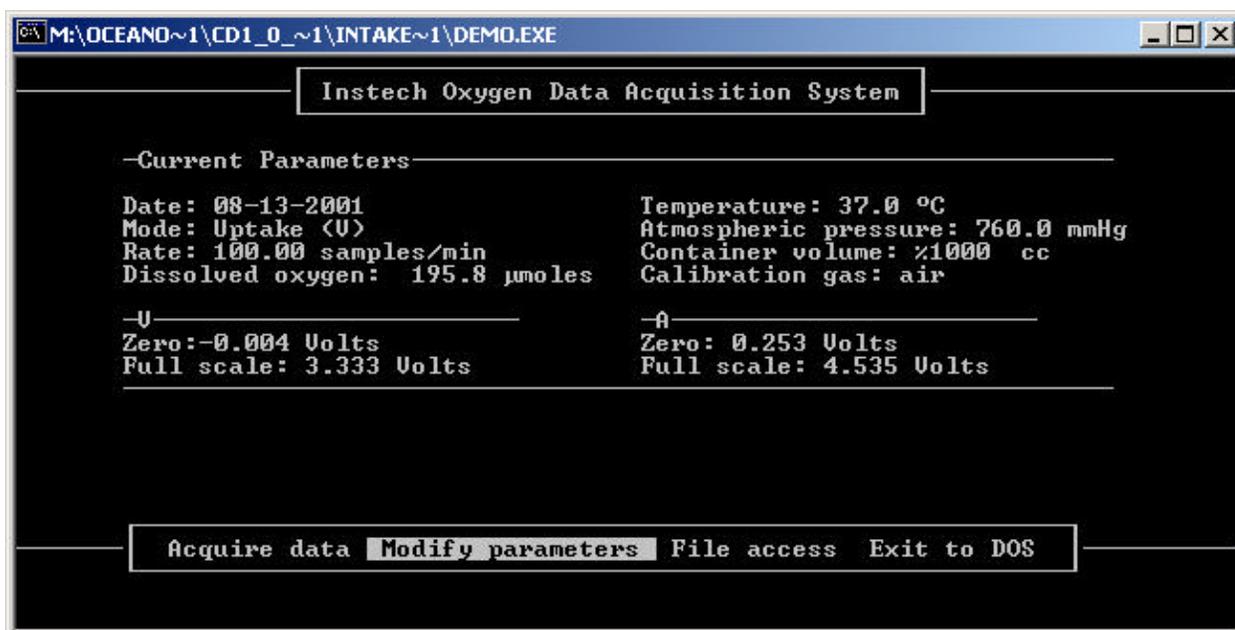
Installing INTAKE DEMO

- Copy the files provided into a separate directory. No special setup is required.
- Create a shortcut to demo.exe for desktop for ease of access.

Using INTAKE DEMO

This DOS program is provided as a tool to help calculate oxygen concentrations or partial pressures

at different temperatures for the calibration process data entry. It was part of discontinued Instech Laboratories acquisition system but is helpful in performing calculations quickly. Disregard all but the “modify parameters” feature. Use the arrow keys to move around the screen. Use Gas -> Units to set units of measure. Enter temperature values to calculate concentration. Volume can be set to 1000 ml to read in micromolar.



Other Probes

Probes

There are seven available oxygen probes. The distal tip of each probe is polished and coated with the oxygen-sensing material. The proximal end of each probe has an SMA 905 fitting for coupling to the optical cables.

Ocean Optics PN	Description
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) This is the standard probe (125/FO) supplied with the Instech Laboratories Model 110 and 210.
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 These are available in customs lengths as well.
FOXY-24G	300- μ m aluminum jacketed fiber optic probe with 24-gauge needle tip for penetrating vial septa, designed to couple to a 200- 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-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

Overcoats

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.*

Ocean Optics PN	Description
FOXY-AF	RTV healthcare-grade silicone overcoat for FOXY probes
FOXY-AF-MG	RTV high-strength medical implant-grade silicone overcoat for FOXY probes (provides a thicker and stronger coating than the FOXY-AF)

Additional probes may be ordered directly from Instech Laboratories.

Appendix 1: Theory of Operation

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. Our 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 or temperature monitoring devices should be used.

If you decide to compensate for temperature, the relationship between the Stern-Volmer values and temperature is 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 details of the optical setup: the power of the LED, the optical fibers, loss of light at the probe due to fiber coupling, and backscattering from the sample. It is important to 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. Using the Second Order Polynomial algorithm when calibrating corrects these deviations.

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 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

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

$$I_0 = a_0 + b_0 * T + c_0 * T^2$$
$$K_1 = a_1 + b_1 * T + c_1 * T^2$$
$$K_2 = a_2 + b_2 * T + c_2 * T^2$$

Henry's Law

It is possible to calibrate the FOXY system in gas and then use the FOXY system in liquid or vice versa. In theory, your FOXY 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. (However, Henry's Law does not apply to gases that are extremely soluble in water.) The concentration (mole %) can be calculated if the absolute pressure is known:

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

Since the FOXY 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 FOXY 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 following information illustrates the solubility of oxygen in water at different temperatures.

$$\ln(X) = a + b/T^* + c \ln(T^*)$$

Temperature range: 0° C - 75° C

X is the mole fraction

T* is the T/100 in Kelvin

- a -66.7354
- b 87.4755
- c 24.4526

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 -- 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 (+/-1° C) temperature. If this is not practical, then you should calibrate your FOXY 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 FOXY probe. (Optional thermistor and K-type thermocouple accessories are available.) OOISensors Software corrects for changes in data due to temperature fluctuations. At the present time, automatic compensation only works for gaseous measurements. (Check with Instech Laboratories for possible work-around when making dissolved oxygen measurements).

Scattering Media with uncoated 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. Also, 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, N₂ can be used for the low value (0%) and either air (20.9%) or O₂ (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 will consume O₂ rapidly, and can be used to prepare a 0% concentration. Air-saturated values for various solvents and salt solutions can be found in textbooks.

Calibration Data

Once you have calibrated your sensor system, the calibration data is stored in two files. It is stored in the **OOISensors.cfg** file, which is the application configuration file. The calibration data is called from this binary file each time you use your sensor system and software.

Calibration data is also stored in an ASCII file (or text file) so that you use read the data and even import it into other application programs such as Microsoft Word and Excel. This ASCII file is called **chXFoxy.cal**, where "**x**" stands for the spectrometer channel ("0" for master spectrometer, "1" for spectrometer channel 1, "2" for spectrometer channel 2 and so on). The **chXFoxy.cal** file is not used by the OOISensors application; it is strictly for analyzing calibration data. (If you have temperature data in this file, temperature will be displayed as Kelvin.)

Re-calibration

FOXY probes do need re-calibration from time to time. The following factors necessitate re-calibration:

1. If you use a FOXY probe in a harsh environment that degrades the probe coating.
2. If you expose a FOXY probe to the LED source for long periods of time, causing faster photobleaching of the ruthenium compound.
3. If you re-condition your FOXY probe.
4. If you sterilize your FOXY probe with processes such as autoclave or gamma radiation.

Appendix 2: Hardware Descriptions

S2000-series Pin-outs and Jumpers

The average user would not normally need to know about the interconnect scheme of the S2000, as the cables supplied with all of the units need only be plugged into the matching connectors on the hardware. However, if the need arises to design and fabricate your own cabling system, the following tables supply the necessary information.

J1 (D-25) Interface Cable (connects the S2000 master with the A/D board)

J1 Pin	Function	A/D Pin Connection
1	Analog Channel 0	37
2	Analog Channel 1	36
3	Analog Channel 2	35
4	Analog Channel 3	34
5	Analog Ground	19
6	Reserved	
7	N/C	
8	N/C	
9	Digital Ground	7
10	A/D Trigger	25
11	Master Clock	20
12	Digital Ground	Not in Cable
13	+5VDC	1
14	Analog Channel 4	33
15	Analog Channel 5	32
16	Analog Channel 6	31
17	Analog Channel 7	30
18	Analog Ground	Not in Cable
19	N/C	
20	Continuous Strobe In	8 (or use internal jumpers)
21	External Software Trigger Out (DO3)	5
22	Spectrometer Mode Input S1	4
23	Integration Time Clock In	2
24	Strobe Enable, Spectrometer Mode Input S0	23
25	Enable Read In	3

J2 (D-SUB-15) Accessory Connector

J2 Pin	DB-15 (Female)	Description															
1	Single Strobe	TTL output signal used to pulse a strobe that is high at the start of each integration period.															
2	Continuous Strobe	TTL output signal used to pulse a strobe that is divided down from Master Clock signal.															
3	V _{cc}	The positive supply voltage +5VDC.															
4	External Hardware Trigger	TTL trigger signal (rising edge trigger input) used in the External Hardware Trigger mode.															
5	External Synchronization Trigger	TTL signal used to define the integration time (time between rising edges) when using the External Synchronization Trigger mode.															
6	Channel 7	The analog input for spectrometer slave channel 7.															
7	Channel 6	The analog input for spectrometer slave channel 6.															
8	D3 or External Software Trigger	Active high TTL input signal used to trigger the acquisition system in the External Software Trigger mode. The input from J1-8 is passed unbuffered to this line.															
9	Channel 1	The analog input for spectrometer slave channel 1.															
10	GND	Ground (supply voltage return) or case ground.															
11	Channel 4	The analog input for spectrometer slave channel 4.															
12	Channel 5	The analog input for spectrometer slave channel 5.															
13	S0 and S1	<p>TTL inputs used to determine the triggering mode:</p> <table border="0"> <tr> <td>S1</td> <td>S0</td> <td>Mode</td> </tr> <tr> <td>L</td> <td>X</td> <td>Normal or Continuous Scan</td> </tr> <tr> <td>L</td> <td>X</td> <td>External Software Trigger</td> </tr> <tr> <td>H</td> <td>L</td> <td>External Synchronization Trigger</td> </tr> <tr> <td>H</td> <td>H</td> <td>External Hardware Trigger</td> </tr> </table> <p>X = does not matter</p> <p>In the first 2 modes, S0 is also used to enable/disable light sources.</p>	S1	S0	Mode	L	X	Normal or Continuous Scan	L	X	External Software Trigger	H	L	External Synchronization Trigger	H	H	External Hardware Trigger
S1	S0	Mode															
L	X	Normal or Continuous Scan															
L	X	External Software Trigger															
H	L	External Synchronization Trigger															
H	H	External Hardware Trigger															
14	Channel 3	The analog input for spectrometer slave channel 3.															
15	Channel 2	The analog input for spectrometer slave channel 2.															

H1 Header Pins (Analog)

Pin	Description
1	Analog Channel 0
2	Analog Channel 1
3	Analog Channel 2
4	Analog Channel 3
5	Analog Channel 4
6	Ground
7	Reserved
8	Analog Channel 7
9	Analog Channel 6
10	Analog Channel 5

H2 Header Pins (Digital)

Pin	Description
D	N/C
C	A/D Trigger
B	Digital In 3 (D3)
A	S1
1	Ground
2	+5 VDC
3	Phi A/D clock
4	Phi Read Out Gate
5	Reserved
6	Temperature (optional)
7	Read Enable
8	S0
9	Strobe Single flash
10	Strobe Multiple Flash
11	Integration Clock
12	Master Clock

H1 and H2 Header Blocks connect a master spectrometer channel to one or more additional spectrometer channels.

USB2000-series Pin-outs

Listed below is the pin description for the USB2000 Accessory Connector (J2) located on the front vertical wall of the unit. (To order multiple mating connectors, contact Samtec, Inc. for item number IPS1-105-01-S-D. Visit Samtec's web site at www.samtec.com.)

Pin #	Description
1	V _{USB} or 5V _{in}
2	RS232 Tx
3	RS232 Rx
4	Lamp Enable
5	Continuous Strobe
6	Ground
7	External Trigger In
8	Single Strobe
9	I ² C SCL
10	I ² C SDA

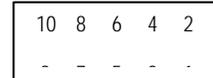


Diagram of Pins on the Accessory Connector

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. The R-LS-450 can be configured to operate in continuous wave mode through manual operation and through the software. It can also be configured to operate in pulsed mode through manual operation and through the software.

The R-LS-450 is shipped with the following pins jumpered: Jumper over pins in JP1, a jumper over the Manual pins in JP3, and a jumper over the 2¹⁰ pins in JP2 for the fastest pulse rate available.

Operation with the R-LS-450 Board

You can configure the lamp's performance through 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 lists the many different choices you have for configuring the R-LS-450 for your application. You need to determine the best mode of operation for your setup and configure your system appropriately.

S1 Switch

The S1 Switch is a three-position switch on the R-LS-450. The switch can be positioned in continuous wave operation, no operation, and pulsed operation.

Jumper Block 1 (JP1)

There is only 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 to even control the pulse rate through the Flash Delay feature in OOISensors Software. This feature is only available with an ADC1000 A/D converter and with a "J-series" or later version of the S2000. (To find out if you have a J-series or later S2000, see the third letter in your S2000 serial number.)

Jumper Block 2 (JP2)

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

- ◆ A jumper over the CW pins makes the R-LS-450 operate continuously, which means that there is no pulsing of the light source. Other jumper blocks must be configured correctly. However, this configuration is not recommended for use with the FOXY system.
- ◆ 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 you are using to interface to your S2000. (See table below for pulse rates.)
- ◆ A jumper over the CS pins allows you to control the pulse rate via the OOISensors Software. (See Using JP3 on the S2000 on the next page for more information.)

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 here determines the source of control for the R-LS-450: manual or remote control. A jumper over the Remote pins means that you can control the R-LS-450 through the software (if other jumper blocks are configured correctly).

R-LS-450 Operating Matrix

This matrix will help you configure 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)

SAD500 Specifications

A/D resolution:	12-bit
A/D sampling frequency:	500 kHz (maximum)
Communication port:	RS-232
Baud rate:	2400-115,200
Input voltage:	10 – 24V
Input current:	130 mA without spectrometer
Interface cable:	6-pin DIN connector to PC, 25-pin connector to spectrometer
Multiple-channel capability:	supports up to 8 spectrometer channels
Spectrometer integration time:	5 milliseconds to 60 seconds (S2000 spectrometers)

125/FO Probe Specifications

Fiber core:	1000 μ m silica
Fiber cladding:	Silica
Fiber jacketing:	stainless steel
Outer diameter:	1/8"
Length:	2.5"
Amount of pressure that can be applied:	300 psi
Probe temperature range:	-80° C to +120° 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:	when probe is replaced or cleaned by autoclave
Probe lifetime:	1 year

Appendix 3:

Chemical Effects on FOXY Probes

Chemicals that Interfere with FOXY Measurements	Supporting Data Available
Strong Bases pH> 10	Yes
Styrene	Yes
Ethanol	Yes
Liquid acetone	No
Acetonitrile	Yes
HF	No

Benign Chemicals with FOXY Measurements	Supporting Data Available
50% Methanol (overcoat probe results)	Yes
Acids	No
Hexane	Yes
Sodium Sulfite	Yes
SF6 (test slide results)	Yes
NF ₃ (test slide results)	Yes