Endospore Detection System

EDS2000

Installation and Operation Manual

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Table of Contents

About This Manual

Document Purpose and Intended Audience

This document provides you with instructions to get your EDS2000 system up and running.

Document Summary

Chapter	Description
Chapter 1: *Introduction*	Introduces the product features. Also contains a list of items included in the shipment and a list of computer requirements.
Chapter 2: *Installation and Startup*	Provides procedures for installing the EDS2000 and starting the OOIBase32-Bacillus software.
Chapter 3: *Using OOIBase32-Bacillus*	Contains information for using the OOIBase32-Bacillus software.
Chapter 4: *Troubleshooting*	Contains typical problems and suggested resolutions.

Product-Related Documentation

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

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

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

Upgrades

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

Chapter 1

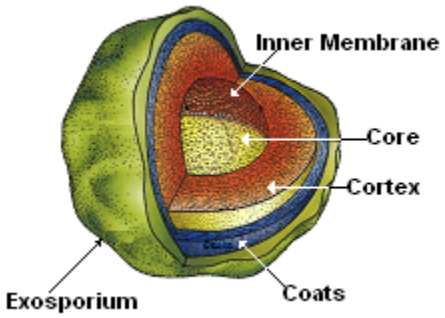
Introduction

Overview

The following sections provide information on how the EDS2000 Endospore Detection System (ED2000) works, as well as shipment and compatibility specifications.

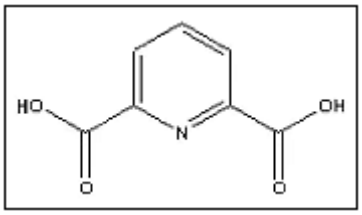
How the EDS2000 Works

The EDS2000 tests for the presence of dipicolinic acid (DPA). DPA is unique to bacterial endospores and makes up approximately 10% of their dry weight. The DPA is found in the cortex, surrounding the core, and is thought to offer protection from UV radiation. DPA is not found in vegetative cells.



Bacterial Endospore

Terbium III is a lanthanide cation that forms a photoluminescent complex with DPA. The photoluminescence is unusual in that four specific visible wavelengths of light are emitted after absorption of UV radiation. In addition, the photoluminescent complex has a long decay time (several milliseconds). The EDS2000 can distinguish DPA from other substances by using both the spectral pattern of the emission, and the decay lifetime.



Tb^{+3}

Tb^{+3} exhibits photoluminescence even in the absence of DPA. At low concentrations, this is minimized. The storing of a reagent blank spectrum corrects for the slight fluorescence of the Tb^{+3} solution. In the presence of DPA, photoluminescence is greatly enhanced.

The detection limit is approximately 100,000 spores. These figures are based on tests with *Bacillus globigii*, a surrogate for *Bacillus anthracis*. The test is useful for the rapid screening of substances that are suspected to be concentrated forms of the anthrax spore. It will report positive for any bacterial endospore, and it will report negative if the number of spores is less than the detection limit.

References:

Bacterial Spore Detection and Determination by Use of Terbium Dipicolinate Photoluminescence. 1997 David L. Rosen *Analytical Chemistry* volume 69, pages 1082-1085.

Bacterial Endospore Detection Using Terbium Dipicolinate Photoluminescence in the Presence of Chemical and Biological Materials. 1998 Paul M. Pellegrino, Nicholas F. Fell, Jr., David L. Rosen and James B. Gillespie *Analytical Chemistry* volume 70, pages 1755-1760.

Ocean Optics has an exclusive license agreement with United States Army Research Laboratory to use the terbium reagent for endospore detection.

Unpacking the EDS2000 System

The Endospore Detection System consists of a gated spectrofluorometer and accessory batteries in a splash proof carrying case. The system components are pre-assembled and packed in protective foam. It is not necessary to unpack or disassemble these components.

The hardware components included in the yellow carrying case are as follows:

- (1) Modified USB2000-FL 2048-element CCD gated spectrometer, 350-1000 nm, 10 nm FWHM optical resolution, with SMA 905 fiber optic connection
- (1) Pulsed Xenon Flash Lamp
- (1) Cuvette cover
- (1) 12-VDC lead acid gel cell battery
- (1) 120-VAC to 12VDC charger, prewired to the battery
- (1) AC power cord
- (1) 12-VDC 120 VAC transformer

(1) Each (in sealed UV-transmissive polymer cuvettes):

- Prepackaged reagent blank (Blank - Blue cap)
- Dipicolinic acid standard (DPA - Green cap)
- *B. globigii* spores (BG - Red cap)

Other components included with the shipment consist of the following:

- (2) Boxes (100 in each box) of EDS-TEST endospore detection test reagents in disposable cuvettes
- (1) Endospore Detection System Software CD
- (1) USB2000 Calibration diskette and Calibration Data Sheet
- (1) Ocean Optics Software and Resources CD

Computer Requirements

The EDS2000 shipment does not include a computer.

You can use the EDS2000 with any notebook or desktop PC computer with the following minimum specifications:

- Windows 98/Me/2000/XP
- 32 MB of RAM
- One available USB port

Note

Windows 95 and Windows NT do not support USB connectivity.

To determine if your computer is USB-ready and compatible with the USB2000 Spectrometer, download the Intel USB System Check utility from:

ftp://ftp.oceanoptics.com/pub/usbready/usbready.exe

You must ensure that you have USB compatibility and availability on your system in order to use the EDS2000.

Ordering Information - Accessories

The following accessories or replacement parts are available for the EDS2000:

Part Number	Description	Cost
EDS-TEST	Endospore Test Kit (100/box)	$300.00

To order, contact your Ocean Optics sales representative at (727) 733-2447.

Documentation

The EDS2000 consists of standard Ocean Optics products configured for spectrofluorimetry. The only exception is the USB2000-FLG spectrometer, which has special firmware that enables gated emission spectroscopy. The EDS2000 software is an add-on application to OOIBase32 general operating software. All of the features of OOIBase32 are supported. Detailed descriptions and instructions for the various components are available on the Ocean Optics *Software and Technical Resources* CD. See *Product-Related Documentation* for more information.

Chapter 2

Installation and Startup

The following sections provide instruction on installing Bacillus Endospore Detection Software, as well as hardware setup and equipment operation.

Installing the Bacillus Endospore Detection Software

Note

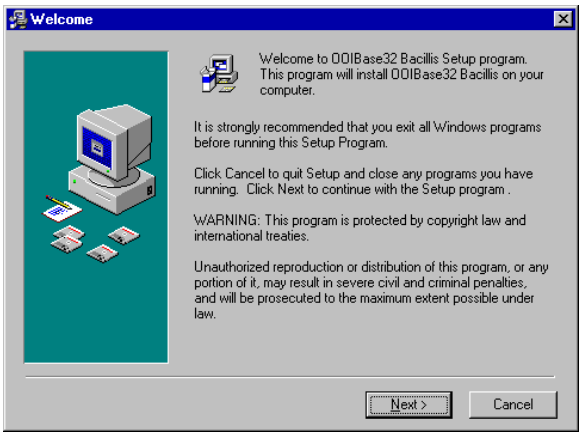
You **MUST** install the Bacillus Endospore Detection Software **BEFORE** connecting the spectrometer to your computer's USB port. See *Windows Cannot Locate/Install Drivers for Spectrometer* in Chapter 4: *Troubleshooting* for more information.

The EDS2000 system contains a specially designed version of Ocean Optics' OOIBase32 operating software. Consult the *OOIBase32 Spectrometer Operating Software, Operating Instructions* on the Ocean Optics *Software and Technical Resources* CD for more information on OOIBase32.

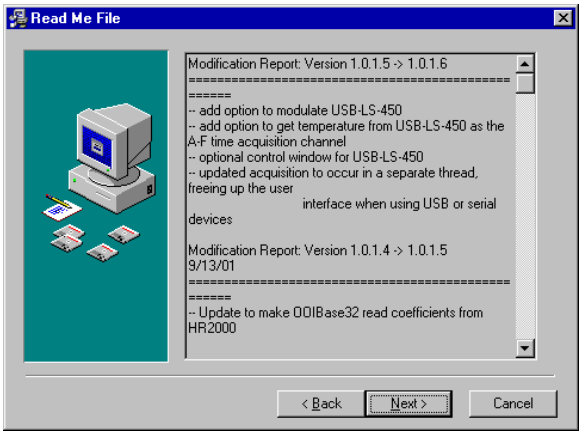
► *Procedure*

To install the Bacillus Endospore Detection Software, perform the following steps:

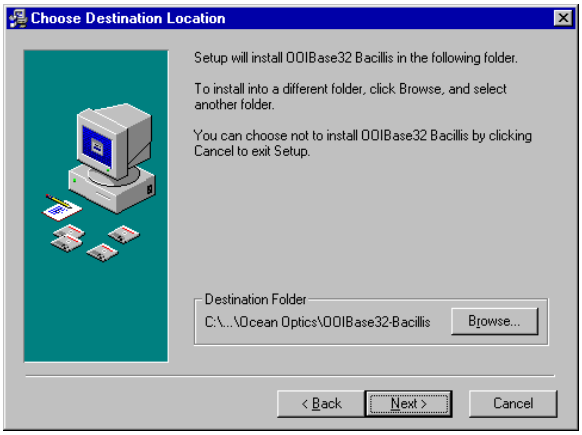
1. Ensure that no other applications are currently running on the PC.
2. Insert the Bacillus Endospore Detection System CD into the CD-ROM drive (if you do not have this CD, contact Ocean Optics Technical Support). The **Welcome** screen appears.



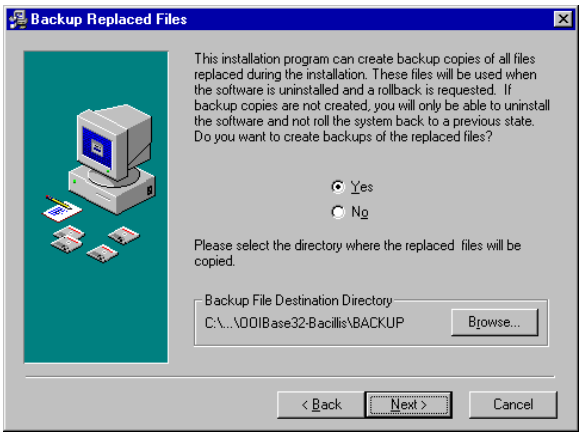
3. Click the **Next** button. The **Read Me File** screen appears.



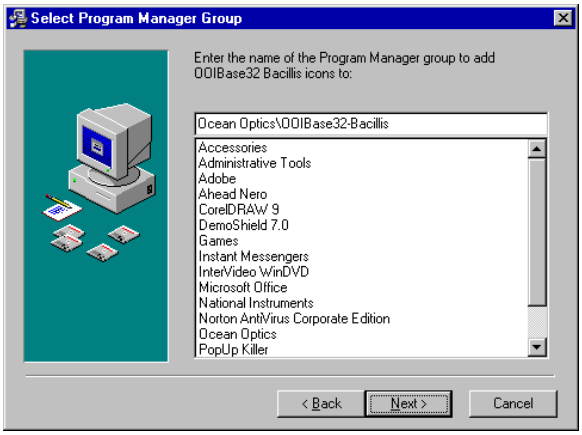
4. Click the **Next** button. The **Choose Destination Location** screen appears.



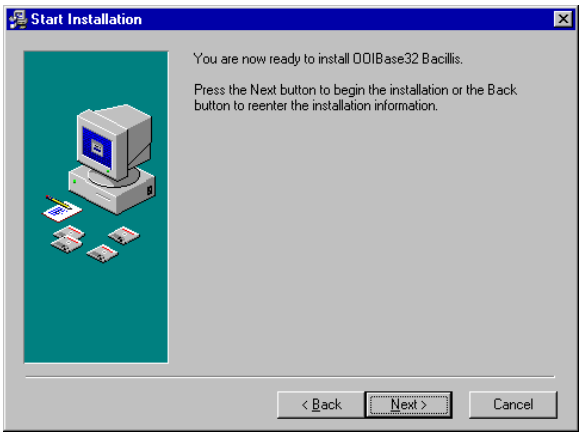
5. Click **Next** to accept the default destination location (recommended). The **Backup Replaced Files** screen appears.



6. Select the **Yes** radio button to backup files replaced during this installation. You will be prompted to specify a file backup destination. Otherwise, select the **No** radio button to skip this step. Click the **Next** button to proceed. The Program Manager Group window appears.



7. Click the **Next** button. The **Start Installation** window appears.



8. Follow all prompts regarding the Spectrometer Configuration disk that came with your system. The Spectrometer Configuration disk contains information specific to the USB2000 Spectrometer included with the EDS2000 system.

9. Click **Finish** when the installation is completed.

10. Reboot the system when prompted.

Connecting the Spectrometer to the PC

Once software installation is completed, you can connect the spectrometer to the PC.

Note

If you connected the spectrometer to the PC prior to installing the Bacillus Endospore Detection Software, see *Windows Cannot Locate/Install Drivers for Spectrometer* in Chapter 4: *Troubleshooting*.

► *Procedure*

To connect the spectrometer to the PC, perform the following steps:

1. Locate the coiled USB cable stored in the compartment above the EDS2000. The square end of the USB cable should already be plugged into the side of the EDS2000.

2. Insert the rectangular end of the USB cable into an available USB port on the PC.

   Windows automatically locates the USB2000 Spectrometer and installs the appropriate device drivers.

Note

If you are installing the EDS2000 on a Windows XP machine, you may encounter a "Device not verified" warning during device identification and installation. This is an expected event. If this warning appears, click **Continue Anyway** to proceed.

Once the device and device drivers are installed, proceed to *Connecting the Pulsed Xenon Lamp*.

Connecting the Pulsed Xenon Lamp

This section contains information on connecting and operating the Pulsed Xenon lamp included with your EDS2000 system.

WARNING

The Pulsed Xenon lamp used in the EDS2000 system emits UV light that is harmful to your eyes.

Ensure that the cuvette cover is in place when operating the lamp. Do not look into the light source without the use of protective eyewear.

You can operate the Pulsed Xenon lamp with power from the included 12 VDC battery or from an external 120 VAC power source.

Powering the EDS2000

The EDS200 is capable of using either a battery (supplied) or a 12-volt power supply (included).

Battery Operation

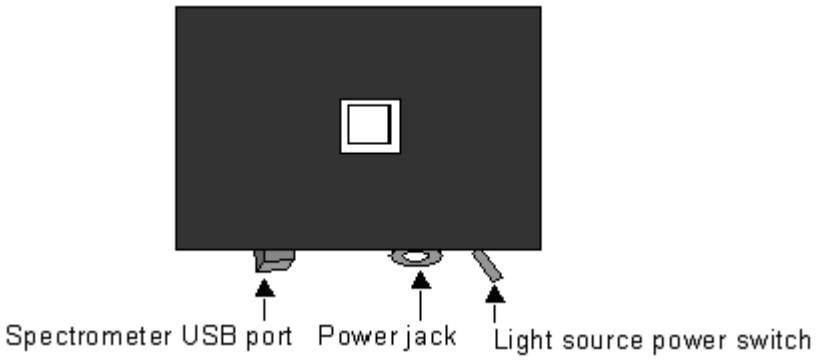
The battery is already connected to the light source through a power jack located on the front of the blue EDS2000 enclosure. The battery charger can be connected to a 120 VAC power source during EDS2000 operation, or to charge the battery when the unit is not being used. To charge the battery, plug the power cord into the power receptacle on the EDS2000, then connect the other end to a wall power socket. The battery will fully charge in approximately 2-3 hours, and will provide 4-6 hours of operation time.

Note

The battery on the EDS2000 will not power a notebook PC.

AC Power Operation

A 12-volt power supply is also provided for stand-alone use of the integrated system contained in the blue enclosure. To remove the system for use outside of the carrying case, unplug the battery from the front of the blue enclosure and remove the blue enclosure from the carrying case. The power supply can then be used to power the xenon lamp by plugging the system into a wall socket.



EDS2000 Input/Control Layout

Lamp Operation

The Pulsed Xenon lamp included with your EDS2000 is configured to operate under software control. The lamp will not operate until the software is activated.

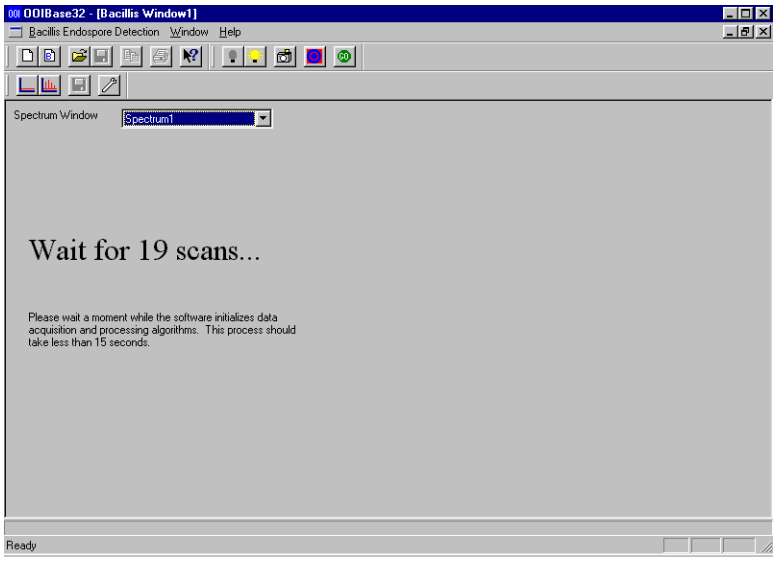
Starting the Software

This section provides instructions for starting the OOIBase32-Bacillus software.

► *Procedure*

To launch the Ocean Optics OOIBase32-Bacillus software program, perform the steps below:

1. Click Start and navigate to Programs | Ocean Optics | OOIBase32-Bacillus.
2. Click the OOIBase32-Bacillus icon to launch the software. The **OOIBase32-Bacillus Warm-Up Mode** screen appears. Additionally, you should hear the Pulsed Xenon light source emit a series of clicks, as the strobe fires a series of 20 bursts (10 flashes per burst) during the warm-up mode.



Once the OOIBase32-Bacillus program exits Warm-Up mode, you can use the software. See Chapter 3: *Using OOIBase32-Bacillus*.

If you have difficulties with the system after the software is started, please refer to Chapter 4: *Troubleshooting*.

Chapter 3

Using OOIBase32-Bacillus

OOIBase32-Bacillus Toolbar

The OOIBase32-Bacillus software is similar to Ocean Optics' standard OOIBase32 software. However, the OOIBase32-Bacillus software contains a special Bacillus window, which contains a customized Bacillus toolbar.

Bacillus Toolbar

The Bacillus toolbar contains the following icons:

Icon	Name	Function
	Store Background	Stores the spectra of the background solution
	Store Standard	Stores the standard spectra in an overlay mode
	Save Data File	Saves the current data
	Configure Settings	Configures the goodness of fit criteria, text message for each level, and values for the standards

Testing Samples

Testing samples with the EDS2000 system involves the following procedures:

- Storing Background and Standard Spectra
- Testing Samples
- Logging Results

Store Background and Standard Spectra

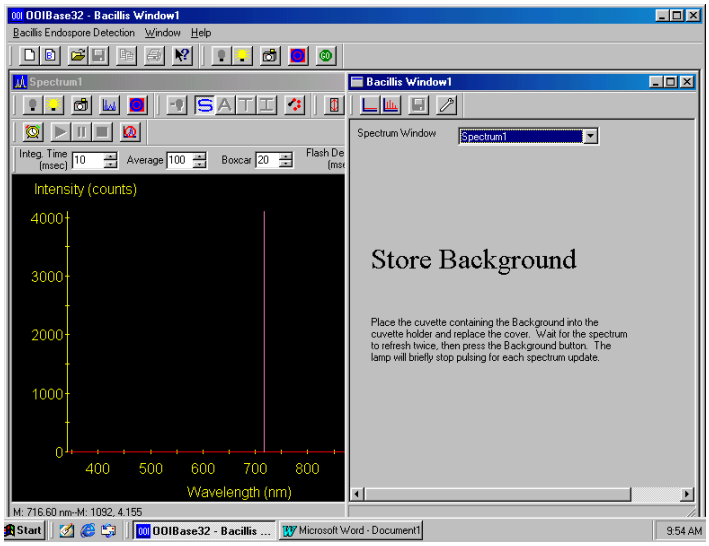
Once the system has warmed up, the flash rate changes to the match the acquisition parameters set in the OOIBase32-Bacillus software. You need to store background and standard spectra before testing your samples.

Perform the steps below to store background and standard spectra:

Storing Background Spectra

► *Procedure*

1. Ensure that the system has fully warmed up. Once the system is warmed up, the **Store Background** message appears.



2. Place the cuvette labeled **Blank** (blue cap) into the cuvette holder. The cuvette holder on the EDS2000 is designed so that the cuvette can only be placed in the proper orientation.

3. Install the cuvette cover.

4. Wait a few seconds (until at least 2 bursts have occurred).

5. Click the **Store Background** icon.

The software stores the spectrum of the background solution, and automatically subtracts this spectrum in real time from all new spectra acquired by the spectrometer.

You can retake the background spectrum reading at any time by repeating the steps above. You should check the background periodically, and retake the background spectrum reading as necessary to correct for any drift in the system. One of the parameters reported by the OOIBase32-Bacillus software is baseline drift. If this number exceeds approximately 10 counts, you should store a new background.

It is important that you use the reagent blank when acquiring background spectra readings. You can use any reagent filled tests as the blank, or you can use the sealed cuvette with the blue cap (Blank) that was packaged with the system.

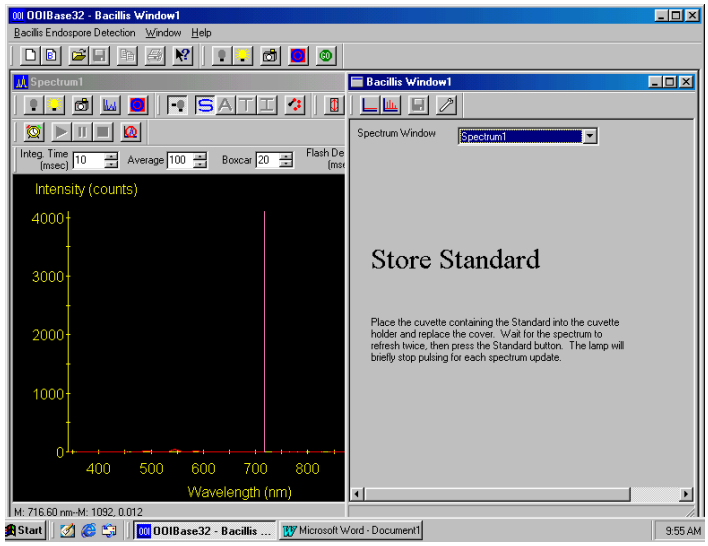
The Tb solution has a slight background photoluminescence that must be subtracted from incoming spectra. If this does not occur, false positives may be reported. If the Tb in the reagent is significantly diluted by the samples, for example if a liquid sample is being tested, then a similarly diluted reagent blank should be prepared using de-ionized water.

Once you store the background spectrum, the Bacillus window displays the **Store Standard** message. Proceed to the next section for instructions on storing Standard spectra .

Storing Standard Spectra

► *Procedure*

1. Ensure that the **Store Standard** message is on the screen.



2. Place the cuvette labeled **DPA** (green cap) into the cuvette holder. The cuvette holder on the EDS2000 is designed so that the cuvette can only be placed in the proper orientation.

3. Install the cuvette cover.

4. Wait a few seconds (until at least 2 bursts have occurred).

5. Click on the **Store Standard** icon. OOIBase32-Bacillus stores the spectrum in an overlay mode and automatically rescales the graph so that the standard spectrum fills most of the view.

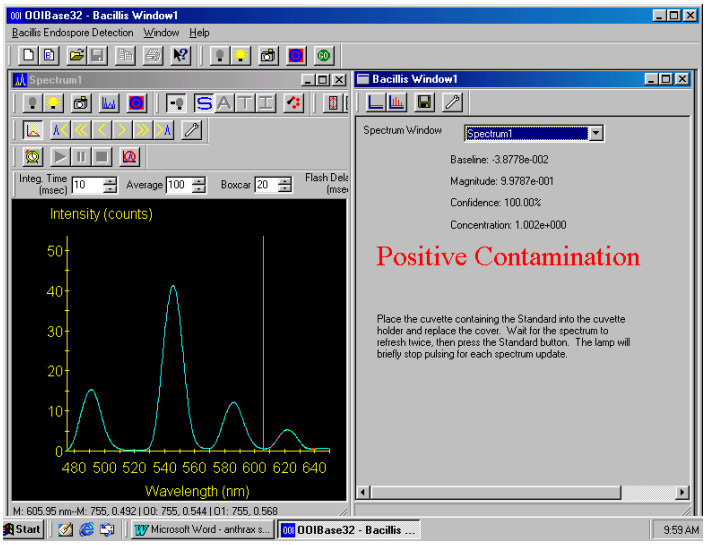
The standard supplied with the kit is 25 x 10^{-6} molar DPA in 100 uM $TbCl_3$ in 0.01 N HCl. The photoluminescence from this sample should result in four peaks, with the largest peak occurring at 546nm.

Once you store the standard spectrum, OOIBase32-Bacillus compares the active trace from the spectrometer to this saved standard using a regression analysis. The goodness of fit (expressed as a percentage = 100 x r^2) is used to evaluate the sample as either positive, maybe, or below detection limits (see example in Figure 3-2). The active trace and the predicted trace based on the regression coefficients times the active trace are displayed in the window as well.

Immediacy of Response

While the standard cuvette is still in the sample holder, the software immediately reports a positive and the sample, standard, and predicted traces overlay one another.

The goodness of fit criteria are preset at >90% for a “Positive Contamination” and greater than 70% for a “Possible Contamination”. Values lower than 70% result in a “Below LOD” for below the limit of detection, or “Error, Negative Magnitude”



Example of Positive Response

The goodness of fit criteria, the text message for each level, and the values for the standards can be entered in the Configure Settings window, which is opened by clicking the Configure Settings icon in the Bacillus toolbar.

Testing Your Samples

The EDS-TEST consists of a UV transmissive plastic cuvette containing 1 ml of the reagent. The sample containing the suspected anthrax must be added to the contents of the cuvette. For liquids, add a drop or two of the sample. For powders, use a swab wet with a small amount of the reagent to pick up the sample. Transfer it to the cuvette contents by swirling the swab in the cuvette.

Caution

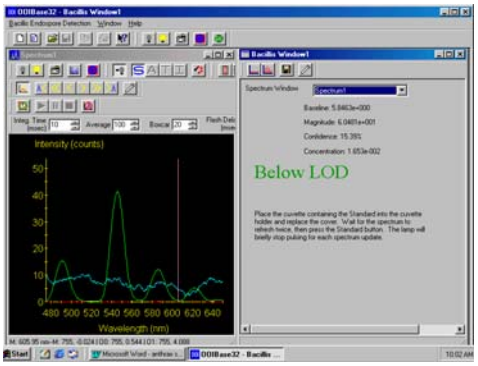
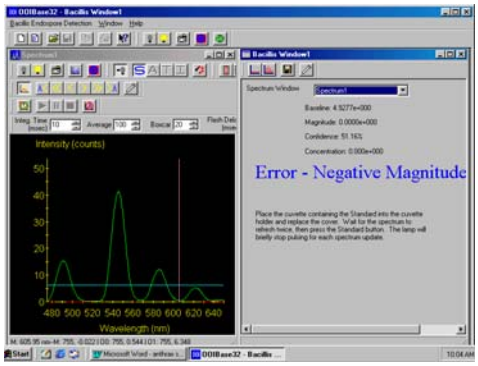
Do not contaminate the outside of the cuvette. Be sure to replace the cuvette cap after the sample is introduced, and place the used swab in an appropriate biohazard waste receptacle.

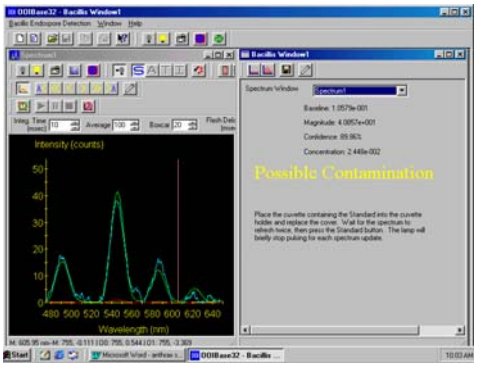
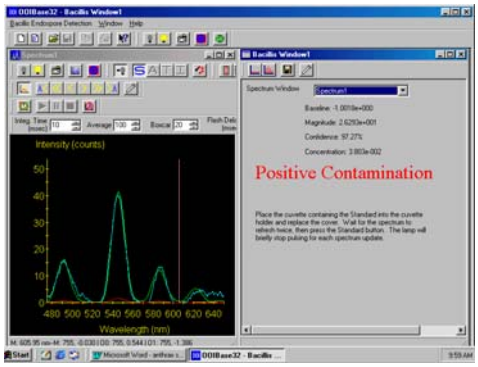
Once the cuvette is sealed, you can disinfect it with bleach. Do not use acetone or other organic solvents. You can label the upper half of the cuvette, but keep the lower optical half of the cuvette clear of labels, dirt, and other obstacles.

► *Procedure*

Perform the steps below to test your samples:

1. Place the sample in the cuvette holder. The cuvette holder on the EDS2000 is designed so that the cuvette can only be placed in the proper orientation.
2. Install the cuvette cover.
3. Wait a few seconds (until at least 2 bursts have occurred). Once the spectra have refreshed at least twice (2 bursts), one of four messages will appear:

	
Below LOD (Limit of Detection)	**Error – Negative Magnitude**

	
Possible Contamination	**Positive Contamination**

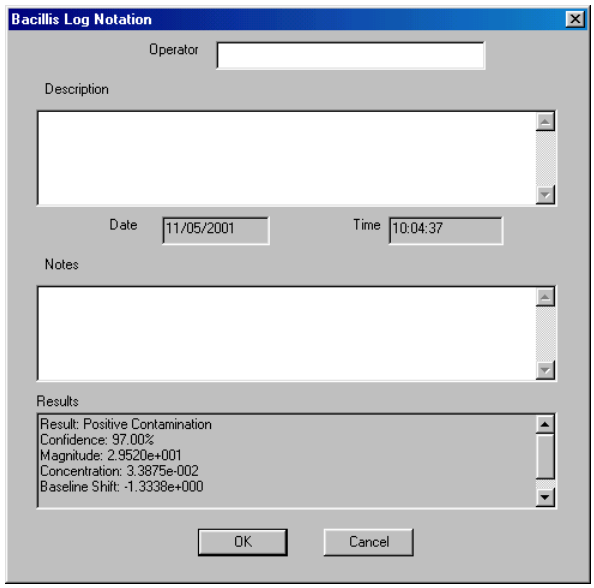
- **Below LOD** or **Error – Negative Magnitude**: Reported for samples that do not display the characteristic photoluminescence peaks of DPA.
- **Positive Contamination** or **Possible Contamination**: Reported if the peaks are there and the shapes match the standard to better than 90% (for Positive reading) or 70% (for Possible reading).

Note

The EDS2000 is designed for the rapid screening of suspicious materials. Further testing of samples for the presence of low numbers of endospores below the limit of detection or for other biological or chemical agents is recommended.

Logging Your Results

Once you test your samples, you can save the data in a file. Click the Save Data File icon ( ) in the Bacillus toolbar. When you click on this icon, you are prompted for a file name and destination directory for the data file. Enter this information and continue to bring up the Bacillus Log Notation screen.



Bacillus Log Notation Screen

From this screen, you can save the sample, blank, and standard spectra in a data file. This data file also contains the regression statistics, and any additional information that you enter into the **Operator**, **Description**, and **Notes** text boxes.

Type your sample information into the text boxes and click **OK** when you are done. The file is saved in ASCII format and can be viewed using Notepad or imported into EXCEL or any other program capable of reading text files.

Chapter 4

Troubleshooting

Overview

This chapter contains troubleshooting procedures for issues you may encounter when using the EDS2000 system.

Windows Cannot Locate/Install Drivers for Spectrometer

Issue

Windows cannot locate or install the correct drivers for the USB2000 spectrometer.

Probable Cause

This typically occurs if you connect the spectrometer to the PC before installing the OOIBase software.

Resolution

Follow the applicable steps below to remove the incorrectly installed device, device driver, and installation files.

Note

If these procedures do not correct your device driver problem, you must obtain the *Correcting Device Driver Issues* document from the Ocean Optics website at: **http://www.oceanoptics.com/technical/engineeringdocs.asp**.

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 may also appear under the Universal Serial Bus Controller option. Be sure to check this location if you cannot locate the unknown device.

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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. Remove the USB or PCI device from your computer.

You must now remove files associated with the improperly installed device from the computer.

Remove Files

Windows 98:

1. Open Windows Explorer.

2. Navigate to the **Windows | INF** directory. If the INF directory is not visible, you must disable the **Hide System Files and Folders** option 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:

1. Open Windows Explorer.
2. Navigate to the **Windows | INF** directory. If the INF directory is not visible, you must disable the **Hide System Files and Folders** option 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.
3. Navigate to the **Windows | System32 | Drivers** directory.
4. Delete the **EZUSB.SYS** file.
5. Reinstall your Ocean Optics application and reboot the system when prompted.
6. Plug in the USB device.

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

Windows XP:

1. Open Windows Explorer.
2. Navigate to the **Windows | INF** directory. If the INF directory is not visible, you must disable the **Hide System Files and Folders** option in **Windows Folder Options**.
4. You can access **Windows Folder Options** from Windows Explorer, under the **View | Options** menu selection.
5. Delete the **OOI_USB. INF** and **OOI_USB.PNF** files in the INF directory.
6. Navigate to the Windows | System32 | Drivers directory.
7. Delete the EZUSB.SYS file.
8. Reinstall your Ocean Optics application and reboot the system when prompted.
9. Plug in the USB device.

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

USB2000 is Installed Correctly, but is not Recognized by OOIBase32-Bacillus

Issue

Windows recognized the USB2000 and installed the drivers correctly, but the OOIBase32-Bacillus software does not recognize the spectrometer.

Probable Cause

The default spectrometer type specified in OOIBase32-Bacillus is incorrect.

Resolution

Part 1:

Perform the steps below to manually configure the spectrometer type in OOIBase32-Bacillus:

1. Start the OOIBase32-Bacillus software.
2. Choose **Spectrometer | Configure** from the OOIBase32-Bacillus menu bar.
3. Click on the **A/D Interface** tab.
4. Ensure that **Spectrometer Type** is set to **S2000** and the **A/D Interface** is set to **USB2000.**
5. Click **OK**.
6. Select **Spectrometer | Save Configuration As…** from the OOIBase32-Bacillus menu bar and save the modified configuration with a unique name.
7. Click **Yes** when prompted to make the new configuration the default spectrometer configuration.
8. Exit the OOIBase32-Bacillus software.
9. Unplug the USB2000 from the computer's USB port, then reattach.
10. Wait 15 seconds, then restart the OOIBase32-Bacillus software.

OOIBase32-Bacillus should now recognize the USB2000 spectrometer. If you are still experiencing problems, proceed to Part 2 on the next page.

Part 2:

Perform the steps below to check and correct the settings in the Default.spec file:

1. Open Windows Explorer and navigate to the OOIBase32-Bacillus installation directory. The default location is:

   C:\Program Files\Ocean Optics\OOIBase32-Bacillus

2. Locate the Default.spec file and open the file with Notepad (or any text editor).
3. Save a backup of the Default.spec file before editing the settings (optional).
4. Locate the line that begins with "ADCType=" and ensure that it reads ADCType=USB2000.
5. Locate the line that begins with "Initialized=" and modify the line to read Initialized=0.
6. Save the Default.spec file.
7. Restart the OOIBase32-Bacillus software

OOIBase32-Bacillus should now recognize the USB2000 spectrometer. If you are still experiencing problems, proceed to Part 3 below.

Part 3:

Perform the steps below to check and correct the settings in the Ooidrv.ini file:

1. Open Windows Explorer and navigate to the C:\Windows directory.
2. Locate the Ooidrv.ini file and open the file with Notepad (or any text editor).
3. Locate the line that begins with "Initialized=" and ensure that it reads Initialized=0.
4. Save the Ooidrv.ini file.
5. Restart the OOIBase32-Bacillus software.

Note

You do not need to restart the computer after making these changes. When you set Initialized=0, the driver opens a dialog box when you start OOIBase32-Bacillus. You still need to verify the settings in this dialog box, then click **OK** to save the changes.

If the procedures in this chapter do not resolve your problem, please contact Ocean Optics for technical support.

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