



HR4000 and HR4000CG-UV-NIR Series  
High-Resolution Fiber Optic Spectrometers  
HR4000 / HR4000CG-UV-NIR

## 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](mailto:Info@OceanOptics.com) (General sales inquiries)  
[Info@OceanOpticsBV.com](mailto:Info@OceanOpticsBV.com) (European sales inquiries)  
[Orders@OceanOptics.com](mailto:Orders@OceanOptics.com) (Questions about orders)  
[TechSupport@OceanOptics.com](mailto:TechSupport@OceanOptics.com) (Technical support)

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# About This Manual

## Document Purpose and Intended Audience

This document provides the user of HR4000 Series Spectrometers (both HR4000 and HR4000CG-UV-NIR) with instructions for setting up, calibrating and performing experiments with their spectrometer.

## What's New in this Document

This version of the *HR4000 and HR4000CG-UV-NIR Series High-Resolution Fiber Optic Spectrometers HR4000 / HR4000CG-UV-NIR Installation and Operation Manual* updates the customer service hours and the Ocean Optics B.V. office location.

## Document Summary

Chapter	Description
Chapter 1: <a href="#">Introduction</a>	Contains descriptive information about the HR4000 Spectrometer and how sampling works. It also provides a list of system requirements, interface options, and shipment components.
Chapter 2: <a href="#">Installing the HR4000</a>	Provides installation and configuration instructions.
Chapter 3: <a href="#">Troubleshooting</a>	Contains recommended steps to isolate and correct common problems.
Chapter 4: <a href="#">Sample Experiments</a>	Offers instructions for preparing for and taking measurements with the HR4000 Series Spectrometer.
Appendix A: <a href="#">Calibrating the Wavelength of the HR4000</a>	Provides instructions for calibrating the HR4000 Series Spectrometer.
Appendix B: <a href="#">Specifications</a>	Contains technical specifications and connector pinouts for the HR4000 Series Spectrometer.
Appendix C: <a href="#">HR4000CG-UV-NIR Spectrometer</a>	Contains features and specifications unique to the HR4000CG-UV-NIR Spectrometer.

## Product-Related Documentation

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

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

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

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

## Upgrades

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



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# Chapter 1

# Introduction

## Product Overview

The HR4000 High-Resolution Miniature Fiber Optic Spectrometer provides optical resolution as good as 0.025 nm (FWHM). The HR4000 is responsive from 200-1100 nm, but the specific range and resolution depends on your grating and entrance slit selections.

The HR4000 is perfect for applications where high resolution is necessary, such as absorbance of gases or atomic emission lines (for solution chemistry or for color measurements, the S2000, PC2000 and USB2000 are more appropriate spectrometers).

Data programmed into a memory chip on each HR4000 includes wavelength calibration coefficients, linearity coefficients, and the serial number unique to each spectrometer. Our spectrometer operating software simply reads these values from the spectrometer — a feature that enables hot swapping of spectrometers among PCs.

The HR4000 Spectrometer connects to a notebook or desktop PC via USB port or serial port. When connected to the USB port of a PC, the HR4000 draws power from the host PC, eliminating the need for an external power supply.



**Ocean Optics HR4000 High-Resolution Fiber Optic Spectrometer**

# System Requirements

You can use the HR4000'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 handheld PCs)
- Ocean Optics OOIBase32 software application installed and configured for use with the HR4000 (OOIPS2000 software if using a handheld PC). Consult the [Configuring the HR4000 in OOIBase32](#) section of *Chapter 2: Installing the HR4000* for specific configuration instructions.

Alternately, the HR4000 has serial port adaptability 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 HR4000, as well as a serial port adapter.

## EEPROM Utilization

An EEPROM memory chip in each HR4000 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.

## 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.

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### Note

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

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## Sampling System Overview

### How Sampling Works

Ocean Optics components function in a sampling system as follows:

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 HR4000. 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 HR4000 has both USB and serial port connectors (with the use of an adapter), enabling you to connect the spectrometer to a desktop or notebook PC via a USB port or to a desktop, notebook, or to a handheld 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 HR4000 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 HR4000 to serial port on desktop or notebook PC; comes with 5 VDC power supply (required when connecting to serial port)
Handheld 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 HR4000 to serial port on handheld PC; comes with 5 VDC power supply (required when connecting to serial port)

## HR4000 Breakout Box

Ocean Optics also offers the HR 4000 Breakout Box (HR4-BREAKOUT), a passive module that separates the signals from their 30-pin port to an array of standard connectors and headers, enabling easy access to a variety of features found in Ocean Optics' HR4000 Spectrometer. In addition to the accessory connector, the breakout box features a circuit board based on a neutral breadboard pattern that allows custom circuitry to be prototyped on the board itself.

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### Note

The HR4000 Breakout Box is compatible with HR4000 Spectrometers with Revision B or greater. HR4000 Spectrometers with serial numbers beginning with HR4A are *not* compatible with the HR4000 Breakout Box.

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## Shipment Components

The following information and documentation ships with the HR4000 Spectrometer:

❑ **Packing List**

The packing list is inside a plastic bag attached to the outside of the shipment box (the invoice arrives separately). It 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-Related Documentation](#) for instructions on accessing OOIBase32 documentation).

---

### Note

Please save the Wavelength Calibration Data Sheet for future reference.

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❑ **Software and Technical Resources CD**

Each order ships with the Ocean Optics *Software and 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 the 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.

# Installing the HR4000

## Overview

You must install the OOIBase32 software application prior to connecting the HR4000 Spectrometer to the PC. The OOIBase32 software installation installs the drivers required for HR4000 installation. If you do not install OOIBase32 first, the system will not properly recognize the HR4000.

If you have already connected the HR4000 to the PC prior to installing OOIBase32, consult *Chapter 3: [Troubleshooting](#)* for information on correcting a corrupt HR4000 installation.

## HR4000 Installation

This section contains instructions for connecting the HR4000 via both USB and serial modes.

### USB Mode

To connect the HR4000 to a PC via the USB port, the PC must be running the Windows 98/ME/2000/XP operating system.

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#### Note

The USB port on a PC can power up to five HR4000 spectrometer channels. Systems with more than five channels require a powered USB hub.

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#### ► Procedure

Follow the steps below to connect the HR4000 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 HR4000.
3. Insert the square end of the cable into the side of the HR4000.
4. Insert the rectangular end of the cable into the USB port of the PC.

If you installed OOIBase32 prior to connecting the HR4000, the **Add New Hardware Wizard** appears and installs the HR4000 drivers. If the drivers do not successfully install (or if you connected the HR4000 to the PC before installing OOIBase32), consult *Chapter 3: [Troubleshooting](#)*.

## Serial Port Mode

To use the serial port capacity of the HR4000 Spectrometer, the PC must be running a 32-bit version of the Windows operating system (or Windows CE 2.11 or higher for handheld PCs).

### ► Procedure

Follow the steps below to connect the HR4000 to the PC via serial port:

1. Connect the serial cable adapter block to the appropriate pins of the HR4000's 30-Pin Accessory Connector.
2. Connect one end of the 9-pin serial cable to the adapter block on the HR4000, 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 HR4000 (some PCs may not have numbered ports; handheld PCs typically have only one serial port).
4. Plug the 5 VDC external power supply into an outlet and connect it to the HR4000.

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### 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 (refer to [Product-Related Documentation](#) for instructions on accessing OOIBase32 documentation).

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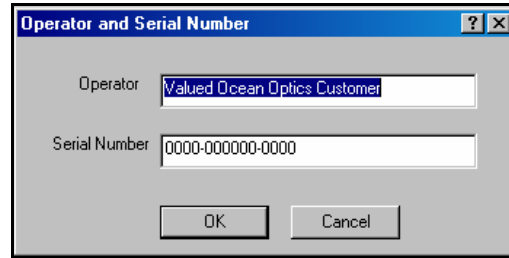
## Configuring the HR4000 in OOIBase32

Once you install the HR4000, you must configure OOIBase32's **Configure Spectrometer** options so that OOIBase32 recognizes the HR4000 Spectrometer. Consult the *OOIBase32 Spectrometer Operating Software Operating Instructions* for detailed instructions on configuring the spectrometer in OOIBase32 (see [Product-Related Documentation](#)).

The following sections contain instructions on initially configuring the HR4000 the first time you start OOIBase32.

## Operator and Serial Number Dialog Box

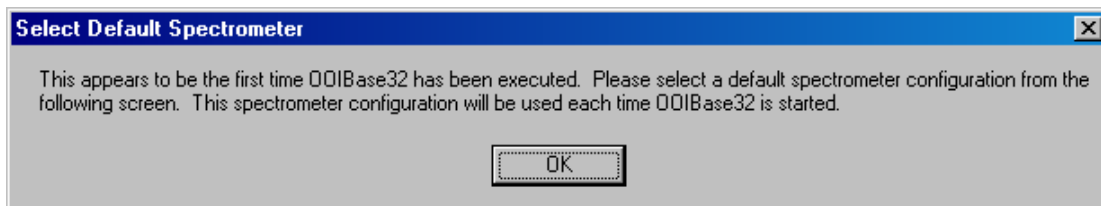
The **Operator and Serial Number** screen prompts you to enter a user name and software serial number into OOIBase32. Some data files created by OOIBase32 during sampling procedures use this information in the file headers.



## Default Spectrometer Configuration File

The **Default Spectrometer Configuration File** screen prompts you to select a spectrometer configuration (.SPEC) file for use with the HR4000. The unique serial number of the HR4000 precedes the file extension (for example, HR4A0162.SPEC).

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



## Configure Hardware Screen

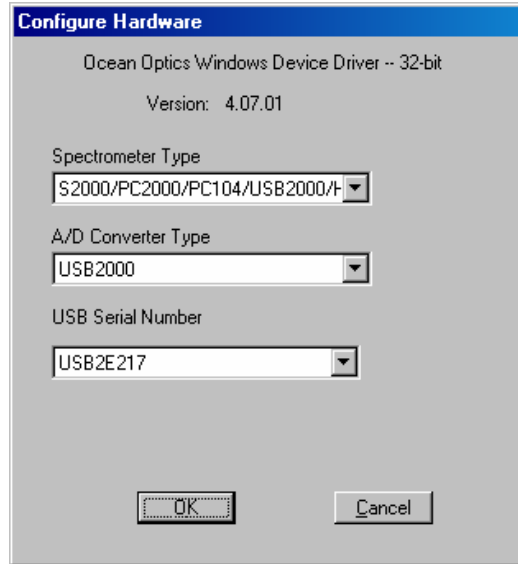
The **Configure Hardware** screen prompts you to enter spectrometer-specific information into OOIBase32 the first time you run the program. Typically, you need only enter this information the first time you run OOIBase32. However, you can alter the hardware configuration at any time using the **Spectrometer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

---

### Note

You do not need to configure the spectrometer hardware in the OOIPS2000 handheld PC operating software. Most handheld 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 HR4000 configuration with OOIPS2000.

---



## Configuring Hardware in USB Mode

### ► Procedure

1. Specify S4000 in the **Spectrometer Type** drop-down menu.
2. Specify HR4000 in the **A/D Converter Type** drop-down menu.
3. Specify the serial number of the HR4000 under the **USB Serial Number** drop-down menu.

---

### Note

The system pre-fills this drop-down menu with the serial numbers of all discovered HR4000 Spectrometers.

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4. Click the **OK** button to accept the selected options.
5. The spectrometer should now be able to acquire data and respond to light. Exit and restart OOIBase32 to save configuration data to disk.

## Configuring Hardware in Serial Port Mode

### ► Procedure

1. Select the **S4000** 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 for the HR4000 in the **SAD500 Serial Port** drop-down menu. See *Chapter 3: [Troubleshooting](#)* for information on identifying serial ports.
4. Select the HR4000 operating speed from the **SAD500 Baud Rate** drop-down menu (115,200 baud is recommended).



5. Specify the pixel resolution (from 1 to 500) in the **SAD500 Pixel Resolution** box. This value specifies that every  $n$ th pixel of the spectrometer will transmit from the HR4000 to the PC.

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### Note

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

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6. Enable the **Compress SAD500 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 HR4000 compresses every scan that it transmits. This greatly increases the data transfer speed of the HR4000.
7. Click the **OK** button to complete the setup.
8. The spectrometer should now be able to acquire data and respond to light. Exit and restart OOIBase32 to save configuration data to disk.

## Spectrometer Configuration Screen

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

### ► Procedure

1. Select **Spectrometer | Configure** from the menu and set system parameters.
2. Select the **Wavelength Calibration** tab. If you have connected your spectrometer to the PC's USB port, OOIBase32 pre-fills the coefficients for the HR4000 Spectrometer 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.
3. Verify that the calibration coefficients match the coefficients from the Wavelength Calibration Data Sheet that accompanied the spectrometer. If necessary, modify these values using the USB Programmer utility.
4. Additionally, ensure that you select both the **Master** and **Channel Enabled** boxes.
5. In the **A/D Interface** tab, enter the same values as in the **Configure Hardware** screen. OOIBase32 stores this information for future use once you close the program.

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### Note

Information on using the HR Series spectrometers with OOIPS2000 operating software for the handheld PC is located at <http://www.oceanoptics.com/technical/palmspec.pdf>.

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## Connect Spectroscopic Accessories

To find operating instructions for HR4000-compatible products (such as light sources, sampling chambers, and probes), consult the *Software and Technical Resources* CD or the Ocean Optics website at <http://www.oceanoptics.com/technical/operatinginstructions.asp>.

## External Triggering Options

You can trigger the HR4000 using a variety of External Triggering options through the 30-pin Accessory Connector on the spectrometer. See the External Triggering Options document located at <http://www.oceanoptics.com/technical/externaltriggering.pdf>. This document contains instructions for configuring External Triggering options for the HR4000.

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### Note

Only the external software triggering option is available when using a handheld PC.

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# Troubleshooting

## Overview

The following sections contain information on troubleshooting issues you may encounter when using the HR4000 Spectrometer.

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### Note

For issues encountered when using a handheld PC, consult the OOIPS2000 manual.

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## HR4000 Connected to PC Prior to OOIBase32 Installation

If you connected your Ocean Optics USB 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 below to remove the incorrectly installed device, device driver, and installation files.

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

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## Remove the Unknown Device from Windows Device Manager

### ► Procedure

1. Open Windows Device Manager. Consult the Windows operating instructions for your computer for directions, if needed.
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.

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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. Disconnect the HR4000 from your computer.
6. Locate the section in this chapter that is appropriate to your operating system and perform the steps in the following [Remove Improperly Installed Files](#) section.

## Remove Improperly Installed Files

### ► Procedure

1. Open Windows Explorer.
2. Navigate to the **Windows | INF** directory.

---

### Note

If the INF directory is not visible, you must disable the Hide System Files and Folders and Hide File Extensions for Known File Types options in Windows Folder Options. Access Windows Folder Options from Windows Explorer, under the **Tools | Folder Options** menu selection.

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3. Delete the **OOI\_USB.INF** in the INF directory. If your computer is running either the Windows 2000 or XP operating system, you must also delete the **OOI\_USB.PNF** 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 is now 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 procedure to troubleshoot the serial port connection.

### ► Procedure

1. Cycle the power on the HR4000 and restart the OOIBase32 software. This ensures that the software and the HR4000 synchronize properly.
2. Determine the serial port (COM port) number:

Operating System	Instructions
Windows 95/98/ME/XP	<ol style="list-style-type: none"> <li>1. Right-click on <b>My Computer</b></li> <li>2. Select <b>Properties</b></li> <li>3. Click the <b>Device Manager</b> tab</li> </ol>
Windows 2000	<ol style="list-style-type: none"> <li>1. Select <b>Start   Settings   Control Panel   System</b></li> <li>2. Select the <b>Hardware</b> tab</li> <li>3. Click the <b>Device Manager</b> button.</li> </ol>
Windows NT	Select <b>Start   Programs   Administrative Tools (common) NT Diagnostics</b>

3. Double-click on the **Ports (COM & LPT)** option to display COM port numbers. Ensure that no warning icon appears next to the HR4000's COM port.
4. Verify that the HR4000's COM port 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 to be used to interface to your HR4000 already has an older version of OOIBase32 software installed, you must 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 website at <http://www.oceanoptics.com/technical/softwaredownloads.asp>.

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

# Sample Experiments

## Overview

The following sections contain information on conducting sample experiments using the HR4000 and OOIBase32. For information on experiments with OOIPS2000, consult the *OOIPS2000 Operating Instructions* (see [Product-Related Documentation](#)).

## Preparing for Experiments

Follow the procedure below to configure the HR4000 and OOIBase32 for experiments.

### ► **Procedure**

1. Double-check that you have correctly installed the HR4000, installed OOIBase32, and configured the light source and other sampling optics.
2. Open the OOIBase32 application.
3. Select **Spectrometer | Configure** from the menu bar, and double-check that **A/D Interface** settings are correct.
4. Check your spectrometer setup configurations in OOIBase32:
  - a. Locate the Wavelength Calibration Data sheet that came with the HR4000.
  - b. Select **Spectrometer | Configure** from the menu and choose the **Wavelength Calibration** page.
  - c. 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.
5. 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.

# Taking Measurements

There are four basic optical measurements from which to choose:

- Absorbance (see [Absorbance Experiments](#))
- Transmission (see [Transmission Experiments](#))
- Reflection (see [Reflection Experiments](#))
- Relative irradiance (see [Relative Irradiance Experiments](#))

The type of measurement you 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 scan and a dark spectrum scan. After you take a reference scan and a dark spectrum scan, 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 new reference and dark spectrum scans.

---

## 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 ( $A_\lambda$ ) using the following equation:

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



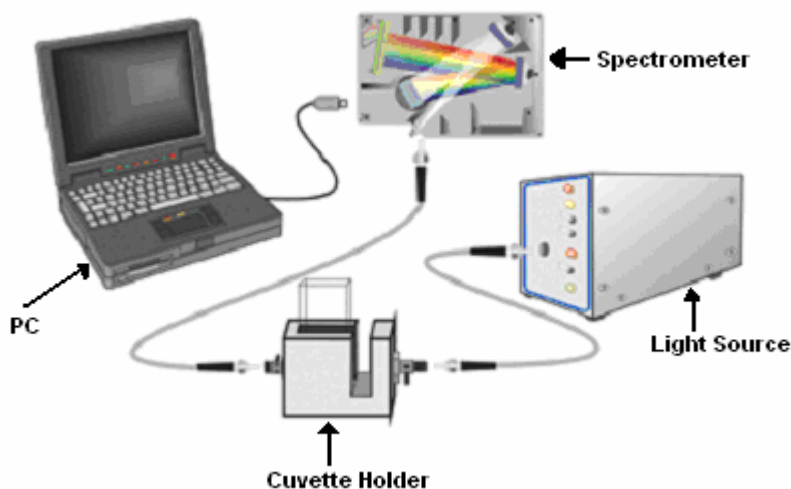
Where:

$S_\lambda$  = the sample intensity at wavelength  $\lambda$

$D_\lambda$  = the dark intensity at wavelength  $\lambda$

$R_\lambda$  = the reference intensity at wavelength  $\lambda$

The figure below shows a typical absorbance setup. The light source sends light via an input fiber into a cuvette in a cuvette holder. The light interacts with the sample. The output fiber carries light from the sample to the spectrometer, which is connected to the PC.



**Typical Absorbance Setup**

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.

► **Procedure**

To take an absorbance measurement using OOIBase32, follow the steps below:

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 14,000 counts. If necessary, adjust the integration time until the intensity is approximately 14,000 counts.
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.

---

4. 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. After the lamp warms up again, store a new reference scan.

---

You must take a dark spectrum scan before measuring absorbance.

6. 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<sub>λ</sub>) relative to a standard substance (such as air). OOIBase32 calculates %T<sub>λ</sub> (or %R<sub>λ</sub>) with the following equation:

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

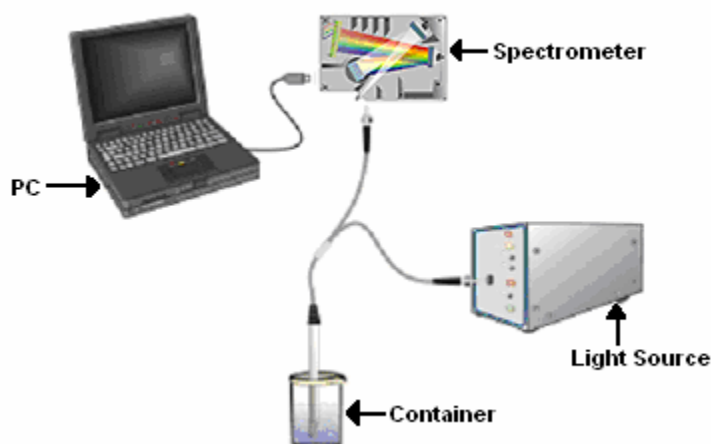
Where:

$S_\lambda$  = the sample intensity at wavelength  $\lambda$

$D_\lambda$  = the dark intensity at wavelength  $\lambda$

$R_\lambda$  = the reference intensity at wavelength  $\lambda$

The following figure shows a typical transmission setup. The light source sends light via the input leg of a transmission probe into a container. The light interacts with the sample. The output leg of the transmission probe carries the information to the spectrometer, which transmits the information to the PC.



**Typical Transmission Setup**

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

### ► **Procedure**

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 14,000 counts. If necessary, adjust the integration time until the intensity is approximately 14,000 counts.
3. 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.

---

#### 4: Sample Experiments

---

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 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 (% $R_{\lambda}$ ) relative to the reflection from a standard reference substance:

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

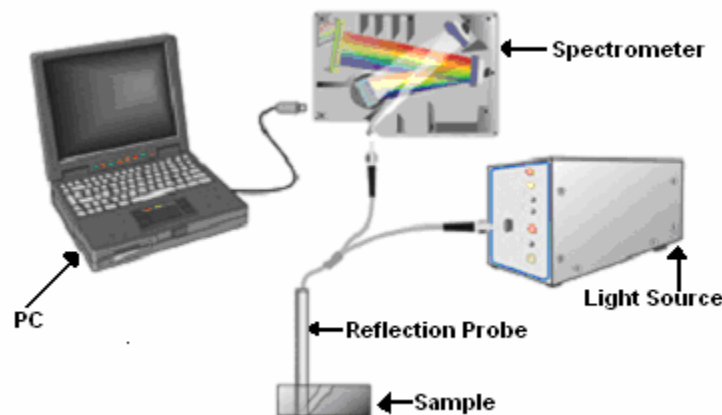
Where:

$S_\lambda$  = the sample intensity at wavelength  $\lambda$

$D_\lambda$  = the dark intensity at wavelength  $\lambda$

$R_\lambda$  = the reference intensity at wavelength  $\lambda$

The following figure shows a typical reflection setup. A light source sends light via the input leg of a reflection probe onto a sample. 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, which is connected to the PC.



**Typical Reflection Setup**

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.

► **Procedure**

Perform the following steps to take reflection measurements 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 14,000 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.

- Put the sample 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:

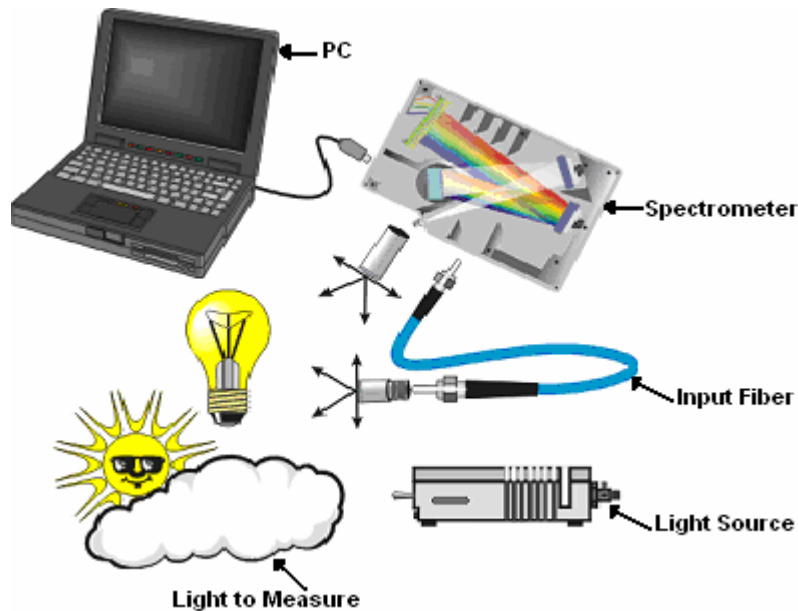
$B_{\lambda}$  = the relative energy of the reference (calculated from the color temperature) at wavelength  $\lambda$

$S_{\lambda}$  = the sample intensity at wavelength  $\lambda$

$D_{\lambda}$  = the dark intensity at wavelength  $\lambda$

$R_{\lambda}$  = the reference intensity at wavelength  $\lambda$

The following figure shows a typical relative irradiance setup. A light source with a known color temperature (such as the LS-1 or LS-1-LL) is used to take a reference spectrum. The light to measure 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.



### Typical Relative Irradiance Setup

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.

#### ► Procedure

Perform the following steps to take a relative irradiance 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 14,000 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.

#### 4: Sample Experiments

---

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 Online Help System**.

### ► Procedure

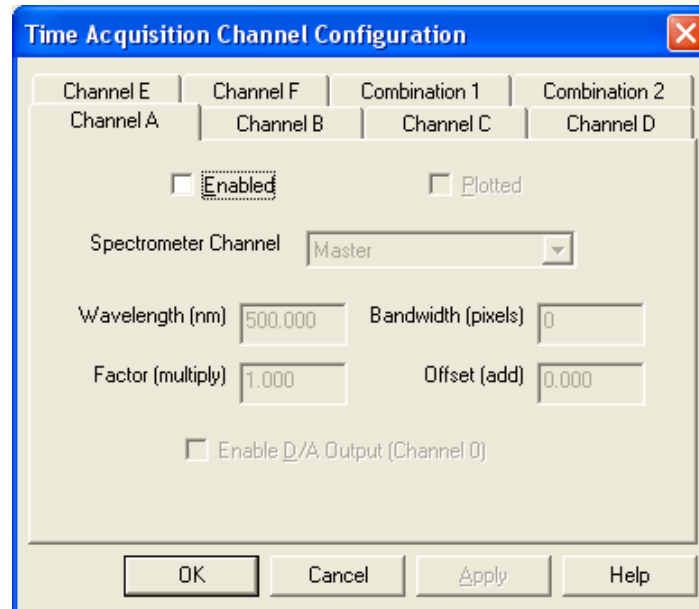
Follow the steps below to perform a time series experiment in OOIBase32:

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 Configuration Screen](#) section below.



# Configuring the Time Acquisition Channel Configuration Screen



## ► Procedure

Perform the following steps on the **Time Acquisition Channel Configuration** screen:

1. 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
2. Select **Plotted** to see a real-time graph of the acquired data in a spectral window.
3. Select a **Spectrometer Channel** for the time acquisition process
4. Specify the analysis wavelength in the **Wavelength (nm)** box.
5. Specify the number of pixels around the analysis wavelength to average in the **Bandwidth (pixels)** box.
6. 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: **Results = (Factor \* Data) + Offset**

7. 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.
8. 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.

## Configuring for a Combination of Two Time Channels

Configure a time acquisition process for a combination of two time channels (if desired) by selecting the **Combination 1** tab on the **Time Acquisition Channel Configuration** screen.

### ► Procedure

Perform the steps below to configure a combination:

1. Select **Enabled** to set the time acquisition calculation for the wavelength.
2. Enable **Plotted** to see a real-time graph of the acquired data in a spectral window.
3. Specify Time Channel A through F for the First Channel.
4. Select the mathematical operation to produce the data for Combination 1.
5. Specify Time Channel A through F for the Second Channel.
6. 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.

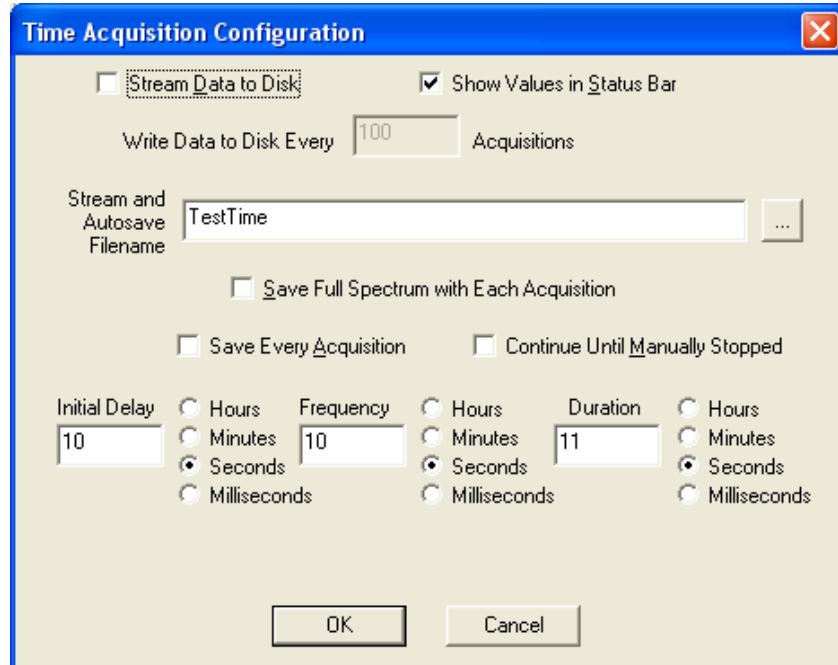
The equation for the Factor and Offset functions is: **Results = (Factor \* Data) + Offset**

7. 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.
8. Click the **Apply** button to apply the changes, and then click the **OK** button to close the Time Acquisition Channel Configuration screen.
9. Proceed to the [Configuring the Time Acquisition Configuration Screen](#) section below.

## Configuring the Time Acquisition Configuration Screen

### ► Procedure

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 saving data. 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 3684 acquisitions in a spectral window. If OOIBase32 collects more than 3684 acquisitions, it only displays the last 3684. To store more than 3684 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.
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 HR4000

## Overview

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 HR4000.

## 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:

$\lambda$  = the wavelength of pixel  $p$

$I$  = the wavelength of pixel 0

$C_1$  = the first coefficient (nm/pixel)

$C_2$  = the second coefficient (nm/pixel<sup>2</sup>)

$C_3$  = the third coefficient (nm/pixel<sup>3</sup>)

$R_\lambda$  = the reference intensity at wavelength  $\lambda$

You will be calculating the value for  $I$  and the three  $C$ s.

# Calibrating the Spectrometer

## Preparing for Calibration

To recalibrate the wavelength of your spectrometer, you need the following components:

- A light source capable of producing spectral lines

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### Note

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

- 
- An HR4000 spectrometer
  - An optical fiber (for spectrometers without a built-in slit, a 50- $\mu\text{m}$  fiber works best)
  - A spreadsheet program (Excel or Quattro Pro, for example) or a calculator that performs third-order linear regressions

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### Note

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

## Calibrating the Wavelength of the Spectrometer

### ► Procedure

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 in the following figure. 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 # <sup>2</sup>	Pixel # <sup>3</sup>	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

- 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**
- 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. Numbers of importance are noted.

**Regression Statistics**

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

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

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 HR4000 onto an EEPROM memory chip in the HR4000.

You can overwrite old calibration coefficients on the EEPROM if you are using the HR4000 via the USB port. If you are using the HR4000 via the serial port, consult the [Saving the New Calibration Coefficients: Serial Mode](#) section later in this appendix.

### ► Procedure

To save wavelength calibration coefficients using the USB mode, perform the following steps:

1. Ensure that the HR4000 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**. Select **USB EEPROM Programmer**.
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 desired HR4000 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 HR4000.



# Saving the New Calibration Coefficients: Serial Mode

If you are connecting the HR4000 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.

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## Note

You cannot save the calibration coefficients to the EEPROM memory chip on the HR4000 when using the serial mode.

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### ► Procedure

To save Wavelength Calibration Coefficients using the Serial mode, perform the following steps:

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.



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# Appendix B

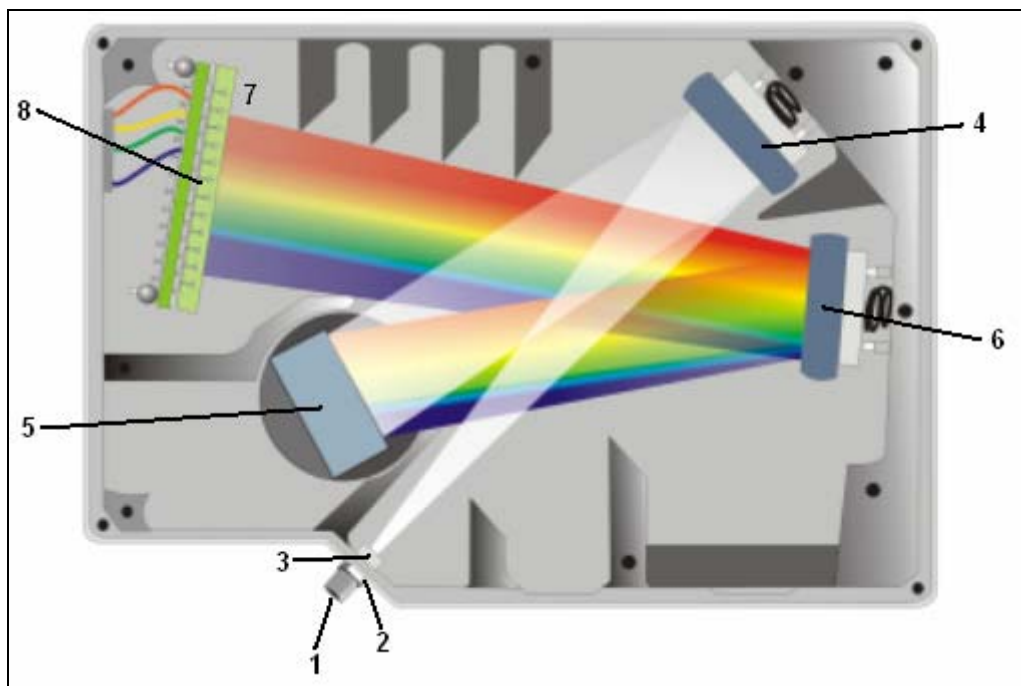
# Specifications

## Overview

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

## How the HR4000 Works

Below is a diagram of how light moves through the optical bench of an HR4000 Spectrometer. The optical bench has no moving parts that can wear or break; all the components are fixed in place at the time of manufacture. Items with an asterisk (\*) are user-specified.



**HR4000 Spectrometer with Components**

See [HR4000 Components Table](#) on the following page for an explanation of the function of each numbered component in the HR4000 Spectrometer in this diagram.

## HR4000 Components Table

Ocean Optics permanently secures all components in the HR4000 at the time of manufacture. Only Ocean Optics technicians can replace interchangeable components, where noted.

Item	Name	Description
1	SMA Connector	Secures the input fiber to the spectrometer. Light from the input fiber enters the optical bench through this connector.
2	Slit	<p>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.</p> <p>You can also use the HR4000 without a Slit. In this configuration, the diameter of the fiber connected to the HR4000 determines the size of the entrance aperture.</p> <p>Only Ocean Optics technicians can change the Slit.</p>
3	Filter	<p>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.</p> <p>Only Ocean Optics technicians can change the Filter.</p>
4	Collimating Mirror	<p>Focuses light entering the optical bench towards the Grating of the spectrometer.</p> <p>Light enters the spectrometer, passes through the SMA Connector, Slit, and Filter, and then reflects off the Collimating Mirror onto the Grating.</p>
5	Grating	<p>Diffraction 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.</p> <p>Only Ocean Optics technicians can change the Grating.</p>
6	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	<p>An optional component that attaches to the CCD Detector. It focuses light from a tall slit onto the shorter CCD Detector elements.</p> <p>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.</p> <p>Only Ocean Optics technicians can add or remove the L2 Detection Collection Lens.</p>
8	CCD Detector (UV or VIS)	<p>Collects the light received from the Focusing Mirror or L2 Detector Collection Lens and converts the optical signal to a digital signal.</p> <p>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.</p>

# HR4000 Specifications

The following sections provide specification information for the CCD detector in the HR4000, as well as the HR4000 Spectrometer itself. HR4000CG-UV-NIR specifications are listed in *Appendix C: [HR4000CG-UV-NIR Spectrometer](#)*.

## CCD Detector Specifications

Specification	Value
Detector	Toshiba TCD1304AP linear CCD array
No. of elements	3648 pixels
Sensitivity	100 photons per count at 800 nm
Pixel size	8 $\mu\text{m}$ x 200 $\mu\text{m}$
Pixel well depth	-100,000 electrons
Signal-to-noise ratio	300:1 (at full signal)
A/D resolution	14 bit
Dark noise	8 RMS counts
Corrected linearity	>99.8%
Maximum pixel rate	Rate at which pixels are digitized is 1 MHz

## HR4000 Spectrometer

Specification	Value
Dimensions	148.6 mm x 104.8 mm x 45.1 mm
Weight	570 g
Power consumption	450 mA @ 5 VDC
Detector	3648-element linear silicon CCD array
Detector range	200-1100 nm
Gratings	14 gratings available
Entrance aperture	5, 10, 25, 50, 100 or 200 $\mu\text{m}$ wide slits

## B: HR4000 Specifications

Specification	Value
Order-sorting filters	Installed longpass and bandpass filters
Focal length	f/4, 101 mm
Optical resolution	Depends on grating and size of entrance aperture
Stray light	<0.05% at 600 nm; <0.10% at 435 nm
Dynamic range	$2 \times 10^9$ (system); 2000:1 for a single acquisition
Fiber optic connector	SMA 905 to single-strand optical fiber (0.22 NA)
Data transfer rate	Full scans into memory every 4 milliseconds with USB 2.0 port, every 600 milliseconds with the serial port
Integration time	10 microseconds to 65 seconds
Interfaces	USB 2.0, 480 Mbps (USB 1.1 compatible); RS-232 (2-wire); SPI (3-wire); I <sup>2</sup> C Inter-Integrated Circuit 2-wire serial bus
Operating systems	Windows 98/Me/2000/XP, Mac OS X, and Linux when using the USB port Any 32-bit Windows operating system when using the serial port
Onboard GPIO	10 user-programmable digital I/Os
Analog channels	One 13-bit analog input and one 9-bit analog output

## System Compatibility

The following sections provide information on hardware and software requirements for the HR4000.

### Compatibility for Desktop or Notebook PCs

To use the HR4000, 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 HR4000 to a PC via USB port)

OR

Any 32-bit version of Windows (when connecting the HR4000 to a PC via serial port)

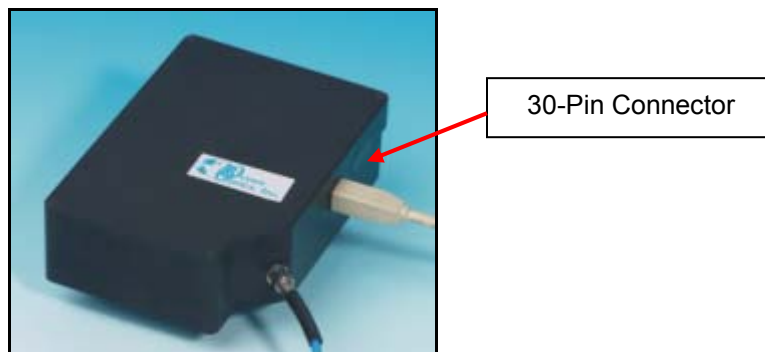
## Compatibility for Handheld PCs

To use the HR4000 with your handheld PC, the computer must meet the following minimum requirements:

- Handheld PC running Windows CE 2.11 or later
- 32 MB RAM
- OOIPS2000 Spectrometer Operating Software
- Serial port connectivity

## 30-Pin Accessory Connector Pinout

The HR4000 features a 30-pin Accessory Connector, located on the side of the unit as shown:



Location of HR4000 30-Pin Accessory Connector

## 30-Pin Accessory Connector Pinout Diagram

When facing the 30-pin Accessory Connector on the front of the vertical wall of the HR4000, pin numbering is as follows:

USB Port	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29

30-Pin Accessory Connector Pinout Diagram

## 30-Pin Accessory Connector – Pin Definitions and Descriptions

The following table contains information regarding the function of each pin in the HR4000's 30-Pin Accessory Connector:

## B: HR4000 Specifications

Pin #	Function	Input/Output	Description
1	RS232 Rx	Input	RS232 receive signal – Communicates with a PC over DB9 Pin 3
2	RS232 Tx	Output	RS232 transmit signal – Communicates with a PC over DB9 Pin 2
3	GPIO (2)	Input/Output	General purpose software-programmable, digital input/output (channel number)
4	V5_SW	Output	Regulated 5 Volt power pin – Supplies 50 mA (maximum)
5	Ground	Input/Output	Ground
6	I <sup>2</sup> C SCL	Input/Output	I <sup>2</sup> C clock signal for communication to other I <sup>2</sup> C peripherals
7	GPIO (0)	Input/Output	General purpose software-programmable, digital input/output (channel number)
8	I <sup>2</sup> C SDA	Input/Output	I <sup>2</sup> C data signal for communication to other I <sup>2</sup> C peripherals
9	GPIO (1)	Input/Output	General purpose software-programmable, digital input/output (channel number)
10	Ext. Trigger In	Input	TTL input trigger signal – See External Triggering Options document for info.
11	GPIO (3)	Input/Output	General purpose software-programmable, digital input/output (channel number)
12	V <sub>CC</sub> , V <sub>USB</sub> , or 5V <sub>IN</sub>	Input or Output	Input power pin for HR4000 – When operating via USB, this pin can power other peripherals – Ensure that peripherals comply with USB specifications
13	SPI Data Out	Output	SPI Master Out Slave In (MOSI) signal for communication to other SPI peripherals
14	V <sub>CC</sub> , V <sub>USB</sub> , or 5V <sub>IN</sub>	Input or Output	Input power pin for HR4000 – When operating via USB, this pin can power other peripherals – Ensure that peripherals comply with USB specifications
15	SPI Data In	Input	SPI Master In Slave Out (MISO) signal for communication to other SPI peripherals
16	GPIO (4)	Input /Output	General purpose software-programmable, digital input/output (channel number)
17	Single Strobe	Output	TTL output pulse used as a strobe signal – Has a programmable delay relative to the beginning of the spectrometer integration period
18	GPIO (5)	Input/Output	General purpose software-programmable, digital input/output (channel number)



Pin #	Function	Input/Output	Description
19	SPI Clock	Output	SPI clock signal for communication to other SPI peripherals
20	Continuous Strobe	Output	TTL output signal used to pulse a strobe – Divided down from the master clock signal
21	SPI Chip Select	Output	SPI Chip/Device Select signal for communication to other SPI peripherals
22	GPIO (6)	Input/Output	General purpose software-programmable, digital input/output (channel number)
23	Analog In (0-5V)	Input	13-bit analog-to-digital input with a 0-5V range
24	Analog Out (0-5V)	Output	9-bit programmable output voltage with a 0-5V range
25	Lamp Enable	Output	TTL signal driven Active HIGH when the Lamp Enable command is sent to the spectrometer
26	GPIO (7)	Input/Output	General purpose software-programmable, digital input/output (channel number)
27	Ground	Input/Output	Ground
28	GPIO (8)	Input/Output	General purpose software-programmable, digital input/output (channel number)
29	Ground	Input/Output	Ground
30	GPIO (9)	Input/Output	General purpose software-programmable, digital input/output (channel number)

## 30-Pin J2 Accessory Connector - Part Numbers

The part numbers for the 30-pin accessory connector on the HR4000 Spectrometer are as follows:

- The connector is Pak50™ model from 3M Corp. Headed Connector – Part Number **P50-030P1-RR1-TG**.
- The mating connector is Part Number **P50-030S-TGF**.
- Mating the two components requires two 1.27 mm (50 mil) flat ribbon cables (3M 3365 Series is recommended).

If you are customizing your HR4000 Spectrometer system or configuring External Triggering, you may need these part numbers to complete your setup.

# HR4000 15-Pin Accessory Cable Pinout

Pin #	Description	Pin #	Description
1	Single_strobe	9	GPIO-9
2	ContStrobe	10	GND_SIGNAL
3	V5_SW	11	SDA
4	ExtTrigIn	12	SCL
5	ExtTrigIn	13	LampEnable
6	GPIO-8	14	A_IN
7	A_OUT	15	GPIO-7
8	ExtTrigIn		

# HR4000CG-UV-NIR Spectrometer

The HR4000CG-UV-NIR Composite Grating Spectrometer has a new proprietary grating and order-sorting filter to provide a 200-1100 nm wavelength range with 0.5 nm optical resolution in one spectrometer.

The HR4000-CG-UV-NIR is functionally similar to the standard HR4000 Spectrometer. Follow the instructions in *Chapter 2: [Installing the HR4000](#)* to configure the HR4000CG-UV-NIR.

## HR4000CG-UV-NIR Features

The HR4000CG-UV-NIR contains the following features that differ from the standard HR4000:

### New HC-1 Landis Composite Grating

The HR4000CG-UV-NIR uses the new HC-1 Landis grating designed to provide a 200-1100 nm wavelength range. The HC-1 is fixed in place at the time of manufacture.

### Variable Order-Sorting Filter

The HR4000CG-UV-NIR contains a new OFLV-200-1000 variable order sorting filter to eliminate second and third order effects.

## HR4000CG-UV-NIR Spectrometer Specifications

Specification	Value
Dimensions:	148.6 mm x 104.8 mm x 45.1 mm
Weight	570 g
Power consumption	450 mA @ 5 VDC
Detector	3648-element linear silicon CCD array
Wavelength range	200-1100 nm
Optical resolution	0.75 nm FWHM

**C: HR4000-UV-NIR Spectrometer**

Specification	Value
Gratings	HC-1, 300 lines per nm grating
Entrance aperture	5 $\mu$ m-wide slit
Order-sorting filters	OFLV-200-1100 installed
Focal length	f/4, 101 mm
Dynamic range	2 X 10 <sup>9</sup> (system); 2000:1 for a single acquisition
Stray light	<0.05% at 600 nm; <0.10% at 435 nm
Fiber optic connector	SMA 905 to single-strand optical fiber (0.22 NA)
Data transfer rate	Full scans into memory every 4 milliseconds with the USB 2.0, every 18 ms with the USB 1.1, every 600 milliseconds with the serial port
Integration time	Continuous – 4 milliseconds to 20 seconds Shutter – 10 microseconds to 4 milliseconds
Operating systems	Windows 98/Me/2000/XP, MAC OS X, and Linux when using the USB port Any 32-bit Windows operating system when using the serial port
Onboard GPIO	10 user-programmable digital I/Os
Analog channels	One 13-bit analog input and one 9-bit analog output

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