



USB2000 Fiber Optic Spectrometer

Installation and Operation Manual

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Offices:

Ocean Optics, Inc.

830 Douglas Ave., Dunedin, FL., USA 34698

Phone 727.733.2447

Fax 727.733.3962

8 a.m.– 8 p.m. (Mon–Thu), 8 a.m.– 6 p.m. (Fri) EST

Ocean Optics B.V. (Europe)

Geograaf 24, 6921 EW DUIVEN, The Netherlands

Phone 31-(0)26-3190500

Fax 31-(0)26-3190505

E-mail:	Info@OceanOptics.com	(General sales inquiries)
	Info@OceanOpticsBV.com	(European sales inquiries)
	Orders@OceanOptics.com	(Questions about orders)
	TechSupport@OceanOptics.com	(Technical support)

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1 USB2000 Introduction

The following chapter contains introductory information about the USB2000 Spectrometer, shipment information, and spectrometer connectivity.

Product Overview

The USB2000 Spectrometer connects to a notebook or desktop PC via USB port or serial port. When connected to the USB port of a PC, the USB2000 draws power from the host PC, eliminating the need for an external power supply.



Figure 1-1: Ocean Optics USB2000 Fiber Optic Spectrometer

System Requirements

You can use the USB2000's USB connectivity with any PC that meets the following requirements:

- Windows 98/Me/2000/XP operating system (or Windows CE 2.11 or later for palm-sized PCs)
- Ocean Optics OOIBase32 software application installed and configured for use with the USB2000 (OOIPS2000 software if using a palm-sized PC). Consult the "Configuring the USB2000 in OOIBase32" section of *Chapter 3: Installing the USB2000* for specific configuration instructions.

Alternately, the USB2000 has a serial port for connecting to PCs, PLCs, and other devices that support the RS-232 communication protocol. However, this connection method requires an external power supply to power the USB2000.

EEPROM Utilization

An EEPROM memory chip in each USB2000 contains wavelength calibration coefficients, linearity coefficients, and a serial number unique to each individual spectrometer. The OOIBase32 software application reads these values directly from the spectrometer, enabling the ability to "hot-swap" spectrometers between PCs without entering the spectrometer coefficients manually on each PC.

USB2000 Introduction

About OOIBase32

OOIBase32 is the latest generation of operating software for all Ocean Optics spectrometers and is available free to all customers. OOIBase32 is a user-customizable, advanced acquisition and display program that provides a real-time interface to a variety of signal-processing functions. With OOIBase32, you have the ability to perform spectroscopic measurements (such as absorbance, reflectance, and emission), control all system parameters, collect and display data in real time, and perform reference monitoring and time acquisition experiments.

Note: When using a palm-sized PC, you will use the OOIPS2000 software instead of OOIBase32. The functionality of OOIPS2000 is similar to OOIBase32, but it is specifically for the palm-sized PC.

Sampling System Overview

Ocean Optics fiber optic spectrometer systems consist of low-cost, modular data acquisition components. A typical USB2000-based sampling system contains four core elements:

- USB2000 Spectrometer
- OOIBase32 operating software
- Light source
- Sampling optics (varying, depending on application requirements)

How Sampling Works

The following list explains the function of Ocean Optics sampling components in the sampling process:

1. The user stores reference and dark measurements to correct for instrument response variables.
2. The light from the light source transmits through an optical fiber to the sample.
3. The light interacts with the sample.
4. Another optical fiber collects and transmits the result of the interaction to the spectrometer.
5. The spectrometer measures the amount of light and transforms the data collected by the spectrometer into digital information.
6. The spectrometer passes the sample information to OOIBase32.
7. OOIBase32 compares the sample to the reference measurement and displays processed spectral information.

Modular Sampling Accessories

Ocean Optics offers a complete line of spectroscopic accessories for use with the USB2000. Most of our spectroscopic accessories have SMA connectors for application flexibility. Accordingly, changing the sampling system components is as easy as unscrewing a connector and replacing an accessory.

Interface Options

The USB2000 has both USB and serial port connectors, enabling you to connect the spectrometer to a desktop or notebook PC via a USB port or to a desktop, notebook, or to a palm-sized PC via a serial port.

Computer Interface	Operating System Requirements	Part Needed	Description of Part
Desktop or Notebook PC via USB Port	Windows 98/Me/2000/XP	USB-CBL-1 (included)	Cable that connects from USB port on USB2000 to USB port on desktop or notebook PC
Desktop or Notebook PC via Serial Port	Any 32-bit Windows operating system	USB-ADP-PC (not included)	Adapter block that enables connection from serial port on USB2000 to serial port on desktop or notebook PC; comes with 5 VDC power supply (required when connecting to serial port)
Palm-sized PC via Serial Port	Windows CE 2.11 or higher	USB-ADP-H (not included)	Adapter block that enables connection (with standard 9-pin serial cable) from serial port on USB2000 to serial port on palm-sized PC; comes with 5 VDC power supply (required when connecting to serial port)

Shipment Components

The following information and documentation ships with the USB2000 Spectrometer:

Packing List

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

Wavelength Calibration Data Sheet

Each spectrometer is shipped with a Wavelength Calibration Data Sheet that contains information unique to your spectrometer. OOIBase32 Operating Software reads this calibration data from your spectrometer when it interfaces to a PC via the USB port. Any other interface requires that you manually enter the calibration data in OOIBase32 (select **Spectrometer | Configure | Wavelength Calibration** tab). See the OOIBase32 documentation for more information (refer to [Product Overview](#) for instructions on accessing OOIBase32 documentation).

Note: Please save the Wavelength Calibration Data Sheet for future reference.

Software and Technical Resources CD

Each order ships with the Ocean Optics *Software and Technical Resources* CD. This disc contains software, operating instructions, and product information for all Ocean Optics software, spectrometers, and spectroscopic accessories. You need Adobe Acrobat Reader version 6.0 or higher to view these files. Ocean Optics includes Adobe Acrobat Reader on the *Software and Technical Resources* CD.

With the exception of OOIBase32 Spectrometer Operating Software, all Ocean Optics software requires a password during the installation process. You can locate passwords for the other software applications on the back of the *Software and Technical Resources* CD package.

Other Documentation

You can find detailed instructions for the OOIBase32 Spectrometer Operating Software at <http://www.oceanoptics.com/technical/operatinginstructions.asp>.

Note: You can find instructions for the OOIPS2000 Operating Software for the handheld PC at the following location: <http://www.oceanoptics.com/products/ooips2000.asp>.

To find operating instructions on some of Ocean Optics' most popular spectroscopic accessories (including light sources, sampling chambers, sampling optics, and probes), consult the operating instructions for Ocean Optics products from the *Software and Technical Resources* CD that ships with the product, or visit our website at <http://www.oceanoptics.com/technical/operatinginstructions.asp>.

Modification and Repair Policy

In the event that you need to return an item for modification or service, you must obtain a Return Merchandise Authorization (RMA) number prior to shipping the item back to Ocean Optics. Contact an Ocean Optics Applications Scientist for specific instructions on returning an item.

2 USB2000 Specifications

This chapter contains information on spectrometer operation, specifications, and system compatibility. It also includes accessory connector pinout diagrams and pin-specific information.

How the USB2000 Works

The following diagram illustrates the movement of light through the optical bench of the USB2000 Spectrometer. You can customize the items marked with an asterisk (*) when ordering the USB2000.

Note: The optical bench has no moving parts that can wear or break. Ocean Optics secures all components in place permanently at the time of manufacture.

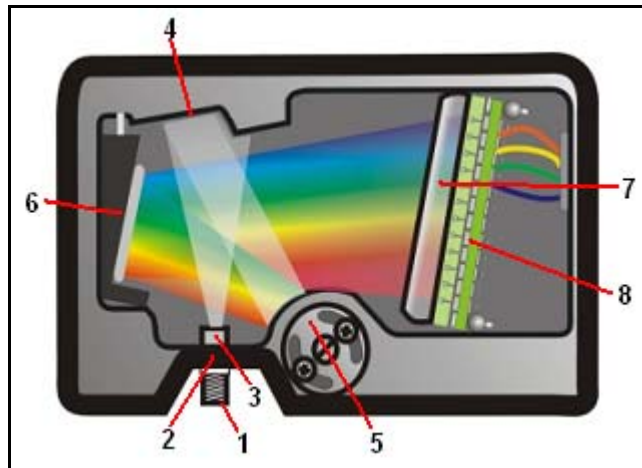


Figure 2-1: USB2000 Spectrometer with Components

The USB Component Table on the following page explains the function of each numbered component in the USB2000 Spectrometer diagram (Figure 2-1).

USB2000 Component Table

Ocean Optics permanently secures all components in the USB2000 at the time of manufacture. Only Ocean Optics Technicians can replace interchangeable components, where noted.

Item	Name	Description
1	SMA Connector	The SMA Connector secures the input fiber to the spectrometer. Light from the input fiber enters the optical bench through this connector.
2	Slit*	The Slit is a dark piece of material containing a rectangular aperture, which is mounted directly behind the SMA Connector. The size of the aperture regulates the amount of light that enters the optical bench and controls spectral resolution. You can also use the USB2000 without a Slit . In this configuration, the diameter of the fiber connected to the USB2000 determines the size of the entrance aperture. Only Ocean Optics technicians can change the Slit .
3	Filter*	The Filter is a device that restricts optical radiation to pre-determined wavelength regions. Light passes through the Filter before entering the optical bench. Both bandpass and longpass filters are available to restrict radiation to certain wavelength regions. Only Ocean Optics technicians can change the Filter .
4	Collimating Mirror	The Collimating Mirror focuses light entering the optical bench towards the Grating of the spectrometer. Light enters the spectrometer, passes through the SMA Connector, Slit, and Filter, and then reflects off the Collimating Mirror onto the Grating.
5	Grating*	The Grating diffracts light from the Collimating Mirror and directs the diffracted light onto the Focusing Mirror. Gratings are available in different groove densities, allowing you to specify wavelength coverage and resolution in the spectrometer. Only Ocean Optics technicians can change the Grating .
6	Focusing Mirror	The Focusing Mirror receives light reflected from the Grating and focuses the light onto the CCD Detector or L2 Detector Collection Lens (depending on the spectrometer configuration).
7	L2 Detector Collection Lens*	The L2 Detector Collection Lens (optional) attaches to the CCD Detector. It focuses light from a tall slit onto the shorter CCD Detector elements. The L2 Detector Collection Lens should be used with large diameter slits or in applications with low light levels. It also improves efficiency by reducing the effects of stray light. Only Ocean Optics technicians can add or remove the L2 Detection Collection Lens .
8	CCD Detector (UV or VIS)	The CCD Detector collects the light received from the Focusing Mirror or L2 Detector Collection Lens and converts the optical signal to a digital signal. Each pixel on the CCD Detector responds to the wavelength of light that strikes it, creating a digital response. The spectrometer then transmits the digital signal to the OOIBase32 application.

USB2000 Specifications

The following sections provide specification information for the CCD Detector in the USB2000, as well as the USB2000 Spectrometer itself.

CCD Detector Specifications

Detector:	Sony ILX511 linear silicon CCD array
Number of elements:	2048 pixels
Pixel size:	14 μm x 200 μm
Pixel well depth:	62,500 electrons
Signal-to-noise ratio:	250:1 (at full signal)
A/D resolution:	12 bit
Dark noise:	2.5 RMS counts
Corrected linearity:	>99.8%

USB2000 Spectrometer Specifications

Dimensions:	89.1 mm x 63.3 mm x 34.4 mm
Weight:	190 g (without cable)
Power consumption:	90 mA @ 5 VDC
Detector range:	200-1100 nm
Detector:	2048-element linear silicon CCD array
Gratings:	14 gratings; UV through Shortwave NIR
Entrance aperture:	5, 10, 25, 50, 100 or 200 mm wide slits or fiber (no slit)
Order-sorting filters:	Installed longpass and bandpass filters
Focal length:	f/4, 42 mm (input); 68 mm (output)
Optical resolution:	~0.3-10.0 nm FWHM (depending on grating and size of entrance aperture)
Dynamic range:	2×10^8 (system); 2000:1 for a single scan
Stray light:	<0.05% at 600 nm; <0.10% at 435 nm; <0.10% at 250 nm
Sensitivity (estimate):	400 nm – 90 photons/count; 600 nm – 41 photons/count; 800 nm – 203 photons/count
Fiber optic connector:	SMA 905 to single-strand optical fiber (0.22 NA)
Data transfer rate:	Full scans into memory every 13 milliseconds
Integration time:	3 milliseconds to 65 seconds
Fiber optic connector:	SMA 905 to single-strand optical fiber (0.22 NA)
Operating systems:	Windows 98/Me/2000/XP when using the USB interface on a desktop or notebook PCs Any 32-bit Windows operating system when using the serial port on desktop or notebook PCs Windows CE 2.11 and above when using the serial port on palm-sized PCs

USB2000 Specifications

System Compatibility

The following sections provide information on hardware and software requirements for the USB2000:

Compatibility for Desktop or Notebook PCs

To use the USB2000, you must have a PC that meets the following minimum requirements:

- IBM-compatible PC with Pentium (or higher) processor
 - 32 MB RAM
 - OOIBase32 Spectrometer Operating Software
 - Windows 98/Me/2000/XP operating system (when connecting the USB2000 to a PC via USB port)
- or**
- Any 32-bit version of Windows (when connecting the USB2000 to a PC via serial port)

Compatibility for Palm-sized PCs

In order to use the USB2000 with your palm-sized PC, the computer must meet the following requirements:

- Palm-sized PC running Windows CE 2.11 or higher
- 32 MB
- OOIPS2000 Spectrometer Operating Software
- Serial port connectivity

10-pin Accessory Connector Pinout

The USB2000 features a 10-pin Accessory Connector, located on the side of the unit as follows:

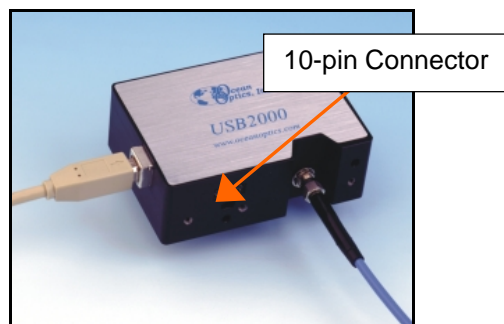


Figure 2-2: Location of USB2000 10-pin Connector

10-Pin Accessory Connector Pinout Diagram

When facing the 10-pin Accessory Connector on the USB2000, pin numbering is as follows:

10	8	6	4	2
9	7	5	3	1

Figure 2-3: 10-Pin Accessory Connector Pinout Diagram

10-Pin Accessory Connector – Pin Definitions

The following table contains information regarding the function of each pin in the USB2000's 10-pin accessory connector:

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

10-Pin J2 Accessory Connector – Part Number and Compatibility

The part numbers for the USB2000 Spectrometer 10-pin accessory connector are as follows:

- The connector is Samtec model IPT1-105-01-S-D-RA.
- The mating right-angle connector is Samtec model IPS1-105-01-S-D-RA.

If you are customizing your USB2000 Spectrometer system or configuring an External Triggering configuration, you may need these part numbers to complete your setup.

3 Installing the USB2000

This chapter contains instructions in parallel for connecting the USB2000 via both USB and serial modes.

Note: You must install the OOIBase32 software application prior to connecting the USB2000 Spectrometer to the PC. The OOIBase32 software installation installs the drivers required for USB2000 installation. If you do not install OOIBase32 first, the system will not properly recognize the USB2000.

If you have already connected the USB2000 to the PC prior to installing OOIBase32, consult *Chapter 4: Troubleshooting* for information on correcting a corrupt USB2000 installation.

USB2000 Installation

USB Mode

To connect the USB2000 to a PC via the USB port, the PC must be running the Windows 98/ME/2000/XP operating system.

Note: The USB port on a PC can power up to five USB2000 spectrometer channels. Systems with more than five channels require a powered USB hub.

Follow the steps below to connect the USB2000 to a PC via the USB port:

1. Install OOIBase32 on the destination PC.
2. Locate the USB cable (USB-CBL-1) provided with the USB2000.
3. Insert the square end of the cable into the side of the USB2000.
4. Insert the rectangular end of the cable into the USB port of the PC.

If you installed OOIBase32 prior to connecting the USB2000, the **Add New Hardware Wizard** appears and installs the USB2000 drivers. If the drivers do not successfully install (or if you connected the USB2000 to the PC before installing OOIBase32), consult *Chapter 4: Troubleshooting*.

Serial Port Mode

To use the serial port capacity of the USB2000 Spectrometer, the PC must be running a 32-bit version of the Windows operating system (or Windows CE 2.11 or higher for palm-sized PCs).

Follow the steps below to connect the USB2000 to the PC via serial port:

1. Connect the serial cable adapter block to the USB2000.
2. Connect one end of the 9-pin serial cable to the adapter block on the USB2000, and then connect the other end to a serial port on the PC.
3. Note the number of the serial port (COM Port) to which you connected the USB2000 (some PCs may not have numbered ports; palm-sized PCs typically have only one serial port).
4. Plug the 5 VDC external power supply into an outlet and connect it to the USB2000.

Note: Connecting the spectrometer to the PC's serial port requires that you manually enter the calibration coefficients from the Wavelength Calibration Data Sheet into OOIBase32 software (select **Spectrometer | Configure | Wavelength Calibration** tab). See the OOIBase32 documentation for more information.

Configuring the USB2000 in OOIBase32

Once you install the USB2000, you must configure OOIBase32's **Configure Spectrometer** options so that OOIBase32 recognizes the USB2000 Spectrometer.

Note: Consult the *OOIBase32 Spectrometer Operating Software Manual* for detailed instructions on configuring the spectrometer in OOIBase32. You can find these instructions on the Software and Technical Resources CD or on the Ocean Optics web site at the following location:
<http://www.oceanoptics.com/technical/ooibase32bit.pdf>.

The following sections contain instructions on initially configuring the USB2000 the first time you start OOIBase32:

Operator and Serial Number Dialog Box

The **Operator and Serial Number** screen prompts you to enter a user name and software serial number into OOIBase. Some data files created by OOIBase32 during sampling procedures use this information in the file headers.

Default Spectrometer Configuration File

The **Default Spectrometer Configuration File** screen prompts you to select a spectrometer configuration (.SPEC) file for use with the USB2000. The unique serial number of the USB2000 precedes the file extension (for example, USB2J0162.SPEC).

Navigate to the OOIBase32 installation directory and select the spectrometer configuration file.

Configure Hardware Screen

The **Configure Hardware** screen prompts you to enter spectrometer-specific information into OOIBase32 the first time you run the program. Typically, you will only enter this information the first time you run OOIBase32. However, you can alter the hardware configuration at any time using the Spectrometer Configuration screen. Select **Spectrometer | Configuration** from the OOIBase32 menu bar to access the Spectrometer Configuration screen.

Follow the steps on the next page to configure the USB2000:

Note: You do not need to configure the spectrometer hardware in the OOIPS2000 palm-sized PC operating software. Most palm-sized PCs have only one serial connector and do not have USB ports. Thus, OOIPS2000 will communicate with the spectrometer via this port at a fixed baud rate. There is no way to customize the USB2000 configuration with OOIPS2000.

Installing the USB2000

Configure Hardware Screen - Continued

USB Mode

1. Specify USB2000 in the **Spectrometer Type** drop-down menu.
2. Specify USB2000 in the **A/D Converter Type** drop-down menu.
3. Specify the serial number of the USB2000 under the **USB Serial Number** drop-down menu.

Note: The system pre-fills this drop-down menu with the serial numbers of all discovered USB2000 Spectrometers.

4. Click the **OK** button to accept the selected options.

Serial Port Mode

1. Select the USB2000 option from the **Spectrometer Type** drop-down menu.
2. Select the Serial (RS-232) A/D option from the **A/D Converter Type** drop-down menu. This selection enables serial-specific options in the lower portion of the Configure Hardware screen.
3. Select the COM port that the USB2000 is connected to in the **Serial Port** drop-down menu. Consult *Chapter 4: Troubleshooting* for information on identifying serial ports.
4. Select the speed at which the USB2000 will operate from the **Baud Rate** drop-down menu (115,200 baud is recommended).
5. Specify the pixel resolution (from 1 to 500) in the Pixel Resolution box. This value specifies that every n th pixel of the spectrometer will transmit from the USB2000 to the PC.

Note: You can sacrifice pixel resolution to gain speed. The transfer of one complete spectra requires ~0.3 seconds at 115,200 baud.

6. Enable the **Compress Data** function to minimize the amount of data transferred over the RS-232 connection. The transmission of spectral data over the serial port is a relatively slow process. Enabling this function ensures that the USB2000 compresses every scan that it transmits. This greatly increases the data transfer speed of the USB2000.
7. Click the **OK** button to complete setup.

The spectrometer should now be able to acquire data and respond to light. Exit and restart OOIBase32 to save configuration data to disk.

Installing the USB2000

Spectrometer Configuration Screen

The Spectrometer Configuration screen prompts you to configure specific channel-level spectrometer information, if necessary.

Select **Spectrometer | Configure | Wavelength Calibration tab** from the menu and set system parameters. If you have connected your spectrometer to the PC's USB port, OOIBase32 pre-fills the coefficients for the USB2000 from information on a memory chip in the spectrometer. Otherwise, you must manually type the coefficients as they are printed on the Wavelength Calibration Data Sheet that accompanied your spectrometer. Verify that the calibration coefficients match the coefficients from the Wavelength Calibration Data Sheet. If necessary, modify these values using the USB Programmer utility. Additionally, ensure that you select both the **Master** and **Channel Enabled** boxes.

In the **A/D Interface** tab, enter the same values as in the Configure Hardware dialog box. OOIBase32 stores this information for future use once you close the program.

Note: For information on using the USB2000 with the OOIPS2000 Operating Software for the palm-sized PC, visit <http://www.oceanoptics.com/technical/palmspec.pdf>.

Connect Spectroscopic Accessories

To find operating instructions for USB2000-compatible products (such as light sources, sampling chambers, and probes), consult the *Software and Technical Resources* CD or the Ocean Optics web site at:

<http://www.oceanoptics.com/technical/operatinginstructions.asp>

External Triggering Options

You can trigger the USB2000 using a variety of External Triggering Options through the 10-pin Accessory Connector on the spectrometer.

Consult the External Triggering Options document located at the following address:

<http://www.oceanoptics.com/technical/externaltriggering.pdf>

If you do not have web access, you can retrieve this document from the *Software and Technical Resources* CD included with your spectrometer shipment.

The External Triggering PDF contains instructions on configuring External Triggering Options for the USB2000.

Note: Only the external software triggering option is available when using a palm-sized PC.

4 Troubleshooting

The following sections contain information on troubleshooting issues you may encounter when using the USB2000 Spectrometer.

Note: For issues encountered when using a palm-sized PC, consult the OOIPS2000 manual.

USB2000 Connected to PC Prior to OOIBase32 Installation

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

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

Note: If these procedures do not correct your device driver problem, you will need to obtain the "Correcting Device Driver Issues" document from the Ocean Optics website. Visit the following web address:

<http://www.oceanoptics.com/technical/engineering/correctingdevicedriverissues.pdf>

Remove the Unknown Device from Windows Device Manager

1. Open Windows Device Manager as follows:

Windows 98/ME:

- Go to the desktop and right-click on **My Computer**.
- Select **Properties** from the pop-up menu.
- Click on the **Device Manager** tab.

Windows 2000/XP:

- Click **Start | Settings | Control Panel | System**.
- Select the **Hardware** tab.
- Click on the **Device Manager** button.

2. Locate the **Other Devices** option and expand the **Other Devices** selection by clicking on the "+" sign to the immediate left.

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

Troubleshooting

3. Locate the unknown device (marked with a large question mark). Right-click on the **Unknown Device** listing and select the **Uninstall** or **Remove** option.
4. Click the **OK** button to continue. A warning box appears confirming the removal of the Unknown Device. Click the **OK** button to confirm the device removal.
5. Disconnect the USB2000 from your computer.
6. Locate the section in this chapter that is appropriate to your operating system and perform the steps in the “Remove Improperly Installed Files” section.

Windows 98:

Remove Improperly Installed Files:

1. Open Windows Explorer.
2. Navigate to the **Windows | INF** directory. If the INF directory is not visible, you will need to disable the “Hide System Files and Folders” option on in Windows Folder Options.

Note: If the INF directory is not visible, you will need to disable the “Hide System Files and Folders” and “Hide File Extensions for Known File Types” options in Windows Folder Options.

You can access Windows Folder Options from Windows Explorer, under the **View | Options** menu selection.

3. Delete the **OOI_USB.INF** file in the INF directory.
4. Navigate to the **Windows | System32 | Drivers** directory.
5. Delete the **EZUSB.SYS** file.
6. Reinstall your Ocean Optics application and reboot the system when prompted.
7. Plug in the USB device.

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

Windows 2000/XP:

Remove Improperly Installed Files:

1. Open Windows Explorer.
2. Navigate to the **Windows | INF** directory. If the INF directory is not visible, you will need to disable the “Hide System Files and Folders” option on in Windows Folder Options.

Note: If the INF directory is not visible, you will need to disable the “Hide System Files and Folders” and “Hide File Extensions for Known File Types” options in Windows Folder Options.

You can access Windows Folder Options from Windows Explorer, under the **Tools | Folder Options** menu selection.

3. Delete the **OOI_USB.INF** and **OOI_USB.PNF** files in the INF directory.
4. Navigate to the **Windows | System32 | Drivers** directory.
5. Delete the **EZUSB.SYS** file.
6. Reinstall your Ocean Optics application and reboot the system when prompted.
7. Plug in the USB device.

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

Troubleshooting the Serial Port Configuration

Occasionally, you may encounter problems with the serial port connection and/or software. Perform the following steps to troubleshoot the serial port connection:

1. Cycle the power on the USB2000 and restart the OOIBase32 software. This ensures that the software and the USB2000 are synchronized.
2. Determine the serial port (COM port) number:

Operating System	Instructions
Windows 95/98/ME	<ol style="list-style-type: none">1. Right-click on My Computer2. Select Properties3. Click on the Device Manager tab
Windows 2000/XP	<ol style="list-style-type: none">1. Select Start Settings Control Panel System2. Select the Hardware tab3. Click the Device Manager button.
Windows NT	<ol style="list-style-type: none">1. Select Start Programs Administrative Tools (common) NT Diagnostics

3. Double-click on the Ports (COM & LPT) option to display COM port numbers. Ensure that no warning icon appears next to the USB2000's COM port.
4. Verify that the COM port to which the USB2000 is interfaced is active. If the ports on the PC are not labeled and you do not know the COM port number, use trial-and-error to find the correct COM port.

Open OOIBase32 and view the displayed graph. If the correct COM port is selected, you will see a dynamic trace responding to light near the bottom of the graph. If the correct COM port is not selected, you will see a straight line at zero counts.

5. Disable virus protection to ensure timely and complete data transfer (optional – some computers require this step).

Older Version of OOIBase32 Installed

If the PC you want to use to interface to your USB2000 already has an older version of OOIBase32, you need to install the latest version of OOIBase32. You can download the latest version of OOIBase32 from the *Software and Technical Resources* CD or from the Ocean Optics web site at the following URL:

<http://www.oceanoptics.com/technical/softwaredownloads.asp>.

You do not need to uninstall previous versions of OOIBase32 when upgrading to the latest version.

5 Sample Experiments

The following sections contain information on conducting sample experiments using the USB2000 and OOIBase32. For information on experiments with OOIPS2000, consult the OOIPS2000 Operating Instructions.

Preparing for Experiments

Follow the steps below to configure the USB2000 and OOIBase32 for experiments:

1. Double-check that you have correctly installed the USB2000, installed OOIBase32, and configured the light source and other sampling optics.
2. Open the OOIBase32 application, select **Spectrometer | Configure** from the menu bar, and double-check that **A/D Interface** settings are correct.
3. Check your spectrometer setup configurations in OOIBase32:

Locate the Wavelength Calibration Data sheet that came with the USB2000. Select **Spectrometer | Configure** from the menu and choose the **Wavelength Calibration** page. For each spectrometer channel in the system, enable the channel and make sure the First Coefficient, Second Coefficient, Third Coefficient and Intercept correspond to those of the system.

4. Adjust the acquisition parameters using the **Acquisition Parameters** dialog bar or select **Spectrum | Configure Data Acquisition** from the menu.

If you have followed the previous steps and started OOIBase32, the spectrometer is already acquiring data. Even with no light in the spectrometer, there should be a dynamic trace displayed in the bottom of the graph. If you allow light into the spectrometer, the graph trace should rise with increasing light intensity. This means the software and hardware are correctly installed.

Once you install the hardware, configure the software, and establish your sampling system, you are ready to take measurements.

There are four basic optical measurements from which to choose:

- Absorbance
- Transmission
- Reflection
- Relative irradiance

The type of measurement you will take determines the configuration of the sampling optics for your system. Furthermore, your choice of reference and data analysis determines how the OOIBase32 presents the results.

Note: For each measurement, you must first take a reference and dark spectrum. After you take a reference and a dark spectrum, you can take as many measurement scans as needed. However, if you change any sampling variable (integration time, averaging, smoothing, angle, temperature, fiber size, etc.), you must store a new dark and reference spectrum.

Sample Experiments

Application Tips

If the signal you collect is saturating the spectrometer (intensity greater than 4000 counts), you can decrease the light level on scale in scope mode by:

- Decreasing the integration time
- Attenuating the light going into the spectrometer
- Using a smaller diameter fiber
- Using a neutral density filter with the correct optical density

If the signal you collect has too little light, you can increase the light level on scale in scope mode by:

- Increasing the integration time
- Using a larger diameter fiber
- Removing any optical filters

Absorbance Experiments

Absorbance spectra are a measure of how much light a sample absorbs. For most samples, absorbance is linearly related to the concentration of the substance. OOIBase32 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_λ is the sample intensity at wavelength λ , D_λ is the dark intensity at wavelength λ , R_λ is the reference intensity at wavelength λ .

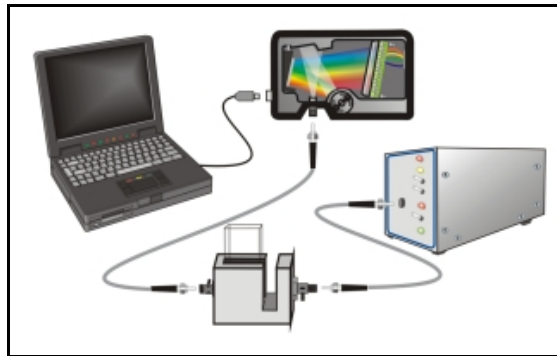


Figure 6-1: Typical absorbance setup. The light source (far right) sends light via an input fiber into a cuvette in a cuvette holder (bottom center). The light interacts with the sample. The output fiber carries light from the sample to the spectrometer (top center), which is connected to the PC (far left).

Absorbance is also proportional to the concentration of the substance interacting with the light (this is known as Beer's Law). Common absorption applications include the quantification of chemical concentrations in aqueous or gaseous samples.

Follow the steps below to take an absorbance measurement using OOIBase32:

1. Place OOIBase32 in scope mode by clicking the scope mode icon on the toolbar or selecting **Spectrum | Scope Mode** from the menu bar.
2. Ensure that the entire signal is on scale. The intensity of the reference signal should peak at about 3500 counts. If necessary, adjust the integration time until the intensity is approximately 3500 counts.

(Continued)

Sample Experiments

3. Place a sample of the solvent into a cuvette and take a reference spectrum. You must take a reference spectrum before measuring absorbance.

Note: Do not put the sample itself in the path when taking a reference spectrum, only the solvent.

Click the **Store Reference** spectrum icon on the toolbar or select **Spectrum | Store Reference** from the menu bar to store the reference. This command merely stores a reference spectrum in memory. You must select **File | Save | Reference** from the menu bar to permanently save the spectrum to disk.

4. Block the light path to the spectrometer. Then, take a dark spectrum by clicking the **Store Dark Spectrum** icon on the toolbar or by selecting **Spectrum | Store Dark** from the menu bar. This command merely stores a dark spectrum in memory. You must select **File | Save | Dark** from the menu to permanently save the spectrum to disk.

Note: If possible, do not turn off the light source when taking a dark spectrum. If you must turn off your light source to store a dark spectrum, allow enough time for the lamp to warm up again before continuing your experiment. After the lamp warms up again, store a new reference (Step 3).

You must take a dark spectrum before measuring absorbance.

5. Put the sample in place and ensure that the light path is clear. Then, take an absorbance measurement by clicking on the **Absorbance Mode** icon on the toolbar or selecting **Spectrum | Absorbance Mode** from the menu. To permanently save the spectrum to disk, click the **Save** icon on the toolbar or select **File | Save | Processed** from the menu bar.

Note: If you change any sampling variable (integration time, averaging, smoothing, angle, temperature, fiber size, etc.), you must store a new dark and reference spectrum.

Transmission Experiments

Transmission is the percentage of energy passing through a sample relative to the amount that passes through the reference. Transmission Mode also displays the portion of light *reflected* from a sample, since transmission and reflection measurements use the same mathematical calculations. The transmission is expressed as a percentage (%T_λ) relative to a standard substance (such as air). OOIBase32 calculates %T_λ (or %R_λ) with the following equation...

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

...where S_λ is the sample intensity at wavelength λ, D_λ is the dark intensity at wavelength λ, R_λ is the reference intensity at wavelength λ.

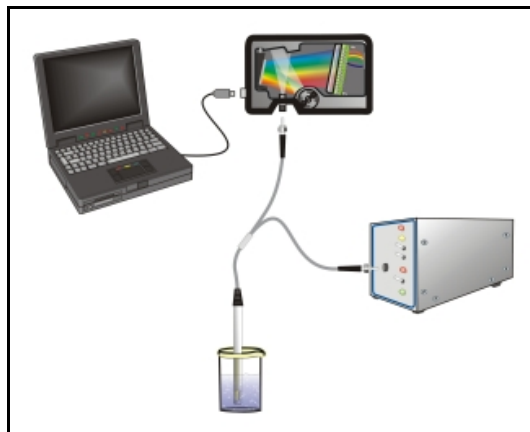


Figure 6-2: Typical transmission setup. The light source (far right) sends light via the input leg of a transmission probe into a container (bottom center). The light interacts with the sample. The output leg of the transmission probe carries the information to the spectrometer (top center), which transmits the information to the PC (far left).

Common transmission applications include measuring light through solutions, optical filters, optical coatings, and other optical elements (such as lenses and fibers).

Perform the following steps to take a transmission measurement using OOIBase32:

1. Place OOIBase32 in scope mode by clicking the **Scope Mode** icon on the toolbar or by selecting **Spectrum | Scope Mode** from the menu bar.
2. Ensure that the entire signal is on scale. The intensity of the reference signal should peak at about 3500 counts. If necessary, adjust the integration time until the intensity is approximately 3500 counts.

(Continued)

Sample Experiments

6. Place a sample of the solvent into a cuvette and take a reference spectrum. You must take a reference spectrum before measuring transmission.

Note: Do not put the sample itself in the path when taking a reference spectrum, only the solvent.

Click the **Store Reference** spectrum icon on the toolbar or select **Spectrum | Store Reference** from the menu bar to store the reference. This command merely stores a reference spectrum in memory. You must select **File | Save | Reference** from the menu bar to permanently save the spectrum to disk.

3. Block the light path to the spectrometer. Then, take a dark spectrum by clicking the **Store Dark Spectrum** icon on the toolbar or by selecting **Spectrum | Store Dark** from the menu bar. This command merely stores a dark spectrum in memory. You must select **File | Save | Dark** from the menu to permanently save the spectrum to disk.

Note: If possible, do not turn off the light source when taking a dark spectrum. If you must turn off your light source to store a dark spectrum, allow enough time for the lamp to warm up again before continuing your experiment.

You must take a dark spectrum before measuring transmission.

4. Put the sample in place and verify that the light path is clear. Then, take a transmission measurement by clicking the **Transmission Mode** icon on the toolbar or selecting **Spectrum | Transmission Mode** from the menu bar. To save the spectrum to disk, click the **Save** icon on the toolbar or select **File | Save | Processed** from the menu bar.

Note: If you change any sampling variable (integration time, averaging, smoothing, angle, temperature, fiber size, etc.), you must store a new dark and reference spectrum.

Reflection Experiments

Reflection is the return of radiation by a surface, without a change in wavelength. Reflection can be:

- Specular (the angle of incidence is equal to the angle of reflection)
- Diffuse (the angle of incidence is not equal to the angle of reflection)

Every surface returns both specular and diffuse reflections. Some surfaces may return mostly specular reflection, while others may return mostly diffuse reflection. Specular reflection increases proportionately with the amount of gloss on a surface.

Reflection is expressed as a percentage (% R_λ) relative to the reflection from a standard reference substance...

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

...where S_λ is the sample intensity at wavelength λ , D_λ is the dark intensity at wavelength λ , R_λ is the reference intensity at wavelength λ .



Figure 6-3: Typical reflection setup. A light source (far right) sends light via the input leg of a reflection probe onto a sample (bottom center). A reflection probe holder holds the probe in either a 90 or 45-degree angle from the surface. The output leg of the reflection probe carries light from the sample to the spectrometer (top center), which is connected to the PC (far left).

Common reflection applications include measuring the properties of mirrors and coatings. Other applications include measuring the visual properties of the color in paints, plastics, and food products.

Sample Experiments

Perform the following steps to take reflection measurements using OOIBase32:

1. Place OOIBase32 is in scope mode by clicking the **Scope Mode** icon on the toolbar, or by selecting **Spectrum | Scope Mode** from the menu bar.
2. Ensure that the entire signal is on scale. The intensity of the reference signal should peak at about 3500 counts.
3. Take a reference spectrum with the WS-1 Diffuse Reflectance Standard or the STAN-SSH High-reflectivity Reference Standard. You must take a reference spectrum before measuring reflection.

Click the **Store Reference** spectrum icon on the toolbar or select **Spectrum | Store Reference** from the menu bar to store the reference. This command merely stores a reference spectrum in memory. You must select **File | Save | Reference** from the menu bar to permanently save the spectrum to disk.

4. Block the light path to the spectrometer. Then, take a dark spectrum by clicking the **Store Dark Spectrum** icon on the toolbar or by selecting **Spectrum | Store Dark** from the menu bar. This command merely stores a dark spectrum in memory. You must select **File | Save | Dark** from the menu to permanently save the spectrum to disk.

Note: If possible, do not turn off the light source when taking a dark spectrum. If you must turn off your light source to store a dark spectrum, allow enough time for the lamp to warm up again before continuing your experiment.

You must take a dark spectrum before measuring transmission.

5. Put the sample is in place and ensure that the light path is clear. Then, take a reflection measurement by clicking on the **Transmission Mode** icon on the toolbar or selecting **Spectrum | Transmission Mode** from the menu bar (since the mathematical calculations used to calculate transmission and reflection are identical). To save the spectrum to disk, click the **Save** icon on the toolbar or select **File | Save | Processed** from the menu bar.

Note: If you change any sampling variable (integration time, averaging, smoothing, angle, temperature, fiber size, etc.), you must store a new dark and reference spectrum.

Relative Irradiance Experiments

Irradiance is the amount of energy at each wavelength emitted from a radiant sample. In relative terms, it is a comparison of the fraction of energy the sample emits and the energy the sampling system collects from a lamp with a blackbody energy distribution (normalized to 1 at the energy maximum). OOIBase32 calculates relative irradiance with the following equation...

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

...where B_{λ} is the relative energy of the reference (calculated from the color temperature) at wavelength λ , S_{λ} is the sample intensity at wavelength λ , D_{λ} is the dark intensity at wavelength λ , R_{λ} is the reference intensity at wavelength λ .



Figure 6-4: Typical Relative Irradiance Setup. A light source with a known color temperature (such as the LS-1 or LS-1-LL - lower right) is used to take a reference spectrum. The light to measure (lower left) is accumulated through a CC-3 Cosine Corrector (or FOIS integrating sphere) into an input fiber, which carries the light information to the spectrometer. The spectrometer then transmits the information to the PC, which compares the measured spectra against the reference spectrum, thus removing wavelength-dependent instrument response from the measurement.

Common applications include characterizing the light output of LEDs, incandescent lamps, and other radiant energy sources such as sunlight. Relative irradiance measurements also include fluorescence measurements, which measure the energy given off by materials that have been excited by light at shorter wavelengths.

Sample Experiments

Perform the following steps to take a relative irradiance measurement using OOIBase32:

1. Place OOIBase32 is in scope mode by clicking the **Scope Mode** icon on the toolbar, or by selecting **Spectrum | Scope Mode** from the menu bar.
2. Ensure that the entire signal is on scale. The intensity of the reference signal should peak at about 3500 counts.

Note: The light source must be a blackbody of known color temperature.

3. In the **Reference Color Temperature** dialog box, enter the color temperature of the light source (in Kelvin) and click the **OK** button.
4. Take a reference spectrum using a light source with a black body of a known color temperature, such as the LS-1.

Click the **Store Reference** spectrum icon on the toolbar or select **Spectrum | Store Reference** from the menu bar to store the reference. This command merely stores a reference spectrum in memory. You must select **File | Save | Reference** from the menu bar to permanently save the spectrum to disk.

5. Block the light path to the spectrometer. Then, take a dark spectrum by clicking the **Store Dark Spectrum** icon on the toolbar or by selecting **Spectrum | Store Dark** from the menu bar. This command merely stores a dark spectrum in memory. You must select **File | Save | Dark** from the menu to permanently save the spectrum to disk.

Note: If possible, do not turn off the light source when taking a dark spectrum. If you must turn off your light source to store a dark spectrum, allow enough time for the lamp to warm up again before continuing your experiment.

You must take a dark spectrum before measuring relative irradiance.

6. Position the fiber at the light source you wish to measure. Then, choose the **Irradiance** mode icon on the toolbar or select **Spectrum | Relative Irradiance Mode** from the menu bar.
7. Click the **Save** icon on the toolbar or select **File | Save | Processed** from the menu bar to save the spectrum to disk.

Note: If you change any sampling variable (integration time, averaging, smoothing, angle, temperature, fiber size, etc.), you must store a new dark and reference spectrum.

Time Acquisition Experiments

OOIBase32 allows you to perform time acquisition experiments. Time acquisition experiments track processes, perform kinetic analyses, and monitor spectral events all as a function of time. You can collect, as a function of time, spectral data from up to six single wavelengths (designated as Channels A through F) and up to two mathematical combinations of these wavelengths (designated as Combinations 1 and 2). Additionally, you can acquire data in any mode (transmission, absorbance, etc.).

For more details about this and other OOIBase32 functions, refer to the *OOIBase32 Spectrometer Operating Software Manual*.

Follow the steps below to perform a time series experiment in OOIBase32:

1. Enter scope mode and store a reference spectra and dark spectra.
2. Choose the measurement mode (absorbance, transmission, etc.) and select **Time Acquisition | Configure | Configure Time Channels** from the menu bar to access the Time Acquisition Channel Configuration screen.

Proceed to the Configuring the Time Acquisition Channel Configuration Screen section below.

Configuring the Time Acquisition Channel Configuration Screen

1. Perform the following steps on the Time Acquisition Channel Configuration screen:
 - a. Select **Enabled** to set the time acquisition calculation for the wavelength. The time acquisition process will not calculate data if you do not select this option for at least
 - b. Select **Plotted** to see a real-time graph of the acquired data in a spectral window.
 - c. Select a **Spectrometer Channel** for the time acquisition process
 - d. Specify the analysis wavelength in the **Wavelength (nm)** box.
 - e. Specify the number of pixels around the analysis wavelength to average in the **Bandwidth (pixels)** box.
 - f. Select a multiplicative factor to apply to the data before plotting or storing. Then, select an additive constant or offset to apply to the data. OOIBase32 applies the additive constant or offset after applying the factor but before plotting or storing data.

The equation for the Factor and Offset functions is:

$$\text{Results} = (\text{Factor} * \text{Data}) + \text{Offset}$$

2. Configure a time acquisition process for the second single wavelength (if desired). Select the Channel B page and repeat Steps 1-3 for Channel B.

To configure a time acquisition process for the third, fourth, fifth, and sixth single wavelengths, select the Channel C, Channel D, Channel E, and Channel F pages, respectively, and set the necessary parameters.

(Continued)

Sample Experiments

3. Configure a time acquisition process for a combination of two time channels (if desired) by selecting **Combination 1**.

Perform the steps below to configure a combination:

- g. Select **Enabled** to set the time acquisition calculation for the wavelength.
- h. Enable **Plotted** to see a real-time graph of the acquired data in a spectral window.
- i. Specify Time Channel A through F for the First Channel.
- j. Select the mathematical operation to produce the data for Combination 1.
- k. Specify Time Channel A through F for the Second Channel.
- l. Select a multiplicative factor to apply to the data before plotting or storing. Then, select an additive constant or offset to apply to the data. OOIBase32 applies the additive constant or offset after applying the factor but before plotting or storing data.

The equation for the Factor and Offset functions is:

$$\text{Results} = (\text{Factor} * \text{Data}) + \text{Offset}$$

4. Configure a time acquisition process for the Combination 2 page, if desired. This page is virtually identical to the Combination 1 page, with the exception that you can choose Combination 1 for the first or second channel in Combination 2.
5. Click the **Apply** button to apply the changes, and then click the **OK** button to close the Time Acquisition Channel Configuration screen.

Proceed to the Configuring the Configure Acquisition Screen section below.

Configuring the Time Acquisition Configuration Screen

1. Select **Time Acquisition | Configure | Configure Acquisition** from the menu bar to open the Time Acquisition Configuration screen.
2. Enable **Stream Data to Disk** to save time acquisition data.
3. Enter a value in the **Write Data to Disk Every X Acquisitions** box to set the frequency for data saves. OOIBase32 saves data more frequently if the number is smaller, or less frequently if the number is larger. Entering a large number enhances the performance of the time acquisition process.

Note: At specified time intervals, OOIBase32 stores data into time acquisition channels or combination channels. OOIBase32 can plot the data in a spectral window, or stream the data to disk, or both. OOIBase32 can display up to 2048 acquisitions in a spectral window. If OOIBase32 collects more than 2048 acquisitions, it only displays the last 2048. To store more than 2048 acquisitions, you must stream the data to disk.

Writing data to the disk is a slow process (relative to the speed of some spectral acquisitions) and causes a decrease in system performance. However, writing data to disk more frequently gives a larger margin of safety.

4. Enable **Show Values in Status Bar** to see the time acquisition values in the status bar. These values replace the cursor values.
5. Name the **Stream Filename** for the time acquisition process. Clicking on the ellipsis to the right of this box opens a file save dialog box, allowing you to navigate to a designated folder.

(Continued)

Sample Experiments

6. Enable **Save Every Acquisition** to store data for every spectral acquisition during a time acquisition process (optional).

Note: OOIBase32 has options to either store data for each acquisition, or to collect data only after a specified delay. Several factors affect the minimum time acquisition frequency, including integration time, number of spectrometer channels, samples averaged, and computer speed. If you instruct OOIBase32 to store data every 100 milliseconds, the delay between data acquisitions will be 100 milliseconds or more, depending on your experimental configuration. OOIBase32 spends a large amount of time calculating, rendering, and displaying the spectra in a spectral window. You can suspend the graph display, which greatly improves the performance of OOIBase32.

7. Enter an **Initial Delay** to set the delay preceding the time acquisition process. Keep in mind that the delay countdown does not begin until you start the time acquisition process. Be sure to select Hours, Minutes, Seconds, or Milliseconds immediately to the right of the initial delay entry.
8. Enter a value to set the **Frequency** of the data collected in a time acquisition process. OOIBase32 stamps data from a time acquisition with a time accurate to one millisecond. Be sure to select Hours, Minutes, Seconds, or Milliseconds immediately to the right of the frequency entry. You can enable the **Save Every Acquisition** box to store the acquisitions that occur at this frequency. See Step 6 for more information.
9. Enter a value to set the **Duration** for the entire time acquisition process. Be sure to select Hours, Minutes, Seconds, or Milliseconds to the right of the duration entry. Click the **OK** button to close the Time Acquisition Configuration dialog box. Then, enable **Continue Until Manually Stopped**, which instructs OOIBase32 to store data until you manually stop the acquisition process (optional).

Appendix A: Calibrating the Wavelength of the USB2000

This Appendix describes how to calibrate the wavelength of your spectrometer. Though each spectrometer is calibrated before it leaves Ocean Optics, the wavelength for all spectrometers will drift slightly as a function of time and environmental conditions. Ocean Optics recommends periodically recalibrating the USB2000.

About Wavelength Calibration

You are going to be solving the following equation, which shows that the relationship between pixel number and wavelength is a third-order polynomial...

$$\lambda_p = I + C_1 p + C_2 p^2 + C_3 p^3$$

...where λ is the wavelength of pixel p , I is the wavelength of pixel 0, C_1 is the first coefficient (nm/pixel), C_2 is the second coefficient (nm/pixel²), and C_3 is the third coefficient (nm/pixel³). You will be calculating the value for I and the three C s.

Calibrating the Spectrometer

Preparing for Calibration

To re-calibrate the wavelength of your spectrometer, you will need the following:

- A light source capable of producing spectral lines

Note: Ocean Optics' HG-1 Mercury-Argon lamp is ideal for recalibration. If you do not have an HG-1, you will need a light source that produces several (at least 4-6) spectral lines in the wavelength region of your spectrometer.

- A USB2000 spectrometer
- An optical fiber (for spectrometers without a built-in slit, a 50- μ m fiber works best)
- A spreadsheet program (Excel or Quattro Pro, for example) or a calculator that performs third-order linear regressions

Note: If you are using Microsoft Excel, choose **Tools | Add-Ins** and check **AnalysisToolPak** and **AnalysisToolPak-VBA**.

Appendix A

Calibrating the Wavelength of the Spectrometer

Perform the steps below to calibrate the wavelength of the spectrometer:

1. Place OOIBase32 into **Scope Mode** and take a spectrum of your light source. Adjust the integration time (or the A/D conversion frequency) until there are several peaks on the screen that are not off-scale.
2. Move the cursor to one of the peaks and position the cursor so that it is at the point of maximum intensity.
3. Record the pixel number that is displayed in the status bar or legend (located beneath the graph). Repeat this step for all of the peaks in your spectrum.
4. Use the spreadsheet program or calculator to create a table like the one shown below. In the first column, place the exact or true wavelength of the spectral lines that you used.

In the second column of this worksheet, place the observed pixel number. In the third column, calculate the pixel number squared, and in the fourth column, calculate the pixel number cubed.

Independent Variable	Dependent Variables			Values Computed from the Regression Output	
True Wavelength (nm)	Pixel #	Pixel # ²	Pixel # ³	Predicted Wavelength	Difference
253.65	175	30625	5359375	253.56	0.09
296.73	296	87616	25934336	296.72	0.01
302.15	312	97344	30371328	302.40	-0.25
313.16	342	116964	40001688	313.02	0.13
334.15	402	161604	64964808	334.19	-0.05
365.02	490	240100	117649000	365.05	-0.04
404.66	604	364816	220348864	404.67	-0.01
407.78	613	375769	230346397	407.78	0.00
435.84	694	481636	334255384	435.65	0.19
546.07	1022	1044484	1067462648	546.13	-0.06
576.96	1116	1245456	1389928896	577.05	-0.09
579.07	1122	1258884	1412467848	579.01	0.06
696.54	1491	2223081	3314613771	696.70	-0.15
706.72	1523	2319529	3532642667	706.62	0.10
727.29	1590	2528100	4019679000	727.24	0.06
738.40	1627	2647129	4306878883	738.53	-0.13
751.47	1669	2785561	4649101309	751.27	0.19

(Continued)

Appendix A

5. Use the spreadsheet or calculator to calculate the wavelength calibration coefficients. In the spreadsheet program, find the functions to perform linear regressions.
 - If using Quattro Pro, look under **Tools | Advanced Math**
 - If using Excel, look under **Analysis ToolPak**
6. Select the true wavelength as the dependent variable (Y). Select the pixel number, pixel number squared, and the pixel number cubed as the independent variables (X). After executing the regression, you will obtain an output similar to the one shown below.

Regression Statistics

Multiple R	0.999999831
R Square	0.999999663
Adjusted R Square	0.999999607
Standard Error	0.125540214
Observations	22

	<u>Coefficients</u>	<u>Standard Error</u>	
Intercept	190.473993	0.369047536	Intercept
X Variable 1	0.36263983	0.001684745	First coefficient
X Variable 2	-1.174416E-05	8.35279E-07	Second coefficient
X Variable 3	-2.523787E-09	2.656608E-10	
	Third coefficient		

The figure above notes the numbers of importance.

7. Record the Intercept, as well as the First, Second, and Third Coefficients. Additionally, look at the value for R squared. It should be very close to 1. If not, you have most likely assigned one of your wavelengths incorrectly.

Keep these values at hand.

Saving the New Calibration Coefficients: USB Mode

Ocean Optics programs wavelength calibration coefficients unique to each USB2000 onto an EEPROM memory chip in the USB2000.

You can overwrite old calibration coefficients on the EEPROM if you are using the USB2000 via the USB port. If you are using the USB2000 via the serial port, consult the *Saving the New Calibration Coefficients: Serial Mode* section later in this Appendix.

To Save Wavelength Calibration Coefficients Using the USB Mode

1. Ensure that the USB2000 is connected to the PC and that you have closed all other applications.
2. Point your browser to <http://www.oceanoptics.com/technical/softwaredownloads.asp> and scroll down to **Microcode**. Click on the **USB EEPROM Programmer** selection.
3. Save the setup file to your computer.
4. Run the **Setup.exe** file to install the software. The Welcome screen appears.
5. Click the **Next** button. The Destination Location screen appears.
6. Accept the default installation location, or click the **Browse** button to specify a directory. Then, click the **Next** button. The Program Manager Group screen appears.
7. Click the **Next** button. The Start Installation screen appears.
8. Click the **Next** button to begin the installation. Once the installation finishes, the Installation Complete screen appears.
9. Click the **Finish** button and reboot the computer when prompted.
10. Navigate to the **USB EEPROM Programmer** from the Start menu and run the software.
11. Click on the appropriate USB2000 device displayed in the left pane of the USB Programmer screen.
12. Double-click on each of the calibration coefficients displayed in the right pane of the USB Programmer screen and enter the new values acquired in Steps 5 and 6 of the *Calibrating the Wavelength of the Spectrometer* section in this Appendix.
13. Repeat Step 12 for all of the new values.
14. Click on the **Save All Values** button to save the information, and then **Exit** the USB Programmer software.

The new wavelength calibration coefficients are now loaded onto the EEPROM memory chip on the USB2000.

Appendix A

Saving the New Calibration Coefficients: Serial Mode

If you are connecting the USB2000 Spectrometer to the serial port of the PC, you need to save the new wavelength calibration coefficients to the .SPEC file that OOIBase32 accesses when opened.

Note: You cannot save the calibration coefficients to the EEPROM memory chip on the USB2000 when using the serial mode.

To Save Wavelength Calibration Coefficients Using the Serial Mode

1. Open the OOIBase32 application.
2. Select **Spectrometer | Configure** from the OOIBase32 menu bar. The Configure Spectrometer screen appears.
3. Select the **Wavelength Calibration** tab to update the wavelength coefficients within OOIBase32.
4. Enter in the new values acquired from Steps 5 and 6 of the *Calibrating the Wavelength of the Spectrometer* section in this Appendix.
5. Click the **OK** button to save the information in OOIBase32.

Appendix B: USB2000-FLG Spectrometer

The USB2000-FLG Spectrometer is preconfigured for use with fluorescence applications from 380-1050 nm. It contains proprietary Silver AgPlus Mirrors which provide an increase in sensitivity of 10-35% over the standard USB2000 Spectrometer.

The USB2000-FLG contains custom firmware that allows you to program a variable between a Single Strobe signal and the commencement of an acquisition. You can enable this mode entirely through software configuration, and you can easily control the mode via the OOIBase32 interface.

About the USB2000-FLG

On the USB2000-FLG, Ocean Optics has altered the functionality of the External Hardware Trigger function and reprogrammed the Single Strobe signal function. These alterations of the USB2000 enable you to operate in the Variable Delay Mode without the presence of an external trigger signal. The USB2000-FLG will simulate the signal to trigger the appropriate circuitry in the spectrometer.

The table below summarizes the capacity of the USB2000-FLG in Variable Delay Mode:

Parameter	Value in Normal Acquisition Mode	Value in Variable Delay Mode
Integration time	User controlled	5 ms fixed
Strobe delay	0 μ s	2 – 511 μ s + 3 μ s
Jitter	< 25 ns	< 25 ns

Using the USB2000-FLG

1. Install the OOIBase32 software on your PC.

Note: Ensure that you have installed the OOIBase32 software prior to connecting the USB2000-FLG Spectrometer to your PC.

2. Connect the USB2000-FLG Spectrometer to the USB port of your PC.
3. Start OOIBase32 and ensure that the spectrometer is acquiring spectra.
4. Verify that the driver version is 4.0.4.9 or later. Open the **Help | About** option in OOIBase32 to obtain this information. If the driver meets this requirement, proceed to Step 6. Otherwise, proceed to Step 5.
5. Overwrite (if necessary) the outdated version of the driver with the one included with the USB2000-FLG. Open Windows Explorer, browse to the OOIBase32 directory (C:\Program Files\Ocean Optics\OOIBase32 is the default installation location) and overwrite the existing ooidrv32.dll with the new file.
6. Enter the desired delay value in the Strobe Freq. box (See Figure B-1 on the following page).
7. Enter Variable Delay Mode by placing the USB2000-FLG in External Hardware Trigger mode. In OOIBase32, select **Spectrum | Configure Data Acquisition | External Trigger**, and then select the **Hardware Trigger** option. You do not need to apply a trigger signal.

(Continued)

Appendix B

- Enable Single Strobe output by checking the Strobe Enable check box (See Figure B-1 on the following page).

The strobe signal is a rising edge trigger signal that is TTL High for the entire delay period. See Figure B-2 for a timing diagram.

The USB2000-FLG is now configured for operation in Variable Delay Mode.

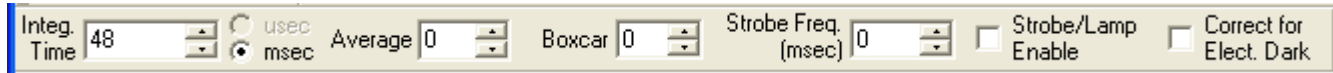


Figure B-1: The Acquisition Parameters Toolbar.

Note the location of the Strobe Freq (entered in μs) and Strobe/Lamp Enable options.

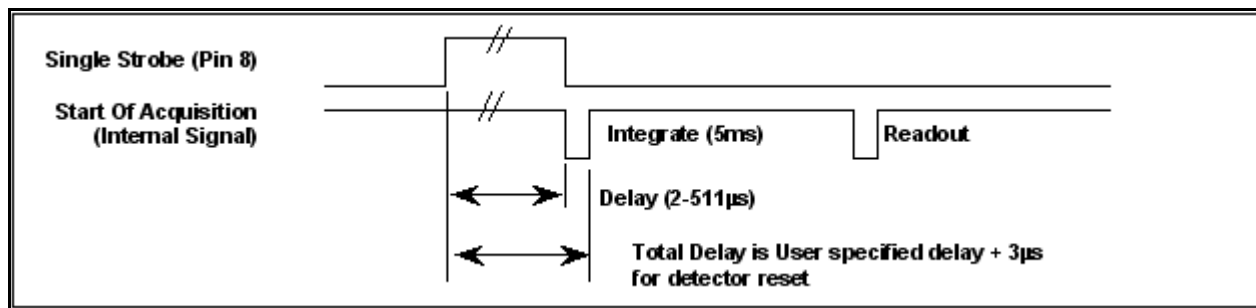


Figure B-2: Timing Diagram for Variable Delay Mode

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