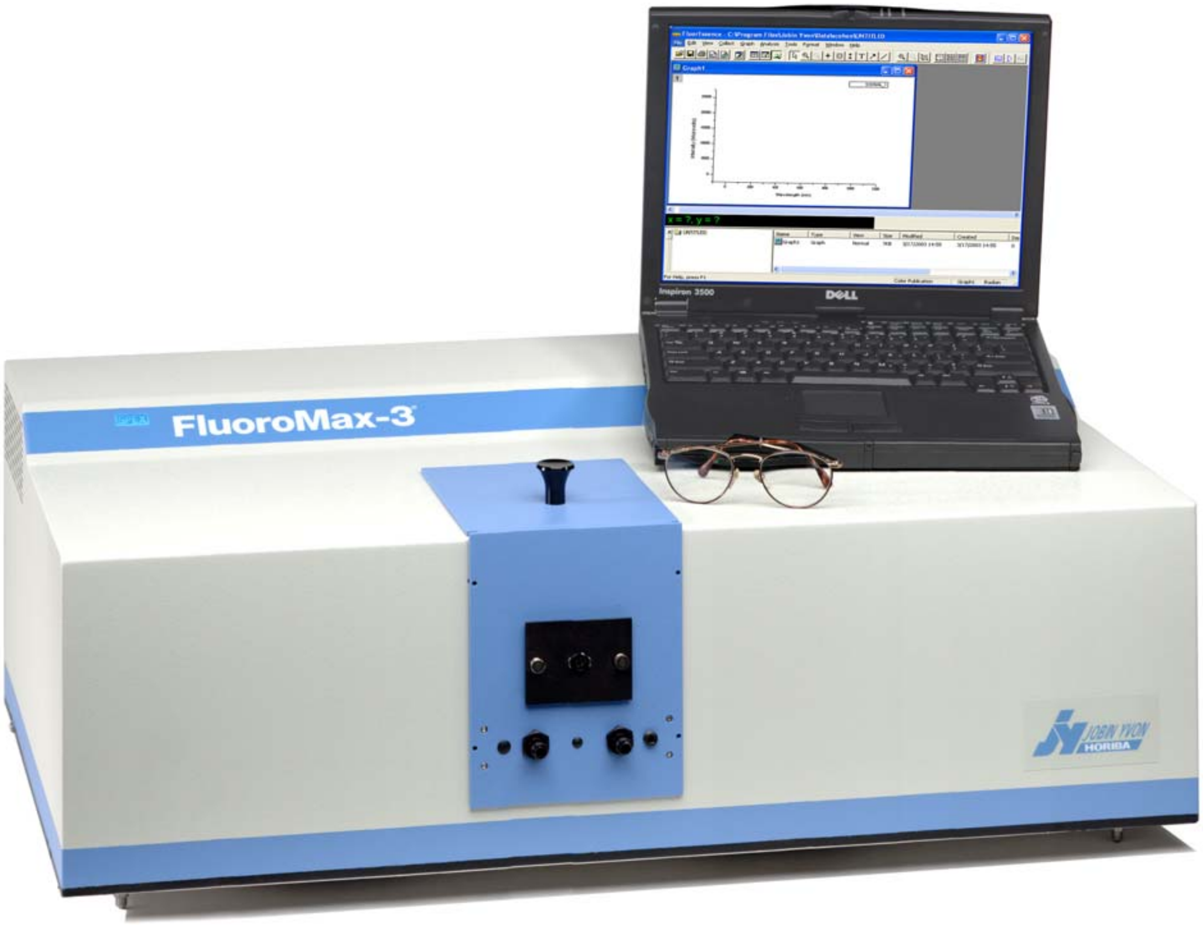
FluoroMax®-3 & FluoroMax®-P with FluorEssence™



Operation Manual

http://www.jobinyvon.com

Rev. 3.1

HORIBA JOBIN YVON

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April 2006

Part Number 81038

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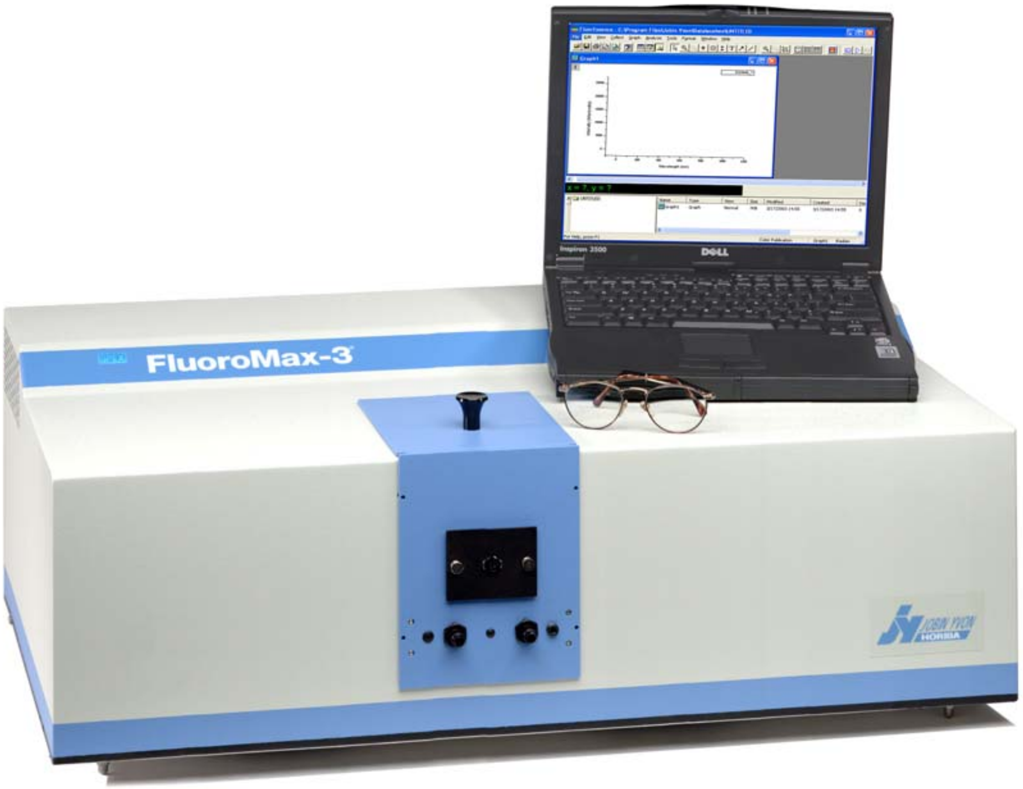
0: Introduction

About the FluoroMax®-3 and FluoroMax®-P



Both the FluoroMax®-3 and FluoroMax®-P are self-contained, fully automated spectrofluorometer systems. Data output is viewed on a PC, while printouts may be obtained via an optional plotter or printer. All FluoroMax®-3 and FluoroMax®-P functions are under the control of FluorEssence™ spectroscopy software. The main parts of the FluoroMax®-3 and FluoroMax®-P spectrofluorometer systems are:

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

The difference between the FluoroMax®-3 and FluoroMax®-P is that the FluoroMax®-P contains a phosphorimeter for phosphorescence measurements. This manual explains how to operate and maintain a FluoroMax®-3 and FluoroMax®-P spectrofluorometer. The manual also describes measurements and tests essential to obtain accurate data. For a complete discussion of the almost limitless power provided by FluorEssence™, refer to the *FluorEssence™ User's Guide* (especially regarding software installation) and the on-line help for Origin® and FluorEssence™, which accompany the system.

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	How the FluoroMax®-3 and -P work.
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 Acquisition	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 the system's sensitivity across the spectral range.
9: FluoroMax®-P Phosphorimeter Operation	Theory, operation, applications, and troubleshooting of the phosphorimeter, available on the FluoroMax®-P system.
10: Automated Polarizers	Installation, operation, and troubleshooting of the optional automated polarizers.
11: Technical Specifications	Instrument specifications and computer requirements.
12: Components & Accessories	Accessories available for the FluoroMax®-3, and how to use them.
13: Glossary	Some useful technical terms related to fluorescence spectroscopy.
14: Bibliography	Other important sources of information.
15: CE Declaration of Conformity	
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Disclaimer

By setting up or starting to use any HORIBA Jobin Yvon product, you are accepting the following terms:

You are responsible for understanding the information contained in this document. You should not rely on this information as absolute or all-encompassing; there may be local issues (in your environment) not addressed in this document that you may need to address, and there may be issues or procedures discussed that may not apply to your situation.

If you do not follow the instructions or procedures contained in this document, you are responsible for yourself and your actions and all resulting consequences. If you rely on the information contained in this document, you are responsible for:

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

As a condition of purchase, you agree to use safe operating procedures in the use of all products supplied by HORIBA Jobin Yvon, including those specified in the MSDS provided with any chemicals and all warning and cautionary notices, and to use all safety devices and guards when operating equipment. You agree to indemnify and hold HORIBA Jobin Yvon harmless from any liability or obligation arising from your use or misuse of any such products, including, without limitation, to persons injured directly or indirectly in connection with your use or operation of the products. The foregoing indemnification shall in no event be deemed to have expanded HORIBA Jobin Yvon's liability for the products.

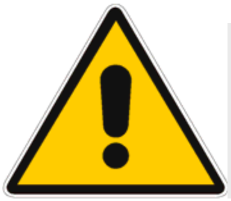
HORIBA Jobin Yvon products are not intended for any general cosmetic, drug, food, or household application, but may be used for analytical measurements or research in these fields. A condition of HORIBA Jobin Yvon's acceptance of a purchase order is that only qualified individuals, trained and familiar with procedures suitable for the products ordered, will handle them. Training and maintenance procedures may be purchased from HORIBA Jobin Yvon at an additional cost. HORIBA Jobin Yvon cannot be held responsible for actions your employer or contractor may take without proper training.

Due to HORIBA Jobin Yvon's efforts to continuously improve our products, all specifications, dimensions, internal workings, and operating procedures are subject to change without notice. All specifications and measurements are approximate, based on a standard configuration; results may vary with the application and environment. Any software manufactured by HORIBA Jobin Yvon is also under constant development and subject to change without notice.

Any warranties and remedies with respect to our products are limited to those provided in writing as to a particular product. In no event shall HORIBA Jobin Yvon be held liable for any special, incidental, indirect or consequential damages of any kind, or any damages whatsoever resulting from loss of use, loss of data, or loss of profits, arising out of or in connection with our products or the use or possession thereof. HORIBA Jobin Yvon is also in no event liable for damages on any theory of liability arising out of, or in connection with, the use or performance of our hardware or software, regardless of whether you have been advised of the possibility of damage.

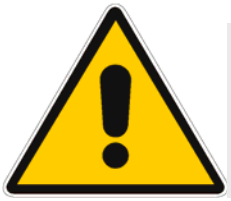
Safety summary

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 Jobin Yvon 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:



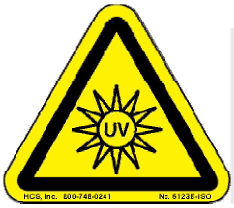
Warning:

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 Jobin Yvon Inc. is not responsible for damage arising out of improper use of the equipment.



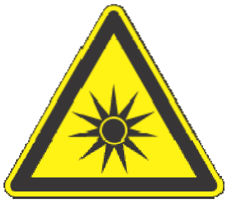
Caution:

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 Jobin Yvon Inc. is not responsible for damage arising out of improper use of the equipment.



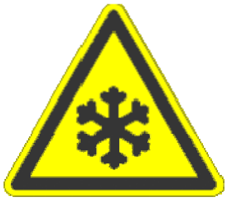
Caution:

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



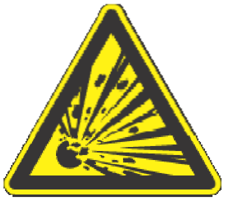
Caution:

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.



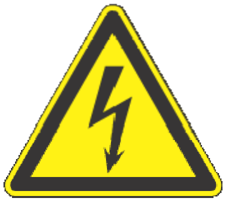
Caution:

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



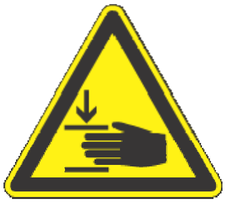
Warning:

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



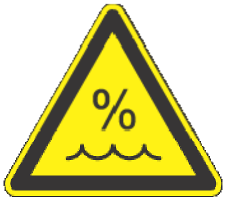
Warning:

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



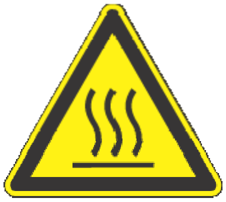
Warning:

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.



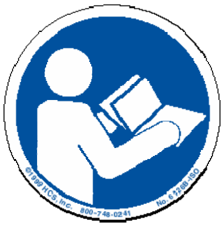
Caution:

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

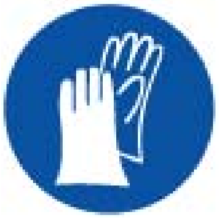


Warning:

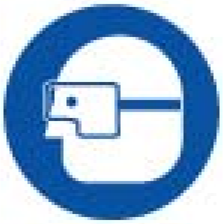
Hot! This symbol warns the user that hot equipment may be present, and could create a risk of fire or burns.



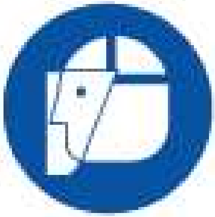
Read this manual before using or servicing the instrument.



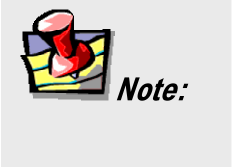
Wear protective gloves.



Wear appropriate safety goggles to protect the eyes.

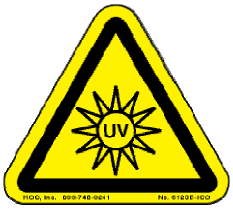


Wear an appropriate face-shield to protect the face.



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*

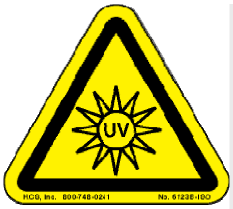
Do not aim the UV light at anyone.
Do not look directly into the light.
Always wear protective goggles, full-face shield and skin protection clothing and gloves when using the light source.

- Light is subdivided into visible light, ranging from 400 nm (violet) to 700 nm (red); longer infrared, “above red” or > 700nm, also called heat; and shorter ultraviolet radiation (UVR), “below violet” or < 400nm. 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!



Warning: *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 International 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 "glass-blowers' " 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

Safety-training requirements

Every user of the FluoroMax®-3 and FluoroMax®-P must know general and specific safety procedures before operating the instrument. For example, proper training includes (but is not limited to):

- Understanding the risks of exposure to ultraviolet, visible, and infrared light, and how to avoid unsafe exposures to these types of radiation
- Handling xenon-lamp bulbs, and their dangers
- Safe handling for all chemicals and other samples used in the instrument

Safety-training may be purchased from HORIBA Jobin Yvon. Contact your Spex® Fluorescence Representative or the Fluorescence Service Department for details.

Surface requirements

- A sturdy table- or bench-top.
- Surface must hold 90 kg (200 lbs.).
- Surface should be about 27" × 72" (69 cm × 183 cm) to hold spectrofluorometer, computer, and accessories comfortably.
- Overhead clearance should be at least 36" (91 cm).

Environmental requirements

- Temperature 59–86°F (15–30°C)
- Maximum temperature fluctuation ± 2°C
- Ambient relative humidity < 75%

Caution: *Excessive humidity can damage the optics.*

- Low dust levels
- No special ventilation

Warning: *For adequate cooling, do not cover, block, or obstruct the vents on the left side and underside of the instrument.*

Electrical requirements

- 110 VAC ± 5%, 60 Hz; or 220 VAC ± 5%, 50 Hz
- Have enough outlets available for:
  - Host computer (PC)
  - Monitor
  - Optional printer
  - FluoroMax$^{®}$-3
  - Each of certain accessories, such the MicroMax, temperature bath, etc.

Warning: *HORIBA Jobin Yvon Inc. is not liable for damage from line surges and voltage fluctuations. A surge protector is strongly recommended for minor power fluctuations. For more severe voltage variations, use a generator or uninterruptible power supply.* ***Improper line voltages can damage the equipment severely.***

Warning: *The FluoroMax$^{®}$-3 is equipped with a three-conductor power cord that is connected to the system frame (earth) ground. This ground provides a return path for fault current from equipment malfunction or external faults. For all instruments, ground continuity is required for safe operation. Any discontinuity in the ground line can make the instrument unsafe for use.* ***Do not operate this system from an ungrounded source.***

Note: *HORIBA Jobin Yvon Inc. recommends connecting the host computer, monitor, and printer to a single surge-protector, to make start-up more convenient, and to conserve AC outlets. Connect the FluoroMax$^{®}$-3 to a separate line, if possible, to isolate the xenon-lamp power supply inside the FluoroMax$^{®}$-3.*

Unpacking and installation

Introduction

The FluoroMax®-3 spectrofluorometer system is delivered in a single packing carton. If a host computer (PC) is ordered as a part of the system, the PC is delivered in a few clearly labeled boxes. All accessories, cables, software, and manuals ordered with the system are included with the delivery.

Examine the shipping boxes carefully. Any evidence of damage should be noted on the delivery receipt and signed by representatives of the receiving and carrier companies. Once a location has been chosen, unpack and assemble the equipment as described below. To avoid excessive moving and handling, the equipment should be unpacked as close as possible to the selected location.

Note: *Many public carriers will not recognize a claim for concealed damage if it is reported later than 15 days after delivery. In case of a claim, inspection by an agent of the carrier is required. For this reason, the original packing material should be retained as evidence of alleged mishandling or abuse. While HORIBA Jobin Yvon Inc. assumes no responsibility for damage occurring during transit, the company will make every effort to aid and advise.*

Caution: *The spectrofluorometer system is a delicate instrument. Mishandling may seriously damage its components.*

FluoroMax®-3 carton contents

Quantity	Item	Part number
1	FluoroMax®-3	
1	Null modem communications cable	400144
1	*FluoroMax®-3 Operation Manual*	81038
1	Laminated safety summary card	81106
1	Set of Allen wrenches	53057
1	Single-cell sample-holder	351697
1	Power cord (110 V)	98015
	(220 V)	98020
1	FluorEssence™ software package	

1 Unpack and set up the FluoroMax®.

a Carefully open the FluoroMax®-3 shipping carton.

b Remove the foam-injected top piece and any other shipping restraints in the carton.

c With assistance, carefully lift the instrument from the carton, and rest it on the side of the laboratory bench where the system will stay.

Warning: *Watch your fingers!*

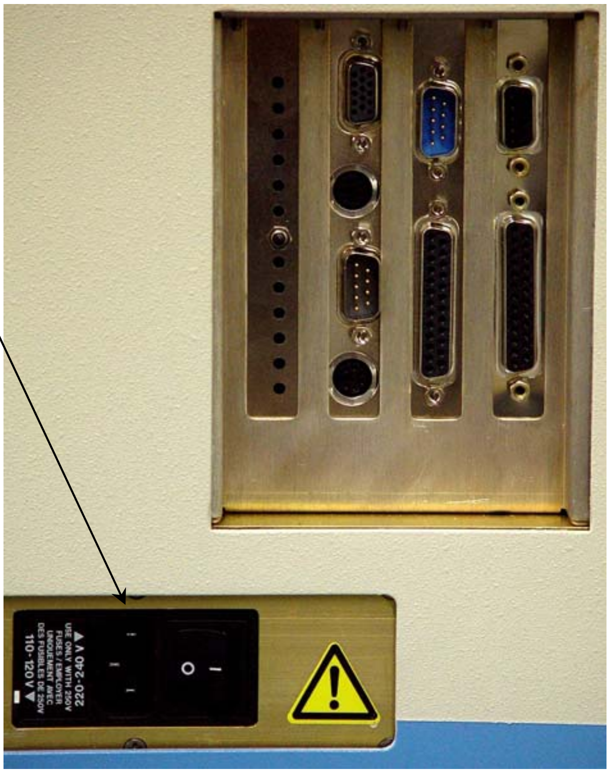
d Place the instrument in its permanent location.

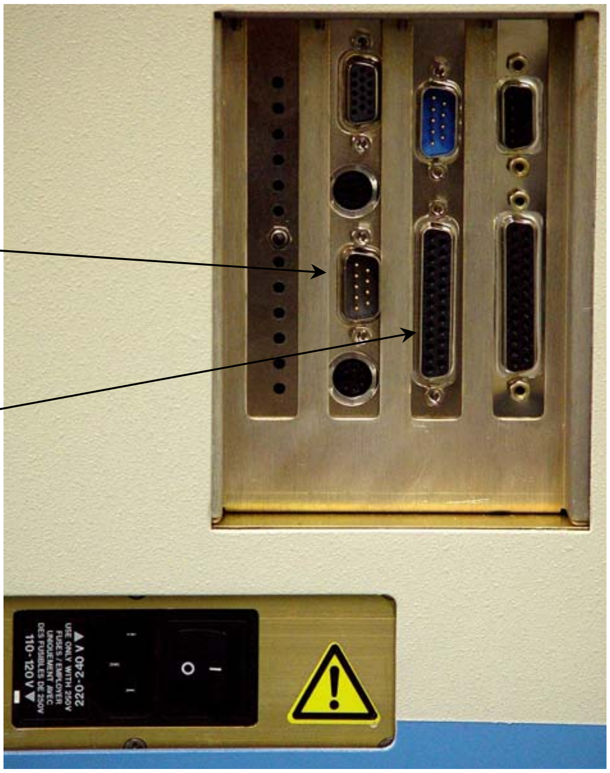
e Level the spectrofluorometer.
Adjust the four leveling feet on the bottom of the instrument.

f Inspect for previously hidden damage.
Notify the carrier and HORIBA Jobin Yvon Inc. if any is found.

g Check the packing list to verify that all components and accessories are present.

h Plug one end of the power cord into the proper receptacle on the left side (while facing the unit) of the spectrofluorometer.




220-240 V
USE ONLY WITH 250V FUSES / EMPLOYER UNIQUEMENT AVEC DES FUSIBLES DE 250V
110-120 V

i Plug one end of the 9-pin communications cable into this 9-pin connector (COM1) on the FluoroMax®-3's right panel.

j With an optional trigger accessory, plug one end of the trigger cable into this 25-pin connector on the FluoroMax®-3.

Allow the unconnected ends of the cables to dangle freely; they will be connected in later steps.

2 Set up the computer.

The information gathered by the spectrofluorometer system is displayed and controlled through the host PC via FluorEssence™ software. The host PC may be purchased from HORIBA Jobin Yvon Inc. or another supplier.

a Set up the host PC reasonably close to the FluoroMax®-3 system. The limitation is the length of the null modem communications cable. The recommended location for the PC is just to the right of the spectrofluorometer, but other positions are possible.

b Follow the instructions for the host PC to set up the computer system, including the CPU, monitor, keyboard, mouse, speakers, printers, etc.

3 Connect the FluoroMax® to the computer.

a Attach the free end of the communication cable to COM1 (first serial port) of the computer.

If COM1 is not available, then use an unused serial port (COM) on the host computer. If only a 25-pin connector is available, use a standard 25-pin–9-pin converter, or contact Spex® Fluorescence Service for assistance. During software installation, the serial port setting used on the host computer to communicate with the FluoroMax®-3 is entered.

b With all devices OFF, plug the power cords from the monitor, computer, FluoroMax®, and the printer into properly grounded receptacles.

c If a Trigger-box accessory is included, attach the free end of the Trigger-box cable to the Trigger box.

d Install any accessories that arrived with the system, using the instructions that accompany the accessories.

See Chapter 12 for a detailed list of accessories.

4 Install the FluorEssence™ software.

The spectrofluorometer system is controlled by FluorEssence™ spectroscopy software operating within the Windows™ environment. If the computer and software were purchased from HORIBA Jobin Yvon Inc., the software installation is complete. If the computer is not from HORIBA Jobin Yvon Inc., perform the installation. Contact a HORIBA Jobin Yvon Inc. Sales Representative for recommended specifications for a suitable host computer.

Before the FluorEssence™ software can be installed, however, Windows™ must be installed already and operating properly. Refer to the Windows™ manual that came with the computer for installation instructions.

The FluorEssence™ software is supplied on one CD-ROM. Follow the *FluorEssence™ User's Guide* for details on installation.

Note: *Be sure to agree to the terms of the software license before using the software.*

Users outside of the USA:

Users outside of the USA receive a softkey device that connects to the printer port of the host computer for software security. The softkey should be left in place on the host computer at all times.

Note: *Copying, disassembly, or removal of the softkey is illegal.*

Software emulation

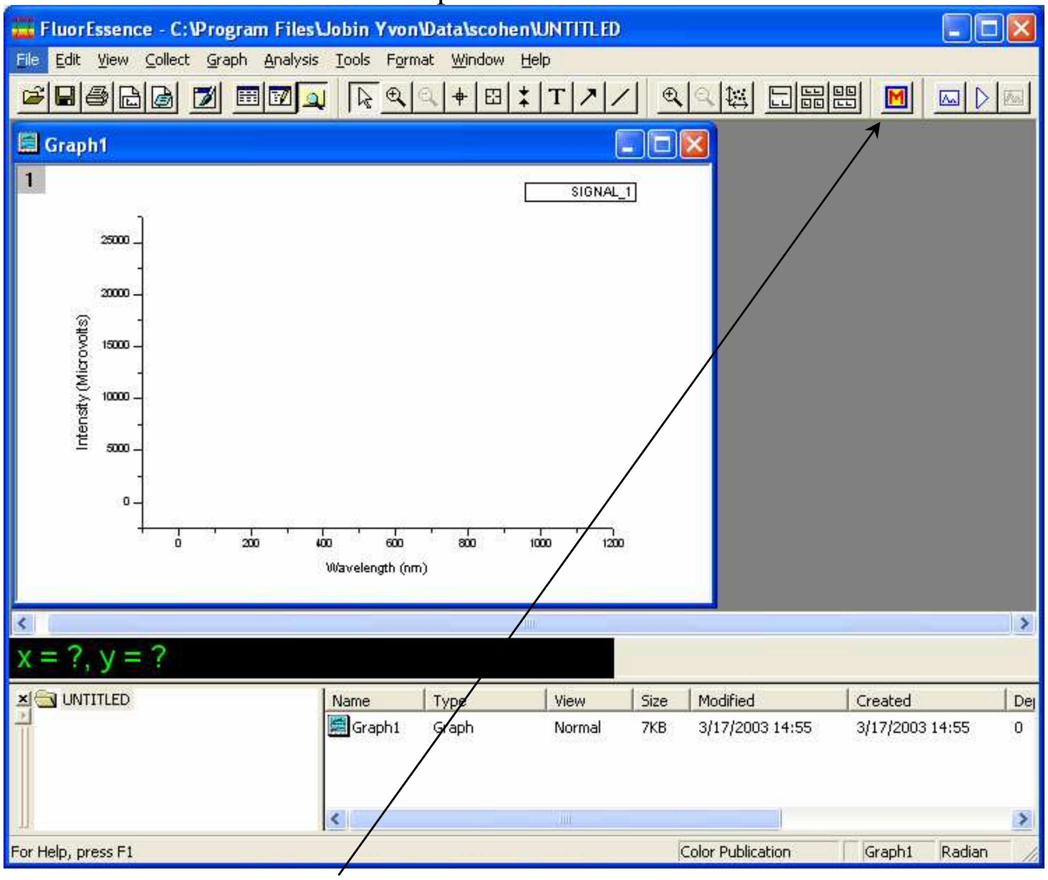
Emulating the FluorEssence™ software means letting the computer act as though the FluoroMax® is properly connected, even if it isn't.

1 Disconnect the communications cable from the host computer to the FluoroMax®.

Note: *Be sure the FluorEssence™ USB key is inserted into a free USB port on the host computer. Without the key, FluorEssence™ will not run properly, even in emulation mode.*

2 Double-click the FluorEssence icon to start FluorEssence™.

The main **FluorEssence** window opens:


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
Graph1
1
SIGNAL_1
25000
20000
15000
10000
5000
0
Intensity (Microvolts)
0
200
400
600
800
1000
1200
Wavelength (nm)
x = ?, y = ?
UNTITLED
For Help, press F1
Color Publication
Graph1
Radian

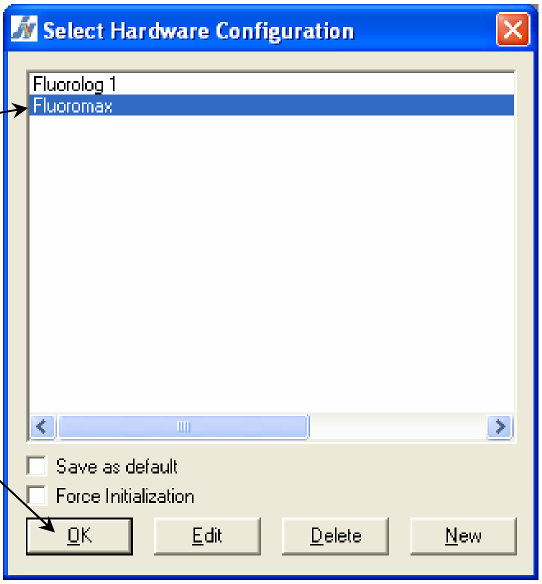
Name	Type	View	Size	Modified	Created	De
Graph1	Graph	Normal	7KB	3/17/2003 14:55	3/17/2003 14:55	0

3 Click the Monos button to open a hardware configuration.

The **Select Hardware Configuration** window opens.

4 Choose the desired instrument you wish to emulate.

5 Click OK.



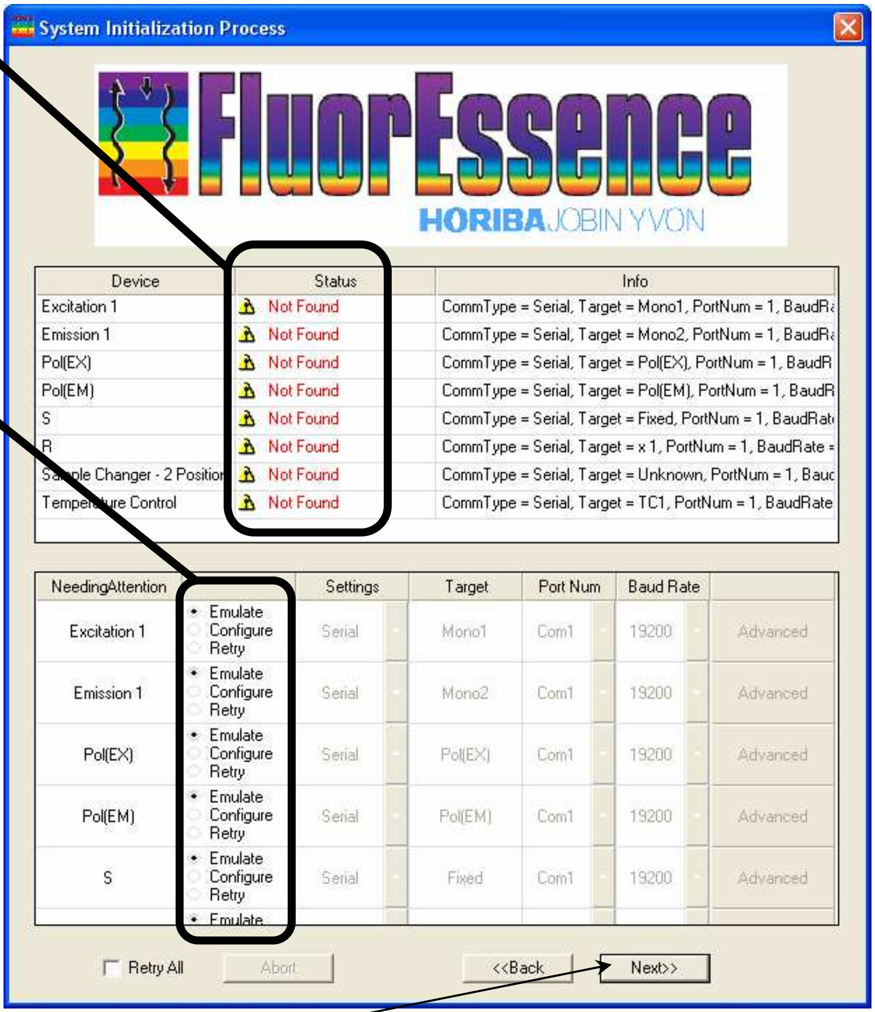
Select Hardware Configuration

Fluorolog 1
Fluoromax

☐ Save as default
☐ Force Initialization

The **System Initialization Process** window opens:

Under the Status column, warning symbols appear for the hardware devices, noting that they were Not Found. Thus FluorEssence™ chooses the Emulate radio button as the default action for each device.



System Initialization Process

Device	Status	Info
Excitation 1	⚠ Not Found	CommType = Serial, Target = Mono1, PortNum = 1, BaudR
Emission 1	⚠ Not Found	CommType = Serial, Target = Mono2, PortNum = 1, BaudR
Pol(EX)	⚠ Not Found	CommType = Serial, Target = Pol(EX), PortNum = 1, BaudR
Pol(EM)	⚠ Not Found	CommType = Serial, Target = Pol(EM), PortNum = 1, BaudR
S	⚠ Not Found	CommType = Serial, Target = Fixed, PortNum = 1, BaudRat
R	⚠ Not Found	CommType = Serial, Target = x 1, PortNum = 1, BaudRate =
Sample Changer - 2 Positio	⚠ Not Found	CommType = Serial, Target = Unknown, PortNum = 1, Baud
Temperature Control	⚠ Not Found	CommType = Serial, Target = TC1, PortNum = 1, BaudRate

NeedingAttention		Settings	Target	Port Num	Baud Rate	
Excitation 1	● Emulate ○ Configure ○ Retry	Serial	Mono1	Com1	19200	Advanced
Emission 1	● Emulate ○ Configure ○ Retry	Serial	Mono2	Com1	19200	Advanced
Pol(EX)	● Emulate ○ Configure ○ Retry	Serial	Pol(EX)	Com1	19200	Advanced
Pol(EM)	● Emulate ○ Configure ○ Retry	Serial	Pol(EM)	Com1	19200	Advanced
S	● Emulate ○ Configure ○ Retry	Serial	Fixed	Com1	19200	Advanced
	● Emulate					

☐ Retry All

6 Click Next>>.

The **Fluorescence Main Experiment Menu** appears. FluorEssence™ is now emulating the instrument.

2: System Description

Warning: *Do not open the instrument without proper training and having read this operation manual. The instrument contains dangerous voltages, ultraviolet, visible, and infrared radiation, and fragile light-sources. In addition, tampering with the optical components can irreversibly damage them.*

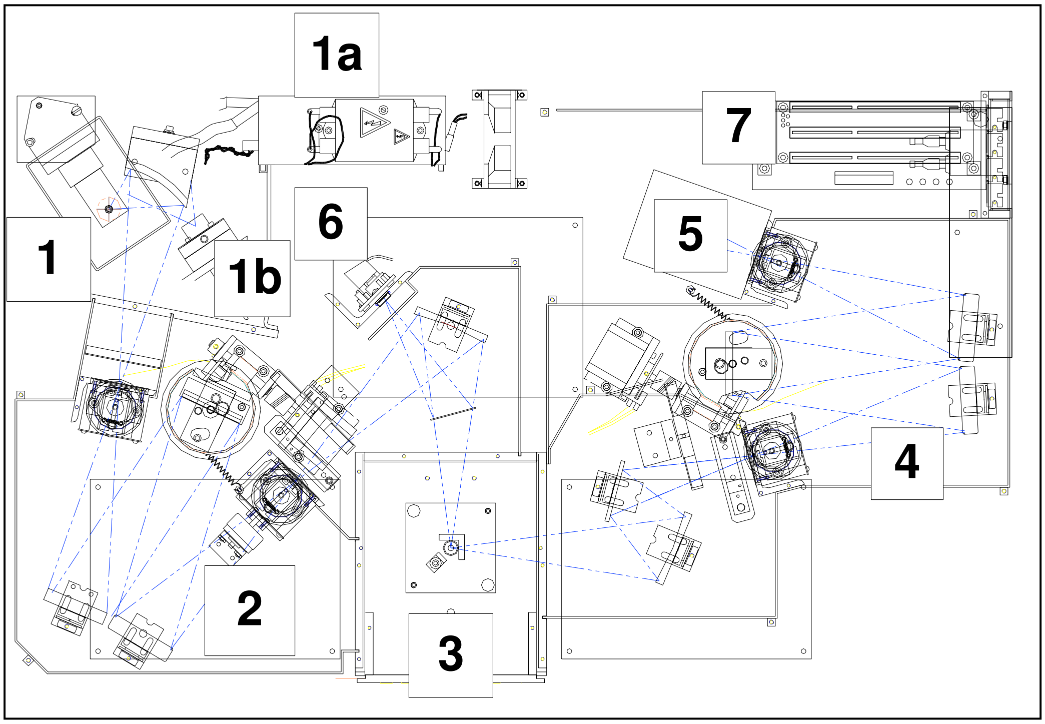
Introduction

A spectrofluorometer is an analytical instrument used to measure and record the fluorescence of a sample. While recording the fluorescence, the excitation, emission, or both wavelengths may be scanned. With additional accessories, variation of signal with time, temperature, concentration, polarization, or other variables may be monitored.

Basic theory of operation

A continuous source of light shines onto an excitation monochromator, which selects a band of wavelengths. This monochromatic excitation light is directed onto a sample, which emits luminescence. The luminescence is directed into a second, emission monochromator, which selects a band of wavelengths, and shines them onto a detector. The signal from the detector is reported to a system controller and host computer, where the data can be manipulated and presented, using special software.

Optical layout

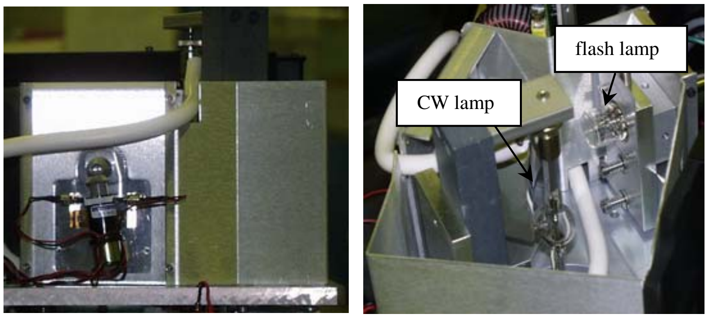


1	Xenon arc-lamp and lamp housing
1a	Xenon-lamp power supply
1b	Xenon flash lamp (FluoroMax®-P only)
2	Excitation monochromator
3	Sample compartment
4	Emission monochromator
5	Signal detector (photomultiplier tube and housing)
6	Reference detector (photodiode and current-acquisition module)
7	Instrument controller
	Host computer (not on diagram)

Illuminator (xenon arc-lamp, 1)

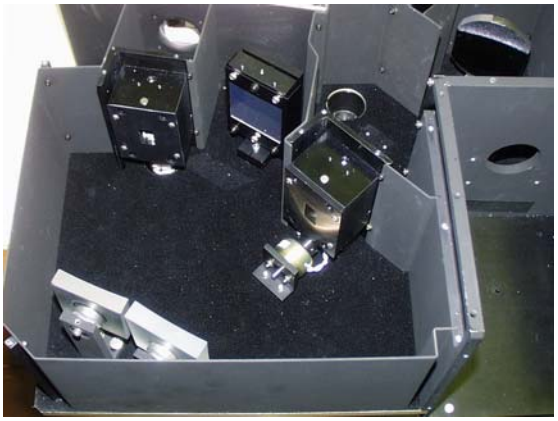
The continuous light source is a 150-W ozone-free xenon arc-lamp. Light from the lamp is collected by a diamond-turned elliptical mirror, and then focused on the entrance slit of the excitation monochromator. A portion of the light is directed upward to the Spex® logo on the instrument cover, to provide a “lamp on” indicator on the front panel. The lamp housing is separated from the excitation monochromator by a quartz window. This vents heat out of the instrument, and protects against the unlikely occurrence of lamp failure.

In the FluoroMax®-P, a second source, a xenon flash lamp, is available also. A FluorEssence™-controlled motor and gearbox rotate the mirror in the illumination area, in order to switch between the CW source and the flash lamp.

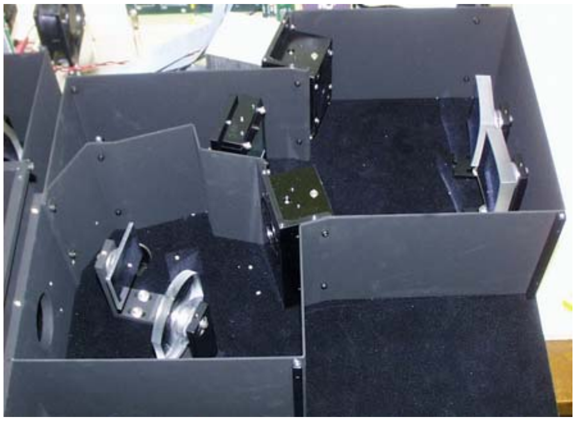


Left: motor and gearbox that rotate the mirror to switch lamps. Right: Twin sources in the FluoroMax®-P.

Monochromators (2 and 4)



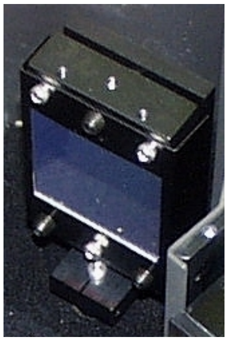
Excitation monochromator.



Emission monochromator.

The FluoroMax®-3 contains Czerny-Turner monochromators for excitation and emission. The Czerny-Turner design uses all-reflective optics to maintain high resolution over the entire spectral range, and minimize spherical aberrations and re-diffraction.

Gratings



The essential part of a monochromator is a reflection grating. A grating disperses the incident light by means of its vertical grooves. A spectrum is obtained by rotating the gratings, and recording the intensity values at each wavelength. The gratings in the FluoroMax®-3 contain 1200 grooves mm^{-1}, and are blazed at 330 nm (excitation) and 500 nm (emission). *Blazing* is etching the grooves at a particular angle, to optimize the grating's reflectivity in a particular spectral region. The wavelengths selected are optimal for excitation in the UV and visible, and for emission in the high-UV to near-IR. Each grating is coated with MgF_2 for protection against oxidation. The system uses a direct drive for each grating, to scan the spectrum at up to 200 nm s^{-1}, with accuracy better than 0.5 nm, and repeatability of 0.3 nm.

Slits

The entrance and exit ports of each monochromator have continuously adjustable slits controlled by FluorEssence™. The width of the slits on the excitation monochromator determines the bandpass of light incident on the sample. The emission monochromator's slits control the intensity of the fluorescence signal recorded by the signal detector. When setting slit width, the trade-off is intensity of signal versus spectral resolution. The wider the slits are, the more light falls on the sample and detector, but the resolution decreases. The narrower the slits are, the higher the resolution gets, but at the expense of signal. Set the slits for intensity toward the higher end of the detector's linear response, with sharp-enough resolution to discern desired spectral features.

Slits may be set in bandpass units, or the physical width of the slit (mm). Under bandpass units, each monochromator's slits are set simultaneously, for the bandpass is determined by the dispersion of the monochromator:

$$\text{bandpass (in nm)} = \text{slit width (in mm)} \times \text{dispersion (in nm mm}^{-1}\text{)}$$

The dispersion of FluoroMax®-3 monochromators is 4.25 nm mm^{-1} for gratings with 1200 grooves mm^{-1}. Below is a table showing standard slit widths with their corresponding bandpasses.

Slit width (mm)	Bandpass (nm)	Rounded bandpass (nm)
0.50	2.125	2
1.175	4.994	5
2.00	8.500	8.5

Shutters

An excitation shutter, standard on the FluoroMax®-3, is located just after the excitation monochromator's exit slit. The shutter protects samples from photobleaching or photodegradation from prolonged exposure to the light source. FluorEssence™ controls the shutter, and can set the shutter to automatic or photobleach modes. An emission shutter is an optional accessory, placed just before the emission monochromator's entrance, and protects the detector from bright light.

Caution: *Operation of the instrument when the excitation shutter is disabled may expose the user to excessive light. Wear light-blocking goggles or face-shield, and light-blocking clothing and gloves.*

Sample compartment (3)

A toroidal mirror focuses the beam from the excitation monochromator on the sample. About 8% of this excitation light is split off, using a beam-splitter, to the reference photodiode. Fluorescence from the sample is then collected and directed to the emission spectrometer.

The sample compartment accommodates various optional accessories, as well as fiber-optic bundles to take the excitation beam to a remote sample (or the MicroMax), and return the emission beam to the emission monochromator. See Chapter 7 for a list of accessories.

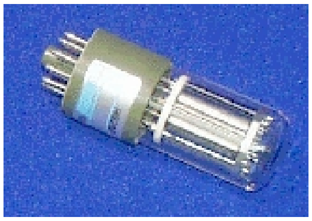
To insert or remove a sample platform,

1. If a multiple-sample turret is installed, shut off the system.
2. Remove the four screws on the front of the sample platform.
3. Slide out the old platform.
4. Slide in the new platform.
5. If the platform has a rotatable turret or magnetic stirrer, slide the 15-pin connector gently and securely onto the 15-pin receptacle in the sample compartment.
6. Re-attach the four screws on the front of the sample platform.

Detectors (5 and 6)

Each FluoroMax®-3 contains two detectors:

- Signal detector: The signal detector is a photon-counting detector. This detector is an R928P photomultiplier tube, which sends the signal to a photon-counting module. The detector's response ranges from 180–850 nm, with dark counts < 1000 counts per second (cps). The linear range for photon counting is 0–2 million cps. The working range is up to about 1–2 million cps. Above 2 million cps, photon-pulse pile-up reduces the signal-to-noise ratio, and causes the detector to lose its linear response.

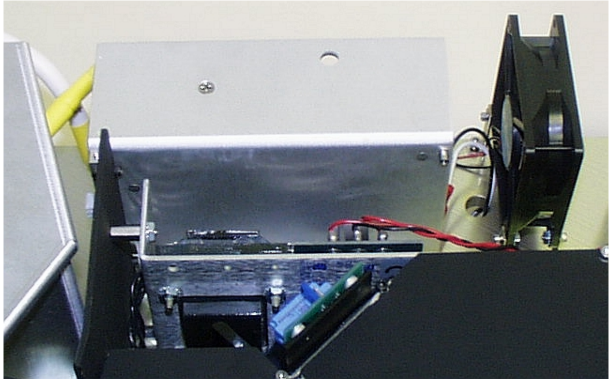


- Reference detector: The reference detector monitors the xenon lamp, in order to correct for wavelength- and time-dependent output of the lamp. This detector is a UV-enhanced silicon photodiode, which is just before the sample compartment. It requires no external bias, and has good response from 190–980 nm. The output is collected by a current-input module, with a range from 0–12 µA.

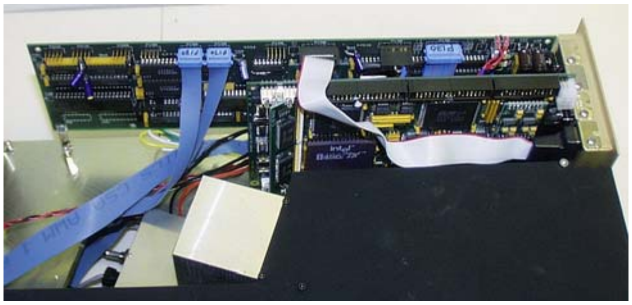
Both the reference and signal detectors have correction-factor files run for them, to correct for wavelength dependencies of each optical component. The files are created at HORIBA Jobin Yvon Inc. for every instrument, and may be applied to data through FluorEssence™. See Chapter 8 for more details.

Electronics and controllers (1a and 7)

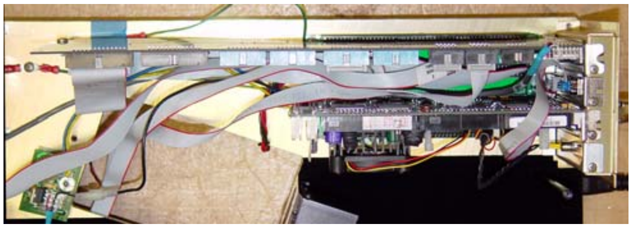
The right rear and bottom of the FluoroMax®-3 houses the electronics for running the lamp, instrument, scans, and measurements.



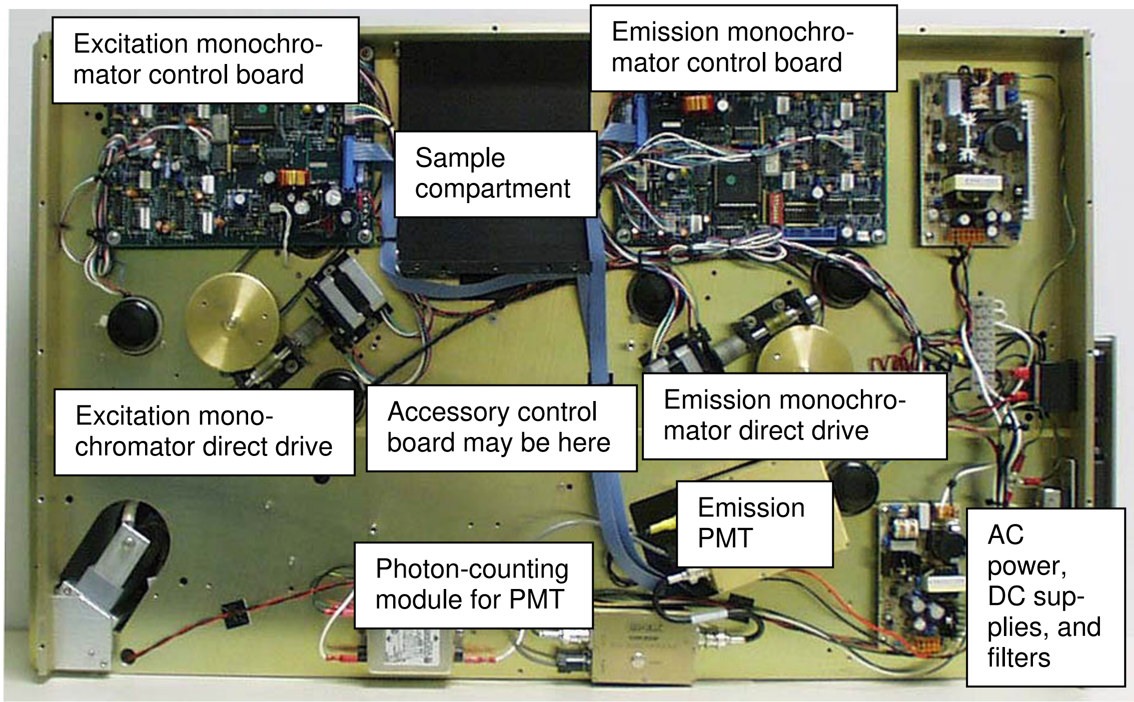
Lamp power supply (1a).



Older generation of slave-controller electronics above the optical platform



Newer generation of slave-controller electronics above the optical platform


Excitation monochromator control board
Emission monochromator control board
Sample compartment
Excitation monochromator direct drive
Accessory control board may be here
Emission monochromator direct drive
Emission PMT
Photon-counting module for PMT
AC power, DC supplies, and filters

Electronics underneath the optical platform.

Xenon-lamp power supply (1a)	This supply is a tunable 180-W-maximum power supply. It supplies a large start-up voltage to the 150-W xenon lamp, then holds the lamp steady at 12 V. The voltage is filtered, to stabilize the illumination as much as possible. The lamp is started with the left rocker-switch on the front-right panel of the instrument.
Slave controller	Located on the right rear of the FluoroMax®-3, it is composed of a dedicated motherboard that holds control and data-acquisition cards. A set of drivers is held in firmware on the motherboard, for low-level instrument control. This controller is linked to the host computer via a null modem serial link. The controller houses the CTI (**c**ounter, **t**imer, **i**ntegrator) card, for all instrument control and data-acquisition.
Monochromator 180F boards and optional accessory board	Underneath the optical platform, there are several control boards. They control the monochromators and any optional accessories connected to the sample compartment. See Chapter 12 for more details.

Computer system and software (not on diagram)

Not shown on the schematic is the host computer with FluorEssence™ software. The technical specifications chapter lists the computer requirements. An optional printer or network card is useful for printing. FluorEssence™ software for Windows™ controls all interaction with the spectrofluorometer. For information on FluorEssence™, see the *FluorEssence™ User's Guide* and the on-line help files within FluorEssence™.

3: System Operation

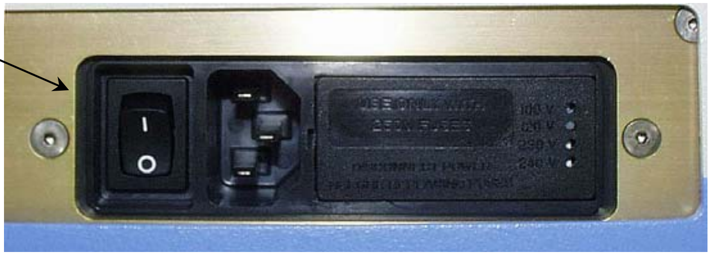
Introduction

This chapter explains how to turn on the FluoroMax®-3 system, check its calibration, and, if necessary, recalibrate the monochromators. While doing these procedures, how to define a scan, run a scan, and optimize system settings to obtain the best results is explained.

Controls and indicators

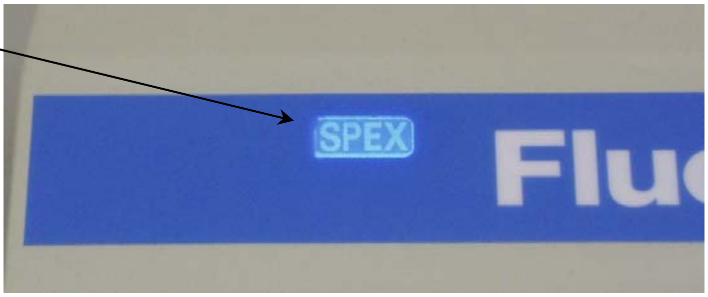
Power switch

The power switch is located on the lower right-hand side of the instrument. When switched on, the xenon lamp arcs immediately, and the FluoroMax®-3 turns on.



Lamp-on indicator

Once the lamp is operating normally, the Spex® logo is illuminated on the upper front panel.



Hour meter

An hour meter is mounted on the lower right-hand side, to monitor total usage of the xenon lamp.

ENM
0 0 5 2 0
HOURS
1/10

Note: *Each time the xenon lamp is ignited adds one more hour to lamp use. HORIBA Jobin Yvon Inc. suggests leaving the lamp on between brief periods of inactivity.*

Turning on the system

Warning: *When the xenon lamp is ignited, a large voltage is applied across the lamp. This voltage spike can return along the electrical power cord, causing damage to computer equipment, if the host computer is already on. Therefore, always follow the sequence below for switching on the system.*

An extremely rare occurrence is the explosion of the xenon lamp upon ignition. Therefore, take care in case tiny lamp shards exit the ventilation fans.

1 Turn on the FluoroMax®-3.

Turn the power switch to the ON (1) position. When the xenon lamp is lit, the Spex® logo is illuminated.

2 Turn on instrument accessories

Accessories include a temperature bath, MicroMax, etc.

3 Turn on all peripheral devices for the host PC.

Peripherals include any printers or plotters.

4 Start the host computer.

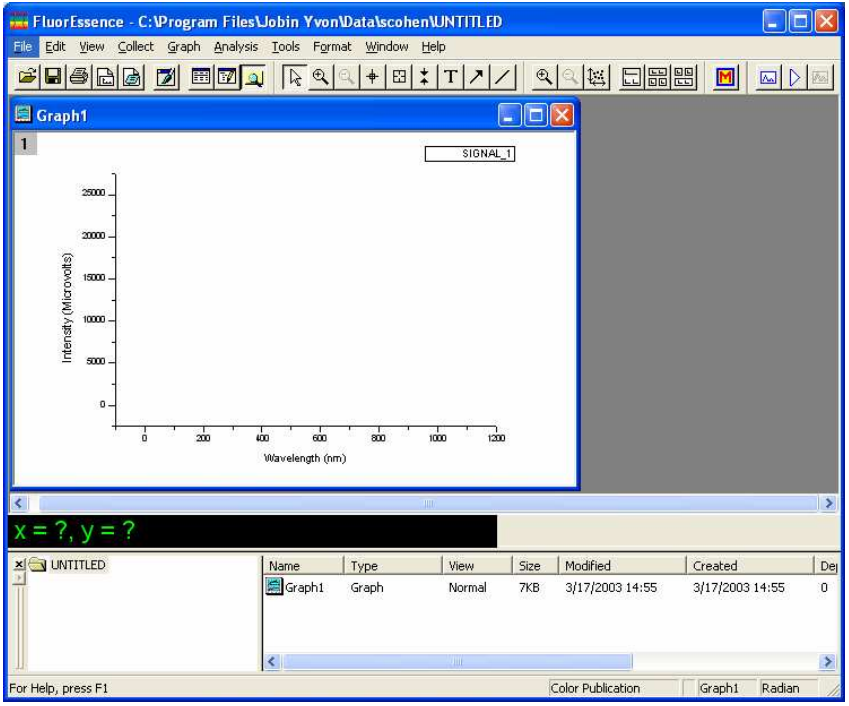
a Switch on the host computer.

b Click on the FluorEssence™ icon in Windows™.

FluorEssence

The instrument initializes, then the **FluorEssence** window appears.

If there are any difficulties, see the troubleshooting chapter.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
Graph1
1
SIGNAL_1
25000
20000
15000
10000
5000
0
Intensity (Microvolts)
0
200
400
600
800
1000
1200
Wavelength (nm)
x = ?, y = ?
UNTITLED

Name	Type	View	Size	Modified	Created	De
Graph1	Graph	Normal	7KB	3/17/2003 14:55	3/17/2003 14:55	0

For Help, press F1
Color Publication
Graph1
Radian

Checking system performance

Introduction

Upon installation and as part of routine maintenance checks, examine the performance of the FluoroMax®-3. HORIBA Jobin Yvon recommends checking the system calibration before each day of use with the system. Scans of the xenon-lamp output and the Raman-scatter band of water are sufficient to verify system *calibration*, *repeatability*, and *throughput*.

- *Calibration* is the procedure whereby the drive of each monochromator is referenced to a known spectral feature. One verifies the excitation and emission monochromators' calibration at a particular wavelength in this step.
- *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 FluoroMax®-3 is an *autocalibrating* spectrofluorometer. This means the system initializes its monochromators' drives, locates the home position of the 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 the calibration prior to the day's session with the instrument. For the calibration checks detailed here, a single-sample mount or automated sample changer should be 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 the calibration checks.

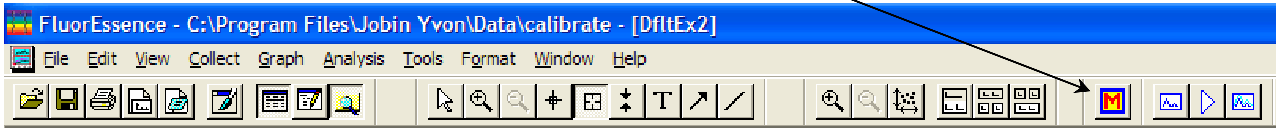
Note: *HORIBA Jobin Yvon Inc. is not responsible for customer errors in calibration. To be sure that your instrument is properly calibrated, call Spex® Fluorescence Service for assistance. We can arrange a visit and calibrate your instrument for a fee.*

Excitation calibration check

This calibration check verifies the wavelength calibration of your 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.

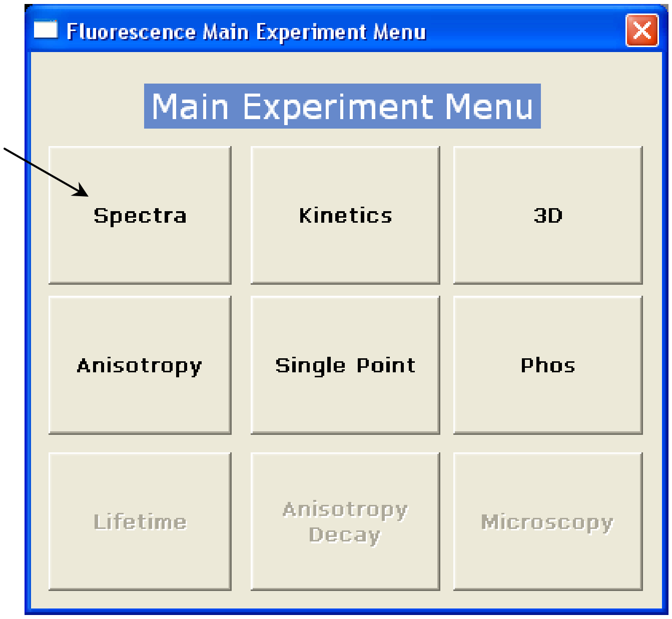
1 Close the lid of the sample compartment.

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


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File Edit View Collect Graph Analysis Tools Format Window Help
M

The **Fluoresence Main Experiment Menu** appears:

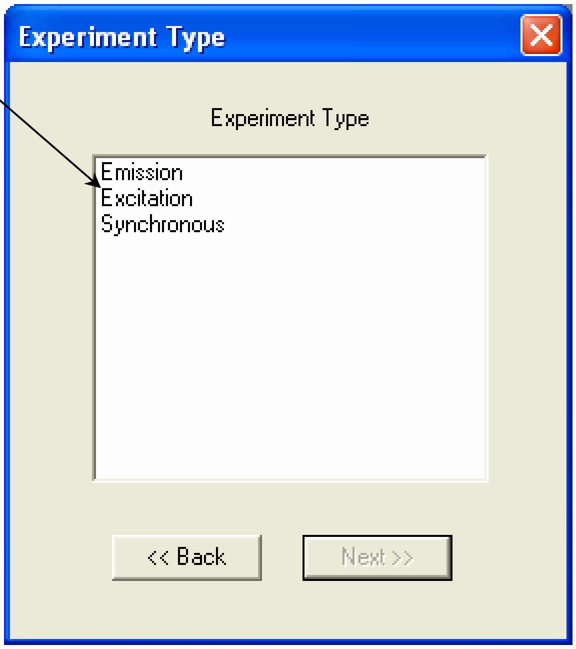
3 Choose Spectra.


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

The **Experiment Type** window appears:

4 Choose Excitation.

The xenon-lamp scan experiment automatically loads.


Experiment Type
Experiment Type
Emission
Excitation
Synchronous
<< Back
Next >>

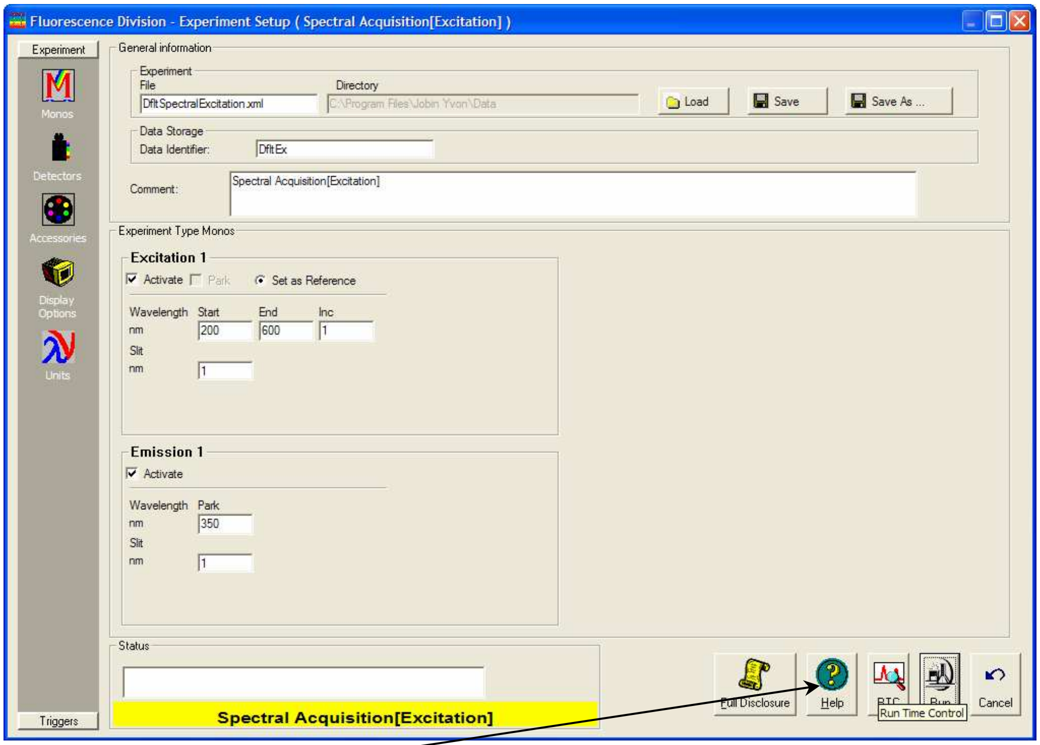
5 Use the default parameters or adjust them.

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
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA



Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])

General information

Experiment File: DfltSpectralExcitation.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage

Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Experiment Type Monos

Excitation 1

☑ Activate ☐ Park ● Set as Reference

Wavelength nm: Start 200, End 600, Inc 1

Slit nm: 1

Emission 1

☑ Activate

Wavelength nm: Park 350

Slit nm: 1

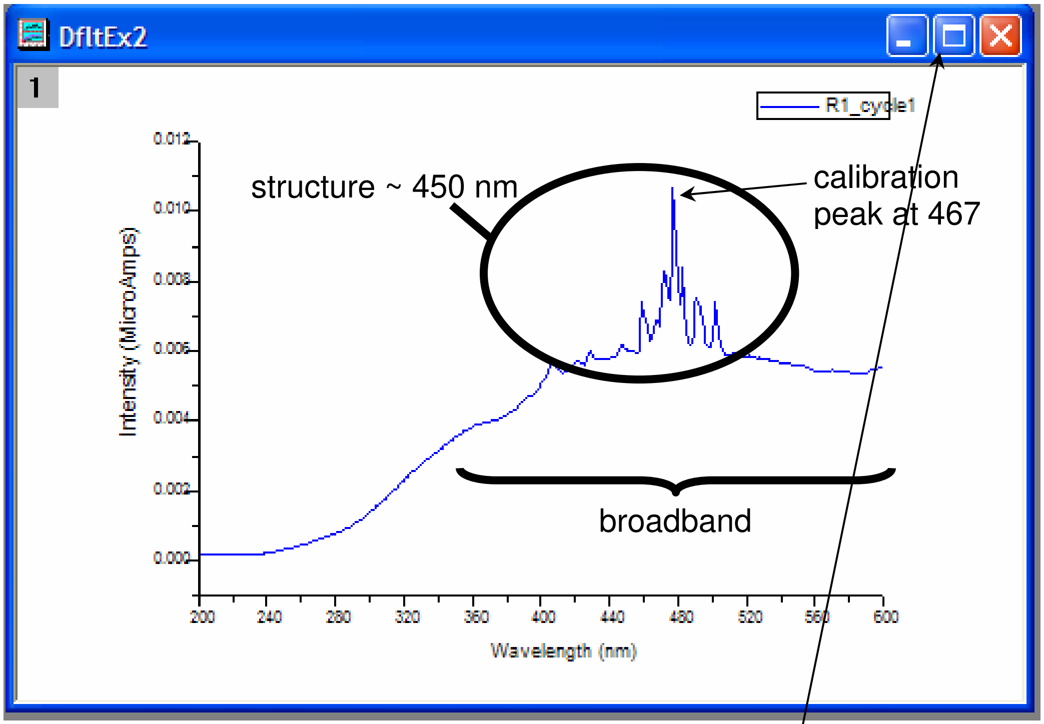
Status

Spectral Acquisition[Excitation]

Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Full Disclosure
Help
RTC
Run
Run Time Control
Cancel

6 Click Run.

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



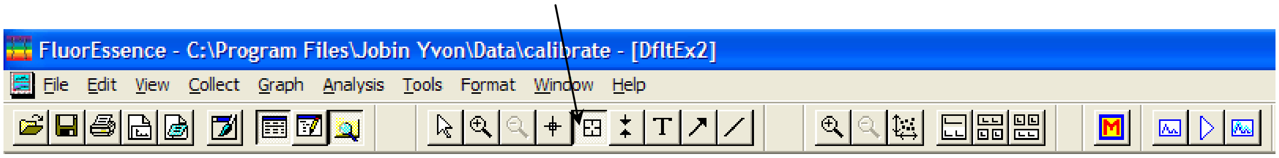
This is an uncalibrated FluoroMax® lamp scan. The main peak ought to be at 467 nm, but here appears near 480 nm.

***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.

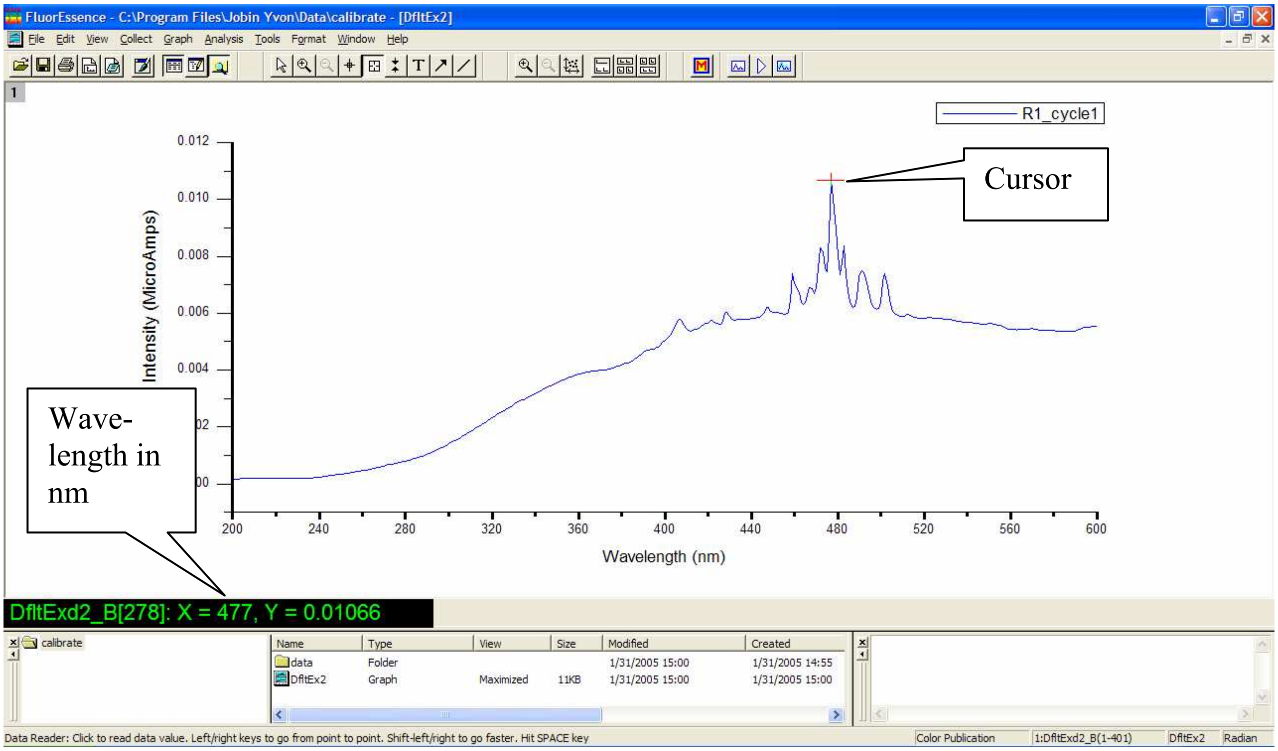
b Click the cursor button to start the Cursor function.


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
T
M

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.

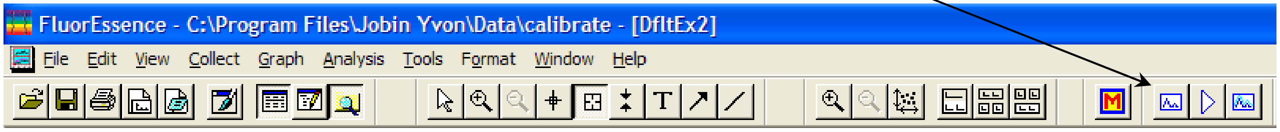

FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File Edit View Collect Graph Analysis Tools Format Window Help
R1_cycle1
Cursor
Intensity (MicroAmps)
0.012
0.010
0.008
0.006
0.004
Wave-length in nm
200 240 280 320 360 400 440 480 520 560 600
Wavelength (nm)
calibrate
Name Type View Size Modified Created
data Folder 1/31/2005 15:00 1/31/2005 14:55
DfltEx2 Graph Maximized 11KB 1/31/2005 15:00 1/31/2005 15:00
Color Publication 1:DfltExd2_B(1-401) DfltEx2 Radian

DfltExd2_B[278]: X = 477, Y = 0.01066

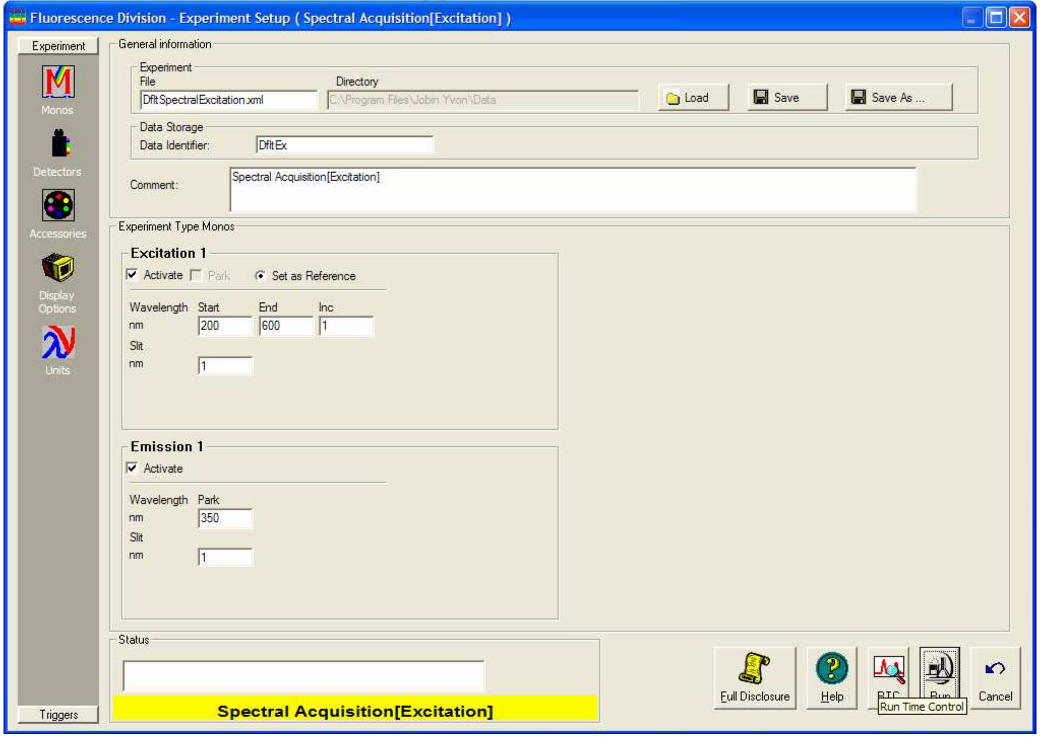
Data Reader: Click to read data value. Left/right keys to go from point to point. Shift-left/right to go faster. Hit SPACE key

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.


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File Edit View Collect Graph Analysis Tools Format Window Help

The **Experiment Setup** window appears.


Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Full Disclosure
Help
RTC
Run
Run Time Control
Cancel

General information

Experiment
File: DfltSpectralExcitation.xml
Directory: C:\Program Files\Jobin Yvon\Data

Data Storage
Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Experiment Type Monos

Excitation 1
☑ Activate ☐ Park ● Set as Reference
Wavelength nm: Start 200, End 600, Inc 1
Slit nm: 1

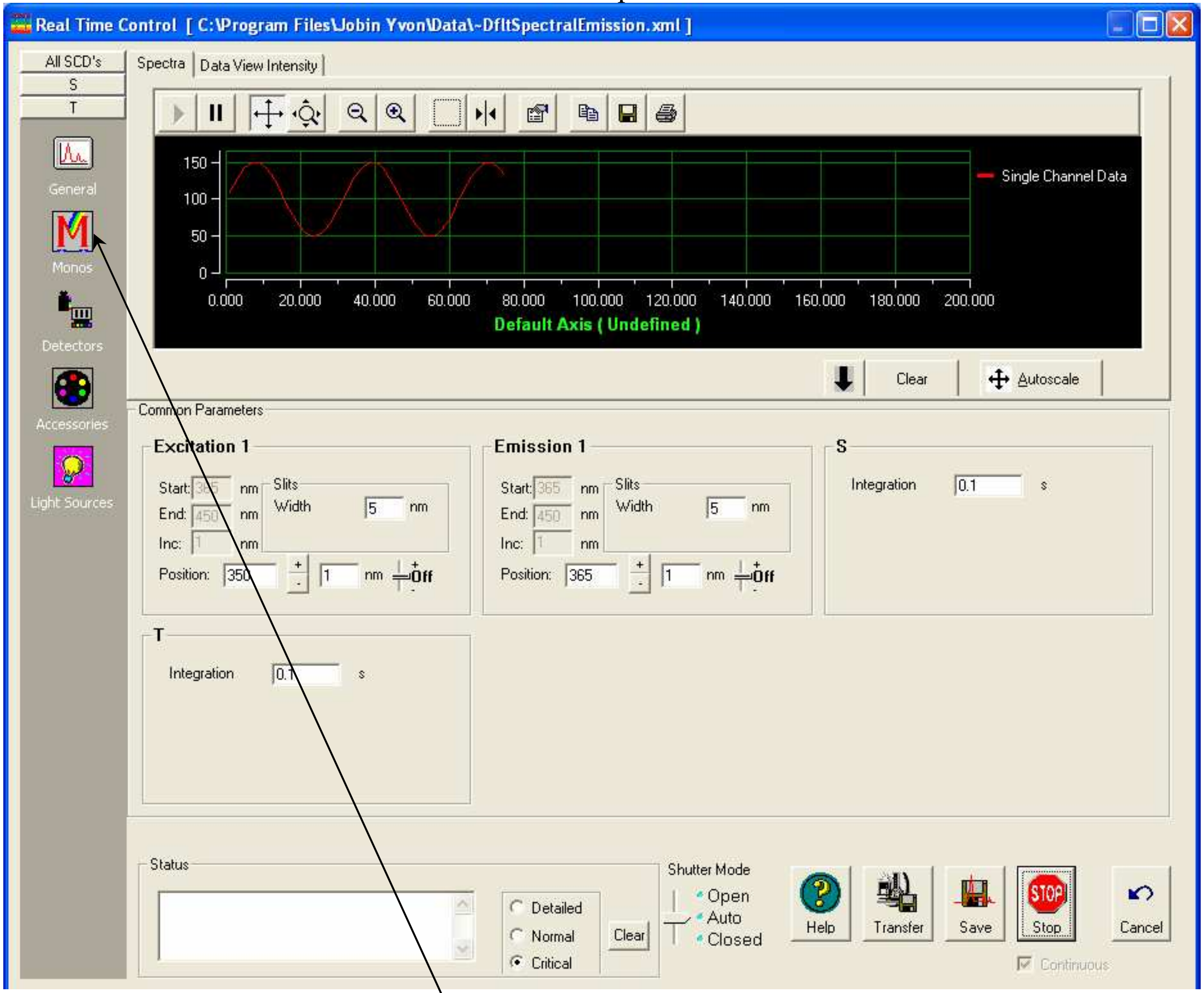
Emission 1
☑ Activate
Wavelength nm: Park 350
Slit nm: 1

Status

Spectral Acquisition[Excitation]

g Click the RTC button on the lower right.

The **Real Time Control** window opens.


All SCD's
S
T
General
Monos
Detectors
Accessories
Light Sources
Spectra
Data View Intensity
150
100
50
0
0.000
20.000
40.000
60.000
80.000
100.000
120.000
140.000
160.000
180.000
200.000
Default Axis (Undefined)
Single Channel Data
Clear
Autoscale
Help
Transfer
Save
STOP
Stop
Cancel
Clear

Real Time Control [C:\Program Files\Jobin Yvon\Data\~DfltSpectralEmission.xml]

Common Parameters

Excitation 1

Start: 365 nm
End: 450 nm
Inc: 1 nm
Slits Width 5 nm
Position: 350 + - 1 nm Off

Emission 1

Start: 365 nm
End: 450 nm
Inc: 1 nm
Slits Width 5 nm
Position: 365 + - 1 nm Off

S

Integration 0.1 s

T

Integration 0.1 s

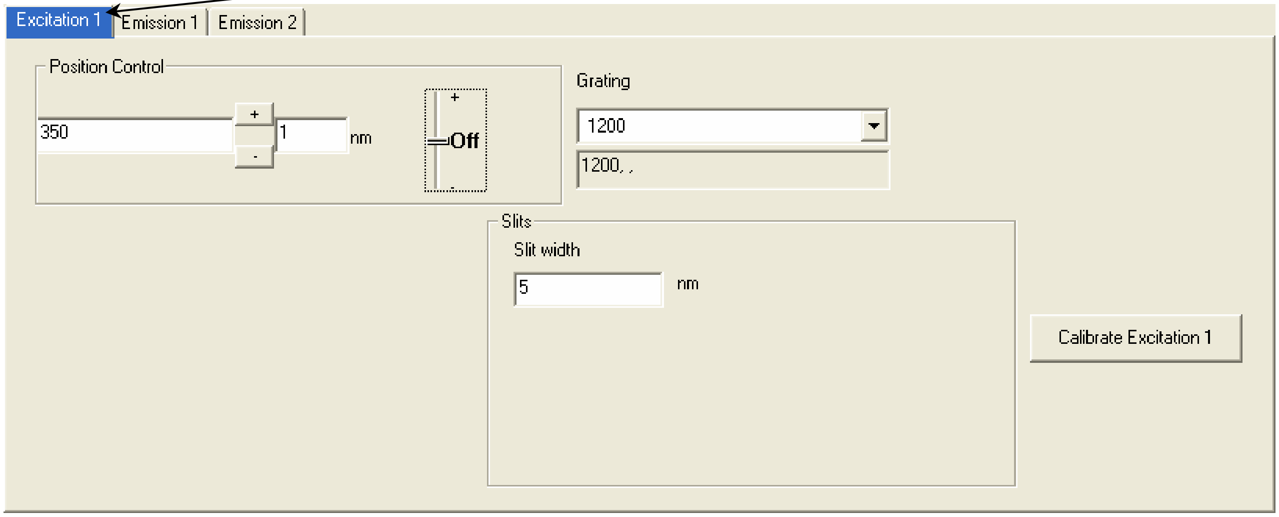
Status

○ Detailed
○ Normal
● Critical

Shutter Mode
Open
Auto
Closed

☑ Continuous

h Click the Monos icon to view the monochromators' index card, then click the excitation monochromator tab.


Excitation 1
Emission 1
Emission 2
Calibrate Excitation 1

Position Control
350 + - 1 nm
Off

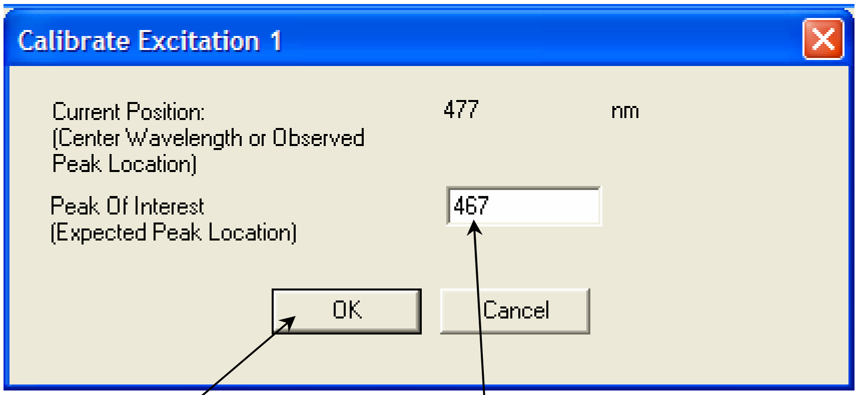
Grating
1200
1200, ,

Slits
Slit width
5 nm

i Enter the current, observed position of the peak in the Position Control (here, 477 nm).

j Click the Calibrate Excitation 1 button.

The **Calibrate** window opens:


Calibrate Excitation 1
OK
Cancel

Current Position: (Center Wavelength or Observed Peak Location) 477 nm

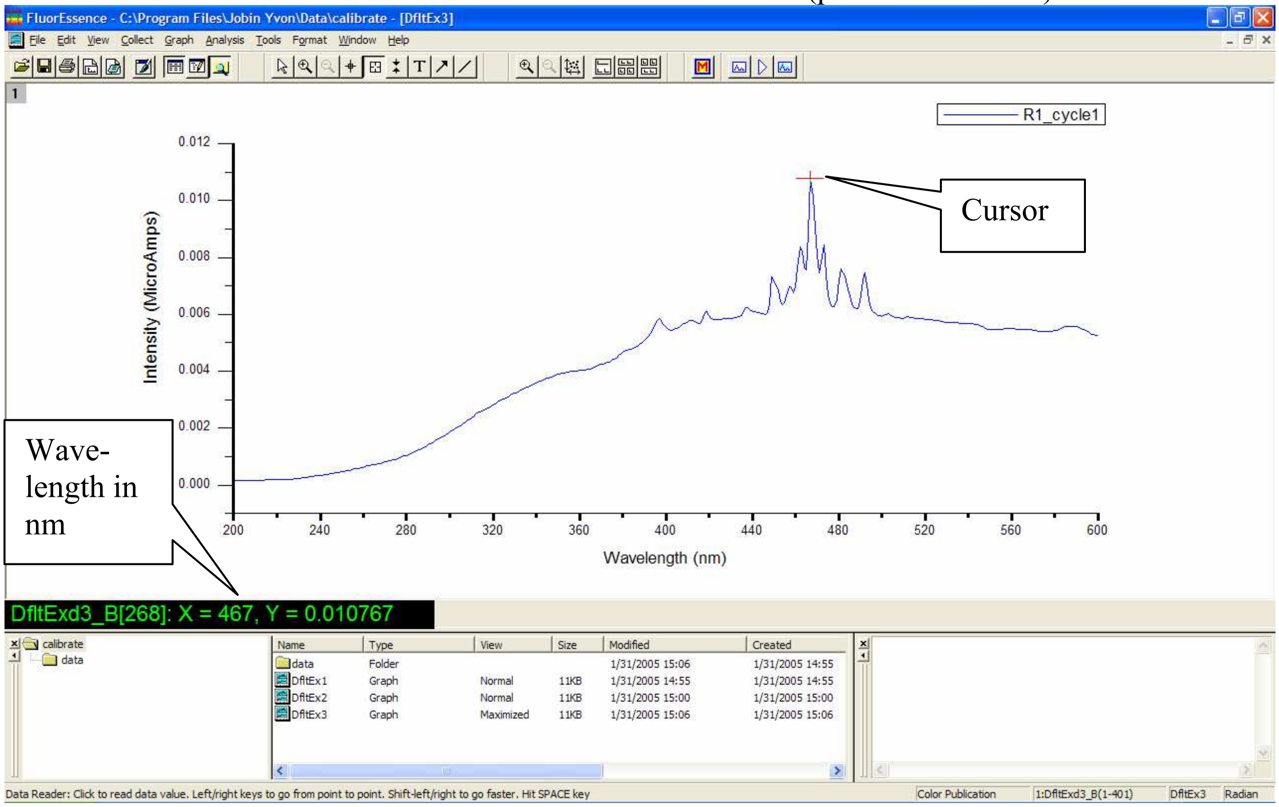
Peak Of Interest (Expected Peak Location) 467

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

l Click OK.

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

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


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx3]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
1
R1_cycle1
Cursor
Intensity (MicroAmps)
0.012
0.010
0.008
0.006
0.004
0.002
0.000
200
240
280
320
360
400
440
480
520
560
600
Wavelength (nm)
Wave-length in nm
calibrate
data
Color Publication
1:DfltExd3_B(1-401)
DfltEx3
Radian

```
DfltExd3_B[268]: X = 467, Y = 0.010767
```

Name	Type	View	Size	Modified	Created
data	Folder			1/31/2005 15:06	1/31/2005 14:55
DfltEx1	Graph	Normal	11KB	1/31/2005 14:55	1/31/2005 14:55
DfltEx2	Graph	Normal	11KB	1/31/2005 15:00	1/31/2005 15:00
DfltEx3	Graph	Maximized	11KB	1/31/2005 15:06	1/31/2005 15:06

Data Reader: Click to read data value. Left/right keys to go from point to point. Shift-left/right to go faster. Hit SPACE key

Emission calibration check

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

This calibration check verifies the wavelength calibration of the emission monochromator with the emission photomultiplier tube. It is an emission scan of the Raman-scatter band of water performed in right-angle mode. Perform this check after the xenon-lamp scan. When completed, the performance of the system has been verified.

The water sample should be research-quality, triple-distilled or deionized water. HPLC-grade (18-MΩ spec.) or equivalent water is suggested for the Raman scan. Impure samples of water will cause elevated background levels as well as distorted spectra with (perhaps) some unwelcome peaks.

Use a 4-mL quartz cuvette.

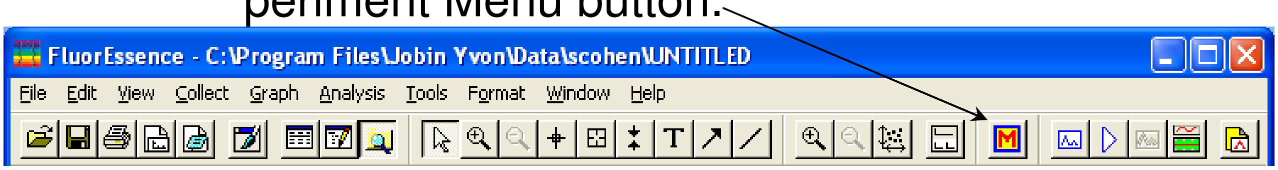
Note: *Avoid glass or acrylic cuvettes: they may exhibit UV fluorescence or filtering effects.*

1. Insert the water sample into the sample compartment.

   With an automated sample changer, note the position number in which the sample cell is placed.

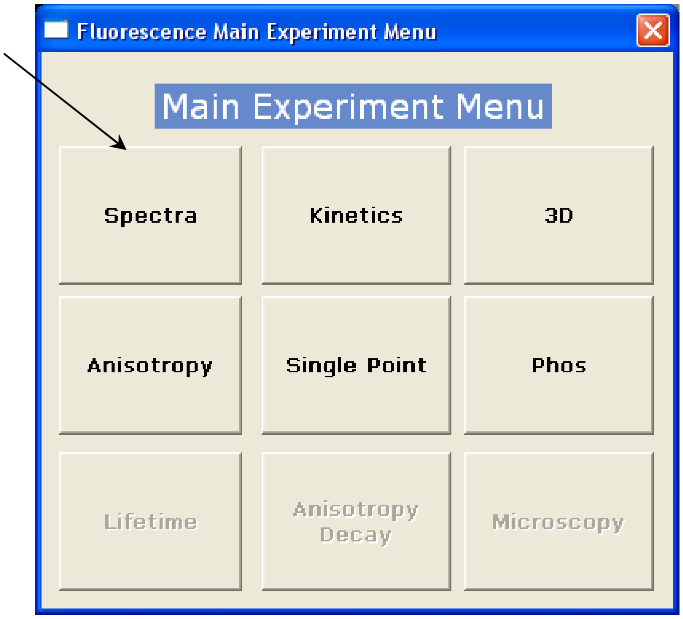
2. Close the lid of the sample chamber.

3. In the main **FluorEssence** toolbar, choose the Experiment Menu button.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
T
M

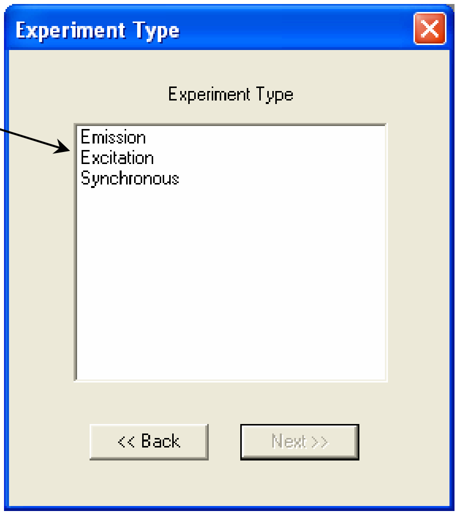
The **Fluorescence Main Experiment Window** opens.

4 Choose Spectra.

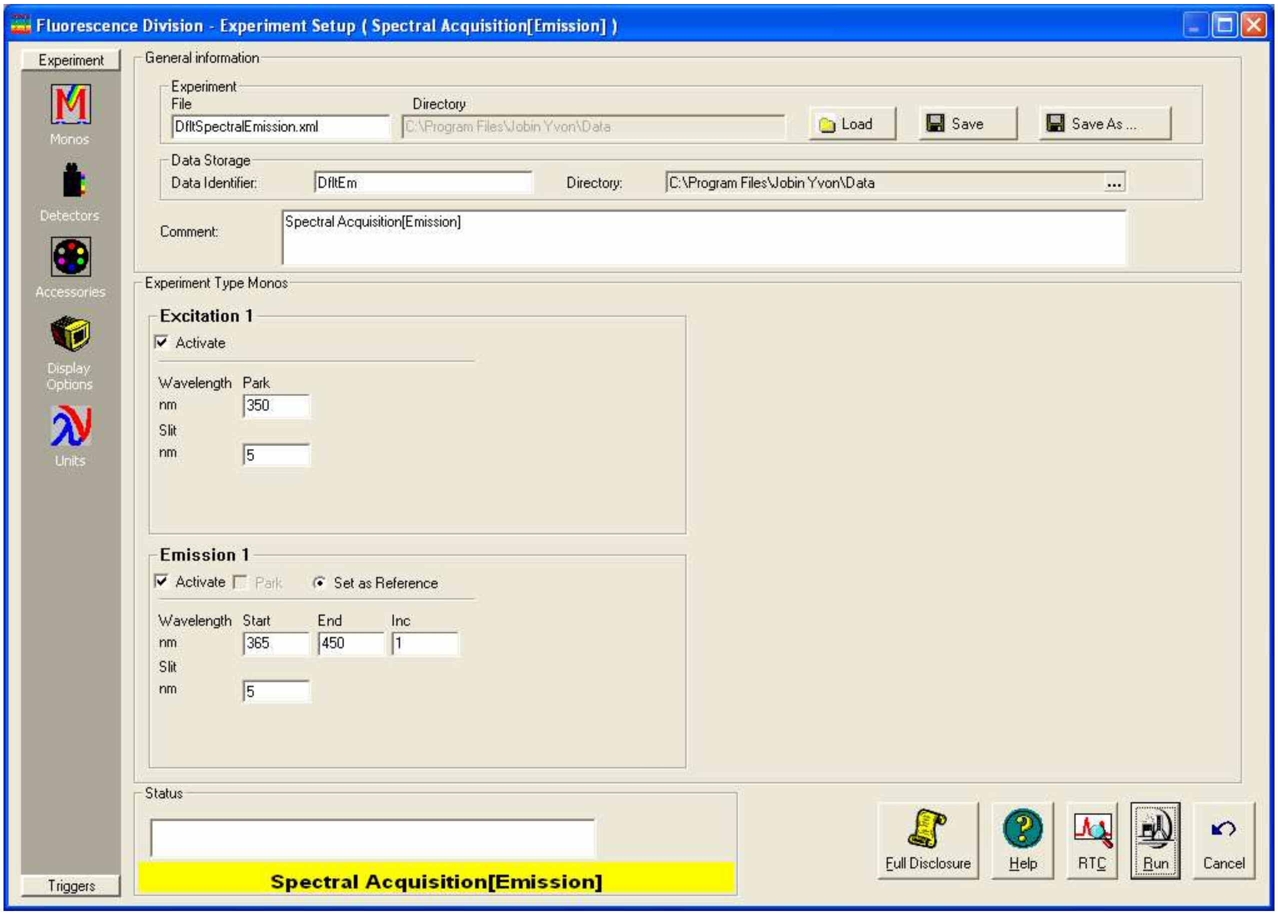

Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

The **Experiment Type** window appears:

5 Choose Emission.


Experiment Type
Experiment Type
Emission
Excitation
Synchronous
<< Back
Next >>

The water-Raman experiment automatically loads.


Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Load
Save
Save As ...
...
Full Disclosure
Help
RTC
Run
Cancel

Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])

Experiment
File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\Data

Data Storage
Data Identifier: DfltEm
Directory: C:\Program Files\Jobin Yvon\Data

Comment: Spectral Acquisition[Emission]

Experiment Type Monos

Excitation 1
☑ Activate
Wavelength nm Park: 350
Slit nm: 5

Emission 1
☑ Activate ☐ Park ● Set as Reference
Wavelength nm Start: 365 End: 450 Inc: 1
Slit nm: 5

Status
Spectral Acquisition[Emission]

6 Use the default parameters or adjust them.

Monochromator parameters for the water-Raman scan:

Monochromator (1200 grooves/mm)	Initial wavelength	Final wavelength	Increment	Slits (bandpass)
Excitation	350 nm	--	--	5 nm
Emission	365 nm	450 nm	1 nm	5 nm

Detector parameters for the water-Raman scan:

Detector (Signal)	Integration time	Units
Signal (S1)	100 ms	CPS
Reference (R1)	100 ms	mA

7 Click Run.

The **Intermediate Display** opens. The water-Raman scan runs.

A new FluoroMax® should display a water-Raman peak intensity of at least 300 000 counts s^{-1}.

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 water-Raman peak intensity may indicate a need to replace the xenon lamp.*

8 If the water-Raman peak is not at 397 nm, calibrate the emission monochromator as shown for the excitation-monochromator calibration on pages 3-7 to 3-9.

Calculation of water-Raman signal-to-noise ratio

Introduction

The water-Raman test is a good measure of relative sensitivity between different instruments, if the experimental conditions used to compare the systems are the same. Unfortunately, there are different ways of handling the data, all of which are valid but which will give quite different values. Therefore, it is important not only to know how the water-Raman *S*/*N* values are measured, but also how the data were treated. The water Raman *S*/*N* test method combines a value for system sensitivity (a signal) with a value for system noise (no signal) to show the overall performance of the instrument.

Definitions

At HORIBA Jobin Yvon, we define the *S*/*N* ratio as the difference of peak and background signal, divided by the square root of the background signal.

$$\frac{S}{N} = \frac{S_{peak} - S_{background}}{\sqrt{S_{background}}} \quad \text{Our method}$$

The peak signal is measured at the water-Raman peak (397 nm for 350 nm excitation) and the noise in a region (450 nm) where no Raman signal is present. An "ideal" system would give a signal value of zero at 450 nm. Thus the equation becomes

$$\frac{S}{N} = \frac{S_{397\,\mathrm{nm}} - S_{450\,\mathrm{nm}}}{\sqrt{S_{450\,\mathrm{nm}}}}$$

Another commonly used method is to divide the difference (peak signal minus background signal) by the rms value of the noise on the background signal. This second method is used by a few other manufacturers:

$$\frac{S}{N} = \frac{S_{peak} - S_{background}}{N_{rms,background}} \quad \text{Other manufacturers' method}$$

Example

Some actual data from a Fluorolog® FL3-11 system (a typical system, a few years old) serve to show the difference between the two methods. The experimental conditions were as follows:

Excitation	350 nm with 5 nm bandpass
Emission	360–450 nm with 5 nm bandpass
Increment	1 nm
Integration time	1 s

No smoothing of data points

Standard room-temperature, red-sensitive detector

Note: *Make sure the test is carried out with the actual detector you will be using. All Spex® systems' parameters are specified with a R928P photomultiplier tube at room temperature.*

Results

The measurements provided the following data:

S_{peak}	peak signal at 397 nm	501 500 cps
$S_{background}$	background noise at 450 nm	10 500 cps
$N_{\text{p–p}}$	Peak-to-peak noise of background at 450 nm*	223 c

*Measured with a separate kinetic scan

$N_{\text{p–p}}$ gives an rms noise of the background signal of

$$\begin{aligned} N_{rms,background} &= \frac{223\,\text{c}}{5} \\ &= 44.6 \end{aligned}$$

The HORIBA Jobin Yvon method gives a water-Raman S/N of

$$\begin{aligned} \frac{S}{N} &= \frac{S_{peak} - S_{background}}{\sqrt{S_{background}}} \\ &= \frac{501\,500 - 10\,500}{\sqrt{10\,500}} \\ &= 4790 \end{aligned}$$

The other method similarly gives a water-Raman S/N of

$$\begin{aligned} \frac{S}{N} &= \frac{S_{peak} - S_{background}}{N_{rms,background}} \\ &= \frac{501\,500 - 10\,500}{44.6} \\ &= 11\,000 \end{aligned}$$

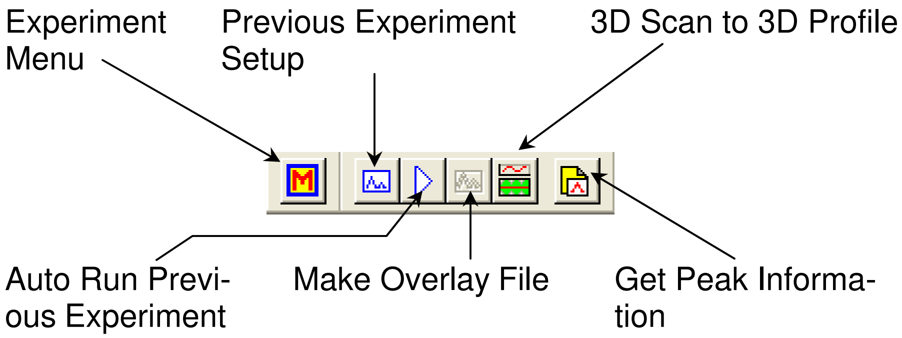
HORIBA Jobin Yvon feels that the first method is correct although it gives a lower S/N. The second method only takes into account the detector noise and the shot noise of the electronics. By using the background total intensity as a measure of noise, the HORIBA Jobin Yvon method is more representative of a real “live” experiment where noise is also influenced by factors like the quality of the optics and scattered light in the system. These additional factors influence the ability to measure a very low signal from a sample and should not be ignored.

Notes on excitation and emission calibration

- Two experiments, `lamp.exp` and `water.exp`, have been defined and saved. They can be run, after the system is switched on each day, to check the calibration and performance of the FluoroMax®-3.
- HORIBA Jobin Yvon Inc. recommends monitoring the number of hours of xenon-lamp use, via the hour meter.
- Additionally, you may want to record the water-Raman intensity daily or weekly.
- The lamp is rated for 1200–1500 h, but if the Raman intensity starts to drop, you may wish to change the lamp sooner.

4: Data Acquisition

This chapter presents an introduction to the six special buttons used in FluorEssence™ to record and present data with the FluoroMax®-3. These buttons, located in FluorEssence™'s main window, are:


Experiment Menu
Previous Experiment Setup
3D Scan to 3D Profile
Auto Run Previous Experiment
Make Overlay File
Get Peak Information

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

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

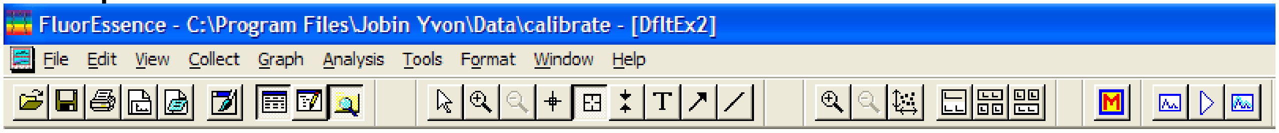
Experiment Menu button

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.

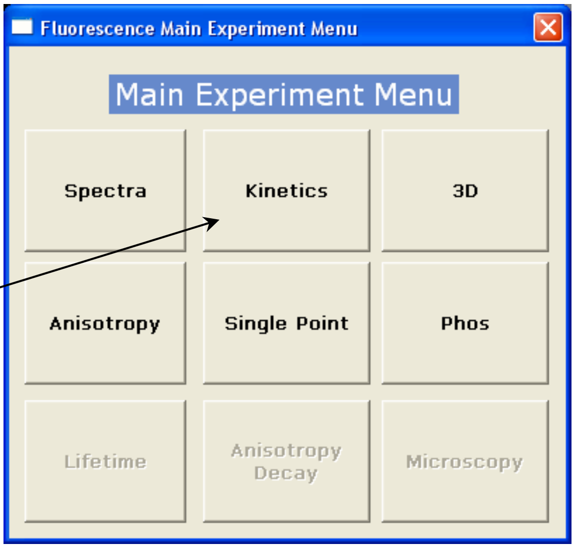
Calibration scans for the FluoroMax® use default parameters:

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

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


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
T
M

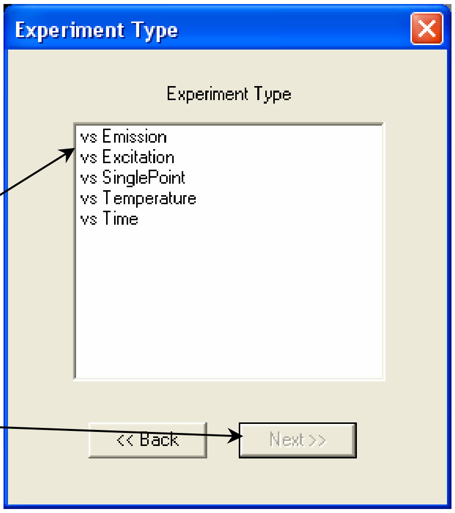
The **Fluorescence Main Experiment Menu** appears:


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

Note: *Certain scan types are not allowed with the FluoroMax®, and are grayed out.*

2 Choose an experiment type.

The **Experiment Type** menu appears.

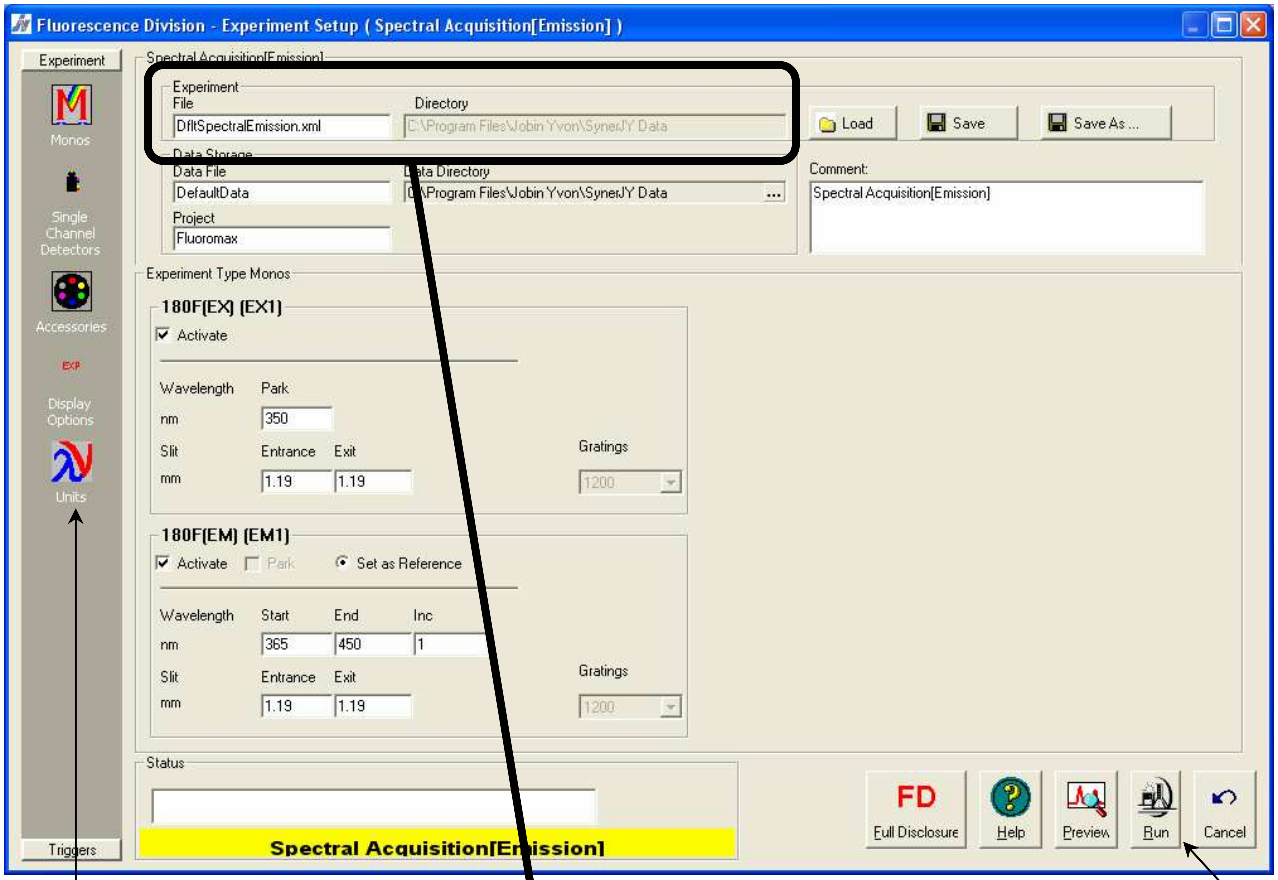

Experiment Type
Experiment Type
vs Emission
vs Excitation
vs SinglePoint
vs Temperature
vs Time
<< Back
Next >>

Note: *The list of subtypes depends upon the general type of experiment chosen.*

3 Choose a subtype of experiment.

4 Click Next >>.

The **Experiment Setup** window appears:


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Single Channel Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
FD
Full Disclosure
Help
Preview
Run
Cancel

Spectral Acquisition[Emission]

Experiment
File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\SynerJY Data

Data Storage
Data File: DefaultData
Data Directory: C:\Program Files\Jobin Yvon\SynerJY Data
Project: Fluoromax

Comment: Spectral Acquisition[Emission]

Experiment Type Monos

180F(EX) [EX1]
☑ Activate
Wavelength nm Park: 350
Slit mm Entrance: 1.19 Exit: 1.19
Gratings: 1200

180F(EM) [EM1]
☑ Activate ☐ Park ● Set as Reference
Wavelength nm Start: 365 End: 450 Inc: 1
Slit mm Entrance: 1.19 Exit: 1.19
Gratings: 1200

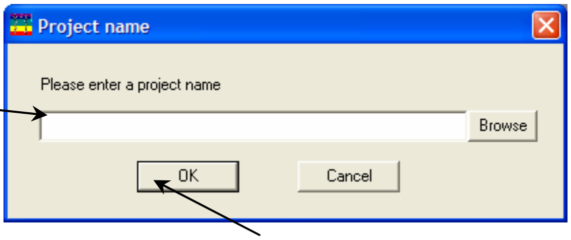
Status
Spectral Acquisition[Emission]

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

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

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

8 Click Run.
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:


Project name
Browse
OK
Cancel

Please enter a project name

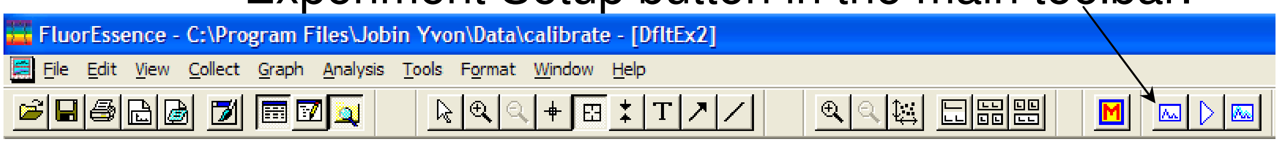
9 Enter a name for the entire project, or browse for an existing project name with the Browse button, then click OK.
All data are moved to Origin$^{®}$'s graph window for post-processing.

Previous Experiment Setup button

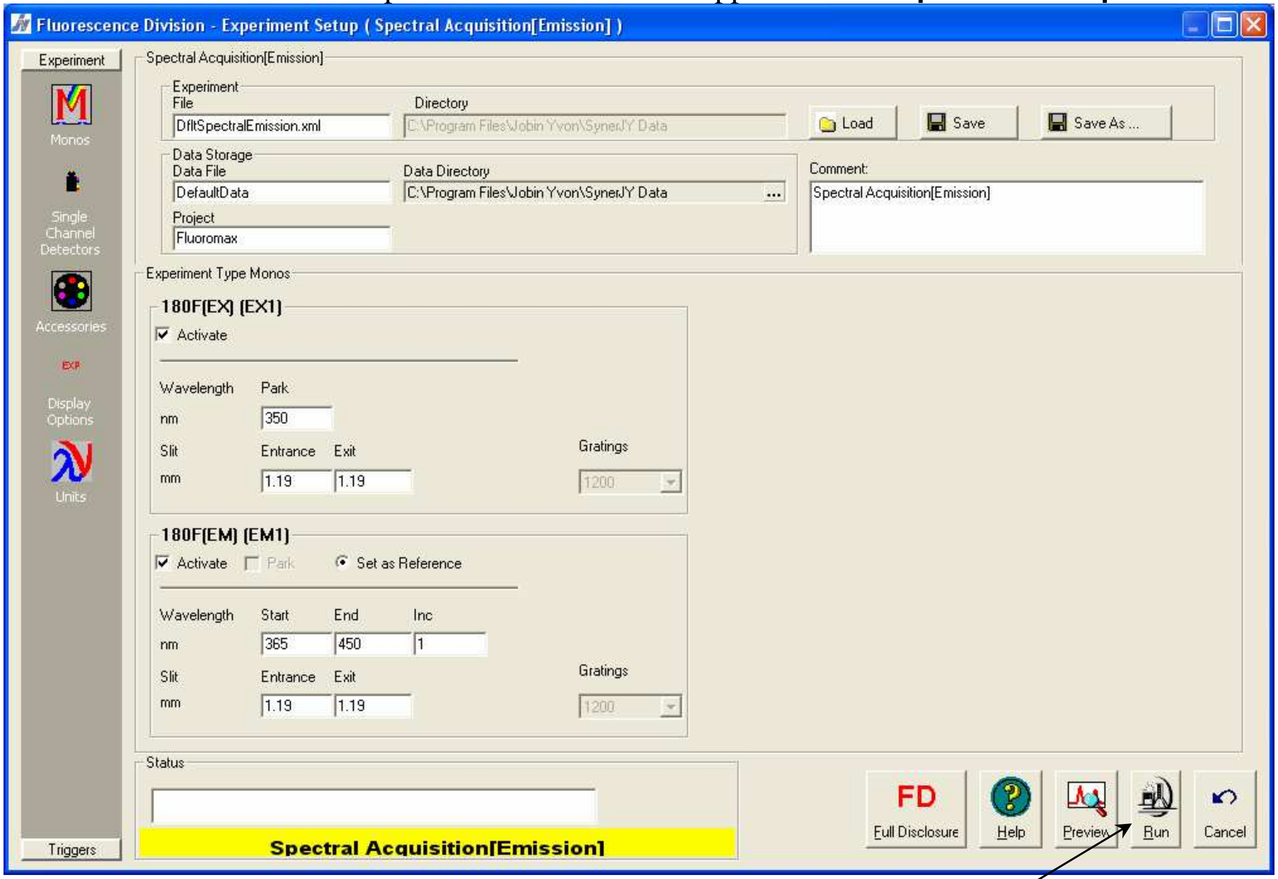
The **Previous Experiment Setup** button resets the experiment to the previous experiment used, with minor modifications to the hardware possible.

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

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


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File Edit View Collect Graph Analysis Tools Format Window Help

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


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Single Channel Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
FD Full Disclosure
Help
Preview
Run
Cancel

Spectral Acquisition[Emission]

Experiment
File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\SynerJY Data

Data Storage
Data File: DefaultData
Data Directory: C:\Program Files\Jobin Yvon\SynerJY Data
Project: Fluoromax

Comment: Spectral Acquisition[Emission]

Experiment Type Monos

180F[EX] [EX1]
☑ Activate
Wavelength nm Park: 350
Slit mm Entrance: 1.19 Exit: 1.19
Gratings: 1200

180F[EM] [EM1]
☑ Activate ☐ Park ● Set as Reference
Wavelength nm Start: 365 End: 450 Inc: 1
Slit mm Entrance: 1.19 Exit: 1.19
Gratings: 1200

Status
Spectral Acquisition[Emission]

2 Modify the parameters as required.

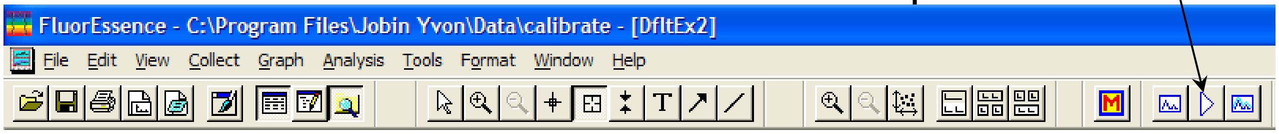
3 Click Run to run the experiment.

Auto Run Previous Experiment button ▷

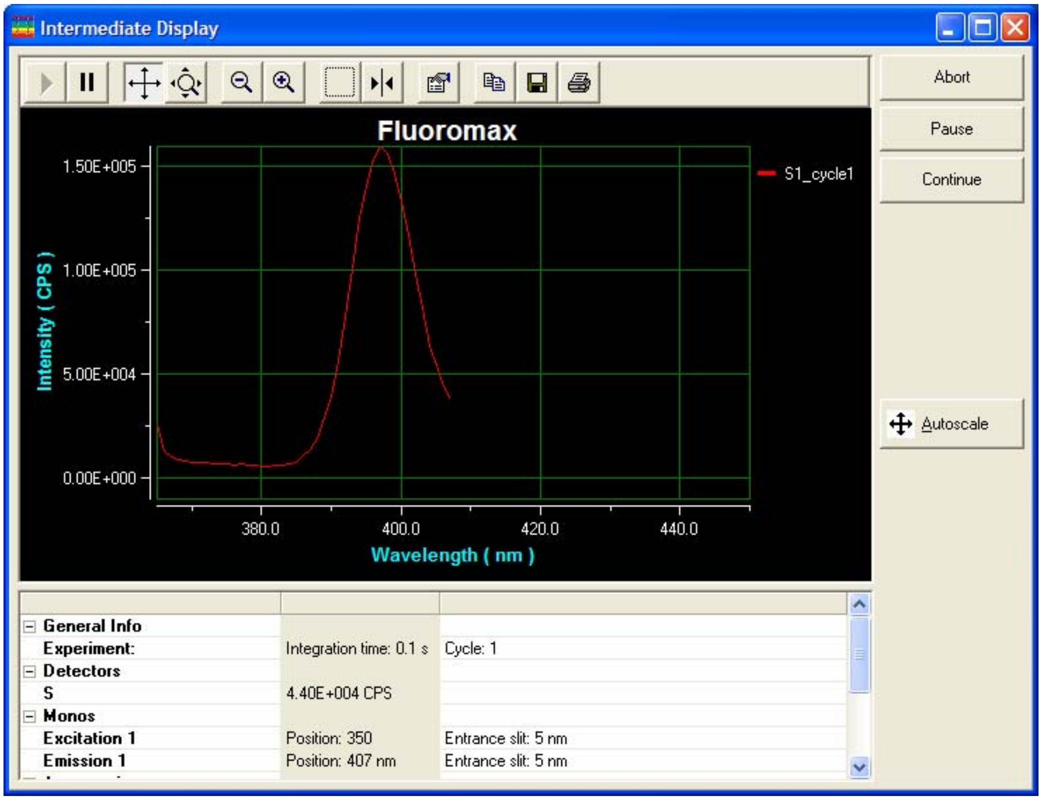
The Auto Run Previous Experiment button reruns the last experiment loaded without modifications.

***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.

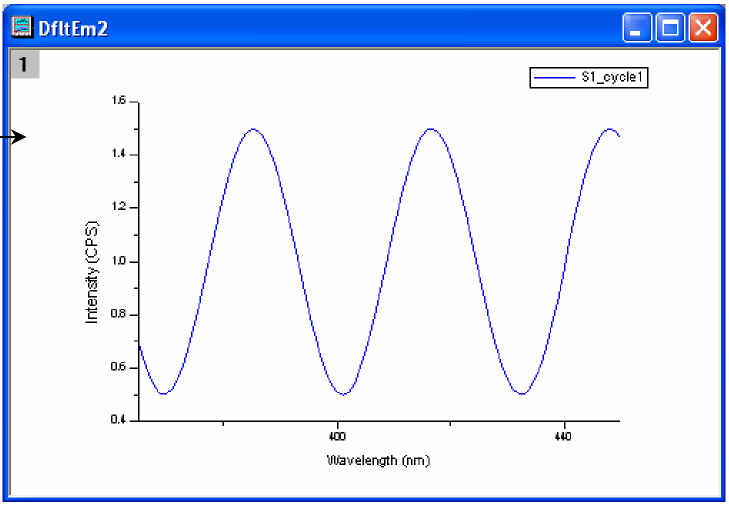

FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help

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


Intermediate Display
Abort
Pause
Continue
Autoscale
Fluoromax
S1_cycle1
1.50E+005
1.00E+005
5.00E+004
0.00E+000
Intensity (CPS)
380.0
400.0
420.0
440.0
Wavelength (nm)

General Info		
Experiment:	Integration time: 0.1 s	Cycle: 1
Detectors		
S	4.40E+004 CPS	
Monos		
Excitation 1	Position: 350	Entrance slit: 5 nm
Emission 1	Position: 407 nm	Entrance slit: 5 nm

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

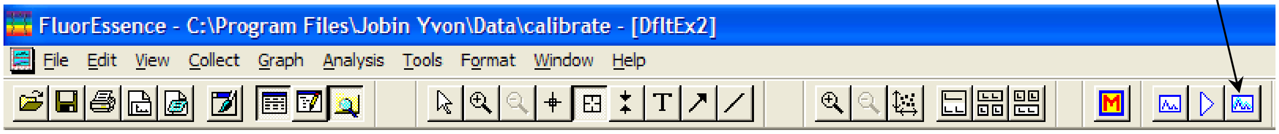

DfltEm2
1
S1_cycle1
1.6
1.4
1.2
1.0
0.8
0.6
0.4
Intensity (CPS)
400
440
Wavelength (nm)

Make Overlay File button

The Make Overlay File button creates an `*.SPC` file for use as an overlay file.

***Note:** The Make Overlay File button is active only with an active graph.*

2 Click the Auto Run Previous Experiment button in the toolbar.

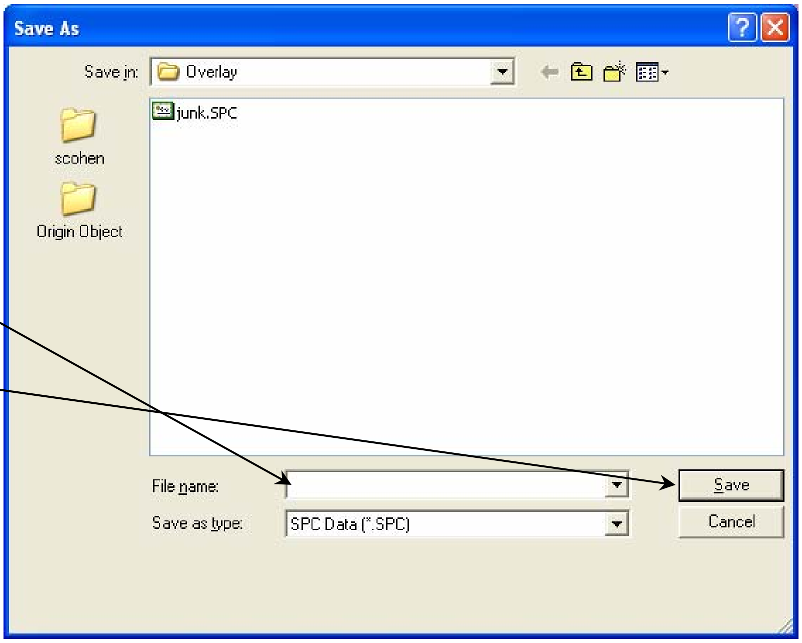

FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
T
M

The **Save As** window appears.

3 Enter a file name in the File name field.

4 Click Save.

The overlay file is saved.


Save As
junk.SPC
scohen
Origin Object
File name:
Save
Cancel

Save in: Overlay

Save as type: SPC Data (*.SPC)

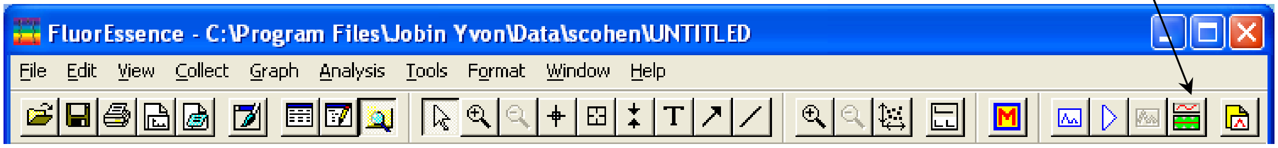
3D Scan to 3D Profile button

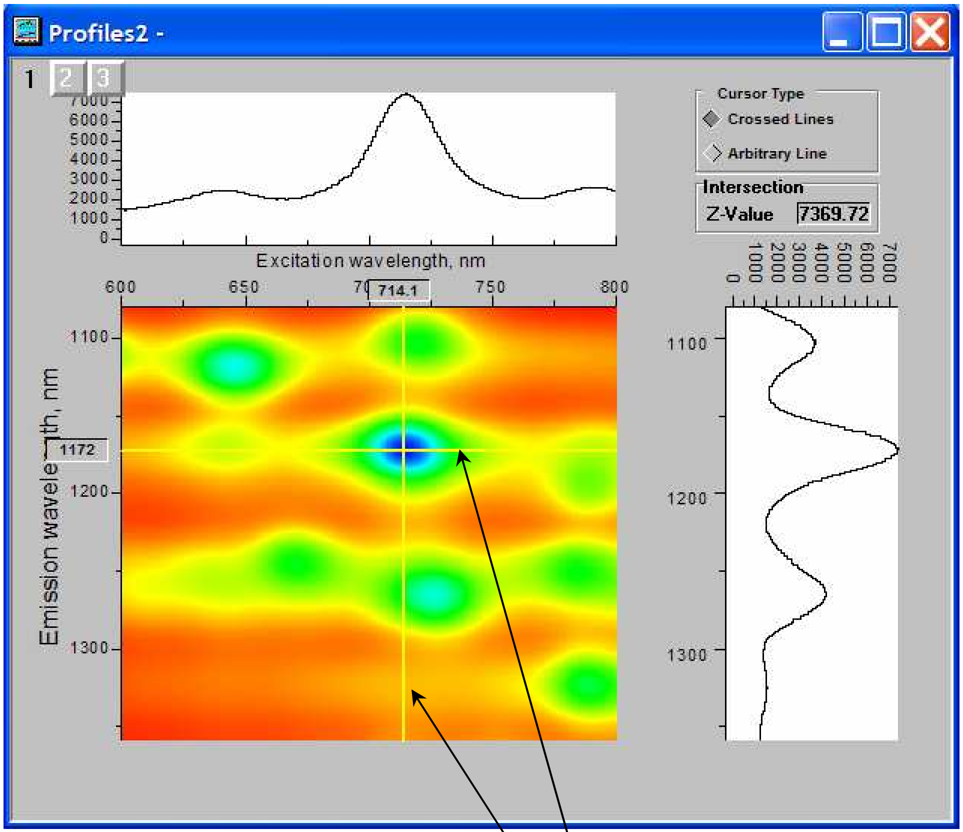
The 3D Scan to 3D Profile button extracts emission profiles from an excitation-emission matrix.

Note: *The 3D Scan to 3D Profile button only operates with excitation-emission matrix data.*

1 Open excitation-emission matrix data.

2 Click the 3D Scan to 3D Profile button in the toolbar.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
T


Profiles2 -
1
2
3
7000
6000
5000
4000
3000
2000
1000
0
Excitation wavelength, nm
600
650
70
714.1
750
800
1100
1172
1200
1300
Emission wavelength, nm
1100
1200
1300
0
1000
2000
3000
4000
5000
6000
7000

Cursor Type
◆ Crossed Lines
◇ Arbitrary Line

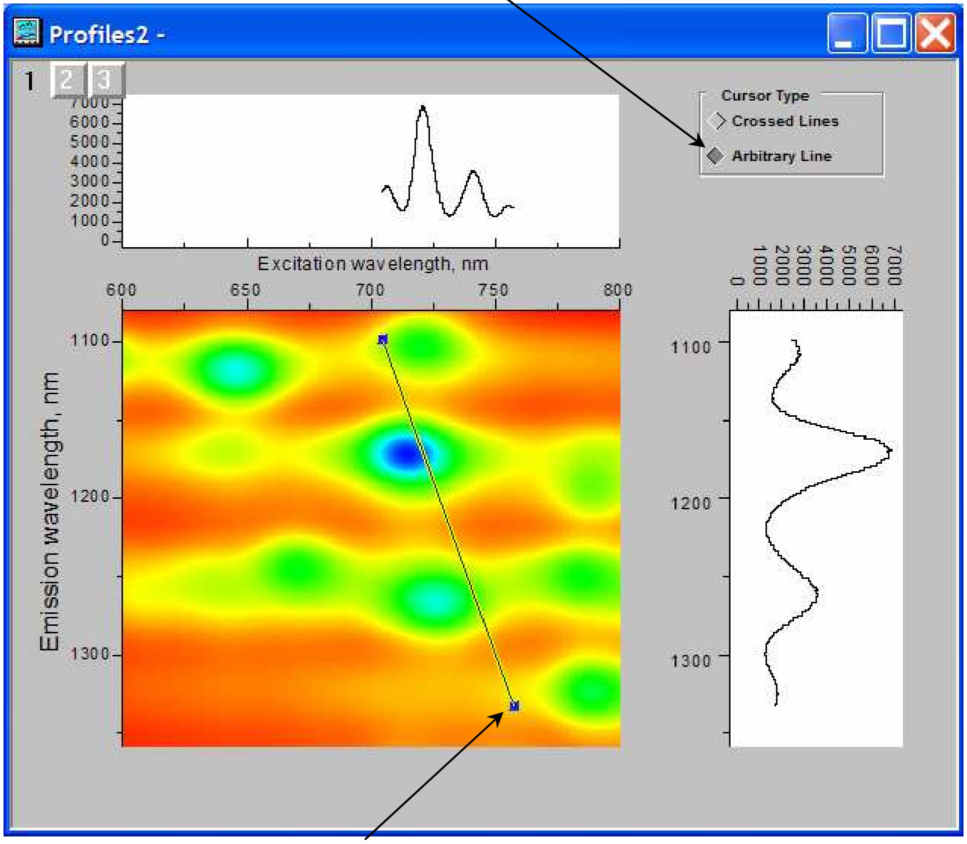
Intersection
Z-Value 7369.72

The **Profiles** window appears.

3 Grab and move the emission and excitation profile lines to see the profiles above and to the right of the data-matrix.

The Z-Value field shows the intensity where the excitation and emission profiles intersect.

4 Click the Arbitrary Line button to choose an arbitrary profile.


Profiles2 -
1
2
3
Cursor Type
Crossed Lines
Arbitrary Line
7000
6000
5000
4000
3000
2000
1000
0
Excitation wavelength, nm
600
650
700
750
800
Emission wavelength, nm
1100
1200
1300

Grab an end of the profile line and move to the desired location on the matrix. The profiles are updated.

5 To return to perpendicular profiles, click the Crossed Lines button.

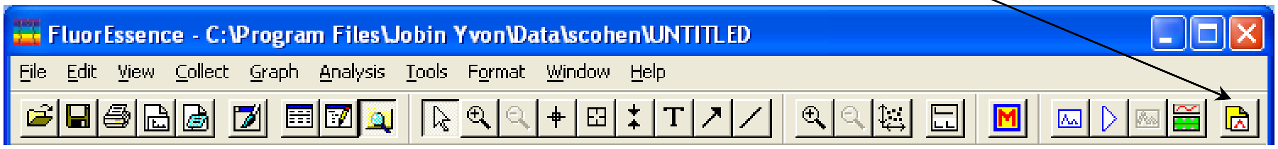
Get Peak Information button

The Get Peak Information button shows the full width at half-maximum for the chosen peak, plus other parameters for that peak.

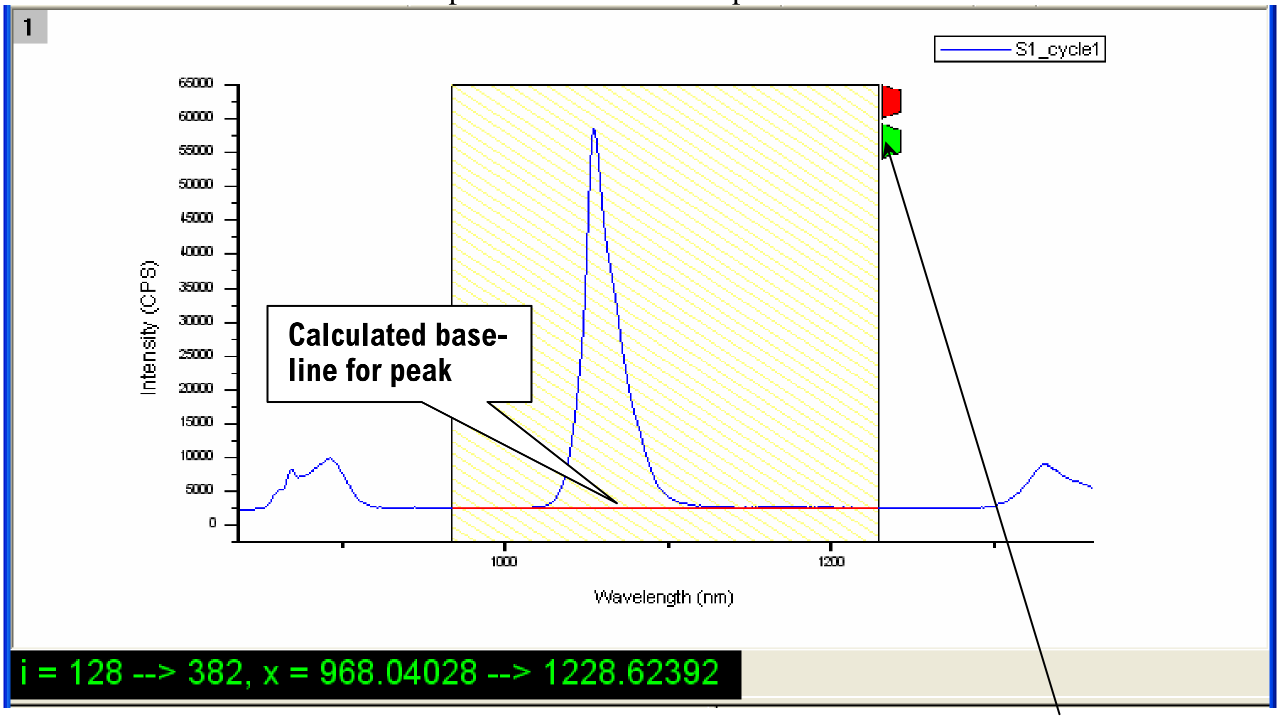
Note: *The Get Peak Information button only operates with an open data-set.*

1 Open a data-set.

2 Click the Get Peak Information button in the toolbar.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help

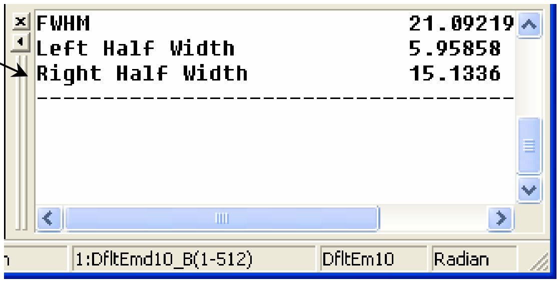
A shaded area with two tabs appears over the peak, and a red line calculates a baseline for the peak. Here is an example:


1
S1_cycle1
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Intensity (CPS)
1000
1200
Wavelength (nm)
Calculated base-line for peak

```
i = 128 --> 382, x = 968.04028 --> 1228.62392
```

3 Click the lower, green tab to calculate the parameters for the peak.

The information about the peak appears in the area at the lower right.



```
FWHM                         21.09219
Left Half Width               5.95858
Right Half Width             15.1336
---------------------------------------
```

1:DfltEmd10_B(1-512)
DfltEm10
Radian

4 Click the upper, red tab to remove the peak information.

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.

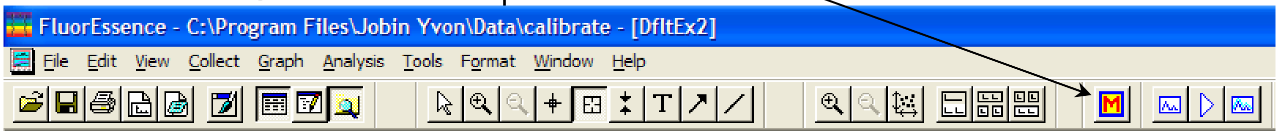
1 Find the preliminary emission maximum.

The object of this step is to acquire a preliminary emission scan, based on a "best-guess" excitation wavelength. 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.

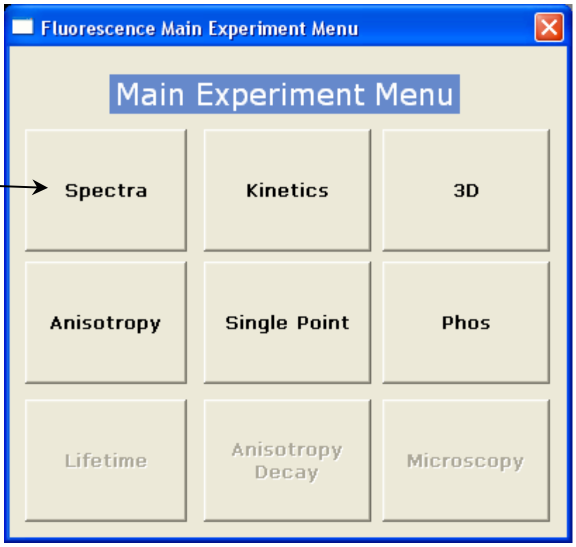
a Be sure all system components are on, and the FluoroMax®-3 is calibrated as explained in Chapter 3.

b Set up the sample with the fiber-optic probe, with minimal stray-light interference.

c Click the Experiment Menu button:


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File Edit View Collect Graph Analysis Tools Format Window Help
M

The **Fluorescence Main Experiment Menu** appears:

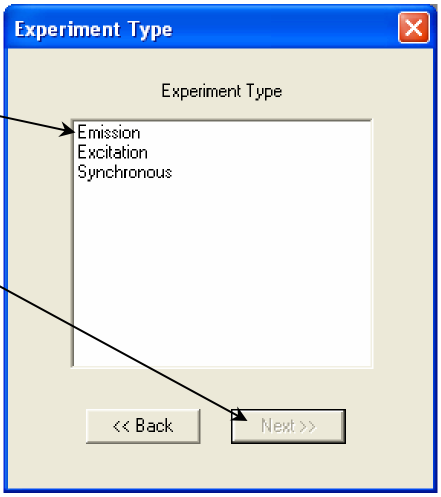

Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

d Choose Spectra.

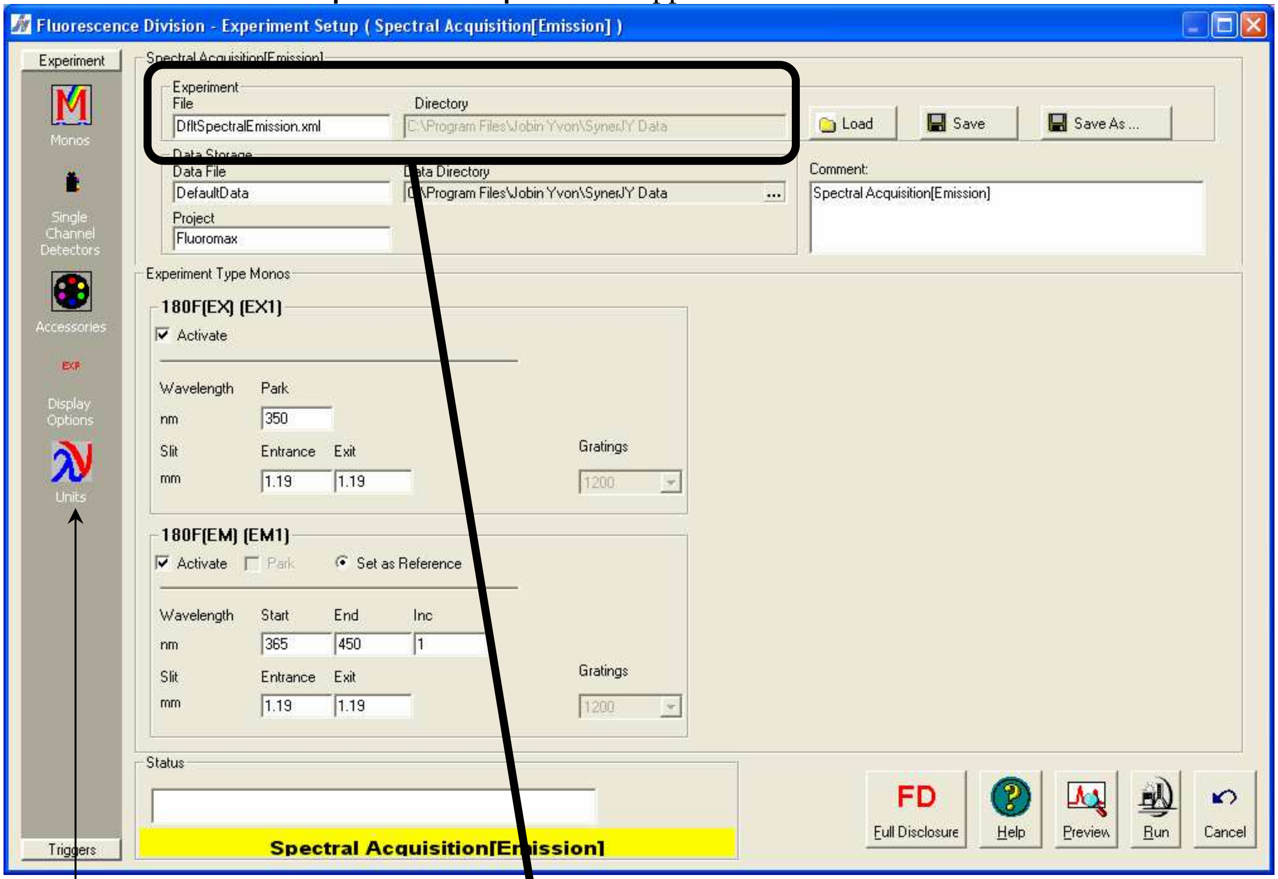
The **Experiment Type** menu appears.

e Choose Emission.

f Click Next >>.


Experiment Type
Experiment Type
Emission
Excitation
Synchronous
<< Back
Next >>

The **Experiment Setup** window appears:


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Single Channel Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
FD Full Disclosure
Help
Preview
Run
Cancel

Spectral Acquisition[Emission]

Experiment File: DfltSpectralEmission.xml

Directory: C:\Program Files\Jobin Yvon\SynerJY Data

Data Storage

Data File: DefaultData

Data Directory: C:\Program Files\Jobin Yvon\SynerJY Data

Project: Fluoromax

Comment: Spectral Acquisition[Emission]

Experiment Type Monos

180F[EX] [EX1]

☑ Activate

Wavelength nm Park: 350

Slit mm Entrance: 1.19 Exit: 1.19

Gratings: 1200

180F[EM] [EM1]

☑ Activate ☐ Park ● Set as Reference

Wavelength nm Start: 365 End: 450 Inc: 1

Slit mm Entrance: 1.19 Exit: 1.19

Gratings: 1200

Status

Spectral Acquisition[Emission]

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

h Verify that experimental parameters are correct.

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

i Set the scan parameters.

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 Jobin Yvon suggests an increment of 1.0 nm, an integration time of 0.1–0.5 s, and one scan. If unsure of an excitation wavelength, try 300 nm, at which many samples absorb light. Use S (signal detector) for the acquisition mode. Don’t forget a data file name.

> ***Note:*** *To minimize Rayleigh scatter, offset the start position by at least 15 nm from the excitation wavelength, with a bandpass of 5 nm. For example, for an excitation wavelength of 300 nm, use 315 nm as the start. Set the ending wavelength to 550 nm. Use an increment of 2 nm and an integration time of 0.1 s.*

j Insert the sample into the sample compartment, and close the sample-compartment’s cover.

k Click Run.

The scan starts.

l With the spectrum on the screen, note the greatest intensity.

If the signal exceeds 12 µA, then the emission detector is saturating, so close the slits more. If there is no obvious peak, increase the excitation wavelength, starting, and ending by 25 nm, and retry a scan.

m With an acceptable emission peak, record its wavelength.

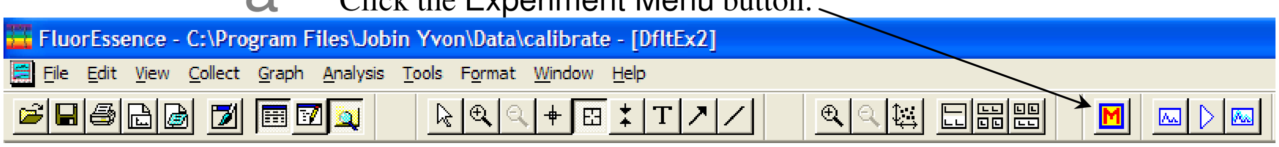
This is the emission maximum.

Otherwise, repeat steps i through l until an obvious emission peak appears.

2 Find the optimal excitation wavelength.

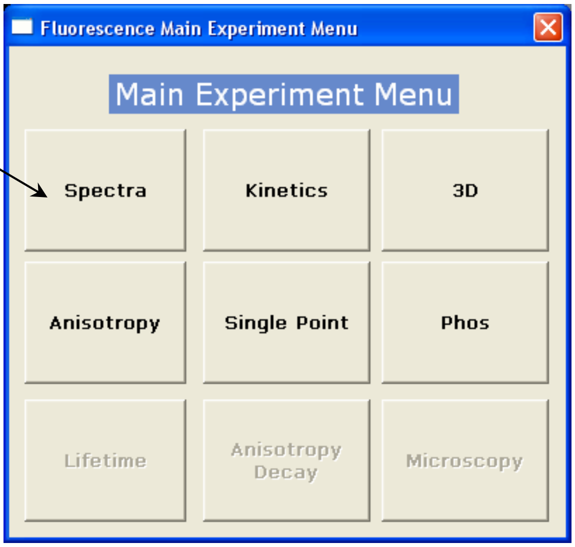
This procedure uses the emission maximum to determine the optimum excitation wavelength, and is similar to step 1.

a Click the Experiment Menu button:


FluorEssence - C:\Program Files\Jobin Yvon\Data\calibrate - [DfltEx2]
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
M

The **Fluorescence Main Experiment Menu** appears:

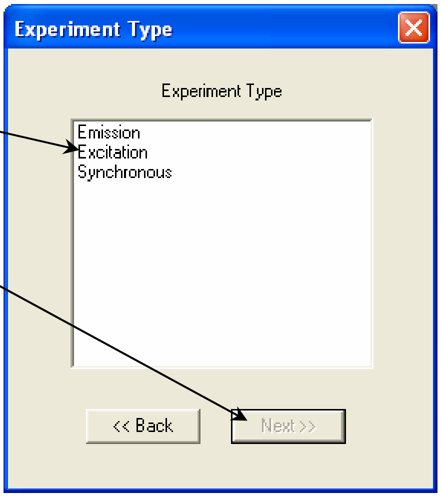
b Choose **Spectra**.


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

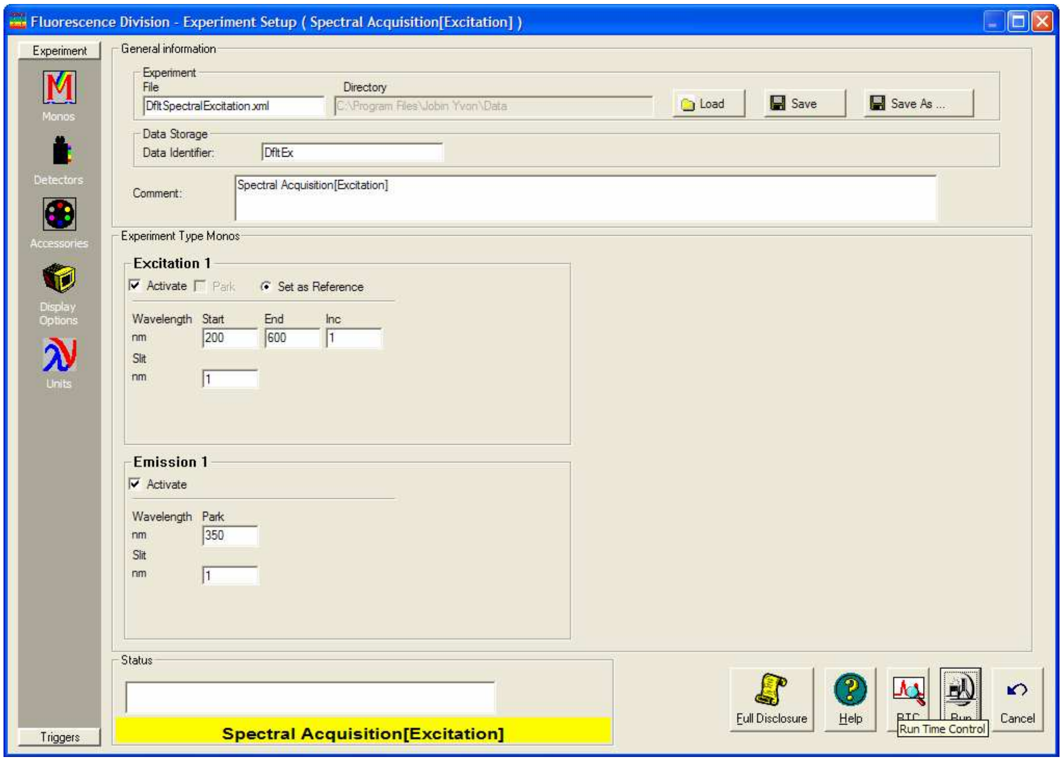
The **Experiment Type** menu appears.

c Choose **Excitation**.

d Click **Next >>**.


Experiment Type
Experiment Type
Emission
Excitation
Synchronous
<< Back
Next >>

The **Experiment Setup** window appears:


Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Experiment
Load
Save
Save As ...
Data Storage
Experiment Type Monos
Excitation 1
Emission 1
Status
Full Disclosure
Help
RTC
Run
Run Time Control
Cancel

File: DfltSpectralExcitation.xml
Directory: C:\Program Files\Jobin Yvon\Data
Data Identifier: DfltEx
Comment: Spectral Acquisition[Excitation]

Excitation 1
☑ Activate ☐ Park ● Set as Reference
Wavelength nm: Start 200, End 600, Inc 1
Slit nm: 1

Emission 1
☑ Activate
Wavelength nm: Park 350
Slit nm: 1

Spectral Acquisition[Excitation]

e Set the scan parameters.
Use the emission maximum determined above for the excitation, use 250 nm for starting, enter the emission maximum minus 15 nm for the end of the scan, and select two acquisition modes, S and S/R. S collects raw signal from the emission detector, and S/R ratioes the signal to the reference detector.

f Set excitation and emission slits identical to the emission scan.
Be sure that the emission scan did not exceed 20 µA in the emission scan.

g Enter the Experiment File name.

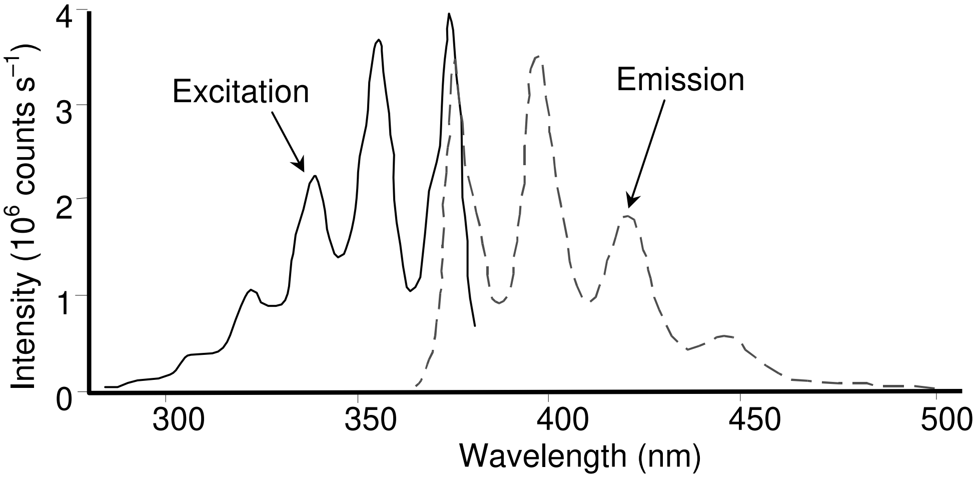
h Click Run.
The scan starts.
The resulting spectrum shows maximum excitation wavelength. If the raw S-channel signal > 20 µA, reduce slits, and rescan.

i Note the excitation peak.
This is the optimum excitation peak position.

3 Find the optimal emission peak.

a Use the optimum excitation wavelength determined in step 2.

b When complete, both excitation and emission peaks are found.
Optimized excitation and emission spectra of a 1×10^{-8}-*M* anthracene solution are shown below. Because the acquisition modes were different for the excitation and emission scans, the data intensity had to be normalized. After normalization, the excitation and emission scans are virtually mirror images of one another.



Normalized excitation and emission spectra of a 1 × 10^{-8}-*M* anthracene solution.

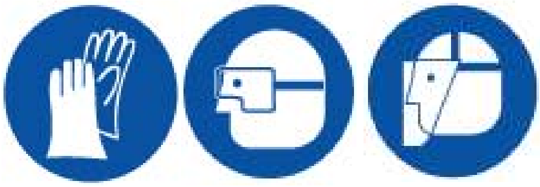
5: Optimizing Data

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 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

Warning: *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 Jobin Yvon Inc. 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 Jobin Yvon recommends a capillary cell such as our 50-µL or 250-µL optional micro-sample capillary cells, which are specifically designed for a small volume. A 1-mL cell (5 mm × 5 mm cross-section) is also available.

Solid samples

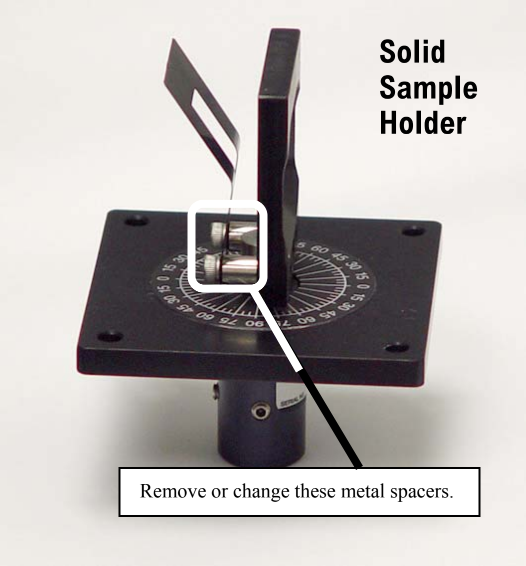
Solid samples usually are mounted in the Model 1933 Solid Sample Holder, with the fluorescence collected from the front surface of the sample. The mounting method depends on the form of the sample. See the section on “Highly opaque samples” for more information on sample arrangement in the sample compartment.

- 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.



Remove or change these metal spacers.

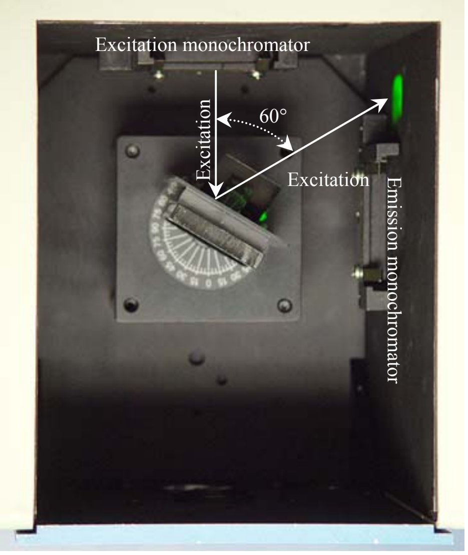
Biological samples

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.

Running a scan on a sample

Precautions with the Solid Sample Holder

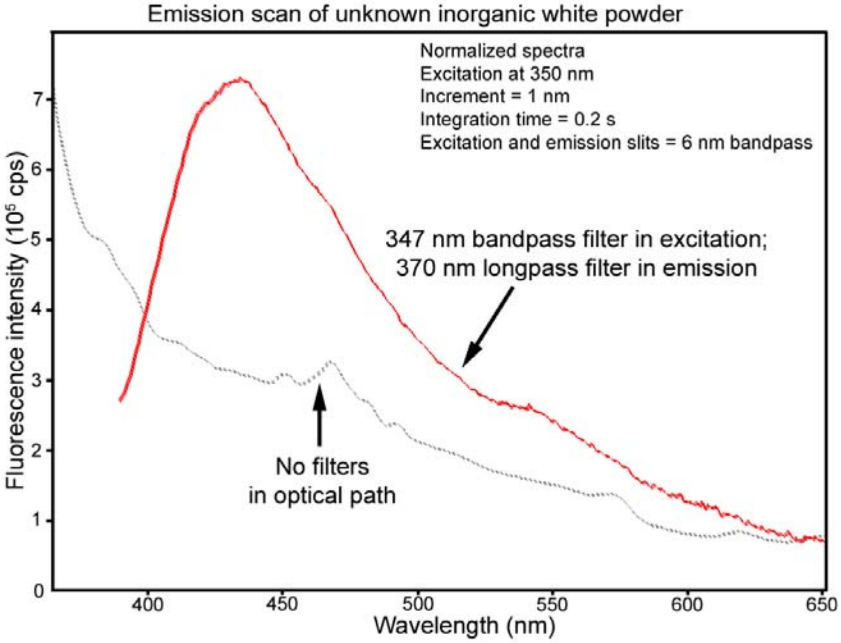
Avoid placing the front face of the sample so that the excitation beam is reflected directly into the emission monochromator. If the sample is rotated at 45° from excitation, this may occur, increasing interference from stray light. Instead, set up the sample with a 30° or 60°-angle to the excitation, 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: *Always remember that the focal point of the excitation beam must be on the sample itself.*

Use filters in the optical path.

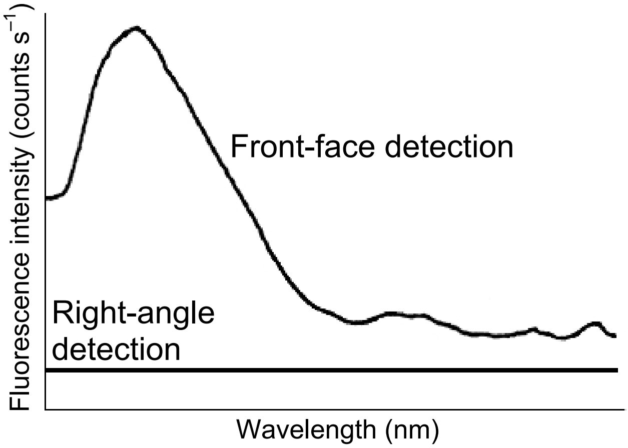
Stray light from the excitation beam can interfere with the emission from the sample. To reduce the deleterious effects of stray light, place a filter that removes excitation wavelengths from the emission beam in the emission optical path. Here is an example of scans with and without filters on a FluoroMax®-3, using an unknown white powder as the sample. A 347 nm band-pass filter allows only the desired excitation to reach the sample, while a long-pass filter in the emission side lets only fluorescence, and no stray excitation into the detector. Notice how the shape of the spectrum changes drastically when filters are added.



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, set 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.

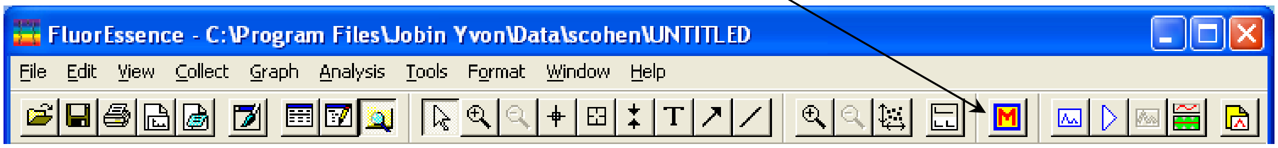


Comparison of fluorescence emission signal from sickle-cell hemoglobin using right-angle versus front-face detection. The β-37 tryptophan is primarily responsible for this fluorescence.

Measuring the *G* factor

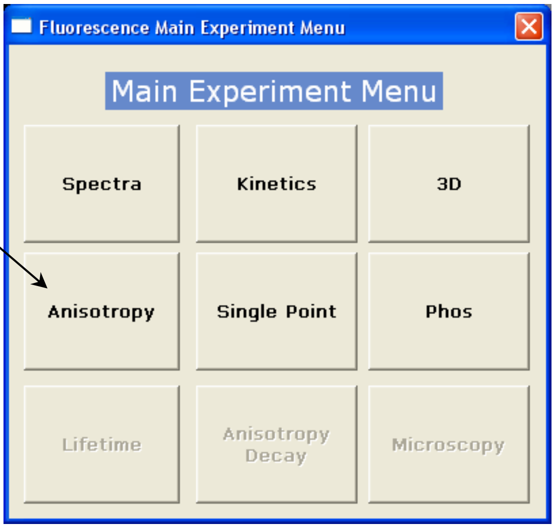
Include the grating factor, or *G* factor, whenever polarization measurements are taken. The *G* factor corrects for variations in polarization wavelength-response for the emission optics and detectors. A pre-calculated *G* factor may be used when all other experimental parameters are constant. In other 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 menu, click the Experiment Menu button.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help

The **Fluorescence Main Experiment Menu** appears.

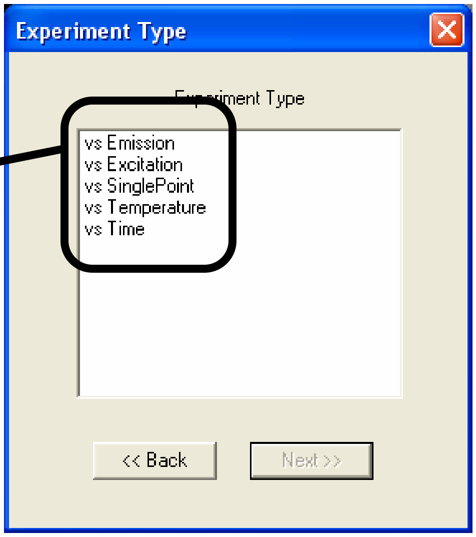
2 Click Anisotropy.


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

The **Experiment Type** window appears.

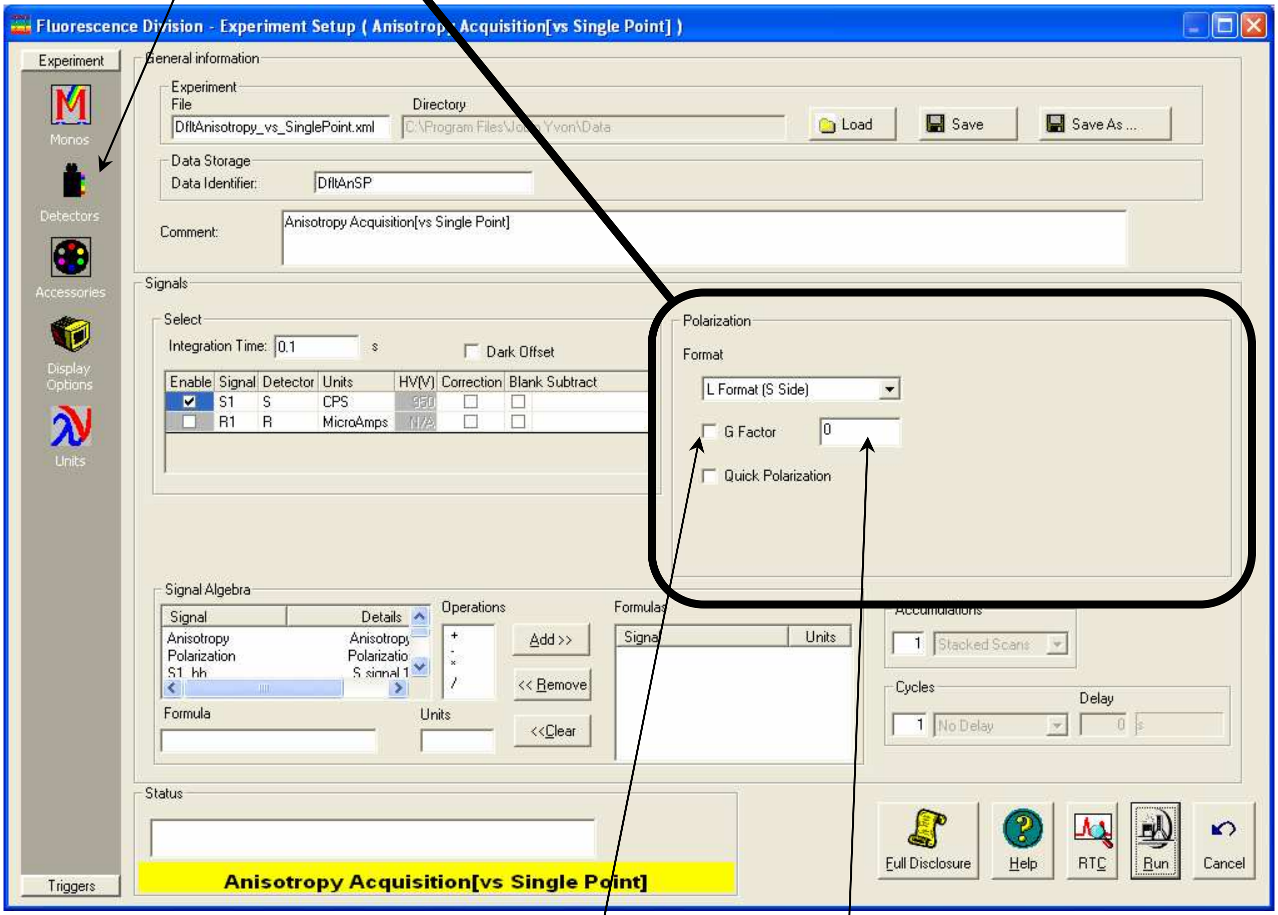
3 Choose the type of Anisotropy experiment.

The **Experiment Setup** window opens. To determine polarization at particular excitation/emission wavelength-pairs, choose vs SinglePoint.


Experiment Type
Experiment Type
vs Emission
vs Excitation
vs SinglePoint
vs Temperature
vs Time
<< Back
Next >>

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

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



Fluorescence Division - Experiment Setup (Anisotropy Acquisition[vs Single Point])

General information

Experiment File: DfltAnisotropy_vs_SinglePoint.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage

Data Identifier: DfltAnSP

Comment: Anisotropy Acquisition[vs Single Point]

Signals

Integration Time: 0.1 s

☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☑	S1	S	CPS	950	☐	☐
☐	R1	R	MicroAmps	N/A	☐	☐

Polarization

Format: L Format (S Side)

☐ G Factor 0

☐ Quick Polarization

Signal Algebra

Signal	Details
Anisotropy	Anisotropy
Polarization	Polarizatio
S1_hh	S signal 1

Accumulations: 1 Stacked Scans

Cycles: 1 No Delay

Delay: 0 s

Status

Anisotropy Acquisition[vs Single Point]

Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Select
Operations
+
-
*
/
Add >>
<< Remove
<<Clear
Formula
Units
Formulas
Signal
Units
Full Disclosure
Help
RTC
Run
Cancel

2 **Click the G Factor checkbox to include a *G* factor in your measurements.**

3 **Enter a value for the *G* factor in the field if you want to use a pre-determined value. To automatically measure the *G* factor, use the default value of 0.**

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

Note: *For detailed information on the* G *factor, see the* Polarizers Operation Manual*.*

Improving the signal-to-noise ratio

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

The quality of acquired data is determined largely 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* ratio and the advantages and disadvantages of 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,

$$\begin{aligned} S / N &= t^{1/2} \\ &= 1^{1/2} \\ &= 1 \end{aligned}$$

For an integration time of 2 seconds,

$$\begin{aligned} S / N &= t^{1/2} \\ &= 2^{1/2} \\ &\approx 1.414 \end{aligned}$$

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:

a Find the maximum fluorescence intensity by acquiring a preliminary scan, using an integration time of 0.1 s and a bandpass of 5 nm.

b From this preliminary scan, note the maximum intensity, and select the appropriate integration time from the table below.

Signal intensity (counts per second)	**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

***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 integration time through **Experiment Setup** for a specific experiment, or **Real Time Control** to view the effects of different integration times on a spectrum. See the FluorEssence™ on-line help to learn more about setting the integration time.

Scanning a sample multiple times

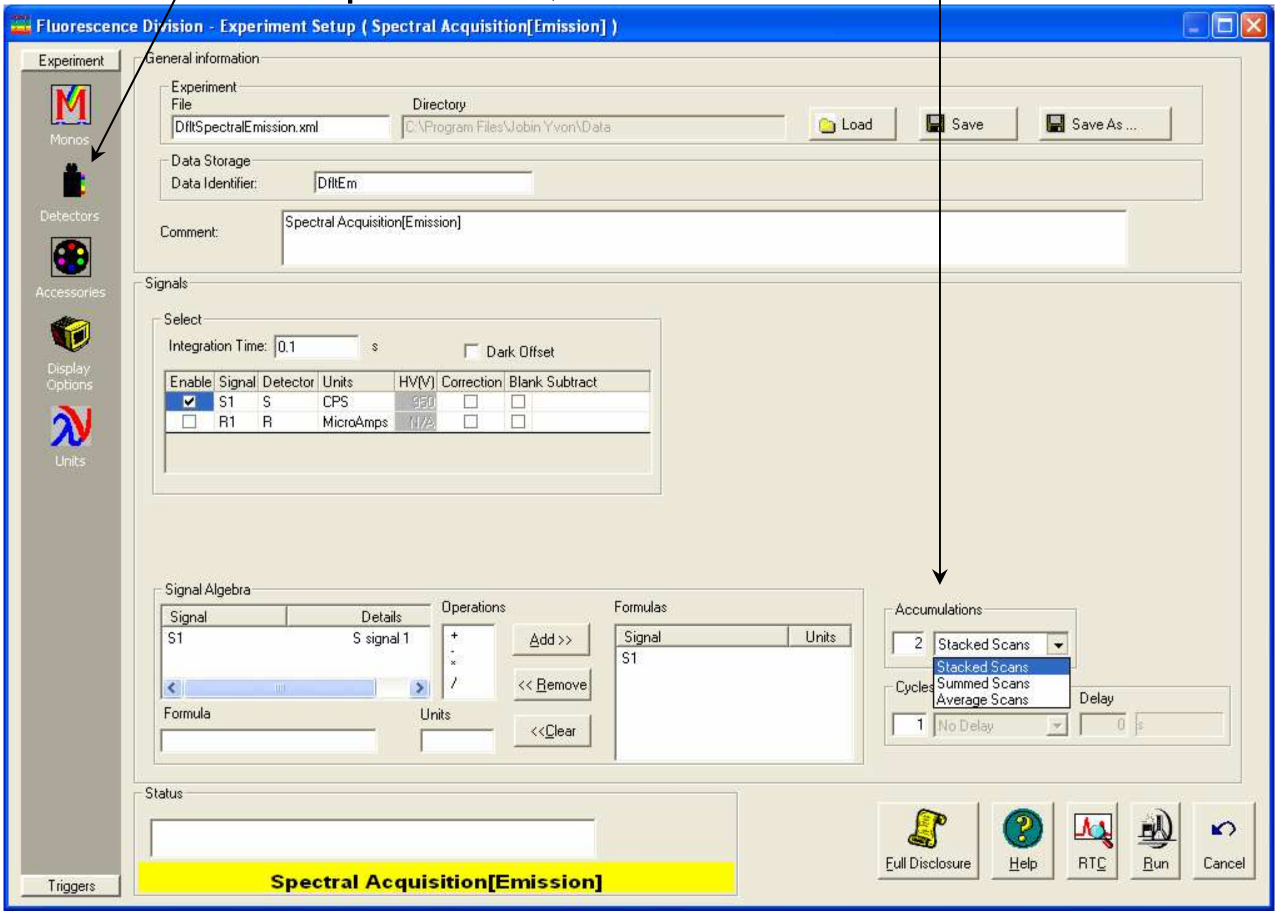
Scanning a sample more than once, and averaging the scans together, enhances the *S*/*N*. In general, the *S*/*N* improves by $n^{1/2}$, where n is the number of scans.

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.


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Experiment
Load
Save
Save As ...
Data Storage
Signals
Select
Signal Algebra
Operations
+
-
×
/
Add >>
<< Remove
<<Clear
Formulas
Status
Full Disclosure
Help
RTC
Run
Cancel

File: DfltSpectralEmission.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Identifier: DfltEm

Comment: Spectral Acquisition[Emission]

Integration Time: 0.1 s

☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☑	S1	S	CPS	950	☐	☐
☐	R1	R	MicroAmps	N/A	☐	☐

Signal	Details
S1	S signal 1

Formula:

Units:

Signal	Units
S1	

Accumulations: 2 Stacked Scans

Stacked Scans
Summed Scans
Average Scans

Cycles: 1 No Delay

Delay: 0 s

Spectral Acquisition[Emission]

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

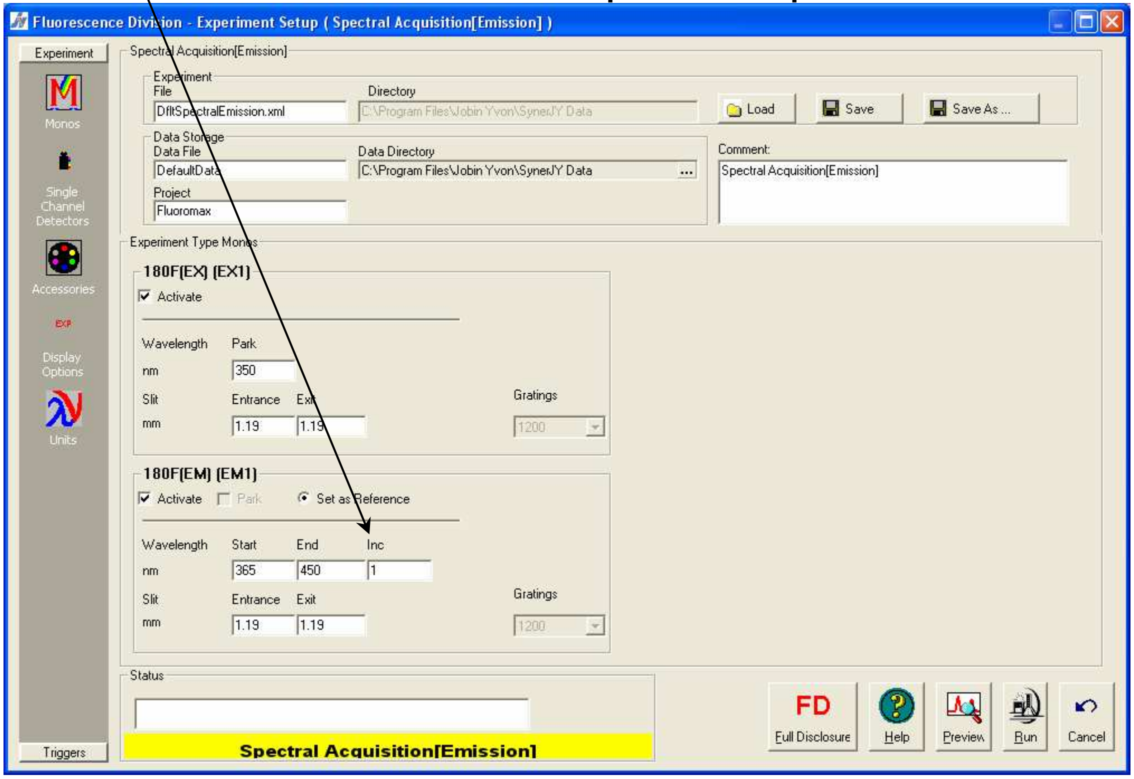
Using the appropriate wavelength increment

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 photobleaching of the sample. 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 the react or degrade. Time increments often range from 0.1–20 s.

Set increments under the Monos icon in the **Experiment Setup** window.


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])

Spectral Acquisition[Emission]

Experiment
File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\SynerJY Data

Data Storage
Data File: DefaultData
Data Directory: C:\Program Files\Jobin Yvon\SynerJY Data
Project: Fluoromax
Comment: Spectral Acquisition[Emission]

Experiment Type Monos

180F(EX) (EX1)
☑ Activate
Wavelength nm: Park 350
Slit mm: Entrance 1.19 Exit 1.19
Gratings: 1200

180F(EM) (EM1)
☑ Activate ☐ Park ● Set as Reference
Wavelength nm: Start 365 End 450 Inc 1
Slit mm: Entrance 1.19 Exit 1.19
Gratings: 1200

Status
Spectral Acquisition[Emission]

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:

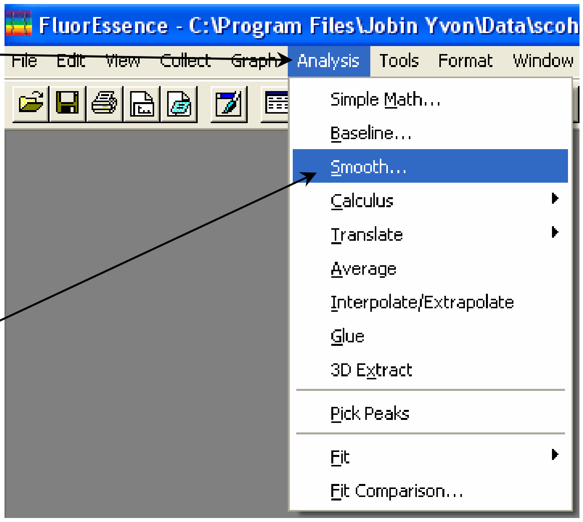
$$\text{bandpass (nm)} = \text{slit width (mm)} \times \text{dispersion (nm/mm)}$$

A FluoroMax®-3, which has a single-grating monochromator and 1200 grooves/mm gratings, has a dispersion of 4.25 nm/mm.

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®.

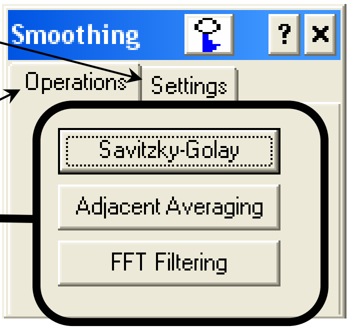

FluorEssence - C:\Program Files\Jobin Yvon\Data\scoh
File Edit View Collect Graph Analysis Tools Format Window
Simple Math...
Baseline...
Smooth...
Calculus
Translate
Average
Interpolate/Extrapolate
Glue
3D Extract
Pick Peaks
Fit
Fit Comparison...

1 Select Analysis from the main FluorEssence™ menu.

A drop-down menu appears.

2 Choose Smooth....

The **Smoothing** dialog box opens.


Smoothing
Operations
Settings
Savitzky-Golay
Adjacent Averaging
FFT Filtering

3 Under the Settings tab, choose the appropriate parameters.

4 Under the Operations tab, choose FFT Filtering (fast-Fourier transform), Adjacent Averaging, or Savitsky-Golay smoothing.

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 fluorescent or phosphorescent properties of a sample depends upon several factors:

- Equipment specifications
- Sample characteristics
- Timing considerations.

To ensure that the spectra collected indicate the actual properties of the sample and not external conditions, data often must be corrected. To *correct* data means to subtract information from the data not directly related to the properties of the sample. Gratings, detectors, and other 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 accurate intensity comparisons, such as those required for quantum-yield determinations, response characteristics must be eliminated. Supplied with the instrument are sets of excitation and emission correction factors to eliminate response characteristics. These files[1], `xcorrect` and `mcorrect`, are included with the software.

Note: *The excitation range is 240–600 nm; the emission range is 290–850 nm.*

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

To use radiometric correction factors, either:

- Select the ones supplied with the program, or
- Select a set generated at your facility during or after acquisition, discussed in the following section. Acquiring radiometric correction factors is explained in Chaper 8: *Producing Correction Factors.*

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

---

[1] Filenames include a three-letter extension. For the sake of clarity, we have omitted the extensions in this section. Refer to the software manual for specifics regarding extensions.

During acquisition

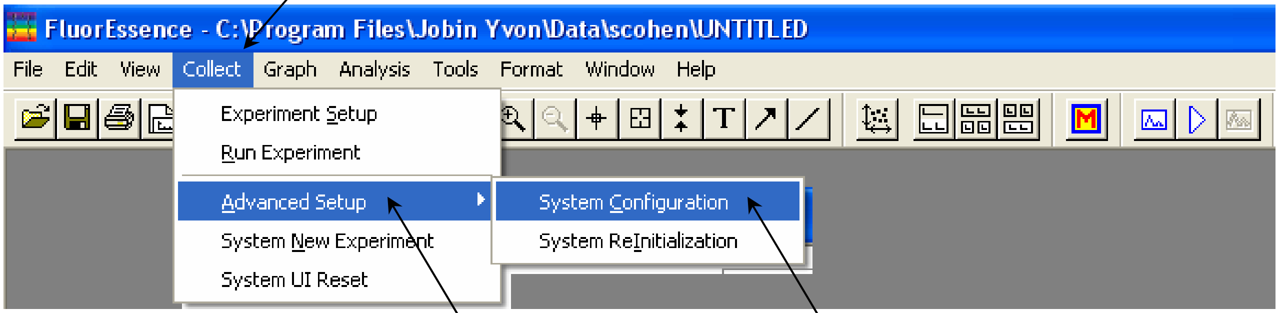
Data can be acquired either as raw data or as corrected data. A spectrum composed of raw data exhibits the effects of system parameters, while a corrected spectrum displays only the properties related to the sample.

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

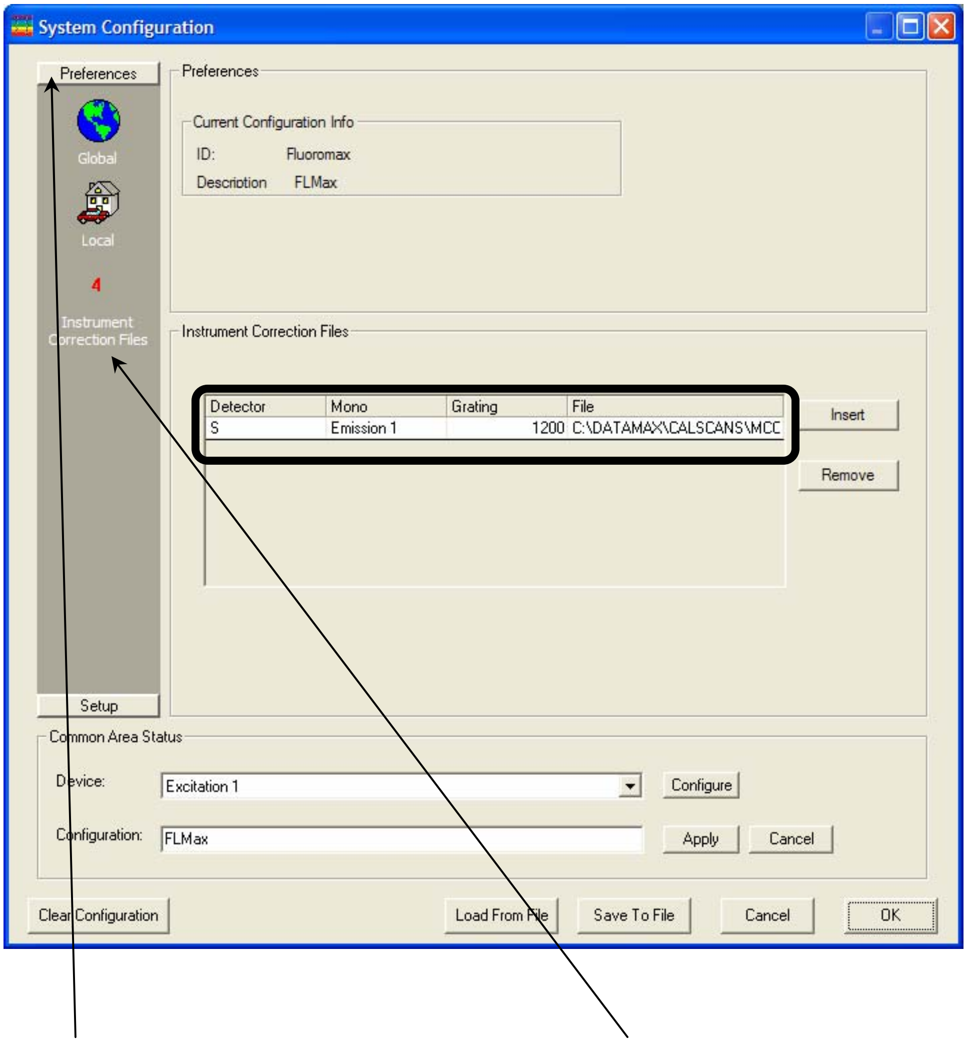
Caution: *The `mcorrect`, `xcorrect`, and `tcorrect` files are custom-generated at the factory for each particular instrument, and cannot be swapped.*

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.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
Help
Experiment Setup
Run Experiment
Advanced Setup
System New Experiment
System UI Reset
System Configuration
System ReInitialization

b Choose **Advanced Setup**, then **System Configuration**.
The **System Configuration** window appears.


System Configuration
Preferences
Global
Local
4
Instrument Correction Files
Setup
Insert
Remove
Configure
Apply
Cancel
Clear Configuration
Load From File
Save To File
Cancel
OK

Preferences

Current Configuration Info

ID: Fluoromax

Description FLMax

Instrument Correction Files

Detector	Mono	Grating	File
S	Emission 1	1200	C:\DATAMAX\CALSCANS\MCC

Common Area Status

Device: Excitation 1

Configuration: FLMax

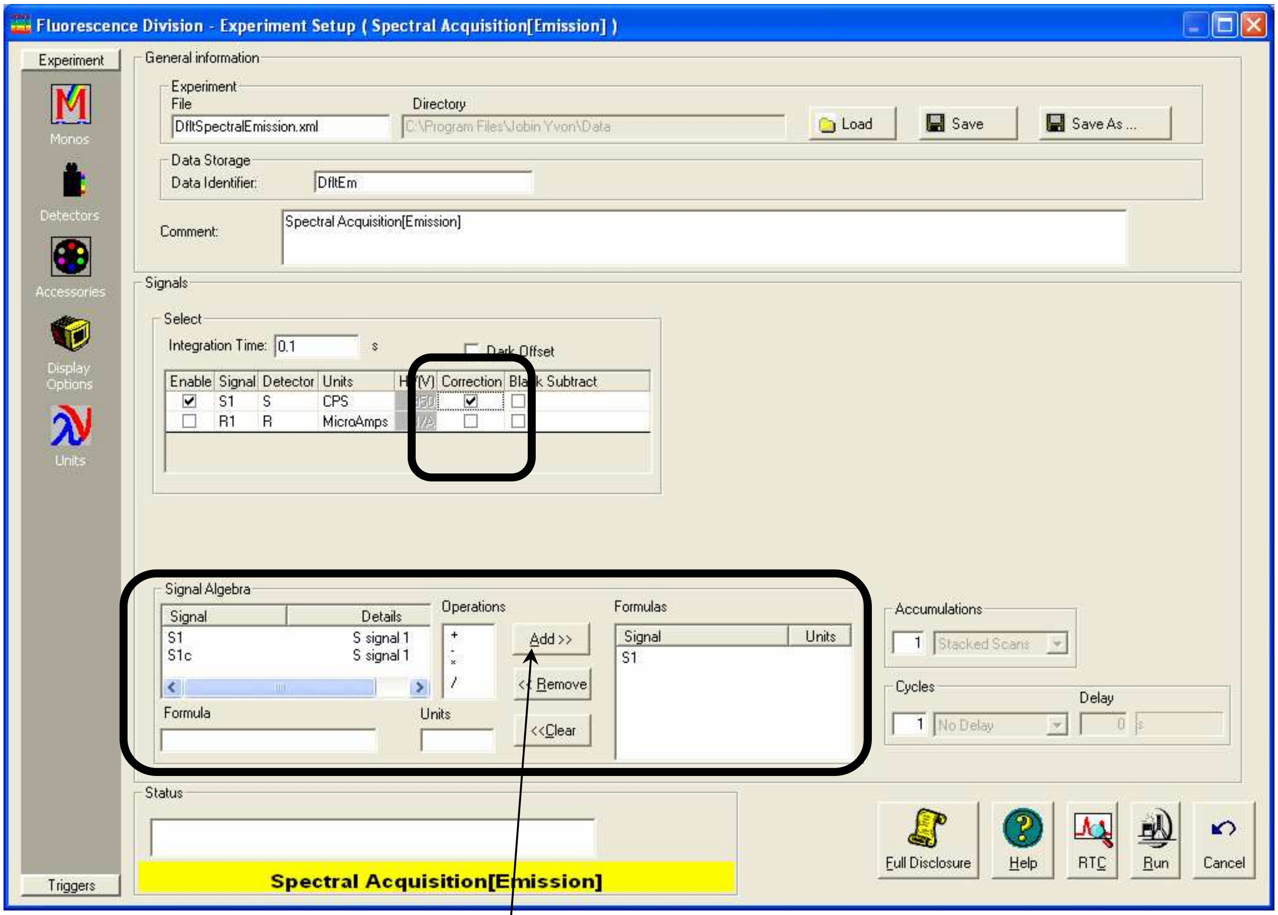
c Choose Preferences, then the Instrument Correction Files icon. The Instrument Correction Files area should display a correction file for the Detector (S or R). If not, click the Insert button, and browse for the desired correction file.

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:


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Experiment
Load
Save
Save As ...
Data Storage
Signals
Select
Signal Algebra
Operations
+
-
*
/
Add >>
<< Remove
<<Clear
Formulas
Accumulations
Stacked Scans
Cycles
Delay
No Delay
Status
Full Disclosure
Help
RTC
Run
Cancel

File: DfltSpectralEmission.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Identifier: DfltEm

Comment: Spectral Acquisition[Emission]

Integration Time: 0.1 s

☐ Dark Offset

Enable	Signal	Detector	Units	H (V)	Correction	Bla k Subtract
☑	S1	S	CPS	50	☑	☐
☐	R1	R	MicroAmps	/A	☐	☐

Signal	Details
S1	S signal 1
S1c	S signal 1

Formula:

Units:

Signal	Units
S1	

Accumulations: 1

Cycles: 1

Delay: 0 s

Spectral Acquisition[Emission]

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

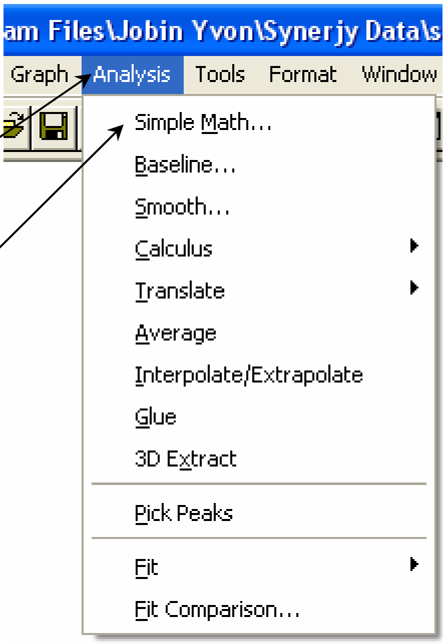
The corrected signal appears in the Formulas table.

d Run the experiment with the corrected signal.

After acquisition

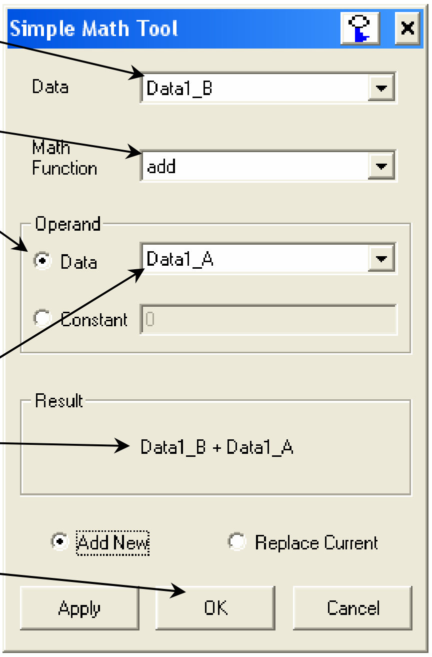
To apply the correction factors after the data have been acquired, multiply the data file by the appropriate correction factor file (`mcorrect` for the S detector or `xcorrect` for the R detector).

1. Make sure the graph is open and trace to be corrected is active in the main **FluorEssence** window.
2. Choose Analysis.
   A drop-down menu appears.
3. Select Simple Math.
   This opens the **Math Functions** dialog box. The chosen trace should appear in the Data field. If not, browse for it with the down arrow.


am Files\Jobin Yvon\Synerjy Data\s
Graph
Analysis
Tools
Format
Window
Simple Math...
Baseline...
Smooth...
Calculus
Translate
Average
Interpolate/Extrapolate
Glue
3D Extract
Pick Peaks
Fit
Fit Comparison...

4. Select multiply.
5. Choose the Data radio button in the Operand area.
6. Browse for the appropriate correction-factor file (`mcorrect` or `xcorrect`).
   The proper formula should appear in the Result area.
7. Click OK.

The trace that appears on the screen is a result of the mathematical operation, giving a corrected spectrum.


Simple Math Tool

Data: Data1_B

Math Function: add

Operand

● Data: Data1_A

○ Constant: 0

Result

Data1_B + Data1_A

● Add New ○ Replace Current

Apply
OK
Cancel

6: Maintenance

Introduction

The FluoroMax®-3 requires little maintenance. To remove dust and fingerprints, wipe the outside panels with a clean, damp cloth. The lamp is the only component that must be replaced routinely. Regular examination of the xenon-lamp scan and water Raman spectrum serves as early indicators of the system's integrity. See Chapter 3 for these tests.

Lamp replacement

When to replace the lamp

Obtaining good spectral results depends on the xenon lamp. Keep track of lamp usage with the hour meter. After 1200–1500 h of use, the lamp output decreases significantly, indicating that the lamp should be replaced. A new lamp produces a peak intensity of 300 000 cps for a water-Raman scan; when the current lamp's output drops below 100 000 cps, replace it. Replacing the lamp within the recommended time may prevent a catastrophic failure. Each time the lamp is turned on constitutes one full hour of use. Therefore, HORIBA Jobin Yvon Inc. suggests leaving the lamp on during brief periods of inactivity.

Parts and tools required

Xenon lamp

The replacement xenon lamp is packed in the manufacturer's box. Read all instructions and precautions before removing the lamp from the protective cover, and inserting it into the FluoroMax®-3.

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

Phillips screwdriver

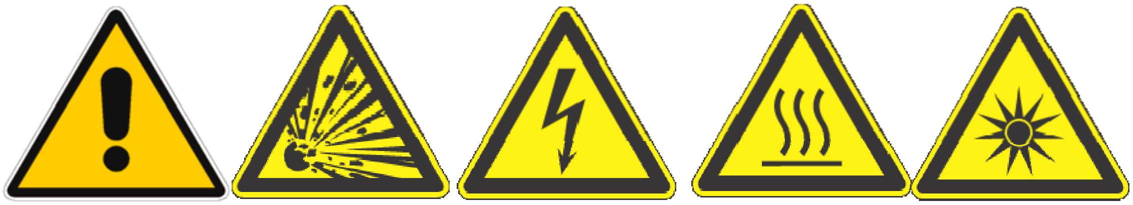
3/32" Allen key

7/64" Allen key

1/8" Allen key

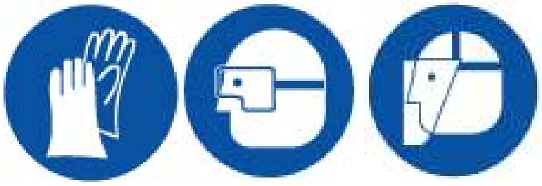
9/64" Allen key

Warning: *Xenon lamps, by nature, are an explosion hazard. Be sure that the power is off, and all AC (mains) power is disconnected from the system. Read and follow all the cautions 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.

Changing the lamp

1 Switch off and prepare the FluoroMax®-3.

a Be sure that the FluoroMax®-3 and the host computer are turned off.

b Remove the AC (mains) power cord from the FluoroMax®-3.

c Disconnect the RS-232 cable, optional trigger-box cable, power cord, and any other cables attached to the spectrofluorometer.

2 Remove the sample mount from the front of the FluoroMax®-3.

a Remove the four Phillips screws that secure the sample mount to the instrument.

b Gently slide the sample mount out of the instrument.

Some sample mounts have a 15-pin connector at the inside end for automated accessories.

3 Remove the instrument cover.

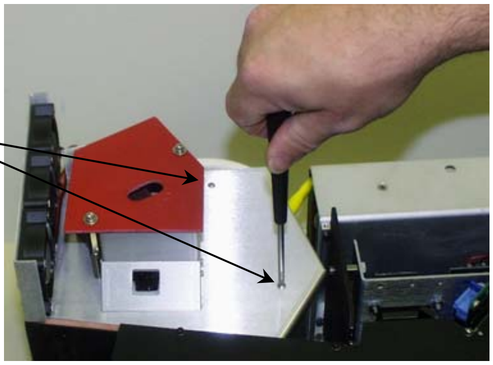
a Remove the seven Phillips screws (two on each side, and three in the back) from the cover.

b Lift the cover vertically off the instrument by grasping opposite corners of the cover and raising upward.

If the cover sticks, gently work each side upward until the cover slides smoothly off.

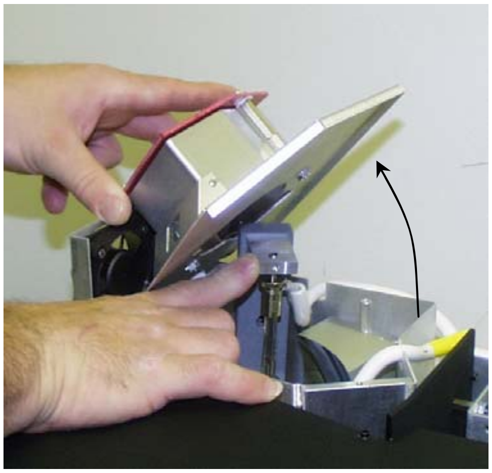
4 Remove the lamp-housing cover at the rear of the instrument.

a Remove the two Phillips screws from the lamp-housing cover.



b Gently rotate the lamp-housing cover with its cooling fans attached.

c Rotate the cover backwards, and set it behind the instrument so that electrical connections are not strained.



The lamp is held in place by spring tension and the height adjustment on top of the lamp. The anode and cathode connections are attached to the lamp via thumbscrews on top and bottom of the lamp.

5 Prepare the replacement lamp.

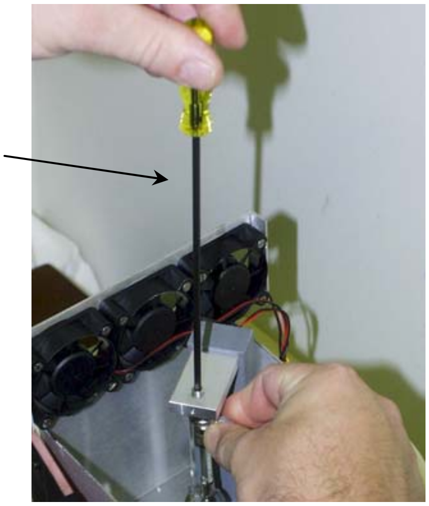
a Place the new xenon lamp (still in its protective cover) on top of the excitation monochromator.

b Remove the top half of the new lamp's protective cover.

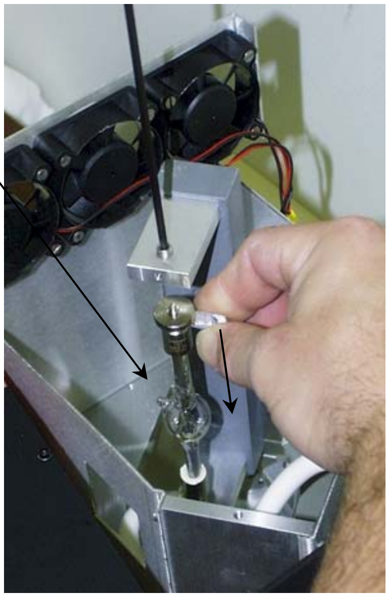
Keep this top half handy for later.

6 Remove the old xenon lamp.

a While holding the metal anode (top) portion of the lamp, loosen the height adjustment above the lamp with a 1/8" Allen key, until the lamp is removable.



b Press down against the spring action. Notice how the nipple faces away from the collection mirror (not visible here behind the hand).

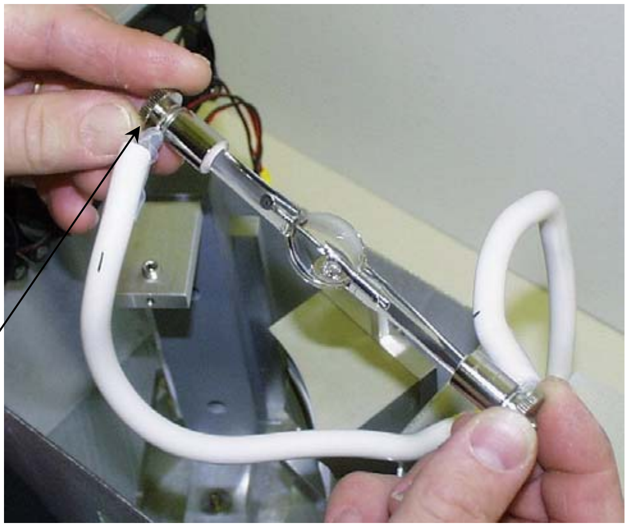


Caution: *Improper connections to the lamp severely affect lamp performance and affect the power supply. Carefully note the anode and cathode connections to the lamp. The anode (+) is on top; the cathode (–) is on the bottom. The nipple on the lamp's glass envelope marks the anode (+) side.*

c Gently tilt the lamp away from the top post.

d Lift the lamp out of the lamp housing far enough to remove the anode and cathode connections.

e Remove the thumbscrew at the anode end (top) of the lamp, leaving the post ex-



posed.

f Remove the anode cable.

g Remove the thumbscrew at the cathode end (bottom) of the lamp, leaving the post exposed.

h Remove the cathode cable.

7 Place the old lamp in the top protective cover from the new lamp.

8 Put the old lamp (in the top cover) in a safe place.

Warning: *Do not touch any portion of the lamp except the metal cathode and anode.*

9 Attach connections to the new lamp.

a Attach the cathode connection to the new lamp.

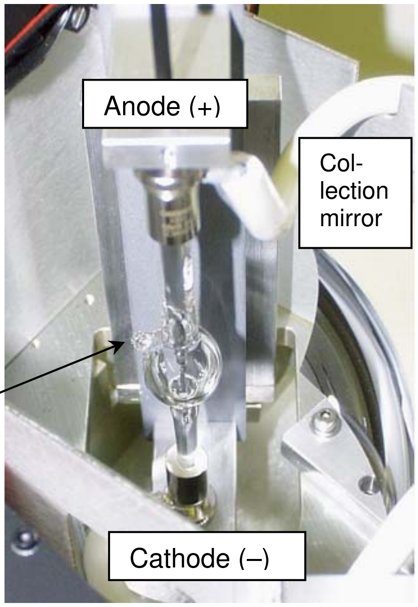
b Secure the connection with the new lamp's thumbscrew.

c Attach the anode connection to the new lamp.

d Secure the connection with the new lamp's thumbscrew.

e Recheck that the connections are correct.

10 Insert the new xenon lamp.



a Insert the new lamp in the bottom holder.

b Press the lamp down into the holder to compress the spring.

c Tilt and raise the anode end of the lamp into the top holder.

d Place the lamp so that the nipple on the glass envelope is opposite the collection mirror.

e Set the height adjustment, using the 1/8" Allen key. Try to return the new lamp to the approximate position of the old lamp.

11 Replace the lamp-housing cover.

12 Replace the two screws on the lamp-housing cover.

13 Reconnect all cables (power, accessories, etc.) to the FluoroMax®-3.

Note: *Do not replace the FluoroMax®-3 cover until the lamp is correctly adjusted.*

Adjusting the new xenon lamp

Choices

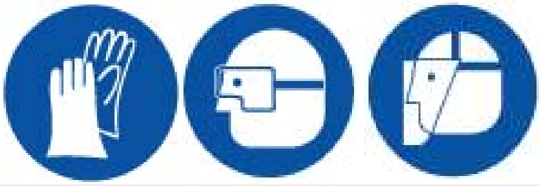
There are two choices after lamp installation:

- Let the lamp "burn in", i.e., run, for 6 h before adjustment of its position.
- Set the coarse lamp adjustments immediately. After a 6-h burn-in, set the fine adjustments.

For lamp replacement, the major adjustment is to optimize the height screw that was loosened to remove the old xenon lamp.

Procedure

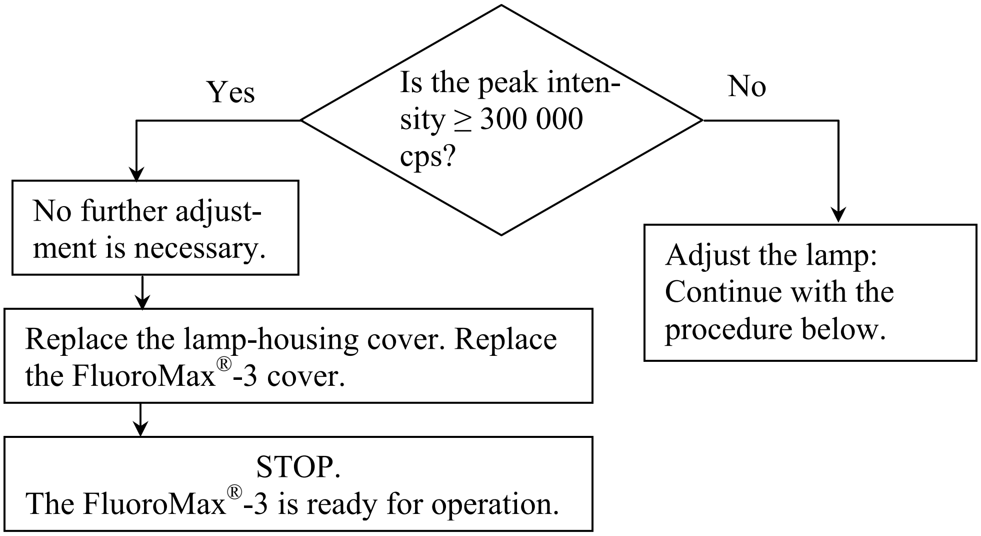
1 With the instrument cover still removed, turn on the FluoroMax®-3.



Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open, so wear eye- and skin-protection, such as light-blocking goggles and light-blocking clothing.*

2 Insert a clean cuvette filled with de-ionized water in the sample compartment.

3 With the room lights off and ambient stray light minimized, acquire a xenon-lamp scan and water Raman scan, as explained in Chapter 3.


Is the peak intensity ≥ 300 000 cps?
Yes
No
No further adjustment is necessary.
Adjust the lamp: Continue with the procedure below.
Replace the lamp-housing cover. Replace the FluoroMax®-3 cover.
STOP.
The FluoroMax®-3 is ready for operation.

This should confirm that the instrument is roughly calibrated.

4 Open **Real Time Control**.

a Move the excitation monochromator to 350 nm, and the emission monochromator to 397 nm.

b Set the slits to 5-nm bandpass.

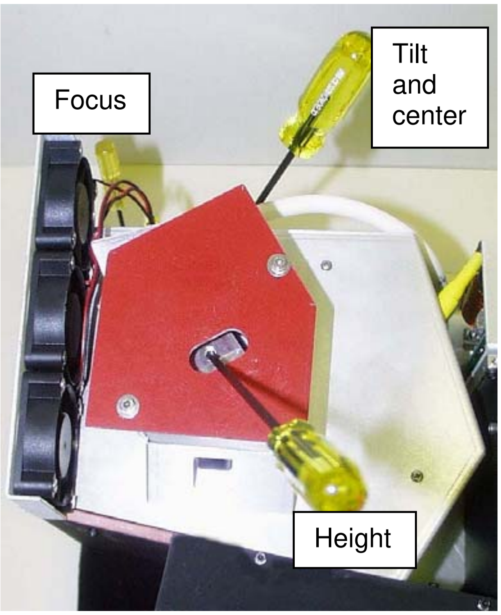
c Open the excitation shutter and emission shutter.

d Set the integration time to 0.5 s.

5 Adjust the xenon lamp's position.

There are three adjustments to optimize the lamp position:

Adjustment	Allen key required
Focus	3/32"
Tilt and Center	9/64"
Height	1/8"


Focus
Tilt and center
Height

a Slowly work each adjustment to optimize the signal.

At maximum signal (> 300 000 cps, depending upon the spectrometer), the lamp is optimized.

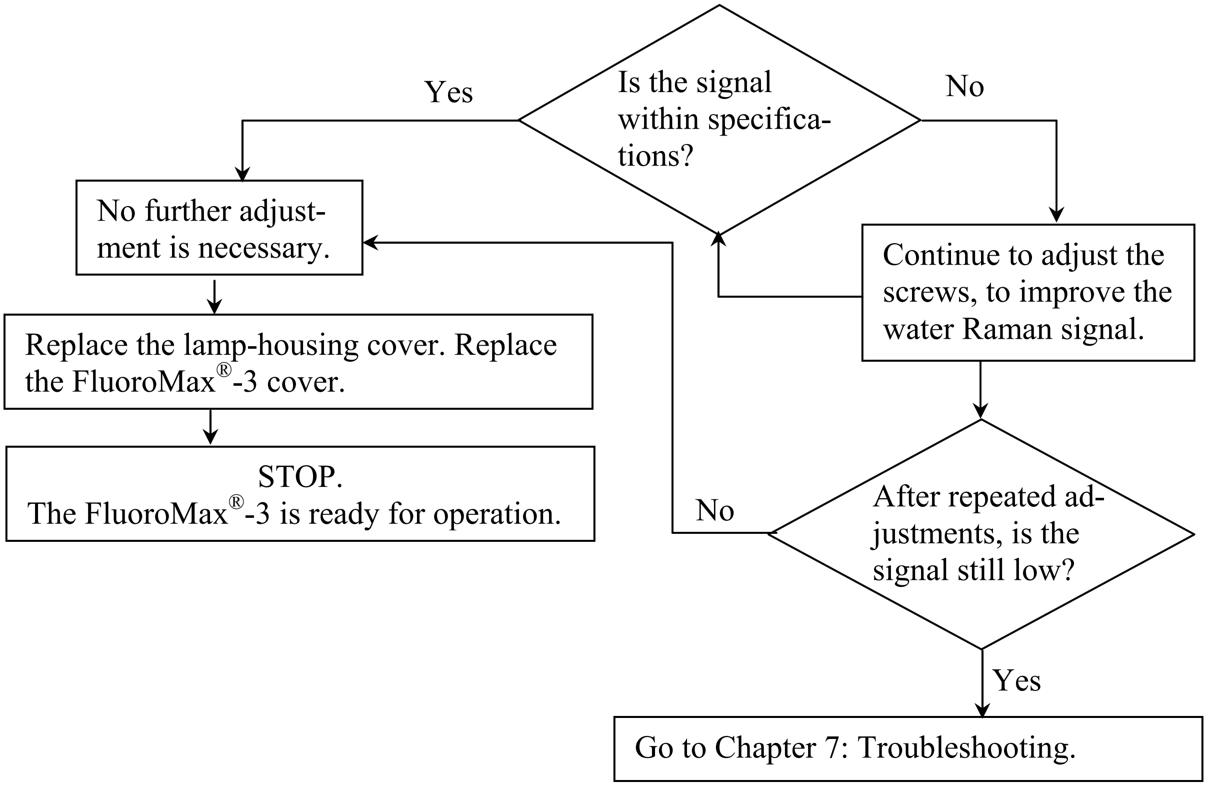
b Remove the Allen keys.

6 Acquire another water Raman scan.

a Use the same parameters as in step 3.

b Note the peak intensity.

c Use the flowchart below.

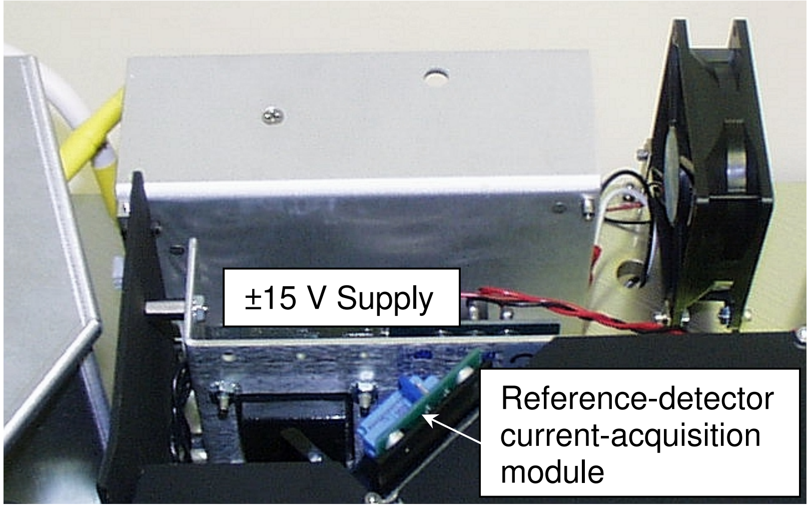

Is the signal within specifications?
Yes
No
No further adjustment is necessary.
Replace the lamp-housing cover. Replace the FluoroMax®-3 cover.
STOP.
The FluoroMax®-3 is ready for operation.
Continue to adjust the screws, to improve the water Raman signal.
After repeated adjustments, is the signal still low?
No
Yes
Go to Chapter 7: Troubleshooting.

Electronics

In case of the rare chance of system failure, this section is provided to help the user understand the electronics components.

Warning: *The information in this section does not constitute permission to adjust, manipulate, or remove any electrical or other components in the FluoroMax®-3. This information is provided for reference purposes only. Contact Spex® Fluorescence Service in case of system failure or suspected failure, before attempting repairs or testing with meters. DO NOT ATTEMPT ANY REPAIRS OR INSTRUMENT EVALUATION WITHOUT THE EXPRESS PERMISSION OF SPEX® FLUORESCENCE SERVICE.*

Xenon-lamp power supply



The xenon lamp's power supply has a capacity of 150–300 W, and is found in the center back of the instrument. In order to suppress the emf spike emitted from the lamp's power supply during arcing and start-up of the lamp—several thousand volts—this contains specialized line filters.

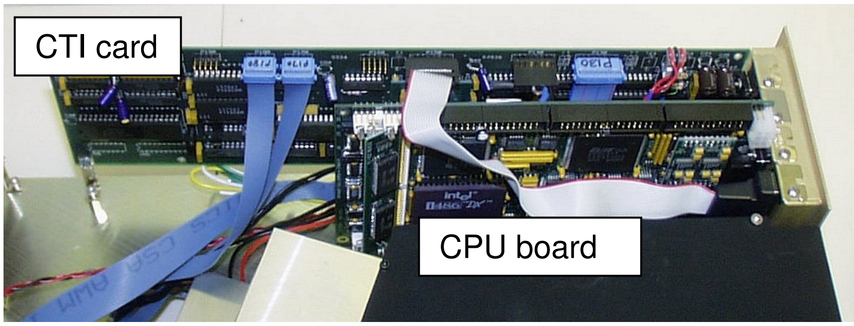
The power supply is preset at the factory for the correct current and voltage to supply the xenon lamp with 150 W. Poor lamp performance or difficulty maintaining a stable lamp arc may be the result of an improperly set power supply. Contact Spex® Fluorescence Service for more details.

CTI card and slave controller

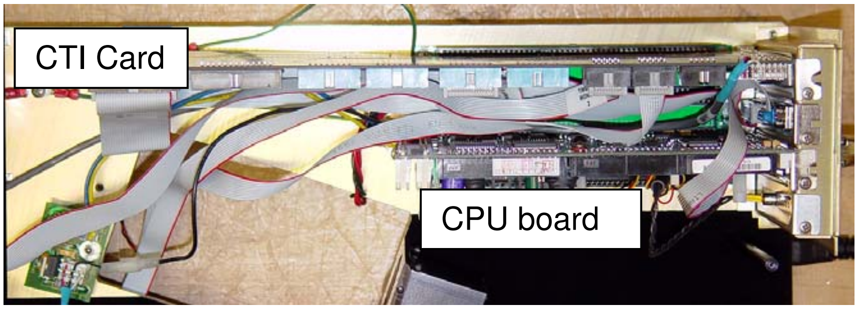
The main instrument board and CPU board for the slave controller are located in the top rear of the FluoroMax®-3, mounted on a motherboard. The CPU board contains a firmware version of the low-level system drivers.

The CTI card is used for all instrument control, data collection, and system timing. Connections from this board go to the monochromator-drive boards, detector acquisition-modules, and optional trigger input from a TRIG-15/25 accessory. The board has an internal ±15 V DC supply.

Older version

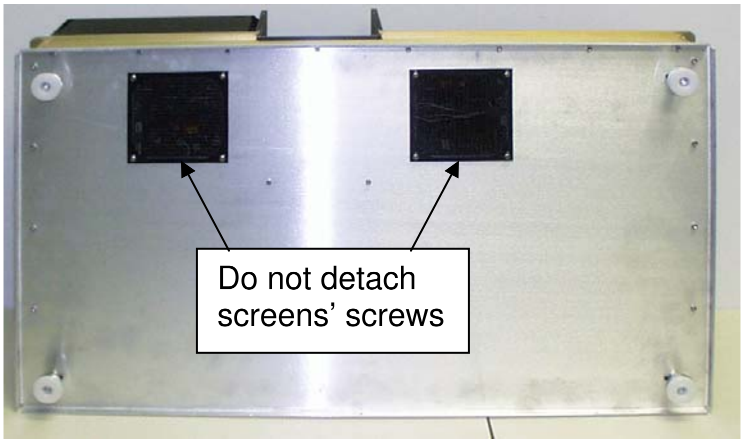


Newer version



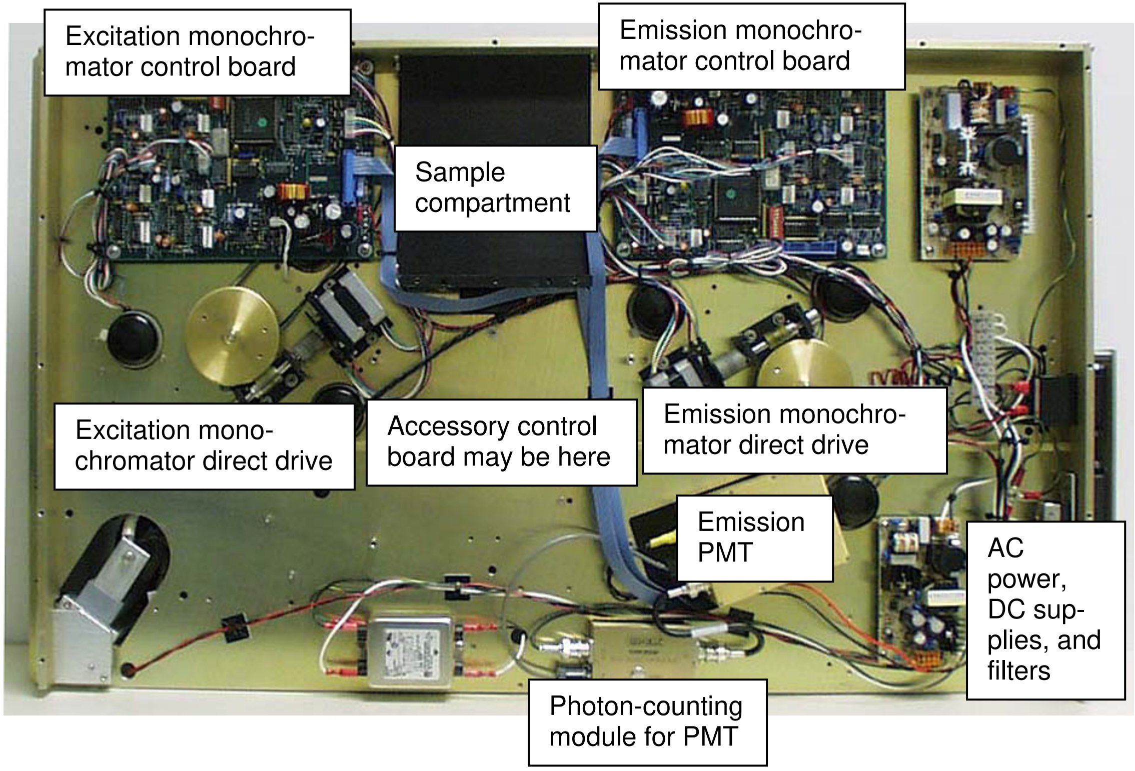
Monochromator drives and accessory controllers

The monochromator drives, drive electronics, and accessory electronics are found on the underside of the optical platform. Electrical connections for the emission detector and the basic system-electronics for AC-power conversion are also underneath the instrument.



To gain access to the drive electronics, remove the bottom cover of the instrument. Remove all screws on the bottom cover except those that attach the dust screens for the exhaust fans. The leveling feet do not need to be removed before detaching the bottom cover.

There are two or three 180F-drive boards underneath the instrument. Each has a specific role: one board controls the drives, slits, shutter, and automated accessories in the excitation monochromator. Another board controls the same components of the emission monochromator. The third board is an optional sample-compartment-accessory control board for options such as a sample changer. (See photograph on next page)


Excitation monochromator control board
Emission monochromator control board
Sample compartment
Excitation monochromator direct drive
Accessory control board may be here
Emission monochromator direct drive
Emission PMT
AC power, DC supplies, and filters
Photon-counting module for PMT

The emission photomultiplier tube is coupled to a photon-counting acquisition module, called a DM302. The photomultiplier tube's housing contains a high-voltage supply preset for the standard R928P photomultiplier tube. The DM302 is connected to the S-channel input on the CTI board at P140. The reference detector is a silicon photodiode, which has a current-acquisition module directly behind it. This module is connected to the CTI board at P130. The silicon photodiode does not require high-voltage bias.

Each direct drive has the main-gear drive and stepper motor accessible on the bottom platform. Power and optical sensors are interfaced with the respective 180F drive board.

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7: Troubleshooting

The FluoroMax®-3 system has been designed to operate reliably and predictably. If there is a problem, examine the chart below, and try the steps 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.
	FluoroMax®-P is in phosphorimeter layout.	Change to a steady-state layout.
	Monochromator is miscalibrated.	Check and recalibrate monochromator.
	Sample turret is not in correct position.	Using FluorEssence™, set the position and open the cover to verify the position.
Signal intensity is low.	CW lamp is not aligned.	Align the lamp.
	Slits are improperly set.	Adjust the slit widths.
	Shutter(s) is(are) not completely open.	Open the shutter(s).
	Lamp power supply is set to the wrong current rating.	Call the Spex® Fluorescence Service Department.
	Lamp is too old.	Replace lamp. (150-W lamp has lamp lifetime 1200–1500 h.)
	Shutter(s) closed.	Open all shutters.
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.	Monochromators are set to wrong wavelength.	Select appropriate wavelength based on excitation and emission of sample.
	Detectors are saturated.	Adjust slits. (Signal detector is linear to 2×10^6 cps in photon-counting mode. Reference detector saturates at 12 µA.)
	Optical density effects and self-absorption.	Sample is too concentrated. Dilute sample by a factor of 10 or 100 and retry experiment.
	Detectors are saturated.	Reduce slit settings.
No signal.	Lamp is not on.	Bad lamp: change xenon lamp.
Erratic signal.	Lamp unstable.	Let lamp warm up 20 min before use.
	Light leaks.	Check dark value to determine.
	Sample has particles that scatter light irregularly.	Filter sample, or let particles settle before running scan.

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.	Second-order effects from the spectrometer.	Use cut-on filters to eliminate 2nd-order peak.
Stray light in emission scan (also see example in this chapter).	Scattered light off the excitation wavelength.	Place bandpass filters in excitation light path. Decrease emission-spectrometer slit widths.
	Dirty cuvette.	Clean the cuvette as described in Chapter 5.
	Solid-sample holder in sample compartment.	Rotate the holder to prevent direct scatter from entering the emission spectrometer.
Corrected excitation spectrum curves upward ~240–270 nm.	Dark count is divided by low reference signal.	Use Dark Offset checkbox; retry scan.
Noisy spectrum with magnetic stirrer.	Stirring speed is too fast.	Use slower stirring speed.
	Stirring bar is too large; light beam is striking it.	Use a smaller stirring bar (available from BelArt Products, Pequannock, NJ).
Communication problems between computer and instrument.	Cables are improperly connected.	Check communications cables' connections.
	BIOS is reset	Check the BIOS: See section "Checking the FluoroMax®'s BIOS" later in this chapter.
	Computer's or SpectrAcq's I/O-controller is failing.	Replace I/O controller: Call Spex® Fluorescence Service.
Hardware Init. error appears.	Broken IR sensor in monochromator.	Replace IR sensor: Call Spex® Fluorescence Service.
Sample turret is not operating.	Software is not enabled.	Check status.
	Cables are connected improperly.	Check cable connections.
FluoroMax®-P resets itself in phosphorimeter mode	Flash lamp's life is expired, drawing excess current, causing transient emf interference.	Replace xenon flash lamp.
"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™.

Checking the FluoroMax®'s BIOS

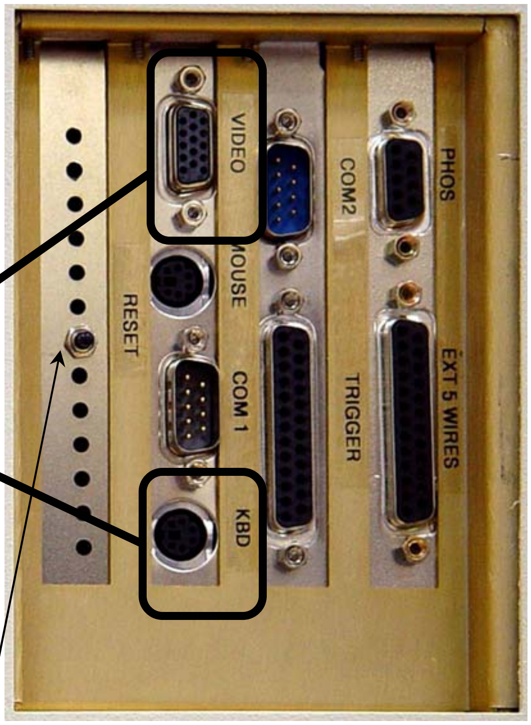
1 Check by listening to the boot-up sequence.

a About 30 s after switching on the FluoroMax®, the instrument beeps.

b After this initial beep, about 1 min later the instrument emits two more very quiet chirps.

> ***Note:*** *You can hear these sounds only if the laboratory environment is quiet, and your hearing is good.*

2 Set up the BIOS on the computer board.



a Switch off the FluoroMax®.

b On the right side of the FluoroMax®, attach an SVGA monitor to the VIDEO connector, and a keyboard to the KBD connector.

c Switch on the FluoroMax®.
A number of messages scroll down the monitor. The last message should be something similar to "`CMOS checksum error`". If so, continue to the next step. If not, call the Spex® Fluorescence Service Department.

d Press the RESET button on the right side of the FluoroMax®.
The FluoroMax® reboots. The following message appears at the bottom of the monitor: "`Press DEL to enter SETUP`".

e Press the `DELETE` key.
The `CMOS SETUP Utility` screen appears.

f Check the following settings; change them as necessary. Leave the other settings as they are.
Use the ↑ and ↓ keys to move the cursor, and the `Page Down` key to change values.

Section: “`STANDARD CMOS SETUP`”

Settings to change: `Drive C : None`
`Drive D : None`
`Halt On : No Errors`

Once these values are set, press `ESC` to return to the main screen.

Section: “`BIOS FEATURES SETUP`”

Settings to change: `Boot Sequence : A,C,SCSI`
`Boot Up Floppy Disk : Enabled`
`Boot Up NumLock Status : On`

Section: “`CHIPSET FEATURES SETUP`”

Settings to change: `VGA Shared Memory Size : 0.5 MB`

Section: “`POWER MANAGEMENT SETUP`”

Settings to change: `Power Management : Disable`

Section: “`PNP/PCI CONFIGURATION`”

Settings to change: `PNP OS Installed : No`

Section: “`INTEGRATED PERIPHERALS`”

Settings to change: `Internal PCI/IDE : Disabled`

g When all the above values are set, press `F10` to save.

h Press `Y` and then `ENTER` to confirm this.

The FluoroMax® reboots, using the new settings. The monitor displays a sequence of messages as the computer boots up. The last message is “in autobaud at 19200”.

Note: *If the above sequence fails to improve communication between host computer and FluoroMax®, contact the Spex® Fluorescence Service Department.*

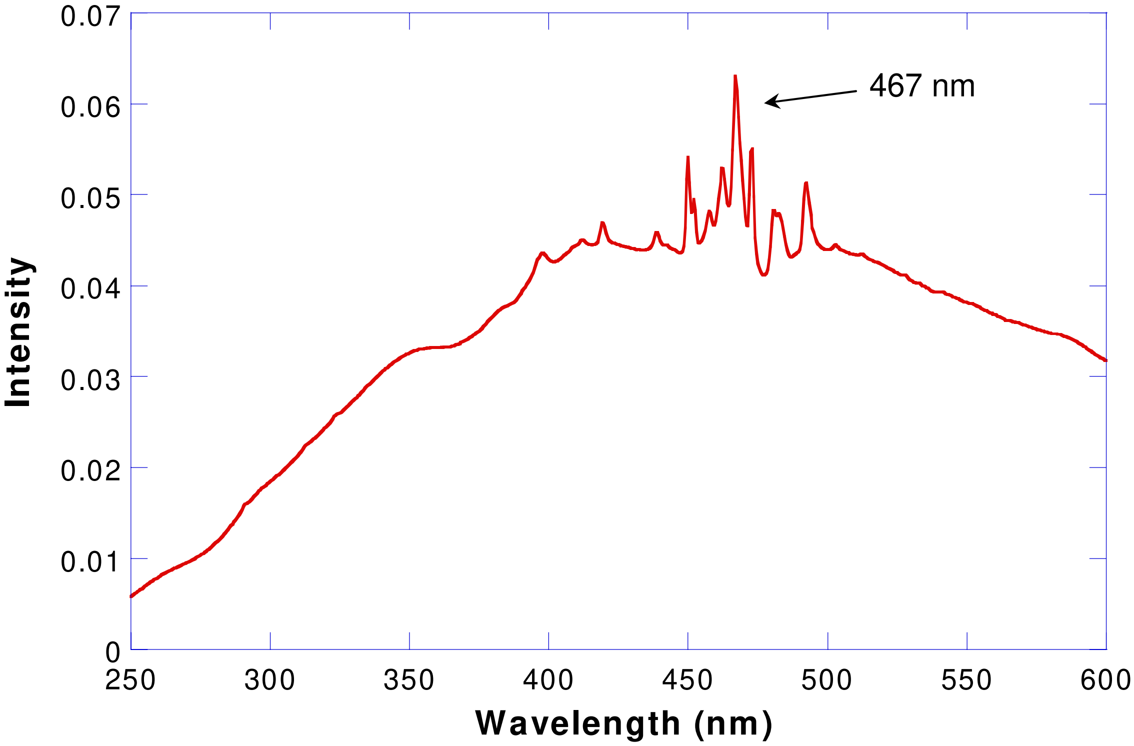
Using diagnostic spectra

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

Note: *Not all spectra shown in this section were produced using the FluoroMax®-3. The spectra are presented to show different possible system or sample problems, and may not reflect the performance of your particular FluoroMax®-3.*

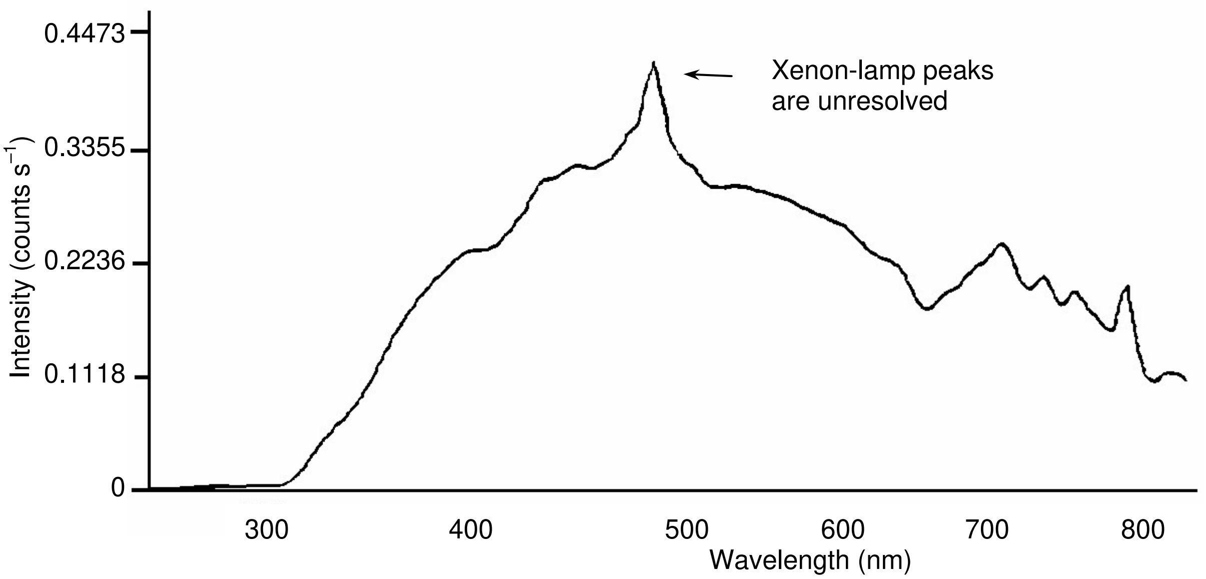
Lamp scan

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 lamp scan run with a known good lamp.



Scan of good quality 150-W xenon lamp in FluoroMax®-3.

The following lamp scan spectrum shows poor resolution in the area around the peak.



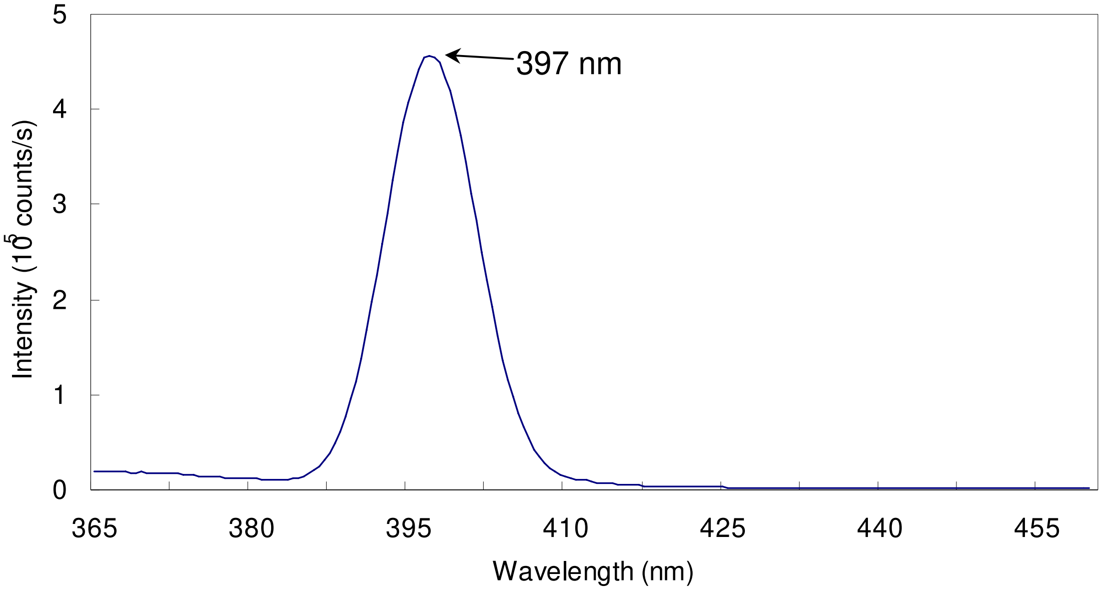
Poor lamp scan of 150-W Xe lamp. Note low resolution in the area near the 467-nm peak.

This lack of spectral resolution appears because the slit widths are set too wide. To resolve this problem, narrow the slit widths.

Water Raman spectra

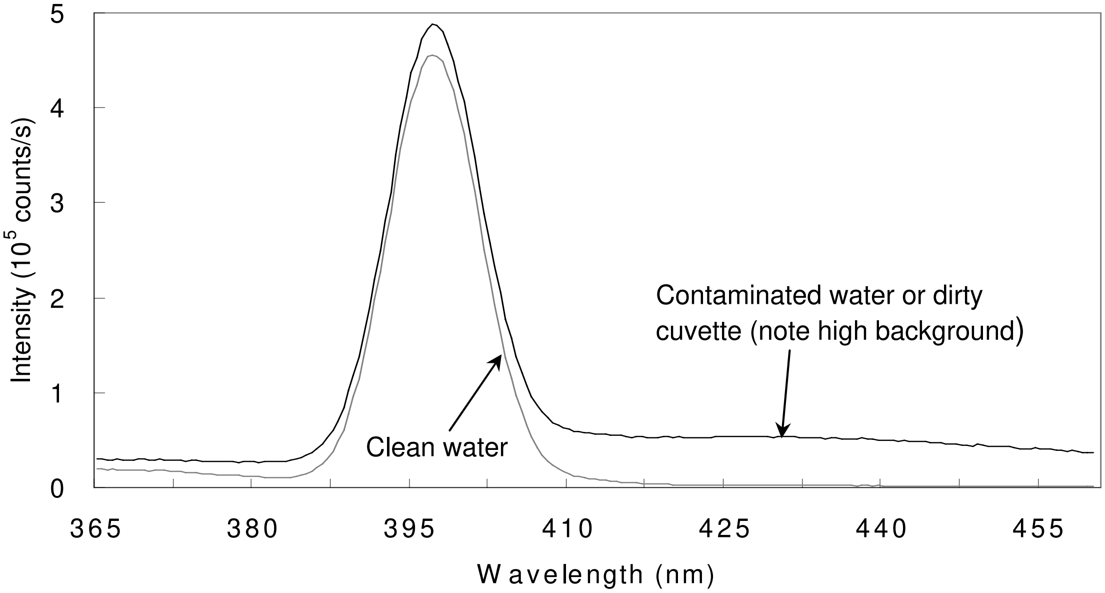
Contaminated water

Running a water Raman scan helps identify abnormalities caused by accessory problems or miscalibration. The following spectrum is normal:



Clean water Raman scan.

Below is a normal water Raman spectrum superimposed on one that exhibits a problem. In this instance, the water was contaminated, resulting in a high background.



Contaminated water in a water Raman scan.

If a spectrum similar to this is obtained after running a water Raman scan,

1 Rotate the cuvette 90° and rerun the scan.

If the problem goes away, then the problem was due to the cuvette surface. Clean or use a different cuvette.

Or

1 Clean the cuvette.

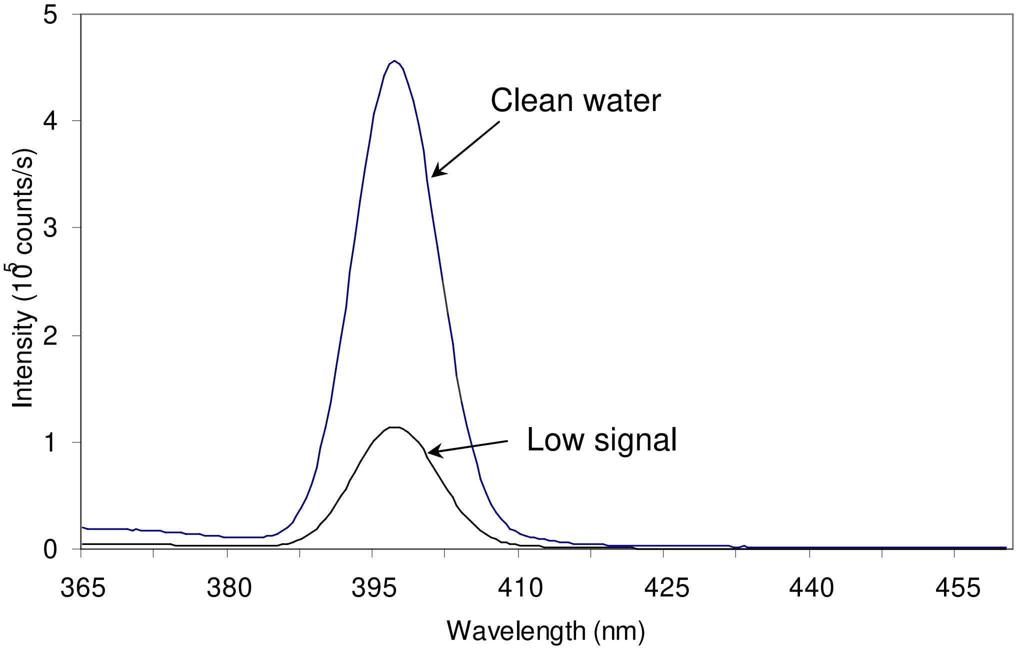
2 Fill with fresh, double-distilled, deionized water.

If the problem goes away, then the problem was due to contaminated water.

> ***Note:*** *If the instrument is out of calibration by more than 50 nm, the Rayleigh scatter band (intense) can be mistaken at first glance for the water Raman peak (much weaker). Try scanning to 600 nm (instead of 450 nm) to help ensure that the water Raman peak is found.*

Light not striking cuvette

The following graph shows a normal water-Raman scan with a superimposed problem scan.



Low intensity during a Raman scan.

Here the problem is low intensity of the water signal when compared with the superimposed typical water Raman scan. To resolve this problem:

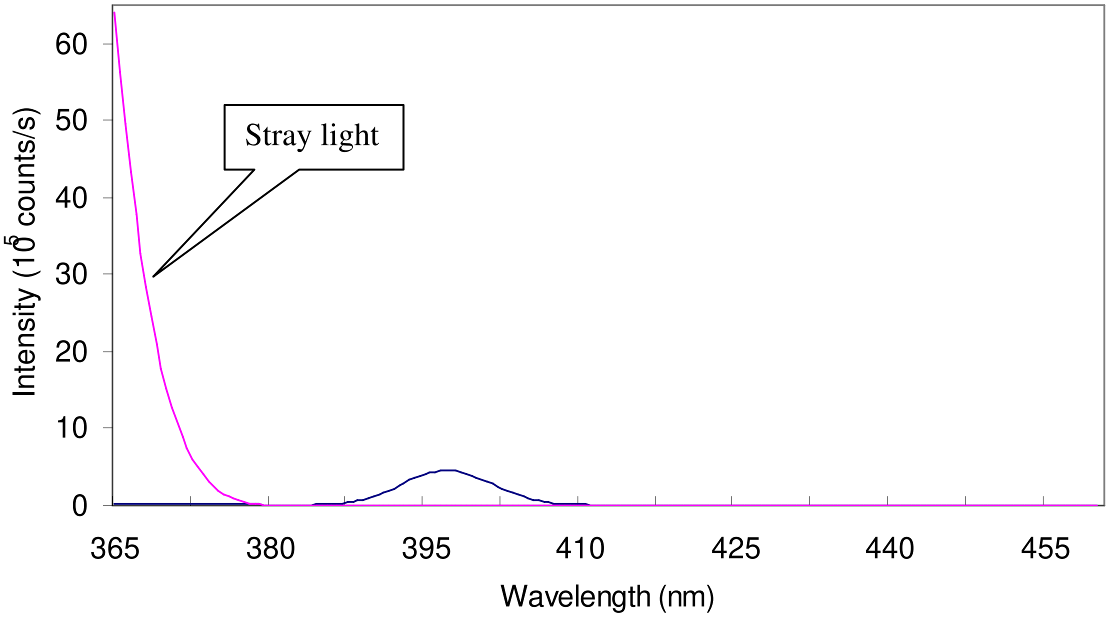
1 Make sure the cuvette is filled to the proper level.

Light should fall on the sample, and the meniscus should not be in the light path.

2 Make sure that the excitation and emission slits are set to the proper widths.

Stray light

In the following diagram, notice the high level of stray-light below 380 nm in the water Raman spectrum.



High stray light in a water Raman scan.

To correct this problem,

1 Inspect the cuvette surface for fingerprints and scratches.

2 Clean the cuvette or use a new one.

3 Verify that the excitation and emission slits are set correctly for a water Raman scan.

4 Verify that the excitation spectrometer is at the correct position.

Further assistance...

Read all software and accessory manuals before contacting the Spex® Fluorescence 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 Chapter 3, *System Operation*, run a lamp scan and a water Raman scan to make sure the system is properly calibrated.

   Print the spectrum for each and note the peak intensities.

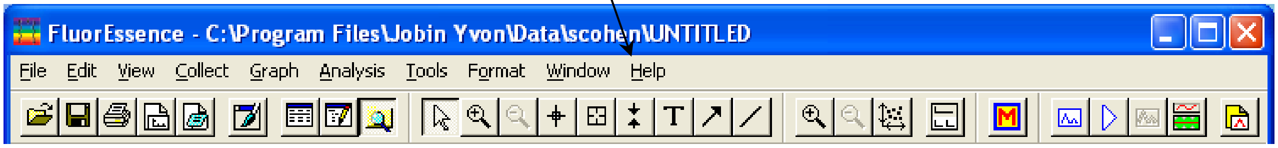
4. Check this chapter to see if the problem is discussed.

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.

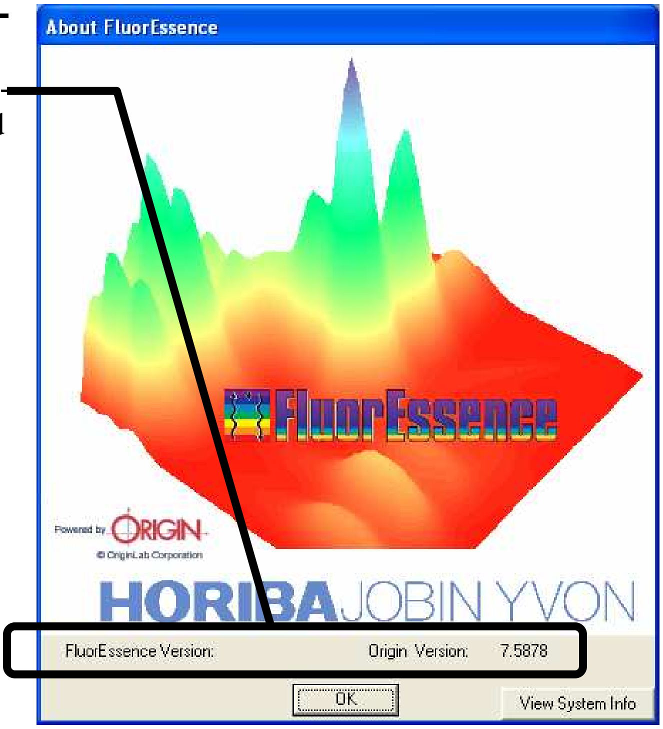
7. In FluorEssence™, in the **FluorEssence** main window's toolbar, choose Help.


FluorEssence - C:\Program Files\Jobin Yvon\Data\scohen\UNTITLED
File
Edit
View
Collect
Graph
Analysis
Tools
Format
Window
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.

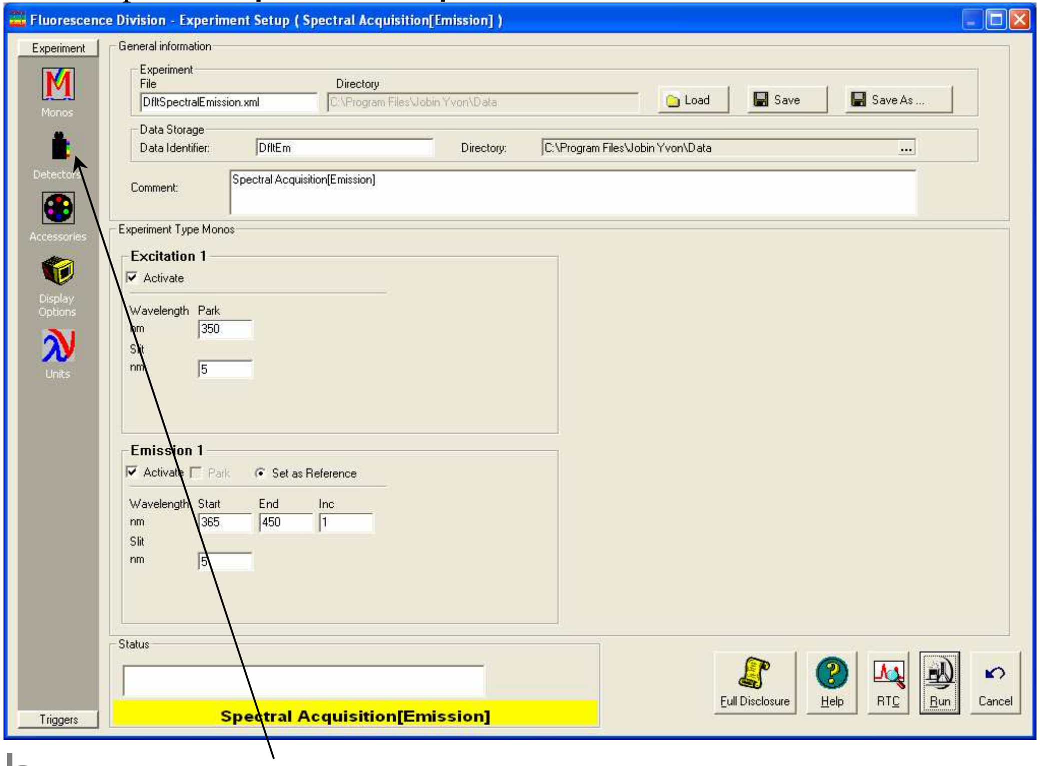

About FluorEssence
FluorEssence
Powered by ORIGIN
© OriginLab Corporation
HORIBA JOBIN YVON
OK
View System Info

FluorEssence Version:

Origin Version: 7.5878

9 Determine the SpectrAcq firmware version.

a Open the **Experiment Setup** window:


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Load
Save
Save As ...
Experiment Type Monos
Status
Full Disclosure
Help
RTC
Run
Cancel

Experiment File: DfltSpectralEmission.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage Data Identifier: DfltEm

Directory: C:\Program Files\Jobin Yvon\Data

Comment: Spectral Acquisition[Emission]

Excitation 1

☑ Activate

Wavelength nm Park 350

Slit nm 5

Emission 1

☑ Activate ☐ Park ● Set as Reference

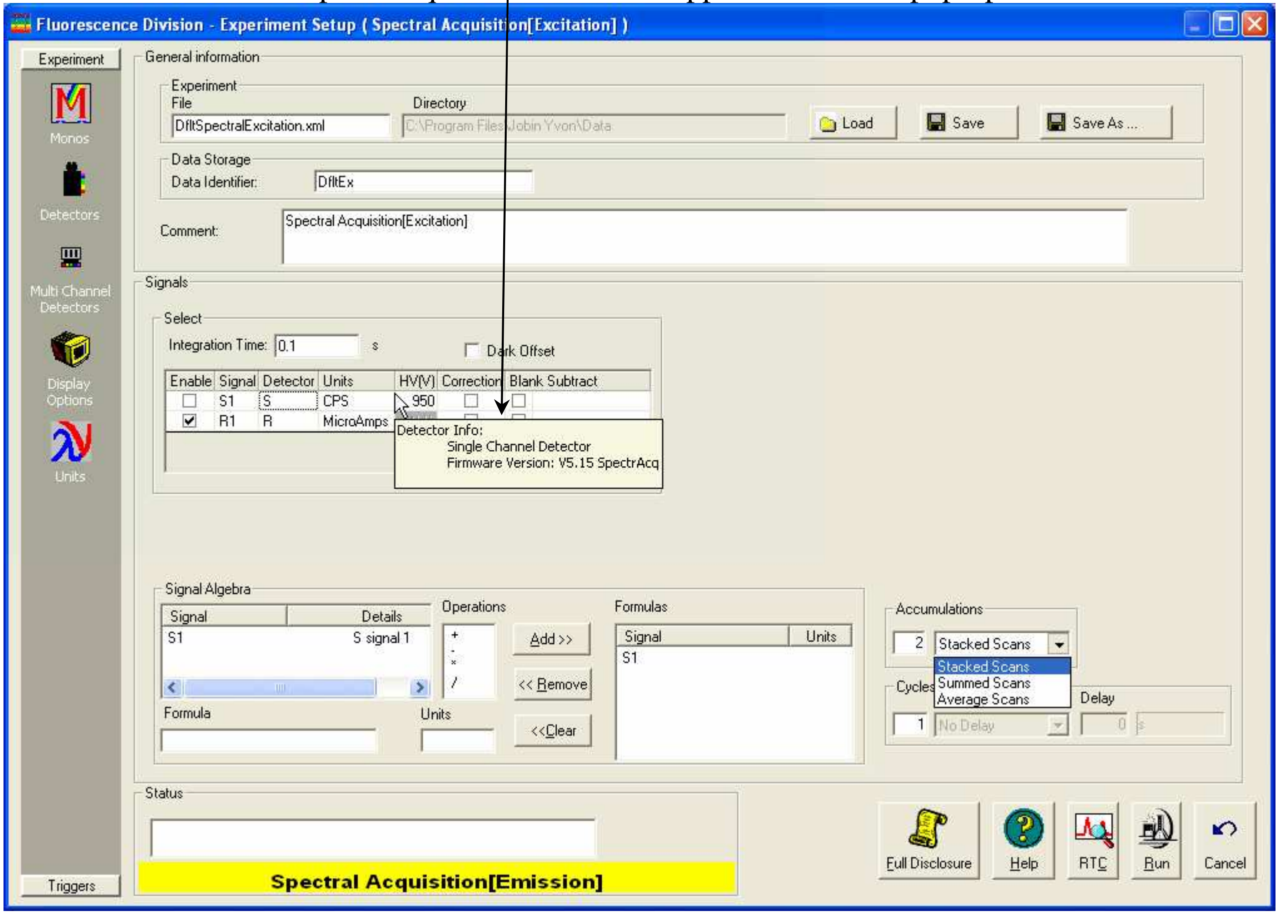
Wavelength nm Start 365 End 450 Inc 1

Slit nm 5

Spectral Acquisition[Emission]

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:



Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])

General information

Experiment File: DfltSpectralExcitation.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage

Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Signals

Select

Integration Time: 0.1 s

☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☐	S1	S	CPS	950	☐	☐
☑	R1	R	MicroAmps			

Detector Info:
Single Channel Detector
Firmware Version: V5.15 SpectrAcq

Signal Algebra

Signal	Details
S1	S signal 1

Formulas

Signal	Units
S1	

Accumulations: 2 Stacked Scans

Cycles: 1 No Delay

Delay: 0 s

Status

Spectral Acquisition[Emission]

Experiment
Monos
Detectors
Multi Channel Detectors
Display Options
Units
Triggers
Load
Save
Save As ...
Operations
+
-
×
/
Add >>
<< Remove
<<Clear
Formula
Units
Stacked Scans
Summed Scans
Average Scans
Full Disclosure
Help
RTC
Run
Cancel

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

8: Producing Correction Factors

Introduction

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 comparisons, 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 FluoroMax®-3 are sets of excitation and emission correction factors designed to eliminate response characteristics. These files, `xcorrect.spc` and `mcorrect.spc`, are included with the software and should be copied to the hard disk. The excitation correction range is from 240–600 nm, and the correction range for emission spectra is from 290–850 nm.

Generating emission correction factors

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

Required kits

Emission correction factors should be updated periodically or whenever different gratings or signal detectors are installed. The correction factors can be updated either at the user's location, or by a representative from the Spex® Fluorescence Service Department. To arrange for a visit and a fee estimate, call Spex® Fluorescence Service Department. To update the correction factors without a service visit, call Spex® Fluorescence Service Department for instructions and further information.

One way to generate correction factors for the instrument is to scan 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 can then be applied to the raw fluorescence data. The emission correction factor file `mcorrect.spc` was acquired in this manner.

To generate emission correction factors, several items are needed: a standard lamp, appropriate holders, and a scatter assembly. HORIBA Jobin Yvon Inc. offers two kits: the Model 1908 Standard Lamp Accessory, and the Model 1908MOD Scatter Assembly. The Model 1908 is a complete correction factor kit, while the Model 1908MOD Scatter Assembly is provided for users who already have a calibrated standard lamp and a constant-current source.

The Model 1908 Standard Lamp Assembly is a complete correction factor kit, which includes the following items:

- 200-watt quartz tungsten-halogen filament lamp with irradiance values
- Constant-current power supply with lamp holder
- 1908MOD scatter assembly

The Model 1908MOD scatter assembly includes:

- Lamp-mount assembly and mask with square center
- Scatter block with neutral-density filter and reflectance plate

Calculating emission correction factors

Introduction

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.

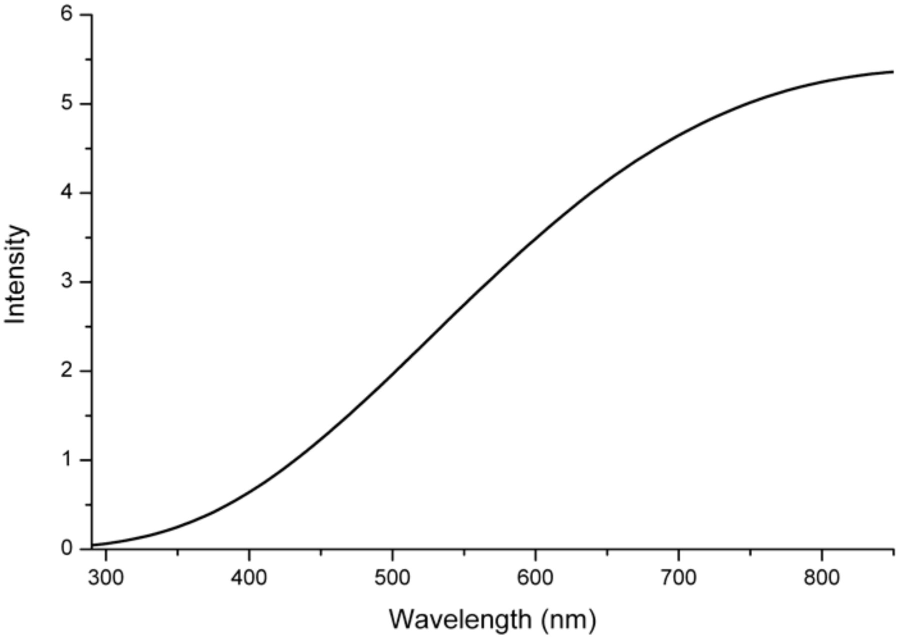
Irradiance values for a standard lamp, packaged with the lamp, usually are expressed in 10^{-6} W·cm^{-2}·nm. With photon-counting systems like the FluoroMax®-3 spectrofluorometer, however, data usually are collected in units of photons·s^{-1}·cm^{-2}·nm. To convert the units, multiply each irradiance 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.

1 Load the irradiance values.

a Enter the irradiance values into a spreadsheet.

Use the Origin® project supplied by HORIBA Jobin Yvon.

b Save the file as `IRR`.



Now you have the two files: `IRR` and `stdlamp2`. These files are required to calculate the emission correction factors for the FluoroMax®-3 system.

2 Calculate the correction factors.

a Using the **Simple Math Tool** window under the Analysis menu, divide `IRR` by `stdlamp2`, and name the resulting file `mcorrect`.

$$\texttt{mcorrect} = \frac{\texttt{IRR}}{\texttt{stdlamp2}}$$

***Note:** Naming the file `mcorrect` overwrites the `mcorrect` file supplied with the software.*

3 Normalize the new `mcorrect` file.

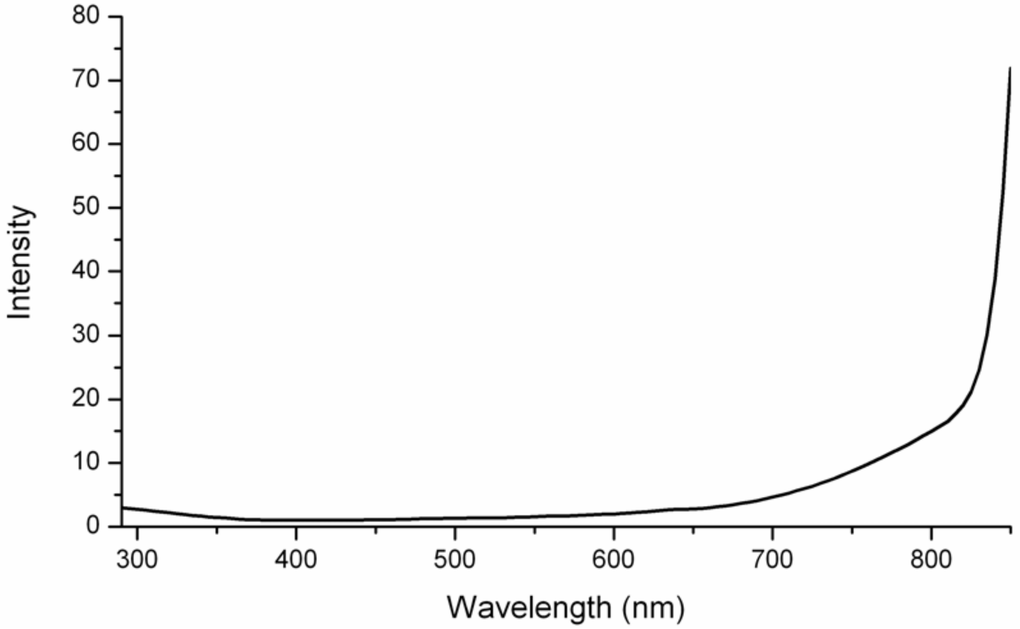
a Display the new `mcorrect` file and find the minimum signal intensity.

To find the minimum quickly, right-click on the column of data's header, choose Statistics on Columns, and find the Min(Y) value.

b Using the **Simple Math Tool** window under the Analysis menu, divide the `mcorrect` file by this minimum signal intensity, a constant.

c Save this new file as `mcorrect`.

(That is, overwrite the existing `mcorrect` file.) This normalizes the correction factor file so that the minimum intensity of `mcorrect` will be 1 count s^{-1}. `mcorrect` contains the emission correction factors for the system. The correction-factor file should look similar to this:



`mcorrect` file.

Once the emission correction factors have been found, determination of the excitation correction factors may be necessary. The following procedures describe how to obtain excitation correction factors using the photomultiplier and the photodiode. Follow the procedure that applies to your configuration.

Calculating excitation correction factors

The photodiode reference detector handles the bulk of excitation correction from 240–600 nm when a ratio-acquisition mode is selected (i.e., *S*/*R*). More accurate measurements require that compensation be applied for the difference in optical path between the detector and the sample. This can be accomplished by a simple excitation scan with rhodamine-B placed in the sample position.

Note: *To calibrate the reference detector out to 800 nm, use HORIBA Jobin Yvon's calibrated photodiode accessory.*

1 Fill a cuvette with a solution of rhodamine-B.

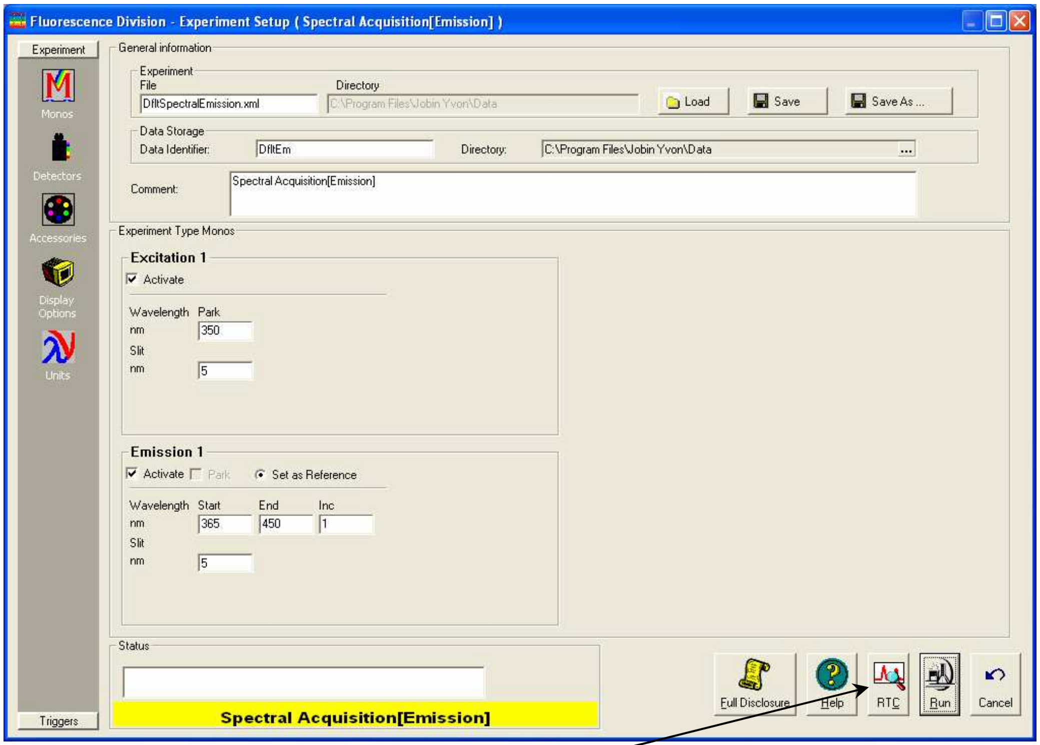
Use 8 g L^{-1} of laser-grade rhodamine-B in 1,2-propanediol.

Warning: *Read the Materials Safety Data Sheets (MSDS) before using rhodamine-B and 1,2-propanediol.*

2 Place the cuvette in the sample compartment.

3 Check the hardware to be sure that you do not over-range the detector.

a Open the **Experiment Setup** window for an emission scan.


Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Load
Save
Save As ...
Experiment Type Monos
Full Disclosure
Help
RTC
Run
Cancel

Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])

Experiment File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\Data

Data Storage
Data Identifier: DfltEm
Directory: C:\Program Files\Jobin Yvon\Data

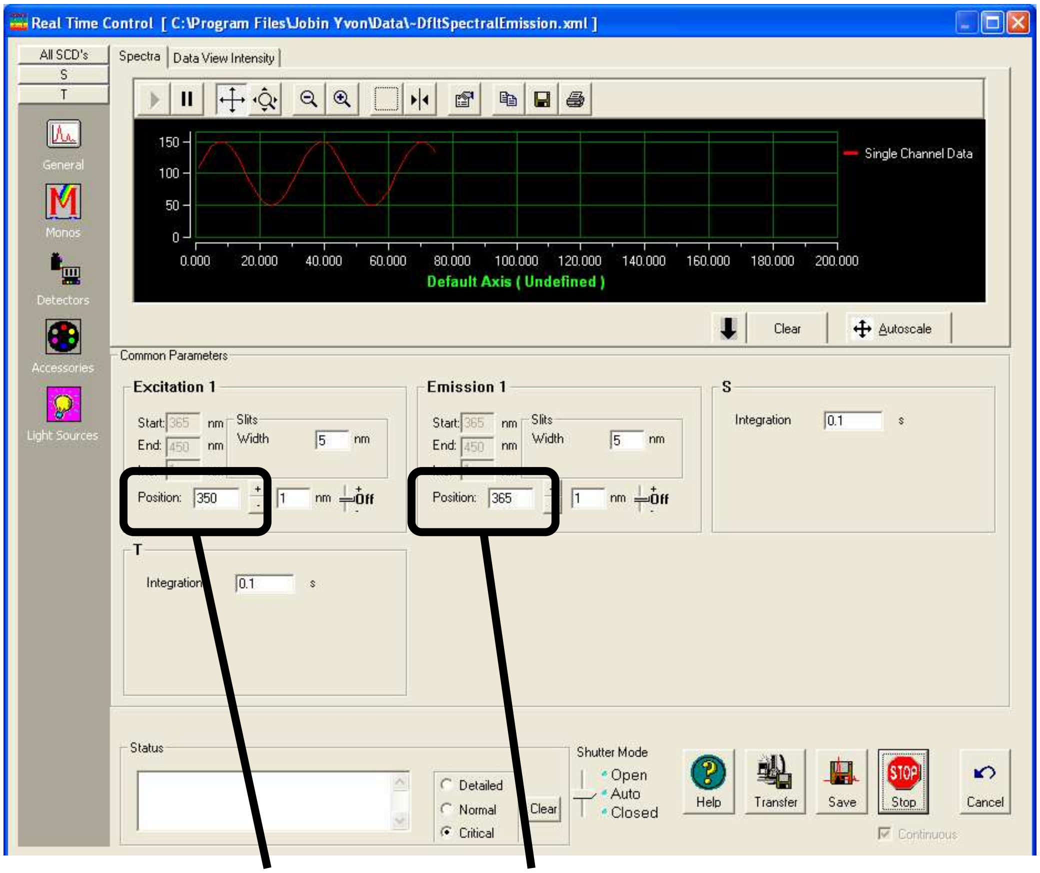
Comment: Spectral Acquisition[Emission]

Excitation 1
☑ Activate
Wavelength nm Park: 350
Slit nm: 5

Emission 1
☑ Activate ☐ Park ● Set as Reference
Wavelength nm Start: 365 End: 450 Inc: 1
Slit nm: 5

Status: **Spectral Acquisition[Emission]**

b Open the **Real Time Control**.


All SCD's
S
T
General
Monos
Detectors
Accessories
Light Sources
Spectra
Data View Intensity
150
100
50
0
0.000
20.000
40.000
60.000
80.000
100.000
120.000
140.000
160.000
180.000
200.000
Default Axis (Undefined)
Single Channel Data
Clear
Autoscale
Help
Transfer
Save
Stop
Cancel

Real Time Control [C:\Program Files\Jobin Yvon\Data\-DfltSpectralEmission.xml]

Common Parameters

Excitation 1
Start: 365 nm
End: 450 nm
Slits Width: 5 nm
Position: 350
1 nm Off

Emission 1
Start: 365 nm
End: 450 nm
Slits Width: 5 nm
Position: 365
1 nm Off

S
Integration: 0.1 s

T
Integration: 0.1 s

Status
○ Detailed
○ Normal
● Critical
Clear

Shutter Mode
Open
Auto
Closed

☑ Continuous

c Set the excitation and emission monochromators to 467 nm and 630 nm, respectively.

The largest lamp peak occurs at 467 nm.

d Set the slit on the excitation monochromator to 0.5 mm.

e Make sure the shutter is open.

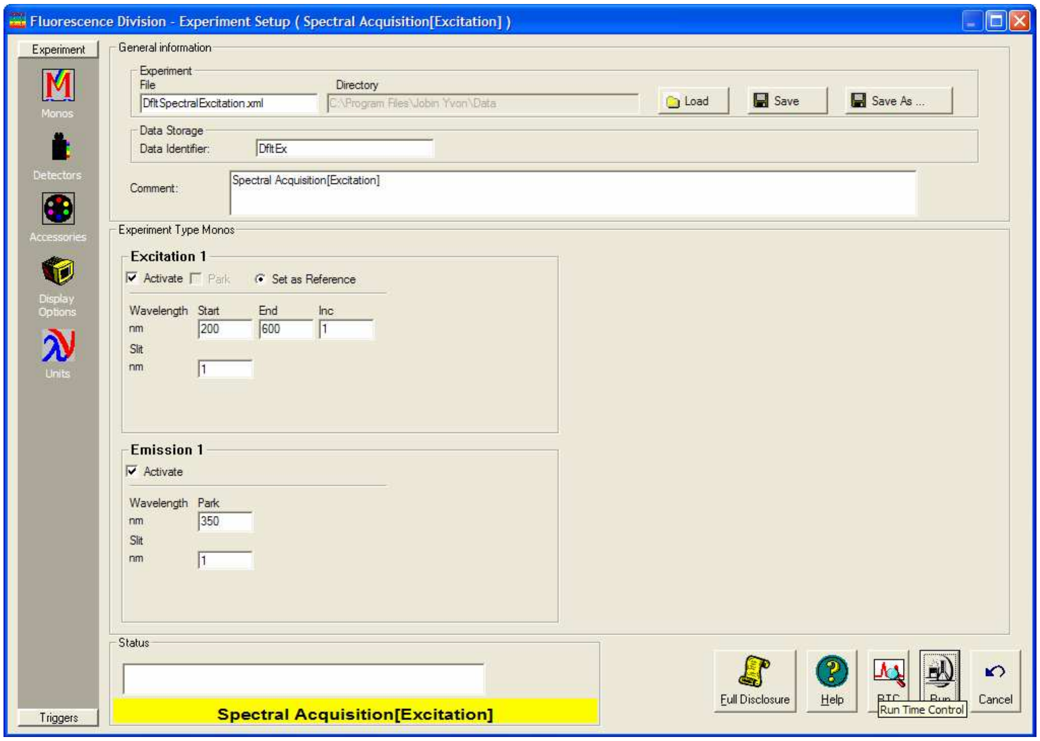
f Adjust the slit on the emission monochromator (Slits Width field) to obtain a signal intensity of (1 ± 0.1) × 10^6 cps.

Note the slit-width: the slit-width discovered in this step will be used to run the scan.

g Close the **Real Time Control** and revert to the **Experiment Setup** dialog box.

h Close the dialog box and open the **Experiment Setup** dialog box for an excitation scan instead.

4 Set up the excitation-correction scan.


Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Full Disclosure
Help
RTC
Run
Run Time Control
Cancel

Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])

General information

Experiment File: DfltSpectralExcitation.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage

Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Experiment Type Monos

Excitation 1

☑ Activate ☐ Park ● Set as Reference

Wavelength nm: Start 200, End 600, Inc 1

Slit nm: 1

Emission 1

☑ Activate

Wavelength nm: Park 350

Slit nm: 1

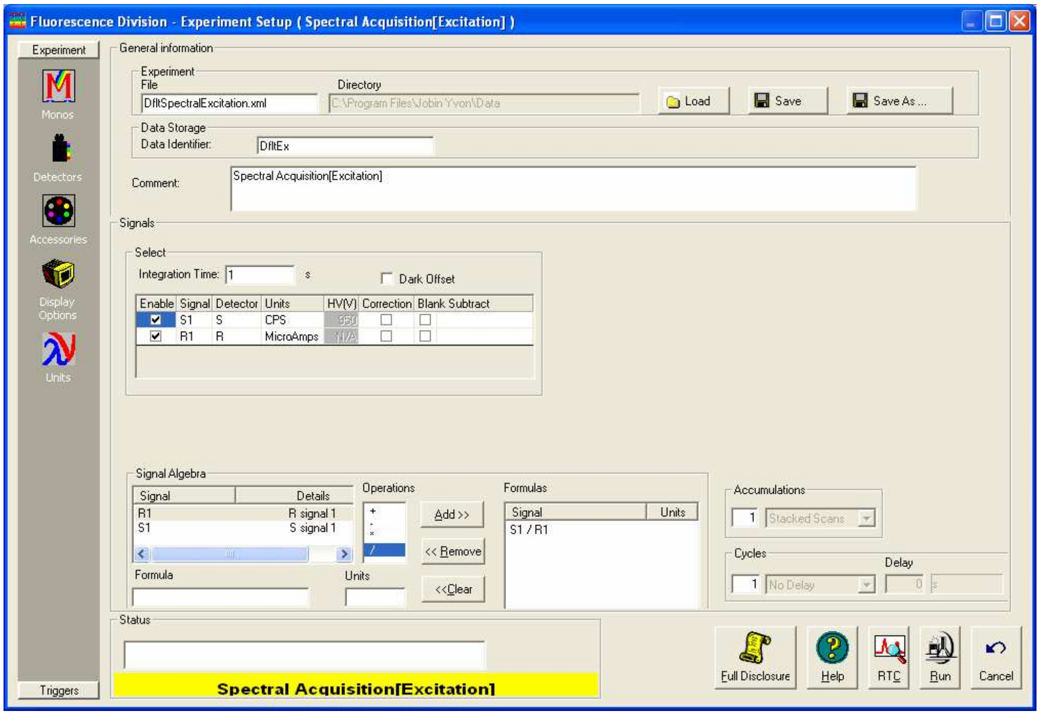
Status

Spectral Acquisition[Excitation]

a Enter the scan parameters:

Excitation Wavelength Start (nm)	`240`
Excitation Wavelength End (nm)	`600`
Inc (nm)	`5`
Emission Wavelength Park	`650`
Excitation Slit (nm)	`0.5`
Emission Slit (nm)	Use value determined in step 3f.

b Click the Detectors icon .


Fluorescence Division - Experiment Setup (Spectral Acquisition[Excitation])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Load
Save
Save As ...
Signal Algebra
Operations
+
-
×
/
Add >>
<< Remove
<<Clear
Status
Full Disclosure
Help
RTC
Run
Cancel

Experiment File: DfltSpectralExcitation.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Signals — Select

Integration Time: 1 s

☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☑	S1	S	CPS	950	☐	☐
☑	R1	R	MicroAmps	N/A	☐	☐

Signal	Details
R1	R signal 1
S1	S signal 1

Formula:

Units:

Formulas

Signal	Units
S1 / R1	

Accumulations: 1 Stacked Scans

Cycles: 1 No Delay

Delay: 0 s

Spectral Acquisition[Excitation]

c Enable both S1 and R1 detectors.

The codes S1 and R1 appear in the Formulas table.

d Remove both S1 and R1 from the Formulas table.

e In the Signal column, click S1. In the Operations column, click the division sign, /. In the Signal column, click R1.

In the Formula box, S1/R1 appears.

f Click Add >> to add this formula to the Formulas table.

g Enter an Integration Time of 1 second.

5 Click Run to execute the scan.

6 Save the file as `xcorrect`.

Note: *Doing so replaces the* `xcorrect` *file that was shipped with the system.*

7 Normalize the data in the same way as in the previous section.

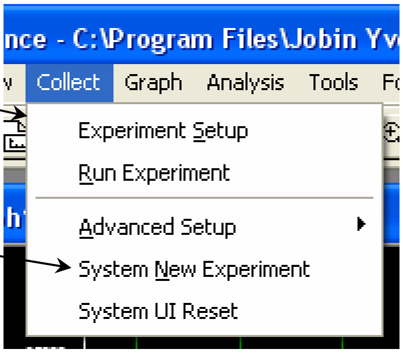
As with the corrected emission factors, find the minimum data point and divide the file by that value.

8 Save the normalized file as `xcorrect`.

This overwrites the existing excitation correction-factor file.

Using correction-factor files

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


nce - C:\Program Files\Jobin Yv
Collect
Graph
Analysis
Tools
Experiment Setup
Run Experiment
Advanced Setup
System New Experiment
System UI Reset

1 In the FluorEssence toolbar, choose Collect.

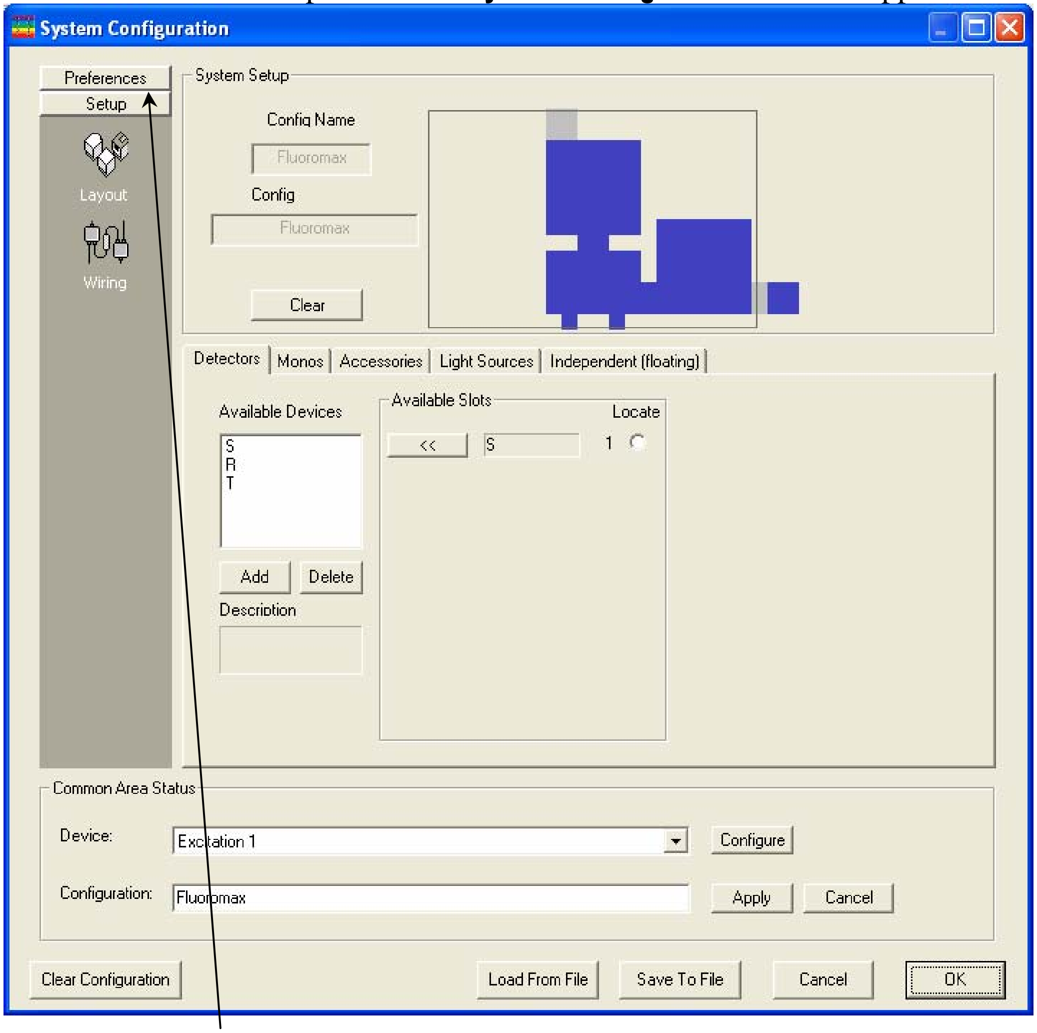
A drop-down menu appears.

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.


System Configuration
Preferences
Setup
Layout
Wiring
System Setup
Clear
Detectors
Monos
Accessories
Light Sources
Independent (floating)
Available Devices
S
R
T
Add
Delete
Description
Available Slots
Locate
<<
S
1
Common Area Status
Configure
Apply
Cancel
Clear Configuration
Load From File
Save To File
Cancel
OK

Config Name: Fluoromax

Config: Fluoromax

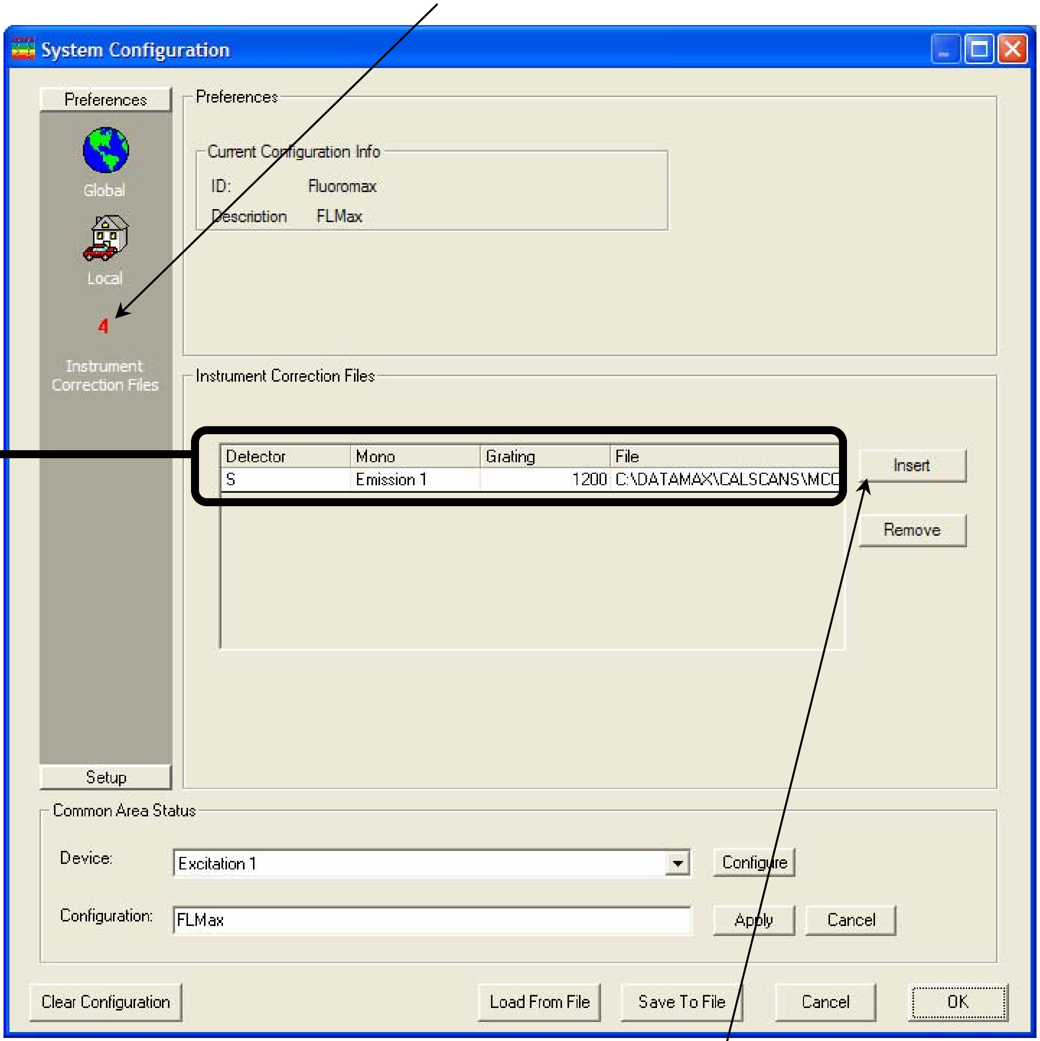
Device: Excitation 1

Configuration: Fluoromax

4 Choose Preferences.

The Preferences area appears.

5 Choose the Instrument Correction Files icon.


System Configuration
Preferences
Global
Local
4
Instrument Correction Files
Setup
Preferences
Current Configuration Info
Instrument Correction Files
Insert
Remove
Common Area Status
Configure
Apply
Cancel
Clear Configuration
Load From File
Save To File
Cancel
OK

ID: Fluoromax

Description FLMax

Detector	Mono	Grating	File
S	Emission 1	1200	C:\DATAMAX\CALSCANS\MCO

Device: Excitation 1

Configuration: FLMax

The Instrument Correction Files area appears.

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

7 In sequence,

Note: *The signal detector, S, uses only the `mcorrect` file; the reference detector, R, uses only the `xcorrect` file.*

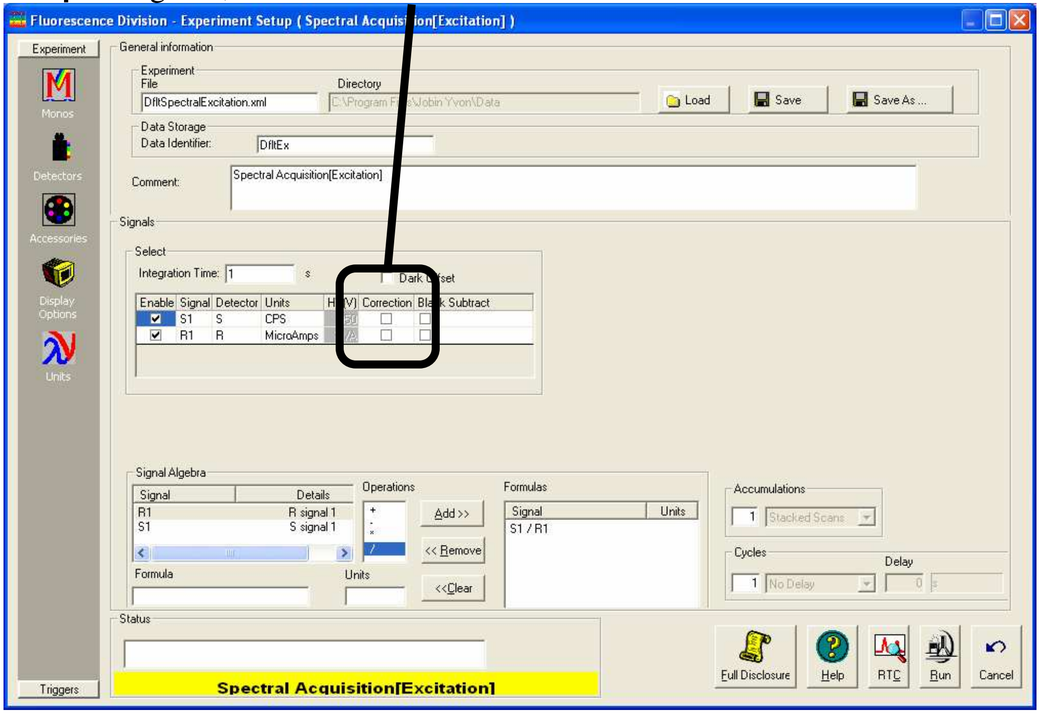
a Choose the Detector column, and select the appropriate detector from the drop-down list,

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

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 OK.

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


Fluorescence Division - Experiment Setup (Spectral Acquisi ion[Excitation])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Add >>
<< Remove
<<Clear
Full Disclosure
Help
RTC
Run
Cancel

General information

Experiment File: DfltSpectralExcitation.xml

Directory: C:\Program Fi s\Jobin Yvon\Data

Data Storage Data Identifier: DfltEx

Comment: Spectral Acquisition[Excitation]

Signals

Select

Integration Time: 1 s

☐ Dark fset

Enable	Signal	Detector	Units	H (V)	Correction	Bla k Subtract
☑	S1	S	CPS	50	☐	☐
☑	R1	R	MicroAmps	/A	☐	☐

Signal Algebra

Signal	Details
R1	R signal 1
S1	S signal 1

Operations: + - × /

Formula

Units

Formulas

Signal	Units
S1 / R1	

Accumulations: 1 Stacked Scans

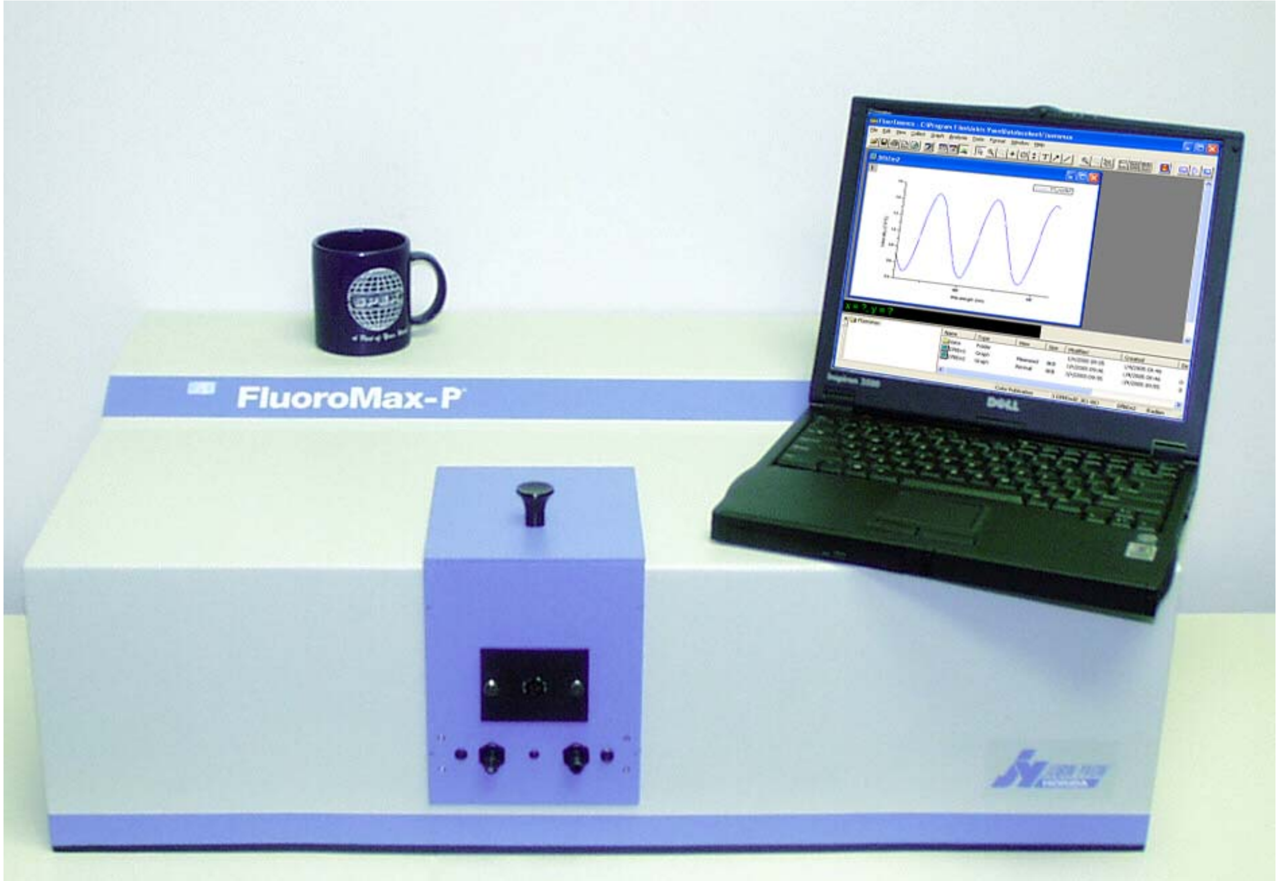
Cycles: 1 No Delay

Delay: 0 s

Status

Spectral Acquisition[Excitation]

9: FluoroMax®-P Phosphorimeter Operation



Introduction

The FluoroMax®-P includes a phosphorimeter, that is, a programmable pulsed source and selectable signal gating from the reference detector. Switching between the pulsed lamp and continuous lamp is computer-controlled. Apart from this, the FluoroMax®-P is identical in operation in all other respects to the FluoroMax®-3.

Theory of operation

A second source of illumination, a pulsed xenon lamp, is used for phosphorescence measurements. Samples are excited with pulsed light; the emitted phosphorescence is measured using an R928P photon-counting detector.

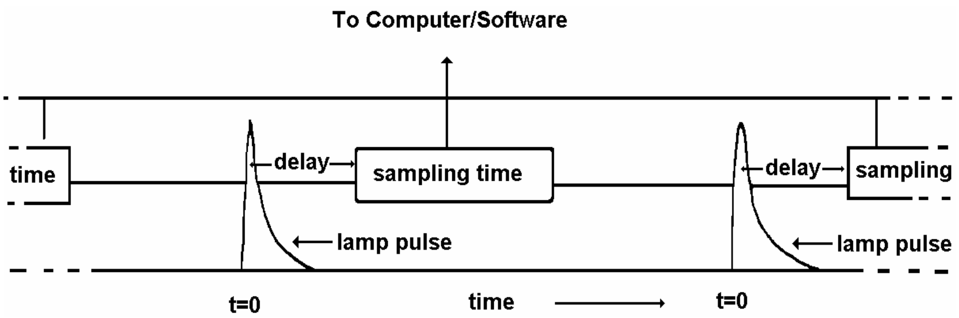
Sequence of data acquisition

The illuminator housing, or flash lamp, operates at up to 25 Hz. The control module triggers each lamp pulse. When the start of the light output is detected, a signal is sent to the control module for timing purposes. The control module houses the signal-gating circuitry that intercepts the signal from the pulse-counting emission photomultiplier tube, collects a selected, time-delimited portion of the signal, and later passes it to the software. The maximum signal detectable per flash varies with the integration time:

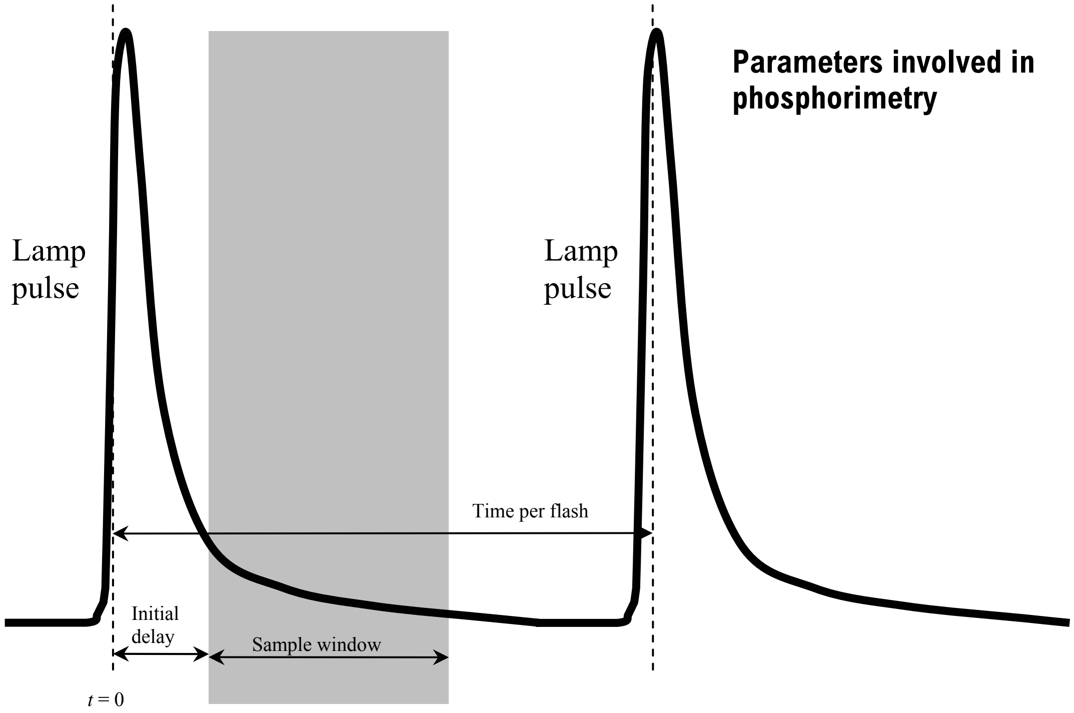
Integration time	Maximum signal (counts) per flash
100 μs	400
1 ms	4000
10 ms	40 000
100 ms	400 000
1 s	4 000 000

With, for example, 100 flashes of integrated over 10 ms each, the maximum detectable signal is:

100 flashes × 40 000 counts = 4 000 000 counts total



A typical sequence of data-acquisition starts with a flash from the pulsed lamp, sensed by the control module as time $t = 0$. The light enters the excitation monochromator, where it is dispersed. Monochromatic light from the monochromator excites the sample. Luminescence emission from the sample then passes through the emission monochromator to the photomultiplier-tube detector. The control module includes a gate-and-delay generator, allowing the signal at the detector to be integrated only during a specific period after the flash (the Initial Delay), for a pre-determined length of time (the Sample Window). Any signal arriving before or after the gating is ignored. This sequence of excitation, delay, and collection is repeated for each lamp pulse. The total signal is accumulated for a pre-determined number of exciting pulses (flashes) and saved to disk. The data then can be manipulated using the Analysis menu.



Parameters involved in phosphorimetry

This sequence of excitation, delay, and sampling, is repeated for each lamp flash. The signal is accumulated for a predetermined number of excitation pulses, then FluorEssence™ collects the total signal. After collection, FluorEssence™ displays the intensity of the luminescence as a function of time or wavelength. The *x*-axis is based on one of the four scan options:

- Excitation
- Emission
- Synchronous
- Phosphorescence delay

Phosphorimeter parameters

Four FluorEssence™ parameters govern the sequence in a phosphorimeter experiment. These parameters automatically appear on the phosphorimeter experiment-acquisition dialog box.

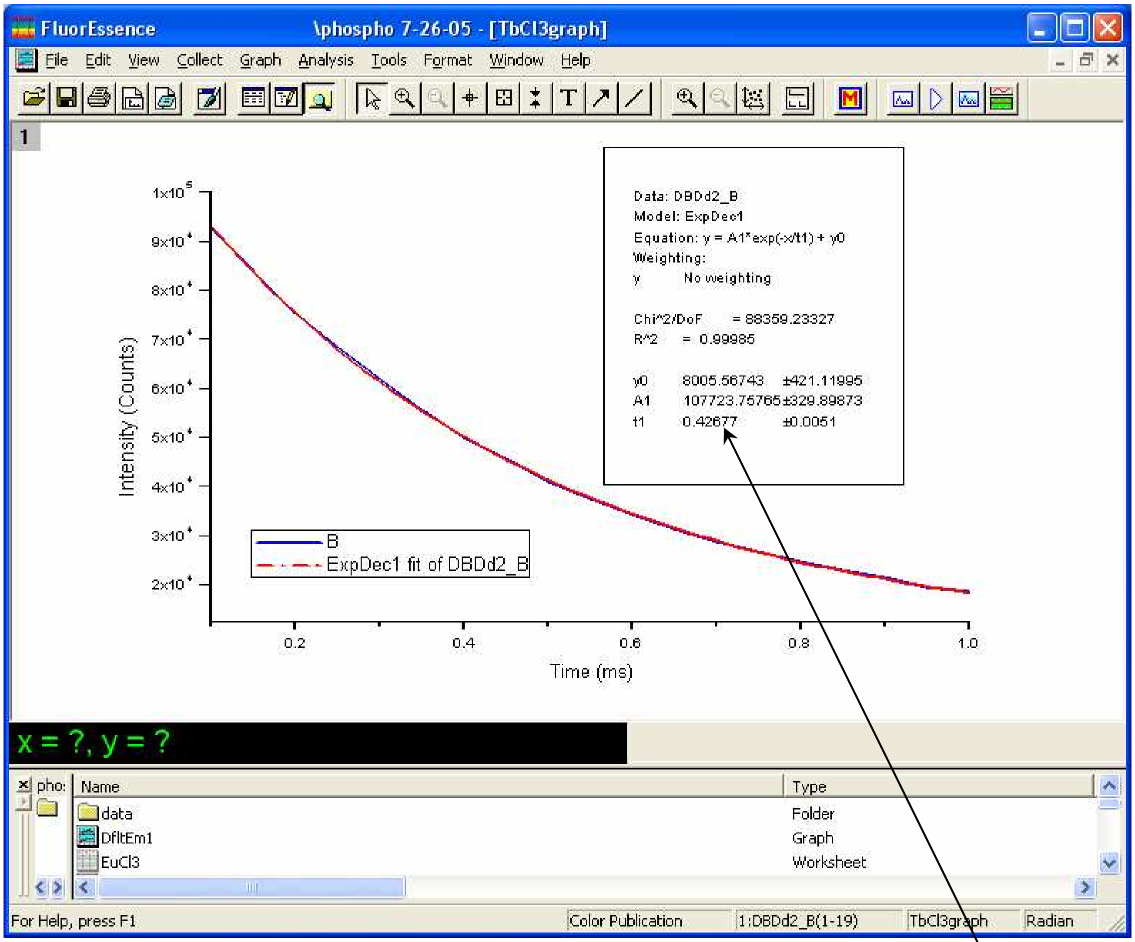
Initial Delay	Sets the time, in ms, between the start of the lamp flash and the onset of data-acquisition (opening of the Sample Window). Initial Delay can range from 0–10 000 ms, in increments of 0.001 ms. Accuracy of Initial Delay is better than ± 0.001 ms. Set Initial Delay long enough so that fluorescence emission and lamp decay are complete, so that the resulting spectrum represents phosphorescence only. The full-width at half-maximum lamp-pulse width is 3 µs, but there is a long decay time for the light output. Lamp intensity falls to less than 1% of peak output after 45 µs. Setting the delay to > 50 µs effectively removes any interference from the lamp.

	Initial Delay can be varied with time to yield a decay curve. Spectra can be scanned to isolate different phosphorescing components based on the lifetime of the luminescent species in the sample. Together, these two techniques can be used to create three-dimensional plots. For example, successive scans with varying delay times can be plotted. To record fluorescence and phosphorescence emission, set Initial Delay to zero.
Sample Window	Sets the duration of signal acquisition, in ms. The Sample Window opens when the Initial Delay ends. When the Sample Window opens, the signal is counted and integrated. After the Sample Window closes, any signal is ignored. The Sample Window may be set from 0.01 to 10 000 ms. If the lifetime of the phosphorescence is known, set the Sample Window to 5–10 times the lifetime. If the phosphorescence lifetime is unknown, make the Sample Window a small fraction of the anticipated lifetime, and then increase it until acceptable results are observed. If the Sample Window is too long, the detector will record spurious background signal. If the Sample Window is too short, components of the lifetime decay may be missed. With two or more species decaying simultaneously, try varying the Initial Delay and the Sample Window.
Time per flash	Sets the total cycle length per flash, including on time, decay time, and dead time between flashes. The Time per flash is the reciprocal of the repetition rate of the lamp pulses. The allowable repetition rate is 0.03–25 Hz. The Time per flash must be slow enough to let the Sample Window close before another flash begins. Accuracy of the repetition rate is ± 1 ms. The Time per flash is governed by Time per Flash ≥ Initial Delay + Sample Window + 20 ms
Flash count	Sets the number of lamp pulses that contribute to each data point. The range for Flash count is 1 to 999. The signal collected per flash is integrated over the total Flash counts before FluorEssence™ stores it. The more flashes accumulated, the higher the signal-to-noise ratio becomes.

For more information about FluorEssence™ phosphorimeter commands, consult the FluorEssence™ on-line help.

Applications for the phosphorimeter

Phosphorescence decay curve


FluorEssence
\phospho 7-26-05 - [TbCl3graph]
File Edit View Collect Graph Analysis Tools Format Window Help
1
Intensity (Counts)
1x10⁵
9x10⁴
8x10⁴
7x10⁴
6x10⁴
5x10⁴
4x10⁴
3x10⁴
2x10⁴
0.2
0.4
0.6
0.8
1.0
Time (ms)
B
ExpDec1 fit of DBDd2_B
x = ?, y = ?
pho:
For Help, press F1
Color Publication
1:DBDd2_B(1-19)
TbCl3graph
Radian

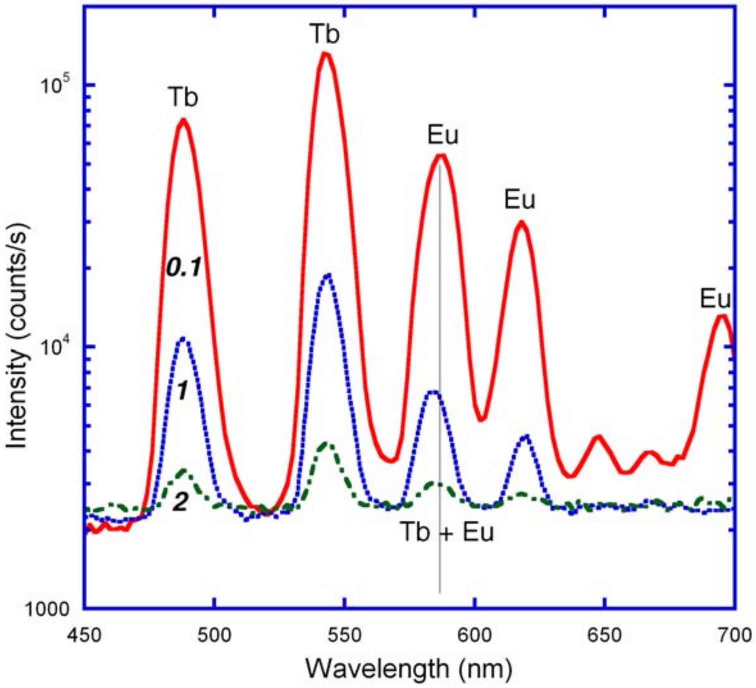
```
Data: DBDd2_B
Model: ExpDec1
Equation: y = A1*exp(-x/t1) + y0
Weighting:
y         No weighting

Chi^2/DoF      = 88359.23327
R^2     =  0.99985

y0        8005.56743     ±421.11995
A1        107723.75765±329.89873
t1        0.42677          ±0.0051
```

Name	Type
data	Folder
DfltEm1	Graph
EuCl3	Worksheet

With the Phos experiment type in the **Fluorescence Experiment Menu**, create a phosphorescence-decay curve, as in the screenshot from FluorEssence™ shown above. This is an example using data from $TbCl_3$(*aq*), fitted to a single-exponential curve-fit in Origin®. All parameters are automatically displayed, including the fitted lifetime of 427 µs.

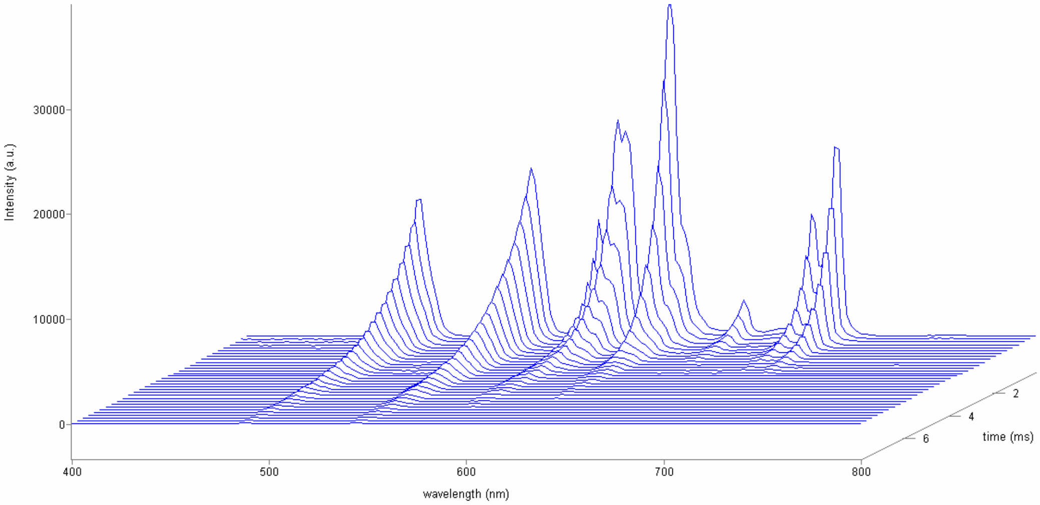
Isolate components in a mixture based on lifetimes

To the right are three scans of an aqueous mixture of terbium and europium chlorides that isolate different phosphorescent components based on their lifetimes. For example, a sample containing two phosphorescent species with different lifetimes (Tb = 421 µs; Eu = 113 µs) can be resolved into its components by varying the Initial Delay parameter, here shown as bold italic numerals, ***0.1*** ms, ***1*** ms, and ***2*** ms initial delay. Notice the wavelength shift in the 593 nm peak (marked with a gray line) from initially mostly europium fluorescence (short lifetime) to a mixture of terbium (longer lifetime) and europium fluorescence at later delays. Also notice the Eu peak near 690 nm at 0.1 ms that vanishes at later times. This experiment was performed using the Emission subtype of Phos experiment.



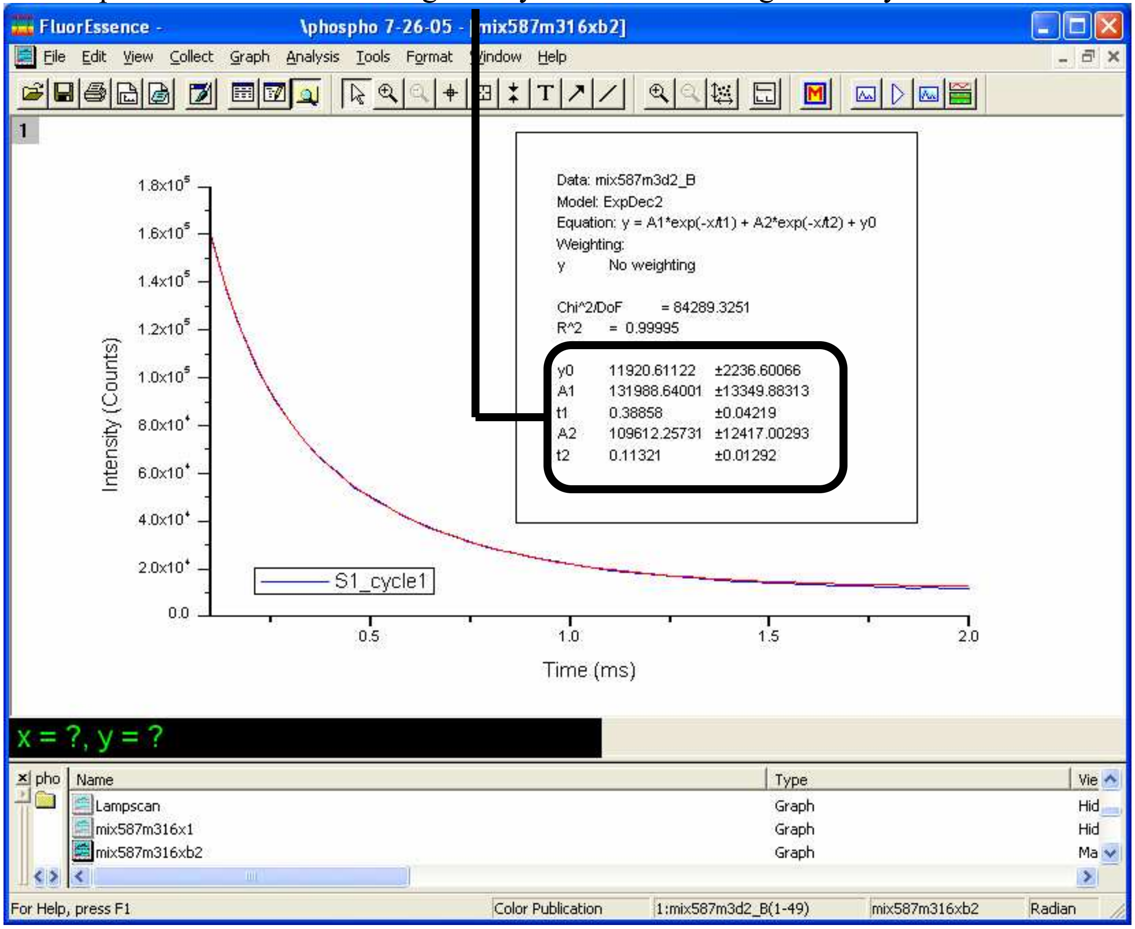
Three-dimensional plots

Using the phosphorescence delay curve with the Initial Delay technique, a three-dimensional graph can be created, as shown below. This graph shows successive scans of a mixture of Tb-L and Eu-L (where L = benzophenone antenna chromophore) with varying time delays, segregating species into contours representing a particular slice of time.



Kinetic analysis of mixtures

Often a sample containing a mixture of components can be analyzed through fitting its phosphorescence-decay curve. Here is a phosphorescence decay of an aqueous mixture of $EuCl_3$ and $TbCl_3$, whose different lifetimes have been extracted by FluorEssence™'s dual-exponential fit. Curve-fitting merely involves choosing the analytical model.


FluorEssence - \phospho 7-26-05 - [mix587m316xb2]
File Edit View Collect Graph Analysis Tools Format Window Help
1
Intensity (Counts)
1.8x10⁵
1.6x10⁵
1.4x10⁵
1.2x10⁵
1.0x10⁵
8.0x10⁴
6.0x10⁴
4.0x10⁴
2.0x10⁴
0.0
0.5
1.0
1.5
2.0
Time (ms)
S1_cycle1
x = ?, y = ?
pho
Name
Type
Vie
Lampscan
mix587m316x1
mix587m316xb2
Graph
Graph
Graph
Hid
Hid
Ma
For Help, press F1
Color Publication
1:mix587m3d2_B(1-49)
mix587m316xb2
Radian

```
Data: mix587m3d2_B
Model: ExpDec2
Equation: y = A1*exp(-x/t1) + A2*exp(-x/t2) + y0
Weighting:
y        No weighting

Chi^2/DoF       = 84289.3251
R^2      =  0.99995

y0       11920.61122    ±2236.60066
A1       131988.64001   ±13349.88313
t1       0.38858        ±0.04219
A2       109612.25731   ±12417.00293
t2       0.11321        ±0.01292
```

Operation of the phosphorimeter

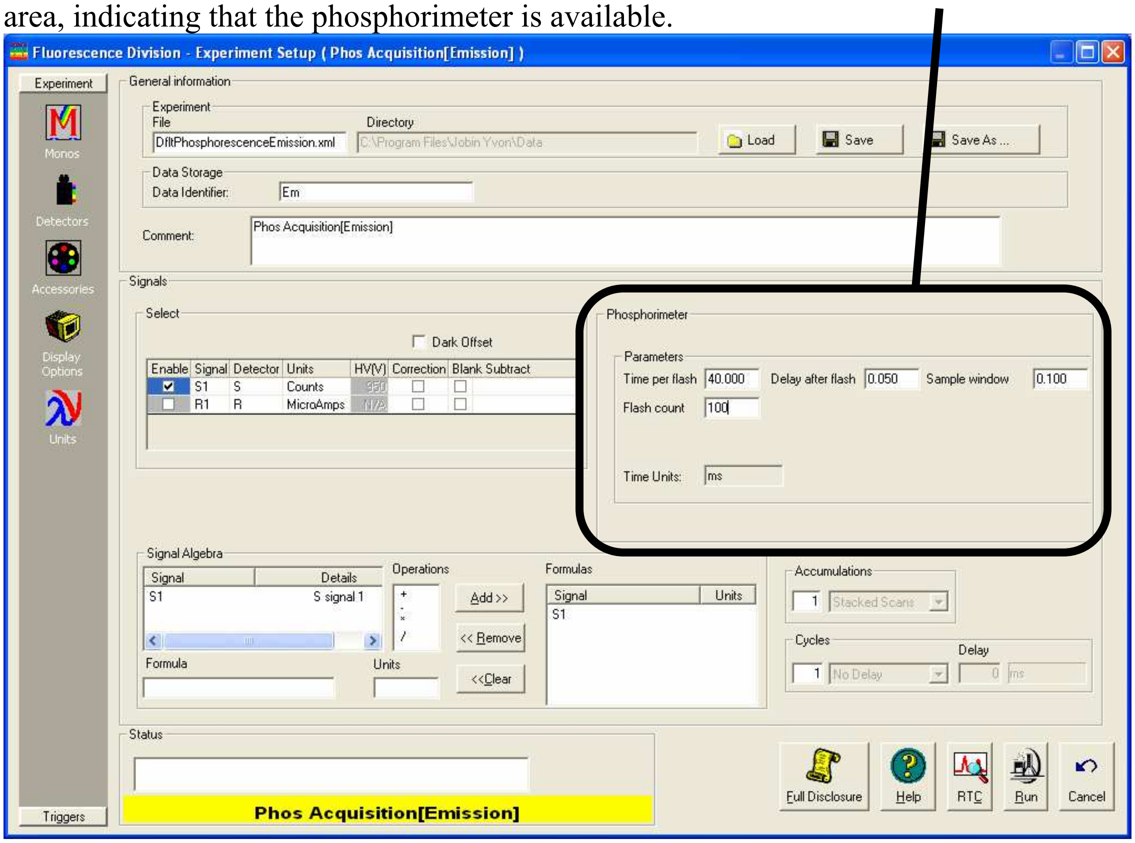
Start-up

Load an appropriate instrument configuration that includes the phosphorimeter.

FluorEssence™ features

Experiment Setup window

The **Experiment Setup** window, under the Detectors icon, includes a Phosphorimeter area, indicating that the phosphorimeter is available.



Fluorescence Division - Experiment Setup (Phos Acquisition[Emission])

General information

Experiment File: DfltPhosphorescenceEmission.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage Data Identifier: Em

Comment: Phos Acquisition[Emission]

Signals

Select ☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☑	S1	S	Counts	950	☐	☐
☐	R1	R	MicroAmps	N/A	☐	☐

Phosphorimeter

Parameters

Time per flash: 40.000

Delay after flash: 0.050

Sample window: 0.100

Flash count: 100

Time Units: ms

Signal Algebra

Signal	Details
S1	S signal 1

Formulas

Signal	Units
S1	

Accumulations: 1 Stacked Scans

Cycles: 1 No Delay

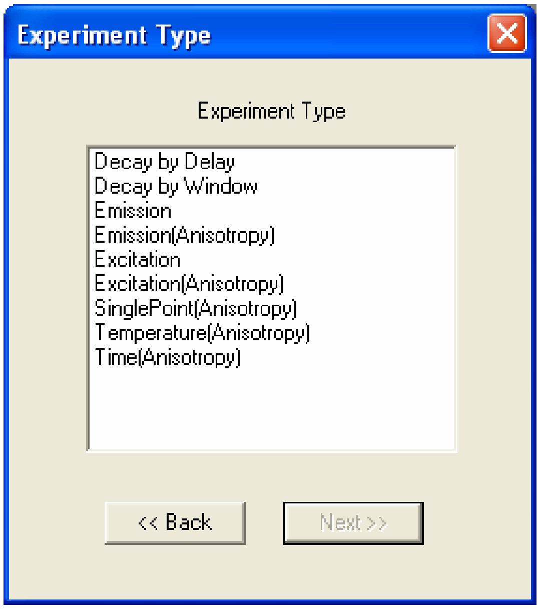
Delay: 0 ms

Status: Phos Acquisition[Emission]

Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Operations
+
-
*
/
Add >>
<< Remove
<<Clear
Formula
Units
Full Disclosure
Help
RTC
Run
Cancel

Experiment Type window

After choosing the Phos experiment type in the **Fluorescence Main Experiment Menu**, up to nine experiment types are available for the phosphorimeter, depending on the instrument configuration:



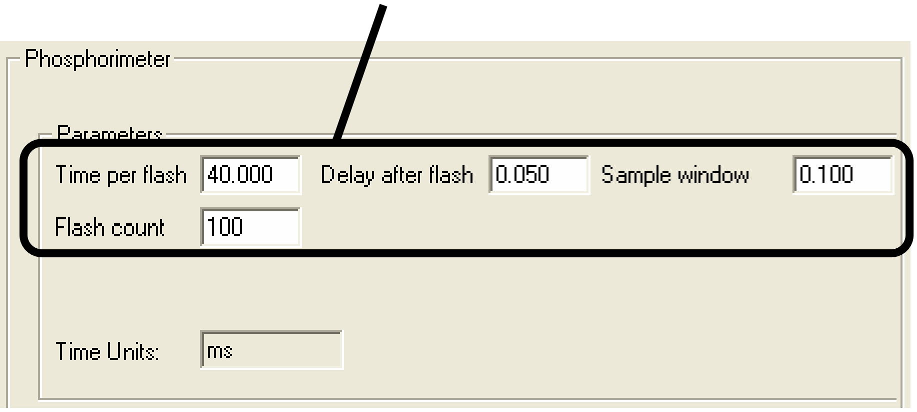
Experiment Type

Decay by Delay
Decay by Window
Emission
Emission(Anisotropy)
Excitation
Excitation(Anisotropy)
SinglePoint(Anisotropy)
Temperature(Anisotropy)
Time(Anisotropy)

Excitation

Emission

These are similar to the standard excitation, and emission synchronous scans. Among the changes are that the **Integration Time** field is removed, and instead are data-entry fields for **Sample window**, **Delay after flash**, **Time per flash**, and **Flash count**. These four fields are described above, in the Theory of Operation section, and shown below.

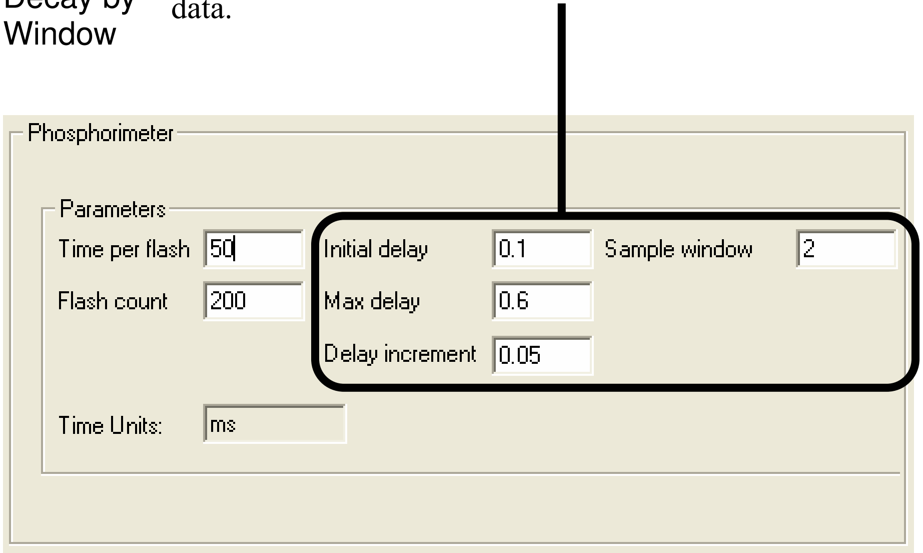

Phosphorimeter
Parameters
Time per flash: 40.000
Delay after flash: 0.050
Sample window: 0.100
Flash count: 100
Time Units: ms

Above is a **Phosphorimeter** area with a typical set of parameters for the emission of $EuCl_3$. Use an excitation monochromator set to 393 nm; the emission monochromator should start at 570 nm, end at 750 nm, with an increment of 1 nm.

Decay by Delay

Decay by Window

These produce a decay of phosphorescence over time. **Decay by Delay** varies the flash's **Delay after flash** in order to construct the decay curve. **Decay by Window** varies the length of the **Sample window** while taking data.

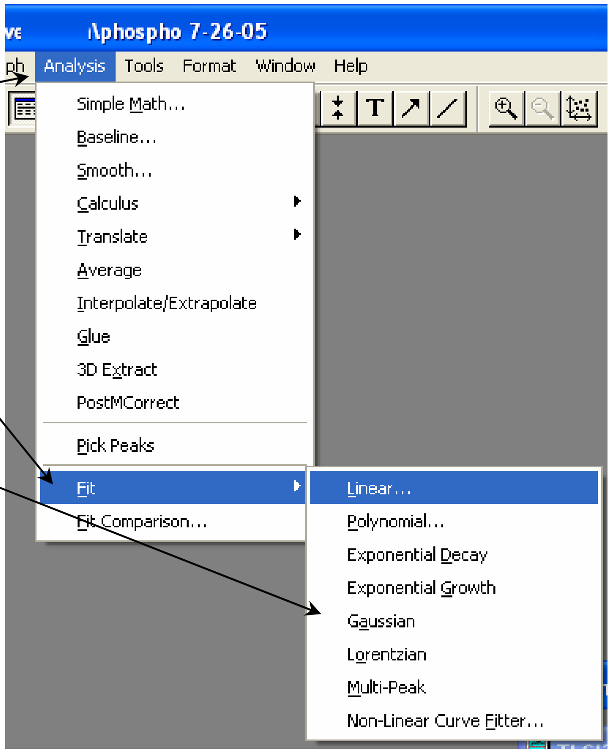

Phosphorimeter
Parameters
Time per flash: 50
Initial delay: 0.1
Sample window: 2
Flash count: 200
Max delay: 0.6
Delay increment: 0.05
Time Units: ms

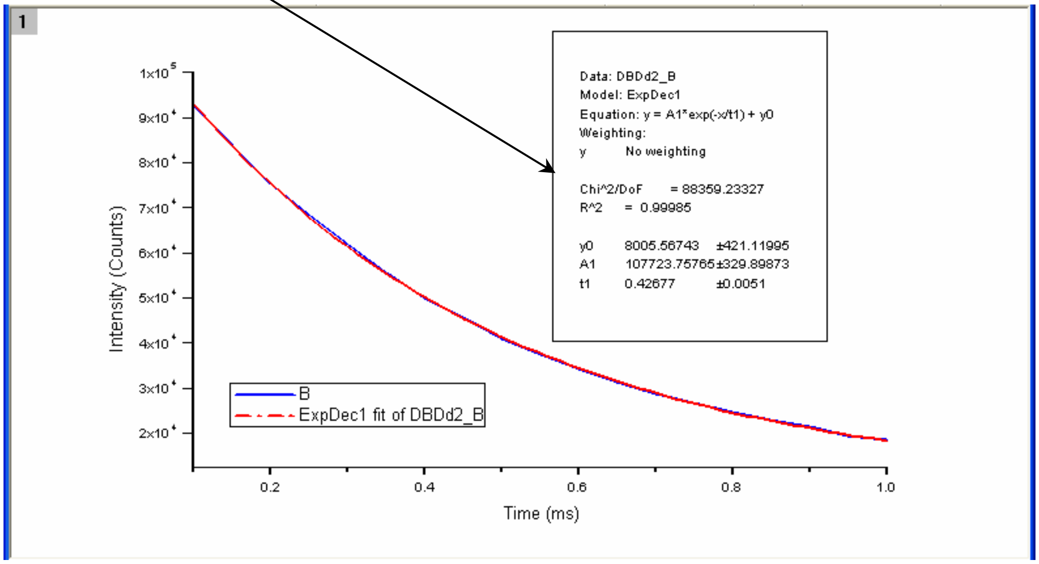
Above is a **Phosphorimeter** area showing typical parameters for the phosphorimeter decay of $EuCl_3$. Set the emission monochromator to 590 nm and the excitation monochromator to 393 nm.

Anisotropy phosphorimeter scans run the automated polarizers as well.

Processing phosphorimeter data

1. Open the graph to be processed.
2. Click on the data points to be processed.
3. In the toolbar, choose Analysis.
   A drop-down menu appears.
4. Choose Fit.
5. From the sub-menu, choose the type of analytical curve to use.
6. Follow the instructions for that type of curve.
   The fit plus parameters appear on the graph.


\phospho 7-26-05
ph
Analysis
Tools
Format
Window
Help
Simple Math...
Baseline...
Smooth...
Calculus
Translate
Average
Interpolate/Extrapolate
Glue
3D Extract
PostMCorrect
Pick Peaks
Fit
Fit Comparison...
Linear...
Polynomial...
Exponential Decay
Exponential Growth
Gaussian
Lorentzian
Multi-Peak
Non-Linear Curve Fitter...



```
Data: DBDd2_B
Model: ExpDec1
Equation: y = A1*exp(-x/t1) + y0
Weighting:
y          No weighting

Chi^2/DoF      = 88359.23327
R^2     =  0.99985

y0       8005.56743     ±421.11995
A1       107723.75765±329.89873
t1       0.42677           ±0.0051
```

Lamp replacement

The xenon flash lamp typically has a half-intensity life of at least 10 million flashes. Follow the procedure below for replacement and alignment.

Warning: *High voltage exists within the FluoroMax®-P. To avoid fatal shocks, before removing the lamp cover, unplug the FluoroMax®-P's power cord, and wait at least 15 min while the internal capacitors discharge.* ***Never operate the lamp with the cover removed!***

Caution: *Intense ultraviolet, visible, or infrared light may be present when the instrument is open, so wear eye- and skin-protection, such as light-blocking goggles and light-blocking clothing.*

Warning: *Xenon lamps are an explosion hazard. Be sure that the power is off, and all AC (mains) power is disconnected from the system. Read and follow all the cautions 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.

1 In FluorEssence™, be sure the FluoroMax®-P instrument configuration is loaded.

The flip-mirror automatically rotates to the flash lamp, giving you more room to work.

Caution: *Never rotate the flip mirror inside the lamp housing manually. This can strip the gears in the gearbox.*

2 Switch off and prepare the FluoroMax®-P.

a Be sure that the FluoroMax®-P and the host computer are turned off.

b Remove the AC (mains) power cord from the FluoroMax®-P.

c Disconnect the RS-232 cable, optional trigger-box cable, power cord, and any other cables attached to the spectrofluorometer.

3 Remove the sample mount from the front of the FluoroMax®-P.

a Remove the four screws that secure the sample mount to the instrument.

b Gently slide the sample mount out of the instrument.

Some sample mounts have a 15-pin connector at the inside end for automated accessories.

4 Remove the instrument cover.

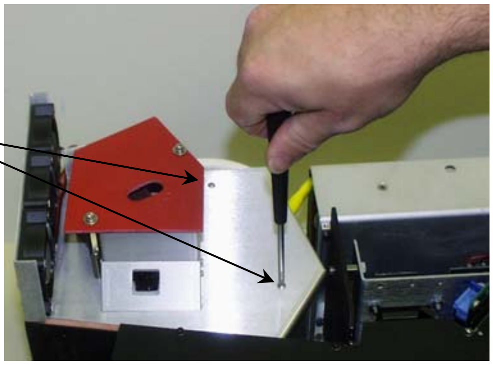
a Remove the seven Phillips screws (two on each side, and three in the back) from the cover.

b Lift the cover vertically off the instrument by grasping opposite corners of the cover and raising upward.

If the cover sticks, gently work each side upward until the cover slides smoothly off.

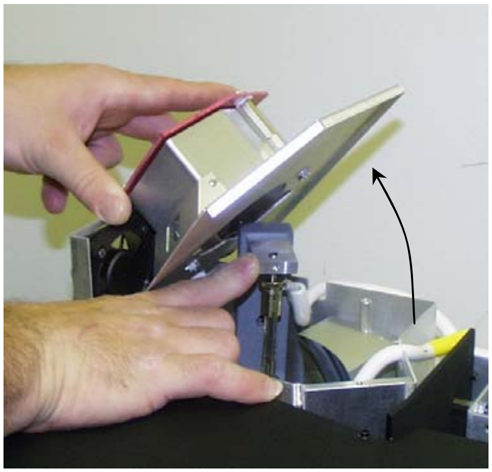
5 Remove the lamp-housing cover at the rear of the instrument.

a Remove the two screws from the lamp-housing cover.



b Gently rotate the lamp-housing cover with its cooling fans attached.

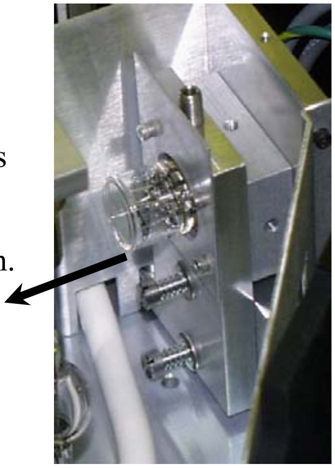
c Rotate the cover backwards, and set it behind the instrument so that electrical connections are not strained.



6 Remove the flash lamp.

a Follow all safety precautions on the new lamp's box.

b Pull the old flash lamp out with a steady motion.

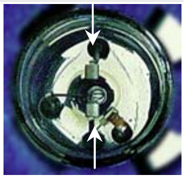


c Discard the old flash lamp in a safe and appropriate manner.

7 Insert the new flash lamp.

> ***Warning:*** *Never touch the flash lamp's glass bulb with bare hands. The oils from your hands can weaken the bulb and cause catastrophic failure. Handle the flash lamp with tissues, cloth gloves, or soft cloths only.*

a Align the pins with the lamp socket.
The cathode and anode should be vertical, as shown here.→



b Push the lamp in firmly until it is seated securely and properly.

> ***Note:*** *If the lamp tilts during insertion, check the pins—they should be straight. If they are not straight, re-align the pins before insertion.*

8 Replace the lamp-housing cover.

9 Secure the two screws on the lamp-housing cover.

10 Replace the instrument cover, and reseat the seven Phillips screws.

11 Reconnect all cables (power, accessories, etc.) to the FluoroMax®-P.

10: 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 $\sim 10^{9}$ 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 ($<r>$) 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 oriented polarizers (H) are said to be at 90°. Polarization and anisotropy are expressed as follows:

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

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

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:

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

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:

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

Anisotropy in a spectrofluorometer is defined as:

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

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*.

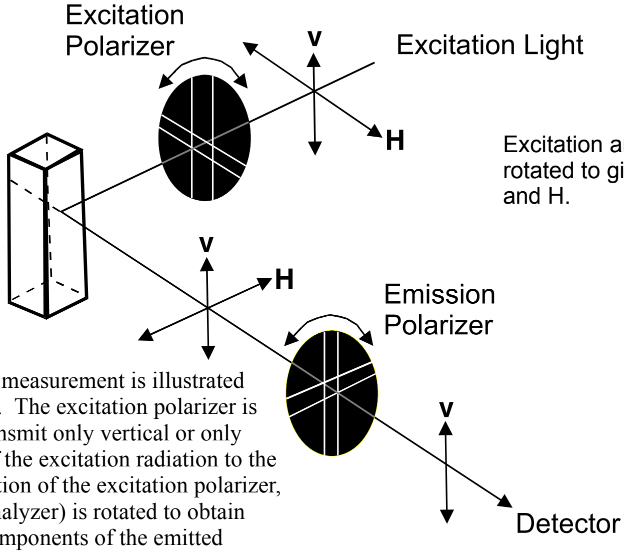
Experimentalists often multiply polarization units by 1000 to yield *millipolarization* units, m*P*, for very small changes in the polarization.

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 FluoroMax®-3 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.

A schematic diagram of the L-format geometry 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.

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 lifetime, 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

Spex® 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.

Warning: *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:

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

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

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

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

1. Go to the **Real Time Control**.
2. Place the scatterer in the sample-cuvette position.
3. Place the sample-compartment cover on the sample compartment.
4. Set all monochromators to 400 nm.
5. Set all slits to 0.5 mm.

6 Apply the proper voltage on S (950 V for an R928P photomultiplier).

7 Open the excitation shutter (if applicable).

8 Click the Accessories icon.

9 Set both polarizers to VV (0°).

10 Check the signal on S. Set slits evenly for each monochromator to result in 1–1.5 million cps on S.

11 Record the four permutations of the polarizers: VV, HV, VH, and HH.

12 Subtract the dark counts from each permutation. For example, VV′ = VV – dark counts.

13 Calculate the polarization ratio using the dark-subtracted data (e.g., VV′).

14 If the polarization ratio ≥ 100, then the polarizers are satisfactorily aligned. If not, then polarizers should be re-aligned.

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

Re-alignment of polarizers

FluoroMax®-3 autopolarizers may be aligned using a software routine called **Polarizer Alignment** in **Experiment Setup**.

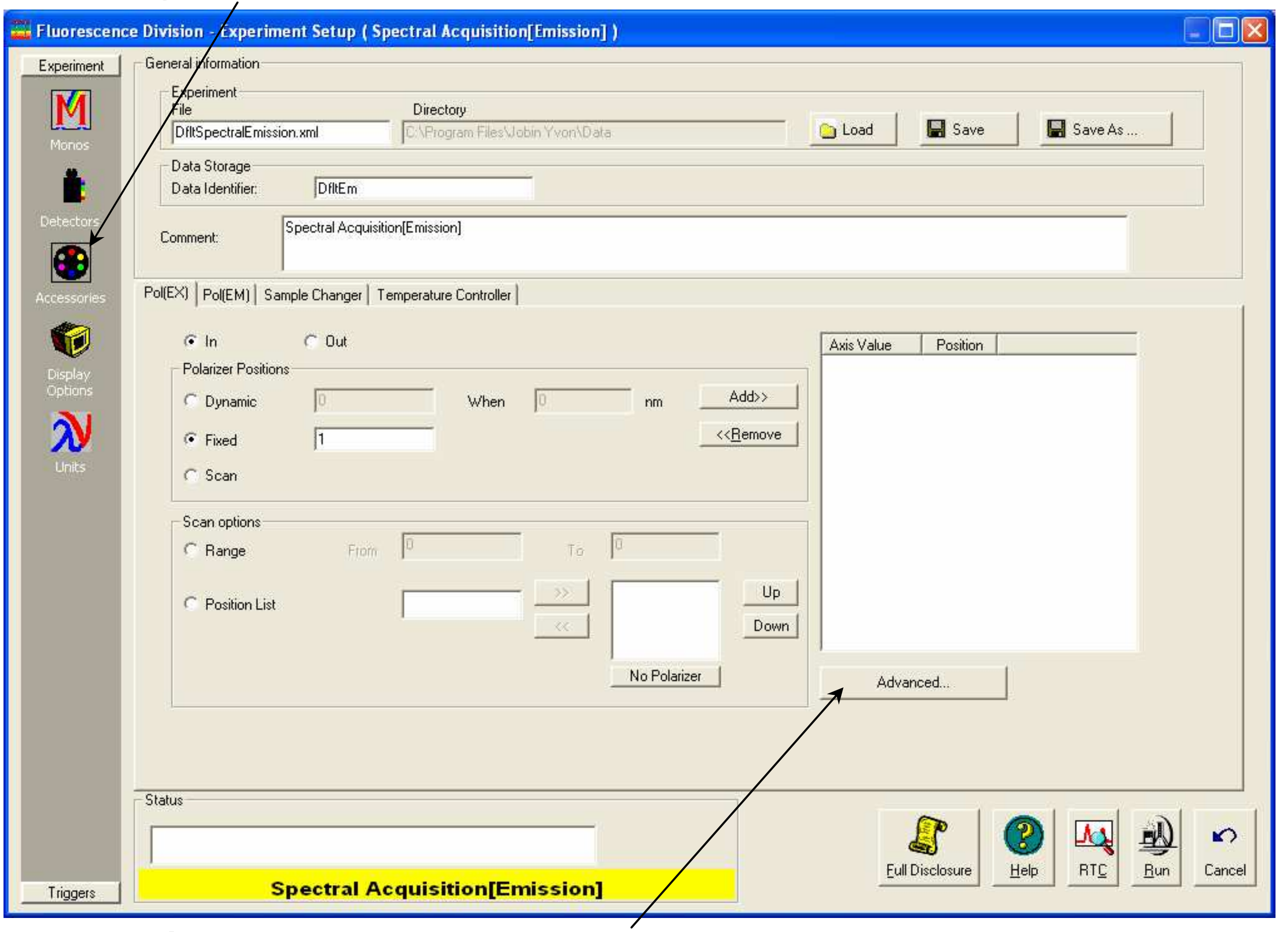
Using Polarizer Alignment

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.



Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])

File: DfltSpectralEmission.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Identifier: DfltEm

Comment: Spectral Acquisition[Emission]

● In ○ Out

Polarizer Positions: ● Fixed 1

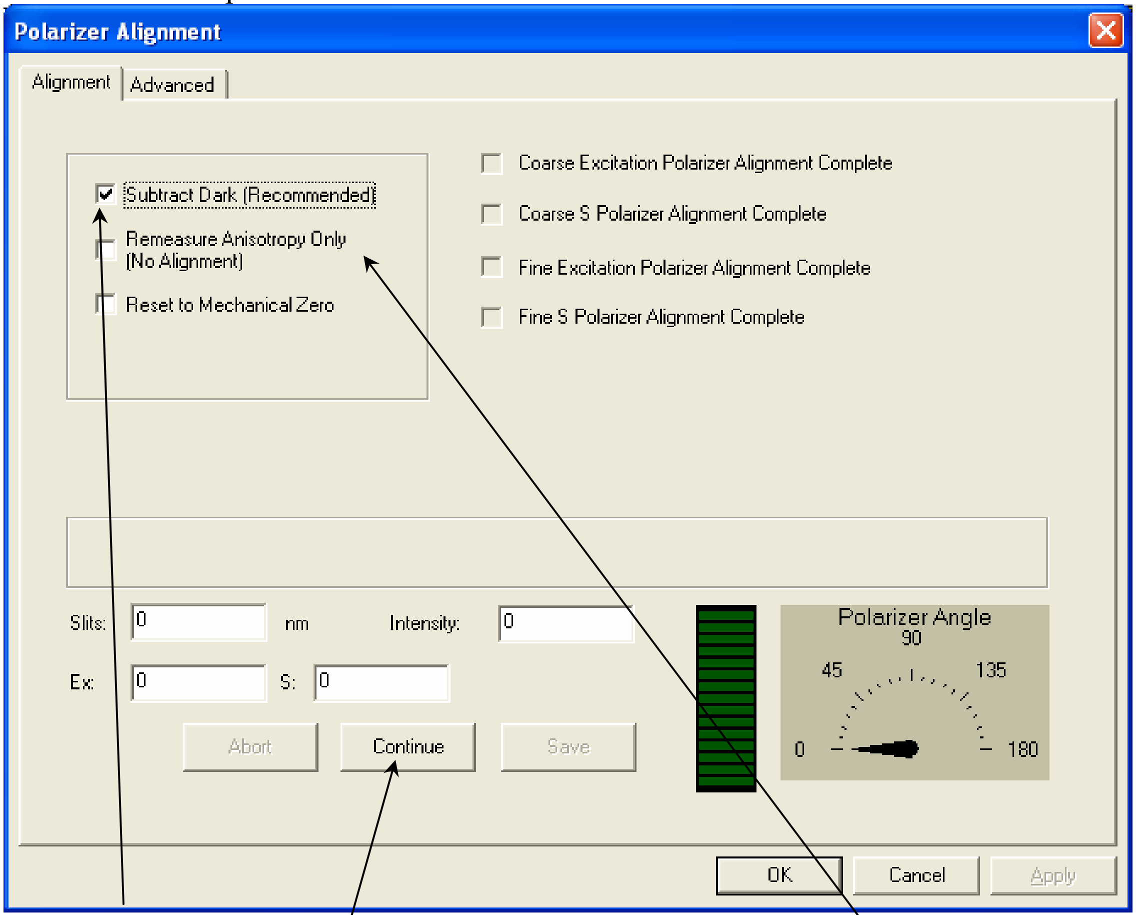
Status: Spectral Acquisition[Emission]

Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Experiment
Data Storage
Load
Save
Save As ...
Pol(EX)
Pol(EM)
Sample Changer
Temperature Controller
○ Dynamic
When
nm
○ Scan
Add>>
<<Remove
Scan options
○ Range
From
To
○ Position List
>>
<<
Up
Down
No Polarizer
Axis Value
Position
Advanced...
Full Disclosure
Help
RTC
Run
Cancel

4 Click the Advanced... button.

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

Choose various options:


Polarizer Alignment
Alignment
Advanced
Polarizer Angle
90
45
135
0
180
Abort
Continue
Save
OK
Cancel
Apply

☑ Subtract Dark (Recommended)
☐ Remeasure Anisotropy Only (No Alignment)
☐ Reset to Mechanical Zero

☐ Coarse Excitation Polarizer Alignment Complete
☐ Coarse S Polarizer Alignment Complete
☐ Fine Excitation Polarizer Alignment Complete
☐ Fine S Polarizer Alignment Complete

Slits: 0 nm Intensity: 0
Ex: 0 S: 0

- 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 check-box.*

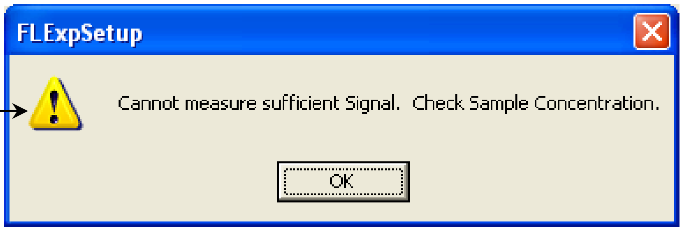
6 Place the LUDOX® or glycogen in the sample holder.

7 Click the Continue button.

Warning: *Read the Materials Safety Data Sheets (MSDS) before using colloidal silica or glycogen.*

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.


FLExpSetup
OK

Cannot measure sufficient Signal. Check Sample Concentration.

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

8. Approve or retry the measurement based on satisfaction with the result.
9. To quit, hit the Cancel button at 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.*

> ***Caution:*** *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 occur at the calibration position of the polarizers.*

2. Loosen the screws holding 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.

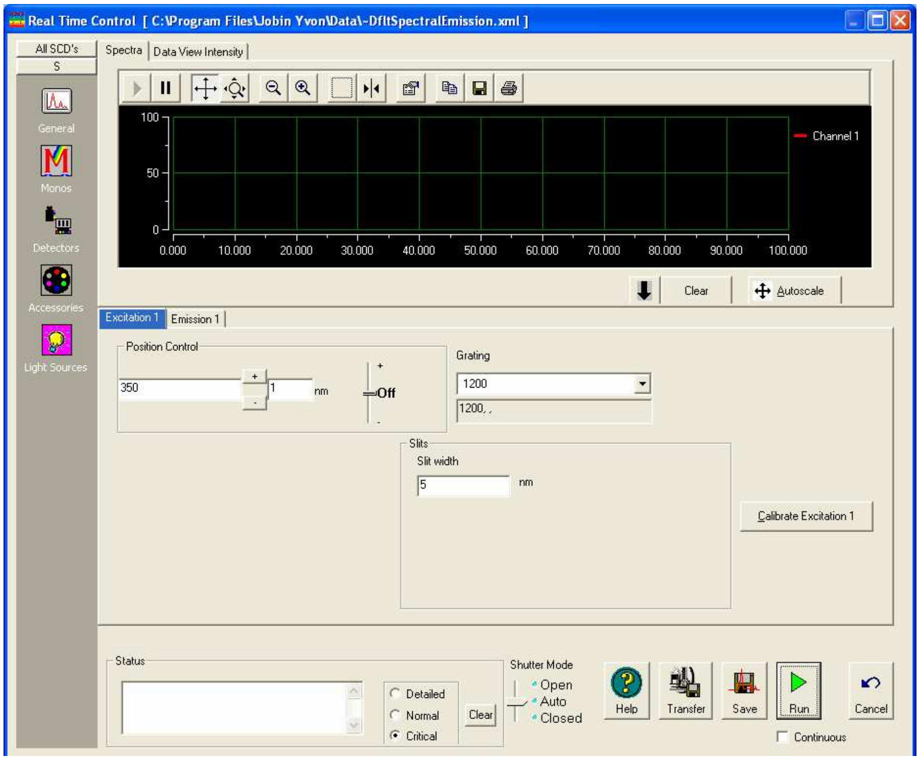
   They should not slip, but should allow easy manual rotation.
4. Set the polarizer crystals’ position.

   They should 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.

Warning: *Read the Materials Safety Data Sheets (MSDS) before using colloidal silica or glycogen.*

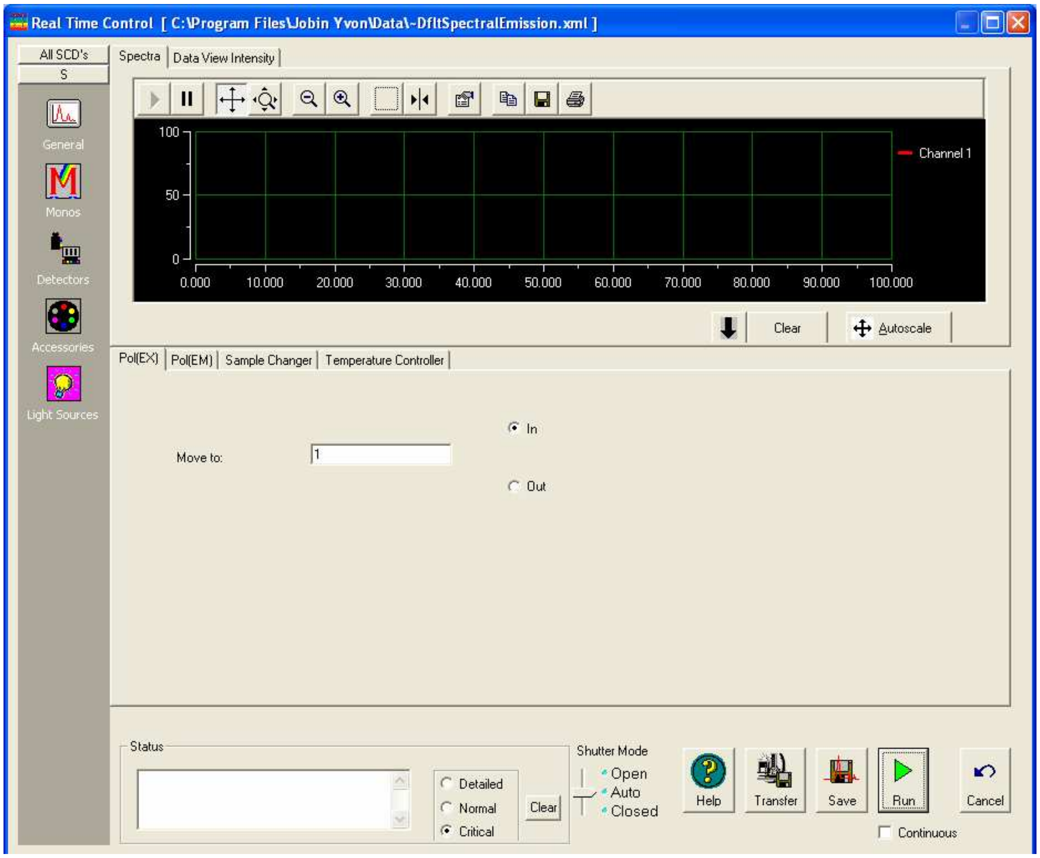
7 Start the software (if not yet running) and go to **Real Time Control**:


Real Time Control [C:\Program Files\Jobin Yvon\Data\-DfltSpectralEmission.xml]

Position Control: 350 nm, 1
Grating: 1200
Slit width: 5 nm
Shutter Mode: Open, Auto, Closed
All SCD's
S
General
Monos
Detectors
Accessories
Light Sources
Spectra
Data View Intensity
100
50
0
0.000
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
Channel 1
Clear
Autoscale
Excitation 1
Emission 1
Off
1200, ,
Slits
Calibrate Excitation 1
Status
Detailed
Normal
Critical
Clear
Help
Transfer
Save
Run
Cancel
Continuous

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

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


Real Time Control [C:\Program Files\Jobin Yvon\Data\~DfltSpectralEmission.xml]
All SCD's
S
General
Monos
Detectors
Accessories
Light Sources
Spectra
Data View Intensity
100
50
0
0.000
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
Channel 1
Clear
Autoscale
Pol(EX)
Pol(EM)
Sample Changer
Temperature Controller
Status
Clear
Shutter Mode
Open
Auto
Closed
Help
Transfer
Save
Run
Cancel

Move to: 1

● In

○ Out

○ Detailed

○ Normal

● Critical

☐ Continuous

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 Spex® 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.

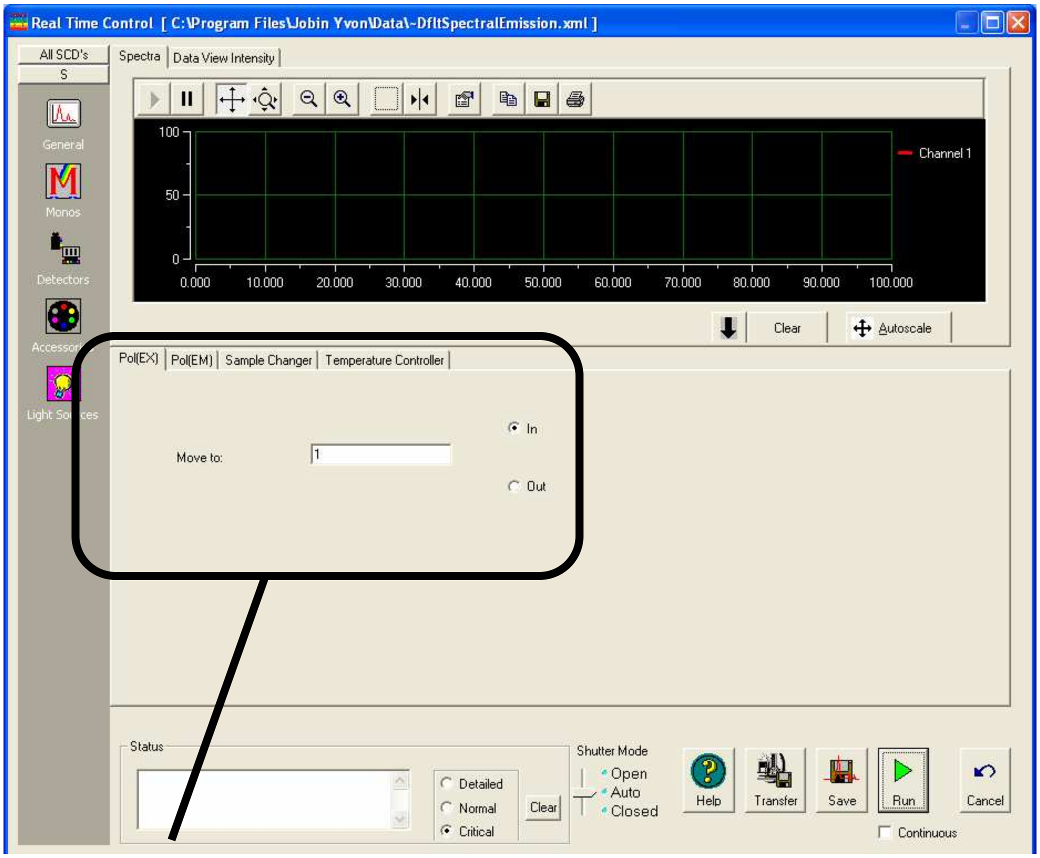
Applications for polarizers

- 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 or the `POLAR.AB` macro acquisition. This provides information about rotational sensitivity of the excitation spectrum by measuring $\langle r \rangle$ 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 [C:\Program Files\Jobin Yvon\Data\-DfltSpectralEmission.xml]
All SCD's
S
General
Monos
Detectors
Accessories
Light Sources
Spectra
Data View Intensity
100
50
0
0.000
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
Channel 1
Clear
Autoscale
Pol(EX)
Pol(EM)
Sample Changer
Temperature Controller
Status
Detailed
Normal
Critical
Clear
Shutter Mode
Open
Auto
Closed
Help
Transfer
Save
Run
Cancel
Continuous

Move to: 1

● In

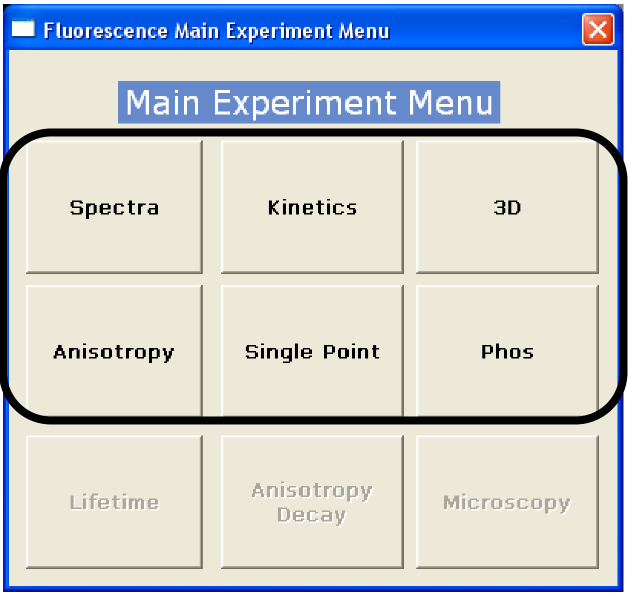
○ Out

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.

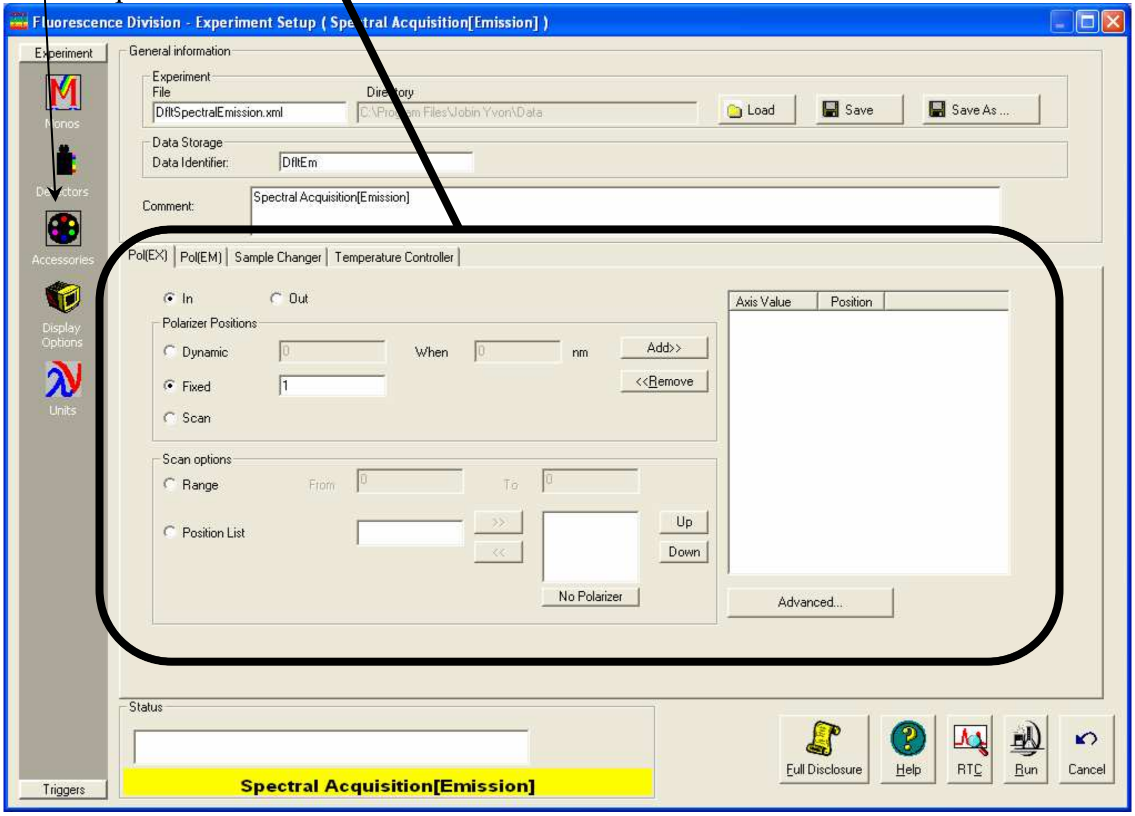
> ***Note:*** **Real Time Control** *is only intended for real-time setup of a scan. Use* **Experiment Setup** *to work at fixed wavelengths.*

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:**


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

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


Fluorescence Division - Experiment Setup (Spectral Acquisition[Emission])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
General information
Load
Save
Save As ...
Pol(EX)
Pol(EM)
Sample Changer
Temperature Controller
Add>>
<<Remove
>>
<<
Up
Down
No Polarizer
Axis Value
Position
Advanced...
Full Disclosure
Help
RTC
Run
Cancel

Experiment
File: DfltSpectralEmission.xml
Directory: C:\Program Files\Jobin Yvon\Data

Data Storage
Data Identifier: DfltEm

Comment: Spectral Acquisition[Emission]

● In ○ Out

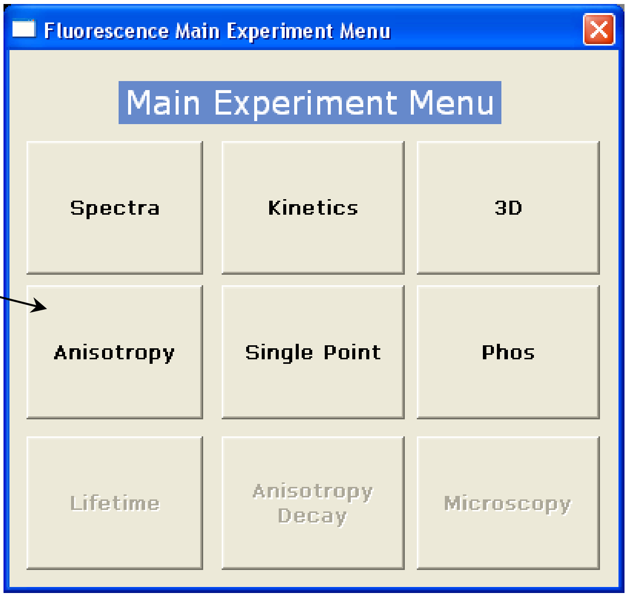
Polarizer Positions
○ Dynamic 0 When 0 nm
● Fixed 1
○ Scan

Scan options
○ Range From 0 To 0
○ Position List

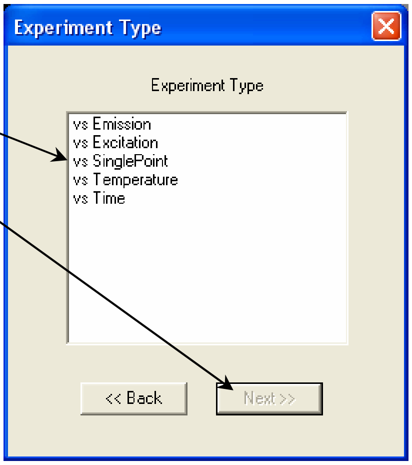
Status
Spectral Acquisition[Emission]

Constant Wavelength Analysis

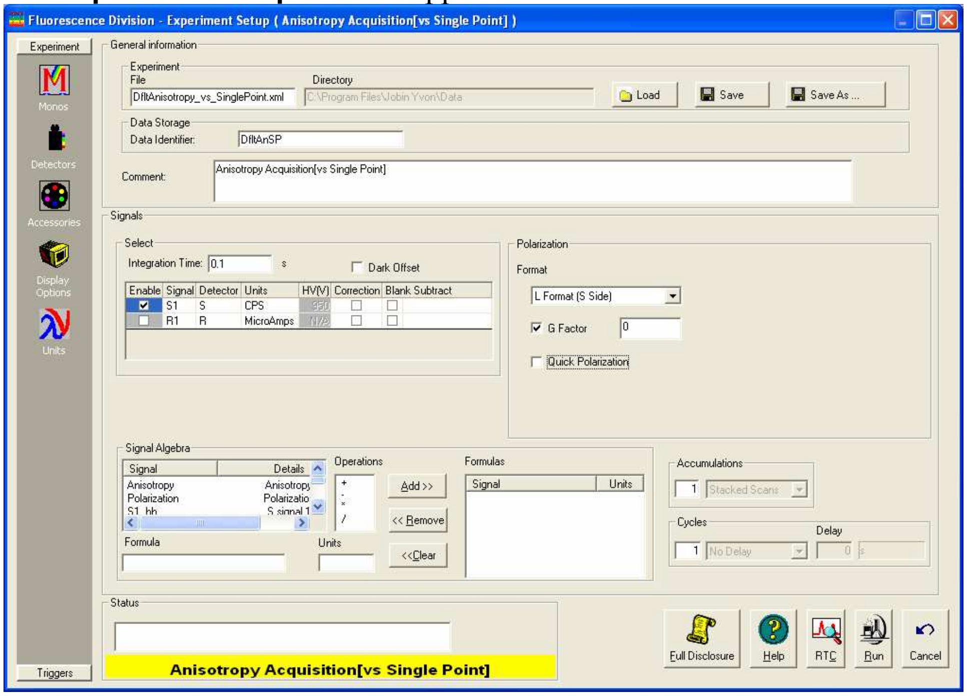
To do a constant-wavelength analysis experiment, that is, to take polarization acquisitions at fixed excitation/emission wavelength-pairs, choose Anisotropy from the **Fluorescence Main Experiment Menu**.


Fluorescence Main Experiment Menu
Main Experiment Menu
Spectra
Kinetics
3D
Anisotropy
Single Point
Phos
Lifetime
Anisotropy Decay
Microscopy

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


Experiment Type
Experiment Type
vs Emission
vs Excitation
vs SinglePoint
vs Temperature
vs Time
<< Back
Next >>

The **Experiment Setup** window appears.


Fluorescence Division - Experiment Setup (Anisotropy Acquisition[vs Single Point])
Experiment
Monos
Detectors
Accessories
Display Options
Units
Triggers
Load
Save
Save As ...
Add >>
<< Remove
<<Clear
Full Disclosure
Help
RTC
Run
Cancel

General information

Experiment File: DfltAnisotropy_vs_SinglePoint.xml

Directory: C:\Program Files\Jobin Yvon\Data

Data Storage

Data Identifier: DfltAnSP

Comment: Anisotropy Acquisition[vs Single Point]

Signals

Select

Integration Time: 0.1 s

☐ Dark Offset

Enable	Signal	Detector	Units	HV(V)	Correction	Blank Subtract
☑	S1	S	CPS	950	☐	☐
☐	R1	R	MicroAmps	N/A	☐	☐

Polarization

Format: L Format (S Side)

☑ G Factor 0

☐ Quick Polarization

Signal Algebra

Signal	Details
Anisotropy	Anisotropy
Polarization	Polarizatio
S1_hh	S signal 1

Operations: + - × /

Formula:

Units:

Formulas

Signal	Units

Accumulations: 1 Stacked Scans

Cycles: 1 No Delay

Delay: 0 s

Status

Anisotropy Acquisition[vs Single Point]

Make sure to use the appropriate Signal in the Signal Algebra area. Add >> it to the Formulas table. Click the checkbox to measure G factor(s) during the scan, or specify *G*-factor(s) beforehand in the field.

Click Run when ready.

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.

Warning: *Refer to the Materials Safety Data Sheet (MSDS) for detailed information on 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 water Raman 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 Fluorescence Service at HORIBA Jobin Yvon 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 Fluorescence Service Department by phone at (732) 494-8660, or fax at (732) 549-5125. Outside the United States, call the local distributor. You may also reach us by e-mail at info@JobinYvon.com

When you contact the Fluorescence 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, water Raman 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 × 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 water Raman 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 water Raman 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.

11: Technical Specifications

Each FluoroMax®-3 system consists of:

- An excitation source
- An excitation monochromator
- A sampling module with reference detector
- An emission monochromator
- An emission detector.

A FluoroMax®-P system adds:

- A motorized mirror to change between light sources
- A pulsed xenon lamp
- A phosphorimeter-control module

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 FluoroMax®-3 and FluoroMax®-P series of spectrometers follow.

Spectrofluorometer system

FluoroMax®-3 and FluoroMax®-P

The FluoroMax®-3 and FluoroMax®-P spectrofluorometers consist of components controlled by the specialized software. The basic (standard) FluoroMax®-3 and FluoroMax®-P spectrofluorometer systems contain of the following components:

Excitation Source	150-W xenon, continuous output, ozone-free lamp
Optics	All-reflective, for focusing at all wavelengths and precise imaging for microsamples.
Dispersion	4.25 nm mm^{-1}
Monochromators	Single-grating excitation and emission spectrometers (standard). Monochromators are *f*/3.5 Czerny-Turner design with classically-ruled gratings and all-reflective optics, using 1200-grooves/mm gratings: **Resolution** **0.3 nm** **Maximum scan speed** **200 nm s^{-1}** **Accuracy** **±0.5 nm** **Step Size** **0.0625–100 nm** **Range** **0–950 nm (physical)** **Gratings** ***Excitation*** ***330-nm blaze (220–600 nm optical range)*** ***Emission*** ***500-nm blaze (290–850 nm optical range)***
Sample Module	The sample module also has a removable gap-bed assembly for sampling accessory replacement.
Detectors	• Calibrated photodiode for excitation reference correction from 200–980 nm. • Emission detector is an R928P for high sensitivity in photon-counting mode (180–850 nm). High voltage = 950 V, linearity to 2×10^6 counts s^{-1}, < 1000 dark counts s^{-1}.
Sensitivity	Double-distilled, de-ionized, ICP-grade water-Raman scan 2500:1 *S*/*N* at 397 nm, 5-nm bandpass, 1-s integration time, background noise first standard deviation at 450 nm. 300 000 counts s^{-1} using these conditions.
Excitation shutter	Computer-controlled
Integration time	0.001–160 s
Slit width	0–30 nm bandpass, continuously adjustable via host computer
Dimensions (instrument)	32.5" wide × 10.5" high × 19" long 82.6 cm wide × 26.7 cm high × 48.3 cm long

Dimensions (sample compartment only)	5.5" wide × 7" high × 7" long 14.0 cm wide × 17.8 cm high × 17.8 cm long
Weight	75 lbs (34 kg)
Ambient temperature range	15–30°C (59–86°F)
Maximum relative humidity	75%
Power	5 A, 120 V, 60 Hz; or 2.5 A, 240 V, 50 Hz single-phase AC

Phosphorimeter (FluoroMax®-P only)

The following components and specifications also apply to the FluoroMax®-P.

Source	UV xenon flash tube
Flash rate	0.05–25 Hz
Flash duration	3 µs at full-width half-maximum. Low-intensity tail extends > 30 µs.
Delay after flash	50 µs to 10 s, in increments of 1 µs.
Flashes per data point	1–999
Sample window	10 µs to 10 s, in increments of 1 µs.

Minimum computer requirements

Microprocessor	Pentium IV or higher recommended
Operating system and environment	Windows™ 2000 or XP Pro
Floppy drive	1.4 MB, 3½" floppy-disk drive
Hard disk	At least 80 GB of free storage
CD-ROM drive	Required
Memory	128 MB RAM (256 MB Recommended)
Video display	Video resolution of at least 1024 × 768
Keyboard	A 104-key keyboard, plus USB or PS/2 mouse
Available ports	One available USB port One available Ethernet Network Interface Card (NIC) connection (no hubs) One serial port capable of 115 kilobaud for the SpectrAcq One serial port for optional plotter or mouse

Note: *Additional COM ports may be required to control accessories such as the MicroMax, temperature bath, etc.*

Software

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

12: Components & Accessories

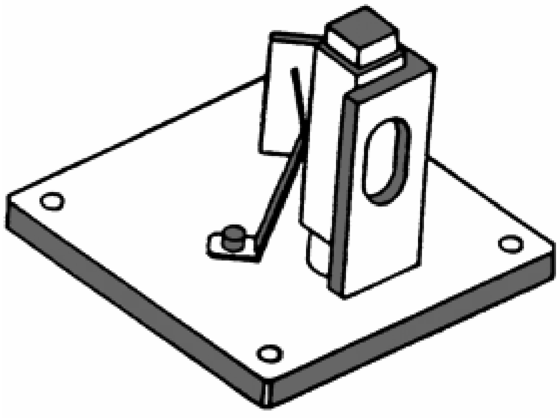
Accessories for the FluoroMax®-3 can be added to obtain optimum results for a variety of applications. The following list represents all the accessories and components, in alphabetical order, available for the FluoroMax®-3 spectrofluorometers. A brief description of each is included in the following 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 Sales Representative.

Itemized list of FluoroMax®-3 accessories

Item	Model	Page
Accessory, absorption/transmission	1940	12-3
Adapter, micro cell (See Cell, micro)	1923A	
Adapter, micro cell (See Cell, micro)	1924A	
Assembly, liquid-nitrogen Dewar	FL-1013	12-6
Assembly, scatter block	1908MOD	12-7
Assembly, standard lamp	1908	12-7
Cell, HPLC flow	1955	12-8
Cell, micro	1923	12-8
Cell, micro	1924	12-8
Cell, quartz	1925	12-8
Cell, sample	1920	12-8
Cell, sample (reduced volume)	QC-SK	12-8
Dewar, liquid-nitrogen (See Assembly, liquid-nitrogen Dewar)	1932D	
Fiber-optic mount	F-3000	12-9
Filter, cut-on (1" × 2")	1938	12-10
Filter, cut-on (2" × 2")	1939	12-10
Holder, filter	FL-1010	12-11
Holder, four-position variable temp. control w/ magnetic stirrer	FL-1011	12-12
Holder, dual-position variable temp. control w/ magnetic stirrer	FL-1012	12-14
Holder, solid-sample	1933	12-16
Injector, autotitration	F-3005/6	12-18
Injector port (see Port, injector)	FL-1015	
Interface, microscope		12-19
Lamp, xenon replacement, 150-W	1905-OFR	12-20
Peltier drive, sample heater/cooler	F-3004	12-21
Phosphorimeter upgrade	FM-2005	12-22
Plate reader, MicroMax 384 microwell	MicroMax 384	12-23
Polarizer, L-format	FL-1044	12-24
Port, injector	FL-1015	12-25
Quantum-Yield accessory		12-26
Stopped-flow accessory	SFA-20/SPEX	12-27
TCSPC upgrade	FM-2013	12-28
Temperature bath	F-1000/1	12-29
Trigger accessory, external	TRIG-15/25	12-30
Windows for the FluoroMax®-3 sample compartment	FM-2007	12-31

Model 1940 Absorption/Transmission Accessory



The Model 1940 Absorption/Transmission Accessory slightly displaces the sample from its normal position and directs the transmitted light into the collection optics with a mirror mounted at 45°.

Installation

1. Remove the sample holder currently in place.
2. Position the Model 1940 on the posts.
3. Tighten the two thumbscrews.

Before transmission or absorption spectra are acquired, the emission and reference signals must be optimized.

Signal optimization

1. Place the cuvette containing the blank in the sample holder.
2. Set the emission monochromator position to 0 nm.
3. Select acquisition mode *S/R*.
4. Run an excitation scan over the absorption range of the sample.

Note: *If uncertain of the absorption range, enter a range of 250 nm to 400 nm, and scan the excitation spectrometer. Detailed instructions for setting parameters are contained in the on-line help. The S/R acquisition mode compensates for fluctuations in lamp intensity, and wavelength dependency of the lamp and excitation spectrometer.*

5 Note the emission signal at maximum transmission of the blank.

6 Adjust the slits to maximize the signal without saturating the detector.

Note: *The linear range of the R928P detector operated in the photon-counting mode is 2 million cps.*

7 Similarly, locate the maximum reference signal of the lamp spectrum.

8 Set the excitation monochromator to that position.

Caution*: Only when both emission and reference signals are within prescribed limits, proceed with the measurements.*

9 Adjust the high voltage HV2, as needed, so that the reference signal is ~ 1 µA (saturation of the reference detector is 10 µA).

10 Acquire transmission spectra.

Either

a Position the emission monochromator at 0 nm, and use the S/R acquisition mode.

b Acquire an excitation scan of the blank.

c Acquire an excitation scan of the sample.

Note: *Use S/R because the ratio of the sample (here, “S”) to the blank (here “R”) removes R (here, the blank).*

Or

When the monochromators are synchronously scanned using an offset of 0 nm, the emission slits should be opened five times as wide as the slits of the excitation monochromator.

a Acquire a synchronous scan with an offset of 0 nm over the absorption range of the blank.

b Acquire a synchronous scan with an offset of 0 nm over the absorption range of the sample.

Note: *If the detectors are saturated, close the slits a little or install a neutral-density filter.*

11 Calculate the absorption.

The absorption spectrum can be determined from the following equation:

$$A = \log(S/R)_{\text{blank}} - \log(S/R)_{\text{sample}}$$

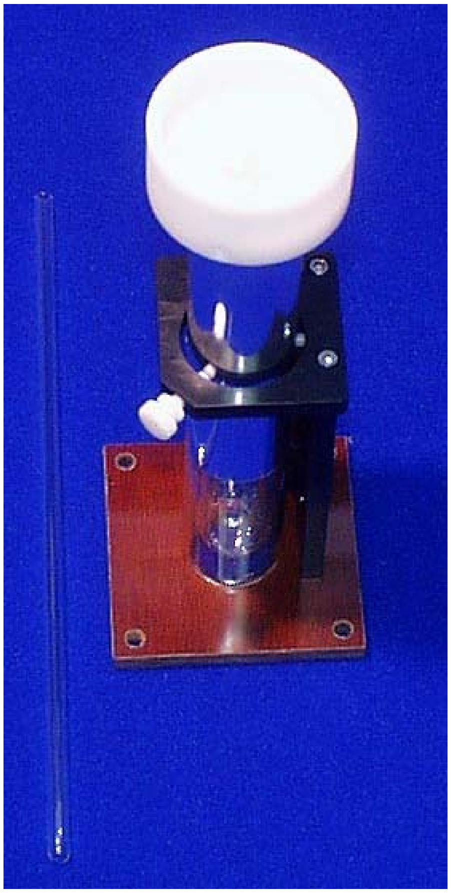
FL-1013 Liquid Nitrogen Dewar Assembly

***Warning**: 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.

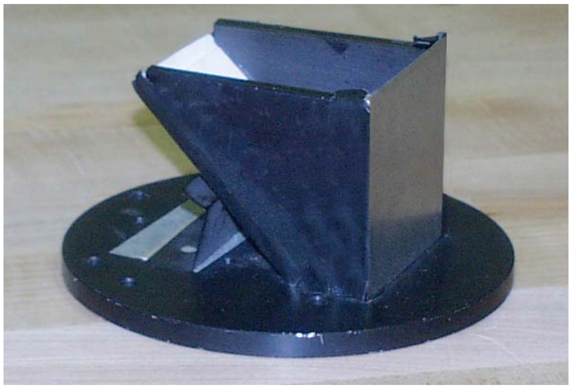
***Note:** If condensation appears on the outside of the Dewar flask, it must be re-evacuated.*



FL-1013 Liquid Nitrogen Dewar Assembly.

Model 1908MOD Scatter Block Assembly

The 1908MOD Scatter Block Assembly includes a white scatter block assembly and a clamp holder for use with a user-supplied standard lamp and regulated power supply.



Model 1908MOD Scatter Block Assembly.

Model 1908 Standard Lamp Assembly

The Model 1908 Standard Lamp Assembly is a complete correction factor kit used to generate radiometric emission correction factors for the spectrofluorometer systems. The assembly includes:

- an NIST-traceable, calibrated, 200-W quartz–tungsten halogen lamp with irradiance values.
- Regulated 6.5-A power supply with lamp holder
- 1908MOD Scatter Block Assembly (see above)

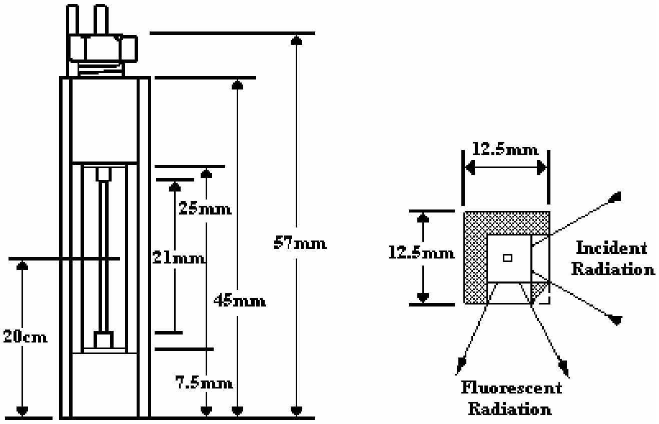
Emission correction factors compensate for the response of the photomultiplier detector as well as the wavelength dependency of the gratings in the emission spectrometer. Emission correction factors should be updated periodically and whenever different gratings or a new signal detector is installed.

Model 1908 Standard Lamp Assembly.

Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open, so wear eye- and skin-protection, such as light-attenuating goggles and light-blocking clothing.*

Sample cells

Model 1955 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.

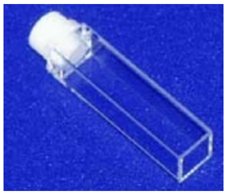
Model 1923 Micro Cell (with Model 1923A Adapter

This non-fluorescing fused silica cylindrical cell holds 50 µL. This cell does not accept a magnetic stirrer. The 1923A Adapter is required to be able to mount in a standard 10 mm × 10 mm cell holder.

Model 1924 Micro Cell (with Model 1924A Adapter

This non-fluorescing fused silica cylindrical cell holds 250 µL. A magnetic stirrer cannot be used with this cell. The 1924A Adapter is required to enable mounting in the standard 10 mm × 10 mm cell holder.

Model 1925 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.

Model 1920 Sample Cell

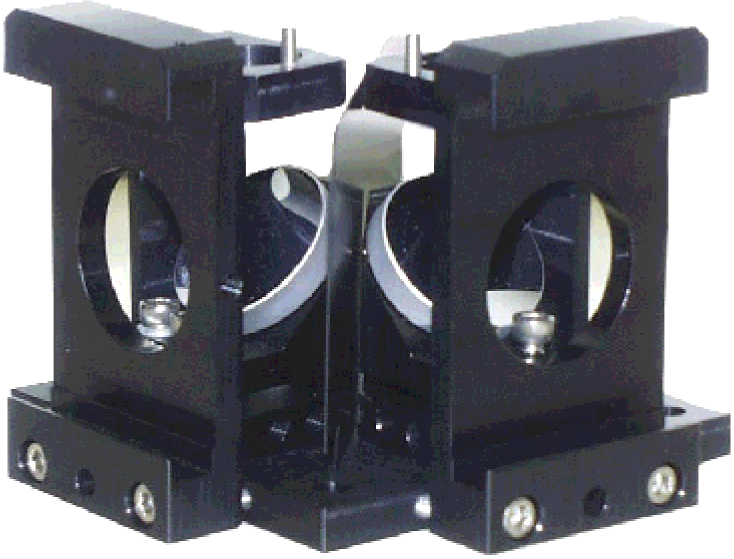
This 2-mL to 4-mL non-fluorescing fused silica cell, can accept a magnetic stirrer, has a 10-mm path length, and includes a white Teflon® cap that prevents sample evaporation.

Model QC-SK Reduced Volume Sample Cell

This non-fluorescing fused silica cell is selected for samples with a maximum volume of 1 mL. 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-3000 Fiber Optic Mount

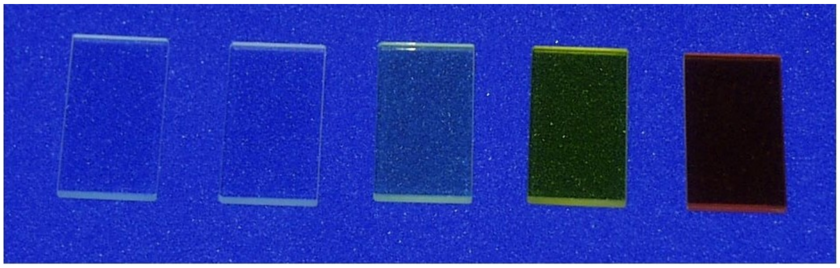
Now you can study marine environments, skin and hair, or other large samples *in situ*! For those users who want to examine samples unable to be inserted into the sample compartment, the F-3000 Fiber Optic Mount (plus fiber-optic bundles) allows remote sensing of fluorescence. The F-3000 couples to the T-box; 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 the local Sales Representative for details.



F-3000 Fiber Optic Mount.

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.*

Model 1938 Cut-On Filter



The Model 1938 Cut-On Filter Set consists of five 1" × 2" filters. To position the filter properly, the Model FL-1010 Filter Holder is required.

Cut-on filters are used to eliminate 2nd-order effects of the gratings. For example, if sample excitation is at 300 nm, a 2nd-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 2nd-order peak of the excitation spectrometer. 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 2nd-order effects are common.

The sample compartment has slots to hold the Model FL-1010 filter holder in the emission and excitation light-path positions. To eliminate 2nd-order effects from an excitation spectrum, install the filter holder and the appropriate cut-on filter in the excitation light path.

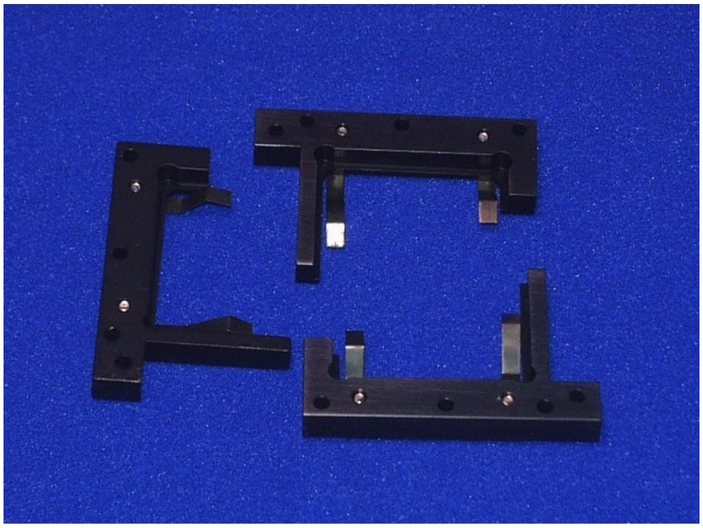
Model 1939 Cut-On Filter

The Model 1939 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.

Cut-on filters are used to eliminate 2nd-order effects of the gratings. For example, if sample excitation is at 300 nm, a 2nd-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 2nd-order peak of the excitation spectrometer. 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 2nd-order effects are common.

The sample compartment has slots to hold the Model FL-1010 filter holder in the emission and excitation 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.

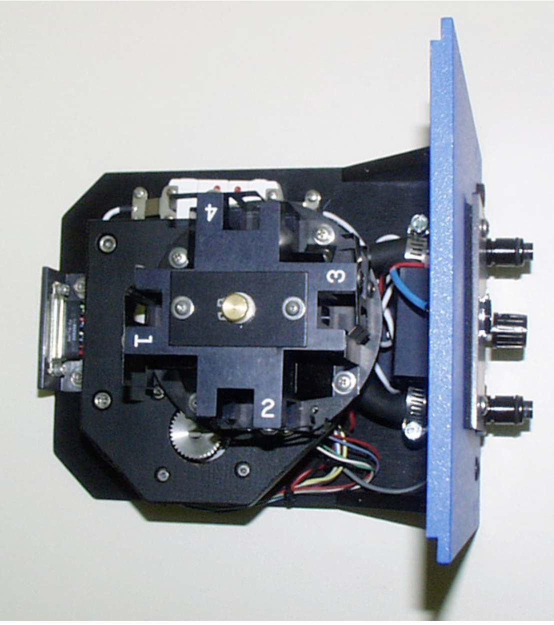
FL-1010 Cut-On Filter Holder



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 either Model 1939 Cut-On Filter or Model 1938 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-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.

Warning: *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.*

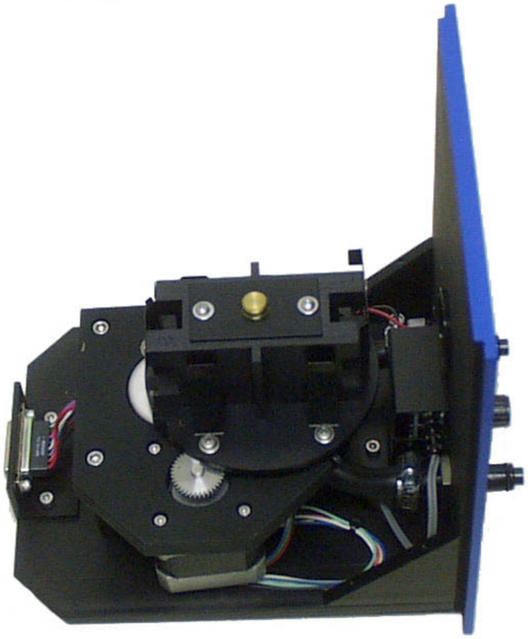
6 Run your experiment as usual.

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-1012 Dual-Position Thermostatted Cell Holder.

Warning: *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.

Caution: *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.

3. Allow the sample to reach the desired temperature.

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

4. Turn on the magnetic stirrer.

5. Select the appropriate speed.

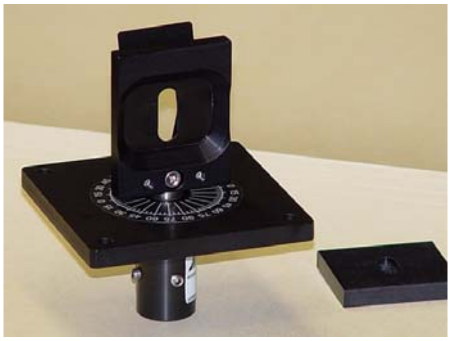
   The speed at which the sample should be mixed depends on the viscosity of the sample.

6. Run your experiment as usual.

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

Model 1933 Solid Sample Holder

The Model 1933 Solid Sample Holder is designed for samples such as thin films, powders, pellets, microscope slides, and fibers. The holder consists of a base with a dial indicating angle of rotation, upon which a bracket, a spring clip, and a sample block rest.



Model 1933 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:

1. Fill the well of the block.

> ***Warning***: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

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 perpendicular to the excitation light.

Note: *When the sample is perpendicular the light is collected at an angle of 22.5°. This orientation minimizes stray and reflected light off the surface of the sample.*

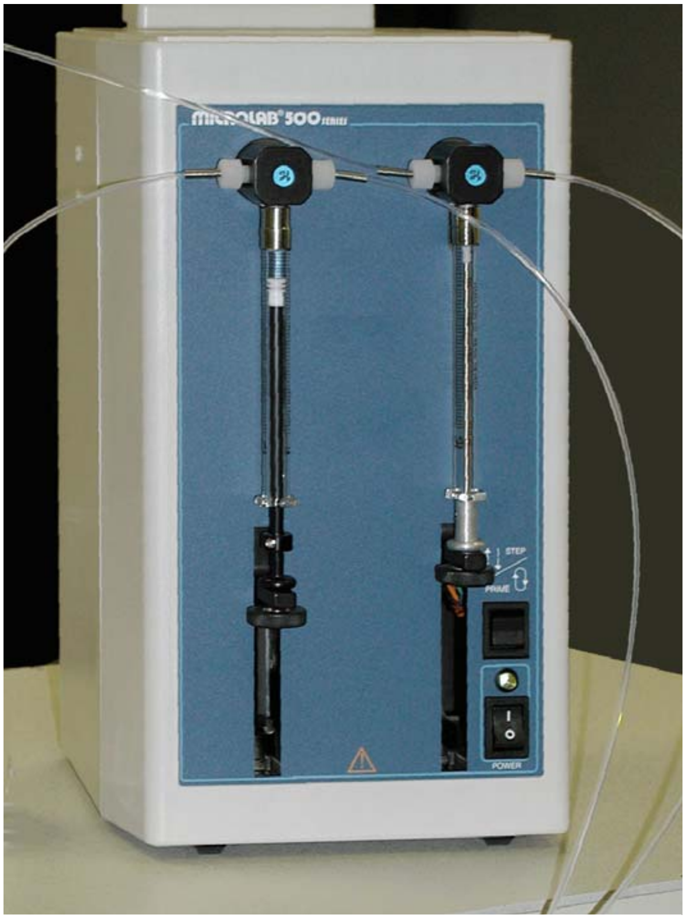
For samples such as thin films, microscope slides, fibers, or other materials:

1. Place the material on the block on the side opposite that of the well.
2. Insert the block between the bracket and spring clip.

   The sample should be perpendicular to the excitation light and fluorescence collected.

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.

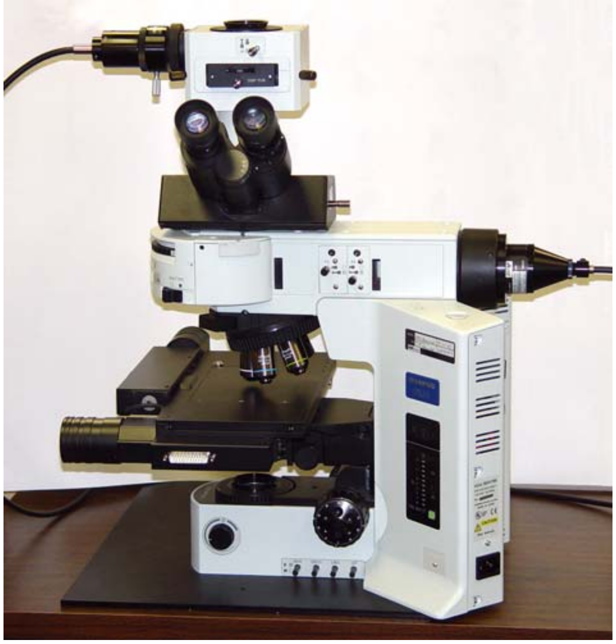


F-3005/6 AutoTitrator Injector.

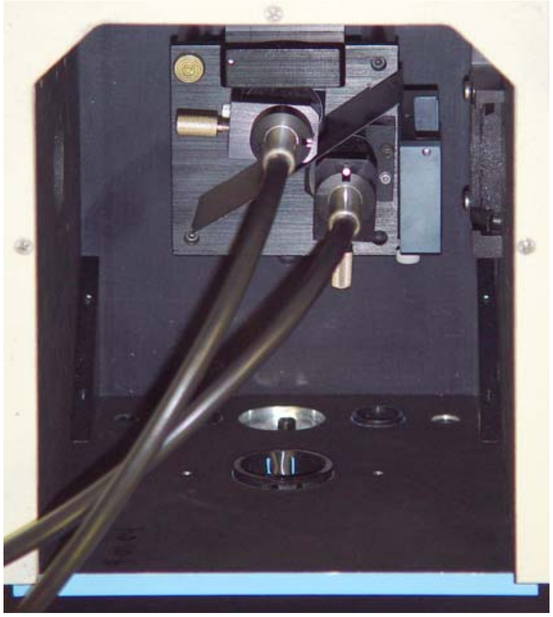
***Warning**: Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

Microscope Interface

The Microscope Interface eases the use of the FluoroMax®-3 systems 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 FluoroMax®.



Microscope Interface

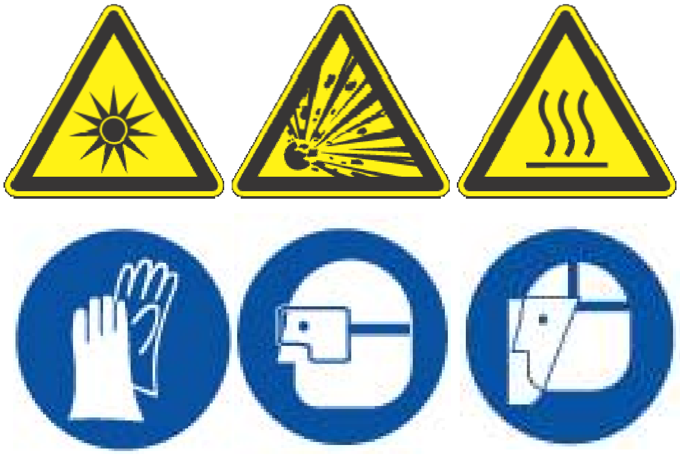


Fiber-optics in sample compartment

***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.*

Model 1905-OFR 150-W Xenon Lamp

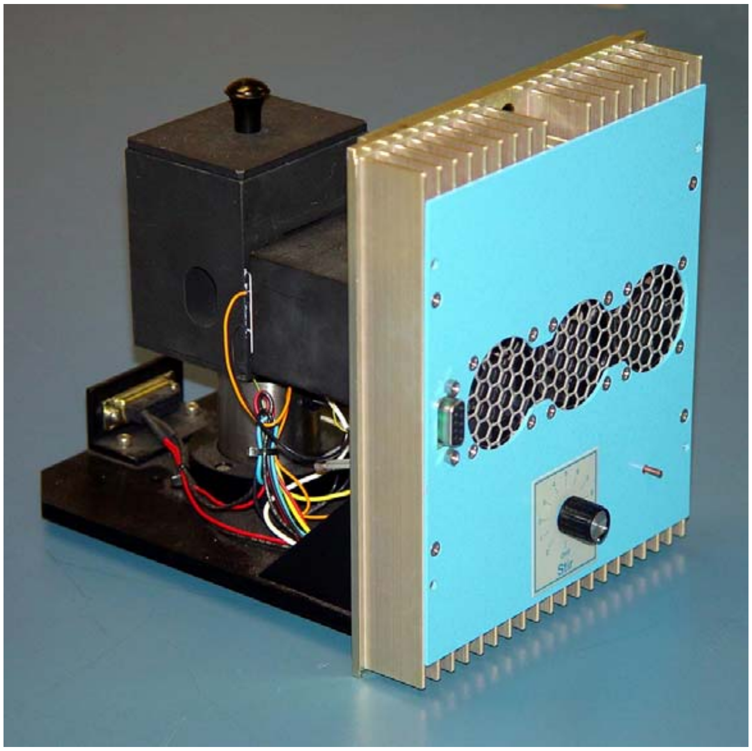
The Model 1905 150-W xenon lamp delivers light from 240 nm to 850 nm for sample excitation. The lamp has an approximate life of 1500 hours, and is ozone-free.



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.*

F-3004 Sample Heater/Cooler Peltier Thermocouple Drive

For rapid control of the sample's temperature in the FluoroMax®-3'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.

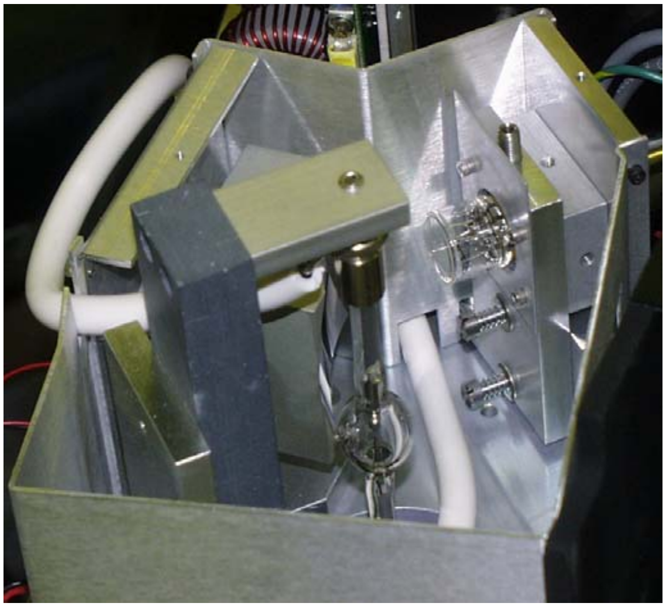


F-3004 Sample Heater/Cooler Peltier Thermocouple Drive.

MODEL LFI-3751
STATUS
TEMP LIMIT
T LIMIT
LIMIT I
REMOTE RS-232
SENSOR ERROR
HOLD FOR LOCAL
°C
kΩ
AMPS
VOLTS
ACT T
SET T
TE I
TE V
LIM I
AUX T
P
I
D
A
B
C
HOLD SHIFT FOR RS-232 ADDRESS
SHIFT
SENSOR
OUTPUT
ON
POWER
5 Amp - 40 Watt
Temperature Controller
Wavelength Electronics

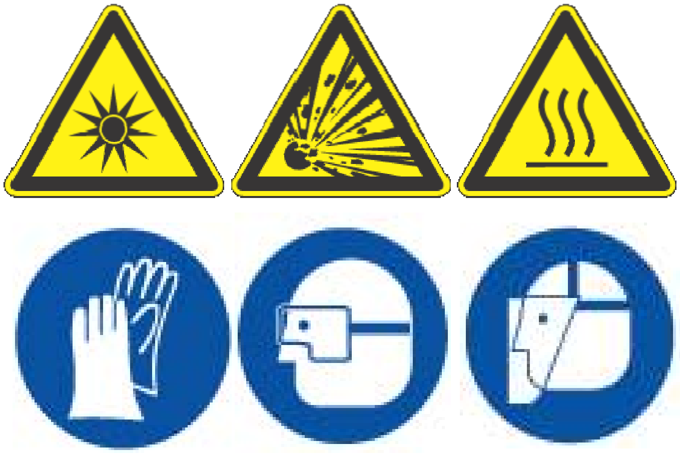
Peltier controller.

FM-2005 Phosphorimeter upgrade



To perform phosphorescence measurements using the FluoroMax®-3, order the FM-2005 upgrade. This accessory, which converts the FluoroMax®-3 into a FluoroMax®-P, offers automated switching via a motorized mirror between the existing CW xenon lamp in the FluoroMax® system, and a new pulsed xenon lamp installed in the FluoroMax®-3 housing. No manual adjustments are necessary.

The FM-2005 provides FluorEssence™-controlled flashlamp delays, number of flashes per data point, signal gating, and acquisition time, all maintaining the accuracy and precision you have come to expect from Spex® products. Acquire long phosphorescence lifetimes, while eliminating fast fluorescence interference automatically.



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.*

MicroMax 384 Microwell Plate Reader

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 FluorEssence™ 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 rapid scan speed. Various scan types are possible:

- Single-Point Analysis
- Emission
- Synchronous
- Excitation
- Time-Base
- Multigroup

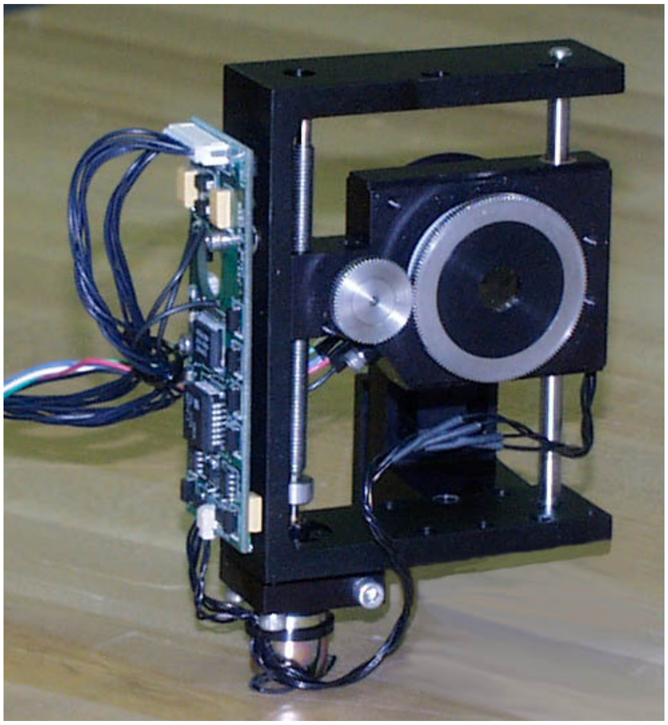
Signals are transmitted between the FluoroMax®-3 and the MicroMax via a fiber-optic bundle.

***Warning**: Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

***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.*

FL-1044 L-Format Polarizer

For L-format spectrofluorometers such as the FluoroMax®-3, 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.



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 Trigger Box/Event Marker.

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.

***Warning**: Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

Quantum-Yield accessory

The integrating sphere is used in the FluoroMax®-3 spectrofluorometer to study fluorescence from solid and liquid samples. The sphere has an internal diameter of 4″ (10 cm). Of special interest is the measurement of photoluminescence quantum yields of such materials, especially for thin solid films. Measurement of quantum yields of solids requires more complicated apparatus when an integrated sphere is unavailable. The integrating sphere's base mounts directly on the gap-bed, and is inserted into the spectrofluorometer's sample compartment. A special mount is provided to hold the sample inside the integrating sphere.

Quantum-yield accessory.

Warning*: Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

SFA-20/SPEX Stopped-flow accessory

SFA-20 RAPID KINETICS ACCESSORY
HI-TECH

The SFA-20/SPEX series of stopped-flow rapid-kinetics accessories offers versatility for spectroscopic monitoring of fast reactions in solution.

In addition to the conventional two-syringe mixing system, there is also a three-syringe version with two sequential mixers in the cell, giving you an option to do double-mixing. Further choices include a micro-volume version, reducing the volumes of reagents required to load the instrument, and thus improve sample economy.

The SFA-series stopped-flow accessory permits observation of the reaction rate of two reactants forced through a mixing chamber, and into an observation cell. The reactant solutions are contained in drive syringes whose pistons simultaneously are driven. After leaving the observation cell, the reactants advance a stop syringe, triggering data-acquisition by the spectrofluorometer.

This instrument has been designed to suit the particular needs of FluoroMax® spectrofluorometers. The optical cell matches the beam geometry of this instrument. A cable is supplied so data-acquisition can be externally triggered at stopping, providing a reproducible time-zero registration for all traces, and allowing accurate overlay and averaging.

FM-2013 TCSPC upgrade

Now you can have the speed and versatility you've come to expect in the FluoroMax®-3, 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.

FluoroMax®-3 with FM-2013 TCSPC upgrade.

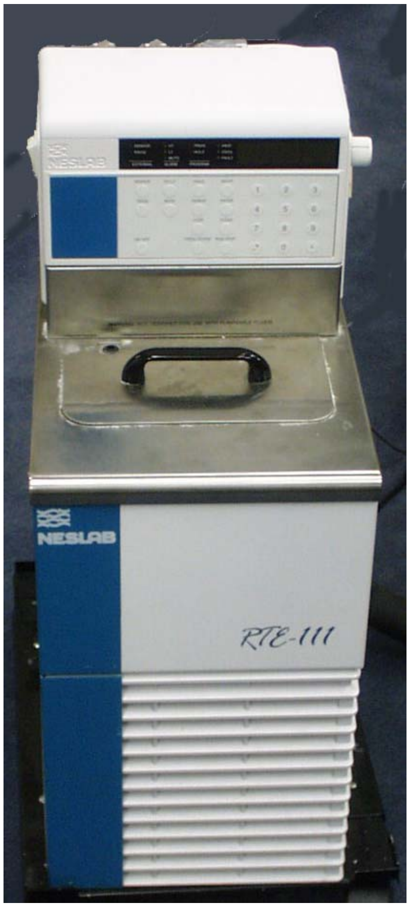
The pulsed source used in the TCSPC upgrade is our NanoLED solid-state pulsed diode, which can be ordered from a full spectrum of wavelengths ranging from deep-UV to near-IR.

The TCSPC upgrade for the FluoroMax®-3 includes all electronics, a special sample compartment, and your choice of NanoLED.

Caution: *Intense ultraviolet, visible, or infrared light and laser beams may be present when the sample compartment is open, so wear eye- and skin-protection, such as light-attenuating goggles and light-blocking clothing.*

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.

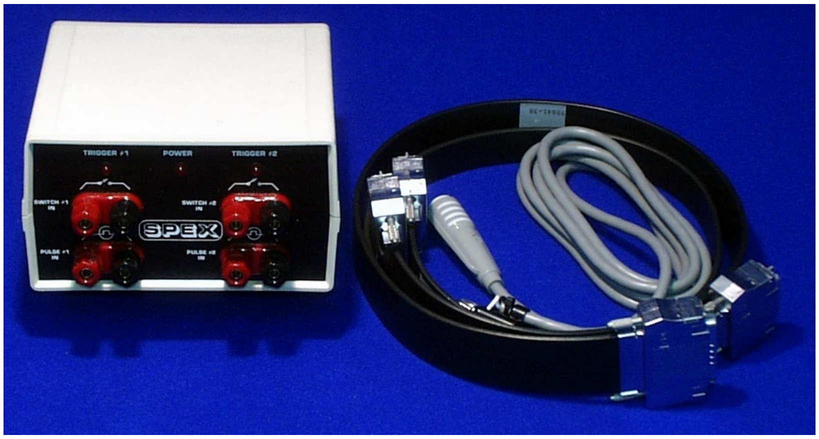
Warning: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of an ethylene-glycol–water mixture*

This instrument uses high-temperature fluids, which can cause severe burns.

Model TRIG-15/25 External Trigger Accessory

Note: *The TRIG-15/25 is only used for older FluoroMax®-3s. Newer models have a trigger built into their timer boards.*

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, by automatically following a voltage pulse (minimum 3 V above ground), or manually 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.



Model 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.

FM-2007 Windows for the FluoroMax®-3 sample compartment

Installing the windows

1 Prepare the instrument.

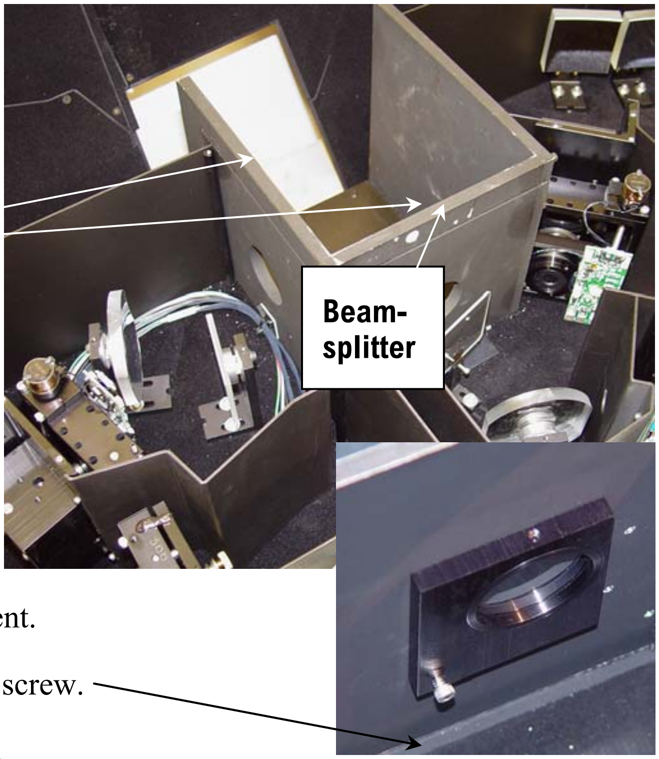
***Note:** If there are no filter holders, mount the FM-2007 Windows on the inside of the sample compartment. Go directly to step 2a.*

***Caution:** Never touch any optical surfaces of the gratings, mirrors, etc.*

a Remove the instrument cover.

b Remove the two black irregularly-shaped covers over the optical path.

***Note:** The window assemblies will be mounted here.*



2 Insert window assemblies.

a Snap one window assembly onto one external side-wall of the sample compartment.

b Insert 6-32 × 3/8" cap screw.

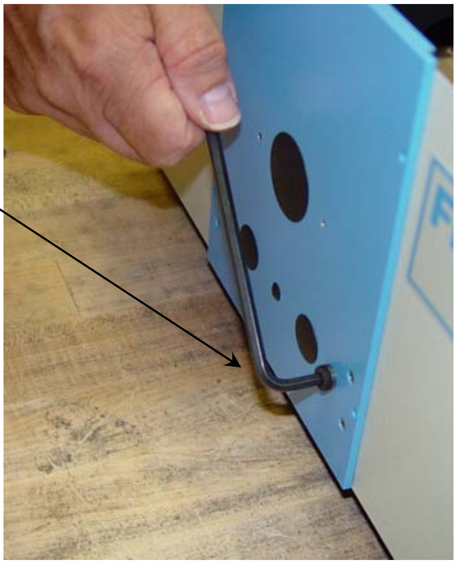
c Tighten the cap screw.

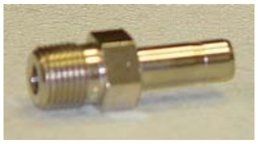
d Repeat steps (a) through (c) for the other window assembly.

***Caution:** Take care to avoid contact with the beam-splitter (with one window assembly) and the optics (with the other assembly).*

3 Install purge port.

a Remove plastic original plug, using a 3/16" Allen key.



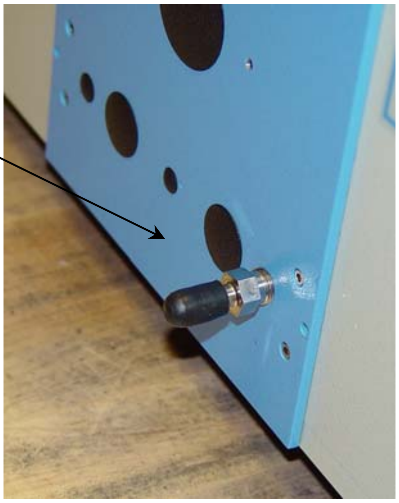


New purge port.

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.*

4 Close the instrument's cover.

13: Glossary

Absorption	The electronic transition from the ground state to the excited singlet state, by absorption of a photon of a particular energy. This process typically occurs in ~10^{-15} s.
Absorbance	The extent of light absorption by a substance, $-\log T$, where T is the transmittance of the sample. Absorbance is also synonymous with optical density, OD. Absorbance is defined as $A = \varepsilon cl = \mathrm{OD} = -\log T$ where ε is the extinction coefficient (M^{-1} cm^{-1}), C is the sample concentration (M), and l is the path length (cm).
Acquisition modes (R, S channels)	The logical input channels used on the spectrofluorometer to input collected signal from the detectors present on the system. The detectors are assigned as: the reference detector connected to channel R, and the emission connected to channel S. These logical channel names are used in the collection of data in most FluorEssence™ applications. The user may create algebraic expressions on these input channels when defining experiments in FluorEssence (e.g., S/R).
Anisotropy (<*r*>)	A measurement of the fluorescence polarization of a samples, defined as the linear-polarizer's component's intensity divided by the total light intensity. The measurement of anisotropy can provide insight into molecular size and shape, as well as the environment that surrounds it.
Autopolarizers	An automated device to hold and precisely rotate a set of polarizers to acquire anisotropy (or polarization) measurements. FluoroMax®-3 systems with autopolarizers contain two automated polarizer mounts, one for the excitation polarizer and one for the emission polarizer. Both are located between the sample compartment and their respective monochromators. Their calibration is maintained by optical sensors that are offset in the software. Autopolarizers on the FluoroMax®-3 may be inserted into and out of the light path in FluorEssence™, in the **Experiment Setup** window, under the Accessories icon. (Automated realignment of the polarizers also may be performed here.)
Bandpass	The range of wavelengths of light passing through the excitation and emission spectrometers, usually expressed in nanometers (nm). This value is dependent on the size of all slits in the monochromator, as well as the dispersion of the monochromator. For spectrofluorometers, both slits on the monochromator should be set equal to properly set the bandpass, if not done automatically through the software. When adjusting the bandpass for samples, the wider the bandpass, the higher the signal intensity, with a trade-off in lower resolution.

Bandpass filter	An optical element, which selectively transmits a range of wavelengths, while absorbing light of higher and lower wavelengths.
Bioluminescence	Emission of light originating from a chemical reaction in a living organism.
Blank subtraction	The removal of the spectral response of the solvent (and sample container) from the sample's spectral response. To accomplish this, an identical scan is run on the solvent just before running the actual sample. Proper use of a blank can remove solvent luminscence artifacts, scattering events, and any artifacts from the sample cuvette or container. In the **Experiment Setup** window, under the Detectors icon, check the Blank Subtract checkbox to acquire a blank.
Blaze wavelength (of gratings)	The wavelength at which a grating is optimized for peak efficiency. As a rough guide, gratings are usable from 2/3 of the blaze wavelength to twice the blaze wavelength. The excitation and emission gratings for the FluoroMax®-3 are blazed for efficiency in the UV and visible.
Chemiluminescence	Emission of light originating from a chemical reaction.
Concentration determination	A function of the Single Point type of scan, that calculates an unknown sample's concentration. The user runs known samples and enters the concentration in order to calibrate the routine. Then an assay may be completed with the measurements based on concentration.
Corrected emission scan	An emission scan that has been corrected for the wavelength response of the emission monochromator and the signal detector. To obtain a corrected emission scan, an emission spectrum is multiplied by the appropriate emission correction-factor file. A set of emission correction factors is supplied with the instrument and stored under the name `MCORRECT.SPC`.
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 excitation spectrometer. To obtain a first-order correction of the excitation scan, the emission detector signal is ratioed to the reference signal (i.e., *S/R*). This provides correction for the lamp and excitation-monochromator spectral response, which is ~95% of the required correction. To obtain a completely correct scan, the excitation scan acquired in the *S/R* acquisition mode is multiplied by excitation correction factors. A set of excitation correction factors (`XCORRECT.SPC`) is included.
Correction factors	A spectral set of multiplicative factors used to compensate for the instrumental wavelength-response from detectors and optics. These correction factors are specific to a particular optical configuration for the instrument, and must be run for each detector in the spectrofluorometer system. Excitation and emission correction factors are included by default with each new spectrofluorometer. Their default

	filenames and `XCORRECT.SPC` and `MCORRECT.SPC`, respectively.
CTI card	This acquisition and control card, located in the rear of the FluoroMax®-3, is the *counter-timer-integrator* board. It handles all spectrofluorometer control, timing, and data acquisition for measurements on the system. The boards carry by default two acquisition channels, and are linked to all monochromator and accessory control boards on the underside of the instrument. The CTI card fits into a slot in the motherboard on the rear of the FluoroMax®-3.
Current input module (DM303)	The current input module collects the current signal from the reference photodiode, digitizes the data, and sends it to the CTI card for data-processing. This module is located directly behind the reference photodiode. It has linear response from 0–10 μA.
Dark counts	Inherent background signal measured in counts s^{-1} (cps) observed on the photomultiplier tube when high voltage is applied. Typically, the R928P photomultiplier tube used for the FluoroMax®-3 system has dark counts of < 1000 cps.
Dark offset	The software correction used to subtract dark counts (or dark signal) on a detector from a spectral acquisition. This option appears as a checkbox in the FluorEssence™ software. Use a corrected signal channel for the acquisition (e.g., S) in order to run the Dark Offset correction.
Datafile	A file used to store spectral data, constant-wavelength analysis data, or other recorded data. In FluorEssence™, the most common datafile is the spectral file (`.SPC`). This is the file-type that contains spectra acquired from a scan run from the **Experiment Setup** menu (e.g., emission scan, time-base scan, single-point, etc.). Datafiles contain all of the information regarding the instrument setup for a scan as well as the spectra (see more information in the *FluorEssence™ User's Guide* and the on-line help files). Multifiles are three-dimensional datafiles that contain an array of spectral files.
Dispersion	The range of wavelengths of light across the field of view of the entrance and exit apertures. Dispersion depends on the focal length of the monochromator, the groove density of the optics, and the *f*-number (speed) of the monochromator. Dispersion is usually expressed in nanometers of spectral coverage per millimeters of slit width (nm/mm).
Emission monochromator	The monochromator located after the sample compartment used to isolate discrete wavelength components of the sample's fluorescence, and may be used to scan the emission from a sample. The emission monochromator on the FluoroMax®-3 is an 0.18-m single monochromator with a Czerny-Turner design: the monochromator includes a collimating mirror, the reflection grating (blazed at 500 nm), and a focusing mirror, with slit apertures at the entrance and exit. The emission-photomultiplier detector is connected to the exit of this

monchromator to measure the fluorescence emission.

Emission scan	An acquisition that shows the spectral distribution of light emitted by a sample. During an emission scan, the excitation spectrometer remains at a fixed wavelength while the emission spectrometer scans a user-selected region.
Energy transfer	The inter-molecular or intra-molecular 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/emission matrix (EEM)	A three-dimensional plot showing the total luminescence from a sample across all useful wavelengths. Total luminescence spectroscopy is devoted to measurements of these EEMs for various materials. *See also:* **Multifile**, **Total Luminescence Spectroscopy**
Excitation monochromator	The monochromator, located between the xenon lamp and the sample compartment, used to isolate discrete wavelength components of the excitation beam. This beam is directed to the sample, during which the excitation monochromator may be used to scan the excitation spectrum from a sample. The excitation monochromator on the FluoroMax®-3 is an 0.18-m single monochromator with Czerny-Turner design. This means that the monochromator includes a collimating mirror, the reflection grating (blazed at 330 nm), and a focusing mirror, with slit apertures at the entrance and exit. An excitation shutter is located directly after the excitation exit slit to protect the sample from photobleaching. The reference detector looks at a fraction of the light exiting the excitation monochromator to correct for the lamp response, if desired.
Excitation scan	A scan that reveals the spectral distribution of light absorbed by the sample. The scan is collected by rotating the excitation grating while holding the emission monochromator fixed. For non-ratiometric acquisitions, acquire the scan using *S/R* to correct for the spectral output of the lamp.
Excited state (S_1)	The energy level to which an electron in the ground level of a molecule is raised after the absorption of a photon of a particular wavelength. Subsequently, fluorescence occurs, if the molecule returns to the ground state via a radiative transfer from the S_1 state to the ground state.
Experiment file	A file that contains specific information on the experimental setup for an acquisition defined in **Experiment Setup**. This file is saved with a default `*.EXP` extension. In addition to basic scan parameters, this file saves system defaults (such as slit units), and some accessory settings for the acquisition. Each acquisition type in the **Fluoresence Experiment Menu** has its own default experiment file (e.g., `DFLT0.EXP` is the default emission-scan definition). Use experiment files to archive scan settings for acquisitions that are performed

	routinely.
Extrinsic fluorescence	Inherent fluorescence of fluorescent probes added to a system to study non-fluorescent molecules. These probes have gained acceptance in a variety of applications.
Filter	An optical element that is used to select certain wavelengths of light. Types of filters include high-pass, low-pass, bandpass, and neutral-density.
Flash lamp	A source that provides pulsed light to excite a sample for phosphorescence or fluorescence measurements. The repetition rates and time response of the source determine the useful range of the source for these measurements. The lamps can be used in either “free running” or “gated” modes depending on the support electronics.
Fluorescence	The emission of light during the transition of electrons from the excited singlet state to the ground state from molecules originally excited by the absorption of light. Fluorescence typically occurs within $\sim 10^{-9}$ seconds.
Fluorescence lifetime (τ)	The average length of time that a molecule remains in the excited state before returning to the ground state.
Fluorophore (fluorescent probe)	A molecule or compound that has a known fluorescence response. These probes have various sensitive areas depending on the peak excitation and emission wavelengths and their fluorescence lifetimes. Fluorophores are used to provide information on concentration, size, shape, and binding, in a particular medium. Good fluorophores are stable over wide pH and temperature ranges.
Front-face detection	A mode of detection in which fluorescence is collected off the front surface of the sample. Front-face detection is usually selected for turbid samples in solution (e.g., blood), samples of high concentration, or solid samples such as powders, thin films, pellets, and cells on a coverslip. Front-face detection collects fluorescence off the sample at a 22.5° angle to minimize reflections and scattering.
Grating	An optical element in a monochromator that uses finely-etched vertical grooves to disperse incident light into its constituent wavelengths. Reflection gratings (grooves etched on a highly reflective surface) are used in the FluoroMax®-3. Gratings are scanned by rotating their optical centers about the optical axis of the instrument, with the incident angle of the entrance beam determining the wavelength of light directed to the exit aperture. Gratings come in a variety of formats and are commonly made by physically *ruling* (scribing) the grating, or holographically *etching* the grating grooves.
Ground state (S_0)	The lowest energy level in a molecule. For fluorescence to occur, a molecule absorbs a photon of light, thereby exciting it to the S_1 level. A fluorescence emission occurs during a transition from an excited state S_1 to the ground state S_0.

High-pass filter	Optical component that passes light of a higher wavelength.
Increment	The spacing between adjacent measurement points in an acquisition. Typically, increments take the form of wavelength (nm) or time (s or ms).
Inner-filter effect	The scattering of the excitation or emission beam from a concentrated sample by the individual molecules in the sample. This reduces the apparent signal intensity from the sample creating an artifact in the data. For this reason, we recommend using concentrations of <0.05 OD in a 1-cm-pathlength cell. Samples measured in higher concentrations should be measured in a reduced-pathlength cell, or in front-face mode.
Integration time	The amount of time that each data point is collected from the detector(s), specified in either seconds or milliseconds. Longer integration times can help improve the signal-to-noise ratio for a measurement, while shorter integration times reduce the amount of time required for a scan.
Internal conversion	Electronic transitions within an excited molecule that do not result in emission. Also called a “non-radiative transition”, this usually involves changes in vibrational levels.
Intersystem crossing	The electronic transition from the excited singlet state to the excited triplet state before returning to the ground state. This transition involves a change of spin that is quantum-mechanically forbidden, giving a much longer timescale than fluorescence. This transition causes phosphorescence on the timescale of microseconds to seconds.
Intrinsic fluorescence	The natural fluorescent properties of molecules.
Jabłonski (energy) diagram	A diagram that illustrates various energy levels and electronic transitions available in a particular molecule. Possible paths for fluorescence, phosphorescence, and non-radiative transfers are shown on this diagram, along with the various vibrational sub-levels available around each energy level.
Laser	A monochromatic light source that provides high excitation intensity.
Linearity	(1) Signal response; the desired response from a light detector is a linear relationship. For example, when detector response is linear, if the light intensity doubles, the detected signal also doubles. Most detectors exhibit non-linear behavior near saturation. On the FluoroMax®-3, the emission photomultiplier tube is linear up to 2 million counts per second. Above this, pulse pileup occurs on the photon-counting module (when multiple photons are counted as one). This results in a non-linear response, and the detector efficiency drops. (2) Spectral positioning accuracy or tracking error of a spectrometer drive mechanism. *See* **Spectral Calibration**.

Low-pass filter	Optical component that passes light of a lower wavelength.
Luminescence	The emission of light from matter excited from a variety of processes, resulting in an electronic transition within the molecule to a lower energy state. *See also*: **Bioluminescence**, **Chemiluminesence**, **Fluorescence**.
MCD shutter	**M**ulti-**c**hannel **d**evice shutter. The Uniblitz shutter is used for its rapid cycle time.
Mercury lamp	A light source that offers discrete narrow lines as opposed to a broad-band radiation (e.g., xenon). Mercury lamps often are used to verify the accuracy and resolution of a spectrometer or spectrograph.
Mirror-image rule	A sample that absorbs light for certain energy-level transitions usually exhibits an emission profile that appears to be the mirror image of the absorption spectrum. The reason is that the same energy-level transitions are used for excitation and emission, with the transitions returning to the ground state as the complement to the those in the excited state.
Molar extinction coefficient (ε)	The absorptivity of a particular substance, in M^{-1} cm^{-1}.
Multifile	The three-dimensional acquisition datafiles collected by the software using matrix scans or temperature scans, stored as an array of datafiles. A multifile is still stored with an `.SPC` extension. Multifiles may be used in their entirety in FluorEssence™ as 3D files, or they may be split up into individual two-dimensional spectra using multifile utilities.
Multigroup scan	This experiment type allows a time-based scan to be acquired across more than one excitation/emission pair. Up to 16 different wavelength pairs may be entered for a multigroup scan. The spectrofluorometer will cycle through each pair, integrating for the specified time, before moving on to the next point. Use the multigroup scan for measuring ratiometric probes (such as Fura-2 or BCECF).
Neutral-density filter	An optical element that absorbs a significant fraction of the incident light. These filters usually are characterized by their optical density, on a logarithmic scale. For example, a filter with OD = 1 transmits 10% of the incident light. Ideally, these filters absorb all wavelengths equally. *See also* **Absorbance**.
Optical-density effects (Inner-filter effect)	Fluorescence intensities are proportional to the concentration over a limited range of optical densities. High optical densities can distort the emission spectra along with apparent intensities. For fluorescence measurements in a 1-cm-pathlength cell, samples should have an OD of 0.05 or less. *See also*: **Inner-filter effect**.
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 oc-

curs within ~10^{-6} to ~1 second. To enhance phosphorescence, samples often are frozen at liquid-nitrogen temperature (77 K).

Photobleaching The reduction in fluorescence from a photosensitive sample overly exposed to excitation light. Not all samples photobleach, but if so, take care to keep the sample out of room light, and to use the excitation shutter and its photobleach modes on the spectrofluorometer to protect the sample from excessive exposure.

Photoelectron An electron released through the interaction of a photon with the active element of a detector. The photoelectron may be released either from a junction to the conduction band of a solid-state detector, or from the photocathode to the vacuum in a PMT. A photoelectron is indistinguishable from other electrons in any electrical circuit.

Photon-counting detection A method of detection used primarily with photomultiplier tubes, in which discrete current pulses from the tube are integrated and "counted up". With this method, noise inherent to the detector can be minimized, resulting in much more sensitive detection than used in traditional current- or voltage-detection modules. A limit to photon-counting is when *pulse pileup* occurs, that is, when two counts occur too fast for the module to count them individually. This creates non-linearity in the detector at high signal-levels.

Polarization (*P*) A measurement of the fluorescence polarization of a sample defined as the linear polarizer's component's intensity divided by the natural light intensity. The measurement of polarization provides insight into molecular size, shape, and the environment surrounding the molecule. Another unit, called millipolarization (mP), is used to monitor small changes in polarization. $P = \mathrm{m}P \times 1000$.

Quantum yield (Fluorescence quantum yield) The efficiency of the absorption of a photon to be emitted (fluoresced). Quantum yields typically are expressed as percents. The fluorescence quantum yield is the percentage of photons absorbed that actually leads to fluorescence. This number is reduced by scattering, quenching, internal conversion, and non-radiative effects, along with several other specialized processes. Measurements of quantum yields usually require the comparison of a sample with a known fluorophore such as Rhodamine-B or $\mathrm{Ru(BPY)_3}$.

Quenching Reduction in the fluorescence intensity of a sample by a variety of chemical or environmental influences. Quenching may be static, dynamic, or collisional in nature.

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. Rayleigh-scattered light is of

	the same energy as the incident light. The scattered radiation's intensity is inversely proportional to the 4th power of the wavelength of incident radiation.
Real Time Control	The FluorEssence™ software application that gives the user full control of the system in real-time, in order to optimize the system setup for a particular measurement. Use **Real Time Control** to find the optimal slit widths for sample measurements, or to check that the excitation beam is striking the sample properly.
Reference detector	The detector used to monitor the output of the xenon lamp. A silicon photodiode with enhanced-UV response is used for the FluoroMax®-3, and is connected to input channel R. Use S/R to correct for the xenon-lamp response during an excitation scan.
Resolution	The ability of a spectrometer or spectrograph to separate two closely spaced peaks. Resolution can be improved by decreasing the number of pixels binned together or the slit widths in the instrument.
Right-angle detection	Collection of fluorescence at 90° to the incident radiation. Right-angle detection is typically selected for dilute and clear solutions in order to minimize the scatter component in the detected emission.
Sample changer (automated)	An automated accessory that automatically positions up to four cuvette samples held in the sample compartment. Use this accessory to run up to four samples at one time for a small assay, or to run blanks with the samples simultaneously. Automated sample changers are thermostatted and possess magnetic stirrers.
Saturation	The effect of having too much signal incident on a particular detector. Saturated detectors give an erroneous result and no longer show any response for small changes in signal. In some cases, saturation can damage a detector's performance, so avoid saturation whenever possible. The R928P photomultiplier tube used on the FluoroMax®-3 saturates at 1×10^7 cps.
Signal channel	*See:* **Acquisition modes**.
Signal photomultiplier	Detector used to measure excitation and fluorescence from the sample, operated in photon-counting mode to provide the highest sensitivity. Different detectors can be used to optimize different wavelength regions.
Signal-to-noise ratio (*S/N*)	The measurement of the signal observed divided by the noise component seen in that signal. Generally, the better the *S/N* is, the better the measurement is. This is accomplished by using photon-counting detection with the proper high-voltage bias for improved sensitivity during fluorescence measurements. The user then optimizes the sample signal to the higher area of the linear range for the detector, typically between 100 000 and 2 000 000 cps. Next, dark offsets or blank subtraction may be used to improve the *S/N*. Finally, increasing the integration time or repeating the same scan several times can improve the signal to noise. For specifications, signal-to-noise may be repre-

sented as signal to peak-to-peak noise, or signal-to-noise at first standard deviation (FSD).

Single Point	The FluorEssence™ scan-type designed for performing single-point measurements at discrete wavelength pairs. The data are acquired as single points at a user-defined set of excitation/emission wavelength pairs for a user-defined number of samples. These data are displayed in either spreadsheet format, or in a plot. This application is for MicroMax or FluoroMax®-3 users who routinely perform assays on a large number of samples.
Singlet state	The spin-paired ground or excited state. The process of absorption generally produces the first excited singlet state, which takes time to fluoresce, and may undergo intersystem crossing to form a triplet state.
Spectral calibration	The accuracy of a monochromator with respect to its wavelength alignment. This is a measure of the monochromator being at the correct wavelength when it is set there. Monochromators are traditionally calibrated using line-spectra sources, such as mercury lamps. Spectrofluorometers may be calibrated by performing two scans, one of the source, and one of a standard (such as water) to calibrate all of the monochromators. For Spex® spectrofluorometers, the xenon-lamp scan is performed on the excitation with the 467.1-nm peak assigned as such in the software. The water Raman band is scanned with 350-nm excitation, and the 397-nm peak is assigned as such in the software for the emission monochromators.
Spectral correction	The removal of the wavelength sensitivity of detectors, optics, sources, and backgrounds from the spectrum taken on a sample. When spectral correction has been properly performed, the true theoretical spectra from a sample should be all the remains. Spectral correction is accomplished with a variety of options on Spex® spectrofluorometers. Excitation and emission correction factor files are provided to remove the wavelength sensitivity of detectors and their optics. The reference detector is present to remove the lamp and excitation optics response. Blank subtraction and dark offset are used to remove background levels and responses.
Spectral response	Most detectors have a higher sensitivity to some wavelengths than to others. The spectral response of a detector is often expressed graphically in a plot of responsivity versus wavelength.
Spectrofluorometer	An analytical instrument used to measure the fluorescence properties of a molecule or substance. The device consists of at least two monochromators, a source, sample compartment and detection electronics. The instruments may be scanned on the excitation, emission or both to provide insight on the characteristics of the sample being studied. Newer spectrofluorometers provide many more automated options, including polarization, temperature, titer plates, pressure, and many more. Today, these instruments are computer-controlled, allowing

	easy control of assays and complex experiments.
Stokes shift	The energy difference between the absorption peak of lowest energy and the fluorescence peak of maximum energy.
Synchronous scan	Scan type characterizing the overlap between the excitation and emission. The excitation and emission spectrometers are scanned at the same time, with a constant offset specified in either nanometers (wavelength units) or in cm^{-1} (energy units).
Technical spectrum	A spectrum acquired on research instrumentation with instrumental bias remaining in the measurement. This spectrum must undergo proper spectral correction in order to match the theoretical spectrum. Spex® spectrofluorometers offer various methods for such correction, including spectral correction, dark offset, blank subtraction, and others.
Temperature scan	A FluorEssence™ Kinetics scan-defintion that consists of a particular scan made across a user-defined temperature range. This scan may be used to monitor a sample's temperature response, or, more specifically, to perform a melting curve for a sample. Temperature scans require an automated bath compatible with FluorEssence™ to be attached to the spectrofluorometer system along with a thermostattable sample mount.
Throughput	The amount of light that passes through the spectrofluorometer for a particular measurement. The throughput usually is measured as the counts per second measured on the water Raman band at 350-nm excitation with 5-nm bandpass. As bandpass increases, so does the throughput. Like bandpass, throughput has an inverse relationship with resolution. When the throughput is increased, the resolution decreases.
Time-based scan	Scan type in which the sample signal is monitored as a function of time, while both the excitation and the emission spectrometers remain at fixed wavelengths. Time-based data are used to monitor enzyme kinetics, dual-wavelength measurements, and determine reaction-rate constants.
Total luminescence spectroscopy (TLS)	Spectroscopy devoted to monitoring changes to the entire excitation/emission matrix of luminescence on a sample. This discipline is best applied to fast kinetics measurements of samples during reactions, temperature curves, or changes in other parameters.
Transmission	Light that passes through a sample without being absorbed, scattered, or reflected. Transmission is usually measured as a percentage of the incident light at a certain wavelength.
Triplet state (T_1)	The spin-paired ground or excited state formed from the excited singlet state, in which electrons are unpaired. The triplet state gives rise to phosphorescence.
Tyndall scattering	Scatter that occurs from small particles in colloidal suspensions.

Variable time kinetics	A special measurement menu in the **Single Point** experiment type. The user defines measurements that occur at specific times, for specific durations, and with different integration times. Those measuring assays can do the actual measurements at the desired times. See the FluorEssence™ on-line help for more information.
Vibrational states	Sublevels within an electronic energy level that are the result of various types of motion of the atoms in a molecule. Transition between these states at a particular energy level does not involve a large change in energy, and typically is a non-radiative transition. In larger electronic transitions such as fluorescence, a molecule drops from the lowest vibrational level of the excited state to the highest vibrational level of the ground state. This emission is termed the *Stokes shift* between the S_1 and ground states.
Xenon lamp	A high-intensity lamp that produces a continuum of light from the ultraviolet to the near-infrared for sample excitation. A xenon lamp is classified as a broadband source.
Xenon-lamp scan	A profile of the lamp output as a function of wavelength. The lamp scan is acquired using 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.

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In addition, the following journals may prove useful:
Analytical Chemistry
Biophysics and Biochemistry
Journal of Fluorescence
Nanotechnology Letters

15: CE Declaration of Conformity

Manufacturer: HORIBA Jobin Yvon

Address: 3880 Park Avenue
Edison, NJ 08820
USA

Product Name: FluoroMax-3
FluoroMax-P

Conforms to the following Standards:

Safety: EN 61010-1/A2
EN 61010-2-061

EMC: EN 50081-1
EN 50011 CLASS A GROUP 2
EN 50082-1
EN 61000-4-2 Performance Criteria A
EN 61000-4-4 2kV, Performance Criteria A

Supplementary Information

The product herewith complies with the requirements of the **Low Voltage Directive 73/23/EEC as amended by 93/68/EEC**.

The CE marking has been affixed on the device according to Annex III of the Low Voltage Directive 73/23/EEC.

The technical file and other documentation are on file with HORIBA Jobin Yvon Inc.

Ray Kaminski
Vice-President, Fluorescence Division

HORIBA Jobin Yvon
Edison, NJ 08820
USA
July 29, 2005

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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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