OOICHEM Software User's Guide

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PRELIMINARY DRAFT

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About This Manual

Document Purpose and Intended Audience

This document provides information for installing and operating OOIChem software with the Maxwell system.

Document Summary

Chapter	Description
Chapter 1: ***Introduction***	Contains descriptive information about OOIChem software and a quick start procedure.
Chapter 2: ***Using OOIChem Software***	Provides installation and configuration instructions for OOIChem software.
Chapter 3: ***Experiment Tutorial***	Contains procedures for taking measurements in Absorbance, Transmission, Relative Irradiance, Concentration, and Kinetics experiments.

Product-Related Documentation

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.

- Detailed instructions for the OOIBase32 Spectrometer Operating Software are located at: **http://www.oceanoptics.com/technical/ooibase32bit.pdf**.
- Instructions for the OOIPS2000 Operating Software for the handheld PC are located at: **http://www.oceanoptics.com/products/ooips2000.asp**.

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

You can also access operating instructions for Ocean Optics products from the *Software and Technical Resources* CD that ships with the product.

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.

OOIChem software will be updated and improved continuously. To obtain free upgrades, visit out web site at **www.oceanoptics.com/products/Software.asp**. To download free upgrades, you will need the following password: hG59mP236I

Chapter 1

Introduction

Company History

Ocean Optics miniature fiber optic spectrometers and accessories have revolutionized the analytical instrumentation market by dramatically reducing the size and cost of optical sensing systems. More than 10,000 Ocean Optics spectrometers have been sold worldwide -- striking evidence of the far-reaching impact of low-cost, miniature components for fiber optic spectroscopy. Diverse fields such as research and development, industrial process control, medical diagnostics, environmental monitoring and of course, education have benefited from access to Ocean Optics technology.

In fact, Ocean Optics has its roots in education. It formed in 1989 when Florida university researchers developed a fiber optic pH sensor as part of an instrument designed to study the role of the oceans in global warming. The researchers soon formed Ocean Optics, Inc. and their ingenious work earned a Small Business Innovation Research grant from the U.S. Department of Energy. While designing the pH-monitoring instrument, the researchers wanted to incorporate with their sensor a spectrometer small enough to fit onto a buoy and were surprised to discover none existed. So, they built their own.

In 1992, the founders of Ocean Optics filled a substantial need in the research community and changed the science of spectroscopy forever by introducing a breakthrough technology: the S1000 Miniature Fiber Optic Spectrometer, nearly a thousand times smaller and ten times less expensive than previous systems. Due to this dramatic reduction in size and cost of optical sensing systems, applications once deemed too costly or impractical using conventional spectrometers were now feasible.

OOIChem Spectrometer Operating Software

OOIChem is a basic acquisition and display software that provides a real-time interface to a variety of spectral-processing functions. OOIChem allows users to perform basic spectroscopic measurements such as absorbance, transmission, relative irradiance, and concentration. OOIChem operates with Windows 95/98/Me/XP and Windows NT/2000. Visit our website at **www.OceanOptics.com/products/software.asp** to download free OOIChem upgrades.

OOIBase32 Spectrometer Operating Software

OOIBase32 is our standard spectrometer operating software that we provide free of charge to all customers. While OOIChem is a basic acquisition and display program, OOIBase32 is user-customizable and a much more advanced acquisition and display program. With OOIBase32 you have the ability to control all system parameters; collect data from up to 8 spectrometer channels simultaneously and display the results in a single spectral window; perform reference monitoring and time acquisition experiments; and use numerous editing, viewing and spectral processing functions. At any time, users can receive free OOIBase32 updates from our web site at **www.OceanOptics.com/products/software.asp**.

Ocean Optics also offers a complete line of light sources, sampling holders, in-line filter holders, flow cells, and other sampling devices; an extensive line of optical fibers and probes; and collimating lenses, attenuators, diffuse reflectance standards and integrating spheres. All components have SMA terminations so that changing the sampling system is as easy as unscrewing a connector and adding a new component or accessory.

This modular approach -- components are easily mixed and matched -- offers remarkable applications flexibility. Users pick and choose from hundreds of products to create distinctive systems for an almost endless variety of optical-sensing applications

Packing List

A packing list comes with each order. It is located inside a plastic bag attached to the outside of the shipment box. The invoice is mailed separately. The items listed on your packing slip include all of the components in your order. However, some items on your packing list are actually items installed *into* your spectrometer, such as the grating and slit. The packing list also includes important information such as the shipping address, billing address, and components on back order.

Quick Start

Step 1: Install OOIChem Software

► *Procedure*

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

1. Insert the software CD into your floppy drive. Execute **Setup.exe**.
2. At the **Welcome** dialog box, click **Next**.
3. At the **Destination Location** dialog box, select a destination directory. Click **Next**.
4. At the **Backup Replaced Files** dialog box, select either **Yes** or **No**. Selecting **Yes** is recommended and enables you to choose a destination directory. Click **Next**.

5. Select a Program Manager Group. Click **Next**. At the **Start Installation** dialog box, click **Next**.

6. At the **Installation Complete** dialog box, choose **Finish**.

7. When prompted to do so, restart your computer when the installation is complete.

Step 2: Configure OOIChem Software

After you restart your computer, navigate to the OOIChem icon and select it. The first time you run OOIChem after installation, you must follow several prompts to configure your system before taking measurements.

Hardware Configuration

The **Configure Hardware** dialog box opens when you first run OOIChem. The parameters in this dialog box are usually set only once – when OOIChem is first installed and the software first opens.

► *Procedure*

1. Under **Spectrometer Type**, choose S4000.

2. Under **A/D Converter Type**, choose HR4000.

3. In the **USB Serial Number** field, select the serial number of the HR4000 spectrometer in your Maxwell system from the drop-down list.

4. For your setup, only these parameters apply to your system. Click **OK**. You can always change these settings once OOIChem is fully operational by selecting **Spectrometer | Hardware Configuration**.

Spectrometer Configuration

At this point, OOIChem should be acquiring data from your spectrometer. There should be a dynamic trace responding to light near the bottom of the displayed graph. Now that OOIChem is running, you need to configure your system. Select **Spectrometer | Spectrometer Configuration** from the menu.

- **Coefficients.** Loaded automatically from the spectrometer.
- **Trigger mode.** Select **No External Trigger**, unless you have wired an external triggering device to the spectrometer for synchronizing data with an external event.
- **Graph and chart display mode.** Choose **Spectrum Only** to only view live spectra from one spectrometer channel. Choose **Spectrum & Kinetics** to view both real-time live spectra in the top half of the graph and to view a chart displaying your kinetics experiment in the bottom half of the graph.
- **Flash Delay.** This function is for use with a strobe light source. The Maxwell system does not come with a strobe light source.

* **Color Temperature.** Enter the color temperature of your reference light source used in relative irradiance measurements.

Acquisition Parameters

Set data acquisition parameters by choosing an integration period and selecting averaging and boxcar smoothing values.

Text Box

Enter the operator name, or any other identifying text here. This text appears in your data files. You can edit this text at any time.

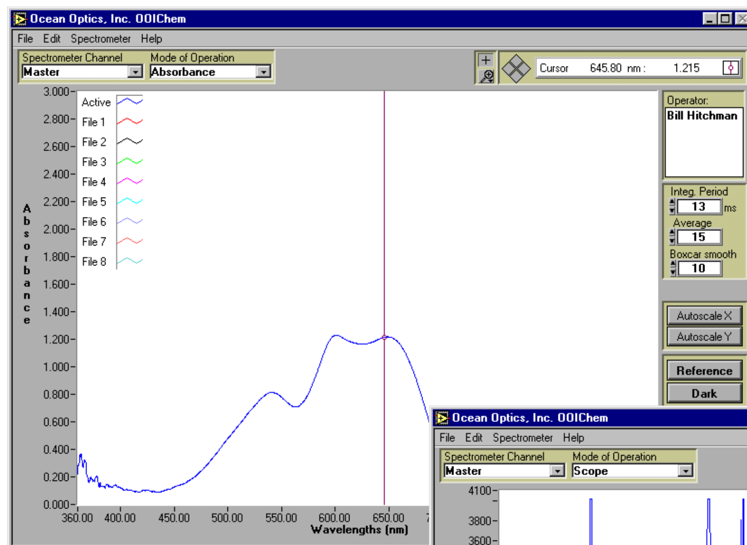
Step 3: Receive Data

Run OOIChem in Scope Mode and take a reference spectrum and a dark spectrum (see *Experiment Tutorial* for details). Choose the absorbance, transmission, or relative irradiance mode to take your sample measurements.

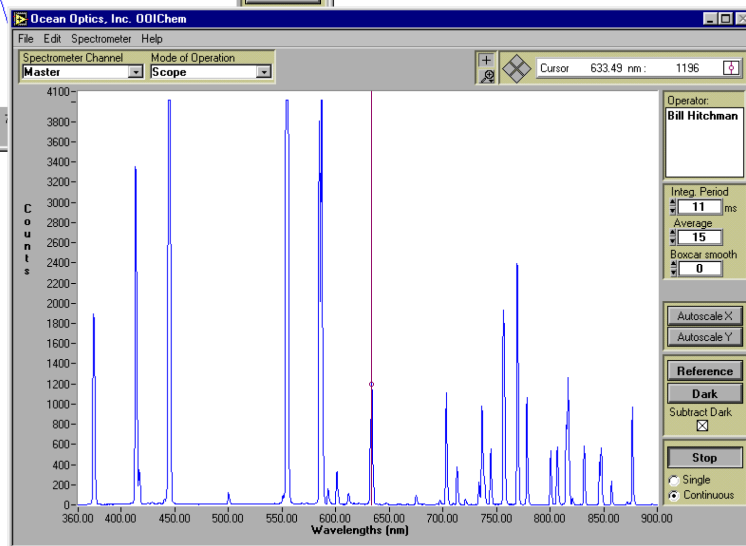
Chapter 2

Using OOIChem Software

OOIChem software provides users with five different modes of operation: Scope, Absorbance, Transmission, Relative Irradiance, and Concentration. In addition, the software allows users to control data acquisition features such as integration period, averaging, and boxcar smoothing -- directly from the spectral graph display. Users can acquire data by taking manual single scans or by running continuous scans, and add into the spectral window as many as 8 previously saved overlay spectra.



Users can also perform kinetics experiments, which allows users to monitor and report up to 4 single wavelengths or up to 2 calculated values from a combination of wavelengths – for example, an absorbance value of 400 minus an absorbance value of 700. A kinetics chart displays the time series. When the experiment is complete, the data can be exported to an ASCII file for additional processing.



Another exciting feature of OOIChem is that users can control the parameters for all system functions such as acquiring data, designing the graph display, and configuring the cursor. Additional features of OOIChem include the ability to save data as ASCII files and to store and retrieve sample spectra.

If you find that you need more advanced spectrometer operating software, we have included, free of charge, OOIBase32, a sophisticated, 32-bit, user-customizable advanced acquisition program. See the included OOIBase32 manual for a list of functions and features.

Display Functions

Several functions are accessed not through the menu but through buttons and task bars directly on the display screen, on the top and to the right of the graph area. (The resolution of your computer's monitor must be 800 x 600 or better to view OOIChem software.) From the display screen, you can choose a mode of operation, configure the cursor, configure the graph, enter acquisition parameters, choose a mode to acquire data, take reference, dark, and sample scans of your sample, and scale the graph.

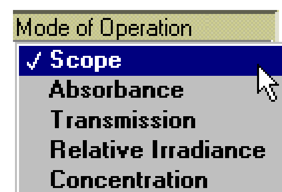
Spectrometer Channel Selection

The Spectrometer Channel area allows you to animate the window for a specific spectrometer channel. The Maxwell is a single spectrometer channel system. Select **Master**.

Mode of Operation

Scope

The signal graphed in Scope Mode is the raw voltage coming out of the A/D converter. Once you open OOIChem and it begins to acquire data, you see the raw voltage from the detector expressed in A/D counts. This spectral view mode is unique to Ocean Optics. It allows you to control signal processing functions before taking absorbance, transmission, and relative irradiance measurements. Be careful when using this mode, as it represents a combination of several factors: the intensity of the light source, the reflectivity of the grating and the mirrors in the spectrometer, the transmission of the fibers, the response of the detector, and the spectral characteristics of the sample.



Scope Mode should primarily be used when selecting signal acquisition parameters such as integration period, averaging and boxcar smoothing; and when taking dark and reference scans.

Absorbance

Selecting this mode switches the spectral window into Absorbance Mode. Before this can occur, both a dark and reference scan must be stored in Scope Mode (see *Experiment Tutorial*). Absorbance is calculated by the following equation. When this equation is evaluated for each pixel of the detector, the absorbance spectrum is produced.

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

Where:

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

Transmission

Selecting this mode switches the spectral window into Transmission Mode. This is also the spectral processing mode used for Reflection spectroscopy, as the math necessary to compute reflection is identical to transmission. Before this can occur, both a dark and reference scan must be stored in Scope Mode (see *Experiment Tutorial*). The transmission of a solution is calculated by the following equation:

$$\%T_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\%$$

Where:

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

Relative Irradiance

Selecting this mode switches the spectral window into Relative Irradiance Mode. The reference spectrum must be made in Scope Mode with a blackbody of known color temperature. (Maxwell users cannot make relative irradiance measurements because the light source that comes with the system is not a blackbody source with a known color temperature. Additional hardware must be purchased.) A dark spectrum is usually obtained by preventing light from entering the fiber that connects to the spectrometer (see *Experiment Tutorial*). Relative irradiance spectra are a measure of the intensity of a light source relative to a reference emission source. Relative irradiance is calculated by the following equation:

$$I_\lambda = B_\lambda \left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \right)$$

Where:

B = Relative energy of the reference calculated from the color temperature (in Kelvin)

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

Concentration

Concentration is the amount of a specified substance in a solution. Graphs of absorbance vs. concentration are known as Beer's Law plots. These are calculated by first measuring the light that is absorbed from a series of solutions with different *known* concentrations. The length of the sample -- such as the path length of our cuvette holder – and the wavelength chosen for monitoring the amount of light absorbed, are constants. Then a linear plot derived from the scans of these standard solutions with known concentrations is obtained. The plot is then used to determine the *unknown* concentrations of solutions. (see *Experiment Tutorial*).

The absorbance of a solution is related to the concentration of the species within it. The relationship, known as Beer's Law , is:

$$A_\lambda = \varepsilon_\lambda \, c \, \ell$$

Where:

A = Absorbance at wavelength λ,

ε = Extinction coefficient of the absorbing species at wavelength λ

c = Concentration

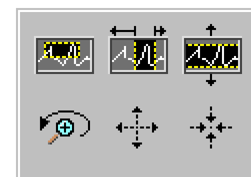
ℓ = Optical pathlength

Cursor Function Bar

+ Sign

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

Magnify Symbols



When the magnify symbol is selected, you can choose from among 6 magnify functions. The function chosen will remain 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:

Magnifies a specific area by clicking and dragging a box around an area

Zooms in on the horizontal scale, but the vertical scale remains the same

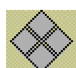
Zooms in on the vertical scale, but the horizontal scale remains the same

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 Diamond

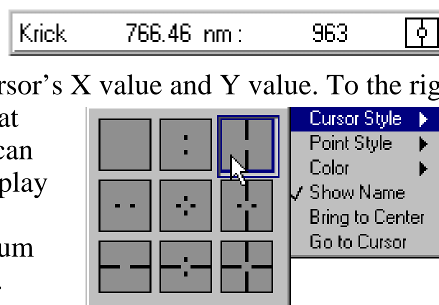


To move the cursor left or right in small increments in the graph area, click on the left and right sections of the move cursor diamond. The top and bottom sections of the diamond will send the cursor to the next or previous channel in your system. (The Maxwell is a single channel system. However, additional channels can be purchased at any time.)

Cursor Properties

Krick 766.46 nm : 963

In this toolbar, you can label the cursor and monitor the cursor's X value and Y value. 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. You can also choose a color for the cursor and whether or not to display the name of the channel the cursor is currently reporting. Finally, you can bring the cursor to the center of the spectrum or center the spectrum around the cursor's current position.



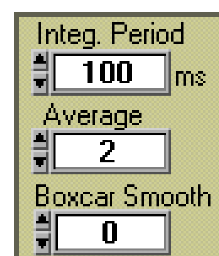
Text Box

This box allows you to enter an operator name and any other text to identify your experiment. This text appears in your data files. You can edit this text at any time.

Acquisition Parameters

Integration Period

Enter a value to set the integration period in milliseconds for an active spectrometer channel. The integration period of the spectrometer is analogous to the shutter speed of a camera. The higher the value specified for the integration period, the longer the detector "looks" at the incoming photons. If your scope mode intensity is too low, increase this value. If the intensity is too high, decrease the value. While watching the graph trace in Scope Mode, adjust the integration period and other acquisition parameters until the signal intensity level is approximately 3500 counts.



Average

Enter a value to implement a sample averaging function that averages the specified number of spectra. The higher the value entered the better the signal-to-noise ratio. The S:N improves by the square root of the number of scans averaged.

Boxcar Smooth

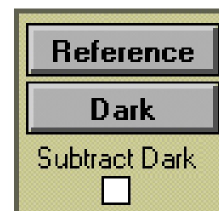
Enter a value to implement a boxcar smoothing technique that averages across spectral data. This method averages a group of adjacent detector elements. A value of 5, for example, averages each data point with 5 points (or bins) to its left and 5 points to its right. The greater this value, the smoother 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.

Reference Scan

Selecting the Reference button activates a prompt to make sure your light is on. You then must choose to either **Store** or **Cancel** your reference scan. A reference spectrum is taken with the light source on and a

blank in the sampling region. Storing a reference spectrum is requisite before the software can calculate absorbance, transmission, and relative irradiance spectra. This command merely stores a reference spectrum. To permanently save the reference spectrum to disk, select **File | Save Spectral Values** from the menu.

Dark Scan



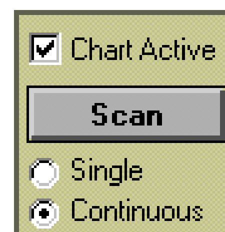
Selecting the Dark button activates a prompt to make sure the light path is blocked. You then must choose to either **Store** or **Cancel** your dark scan. A dark spectrum is taken with the light path to the spectrometer blocked. Storing a dark spectrum is requisite before the software can calculate absorbance, transmission, and relative irradiance spectra. This command merely stores a dark spectrum. To permanently save the reference spectrum to disk, select **File | Save Spectral Values** from the menu.

Subtract Dark

Selecting this box subtracts the current dark spectrum from the spectra being displayed. This command is useful if you are trying to look at a change in an emission spectrum or are trying to eliminate from the spectra fixed pattern noise caused by a very long integration period. The subtract dark spectrum function only acts on spectra displayed in Scope Mode.

Acquire Data Modes

Chart Active



When **Spectrum & Kinetics** is chosen as the **Graph and chart display mode** in the **Spectrometer Configuration** dialog box, the **Chart Active** function becomes visible in the display area above the Scan button. This function is responsible only for the Kinetics chart. By deselecting this function, users can use the scan button and collect data in just the spectrum section of the graph. If the function is enabled, choosing the scan button results in the collection of data in both the spectrum section of the graph and the kinetics section of the graph.

Scan/Stop Button

When in **Single** mode, the Scan button acts as a snapshot. After selecting the Single mode, click on the Scan button to take a scan. The button depresses and Stop replaces Scan. The button will stay depressed until the scan has been completed (the time set in the **Integration Period** box).

When in **Continuous** mode, the Scan button continuously takes scans. After each integration cycle, another scan will immediately begin. The button depresses and Stop replaces Scan. Click on Stop to halt the scanning process and discontinue acquiring data.

Scaling the Graph

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.

File Menu Functions

Save Spectral Values

Select **File | Save Spectral Values** from the menu to save the current spectrum. Text box entries and acquisition parameters are included in the headers of these files. You can then use these files as overlays or import them into other software programs, such as Microsoft Excel.

Save Kinetics Values

Select **File | Save Kinetics Values** from the menu to save kinetics data. Text box entries and acquisition parameters are included in the headers of these files. You can then import them into other software programs, such as Microsoft Excel.

Open Spectrum Overlay

Select **File | Open Spectrum Overlay** 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. You can open up to 8 overlays in the graph.

Open Kinetics Values

Select **File | Open Kinetics Values** from the menu to open a dialog box that allows you to open a previously saved kinetics chart.

Printer Setup

Select **File | Printer Setup** from the menu to select and configure a printer for printing graphical spectra or kinetics data.

Print Spectra and Kinetics

Select **File | Print Spectra** from the menu to print a spectrum, or select **File | Print Kinetics** from the menu to print kinetics data.

Exit

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

Edit Menu Functions

Clear Spectrum Overlays

Select **Edit | Clear Spectrum Overlays** from the menu to remove static spectra from the graph.

Clear Kinetics Values

Select **Edit | Clear Kinetics Values** from the menu to clear both the kinetics values from the chart and to clear the kinetics traces. A message box then appears, asking if you are sure you want to clear the kinetics chart.

Autoscale X

The **Autoscale X** function automatically adjusts the horizontal scale of a current graph so the entire horizontal spectrum fills the display area.

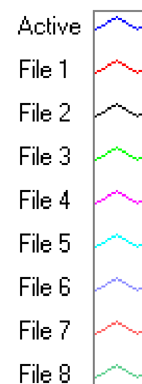
Autoscale Y

The **Autoscale Y** function automatically adjusts the vertical scale of a current graph so the entire vertical spectrum fills the display area.

Show Kinetics Values

When setting up your kinetics experiment, you must first select **Spectrometer | Spectrometer Configuration** from the menu and make sure that **Spectrum & Kinetics** is selected next to **Graph and chart display mode**. Then configure your experiment by selecting **Spectrometer | Kinetics Configuration** from the menu. When you select your wavelengths, the values from these wavelengths will be displayed above the kinetics chart if this function is enabled.

Show Legends



Select **Edit | Show Legends** to enable or disable the legends for the spectral trace, overlays, and kinetics traces. When the legends are displayed, you can opt to configure the traces by simply clicking on the legend trace you want to configure. You have the opportunity to choose from several aesthetic functions such as: the plot design of the spectrum, the point style used in the spectrum, the line style and width desired, color of the plot, and a bar plot design. You can also choose to fill the baseline in the spectrum. Utilize this function to differentiate one spectral trace from another.

Spectrometer Menu Functions

Scan

When the **Single** mode is selected in the display screen, the Scan menu function acts as a snapshot. After selecting the Single mode, select **Spectrometer | Scan** from the menu to take one scan of the sample. When the **Continuous** mode is selected in the display screen, select **Spectrometer | Scan** from the menu to continuously take scans.

Select Concentration Wavelength

This function is used when calculating the unknown concentration of a substance in a solution. You select this function after you take an absorbance measurement of a standard solution with a *known* concentration. Choose the wavelength of the highest peak in your absorbance spectrum. Then select **Spectrometer | Calculate Calibration Curve** from the menu and complete the rest of your concentration experiment. See *Concentration Experiments* for step-by-step instructions on calculating concentrations.

Calculate Calibration Curve

Concentration is the amount of a specified substance in a solution. In order to calculate concentration, you must take absorbance measurements of a series of solutions with different *known* concentrations. The length of the sample and the wavelength chosen for monitoring the amount of light absorbed are constants. Then a linear plot from taking these scans is obtained. This Calibration Curve is used to determine the *unknown* concentrations. See *Concentration Experiments* for step-by-step instructions on calculating concentrations and on using this dialog box.

Enable Strobe

This function allows you to enable or disable the triggering of external strobes through the spectrometer. You would only select **Spectrometer | Strobe Enable** from the menu if you were operating an external strobe source. The Maxwell system does not include a strobe light source. However, the light source that comes with the Maxwell can be turned off and on through the software and this function.

Trigger Mode

With OOIChem Operating Software, you have two methods of acquiring data. In the **No External Trigger Mode** (or Normal Mode), the spectrometer is "free running." That is, 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 scanning, acquiring and transferring of data with an external event.

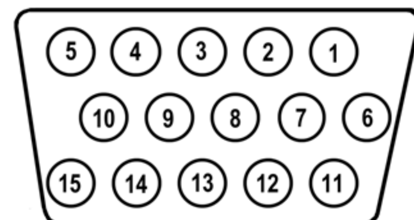
To synchronize data acquisition with an external event, the **External Software Trigger Mode** is available. It involves connecting an external triggering device to the spectrometer and then applying an external trigger to the spectrometer before the software receives the data. In this mode, the spectrometer is "free running," just as it is in the Normal Mode. The spectrometer is continually scanning and collecting data. With each trigger, the data collected during the integration period is transferred to the software. All acquisition parameters, such as the integration period, are still set in the software. You should use this mode if you are using a continuous light source and its intensity is constant before, during and after the trigger.

Note

To use the External Software Triggering 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 triggering mode as it is described here.

► *Procedure*

To use the External Software Trigger Mode:



J2 (D-SUB-15) Accessory Connector (female)

1. Supply a line from your triggering device to Pin 3 of the J2 Accessory Connector on the spectrometer to provide the positive voltage +5VDC to the spectrometer. (See figure for pin location.) We do not advise using an outside source to supply the voltage, as it is based on a referenced ground and your reference may be different from ours. Using Pin 3 to supply voltage ensures that the spectrometer will receive the appropriate voltage for the trigger event.

2. Supply a line from Pin 8 of the J2 Accessory Connector to your triggering device. (See figure for pin location.)

3. Set your acquisition parameters in the software.

4. Select **Spectrometer | Spectrometer Configuration** from the menu and choose **External Software Trigger**.

5. Once you select **External Software Trigger**, it will appear on your computer that your spectrometer is unresponsive. Instead, it is waiting for the trigger. Activate your triggering device.

Graph and Chart Display Mode

If you choose **Spectrum Only**, spectra from one real-time spectrometer channel and up to 8 overlays can be displayed in the graph area. If you choose **Spectrum & Kinetics**, spectra from one real-time spectrometer channel and up to 8 overlays can be displayed in the in the top half of the graph area. A kinetics chart monitoring values from up to 4 wavelengths and 2 of their arithmetic calculations can be displayed in the in the bottom half of the graph area, if the **Chart Active** function is enabled.

Flash Delay

The value entered here sets the delay, in milliseconds, between strobe signals sent out of the spectrometer. This function allows you to enable or disable the triggering of external strobes through the spectrometer. You would only enter a value in this box if you were operating an external strobe source. The Maxwell system does not include a strobe light source.

Color Temperature

This box allows you to enter the color temperature (in Kelvin) of your light source. In order to calculate relative irradiance, the reference spectrum must be made in Scope Mode with a blackbody light source of known color temperature. This data is necessary for the software to complete calculations for relative irradiance measurements. Maxwell users must purchase additional hardware to make relative irradiance measurements.

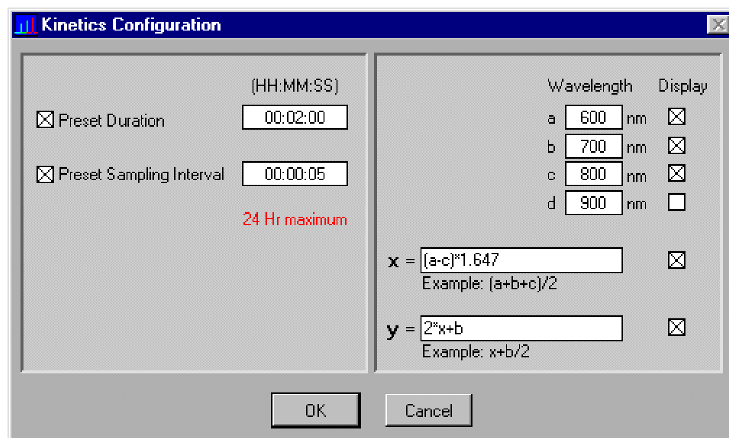
Kinetics Configuration

Select **Spectrometer | Kinetics Configuration** from the menu to configure and establish the parameters for a kinetics experiment. In the **Kinetics Configuration** dialog box, you can collect spectral data as a function of time, from up to 4 single wavelengths and up to two mathematical combinations of these wavelengths.

Data from a kinetics experiment will not be displayed in the graph unless you choose **Spectrometer | Spectrometer Configuration** from the menu and select **Spectrum & Kinetics** next to **Graph and chart display mode**. This way, not only will your kinetics experiment be displayed in the bottom half of the graph area, you will also still see real-time spectra in the top half of the graph area.

Preset Duration

Enter a value to set the length of time for the entire kinetics process. Be sure to select hours, minutes and seconds. Your kinetics experiment cannot exceed a duration of 24 hours.



Preset Sampling Interval

Enter a value to set the frequency of the data collected in a kinetics process. Be sure to select hours, minutes and seconds.

Wavelength

Enter the single wavelengths from which you wish to collect data. You can collect data from up to 4 single wavelengths, characterized as **a**, **b**, **c**, and **d**.

Display

If you want the data graphed from these single wavelengths, or from the mathematical calculations of these wavelengths (described below), select the display box to the right of your values.

Mathematical Calculations

In the boxes next to **x =** and **y =**, you have the opportunity to perform calculations on the data collected from the single wavelengths you specified as **a**, **b**, **c**, and **d**. Also in the box next to **y =**, you can use **x**, which represents the calculation used in the box next to **x =**.

Chapter 3

Experiment Tutorial

Overview

When you are ready to begin your experiment, you should have already installed the Maxwell and OOIChem software. Now you are ready to take your measurements. Because of the components making up your Maxwell system, it is ideal for absorbance and transmission. The Maxwell system can also make relative irradiance measurements. If, however, you wish to use your system for other measuring functions, additional products might be required. Contact an Ocean Optics Applications Scientist for options.

Absorbance Experiments

Absorbance spectra are a measure of how much light is absorbed by a sample. The software calculates absorbance (A_λ) using the following equation:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

Where:

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

Common applications include the quantification of chemical concentrations in aqueous or gaseous samples.

► *Procedure*

To take an absorbance measurement:

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is required before the software can calculate absorbance spectra.

2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate absorbance spectra.

3. Begin an absorbance measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then select **Absorbance** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

Note

If at any time any sampling variable changes – including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. – you must store a new reference and dark spectrum.

Transmission Experiments

Transmission is the percentage of energy passing through a system relative to the amount that passes through the reference. Transmission Mode is also used to show the portion of light *reflected* from a sample. Transmission and reflection measurements require the same mathematical calculation. The transmission is expressed as a percentage ($\%T_\lambda$) relative to a standard substance (such as air).

The software calculates $\%T_\lambda$ (or $\%R_\lambda$) by the following equation:

$$\%T_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\%$$

Where:

S = Sample intensity at wavelength λ

D = Dark intensity at wavelength λ

R = Reference intensity at wavelength λ

Common applications include measurement of transmission of light through solutions, optical filters, optical coatings, and other optical elements such as lenses and fibers.

Note

For transmission of light through solutions, we offer a transmission dip probe with screw-on, removable tips in 2-mm, 5-mm or 10-mm path lengths. Contact Ocean Optics for more information.

► *Procedure*

To take a transmission measurement:

1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate transmission spectra.

2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate transmission spectra.

3. Begin a transmission measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then select **Transmission** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

Note

If at any time any sampling variable changes – including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. – you must store a new reference and dark spectrum.

Relative Irradiance Experiments

Irradiance is the amount of energy at each wavelength from a radiant sample. In relative terms, it is the fraction of energy from the sample compared to the energy collected from a lamp with a blackbody energy distribution, normalized to 1 at the energy maximum. Relative irradiance is calculated by the following equation:

$$I_\lambda = B_\lambda \left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \right)$$

Where:

B = Relative energy of the reference calculated from the color temperature (in Kelvin)
S = Sample intensity at wavelength λ
D = Dark intensity at wavelength λ
R = Reference intensity at wavelength λ

Common applications include characterizing the light output of LEDs, incandescent lamps and other radiant energy sources such as sunlight. Also included in irradiance measurements is fluorescence, in which case the spectrometer measures the energy given off by materials that have been excited by light at a shorter wavelength.

Note

The components that came with the Maxwell will not allow you to make relative irradiance measurements. To make relative irradiance measurements, the reference spectrum must be made in Scope Mode with a blackbody light source of known color temperature. This color temperature is needed in order to calculate relative irradiance. The light source that comes with the Maxwell is not a blackbody light source with a known color. To purchase a blackbody light source and other necessary hardware, contact Ocean Optics.

► *Procedure*

To take a relative irradiance measurement:

1. Select **Spectrometer | Spectrometer Configuration** from the menu. Next to **Color Temp**, make sure the color temperature in Kelvin of the reference lamp you are going to use is entered here. Click **OK**.

2. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale by adjusting acquisition parameters. Take a reference spectrum of your reference lamp. Take the reference reading by clicking the **Reference** button in the software display area. (This command merely stores a reference spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a reference spectrum is requisite before the software can calculate relative irradiance spectra.

3. While still in **Scope Mode**, take a dark spectrum by first completely blocking light from going to your spectrometer. Take the dark reading by clicking the **Dark** button in the software display area. (This command merely stores a dark spectrum. To save a spectrum, you must select **File | Save Spectral Values** from the menu.) Storing a dark spectrum is requisite before the software can calculate relative irradiance spectra.

4. Begin a relative irradiance measurement by first positioning the fiber at the light or emission source you wish to measure. Then select **Relative Irradiance** under **Mode of Operation** in the software display area. Click on the **Scan** button in the display area to take a scan. If **Single** is selected, only one scan will be taken. If **Continuous** is selected, the spectrometer will continuously take scans until you click on the **Stop** button. To save the spectrum, select **File | Save Spectral Values** from the menu.

Note

If at any time any sampling variable changes – including integration period, averaging, boxcar smoothing, distance from light source to sample, etc. – you must store a new reference and dark spectrum.

Concentration Experiments

The absorbance of a solution is related to the concentration of the species within it. The relationship, known as Beer's Law , is:

$$A_\lambda = \varepsilon_\lambda \, c \, \ell$$

Where:

A = Absorbance at wavelength λ,

ε = Extinction coefficient of the absorbing species at wavelength λ

c = Concentration

ℓ = Optical pathlength

Concentration is the amount of a specified substance in a solution. Graphs of absorbance vs. concentration are known as Beer's Law plots. These are prepared by measuring the light absorbed by a series of solutions with different *known* concentrations. The length of the sample -- such as the path length of our cuvette holder -- and the wavelength chosen for monitoring the amount of light absorbed are constants. A linear plot from taking scans of these standard solutions with known concentrations is then obtained. The plot is then used to determine the *unknown* concentrations of substances in solutions.

To discover the unknown concentration of a substance in a solution, you must first take spectral scans of a series of solutions with different known concentrations of the same substance. You begin this process by taking an absorbance spectrum of the solution with the highest known concentration.

► *Procedure*

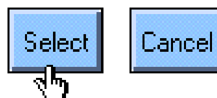
1. Select **Scope** under **Mode of Operation** in the software display area. Make sure the signal is on scale. Adjust acquisition parameters so that the peak intensity of the reference signal is about 3500 counts. Take a reference spectrum by first making sure nothing is blocking the light path going to your spectrometer. The solution with the highest known concentration you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Reference** button in the software display area. To save the spectrum, select **File | Save Spectral Values** from the menu.

2. While still in **Scope Mode**, take a dark spectrum by first completely blocking the light path going to your spectrometer. (If possible, do not turn off the light source. If you must turn off your light source to store a dark spectrum, make sure to allow enough time for the lamp to warm up before continuing your experiment.) Take the dark reading by clicking the **Dark** button in the software display area. To save the spectrum, select **File | Save Spectral Values** from the menu.

3. Take the solution with the highest known concentration and put it in the cuvette holder. Make sure nothing is blocking the light going to your sample. Then select **Absorbance** under **Mode of Operation**. Click on the **Scan** button in the display area to take a scan. Make sure **Single** is selected. To save the spectrum, select **File | Save Spectral Values** from the menu.

4. Now select the wavelength for monitoring the concentration of your solutions by choosing **Spectrometer | Select Concentration Wavelength** from the menu. Move the cursor to the highest absorbance peak of the spectrum of the solution with the highest known concentration and choose **Select**.

Move cursor to desired wavelength and click :

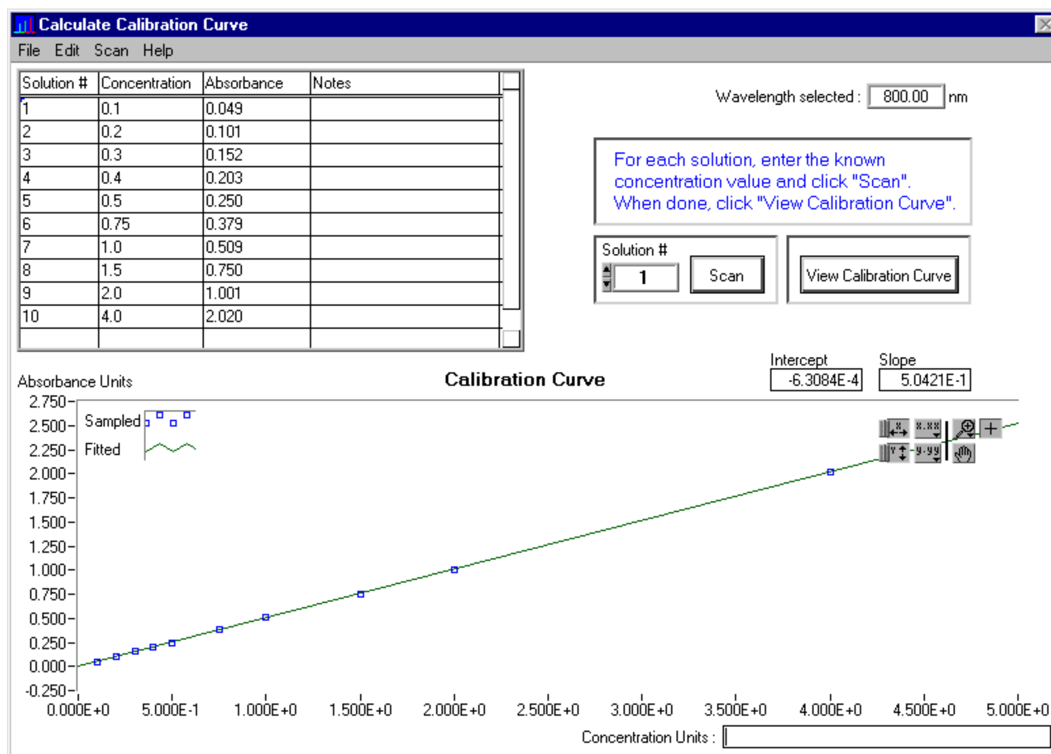


5. Remove the solution with the highest known concentration. Select **Spectrometer | Calculate a Calibration Curve** from the menu. The Calculate Calibration Curve dialog box opens. Now you will begin taking scans of the rest of your series of standard solutions with known concentrations, from the lowest known concentration to highest, all while working in this dialog box.

6. If you wish, you can take a new reference and a new dark scan for each solution by choosing **Scan | Dark** and **Scan | Reference** from the menu of this dialog box. However, in this case, it is not necessary. If no reference or dark scan is taken at this point, the software will use the reference and dark scans taken in Steps 1 and 2 to calculate absorbance.

7. Take the solution with the lowest known concentration and put it in the cuvette holder. Enter the known concentration of the standard solution in the chart in the **Concentration** column, next to **Solution #1**.

8. Click the **Scan** button or select **Scan | Solution** from the menu. The absorbance value will appear next to the concentration for Solution #1. At any point, you can select **Edit | Clear** from the menu to clear the dialog box of all data.



9. Take Solution #1 out of the cuvette holder and put in another standard solution with the next highest known concentration. Enter the known concentration of the standard solution in the chart in the **Concentration** column, next to **Solution #2**.

10. Click the **Scan** button or select **Scan | Solution** from the menu. The absorbance value will appear next to the concentration for Solution #2.

11. You may continue to scan solutions with known concentrations. You must scan at least 2 in order to achieve a calibration curve.

12. When you have completed taking scans of your solutions with known concentrations, click the **View Calibration Curve** button. You will then have the **Intercept** and **Slope** of your curve. The Slope is the ε necessary to compute Beer's Law and to find the *unknown* concentration of a solution.

13. At this time, you may also select a label for your concentration values, such at **Moles per Liter**, in the **Concentration Units** box. This is only a label and does not affect the data in any way.

14. Select **Edit | Show Legend** from the menu to display the legend for the calibration curve. The legend allows you to choose a plot design, point style, line style, line width and plot color. Select **Edit | Show Palette** from the menu to display a variety of options for configuring the curve. The palette provides features such as autoscaling, graph formatting, value precision, mode mapping, and graph positioning.

15. You can select **File | Print** from the menu for the dialog box to print the dialog box. To save the current calibration curve data, select **File | Save** from the menu for the dialog box.

16. Select **File | Close** from the menu for the dialog box to close this dialog box and return to the main window.

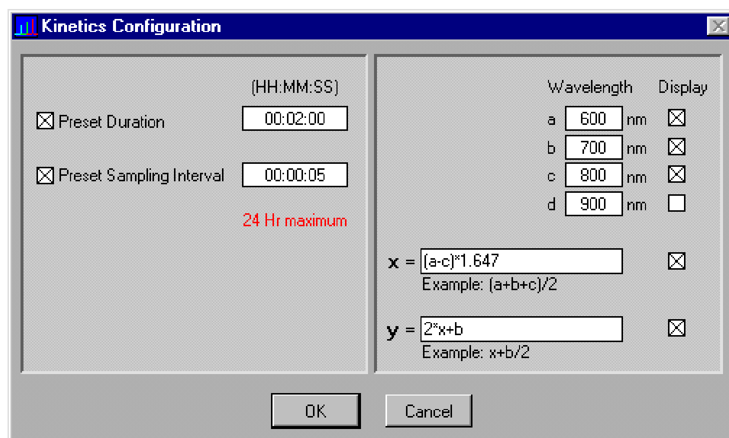
17. When a message box asks if you would like to use this calibration curve when calculating concentration values, select **Yes**.

18. Now that you are back in the main display window, place the solution with the *unknown* concentration of a substance in the cuvette holder.

19. Under **Mode of Operation**, select **Concentration**. Click the **Scan** button to receive your concentration values.

Kinetics Experiments

Select **Spectrometer | Kinetics Configuration** from the menu to configure and establish the parameters for a kinetics experiment. In the **Kinetics Configuration** dialog box, you can collect spectral data as a function of time, from up to 4 single wavelengths and up to two mathematical combinations of these wavelengths.



A kinetics experiment will not be displayed in the graph unless you choose **Spectrometer | Spectrometer Configuration** from the menu and choose **Spectrum & Kinetics** next to **Graph and chart display mode**. This way, not only will your kinetics experiment be displayed in the bottom half of the graph area, you will also still see a spectrometer channel's real-time spectra in the top half of the graph area.

► *Procedure*

To run a kinetics experiment:

1. In the graph area, select acquisition parameters, such as integration period, averaging, and boxcar smoothing values. Do not change these parameters for the duration of the kinetics experiment.

2. Select **Spectrometer | Kinetics Configuration** from the menu to open the Kinetics Configuration dialog box.

3. Enter a **Preset Duration** value to set the length of time for the entire kinetics process. Be sure to select hours, minutes and seconds. The duration of your kinetics experiment cannot exceed 24 hours.

4. Enter a **Preset Sampling Interval** value to set the frequency of the data collected in a kinetics process. Be sure to select hours, minutes and seconds. Data from a timed acquisition is stamped with a time that is accurate to 1 millisecond.

5. Under **Wavelength**, enter the single wavelengths from which you wish to collect data. You can collect data from up to 4 single wavelengths, characterized as **a**, **b**, **c**, and **d**.

6. If you want the data graphed from these single wavelengths, or from the mathematical calculations (described in the next step), select the **Display** box to the right of your values.

7. In the boxes next to **x =** and **y =**, you have the opportunity to perform calculations on the data collected from the single wavelengths you specified as **a**, **b**, **c**, and **d**. In the **y =** box, you can also use **x**, which represents the calculation used in **x =**.

8. Click **OK** to confirm the parameters and close the dialog box.

9. Click the **Scan** button to begin the kinetics experiment. Make sure that **Continuous** is selected. The top half of the graph displays a real-time full wavelength spectrum. The bottom half of the graph displays the data for the selected wavelengths and their derived arithmetic calculations. Each data set is stored with a time stamp.

10. Click the **Stop** button to stop the experiment. However, if a **Preset Duration** time was selected, the experiment will automatically stop after the designated time has passed.

11. Select **File | Save Kinetics Values** from the menu to save a tab-delimited ASCII file with the spectrometer's serial number, active channel and acquisition parameters in a header. This file can be opened with any text or spreadsheet editor.

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