The Curie
UV-VIS Emission Spectrofluorometer

Installation and Operation Manual
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About This Manual

Document Purpose and Intended Audience

This document provides you with an installation section to get your system up and running. In addition to Curie installation and operation instructions, this manual also includes information for locating the OOIBase32 installation instructions (see *Product-Related Documentation*).

Document Summary

Chapter	Description
Chapter 1: *Introduction*	Provides a list of system components, and operating requirements. Also contains instructions for connecting the Curie system to a PC.
Chapter 2: *Configuration*	Contains instructions for configuring the Curie system with the OOIBase32 application software.
Chapter 3: *Using the Curie System*	Contains instructions for performing experiments using the Curie system.
Chapter 4: *Troubleshooting*	Provides a list of possible problems that you may encounter when using your Curie system and suggested solutions.
Appendix A: *Specifications*	Provides product specifications for the Curie system.
Appendix B: *Calibrating the Wavelength of the Curie Spectrometer*	Provides instructions for calibrating the wavelength of the Curie system.
Appendix C: *Relative Irradiance Mode*	Contains information about Relative Irradiance Mode.
Appendix D: *Filter Sets*	Contains specifications for the filters supplied with the Curie.

Product-Related Documentation

- *OOIBase32 Spectrometer Operating Software, Operating Instructions*
- *Correcting Device Driver Issues*
- *External Triggering Options*

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.

You can also access operating instructions for 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 Ocean Optics Curie UV-VIS Emission Spectrofluorometer is a self-contained fluorescence system (the Curie system) that represents a new level of innovation and simplicity in spectroscopy. This standalone system contains all the components required to make fluorescence measurements in a single package with a remarkably compact footprint.

The Curie is a high-sensitivity cuvette spectrofluorometer for measuring fluorophores in liquids. The Curie is a versatile lab system distinguished by internal filtering technology that helps to discriminate between powerful excitation source wavelengths and the weak spectral emissions from samples, so that additional correction for excitation and emission is unnecessary and data is more reliable.

Features of the Curie System

Your Curie system offers the following features:

- A high-sensitivity 2048-element CCD-array detector.
- Full spectral analysis (i.e., 2048 wavelengths over the 200–850 nm spectral range). The Curie system is preloaded with a microcode that allows you to select a delay between activation of the excitation source and the start of the emission fluorometer's integration time (available in future versions of the Curie). This gated-mode operation is ideal for measuring fluorophores that have long fluorescence lifetimes, such as lanthanides.

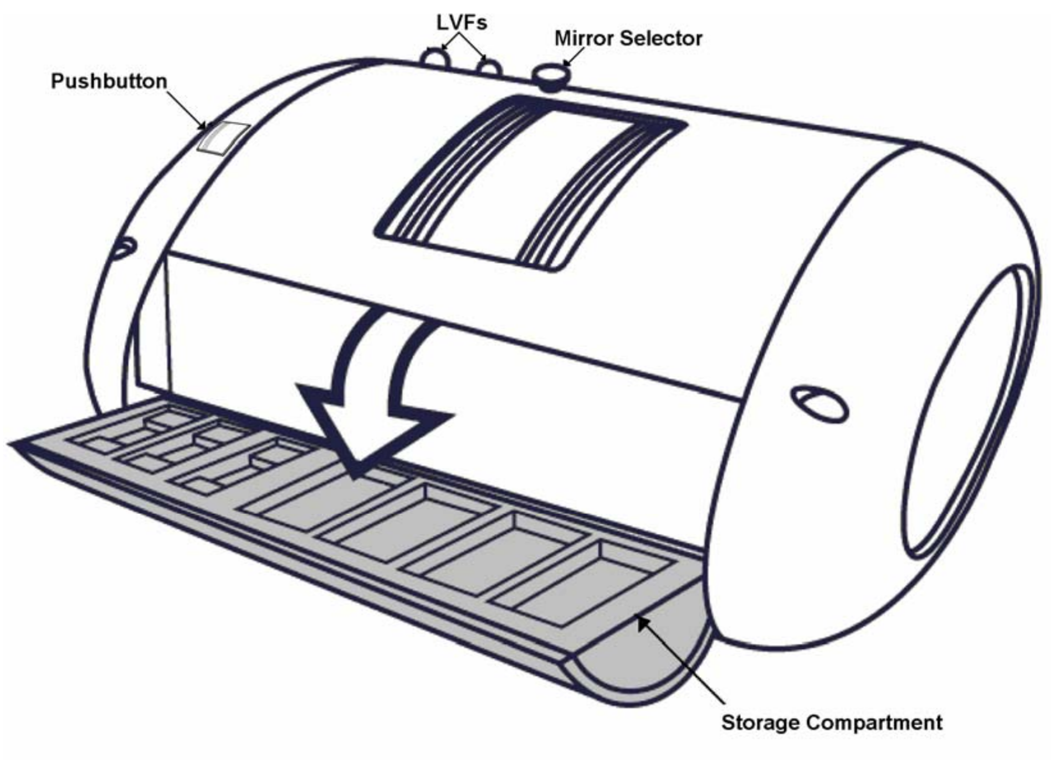
- Novel filtering technology. The Curie system is the only emission fluorometer with built-in linear variable filters (LVFs). These LVFs are ideal for spectrally shaping the excitation energy from the onboard pulsed-xenon excitation source, and eliminate the need for scanning monochromators. Each filter's transmission or blocking band can be moved throughout 230–500 nm or 300–750 nm wavelengths.
- A pushbutton to enable the software trigger for automatic spectral saving.

Curie System Contents

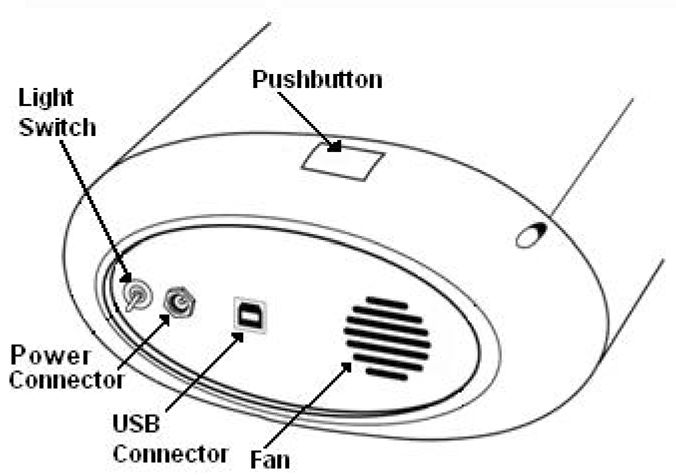
The Curie system contains a USB2000 spectrometer that features a microcode optimized for gated fluorescence measurements and a pulsed xenon light source. It also comes equipped with a set of Linear Variable Filters (LVFs) mounted internally on sliding rails. The LVFs allow you to specify the excitation wavelength range for the measurement and eliminate the need for scanning monochromators found in other systems.

The Curie also features two user-selectable mirrors; one for use with the UV LVF filters optimized for UV light (a cold mirror) and one standard mirror for use with the VIS LVF filters. A knob on the top of the unit allows you to select the mirror to use for a specific measurement. A pushbutton allows you to easily save a spectrum.

A storage compartment is provided on the side of the Curie for storing filters and cuvettes for your experiments. A switch allows you to operate the light source and the fan.



Curie System Front View



Curie System Left Panel

Other Items Included with Shipment

Your Curie Fluorescence System from Ocean Optics should also contain the following items:

- Packing List: The packing list is located inside a plastic bag attached to the outside of the shipment box (the invoice is mailed separately). The items listed on the packing slip include all of the Curie components that have been shipped to you, as well as important information such as the shipping and billing addresses, and any components that may be on back order.
- OOIBase32 software (on the *Software and Technical Resources CD*)
- Curie software (on the Custom Software CD)
- USB cable (USB-CBL-1)
- 12 VDC power supply (WT-12V)
- One pack of disposable cuvettes (CVD-UV1S-SAM)
- One CVD-DIFFUSE
- One filter set containing commonly used filters. See Appendix D, *Filter Sets* for more information.
- One quartz cuvette (CV-FL-Q-10)
- Wavelength Calibration Data Sheet: This data sheet contains information unique to the individual spectrometer contained in your Curie system. The operating software reads this calibration data from your spectrometer when it interfaces to a PC through the USB port. Should you need to reenter it at any time, select **Spectrometer | Configure | Wavelength Calibration** tab in the OOIBase32 software. See the OOIBase32 documentation for more information (refer to *Product-Related Documentation* for instructions on accessing OOIBase32 documentation).

Note

Please keep the Wavelength Calibration Data sheet for future reference.

- *Software and Technical Resources CD*: Each Curie system order comes with Ocean Optics' *Software and Technical Resources CD*. This CD contains all Ocean Optics software and manuals for software operation, spectrometers, and spectroscopic accessories.

  Documentation is provided in Portable Document Format (PDF). You need the Adobe Acrobat Reader (version 6.0 or higher) to view these files. The Adobe Acrobat Reader is included on the CD and will install automatically (if needed) when you attempt to view a document.

  With the exception of OOIBase32 Spectrometer operating software, all Ocean Optics software is password-protected. Passwords for purchased software are located on the back of the *Software and Technical Resources CD* package.

Operating Requirements

You must have the following components to use the Curie Fluorescence System:

- Ocean Optics Curie Self-contained Fluorescence System and included accessories (including the 12 VDC power supply)
- Windows-based PC with USB connectivity
- USB device cable (included)
- OOIBase32 operating software (included)

Recommended Additions

The following products, available from Ocean Optics, are recommended additions to your system:

- Tungsten light source enabling relative irradiance measurements (LS-1-LL)
- PS-HG1-ADP cuvette adapter for LS-1-LL for performing in-house relative irradiance measurements
- Fiber Optic Cable (QP50-2-VIS/NIR) for performing in-house relative irradiance measurements and wavelength calibration
- HG-1 Mercury Argon Calibration Source for Curie wavelength calibration
- Annual Service Package (ASP)

Installing OOIBase32 Software

Caution

Do NOT connect the Curie System to your PC until after you have installed the OOIBase32 software. Follow the instructions below or in the OOIBase32 manual (see *Product-Related Documentation*) to properly connect and configure your system.

► *Procedure*

To install OOIBase32 software,

1. Close all other applications running on the PC.

2. Start the OOIBase32 installation process.

Installing from CD:

   a. Insert the *Software and Technical Resources* CD containing the OOIBase32 software. The CD interface automatically launches.

   b. Click on **Install Ocean Optics Software**.

   c. Click on **OOIBase32 Operating Software**. The installation process begins.

Installing from the Web:

   a. Go to http://www.oceanoptics.com/technical/softwaredownloads.asp.

   b. Right-click on **OOIBase32™ Spectrometer Operating Software** and select **Save Target As…** to download the executable to your machine.

   c. Double-click on the downloaded file. The installation process begins.

3. Click the **Next** button at the **Welcome** screen. The **Read Me File** screen appears.
4. Read the Read Me file and click the **Next** button. The **Choose Destination Location** screen appears.
5. Click the **Browse** button to customize your installation location, or click the **Next** button to proceed. The **Backup Replaced Files** screen appears.
6. Click the **Yes** button to back up replaced files (OOIBase32 prompts you for a backup location), or click the **No** button to proceed. The **Select Program Manager Group** screen appears.
7. Select a program manager group, and then click the **Next** button. The **Start Installation** screen appears.
8. Click the **Next** button to begin installation. The OOIBase32 Platinum password screen appears.
9. Enter your OOIBase32 Platinum password here, if necessary. Otherwise, click the **OK** button to start the install of the free version of OOIBase32.
10. Click the **Finish** button when the installation completes.
11. Click the **OK** button to restart your computer.

You have now installed the OOIBase32 software.

Installing Custom Curie Software to Activate Relative Irradiance Mode

Caution

Do NOT connect the Curie System to your PC until after you have installed the OOIBase32 software. Follow the instructions below or in the OOIBase32 manual (see *Product-Related Documentation*) to properly connect and configure your system.

Prior to shipping, your Curie system was calibrated at the factory to allow for operation in Relative Irradiance mode. Relative Irradiance mode compensates for the grating efficiency and detector sensitivity of a specific spectrophotometer (see the OOIBase32 manual for more information). It is recommended that you run your experiments in this mode to achieve true peak shapes and ratios. Follow the protocol below to install the calibration performed on your Curie instrument. Once you have followed this protocol, your Curie will be operating in Relative Irradiance mode when you open the OOIBase32 software.

To escape Relative Irradiance mode, click **S** ( ) on the OOIBase toolbar. Your system returns to raw signal mode. You can then switch back to Relative Irradiance mode by clicking on the **I** ( ) on the OOIBASE toolbar.

► *Procedure*

1. Install the OOIBase32 software (see *Installing OOIBase32 Software* for installation instructions).
2. After OOIBase32 has installed completely and your computer has restarted, insert the Custom Software CD.
3. Copy all of the files on the Custom Software CD into your C:\Program Files\Ocean Optics\OOIBase32 directory. Click **OK** when prompted to overwrite the files currently in that directory.
4. Double click on the **ConfigurationTree.reg** file. Click **OK** when prompted to allow the file to write to the registry.

   The OOIBase32 software is now ready to run. You can access OOIBase32 via your **Start** toolbar or from the C:\Program Files\Ocean Optics\OOIBase32 directory.

Note

When the software from the Custom Software CD is run, two **File Open Mismatch** screens appear (one for the Reference file and one for the Electrical Dark file) informing you that the acquisition parameters in your Curie file do not match the current parameters. Select **Yes** to use the parameters from the Curie file. You can change these parameters later, if desired (see *Configuring Data Acquisition Parameters*).

Connecting the Curie to a PC

The Curie connects to a PC via a standard USB device cable. USB 1.1 is currently supported.

Caution

Before connecting the Curie to your PC, be sure to install the OOIBase32 Spectrometer Operating Software that comes on the *Software and Technical Resources CD*. This software contains the necessary USB drivers for the Curie system. You should also install the Curie Custom Software to allow for measurements in Relative Irradiance mode.

► *Procedure*

To connect the Curie system to your PC,

1. Install the OOIBase32 Spectrometer Operating Software from the *Software and Technical Resources CD* (see *Installing OOIBase32 Software*). To activate Relative Irradiance mode, you should also install the Curie Custom Software from the Custom Software CD (see *Installing Custom Curie Software to Activate Relative Irradiance Mode*).
2. Once the software is installed, insert the rectangular end of the USB device cable into any USB port on your PC and the square end into the USB connector on the side of the Curie. The system automatically discovers and installs the appropriate USB drivers for the Curie.

Note

The Curie only supports USB connectivity. You cannot use the RS-232 serial port standard to connect the Curie to your PC.

Connecting the Power Cord

A 12 VDC power supply (WT-12V) is supplied with your Curie system and is needed to power its pulsed xenon light source. Plug one end of the power cord into the round power receptacle on the side of the Curie and the other end into an appropriate power source. Once you have plugged in the power supply, turn the light source on by placing the power switch on the end of the Curie in the up (on) position.

Chapter 2

Configuration

Introduction

This chapter provides instructions for configuring the OOIBase32 Configure Spectrometer options so that the application recognizes your connected Curie system.

Configuring the Curie in OOIBase32

Once the Curie system is installed (see *Installing OOIBase32 Software*), you must configure OOIBase32's Configure Spectrometer options so that the application recognizes the connected Curie system and its components.

Note

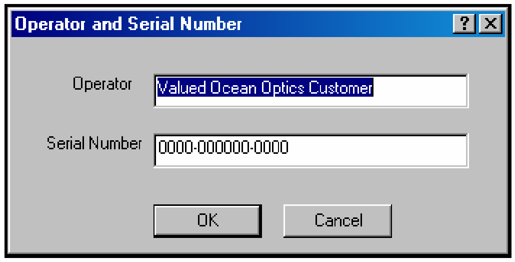
See the *OOIBase32 Spectrometer Operating Software, Operating Instructions* for detailed instructions on configuring your spectrometer in OOIBase32. See *Product-Related Documentation* for information on locating this document.

Initial Start-up

The following sections provide information for initially configuring the Curie system in OOIBase32. With your Curie attached to the computer via the USB port and plugged into the wall power outlet, start the OOIBase32 software application.

Operator and Serial Number Dialog Box

The **Operator and Serial Number** dialog box prompts you to enter a user name and software serial number into OOIBase32. Some of the data files use this information in the data file headers. Since OOIBase32 is free software, it requires no serial number for installation. You can leave the field as is.



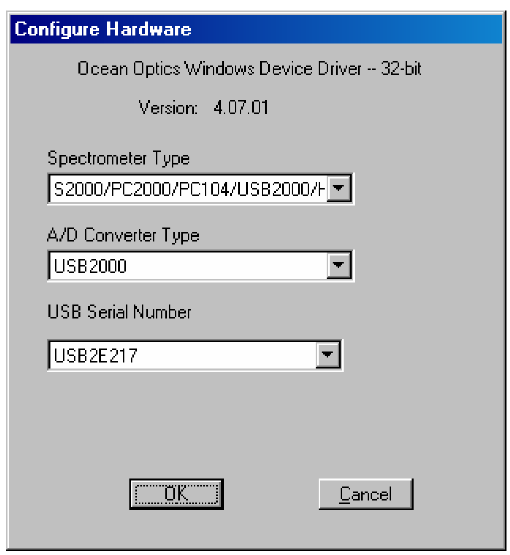
Default Spectrometer Configuration File

The **Default Spectrometer Configuration File** screen prompts you to select a default .SPEC file to use with the Curie system. The .SPEC file extension is preceded by the unique serial number of the spectrometer in your Curie system (for example, USBA001.SPEC).

Navigate to the OOIBase32 installation directory and select the default .SPEC file, then proceed. Do not specify a .SPEC file located on removable media (such as a floppy disk). The **Configure Hardware** screen appears.

Configure Hardware Screen

The **Configure Hardware** screen prompts you to enter spectrometer-specific information into OOIBase32. Typically, you only need to enter this information once upon first running the OOIBase32 application. However, you can access this screen at any time by selecting **Configure | Hardware** from the OOIBase32 menu bar.



► *Procedure*

To configure the Curie system, do the following:

1. In the **Spectrometer Type** field, select **S2000/PC2000/PC104/USB2000/H2000** from the drop-down list.
2. In the **A/D Converter Type** field, select **USB2000** from the drop-down list.

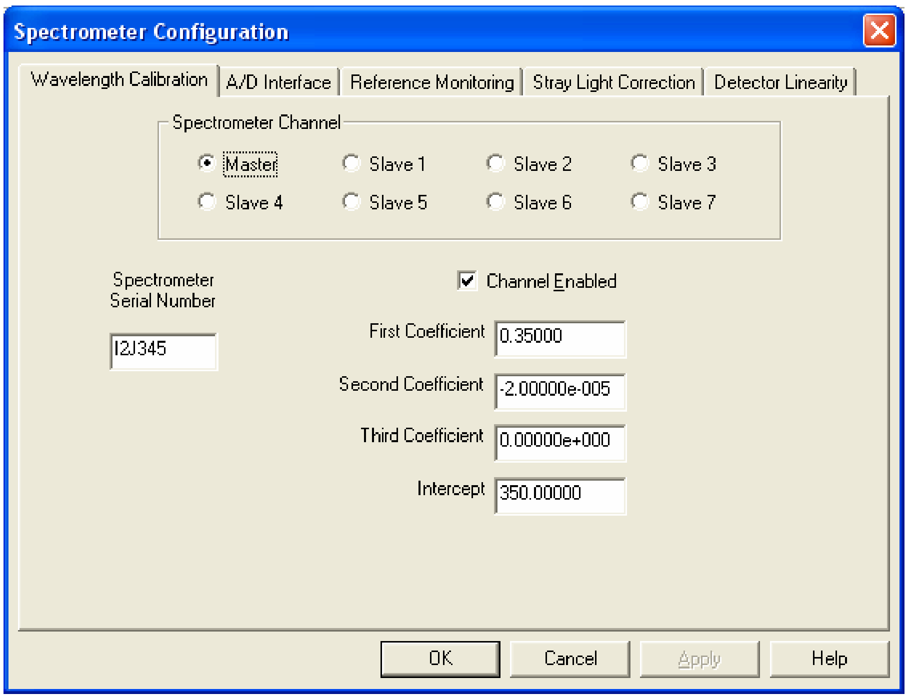
3. In the **USB Serial Number** field, select the serial number of the USB2000 spectrometer in your Curie system from the drop-down list.
4. Click **OK**.

Spectrometer Configuration Dialog Box

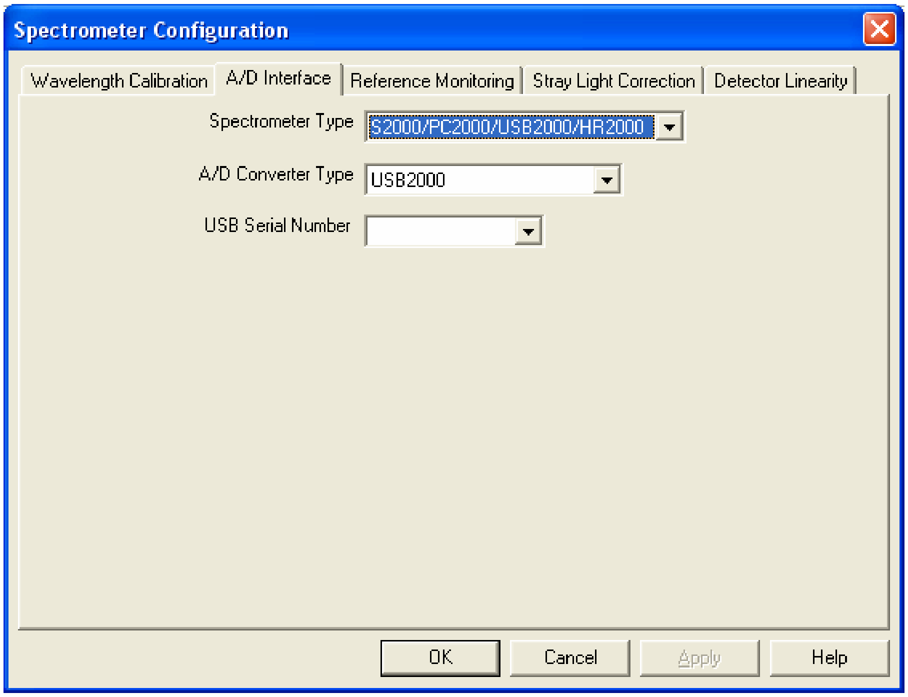
► *Procedure*

To set the system parameters, do the following:

1. Select **Spectrometer | Configure** from the menu. The **Spectrometer Configuration** screen appears.



2. On the **Wavelength Calibration** tab, ensure that the calibration coefficients (read from a memory chip in the Curie spectrometer) match the coefficients listed on the Wavelength Calibration data sheet that came with your spectrometer.
3. Ensure that both the **Master** and **Channel Enabled** are selected.
4. Select the **A/D Interface** tab.



5. On the **A/D Interface** tab, enter the same values that you entered in the **Configure Hardware** screen (see *Configure Hardware Screen*). When you exit the OOIBase32 application, this data is stored in the .SPEC file.

6. Click **OK** to save the data and close the Spectrometer Configuration screen.

   Upon exiting OOIBase32, the software stores this configuration information in a spectrometer configuration file named [your serial number].SPEC. Upon restart, OOIBase32 loads this as the default .SPEC file. You can change the name of this file by selecting **Spectrometer | Save Configuration As** from the menu and changing the name of the saved .SPEC file.

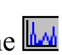
Enabling the Pushbutton

The Curie features a pushbutton software trigger. To use the pushbutton feature to save spectral data, you must enable Software Trigger mode in the OOIBase32 software. Note that when the pushbutton feature is enabled, the spectrum on the screen will only update after the pushbutoon is pushed. The display is frozen until the pushbutton is used.

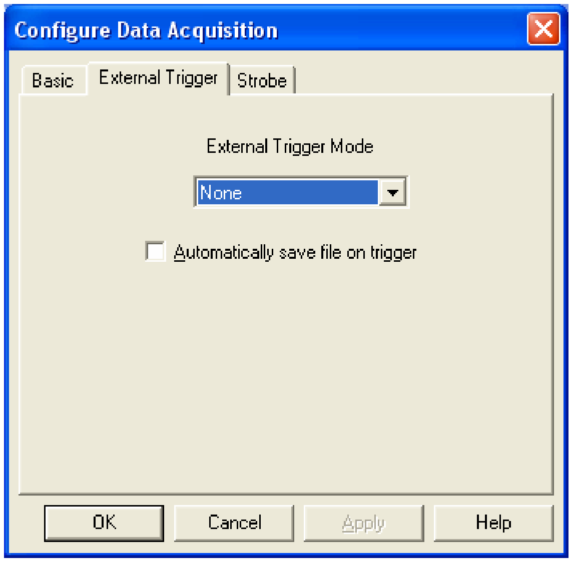
See *Autoincrementing Filenames* to save all spectra in the spectral window automatically and name the file with the base name and numerical index that you specify.

► *Procedure*

To enable the pushbutton feature in the OOIBase32 software, do the following:

1. Select **Spectrum | Configure Data Acquisition** from the menu or click the  button. The **Configure Data Acquisition** screen appears.

2. Select the **External Trigger** tab.



3. In the **External Trigger Mode** field, select **Software Trigger** to enable the pushbutton.
4. To save files when the pushbutton is pushed, enable the **Automatically save file on trigger** option.
5. Click **OK**.

Autoincrementing Filenames

When the pushbutton feature is enabled, the Autoincrement Filenames option allows you to choose a name and save spectra automatically when you push the button on the Curie system or when you click the Save command in OOIBase32. Select **File | Autoincrement Filenames | Enabled** to enable this feature.

When you enable this feature, choosing any save command automatically saves all spectra in the spectral window and names the file with a base name and numerical index you specify.

The following table illustrates a sample file name structure:

Test	The base name that you specified.
00012	A sequential numerical index beginning from a user-specified number.
Master	The spectrometer channel name, which OOIBase32 automatically adds to the filename.
Irradiance	The file extension, which OOIBase32 automatically adds to the filename. In this instance, it indicates that OOIBase32 saved the data while in relative irradiance mode.

In this example, the specified values result in an autoincremented filename of **Test.00012.Master.Irradiance**.

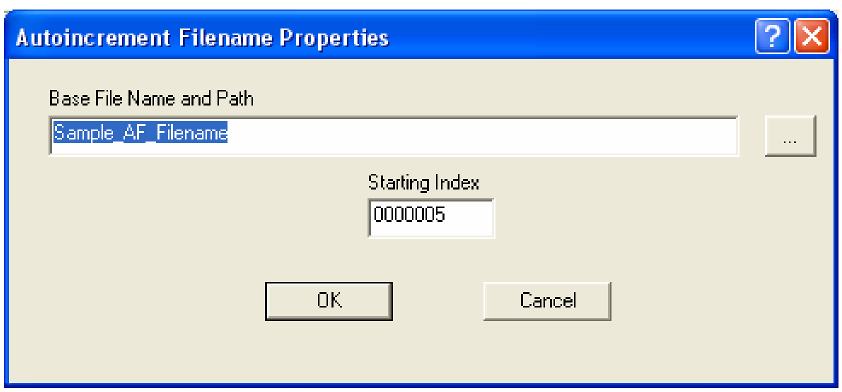
Note

If you do not enable the Autoincrement Filenames function, a save file dialog box will open every time you push the button (if enabled) or choose a save command.

► *Procedure*

To configure the Autoincrement Filenames function, do the following:

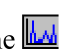
1. Select **File | Autoincrement Filenames | Enabled** from the OOIBase32 menu to enable the feature.
2. Select **File | Autoincrement Filenames | Show Name** to enable the Show Name option. When you enable both this option and the Autoincrement Filenames option, the filename of the next saved file displays in the title bar of OOIBase32.
3. Select **File | Autoincrement Filenames | Configure** to configure the following parameters:
   a. Base name for autoincremented files.
   b. Starting index for autoincremented files. For example, if you enter “1” here, the number in the saved filename will appear as 00001. The next saved file will have 00002 in the filename, etc.

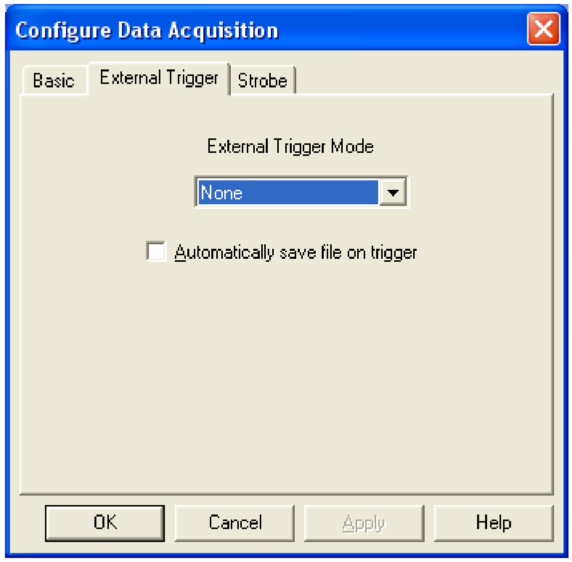


Disabling the Pushbutton

► *Procedure*

To disable the pushbutton feature,

1. Select **Spectrum | Configure Data Acquisition** from the menu or click the  button. The **Configure Data Acquisition** screen appears.
2. Select the **External Trigger** tab.



3. In the **External Trigger Mode** field, select **None** to disable the pushbutton, and click **OK**.
4. Push the pushbutton on the Curie. The pushbutton is now inactive.

Note

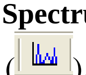
If you are unable to access the menu after clicking on it, push the pushbutton on the Curie and try accessing the menu again.

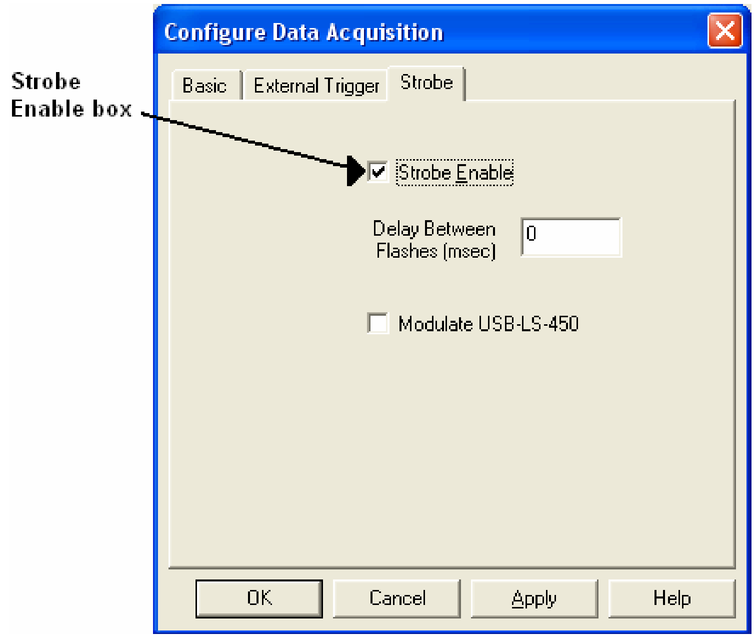
Turning on the Light Source

There are two ways that you can turn on the lamp using OOIBase 32 software:

- Method 1: Check the **Strobe Enable** box on the Strobe tab in the **Configure Data Acquisition** screen.

► *Procedure*

1. Select **Spectrum | Configure Data Acquisition** from the menu or click the Data Acquisition button (). The **Configure Data Acquisition** screen appears.
2. Select the Strobe tab.
3. Check the **Strobe Enable** box.



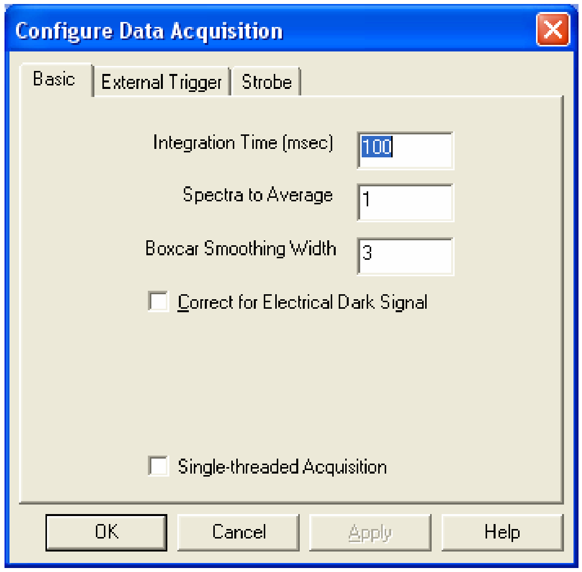
- Method 2: Check the **Strobe/Lamp Enable** box on the Acquisition Parameters toolbar.

Once you enable the strobe via any of the options listed above, the lamp activates and a trace displays on the graph when the CVD-DIFFUSE is placed in the cuvette holder. See *Using the CVD-DIFFUSE* for more detailed information on using the CVD-DIFFUSE with your Curie system. Adding a sample in the cuvette holder should change the graph accordingly. If these results occur, your hardware and software have been installed correctly.

Configuring Data Acquisition Parameters

► *Procedure*

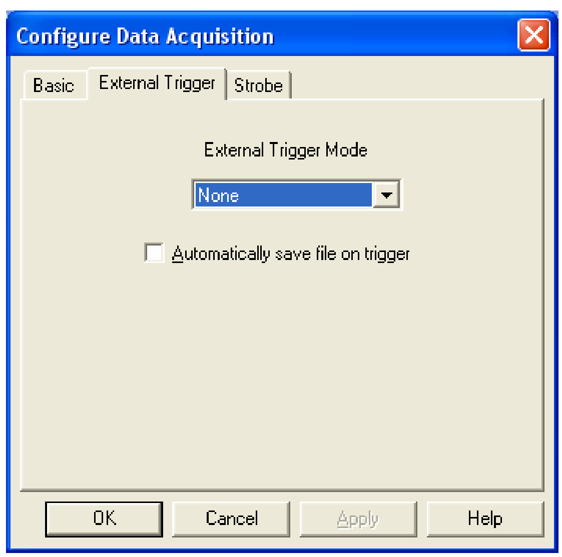
1. Using OOIBase32, select **Spectrum | Configure Data Acquisition** from the menu or click the  button. The **Configure Data Acquisition** screen appears with the **Basic** tab selected.
2. On the **Basic** tab, configure the integration time, averaging, and boxcar smoothing values. See the *OOIBase32 Spectrometer Operating Software, Operating Instructions* for more information. See *Performing a Fluorescence Experiment* for more detailed information on choosing the optimal data acquisition parameters for your measurement.



3. On the **External Trigger** tab, configure your external triggering mode to activate your pushbutton and data save option, if necessary. Then, click **OK**.

Note

See *Enabling the Pushbutton* to set the External Trigger when using the Curie pushbutton feature.



4. Flip the light switch on the side of the Curie to the ON position.

5. On the **OOIBase32** main screen, check the **Strobe/Lamp Enable** option. The pulsed xenon lamp activates, and a fluctuating trace appears on the graph when the CVD-DIFFUSE is placed in the cuvette holder. See *Using the CVD-DIFFUSE* for more detailed information on using the CVD-DIFFUSE with your Curie system. If you put a sample into the cuvette holder, the graph trace should change accordingly. When this occurs, you will know that the software and hardware are correctly installed.

Chapter 3

Using the Curie System

Introduction

This chapter provides you with instructions for performing experiments using the Curie system with the OOIBase32 application.

Performing Experiments with the Curie System

The Curie system allows you to detect picomolar-range concentrations of fluorophores in solution with emission from 200–850 nm.

Preparing for Experiments

The following procedures walk you through the steps necessary to carry out a fluorescence measurement with your Curie system and OOIBase32 software.

Before performing an experiment, ensure the following:

- Both the Curie system and the OOIBase32 application with Curie custom software have been correctly installed.
- The Wavelength Calibration Curie system configurations in OOIBase32 are correct. The calibration coefficients (read from a memory chip in the USB2000 spectrometer) must match the coefficients listed on the Wavelength Calibration data sheet that came with your spectrometer (see *Spectrometer Configuration Dialog Box*, Step *2*).
- Both the Master and Channel Enabled options are enabled (see *Spectrometer Configuration Dialog Box*, Step *3*). The First Coefficient, Second Coefficient, Third Coefficient, and Intercept should correspond to those of your Curie system.
- The minimum integration time is set to 20 milliseconds or greater. If you set the integration time below 20 milliseconds, the lamp is driven too fast and the spectrum is more unstable.
- The A/D Interface settings are correct (see *Spectrometer Configuration Dialog Box*, Steps *4* and *5*).
- Your Curie is plugged in and turned on (the switch on the end of the Curie is in the up or on position).

Once you have installed and configured your hardware and software, and have set up your system, you are ready to take a fluorescence measurement.

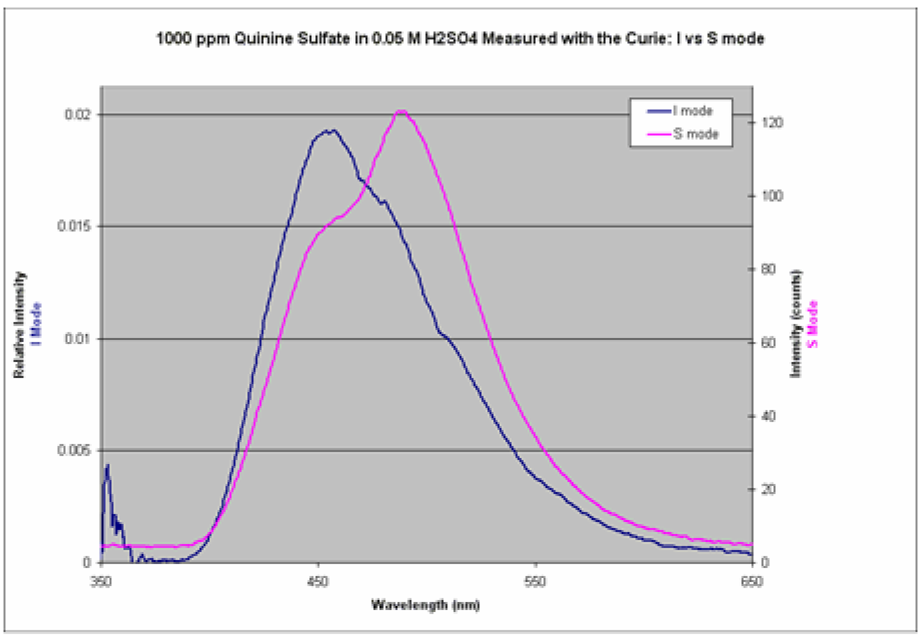
Performing a Fluorescence Experiment

You can take a fluorescence reading in either Relative Irradiance mode (I) or Scope mode (S).

Relative Irradiance mode – Relative Irradiance mode is the preferred mode for fluorescence experiments. Relative irradiance spectra are a measure of the intensity of a light source relative to a reference emission source. There are two methods of performing a fluorescence experiment in Relative Irradiance mode: use the custom Curie software provided with your Curie system to start OOIBASE32 in Relative Irradiance mode using the calibration files generated during factory calibration, or perform your own radiometric calibration using a blackbody of known color temperature. See Appendix C, *Relative Irradiance Mode*, for more information.

- Scope mode – Scope mode is the preferred mode for choosing the excitation wavelengths with the linear variable filters and configuring your acquisition parameters. The signal graphed in Scope mode is the raw voltage coming out of the A/D converter. This spectral view mode provides complete control of signal processing functions before taking absorbance, transmission, reflection, and relative irradiance measurements. This mode reflects the intensity of the light source, the reflectivity of the grating and mirrors in the spectrometer, the response of the detector, and the spectral characteristics of the sample. Use Scope mode when configuring your setup, adjusting the integration time, and choosing your excitation wavelengths with linear variable filters.

An example of Curie spectra for quinine sulfate measured in Scope mode (S) and Relative Irradiance mode (I) is shown below.



As shown in the figure, the shape of the fluorescence spectrum is impacted by the measurement mode chosen. The preferred mode for fluorescence measurements is Relative Irradiance mode because the impact of variables such as the intensity of the light source, the reflectivity of the grating and mirrors in the spectrometer, the response of the detector, and the spectral characteristics of the sample do not affect the shape of the spectrum. When the Curie custom software is installed and used, the software is automatically placed in Relative Irradiance mode using the factory calibration performed for your Curie (see *Installing Custom Curie Software to Activate Relative Irradiance Mode* for instructions on installing the Curie software from the Custom Software CD).

The first step in measuring fluorescence from your sample is to choose the excitation wavelength for your measurement. See *Selecting the Excitation Wavelength* for the options available for selecting the excitation wavelength range.

Selecting the Excitation Wavelength

The pulsed xenon light source included in your Curie system is a broadband light source capable of providing excitation energy throughout the UV/VIS (200 to 850 nm) region. To avoid masking your fluorescence signal with the broadband energy from the light source, the wavelength range for excitation can be chosen using the novel, LVF technology or discrete bandpass filters.

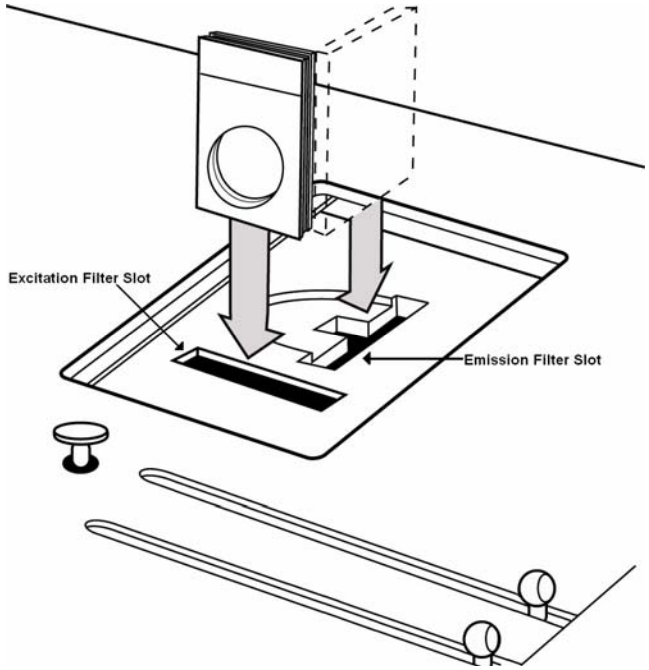
The Curie offers you three filtering options to select the excitation wavelength:

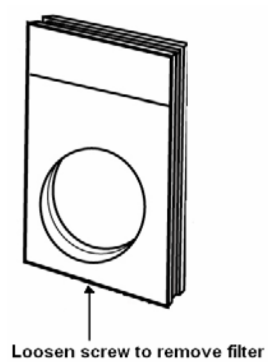
- Linear variable bandpass filters – See *Selecting the Excitation Wavelength Range with LVF Filters*.
- Discrete bandpass filters – See *Selecting the Excitation Wavelength Range with Discrete Bandpass Filters*.
- No filters – For fluorescence measurements made without filters, there is a possibility that the broadband light source will be scattered into the detector and overlap the fluorescence emission. You can check this by using a cuvette containing solvent only (no fluorophore) to see if your excitation energy is scattered into the detector. The presence of peaks in the spectrum for your solvent alone would suggest that your solvent has a background fluorescence or that excitation energy is being scattered into your detector.

Selecting the Excitation Wavelength Range with Discrete Bandpass Filters

The Curie system provides two slots to hold the filters, one for an excitation filter and one for an emission filter. Note that the linear variable filters must be in the **No LVF** position (thumbscrews positioned closest to the end cap) to access the excitation filter slot.

If you have a 1-inch diameter bandpass filter that you would prefer to use for your measurements, you can place it in the empty filter holder provided or replace the existing filter in one of the other filter holders. To replace an existing filter, loosen the setscrew at the bottom of the filter holder to remove the filter, then insert your own filter and tighten the screw. Your filter must be 1 inch in diameter to fit in the supplied filter holder.





Selecting the Excitation Wavelength Range with LVF Filters

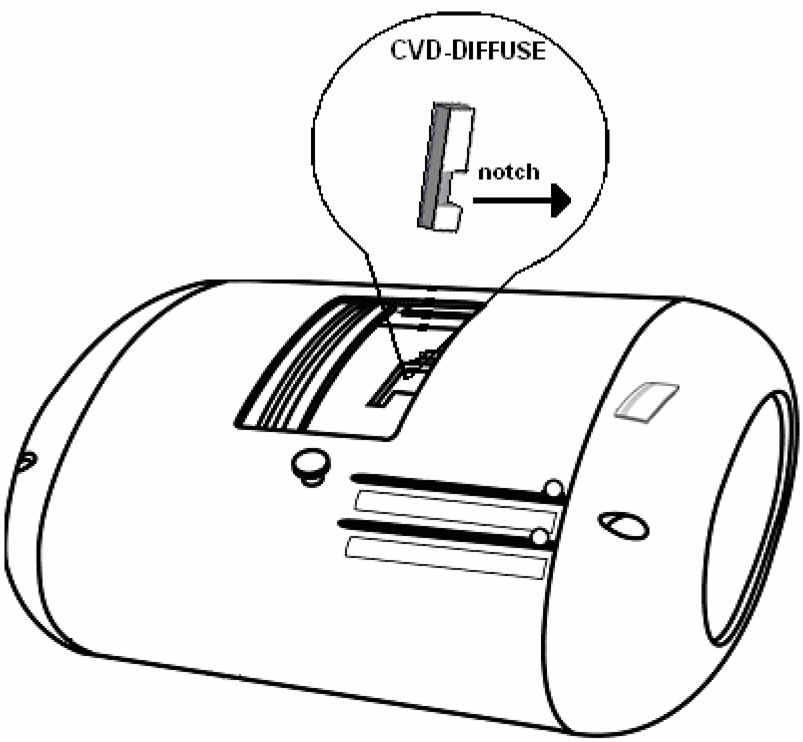
To set the excitation wavelength range with the LVF filters, place the software in Scope mode. If you are running Curie custom software, click on the toolbar to escape Relative Irradiance mode. Your system returns to raw signal mode. You can then switch back to Relative Irradiance mode for your measurement after the excitation wavelength range is chosen by clicking on the on the toolbar.

The sections below describe the steps necessary to select your excitation wavelength range with the intergrated LVF filters. You must use the CVD-DIFFUSE located in the storage compartment door of your Curie to deflect the filtered light energy into the detector (see *Using the CVD-DIFFUSE*). Based on the wavelength range necessary for your emission, you must also determine which set of LVF filters to use (only one set of LVF filters is used at a time – see LVF filter description below) and which mirror you need to use (see *Selecting the Mirror for Use with the LVFs*).

- The UV LVF filter can be used to select excitation energy in the 230 to 500 nm range. The bandwidth is fixed and varies from 30 to 40 nm. For UV excitation light, adjust the UV LVF filter to select your wavelength range. The UV LVF filter is used with the UV (Cold) mirror (Mirror Adjustment knob turned so that the UV label is closest to the cuvette holder – see *Selecting the Mirror for Use with the LVFs*).
- The VIS LVF filter can be used to select excitation energy in the 300 to 750 nm range. The bandwidth is fixed and varies from 30 to 40 nm. For VIS excitation light, adjust the VIS filter to select your wavelength range. The VIS LVF filter is used with the standard mirror (Mirror Adjustment knob turned so that the VIS label is closest to the cuvette holder – see *Selecting the Mirror for Use with the LVFs*).

Using the CVD-DIFFUSE

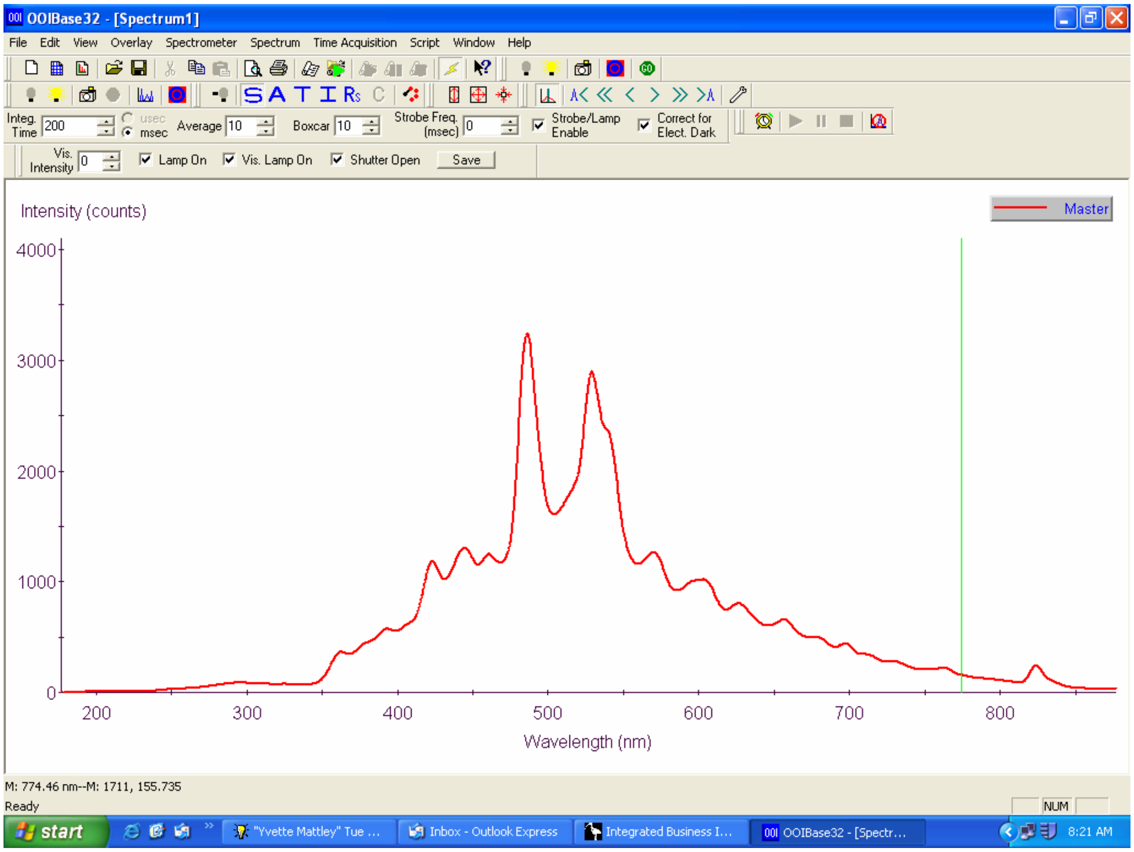
A white, cuvette shaped CVD-DIFFUSE is provided with your Curie system (stored in the side storage door of the Curie) to help you select the excitation wavelength for your measurement. With the detector located at 90 degrees relative to the light source, the CVD-DIFFUSE provides a way to deflect the light energy into the detector. The CVD-DIFFUSE is a 1-cm piece of PTFE material used to deflect the light from the light source into the spectrometer. Insert the CVD into the Curie's cuvette holder as shown in the figure below.



Selecting the Mirror for Use with the LVFs

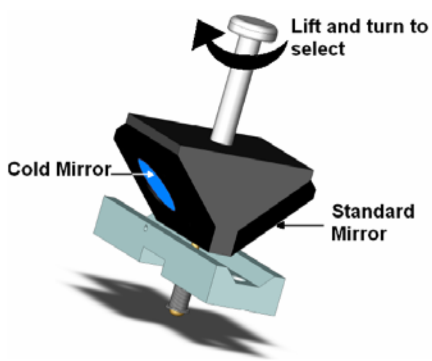
There are two mirrors available in the Curie system:

- Standard mirror – (Mirror Adjustment Knob turned with VIS label closest to the cuvette holder). Reflects visible and UV light from the light source into the cuvette holder. Use this mirror setting with the VIS LVF filter. In the figure below, the spectrum of the xenon lamp reflected off of the standard mirror is shown.



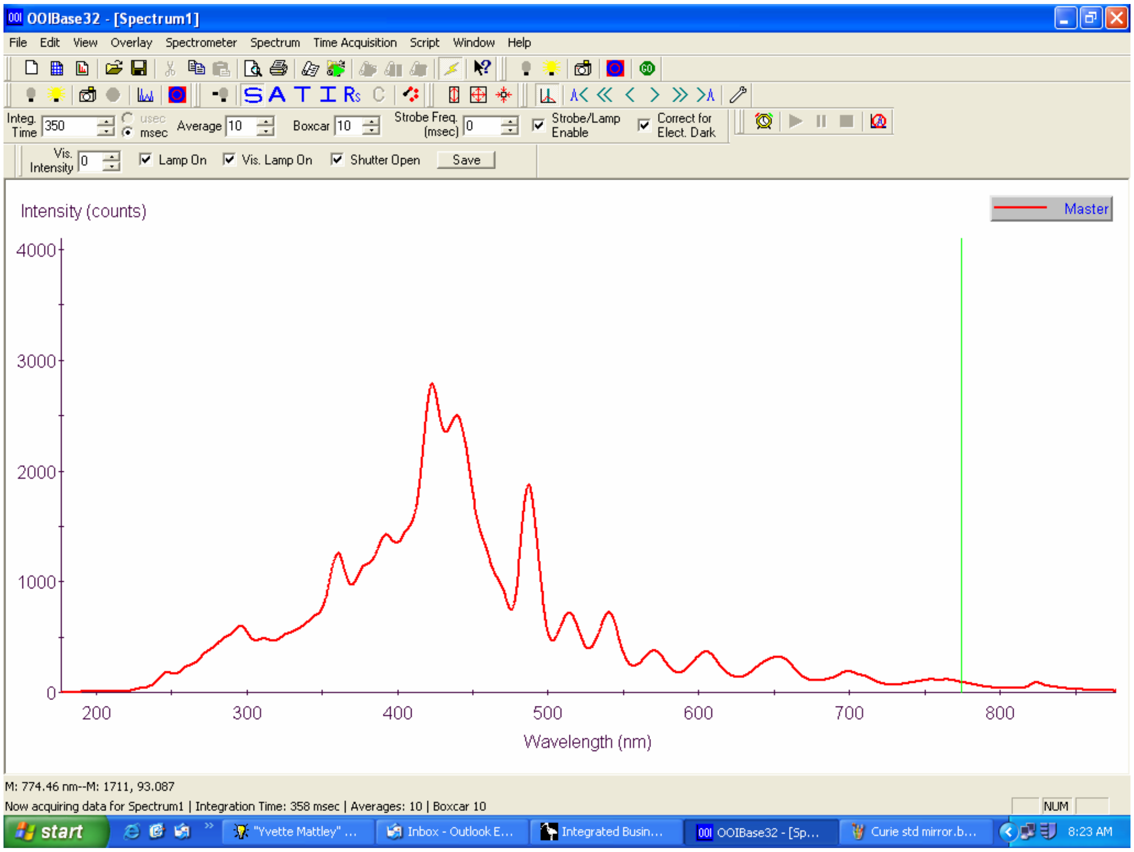
Xenon Lamp Reflected Off of Standard Mirror

- Cold mirror – (Mirror Adjustment Knob turned with UV label closest to the cuvette holder). Reflects only the UV light from your light source into the cuvette holder. The Cold mirror option is included in your Curie system because the UV LVF filter does not block light above 500 nm. If your emission occurs above 500 nm, use the cold mirror with the UV LVF filter to select your excitation wavelength range. The cold mirror setting is used with the UV LVF filter to keep excitation energy above 500 nm from overlapping with your emission.



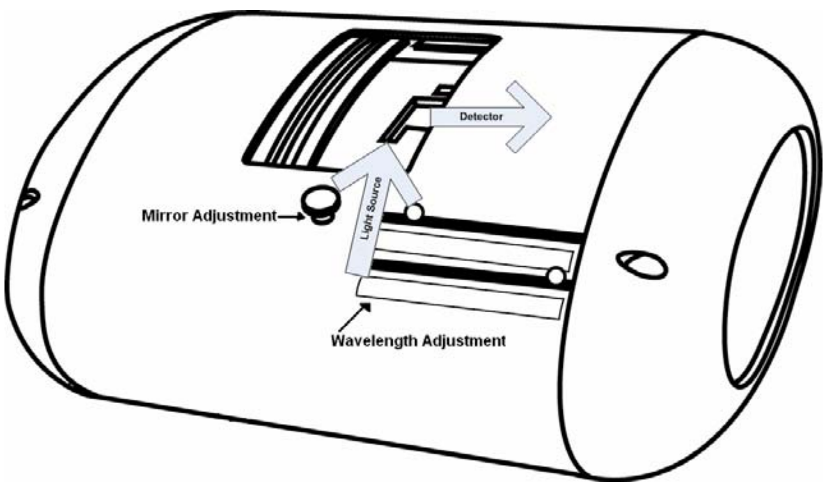
Mirror Mechanism (located inside Curie)

In the figure below, the spectrum of the xenon lamp reflected off of the cold mirror is shown. Note that less visible light is reflected from the cold mirror.



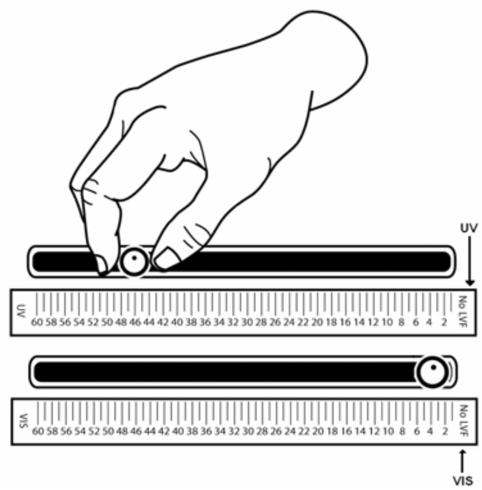
Xenon Lamp Reflected Off of Cold Mirror

To select the mirror for your measurement, lift and turn the mirror adjustment knob on the Curie clockwise to select the desired mirror. When the UV LVF is used, choose the cold mirror by turning the mirror knob until the UV label on the mirror knob is closest to the cuvette holder.

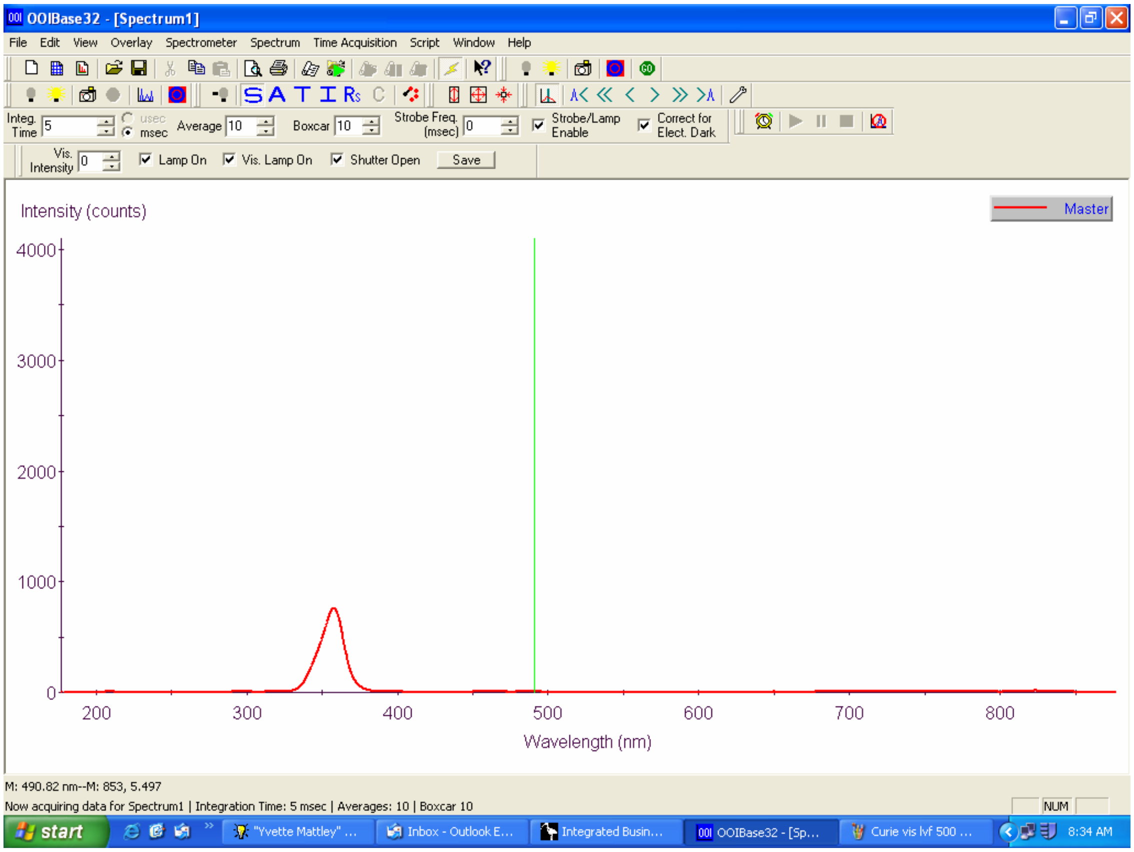


Adjusting the LVFs to Select the Excitation Wavelength Range

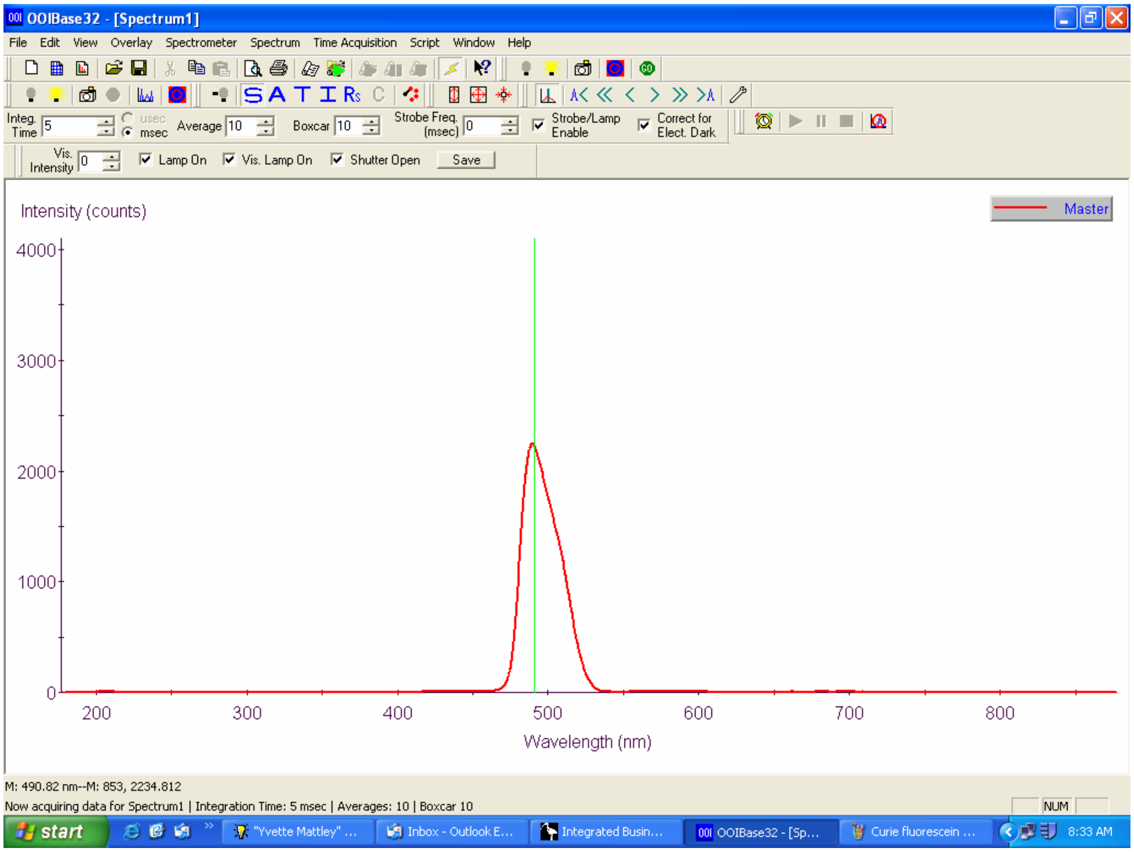
With the software in Scope mode, the CVD-DIFFUSE in the cuvette holder and the correct mirror chosen, loosen the thumbscrews and slide the handles (one for UV and one for VIS) on the Curie to select the excitation wavelength range. You can only adjust and use one LVF time; the other LVF must be in the **No LVF** position.



In the figures below, the spectra for light passing through the UV LVF filter set at 350 nm and the VIS LVF filter set at 500 nm are shown.



Curie UV LVF 350 nm



Curie VIS LVF 500 nm

Once you have chosen your excitation wavelength range, make a note of the location on the LVF scale and lock the filter in place with the thumbscrew. Before making your fluorescence measurement, switch back to Relative Irradiance mode by clicking on the  on the OOIBASE toolbar.

Measuring Fluorescence with your Curie System

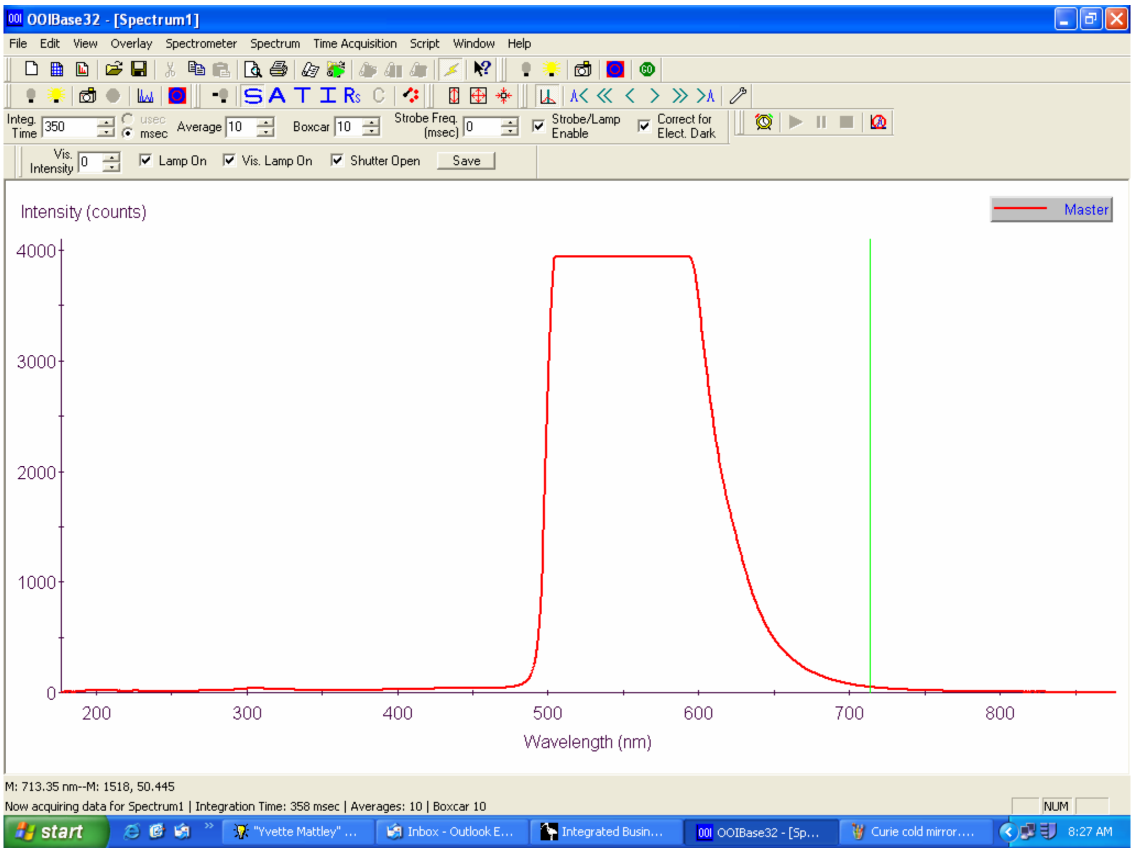
After you have chosen your excitation wavelength range, you are ready to make your measurements in Relative Irradiance mode. Remove the CVD-DIFFUSE from the cuvette holder, place the software in Relative Irradiance mode by clicking on the  on the OOIBASE toolbar and place your sample in the cuvette holder. Depending on the spectrum that you see, the data acquisition parameters and filtering can be adjusted to provide optimal data.

Application Tips

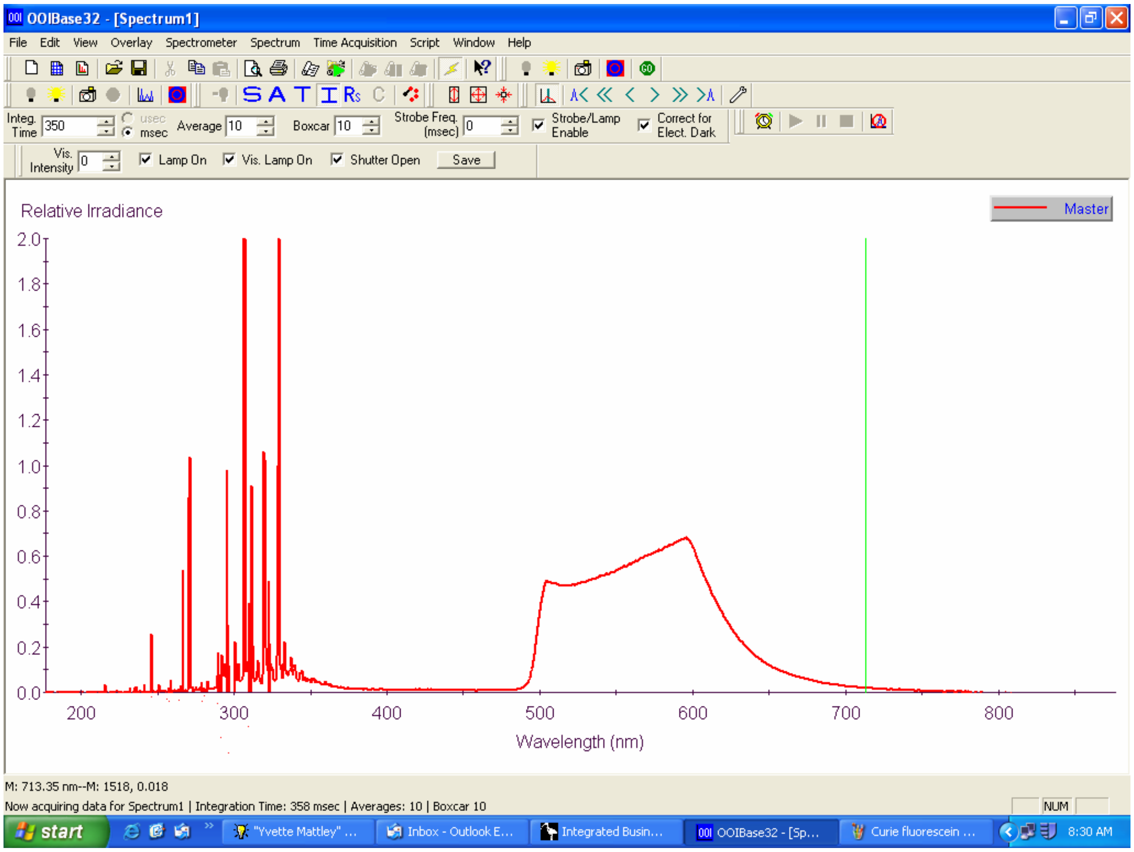
If the signal you collect . . .	You can . . .	By . . .
Saturates the spectrometer (peaks are off the scale)	Decrease the light level on scale in Scope mode	• Decreasing the integration time, or • Incorporating LVFs into your experiment

If the signal you collect . . .	You can . . .	By . . .
Has too little light	Increase the light level on scale in Scope mode	• Increasing the integration time, or • Removing LVFs from the light path

Examples of Saturated Signal

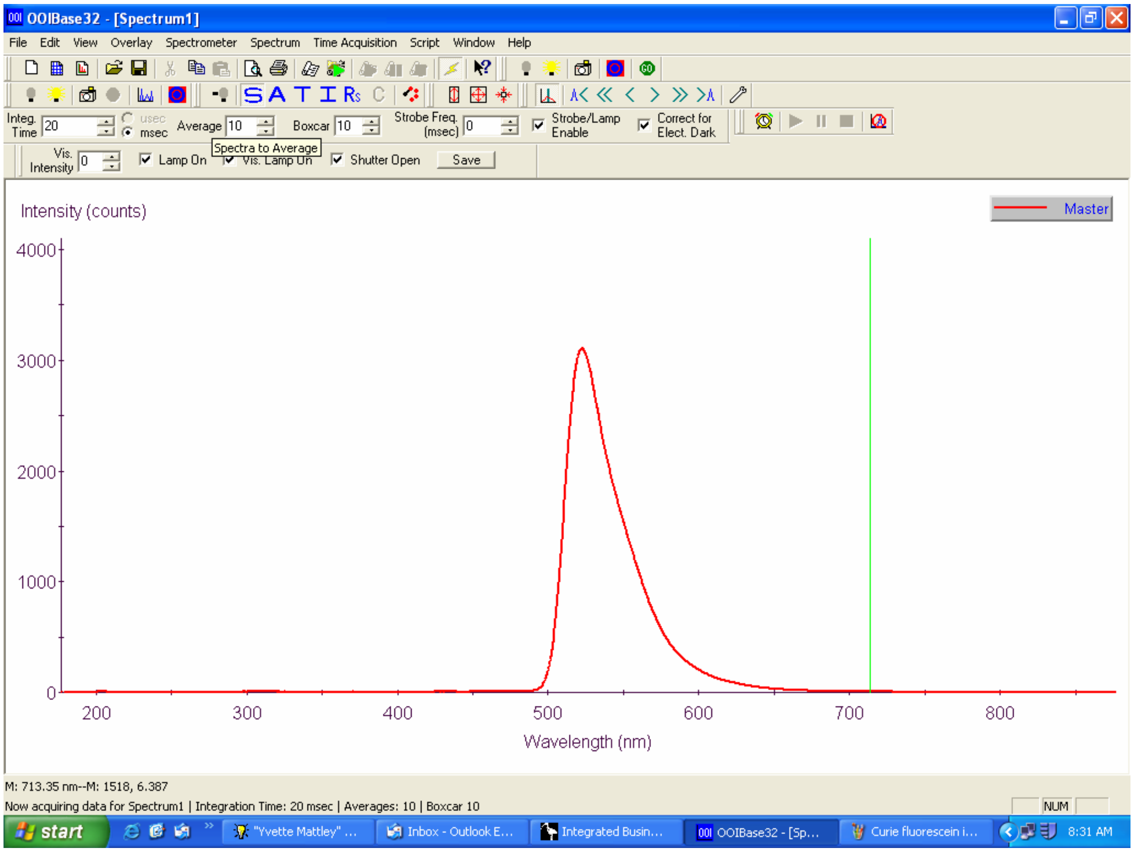


Saturated Fluorescein Signal in Scope Mode

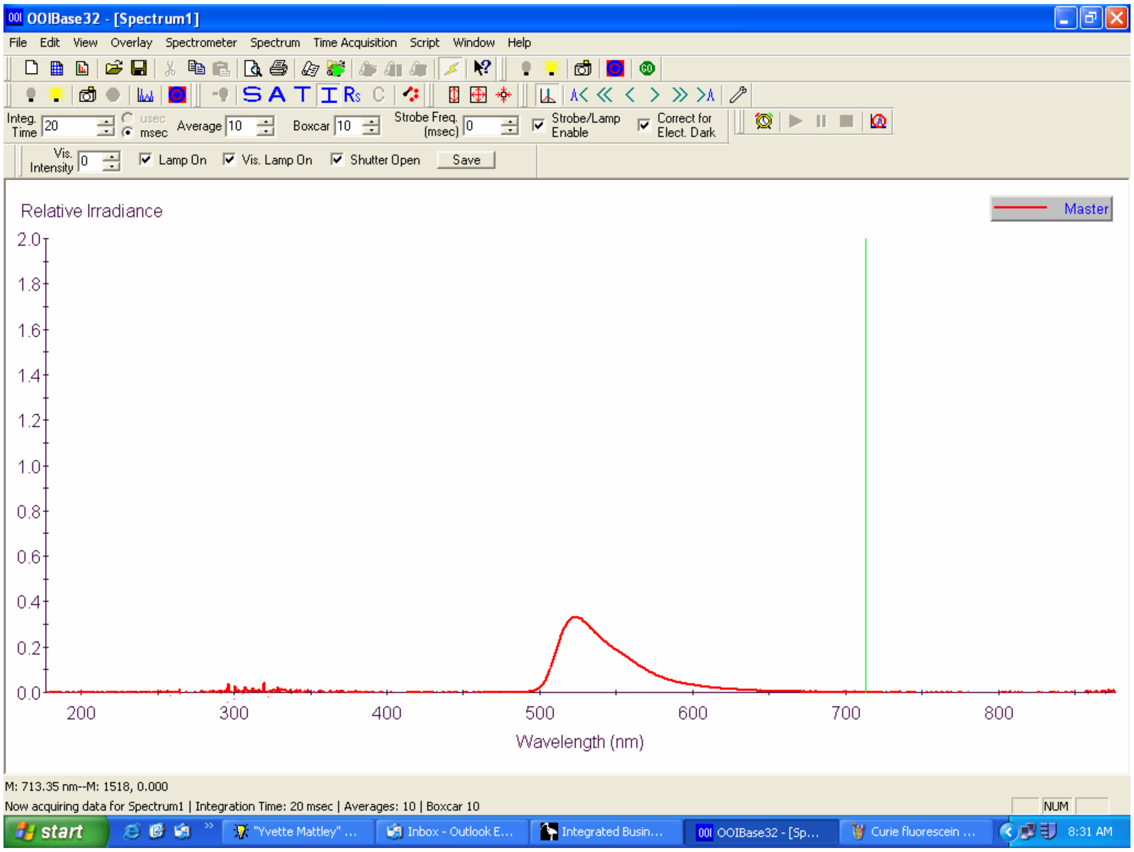


Saturated Fluorescein Signal in Relative Irradiance Mode

Examples of Unsaturated Signal



Unsaturated Fluorescein Signal in Scope Mode



Unsaturated Fluorescein Signal in Relative Irradiance Mode

Chapter 4

Troubleshooting

Introduction

This chapter contains the steps you need to take to solve possible problems that you may encounter with your Curie system installation.

Problem 1: Curie System Connected to PC Prior to OOIBase32 Installation

If your Curie system was connected to the computer prior to installing your OOIBase32 software application, you may encounter installation issues that you must correct before your Ocean Optics device will operate properly. Perform the following steps:

1. Remove the unknown device from the Windows Device Manager.
2. Remove improperly installed files.

Note

If these steps do not correct your device driver problem, you will need to obtain the *Correcting Device Driver Issues* document from the Ocean Optics website at **http://www.oceanoptics.com/technical/engineering/correctingdevicedriverissues.pdf**.

Removing the Unknown Device from Windows Device Manager

► ***Procedure***

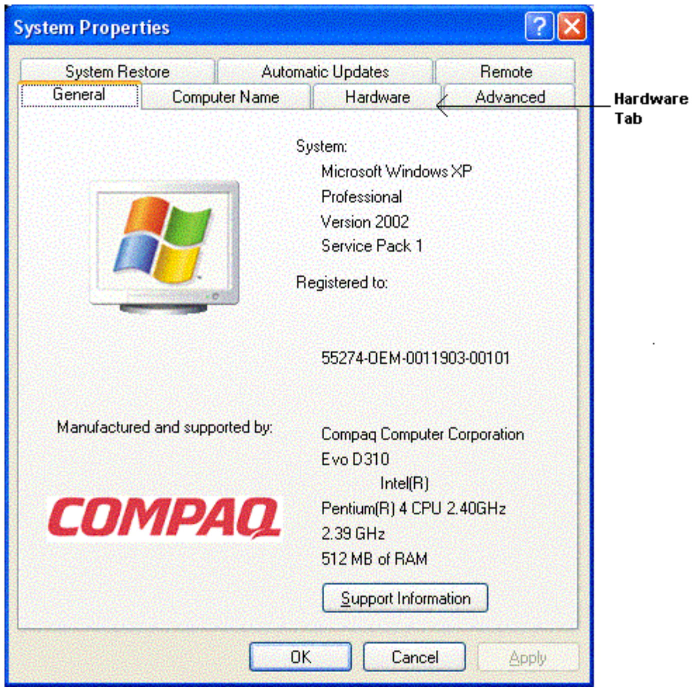
Perform the following procedure to remove the unknown device:

1. Open the Windows Device Manager as follows:
   - **For Windows 98/ME:**
     a. From the desktop, right-click **My Computer.** A pop-up menu appears.
     b. Select Properties.
     c. Select the **Device Manager** tab.

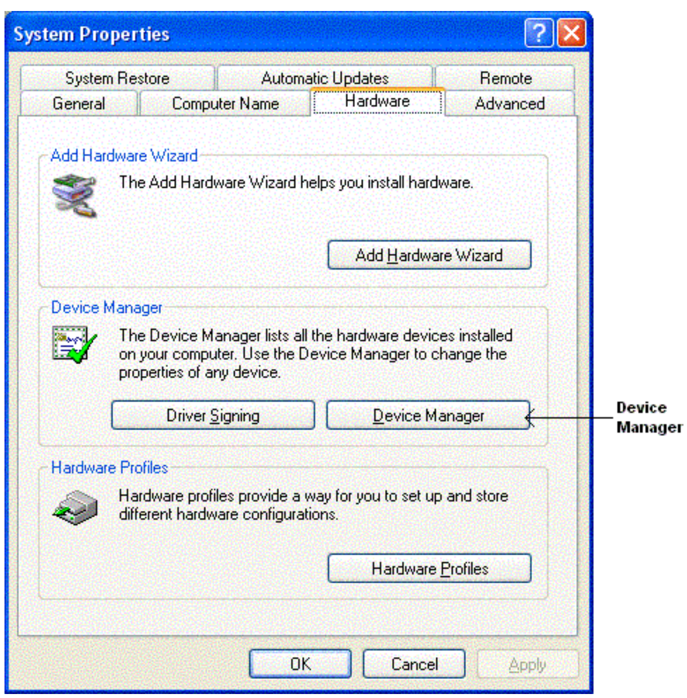
- **For Windows 2000/XP:**

  a. From the desktop, right-click **My Computer** and select **Properties**. The **System Properties** screen appears.

  b. Select the **Hardware** tab.



  c. Click the **Device Manager** button.



2. Locate **Other Devices** and expand the 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 either the **Uninstall** or **Remove** option.
4. Click **OK** to continue. A warning appears confirming the removal of the unknown device.
5. Click **OK** again to confirm the device removal.
6. Disconnect the Curie system from your computer.

Removing Improperly Installed Files

► *Procedure*

To remove improperly installed files, do the following:

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

Note

If the INF directory is not visible, you must disable **Hide protected operating system files** and **Hide extension for unknown file types** in Windows Folder Options.

For Windows 98, access Windows Folder Options from Windows Explorer, under **View | Options**.

For Windows 2000/XP, access Windows Folder Options from Windows Explorer, under **Tools | Folder Options** and select the **View** tab.

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3. Delete the ooi_usb.inf and ooi_usb.PNF files in the INF directory.
4. Navigate to the **Windows | system32 | drivers directory**.
5. Delete the ezusb.sys file.
6. Reinstall your Ocean Optics application and reboot the computer when prompted.
7. Plug in your Curie system.

The computer should now be able to install the correct drivers for your Curie system.

Problem 2: Older Version of OOIBase32 Installed

If the computer you want to use to interface to your Curie system already has an older version of the OOIBase32 application installed, you must install the latest OOIBase32 version instead.

Note

You do not need to uninstall the older version of the OOIBase32 software before you install the latest version.

Obtain the latest version of OOIBase32 from the *Software and Technical Resources CD* included with your Curie system, or from the Ocean Optics website at **http://www.oceanoptics.com/technical/softwaredownloads.asp**.

Appendix A

Specifications

Curie System

Table 1: Curie System Specifications

Specification	Value
System	
Dimensions	33 cm x 24.9 cm x 12.8 cm
Weight	6.75 kg
Temperature Limits	0–60 °C
Humidity Limits	0–90%, noncondensing
Power Consumption	90 mA @ 5 VDC and 0.2 A @ 12 VDC for a total of 2.9 W
Wavelength Range	250 to 800 nm
LVF Range (Excitation Range)	220 to 700 nm
Optical Resolution	~10 nm
Light Source	Pulsed xenon
Warm-up Time	< 5 minutes
A/D Resolution	12 bit
Board Architecture	USB 1.1
Wavelength Accuracy	1 pixel (~0.35 nm)
Photometric Accuracy	<0.1%
Stray Lights	~0.05% at 600 nm, <0.10% at 435 nm

Table 1: Curie System Specifications (Cont'd)

Specification	Value
Optical Bench and Detector	
Detector	2048-element linear silicon CCD array
Number of Elements	2048 pixels
Pixel Size	14 µm x 200 µm
Well Depth	~62,500 electrons
Usable Range	200–1100 nm
Dynamic Range: System Single Aquisition	 2×10^{8} 2000:1
Sensitivity (estimate): At 400 nm At 600 nm At 800 nm	 90 photons/count 41 photons/count 203 photons/count
	2.9×10^{17} joule/count 2.9×10^{17} watts/count (for a 1-second integration time)
Dark Noise	2.5–4.0 (RMS)
Grating	Grating #2
Slit	200 µm slit
Focal Length: Input Output	 42 mm 68 mm
Order Sorting	None
Resolution	~ 10 nm (FWHM)
Bulb Life (hours)	>1 x 10^{8} flashes

Table 1: Curie System Specifications (Cont'd)

Specification	Value
Optical Bench and Detector (Cont'd)	
Stray Light:	
At 600 nm	< 0.05%
At 435 nm	< 0.10%
At 250 nm	< 0.10%
Signal-to-Noise	250:1 (at full signal)
Pulsed Xenon Light Source	
Voltage	11–28 VDC
DC Current	0.2 amps RMS
Peak Current	1.0 amps
Trigger [1]	TTL
Vref (Vo/Vref = 127.5)	3.14–4.7 VDC
[1] Opto-isolated, +5V TTL compatible, 20–50 mA peak input, 10–100 µsec pulse width, leading edge trigger, internal resistor 150 Ω	

Table 2: Electrical Output

Specification	Value
Voltage	400–600 VDC adjustable
Power (Joules/sec)	2 watts maximum (power = Joules x flash rate)
Standard Discharge Capacitor	0.047, 0.10, or 0.22 µfd
Flash Rate (Hz)	$F_{MAX} = 2/E$, where $E = 1/2CV^2$

Table 3: Discharge Capacitor Options

Capacitor (µfd)	Max Input/Flash (mJ)	Max Flash Rate @600 VDC (Hz)	Max Flash Rate @400 VDC (Hz)
0.22	40	50	115
0.10	18	111	250
0.47	8.5	235	530

Table 4: Light Output

Specification	Value
Spectral Range	160–4,000 nm
Stability (CV) [1]	< 3%
Lifetime	>1 x 10^9 Flashes

[1] CV or Coefficient of variation is defined as CV% = (Standard deviation of 20 flashes) / (Mean of 20 flashes)

Compatibility for Desktop or Notebook PCs

To use the Curie Self-contained Fluorescence System with your PC, your PC must meet the following requirements:

- IBM-compatible PC with Pentium or higher microprocessor
- 32 MB RAM
- Ocean Optics' OOIBase32 32-bit Spectrometer operating software
- Windows 98/ME/2000/XP operating system

Appendix B

Calibrating the Wavelength of the Curie Spectrometer

Your Curie system's spectrometer is calibrated before it leaves Ocean Optics and the values are on the CD that you received with your product. However, the wavelength for all spectrometers will drift slightly as a function of time and environmental conditions, requiring you to recalibrate.

About Wavelength Calibration

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

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

Where: λ = Wavelength of pixel p

I = Wavelength of pixel 0

C_1 = First coefficient (nm/pixel)

C_2 = Second coefficient (nm/pixel^2)

C_3 = Second coefficient (nm/pixel^3)

You will be calculating the value for I and the three Cs.

Calibrating the Wavelength of the Spectrometer

Preparing for Calibration

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

- A light source capable of producing spectral lines.

Note

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

- The Curie system
- An optical fiber (maximum 50µm works best)
- PS-HG1-ADP Cuvette Adapter
- 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 select **AnalysisToolPak** and **AnalysisTookPak-VBA**.

Calibrating the Spectrometer

► *Procedure*

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

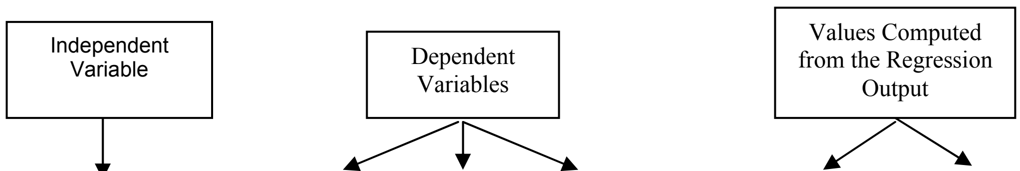
1. Connect the HG-1 to the cuvette adapter via an optical fiber, and then insert the adapter into the cuvette holder of the Curie system so that light from the lamp enters the spectrometer.
2. Place OOIBase32 into Scope mode and take a spectrum of your light source. Adjust the integration time (or the A/D conversion frequency) until there are several peaks on the screen that are not off-scale.
3. Move the cursor to one of the peaks and position the cursor so that it is at the point of maximum intensity.
4. 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.

5. 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.

   In the fourth column, calculate the pixel number cubed.



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

6. Use the spreadsheet or a 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**

7. Select the true wavelength as the dependent variable (Y). Select the pixel number, pixel number squared, and the pixel number cubed as the independent variables (X). After executing the regression, you will obtain an output similar to the one shown below.

Regression Statistics

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

Intercept ↓

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

The figure above notes the numbers of importance.

8. Record the Intercept, as well as the First, Second, and Third Coefficients. Also, the value for R Squared should be very close to 1. If it is not, you have most likely assigned one of your wavelengths incorrectly.

   Keep these values at hand.

Saving the New Calibration Coefficients

Wavelength calibration coefficients unique to each Curie system are programmed into an EEPROM memory chip on the spectrometer in your Curie system.

You can save over old calibration coefficients with new ones. OOIBase32 reads these coefficients from the EEPROM on the spectrometer.

► *Procedure*

To save wavelength calibration coefficients, do the following:

1. Ensure that the Curie system is connected to the PC and that no other applications are running.
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 **Finish** and reboot the computer when prompted.
10. Navigate to the **USB EEPROM Programmer** and run the software.
11. Click on the Curie 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 Spectrometer* section in this Appendix.
13. Repeat Step *12* for all of the new values.
14. Click **Save All Values** 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 spectrometer.

Appendix C

Relative Irradiance Mode

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:

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

Where:

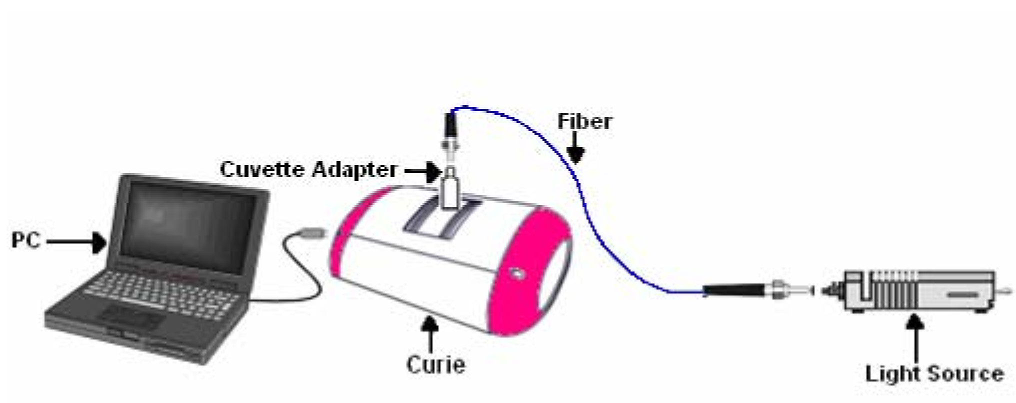
B_λ = Relative energy of the reference (calculated from the color temperature) at wavelength λ

S_λ = Sample intensity at wavelength λ

D_λ = Dark intensity at wavelength λ

R_λ = Reference intensity at wavelength λ

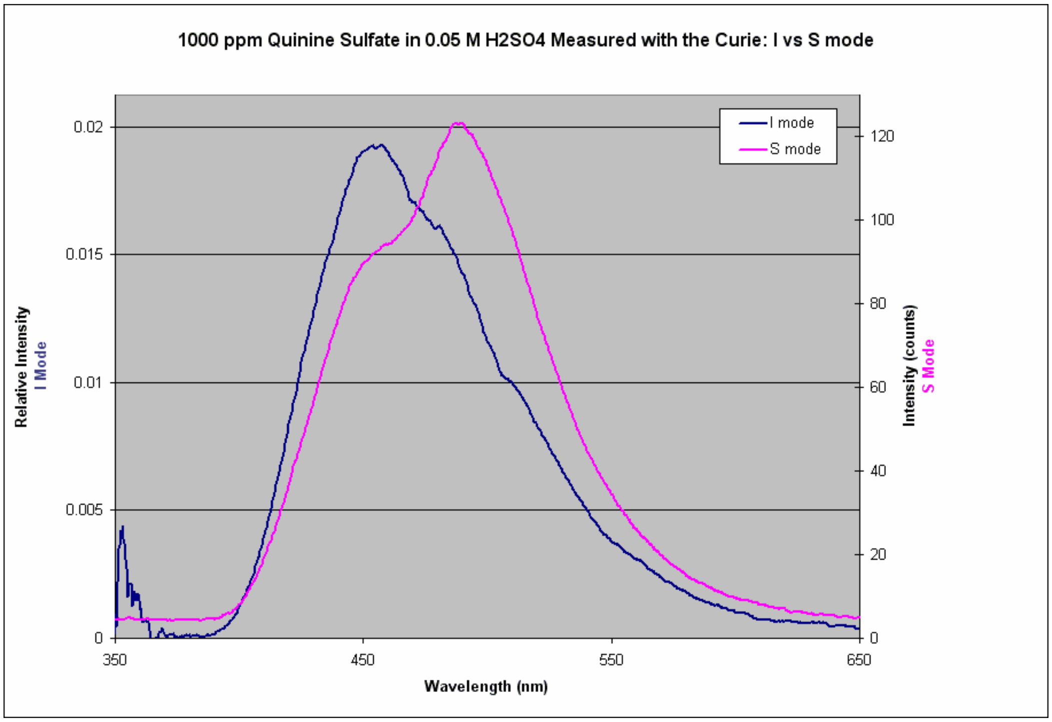
The figure below shows a typical relative irradiance setup: Use a light source with a known color temperature such as the LS-1-LL to take a reference spectrum. The light source is coupled to the cuvette adapter (such as the PS-HG1-ADP) with a fiber. The cuvette adapter is used to reflect light energy into the spectrometer. The spectrometer then transmits the information to the PC, which compares the measured spectra against the reference spectrum, thus removing wavelength-dependent instrument response from the measurement.



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

Before you can access Relative Irradiance Mode, you must take a reference spectrum in Scope Mode of a blackbody of known color temperature. Additionally, you must obtain a dark spectrum by removing the fiber from the reference lamp and preventing light from entering it.

An example of Curie spectra for quinine sulfate measured in Scope mode (S) versus Relative Irradiance mode (I) is shown below.



Calibrating the Spectrometer for Relative Irradiance

Preparing for Calibration

To recalibrate your Curie for relative irradiance, you need the following:

- A light source of known color temperature. Ocean Optics' LS-1-LL is ideal for recalibration.
- The Curie system
- An optical fiber (maximum 50µm works best)
- A cuvette wavelength calibration adapter such as the PS-HG1-ADP
- A spreadsheet program (Excel or Quattro Pro, for example) or a calculator that performs third-order linear regressions

Note

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

Calibrating the Spectrometer

► *Procedure*

Perform the steps below to calibrate for relative irradiance:

1. Disable the light source in the Curie system by using the power switch on the light source or by disabling the spectrometer strobe function on the **Strobe** tab in the **Configure Data Acquisition** screen in the OOIBase32 software.
2. Connect the light source to the cuvette adapter via an optical fiber, and then insert the adapter into the cuvette holder of the Curie system so that light from the lamp enters the spectrometer.
3. Place OOIBase32 into Scope mode by clicking the **Scope Mode** icon ( ) on the toolbar, or by selecting **Spectrum | Scope Mode** from the menu bar, or by typing CTRL + SHIFT + S.
4. Take a reference spectrum of your light source. Adjust the integration time (or the A/D conversion frequency) until the spectrum is on the scale (below 4000 counts).
5. In the **Reference Color Temperature** dialog box, enter the color temperature of the light source (in Kelvin) and click **OK**. For the LS-1-LL, enter **2800**.
6. 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.
7. Turn off the LS-1-LL light source. 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.
8. Place OOIBase32 into Relative Irradiance mode by clicking the **Relative Irradiance Mode** icon ( ) on the toolbar, or by selecting **Spectrum | Relative Irradiance Mode** from the menu bar.

Appendix D

Filter Sets

The following table lists the specifications for the filters included with the Curie.

Wavelength Information

Type	Center Lambda	FWHM	Lambda (50%)	Lambda 100%)	Name
Bandpass	330	140	---	---	U330
Bandpass	407	104	---	---	BG-12
Bandpass	526	53	---	---	VG-9
Longpass	---	---	420	495	GG-420
Longpass	---	---	530	590	OG-530
Longpass	---	---	550	610	OG-550
Longpass	---	---	610	660	RG-610

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