

OOIBase Operating Software

Instruction Manual

Version 1.52

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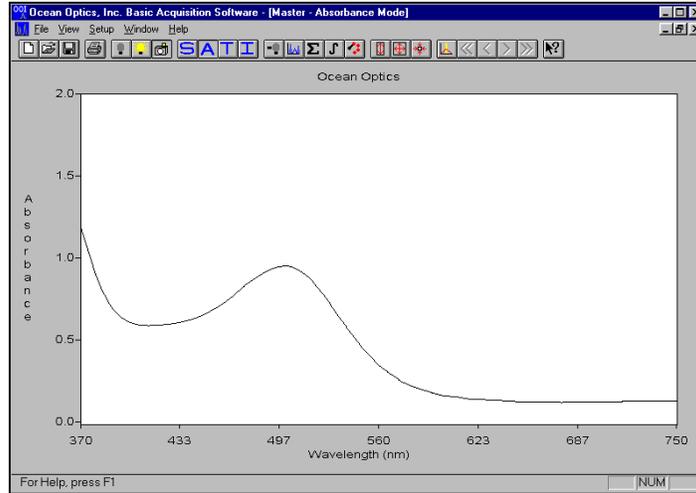


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About OOIBase Operating Software

OOIBase is the standard operating software for Ocean Optics spectrometers. OOIBase for Windows is a basic acquisition and display software that provides a real-time interface to a variety of signal-processing functions. OOIBase allows users to perform basic spectroscopic measurements such as absorbance, reflectance and emission, as well as more sophisticated tasks such as the automation of experiments with a time-series acquisition function. OOIBase allows the user to view, in real-time and entirely on one screen, spectral data from up to 8 spectrometer channels.



Absorbance spectrum of cranberry juice

OOIBase offers users considerably more flexibility than they are likely to experience with other spectrometer operating software. The basic concept for the software is that real-time display of data allows users to evaluate the effectiveness of their experimental setup and data processing selections, make changes to these parameters, instantly see their effect, and save the data. OOIBase offers:

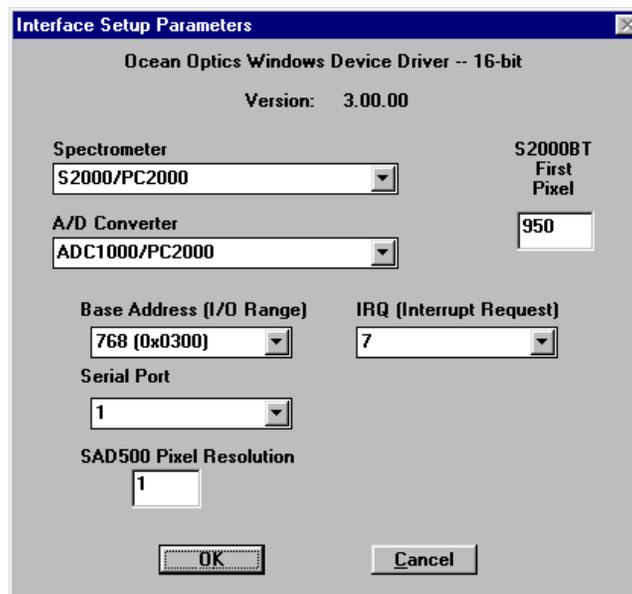
- ❖ Spectrometer control
 - integration time regulation
 - multiple triggering options
 - channel selection
- ❖ Signal Processing including:
 - signal averaging
 - boxcar pixel smoothing
 - electrical dark-signal subtraction
 - stray light correction
- ❖ Spectral Processing: absorbance, transmission, irradiance
- ❖ Time series acquisition for full spectra
- ❖ Real time graphical display with zoom, cursor and cosmetic selections
- ❖ Multi-window support
- ❖ File Export to ASCII format

OOIBase operates with Windows 3.x and Windows 95/98. Windows NT users are easily able to write their own applications using the OOIWinIP Windows or OOILVD LabVIEW drivers.

☺ All Ocean Optics software products comply with Year 2000 requirements. ☺

Installing OOIBase Operating Software

1. Insert the OOIBase floppy disk into a drive.
2. Select **Run** and type **(drive):setup**. Click **OK**.
3. Click **OK** at the “Welcome!” dialog box.
4. Select the **Destination Directory**. Choose the default destination or type where you want the software housed. Click **OK**.
5. In the “Make Backups” dialog box, click **YES**.
6. After the software installation is complete, you will go back out to your desktop.
7. Start OOIBase by selecting **Start | Programs | OOIBase V 1.5 | OOIBase V 1.5**.
8. The first time the software starts, the “Interface Setup Parameters” dialog box opens. This box allows you to specify your Spectrometer, A/D Converter, Base Address (I/O Range), and IRQ (Interrupt Request). Choose the correct parameters for your setup. For the ADC500, ADC1000 or PC2000, choose the same Base Address and IRQ settings as the dip switches on the A/D board. If you left them unchanged, accept the default settings (Base Address of 768 and IRQ of 7). If you have the DAQ-700, you will have to use your computer to find the correct settings of the hardware. For specific instructions on changing the settings for these A/D cards, see the directions that came with your product. If you have the SAD500, ignore the Base Address and IRQ selections. Click **OK**. You can always go back to this dialog box by selecting **Setup | Configure Hardware**



9. If you do not see the “Interface Setup Parameters” dialog box when you start OOIBase, exit OOIBase by selecting **File | Exit**. Then select **Start | Run**, and type **C:\windows\oidrv.ini**. This will open Notepad and allow you to edit the initialization file for our device driver. Find the “Initialized” entry within this file. Make sure this line reads: **Initialized=0**. Save the OOIDRV.INI file and exit Notepad. Restart OOIBase. You should now see the “Interface Setup Parameters” dialog box allowing you to specify your hardware configuration.
10. At this point, OOIBase should be acquiring data from your spectrometer. There should be a dynamic trace responding to light near the bottom of the displayed graph. If your spectrometer is not responding to light (or your computer hangs or crashes), please contact our Technical Support Department.
11. Exit and restart OOIBase. This enables the hardware changes to take effect.

Using OOIBase Operating Software

File Menu Functions

New

This command opens a new window and starts acquiring data from the spectrometer. By default, the Master channel is selected and the graph is placed in Scope Mode.

Open

This command opens a dialog box that allows you to reload a saved set of spectra. If you saved the dark or the reference spectra, they are also restored.

Close

This command immediately closes the current graph window. You are **not** given the opportunity to save the current spectrum when this command is chosen.

Store Dark Spectrum

This command stores the current spectrum as the dark spectrum for the channel activated. This spectrum is taken with the light source off or with the light path blocked. (See **Doing an Experiment** on page 15.) Saving a dark spectrum is requisite before the software can make the calculations of absorbance, transmission, and relative irradiance spectra.

Store Reference Spectrum

This command stores the current spectrum as the reference spectrum for the channel activated. This spectrum is taken with the light source on and a blank in the sampling region. (See **Doing an Experiment** on page 15.) Saving a reference spectrum is requisite before the software can make the calculations of absorbance, transmission, and reflection spectra.

Save Sample Spectrum

This command opens a dialog box that allows you to save a set of spectra as a binary file. If the dark and reference spectra are saved, they are also saved in the binary file. Data acquisition parameters are also saved.

Export Spectrum

This command opens a dialog box that allows you to export the current spectra (sample, reference, and/or dark) into a comma-delimited ASCII file. You are given the opportunity to specify the filename. The following file extensions are the default:

<u>Spectrum</u>	<u>Extension</u>
Reference	.REF
Dark	.DRK
Sample	
Absorbance	.ABS
Transmission	.TRM
Reflection	.RFL
Scope	.SCO

Print Preview

This command opens a window allowing you to view how the current spectrum will look when printed.

Print

This command displays a dialog box allowing you to print the current spectrum. The graph title, grid and cursor are all printed if they are displayed on the screen.

Exit

This command exits OOIBase.

View Menu Functions

Scope Mode

This command switches the current window into Scope Mode. The signal graphed in Scope Mode is the raw voltage coming out of the A/D converter. Once you open OOIBase 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 and allows the user the ability to have complete control of signal processing. 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 only be used to select signal acquisition functions.

Absorbance Mode

This command switches the current window into Absorbance Mode. Before this can occur, both the dark and reference spectra must be stored while in Scope Mode. (See **Doing an Experiment** on page 15 for details.) The absorbance of a solution is related to the concentration of species within it. The relationship, known as Beer's Law, is:

$$A_{\lambda} = \epsilon_{\lambda} c l$$

A = absorbance at wavelength λ , ϵ = extinction coefficient of the absorbing species at wavelength λ ,
c = the concentration of the absorbing species, and l = the pathlength of the absorption.

This relationship is used to determine the concentration of a species in solution. Absorbance is calculated by the following equation. When this equation is evaluated for each pixel of the detector, the absorbance spectrum is produced.

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

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,
 R_{λ} = reference intensity of each wavelength, λ = each wavelength

Transmission Mode

This command switches the current window into Transmission Mode. Before this can occur, both the dark and reference spectra must be made and stored while in Scope Mode. (See **Doing an Experiment** on page 15 for details.) The transmission of a sample is calculated by the following equation:

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

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,
 R_{λ} = reference intensity of each wavelength, λ = each wavelength

Irradiance Mode

This command switches the current window into *relative* Irradiance Mode. The reference spectrum must be made in Scope Mode with a blackbody of known color temperature. A dark spectrum is usually obtained by removing the fiber from the reference lamp and preventing light from entering it. (See **Doing an Experiment** on page 15 for details.) 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:

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

B_{λ} = relative energy of the reference calculated from the color temperature in degrees Kelvin,

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,

R_{λ} = reference intensity of each wavelength, λ = each wavelength

Snapshot

This command freezes the data acquisition for the current spectral window and allows you to continue to view a single spectrum.

Autoscale Y Axis

This command automatically adjusts the vertical scale of a current graph so the selected area fills the graph.

Change Graph Scale

This command opens a dialog box allowing you to manually set both the vertical and horizontal scale of the graph.

Unscale Graph

This command returns the graph to its default scale.

Colors

This command allows the user to choose a color for the frames, background, trace, text, grid, and cursor. It is especially useful if multiple channels are being used. Utilize this function to differentiate one channel's display from another.

Cursor

This command toggles a vertical cursor on the current graph. The cursor can be moved with either the yellow arrows in the toolbar or via the following keystrokes:

<u>Keystroke</u>	<u>Command</u>
Page Up	Moves vertical cursor 25 pixels to the right
Page Down	Moves vertical cursor 25 pixels to the left
→ (right arrow)	Moves vertical cursor 1 pixel to the right
← (left arrow)	Moves vertical cursor 1 pixel to the left
↑ (up arrow)	Moves vertical cursor to beginning pixel
↓ (down arrow)	Moves vertical cursor to end pixel
<, (less than, comma)	Moves vertical cursor to next peak
>. (greater than, period)	Moves vertical cursor to previous peak

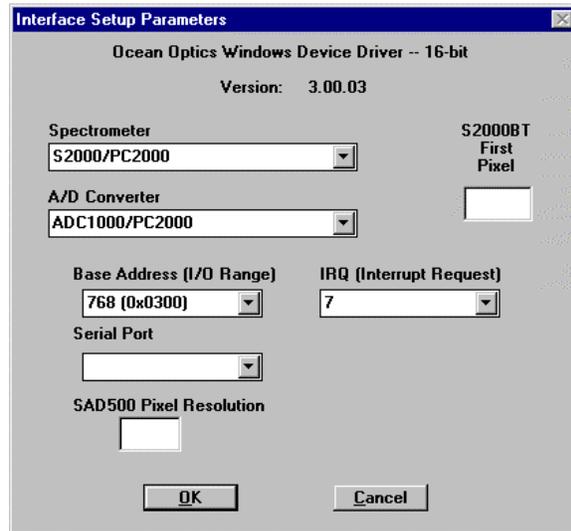
Grid

This command toggles the display of a vertical and horizontal grid on the graph.

Setup Menu Functions

Configure Hardware

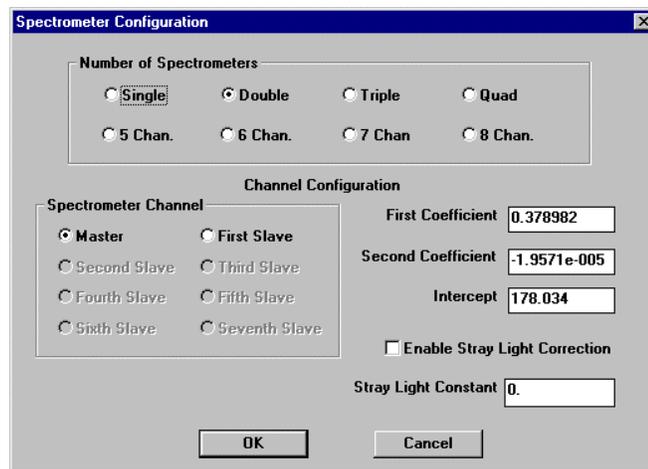
This command opens the “Interface Setup Parameters” dialog box. It allows you to configure the specific type of hardware connecting the spectrometer to the computer. In this box, you specify your **Spectrometer**, **A/D Converter**, **Base Address** and **IRQ**. The parameters in this dialog box are usually set only once -- when OOIBase is first installed and the software first starts. (See **Installing OOIBase Operating Software** on page 3 for further details.)



Configure Spectrometer

This command opens the “Spectrometer Configuration” dialog box. The number of spectrometer channels in your system is selected. The correct numbers for the **First Coefficient**, **Second Coefficient**, and **Intercept** have already been entered from the bottom of the calibration sheet that came with your spectrometer.

Stray light is light detected at a wavelength or wavelengths other than those at which the light *should* be detected. All spectrometers experience the effects of stray light. The **Enable Stray Light Correction** and the **Stray Light Constant** functions, through a mathematical equation, reduce every pixel’s intensity to compensate for the total amount of stray light in the spectrometer.

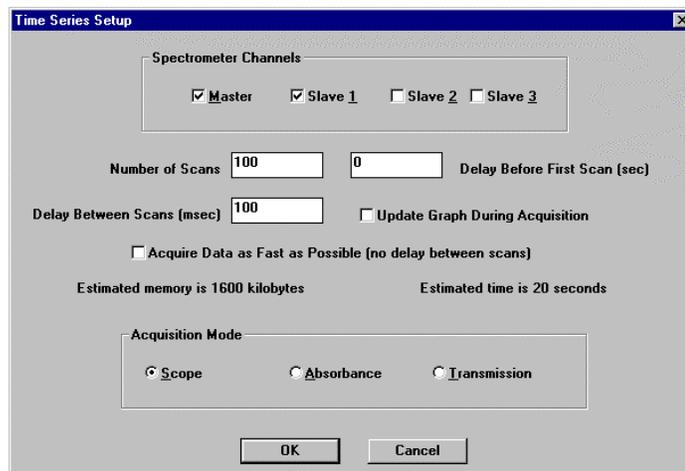


Time Series Acquisition

This command opens the “Time Series Setup” dialog box. It allows you to specify parameters important to the time series data acquisition of full spectra. You can opt to have up to four **Spectrometer Channels** in a time series setup.

Enter the **Number of Scans** and **Delay Between Scans** you want for your setup. The value entered for Number of Scans will affect the amount of memory (see **Estimated memory**) required for your measurements. If these values are too high and require more memory than you have available, your computer may freeze, hang or crash.

The **Delay Before First Scan** function allows you to begin acquiring data at a later time. After entering the time (in



seconds), a dialog box opens with a running countdown of the time left before data acquisition begins. At this point during this countdown, you can click “Start Now” to override the delay. Also, when the data acquisition begins, you can always cancel the procedure, which results in a loss of data.

By selecting **Update Graph During Acquisition**, you can see the live spectra being acquired but the acquisition may take longer. If you select **Acquire Data as Fast as Possible**, you will not be able to use your computer for any other tasks while the time series acquisition is running.

This dialog box also requires that you choose an **Acquisition Mode**. If you choose Absorbance or Transmission, a dialog box opens requiring you to take a reference and dark spectrum – even if you have already done so.

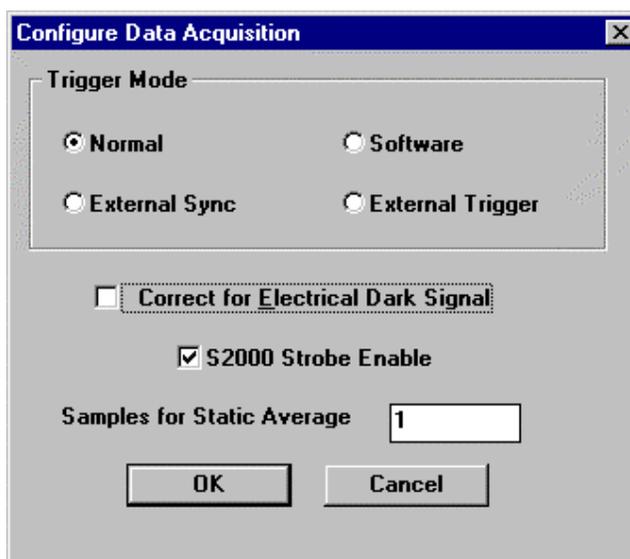
Subtract Dark Spectrum

This command 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 fixed pattern noise from the spectra. The Subtract Dark Spectrum function only acts on the spectra displayed in Scope Mode.

Data Acquisition

This command opens the “Configure Data Acquisition” dialog box (see picture at right). It allows you to specify data acquisition parameters. The choices made in this dialog box control all channels in your system.

Trigger Mode allows you to choose between four modes of operation. The Normal mode is a continuously scanning mode. The Software mode is an external control mode allowing you to signal the spectrometer to take complete data sets. The External Sync mode allows you to control the integration time from an external source, such as a periodic TTL signal. The External Trigger mode is ideal for use with laser and other short-pulse events; however, this mode has a fixed integration time of 55 milliseconds.



The first 24 pixels in the spectrometer do not respond to light but they do produce an electrical signal. When you select the **Correct for Electrical Dark Signal** box, this function subtracts the value of these optical black pixels from the entire signal, reducing the counts of the black pixels to almost zero.

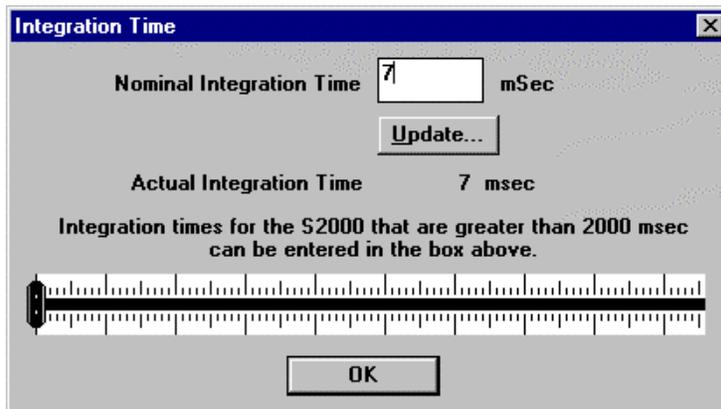
Choosing the **Correct for Electrical Dark Signal** function *does not* replace the **Store Dark Spectrum** function.

You would only select the **S2000 Strobe Enable** if you were operating an external strobe source. To purchase one of our strobe light sources, such as our PX-2 Pulsed Xenon Lamp or our LS-450 Blue LED Pulsed Light Source, contact an Ocean Optics Applications Scientist.

Samples for Static Average is a signal averaging function that averages x number of spectra *before* it gets to OOIBase. The value chosen affects all channels in the system. The higher the value, the better the S:N. The S:N will improve by the square root of the number of scans averaged. Samples for Static Average has no limits; thousands of scans can be averaged if you are patient enough. (Another function averages spectra *after* the scans get to OOIBase. See **Data Analysis** on page 10.)

Integration Time

This command opens the “Integration Time” dialog box (see picture at right). The integration time of the spectrometer is analogous to the shutter speed of a camera. The higher the integration time, 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. You should adjust the integration time so that the greatest amount of light that you anticipate for your application causes a signal of about 3500 counts. While watching the graph trace, adjust the integration time until the signal intensity level is approximately 3500 counts. The integration time specified controls all channels in your system.



Spectrometer Channel

This command opens the “Spectrometer Channel to View” dialog box. It allows you to animate the window for a specific spectrometer channel.

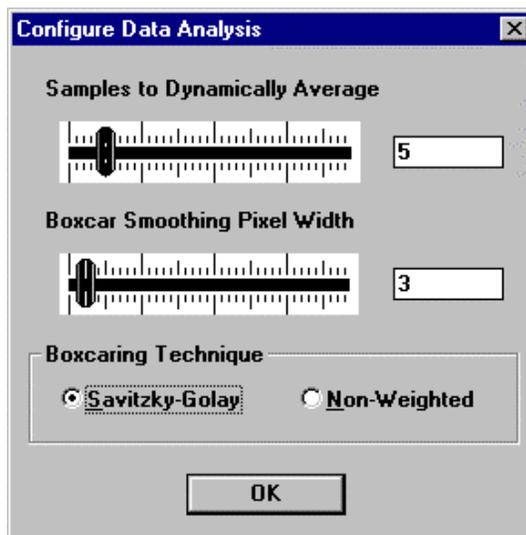
Data Analysis

This command opens the “Configure Data Analysis” dialog box (see picture at right). The functions in this dialog box regulate S:N. The values specified control *only* the activated window.

Samples to Dynamically Average is a signal averaging function that averages x number of spectra *after* it gets to OOIBase. The software collects each spectrum and presents the running average of the value entered in this box. The maximum value is 30 scans. The S:N will improve by the square root of the number of scans averaged. (Another function averages spectra *before* the scans get to OOIBase. Read about this preferred averaging method in **Data Acquisition** on page 9.)

Boxcar Smoothing Pixel Width is a technique that averages across spectral data. This technique averages a group of adjacent detector elements. A value of 5, for example, averages each data point with 5 points to its left and 5 points to its right. The greater this value, the smoother the data and the higher the S:N. The maximum value allowed is 50 pixels. However, if the value entered is too high, a loss in spectral resolution will result. The S:N will improve by the square root of the number of pixels averaged.

Choose **Savitzky-Golay** if you want the Boxcaring Technique to be weighted; otherwise, choose the **Non-Weighted** option.

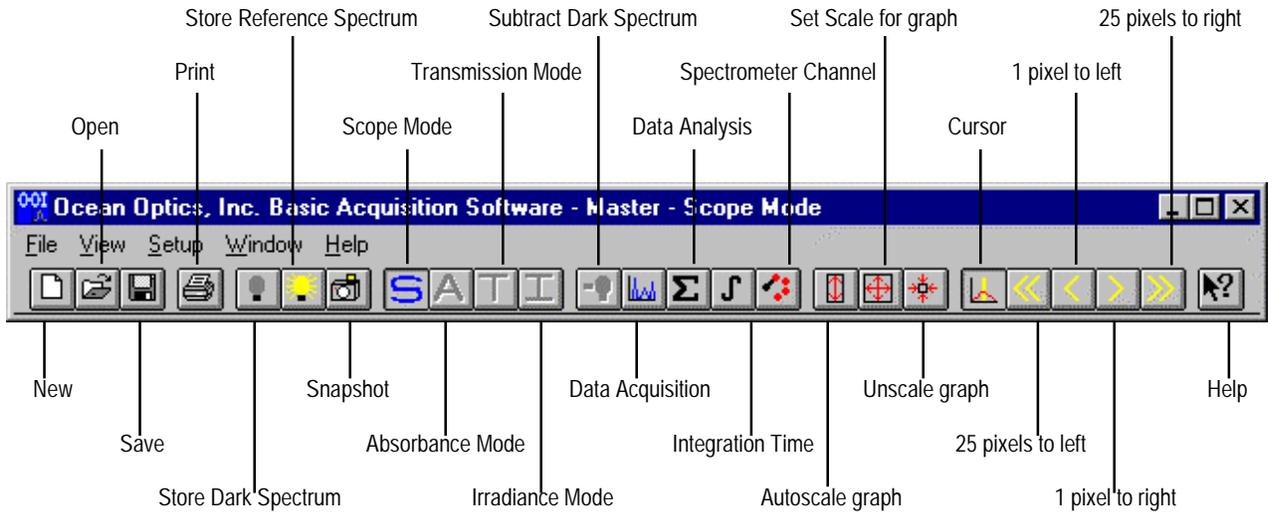


Set Graph Title

This command opens the “Graph Title” dialog box allowing you to specify the title for the current graph. This title is displayed on the screen and is also printed when you select **File | Print**.

Toolbar Functions

Many of the Menu functions can also be accessed through your toolbar. All of the toolbar functions, except for **New**, **Data Acquisition**, **Integration Time**, and **Help**, control only the active window. The toolbar functions include:



Doing an Experiment

By now, your spectrometer and sampling optics should be set-up for your application. Confirm that the parameters for your setup are satisfactory. Assuming that you have followed the necessary steps and started OOIBase, your spectrometer is already acquiring data. With no light in the spectrometer you should see a fluctuating trace on the bottom of the graph. If you turn on your lamp, you should see the graph trace rise with increasing light intensity.

Now you are ready to take your measurements. There are three basic spectral measurements from which to choose: absorbance, transmission, and relative irradiance. Certain measurements require specific components. Your current setup might not be the best configuration for all measuring functions. Contact an Ocean Optics Applications Scientist for options.

For each measurement, a reference and dark spectrum must first be made. After taking a reference and a dark spectrum, you may take as many absorbance, transmission, or relative irradiance measurement scans as you want.

Absorbance Measurement

Absorbance spectra are a measure of how much light is absorbed by a sample. Absorbance spectra are formed by radiation that has penetrated a material medium. For most samples, absorbance is linearly related to the concentration of the substance. The software calculates absorbance using the following equation:

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

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,
 R_{λ} = reference intensity of each wavelength, λ = each wavelength

Absorbance can also be expressed as proportional to the concentration of the substance interacting with the light. Common applications include the quantification of chemical concentrations in aqueous or gaseous samples. To take an absorbance measurement:

1. Make sure you are in Scope Mode, by either clicking the **Scope Mode** icon on the toolbar, or selecting **View | Scope Mode**. Make sure the signal is on scale. The peak intensity of the reference signal should be about 3500 counts. (See **Integration Time** on page 10.) Take a Reference Spectrum by first making sure nothing is blocking the light path going to your sample. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Store Reference Spectrum** icon on the toolbar or selecting **File | Store Reference Spectrum**.
2. While still in **Scope Mode**, take a Dark Spectrum by first completely blocking the light path going to your sample. Do not turn off the light source. Take the dark reading by clicking the **Store Dark Spectrum** icon on the toolbar or selecting **File | Store Dark Spectrum**.
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 choose the **Absorbance Mode** icon on the toolbar or select **View | Absorbance Mode**. To save the spectrum, click the **Save** icon on the toolbar or select **File | Save Sample Spectrum**.

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

Transmission Measurement

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 calculations. The transmission is expressed as a percentage (%T) relative to a standard substance (such as air). The software calculates %T (or %R) by the following equation:

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

S_{λ} = sample intensity of each pixel, D_{λ} = dark intensity of each pixel,

R_{λ} = reference intensity of each pixel, λ = each pixel

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

1. Make sure you are in Scope Mode, by either clicking the **Scope Mode** icon on the toolbar, or selecting **View | Scope Mode**. Make sure the signal is on scale. The peak intensity of the signal should be about 3500 counts. (See **Integration Time** on page 10.) Take a Reference Spectrum by first making sure nothing is blocking the light path going to your sample. The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Store Reference Spectrum** icon on the toolbar or selecting **File | Store Reference Spectrum**.
2. While still in **Scope Mode**, take a Dark Spectrum by first completely blocking the light path going to your sample. Do not turn off the light source. Take the dark reading by clicking the **Store Dark Spectrum** icon on the toolbar or selecting **File | Store Dark Spectrum**.
3. Take a transmission measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then choose the **Transmission Mode** icon on the toolbar or select **View | Transmission Mode**. To save the spectrum, click the **Save** icon on the toolbar or select **File | Save Sample Spectrum**.

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

Relative Irradiance Measurement

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:

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

B_{λ} = relative energy of the reference calculated from the color temperature in Kelvin,

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,

R_{λ} = reference intensity of each wavelength, λ = each wavelength

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 different wavelength. To take a relative irradiance measurement:

1. Make sure you are in Scope Mode, by either clicking the **Scope Mode** icon on the toolbar, or selecting **View | Scope Mode**. Make sure the signal is on scale. The peak intensity of the signal should be about 3500 counts. (See **Integration Time** on page 10.) Take a Reference Spectrum by first making sure nothing is blocking the light path. The light source must be a blackbody of known color temperature. Take the reference reading by clicking the **Store Reference Spectrum** icon on the toolbar or selecting **File | Store Reference Spectrum**.
2. While still in **Scope Mode**, take a Dark Spectrum by blocking the light. Take the dark reading by clicking the **Store Dark Spectrum** icon on the toolbar or selecting **File | Store Dark Spectrum**.
3. Take a relative irradiance measurement by first positioning the fiber at the light source you wish to measure. Then choose the **Irradiance Mode** icon on the toolbar or select **View | Irradiance Mode**. A dialog box opens requiring the light source's color temperature in Kelvin. Enter the color temperature and click **OK**. To save the spectrum, click the **Save** icon on the toolbar or select **File | Save Sample Spectrum**.

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

Reflection Measurement

Reflection is the return of radiation by a surface, without change in wavelength. The reflection may be specular, from a smooth surface; diffuse, from a rough surface or from within the specimen; or total, a combination of the two. Reflection is expressed as a percentage (%R) relative to the reflection from a standard substance (such as our WS-1 white reference material for a diffuse reflection measurement):

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

S_{λ} = sample intensity of each wavelength, D_{λ} = dark intensity of each wavelength,

R_{λ} = reference intensity of each wavelength, λ = each wavelength

Common applications include measuring the reflection properties of mirrors, anti-reflection coatings, and measuring the visual properties of the color in paints, graphics arts, plastics, and food products. To take a reflection measurement:

1. Make sure you are in Scope Mode, by either clicking the **Scope Mode** icon on the toolbar, or selecting **View | Scope Mode**. Take a Reference Spectrum by first making sure nothing is blocking the light path going to your reference. (For diffuse reflection measurements, place the reflection probe over a diffuse white standard. For specular reflection measurements, place the reflection probe over a first-surface mirror.) The analyte you want to measure must be absent while taking a reference spectrum. Take the reference reading by clicking the **Store Reference Spectrum** icon on the toolbar or selecting **File | Store Reference Spectrum**.
2. While still in **Scope Mode**, take a Dark Spectrum by first completely blocking the light path going to your sample. Do not turn off the light source. Take the dark reading by clicking the **Store Dark Spectrum** icon on the toolbar or selecting **File | Store Dark Spectrum**.
3. Take a reflection measurement by first making sure the sample is in place and nothing is blocking the light going to your sample. Then choose the **Transmission Mode** icon on the toolbar or select **View | Transmission Mode**. The Transmission Mode uses the same mathematical formula for both transmission and reflection measurements (%R is calculated in the same way as %T). To save the spectrum, click the **Save** icon on the toolbar or select **File | Save Sample Spectrum**.

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