HORIBA Scientific

NanoLog®
Spectrofluorometer System

Operation Manual
Part number J81096 rev. C

NanoLog®

Operation Manual

Rev. C
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JOBIN YVON Technology

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0: Introduction

About the NanoLog®

The main parts of the NanoLog® spectrofluorometer system are:

- State-of-the-art optical components
- A personal computer
- FluorEssence™ for Windows®, the driving software.

This manual explains how to operate and maintain a NanoLog® spectrofluorometer. The manual also describes measurements and tests essential to obtain accurate data. For more on the almost limitless power provided by FluorEssence™, refer to the *FluorEssence™ User's Guide* and on-line help, and the Origin® on-line help (contains post-processing instructions for data manipulation) which accompany the system.

The combination of time-tested, performance-proven hardware with the powerful data-acquisition and manipulation software yields a system suitable for a wide variety of applications. Equipped with expansion ports and slots, the NanoLog® can grow to meet the changing needs of the user and it will provide years of dedicated service.

Note: *Keep this and the other reference manuals near the system.*

Chapter overview

1: Requirements & Installation	Power and environmental requirements; select the best spot for the instrument.
2: System Description	Overview of the NanoLog® instrument and its major parts.
3: System Operation	Operation of the spectrofluorometer system, and calibration instructions.
4: Data Acquisition	How to use the special FluorEssence™ buttons to acquire and plot data; how to determine peaks in an unknown sample.
5: Optimizing Data	Hints for improving the signal-to-noise ratio, instructions for obtaining corrected data, and other information useful for optimizing data and ensuring reproducibility.
6: System Maintenance	Routine maintenance procedures such as replacing the lamp.
7: Troubleshooting	Potential sources of problems, their most probable causes, and possible solutions.
8: Producing Correction Factors	How to correct for variation in sensitivity across the spectral range.
9: Automated Polarizers	Installation, operation, and troubleshooting of the optional automated polarizers.
10: Applications	Some interesting uses for the NanoLog®.
11: Xenon Lamp Information & Record of Use Form	Information about the xenon lamp, and a form for recording the xenon-lamp usage.
12: Introduction to Lifetime Measurements	Methods of determining the lifetime of a sample using the TCSPC upgrade. The TCSPC upgrade is designed specifically for lifetime applications, and does not affect steady-state measurements.
13: Technical Specifications	Instrument specifications and computer requirements.

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- Following all precautions
- Referring to additional safety documentation, such as Material Safety Data Sheets (MSDS), when advised

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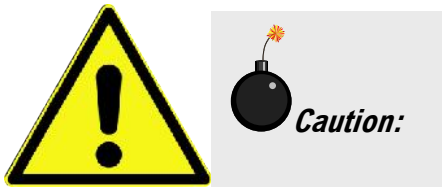
Safety summary

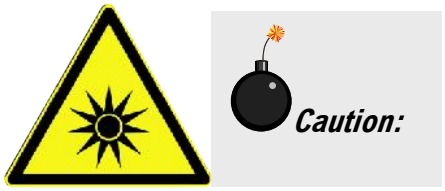
The following general safety precautions must be observed during all phases of operation of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture and intended use of instrument. HORIBA Instruments Incorporated assumes no liability for the customer's failure to comply with these requirements. Certain symbols are used throughout the text for special conditions when operating the instruments:

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or similar that, if incorrectly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met. HORIBA Instruments Incorporated is not responsible for damage arising out of improper use of the equipment.



A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or similar that, if incorrectly performed or adhered to, could result in damage to the product. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met. HORIBA Instruments Incorporated is not responsible for damage arising out of improper use of the equipment.

Ultraviolet light! Wear protective goggles, full-face shield, skin-protection clothing, and UV-blocking gloves. Do not stare into light.



Intense ultraviolet, visible, or infrared light! Wear light-protective goggles, full-face shield, skin-protection clothing, and light-blocking gloves. Do not stare into light.

Extreme cold! Cryogenic materials must always be handled with care. Wear protective goggles, full-face shield, skin-protection clothing, and insulated gloves.

Explosion hazard! Wear explosion-proof goggles, full-face shield, skin-protection clothing, and protective gloves.

Risk of electric shock! This symbol warns the user that un-insulated voltage within the unit may have sufficient magnitude to cause electric shock.

Danger to fingers! This symbol warns the user that the equipment is heavy, and can crush or injure the hand if precautions are not taken.

This symbol cautions the user that excessive humidity, if present, can damage certain equipment.

Hot! This symbol warns the user that hot equipment may be present, and could create a risk of fire or burns.

Read this manual before using or servicing the instrument.

Wear protective gloves.

Wear appropriate safety goggles to protect the eyes.

Wear an appropriate face-shield to protect the face.

Note:

General information is given concerning operation of the equipment.

Risks of ultraviolet exposure

Caution: *This instrument is used in conjunction with ultraviolet light. Exposure to these radiations, even reflected or diffused, can result in serious, and sometimes irreversible, eye and skin injuries.*

Overexposure to ultraviolet rays threatens human health by causing:

- *Immediate painful sunburn*
- *Skin cancer*
- *Eye damage*
- *Immune-system suppression*
- *Premature aging*

Do not aim the UV light at anyone.
Do not look directly into the light.
Always wear protective goggles, full-face shield and skin protection clothing and gloves when using the light source.

- Light is subdivided into visible light, ranging from 400 nm (violet) to 700 nm (red); longer infrared, “above red” or > 700 nm, also called heat; and shorter ultraviolet radiation (UVR), “below violet” or < 400 nm. UVR is further subdivided into UV-A or near-UV (320–400 nm), also called black (invisible) light; UV-B or mid-UV (290–320 nm), which is more skin penetrating; and UV-C or far-UV (< 290 nm).

- Health effects of exposure to UV light are familiar to anyone who has had sunburn. However, the UV light level around some UV equipment greatly exceeds the level found in nature. Acute (short-term) effects include redness or ulceration of the skin. At high levels of exposure, these burns can be serious. For chronic exposures, there is also a cumulative risk of harm. This risk depends upon the amount of exposure during your lifetime. The long-term risks for large cumulative exposure include premature aging of the skin, wrinkles and, most seriously, skin cancer and cataract.

- Damage to vision is likely following exposure to high-intensity UV radiation. In adults, more than 99% of UV radiation is absorbed by the anterior structures of the eye. UVR can contribute to the development of age-related cataract, pterygium, photodermatitis, and cancer of the skin around the eye. It may also contribute to age-related macular degeneration. Like the skin, the covering of the eye or the cornea, is epithelial tissue. The danger to the eye is enhanced by the fact that light can enter from all angles around the eye and not only in the direction of vision. This is especially true while working in a dark environment, as the pupil is wide open. The lens can also be damaged, but because the cornea acts as a filter, the chances

are reduced. This should not lessen the concern over lens damage however, because cataracts are the direct result of lens damage.

Burns to the eyes are usually more painful and serious than a burn to the skin. Make sure your eye protection is appropriate for this work. NORMAL EYEGLASSES OR CONTACTS OFFER VERY LIMITED PROTECTION!

Caution: *UV exposures are not immediately felt. The user may not realize the hazard until it is too late and the damage is done.*

Training

For the use of UV sources, new users must be trained by another member of the laboratory who, in the opinion of the member of staff in charge of the department, is sufficiently competent to give instruction on the correct procedure. Newly trained users should be overseen for some time by a competent person.

Additional risks of xenon lamps

Warning: *Xenon lamps are dangerous. Please read the following precautions.*

Among the dangers associated with xenon lamps are:

- Burns caused by contact with a hot xenon lamp.
- Fire ignited by hot xenon lamp.
- Interaction of other nearby chemicals with intense ultraviolet, visible, or infrared radiation.
- Damage caused to apparatus placed close to the xenon lamp.
- Explosion or mechanical failure of the xenon lamp.

Visible radiation

Any very bright visible light source will cause a human aversion response: we either blink or turn our head away. Although we may see a retinal afterimage (which can last for several minutes), the aversion response time (about 0.25 seconds) normally protects our vision. This aversion response should be trusted and obeyed. NEVER STARE AT ANY BRIGHT LIGHT-SOURCE FOR AN EXTENDED PERIOD. Overriding the aversion response by forcing yourself to look at a bright light-source may result in permanent injury to the retina. This type of injury can occur during a single prolonged exposure. Excessive exposure to visible light can result in skin and eye damage.

Visible light sources that are not bright enough to cause retinal burns are not necessarily safe to view for an extended period. In fact, any sufficiently bright visible light source viewed for an extended period will eventually cause degradation of both night and color vision. Appropriate protective filters are needed for any light source that causes viewing discomfort when viewed for an extended period of time. For these reasons, prolonged viewing of bright light sources should be limited by the use of appropriate filters.

The blue-light wavelengths (400–500 nm) present a unique hazard to the retina by causing photochemical effects similar to those found in UV-radiation exposure.

Infrared radiation

Infrared (or heat) radiation is defined as having a wavelength between 780 nm and 1 mm. Specific biological effectiveness “bands” have been defined by the CIE (Commission Internationale de l’Eclairage or International Commission on Illumination) as follows:

- IR-A (near IR) (780–1400 nm)
- IR-B (mid IR) (1400–3000 nm)
- IR-C (far IR) (3000 nm–1 mm)

The skin and eyes absorb infrared radiation (IR) as heat. Workers normally notice excessive exposure through heat sensation and pain. Infrared radiation in the IR-A that enters the human eye will reach (and can be focused upon) the sensitive cells of the retina. For high irradiance sources in the IR-A, the retina is the part of the eye that is at risk. For sources in the IR-B and IR-C, both the skin and the cornea may be at risk from "flash burns." In addition, the heat deposited in the cornea may be conducted to the lens of the eye. This heating of the lens is believed to be the cause of so called "glassblowers' " cataracts because the heat transfer may cause clouding of the lens.

- Retinal IR Hazards (780 to 1400 nm): possible retinal lesions from acute high irradiance exposures to small dimension sources.
- Lens IR Hazards (1400 to 1900 nm): possible cataract induction from chronic lower irradiance exposures.
- Corneal IR Hazards (1900 nm to 1 mm): possible flashburns from acute high irradiance exposures.

Who is likely to be injured? The user and anyone exposed to the radiation or xenon lamp shards as a result of faulty procedures. Injuries may be slight to severe.

1: Requirements & Installation

Surface requirements

- A sturdy table- or bench-top.
- Table size varies according to the system configuration; an average size of 48″ × 61″ (121 cm × 156 cm) is usually sufficient.

***Caution:* Do not** *split the system between two tables. Using two tables can cause instability, resulting in service requirements or erroneous data.*

Table size for standard systems*

	FL3-2-iHR	FL3-21-iHR	FL3-22-iHR
Length	117 cm	117 cm	121 cm
Width	103 cm	137 cm	156 cm
Height	42 cm	42 cm	42 cm

*****Custom configurations are available. See the *System Description* chapter.

Environmental requirements

- Temperature 72 ± 5°F (22 ± 3°C)
- Humidity level ~70%
- No special ventilation.

Note: *The standard xenon lamp provided with the NanoLog® is ozone-free. The lamp housing contains an electrically powered fan that removes the heat.*

Electrical requirements

- 115 V, 20 A or 220 V, 20 A; factory-set.
- As an extra measure of caution, plug the xenon lamp into a circuit separate from the other components. This guarantees that the electrical surge from the lamp never will interfere with the computer or system.

Note: *For the computer, HORIBA Scientific recommends using a surge suppressor or an uninterruptible power supply (UPS) with a surge suppressor.*

Make sure enough AC outlets are available for the

- Computer
- Printer (optional)
- Monitor
- Xenon lamp
- System controller (SpectrAcq)
- Symphony® detector controller
- Optional 10330 IR PMT power supply
- Any accessories that require an outlet

Use three-prong plugs for proper grounding of the system. If a two-prong adapter is used, for the safety of the operator and to preserve the integrity of the system, the adapter must be attached to the wall outlet properly, according to the manufacturer's instructions. This provides a positive connection to the electrical ground (earth), ensuring that any stray or leakage current is directed to earth ground.

Warning: *A three-prong-to-two-prong adapter* ***is not recommended.***

Installation

Caution: *Customer installation is not recommended. Special tools and several critical alignment verification procedures are required.*

Schedule the initial installation of a NanoLog® by calling the Service Department at 1 (877) 546-7422. Customers outside the United States should contact a local representative. For up-to-the-minute information about products, services, upgrades, frequently-asked questions, etc., visit our web site:

http://www.HORIBA.com/scientific

Subsequent assembly because of moving the instrument should be performed by a HORIBA Scientific engineer for a specified fee.

2: System Description

Overview

General operation

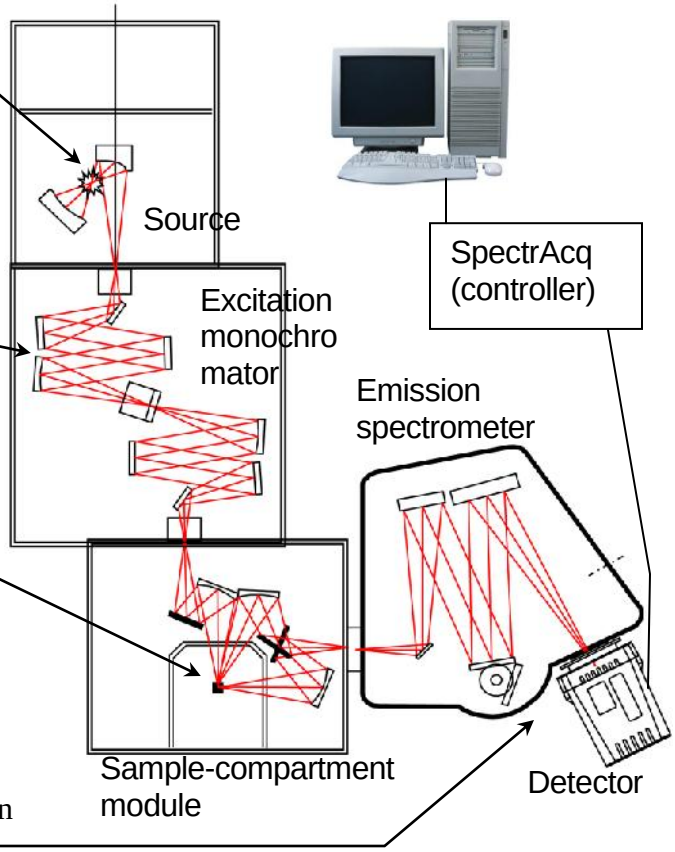
All NanoLog® spectrofluorometers have basic, common features:

a A source of radiation produces photons.

b The beam of light is filtered by an excitation mono-chromator that allows a single wavelength of light to reach the sample.

c In the sample compartment, the sample responds to the incoming radiation.

d The resulting radiation is filtered by an emission spectrometer that feeds the signal to an InGaAs array detector



e By stepping the monochromator or spectrometer or both through a wavelength region, and recording the variation in intensity as a function of wavelength, a spectrum is produced.

f The spectrofluorometer components (monochromator, sample-compartment module, spectrometer, accessories) are connected to a controller (the "SpectrAcq"), which, in turn, transfers information to and from the computer. The computer may be attached to a printer.

Basic components

Monochromator and spectrometer

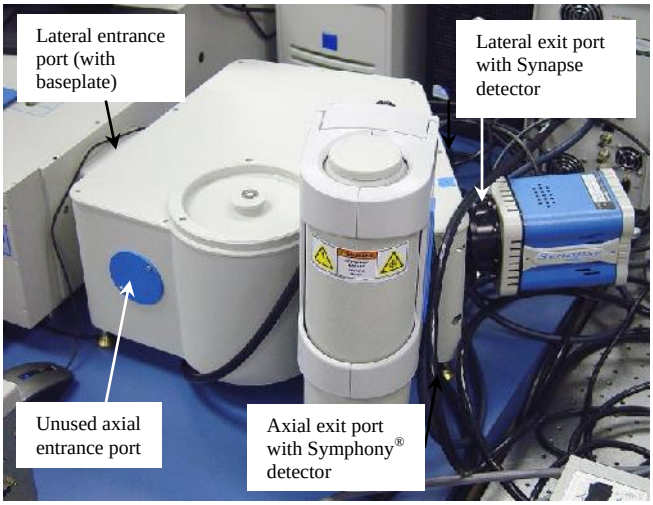
General information

The standard NanoLog® comes equipped with a double-grating monochromator in the excitation position. Double-grating monochromators offer a significant increase in sensitivity, resolution and stray-light rejection over single-grating ones. In the excitation position, the NanoLog® is equipped with a single-grating iHR spectrometer.

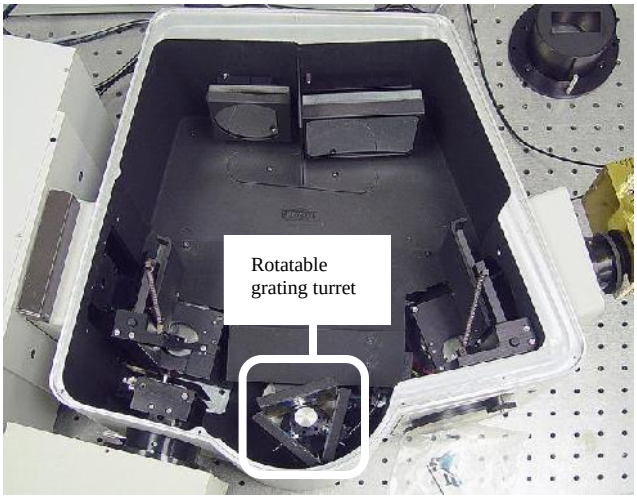
The iHR series of imaging spectrometers is a standard building block in a NanoLog® spectrofluorometer. Using an iHR imaging spectrometer on the emission side of the sample mount offers the option of detection with a CCD, to create an image of the dispersed fluorescence for subsequent analysis. On most iHR spectrometers, there are movable grating-turrets and multiple ports with mirrors to choose, therefore extra information is necessary for operation.

Differences between the iHR and standard monochromators

- Four ports generally are available, rather than two on a standard monochromator. Most iHR spectrometers used with the NanoLog® have two entrance ports ("lateral", i.e., on the side, and "axial", i.e., on the back) and two exit ports (lateral and axial). Usually the lateral entrance port is attached to the sample compartment.



- A FluorEssence™-controlled flip mirror is available to choose between entrance ports and exit ports. Be sure the flip mirror is set to the proper position before running an experiment.
- Three gratings can be mounted on the rotatable grating turret. Choosing which grating to use is under FluorEssence™'s control.



NanoLog® users typically choose the lateral entrance port as the path for luminescence from the sample compartment into the iHR. To attach the iHR to the sample

compartment, a baseplate adapter must be inserted between the sample compartment and the iHR. This baseplate is automatically included with all Nanolog® instruments factory-built with an iHR spectrometer.

Sample compartment

The standard sample compartment is a *T-box*, which provides efficient throughput with a choice of standard right-angle emission collection or optional front-face emission collection. The sample-compartment module comes equipped with a silicon photodiode reference detector to monitor and compensate for variations in the xenon lamp output.

Detector

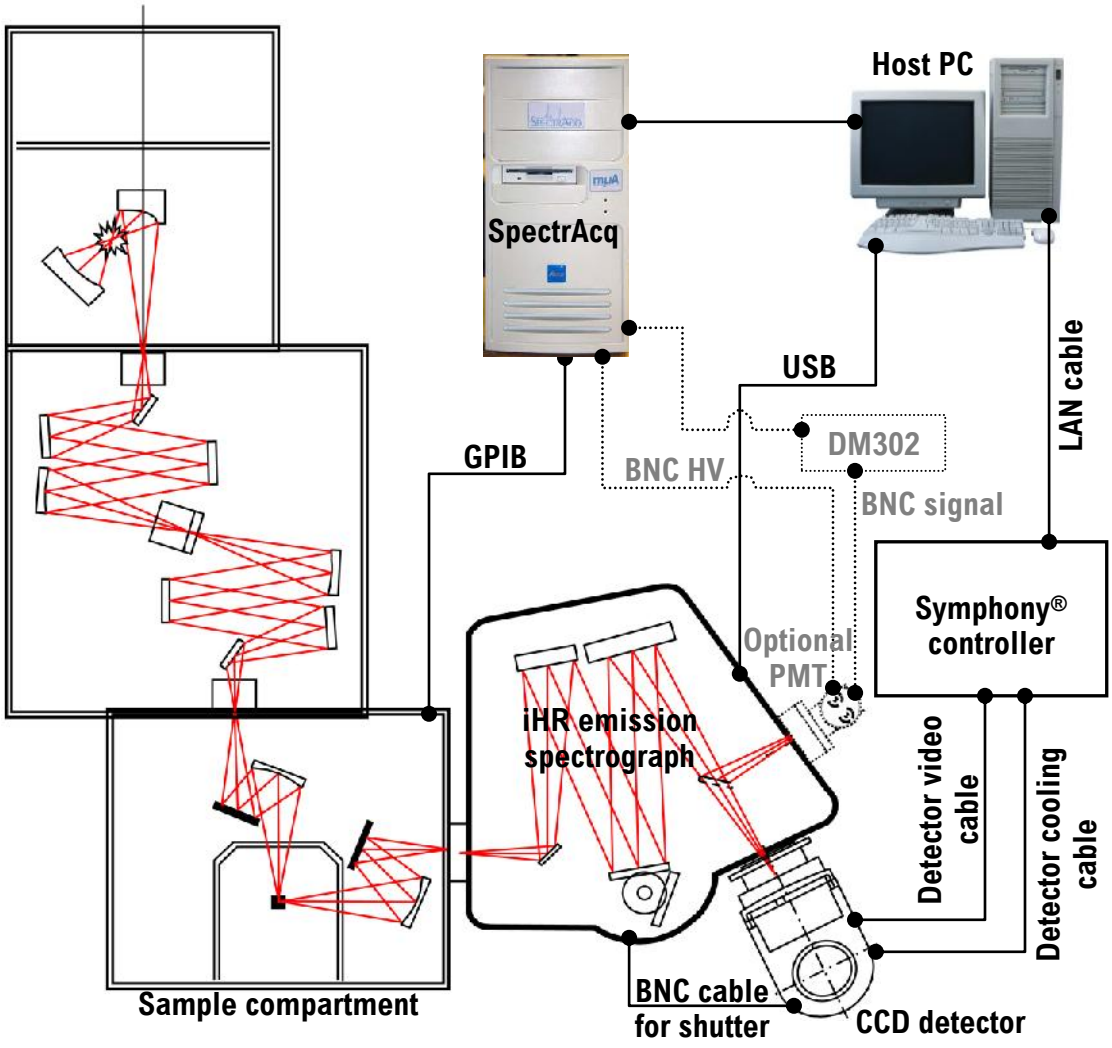
The standard detector offered on the NanoLog® is the Symphony® InGaAs array, which provides rapid and robust spectral characterization in the near-IR. The liquid-nitrogen cooling gives a lower background noise than room-temperature detection.

Accessories

NanoLog® spectrofluorometers offer sampling accessories to increase flexibility, and extend their applications to techniques such as polarization measurements or phosphorescence lifetimes.

System connections

Below is a sketch of the cable connections required between an iHR spectrometer, a CCD detector, and the rest of the system. Other NanoLog® connections are not shown. An optional second photomultiplier tube detector with its connections is shown in dotted lines.



Note: *Check your custom configuration with supplied documents.*

Custom options

Each system can be customized further by selecting different options. Available options are listed below. For additional information, or for a list of the newest options, contact a Fluorescence Product Specialist.

Sources

- Pulsed lamp, including our TCSPC upgrade
- HgXe
- Your laser

Caution: *Always take appropriate protective measures against intense light from external lamps and lasers, including goggles or face-shield, and clothing that blocks the wavelengths that the source emits.*

Detectors

- R928P PMT (for UV-Visible work), with optional cooling
- Cooled 10330 PMT (for IR detection)
- Liquid-nitrogen-cooled 5509 PMT (for visible and near-IR work)

Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of cryogenic materials such as liquid nitrogen.*

- Thermoelectrically cooled TBX-04 detection module
- Single-channel diodes (InGaAs, PbS, etc.) for IR detection
- CCD

Monochromator

- Extra monochromator in the T-position

Refer to Chapter 14, *Components and Accessories*, to see accessories that will help you further tailor a system to your needs.

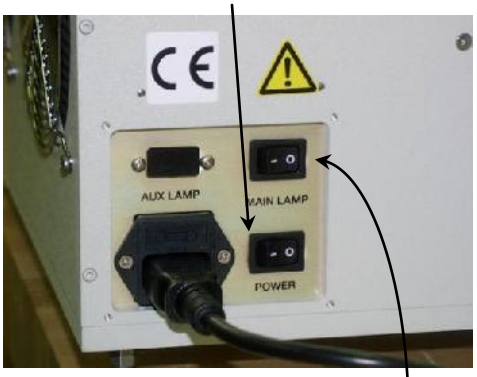
3: System Operation

Turning on the system

1 Start the lamp.

Turn on the lamp before the rest of the NanoLog®, accessories, or peripheral equipment.

a On the back of the lamp, turn on the power switch marked POWER.



b Just above the POWER switch, turn on the MAIN LAMP switch.

Caution: *When the lamp is turned on, a large voltage is put across the lamp, during which a spike can feed back down the electrical line. This spike can cause damage to computer equipment if the equipment is operating at the same time and on the same power circuit as the NanoLog®, when the lamp is started.*

2 Start the accessories.

Turn on any automated accessories (e.g., Temperature Bath, MicroMax, etc.) used with the NanoLog®.

3 Start the SpectrAcq.

a For NanoLog®s with serial numbers 1030 and lower, make sure the boot disk is in the floppy drive.

Caution: *For NanoLog®s with serial numbers 1031 or higher, do **NOT** insert the 3¼" floppy disk into the SpectrAcq. The floppy disk is ONLY for backup purposes, **NEVER** for boot-up.*

b On the rear of the SpectrAcq, switch on the power button to start. Immediately below the mμA logo on the front of the SpectrAcq, the LED indicator lamp should illuminate.

Note: *For Fluorolog®s that use the 3¼" floppy disk to boot up, the boot disk should access for ~ 60 s to load system drivers.*

4 Start the peripheral devices.

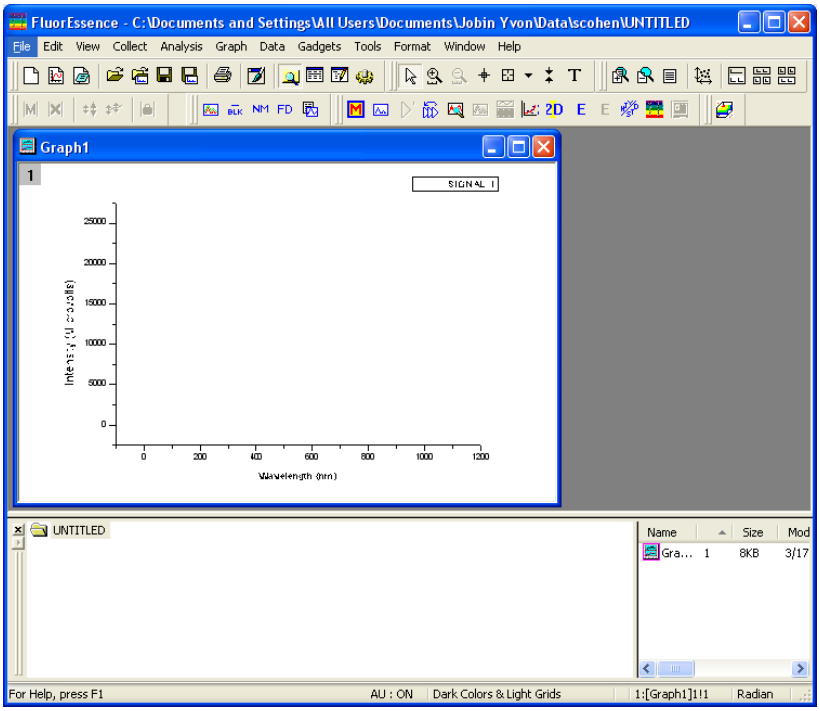
Turn on all peripheral devices, including printers and plotter (i.e., all devices other than the computer).

5 Start the host computer and software.

a Switch on the host computer.

b In Windows®, double-click on the FluorEssence icon to start **FluorEssence™**.

The instrument initializes, then the **FluorEssence** window appears. If there are any difficulties, see the chapter on troubleshooting.



Checking system performance

Introduction

Upon installation and as part of routine maintenance, examine the performance of the NanoLog®. HORIBA Scientific recommends checking the system's calibration before each day of using the system. Scans of the xenon-lamp output and the spectrum of a laser-glass sample are sufficient to verify system *calibration*, *repeatability*, and *throughput*.

- *Calibration* is the procedure in which the monochromator's and spectrometer's drives are referenced to a known spectral feature.
- *Repeatability* is the ability of the system to produce consistent spectra.
- *Throughput* is the amount of signal passing through and detected by the system. The throughput is correlated to the signal-to-noise ratio and sensitivity of the system.

The NanoLog® is an *autocalibrating* spectrofluorometer. This means the system initializes its monochromator's and spectrometer's drives, locates the home position of each drive, and assigns a wavelength value to this position from a calibration file. While the system usually maintains calibration by this method, it is wise to check calibration before the day's session with the instrument. For the calibration checks detailed here, a single-sample mount or automated sample-changer are the only sample-compartment accessories used.

The scans shown herein are examples. A Performance Test Report for your new instrument is included with the documentation. Use the Performance Test Report to validate the spectral shape and relative intensity taken during calibration checks.

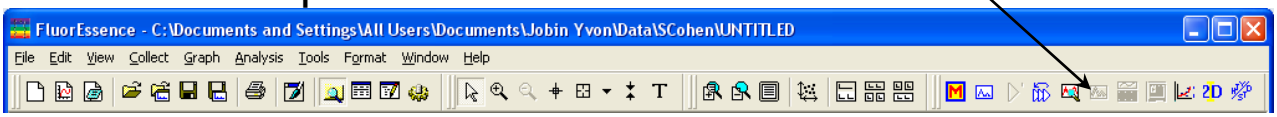
Note: *HORIBA Instruments Incorporated is not responsible for customer errors in calibration. To be sure that your instrument is properly calibrated, call the Service Department for assistance. We can arrange a visit and calibrate your instrument for a fee.*

Excitation calibration check

This calibration check verifies the wavelength calibration of the excitation monochromator, using the reference photodiode located before the sample compartment. It is an excitation scan of the xenon lamp's output, and should be the first check performed.

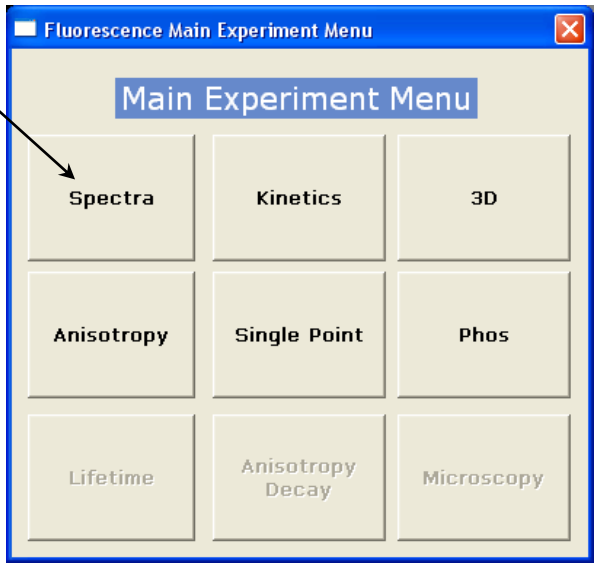
1 Close the sample compartment's lid.

2 In the main **FluorEssence** window, choose the Experiment Menu button.



The **Fluorescence Main Experiment Menu** appears.

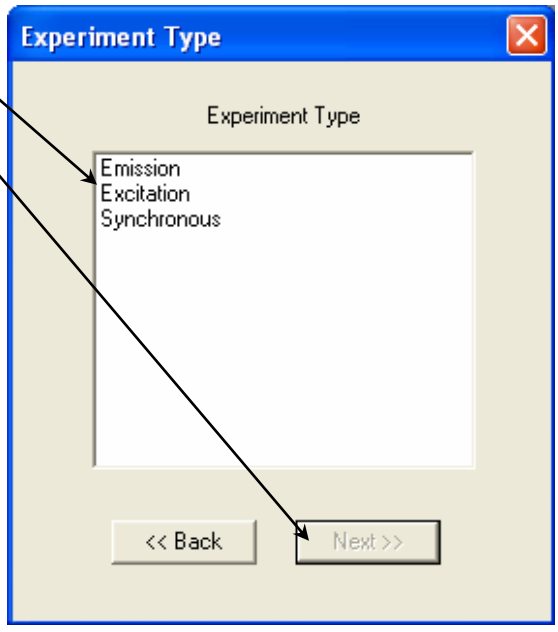
3 Choose Spectra.



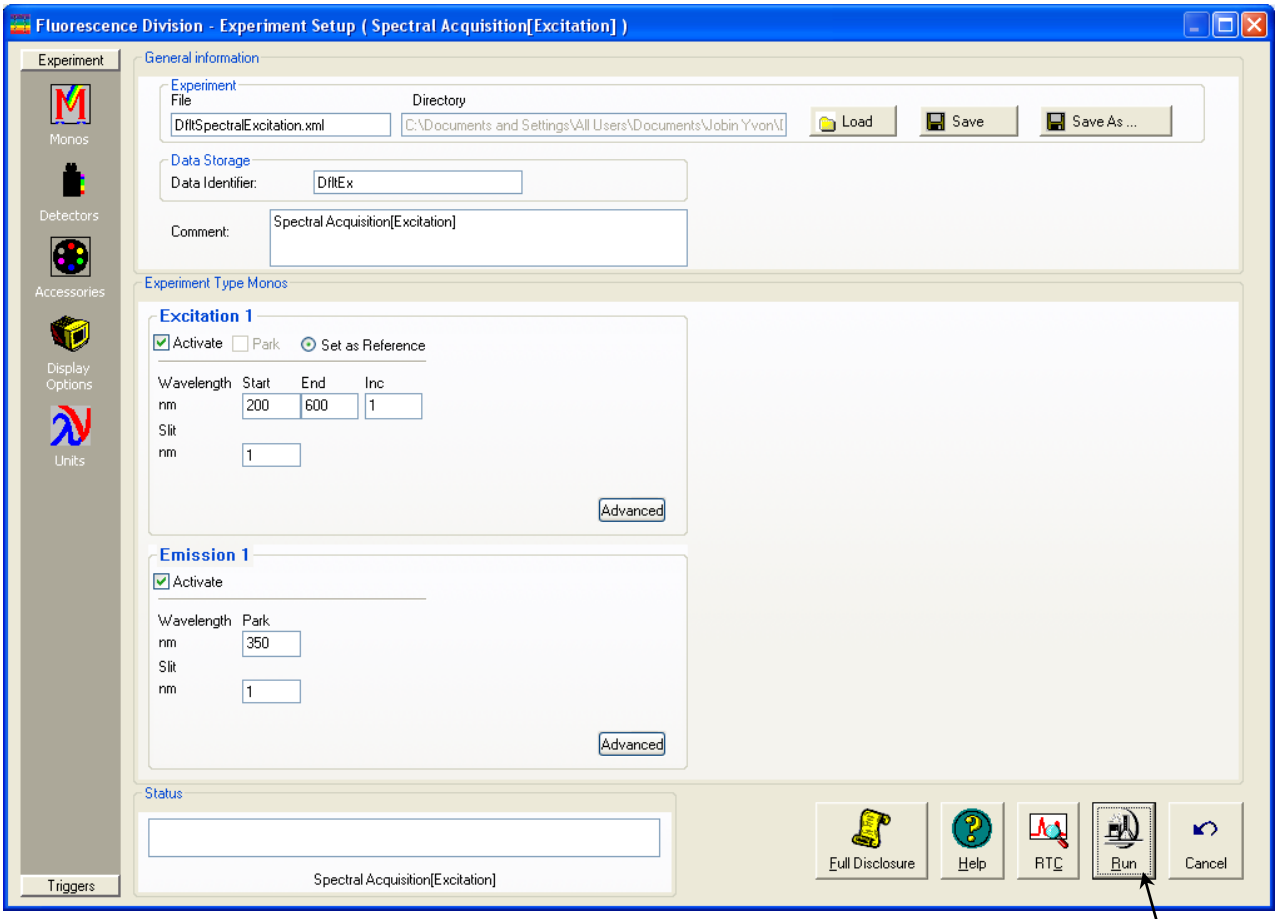
The **Experiment Type** window appears.

4 Choose Excitation, then click the Next >> button.

The xenon-lamp scan experiment automatically loads.



Note: *With an InGaAs or other array detector, the Excitation and Synchronous scans are not available.*



5 Use the default parameters or adjust them.

Default monochromator parameters for the xenon-lamp scan

Monochromator (1200 grooves/mm)	Initial wave-length	Final wave-length	Increment	Slits (bandpass)
Excitation	200 nm	600 nm	1 nm	1 nm
Emission	350 nm	--	--	1 nm

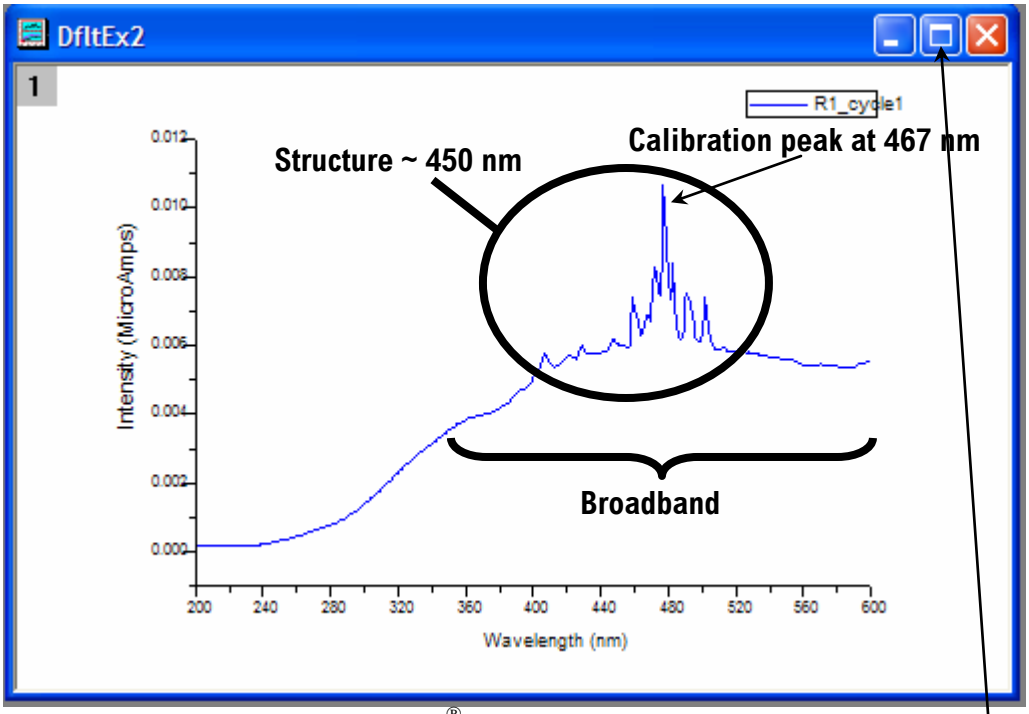
Default detector parameters for the xenon-lamp scan

Detector (Signal)	Integration time	Units
Reference (R1)	0.1 s	mA

6 Click the Run button 

.

The **Intermediate Display** opens. The xenon-lamp scan runs:



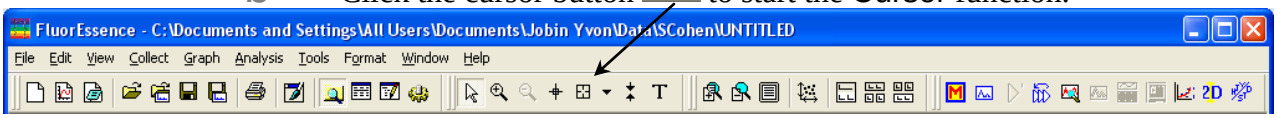
This is an uncalibrated FluoroMax® lamp scan. The main peak ought to be at 467 nm, but here appears near 480 nm.

Note: *Your lamp scan may appear different, depending on the instrument configuration.*

7 Calibrate the excitation monochromator, if required.

a Expand the plot by clicking the Expand button.

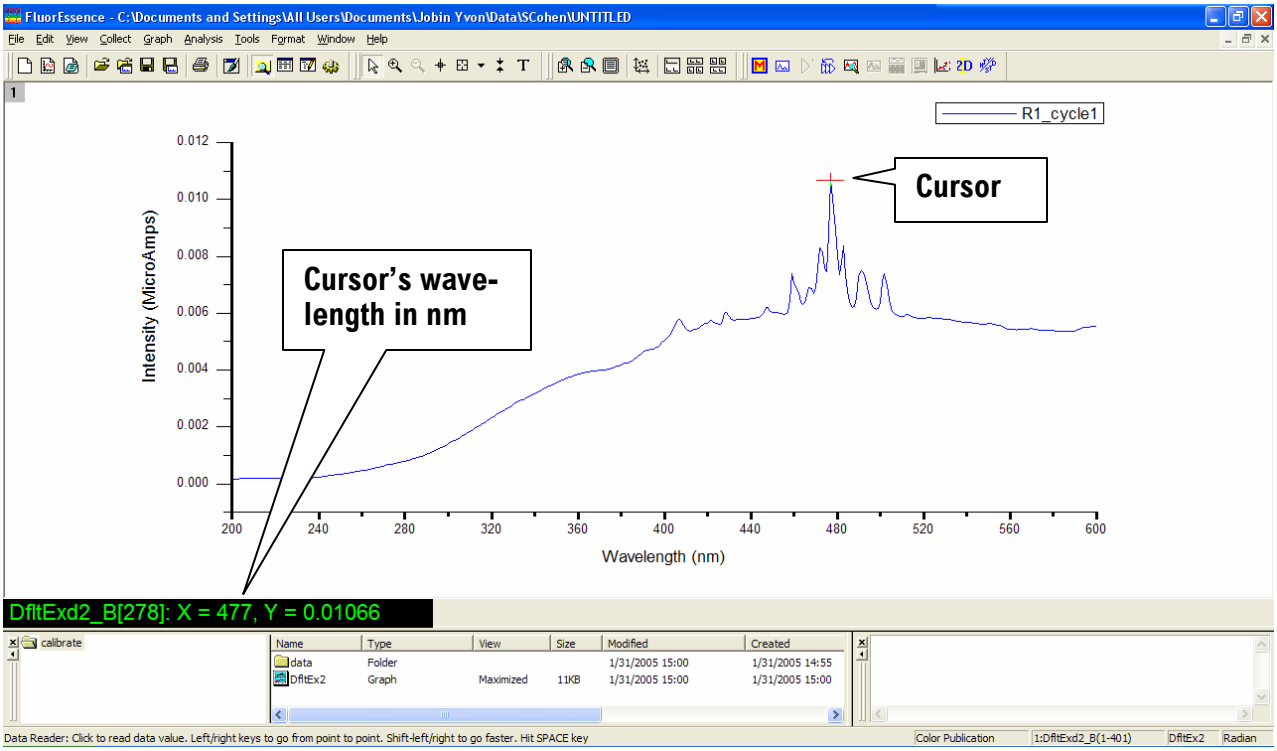
b Click the cursor button  to start the Cursor function.



c Click on the graph near the peak, to place the cursor on the graph.

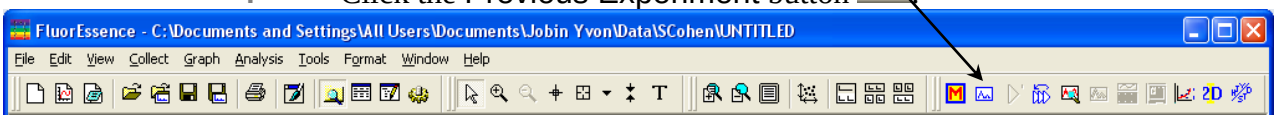
d Using the left and right arrows on the keyboard, move the cursor to the top of the peak.

e Read the *x*-value of this plot: this is the wavelength of the peak.

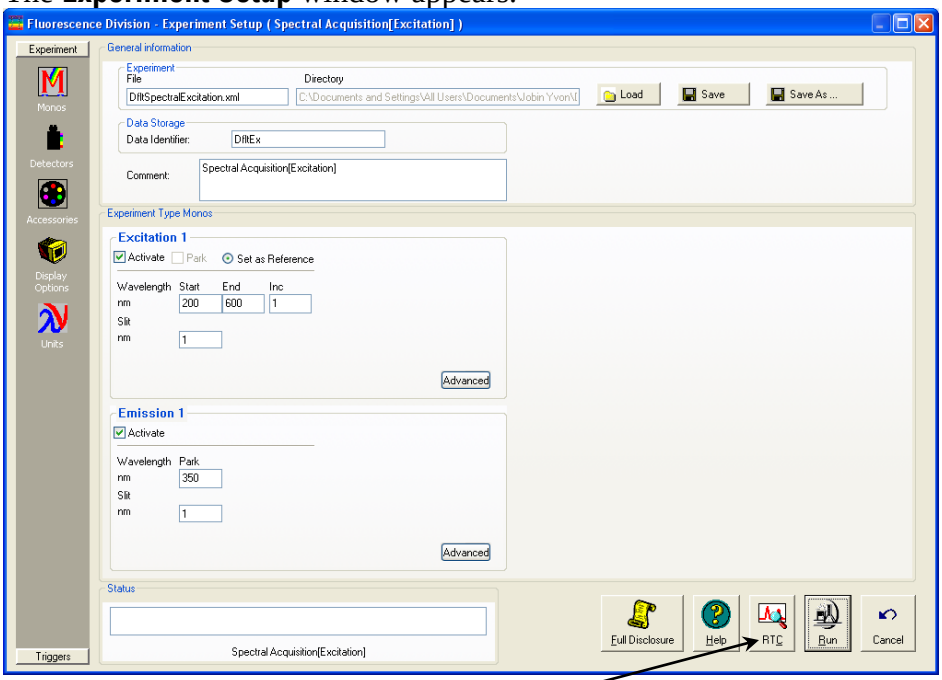


This example shows the peak actually at 477 nm, which is 10 nm too high. **Therefore we must recalibrate the monochromator.**

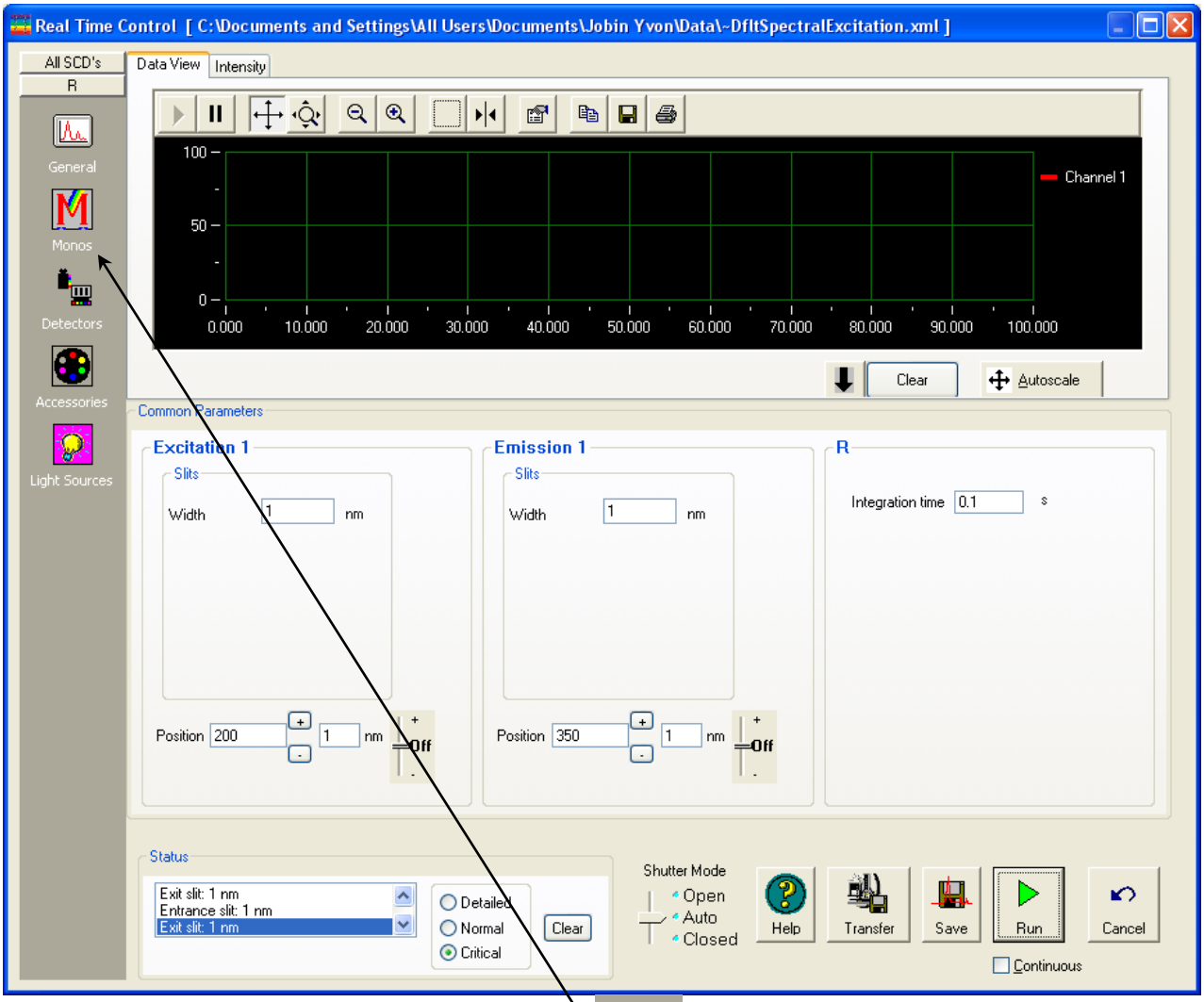
f Click the Previous Experiment button .



The **Experiment Setup** window appears.

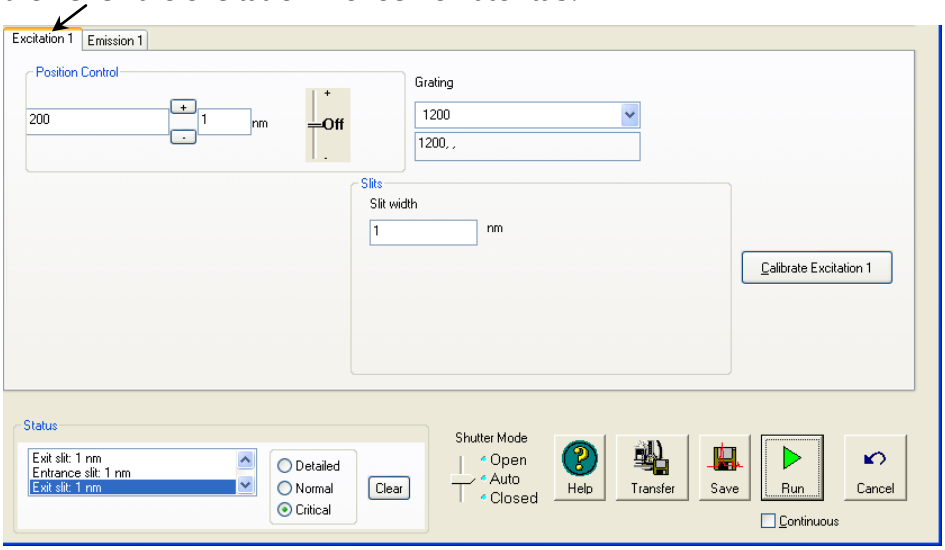


g Click the RTC button on the lower right.
The **Real Time Control** window opens:

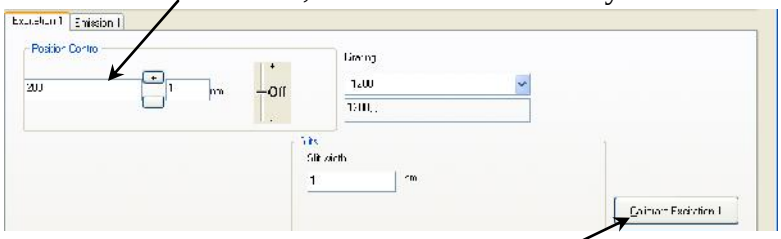


h Click the Monos icon 

to view the monochromators' index card, then click the excitation monochromator tab:



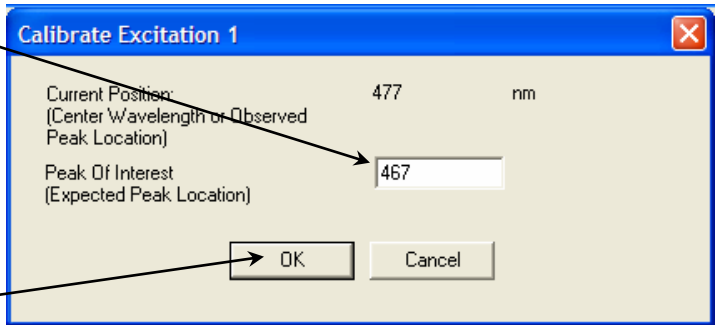
i Enter the current, observed position (here, 477 nm) of the peak in the Position Control field, then hit the `Enter` key.



j Click the Calibrate Excitation 1 button.
The **Calibrate** window opens:

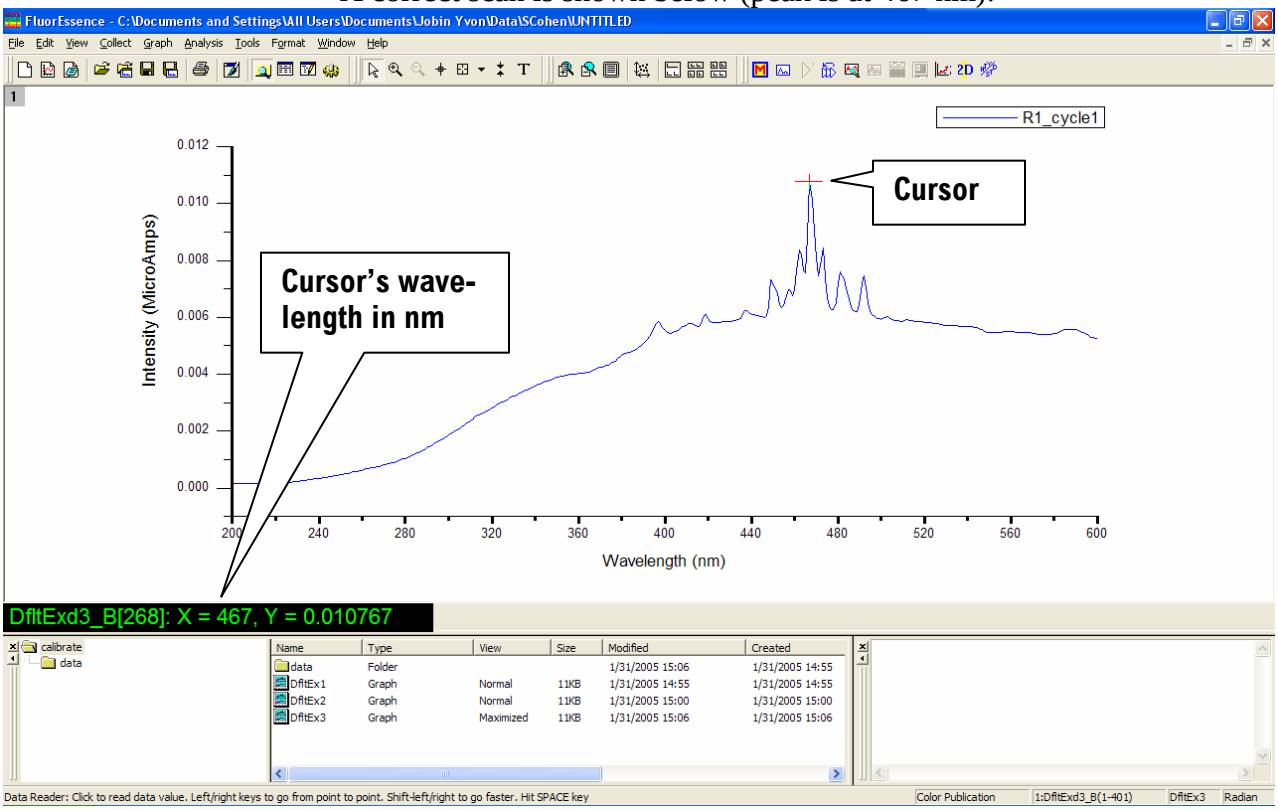
k In Peak Of Interest, enter the actual or expected position of the peak (it ought to be 467 nm).

l Click the OK button.



m At the bottom right of the **Real Time Control** window, click the Cancel button.

n In the **Experiment** window, click the Run button to confirm the correct peak position.
A correct scan is shown below (peak is at 467 nm):

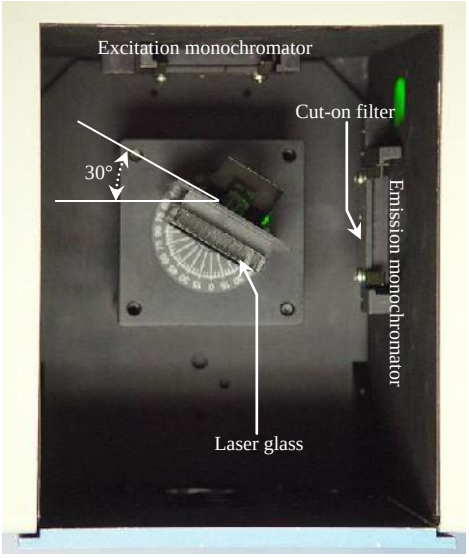


Emission-monochromator calibration check

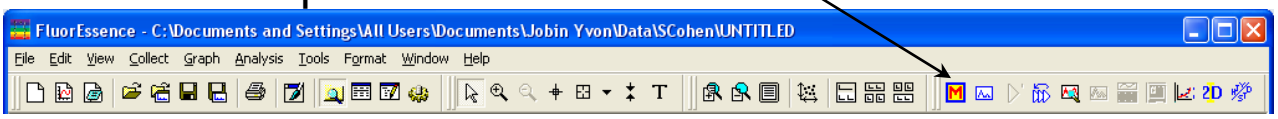
Note: *The emission-monochromator calibration of the instrument is directly affected by the calibration of the excitation monochromator.*

This calibration check verifies the wavelength calibration of the emission spectrometer with the emission detector. It is an emission scan of the two major IR luminescence peaks from a sample of laser-glass. This check should be performed after the xenon-lamp scan. When completed, the performance of the system has been verified.

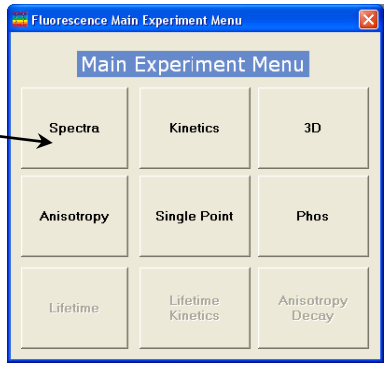
1. Switch the optional FF-RA switch to RA (right-angle mode).
2. Insert the laser-glass into the solid-sample holder, and a RG830 Schott 830 nm cut-on filter in the filter-holder.
3. Insert the solid-sample holder into the sample compartment, and rotate it to 30°.



4. Close the sample compartment's lid.
5. Start FluorEssence™.
   The main **FluorEssence** window appears.
6. In the main **FluorEssence** window, choose the Experiment Menu button.



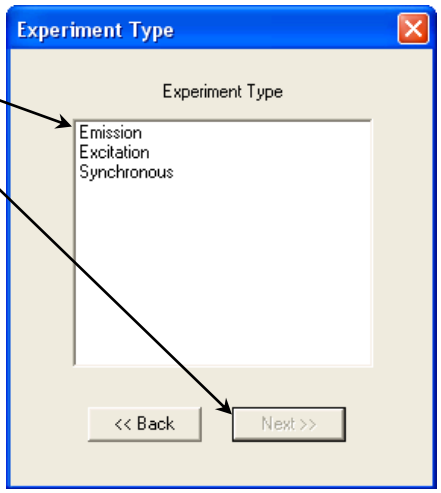
   The **Fluorescence Main Experiment Menu** appears.



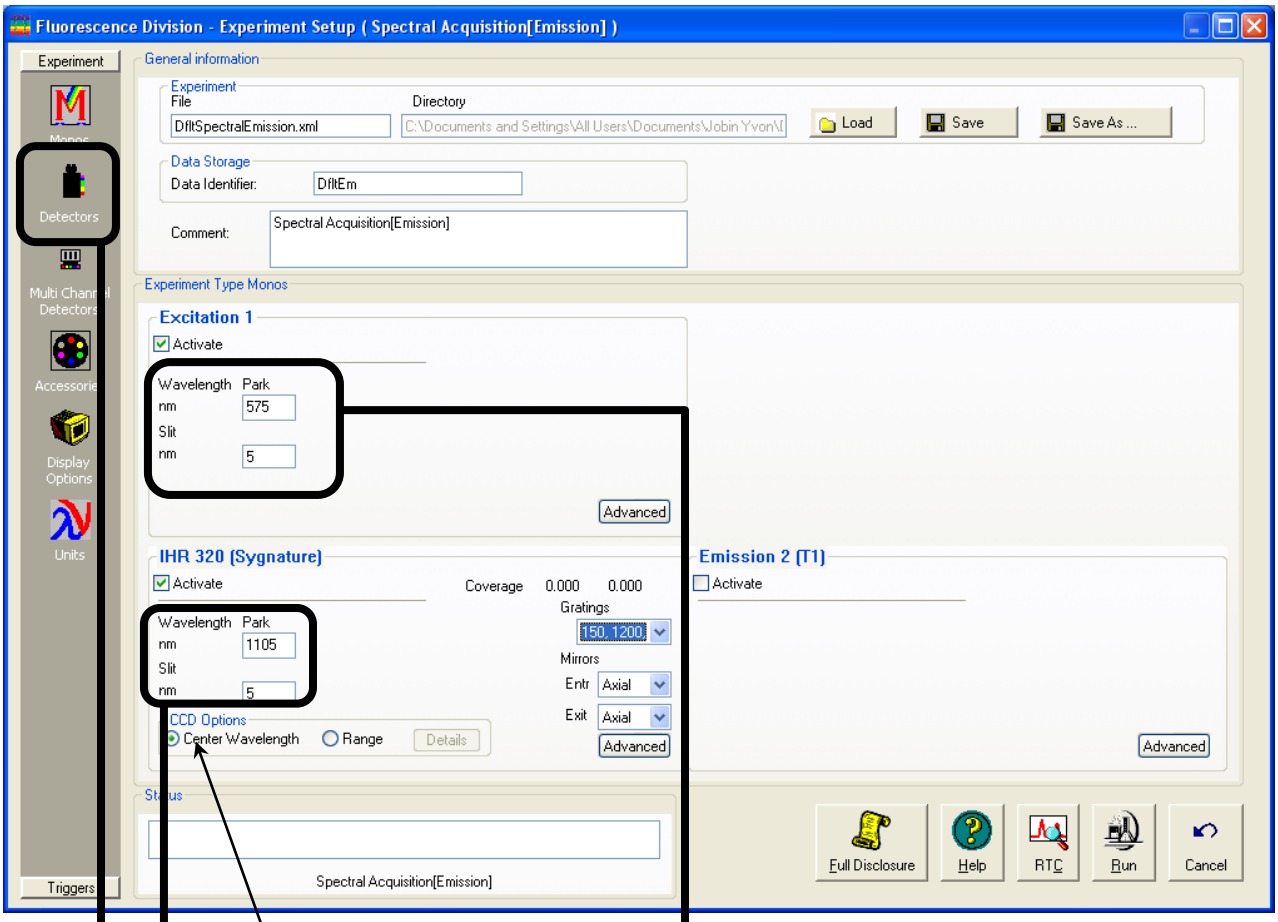
7. Choose Spectra.
   The **Experiment Type** window appears:

8 Choose Emission, then click the Next >> button.

The water-Raman experiment automatically loads.



Note: *The parameters for running the emission calibration are different from a standard water-Raman scan, and so will be changed as follows.*

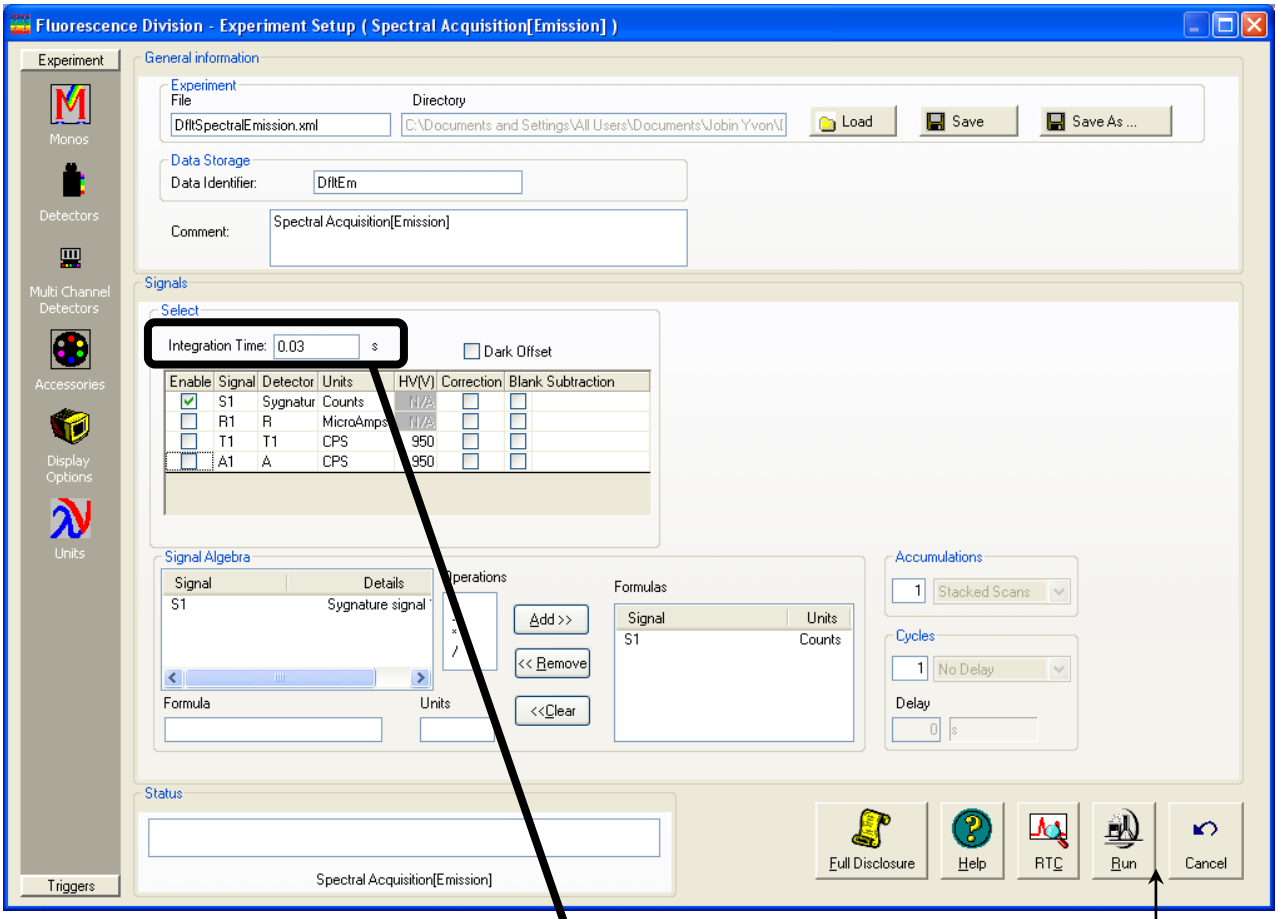


a Change the excitation monochromator Park wavelength to 575 nm, and the excitation Slit width to 5 nm.

b Change the emission spectrometer Start wavelength to 1210 nm (for the 100 gr × 800 nm grating) or 1105 nm (for the 150 gr × 1200 nm grating) and the Slit bandpass to 5 nm.

c Set the CCD Options to the Center Wavelength radio button.

d Click the Detectors icon.

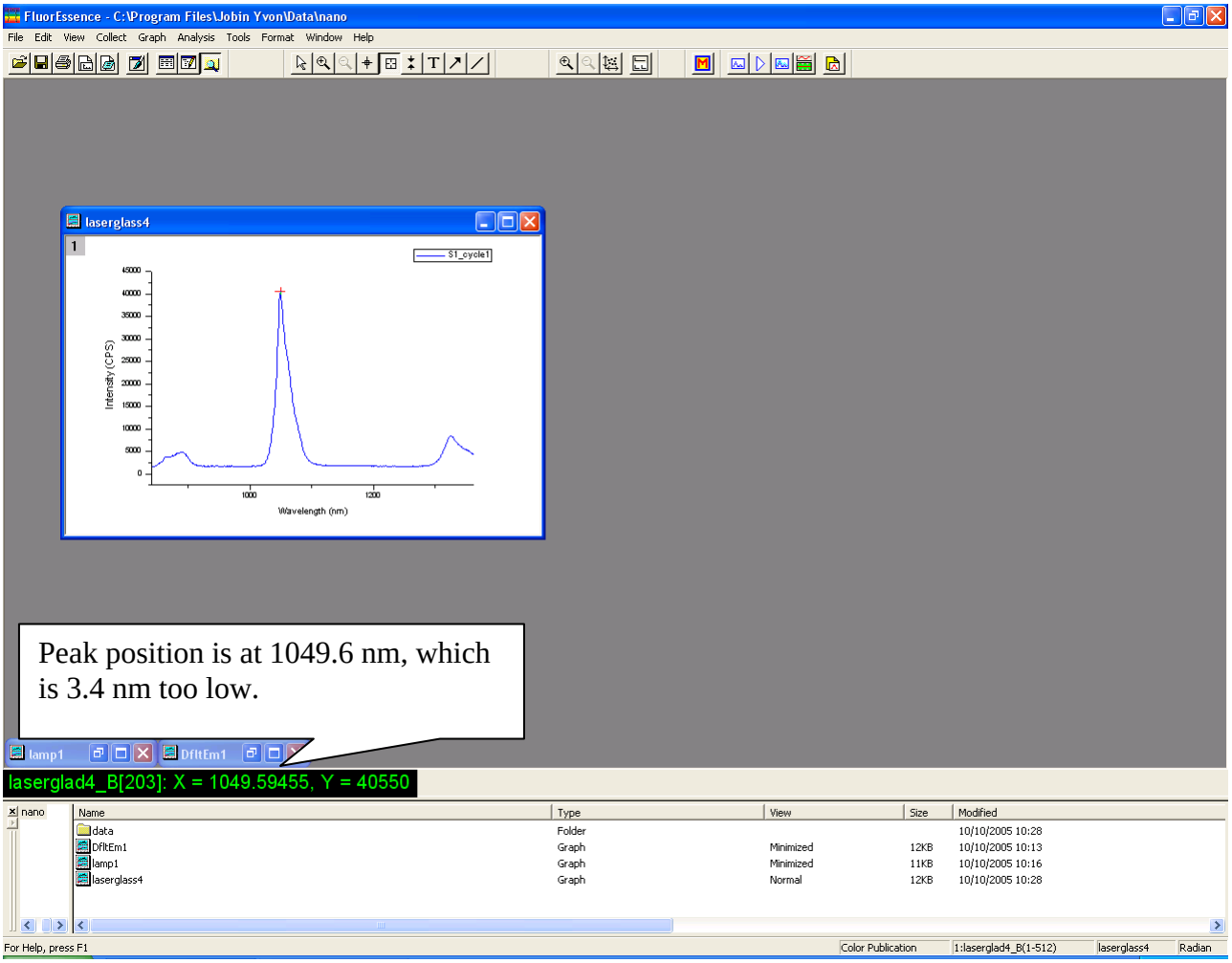


e Change the Integration Time to 0.03 s.

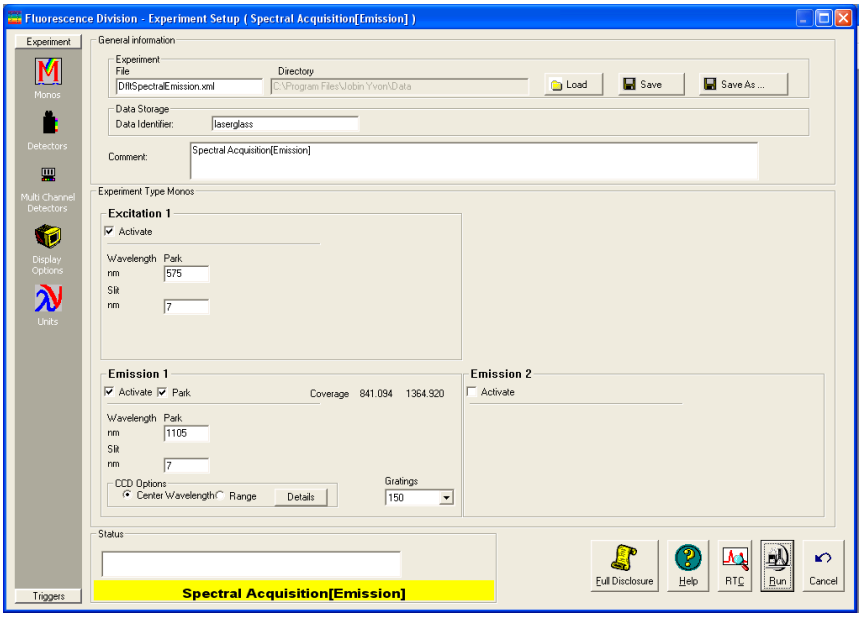
f Click the Run button.

The emission scan starts. The data are transferred to the Origin® window. The peak position on the scan below shows 1049.6 nm, which is 3.4 nm too low. Therefore, we must recalibrate the emission spectrometer.

Note: *Because of the wider groove spacing, precise calibration of the 150 grooves/mm and 100 grooves/mm gratings is mechanically more difficult, so it is harder to get the 1053 nm peak exactly correct. Use a tolerance of ± 1 nm.*

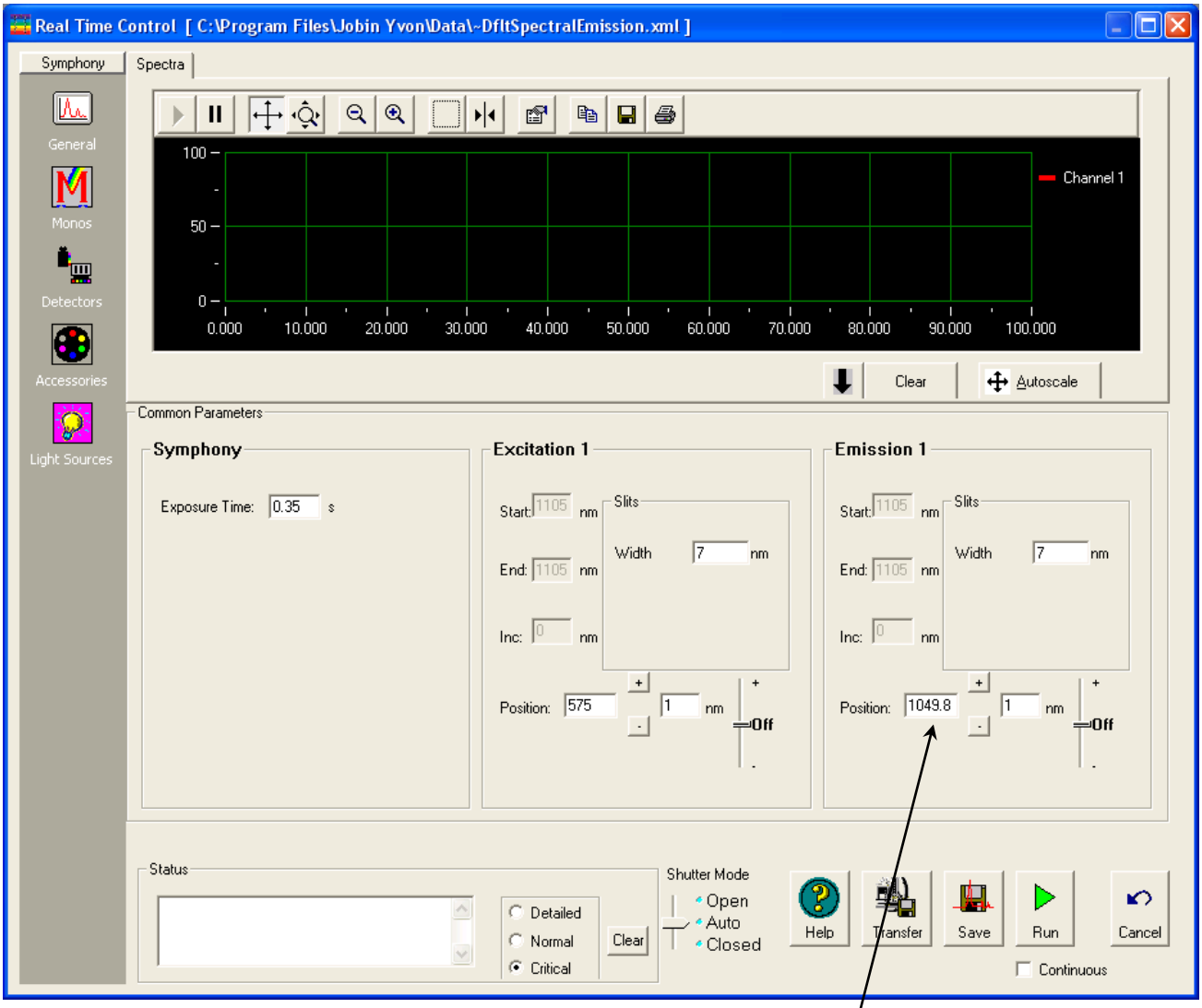


9 Recalibrate the emission spectrometer, if necessary.



a In the **Experiment Setup** window, click the RTC button.

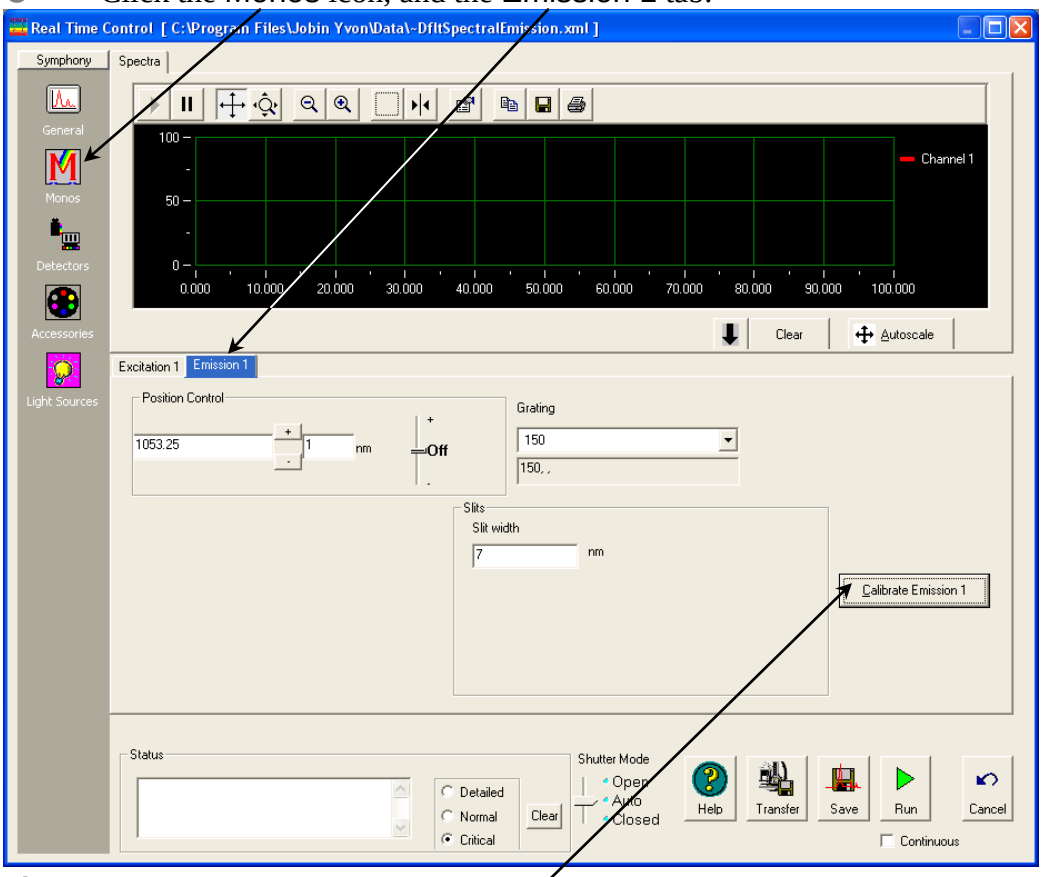
The **Real Time Control** appears.



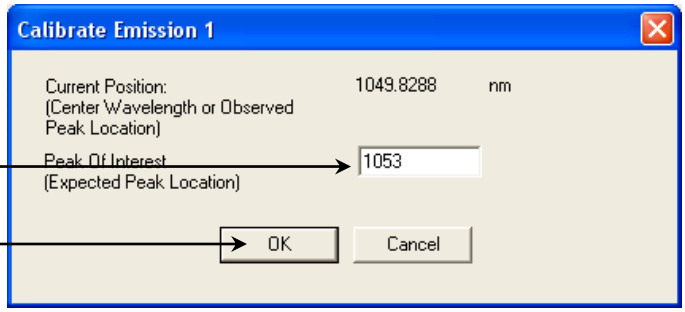
b In the Emission 1 area, set the Position field to 1049.6 nm, the current incorrect position, and hit the `Enter` key.

Note: *In the* ***Real Time Control****'s General screen, all changes must be followed by hitting the* `Enter` *key.*

c Click the Monos icon, and the Emission 1 tab.



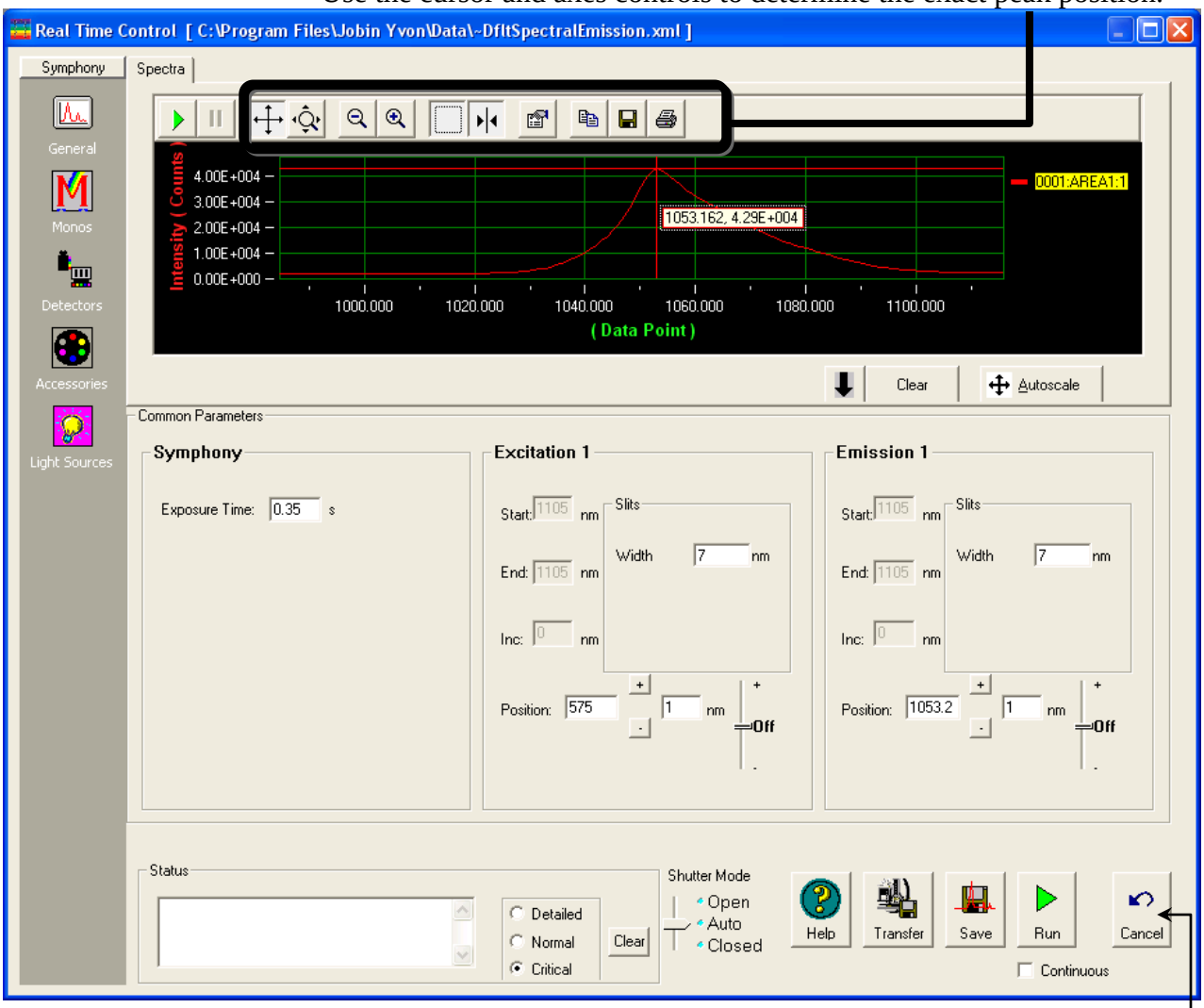
d Click the Calibrate Emission 1 button.
The **Calibrate Emission 1** window opens.



e Enter the correct Peak of Interest position, 1053, then click the OK button.
The **Calibrate Emission 1** window disappears.

f In the **Real Time Control** window, click the Run button.
A scan of the laser-glass peak appears on the black graphing area.

Use the cursor and axes controls to determine the exact peak position.



10 If the observed peak is not at 1053 ± 0.5 nm, re-calibrate the emission spectrometer.

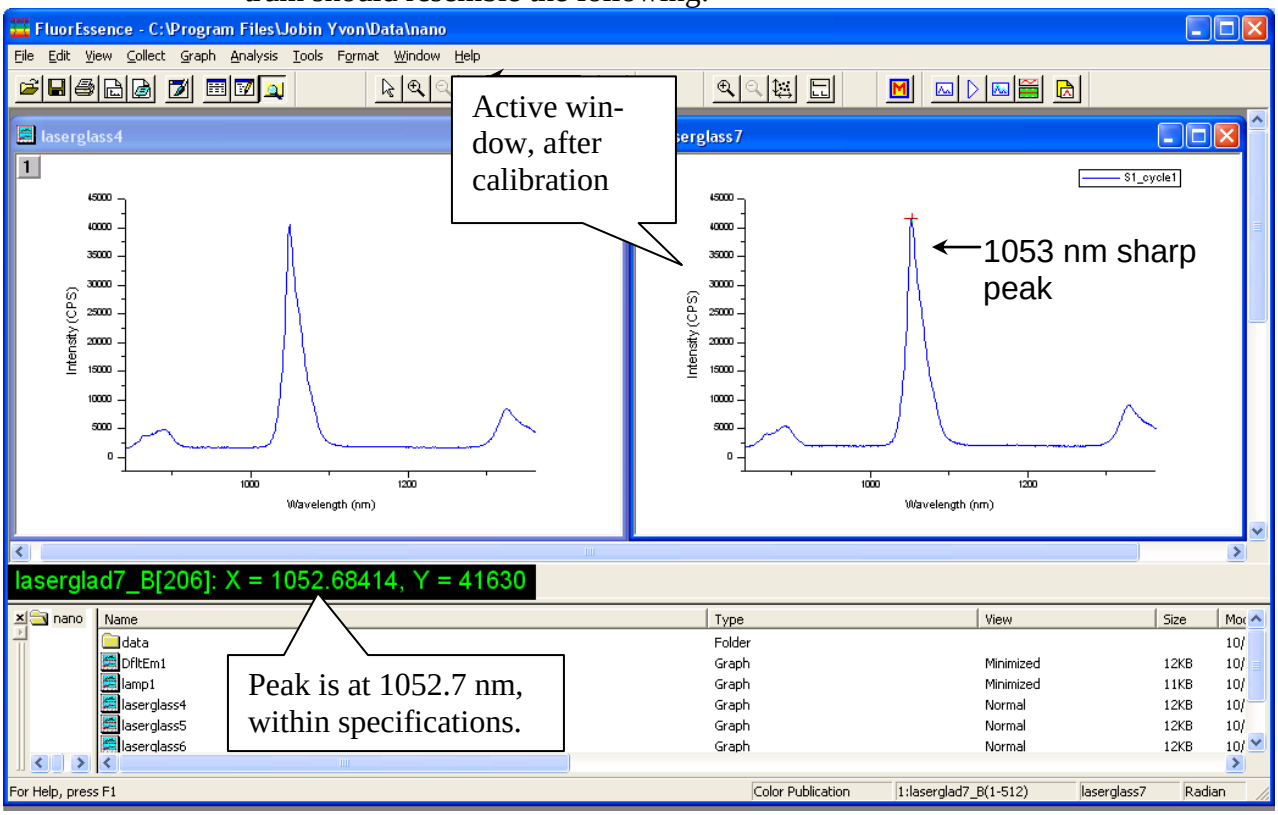
Note: *For 150 grooves/mm and 100 grooves/mm gratings, use a tolerance of 1053 ± 1 nm.*

11 If the peak is at 1053 ± 0.5 nm and the excitation monochromator has been calibrated, then the NanoLog® is within specifications.

12 Click the Cancel button to close the **Real Time Control**.

13 In the **Experiment Setup** window, click the Run button to run and save a scan for diagnostic purposes.

The laser-glass scan runs, and sends the data to the Origin® window. Your spectrum should resemble the following:



14 Calibration is complete.

Note: *Observed throughput (and hence peak intensity) is affected by lamp age and alignment, slit settings, and sample purity. As the xenon lamp ages, the throughput of the system will decline slowly. Therefore, low laser-glass peak intensity may indicate a need to replace the xenon lamp.*

Notes on excitation and emission calibration

- HORIBA Scientific recommends recording the number of hours of xenon-lamp use in a log (see sample sheet in *Xenon Lamp Information & Record of Use Form*).
- Additionally, you may want to record the water-Raman intensity daily or weekly.
- The lamp is rated for 1800–2000 h, but if the laser-glass intensity starts to drop, you may wish to change the lamp sooner.

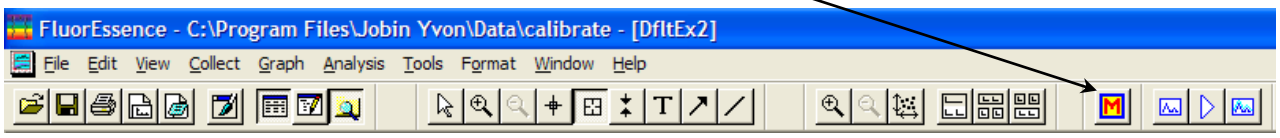
Caution: *To avoid explosion from lamp failure, do not allow xenon lamp to exceed rated lifetime. One clue to imminent failure may be extremely low water-Raman intensity. Please note the hours the xenon lamp has been used.*

Using iHR with FluorEssence™

Experiment Setup window

To run a CCD acquisition with an instrument layout that includes an iHR,

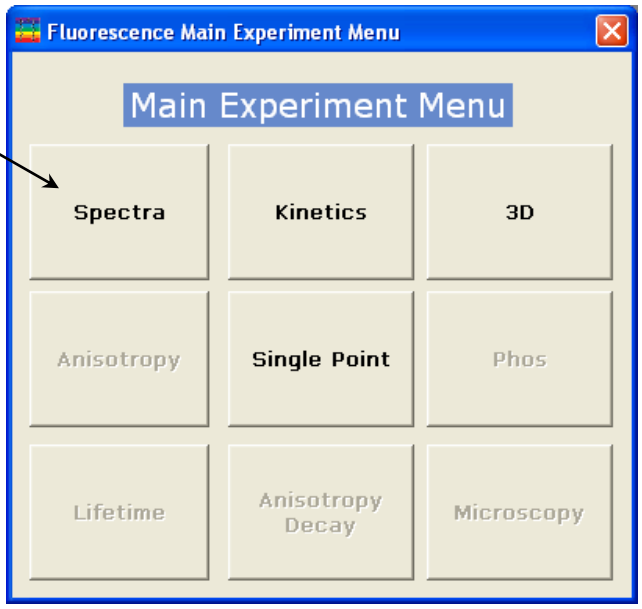
1 On the main **FluorEssence** toolbar, select the Experiment Menu button:



The **Fluoresence Main Experiment Menu** appears:

2 Choose Spectra.

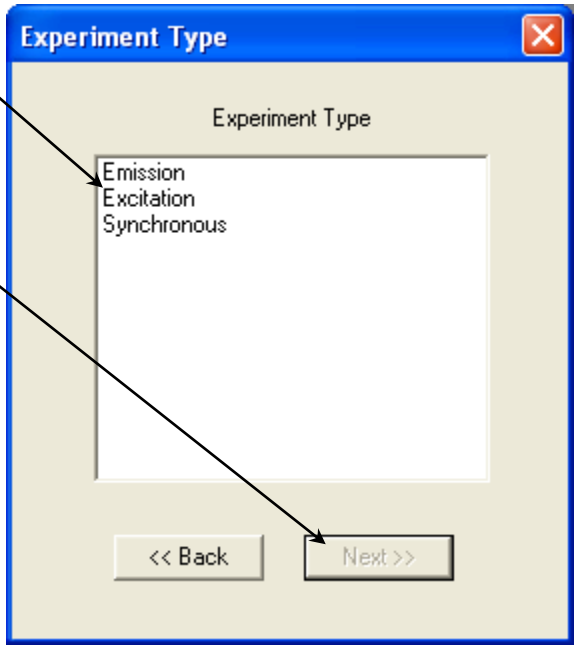
Note: *Certain types of experiments are grayed out.*



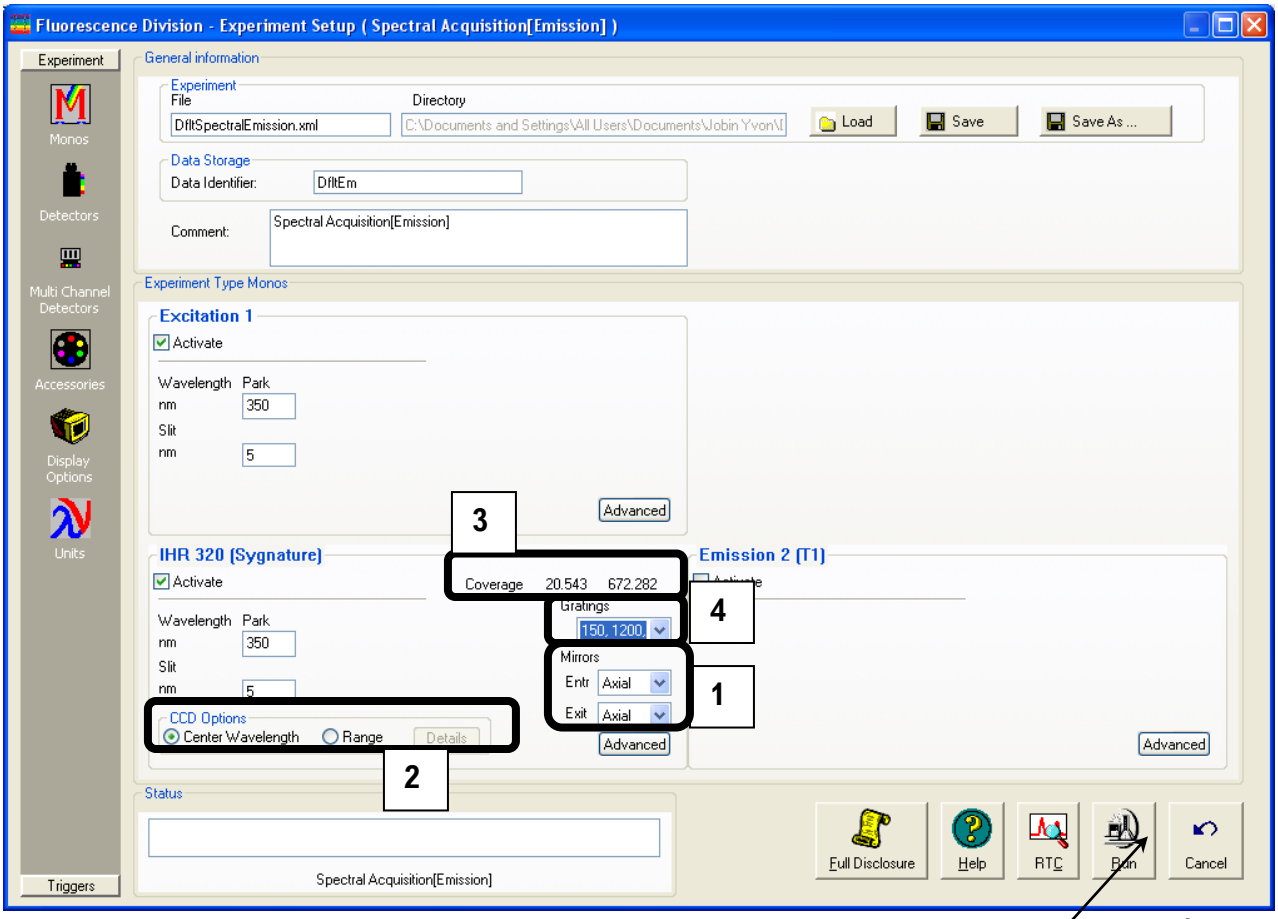
The **Experiment Type** window appears:

3 Choose the experiment type, then click Next >>.

The experiment automatically loads with the **Experiment Setup** window.



4 Use the default parameters or adjust them.



This is an example using an emission scan-type. Under the Monos icon, functions specific to the iHR are available, such as:

1 Mirrors
2 CCD Options
3 Coverage information
4 Gratings

These and other parameters are discussed on the following pages.

5 Complete all required parameters.

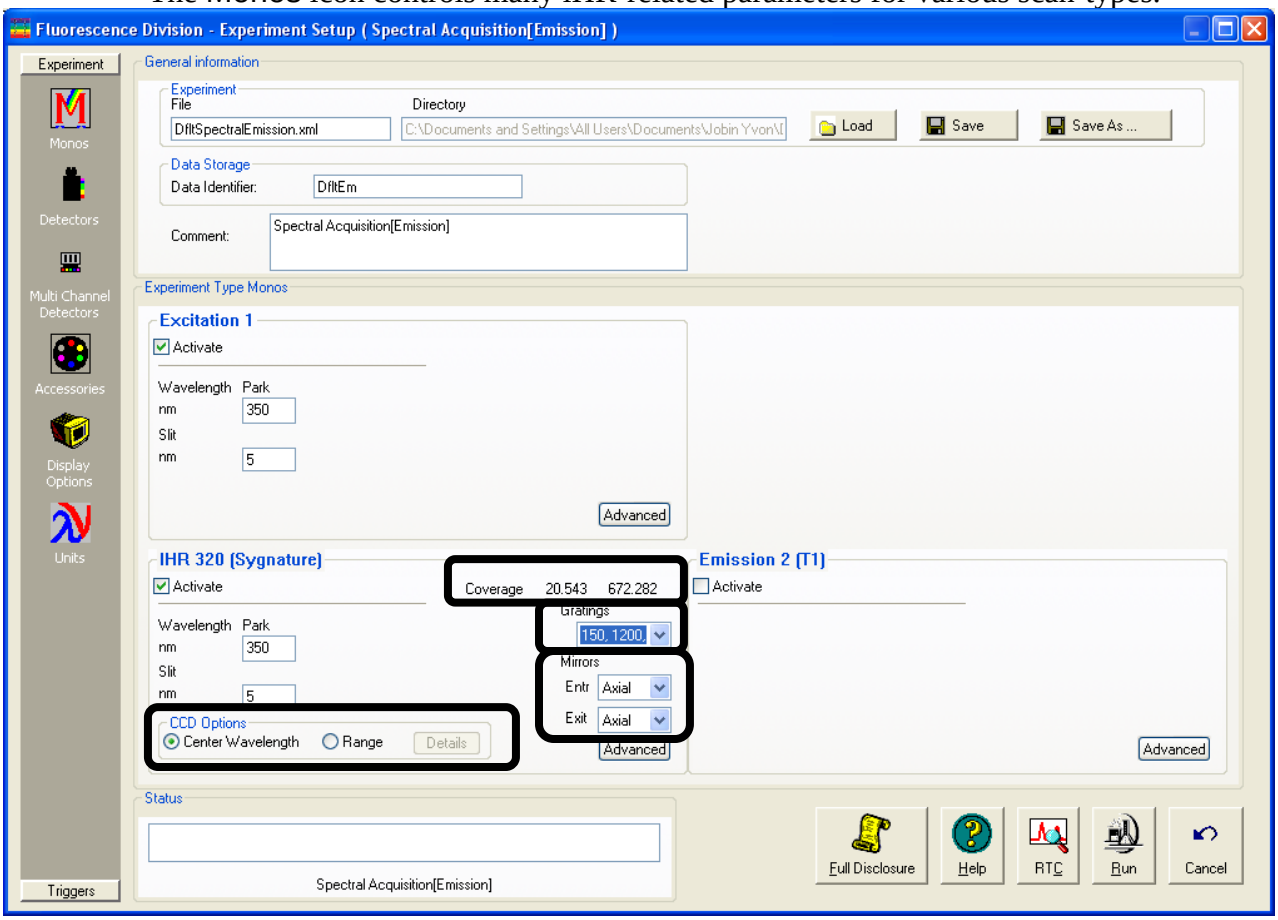
6 Include also detector-related parameters under the Detectors or Multi Channel Detectors icons.

7 Click the Run button.

The experiment begins.

Monos icon

The Monos icon controls many iHR-related parameters for various scan-types.

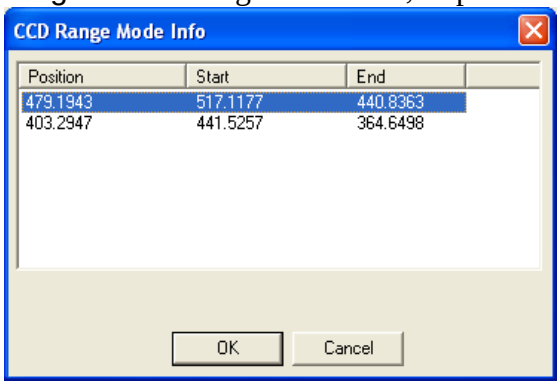


Mirrors

The Mirrors area controls the entrance and exit flip-mirrors' positions. Choose Axial or Lateral positions for each mirror, from drop-down menus.

CCD Options

In the CCD Options area, choose a Range of wavelengths to detect, or pick a Center Wavelength, with the radio buttons. With the Center Wavelength option, the iHR shows a wavelength to Park the detector, instead of Start and End wavelengths. Click the Details button to reveal the **CCD Range Mode Info** window. This shows the exact range of wavelengths the detector will detect.



Coverage

Coverage shows how many wavelength ranges are used on the CCD detector.

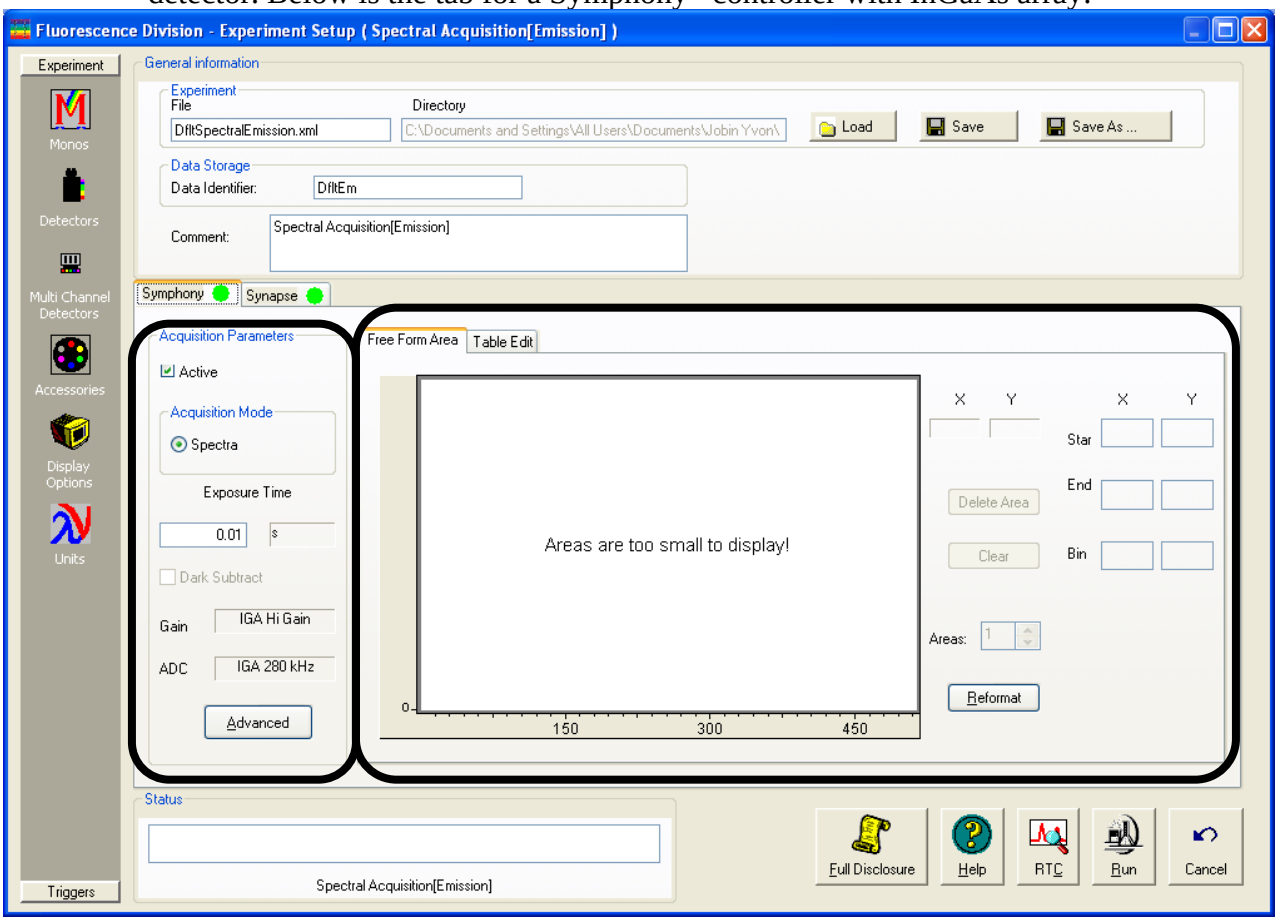
Gratings

The Gratings area allows you to choose, from a drop-down menu, which grating to use.

Note: *To choose a grating, the gratings must have been previously entered using System Configuration. The Turrets checkbox in System Configuration also must be enabled to allow the turret to rotate.*

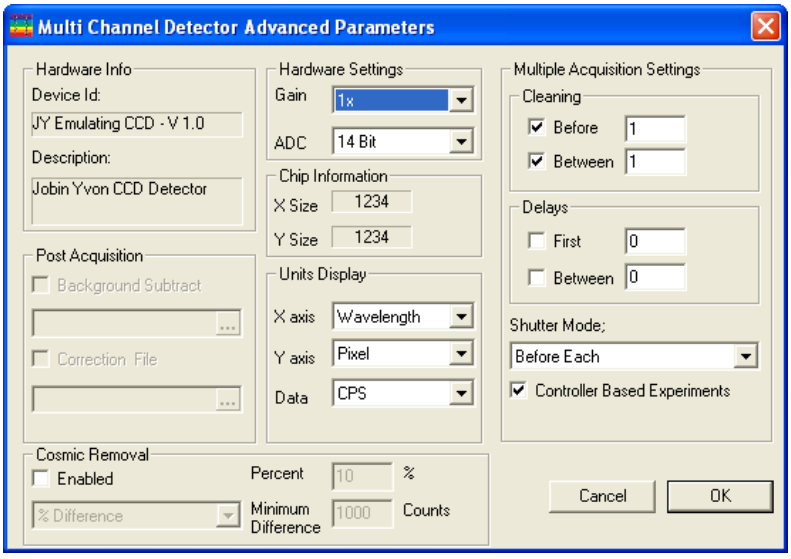
Multi Channel Detectors icon

To adjust parameters related to the CCD detector, click on the Multi Channel Detectors icon in the **Experiment Setup** window. If there is more than one multichannel detector, click the tab for the desired detector. Below is the tab for a Symphony® controller with InGaAs array:

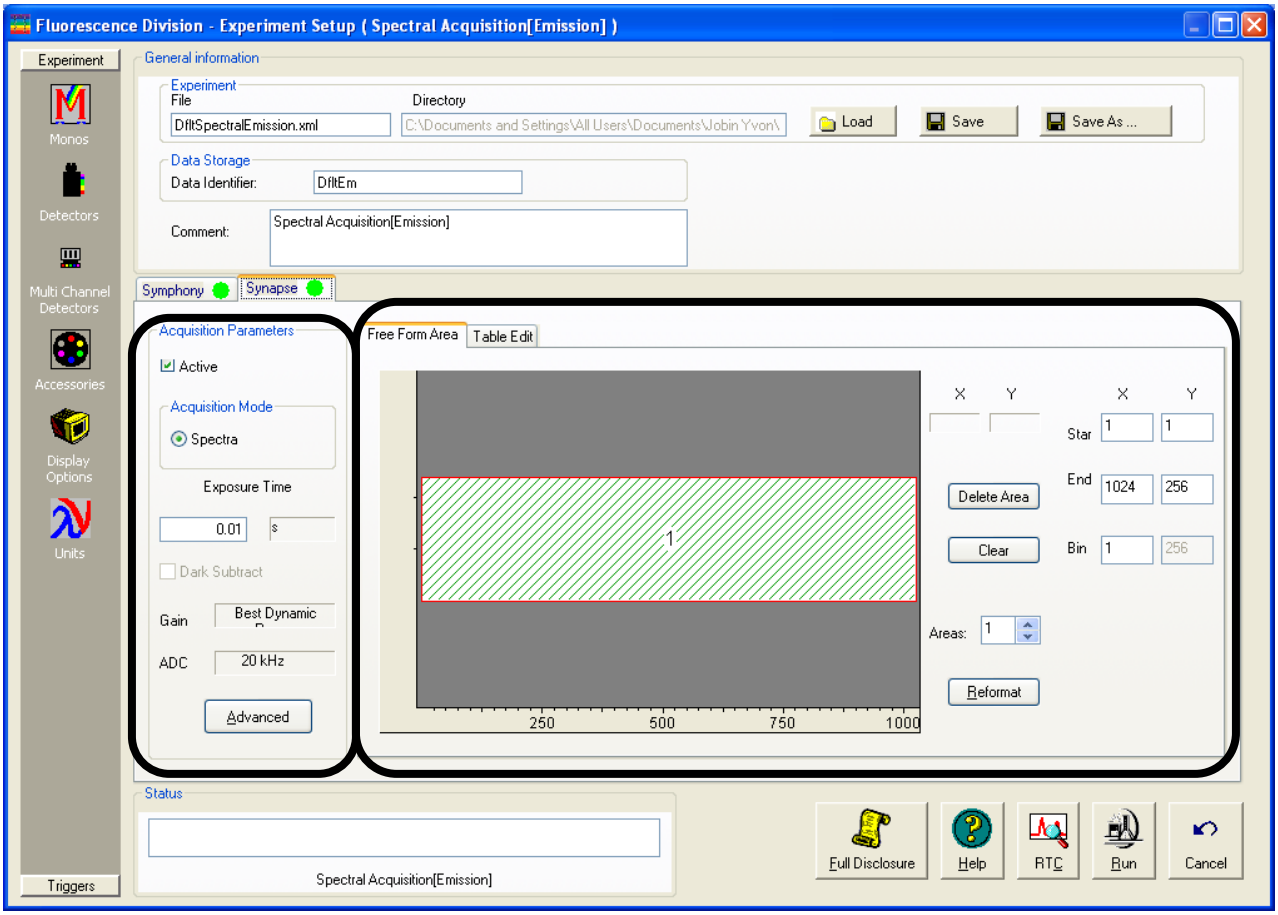


Acquisition Parameters

Under Acquisition Parameters, change the Exposure Time, and view the Gain, and the precision of Analog-to-Digital Conversion (ADC). Clicking the Advanced button opens the **Multi Channel Detector Advanced Parameters** window, to adjust Gain, Units Display, Cosmic Removal, ADC, and other parameters.



Below is the window for a Synapse with CCD detector:

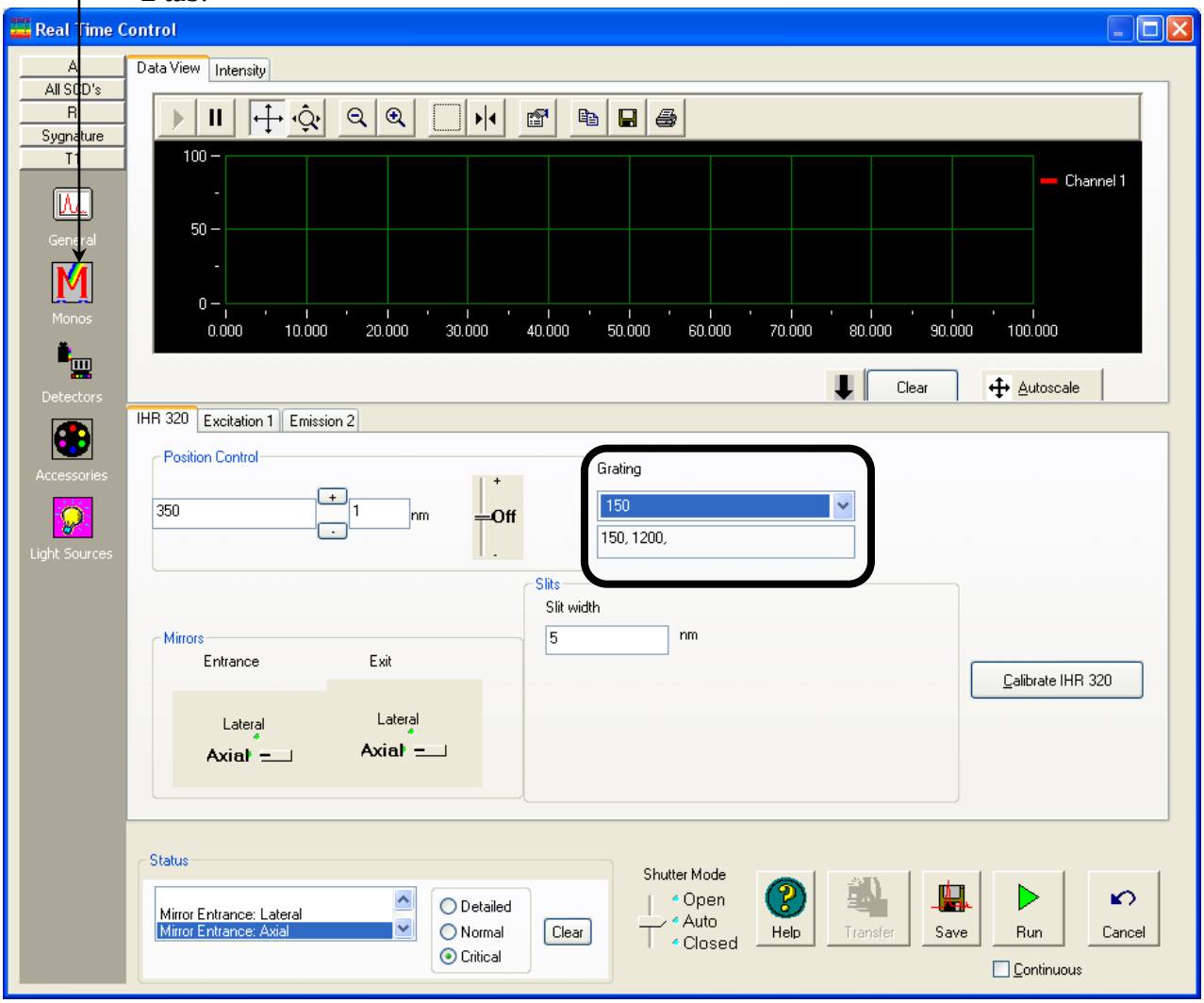


Pixel editing

There are two methods of choosing which pixels are used on the CCD: graphically or tabular. To choose the active pixel areas graphically, click the Free Form Area tab, and draw with the cursor or enter the coordinates under the X and Y columns. Choose the number of active areas with the Areas field. To enter the active areas as a table, choose the Table Edit tab. In both methods, click the Reformat button to clear the existing description and start over.

Real Time Control window

In the **Real Time Control** window, some iHR parameters can be adjusted in real time. To gain access to the iHR in the **Real Time Control**, click the Monos icon. The iHR, when used as an emission spectrometer in L-format, may be listed under the Emission 1 tab.



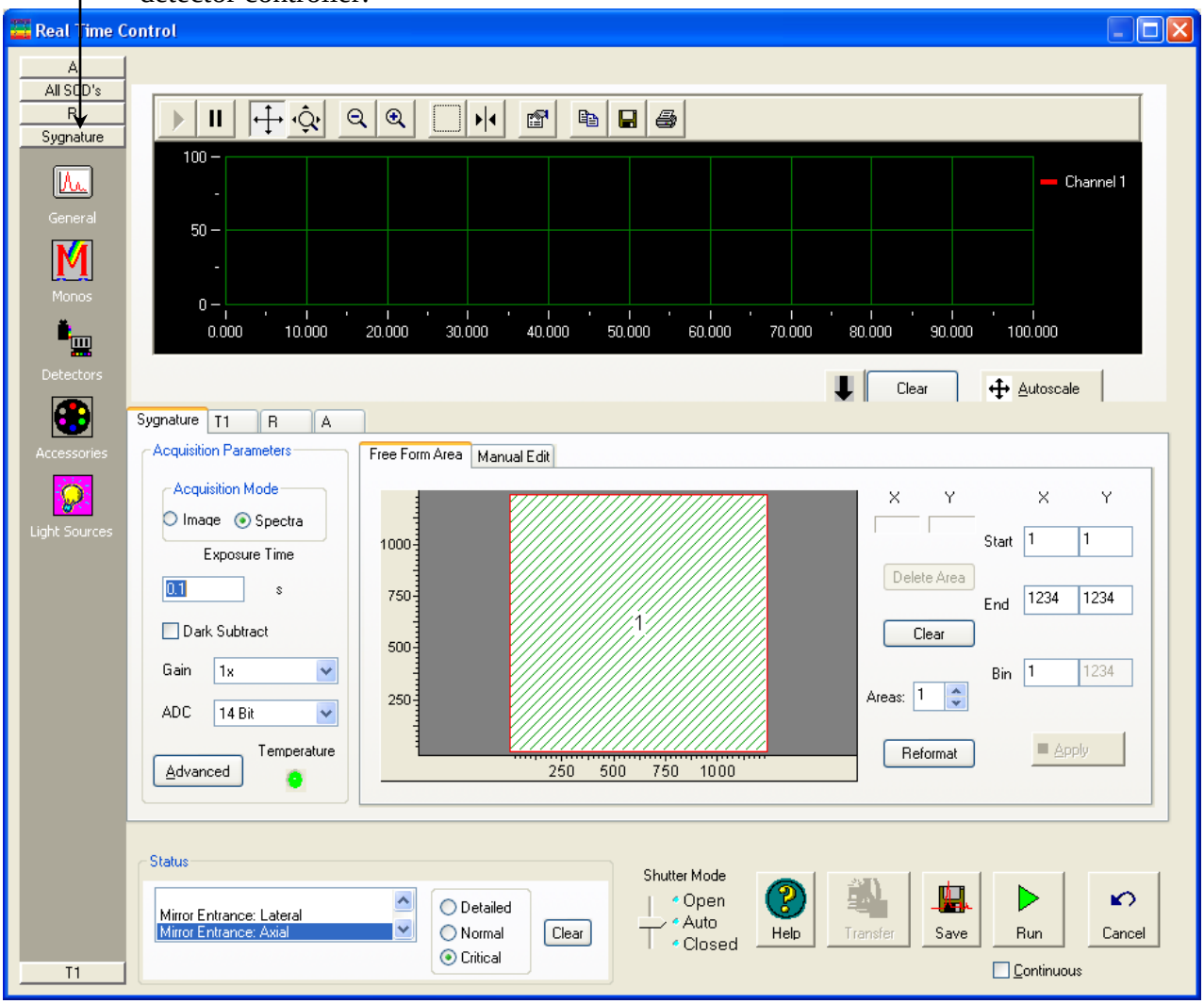
Grating

Rotate the turret in real time by clicking on the Grating area, and choosing the desired grating (listed as grooves/mm) from the drop-down menu.

Note: *To choose a grating, the gratings must have been previously entered using System Configuration. The Turrets checkbox in System Configuration also must be enabled to allow the turret to rotate.*

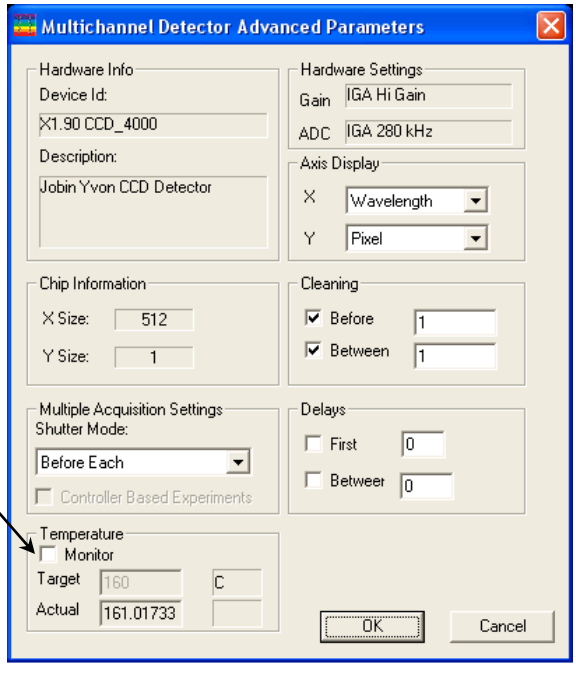
Monitoring temperature of the detector

Click the Synapse or Symphony button to access the functions for the multichannel-detector controller.



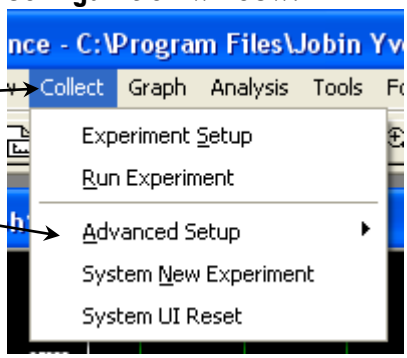
In the Acquisition Parameters area, click the Advanced button to open the **Multichannel Detector Advanced Parameters** window.

Click the Monitor checkbox in the Temperature area to monitor the detector's temperature.



System Configuration window

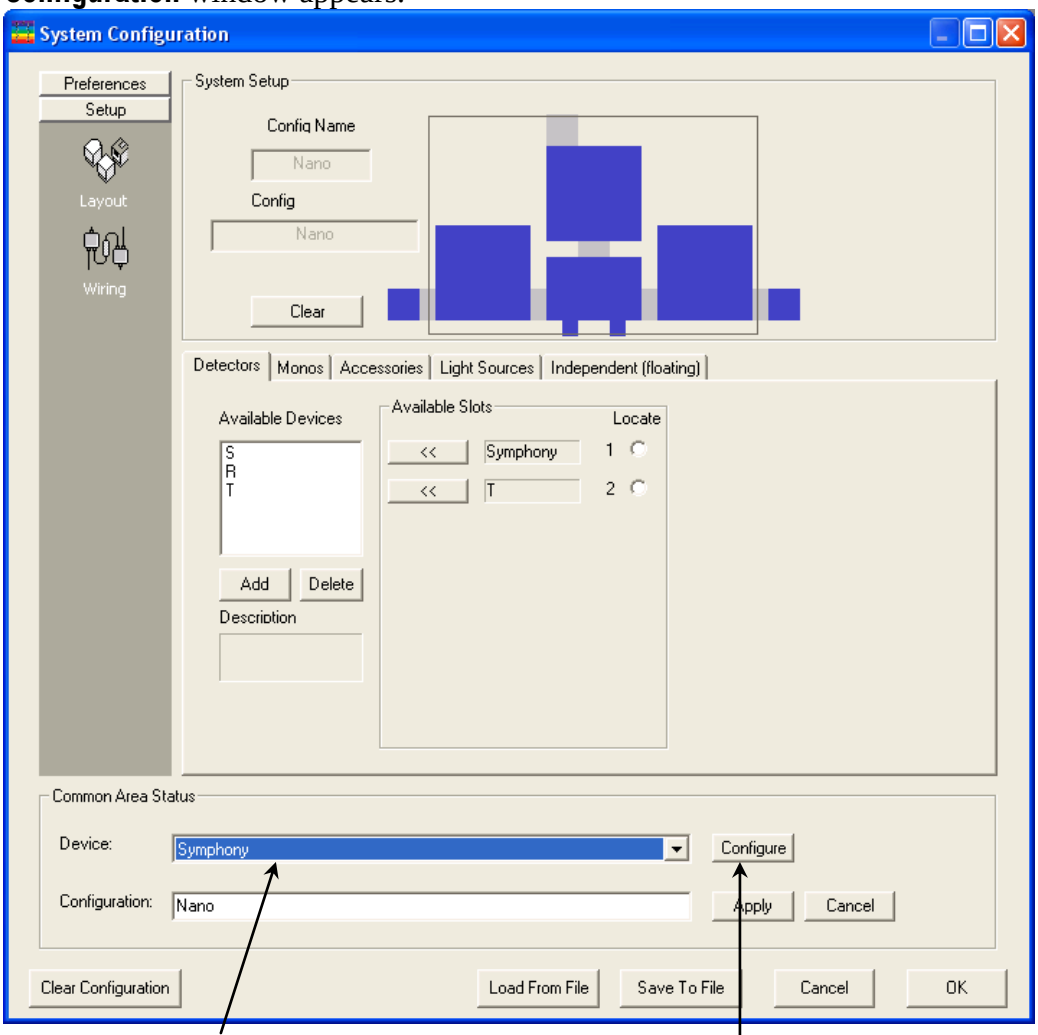
To set the cooled detector's temperature, use the **System Configuration** window.



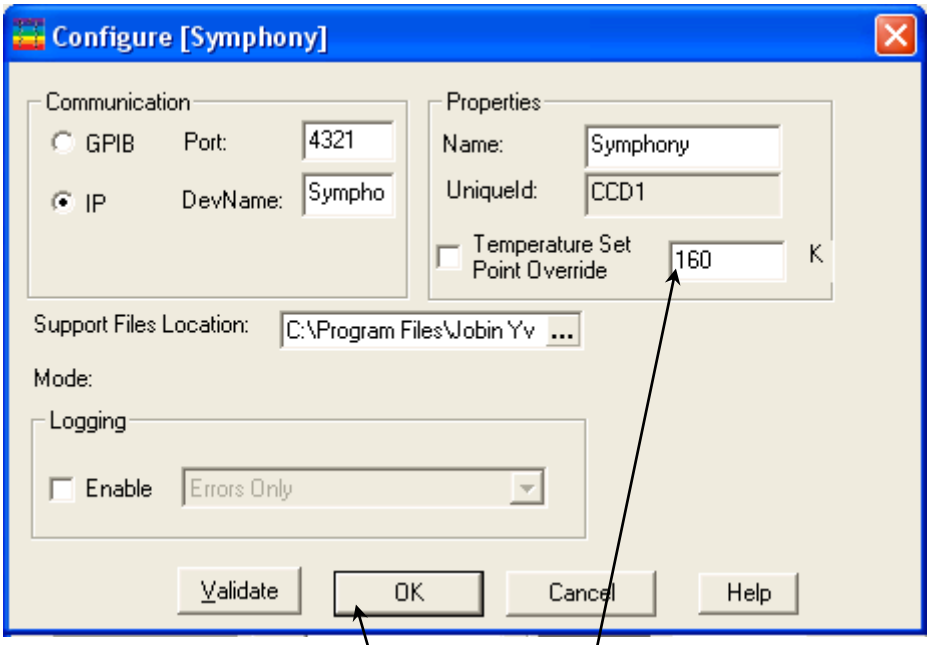
1 In the FluorEssence toolbar, choose Collect.
A drop-down menu appears.

2 Choose Advanced Setup.
A sub-menu appears.

3 Click System Configuration.
If the system has not been initialized, choose the instrument, and let the system initialize. Then redo steps 1–3. The **System Configuration** window appears.



4 From the Device drop-down menu, choose Symphony, then click the Configure button.
The **Configure [Symphony]** window appears.



5 Enter the desired temperature for the detector, then click the OK button.

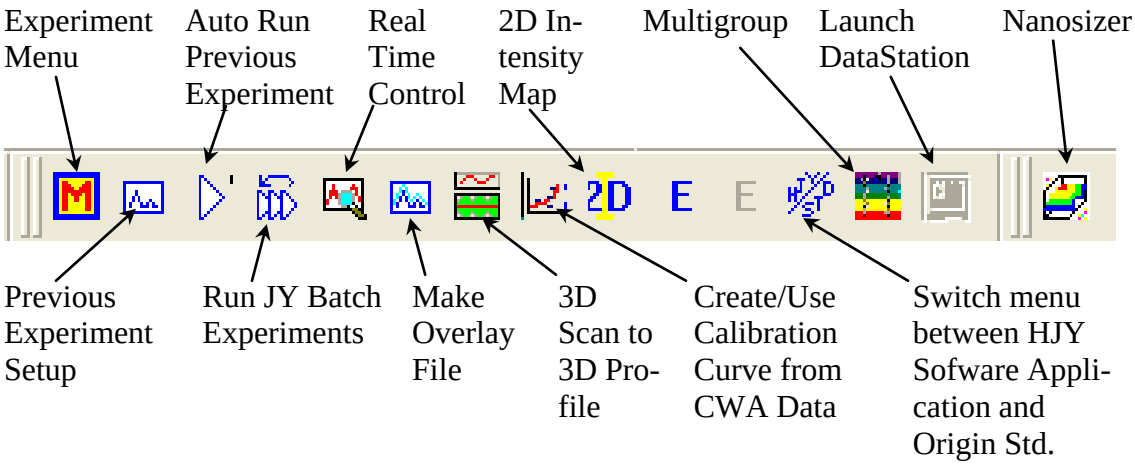
The **Configure [Symphony]** window closes.

6 Click the OK button to confirm and close the **System Configuration** window.

4: Data Acquisition

Introduction to FluorEssence™ and Multigroup

This chapter presents an introduction to some of the special buttons and functions used in FluorEssence™ to record and present data with the NanoLog®. The buttons, located in FluorEssence ™'s main window, are:



For a detailed description of these FluorEssence™ routines, see the *FluorEssence™ User's Guide* and on-line help.

A second program, called Multigroup, offers repeated and sequential fluorescence experiments. Functions such as delays, temperature ramps, and multiple samples and wavelength-groups are allowed within Multigroup. You can start Multigroup from within FluorEssence™, or from a Windows® desktop icon. Multigroup, however, uses only single-point detectors, such as a photomultiplier tube, and does not operate with array detectors.

In addition, methods for determining best excitation and emission wavelengths are presented, in case these wavelengths are unknown for the sample.

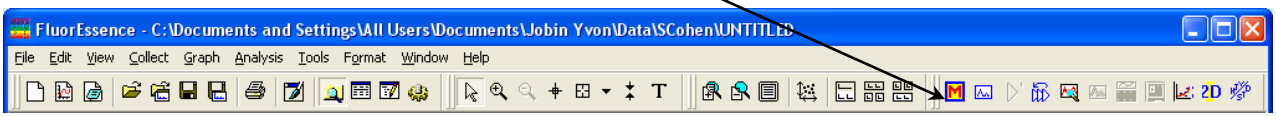
Experiment Menu button

The Experiment Menu button chooses an overall type of experiment to run, such as an emission scan, a phosphorimeter scan, a synchronous scan, etc., based on the instrument and connected accessories, such as a temperature bath, MicroMax, etc. Only those scans that can be run using the available hardware configuration are active; scans that cannot be taken are grayed out.

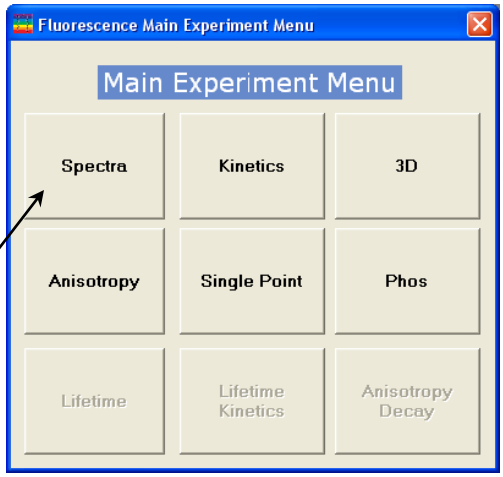
Calibration scans for the NanoLog® use default parameters:

- Excitation monochromator: Spectra/Excitation scan
- Emission monochromator: Spectra/Emission scan

1 To choose an experiment type, click the Experiment Menu button :



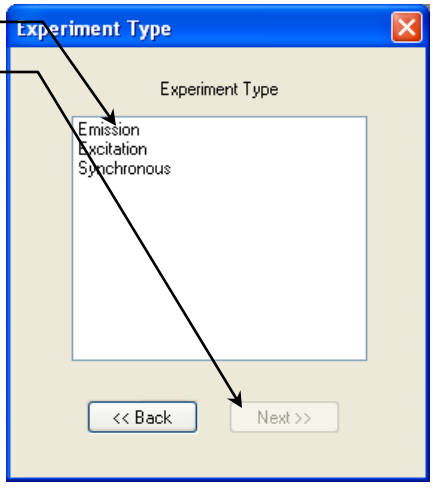
The **Fluorescence Main Experiment Menu** appears:



Note: *Certain scan types are not allowed with the NanoLog®, and are grayed*

2 Choose an experiment type.

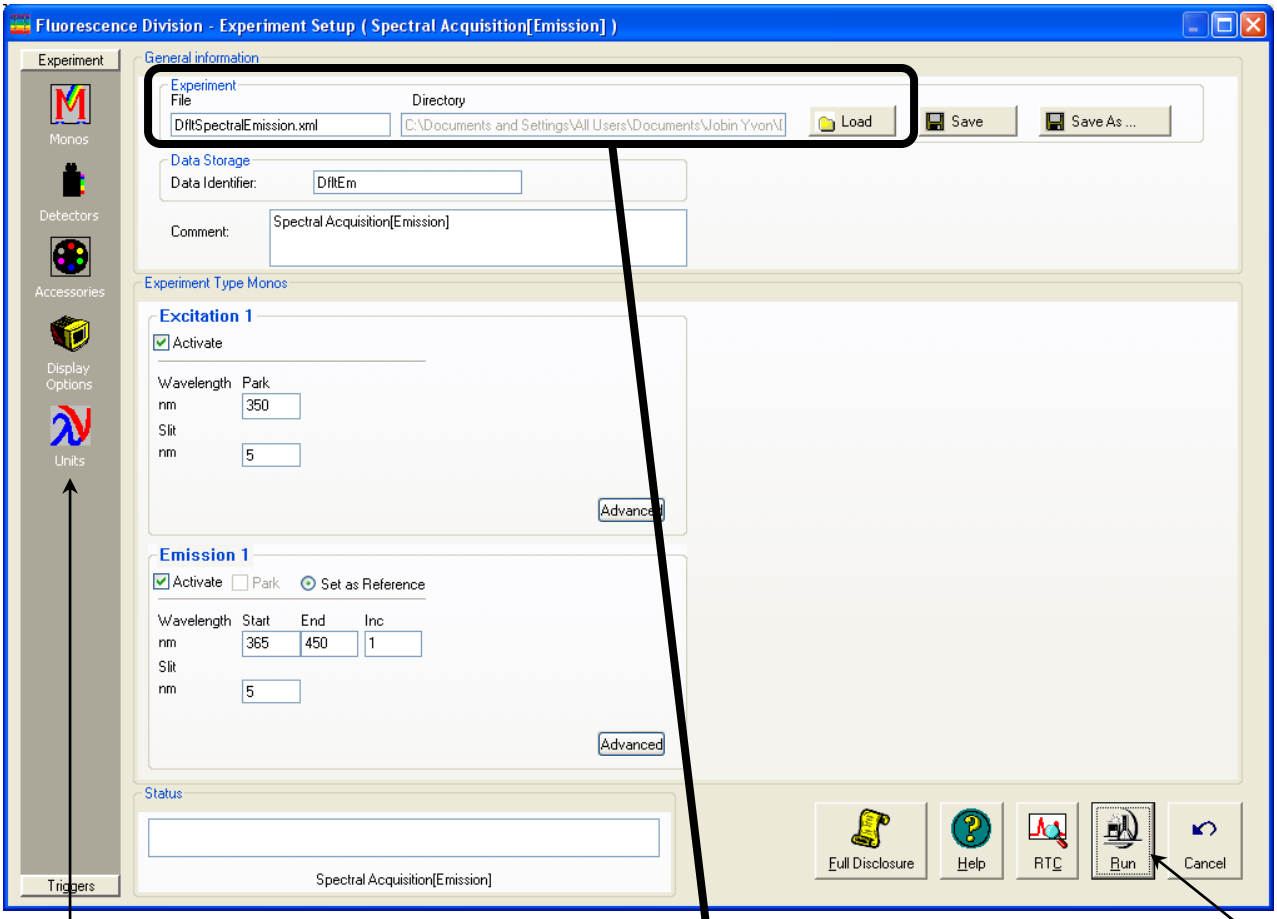
The **Experiment Type** window appears.



3 Choose a subtype of experiment, and click the Next >> button.

Note: *Some experiment types (for example, Kinetics) do not have subtypes, so no* ***Experiment Type*** *window appears. For array detectors, Excitation and Synchronous scans are not available.*

The **Experiment Setup** window appears:



4 Click the Experiment File field, and enter a new file name or select a previously saved file.

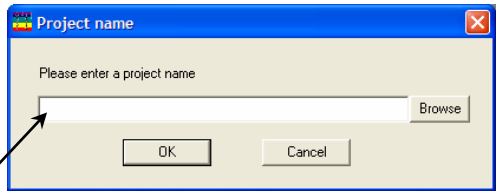
5 Verify that experimental parameters are correct.

Be sure to check all parameters under all icons in the left-hand column.

6 Insert the sample into the sample compartment, and close the sample compartment's cover.

7 Click the Run button.

The collected spectrum is displayed on the **Intermediate Display** screen. After all data are recorded, the **Intermediate Display** vanishes. For a new project, the **Project Name** window appears:



8 Enter a name for the entire project, or browse for an existing project name with the Browse button, then click the OK button.

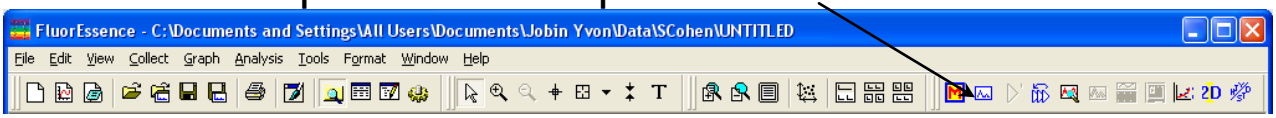
All data are moved to Origin®'s graph window for post-processing.

Previous Experiment Setup button

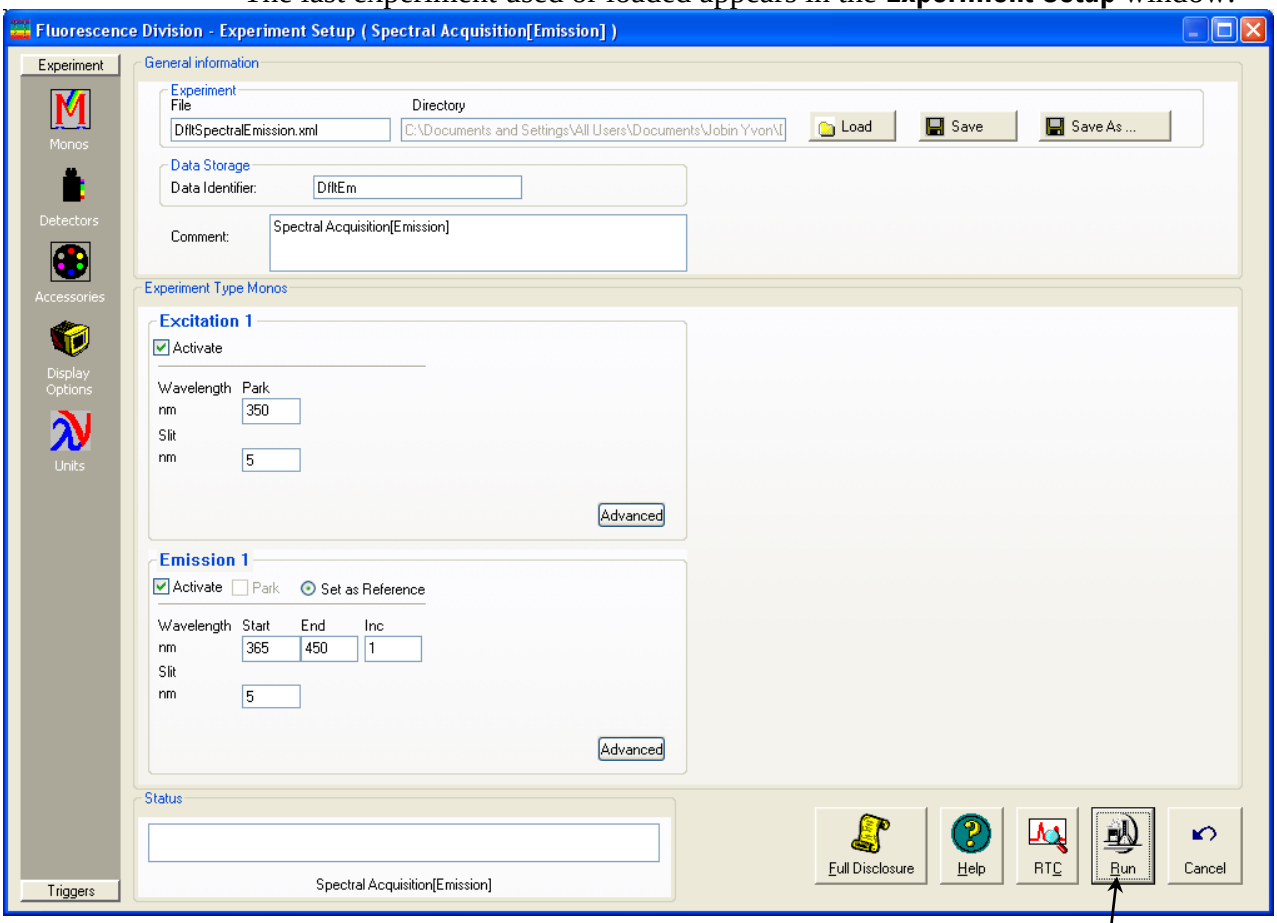
The Previous Experiment Setup button resets the experiment to the previous experiment used, with minor modifications to the hardware possible.

Note: *The Previous Experiment Setup button is active only after an experiment already has been loaded.*

1 After an experiment is loaded, click the Previous Experiment Setup button in the main toolbar:



The last experiment used or loaded appears in the **Experiment Setup** window:



2 Modify the experiment's parameters as required.

3 Click the Run button 

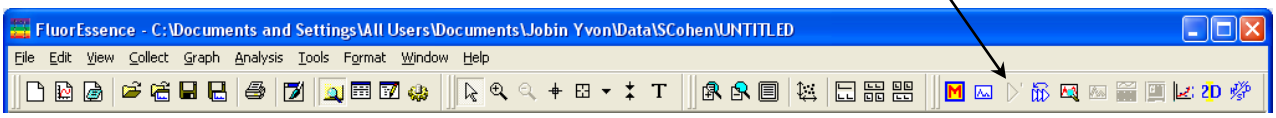
to run the experiment.

Auto Run Previous Experiment button

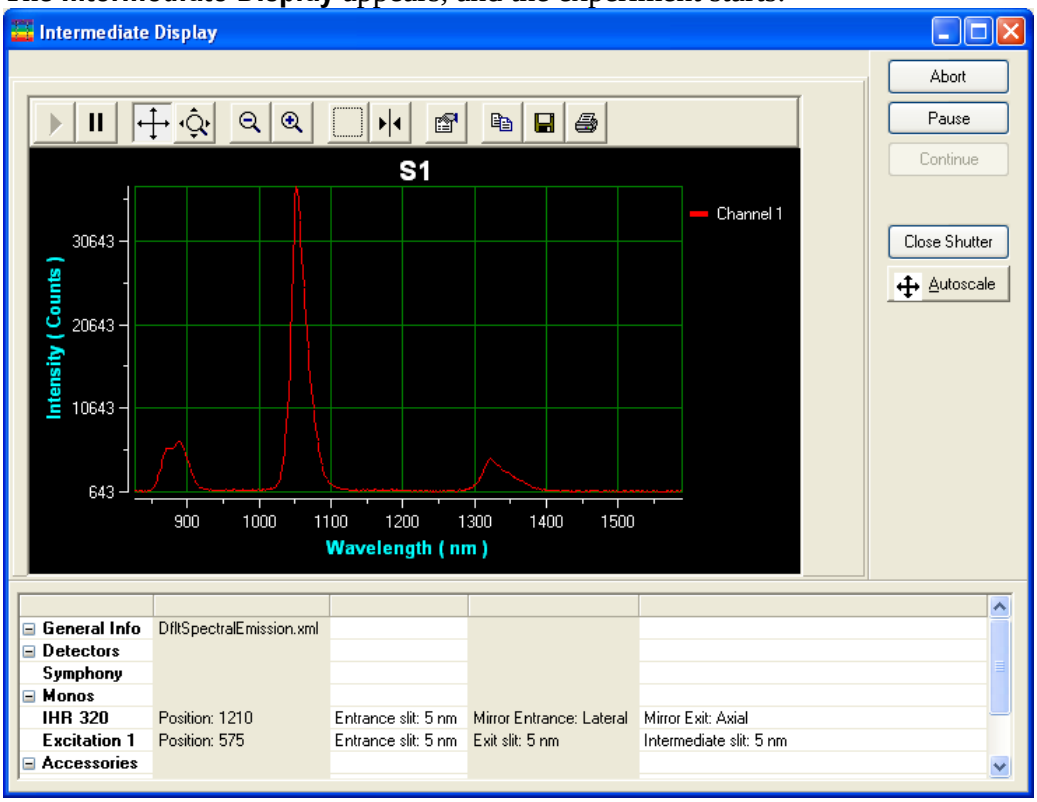
The Auto Run Previous Experiment button reruns the last experiment loaded without modifications.

Note: *The Auto Run Previous Experiment button is active only after an experiment has already been loaded and run.*

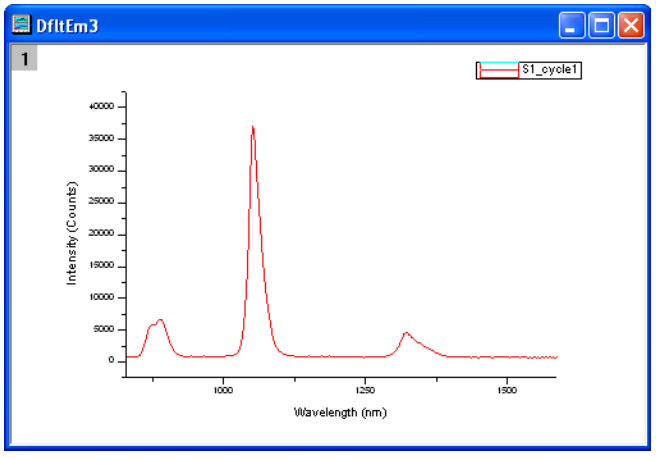
1 Click the Auto Run Previous Experiment button .



The **Intermediate Display** appears, and the experiment starts:



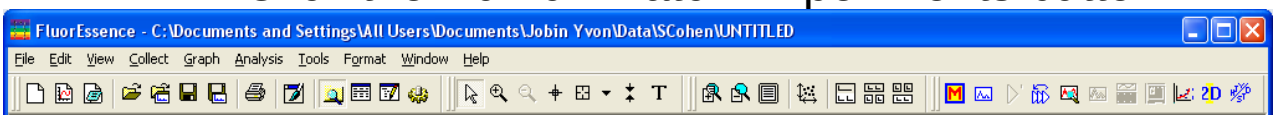
When the experiment is complete, the data are moved into a new Origin® graph window:



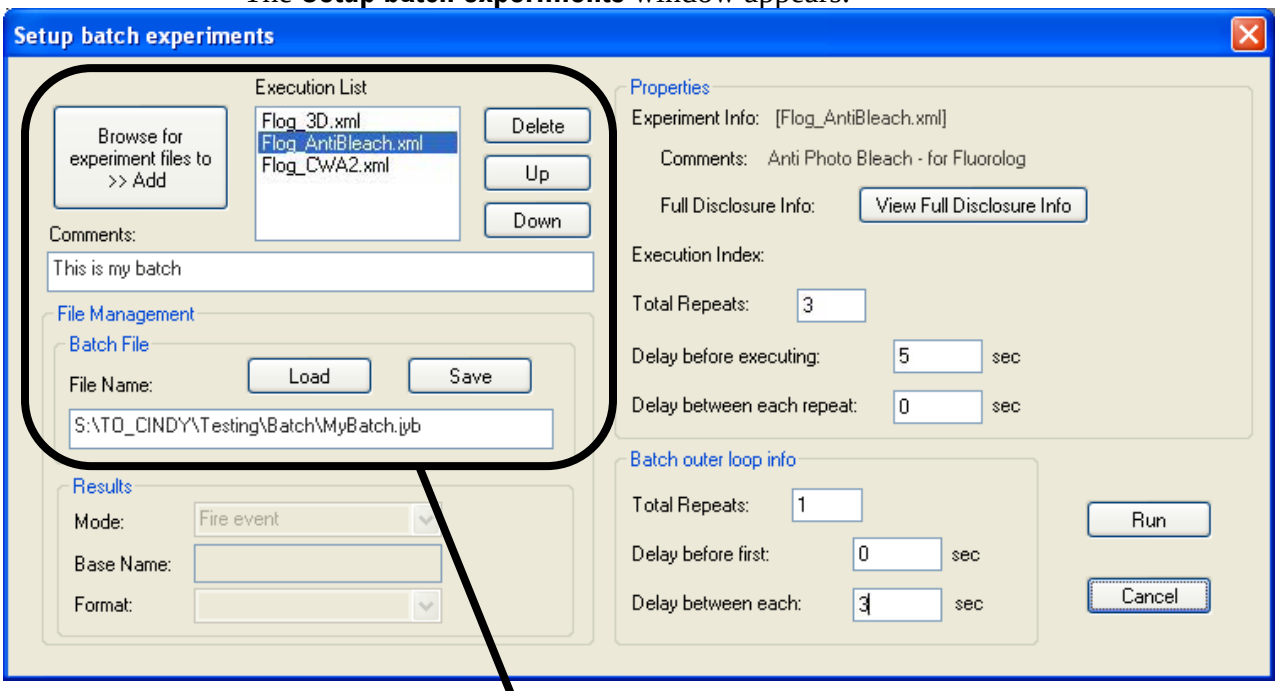
Run JY Batch Experiments

The Run JY Batch Experiments button runs a series of automated experiments, including adjustable repeats and delays between experiments.

1 Click the Run JY Batch Experiments button.



The **Setup batch experiments** window appears.



2 Get the experiment files to create a batch job, or load a previous batch job.

a Load a previously created batch job using the Load button, or browse for experiment files (`.xml` format) using the Browse for experiment files to >> Add button.

b Add each desired experiment file to the Execution List.

c Reorder or remove the files as necessary using the Delete button, the Up button, and the Down button.

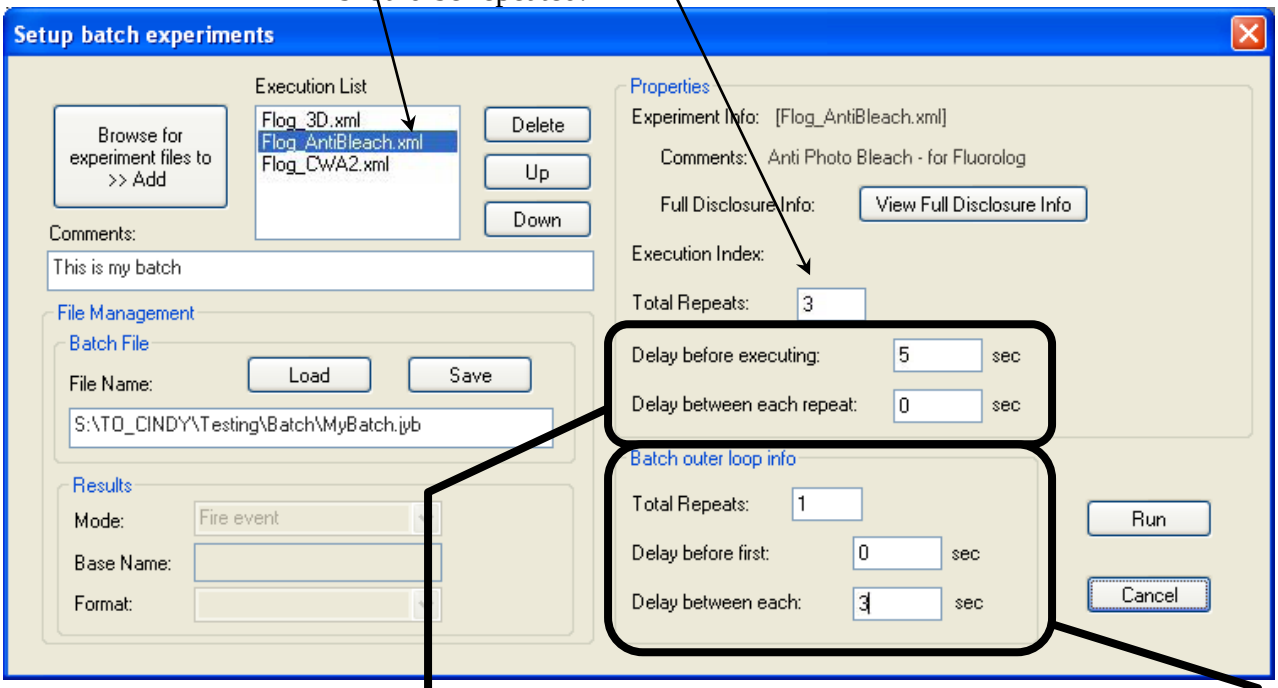
d Add comments about the batch file in the Comments field.

e Save the new batch job in the correct path, in the File Name field, and click the Save button.
The file is saved in a `.jyb` format.

3 Set up each experiment in the batch job.

Select an experiment from the Execution List.

a In the Total Repeats: field, enter the number of times that experiment should be repeated.



b In the Delay before executing: field, enter the number of seconds to wait before executing.

c In the Delay between each repeat list: field, enter the number of seconds to wait before repeating the experiment.

4 Set up an outer loop in the batch job, if desired.

a In the Total Repeats: field, enter the number of times to run the batch job.

b In the Delay before first: field, enter the number of seconds to wait before starting the batch job.

c In the Delay between each: field, enter the number of seconds to wait before rerunning the batch job.

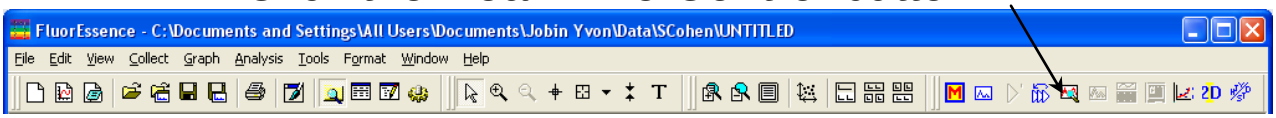
5 Click the Run button to start the batch job.

The batch job executes.

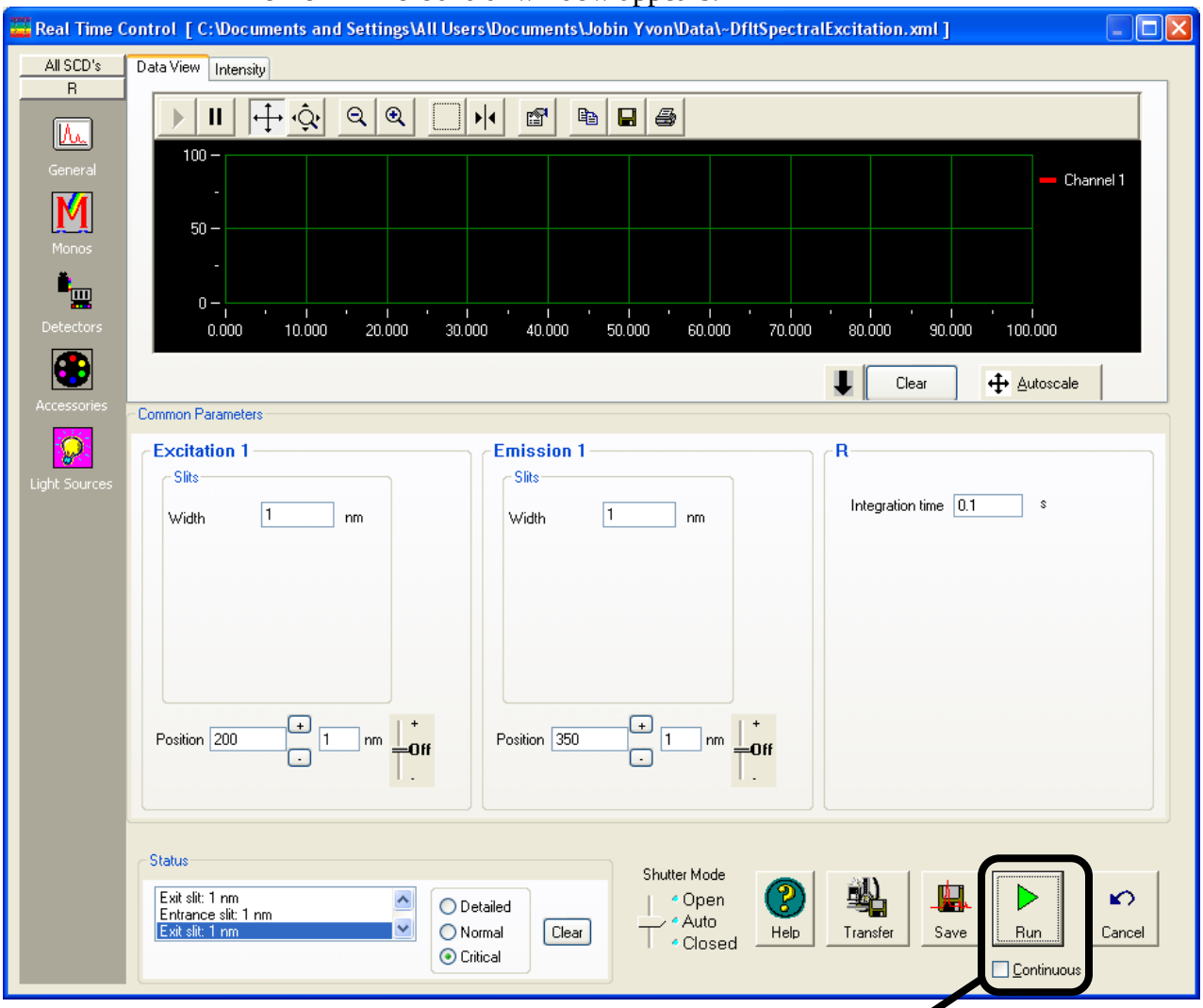
Real Time Control

The Real Time Control button opens the **Real Time Control** window directly, so that the user can adjust experimental parameters in real time, while viewing effects of the adjustments.

1 Click the Real Time Control button .



The **Real Time Control** window appears:



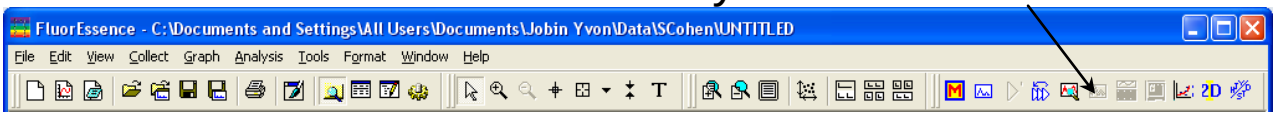
2 Activate the Continuous checkbox, then click the Run button, to monitor the signal.

3 Adjust the desired instrumental parameters as necessary.

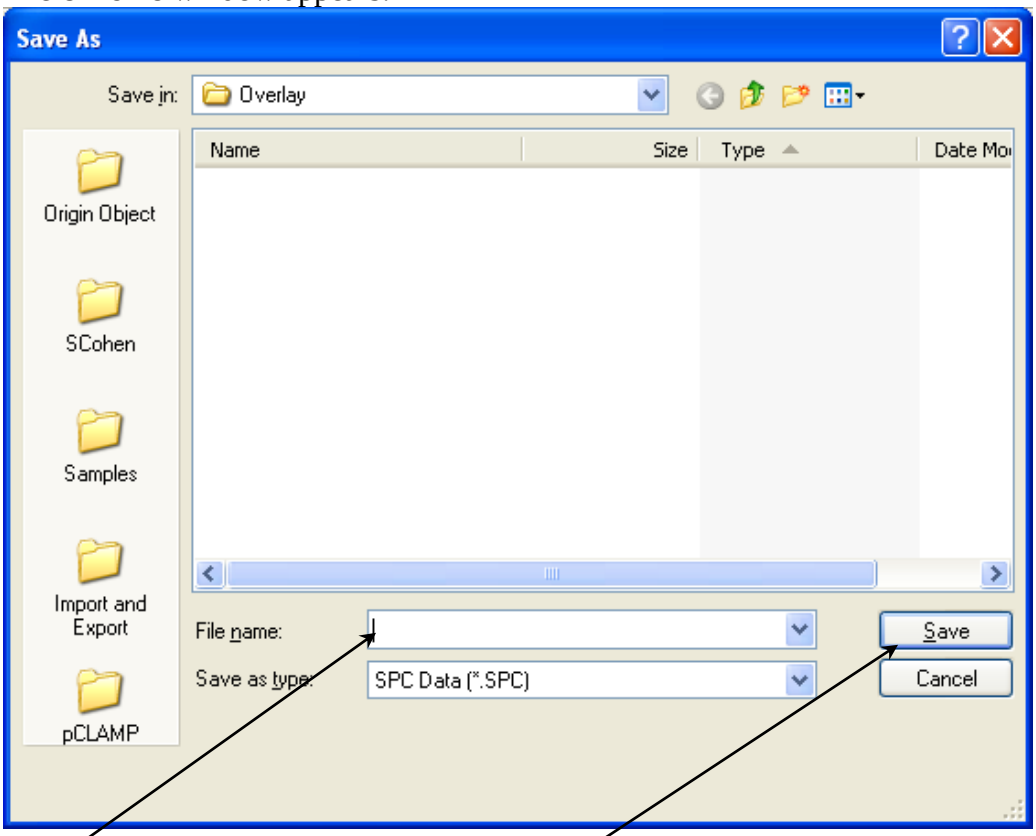
Make Overlay File button

The Make Overlay File button creates an `*.SPC` file for use as an overlay file. This function is useful for blank subtraction also.

1 Click the Make Overlay File button .



The **Save As** window appears:



2 Enter a file name in the File name field.

3 Click the Save button.

The overlay file is saved.

3D Scan to 3D Profile button

The 3D Scan to 3D Profile button takes a 3-D waterfall plot, and converts the data to a contour plot. The button also provides a profile of the data along each axis.

Note: *Because the 3D Scan to 3D Profile button assumes evenly-spaced wavelengths, it is not recommended for multichannel detectors. Use of the button with multichannel detectors can distort your data. Convert to* xyz *using the Origin® Std. menu instead.*

Create/Use Calibration Curve from CWA Data 

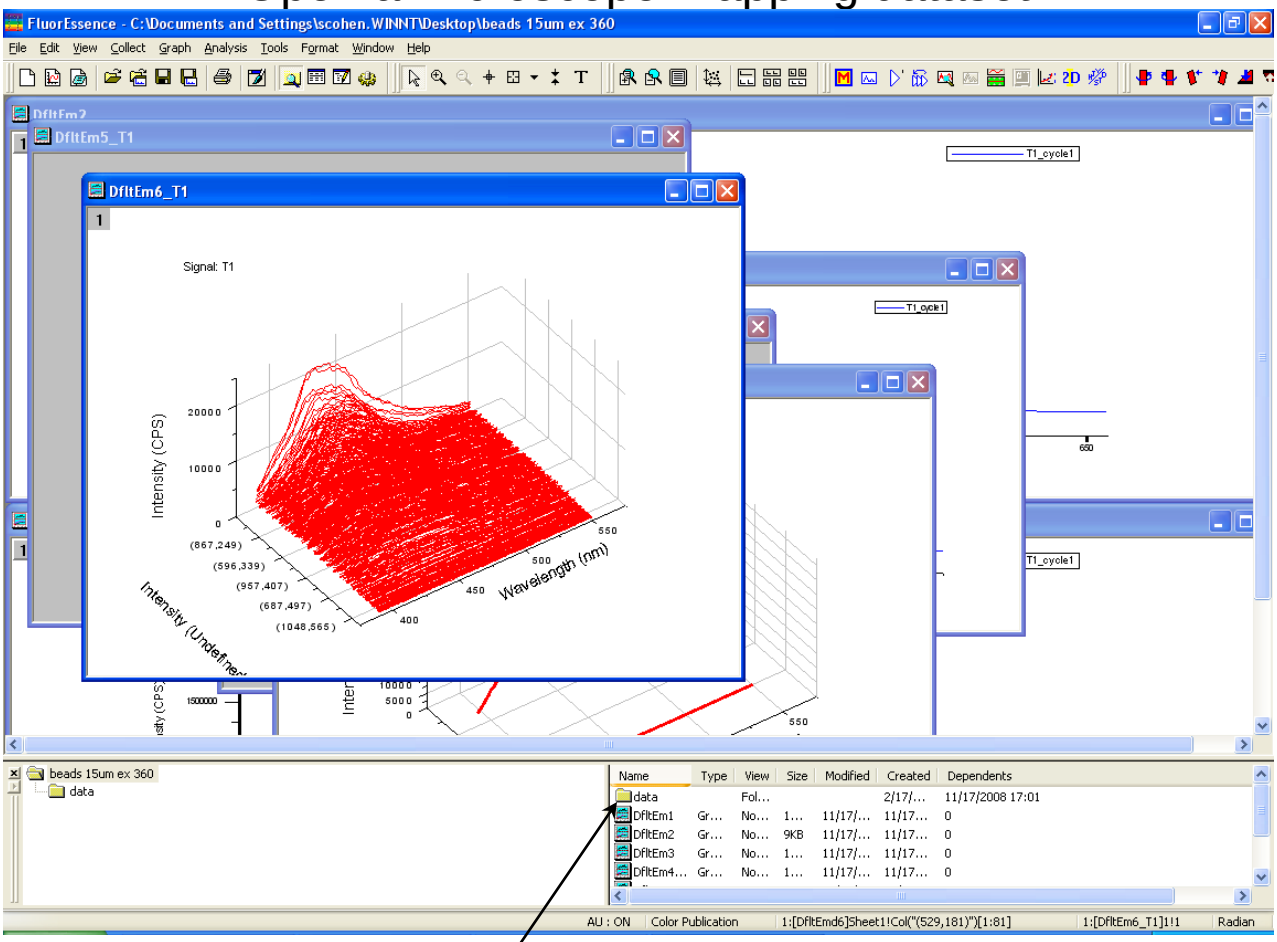
When the user is doing Single Point experiments (especially with the MicroMax or multiple-sample changers), the Create/Use Calibration Curve from CWA Data button creates a calibration curve for analytical measurements.

Note: *The Create/Use Calibration Curve from CWA Data button is not likely to be useful with a Nanolog®.*

2D Intensity Map

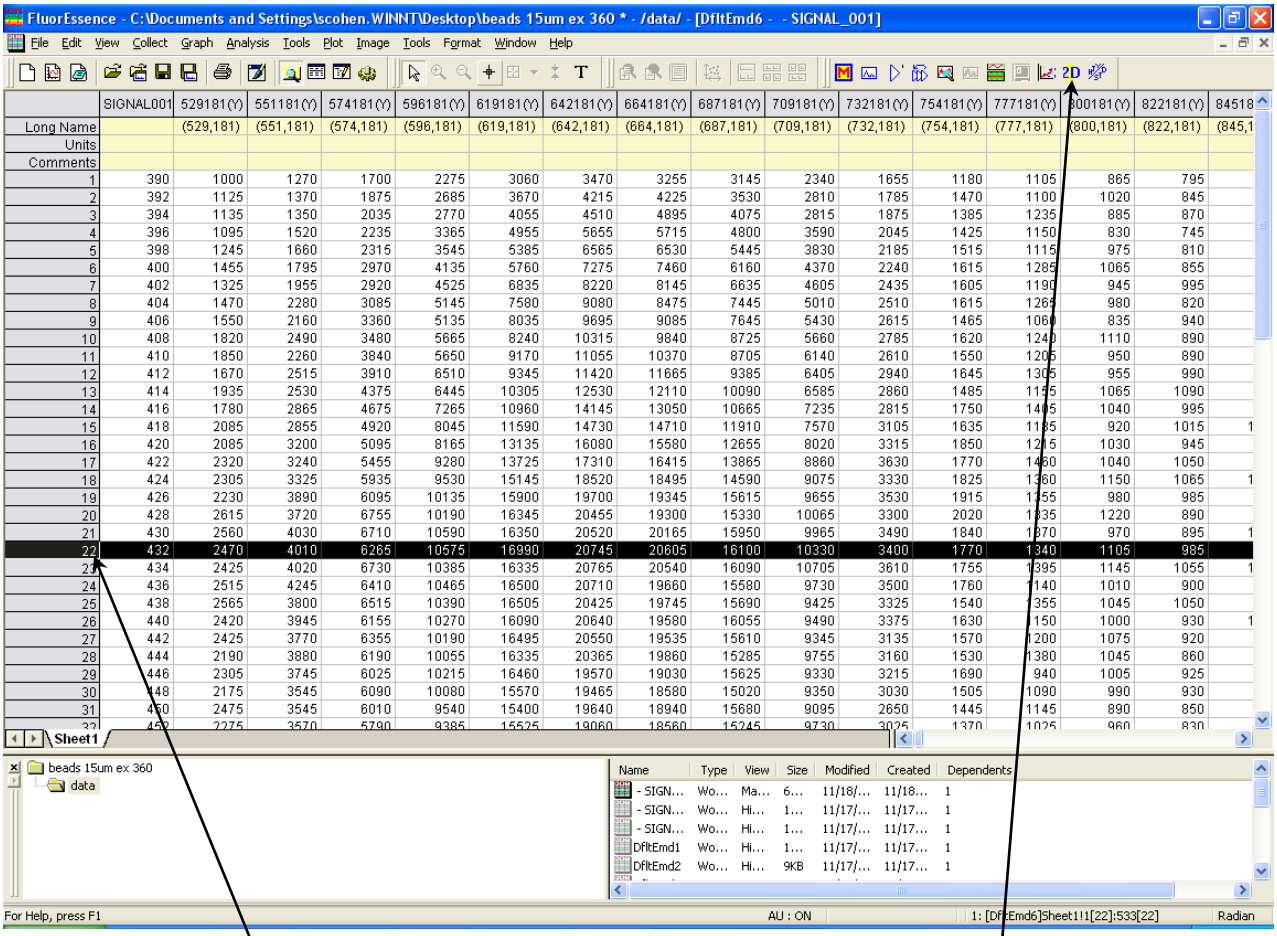
The 2D Intensity Map button creates a two-dimensional intensity map from the active microscope-mapping data.

1 Open a microscope-mapping dataset.



2 Click the data folder in bottom area.

The data spreadsheet appears:



3 Choose an excitation wavelength (row) to examine.

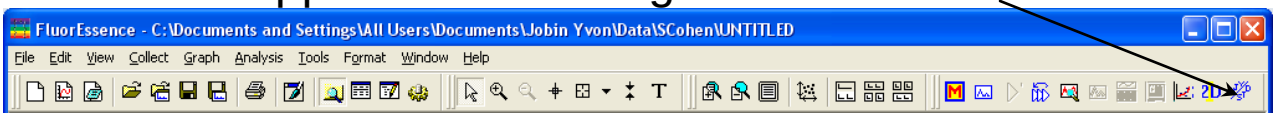
Here we have chosen 432 nm excitation.

4 Click the 2D Intensity Map button 2D.

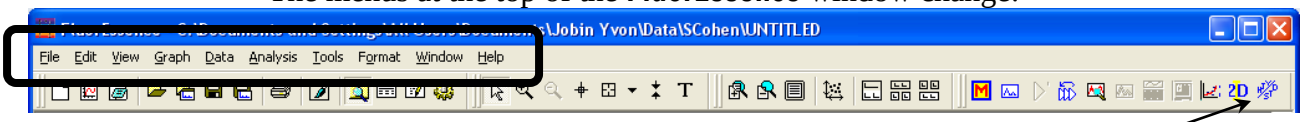
Switch menu between HJY Software Application and Origin Pro

The Switch menu between HJY Software Application and Origin Pro. button switches the menus at the top of the main **FluorEssence** window between FluorEssence™ and Origin® functions. This allows the user to tap the power more fully of Origin® software.

1 Click the Switch menu between HJY Software Application and Origin Pro button .



The menus at the top of the **FluorEssence** window change:



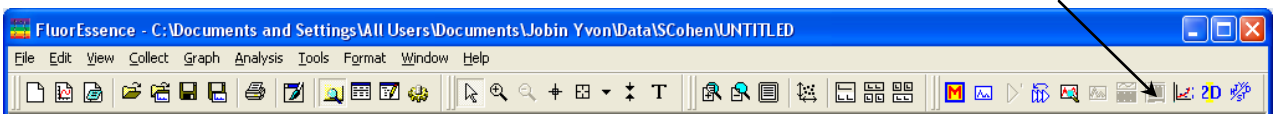
2 Click the Switch menu between HJY Software Application and Origin Pro button again to return to the original menu functions.

Launch DataStation

The Launch DataStation button closes the FluorEssence™ software, and starts DataStation software.

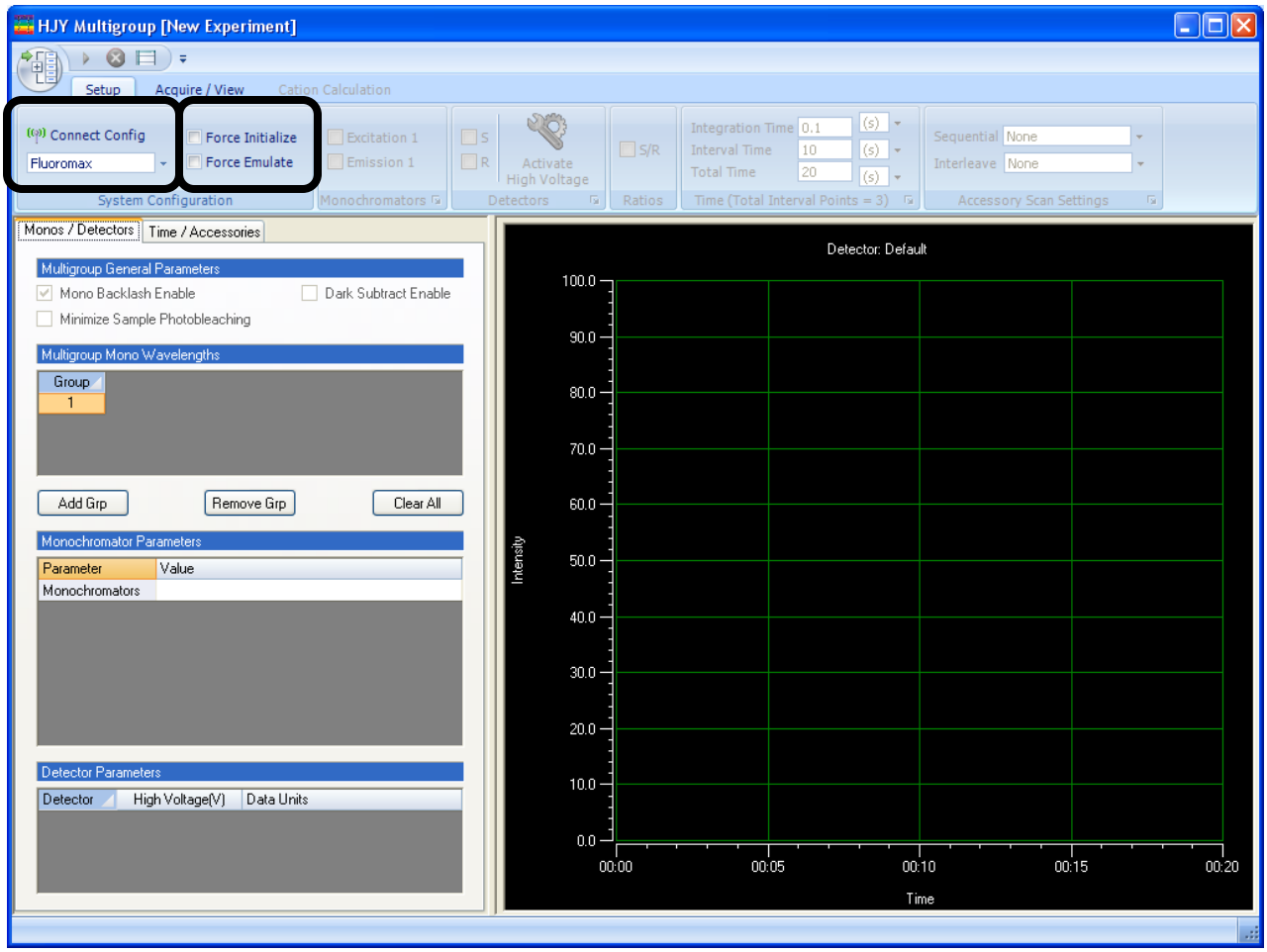
Note: *This function only operates with TCSPC accessories.*

1 Click the Launch DataStation button .



FluorEssence™ shuts down. DataStation starts.

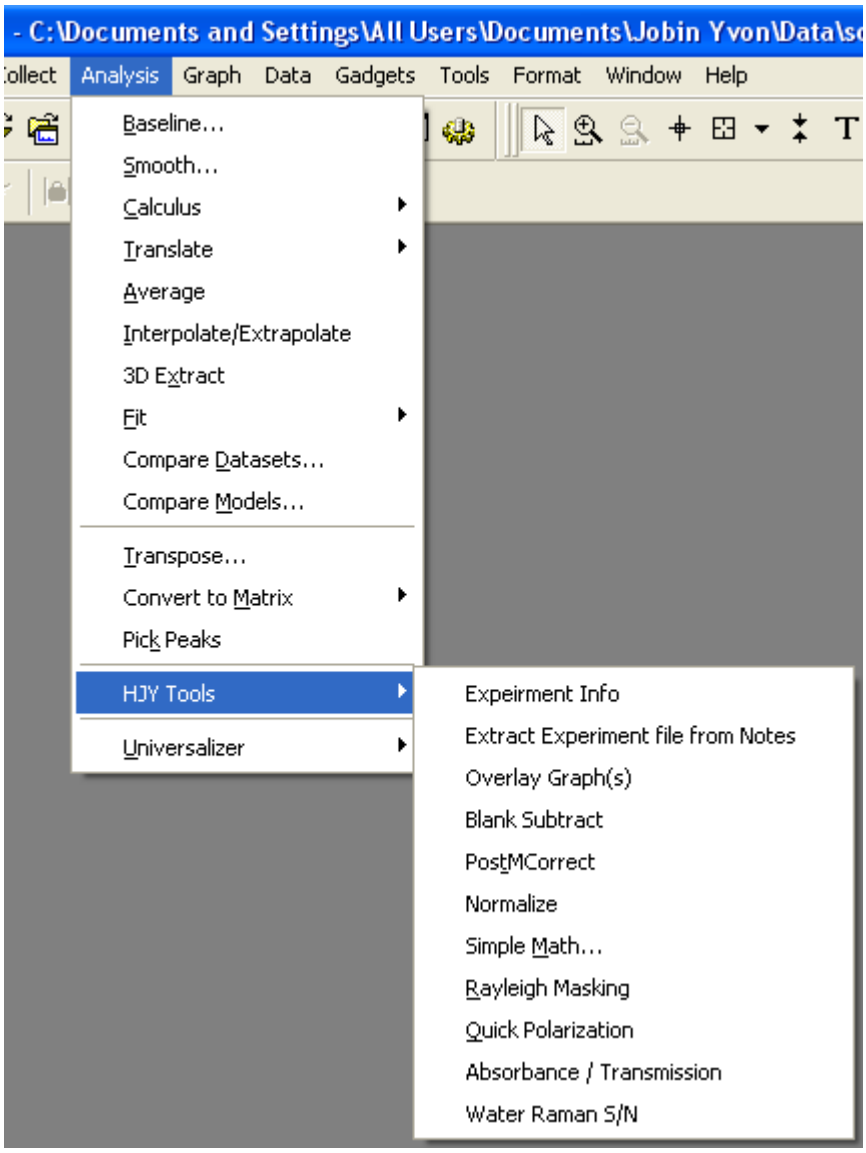
Multigroup



Multigroup offers repeated and sequential fluorescence experiments. Functions not included in FluorEssence™, such as delays, temperature ramps, and multiple samples and wavelength-groups are allowed within Multigroup.

Note: *Multigroup does not work with multichannel-array detectors.*

HJY Tools (in the Analysis menu)



Though not found via specific buttons, certain functions accessible in the Analysis drop-down menu are important for the Nanolog®.

Blank Subtract

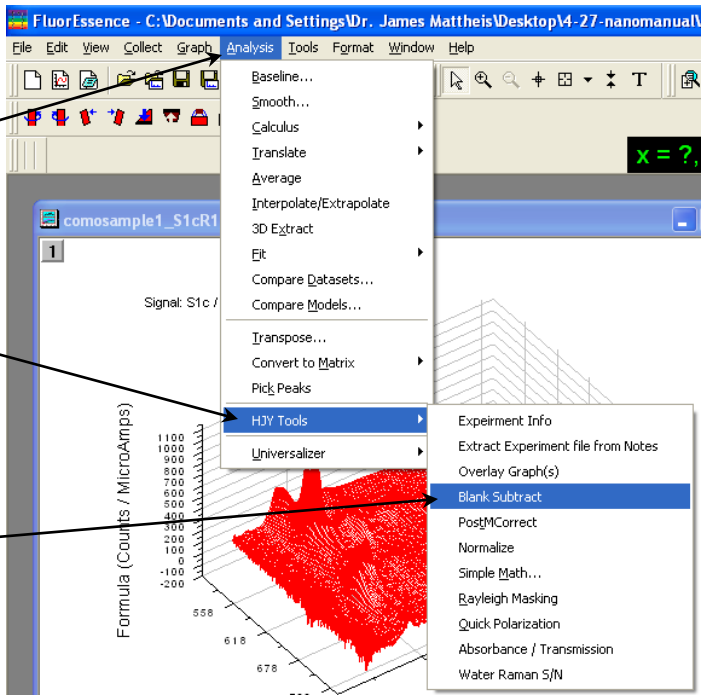
To remove the artifacts from the solvent plus cuvette or other holder, use the Blank Subtract function in the HJY Tools submenu.

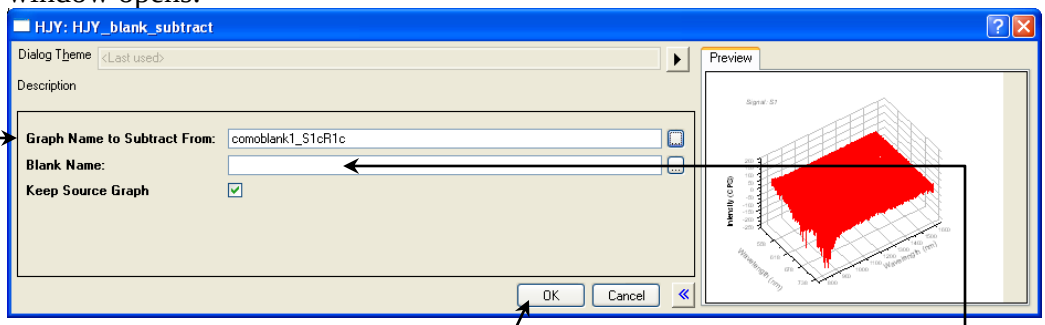
1 Have a scan from a sample and from a blank available.

2 In the toolbar, choose Analysis.
A drop-down menu appears.

3 Choose HJY Tools.
Another drop-down menu appears.

4 Choose Blank Subtract.
The **HJY: HJY_blank_subtract** window opens.

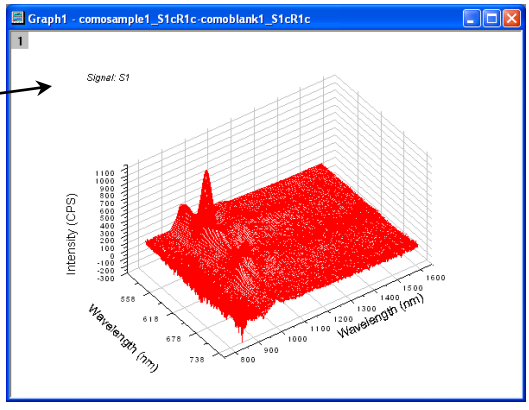




5 Browse for the sample's data in Graph Name to Subtract from field.

6 Browse for the blank's data in Blank Name field.

7 Click the OK button.
A new graph appears, which is the sample data minus the blank data.

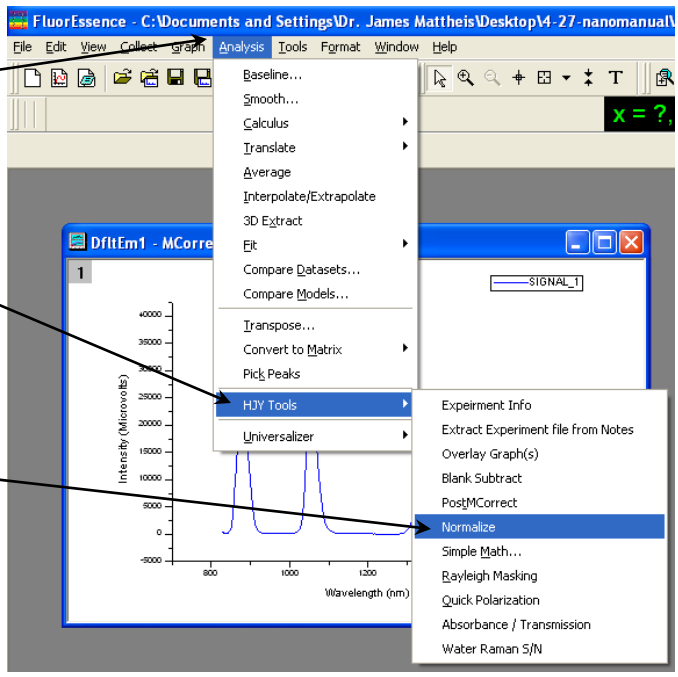


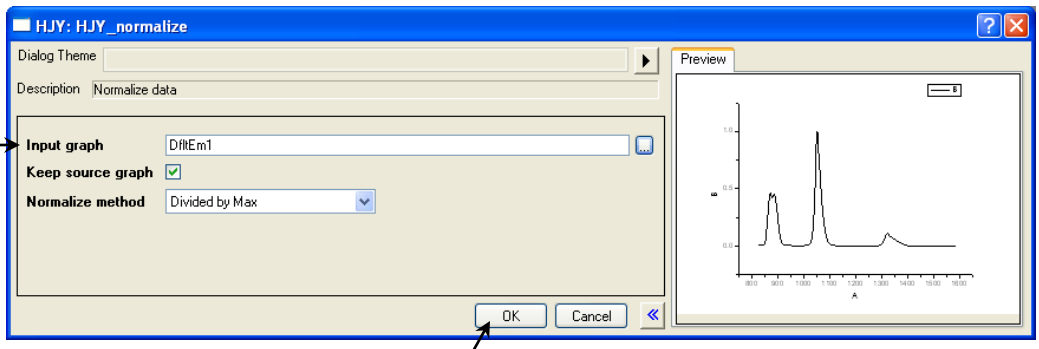
Normalize

The Normalize function sets the maximum value of the spectral scan to 1.00 and scales all other values in the scan accordingly.

1. Have a scan available.
2. In the toolbar, choose Analysis.
   A drop-down menu appears.
3. Choose HJY Tools.
   Another drop-down menu appears.
4. Choose Normalize.

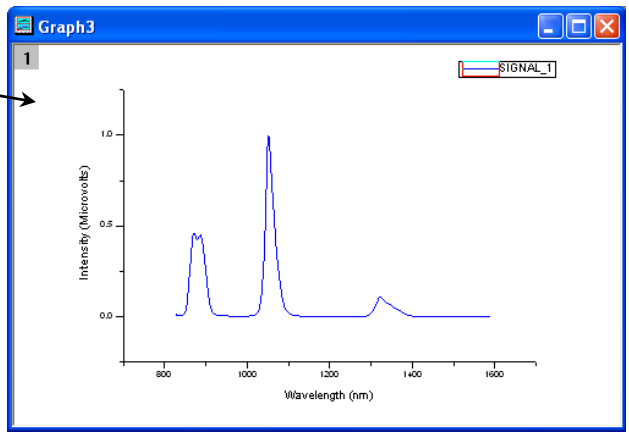
   The **HJY: HJY_normalize** window opens.





5. Browse for the data in the Input graph field.
6. Click the OK button.

   The normalized graph appears.



Running an unknown sample

Often a researcher will scan a sample whose spectral characteristics are unknown. For optimal spectra, the optimal excitation and emission wavelengths must be found.

The optimal excitation wavelength is the wavelength that creates the most intense emission spectrum for a given sample. For many samples, the optimum wavelengths are known. For a sample whose wavelength positions are unknown, the user must determine these wavelengths to obtain the best possible results.

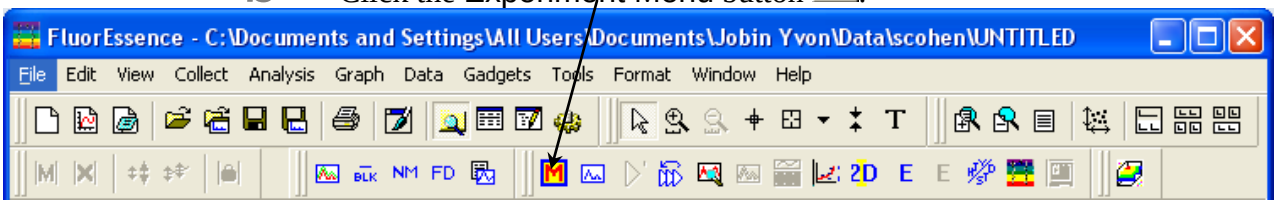
The traditional method consists of running an emission scan to find the peak emission value. Then an excitation scan is run using the determined peak emission value. For single-walled carbon nanotubes, however, a full 3-D scan is more appropriate; this method is given below.

1 Scan for the preliminary emission maxima and maxima.

The object of this step is to acquire a preliminary emission scan, based on a full range of excitation wavelengths. Because the fluorescence emission of samples does not shift with excitation wavelength, the guessed excitation wavelength yields the emission peak, albeit perhaps at lower intensity.

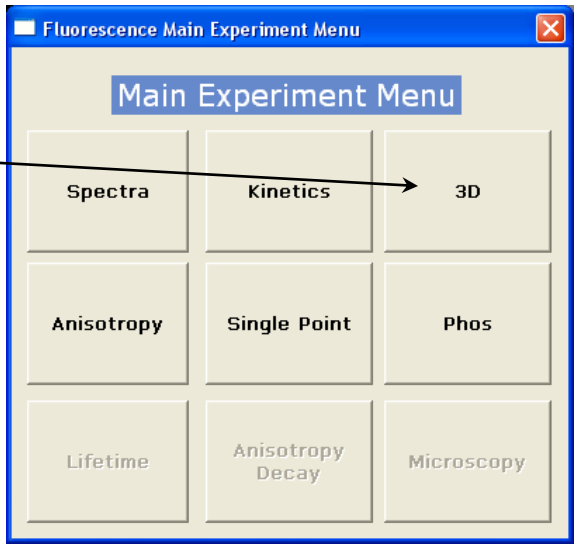
a Be sure all system components are on, and the NanoLog® is calibrated as explained in Chapter 3.

b Click the Experiment Menu button  :

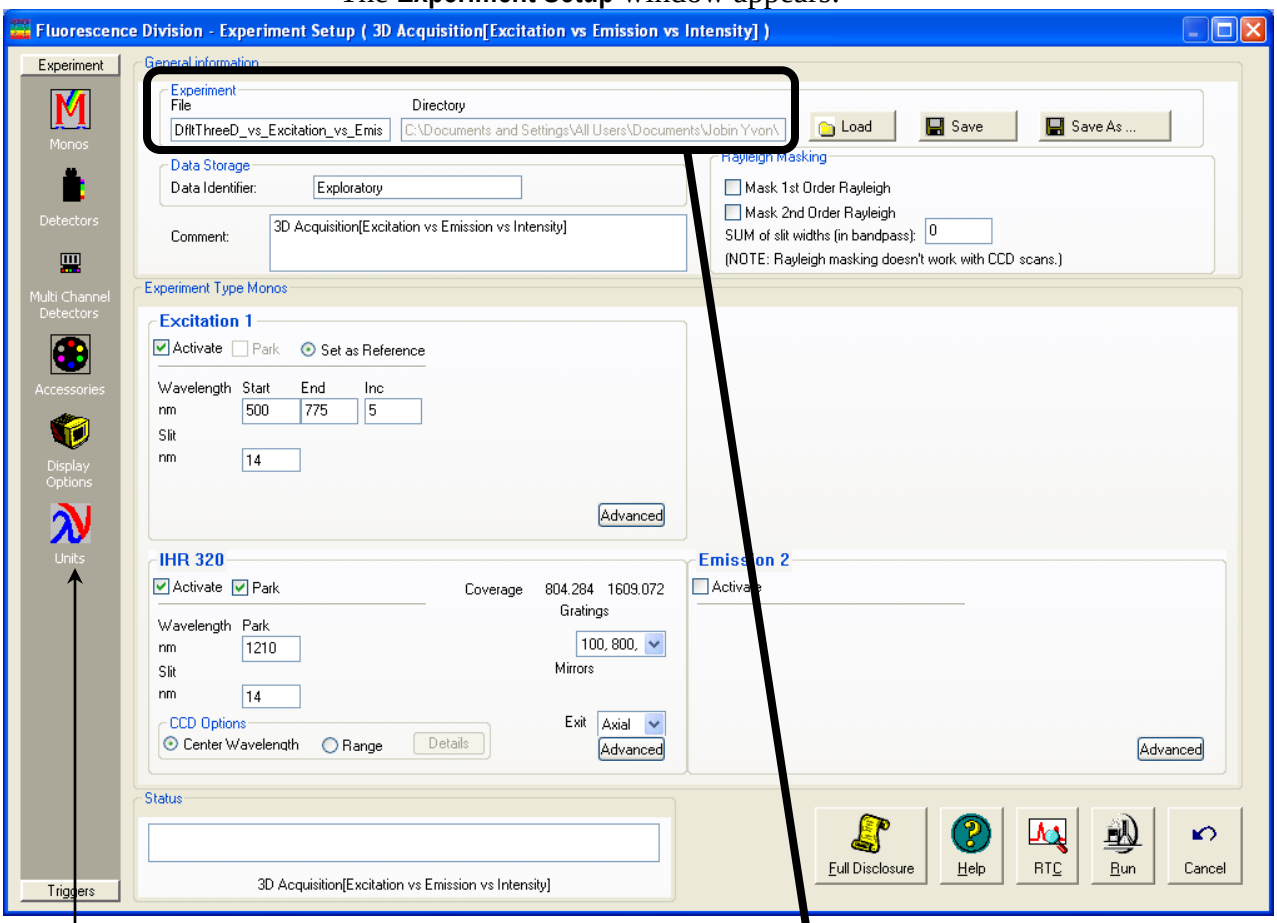


The **Fluorescence Main Experiment Menu** appears:

c Choose 3D.



The **Experiment Setup** window appears:

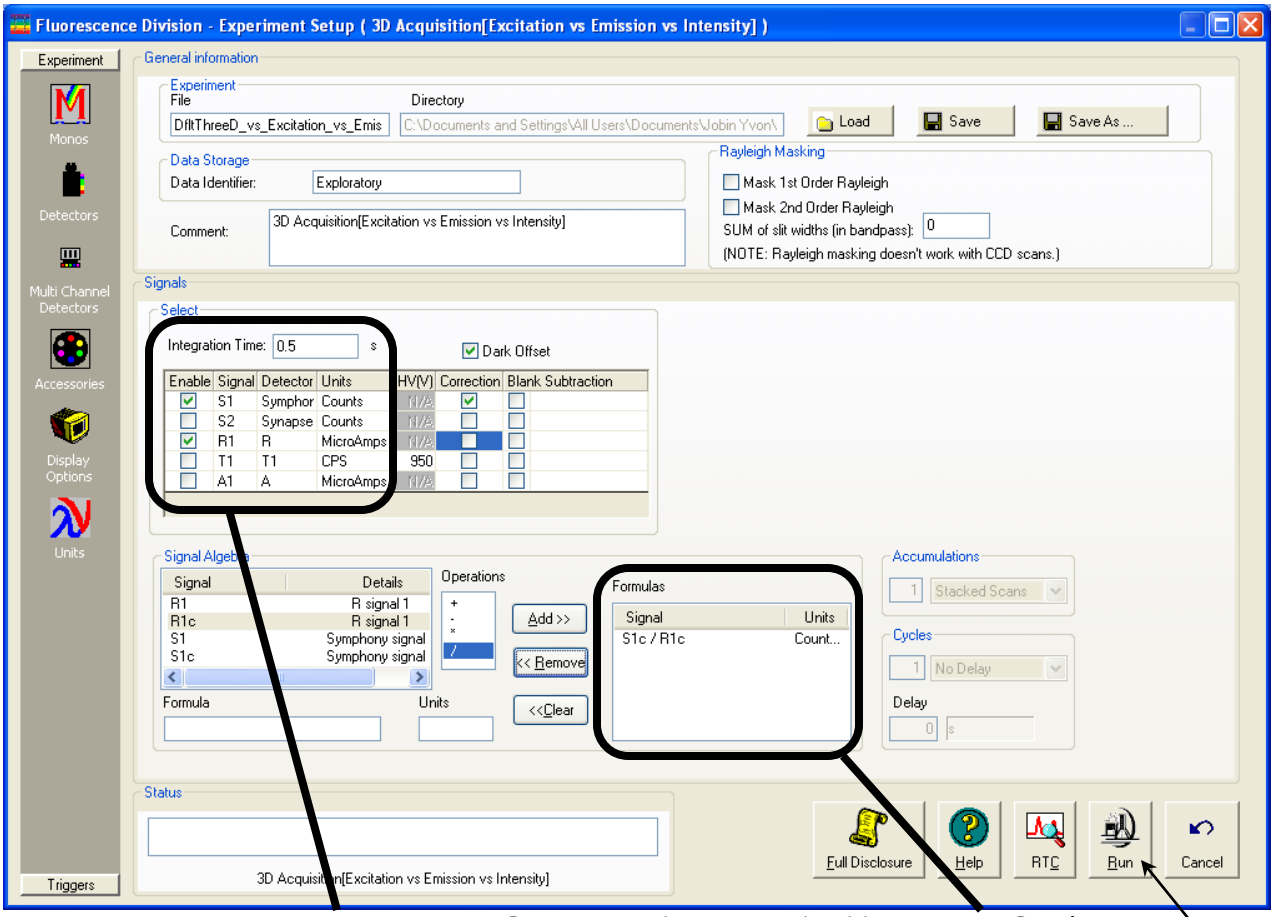


d Click the Experiment File field, and enter a new file name or select a previously saved file.

e Verify that experimental parameters are correct.
Be sure to check all parameters under all icons in the left-hand column.

f Set the scan parameters under the Monos icon as shown above.
Most of these parameters are a trade-off between speed and precision. Choose integration time, increments, and number of scans judiciously, to give an accurate result without excessive time spent. HORIBA Scientific suggests an increment of 5 nm, Starting at 500 nm and Ending at 775 nm for excitation. Park the emission spectrometer at 1210 nm Center Wavelength with a wide slit setting of 14 nm. We recommend the 100 grooves/mm grating blazed at 800 nm, to provide the widest spectral coverage on the emission spectrometer. Don't forget a data file name.

g Set the scan parameters under the Detectors icon as shown below:

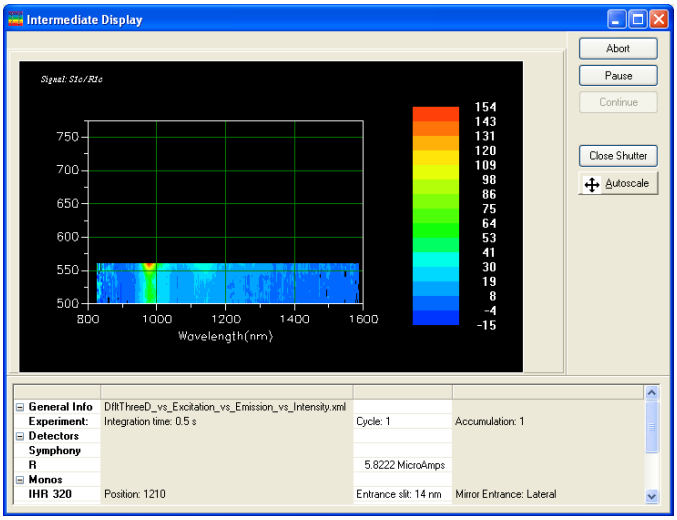


Activate the S1 and R1 detectors' checkboxes. Use S1c/R1c (corrected signal detector divided by the corrected reference detector) for the acquisition mode, in the Formulas table. Try 0.5 s Integration Time.

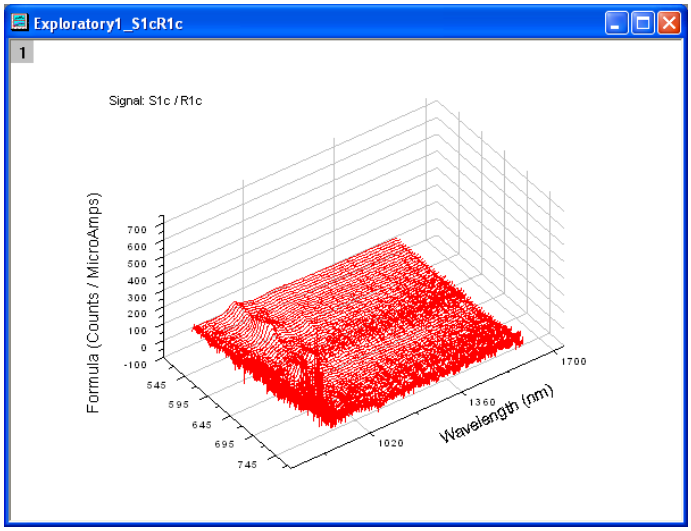
h Insert the sample into the sample compartment, and close the sample-compartment's cover.

i Click the Run button.

The Intermediate Display appears with the scan building up in real time:

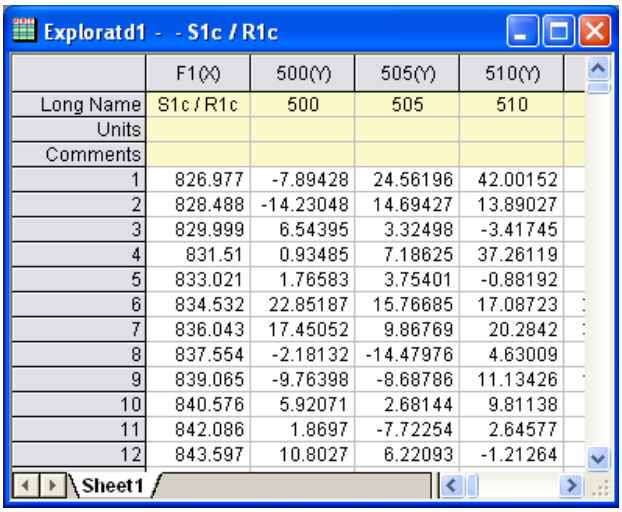


Upon completion of the acquisition, the final data appear:



2 Create a contour plot from the data.

a Examine the spreadsheet of the data:



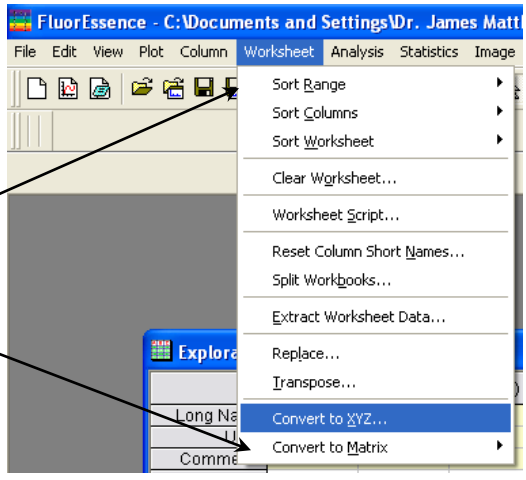
b In the toolbar, choose Format.
A drop-down menu appears.

c In the menu, choose Menu.

d In the menu, choose Origin Standard.
Another drop-down menu appears.
The toolbar changes to the Origin® style.

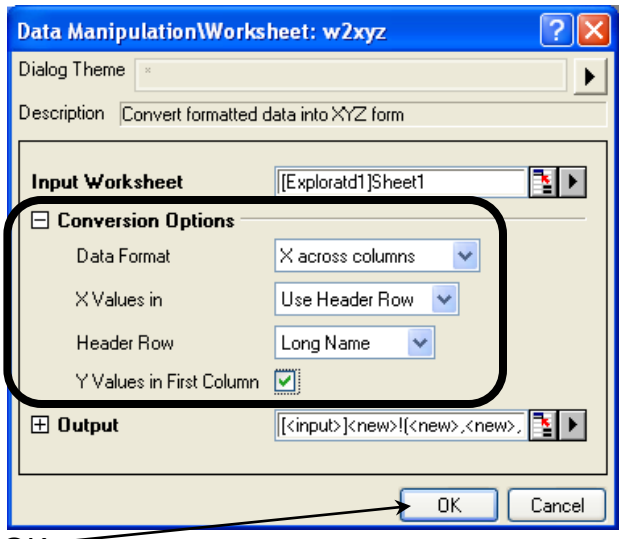
e In the toolbar, choose Worksheet.
A drop-down menu appears.

f In the menu, choose Convert to XYZ....
The **Data Manipulation\Worksheet** window appears:

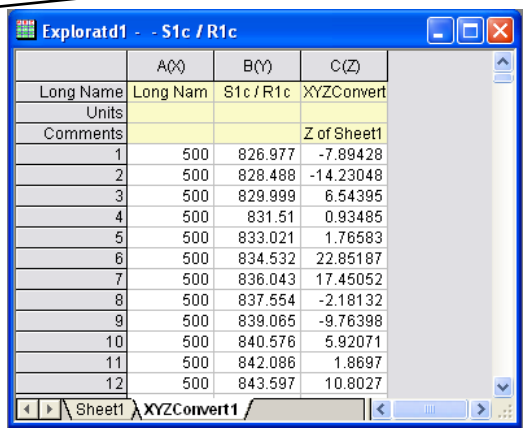


g

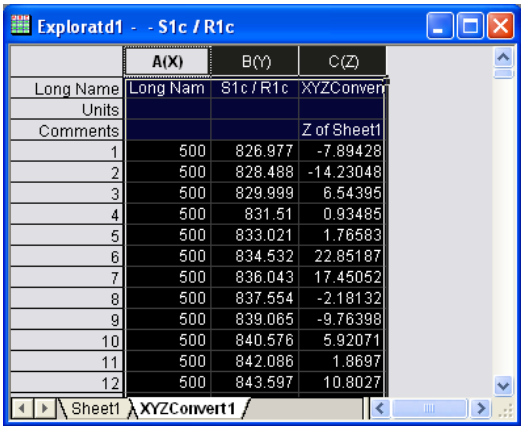
Choose the appropriate parameters:
For Data Format, choose X across columns.
For X Values in, choose Use Header Row.
For Header Row, choose Long Name.
Activate the Y Values in First Column checkbox.



When finished, click the OK button.
The worksheet gets converted:



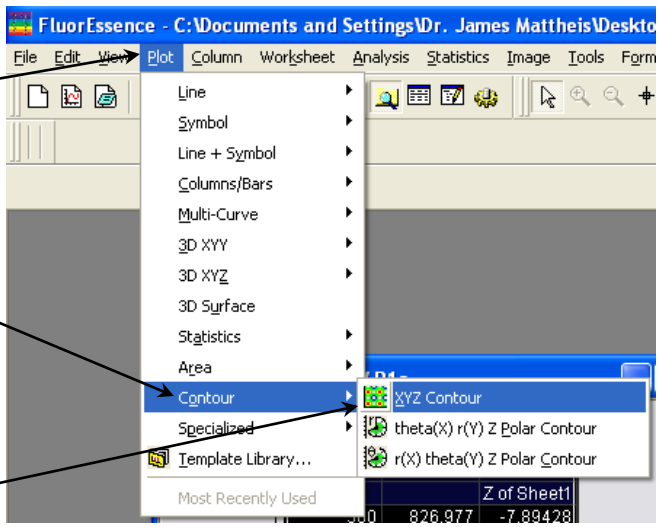
Highlight the three columns:

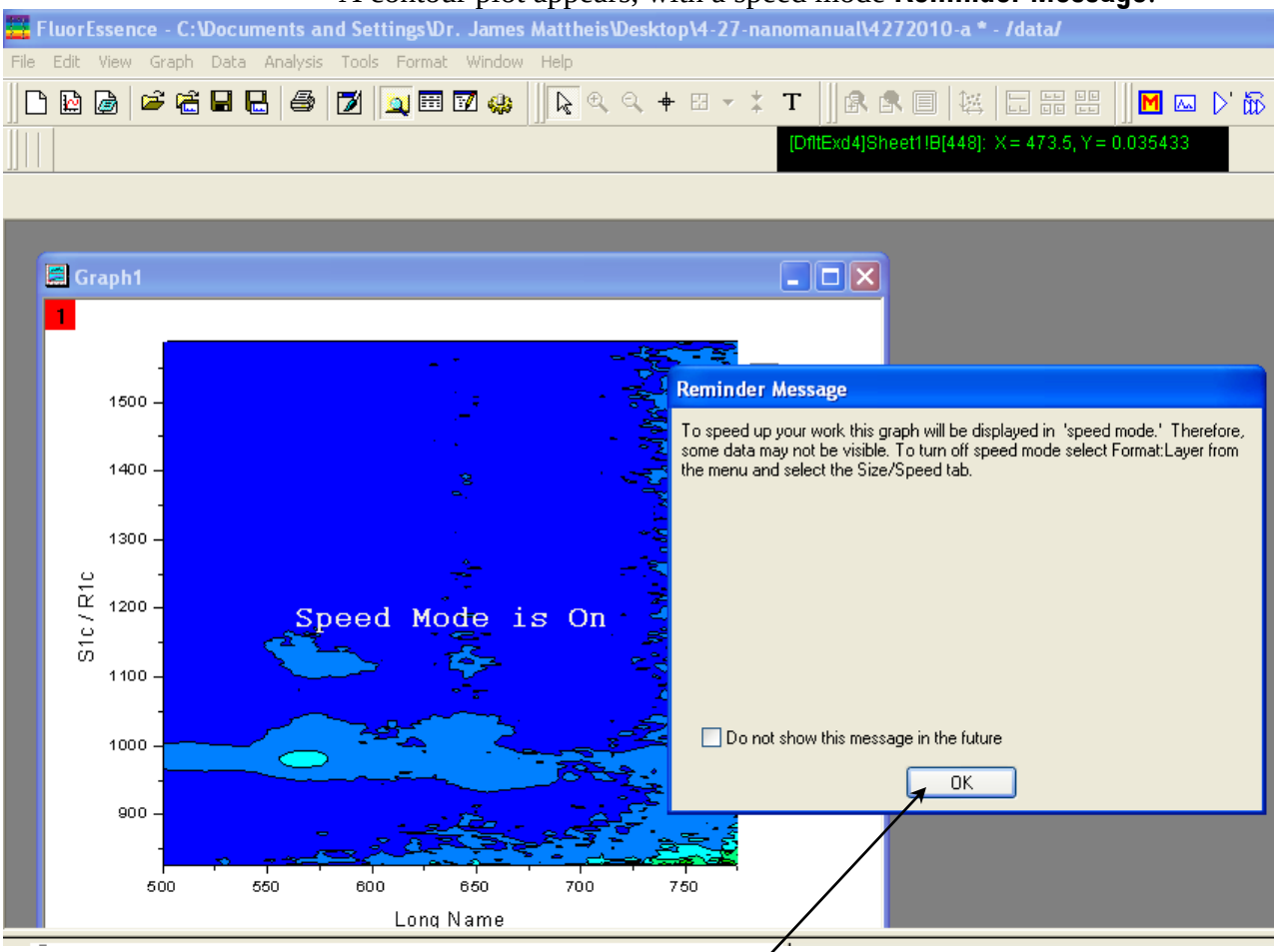


h In the toolbar, choose the Plot command.
A drop-down menu appears.

i In the menu, choose the Contour command.
Another drop-down menu appears.

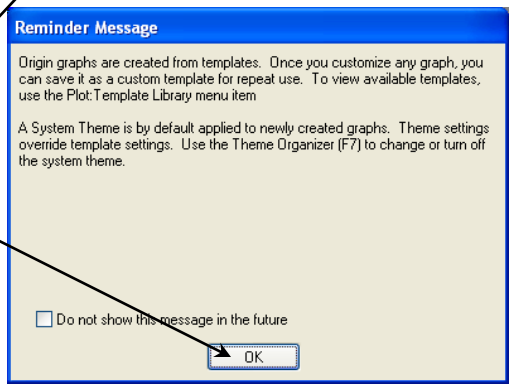
j Choose XYZ Contour.
A contour plot appears, with a speed mode **Reminder Message**:



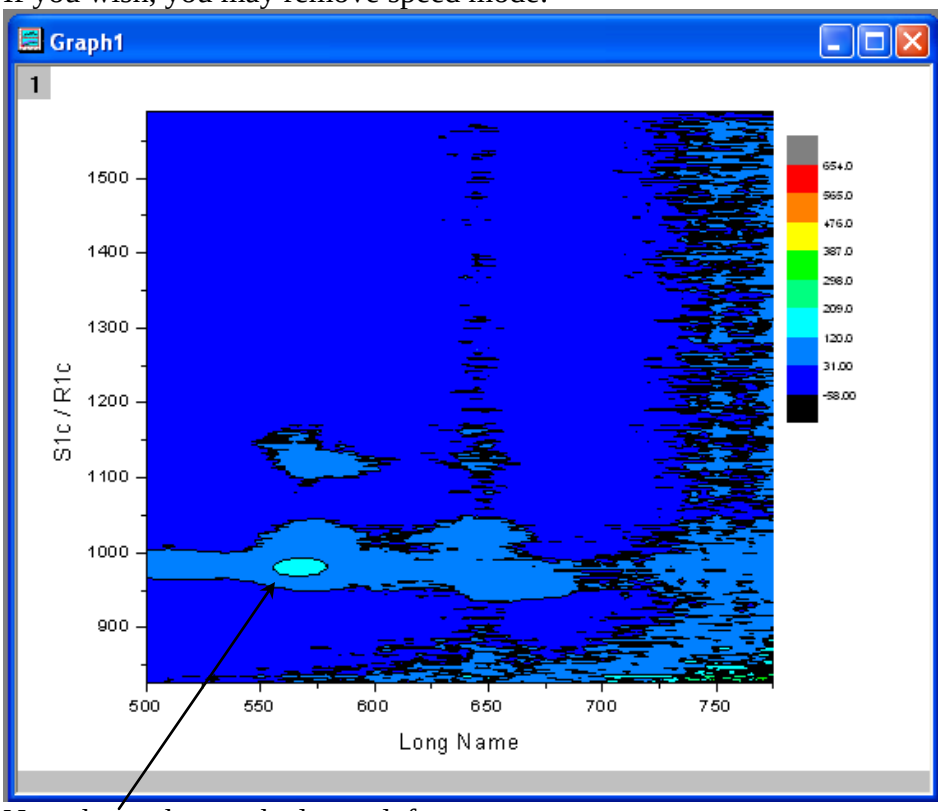


k Click the OK button to continue.
A template **Reminder Message** appears.

l Click the OK button to continue.

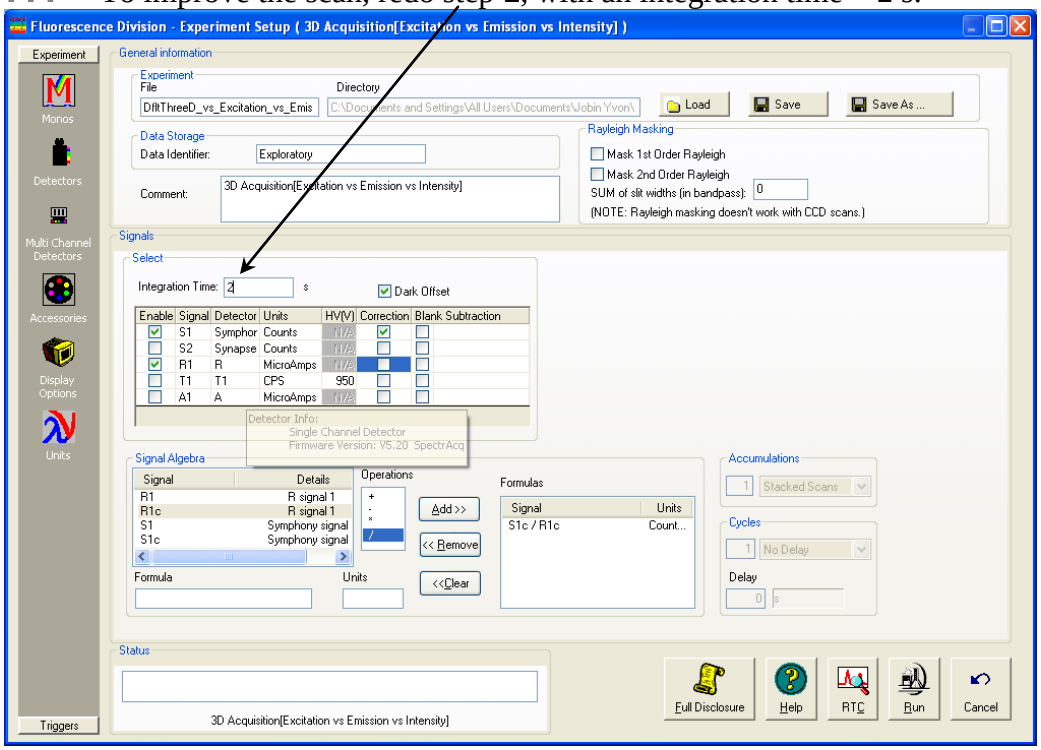


If you wish, you may remove speed mode:



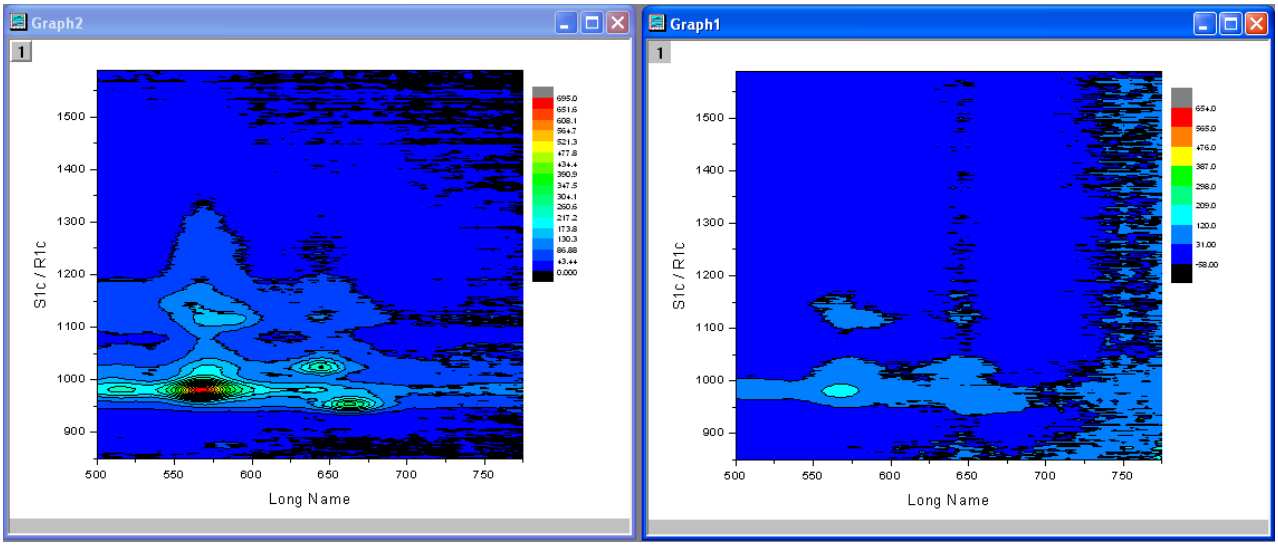
Note the peak near the lower-left corner.

m To improve the scan, redo step 1, with an integration time = 2 s:



n Then redo step 2.

Below is a comparison of the two scans, with longer vs. shorter integration times:



3D scans of unknown single-walled carbon nanotubes. Left: Integration time = 2 s. Right: integration time = 0.5 s.

5: Optimizing Data

Spectra can be enhanced by optimization of data-acquisition. This chapter lists some methods of optimizing sample preparation, spectrofluorometer setup, and data correction to get higher-quality data.

Cuvette preparation

Note: *Clean the sample cells thoroughly before use to minimize background contributions.*

1. Empty all contents from the cuvettes.
2. Fully immerse and soak the cu- cuvettes for 24 h in 50% aqueous nitric acid.
   This cleans the cuvettes' inner and outer surfaces.

Warning: *Nitric acid is a dangerous substance. When using nitric acid, wear safety goggles, face shield, and acid-resistant gloves. Certain compounds, such as glycerol, can form explosive materials when mixed with nitric acid. Refer to the Materials Safety Data Sheet (MSDS) for detailed information on nitric acid.*

3. Rinse with de- ionized water.

4. Clean the cuvettes in the cleaning solution with a test-tube brush.
   Use Alconox® or equivalent detergent as a cleaning solution.
5. Rinse the cuvettes with de-ionized water.
6. Soak the cuvettes in concentrated nitric acid.
7. Rinse them with de-ionized water before use.

Caution: *Soaking the cuvettes for a long period causes etching of the cuvette surface, which results in light-scattering from the cuvettes.*

Sample preparation

Caution: *Always read the Materials Safety Data Sheet before using a sample or reagent.*

The typical fluorescence or phosphorescence sample is a solution analyzed in a standard cuvette. The cuvette itself may contain materials that fluoresce. To prevent interference, HORIBA Scientific recommends using non-fluorescing fused-silica cuvettes that have been cleaned as described above.

Small-volume samples

If only a small sample-volume is available, and the intensity of the fluorescence signal is sufficient, dilute the sample and analyze it in a 4-mL cuvette. If fluorescence is weak or if trace elements are to be determined, HORIBA Scientific recommends a capillary cell such as our 250-µL optional micro-sample capillary cell, which is specifically designed for a small volume. A 1-mL cell (5 mm × 5 mm cross-section) is also available.

Solid samples

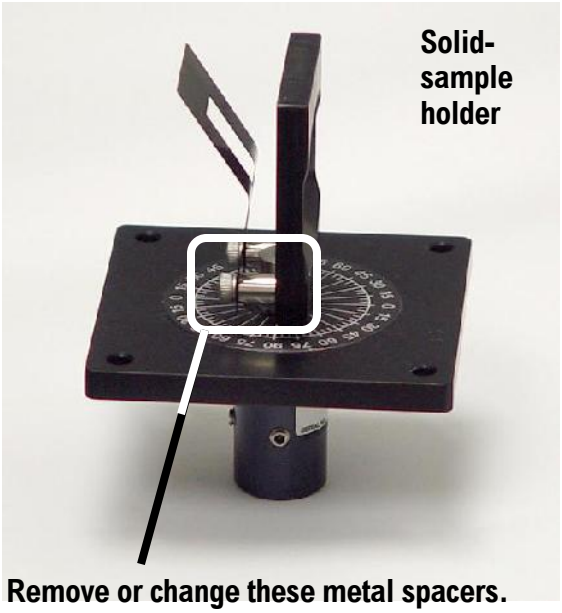
Solid samples usually are mounted in the 1933 Solid Sample Holder, with the fluorescence collected from the front surface of the sample. The mounting method depends on the form of the sample. See the section on "Highly opaque samples" for more information on sample arrangement in the sample compartment.

- Thin films and cell monolayers on coverslips can be placed in the holder directly.
- Minerals, crystals, vitamins, paint chips, phosphors, and similar samples usually are ground into a homogeneous powder. The powder is packed into the depression of the Solid Sample Holder (see next page for diagram). For very fine powder, or powder that resists packing (and therefore falls out when the holder is put into its vertical position), the powder can be held in place with a thin quartz coverslip, or blended with potassium bromide for better cohesion.
- A single small crystal or odd-shaped solid sample (e.g., contact lens, paper) can be mounted with tape along its edges to the Solid Sample Holder. Be sure that the excitation beam directly hits the sample. To keep the excitation beam focused on the sample, it may be necessary to remove or change the thickness of the metal spacers separating the clip from the block.

Note: *Avoid thick coverslips, because the excitation beam may not hit the sample directly with a thick coverslip. Microscope coverslips are useful, except that they are not quartz, and do not transmit UV light.*

Dissolved solids

Solid samples, such as crystals, sometimes are dissolved in a solvent and analyzed in solution. Solvents, however, may contain organic impurities that fluoresce and mask the signal of interest. Therefore, use high-quality, HPLC-grade solvents. If background fluorescence persists, recrystallize the sample to eliminate organic impurities, and then dissolve it in an appropriate solvent for analysis.



Biological samples

For reproducible results, some samples may require additional treatment. For example, proteins, cell membranes, and cells in solution need constant stirring to prevent settling. Other samples are temperature-sensitive and must be heated or cooled to ensure reproducibility in emission signals.

Carbon nanotubes

Details of raw nanotube products synthesized in high-pressure CO reactors, their dispersion in aqueous sodium dodecyl sulfate or other surfactants, sonication, and centrifugation to obtained purified carbon nanotubes, can be found in the following references:

- M.J. O'Connell, *et al.*, "Band Gap Fluorescence from Individual Single-Walled Carbon Nanotubes," *Science*, **297** (2002), 593–596.
- Y. Miyauchi, *et al.*, "Fluorescence spectroscopy of single-walled carbon nanotubes synthesized from alcohol," *Chem. Phys. Lett.*, **387** (2004), 198–203.
- V.C. Moore, *et al.*, "Individually Suspended Single-Walled Carbon Nanotubes in Various Surfactants," *Nano Lett.* **3** (10), 1379–1382 (2003).

Quantum dots

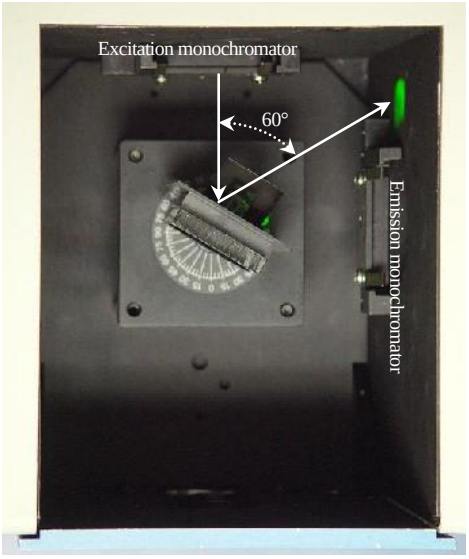
Various methods of synthesizing ZnS-capped CdSe or other quantum dots for fluorescence experiments can be found in references including:

- I.L. Medintz, *et al.*, "Self-assembled nanoscale biosensors based on quantum dot FRET donors," *Nature Materials*, **2** (2003), 630–638.
- J.H. Kim, D. Morikis, and M. Ozkan, "Adaptation of inorganic quantum dots for stable molecular beacons," *Sensors and Actuators B*, **102** (2004), 315–319.
- R.E. Bailey and S. Nie, "Alloyed Semiconductor Quantum Dots: Tuning the Optical Properties without Changing the Particle Size," *J. Am. Chem. Soc.*, **125** (2003), 7100–7106.

Running a scan on a sample

Precautions with the Solid Sample Holder

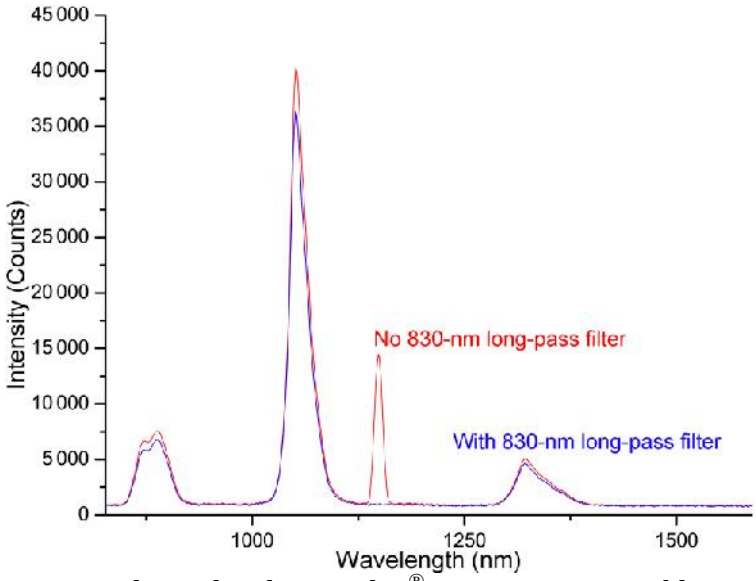
Avoid placing the front face of the sample so that the excitation beam is reflected directly into the emission monochromator. If the sample is rotated at 45° from excitation, this may occur, increasing interference from stray light. Instead, set up the sample with a 30° or 60°-angle to the excitation, preventing the excitation beam from entering the emission slits. The photograph at right illustrates how a 60°-angle to the excitation keeps the incoming excitation light away from the emission monochromator's entrance.



Note: *The focal point of the excitation beam must be on the sample itself.*

Use filters in the optical path.

Stray light from the excitation beam can interfere with the emission from the sample. To reduce the deleterious effects of stray light, place a filter that removes excitation wavelengths from the emission beam in the emission optical path. Here is an example of scans with and without filters on a NanoLog®, using uncharacterized carbon nanotubes as the sample. An 830-nm long-pass filter allows only the desired emission to reach the detector. Stray excitation is eliminated. Notice how the spectrum changes when filters are added.

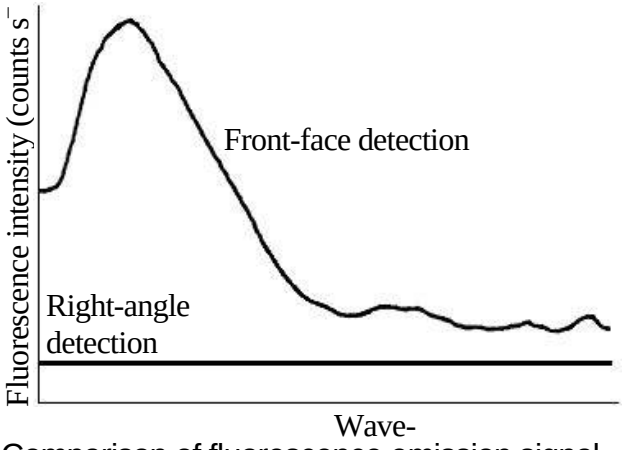


For excitation-emission matrix scans, HORIBA Scientific recommends using a filter to block the 2nd-order Rayleigh line from appearing. The InGaAs array detectors commonly used in the Nanolog® are sensitive to visible light. For example, 500-nm light is detected at 1000 nm. Therefore it is important to use a 2nd-order filter to remove this light.

Highly opaque samples

Highly concentrated and opaque liquids often have problems with self-absorption or complete attenuation of the beam. Intensity measurements with the excitation beam at 90° to the emission beam may not be reproducible or detectable, and the excitation or emission spectra may appear distorted. Try front-face detection: the excitation light is focused to the front surface of the sample, and fluorescence emission is collected from this region at an angle that minimizes reflected and scattered light. With front-face detection, se the front face of the sample at 30° or 60° to the excitation beam.

An example at right shows the difference between detection at right-angles versus front-face.



Comparison of fluorescence emission signal from sickle-cell hemoglobin using right-angle versus front-face detection. The β-37 tryptophan is primarily responsible for this fluorescence.

Preliminary emission maximum

Note: *Follow this procedure if you are using an optional single-channel detector.*

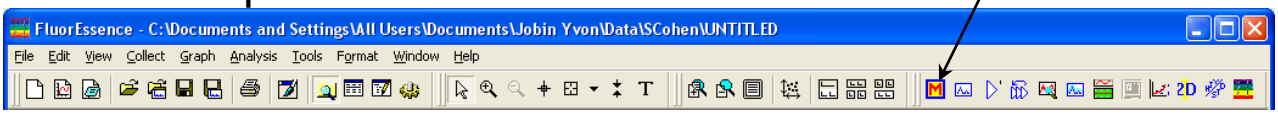
The objective is to acquire a preliminary emission scan using an approximate excitation wavelength. Once the emission peak is determined, this value is used to get the optimal excitation spectrum.

Note: *If you have not verified instrument performance, HORIBA Scientific recommends acquiring a lamp spectrum and a water Raman spectrum, as outlined in the previous chapter, before proceeding.*

1. Verify that all system components are on.
2. Place the sample in the sample compartment.

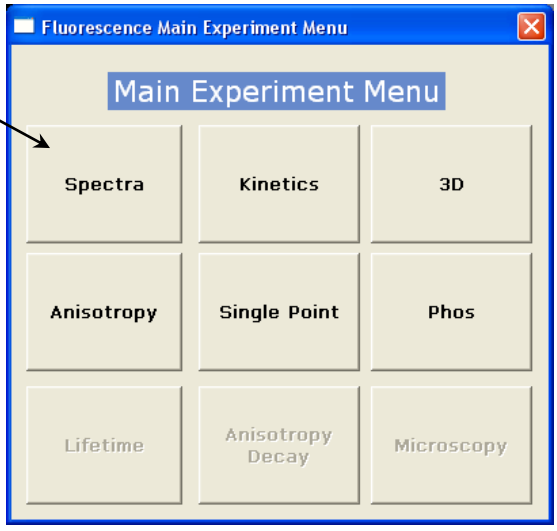
Note: *Make sure the lid is completely closed.*

3. Start FluorEssence™.
4. In the main FluorEssence window, click the Experiment Menu button .



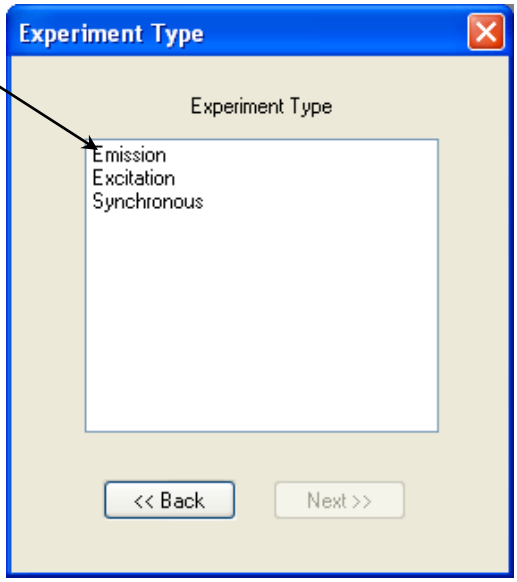
The **Fluorescence Main Experiment Menu** appears.

5. Click Spectra.



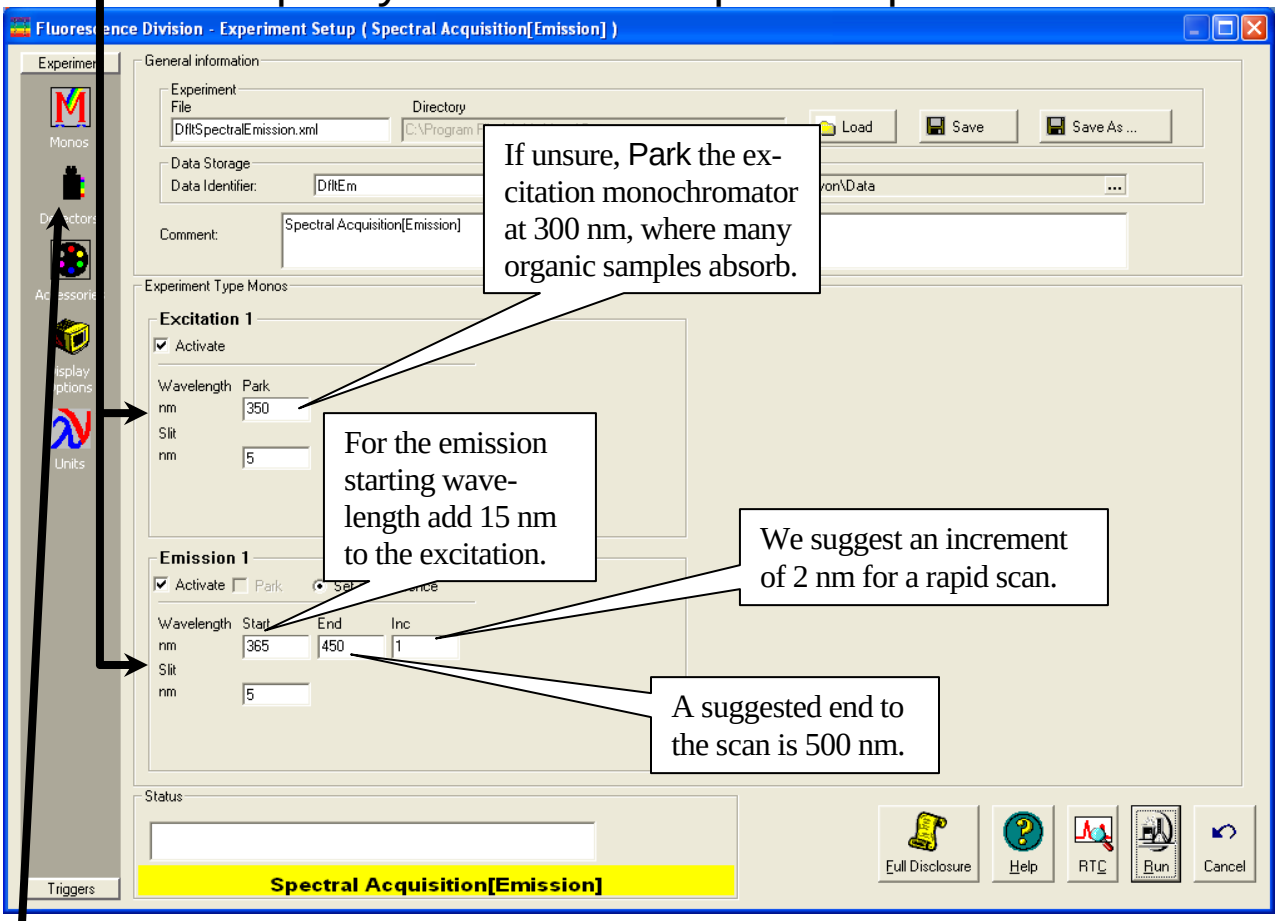
The **Experiment Type** window appears.

6 Choose the Emission sub-type of basic experiment.

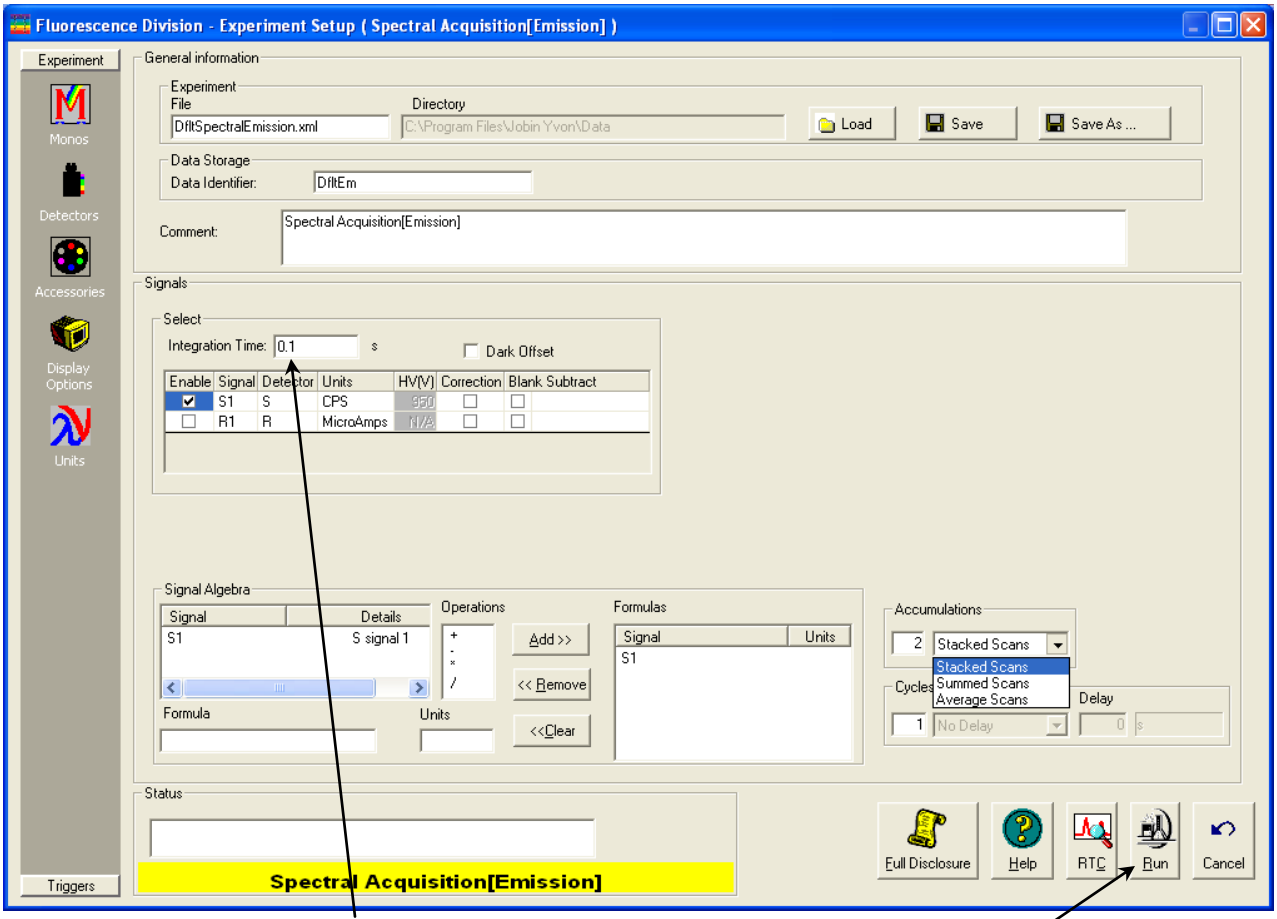


The **Experiment Setup** window opens.

7 Specify other data-acquisition parameters.



8 Click the Detectors icon.



9 Try 0.1 second integration time for a rapid scan.

10 Enter the data-file name, experiment file-name, and any comments to distinguish this experiment from others.

11 Click the Run button.

The experiment executes, then transfers the data to the Origin® window.

12 With the preliminary emission spectrum on the screen, note the greatest intensity.

13 Either

Record the wavelength at which this occurs. This is your emission maximum.

Note: *If the signal level exceeds 2 × 10^6 cps (for PMT) or 65 000 (for CCD or InGaAs array), decrease the slit widths by 50%. If you do not see an obvious peak, increase the excitation wavelength and the starting and ending wavelengths by 25 nm, and acquire another emission scan.*

Or

Repeat this procedure, adjusting parameters, until you find an emission peak.

Excitation scan

The next step is to use the recently discovered emission maximum to determine the optimal excitation/emission wavelength map for the sample. The procedure is very similar to that outlined above.

Improving the signal-to-noise ratio

Introduction

Because of various hardware or software conditions, occasionally it is necessary to optimize the results of an experiment.

The quality of acquired data is largely determined by the signal-to-noise ratio (*S*/*N*). This is true especially for weakly fluorescing samples with low quantum yields. The signal-to-noise ratio can be improved by:

- Using the appropriate integration time,
- Scanning a region several times and averaging the results,
- Changing the bandpass by adjusting the slit-widths, and
- Mathematically smoothing the data.

The sections that follow discuss the alternatives for improving the *S*/*N* and the advantages and disadvantages for each.

Determining the optimum integration time

The length of time during which photons are counted and averaged for each data point is referred to as the *integration time*. An unwanted portion of this signal comes from noise and dark counts (distortion inherent in the signal detector and its electronics when high voltage is applied). By increasing the integration time, the signal is averaged longer, resulting in a better *S/N*. This ratio is enhanced by a factor of $t^{1/2}$, where t is the multiplicative increase in integration time. For example, doubling the integration time from 1 s to 2 s increases the *S/N* by over 40%, as shown below:

For an integration time of 1 second,

$$S/N = t^{1/2}$$
$$= 1^{1/2}$$
$$= 1$$

For an integration time of 2 seconds,

$$S/N = t^{1/2}$$
$$= 2^{1/2}$$
$$\approx 1.414$$

or approximately 42%.

Because *S/N* determines the noise level in a spectrum, use of the appropriate integration time is important for high-quality results.

To discover the appropriate integration time:

1. Find the maximum fluorescence intensity by acquiring a preliminary scan, using an integration time of 0.1 s and a bandpass of 5 nm.
2. From this preliminary scan, note the maximum intensity, and select the appropriate integration time from the table below.

Signal intensity (counts per second) for PMT	Estimated integration time (seconds)
1000 to 5000	2.0
5001 to 50 000	1.0
50 001 to 500 000	0.1
500 001 to 4 000 000	0.05

The CCD saturates at 65 536 counts, but a scheme similar to a PMT holds.

Note: *This table is only a* **guide***. The optimum integration time for other measurements, such as time-base, polarization, phosphorescence lifetimes, and anisotropy, may be different.*

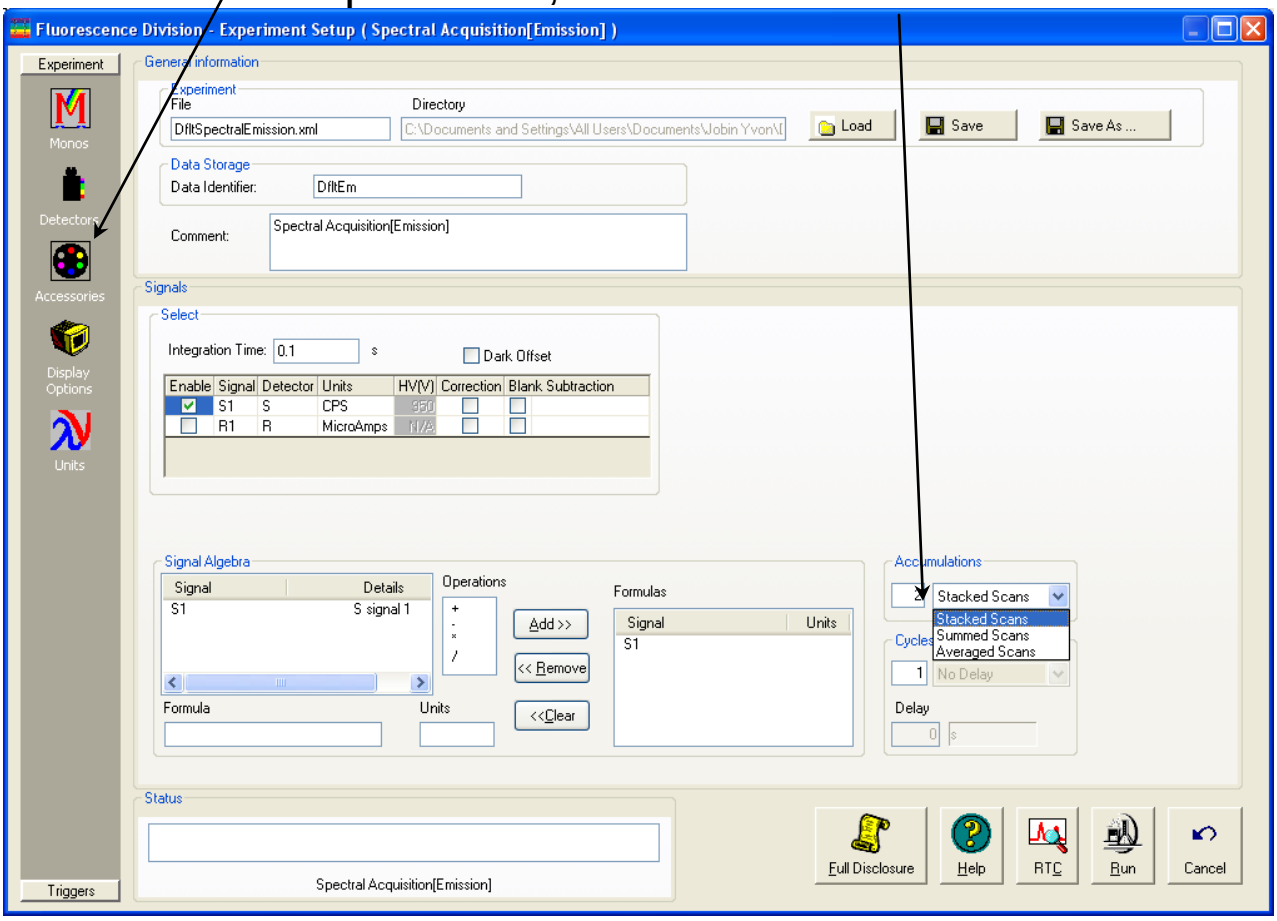
Set the integration time either through the **Experiment Setup** window (under the Detectors icon) for a specific experiment, or **Real Time Control** to view the effects of different integration times in real time. See on-line FluorEssence™ help for more information about the integration time.

Scanning a sample multiple times

Scanning a sample more than once, and averaging the scans together, enhances the *S*/*N*. In general, the *S*/*N* improves by $n^{1/2}$, where *n* is the number of scans.

To scan a sample multiple times,

1. Open the **Experiment Setup** window.
2. Choose the Detectors icon.
3. Specify the number of scans, and how to handle multiple scans, in the Accumulations field.



See FluorEssence™ on-line help for detailed instructions regarding the data-entry fields.

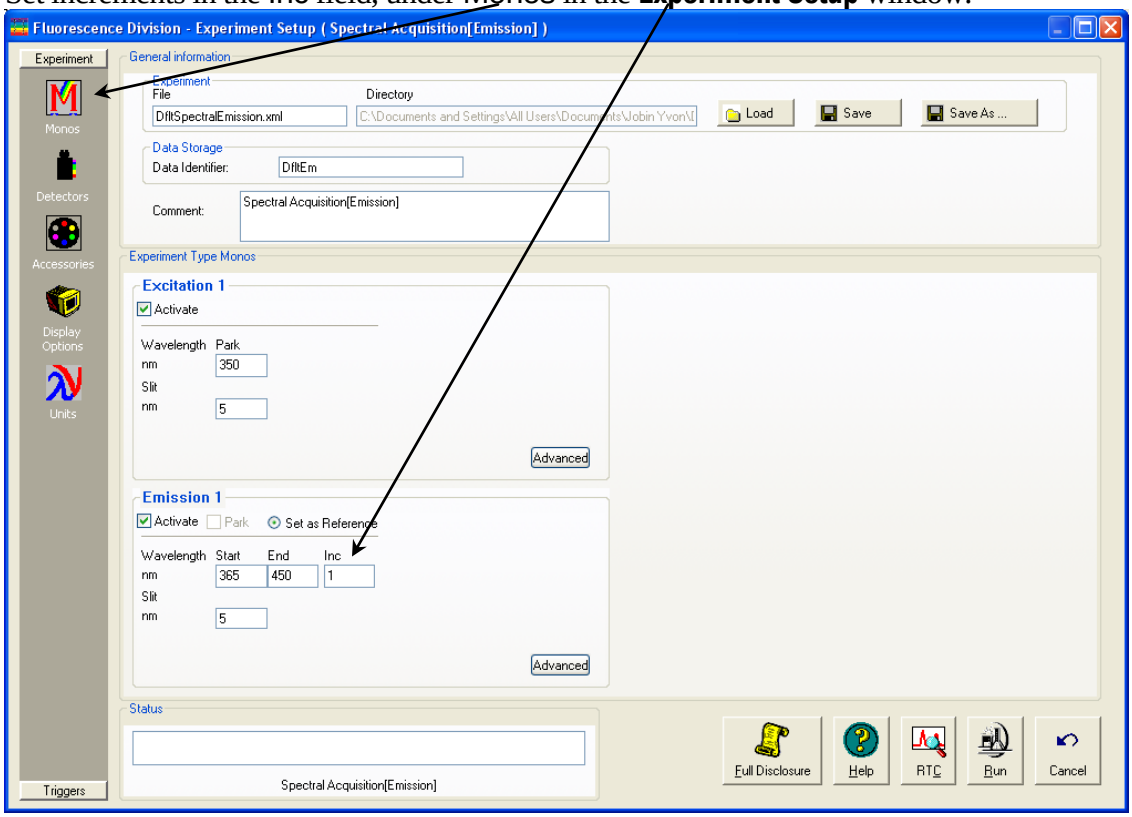
Using the appropriate wavelength increment

The increment in a wavelength scan is the spacing, in nm, between adjacent data points. The spacing between the data points affects the resolution of the spectrum, and total time for acquisition. Consider the required resolution, time needed, and concerns about sample photobleaching. Most samples under fluorescence analysis display relatively broad-band emissions with a Lorentzian distribution, so they do not require a tiny increment.

Common increments range from 0.05–10 nm, depending on the sample and slit size. A first try might be 0.5–1 nm increment. After acquiring the initial spectrum, examine the results. If two adjacent peaks are not resolved (i.e., separated) satisfactorily, reduce the increment. If the spectrum is described by an excessive number of data points, increase the increment, to save time and lamp exposure. A scan taken, using an increment of 0.1 nm, with a peak at full-width at half-maximum (FWHM) of 20 nm, should be characterized with a 1-nm increment instead.

For time-based scans, the increment is the spacing in s or ms between data points. Here, the consideration is the necessary time-resolution. The time increment dictates the total time per data point and for the scan in general. Set this value to resolve any changes in the luminescence of samples as they react or degrade. Time increments often range from 0.1–20 s.

Set increments in the Inc field, under Monos in the **Experiment Setup** window.



See the FluorEssence™ on-line help for more information.

Selecting the appropriate bandpass

The bandpass (wavelength spread) affects the resolution of your spectra. If the bandpass is too broad, narrow peaks separated by a small change in wavelength may be unresolved. For example, for two 2-nm peaks 5 nm apart, and a bandpass of 10 nm, one broad peak, instead of two well-defined ones, is visible.

By adjusting the slit widths, the intensity and bandpass of the light is controlled. The slits of the excitation spectrometer determine the amount of light that passes through the excitation spectrometer to the sample. The emission spectrometer slits control the amount of fluorescence recorded by the signal detector. Signal level is proportional to the square of the slit width, that is,

$$\text{signal level} \propto (\text{slit width})^2$$

Bandpass is calculated using the following formula:

$$\text{bandpass (nm)} = \text{slit width (mm)} \times \text{dispersion (nm/mm)}$$

The dispersion of the NanoLog® spectrofluorometers system depends upon the type of monochromator and spectrometer, and the groove-spacing of the gratings. For example, a NanoLog® with a single-grating monochromator and 1200 groove/mm grating has a dispersion of 4.0 nm/mm, while a NanoLog® with a double-grating monochromator and 1200 groove/mm gratings has a dispersion of 2.0 nm/mm. Below is a table listing various monochromators, installed gratings, and their respective dispersions.

Excitation monochromator	Grating groove-density (grooves/mm)	Monochromator's dispersion
Double	1200	2.1
	600	4.2
Single	1200	4.2
	600	8.4
Emission iHR 320 spectrograph		
	150	20*
	100	30*

*Dispersion varies slightly across near-IR wavelength range.

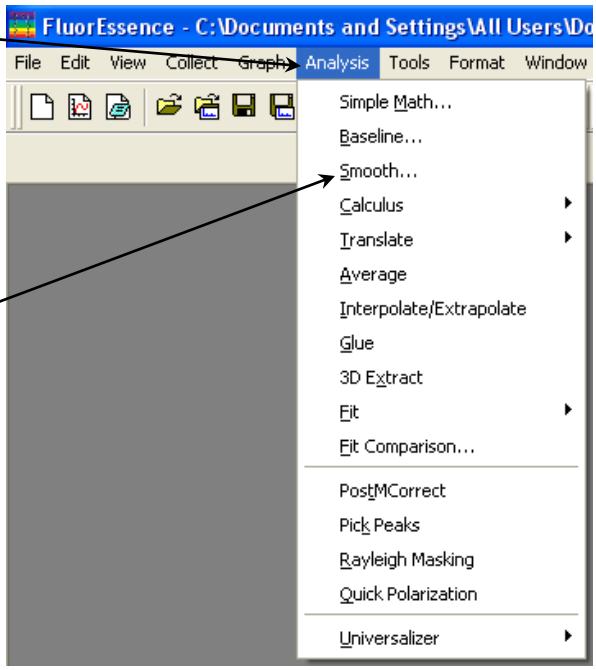
For steady-state fluorescence measurements, set the entrance and exit slits the same for a monochromator. (This occurs automatically when using bandpass units in FluorEssence™.) For biological samples that may photobleach, try narrowing the excitation slits and opening the emission slits wider.

Smoothing data

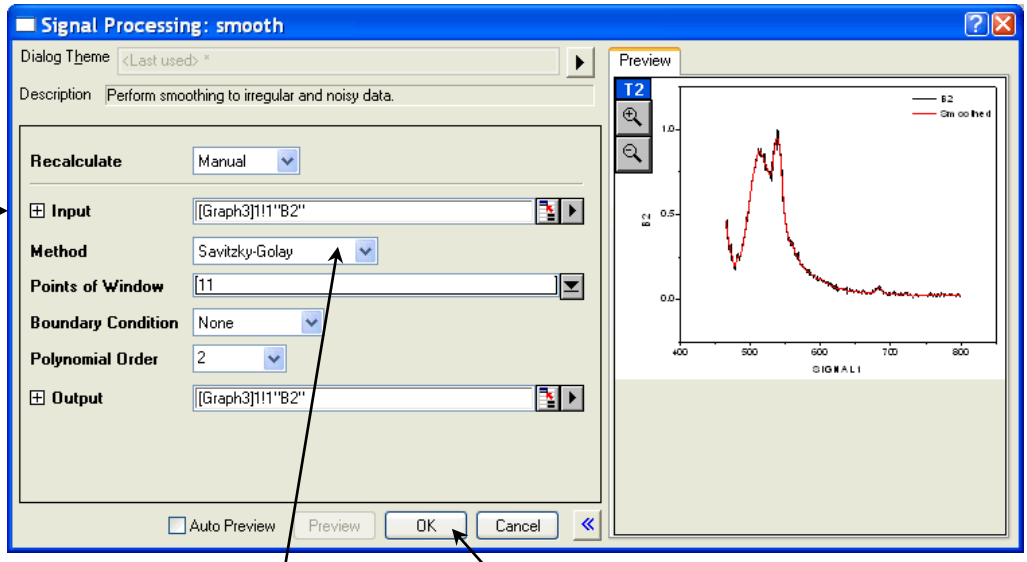
Smoothing the data improves the appearance of the spectrum. Smoothing, as are most post-processing features, is handled by Origin®.

1 Select Analysis from the main FluorEssence™ toolbar.
A drop-down menu appears.

2 Choose Smooth....



The **Smooth** window opens.



3 In the Input drop-down menu, choose the appropriate file to smooth.

4 In the Method drop-down menu, choose FFT Filtering (fast-Fourier transform), Adjacent Averaging, or Savitzky-Golay smoothing.

5 Click the OK button to smooth the data.

See the on-line Origin® help for additional information regarding smoothing data.

In general, start with a 9- or 11-point smooth for a time-base measurement. To select the proper number of points for wavelength-scan types, first locate the area that requires smoothing—usually this is a peak. Determine the number of data points used to make up the peak, and then smooth the data using the number of points closest to this number. To avoid artificially enhancing the data, use the appropriate number of points to smooth the data. For example, selection of too large a number results in the background being smoothed into the peak.

Correcting data

Introduction

Collecting accurate information about the luminescent properties of a sample depends upon several factors:

- Equipment specifications,
- Sample characteristics, and
- Timing considerations.

To ensure that the spectra collected indicate the actual properties of the sample and not external conditions, data must be corrected. To correct data means to subtract information not directly related to the properties of the sample. Gratings, detectors, and monochromator or spectrometer components have response characteristics that vary as a function of wavelength. These characteristics may be superimposed on spectra, thereby yielding a potentially misleading trace. For the Nanolog®, array detectors include their own characteristics, including temperature (which can affect sensitivity to longer wavelengths), center wavelength, bandpass, and dark current. For accurate intensity comparisons, such as those required for quantum-yield calculations, response characteristics must be eliminated. Supplied with the instrument are sets of excitation and emission correction factors to eliminate response characteristics. These files (ignoring their 3-letter extensions), `xcorrect` and `mcorrect` (and `tcorrect` for a T-side detector), are included with the software; copy them to your hard disk.

Note: *The excitation range is 240–600 nm and the emission range is 290–850 nm for the reference detector.*

Items that may be convoluted into a spectrum	How to remove these artifacts
Fluctuations caused by the light source	Monitor lamp-output using the reference detector R, and use the signal ratio S/R to correct lamp-profile or temporal fluctuations
Influence of the sample holder	Run a blank scan (which is later subtracted from the sample scan)
System hardware (e.g., optics, detectors)	Use radiometric correction factors

To use radiometric correction factors, either:

- Select the ones supplied with the software, or
- Select a set generated at your facility during or after data-acquisition, discussed in the following section. Acquiring radiometric correction factors is explained in the chapter *Producing Correction Factors*.

Blank Subtraction and Dark Offset functions are described in the on-line FluorEssence™ help files.

During acquisition

Data can be acquired either as raw data or as corrected data. A spectrum composed of raw data exhibits the effects of system parameters, while a corrected spectrum displays only the properties related to the sample.

Note: *Before applying correction factors, HORIBA Scientific recommends subtracting the dark counts, and the spectrum of the blank, from the data. See the on-line FluorEssence™ help files for specific instructions.*

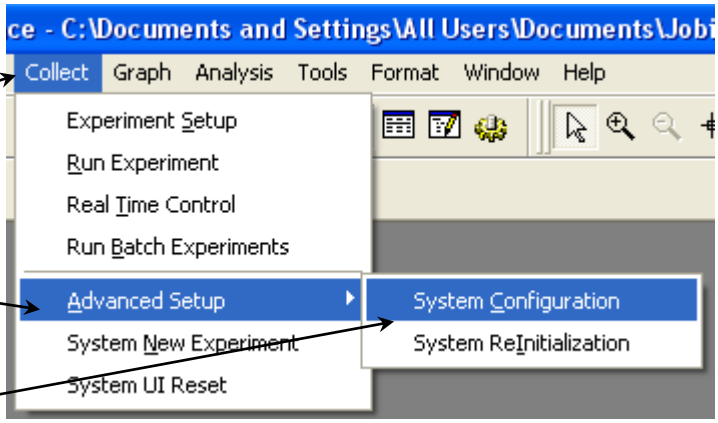
Caution: *The* `mcorrect` *and* `xcorrect` *files are custom-generated at the factory for each particular instrument, and cannot be swapped.*

1 Be sure the instrument configuration has a layout that includes a correction file associated with the appropriate detector.

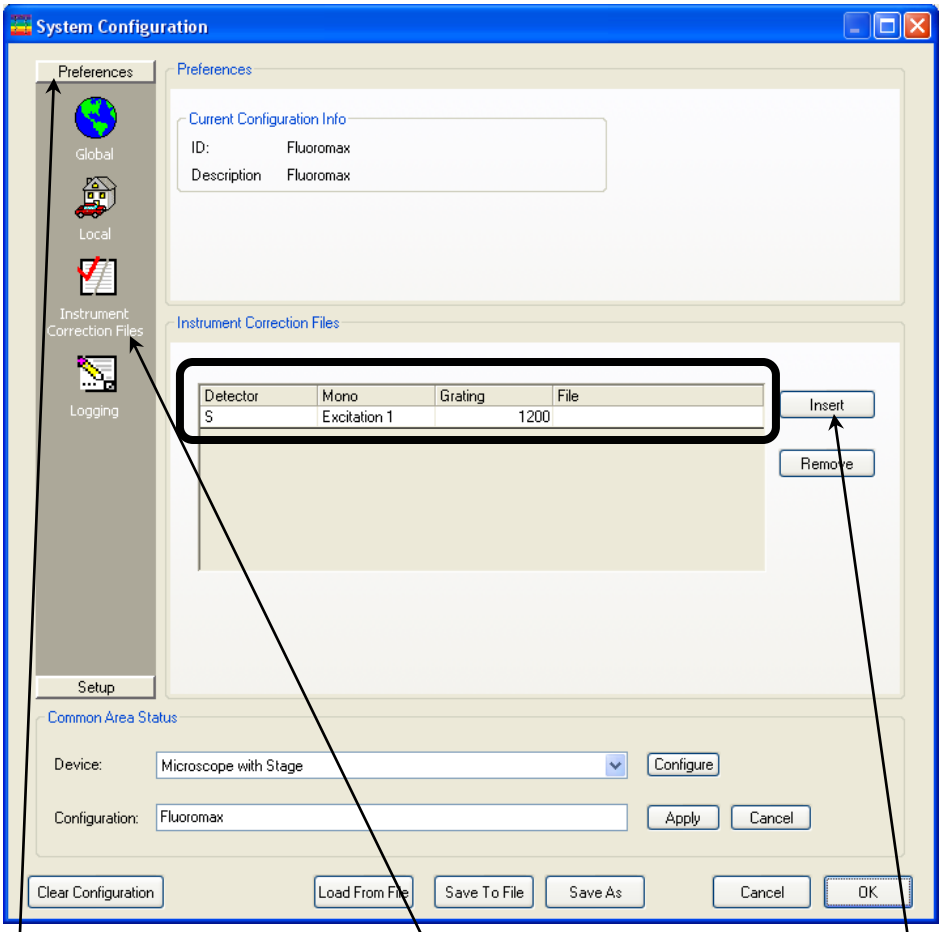
a Choose Collect in the toolbar.

A drop-down menu appears.

b Choose Advanced Setup, then System Configuration.



The **System Configuration** window appears:



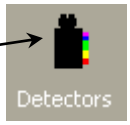
c Choose Preferences, then the Instrument Correction Files icon.

The Instrument Correction Files area should display a correction file for the Detector (S or R). If not, click the Insert button, and browse for the desired correction file.

Detector	Correction-factor file name
S	`mcorrect`
R	`xcorrect`
T (if used)	`tcorrect`

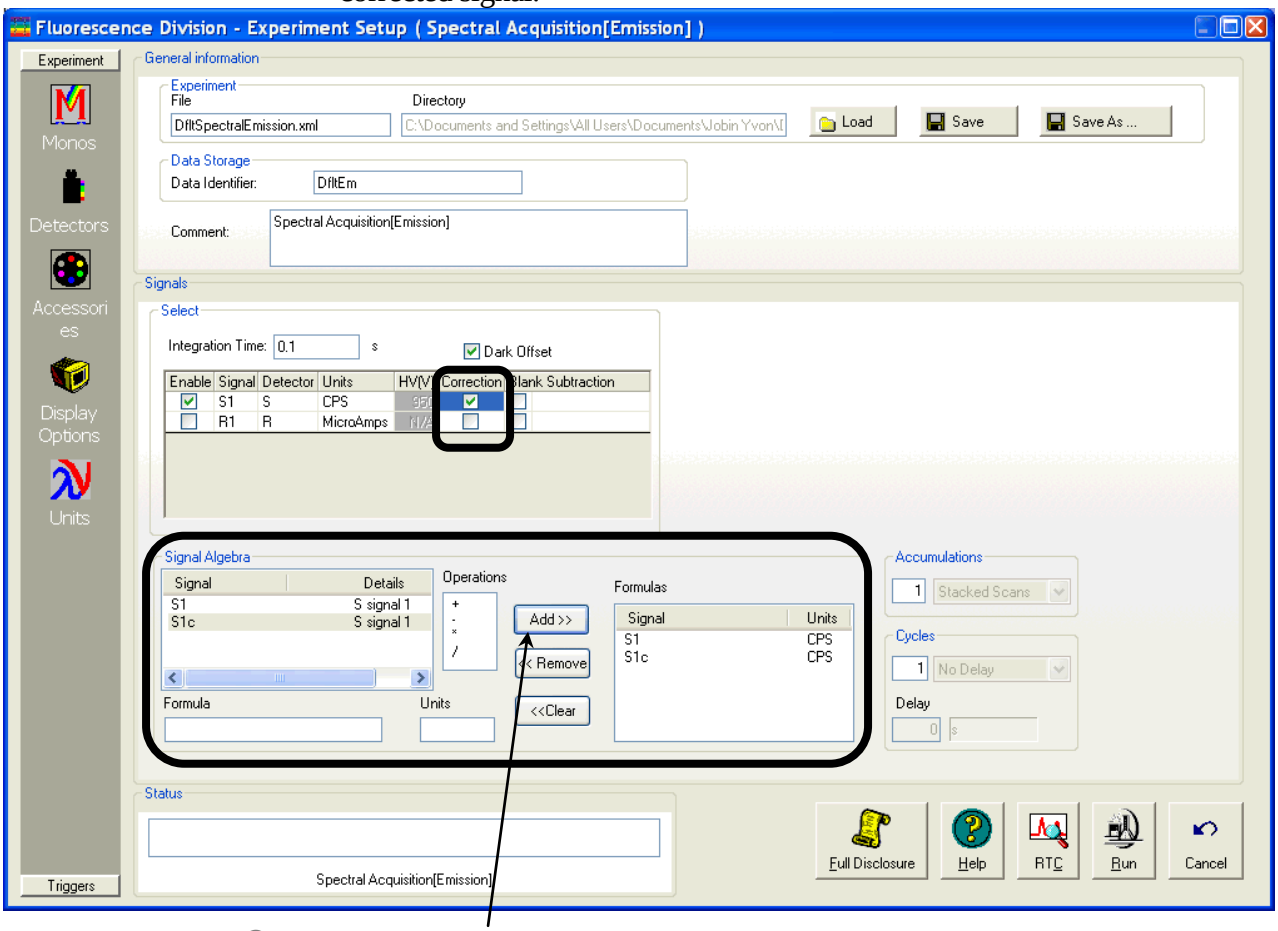
2 Choose correction when setting up the experiment.

a In the **Experiment Setup** window, choose the Detectors icon to display the detectors' parameters.



b Click the Correction checkbox for the detector you want corrected.

In the Signal Algebra area, a signal with appended "c" appears, denoting a corrected signal:



c Click the Add >> button to add the corrected signal to the Formulas table.

The corrected signal appears in the Formulas table.

d Run the experiment with the corrected signal.

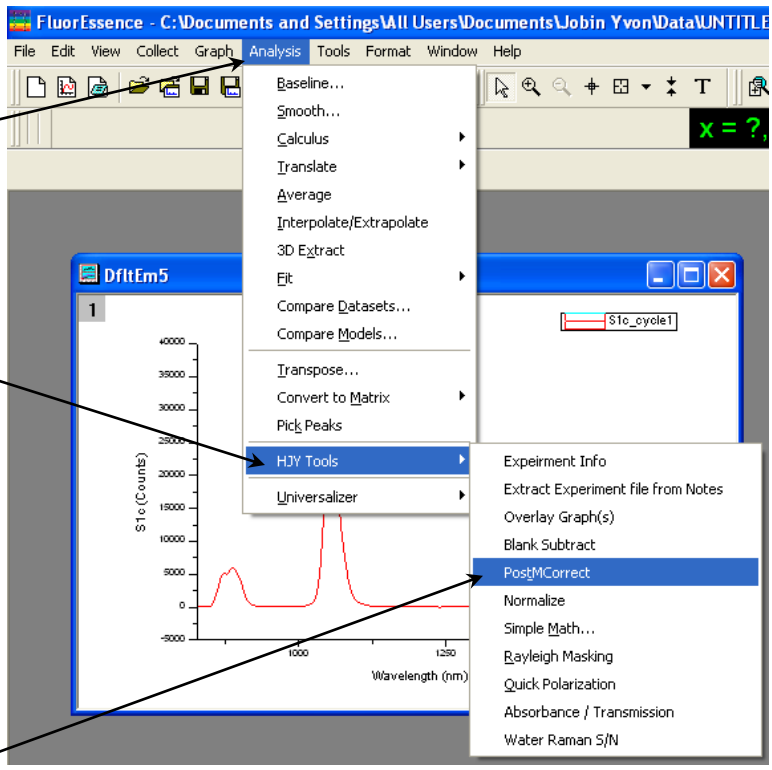
After acquisition

To apply the correction factors after the data have been acquired, use the PostMCorrect function in the HJY Tools menu.

1 Make sure the graph is open, and the trace to be corrected is active in the main **FluorEssence** window.

2 Choose Analysis.
A drop-down menu appears.

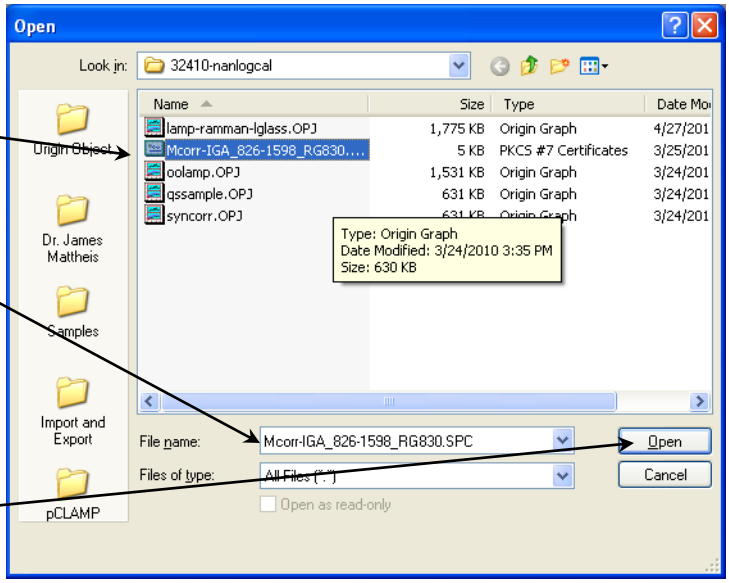
3 Select HJY Tools.
Another drop-down menu appears.



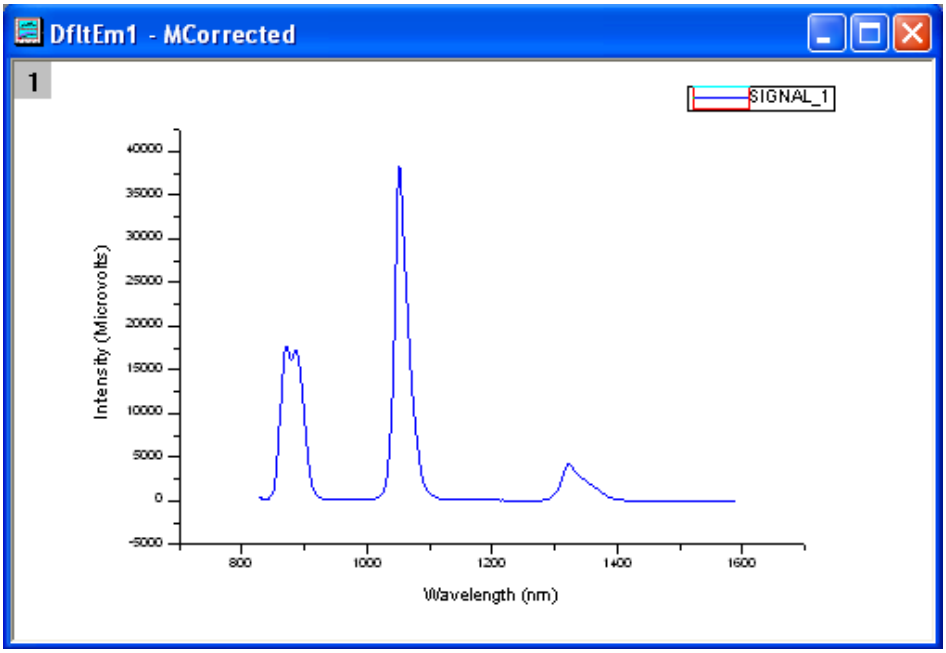
4 Select PostMCorrect.
The **Open** window appears:

In the File name field, choose the appropriate correction file, usually of type `*.spc`.

5 Click the Open button to open the correction file.



The trace that appears is the corrected spectrum using the correction-factor file you chose:



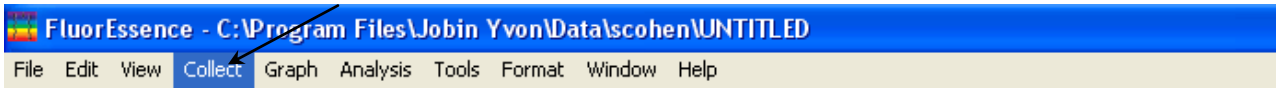
Correcting data on an iHR during acquisition

Data can be acquired either as raw data or as corrected data. A spectrum composed of raw data exhibits the effects of system parameters, while a corrected spectrum displays only the properties related to the sample.

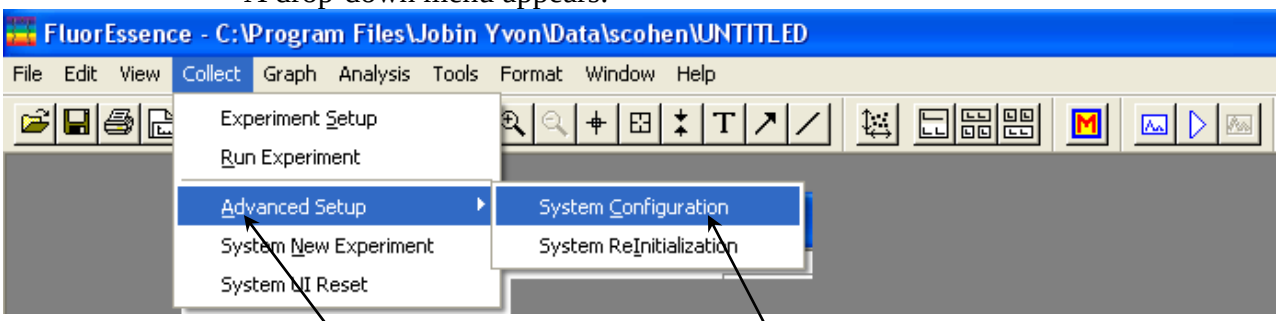
1 Be sure the instrument configuration has a lay-out that includes a correction file associated with the iHR.

Warning: *The iHR's* `mc...`, `xc...`, *and* `tc...` *files are custom-generated at the factory for each particular instrument, and cannot be swapped.*

a Choose **Collect** in the toolbar.

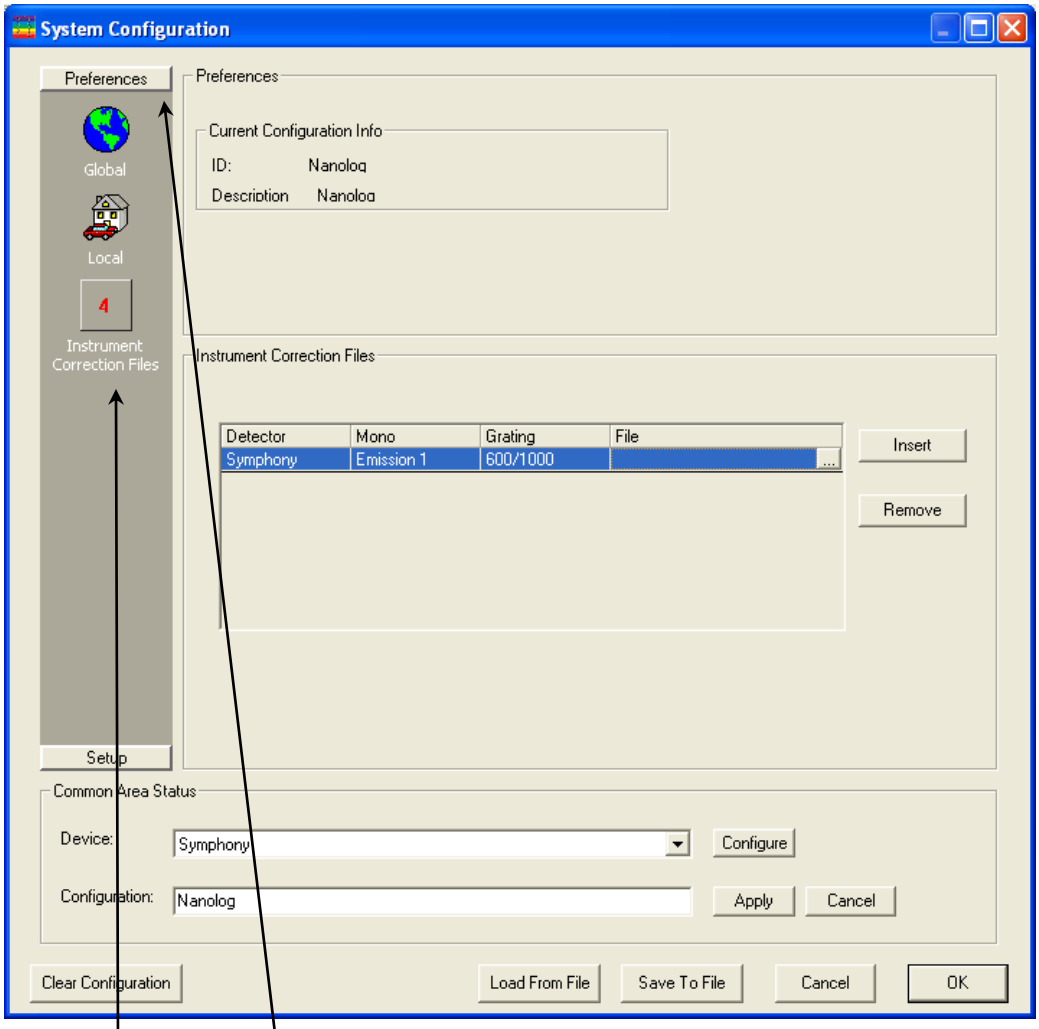


A drop-down menu appears.

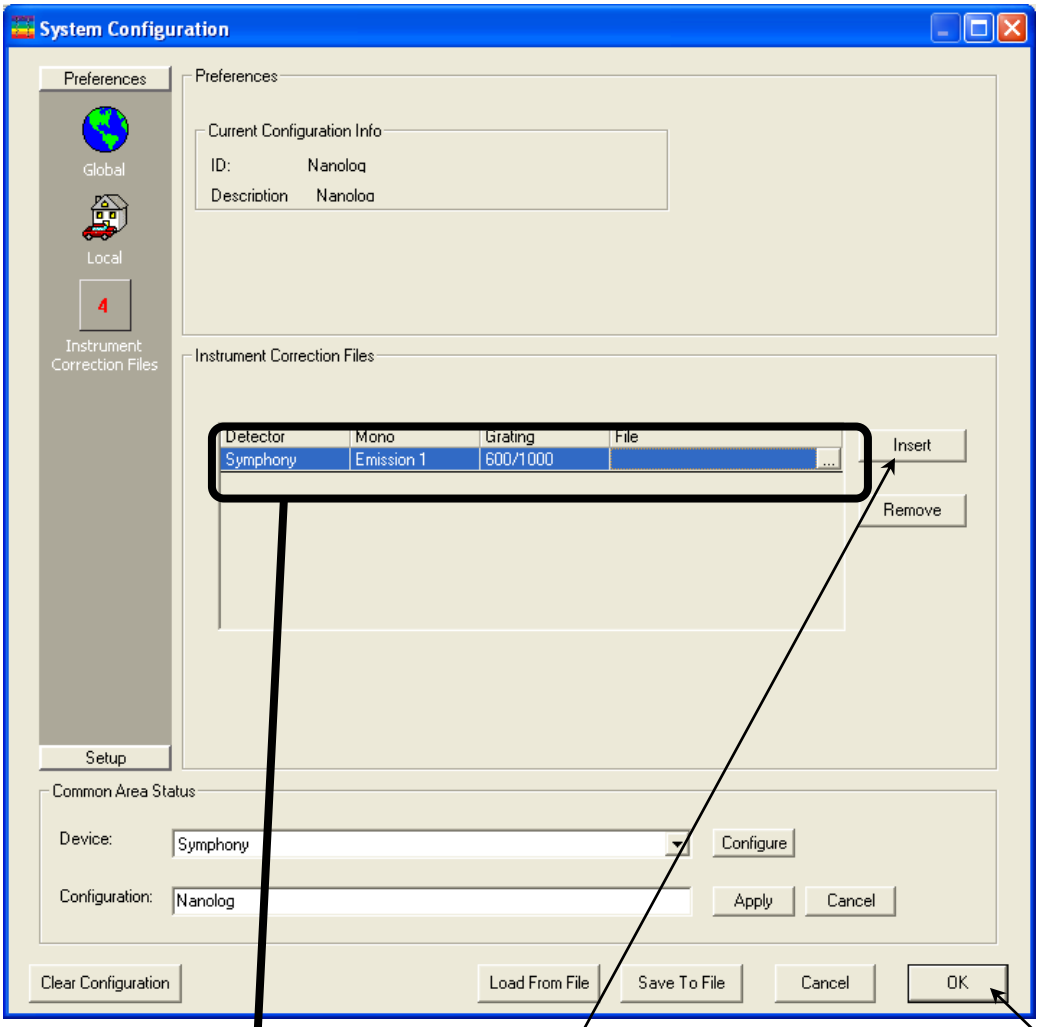


b Choose **Advanced Setup**, then **System Configuration**.

The **System Configuration** window appears.



C Choose Preferences, then the Instrument Correction Files icon.



The Instrument Correction Files area should display a correction file for the Detector (Symphony or other special iHR-related controller and detector). If not, click the Insert button, and browse for the desired correction file using the Browse button. (The correction-factor file is `mc...` generally, as discussed above).

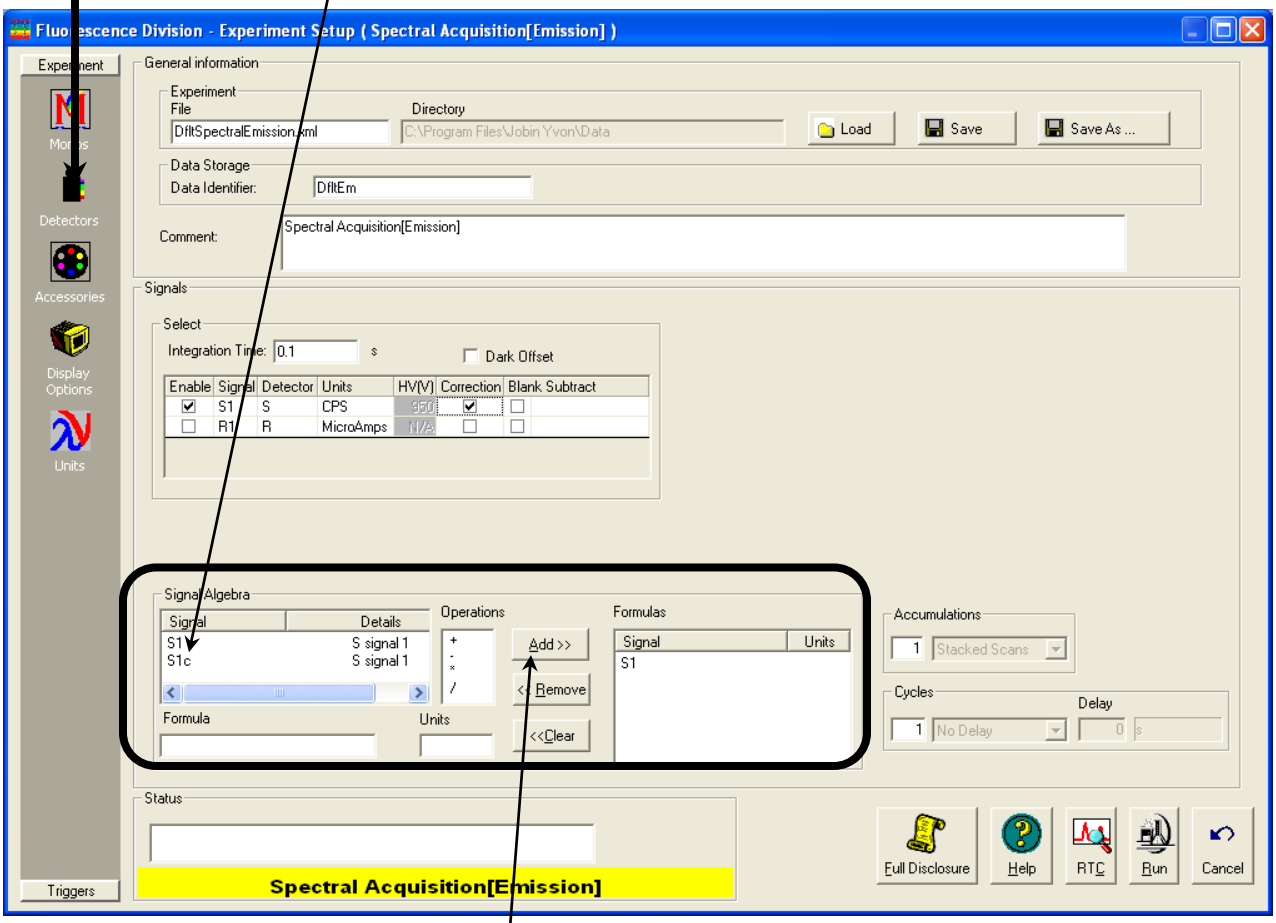
d Click the OK button to close the **System Configuration** window.

2 Choose correction when setting up the experiment.

a In the **Experiment Setup** window, choose the Detectors icon to display the detectors' parameters.

b Click the Correction checkbox for the detector you want corrected.

In the Signal Algebra area, a signal with appended "c" appears, denoting a corrected signal.



c Click the Add >> button to add the corrected signal to the Formulas table.

The corrected signal appears in the Formulas table.

3 Run the experiment with the corrected signal.

6: System Maintenance

External case

The NanoLog® spectrofluorometer requires very little maintenance. The outside panels may be wiped with a damp cloth to remove dust and fingerprints. Blow pressurized air onto the exhaust filters periodically to clean away dust.

Xenon lamp

The lamp is the only component that has to be replaced routinely. Regular examination of lamp and laser-glass spectra serves as an early indicator of the system's integrity. (These two tests are described in Chapter 3, *System Operation*.)

Obtaining good spectral results depends upon the xenon lamp. After 1700–2000 hours of use for the 450-W xenon lamp, the lamp output decreases significantly, indicating that the lamp should be replaced. Replacing the lamp within the specified time may prevent system failure. HORIBA Scientific advises to keep a laboratory notebook near the Nanolog® to record lamp usage. Each time the lamp is turned on, it constitutes one full hour of use; therefore, HORIBA Scientific suggests leaving the lamp on between brief periods of inactivity. Record the hours of use on the form in Chapter 11: *Xenon Lamp Information and Record of Use Form*.

Required tools

Tool	Size	Purpose
Allen wrench	5/32″	Screws for lamp power cables
Allen wrench	5/64″	Height adjustment; centering adjustment
Allen wrench	7/64″	Lamp support arm
Phillips screwdriver	Medium	Screws for top cover of housing

Replacement

The replacement xenon lamp is packed in the manufacturer's box and must be installed in the lamp housing. Read all the packing material including instructions and warnings before attempting to insert the lamp into the lamp housing.

Caution: *Do not remove the protective cover from the replacement xenon lamp until instructed to do so.*

Warning: *Xenon lamps are an explosion hazard. Make sure the power is off and all AC power is disconnected from the system. Read and follow the cautions presented below.*

Hazards

! Xenon arc lamps are an explosion hazard. Wear explosion-proof face-shield and protective clothing when opening the lamp housing and handling the lamp.

! Disconnect the lamp power supply from the AC power line (mains) while handling lamp leads. Lethal high voltages may exist.

! The lamp remains extremely hot for approximately one-half hour after it has been turned off. Do not touch the lamp or the metal unit until the lamp has cooled.

! Never look directly at the xenon arc or its reflection. Intense radiation can permanently damage eyes.

! Do not touch the focusing lens, back-scatter mirror, or the surface of the lamp. Fingerprints will be burned onto these surfaces when the lamp is ignited.

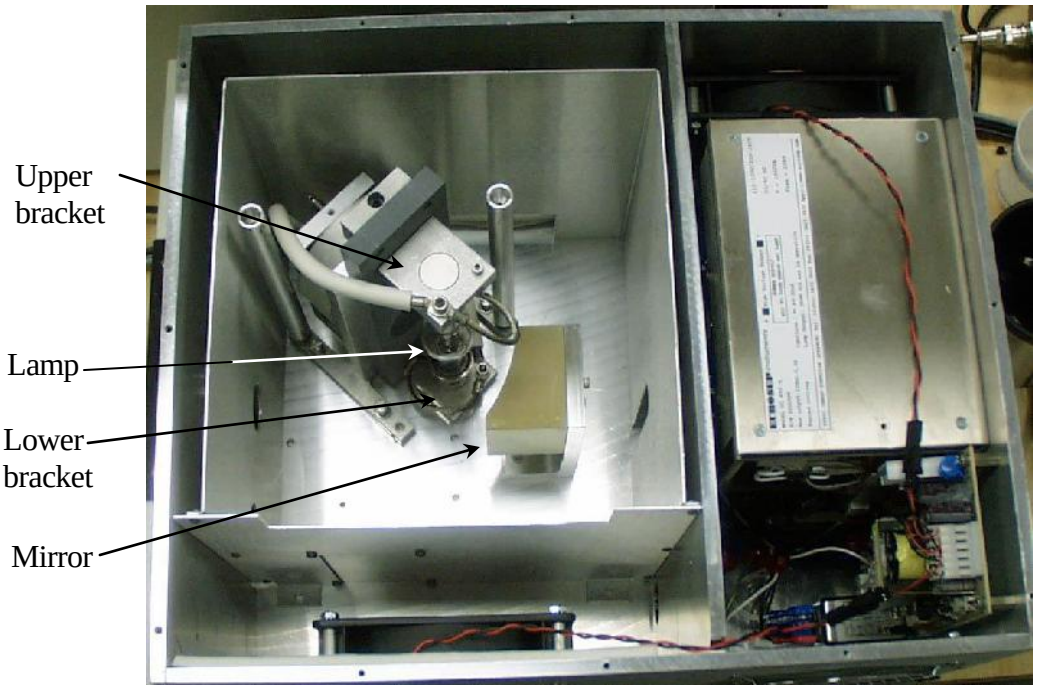
The following instructions are divided into three distinct procedures—each of which may be performed as a stand-alone operation:

- Open the lamp housing
- Remove the existing xenon lamp
- Install a new xenon lamp

To open the lamp housing:

1. Remove the 10 Phillips-head screws on top of the lamp housing.
2. Lift off the cover.

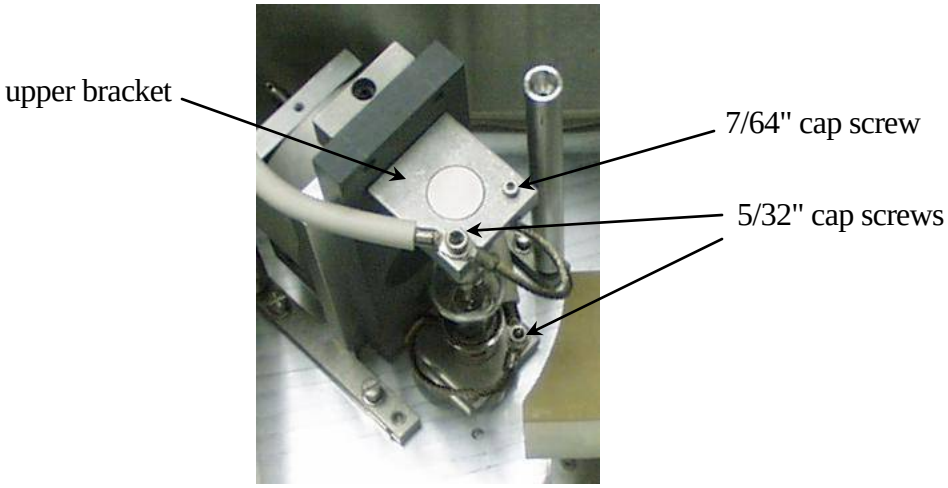
   This exposes the lamp assembly:



Top view of the lamp housing with cover removed.

To remove an existing lamp,

1 Remove the 5/32" cap screws from the upper and lower brackets, freeing the positive and negative power leads.



2 Remove the 7/64" cap screw from the upper bracket.

3 Remove the upper bracket.

4 Remove the lamp.

5 Place the protective cover around the xenon lamp.

6 Lock the cover into place.

Dispose of the spent xenon lamp, following all safety precautions and regulations.

To insert a xenon lamp

1 Be sure the cover of the lamp housing is removed.

Note: *HORIBA Scientific provides new bulbs with leads and connectors of the proper length and size. Other manufacturers' lamps may require extra adjustment before installation.*

2 Pay attention to the polarity of the xenon lamp.

Orient the lamp as shown in this diagram, so that the cathode is above the anode.

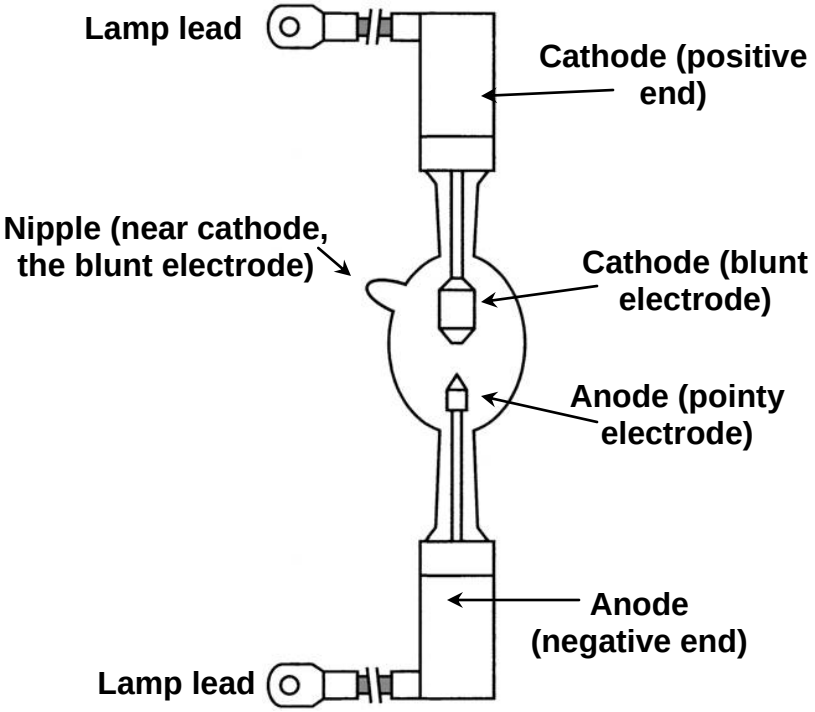


Diagram of lamp emphasizing polarity.

3 Seat the xenon lamp in the bottom bracket.

Make sure that the lamp is inserted all the way into the aluminum bracket. The negative end should be downward.

4 Place the square aluminum upper bracket over the positive terminal of the bulb.

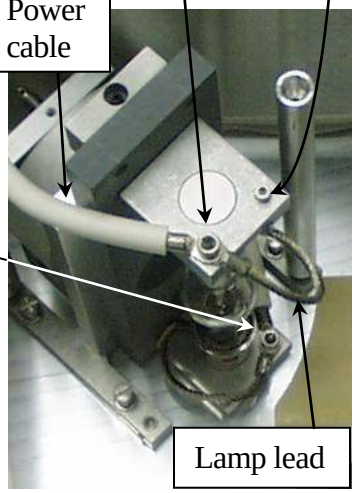
The positive end is marked with a "+", and should be pointing upward.

5 Insert and tighten the 7/64″ cap screw on the upper bracket.

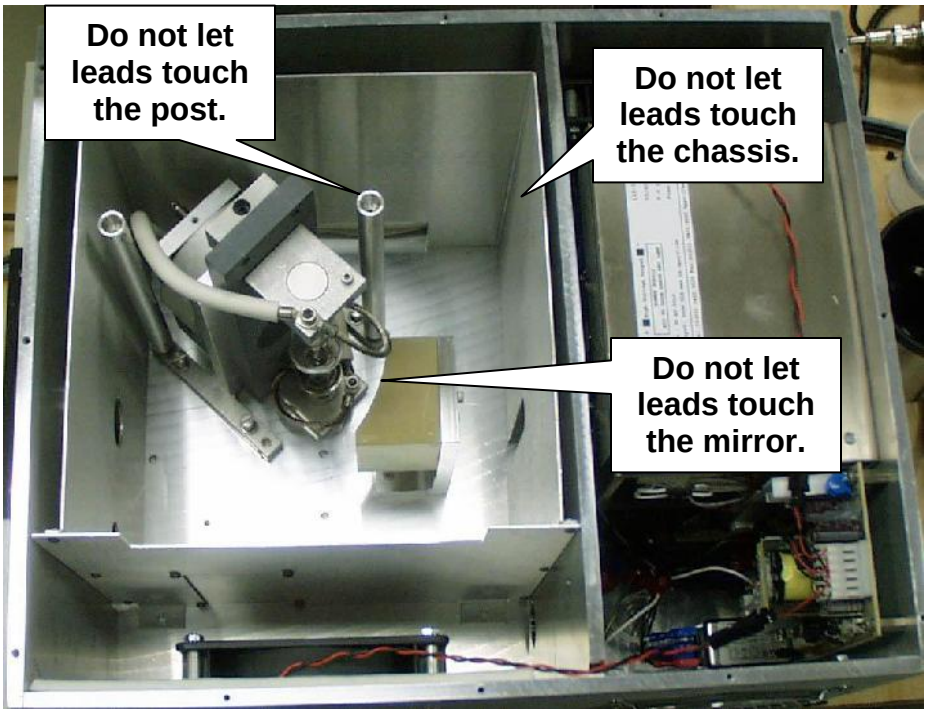
6 Secure the upper lamp lead and upper power cable to the upper bracket, using a 5/32″ cap screw.

7 Secure the lower lamp lead and lower power cable to the lower bracket, using a 5/32″ cap screw.

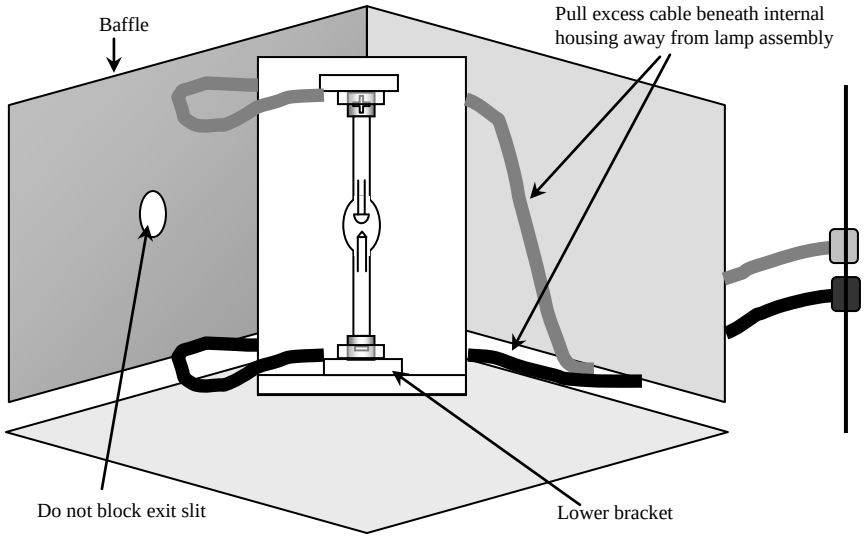
8 Tighten these two 5/32″ cap screws on the upper and lower brackets.



Caution: *Both lamp leads are at a potential with respect to the ground (earth). Do not let bare lamp leads touch* ***any part*** *of the chassis, which is grounded. You can severely damage the power supply if they do.*



9 Pull the excess power cables away from the lamp assembly beneath the baffle.



Note: *Make sure the cables do not block the exit slit of the lamp housing.*

Diagram of lamp in place.

10 Replace the cover of the lamp housing and secure with the ten Phillips-head screws.

11 Plug the power cord from the lamp power supply into an outlet with the proper line voltage.

Warning: DO NOT *operate this system from an un-grounded AC power source.*

Note: *After installation, the lamp should burn in for 24 hours. After the burn-in, the lamp's position may be adjusted to optimize the signal intensity.*

Adjustment

Once the lamp is installed (and after a 24-hour burn-in period), it may need an adjustment to maximize the sensitivity of the NanoLog®. To do this, HORIBA Scientific recommends running a laser-glass scan to check wavelength accuracy, and then monitoring the peak intensity while adjusting the vertical height of the lamp:

1 Take an emission calibration scan.

a Insert the laser glass into the sample compartment as described on page 3-10.

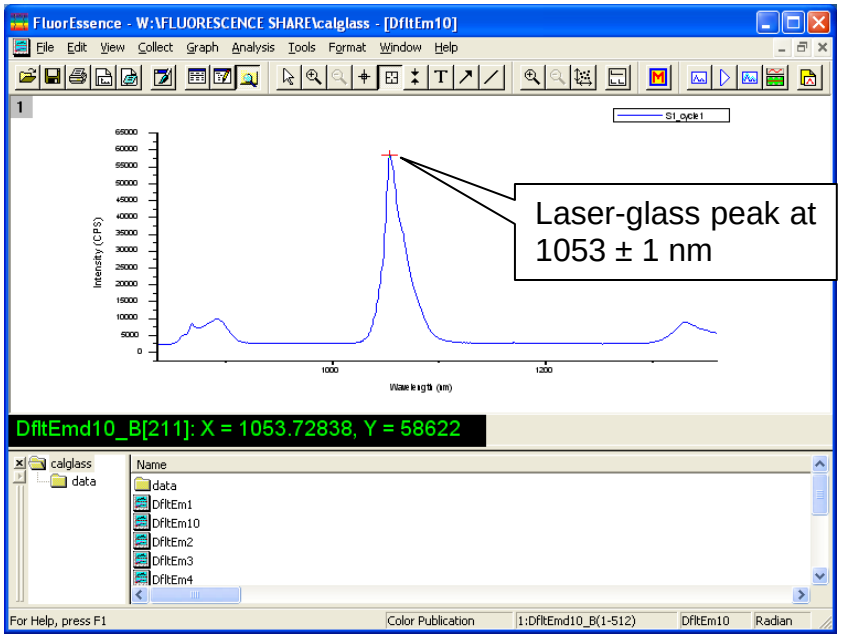
b Set the instrument to RA (right-angle mode).

c Place an 830 nm long-pass filter at the entrance to the emission spectrometer.

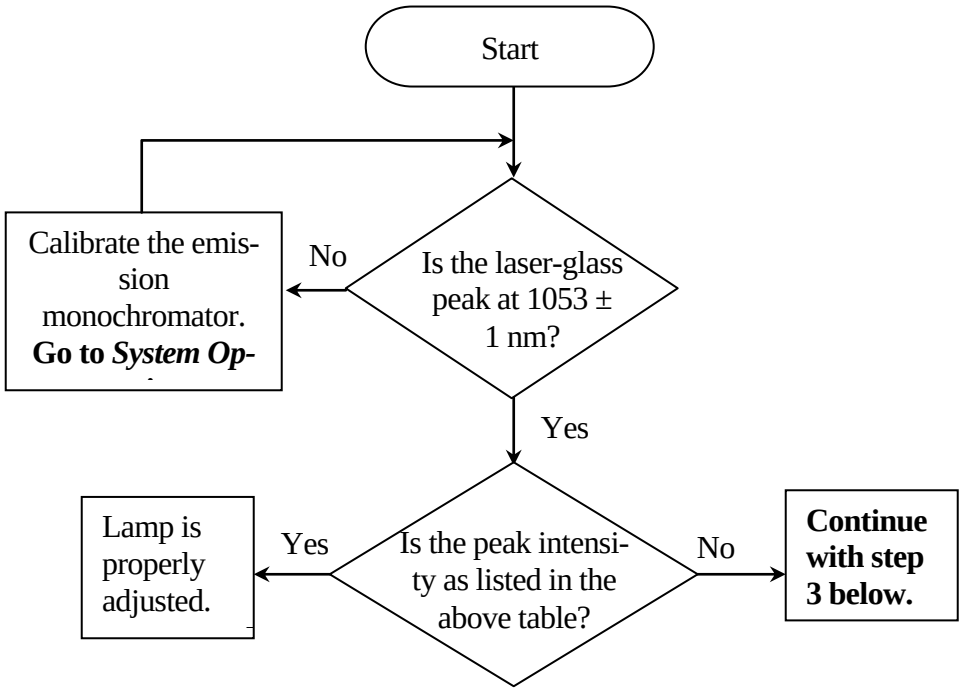
d Take a laser-glass scan (emission calibration scan in Chapter 3) with these parameters:

Excitation wavelength	Park at 575 nm
Emission wavelength	Park at 1105 nm for 150 grooves/mm gratings, or 1210 nm for 100 grooves/mm gratings
Excitation and Emission slits	1 nm bandpass
Integration time	5 s
CCD Options	Center wavelength
Grating	150 grooves/mm blazed at 1200 nm or 100 grooves/mm blazed at 800 nm
Acquisition mode	S1 (Symphony)

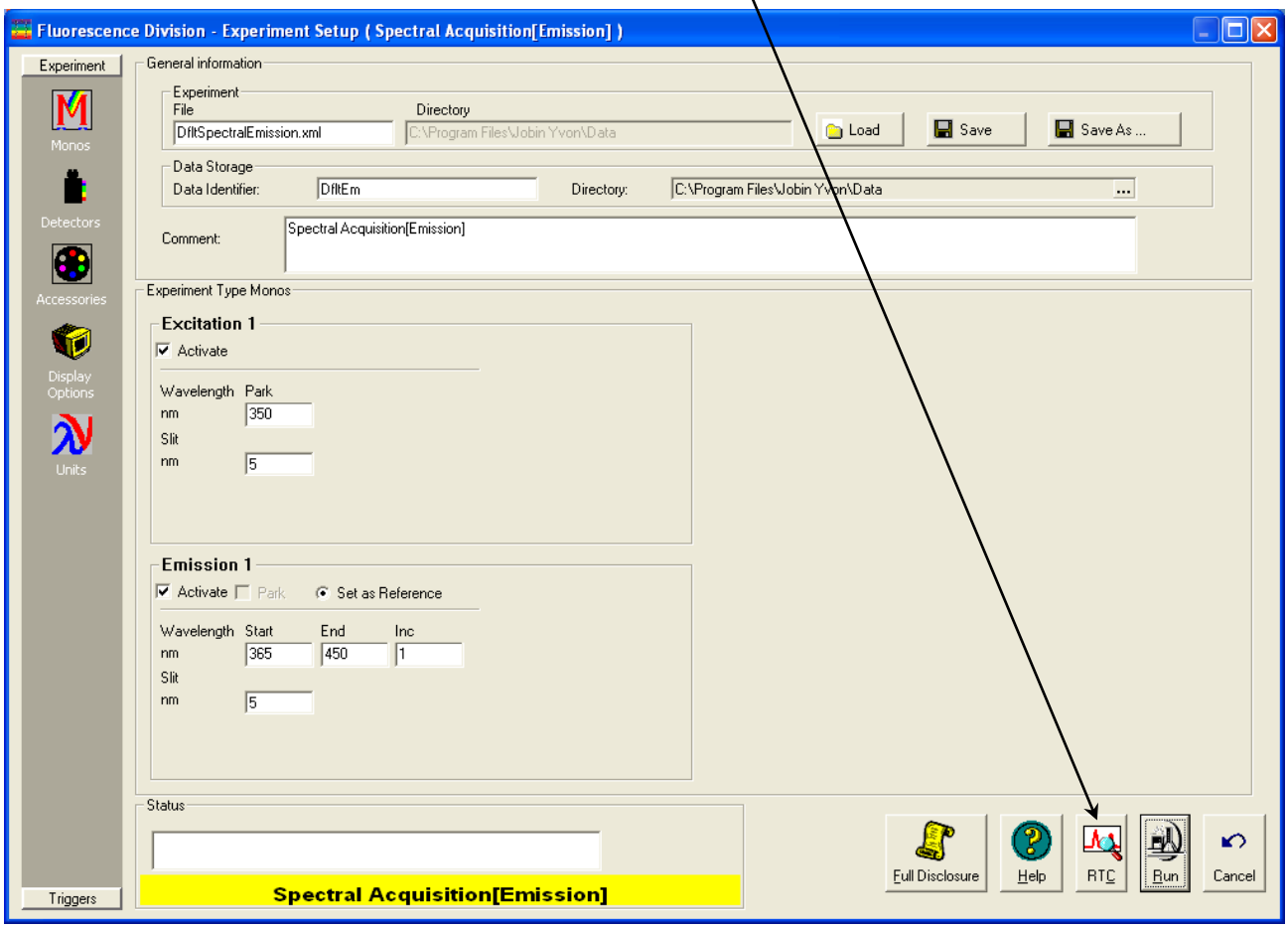
With right-angle mode using a 1933 sample-holder rotated to 30°, the laser-glass peak intensity for the NanoLog® spectrofluorometer should be > 50 000 counts:



2 Follow the flowchart below.



3 Enter the **Real Time Control** from the **Experiment Setup** window.



4 Set the excitation and emission spectrometers to 575 nm and 1053 nm, respectively.

The laser glass's emission appears at 1053 nm when water is excited at 575 nm.

5 Make sure HV1 is on and gain is high.

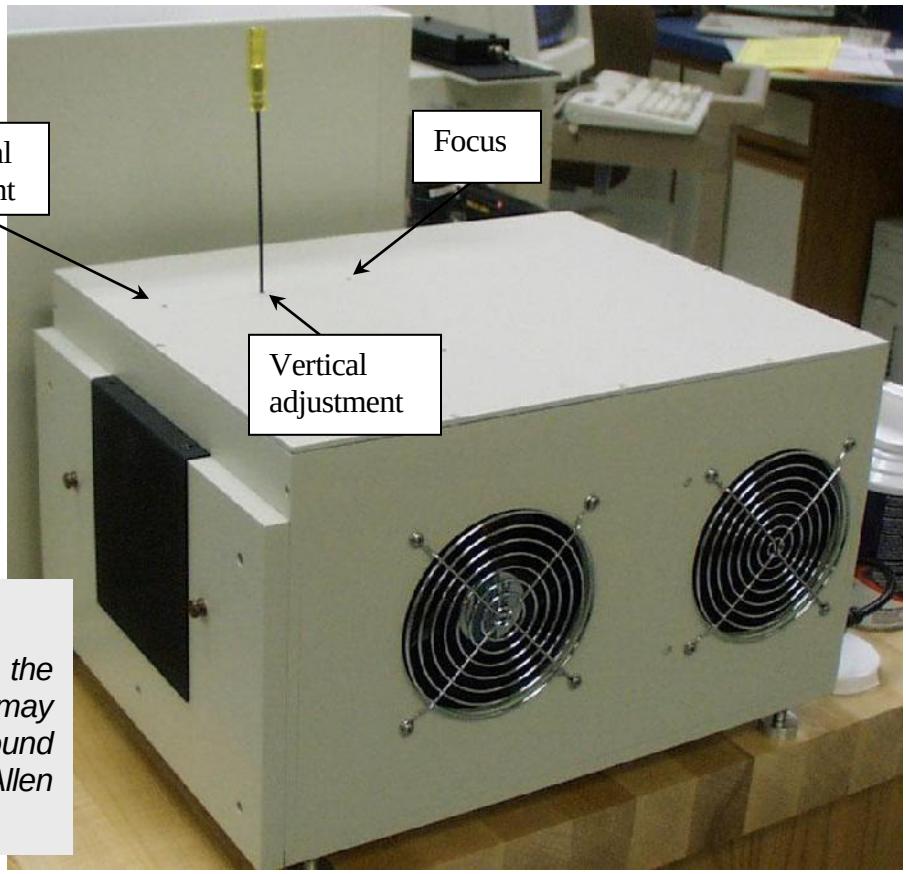
6 Open the programmable excitation shutter.

7 View the incoming data in acquisition mode S1 (Symphony detector).

Watch the signal intensity as you adjust the vertical position of the lamp. Therefore, position the monitor to see the changes while standing by the lamp housing.

Note: *If the lamp is not at the best vertical position, correctly positioning the lamp will increase the signal significantly.*

8 Insert a 5/64″ Allen wrench inside the middle port on the top of the lamp housing.



Note: *To reach the set screw, you may have to fish around with the Allen wrench.*

The Allen wrench is ready to adjust the vertical position of the lamp.

9 View the trace and make vertical adjustments by rotating the wrench either left or right to increase the signal.

10 Return to the **Run Experiment** application.

11 Re-scan the laser-glass spectrum.

This should result in a clean scan at maximum intensity.

Note: *If there is a problem, you may have to adjust the horizontal position of the lamp. This is rarely necessary, and should only be performed as a last resort.*

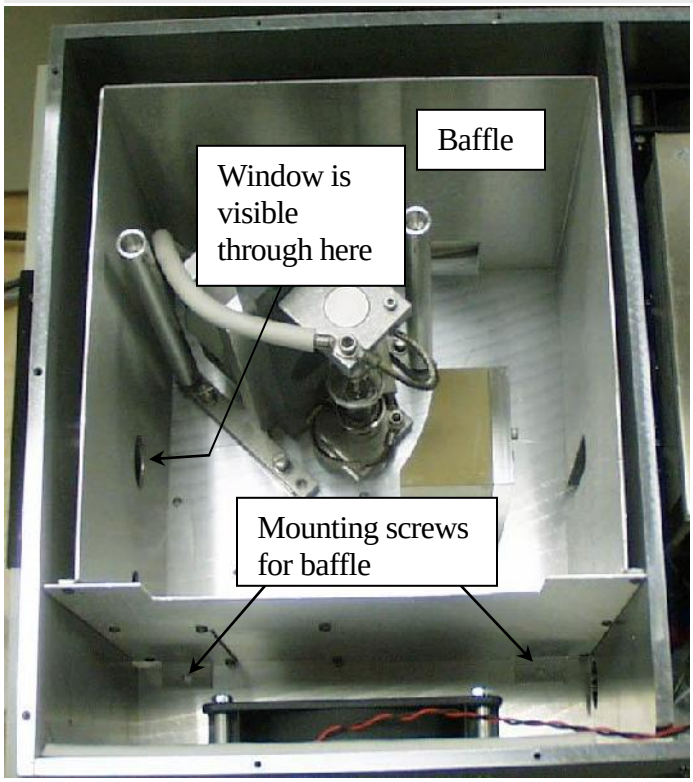
12 Record the date that the new lamp was installed, as well as its maximum intensity.

13 Save the protective cover of the new bulb, for when the bulb must be replaced in the future.

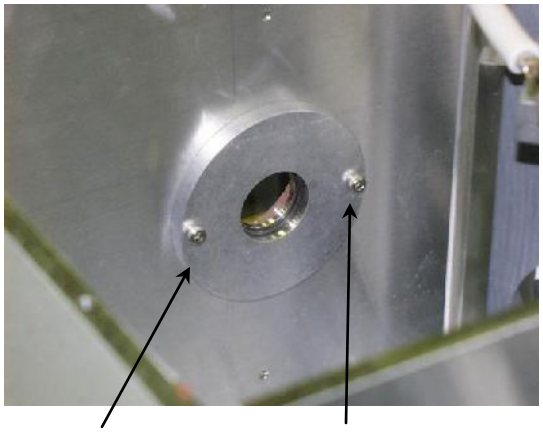
Lamp housing

Clean the window in front of the lamp housing once a year, or more frequently if needed.

Note: *Dust reduces the excitation light transmission, resulting in lower system sensitivity.*



1. Remove 10 Phillips-head screws on the lamp housing's cover.
2. Lift off the cover of the lamp housing.
3. Remove the 2 screws that hold the baffle in place inside the housing.
4. Carefully lift out the baffle from the housing.
5. If desired, remove the 2 cap screws holding the window in place.
6. Clean the window with lens tissue and methanol.



Cap screws on interior wall of lamp housing.

Caution: *Read the Materials Safety Data Sheet (MSDS) before using methanol.*

7 If the cap screws were removed, replace them.

8 Replace the baffle, and re-install the 2 screws holding it in place.

9 Re-install the cover, and secure it with the 10 Phillips-head screws.

Reference signal detector

The reference signal detector is a state-of-the art silicon diode that requires no routine maintenance.

Monochromator gratings

The standard NanoLog® excitation monochromator gratings are 1200 grooves/mm, and are blazed at 500 nm. If an application requires that the system be optimized for a particular region, the gratings can be changed by following a simple procedure.

Required tools

Tool	Size	Purpose
Phillips screwdriver	medium	Remove top cover.
Lid support	--	Supports the lid in an open position.

Replacement

Caution: *Never touch the diffraction surface of a grating.*

Gratings require no routine maintenance. To use a grating with different specifications than those installed in the system, replace the existing ones.

Mirrors in the monochromators have been aligned at the factory. If mirror realignment is necessary, contact the Fluorescence Service Department. Typically, mirror realignment requires special targets and fixtures, and a laser.

Caution: *Do not loosen the mirror mounts.*

Caution: *HORIBA Scientific recommends that you never attempt to clean off any dust on the gratings and mirrors. The effect of cleaning may cause more problems than the dust. The surface is easily marred and cannot be cleaned the way a mirror can.*

Remove the existing grating

1 Close the slits in the excitation monochromator.

2 Turn off all instrument power.

3 Remove the Phillips-head screws holding the lid on the monochromator.

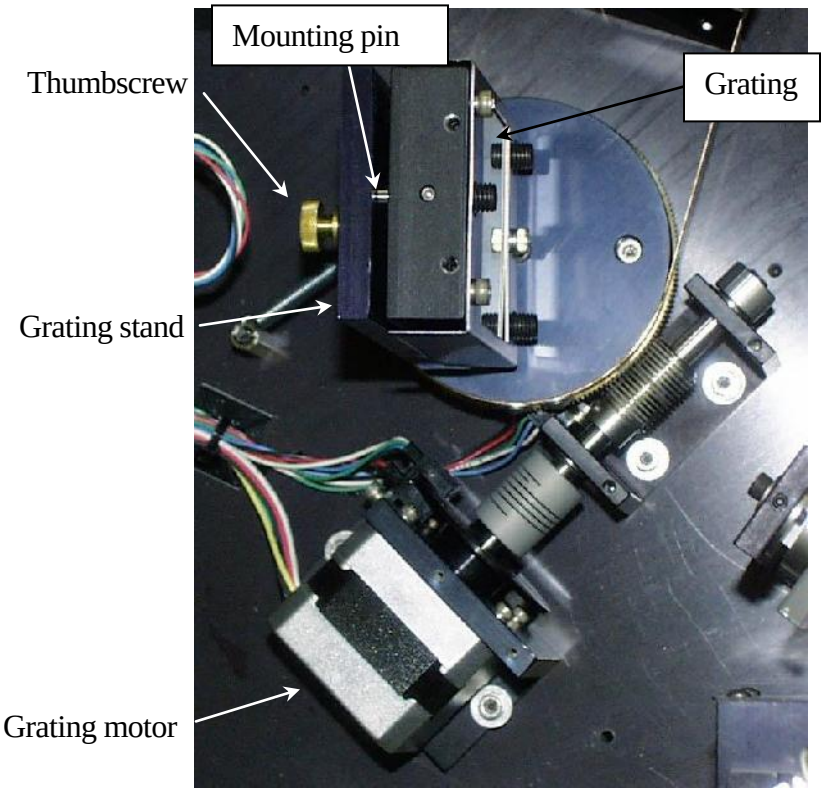
4 Lift the lid slowly until you feel resistance.

This is the maximum length of the cables attached to the lid.

Caution: *A circuit board and critical connections are attached to the lid of the spectrometer. DO NOT attempt to lift the lid completely off.*

5 Insert the lid support at the rear of the monochromator.

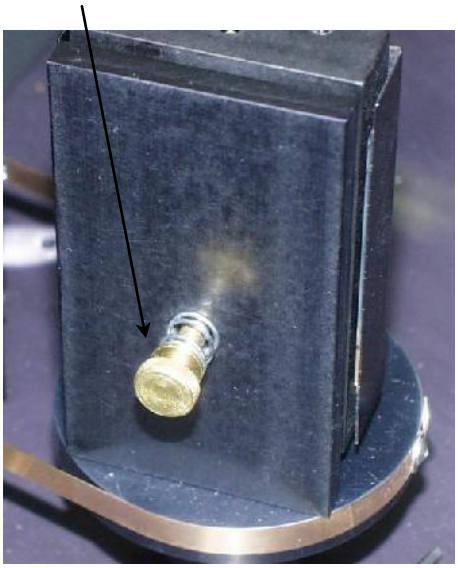
6 Place the lid in the support.



Overhead view of the grating assembly.

7 Release the grating from the stand.

a Loosen the thumbwheel screw securing the grating to the grating stand.



Back view of grating assembly.

b Pull the grating away from the stand.

To insert a new grating,

1. Place the 3 mounting pins on the back of the grating into the matching mounting slots on the grating stand.
2. Tighten the thumbscrew on the back of the grating stand.
3. Lift the lid of the monochromator off of the lid mount.
4. Remove the lid mount.
5. Place the lid on the spectrometer.
6. Secure the lid with the Phillips screws.

No further adjustments or alignments are necessary.

iHR gratings

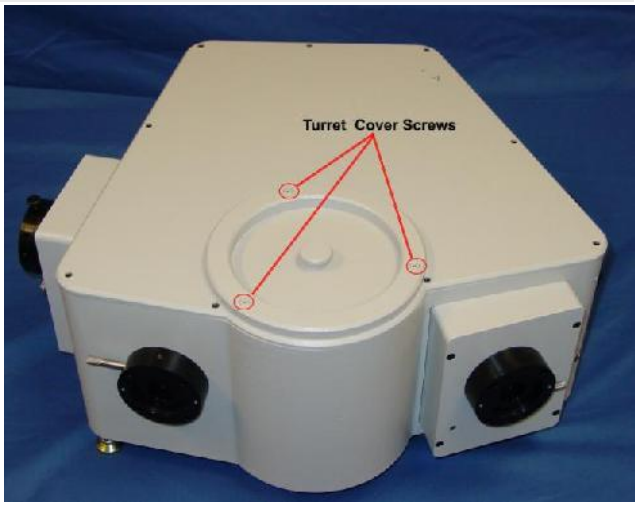
Concerning multiple gratings

Generally, gratings with different groove-densities are installed in an iHR, but gratings with different blaze-angles are also possible for custom applications. Rotation to a new grating is accomplished in **Real Time Control** elsewhere. After installing a new grating on the turret, its groove density must be entered in System Configuration, and its calibration must be performed in **Real Time Control**.

Caution: *Always switch off the power to the iHR before removal of accessories from or addition to a system. Failure to shut off the power to the iHR can damage the SpectrAcq.*

Caution: *Never touch the diffraction surface of a grating.*

Turret removal



1. Using a Phillips screwdriver, loosen the three screws of the iHR turret cover and remove the turret cover.
2. Loosen the thumbscrew.
3. Grasp turret by the thumbscrew.
4. Lift the turret up and out of the instrument.

Caution: *HORIBA Scientific recommends that you never attempt to clean off any dust on the gratings and mirrors. The effect of cleaning may cause more problems than the dust. The surface is easily marred and cannot be cleaned the way a mirror can.*

Turret installation

1. Holding the thumbscrew, lift the turret, being careful not to touch the face of any of the gratings.
2. Place the turret into the spectrometer grating drive, rotating it until it "drops" into place.

   The three points of the turret fit into the three notches on the base of the drive.
3. When the grating turret is securely positioned, tighten the thumbscrew by hand until it is finger-tight.

Caution: *Do not overtighten the thumbscrew. This can cause permanent damage to the iHR's drive mechanism.*

4. Replace the turret cover and tighten the cover screws.

   The turret is self-indexing, so the initial position does not matter.

Mirrors

Mirrors are aligned at the factory and usually do not need realignment.

Caution: *Adjust the mirrors* ***only if a laser is available*** *to check the alignment. Misalignment will destroy the similarity of the right-angle and front-face light paths in the single-beam sampling module. (Front-face capability is an option in the sample compartment.)*

Improperly moving any of the mirrors in the monochromator will reduce resolution capability and refocusing will be required. If the system becomes misaligned, contact the Service Department to arrange for a service visit.

Automated 4-position turret

If a circulating bath is used to regulate the turret's temperature, periodically replace the fluids in the bath.

Note: *Over time, bacteria can grow in the temperature bath, or the water can become hard.*

Cooled detectors

Details of care and maintenance of cooled detectors using Symphony® controllers are given in their respective manuals.

Warning: *Always wear protective clothing, gloves, and goggles when adding liquid nitrogen to cooled detectors. Never overfill a cooled detector with liquid nitrogen, and never let the liquid nitrogen overflow its container.*

7: Troubleshooting

The NanoLog® spectrofluorometer system has been designed to operate reliably and predictably. Should a problem occur, examine the chart below, and try the steps listed on the following pages.

Problem	Possible Cause	Remedy
Light is not reaching the sample.	Excitation shutter closed.	Using the software, open the shutter.
	Slits are not open to the proper width.	Adjust the slits.
	Lamp is not turned on.	Turn on lamp by pressing lamp rocker switch, and then the Start button on the xenon-lamp power-supply front panel.
	Excitation monochromator is miscalibrated.	Check and recalibrate excitation monochromator.
	Sample turret is not in correct position.	Using the software, rotate to correct position and open the cover to verify the position.
	Obstruction in optical path (e.g., beam-block, filter)	Remove obstruction
No signal.	Photomultiplier tube is saturated.	Reduce slit settings.
	High voltage is off.	Turn on high voltage through the software.
	Wrong filter installed in emission path	Change to correct filter.
	Sample is not in the optical path.	Place sample in optical path.
	Bad cables or connections.	Inspect cables and connections, replacing cables as needed.
	Wrong instrument configuration.	Change to correct instrument configuration.
	Bad signal detector.	Run an emission scan to confirm. Call the Service Department.
	Lamp is not on.	Replace bad xenon lamp.
PMT signal intensity is low.	Lamp is not aligned or focused.	Align and focus the lamp.
	Slits are not open to proper width.	Adjust the slits.
	Shutter(s) is(are) not completely open.	Open the shutter(s).
	Lamp power supply is set to the wrong current rating.	Call the Service Department. (450-W Xe lamp current = 25 A.)
	High voltage is improperly set.	Enter proper voltage: Default HV1 = 950 V

[continued on next page]

	Polarizer is in the light path.	In the software, move the polarizer out of the light path.
	Lamp is too old.	Replace lamp. (450-W lamp has lamp lifetime 1500–2000 h.)
	Sample-dependent problems (e.g., too concentrated, front-face vs. right-angle mode)	Correct the sample problem
CCD signal intensity is low.	Lamp is not aligned or focused.	Align and focus the lamp.
	Slits are not open to proper width.	Adjust the slits.
	Shutter(s) is(are) not completely open.	Open the shutter(s).
	Lamp power supply is set to the wrong current rating.	Call the Service Department. (450-W Xe lamp current = 25 A.)
	High voltage is improperly set.	Enter proper voltage: Default HV1 = 950 V
	Polarizer is in the light path.	In the software, move the polarizer out of the light path.
	Lamp is too old.	Replace lamp. (450-W lamp has lamp lifetime 1500–2000 h.)
	Sample-dependent problems (e.g., too concentrated, front-face vs. right-angle mode)	Correct the sample problem
	Gain improperly set.	Click configuration setting.
	Integration time is too low.	Increase integration time.
	Binning is wrong.	Correct binning.
Signal intensity is at least 10 times lower than normal.	Polarizer is in the light path.	In **Real Time Control**, move the polarizer out of the light path.
No change in signal intensity.	Shutter(s) closed.	Open all shutters.
	Monochromator and spectrometer are set to wrong wavelength.	Select appropriate wavelength based on excitation and emission of sample.
	Detectors are saturated	Adjust slits. (R928P signal detector is linear to 2×10^6 cps in photon-counting mode. Reference detector saturates at 200 µA.)
	Optical density effects and self-absorption.	Sample is too concentrated. Dilute sample by a factor of 10 or 100 and retry experiment.
	Obstruction in optical path (e.g., beam-block, filter)	Remove obstruction
Erratic signal.	Lamp is unstable.	Let lamp warm up 20 min before use.
	Light leaks in optical path.	Check dark value to determine.

[continued on next page]

Raman band superimposed on fluorescence scan.	Aqueous solutions and solvents have Raman bands.	Change excitation wavelength to move Raman band away from fluorescence peak, or run a blank scan of the solvent and subtract it from the fluorescence spectrum.
Large off-scale peak at twice the excitation wavelength.	2^{nd}-order effects from the spectrometer.	Use cut-on filters to eliminate the 2^{nd}-order peak.
Noisy signal.	Temperature on cooled detector is too high.	Add liquid nitrogen. Check and adjust temperature setting of cooled detector.
	Signal is too weak.	Try to increase signal strength at the detector.
	Light leak.	Follow directions under "Background signal is too high" for detecting light leaks.
	[continued on next page]	
	Improper grounding.  ***Note:*** *Ground loops often are difficult to diagnose and cure. Experiment patiently. Keep ground wires short, make tight connections, and avoid coated surfaces.*	1 Exit FluorEssence™. 2 Turn off iHR. 3 Rearrange power connections so that spectrograph, source, and detector are connected to the same ground (earth), and if possible, the same power circuits. 4 Add redundant grounds (earths) to various points in the system. Consider a "star" ground (earth) of wires radiating from a single central location, e.g., a grounded metal table surface under the system.
	Electromagnetic interference from lasers or other high-energy apparatus	Construct a faraday cage or shield.
Stray light in emission scan (also see example in this chapter).	Scattered light off the excitation wavelength.	Place bandpass filters in excitation light path.
Noisy spectrum with magnetic stirrer.	Stirring speed is too fast.	Use slower stirring speed.
Hardware Init. error appears.	Broken IR sensor in monochromator.	Replace IR sensor: Call the Service Department.
	[continued on next page]	

"Data file does not exist" or "file read error" message appears.	User is not logged into Windows® XP as administrator or power user.	Log into Windows® XP as administrator and or power user, and restart FluorEssence™.
Background signal is too high	Light leak.	Make sure all covers are in place. Be sure that the area between the sample and the entrance slit is enclosed and light-tight. Test by blocking the entrance slit. Check detector mounting and housing for light leaks.
		Test for stray light: 1 Start at detector, close exit and entrance slits and shutters in order. 2 Be sure all openings and screw holes are plugged. 3 Check that the cover, side, and baseplate fit tightly. 4 In a dark room, shine small flashlight at a suspicious part or joint, and observe detected signal levels.
Corrected excitation spectrum curves upward ~240–270 nm.	Dark count is divided by low reference signal.	Use Dark Offset checkbox; retry scan.
Emission spectrum curves upward consistent with amplitude of corrections	Blank or dark counts not yet subtracted from data.	Subtract blank or dark counts from data.
Communication problems between computer and instrument.	Cables are improperly connected.	Check communications cables' connections.
	Computer's or SpectrAcq's I/O-controller is failing.	Replace I/O controller: Call the Service Department.
"WARNING**: No signal from detector (CCD)" error appears in Status box.**	Bug in FluorEssence™ software.	1. In the **Experiment Setup** window, click the Multichannel Detectors icon. 2. Click the Reformat button. For InGaAs array detectors, ignore the "Areas are too small to display" message.

Note: *To prevent damage to the knife-edges, the slits never close completely. Therefore some light always enters.*

[continued on next page]

Sample turret is not operating.	Software is not enabled.	Check status.
	Cables are improperly connected.	Check cable connections.
	Problem with Symphony® controller's firmware.	Reboot host PC and Symphony® controller.
iHR does not respond to any commands	Cables are improperly connected.	Check external cable connections.
	Problem with USB port.	Check USB cable and ports.
	Software is inappropriate.	Check that system software matches the hardware.
	Improper parameters used with correct command.	Check that the failing command is valid, and that parameters are within correct limits for that function.
iHR responds to some commands, but not all	Software is inappropriate.	Exit FluorEssence™. Check that the software matches the hardware, especially device configuration.
	Improper parameters used with correct command.	Check that the failing command is valid, and that parameters are within correct limits for that function.
iHR wavelength-drive or accessories do not move	Grating may not be working properly	With the power off, remove the turret cover of the iHR and gently move the turret partially through its range of motion. When the system is powered, the device should return to its home position.
	Unconnected cables	Be sure that all cables are connected.
	Improper software or parameters	Check that the failing command is valid, and that parameters are within correct limits for that function.

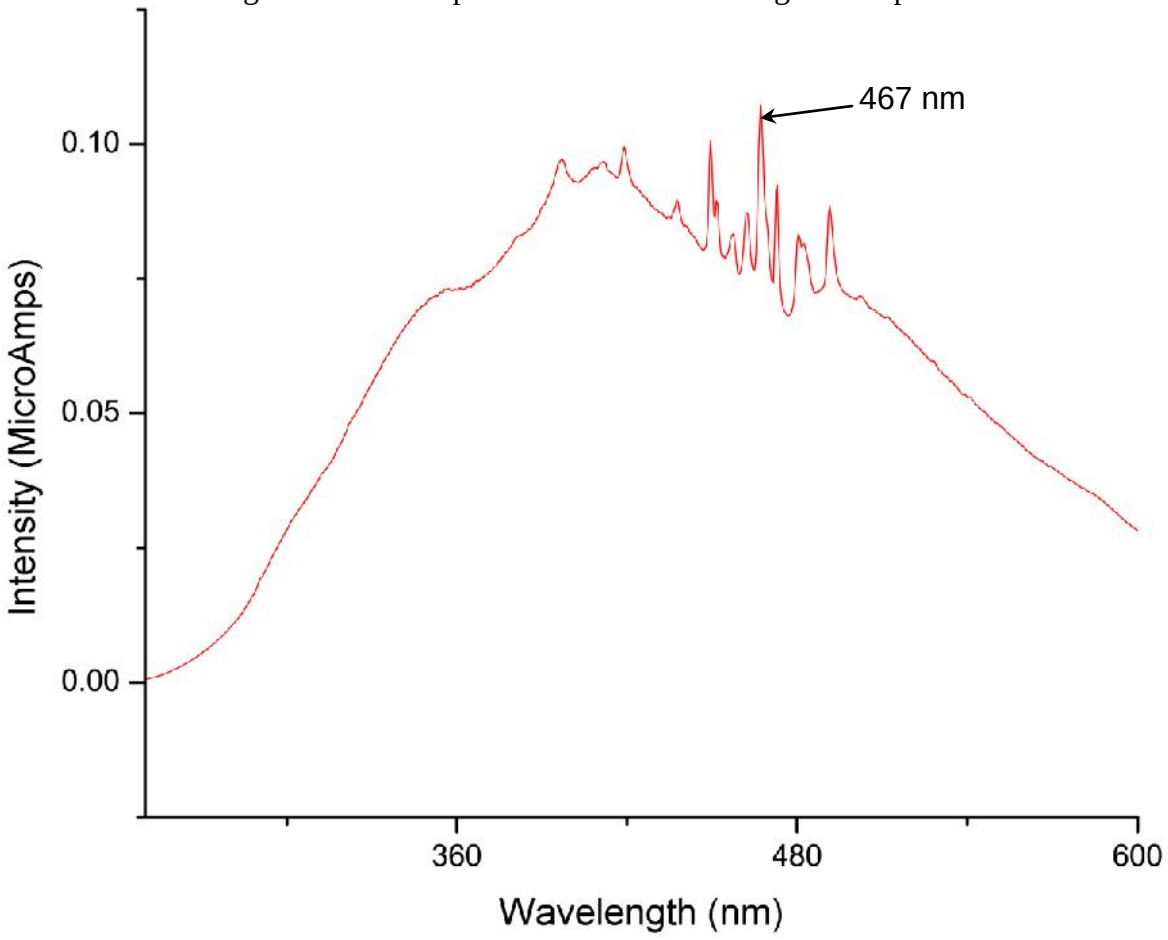
Using diagnostic spectra

Often the spectrum reveals information regarding the hardware or software parameters that should be adjusted. The following spectra occur with explanations regarding problems leading to their appearance.

Note: *Not all spectra shown in this section were produced using the NanoLog®. The spectra are presented to show different possible system or sample problems, and may not reflect the superior performance of the NanoLog®.*

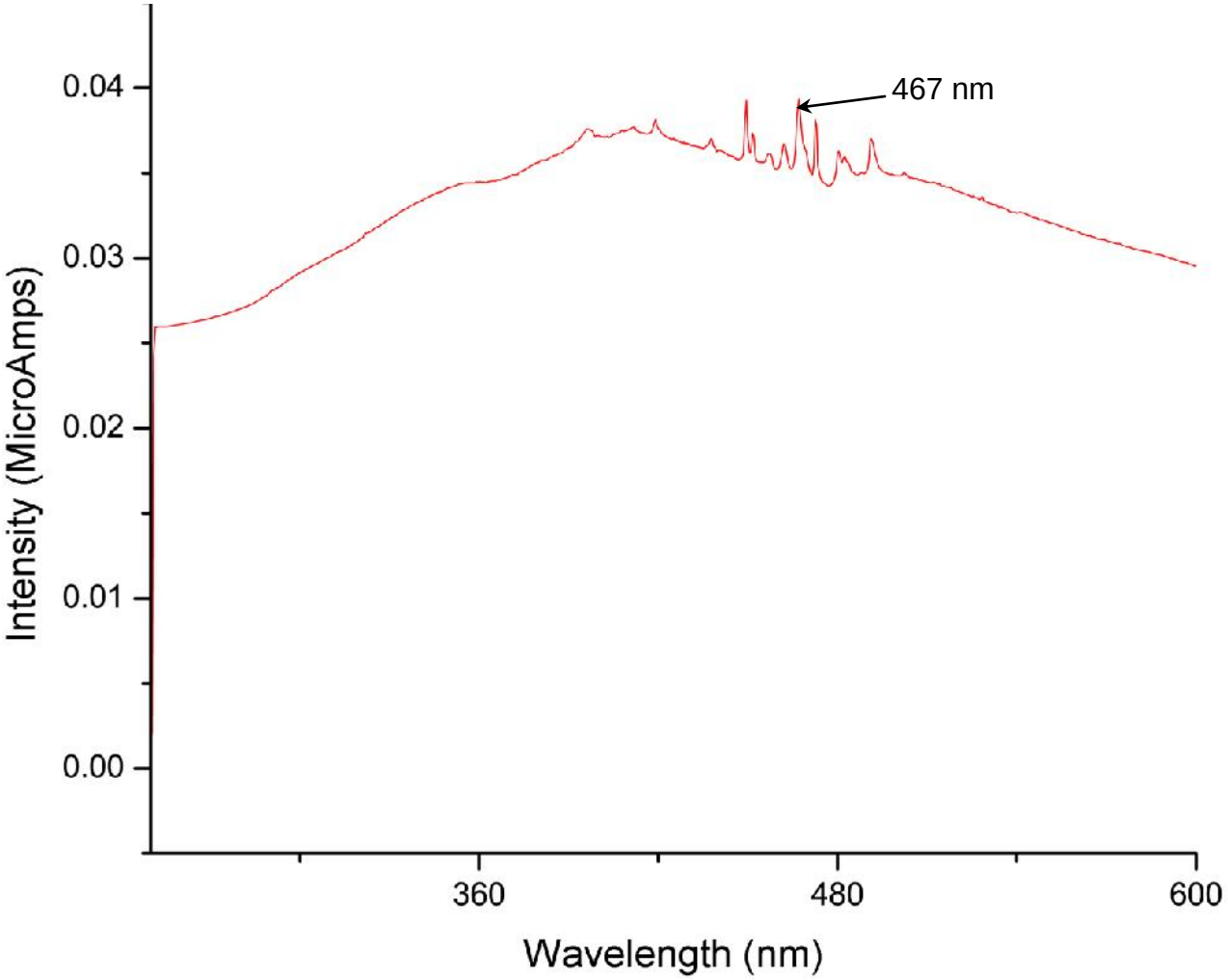
Lamp scan

Running a lamp scan verifies system integrity and indicates whether the correct parameters for the best possible trace are being used. The following spectrum shows the trace resulting from a Xe lamp scan run with a known good lamp.



Scan of good-quality 450-W Xe lamp in Nanolog® with double-grating excitation monochromator.

The following lamp scan spectrum shows stray room light from ambient fluorescent lights.



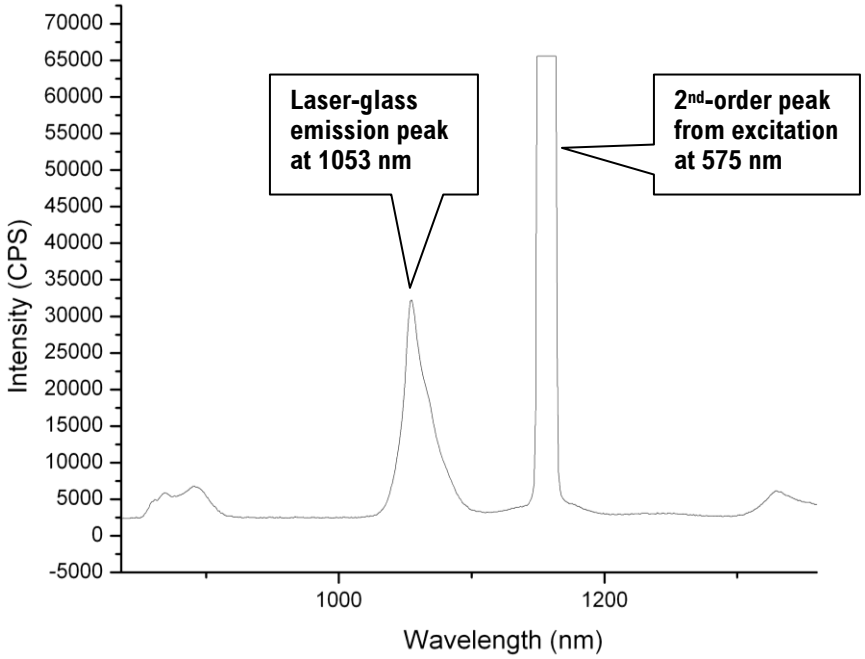
Lamp scan of good-quality 450-W Xe lamp in Nanolog® with double-grating excitation monochromator. Note high background from ambient stray light.

To resolve this problem, be sure the cover is on the sample compartment and seal the enclosure to prevent light-leaks.

Laser-glass spectra

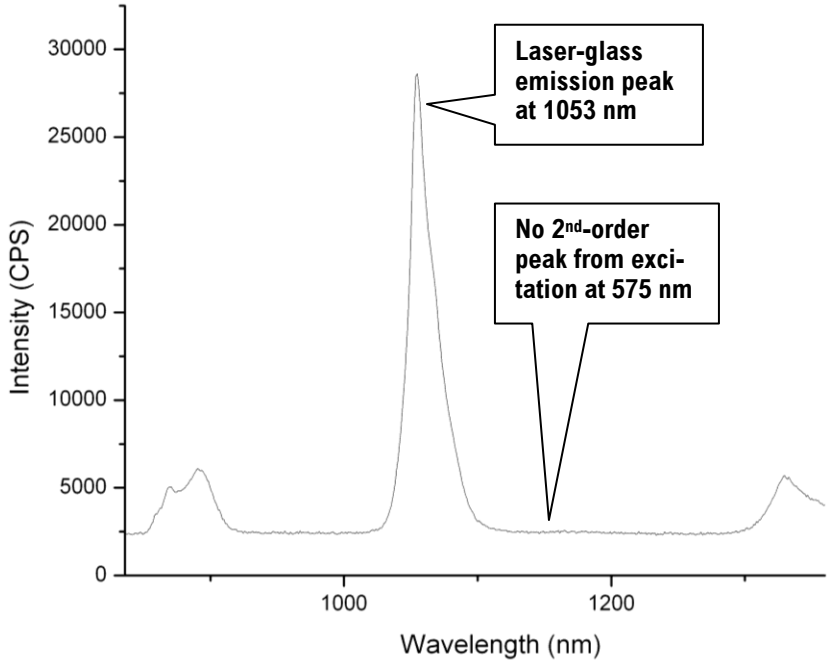
Second-order interference

Running a laser-glass scan helps identify abnormalities as a result of accessory problems or miscalibration. The following spectrum shows the second-order peak from the excitation at 575 nm. Note how the second-order peak has saturated the detector:



Raw laser-glass emission spectrum without an 830 nm cut-on filter.

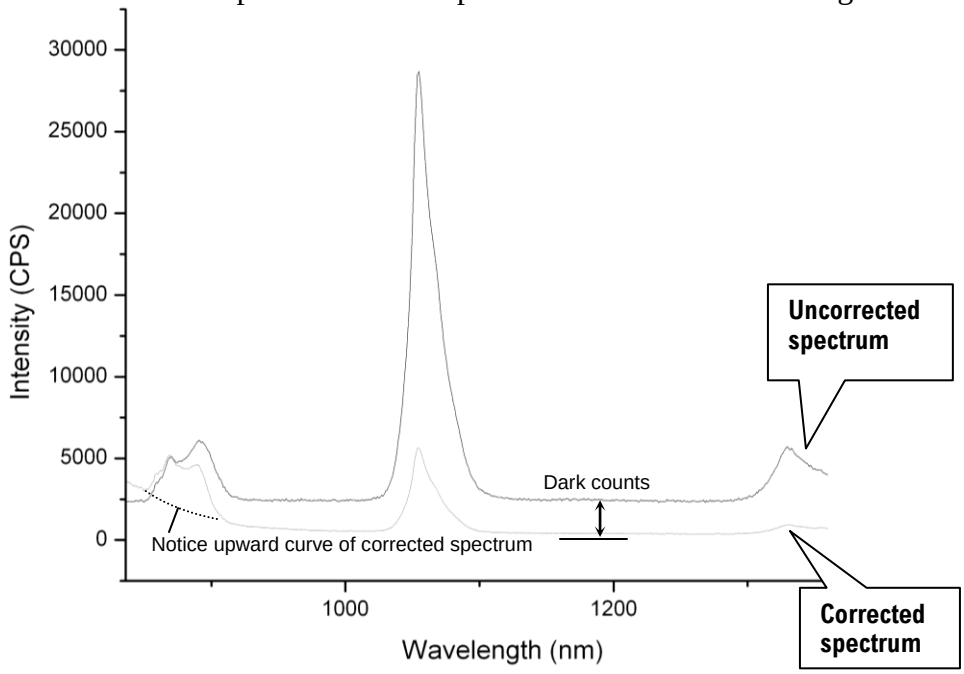
In contrast, the spectrum below includes a cut-on filter removing all wavelengths shorter than 830 nm:



Raw laser-glass emission spectrum with an 830 nm cut-on filter.

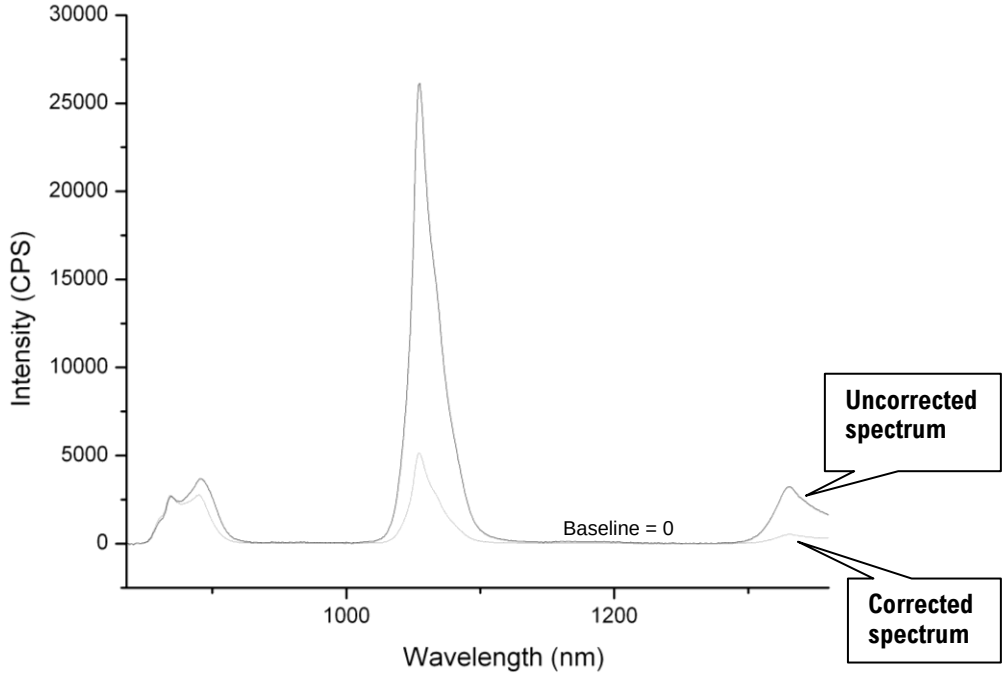
No subtraction of dark counts

If the correction-factor file is used, but no subtraction of dark counts, the following emission spectrum is seen with laser glass. This was taken with an 830 nm cut-on filter to remove second-order effects. Note how the uncorrected spectrum has a dark-count baseline of ~ 2500, and the corrected spectrum curves upward at the shortest wavelengths.



Laser-glass emission spectrum with an 830 nm cut-on filter and instrument correction, but no dark-counts subtraction.

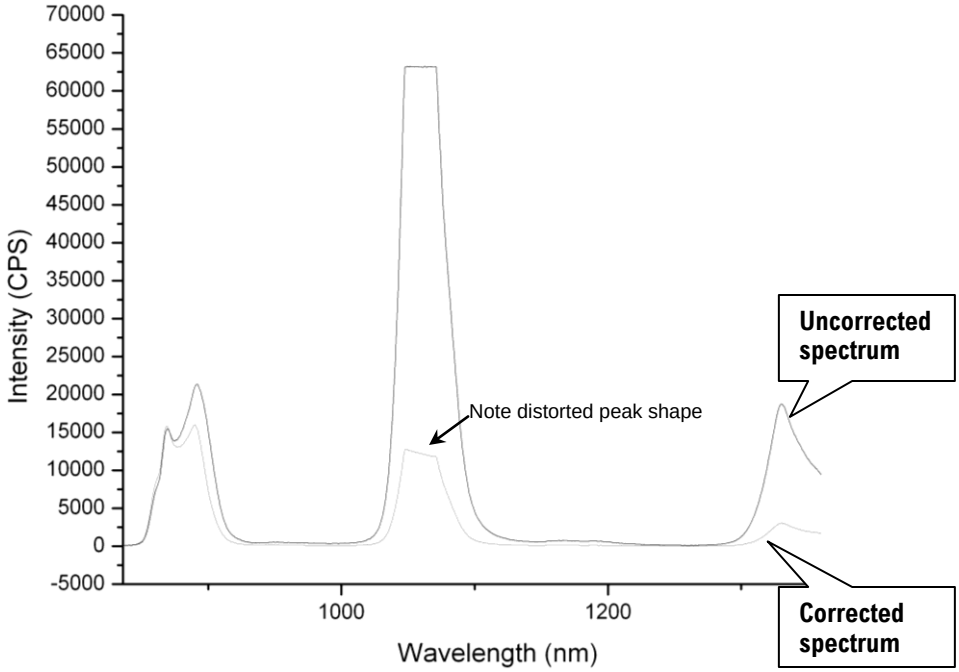
With the dark counts gone, the spectrum has a baseline of 0 and does not curve upward:



Laser-glass emission spectrum with an 830 nm cut-on filter, instrument correction, and dark-counts subtracted.

Saturation of the detector

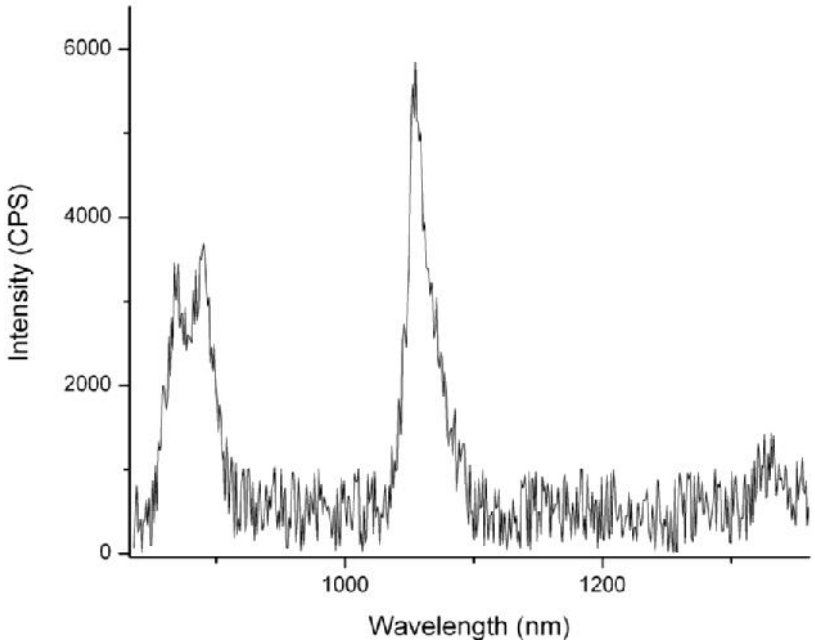
Sometimes the slit-widths or integration time might be too high, causing the detector to become saturated, and the peaks to appear distorted. Notice how both corrected and un-corrected spectral peaks are changed.



Saturated laser-glass emission spectrum with an 830 nm cut-on filter, instrument correction, and dark-counts subtracted.

Detector temperature is too high

If the CCD detector is not properly cooled, the noise-level rises dramatically:



Laser-glass emission spectrum with an 830 nm cut-on filter, instrument correction, and dark-counts subtracted. The CCD is too warm.

Further assistance...

Read all software and accessory manuals before contacting the Service Department. Often the manuals show the problem's cause and a method of solution. Technical support is available for both hardware and software troubleshooting. Before contacting the service department, however, complete the following steps.

1. If this is the first time the problem has occurred, try turning off the system and accessories.

   After a cool-down period, turn everything back on.

2. Make sure all accessories are properly configured, and turned on as needed.

3. Following the instructions in *System Operation*, run a xenon-lamp scan and a laser-glass scan to make sure the system is properly calibrated.

   Print the spectrum for each and note the peak intensities.

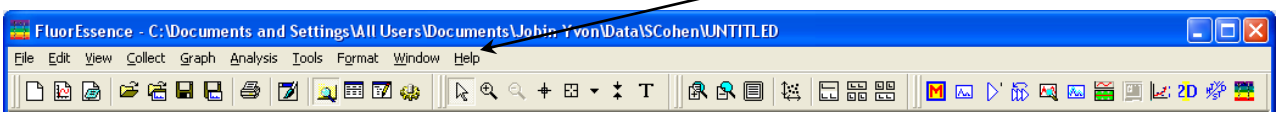
4. Check this chapter to see if the problem is discussed.

5. Try to duplicate the problem and write down the steps required to do so.

   The service engineers will try to do the same with a test system. Depending on the the problem, a service visit may not be required.

6. If an error dialog box appears in FluorEssence™, write down the exact error displayed.

7. In FluorEssence™, in the FluorEssence main window's toolbar, choose Help:

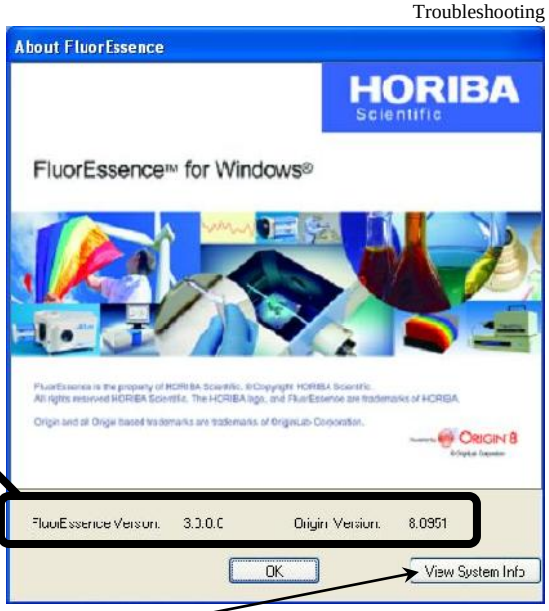


   A drop-down menu appears.

8. Under Help, choose About FluorEssence....

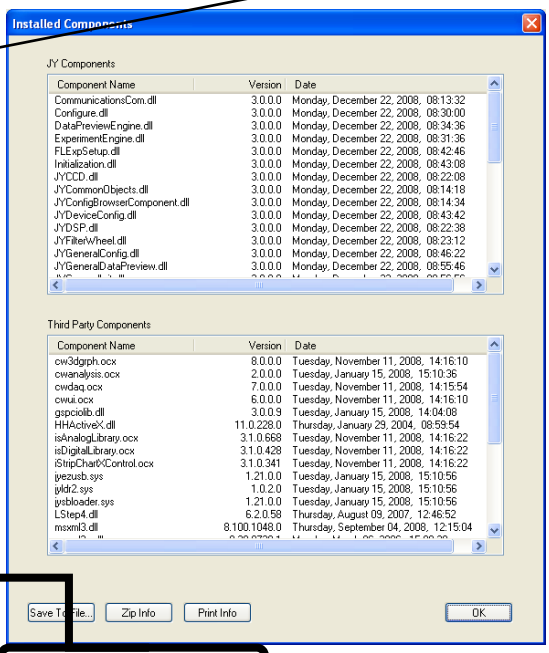
   This opens the **About FluorEssence** window:

The version of the software (both FluorEssence™ and Origin®) is listed here.



9 Click the View System Info button.

The **Installed Components** window appears, displaying all the software required for FluorEssence™.



10 Record the information by clicking the:

- Save To File... button, which saves the information to a file;
- Zip Info button, which compresses the information while saving it;
- Print Info button, which prints out the software information.

11 Click the OK button to close the **Installed Components** window.

12 Click the OK button to close the **About FluorEssence** window.

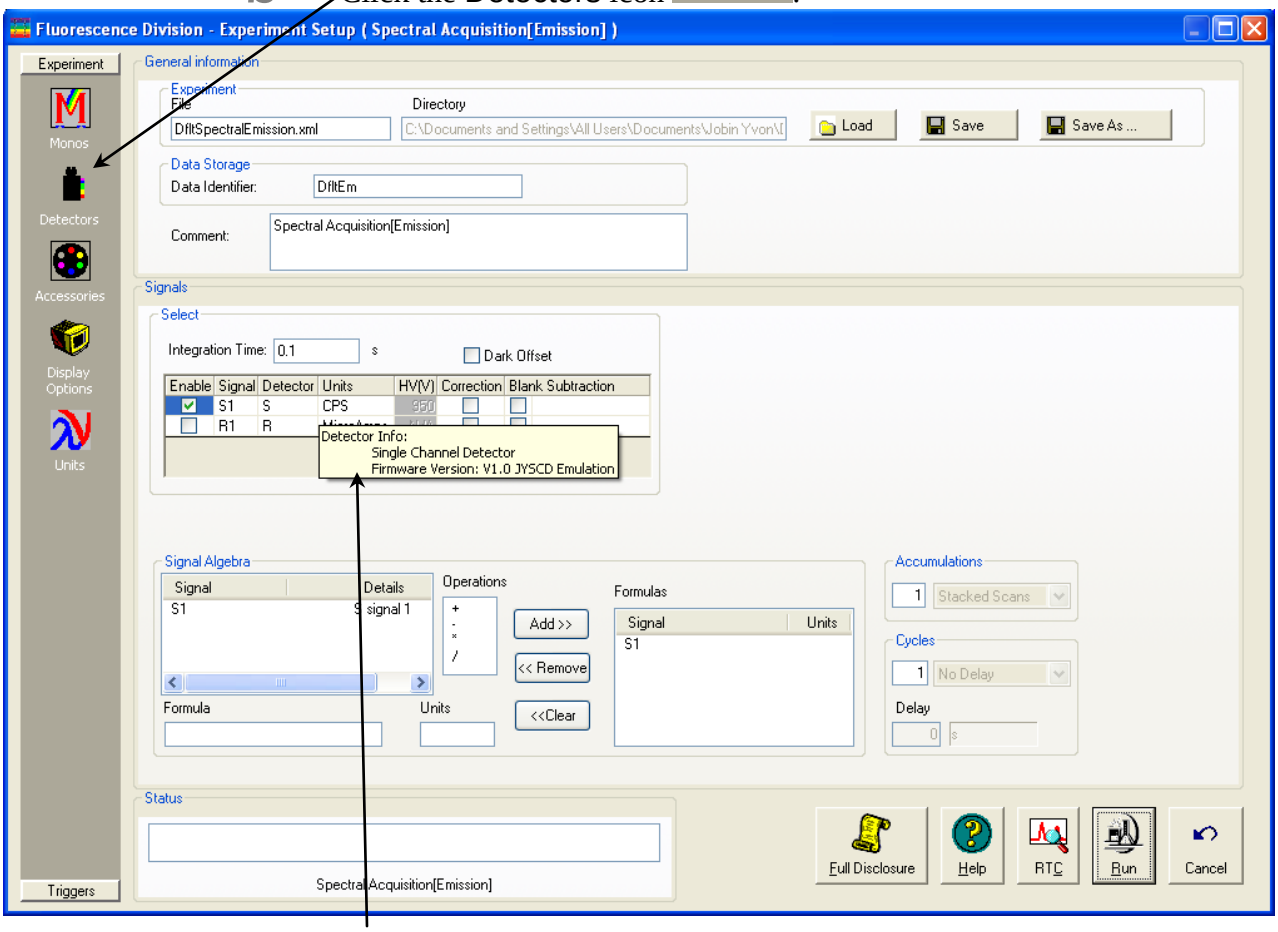
13 Write down the software's version numbers, along with the purchase dates, model numbers, system configuration, and serial numbers of the instrument and its accessories.

14 Determine the SpectrAcq firmware version:

a Open the **Experiment Setup** window:

b Click the Detectors icon 

.



c Move the mouse over the detectors' table in the Select area.
The SpectrAcq firmware version appears in a small pop-up window.

If the problem persists or is unlisted, call the Service Department at (732) 494-8660 × 160.

8: Producing Correction Factors

Introduction

All gratings, detectors and other spectrometer components have response characteristics that are functions of wavelength. These characteristics are superimposed on spectra, and may yield a potentially misleading trace. For accurate intensity and wavelength comparisons over a broad range, such as those required for quantum-yield determinations, spectrometer-response characteristics must be eliminated. Corrections are made for each of these potential problems by using radiometric correction factors.

Supplied with the NanoLog® are sets of excitation and emission correction factors designed to eliminate response characteristics. These files, `xcorrect.spc` and `mcorrect.spc` (and `tcorrect.spc` for T-format instruments), are included with the software and should be copied to the hard disk. The excitation correction range is from 240–1000 nm. The correction range for emission spectra depends on the grating and detector, but can be as low as 280 nm and as high as 1600 nm.

Emission corrections factors should be updated periodically or whenever different gratings or signal detectors are installed. The correction factors can be updated by a representative from the Service Department. To arrange for a visit and a fee estimate, call the Service Department.

One way to generate correction factors for the instrument is to san the spectrum of a standard lamp. Because the actual irradiance values of the standard lamp as a function of wavelength are known, dividing the irradiance values by the lamp spectrum results in a set of relative correction values. These values then can be applied to the raw fluorescence data. The emission correction factor file `mcorrect.spc` was acquired in this manner.

Caution: *Be aware that applying correction factors obtained at one resolution may give erroneous results when applied to spectra acquired at a different resolution.*

Note: *Generate a new correction-factor file only when the gratings or detectors have been replaced with those of different specifications than the original hardware.*

Correction-factor file names for multiple gratings

When the NanoLog® uses an iHR (which contains multiple gratings) or multiple detectors, multiple correction-factor files are required. Each of these has a unique file name, which needs deciphering. Following are rules to guide the correct choice of correction-factor files.

Note: *Upper- or lower-case characters are not important.*

1 The first one or two characters show the type of correction-factor file.

`M` or `MC` = emission correction-factor file
`X` or `XC` = excitation correction-factor file

2 After `M` or `MC`, the next several characters determine the appropriate detector:

Note: *Ignore this rule for `XC…` files.*

`2658` = near-IR detector
`CD` = CCD array
`IGA` = InGaAs array
`928` = R928P photomultiplier tube
`DSS` = InGaAs detector

3 The next set of characters give the grating's groove-density:

`100` = 100 grooves/mm
`15` = 150 grooves/mm
`12` = 1200 grooves/mm
`6` = 600 grooves/mm
`3` = 300 grooves/mm

4 Then may come a separator, `x`.

5 Finally, the last characters may show the blaze of the grating:

`300` = 300 nm
`330` = 330 nm
`500` = 500 nm
`750` = 750 nm
etc.

Here are some examples of deciphering the emission correction-factor file:

`Mcorrect.spc`	The default emission correction-factor file for an instrument, with no iHR, with only one emission grating and detector.
`MC9286x300.spc`	An emission correction-factor file for an R928P PMT, with a grating of 600 grooves/mm and a blaze at 300 mm.
`MCCD12x500.spc`	An emission correction-factor file for a CCD array, with a grating of 1200 grooves/mm and a blaze at 500 nm.
`MC26586x300.spc`	An emission correction-factor file for an IR detector, with a grating of 600 grooves/mm and a blaze at 300 nm.
`Xcorrect.spc`	The default excitation correction-factor file for an instrument, with no iHR, with only one excitation grating.
`XC12x330.spc`	An excitation correction-factor file for a grating with 1200 grooves/mm and a blaze at 330 nm.

Caution: *For CCD arrays, these correction-factor files are only valid under the same conditions, i.e., when used at the same central wavelength as performed in the factory. The files may give invalid corrections when used at other central wavelengths on the CCD array*.

To determine the center wavelength for a particular correction-factor file when used with a CCD array, see the Performance Test Report that accompanies the instrument.

Note: *For slit settings used to generate the correction-factor files, see the Performance Test Report.*

Generating and calculating emission correction factors

Excitation correction factors are measured during production of the instrument. If you have further questions, please contact the Service Department. A new correction-factor file is generated *only* when the gratings or detectors have been replaced with those of different specifications than the original hardware.

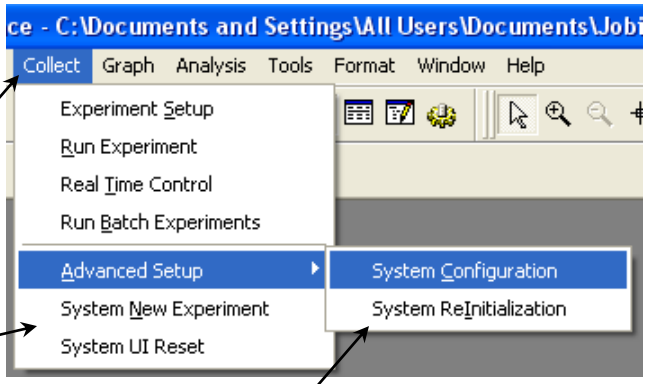
For more information about the theory and application of radiometric correction, consult *Accuracy in Spectrophotometry and Luminescence Measurements*, Mavrodineau, Schultz, and Menis, NBS Spec. Publ. 378 (1973), especially p. 137, "Absolute Spectrofluorometry," by W.H. Melhuish.

Irradiance values for a standard lamp, packaged with the lamp, usually are expressed in 10^{-6} W·cm^{-2}·nm. With photon-counting systems like the NanoLog® spectrofluorometer, however, data usually are collected in units of photons·s^{-1}·cm^{-2}·nm. To convert the units, multiply each irradiance value by the wavelength at which it is valid. (The data will still be off by a factor of *c*, but normalizing the correction factors compensates for this.) Such a mathematical procedure can be done in a spreadsheet program.

To generate correction factors, you may also order our Correction-Factor kit F-3026.

Using correction-factor files

To use the `xcorrect`, `mcorrect`, and optional `tcorrect` files, you must tell FluorEssence™ use them when the Correction check box is activated in the **Experiment Setup** window.



1 In the FluorEssence toolbar, choose Collect.

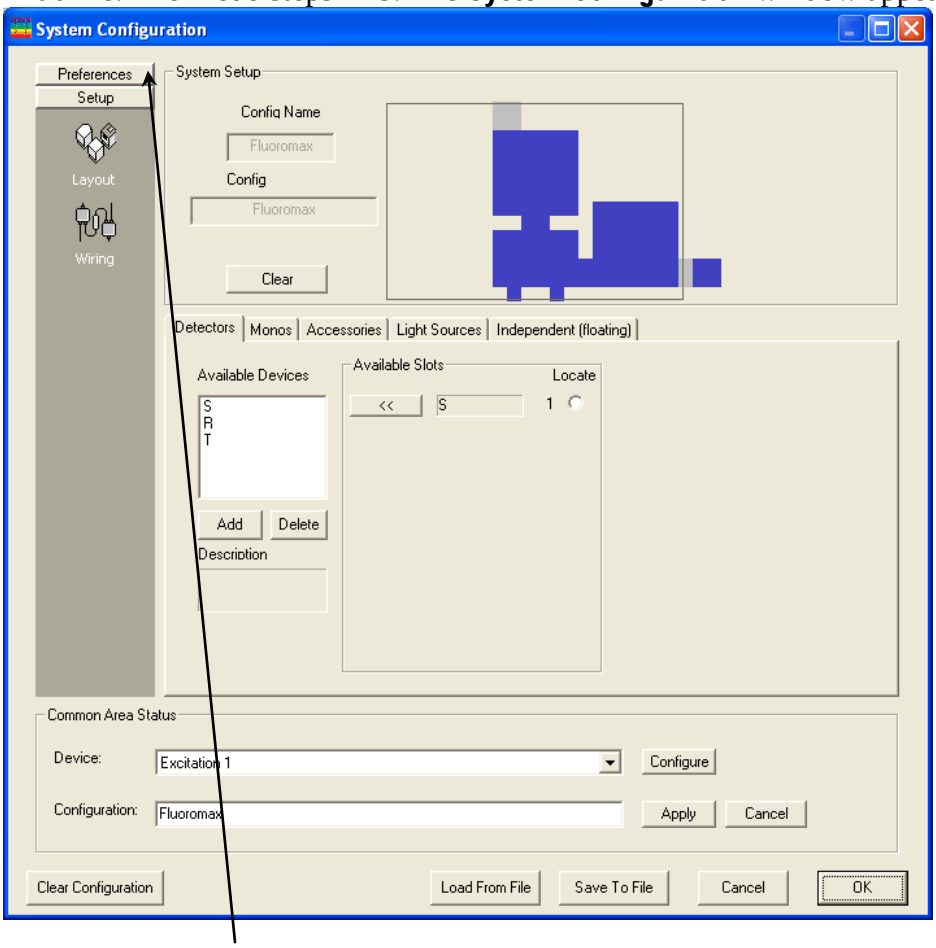
A drop-down menu appears.

2 Choose Advanced Setup.

A sub-menu appears.

3 Click System Configuration.

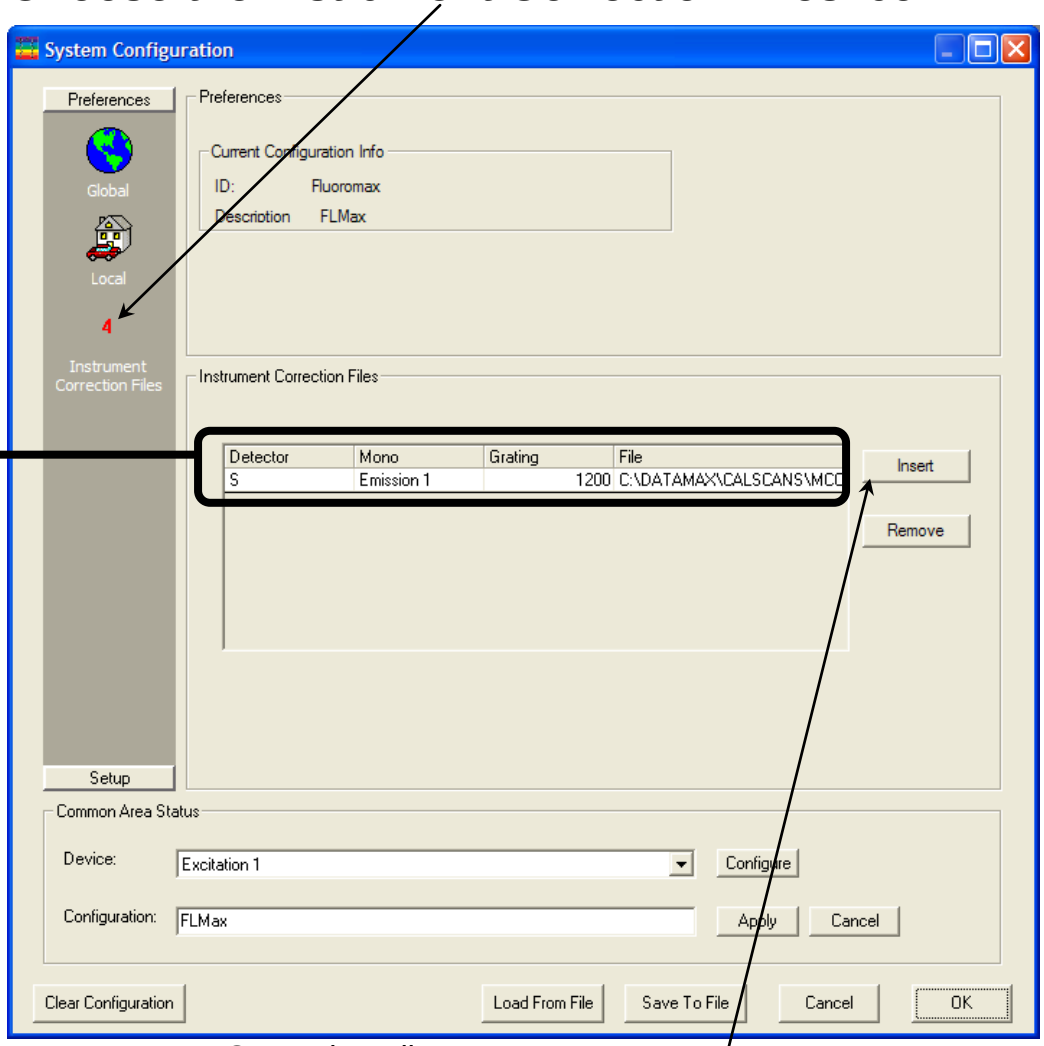
If the system has not been initialized, choose the instrument, and let the system initialize. Then redo steps 1–3. The **System Configuration** window appears.



4 Choose Preferences.

The Preferences area appears.

5 Choose the Instrument Correction Files icon.



The Instrument Correction Files area appears.

6 If there are no active fields in the Instrument Correction Files area, click Insert.

7 In sequence,

a Choose the Detector column, and select the appropriate detector from the drop-down list,

b The Mono column, and the correct monochromator from the drop-down list,

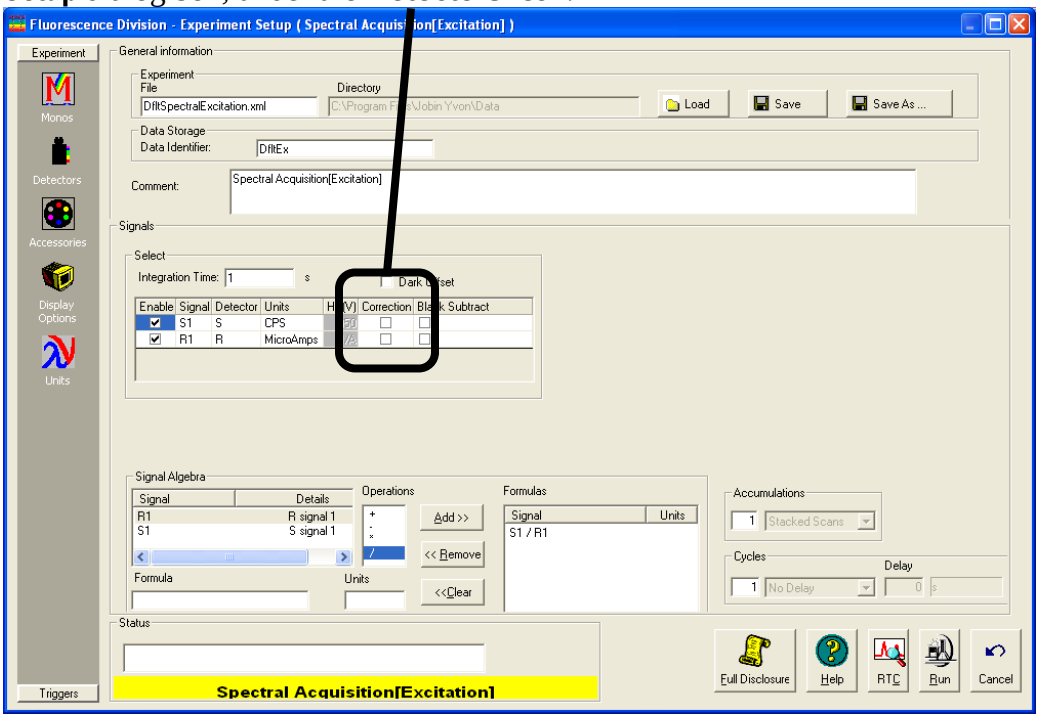
Note: *The signal detector, S, uses the* `mcorrect` *file; the reference detector, R, uses the* `xcorrect` *file; the optional T-side detector, T, uses the* `tcorrect` *file.*

c The Grating column, the correct grating from the drop-down list,

d Then, in the File column, browse for the appropriate correction-factor file.

8 When all necessary detectors have an associated correction-factor file, click the OK button.

The new correction-factor files are now ready to be activated in the **Experiment Setup** dialog box, under the Detectors icon:

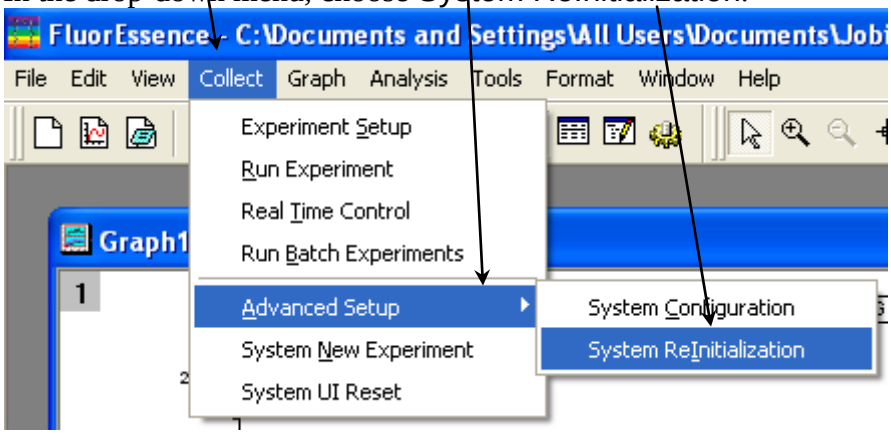


9 Reinitialize the instrument and software.

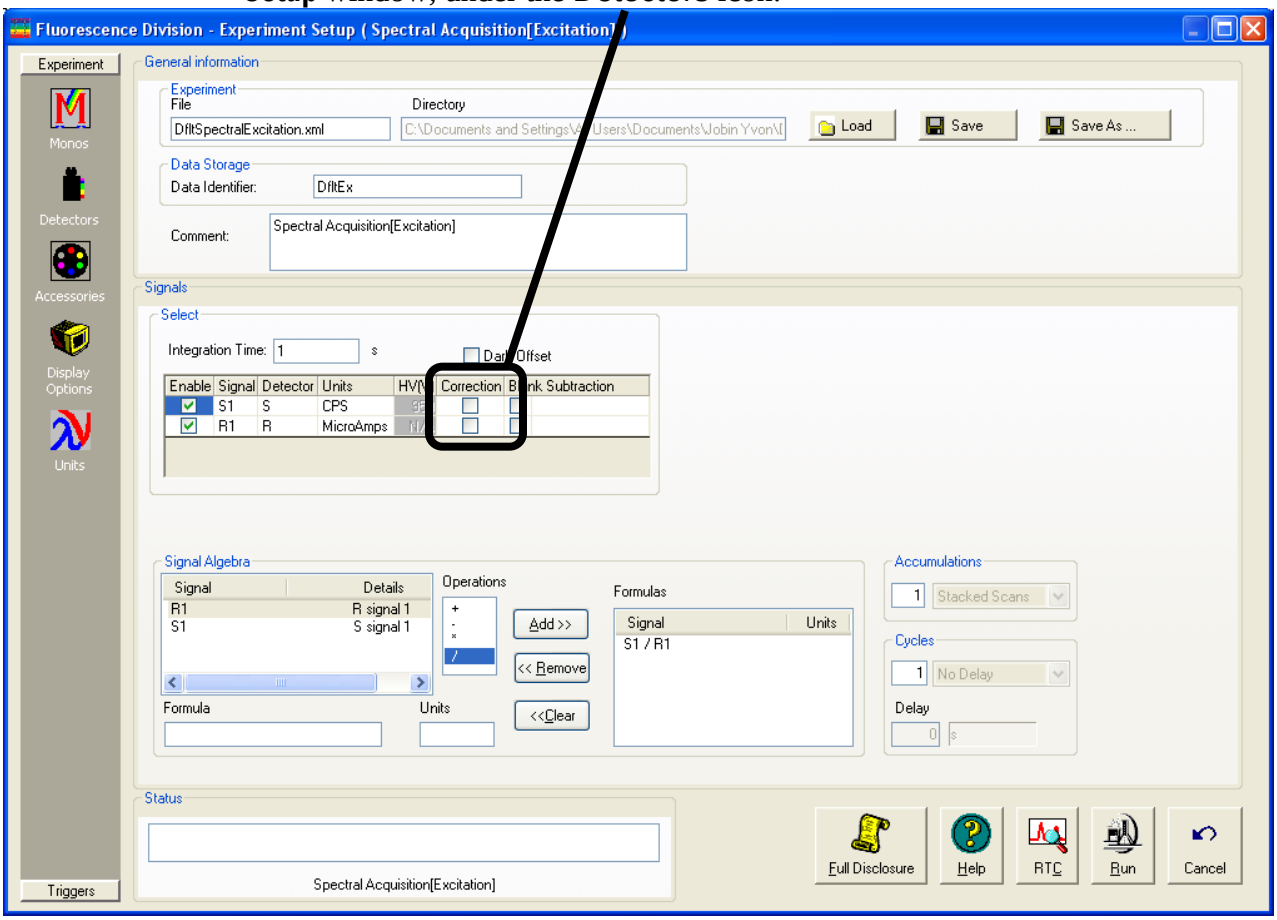
In the FluorEssence™ toolbar, choose Collect.

a In the drop-down menu, choose Advanced Setup.

b In the drop-down menu, choose System ReInitialization.



The new correction-factor files are now ready to be activated in the **Experiment Setup** window, under the Detectors icon:



Excitation correction factors

Excitation correction factors are measured during production of the instrument. If you have further questions, please contact the Service Department.

9: Automated Polarizers

Introduction

Theory

The measurement of polarized emission of fluorescence allows the observation of rotational motions in fluorophores during the lifetime of the excited state. Because the rotation of macromolecules depends on their size, shape, and local environment (i.e., solvent), several kinds of information may be extracted. Polarized-emission measurements often are used to detect small changes in molecular size (*viz.*, aggregation, binding, cleavage) as well as environmental changes (local viscosity, membrane microheterogeneity, and phase transitions).

The first step in these measurements is the *excitation* of a selected group of fluorophores, a fraction of the total ensemble of molecules. This process is known as *photoselection*. Vertically polarized light typically is used to excite a population of molecules whose absorption dipole is oriented in the vertical direction. For photoselection, vertically polarized exciting light usually is produced using a polarizer in the excitation path. A laser whose emission is V-oriented also may be used.

The second step is molecular *rotation*. The molecule, once excited, may rotate during the lifetime of the excited state, typically ~10^9 s. Such rotation will depolarize the fluorescence emission. Measurement of the polarized emission components allows calculation of the type and extent of rotational motions of the molecule.

The third step is measurement of *emission*. The polarized components of fluorescence emission are measured using polarizer(s) in the emission path(s). Measurements of polarization or anisotropy are derived from the intensities of the vertically and horizontally polarized components of the fluorescence emission.

The last step is *calculation*. From the magnitude of the V and H emission components, the extent and type of rotational behavior may be calculated. Both polarization and anisotropy are used to express the rotational behavior. *Polarization* is a ratio, defined as the linearly polarized component's intensity divided by the natural-light component's intensity. *Anisotropy* is also a ratio, defined as the linearly polarized component's intensity divided by the total light intensity. Anisotropy is the preferred expression, because it is additive. Polarization is not additive, but often appears in earlier literature. The measurement is performed in exactly the same manner, differing only in the calculations.

Ideally, polarization (P) and anisotropy ($\langle r \rangle$) are measured using only the vertically polarized excitation with the horizontal and vertical emission components. These measurements are designated I_{VV} and I_{VH}, respectively, where the first subscript indicates the position of the excitation polarizer, and the second, the emission polarizer. Vertically oriented polarizers (V) are said to be at 0° with respect to normal, and horizontally ori-

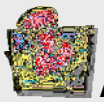
ented polarizers (H) are said to be at 90°. Polarization and anisotropy are expressed as follows:

$$P = \frac{I_{VV} - I_{VH}}{I_{VV} + I_{VH}} \qquad (1)$$

$$\langle r \rangle = \frac{I_{VV} - I_{VH}}{I_{VV} + 2I_{VH}} \qquad (2)$$

In a real optical system, the *G*, or *grating factor*, must be included to correct for the wavelength response to polarization of the emission optics and detectors. The *G* factor is defined as:

$$G = G(\lambda_{EM}) = \frac{I_{HV}}{I_{HH}} \qquad (3)$$



Note: *In some literature, the G factor is defined as the* ***inverse*** *of Equation 3. Therefore, some equations derived in this manual may differ from other sources.*

The *G* factor is primarily a function of the wavelength of the emission spectrometer. The spectral bandpass of the emission also affects *G*. Thus, a pre-calculated *G* factor can be applied to experiments in which instrumental factors (emission wavelength and emission bandpass) are kept constant throughout the entire experiment. In experiments where constant emission wavelength and bandpass is impractical, such as in emission anisotropy spectra, the *G* factor must be measured by recording I_{HH} and I_{HV} during the experiment at each emission wavelength.

Polarization in a spectrofluorometer is defined as:

$$P = \frac{I_{VV} - G*I_{VH}}{I_{VV} + G*I_{VH}} = \frac{\frac{I_{VV}*I_{HH}}{I_{VH}*I_{HV}} - 1}{\frac{I_{VV}*I_{HH}}{I_{VH}*I_{HV}} + 1} \qquad (4)$$

Anisotropy in a spectrofluorometer is defined as:

$$\langle r \rangle = \frac{I_{VV} - G*I_{VH}}{I_{VV} + 2G*I_{VH}} = \frac{\frac{I_{VV}*I_{HH}}{I_{VH}*I_{HV}} - 1}{\frac{I_{VV}*I_{HH}}{I_{VH}*I_{HV}} + 2} \qquad (5)$$

Polarization and anisotropy can be interconverted using these two equations:

$$P = \frac{3\langle r \rangle}{2 + r} \qquad (6)$$

$$\langle r \rangle = \frac{2P}{3-P} \quad (7)$$

For single-photon excitation, the allowed values for the emission anisotropy are governed by:

$$\langle r \rangle = 0.4 \langle P_2(\cos\alpha) \rangle \quad (8)$$

where $P_2(x) = \frac{3x^2 - 1}{2}$ is the second Legendre polynomial, and α is the angle between the molecule's absorption and emission dipoles. The angle α may vary from 0 to 90°. Thus the allowed values for <*r*> and *P* are:

Parameter	α = 0°	α = 90°
P	+0.5	0.333
<*r*>	+0.4	0.2

Values outside of this range indicate scattered light is present in the measurement of <*r*>. If the sample is excited with depolarized light—a less common technique—the measured value of *P* ranges from –1/7 to +1/3 (and <*r*> from –1/11 to +1/4). The individual intensity components (I_{HH}, I_{HV}, I_{VH}, I_{VV}) are also referred to as *raw polarization*.

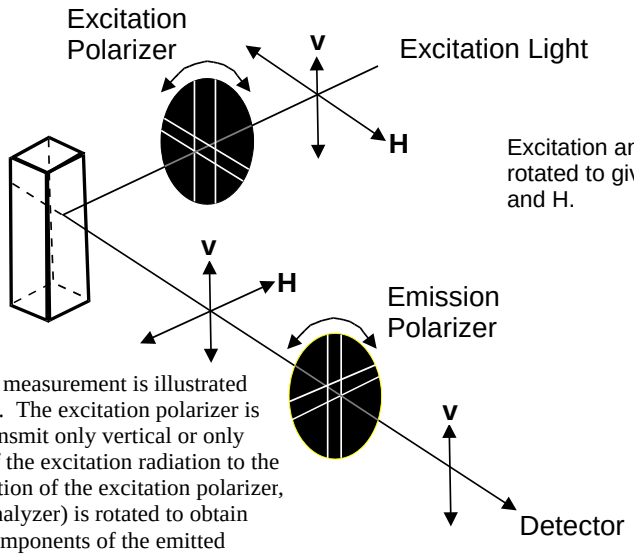
Experimentalists often multiply polarization units by 1000 to yield *millipolarization* units, m*P*, for very small changes in the polarization.

Polarization geometries

Polarization measurements are taken in two basic geometries:

- L-format uses two polarizers, which are both rotated between horizontal and vertical positions for measurements. If the *G* factor is determined beforehand, only two measurements are required: the VV and VH components, found by rotating only the emission polarizer. The standard NanoLog® uses the L-format geometry.
- T-format uses one excitation and two emission polarizers. The excitation polarizer is rotated between horizontal and vertical for measurements, while the emission polarizers are fixed—one horizontal and the other vertical. If the *G* factor is determined beforehand, it is possible to obtain the anisotropy or polarization in one measurement cycle, for the VV and VH components are available simultaneously on the two emission detectors. The *G* factor is measured differently in the T-format technique. NanoLog® spectrofluorometers with a third "T" detector can incorporate T-format polarizers.

A schematic diagram of both geometries is shown on the next page:

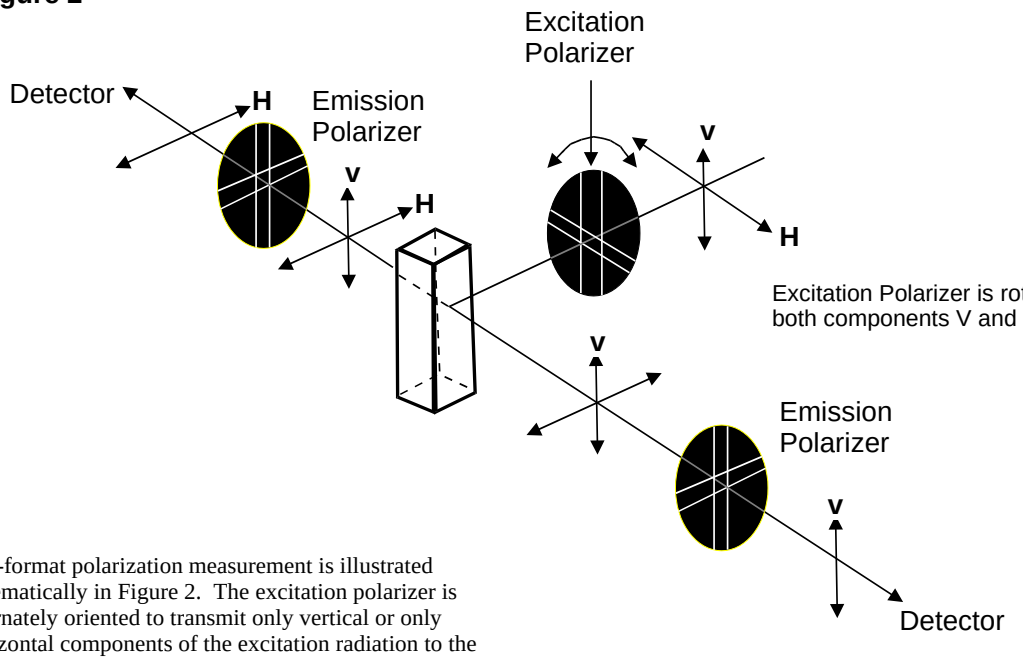


Excitation and Emission Polarizers are rotated to give both components V and H.

An L-format polarization measurement is illustrated schematically in Figure 1. The excitation polarizer is alternately oriented to transmit only vertical or only horizontal components of the excitation radiation to the sample. For each orientation of the excitation polarizer, the emission polarizer (analyzer) is rotated to obtain vertical and horizontal components of the emitted luminescence.

T-Format Polarization

Figure 2



Excitation Polarizer is rotated to give both components V and H.

A T-format polarization measurement is illustrated schematically in Figure 2. The excitation polarizer is alternately oriented to transmit only vertical or only horizontal components of the excitation radiation to the sample. Dual emission paths, each with a polarizer, permit simultaneous measurement of the vertical and horizontal components of the emitted luminescence.

Magic-angle conditions

Some fluorescent compounds exhibit molecular rotations on the same time-scale as their fluorescent lifetimes. This can cause a spectral distortion if the excitation and emission channels of a spectrofluorometer show some polarization bias. Specifically, when the rotational correlation time of a fluorophore is similar to the fluorescence life-time, the effect can be significant. To record spectra that are free of rotational artifacts,

use polarized photoselection conditions that cause the anisotropy to be zero. These polarization angles are called *magic-angle conditions*.

The two magic-angle conditions are:

- Use a single polarizer oriented at 35° in the excitation path with a scrambler plate, or
- Use two polarizers, with excitation at 0° and emission at 55°.

We recommend using the two-polarizer method, exciting with vertically polarized light, and measuring spectra with the emission polarizer set to 55°. Scrambler plates do not offer complete depolarization of the light beam at all wavelengths, and thus are not suitable for all experiments.

To use magic-angle conditions during data collection, set the excitation polarizers to V (0°), and the emission polarizer to magic-angle V (55°) using the Accessories icon in the **Experiment Setup** window. Collect spectra in the normal manner. To use magic-angle conditions for corrected spectra, measure an additional set of correction factors with the polarizers held at the chosen magic-angle settings.

Note: *Most samples do not exhibit an appreciable change in their spectrum when measured under magic-angle conditions. Thus, magic angles need not be used for most samples.*

Installation

HORIBA Scientific's polarizers are made for easy installation and removal from the light path. All of the polarizers use pinned collars to hold the polarizers in their mounts and maintain calibration when the polarizers are removed.

New instrument and complete-polarizer orders are shipped with pre-aligned polarizers marked for excitation ("X") or emission ("M"), and are locked in their collars.

Caution: *When the polarizers are shipped inside an instrument, the polarizers are aligned and calibrated at the factory. Do not remove polarizers from their collars, or else the polarizer must be realigned.*

Store the polarizer crystals in a dust-free environment, in a cabinet or drawer.

Proceed to "Alignment" in this chapter to verify alignment of the polarizers.

Alignment

Checking polarizer alignment

Polarizer alignment is verified by measuring the anisotropy of a dilute scattering solution. Scattered light is highly polarized, and this allows a simple check of the crystal alignment in the instrument. We recommend using a very dilute solution of glycogen or Ludox® (colloidal silica) as the scattering sample.

Caution: *Refer to your Material Safety Data Sheets (MSDS) for hazards regarding the use of glycogen, colloidal silica, or other scatterers.*

The alignment test may be a measurement of the polarization or anisotropy within the software using the Anisotropy scan-type, or use of the Remeasure Anisotropy Only utility (click Advanced..., and the **Polarizer Alignment** window opens). The test also may be performed manually using the **Real Time Control** application. One measures the polarization, anisotropy, or the polarization ratio of scattered light (typically, the excitation and emission monochromators are both set to 400 nm for the measurement). To calculate the *polarization ratio*, use the definition:

$$\text{polarization ratio} = \frac{I_{VV} * I_{HH}}{I_{VH} * I_{HV}} \qquad (9)$$

Alignment is satisfactory when the polarization ratio ≥ 100, or $P \geq 0.98$, or $\langle r \rangle \geq 0.97$.

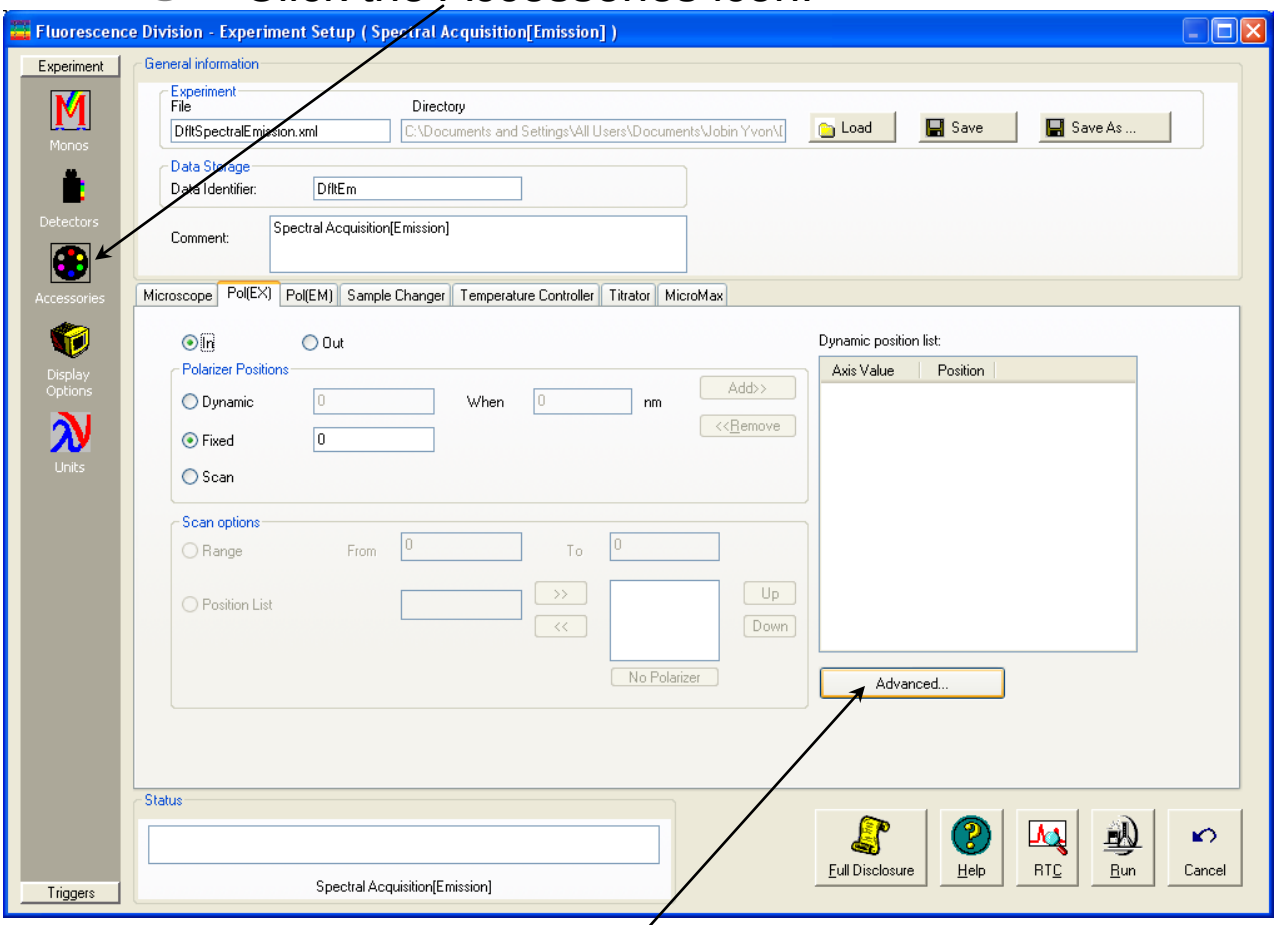
Note: *The polarization ratio can be lowered by using concentrated scatterer. Use only a slight amount of scatterer to align the system.*

The check below assumes a sample of Ludox® or glycogen is used.

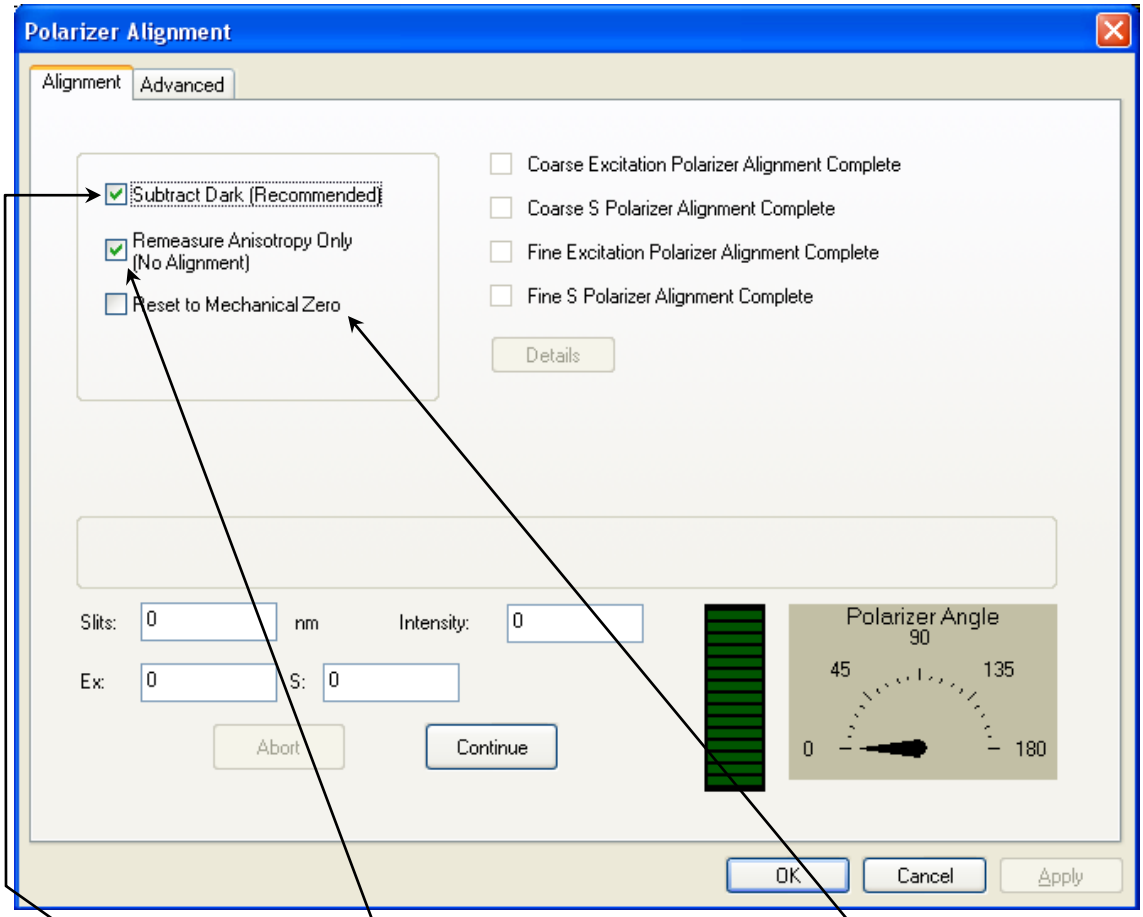
1 Start FluorEssence™.

2 Open the **Experiment Setup** window.
You may use any instrument configuration with polarizers.

3 Click the Accessories icon.



4 Click the Advanced... button.
This opens the **Polarizer Alignment** window:



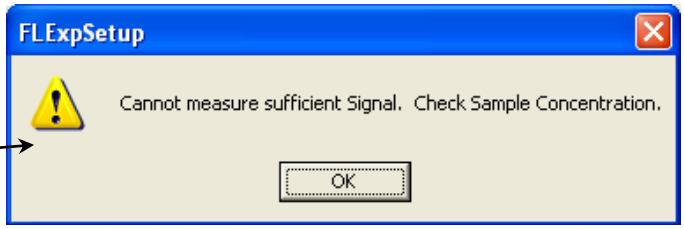
- Activate the Subtract Dark checkbox.
- Activate the Remeasure Anisotropy Only checkbox.
- Activate Reset to Mechanical Zero only if the polarizers are definitely miscalibrated. This deletes the previous calibration.

5 Place the Ludox® or glycogen in the sample holder.

Caution: *Refer to your Material Safety Data Sheets (MSDS) for hazards regarding the use of glycogen or colloidal silica.*

6 Click the Continue button.

The system rotates through the polarizers as shown in the checklist on the window. As each phase is completed, the checkboxes are updated. If the sample is too concentrated or dilute, the software prompts you to correct this.



When complete, the software displays the measured anisotropy for the emission channel (S).

7 Approve or retry the measurement based on satisfaction with the result.

8 To quit, hit the Cancel button at any time during the procedure.

As an alternative, use an Anisotropy scan to acquire the polarization (P) or anisotropy $\langle r \rangle$) to verify alignment. To be aligned, $P \geq 0.98$ or $\langle r \rangle \geq 0.97$.

Re-alignment of polarizers

NanoLog® autopolarizers may be aligned using a software routine called **Polarizer Alignment** in **Experiment Setup**.

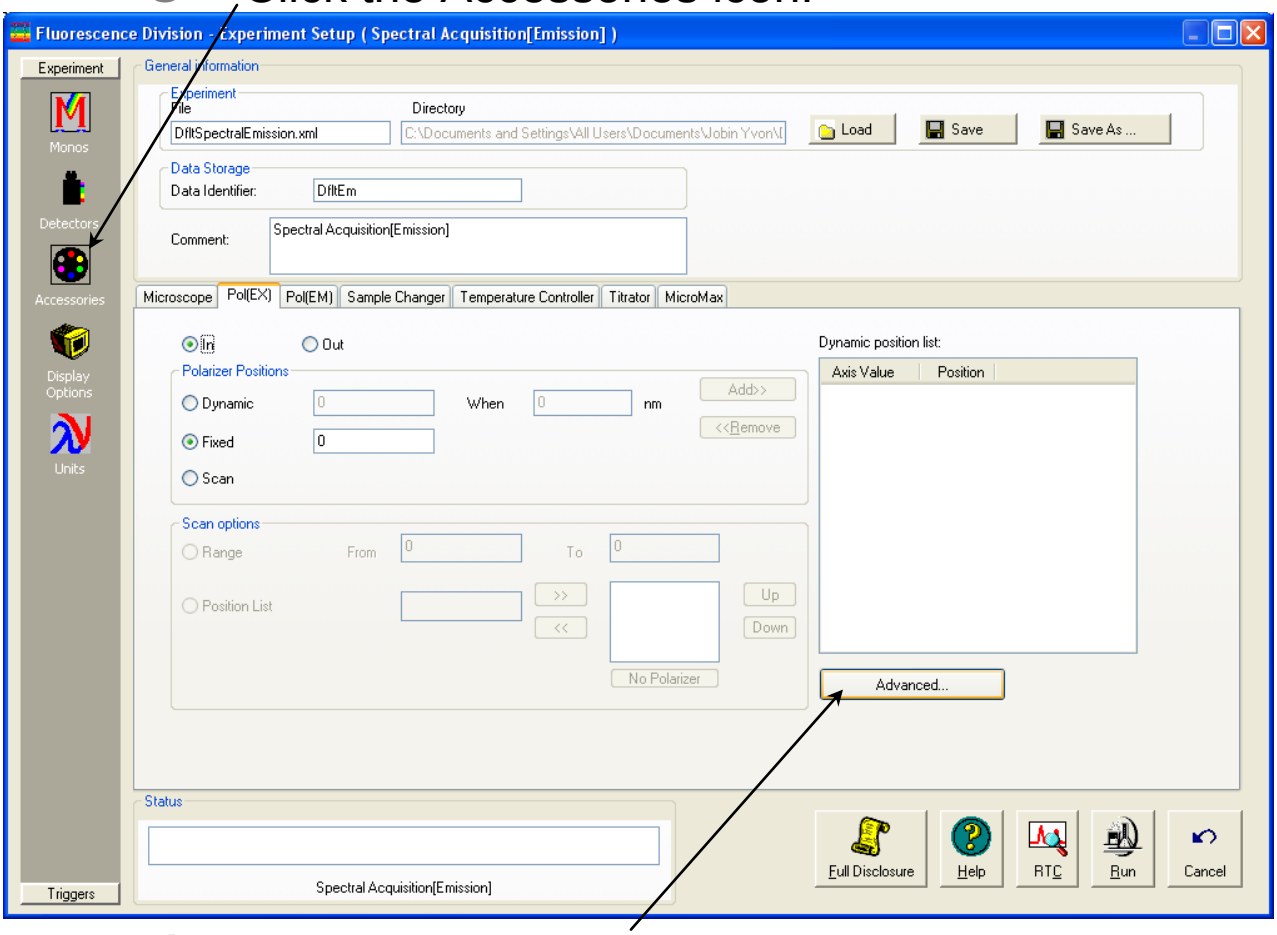
Using Polarizer Alignment

This routine automatically calibrates autopolarizers. Use a sample of Ludox® or glycogen to run the alignment routine. The software rotates the polarizers in 1° increments and locates the optimal positions for each autopolarizer. After completion, the anisotropy for the scattering solution is measured and displayed for user approval of the alignment. If approved, the new calibration positions are saved in the sample-compartment initialization file, and a log file, `POLAR.LOG`, is saved with the results of the calibration procedure. Otherwise, the previous calibration positions are still used.

1 Start FluorEssence™.

2 Open the **Experiment Setup** window.

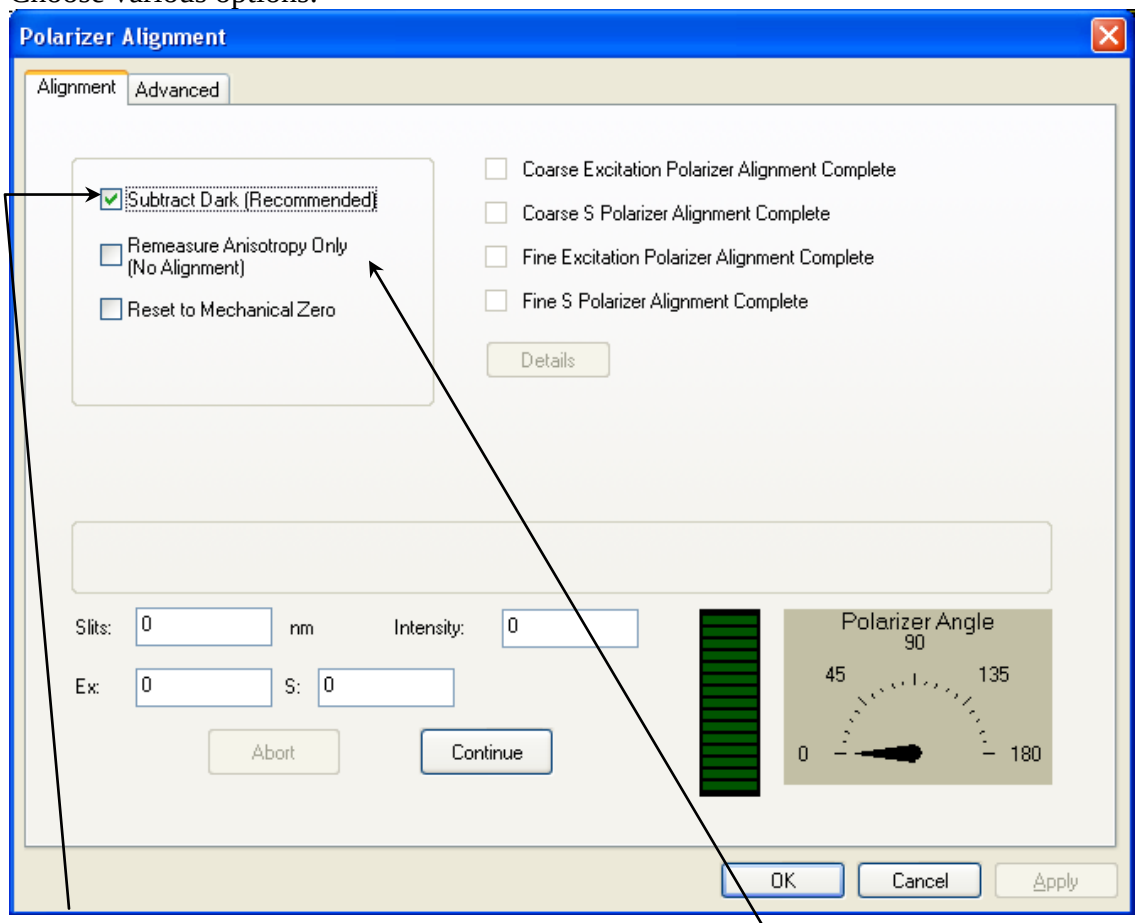
3 Click the Accessories icon.



4 Click the Advanced... button.

This opens the **Polarizer Alignment** dialog box:

Choose various options:



- Subtract Dark (recommended)
- Reset to Mechanical Zero—only if the polarizers are definitely miscalibrated. This deletes the previous calibration.

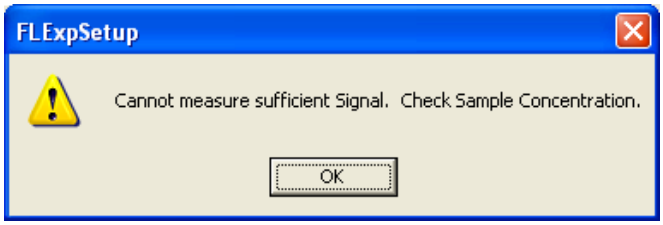
Note: *Do not check the Remeasure* Anisotropy Only *checkbox.*

5 Place the Ludox® or glycogen in the sample holder.

Caution: *Refer to your Material Safety Data Sheets (MSDS) for hazards regarding the use of glycogen or colloidal silica.*

6 Click the Continue button.

The system rotates through the polarizers as shown in the checklist on the window. As each phase is completed, the checkboxes are updated. If the sample is too concentrated or dilute, the software prompts you to correct this.



When complete, the software routine displays the measured anisotropy for each emission channel (S or T).

7 Approve or retry the measurement based on satisfaction with the result.

Acceptable values are anisotropy ≥ 0.98 and polarization ratio ≥ 130.

8 To quit, hit Cancel any time during the procedure.

Alignment

Note: *Adjust the polarizers with the room lights off or the instrument covered with a tarpaulin. Stray light can have a deleterious effect on the photomultiplier tube, or make optimization of the alignment more difficult.*

Warning: *Never attempt to manually realign FL-1044 or FL-1045 polarizers.*

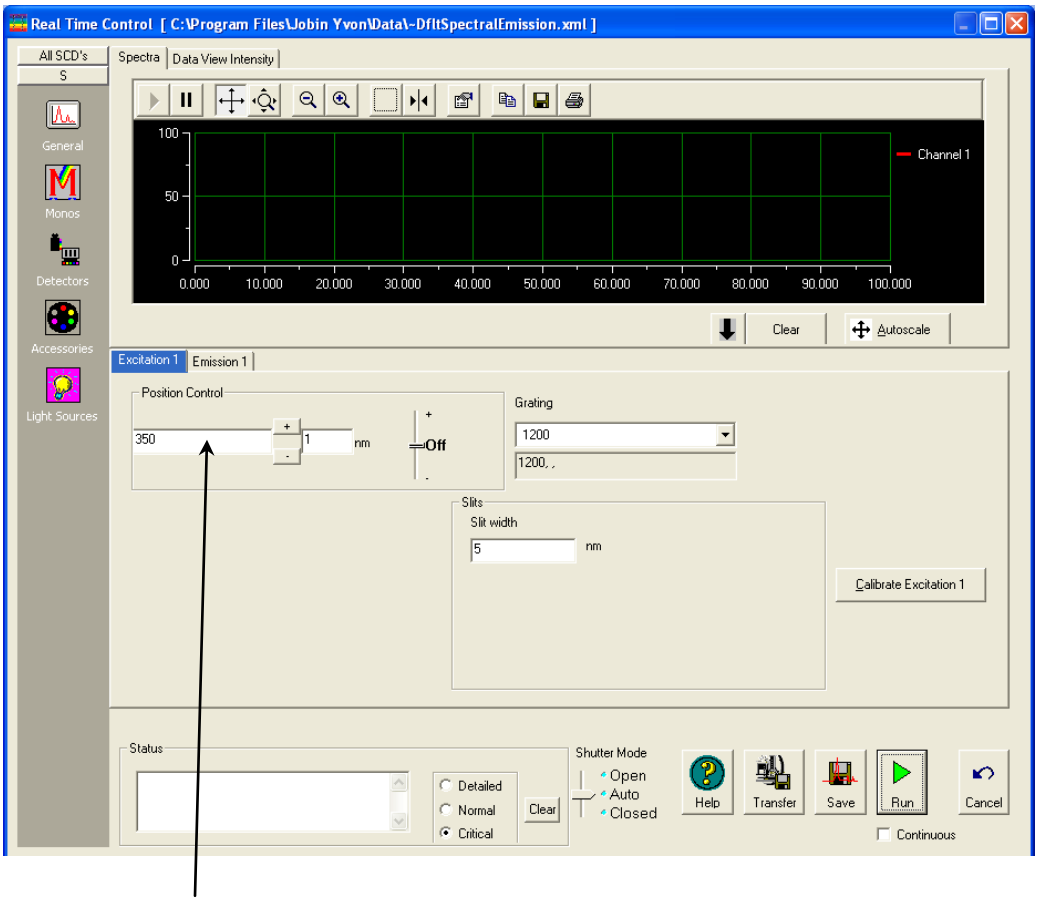
1. Turn off power to the polarizers and 1976 Accessory Controller.

Note: *If the motor rotates during alignment of automated polarizers, immediately stop the procedure. Secure the polarizers in their collars, then re-initialize the polarizers. Otherwise, the alignment may not be performed at the calibration position of the polarizers.*

2. Loosen the screws that hold the polarizers inside their collars. Do not loosen the set screw holding the collar in the mount. FL-1044 and FL-1045 autopolarizers have three set screws: two on one side of the collar, and one on the other side of the collar.
3. Set the tension on these set screws so that they do not slip, but allow easy manual rotation.
4. Set the polarizer crystals so that they protrude from the mounts far enough (~1/4″ or ~6 mm) to allow rotation.
5. Start the polarizers and accessory controller.

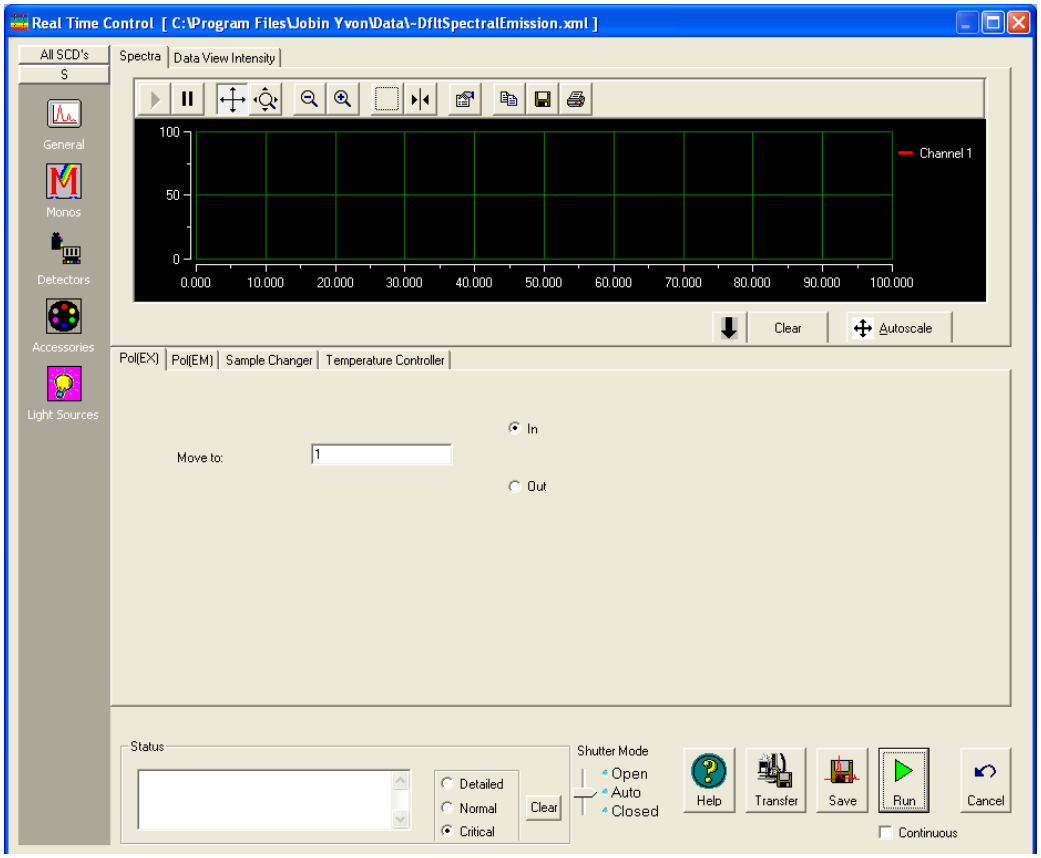
6 Insert the Ludox® or glycogen sample into the sample holder.

7 Start the software (if not yet running) and go to **Real Time Control**:



8 Set all monochromators to 400 nm under the Monos icon.

9 Set polarizers to VV (0°, 0°) under the Accessories icon:



10 Open the excitation shutter (if applicable).

11 Turn on high voltage and set appropriately for S channel (950 V for R928P; 1050 V for R1527).

12 Set slits to 5-nm bandpass for all monochromators.

13 Set scatterer concentration to give 1–1.5 × 10^6 cps on S.

14 Rotate the excitation polarizer to a rough maximum.

15 Set the polarizers to HV (90°, 0°) and rotate the excitation polarizer for the minimum signal on S.

16 Set the polarizers to VH (0°, 90°) and rotate the emission polarizer for the minimum signal on S.

17 Set polarizers to VV. Reset slits for 1–1.5 × 10^6 cps on S channel.

18 Measure polarization ratio (Equation 9). If the polarization ratio > 100, then the alignment is acceptable. Otherwise, repeat steps 15–18.

19 Secure the polarizers in their collars.

20 Verify that all polarizers are properly labeled for their locations in the system:

X = excitation
M = S-side emission

Using automated polarizers

FluorEssence™ software with HORIBA Scientific polarizers provides many choices for polarization measurements. Depending on the accessories, the opportunity exists to remove polarization effects from the sample, measure the polarization characteristics, or analyze the decay of anisotropy using frequency-domain techniques. For further software information, refer to the FluorEssence™ and Origin® on-line help.

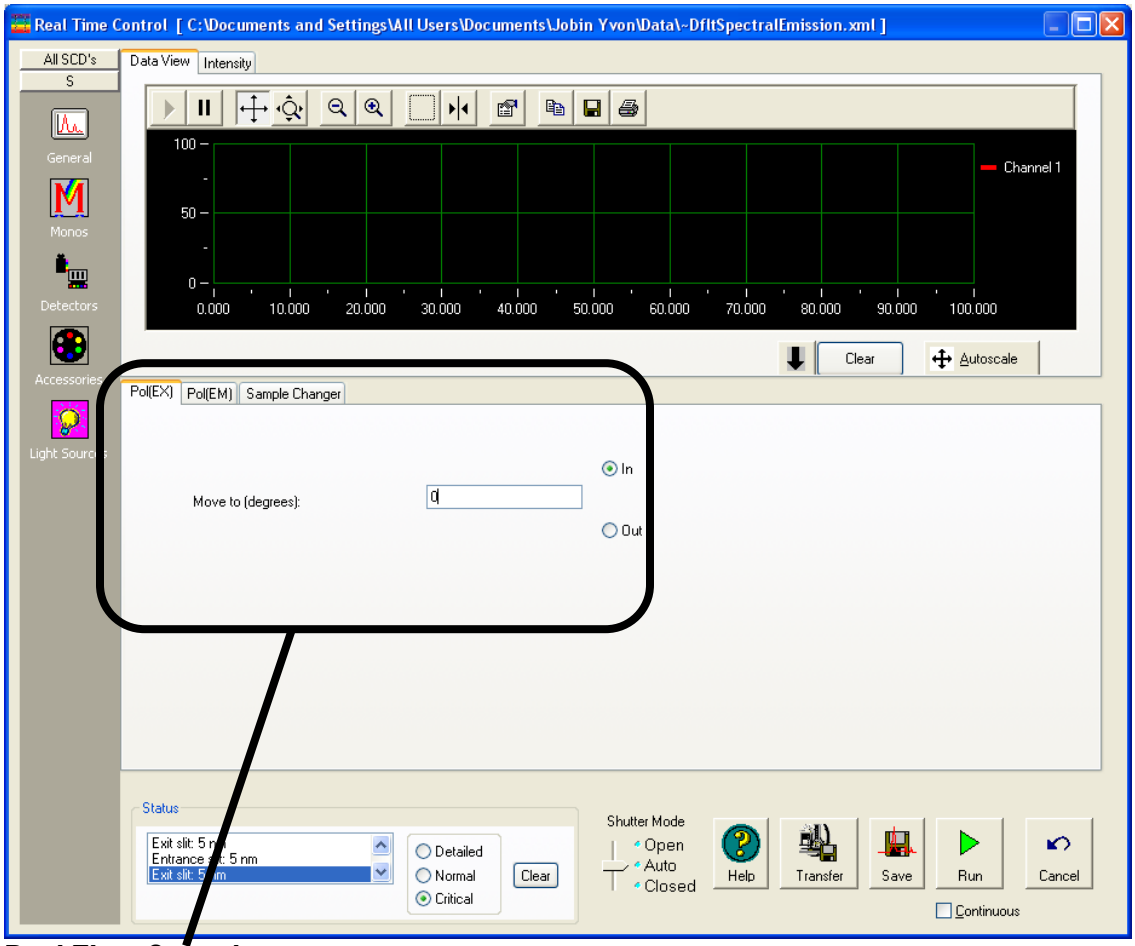
Applications for polarizers

- Measurement of emission anisotropy or polarization at fixed wavelengths. This is used for binding assays, kinetics of molecular size or shape change, temperature effects on rotational motion of fluorophores (e.g., phase transition of phospholipid bilayers).
- Measurement of excitation and emission spectra using magic angles. This helps to eliminate spectral artifacts.
- Measurement of a principal polarization or excitation anisotropy spectrum, using an excitation scan with polarization. This provides information about rotational sensitivity of the excitation spectrum by measuring $<r>$ versus λ_{exc} (with λ_{em} constant). Examine relative molecular dipole-angles at cryogenic temperatures in a viscous solvent.

Using FluorEssence™

To use the autopolarizers, load an instrument configuration with autopolarizers.

Real Time Control

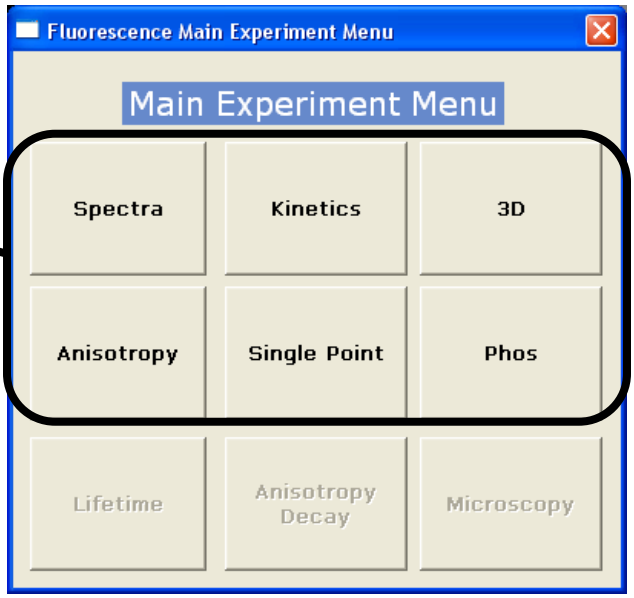


Real Time Control manipulates the polarizers and other instrument settings, to observe and optimize the spectrofluorometer in real time. Under the Accessories icon, each polarizer may be set independently into or out of the optical path under its own index-card tab. A custom angle may be set from 0–180°, in the field provided.

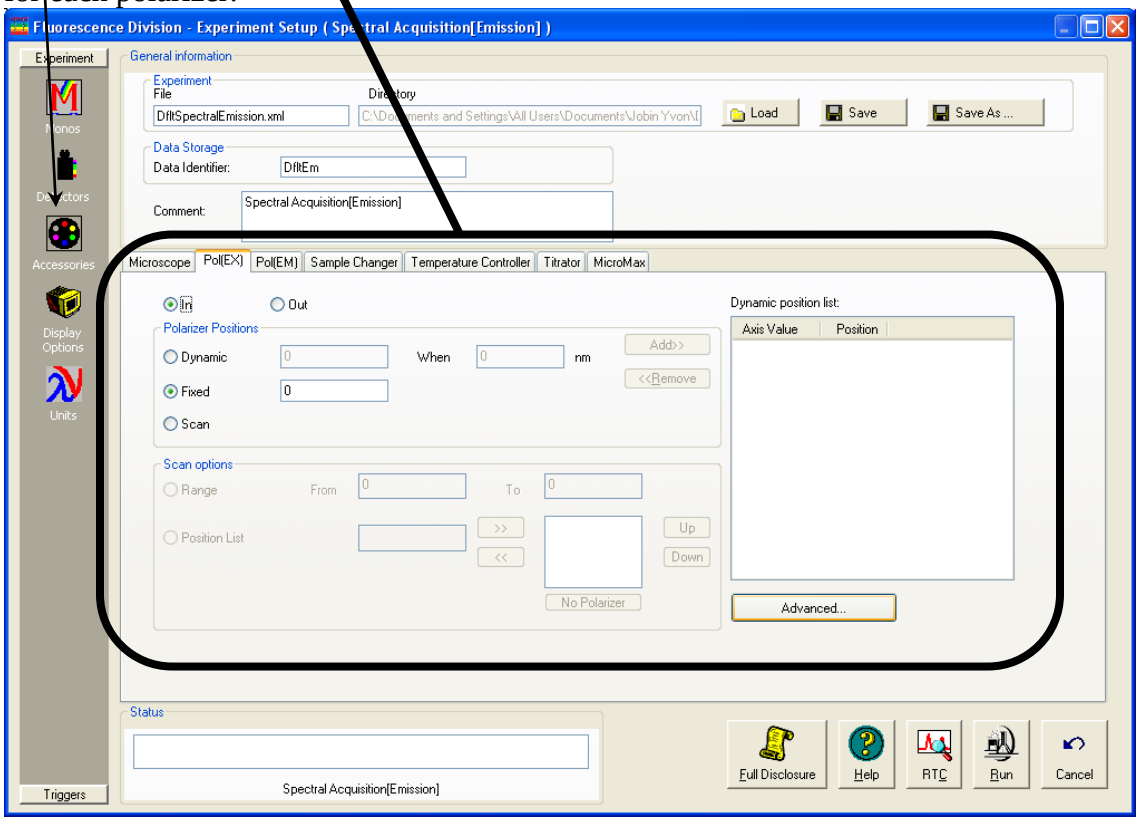
***Note: Real Time Control** is only intended for real-time setup of a scan. Use **Experiment Setup** to work at fixed wavelengths.*

Experiment Setup

Experiment Setup runs all scanning options for the autopolarizers. First choose the type of scan using polarizers in the **Fluorescence Main Experiment Menu:**

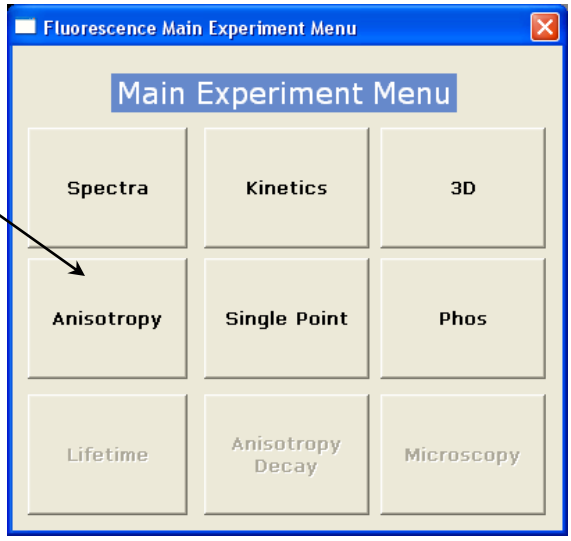


The **Experiment Setup** window appears.
Adjust polarizer parameters under the Accessories icon. One index-card tab appears for each polarizer.

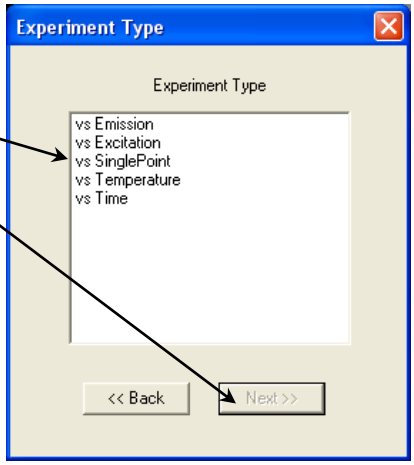


Constant Wavelength Analysis

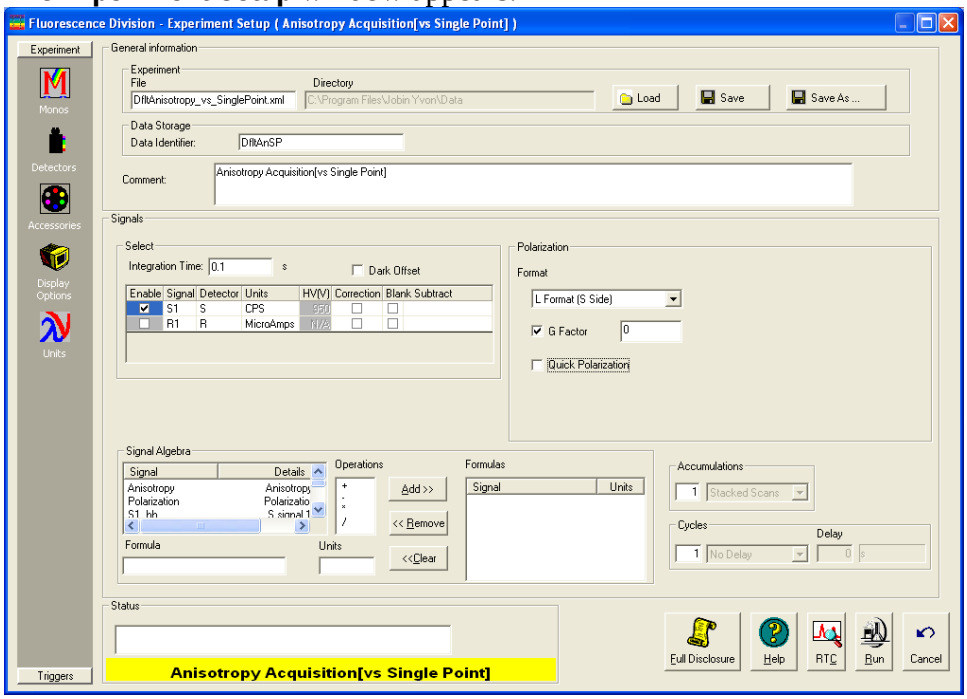
To do a constant-wavelength analysis experiment, that is, to take polarization acquisitions at fixed excitation/emission wavelength-pairs, choose Anisotropy from the **Fluorescence Main Experiment Menu**.



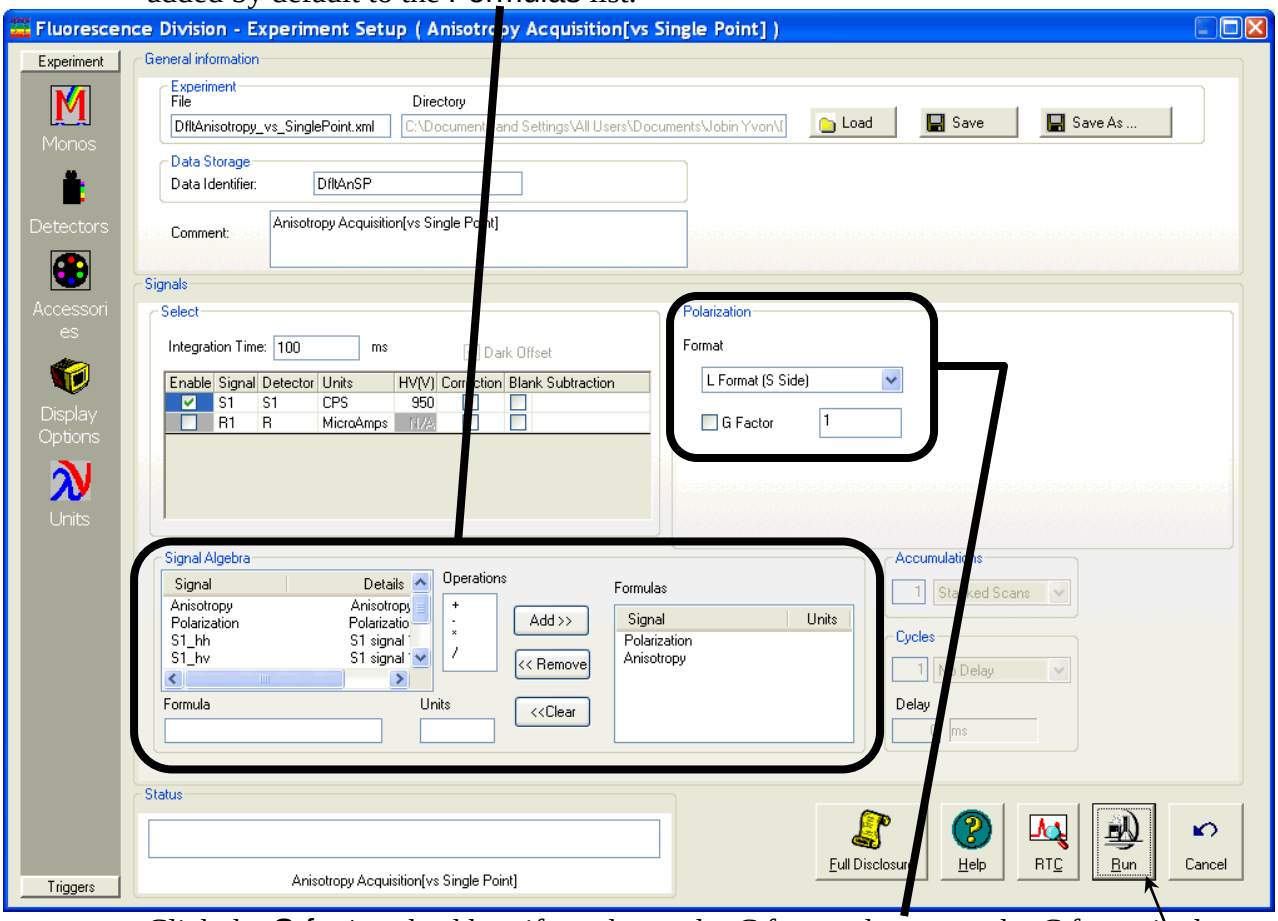
The **Experiment Type** window opens. Choose vs SinglePoint, then click the Next >> button.



The **Experiment Setup** window appears.



Make sure to use the appropriate Signal in the Signal Algebra area. Add >> it to the Formulas table. You can add the detector signals at any of five polarization-angle combinations (VV, VH, HV, HH, VM) to the Formulas list. The polarization and anisotropy ratios are calculated automatically in the Anisotropy-type experiments, and added by default to the Formulas list.



Click the G factor checkbox if you know the *G* factor, then enter the *G* factor in the box. If you do not know the *G* factor, leave the G factor checkbox unchecked, and enter 1. The *G* factor will be measured during the experiment, and automatically used in the anisotropy or polarization ratios.

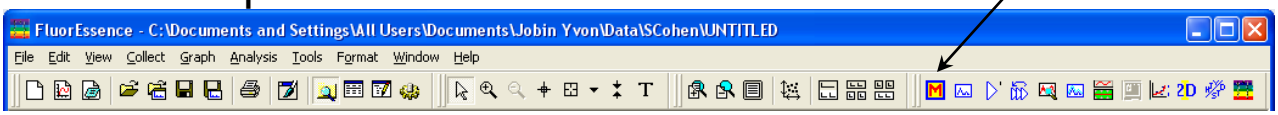
Click the Run button when ready.

Measuring the *G* factor

Note: *Measure the G factor before a polarization experiment.*

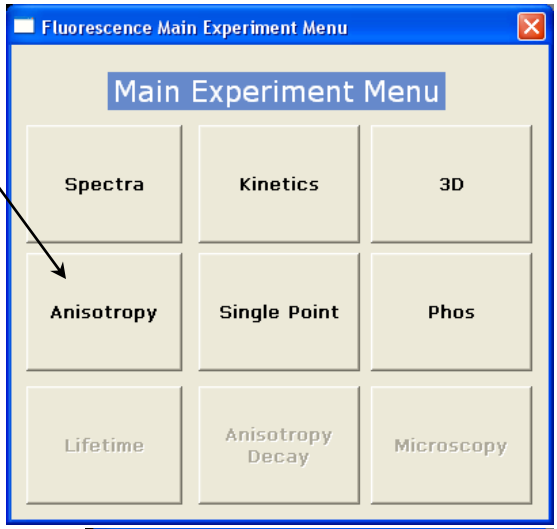
Include the grating factor, or *G* factor, whenever polarization measurements are taken. The *G* factor corrects for variations in polarization wavelength-response for the emission optics and detectors. A pre-calculated *G* factor may be used when all other experimental parameters are constant. In other cases, the system can measure the *G* factor automatically before an experimental run. *G* factors are incorporated into the Anisotropy scan-type:

1 In the FluorEssence main window, click the Experiment Menu button .



The **Fluorescence Main Experiment Menu** appears.

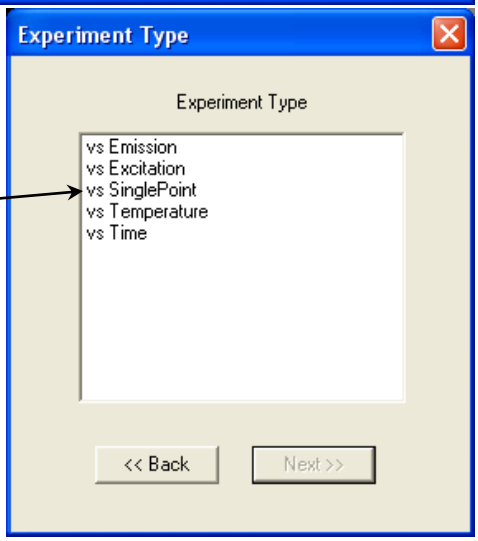
2 Click Anisotropy.



The **Experiment Type** window appears.

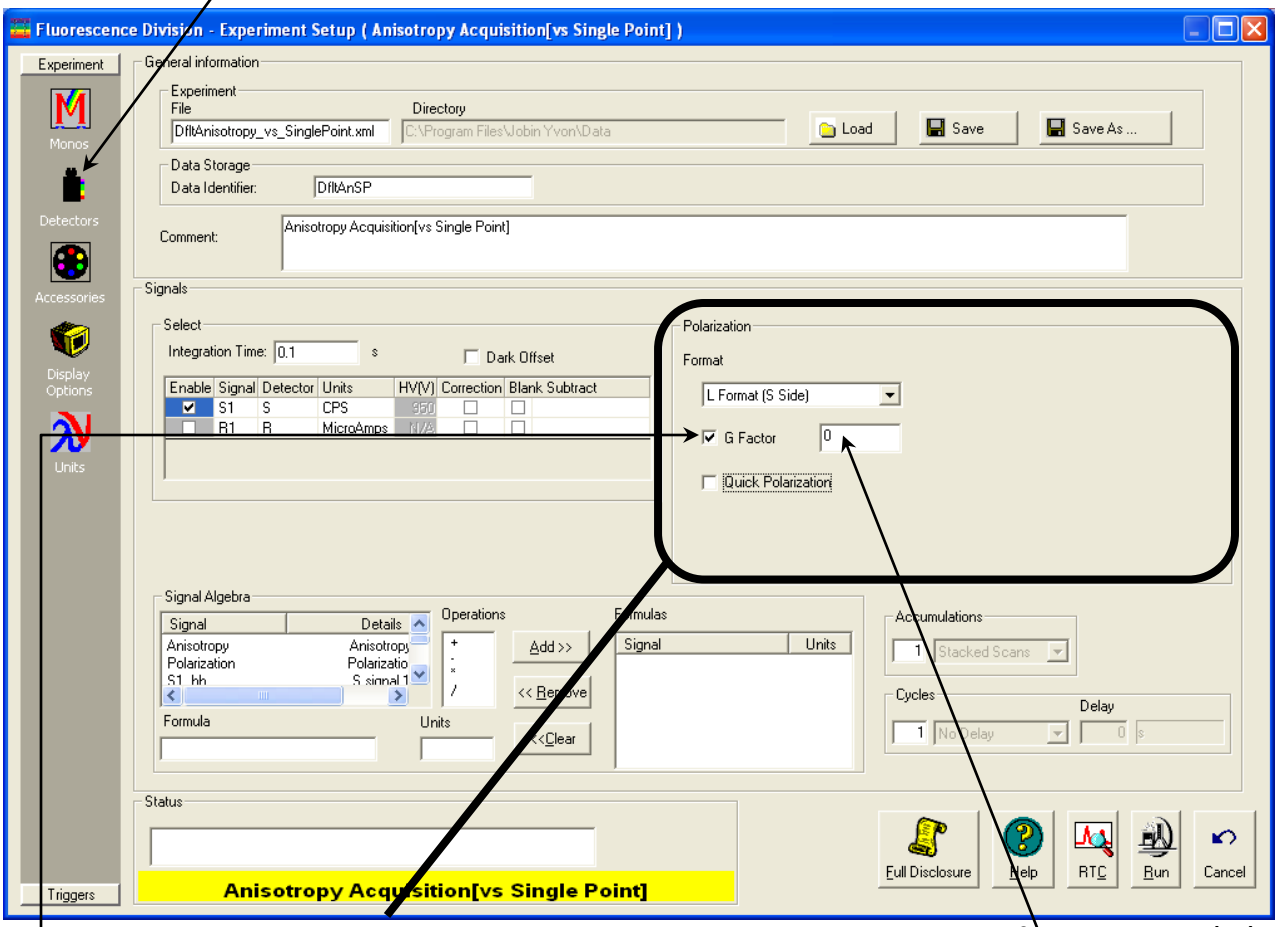
3 Choose the type of Anisotropy experiment.

For example, to determine polarization at particular excitation/emission wavelength-pairs, choose vs SinglePoint.



The **Experiment Type** window opens.

4 In the **Experiment Setup** window, click the Detectors icon.



This shows the parameters related to detectors, including the *G* factor, in the Polarization area.

5 Decide if the software should measure the *G* factor, or you enter your own *G* factor.

a Click the G Factor checkbox to include your pre-measured *G* factor in the scan. Then enter your pre-determined value for the *G* factor in the field. To automatically measure the *G* factor, enter 0 in the field.

b If you leave the G Factor checkbox blank, the software measures the *G* factor for you during the scan.

Note: *For weak signals, enter the G factor, rather than measure it automatically. This may improve the S/N.*

Maintenance

Like all optics, polarizers should be handled with care and stored properly. With proper care, a polarizer should last for many years. Aside from installation, removal, and storage, there is no routine maintenance necessary for a polarizer. Polarizers should be removed and stored when not in use. Store the polarizers in their collars to maintain calibration, in a drawer or cabinet. Wrap the polarizers in lens tissue—to keep them dust-free and for protection—and then place them in a plastic bag. The automated accessories should also be stored in a dust-free environment.

Should the polarizer windows need cleaning, apply a mild solution of methanol, and blow it dry.

Caution: *Refer to your Material Safety Data Sheets (MSDS) for hazards regarding the use of methanol.*

We recommend measuring the anisotropy of scatter (to verify the alignment of the crystals) before any critical experiment. In addition to the standard xenon-lamp spectrum and laser-glass spectra, which serve to verify the wavelength calibration, measurement of the anisotropy of scatter will provide a fast check that the instrument system is ready to perform measurements.

Troubleshooting

For difficulties with polarizers, consult the table below to see if your question is answered here. Otherwise, reach the Service Department at HORIBA Scientific by phone, fax, or e-mail. Before contacting us, please follow the instructions below:

1 Note the problem and record any error messages.

2 See if the problem is listed on the following pages.

If so, try the suggested solutions. Be sure to note carefully the steps taken to remedy the problem and the result. Refer to the appropriate section of this manual (and the software manuals, if necessary).

3 If the problem persists, or is not listed,

Call the Service Department by phone at (732) 494-8660 ×160, or fax at (732) 494-8796. Outside the United States, call the local distributor. You may also reach us by e-mail at service.jy.us@horiba.com.

When you contact the Service Department, have the purchase date, serial number, system configuration, and software version available. Be prepared to describe the malfunction and the attempts, if any, to correct it. Note any error messages observed and have any relevant spectra (sample, polarization ratio, xenon-lamp scan, laser-glass scan) ready for us to assist you.

Problem	Cause	Possible Remedy
Poor polarization data	Improper sample concentration	Adjust sample concentration.
	Photomultiplier saturated; slits improperly set	Check that sample signals are in linear region ($< 2 \times 10^6$ cps on S or T, < 10 µA on R). Reset slits.
	Dirty cuvette	Clean the cuvette.
	Polarizer misaligned	Check polarizer alignment.
	System misaligned	Check system alignment in a generic layout. Run lamp scan and laser-glass scan to check calibration.
Low polarization ratio	Highly concentrated standard	Check Ludox® or glycogen concentration: higher concentrations can cause inner-filter effect, lowering ratio.
	Improperly set slits	Set slits for ~ 1×10^6 cps in VV. Signals much less than this give excessive contribution from dark noise, while signals > 2×10^6 cps are in non-linear region.
	System misaligned	Check system alignment in generic layout. Run lamp scan and laser-glass scan to check calibration.
Autopolarizers do not initialize (they do not move during initialization).	Wrong instrument configuration is loaded	Check that a configuration with autopolarizers is loaded.
	Bad cable connections	With the system power off, recheck cable connections.
Software failure initializing autopolarizers	Wrong instrument configuration is loaded	Check that a configuration with autopolarizers is loaded.
	Bad cable connections	With the system's power off, recheck cable connections.
	Computer hang-up	Exit the software, and reboot the system and host computer.

10: Transferring Data from FluorEssence™ to Nanosizer®

Introduction

Excitation-emission matrices from single-walled carbon nanotube samples are easily analyzed with the Nanosizer® software that comes with your NanoLog®. This chapter shows you how to transfer your data, recorded using FluorEssence™, into the Nanosizer® software.

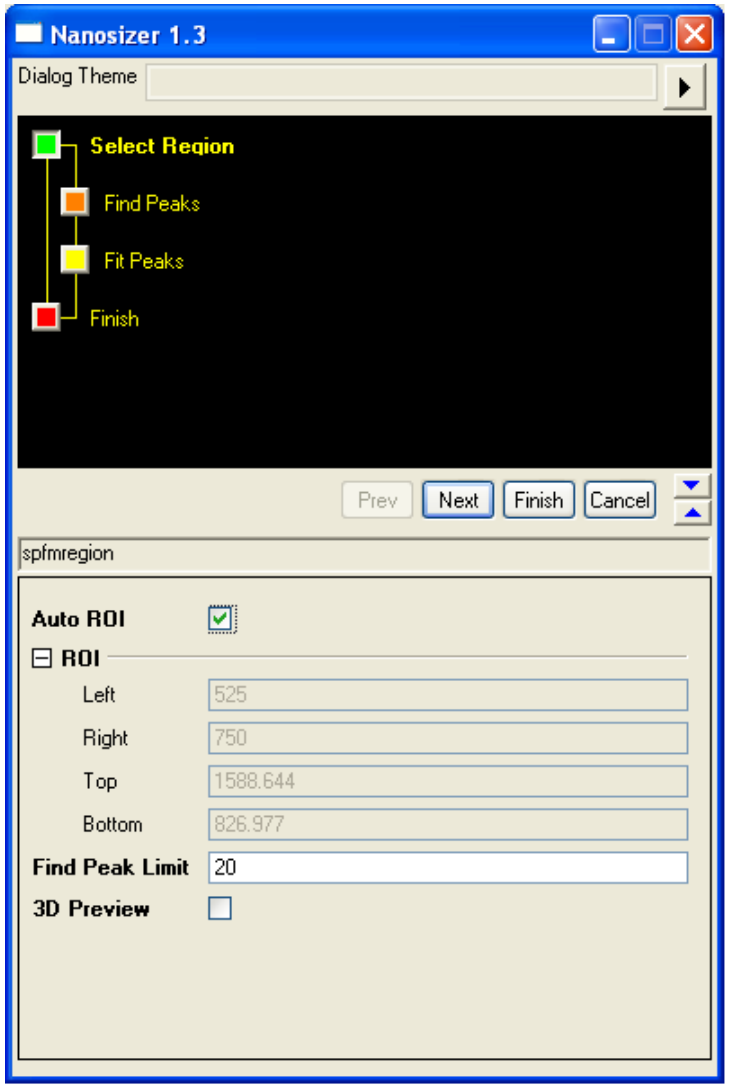
For more information on the Nanosizer® software, see the *Nanosizer® Operation Manual*.

Procedure

1. Have the image of the new data open in FluorEssence™.
2. Double-click directly on the graph to open the actual data instead of the image.
3. In the toolbar, Click the Nanosizer button  .

   The Nanosizer® program starts, and the **Nanosizer** window opens.

For further information on operation of the Nanosizer® software, see the *Nanosizer® Operation Manual.*



11: Xenon Lamp Information & Record of Use Form

Xenon lamps typically are used in fluorescence instruments because they provide a continuous output from 240 nm to 600 nm. In the NanoLog® spectrofluorometer, the standard xenon lamp is ozone-free. The xenon-lamp spectrum exhibits a characteristic peak around 467 nm, which can be used to indicate whether the excitation spectrometer is properly calibrated.

As the xenon lamp ages, water Raman spectra have a progressively lower peak intensity. During its lifetime, the lamp stabilizes at approximately 60% of its original intensity. Keep a record of the time the lamp is in use on the form provided on the following page. From this record, you will be able to determine when the lamp is near the end of its lifetime. The maximum lifetime of the 450-W lamp is 2000 hours.

Warning: *To avoid explosion from lamp failure, do not allow xenon lamp to exceed rated lifetime. One clue to imminent failure may be extremely low water-Raman intensity. Please record the hours the xenon lamp has been used.*

Xenon Lamp Record of Use

Page _____ of _______

In Service Date	Operator	Current Date	Time On	Time Off	Total Time (Hours/Min.)
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			Total	Hours	/

12: Introduction to Lifetime Measurements

Introduction

The standard NanoLog® spectroscopy system is designed to perform steady-state measurements. HORIBA Scientific offers TCSPC on Fluorolog®, which uses the time domain for lifetime detection, to upgrade your NanoLog® system to lifetime capability.

The wide range of pulsed sources with the TCSPC on Fluorolog® system allow you to measure slow decays of phosphorescence to rapid picosecond fluorescence lifetimes. Our NanoLED laser-diode sources typically offer pulses shorter than 100 ps, and the thyratron-gated spark lamp has a pulse-width of about 0.6 ns.

HORIBA Scientific has combined the TCSPC know-how of IBH and combined it with the reliable Fluorolog® series of spectrofluorometers to give you the best in lifetime sensitivity available anywhere in the world. Special DataStation software from IBH gives fluorescence decay curves built up in real time on your computer screen, and easily fits them to a library of decay types.

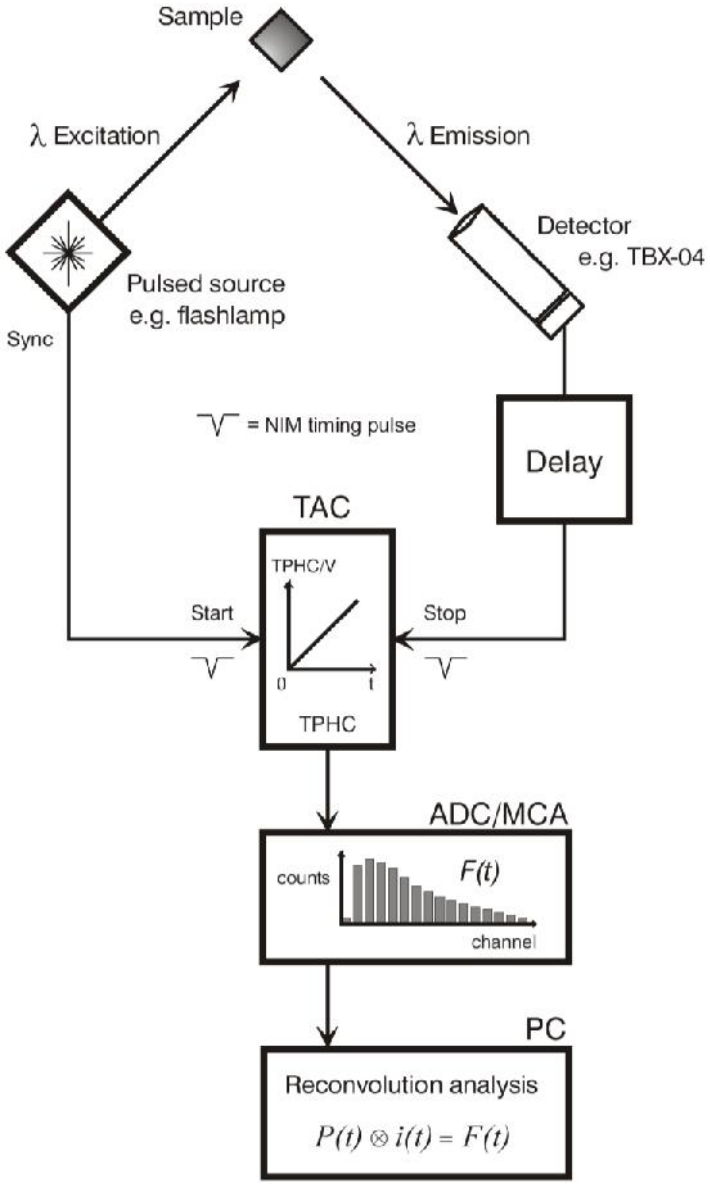
Switch back and forth between steady-state measurements and dynamic experiments easily, from an economical pulsed-diode source to the full broadband spectrum of a flashlamp.

Lifetime measurements

Overview

The first use of TSCPC for measurement of fluorescent lifetimes was mentioned in 1961. TSCPC offers a wide dynamic range, broad temporal range (10^{-12}–10^{-6} s), highest sensitivity, and the method of choice for researchers working with short lifetimes, weak fluorophores, and high repetition-rate light sources (> 10 kHz).

TCSPC relies on the principle that the probability distribution for the emission of a single fluorescence photon is equivalent to the actual intensity-versus-time distribution for all photons emitted. Below is a sketch of the basic experimental set-up in forward-TAC mode.



Sketch of TCSPC in forward-TAC mode.

The arrival of each fluorescence photon, emitted in response to a pulsed light source, is timed with reference to the excitation event by a time-to-amplitude converter (TAC). The TAC produces an analog voltage (time-to-pulse-height conversion, or TPHC), proportional to the elapsed time between start and stop timing signals. An analog-digital converter (ADC) then digitizes the TPHC voltage, and a multi-channel analyzer (MCA) stores the ADC conversion value. The MCA constructs a histogram of photon time-distribution equivalent to the actual fluorescence decay. This histogram usually contains many thousands of photons.

Note that TCSPC-instruments function differently from other transient-recording instruments such as oscilloscopes, boxcars, or multi-channel scalers, for no time-base generation or master oscillator is required. TCSPC-data are not acquired by averaging multiple sweeps along the time-axis.

This MCA data as a function of time, $F(t)$, is fitted to an appropriate physical model, $i(t)$, which is often expressed as a sum of exponentials. Data analysis is described below and in the DAS6 manual. The main point, however, is that on the nanosecond time-scale, the excitation pulse recorded as a prompt profile, $P(t)$, cannot be considered a delta function, with instantaneous rise and fall. The physical model, $i(t)$, therefore, must be reconvoluted interatively with the prompt profile, $P(t)$, before comparison with $F(t)$ and subsequent extraction of fit parameters. The width of $P(t)$ ultimately limits the temporal resolution of the instrument.

Pulse pile-up

In normal operation, a Start pulse generated by the excitation electronics initiates the TAC timing cycle. If no fluorescence photons are detected within the TAC range, the TAC resets and awaits the next Start pulse. If a photon is detected and a valid Stop is presented within the TAC range, then the TAC converts the interval between Start and Stop inputs into a proportional analog voltage (TPHC). Only one conversion can occur per cycle. So, if more than one photon is detected during the same cycle, all but the first are disregarded. This is a fundamental limitation to the TCSPC technique. If enough "late" photons are disregarded, then distortion of the perceived decay occurs, and the recovered lifetime is shorter than the true value. This distortion is called the "pile-up" effect; its influence is minimized by reducing the likelihood that more than one photon is detected in response to any one excitation event for the duration of the TAC range.

The parameter used to describe the throughput of a TCSPC instrument is the start-to-stop ratio, α, which is often quoted as a percentage, defined as

$$\alpha = \frac{S_p}{S_t} \times 100\%$$

where S_t and S_p are the Start and Stop rates, respectively. Although attempts have been made to eliminate or correct for pile-up distortion, simply limiting α to ~ 2% renders pile-up negligible. For measurements of weakly fluorescing samples, an $\alpha \approx 2\%$ is a luxury, and pile-up is irrelevant. With stronger samples, however, attenuation of fluo-

rescence intensity is often necessary, especially at low repetition-rates, so that data-collection rate is limited only by pile-up. For example, when using an excitation source at 1 MHz, the data-collection rate (TAC-out rate) should be limited to 20 kHz.

Dead-time considerations

Each of the electronic components in a TCSPC apparatus has an associated characteristic processing time, or "dead time". During this dead time, no further input is recognized, so that possible conversions may be lost. Total system dead-times are dominated by ADC conversion time and TAC reset time.

All TCSPC experiments operate in one of these two regimes:

Time between excitation events > dead time.	This occurs when low repetition-rate sources such as flash-lamps and nitrogen lasers are used. The electronics have sufficient time to complete processing the event before the next Start signal occurs, and no conversions are lost during the dead time. The relationship between α and collection rate is linear, and the TAC is operated in conventional forward-TAC timing mode.
Time between excitation events < dead time.	This occurs when high repetition-rate sources (> ca. 100 kHz) as diode and mode-locked lasers are used. The electronics cannot process the event in time for the beginning of the next cycle, and counts are inevitably lost. The relationship between α and the collection rate is no longer linear. In this regime, the TAC is operated in reverse-TAC timing mode, where the fluorescence and excitation sync pulses start and stop the TAC respectively. This has the advantage that the TAC cycle is only initiated when there is a fluorescence photon to be timed (except, of course, noise counts), thus minimizing the dead-time contribution from the TAC reset time.

Data analysis

Convolution

Extraction of kinetic parameters from decay-data without reconvolution requires that the prompt (instrumental response function) approximates a delta-function in comparison to the decay. In practice this is rarely true. Timing jitter introduced by the detector, timing electronics, and optical components can broaden the prompt, so that even with an optical pulse of only a few picoseconds, the above condition cannot be met with nanosecond delays. If the timing jitter introduced by the i^{th} component in the instrument is Δt_i, and full-width half-maximum (FWHM) of the excitation pulse is Δt_e, then the resultant prompt FWHM measured for the instrument is approximately

$$\Delta t_m \approx \sqrt{(\Delta t_e)^2 + \sum_i (\Delta t_i)^2}$$

To obtain the optimum time resolution, minimize Δt_m by choosing the fastest electronics and detectors available.

The prompt cannot be considered a delta-function if its width is greater than the width of one MCA data-channel, so the physical model for the decay, $i(t)$, must be reconvoluted with the prompt, $P(t)$, before comparison with the measured decay, $F(t)$. This reconvolution takes the form

$$F(t) = \int_0^t P(t')i(t-t')dt$$

which is often rewritten symbolically as

$$F(t) = i(t) \otimes P(t)$$

where the symbol $\otimes$ represents numerical convolution.

Interative least-squares reconvolution

The statistical method used in DataStation is *iterative least-squares reconvolution*, based on the calculation of the convolution integral using assumed fit-parameters (τ, etc.) to obtain $F'(t)$. $F'(t)$ and the decay data, $F(t)$, are then compared to assess the "goodness of fit", quantified by the parameter χ. $F'(t)$ is then recalculated using a range of fit-parameters until the best possible agreement of $F'(t)$ and $F(t)$ is obtained, with χ^2 minimized. χ^2 is defined as

$$\begin{aligned}\chi^2 &= \sum_{\text{data}} \left(\frac{\text{actual deviation}}{\text{expected deviation}} \right)^2 \\ &= \sum_{\text{data}} \left[\frac{F(i) - F'(i)}{\sigma(i)} \right]^2 \\ &= \sum_{\text{data}} [W(i)]^2\end{aligned}$$

where $\sigma(i)$ is the standard deviation of the i^{th} data point, and $W(i)$ is the weighted residual. If perfect agreement is found between the data and the anticipated model for a particular set of fit parameters, then χ^2 equals the data-set dimension, N. The normalized χ^2 value, χ^2_N, is quoted more often:

$$\chi^2_N = \frac{\chi^2}{N - \nu}$$

where υ is the number of fitted parameters, and the denominator represents the degree of freedom of the fit. For a good fit, $\chi^2_N \approx 1$, although in practice, $\chi^2_N < 1.2$ is considered satisfactory. When $\chi^2_N > 1.2$, this indicates that an inappropriate model was chosen to describe the data, or that a systematic error exists. A useful visual aid for assessing a

fit is to inspect the weighted residuals associated with each point of the decay. For an ideal fit, the residuals are flat and randomly distributed around zero. If they are not, then the shape is often a clue to the origin of the misfit, whether an instrumental artifact (e.g., scatter), or the use of an inappropriate model.

Shift parameter

The calculation of a χ^2 value requires that the calculated zero-time ($t = 0$) points of the prompt and decay are identical. A small mismatch, however, is inevitable because of inherent quantization of the MAC. A shift parameter is therefore included in the fitting algorithm to allow optimum positioning of the reconvoluted model relative to $F(t)$. This also can correct for time-dispersion effects introduced by the color-effect in detectors.

MCS mode

A multi-channel scaler (MCS) can be considered as a series of photon-counters, with one counter for each data-channel in the decay trace. Each counter is gated in sequence as the MCS sweep progresses from the first channel through to the last channel. The gate duration is equal to the time per channel, and is user-selectable to allow a wide range of lifetimes to be measured. (The term "bin" is sometimes used instead of "channel" in some literature.)

Each MCS sweep is synchronized to the excitation source. There are a number of synchronization modes, and these are explained in more detail in the DataStation software manual. Typically a sync-output signal from MCS electronics triggers the excitation source at the beginning of each MCS sweep. The MCS usually is programmed to perform multiple sweeps so that the excitation source flashes each time a new sweep is begins. The data are integrated so that the decay builds from just a few photons per sweep.

The programmed sweep range (analogous to the TAC range in TCSPC mode) is equal to time-per-channel × channels-per-sweep. For example, a channels-per-sweep setting of 200 channels, with a time-per-channel setting of 5 µs, results in a sweep range of 1 ms.

As in TCSPC mode, it is possible to collect photons too quickly. An α indicator is therefore provided to minimize the possibility of pile-up distortion. This is analogous to the indicator in TCSPC mode, except that the α value is calculated at the cursor position. Position the cursor on the data-channel containing the most counts within the range to be analyzed (this is not necessarily the same as the data-channel with the overall peak count). Ideally, α should be < 1%, and certainly < 2% during the measurement.

Types of lifetime scans

The type of scan defines which measurement will be acquired. In lifetime operation, four scan types are available:

- Lifetime
- Lifetime-resolved
- Dynamic depolarization
- Time-resolved.

Recalling an experiment allows the user to retrieve the experimental parameters. Each scan type is defined below.

Lifetime acquisition

The *lifetime acquisition* type of scan determines accurate lifetimes from simple single-component systems as well as complex heterogeneous systems. The measurement records the phase shift and modulation at specified frequencies for an unknown sample relative to a reference standard.

Lifetime-resolved acquisition

A *lifetime-resolved acquisition* scan resolves up to three components of overlapping spectra based on differences in the fluorescence lifetimes. More complex systems can result in improved resolution of one or more spectra but complete resolution requires additional manipulation of data acquisition parameters such as excitation wavelength. An application using lifetime-resolved-acquisition scans can spectrally resolve tyrosine and tryptophan emission spectra from a protein containing both residues. To improve resolution, simply measure the lifetime and obtain the spectral characteristics of the individual components prior to conducting a lifetime-resolved acquisition scan. Generally, this technique works best if a factor of at least 1.5 exists between the lifetimes being resolved spectrally.

Anisotropy-decay acquisition

An *anisotropy-decay acquisition* experiment choice allows the study of rotational properties of fluorescent molecules and probes. As the fluorophore rotates, a change in the polarization occurs. Monitoring this change provides information about the excited state properties of the sample. The anisotropy is affected by Brownian rotation, energy transfer, re-absorption, re-emission and light scattering. Applications involve studying asymmetric complex molecules, environmental perturbations, binding, hindered-rotation phase transitions, and internal viscosities of bilayers.

Time-resolved acquisition

Time-resolved acquisition scans measure the change in the spectral characteristics of the sample during the lifetime of the excited state. The measurement consists of determining the frequency response of the sample over a specified emission range. Applications involve solvent relaxation of the excited state and excimer formation.

Useful materials for characterizing system and samples

The following are materials that HORIBA Scientific has found useful in determining system sensitivity or as standards for lifetime measurements.

Substance	CAS Number	Purpose	Emission Wavelength Range (nm)	Lifetime (ns)
Anthracene (99+%, zone-refined)	120-12-7	Excitation and emission spectral characterization	380–480	4.1 (in MeOH)
9-CA (97%), or 9-Anthracenecarbonitrile	1210-12-4	Single-exponential lifetime standard	380–500	11.8 (in MeOH)
Europium(III) chloride hexahydrate (99.9%)	13759-92-7	Phosphorescence emission and decay standard	580–700	1.40×10^5
Fluorescein (99%)	2321-07-5	Lifetime and sensitivity standard	490–630	4.02 (in pH ≥ 11)
D-glycogen	9005-79-2	Light-scattering standard		0
Laser glass, neodymium phosphate	--	Near-IR sensitivity standard	1042–1066	
LDS 750, or Styryl 7	114720-33-1	Single-exponential lifetime standard	680–700	0.248 (in MeOH, λ_{exc} = 568 nm)
Ludox®, or colloidal silica	7631-86-9	Light-scattering standard		0
$(Me)_2$POPOP, or 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene	3073-87-8	Single-exponential lifetime standard	390–560	1.45 (in EtOH)
β-NADH (β-nicotinamide adenine dinucleotide)	606-68-8 or 104809-32-7	Single-exponential lifetime standard	390–600	0.38 ± 0.05 (in pH = 7.5)
POPOP (99+%), or 1,4-bis(5-phenyloxazol-2-yl)] benzene	1806-34-4	Single-exponential lifetime standard	370–540	1.32 (in MeOH)
PPD (97%), or 2,5-diphenyl-1,3,4-oxadiazole	725-12-2	Single-exponential lifetime standard	310–440	1.20 (in EtOH)
PPO (99%), or 2,5-diphenyloxazole	92-71-7	Single-exponential lifetime standard	330–480	1.40 (in EtOH)
Rose Bengal (90%), or 4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein	632-69-9	Single-exponential lifetime standard	560–680	0.98 ± 0.10
p-Terphenyl (99+%)	92-94-4	Single-exponential lifetime standard	310–410	1.05 (in EtOH)
Water (18-MΩ, de-ionized, triple-distilled)	7732-18-5	water Raman sensitivity test		

13: Technical Specifications

Each NanoLog® system consists of:

- An excitation source
- An excitation spectrometer
- A sampling module with reference detector
- At least one emission spectrometer
- At least one emission detector.

Each system is controlled by an IBM-PC-compatible computer, and may include a printer for hard-copy documentation.

The details and specifications for each component of the NanoLog® series of spectrometers follow.

Spectrofluorometer system

NanoLog®

The NanoLog® spectrofluorometer consists of modules and components controlled by the specialized software. Although the system can be configured in various ways for a variety of applications, the basic (standard) NanoLog® spectrofluorometer system consists of the following components:

Excitation Source

450-W xenon short arc, mounted vertically in an air-cooled housing. Light collection and focusing by an off-axis mirror for maximum efficiency at all wavelengths.

Optional pulsed lamp or laser port interface available.

Excitation or emission monochromator

Single-grating excitation monochromator (standard). Excitation monochromator is *f*/3.6 Czerny-Turner design with kinematic classically-ruled gratings and all-reflective optics. Optional double-grating units are available for highest stray-light rejection and sensitivity.

The following specifications are based on 1200-grooves/mm gratings:

Resolution	**As good as 0.04 nm with iHR spectrograph and 1800 grooves/mm**
Accuracy	**±0.5 nm**
Step size	**0.0625 nm min. to 100 nm max.**
Wavelength repeatability	**0.3 nm**
Slits	**0–7 mm continuously adjustable via computer**
Speed	**150 nm/s**
Range	**0–1300 nm (physical)**
Gratings	
180F or 180DF	***330-nm blaze (220–600 nm range)*** ***500-nm blaze (290–850 nm range) for UV-visible photomultiplier tube***

Sample Module

T-format sample compartment with excitation reference detector R and signal detector S. The T-format design allows a second emission-detection channel T to be incorporated. The sample compartment also has a removable gap-bed assembly for sampling accessory replacement.

Optional front-face collection assembly available.

Emission spectrometer

iHR 320 triple-grating spectrometer (standard).

Focal length	0.32 m
Entrance-aperture ratio	*f*/4.1

Grating size 68 mm × 68 mm

Gratings ***800-nm blaze (600–1600 nm range) for InGaAs array, with 100 grooves/mm***
1200-nm blaze (800 – 2000 nm range) for InGaAs array, with 150 grooves/mm

Image magnification at exit 1.1

Scanning range 0–1500 nm (with 1200-grooves/mm grating); 0–12 000 nm (with 150-grooves/mm grating);

Multichannel coverage 62 nm over 26.7-mm array width (with 1200-grooves/mm grating); 545 nm with 150-grooves/mm grating

Focal plane 30 mm wide × 12 mm high

Spectral dispersion 2.31 nm/mm nominal (with 1200-grooves/mm grating); 20.4 nm/mm nominal (with 150-grooves/mm grating)

Spectral resolution 0.06 nm nominal (with 1200-grooves/mm grating), when scanning as a monochromator with 10 µm slit and single-channel detector

Wavelength accuracy ±0.20 nm

Wavelength repeatability ±0.075 nm

Step size 0.002 nm (with 1200-grooves/mm grating)

Dimensions 16.4″ long × 16.6″ wide × 7.56″ high; 41.7 cm long × 42.2 cm wide × 19.2 cm high

Optical axis height 3.88″; 9.84 cm

Weight 45 lb (20 kg)

Note: *For more information on the iHR spectrometer, see the System Performance chapter in the iHR User Manual.*

Lifetime options Time domain:
Lifetime range: 200 picoseconds to 0.1 milliseconds
Minimum resolution: < 7 picoseconds/channel

High voltage S or T detectors, ≤ 1200 V for R928P

Excitation shutter (standard) Computer-controlled

Integration time 1 ms to 160 s

Sensitivity Double-distilled de-ionized ICP-grade water Raman scan 6000:1 *S/N*

	at 397 nm, 5-nm bandpass, 1 s integration time, background noise first standard deviation at 450 nm.
Dimensions (sample compartment)	5.5″ long × 7″ wide × 7″ high 14 cm long × 17.8 cm wide × 17.8 cm high
Ambient temperature range	15–30°C
Relative humidity maximum	75%
Power requirements	1500 W 50/60 Hz single-phase

Minimum host-computer requirements

Microprocessor	Pentium V or higher recommended
Operating system and environment	Windows 2000, Windows® XP Pro, Windows® Vista, or Windows 7 (in compatibility mode)
Floppy drive	1.4 MB, 3½" floppy-disk drive
Hard disk	At least 1 GB of free storage
DVD-ROM drive	Required
Memory	1 GB RAM
Video display	Video resolution of at least 1280 × 1024
Keyboard	A 104-key keyboard, plus USB or PS/2 mouse
Available ports	Two free USB 2.0 ports

Software

FluorEssence™ software for data-acquisition and manipulation through the Windows® environment.

14: Components & Accessories

The NanoLog® can be configured to obtain optimum results for a variety of applications. The basic system, regardless of configuration, consists of slits, detectors, a xenon lamp with power supply, and a sampling module. These items can be combined in different ways to provide maximum benefits.

The following list represents all the accessories and components (including those mentioned above), in alphabetical order, available for the NanoLog® spectrofluorometers. A brief description of each is included in the following sections. Like the list presented below, the descriptions that follow are alphabetized, except where logical order dictates otherwise.

For additional information or product literature on any of these items, contact your local HORIBA Scientific Sales Representative.

Itemized list of Nanolog® accessories

FL-1013 Liquid Nitrogen Dewar Assembly

Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of cryogenic materials such as liquid nitrogen.*

For phosphorescence or delayed fluorescence measurements, samples are often frozen at liquid-nitrogen temperature (77 K) to preserve the fragile triplet state. The sample is placed in the quartz cell and slowly immersed in the liquid-nitrogen-filled Dewar flask. The white Teflon® cone in the bottom of the Dewar flask keeps the quartz sample-tube centered in the Dewar flask. The Teflon® cover on the top of the Dewar flask holds any excess liquid nitrogen that bubbled out of the assembly. A pedestal holds the Dewar flask in the sampling module. A special stove-pipe sample cover replaces the standard sample lid, so that liquid nitrogen can be added to the Dewar flask as needed. The Dewar flask holds liquid nitrogen for at least 30 min with minimal outside condensation and bubbling.

Included in the FL-1013 Liquid Nitrogen Dewar Assembly, the Dewar flask can be purchased as a spare. The bottom portion, which sits directly in the light path, is constructed of fused silica.

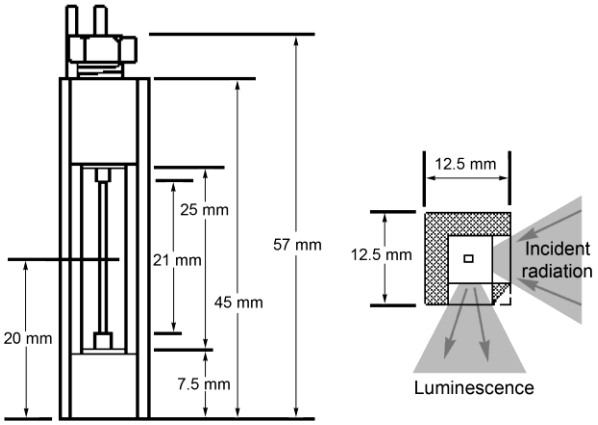
Note: *If condensation appears on the outside of the Dewar flask, it must be re-evacuated.*

FL-1013 Liquid Nitrogen Dewar Assembly.

Sample cells

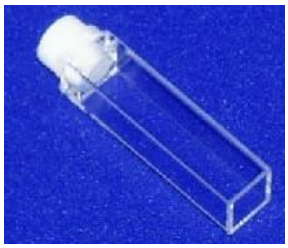
J1955 HPLC Flow Cell

With a sample capacity of 20 µL, this non-fluorescing fused silica cell is ideal for on-line monitoring of fluorescent samples. The cell maintains high sensitivity because it has a large aperture for collecting the excitation light to the sample and fluorescence emission from the sample. The flat sides allow maximum throughput while keeping the scattering of the incident radiation to a minimum. The cell fits in a standard cell holder.



J1925 Quartz Cuvette

With a 4-mL volume, this cell measures 10 mm × 10 mm in cross-section, and comes with a Teflon® stopper to contain volatile liquids.



J1920 Sample Cell

This 2-mL to 4-mL non-fluorescing fused silica cell, can accept a magnetic stirrer, has a 10-mm path length, and includes a white Teflon® cap that prevents sample evaporation.

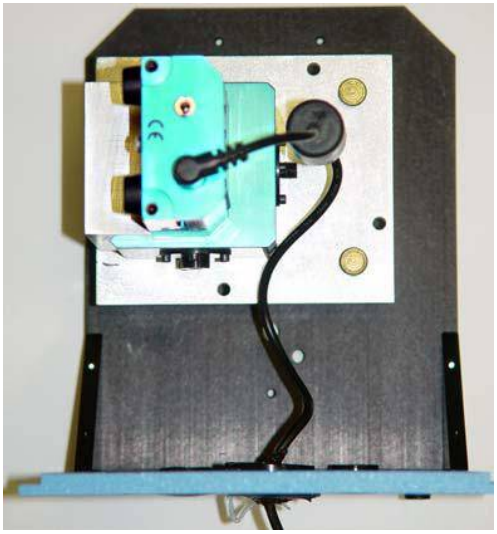
QC–SK Reduced Volume Sample Cell

This non-fluorescing fused silica cell is selected for samples with a maximum volume of 500 µL. The square cross-section of the sample cavity is 5 mm. The precise imaging capability of the excitation light focused onto the sample allows for high sensitivity. The adapter and a "flea" magnetic stirrer are included.

F-3026 Correction-Factor Kit

The F-3026 Correction-Factor Kit provides a convenient, complete correction factor kit that fits inside the sample compartment, and is used to generate radiometric emission correction factors for the NanoLog® system.

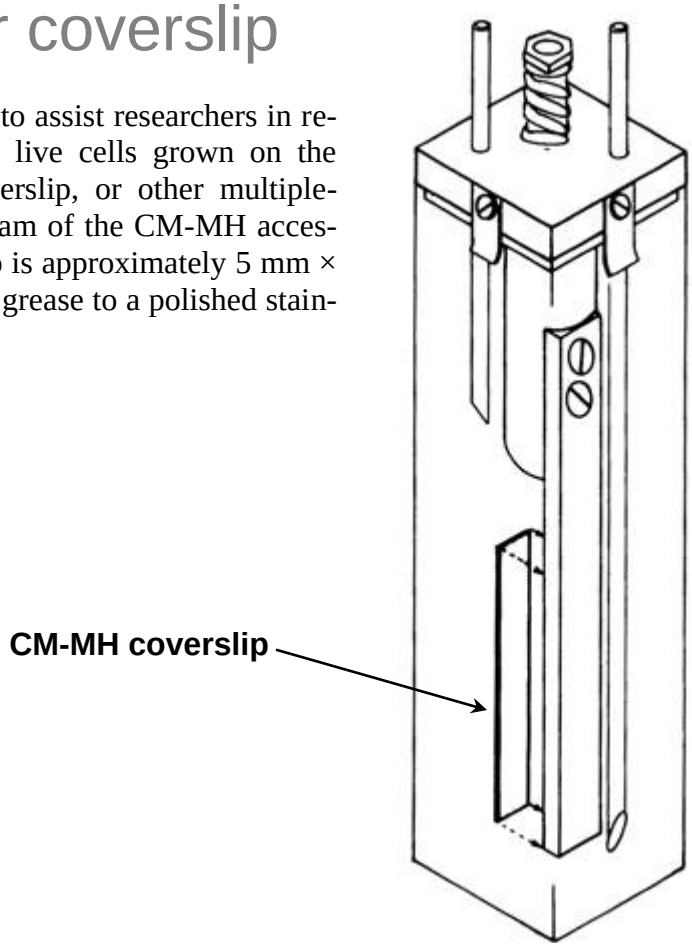
Emission correction factors compensate for the response of the photomultiplier detector as well as the wavelength dependency of the gratings in the emission spectrometer. Emission correction factors should be updated periodically and whenever different gratings or a new signal detector is installed.



F-3026 Correction-Factor Kit.

CM-MH Monolayer coverslip

The CM-MH system is designed to assist researchers in recording cation measurements of live cells grown on the special CM-MH monolayer coverslip, or other multiple-wavelength applications. A diagram of the CM-MH accessory is shown here. The coverslip is approximately 5 mm × 15 mm, and affixed with silicone grease to a polished stainless-steel jig within the CM-MH.



F-3023 Janis cryostat VNF-100

The F-3023 Janis cryostat VNF-100 is a liquid-nitrogen variable-temperature cryostat with the sample located in flowing vapor. Ideal for experiments with samples that are difficult to thermally anchor, e.g., liquids or powders, the F-3023 features a top-loading sample chamber for rapid sample-exchange, and four-way f = 1.0 optical access to the sample chamber. Among the cryostat's features are:

- Quick refill with included funnel assembly.
- High-quality bellows-sealed evacuation valve and a built-in cryopump for maintaining high vacuum.
- Safety pressure-relief valves protecting all independent spaces.
- Variable cooling system which places the sample in flowing N_2 vapor (ideal for low thermal conduction samples), providing excellent cooling power and temperature control.
- Light-weight sample positioner/mount assembly, with multi-pin electrical-feedthrough access to the window region, offering rotation and translation around the cryostat's axis.
- Two silicon diodes installed on the vaporizer (heat exchanger) and sample mount, for controlling and monitoring the system temperature.

Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of cryogenic materials such as liquid nitrogen.*

Multichannel detectors

For multichannel spectral acquisition, many charge-coupled devices and photodiode arrays are available to suit the researcher's needs. Both air-cooled and liquid-nitrogen-cooled CCDs can be inserted into the NanoLog®. Available options include extended-UV and near-IR detection, various pixel sizes and arrays, and maximum-coverage options. Contact a local Sales Representative for more details.

CCD detectors

Our multichannel CCD detectors are InGaAs liquid-nitrogen-cooled arrays.

FL-1095-06

This Symphony® array has a range of 1100–2200 nm.

FL-1094-03

This Symphony® array has enhanced sensitivity, with a range of 800–1700 nm.

Photodiode arrays

These photodiode arrays are matrices of 1024 × 256 pixels, with sensitivity from UV through near-IR (200–1000 nm).

Synapse OE

This array is thermoelectrically cooled.

Symphony® OE

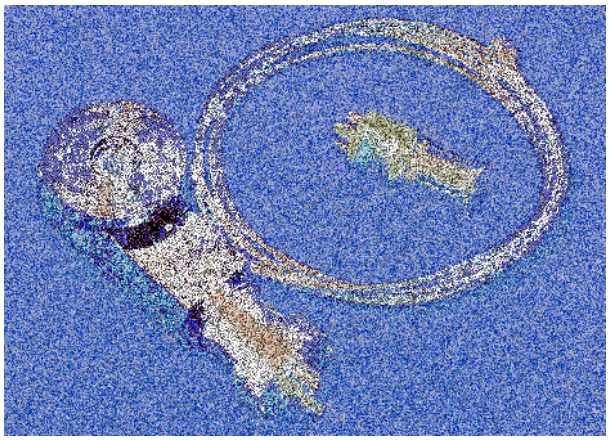
This array is liquid-nitrogen cooled.

Photomultipler tubes

Photomultiplier tubes are single-channel detectors.

FL-1073 visible room-temperature signal detector

One option for the NanoLog® is a room-temperature R928P emission signal detector, usually for the T-side on the sample compartment, and used as the T-channel. This detector is mounted to the emission spectrometer and operated in the photon-counting mode. Dark noise is <1000 counts/s, and the R928P delivers useful output from 190–860 nm.



Room-temperature R928P

As a rule, cooling a detector improves the *S*/*N* ratio by reducing the inherent dark count or noise. For the optional UV-visible signal detector, FL-1073, cooling reduces the dark count from 1000 cps to 20 cps; the useful wavelength band (190–860 nm) remains the same.

The Thermoelectrically Cooled Signal Detector consists of the housing, power supply, 1630 Field Lens Adapter, a black flange, and a silver adapter plate. To cool, a water line and drain are attached to the tubing extending from the housing, and room-temperature water is circulated through the housing.

Thermoelectrically-cooled R928P

FL-1030 thermoelectrically-cooled UV–near-IR photomultiplier tube

For spectral measurements extending into the near-infrared, the FL-1030 Thermoelectrically Cooled PMT is perfect. This detector uses the Hamamatsu R2658 photomultiplier tube. Included in the FL-1030 is the thermoelectrically cooled housing. The detector has a spectral range from 250 nm all the way to 1050 nm. Required for this item is the DM302 Photon Counting Module.

FL-1083 UV–near-IR detector

Our Hamamatsu 5509 photomultiplier tube is a UV-to-near-IR wide-band detector from 300–1700 nm. There are several versions available for the NanoLog®:

FL-1083
For use in standard emission spectrometers with power supply of 110 V 60 Hz.

FL-1083-2
For use in standard emission spectrometers with mains of 220 V 50 Hz.

FL-1083-iHR
For use with iHR spectrometers with power supply of 110 V 60 Hz.

FL-1083-iHR-2
For use with iHR spectrometers with mains of 220 V 50 Hz.

TBX UV-visible detectors

These detectors come in a small package, meaning a faster response, and may be used at room temperature or thermoelectrically cooled.

TBX-04
Response is 185–650 nm

TBX-07
Response is 250–850 nm

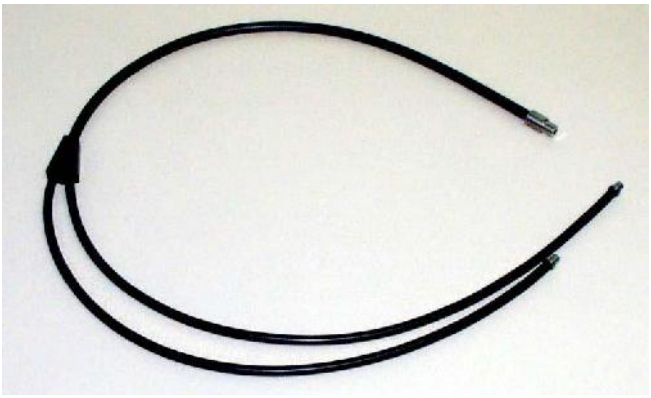
Near-IR detectors

FL-1092
This near-IR detector is based on the Hamamatsu H10330-45. It has a response from 950–1400 nm, and provides a higher signal than the FL-1081. There are two versions: The FL-1092 is for use with standard emission monochromators, while the FL-1092-iHR is designed for iHR emission spectrometers.

FL-1081
This near-IR detector is based on the Hamamatsu H10330-75. It gives lower signal than the FL-1092, but has a wider response: 950–1700 nm. There are two versions: The FL-1081 is for use with standard emission monochromators, while the FL-1081-iHR is for the iHR emission spectrometer.

F-3000 Fiber Optic Mount

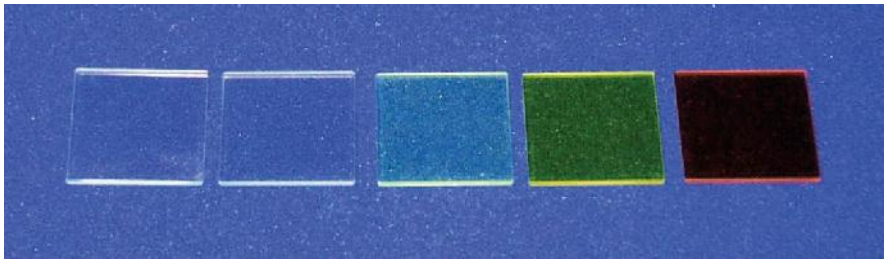
Now you can study marine environments, skin and hair, or other large samples *in situ*! For those users who want to examine samples unable to be inserted into the sample compartment, the F-3000 Fiber Optic Mount (plus fiber-optic bundles) allows remote sensing of fluorescence. The F-3000 couples to the sample compartment; light is focused from the excitation spectrometer onto the fiber-optic bundle, and then directed to the sample. Fluorescence emission from the sample is directed back through the bundle and into the front-face collection port in the sample compartment. Randomized fiber optic bundles ranging in length from 1 meter to 5 meters are available. Contact your local HORIBA Scientific Sales Representative for details.



F-3000 Fiber Optic Mount (above) and J1950 fiber-optic bundle (below).

Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*

J1939 Cut-On UV-Visible Filters

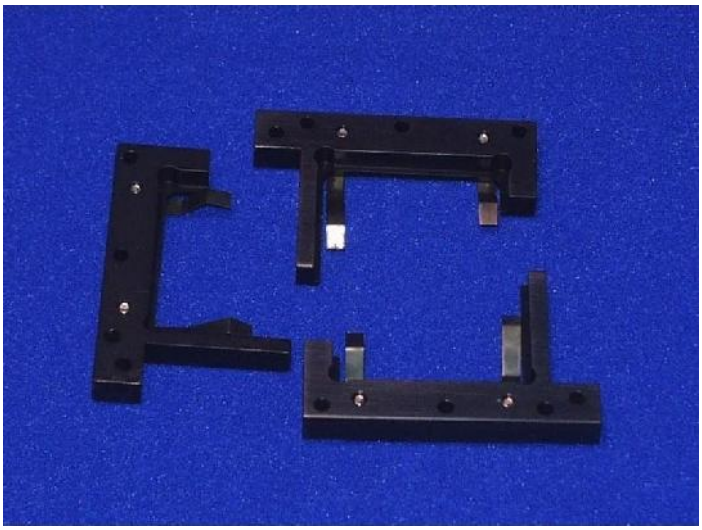


The J1939 Cut-On Filter set consists of five 2″ × 2″ filters with cut-on wavelengths of 350 nm, 399 nm, 450 nm, 500 nm, and 550 nm. To position the filter properly, the FL-1010 filter holder is required. The Nanolog®'s sample compartment has three slots to hold the FL-1010 filter holder in the emission and excitation light-path positions. One slot is in the excitation light path and the other two are the emission light path positions. To eliminate second-order effects from an excitation spectrum, install the filter holder and the appropriate cut-on filter in the excitation light path.

Cut-on filters eliminate second-order effects of the gratings. For example, if sample excitation is at 300 nm, a second-order peak occurs at 600 nm. If the emission spectrum extends from 400 nm to 650 nm, a sharp spike occurs at 600 nm. This peak is the second-order peak of the excitation monochromator. To remove this unwanted peak in the emission spectrum, place a 350 nm filter in the emission slot. Cut-on filters typically are used for phosphorescence measurements, where second-order effects are common.

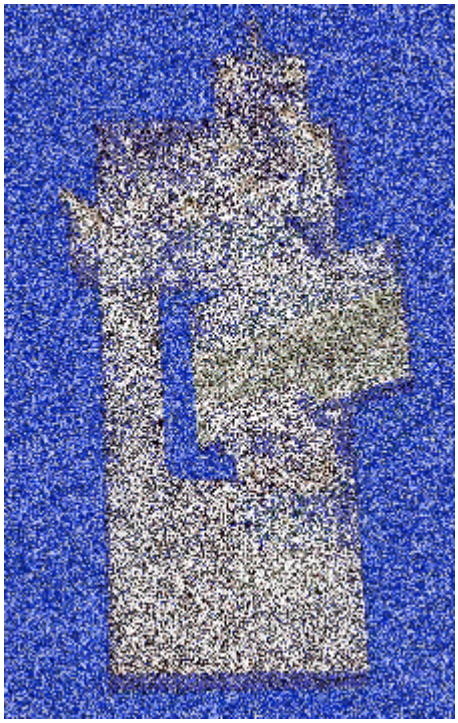
FL-1010 Cut-On Filter Holder

Cut-on filters are used to eliminate second-order effects of the gratings. The sample compartment has three slots that can hold the FL-1010 Filter Holder. Refer to J1939 Cut-On Filter for a detailed description of the placement of the filter holder and the interaction of the cut-on filters and the holder.



FL-1001 Front-Face Viewing Option

Designed to examine fluorescence from the surface of solid samples, the FL-1001 Front-Face Viewing Option includes a swing-away mirror. This allows the researcher to change from front-face and right-angle data collection instantly. In the front-face collection mode, the viewing angle is 22.5°. The FL-1001 is ideal for such samples as pellets, powders, inks, monolayers, dyes, and various solids.



FL-1001 Front-Face Viewing Option.

Gratings

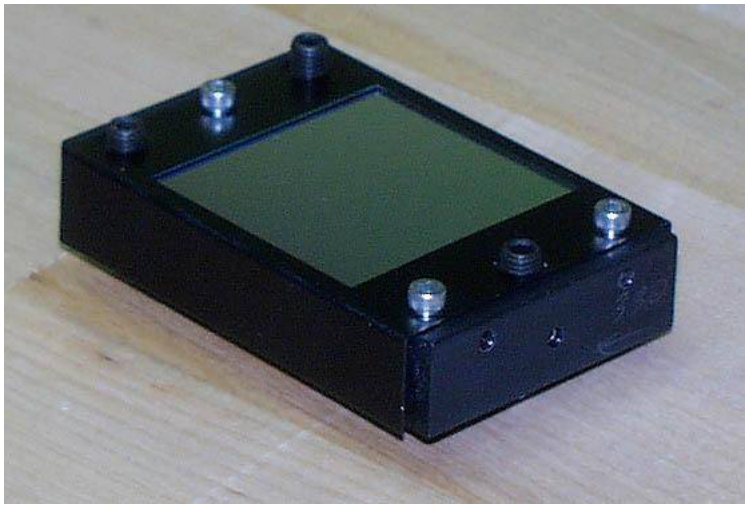
Many gratings are available to replace the standard model in the NanoLog®. Rulings with the following specifications are available:

- 100 grooves/mm
- 150 grooves/mm
- 300 grooves/mm
- 600 grooves/mm
- 1200 grooves/mm

In addition, a number of different blazes can be purchased:

- 250 nm
- 330 nm
- 500 nm
- 750 nm
- 800 nm
- 1000 nm
- 1200 nm

All gratings are classically ruled, and measure 50 mm × 50 mm. For details and model numbers, contact a HORIBA Scientific Sales Representative.

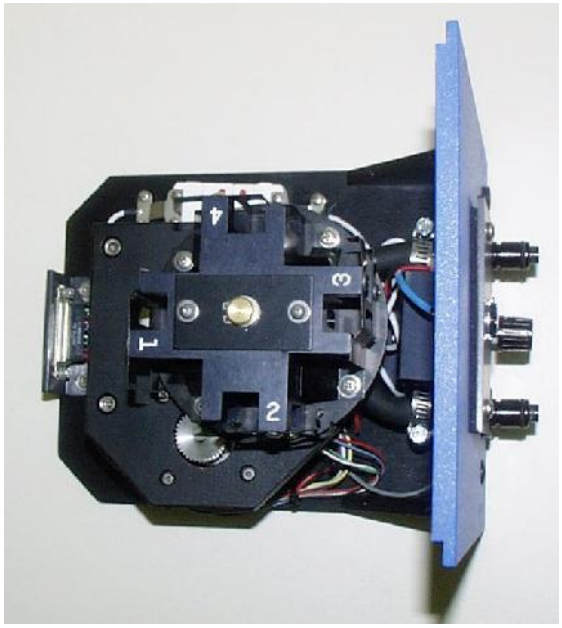


Grating.

FL-1011 Four-Position Thermostatted Cell Holder

The FL-1011 Four-Position Thermostatted Cell Holder keeps a sample at a constant temperature from –20°C to +80°C. The temperature is maintained by an ethylene-glycol–water mixture pumped through from an external circulating temperature bath (not included). The holder also includes a magnetic stirrer, for mixing turbid or viscous samples. Also required is the FM-2003 Sample Compartment Accessory.

FL-1011 Four-Position Thermostatted Cell Holder.



Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of an ethylene-glycol–water mixture.*

Installation

1. Remove the compartment gap-bed.
2. Position the FL-1011 gap-bed drawer.
3. Tighten with four screws.
4. Attach the ¼″ tubing to the brass inlets on the bottom of the holder.

Caution: *Failure to clamp these hoses securely may result in flooding and damage to the optics and electronics of the instrument.*

Use

1. Place the sample in a 10 mm × 10 mm cuvette and insert a magnetic stirring bar.

   (The stirring bar is available from Bel-Art Products, Pequannock, NJ)

2. Place a cuvette in each holder.

   Note: *While the four-position model maintains the temperature of all four samples, only one sample is mixed at a time.*

3. Allow the samples to reach the desired temperature.

4. Turn on the magnetic stirrer.

5. Select the appropriate mixing speed.

   The speed at which the sample should be mixed depends on the viscosity of the sample.

   Note: *Selecting too high a speed may create a vortex, which could affect the reproducibility of the measurement.*

6. Run your experiment as usual.

   Either right-angle or front-face detection can be used.

7. Place the next cuvette in the sample position by lifting up the knob and rotating the holder.

   Be sure to press down, to lock the cuvette into the proper position.

FL-1012 Dual-Position Thermostatted Cell Holder

The FL-1012 Dual-Position Thermostatted Cell Holder keeps a sample at a constant temperature from –20°C to +80°C. The temperature is maintained by an ethylene-glycol–water mixture pumped through from an external circulating temperature bath (not included). The holder also includes a magnetic stirrer, enabling mixing of turbid or viscous samples. Also required is the FM-2003 Sample Compartment Accessory.

FL-1011 Four-Position Thermostatted Cell Holder.

Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of an ethylene-glycol–water mixture.*

Installation

1. Remove the present holder from the posts.
2. Replace with the FL-1012.
3. Tighten the two thumbscrews
4. Attach the ¼″ tubing to the brass inlets on the bottom of the holder.

Warning: *Failure to clamp these hoses securely may result in flooding and damage to the optics and electronics of the instrument.*

Use

1. Place your sample in a 10 mm × 10 mm cuvette and insert a magnetic stirring bar.

   (The stirring bar is available from Bel-Art Products, Pequannock, NJ)

2. Place a cuvette in each holder.

   Note: *While the two-position model maintains the temperature of both samples, only one sample is mixed at a time.*

3. Allow the sample to reach the desired temperature.

4. Turn on the magnetic stirrer.

5. Select the appropriate speed.

   The speed at which the sample should be mixed depends on the viscosity of the sample.

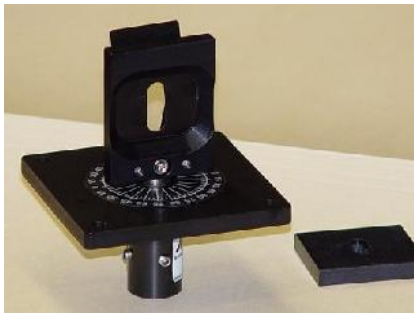
   Note: *Selecting too high a speed may create a vortex, which could affect the reproducibility of the measurement.*

6. Run your experiment as usual.

   Either right-angle or front-face detection can be used.

J1933 Solid Sample Holder

The J1933 Solid Sample Holder is designed for samples such as thin films, powders, pellets, microscope slides, and fibers. The holder consists of a base with graduated dial, upon which a bracket, a spring clip, and a sample block rest.



J1933 Solid Sample Holder with sample block nearby.

Installation

1. Remove the present holder.
2. Position the base on the posts.
3. Tighten the two thumbscrews.

For pellets, crystals, creams, gels, powders, and similar materials:

Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

1. Fill the well of the block.
2. Place a quartz coverslip or Teflon® film over the well.

   This holds the sample in place when vertically positioned.
3. Carefully insert the block between the bracket and spring clip, so that the sample is angled approximately 60° to the excitation light.

   This prevents reflections from entering the emission monochromator, and lets the fluorescence emission to be measured with minimal interference from scattered light.

For samples such as thin films, microscope slides, fibers, or other materials:

1 Place the material on the block on the side opposite that of the well.

Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

2 Carefully insert the block between the bracket and spring clip, so that the sample is angled approximately 60° to the excitation light.

This prevents reflections from entering the emission monochromator, and lets the fluorescence emission to be measured with minimal interference from scattered light.

F-3005/6 Autotitration Injector

For controlled, automatic injection of aliquots into the sample of your choice, the F-3005/6 Autotitration Injector is just the thing, available in both 110-V (F-3005) and 220-V (F-3006) models. The F-3005/6 comes with dual syringes, for complete control over dispensing and aspirating volumes of liquids into and out of the sample cell. A mix function is included. With the injector come 18-gauge Teflon® tubing and two syringes (1 mL and 250 µL). The syringes are interchangeable; aliquot size is controllable to 0.1% of total syringe volume.

F-3005/6 AutoTitrator Injector.

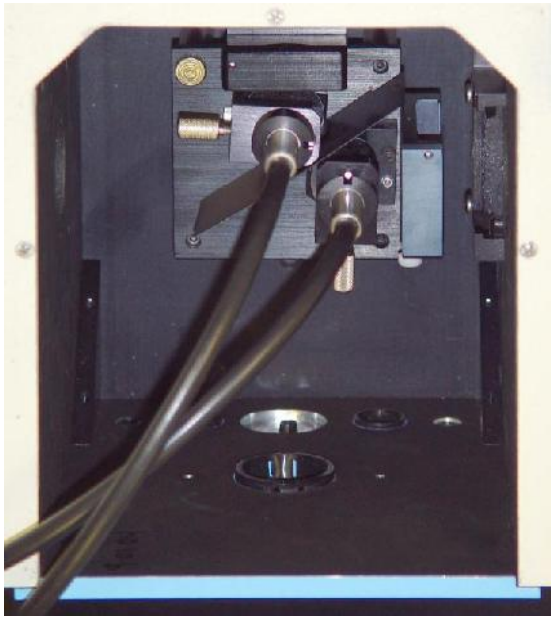
Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

Microscope Interface

The Microscope Interface eases the use of the NanoLog® system for fluorescence-microscopy measurements. The accessory includes fiber-optics to bring excitation light to the microscope's stage and emission light to the emission monochromator, plus a sample-compartment adapter to direct light in and out of the NanoLog®.

Microscope Interface

Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*



Fiber-optics in sample compartment

J1907 450-W Xenon Lamp

The J1907 450-W xenon lamp delivers light from 240 nm to 850 nm for sample excitation. The lamp has an approximate life of 2000 hours, and is ozone-free. The lamp is designed to fit into the FL-1039 Xenon Lamp Housing and the FL-1040 Dual Lamp Housing.

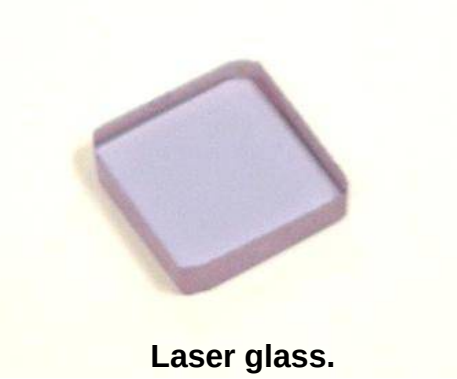
450-W xenon lamp, removed from the protective case.

Caution: *This lamp emits intense light and heat, and contains xenon gas under pressure. Understand all safety precautions before handling or using this xenon-arc lamp.*

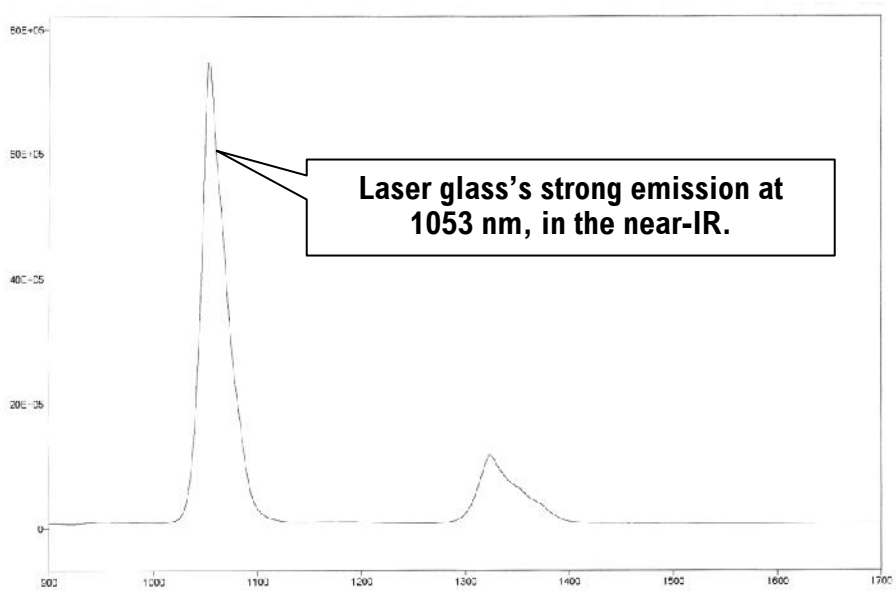
J650378 Laser Glass + RG830 Schott Filter

For calibration of the NanoLog®'s emission spectrometer, a special laser glass is necessary. The pale-violet laser glass, when excited in the visible around 575 nm, emits a sharp spectral peak near 1053 nm, suitable for calibration with a near-IR detector. An example of such an emission spectrum is shown below.

Along with the laser glass is included a Schott RG830 filter, which removes light shorter than 830 nm.



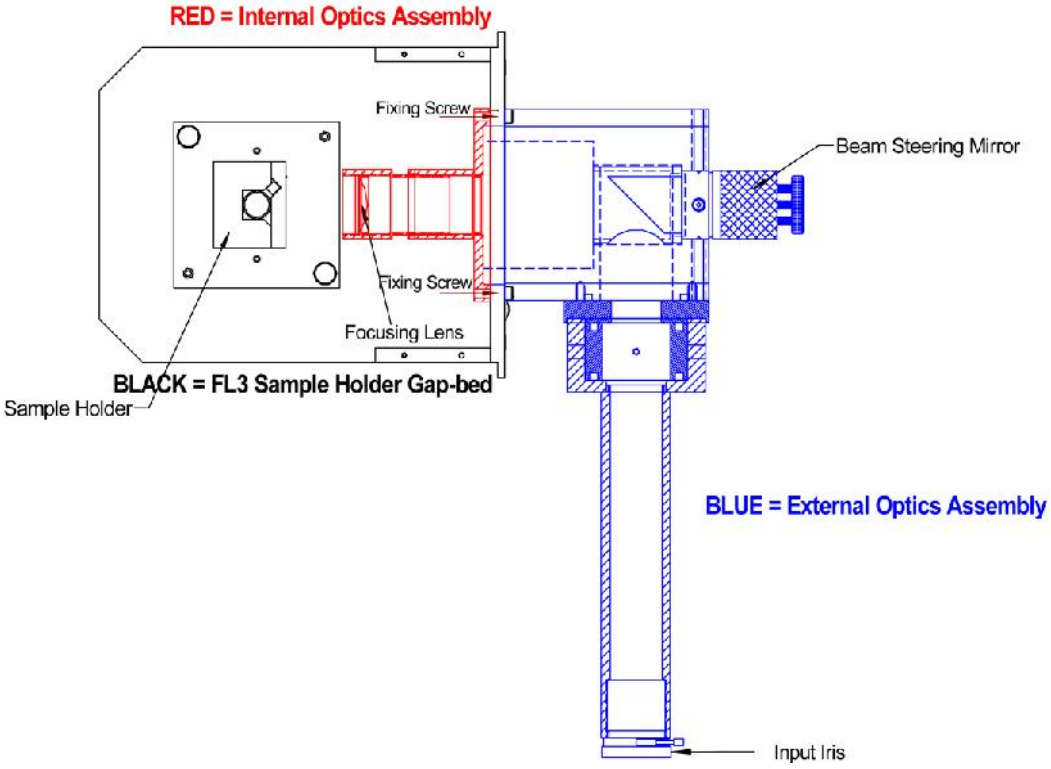
Laser glass.



Laser glass emission.

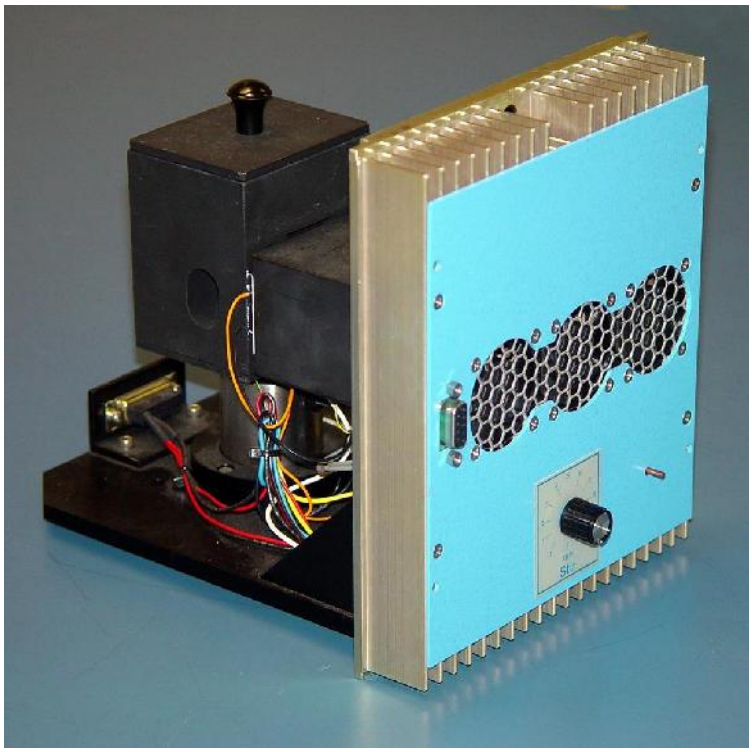
FC-OP-LIO1 and FC-OP-LIO2 Laser Input Optics accessories

For research and analysis using your own external mode-locked laser, such as a Ti:sapphire, add the Laser Input Optics to your NanoLog®. The standard FC-OP-LIO1 accessory includes input iris and tube to the Sample Compartment to exclude ambient light, a beam-steering mirror, a polarizer mount (polarizer not included), filter and lens mount for 1″ (2.5 cm) optics, beam-sampling plate, photodiator and discriminator for optical synchronization, and more. The anisotropy input accessory FC-OP-LIO2 enables you to perform anisotropy and polarization measurements, with the FCOP-LIO1. It includes a Babinet-Soleil compensator, and two extra prism polarizers in mounts. Contact your HORIBA Scientific Sales Representative for details. Note that the laser itself is not included in these accessories.



F-3004 Sample Heater/Cooler Peltier Thermocouple Drive

For rapid control of the sample's temperature in the NanoLog®'s sample compartment, choose the F-3004 Peltier Drive. Instead of messy fluids, the Peltier device heats and cools the sample thermoelectrically and fast! The temperature range is –10°C to +120°C. To prevent condensation of moisture on chilled cuvettes, an injection port for dry nitrogen gas is provided. All software is included, along with a controller and stirring mechanism.



F-3004 Sample Heater/Cooler Peltier Thermocouple Drive.

Peltier controller.

MicroMax 384 Microwell Plate Reader

The MicroMax 384 Microwell Titer-Plate Reader allows multiple samples to be scanned in one experiment. The MicroMax 384 is controlled through the host computer's software via a serial port to the host computer. The titer plate moves beneath a stationary optical beam, and fluorescence measurements are collected with top-reading geometry. Thus, any titer plates—even disposable ones—may be used. Up to 384-well plates may be inserted into the MicroMax 384, with a scan speed < 1 min for all plates. Various scan types are possible:

- Single-Point Analysis
- Emission
- Synchronous
- Excitation
- Time-Base
- Multigroup

Signals are transmitted between the NanoLog® and the MicroMax 384 via fiber-optic bundles.

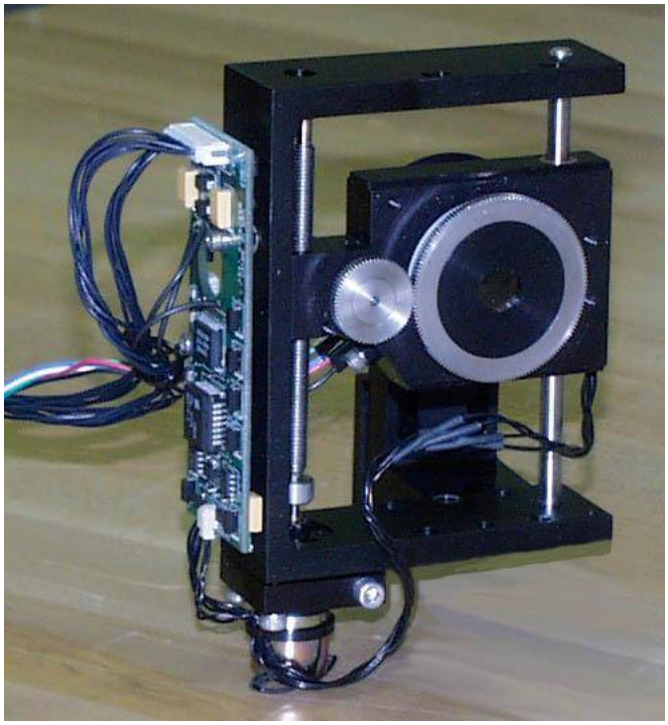
MicroMax 384 Microwell-Plate Reader.

Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*

Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

FL-1044 L-Format Polarizer & FL-1045 T-Format Polarizer

For L-format spectrofluorometers, the FL-1044 dual polarizer is ideal. The kit includes two polarizers, to be placed at the entrance and the exit of the T-box. The polarizers are fully automated, and are adjustable to within 1° rotation. Insertion and removal from the optical path is controlled by the computer. For T-format spectroscopy, researchers should order the FL-1045 third polarization unit in addition to the FL-1044.



Polarizer.

FL-1015 Injector Port

For the study of reaction kinetics, such as Ca^{2+} measurements, the FL-1015 Injector Port is ideal. This accessory allows additions of small volumes via a syringe or pipette to the sample cell without removing the lid of the sample compartment. With the injector in place, a lock-tight seal is achieved, prevented both light and air from reaching the sample. The Injector Port is recommended for use with the TRIG-15/25 External Trigger Accessory.

FL-1015 Injection Port.

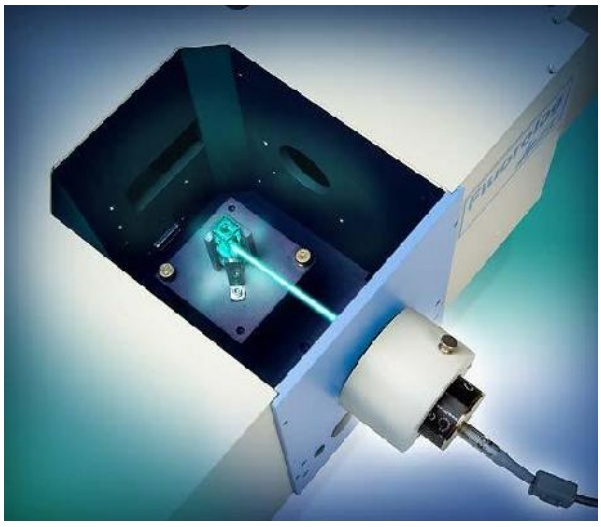
The Injector Port will accommodate most pipettes and syringes, with an injection-hole diameter of 0.125" (3.2 mm). A cap is included to cover the port when not in use.

Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

TCSPC upgrades FL-1054, FL-1057, FL-1065

FL-1054 Triple-Illuminator TCSPC upgrade.

Now you can have The World's Most Sensitive Spectrofluorometer you've come to expect in the NanoLog®, with the bonus of pico- and nanosecond lifetime capability. Time-correlated single-photon counting, or TCSPC, is perfect for dynamic anisotropy, TRES, and virtually any application requiring time-resolution, all with the ultimate, unrivaled sensitivity of digital photon-counting that strips away the noise, rather than adding noise to your signal the way an analog system does.



FL-1057 TCSPC upgrade for the sample compartment.

The pulsed sources used in the TCSPC upgrades are our NanoLED and SpectraLED solid-state pulsed diodes—which can be ordered from a full spectrum of wavelengths ranging from deep-UV to near-IR—our broad-band coaxial nanosecond flashlamp, and submicrosecond xenon spark-lamp. The Triple-Illuminator version (FL-1054) includes all steady-state or pulsed sources, switchable with the turn of a knob, attached to a special module. The economical NanoLED version (FL-1057) is a sample-drawer with pulsed NanoLED mounted on the sample compartment itself.

The FL-1065 is a new FL-1057 with a thermostatted sampling module.

The TCSPC upgrades for the NanoLog® include all electronics, a special sample compartment (for the FL-1057), and your choice of NanoLED or SpectraLED.

Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*

TCSPC Upgrades: FluoroHub

The controlling hardware for TCSPC lifetime acquisitions is the FluoroHub. The FluoroHub comes in two versions:

FluoroHub-A	For lifetimes > 200 ps
FluoroHub-B	For lifetimes > 60 ps

FluoroHub TCSPC controller.

F-1000/1 Temperature Bath

For studies of samples whose properties are temperature-dependent, use the F-1000/1 Temperature Bath. The controller circulates fluids externally, with tubes leading to the sample chamber. The temperature range is from –25°C to +150°C. Sensor and all cables are included with the F-1000/1. The Temperature Bath is available in a 110-V (F-1000) and 220-V (F-1001) version.

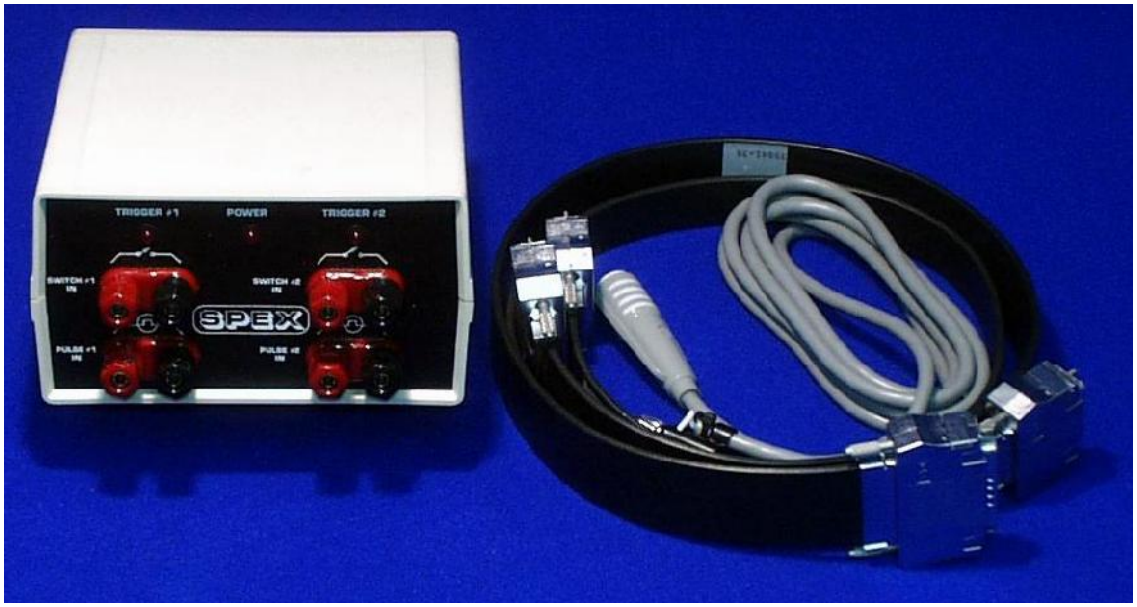
F-1000/1 Temperature Bath.

Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of an ethylene-glycol–water mixture.*

This instrument uses high-temperature fluids, which can cause severe burns.

TRIG-15/25 External Trigger Accessory

The TRIG-15/25 accessory permits the fluorescence system to be operated with almost any external trigger stimulus. Data acquisition can be synchronized with external events, either automatically following a voltage pulse (minimum 3 V above ground), or manually by pushing a button on a trigger-release cable. Multiple trigger events are recorded and stored with the associated data file. A TTL trigger output also is provided, for activating external devices, such as a stopped-flow unit. The front panel has four sets of banana-jack inputs for two independent trigger inputs, Trigger 1 and Trigger 2.



TRIG-15/25 External Trigger Accessory.

There are two sets of jacks for each of these two trigger inputs: an upper set, for manual switch inputs, and a lower set, for pulsed voltage inputs. These two input types can be used simultaneously, but any one event is ignored while the interface is activated by another.

FL-1024 Windows on the NanoLog® sample compartment

The FL-1024 windows are useful when the sample compartment contains a sample chilled with liquid-nitrogen, in order to prevent condensation on the optics. With the windows installed, the sample compartment can be purged with dry nitrogen gas.

Installing the windows

1 Prepare the instrument.

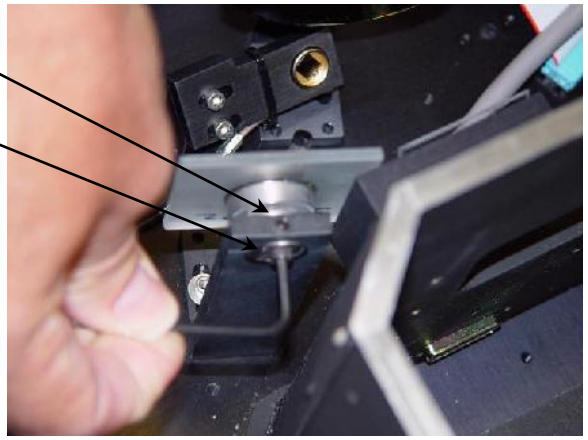
Caution: *Never touch any optical surfaces of the gratings, mirrors, etc.*

a Remove the instrument cover.

b Remove baffles.
Remove 2 cap screws holding each baffle in place.

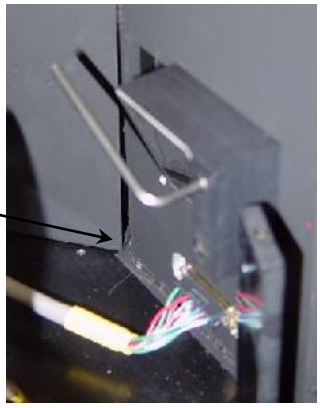
Note: *Remove the baffles for easier access.*

c Remove mirror from its mount.
Loosen set screw on top of mirror.
Remove cap screw from back of mirror.



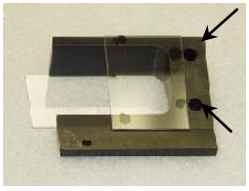
Note: *Remove the mirror temporarily because it is close to the beam-splitter.*

2 Replace the beam-splitter assembly.



a Remove old beam-splitter assembly from external wall of sample compartment.

b Insert two 6-32 × 3/8" cap screws in the side holes of the new beam-splitter assembly.

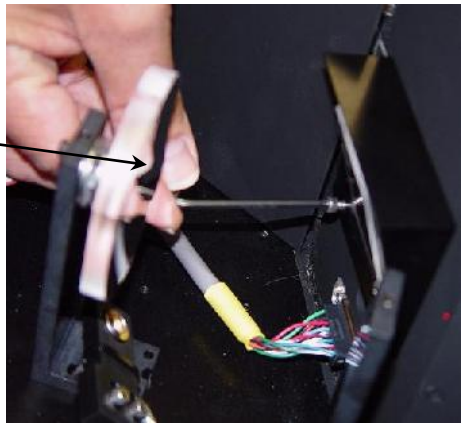


New beam-splitter assembly.

c Mount new assembly on external wall of sample compartment.

d Add third 6-32 × 3/8" cap screw to mount assembly on sample compartment.

e Tighten all 3 cap screws.



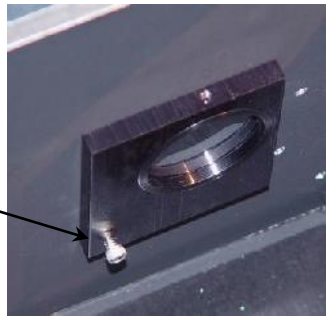
3 Insert window assembly.

a Snap one window assembly onto an external side-wall of sample compartment.

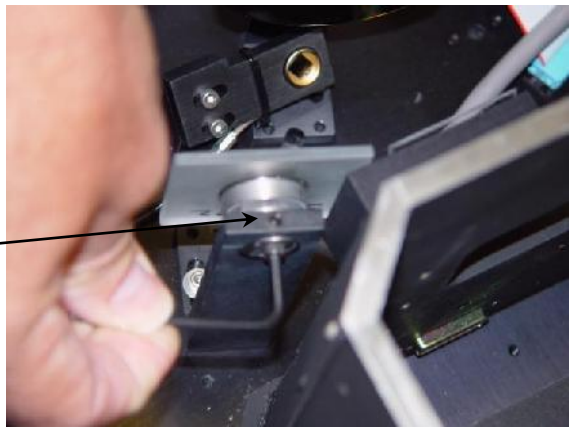
b Insert a 6-32 × 3/8" cap screw.

c Tighten the cap screw.

d Repeat steps (a) through (c) for the other window assembly.



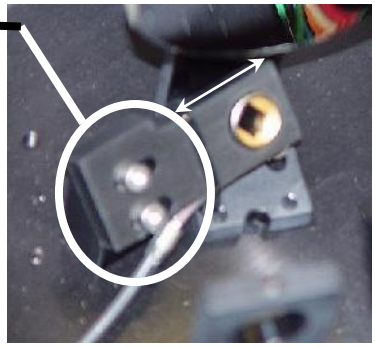
4 Replace mirror and baffles.



a Replace mirror on mount.
Replace cap screw on back of mirror.
Tighten set screw on top of mirror.

b Center the excitation beam's image on photodiode.

Loosen two cap screws.
Slide photodiode sideways in order to center image on it.
Tighten the two cap screws.

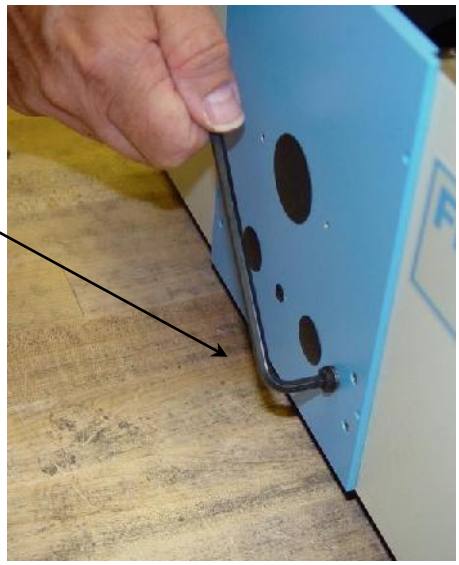


c Replace baffles using two cap screws for each.

5 Install purge port.

a Remove plastic original plug, using a 3/16" Allen key.

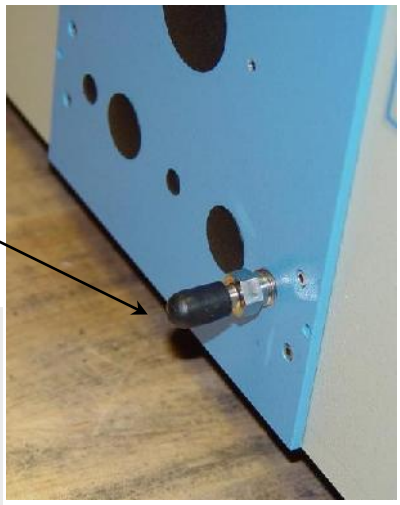
New purge port.



b Screw in manually the new metal purge port.

c Tighten with a 7/16" wrench.

d Remove the plastic cap whenever a hose is to be attached to the new purge port.



Note: *The plastic cap also prevents light from entering the sample compartment.*

6 Close the instrument's cover.

15: Glossary

3D excitation/emission display
This maps a specified emission-scan wavelength range using various excitation wavelengths.

3D synchronous/offset scan
A scan that maps a specified synchronous scan using various offset wavelengths between the spectrometers.

Absorption
Transition, when a photon enters a molecule, from the ground state to the excited singlet state. This process typically occurs in ~10^{-15} s.

Absorbance
The extent of absorption by a substance. Absorbance, A, is –log T, where T is the transmittance of the sample. Absorbance is also synonymous with optical density (OD). Absorbance can be calculated using the Beer-Lambert Law:

$$\text{OD} = A = \varepsilon cl = -\log T$$

ε = the extinction coefficient (M^{-1} cm^{-1})
c = sample concentration (M)
l = path length (cm)

Bandpass
The wavelength range of light passing through the excitation and emission spectrometers. The wider the bandpass, the higher the signal intensity.

Bandpass filter
Optical element that selectively transmits a narrow range of optical wavelengths.

Bioluminescence
Emission of light originating from a chemical reaction in a living organism.

Blaze wavelength
Wavelength at which a grating is optimized for efficiency. Generally, the gratings are efficient to 2/3 before the blaze wavelength to twice the blaze wavelength. The excitation and emission gratings are blazed in the UV and visible respectively.

Chemiluminescence
Emission of light originating from a chemical reaction.

Color effect for pulse technique
Time-dependent wavelength distribution of the lamp pulse.

Color effect for phase-modulation technique
Phase of the excitation and the degree to which it is modulated.

Corrected emission scan
An emission scan corrected for the wavelength characteristics of the emission spectrometer and the response of the signal detector. To obtain a corrected emission scan, an emission spectrum is multiplied by the emission correction factors. A set of emission correction factors is supplied with the instrument and stored as `mcorrect.spc` on the software disks. (Use `tcorrect.spc` for the T-side detector.)

Corrected excitation scan
An excitation scan corrected for the wavelength characteristics of the xenon lamp, the aging of the xenon lamp, and the gratings in the ex-

citation spectrometer. To obtain a corrected excitation scan, the detector signal is ratioed to the reference signal, which provides 90% of the corrected excitation scan. To obtain a completely correct scan, the excitation scan acquired in the manner described above is multiplied by correction factors. A set of excitation correction factors is included on the software disks.

Correction factors Compensates for the wavelength-dependent components of the system, like the xenon lamp, gratings, and signal detector. Emission and excitation correction factors are included with the software and are titled `xcorrect.spc` and `mcorrect.spc`.

Cut-on filter Optical component that passes light of a higher wavelength.

Cut-off filter Optical component that passes light of a lower wavelength.

Dark counts Inherent background signal of the photomultiplier when high voltage is applied. Cooling the detector decreases the dark counts.

Demodulation (less modulation) Usually refers to the demodulated emission relative to the excitation.

Demodulation factor Can be calculated using the following equation:

$$m = \frac{\mathrm{AC}_f / \mathrm{DC}_f}{\mathrm{AC}_x / \mathrm{DC}_x}$$

where AC_f is the amplitude of the emission voltage, DC_f is the excitation offset voltage, AC_x is the amplitude of the excitation voltage, and DC_x is the offset of the emission voltage.

Emission scan Shows the spectral distribution of light emitted by the sample. During an emission scan, the excitation spectrometer remains at a fixed wavelength while the emission spectrometer scans a selected region.

Energy transfer The transfer of the excited energy from a donor to an acceptor. The transfer occurs without the appearance of a photon and is primarily a result of dipole-dipole interactions between the donor and acceptor.

Excitation scan Shows the spectral distribution of light absorbed by the sample. To acquire an excitation scan, the excitation spectrum scans a selected spectral region while the emission spectrum remains at a fixed wavelength.

Extrinsic fluorescence Inherent fluorescence of probes used to study non-fluorescent molecules.

Flash lamp A lamp that provides pulsed-light output used to excite a sample. Can be either “free running” or “gated.”

Fluorescence The emission of light or other electromagnetic radiation during the transition of electrons from the excited singlet state to the ground state. Fluorescence typically occurs within about ~10^{-9} s.

Fluorescence lifetime The average length of time that a molecule remains in the excited

state before returning to the ground state.

Front-face detection A mode of detection in which fluorescence is collected off the front surface of the sample. Front-face detection usually is selected for samples such as powders, thin films, pellets, cells on a cover-slip, and solids.

Grating Optical element in the spectrometer, consisting of finely scribed grooves that disperse white light into a spectrum.

Intrinsic fluorescence The natural fluorescent properties of molecules.

Laser A monochromatic light source that provides high excitation intensity.

Mercury lamp Light source that emits discrete, narrow lines as opposed to a continuum. A mercury lamp can be used to check the spectrometer calibration.

Mirror image rule When the emission profile appears to be the mirror image of the absorption spectrum.

Modulated light Light whose intensity varies in a sinusoidal manner, with a specified frequency.

Optical density A synonym of *Absorbance*. See *Absorbance*.

Optical-density effects Fluorescence intensities are proportional to the concentration over a limited range of optical densities. High optical densities can distort the emission spectra as well as the apparent intensities.

Phase angle The delayed relationship between the modulated emission relative to the excitation.

Phase-modulation method A technique for measuring fluorescence lifetimes, where a sample is excited with light whose intensity is modulated sinusoidally. The emission is a forced response to the excitation.

Phosphorescence The emission of light or other electromagnetic radiation during the transition of electrons from the triplet state to the ground state. Phosphorescence is generally red-shifted relative to fluorescence and occurs within ~10^{-6} s to several seconds. To enhance phosphorescence detection, samples are often chilled to liquid-nitrogen temperature (77 K).

Pockels cell A light modulator that transmits ultraviolet and visible light, and can be operated at variable frequencies. Highly collimated excitation light is required.

Pulse-sampling method A technique for measuring fluorescence lifetimes, in which an initial population of fluorophores is excited by infinitely short pulses of light. An advantage of this technique is the direct recording of time-resolved emission spectra.

Raman scattering Scattering caused by vibrational and rotational transitions. Raman bands generally appear red-shifted relative to the incident electromagnetic radiation. The primary characteristic of Raman scatter is

that the difference in energy between the Raman peak and the incident radiation is constant in energy units (cm^{-1}).

Rayleigh scattering Light scattering from particles whose dimensions are much smaller than the wavelength of incident light. The scattered light is of the same energy as the incident light. Rayleigh scatter shows scatter radiation intensity inversely proportional to the 4^{th} power of the wavelength of incident radiation.

Rayleigh-Tyndall scattering Combination of Rayleigh and Tyndall scatter. These two scattering phenomena cannot be separated. If the molecule's Stokes shift is small, Rayleigh-Tyndall scatter will limit the ultimate resolution.

Red-sensitive photomultiplier A detector that extends fluorescence detection to 850 nm.

Reference photodiode Detector used to monitor the output of the xenon lamp.

Resolution The ability to separate two closely spaced peaks. Resolution can be improved by decreasing the bandpass and the increment (step size).

Right-angle detection Collection of fluorescence at 90° to the incident radiation. Right-angle detection typically is selected for dilute and clear solutions.

Scatter A combination of Raman, Rayleigh, and Rayleigh-Tyndall scattering, which can distort fluorescence spectra with respect to intensities and wavelengths.

Signal photomultiplier Detector used to measure excitation and fluorescence from the sample. It is operated in the photon-counting mode of detection to provide high sensitivity. Different detectors cover different wavelength regions.

Singlet state The spin-paired ground or excited state. The process of absorption generally produces the first excited singlet state that emits fluorescence, or undergoes intersystem crossing to form a triplet state.

Spectrometer The component in a fluorometer system that is scanned to provide the excitation and emission spectra. The spectrometer is chosen for stray-light rejection, resolution, and throughput.

Stokes shift Generally, the energy-difference between the absorption peak of lowest energy and the fluorescence peak of maximum energy.

Synchronous scan Scan that characterizes the overlap between the excitation and emission. The excitation and emission spectrometers are scanned at the same time, with a constant offset specified as either nanometers (wavelength units) or in cm^{-1} (energy units).

TCSPC **T**ime-**c**orrelated **s**ingle-**p**hoton **c**ounting. Technique in which the sample is excited by a pulsed source, and the sample's phosphorescence is collected over the course of many pulses. The arriving photons are timed after the excitation. Gradually a decay-curve is built up and the sample's fluorescent lifetime is calculated.

Time-base scan Scan in which the sample signal is monitored while both the excita-

	tion and the emission spectrometers remain at fixed wavelengths. Time-base data are used to monitor enzyme kinetics, dual wavelength measurements, and determine the reaction rate constant.
Time-resolved emission scan	Scan in which the emission spectra are acquired at various times after the excitation pulse. Provides insight into excited state reactions, charge-transfer-complex formation, solvent dipolar relaxation and other experiments.
Triplet state (T_1)	The spin-paired ground or excited state formed from the excited singlet state when paired electrons become unpaired. The triplet state emits phosphorescence.
Tyndall scattering	Scatter that occurs from small particles in colloidal suspensions.
Xenon lamp	Lamp that produces a continuum of light from the ultraviolet to the near-infrared for sample excitation.
Xenon-lamp scan	A profile of the lamp output as a function of wavelength. The lamp scan is acquired with the reference detector while scanning the excitation spectrometer. The maximum xenon-lamp peak at 467 nm can be used to determine proper calibration of the excitation spectrometer.

16: Bibliography

S.M. Bachilo, *et al.*, "Structure-Assigned Optical Spectra of Single-Walled Carbon Nanotubes," Science **298**, 2361–2366 (2002).

R.E. Bailey and S. Nie, "Alloyed Semiconductor Quantum Dots: Tuning the Optical Properties without Changing the Particle Size," *J. Am. Chem. Soc.* **125**, 7100–7106 (2003).

P.M. Bayley and R.E. Dale, *Spectroscopy and the Dynamics of Molecular Biological Systems*, Academic Press, London, 1985.

R.S. Becker, *Theory and Interpretation of Fluorescence and Phosphorescence*, Wiley-Interscience, New York, 1969.

I.B. Berlman, *Handbook of Fluorescence Spectra in Aromatic Molecules*, 2nd ed., Vols. I & II, Academic Press, New York, 1971.

C.R. Cantor and P.R. Schimmel, *Biophysical Chemistry: Techniques for the Study of Biological Structure and Function*, Vols. 1 & 2, W.H. Freeman, New York, 1980 & 1990.

M. Chalfie and S. Kain, *Green Fluorescent Protein: Properties, Applications, and Protocols (Methods of Biochemical Analysis)*, 2nd ed., Jossey-Bass, New York, 2005.

R.F. Chen, *et al., Biochemical Fluorescence: Concepts*, Vol. I & II, 1964 & 1970.

J.N. Demas, *Excited State Lifetime Measurements*, Academic Press, New York, 1983.

Enrico Gratton, David M. Jameson, and Robert D. Hall, "Multifrequency Phase and Modulation Fluorometry," *Ann. Rev. Biophys. Bioeng.* **13**, 105–124 (1984).

G.G. Guilbault, ed., *Fluorescence—Theory, Instrumentation and Practice*, Marcel Dekker, New York, 1967.

_____, *Practical Fluorescence: Theory, Methods and Techniques*, 2nd ed., Marcel Dekker, 1990.

_____, "Molecular Fluorescence Spectroscopy," *Anal. Chem.* **8**, 71–205 (1977).

D.M. Hercules, ed., *Fluorescence and Phosphorescence Analysis*, Wiley-Interscience, New York, 1966.

J.D. Ingle and S.R. Courch, *Spectrochemical Analysis*, Prentice-Hall, Englewood Cliffs, NJ, 1988.

A. Javier, *et al.*, "Nanosecond exciton recombination dynamics in colloidal CdSe quantum dots under ambient conditions," *App. Phys. Lett.* **83** (7), 1423–1425 (2003).

F.H. Johnson, *The Luminescence of Biological Systems*, Amer. Assoc. Adv. Sci., Washington, D.C., 1955.

J.H. Kim, D. Morikis, and M. Ozkan, "Adaptation of inorganic quantum dots for stable molecular beacons," *Sensors and Actuators B* **102**, 315–319 (2004).

S.V. Konev, *Fluorescence and Phosphorescence of Proteins and Nucleic Acids*, Plenum Press, New York, 1967.

M.A. Konstantinova-Schlezinger, ed. *Fluorometric Analysis*, Davis Publishing Co., New York, 1965.

Joseph R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Springer, New York, 2006.

_____, ed., *Topics in Fluorescence Spectroscopy*, Vols. 1–5, Plenum Press, New York, 1991–1998.

_____, Badri P. Melinal, Enrico Gratton, "Recent Developments in Frequency-Domain Fluorometry," *Anal. Instr.*, **14** (314), 193–223 (1985).

_____, S. Soper, and R. Thompson, *Advances in Fluorescence Sensing Technology IV*, SPIE Proc. Series, Vol. 3602 (1999).

W.T. Mason, ed., *Fluorescent and Luminescent Probes for Biological Activity: A Practical Guide to Technology for Quantitative Real-Time Analysis*, 2nd ed., Academic Press–Harcourt Brace & Co., 1999.

I.L. Medintz, *et al.*, "Self-assembled nanoscale biosensors based on quantum dot FRET donors," *Nature Materials* **2**, 630–638, (2003).

W.H. Melhuish and M. Zander, "Nomenclature, Symbols, Units and Their Usage in Spectrochemical Analysis VI: Molecular Luminescence Spectroscopy*," Pure App. Chem.*, **53**, 1953 (1981).

J.N. Miller, ed., *Standardization & Fluorescence Spectrometry: Techniques in Visible and Ultraviolet Spectrometry*, Vol. 2, Chapman and Hall, 1981.

Y. Miyauchi, *et al.*, "Fluorescence spectroscopy of single-walled carbon nanotubes synthesized from alcohol," *Chem. Phys. Lett.*, **387** (2004), 198–203.

V.C. Moore, *et al.*, "Individually Suspended Single-Walled Carbon Nanotubes in Various Surfactants," *Nano Lett.* **3** (10), 1379–1382 (2003).

M.J. O'Connell, *et al.*, "Band Gap Fluorescence from Individual Single-Walled Carbon Nanotubes," *Science* **297**, 593–596 (2002).

W.G. Richards and P.R. Scott, *Structure and Spectra of Molecules*, John Wiley & Sons, 1985.

A. Schillen, *et al.*, *Luminescence of Organic Substances*, Hellwege Verlag, Berlin, 1967.

S.G. Schulman, ed., *Molecular Luminescence Spectroscopy: Methods and Applications*, Vols. 1–3, Wiley-Interscience, New York, 1985–1993.

A. Sharma and S. Schulman, *Introduction to Fluorescence Spectroscopy*, Wiley Interscience, New York, 1999.

D.A. Skoog, F.J. Holler, and T.A. Nieman, *Principles of Instrumental Analysis*, 5th ed., Brooks Cole, New York, 1998.

N.J. Turro, V. Ramamurthy, and J.C. Scaiano, *Modern Molecular Photochemistry of Organic Molecules*, University Science Books, New York, 2006.

K. Van Dyke, *Bioluminescence and Chemiluminescence: Instruments and Applications*, Vol. 1, CRC Press, Boca Raton, FL, 1985.

T. Vo-Dinh, *Room Temperature Phosphorimetry for Chemical Analysis*, Wiley-Interscience, New York, 1984.

I.M. Warner and L.B. McGown, ed., *Advances in Multidimensional Luminescence*, Vols. 1 & 2, JAI Press, Greenwich, CT, 1991–1993.

E.L. Wehry, ed., *Modern Fluorescence Spectroscopy*, Vol. 1–4, Plenum Press, New York, 1975–1981.

C.E. White and R.J. Argauer, *Fluorescence Analysis: A Practical Approach*, Marcel Dekker, New York, 1970.

J.D. Winefordner, S.G. Schulman, and T.C. O'Haver, *Luminescence Spectrometry in Analytical Chemistry*, Wiley-Interscience, New York, 1972.

In addition, the following journals may prove useful:
Analytical Chemistry
Biophysics and Biochemistry
Journal of Fluorescence
Nano Letters

17 : CE Compliance Information

Declaration of Conformity

Manufacturer:	HORIBA Instruments Incorporated
Address:	3880 Park Avenue Edison, NJ 08820 USA
Product Name:	Fluorolog-3
Product Model Numbers:	FL3-11, FL3-12, FL3-21, FL3-22 FL3C-11, FL3C-12, FL3C-21, FL3C-22
Consisting of Combinations of the Following:	FL-1016 SpectrAcq Controller FL-1039/FL-1040 Illuminator Box FL-1005 Double Monochromator FL-1004 Single Monochromator FL-1073 Un-cooled PMT Assembly FL-1048/FL-1030 Cooled PMT Assemblies FL-1000 Sample Compartment FL-1011, FL-1012, FL-1041, 400313 Sample Drawers FL-1014 Sample Compartment Electronics DM302 Photon Count Module DM303/DM303M Current & Voltage Input Modules FL-1083-XXX, FL-1081, FL-1092 NIR PMT's FL-1096/220ASH Shutter Assemblies FL-1044/FL-1045 Auto Polarizers DSS-IGAXXXX DSS Detectors DSS-15V-TEP DSS Controller

Conforms to the following standards:

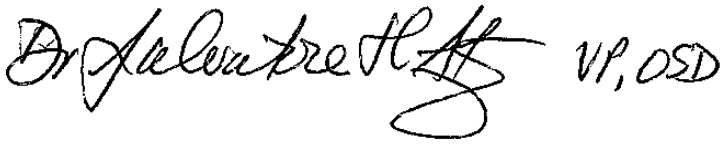
Safety:	EN 61010-1: 2001 EN 61010-1: 2001/AC: 2002
EMC:	EN 61326-1: 2006 (Emissions & Immunity)

Supplementary Information

The product herewith complies with the requirements of the Low Voltage Directive 2006/95/EC and the EMC Directive 2004/108/EC.

The CE marking has been affixed on the device according to Article 8 of the EMC Directive 2004/108/EC.

The technical file and other documentation are on file with HORIBA Instruments Incorporated.



Dr. Salvatore H. Atzeni
Vice-President, Retail Engineering, and CTO
HORIBA Instruments Incorporated
Edison, NJ 08820
USA
November 14, 2011

Applicable CE Compliance Tests and Standards

Test	Standards
Emissions, Radiated/Conducted	EN 55011: 2006
Radiated Immunity	IEC 61000-4-3: 2006
Conducted Immunity	IEC 61000-4-6: 2008
Electrical Fast Transients	IEC 61000-4-4: 2004
Electrostatic Discharge	IEC 61000-4-2: 2008
Voltage Interruptions	IEC 61000-4-11: 2004
Surge Immunity	IEC 61000-4-5: 2005
Magnetic Field Immunity	IEC 61000-4-8: 2009
Harmonics	IEC 61000-3-2: 2006
Flicker	IEC 61000-3-3: 2008
Safety	EN 61010-1: 2001
	EN 61010-1: 2001/AC: 2002

Declaration of Conformity

Manufacturer:	HORIBA Instruments Incorporated
Address:	3880 Park Avenue Edison, NJ 08820 USA
Product Name:	Symphony II CCD / IGA Detector System
Detector Model Numbers:	For CCD sensor: SII-XXX-XXX-XX For IGA sensor: SII-XXX-XXXX-XX SII-XXX-XXX-XX-XX
Power Supply Model Number:	355992

Conforms to the following Standards:

Safety:	**EN 60950-1:2006** **EN 60950-1:2006/A11:2009** **EN 60950-1:2006/A1:2010**
EMC Emissions:	**EN 55022:2006** **EN 55022:2006/A1:2007**
EMC Immunity:	**EN 55024:1998** **EN 55024:1998/A1:2001** **EN 55024:1998/A2:2003**

Supplementary Information

The product herewith complies with the requirements of the Low Voltage Directive 2006/95/EC and the EMC Directive 2004/108/EC.

The CE marking has been affixed on the device according to Article 8 of the EMC Directive 2004/108/EC.

The technical file and documentation are on file with HORIBA Instruments Incorporated.

Nicholas Vézard
Vice-President, OEM Division
HORIBA Instruments Incorporated
Edison, NJ 08820
USA
August 16, 2011

Applicable CE Compliance Tests and Standards

Tests	Standards
Radiated Emissions **Conducted Emissions** **Class A**	**EN 55022:2006** **EN 55022:2006/A1:2007**
Radiated Immunity	**IEC 61000-4-3:2006**
Conducted Immunity	**IEC 61000-4-6:2008**
Electrostatic Discharge	**IEC 61000-4-2:2008**
Voltage Dips & Interrupts	**IEC 61000-4-11:2004**
Electrical Fast Transients	**IEC 61000-4-4:2004**
Surge Immunity	**IEC 61000-4-5:2005**
Magnetic Field Immunity	**IEC 61000-4-8: 2009**
Harmonics	**EN 61000-3-2:2006**
Flicker	**EN 61000-3-3:2008**

Declaration of Conformity

Manufacturer:	HORIBA Instruments Incorporated
Address:	3880 Park Avenue Edison, NJ 08820 USA
Product Name:	iHR Imaging Spectrometer
Model Names:	iHR320, iHR550
Product Options:	All options and customized products based on the above.

Conforms to the following Standards:

Safety:	EN 61010-1: 2001 EN 61010-1: 2001/AC: 2002
EMC:	EN 61326-1: 2006 (Emissions & Immunity)

Supplementary Information

The product herewith complies with the requirements of the Low Voltage Directive 2006/95/EC and the EMC Directive 2004/108/EC.

The CE marking has been affixed on the device according to Article 8 of the EMC Directive 2004/108/EC.

The technical file and documentation are on file with HORIBA Instruments Incorporated.

_____

Salvatore H. Atzeni, Ph.D.
Vice-President, Retail Engineering, and CTO

HORIBA Instruments Incorporated
Edison, NJ 08820
USA
October 7, 2011

Applicable CE Compliance Tests and Standards

Test	Standards
Emissions, Radiated/Conducted	EN 55011: 2006
Radiated Immunity	IEC 61000-4-3: 2006
Conducted Immunity	IEC 61000-4-6: 2008
Electrical Fast Transients	IEC 61000-4-4: 2004
Electrostatic Discharge	IEC 61000-4-2: 2008
Voltage Interruptions	IEC 61000-4-11: 2004
Surge Immunity	IEC 61000-4-5: 2005
Magnetic Field Immunity	IEC 61000-4-8: 2009
Harmonics	IEC 61000-3-2: 2006
Flicker	IEC 61000-3-3: 2008
Safety	EN 61010-1: 2001
	EN 61010-1: 2001/AC: 2002

18: Index

Key to the entries:

Times New Roman font	subject or keyword
Arial font	command, menu choice, or data-entry field
Arial Condensed Bold font	dialog box
`Courier New` font	file name or extension

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[Design Concept]

The HORIBA Group application images are collaged in the overall design.
Beginning from a nano size element, the scale of the story develops all the way to the Earth with a gentle flow of the water.