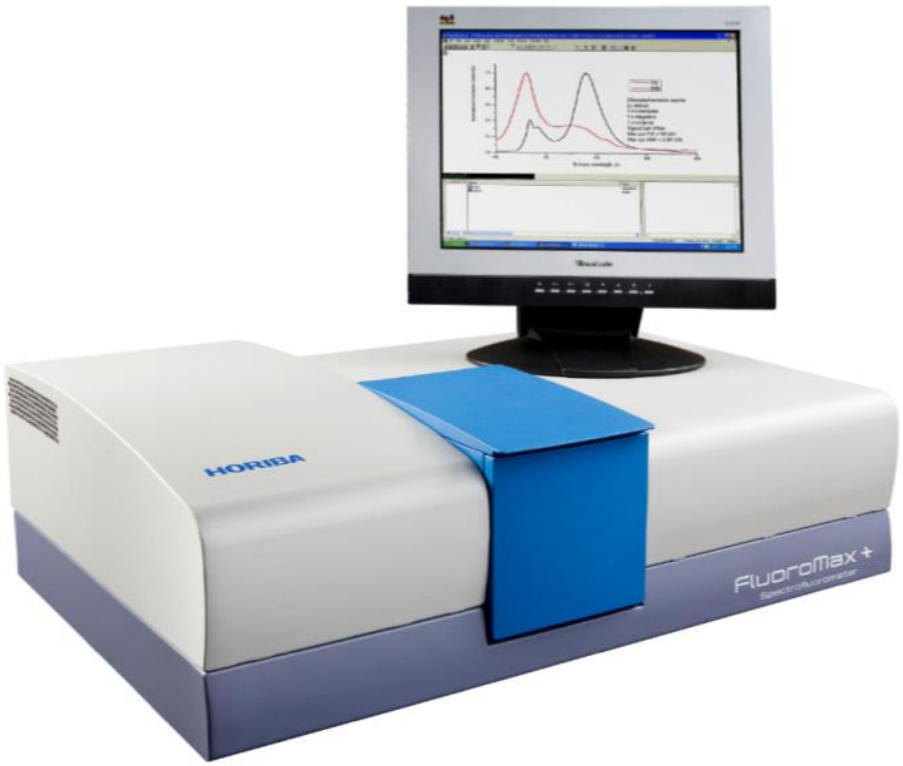
HORIBA Scientific

JOBIN YVON Technology

FluoroMax® Plus & FluoroMax® Plus-P

User manual

Operation Manual



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Revision G

August 2024

Document Revision History

Revision	Date	ECO #	Page	Description
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A	October 2015	3003716	Cover i ii 0-13 9-10 All All	Rev. A, use correct HORIBA Scientific Logo, bottom bar, and watermark Rev. A October 2015 Changes RoHS DOC Director of Fluorescence Division to Carry Davies Remove chapter index pages Remove top and bottom page borders Headers: Add Rev A to PN, change date to 28 October 2015
C	September 2021	4531265	Front Cover 2-3 All Index Table of Content	Updated revision info "… rev. B October, 2020" to "…rev. C, June 2021". Updated Copyright year from 2015 to 2021, and "May 2015" to "June 2021" 'FluoroMax Plus Manual 5700002851 Rev # (Date)' removed from headers. Deleted Index Updated to align with the document
D	December 2022	4892886	22-24 204	Updated compliance information to include UKCA declaration of conformity Changed the slit width from "0–30 nm bandpass" to "0–29 nm bandpass"
E	May 2023	4943834	Cover 204-205	Changes "Rev D" to "Rev E," changed date Changed "Calibrated
F	November 2023	4989978	Cover All 173 174 210	Changes "Rev E" to "Rev F," changed date Updated format. Section 11.2.1: • Monochromators: Range changed from "0-950nm" to "0-1150nm" • Sensitivity: IPC-grade water-Raman scan changed from "3000:1" to "10000:1") • Sensitivity: Removed "400000 counts s^{-1} using these conditions" Section 11.3: Updated minimum host computer requirements (note about administration rights). Section 14: Added reference (Gryczynski, Z., & Gryczynski, I. (2019). Practical Fluorescence Spectroscopy (1st ed.). CRC Press)
G	July 2024	5100303	Cover 193 214-226	Changes "Rev F" to "Rev G," changed date Added a new Section 12.23 Front Mounted Laser Added section 15 Addendum 1

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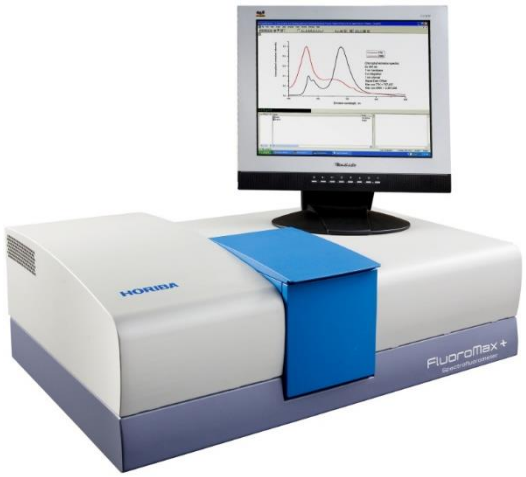
0 INTRODUCTION

0.1 About the FluoroMax® Plus and FluoroMax® Plus-P



State-of-the-art optical components Both the FluoroMax® Plus and FluoroMax® Plus-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. The FluoroMax® Plus series includes an optional second detector to extend working wavelength range or speed. Aside from this difference in hardware, the operation of the FluoroMax® Plus is identical to the FluoroMax®-4. All FluoroMax® Plus and FluoroMax® Plus-P functions are under the control of FluorEssence™ spectroscopy software.

The main parts of the FluoroMax® Plus and FluoroMax® Plus-P spectrofluorometer systems are:

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

The difference between the FluoroMax® Plus and FluoroMax® Plus-P is that the FluoroMax® Plus-P contains a phosphorimeter for phosphorescence measurements. This manual explains how to operate and maintain a FluoroMax® Plus series 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.

What is an Absorbance or % Transmittance Spectrum?

The absorption of light is fundamental to understanding both absorbance, transmittance, and photoluminescence spectroscopy. The Beer-Lambert law states that the optical density, or Absorbance (A) of a solution is proportional to the molar extinction coefficient, (ε $M^{-1}cm^{-1}$), the path length of the cuvette or cell, b (cm) and the concentration, c (mol/L). The absorbance is also calculated by knowing the transmittance, T of the solution.

$$A = -logT = \varepsilon bc$$

Equation 1: Beer-Lambert Law

To measure the transmittance of a solution, we use the incident light from the xenon lamp through the cuvette of the solution of interest. The measured incident light signal as measured through the sample is I. We compare the incident light, I, through the sample to the incident light through a cuvette of a blank solvent. This measured

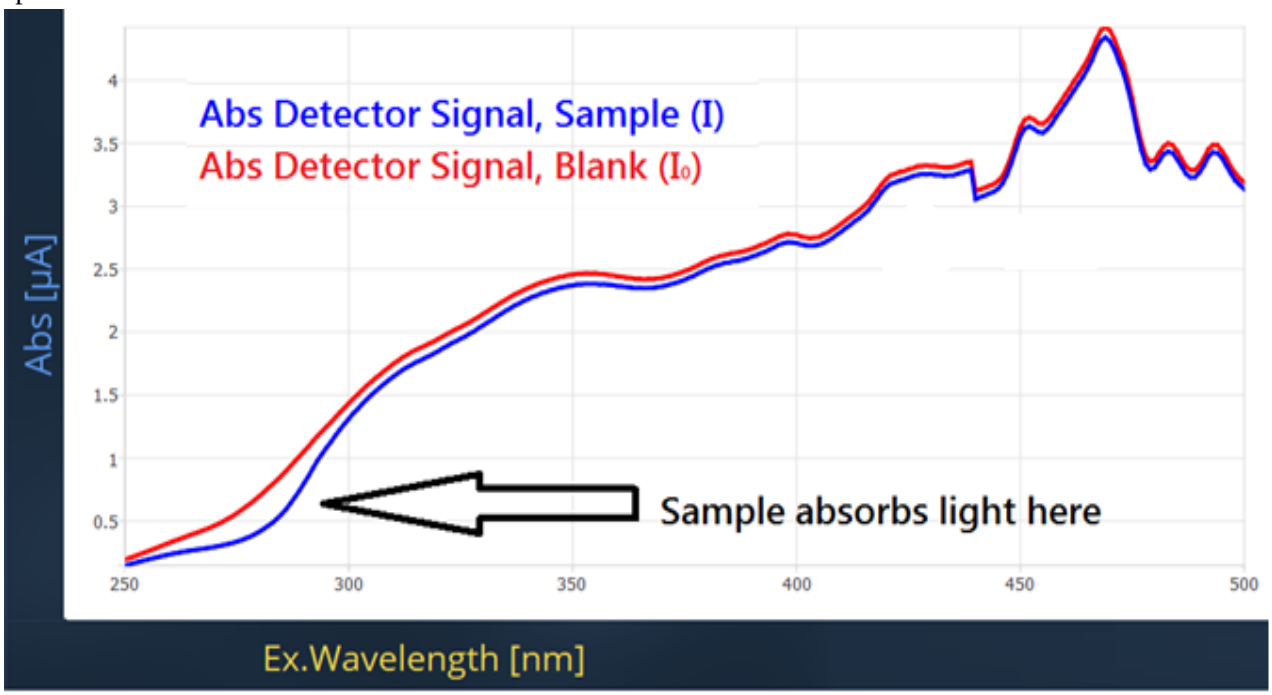
blank signal is called I_0. To calculate the transmission of the sample, T, we can use the other part of the derived Beer-Lambert Law below:

$$T = \frac{I}{I_0} \text{ and } \%T = T*100\%$$

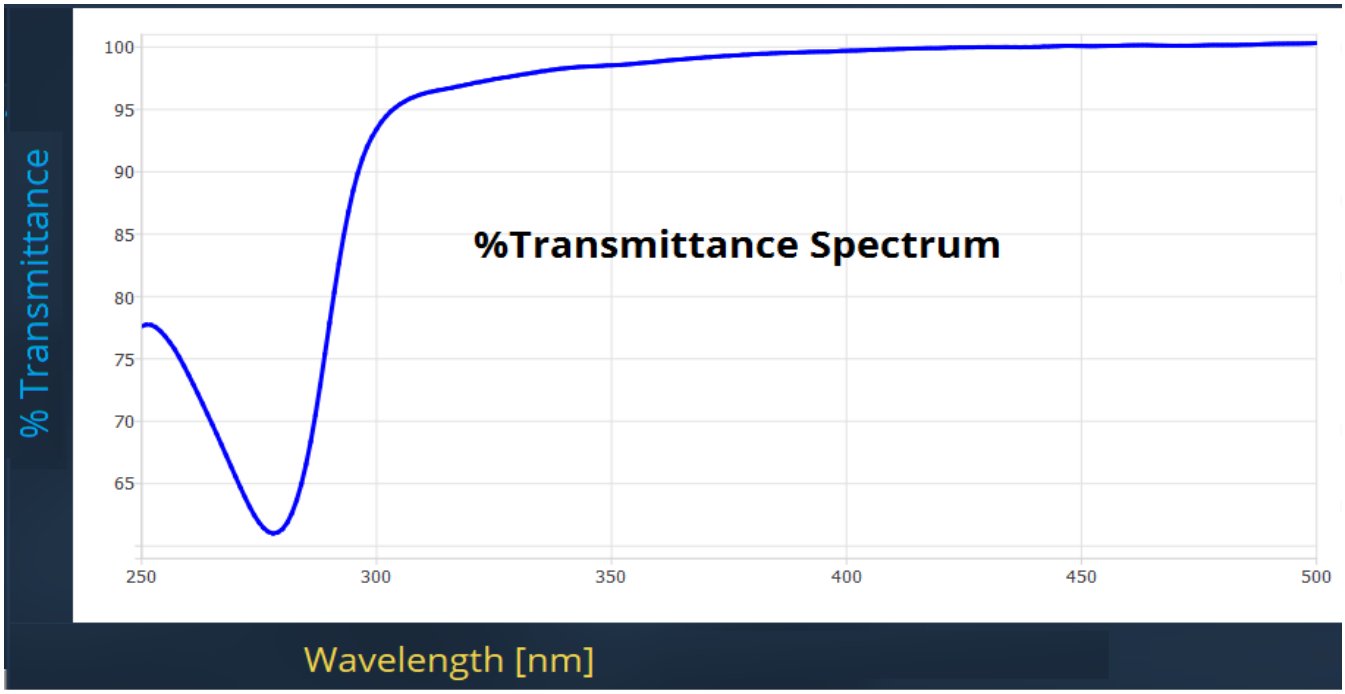
Equation 2: Transmittance (T) of a solution

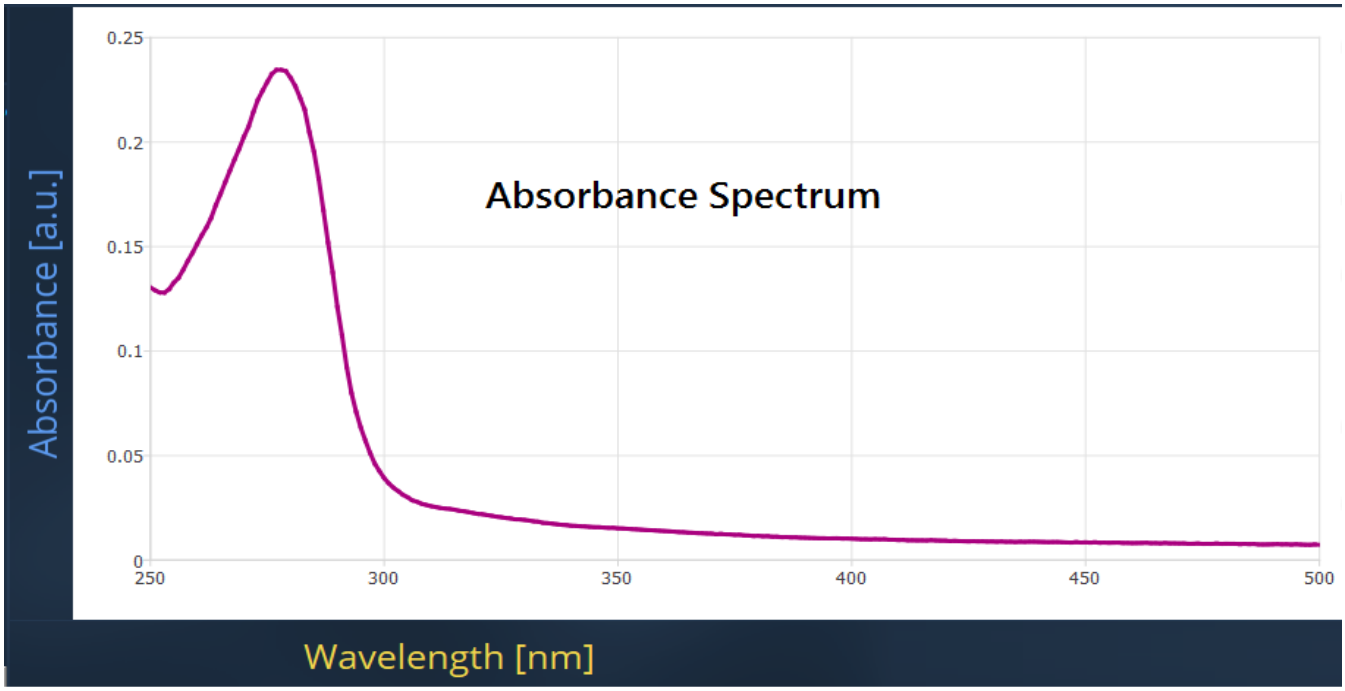
How is Absorbance or Transmittance Measured?

To measure the Absorbance or % Transmittance spectra, the absorbance/transmittance accessory (optional) is a silicon photodiode mounted to the front of the sample compartment so that transmitted light is detected from the excitation monochromator and through a sample or blank cuvette. Scan the excitation monochromator across the wavelength range of the sample spectrum (from high wavelength to low wavelength). In doing this, the light from the xenon lamp is measured by the photodiode on the other side of the sample compartment, which is called the Absorbance detector or A1 signal. When an absorbance or %T spectrum is acquired, you must measure the xenon lamp spectrum measured through the sample and again through the blank. Then, a calculator is used to obtain the Absorbance and % Transmittance spectra by inputting the sample and blank spectra.



In the above Figure, the sample is clearly absorbing in the UV region between 250 and 300 nm. See the resulting %Transmittance and Absorbance spectra below, where there is a peak in the absorbance and a dip in the transmittance at around 280 nm.





Absorbance spectra can change linearly with the concentration of the absorbing species. If the extinction coefficient is known, the concentration can be easily calculated from the absorbance spectrum. The molar extinction coefficients for many compounds are published and are dependent on the solvent in which the chromophore is dissolved.

Knowing the wavelength region for which a sample absorbs is useful for characterizing the optical density, but it is also useful for choosing the appropriate excitation wavelength to use when measuring a fluorescence emission spectrum. Absorbance spectra can also be used to correct for inner-filter effect, which is described in more detail further on in the manual.

What is a Fluorescence Spectrum? (Fluorescence Spectroscopy, 2018)

Steady state fluorescence spectra are measured when molecules, excited by a constant source of light, emit fluorescence, and the emitted photons, or intensity, are detected as a function of wavelength. A fluorescence emission spectrum is when the excitation wavelength is fixed, and the emission wavelength is scanned for a single channel detector (i.e., PMT or IGA) or collected with a CCD to get a plot of intensity vs. emission wavelength. Fluorescence is a type of photoluminescence, which is broadly defined, is emission of photons by a molecule or molecules. There are other types of photoluminescence such as chemiluminescence and electroluminescence.

A fluorescence excitation spectrum is when the emission wavelength is fixed, and the excitation monochromator wavelength is scanned. In this way, the spectrum gives information about the wavelengths at which a sample will absorb and then emit at the single emission wavelength chosen for observation. It is analogous to absorbance spectrum but a much more sensitive technique in terms of

limits of detection and molecular specificity. Excitation spectra are specific to a single emitting wavelength/species as opposed to an absorbance spectrum, which measures all absorbing species in a solution or sample. The emission and excitation spectra for a given fluorophore are mirror images of each other. Typically, the emission spectrum occurs at higher wavelengths (lower energy) than the excitation or absorbance spectrum.

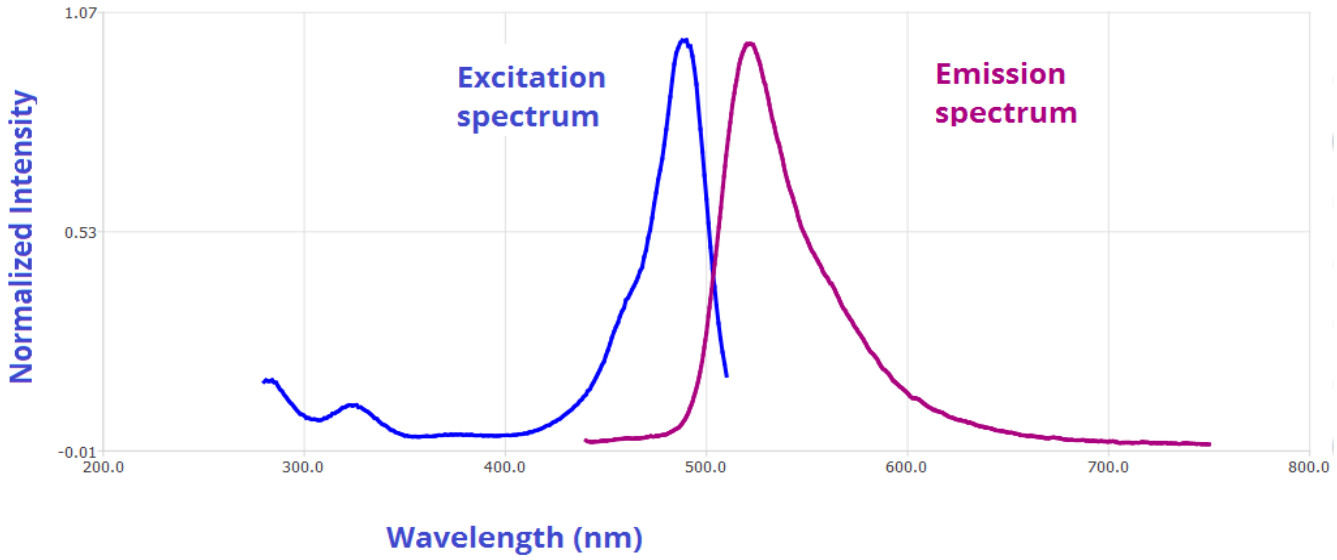


Figure 1: A fluorescence excitation spectrum (blue) and an emission spectrum (purple) are mirror images of each other

These two spectral types (emission and excitation) are used to see how a sample is changing. The spectral intensity and/or peak wavelength may change with variants such as temperature, concentration, or interactions with other molecules around it. This includes other molecules that quench fluorescence and molecules or materials that involve energy transfer. Some fluorophores are also sensitive to solvent environment properties such as pH, polarity, and certain ion concentrations.

What is the Jablonski Diagram?

Figure 2 is showing the Jablonski diagram (Jablonski, 1933), a schematic of the transition of electronic state of a molecule during the fluorescence phenomenon. The left axis shows increasing energy, where a typical fluorescent molecule has an absorbance spectrum. This spectrum shows the energy or wavelengths, where the molecule will absorb light.

If the incident light is at a wavelength where the molecule will absorb the photon, the molecule is then excited from the electronic ground state to a higher excited state, denoted S2 here.
The electrons then go through internal conversion, affected by vibrational relaxation and heat loss to the environment. A photon is then emitted from the lowest lying singlet excited state in the form of fluorescence.

In conventional fluorescence, photons are emitted at higher wavelengths than the photons which are absorbed.

This diagram is extremely important to understand for any fluorescence spectroscopist. When measuring a fluorescence spectrum, one is typically looking at the intensity at which a molecule emits, the wavelength or energy at which it emits, and also the time which the molecule spends in the excited state. This is the fluorescence lifetime, explained further in detail in coming sections.

Any number of things can affect these observables, including energy transfer to and from other molecules, quenching by other molecules, temperature, pH, local polarity, aggregation, or binding. Understanding the mechanisms of these interactions can give one insight into what is being observed with a change in fluorescence spectra or lifetime. There are two non-radiative deactivation processes that compete with fluorescence: internal conversion from the lowest singlet excited to the ground state and intersystem crossing from the excited singlet state to the triplet state. This last process leads to the phenomenon called phosphorescence, explained in further detail later on.

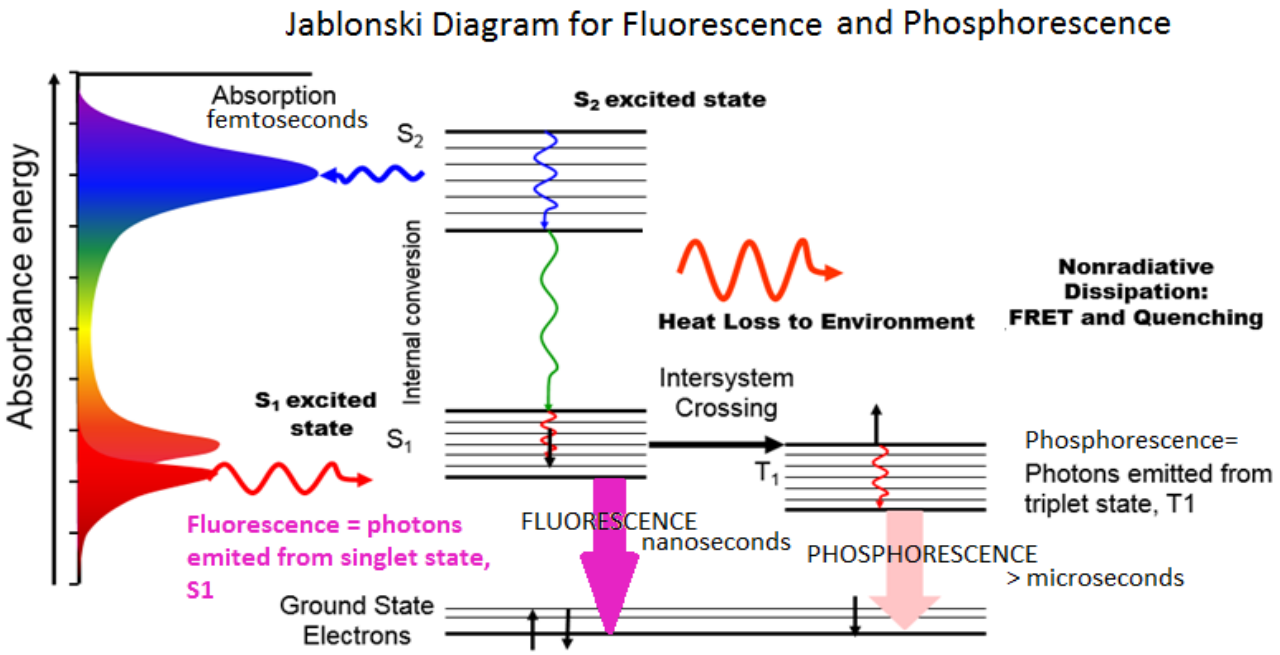


Figure 2: The Jablonski Diagram of molecular absorbance and fluorescence

What is Phosphorescence?

Phosphorescence is a process where the photon is emitted, not from a singlet excited state, but from a forbidden triplet state. The time scale of fluorescence emission is generally in the picosecond to nanosecond range, while phosphorescence typically lasts for microseconds, milliseconds, or even longer…minutes or hours. A pulsed source is typically used, such as a flash lamp or LED to measure phosphorescence spectra and decays on these longer time scales.

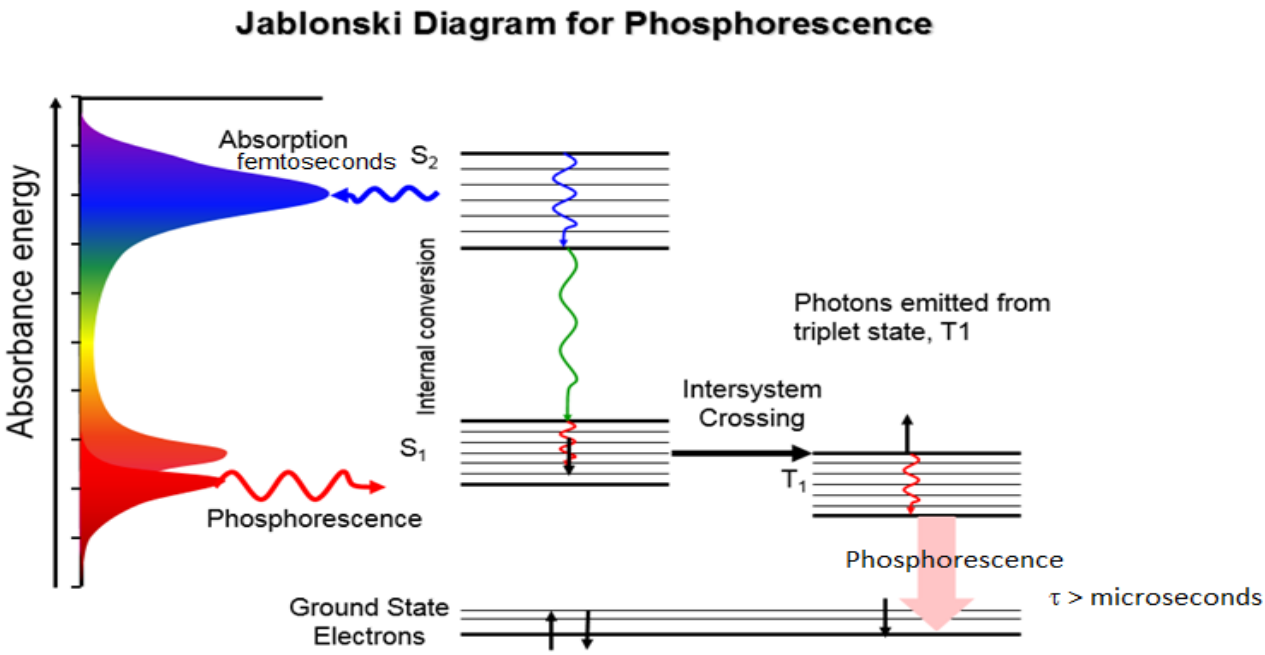
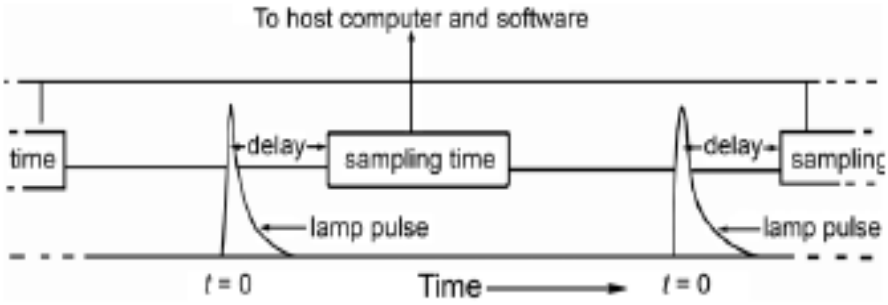


Figure 1: Jablonski diagram for Phosphorescence emission

Phosphorescence measurements use a longer-lived pulsed source, such as a xenon flash lamp (optional). The timing of the flashing lamp is used to measure spectra at different phosphorescence lifetimes. These lifetimes vary from sub microseconds for some molecules, to seconds or even hours for long-lived phosphors. In a phosphorescence lifetime experiment using the box-car method, the xenon flash lamp is set to a flash rate, number of flashes, and an integration window is set. The sample window is integrated over the total decay window of the phosphorescence lifetime incrementally and each integrated data point is added to the acquired graph as the acquisition proceeds. For more information on Phosphorescence Methods, see Chapter 9 of this manual.



More information available at www.horiba.com/fluorescence.

References

Jablonski, A. (1933). Efficiency of Anti-Stokes Fluorescence in Dyes. Nature, 131, 839-840

0.2 Disclaimer

By setting up or starting to use any HORIBA Instruments Incorporated 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 Instruments Incorporated, 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 Instruments Incorporated 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 Instruments Incorporated's liability for the products.

HORIBA Instruments Incorporated 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 Instruments Incorporated'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 Instruments Incorporated at an additional cost. HORIBA Instruments Incorporated cannot be held responsible for actions your employer or contractor may take without proper training.

Due to HORIBA Instruments Incorporated'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 Instruments Incorporated 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 Instruments Incorporated 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 Instruments Incorporated 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.

0.2.1 For Laser Radiation

The FluoroMax® Plus is certified as a CLASS 1 laser product to the requirements of the US Federal Product Performance Standard for Laser Products contained in the regulations in 21 CFR Subchapter J except for specific deviations described in FDA Laser Notice #56.

ⓘ Class 1 laser products are not considered to be hazardous

This instrument can incorporate a variety of certified laser systems up to and including a Class 4 laser system which, by itself, may be hazardous. However, the design of the FluoroMax® Plus includes a protective housing, and safety interlocks such that, once the laser system is installed, there is no exposure or human access to laser radiation during operation, maintenance, or normal service.

UNDER NO CIRCUMSTANCE shall attempts be made to operate the laser system with safety interlocks bypassed or portions of the protective housing removed.

Contact HORIBA service if the incorporated laser system requires bypass of its internal interlocks. ONLY trained HORIBA service personnel are authorized. The laser manufacturer's user manual is supplied with this product.

Protective housing warning labels shall be affixed to specific portions of the protective housing to assist in providing warnings of the potential hazards if protective panels are removed. Discontinue use AND contact HORIBA service immediately if any parts of the protective housing, mechanical interfaces, wires, or cables show signs of wear or damage.

The product complies with the following laser safety standard regulations:

a. EN 60825-1:2014 Safety of Laser Products - Part1: Equipment Classification and Requirements
b. Code of Federal Regulations Title 21: Food and Drugs - Part 1040: Performance Standards For Light-Emitting Products Section1040.10 Laser products and 1040.11 Specific-purpose Laser Products (FDA CDRH), except for deviations pursuant to specific FDA guidance documents.

0.3 Safety Summary

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

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

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

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

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

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

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

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

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

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

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

Read this manual before using or servicing the instrument.

Wear protective gloves.

Wear appropriate safety goggles to protect the eyes.

Wear an appropriate face-shield to protect the face.

Note:

General information is given concerning operation of the equipment.

0.4 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!

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

0.5 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.

0.6 Additional Risks of Xenon Lamps

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

Among the dangers associated with xenon lamps are:

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

Visible Radiation

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

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

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

Infrared Radiation

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

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

The skin and eyes absorb infrared radiation (IR) as heat. Workers normally notice excessive exposure through heat sensation and pain. Infrared radiation in the IR-A that enters the human eye will reach (and can be focused upon) the sensitive cells of the retina. For high irradiance sources in the IR-A, the retina is the part of the eye

that is at risk. For sources in the IR-B and IR-C, both the skin and the cornea may be at risk from "flash burns." In addition, the heat deposited in the cornea may be conducted to the lens of the eye. This heating of the lens is believed to be the cause of so called "glassblowers" cataracts because the heat transfer may cause clouding of the lens.

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

0.7 Compliance Information

CE

0.7.1 CE Declaration of Conformity

The FluoroMax® Plus spectrofluorometer is tested for compliance with both the EMC Directive 89/336/EEC and the Low Voltage Directive for Safety 73/23/EEC and bears the international CE mark as indication of this compliance. HORIBA Instruments Incorporated guarantees the product line's CE compliance only when original HORIBA Instruments Incorporated supplied parts are used.

Manufacturer:	HORIBA Instruments Inc.	
Address:	20 Knightsbridge Rd, Piscataway, NJ 08854 USA	
Authorized Rep:	Pascal Contini	
Address:	16-18 rue du Canal, 91165 Longjumeau Cedex, France	
Product Name:	FluoroMax	
Model Name: **Consisting of Combinations of the Following:**	FluoroMax 4 FluoroMax 4P FluoroMax 4NIR FluoroMax 4PNIR FluoroMax Plus FluoroMax PlusP FluoroMax 4C	FluoroMax 4CP FluoroMax 4CNIR FluoroMax 4CPNIR FluoroMax PlusC FluoroMax Plus CP
Safety:	EN 61010-1: 2010 (3rd Edition)	
EMC:	EN 61326-1: 2013 (Emissions & Immunity)	
RoHS	EN 50581: 2012	

0.7.2 Supplementary Information

- The product herewith complies with the requirements of the Low Voltage Directive 2014/35/EU and the EMC Directive 2014/30/EU.
- The CE marking has been affixed on the device according to Articles 16 and 17 of the EMC Directive 2014/30/EU.
- The product herewith complies with the RoHS Directive 2011/65/EU.

- The technical file and documentation are on file with HORIBA Instruments Inc.

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0.7.3 UKCA Declaration of Conformity

Manufacturer:	HORIBA Instruments Incorporated	
Address:	20 Knightsbridge Road Piscataway, New Jersey 08854-3913 USA	
Authorized Rep:	HORIBA UK Limited	
Address:	Kyoto Cl, Moulton Park Moulton Park Industrial Estate, Northampton NN3 6FL, United Kingdom	
Product Name:	FluoroMax	
Part Numbers:	FluoroMax 4	FluoroMax 4C
	FluoroMax 4P	FluoroMax 4CP
	FluoroMax 4NIR	FluoroMax 4CNIR
	FluoroMax 4PNIR	FluoroMax 4CPNIR
	FluoroMax Plus	FluoroMax PlusC
	FluoroMax PlusP	FluoroMax Plus CP

The above listed product(s) Conform to the following Statutory Requirements and Designated Standards:

UK SI 2016 No. 1101 and amendments:	Electrical Equipment (Safety) Regulations EN 61010-1: 2010 (3rd Edition)
UK SI 2016 No. 1091 and amendments:	Electromagnetic Compatibility Regulations EN 61326-1 (Emissions & Immunity)
UK SI 2012 No. 3032 and amendments:	The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations 2012 EN 63000:2018

0.7.4 Supplementary Information

This declaration is issued under the sole responsibility of the Manufacturer.

23rd September 2022
Date of issue

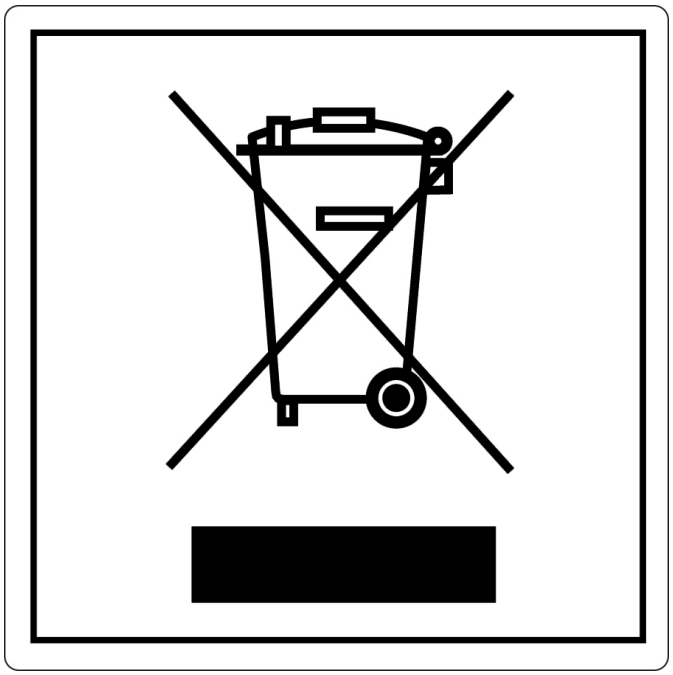
Dr. Salvatore H. Atzeni
General Manager and Executive Vice President
HORIBA Instruments Incorporated
20 Knightsbridge Road
Piscataway, New Jersey 08854

Matt Bryanton
Head of Quality and Business Process
HORIBA UK Limited
Kyoto Cl, Moulton Park
Moulton Park Industrial Estate,
Northampton NN3 6FL, United Kingdom

0.7.5 Applicable CE and UKCA Compliance Tests and Standards

Test	Standards
Emissions, Radiated/Conducted	EN 55011:2009 + A1:2010
Radiated Immunity	EN 61000-4-3: 2006 + A1:2008 + A2:2010
Conducted Immunity	EN 61000-4-6: 2009
Electrical Fast Transients	EN 61000-4-4: 2004 + A1:2010
Electrostatic Discharge	EN 61000-4-2: 2009
Voltage Interruptions	EN 61000-4-11: 2004
Surge Immunity	EN 61000-4-5: 2006
Magnetic Field Immunity	EN 61000-4-8: 2010
Harmonics	EN 61000-3-2: 2006 + A1:2009 + A2:2009
Flicker	EN 61000-3-3: 2008
Safety	EN 61010-1: 2010 (3rd Edition)

0.8 Waste Electric and Electronic Equipment (WEEE)



WEEE Directive Symbol

This symbol on the product or its packaging indicates that this product must not be disposed of with regular waste. It is the user's responsibility to dispose of waste of waste equipment according to local laws. The separate collection and recycling of the waste equipment at the time of disposal will help conserve natural resources and also ensure the waste is recycled in a manner that protects human health and the environment. For information about where the user can drop off the waste equipment for recycling, please contact your local HORIBA Scientific representative.

1 REQUIREMENTS & INSTALLATION

1.1 Safety Training Requirements

Every user of the FluoroMax® Plus and FluoroMax® Plus-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 Scientific. Contact your Sales Representative or the Service Department for details.

1.2 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).

1.3 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

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

1.4 Electrical Requirements

The FluoroMax® Plus series operates from universal AC single-phase input power over the range of 85 to 250 V AC with a line frequency of 50 to 60 Hz. This AC input power is applied to a two-pole fusing power entry module located on the side of the instrument. This module incorporates two 5 × 20 mm IEC approved, 4.0 A, 250 V, Time Delay fuses (Cooper Bussman part number GDC-4A or equivalent) to protect against line disturbances or anomalies outside the system's normal operating range.

Have enough outlets available for:

- Host computer (PC)
- Monitor
- Optional printer
- FluoroMax® Plus or FluoroMax® Plus-P
- Special power supplies for the second detector
- Each of certain accessories, such the MicroMax, temperature bath, etc.

Caution: HORIBA Scientific 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® Plus series 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 Scientific 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® Plus to a separate line, if possible, to isolate the xenon-lamp power supply inside the FluoroMax® Plus.

1.5 Unpacking and Installation

1.5.1 Introduction

The FluoroMax® Plus series 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 Instruments Incorporated assumes no responsibility for damage occurring during transit, the company will make every effort to aid and advice.

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

1.5.2 FluoroMax® Plus Series Carton Contents

QUANTITY	ITEM	PART NUMBER
1	**FluoroMax® Plus or FluoroMax® Plus-P**	
1	**USB cable**	**5500980087**
1	***FluoroMax® Plus Operation Manual***	**5700002851**
1	**Set of Allen wrenches (Allen keys)**	**5500053057**
1	**Single-cell sample-holder**	**5500450423**
1	**Power cord (110 V)** **(220 V)**	**5500098015** **5500098020**
1	**FluorEssence™ software package**	**5500000154**

1.5.3 Directions

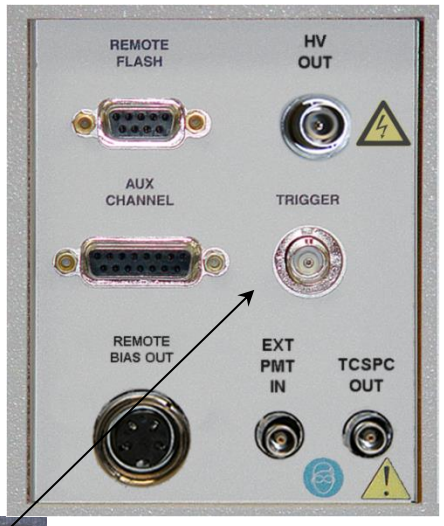
1.5.3.1 Unpack and set up the FluoroMax® Plus

Carefully open the FluoroMax® Plus shipping carton.
Remove the foam-injected top piece and any other shipping restraints in the carton.
With assistance, carefully lift the instrument from the carton, and rest it on the side of the laboratory bench where the system will stay.

Caution: Heavy Lifting Hazard!

Caution: Watch your Fingers!

Place the instrument in its permanent location.
Level the spectrofluorometer. Adjust the four leveling feet on the bottom of the instrument.
Inspect for previously hidden damage. Notify the carrier and HORIBA Scientific if any is found.
Check the packing list to verify that all components and accessories are present.
Plug one end of the power cord into the proper receptacle on the right side (while facing the unit) of the spectrofluorometer.



Plug one end of the USB cable into the USB receptacle.
With an optional trigger accessory, plug one end of the trigger cable into the TRIGGER connector on the FluoroMax® Plus. Allow the unconnected ends of the cables to dangle freely; they will be connected in later steps.

1.5.3.2 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 Scientific or another supplier.

Set up the host PC reasonably close to the FluoroMax® Plus system. The limitation is the length of the USB cable. The recommended location for the PC is just to the right of the spectrofluorometer, but other positions are possible.'

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

1.5.3.3 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 Scientific, the software installation is complete. If the computer is not from HORIBA Scientific, perform the installation. See the minimum host computer requirements listed in section 11 or Contact a HORIBA Scientific 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 a USB memory stick.

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

A USB dongle is supplied with FluorEssence™. This dongle (license) must be connected to the host PC before FluorEssence™ will operate.

1.5.3.4 Connect the FluoroMax® Plus to the computer

a. Attach the free end of the USB cable to a USB receptacle on the host computer.
b. With all devices OFF, plug the power cords from the monitor, computer, FluoroMax® Plus, and the printer into properly grounded (earthed) receptacles.
c. Install any accessories that arrived with the system, using the instructions that accompany the accessories. See Chapter 12 for a detailed list of accessories.

1.5.3.5 Users Outside of the USA:

Users outside 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.

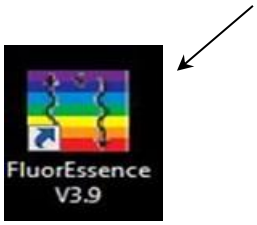
1.6 Software Emulation

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

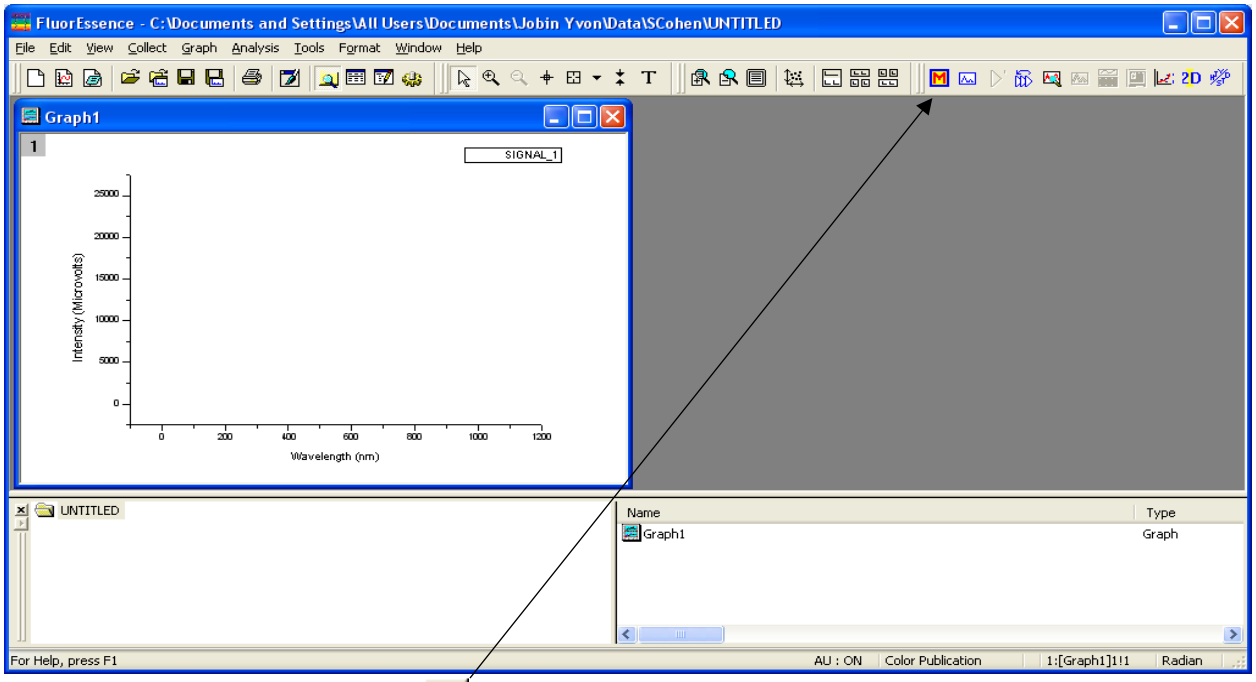
1. Disconnect the communications cable from the host computer to the FluoroMax® Plus.

   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™.



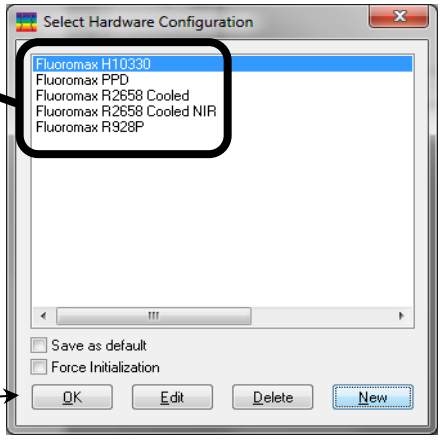
The main FluorEssence window opens:



3. Click the Experiment Menu button  to open a hardware configuration.

The Select Hardware Configuration window opens.

4. Choose the desired instrument you wish to emulate.

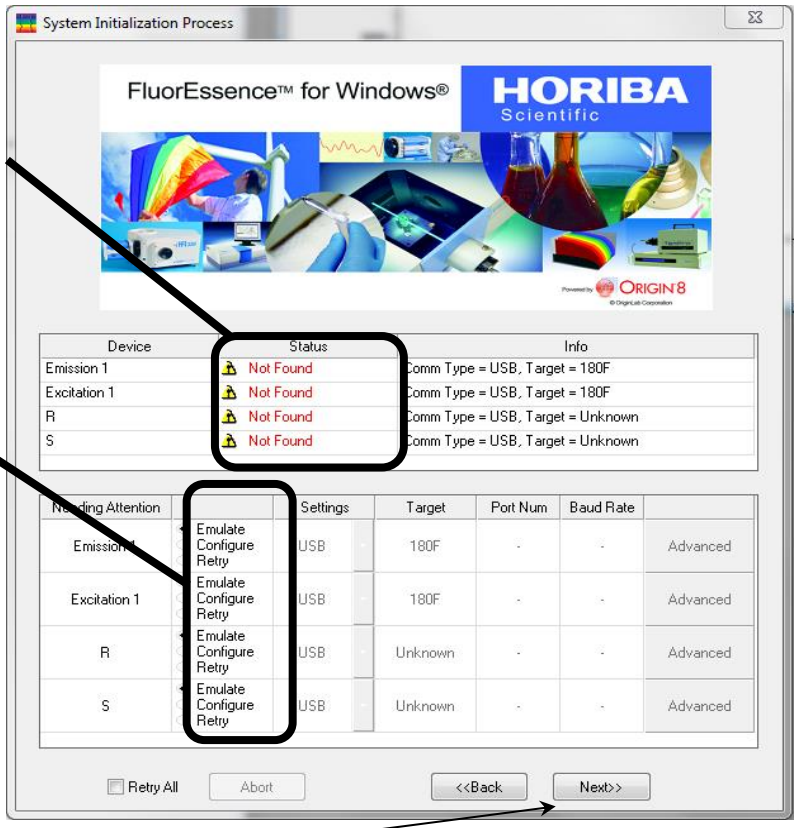


Note: All FluoroMax+ instruments have an R928P, plus only ONE of the other possible configurations shown here.

Click Ok button.

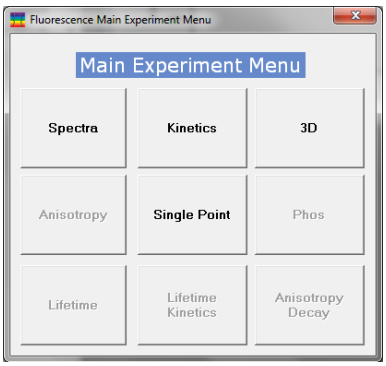
The System Initialization Process window opens:

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.



5. Click the Next>> button.

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



2 SYSTEM DESCRIPTION

Warning: Do not open the instrument without proper training, appropriate protection, 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.

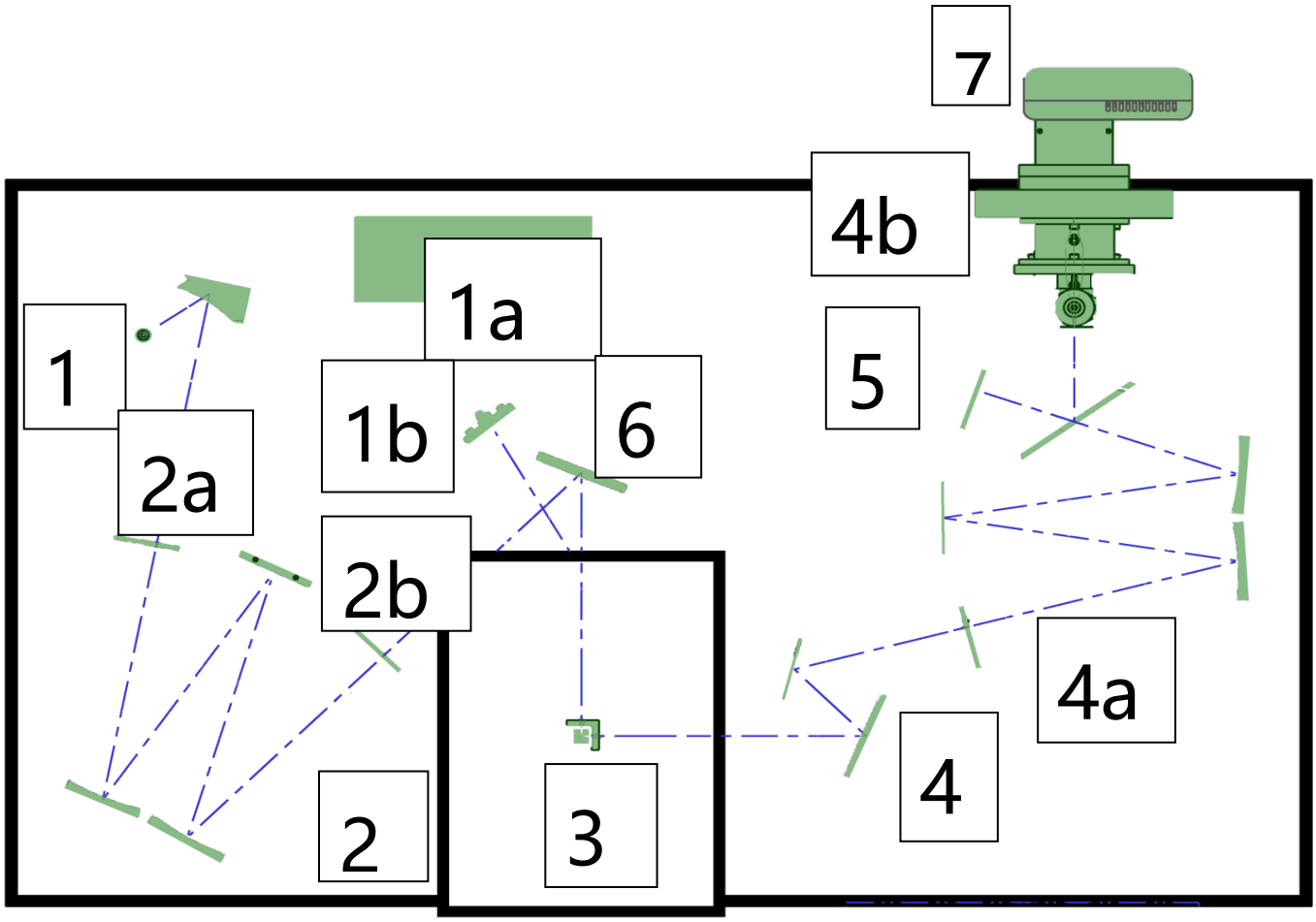
2.1 Introduction

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.

2.2 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.

2.3 Optical Layout

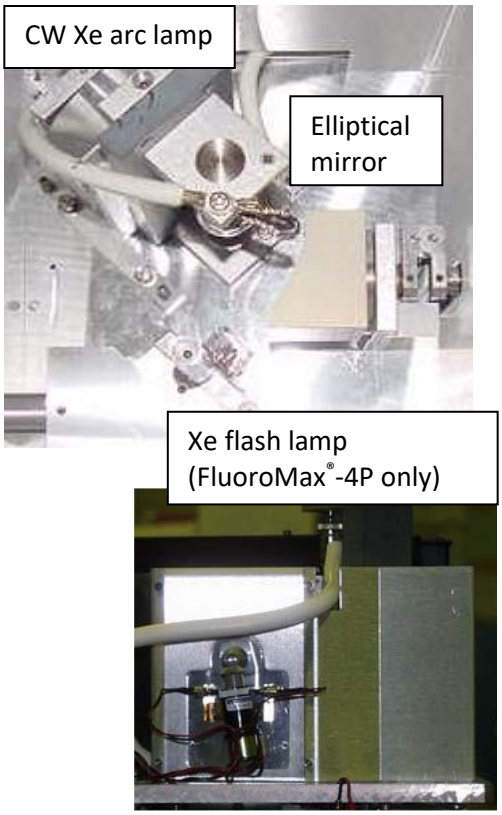


1 Xenon arc-lamp and lamp housing
1a Xenon-lamp power supply
1b Xenon flash lamp (FluoroMax® Plus-P only)
2 Excitation monochromator
2a & 2b Slits
3 Sample compartment
4 Emission monochromator
4a & 4b Slits
5 Internal signal detectors (photomultiplier tube and housing)
6 Reference detector (photodiode and current-acquisition module)
7 External signal detector (exact shape varies with model)
Host computer (not on diagram)

2.3.1 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. 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.

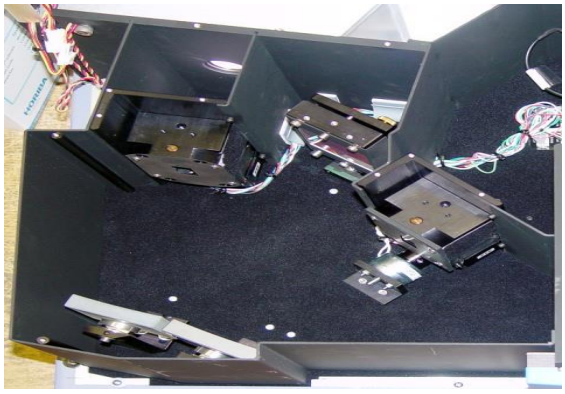
In the FluoroMax® Plus with phosphorimeter, a second source, a xenon flash lamp, is available also. A FluorEssence™-controlled motor and gearbox rotate the mirror in the illumination area, in order to switch between the CW source and the flash lamp.



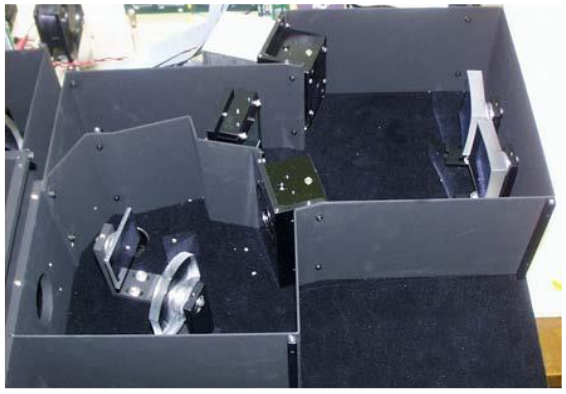
Right: Motor and gearbox that rotate the mirror to switch lamps (optional phosphorimeter only).

2.3.2 Monochromators (2 and 4)

The FluoroMax® Plus 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, eliminate chromatic aberration for high light-throughput, and minimize spherical aberrations and re-diffraction.

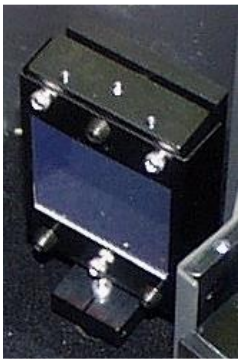


Excitation monochromator.



Emission monochromator.

2.3.3 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® Plus 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 80 nm s^1, with accuracy better than 0.5 nm, and repeatability of 0.3 nm.

If FluoroMax-Plus is configured with either H10330 or R2658 external detector, the emission monochromator will be equipped with a dual grating cassette with back-to-back gratings: 1200 grooves mm^{-1} blazed at 500nm and 600 grooves mm^{-1} blazed at 1 micron.

2.3.4 Slits (2a&2b, 4a&4b)

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 and the spectral resolution. The emission monochromator's slits control the spectral resolution and 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® Plus monochromators is 4.25 nm mm^{-1} for gratings with 1200 grooves mm^{-1} at 540 nm. Below is a table showing standard slit-widths with their corresponding band passes.

SLIT WIDTH (MM)	BANDPASS (NM)	ROUNDED BANDPASS (NM)
0.50	2.125	2
1.175	4.994	5
2.00	8.500	8.5

2.3.5 Shutters

An excitation shutter, standard on the FluoroMax® Plus, 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 anti-photobleaching 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.

2.3.6 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 monochromator.

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 12 for a list of accessories.

2.3.6.1 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.

2.3.7 Detectors (5, 6, and 7)

Each FluoroMax® Plus contains three detectors:

- Signal detector

The standard signal detector is a photon-counting detector (R928P photomultiplier tube). 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 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.

- Second (external) signal detector

Attached to the back of the FluoroMax® Plus chassis is a second detector chosen by the customer. Possible second detectors are: R2658, PPD, or R13456P, cooled R928P or IGA solid state detector.

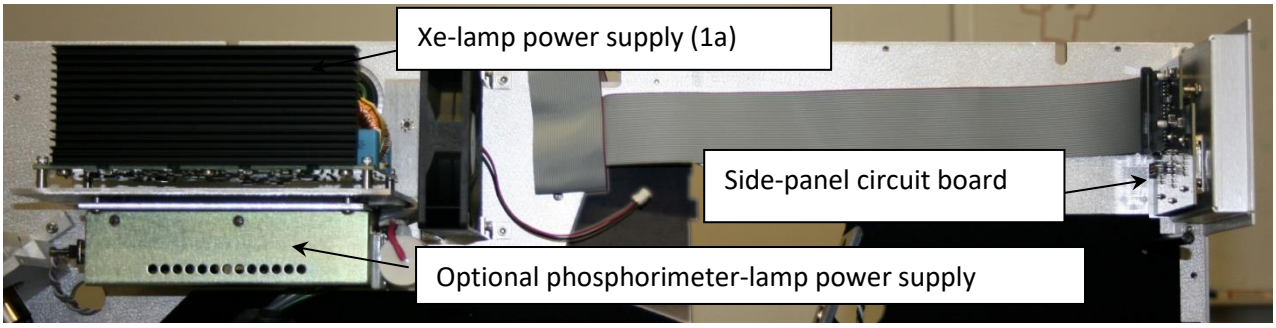
- Reference detector

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 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 Scientific for every instrument and may be applied to data through FluorEssence™. See Chapter 8 for more details.

2.3.8 Electronics and Controllers (1a)

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



Lamp power supply (1a), optional phosphorimeter power supply, and side-panel circuit board.

- 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 15 V. The voltage is filtered, to stabilize the illumination as much as possible. The lamp is started with the rocker-switch on the right panel of the instrument and controlled through software.

- J400906 board

Underneath the optical platform, there is a control board. This controls the monochromators and any optional accessories connected to the sample compartment.

2.3.9 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

3.1 Introduction

This chapter explains how to turn on the FluoroMax® Plus 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.

3.2 Power Switch

The power switch is located on the lower right-hand side of the instrument. When switched on, the xenon lamp arcs initially, and the FluoroMax® Plus turns on, runs through self-diagnostics, then starts the xenon lamp.

Note: HORIBA Scientific suggests leaving the lamp on during brief periods of inactivity.

3.3 Turning on the System

Warning: When the xenon lamp is ignited, a large voltage is applied across the lamp. Therefore, never operate the lamp with the cover removed. 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® Plus.

   Turn the power switch to the ON (1) position.

2. Turn on instrument accessories.

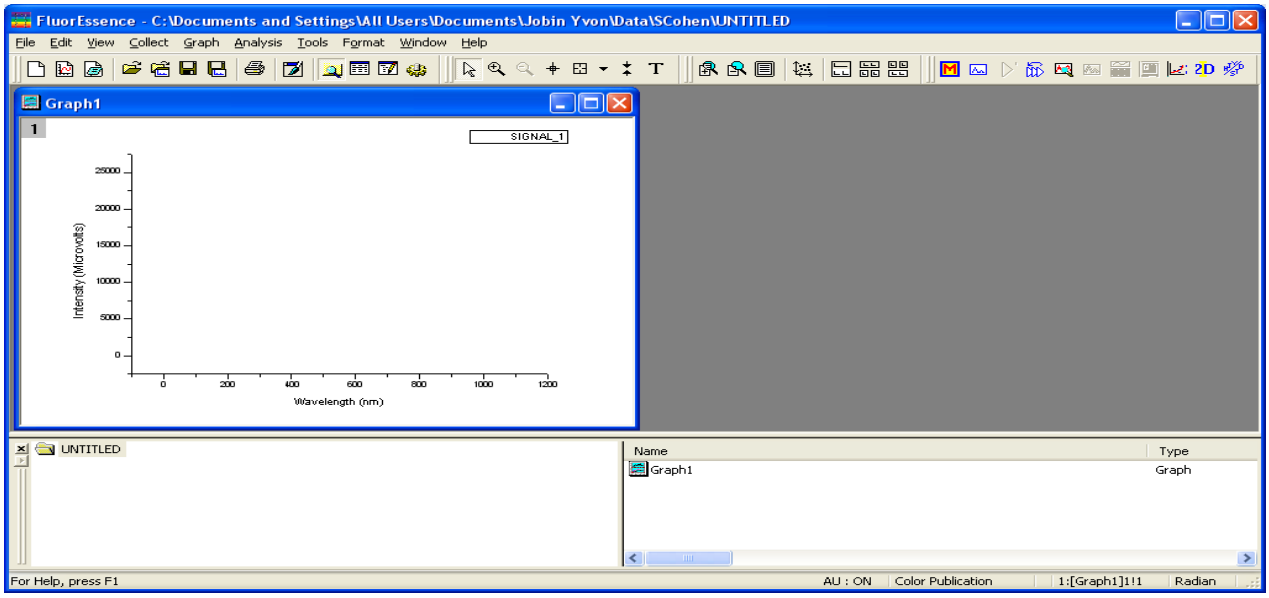
   Accessories include a temperature bath, MicroMax, etc.

3. Turn on all peripheral devices for the host PC.

   Peripherals include any printers or other output devices.

4. Start the host computer.

   Switch on the host computer.
   Click the FluorEssence™ icon on the Windows® desktop.
   The instrument initializes, then the FluorEssence window appears. If there are any difficulties, see the troubleshooting chapter



3.4 Checking System Performance

3.4.1 Introduction

Upon installation and as part of routine maintenance checks, examine the performance of the FluoroMax® Plus. HORIBA Scientific 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® Plus is an auto calibrating spectrofluorometer. This means the system initializes its monochromators' drives, locates the home position of each drive, and assigns a wavelength value to this position from a calibration file. While the system usually maintains calibration by this method, it is wise to check 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.

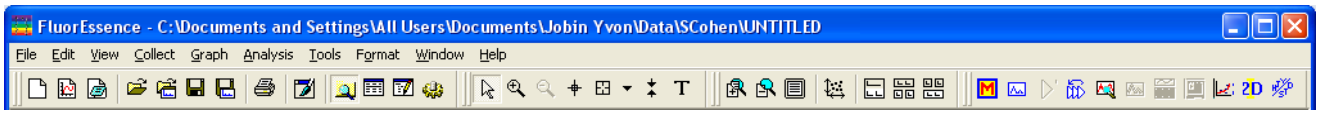
The scans shown herein are *examples*. A Performance Test Report for your new instrument is included with the documentation. Use the Performance Test Report to validate the spectral shape and relative intensity taken during the calibration checks.

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

3.4.2 Excitation-Monochromator Calibration Check

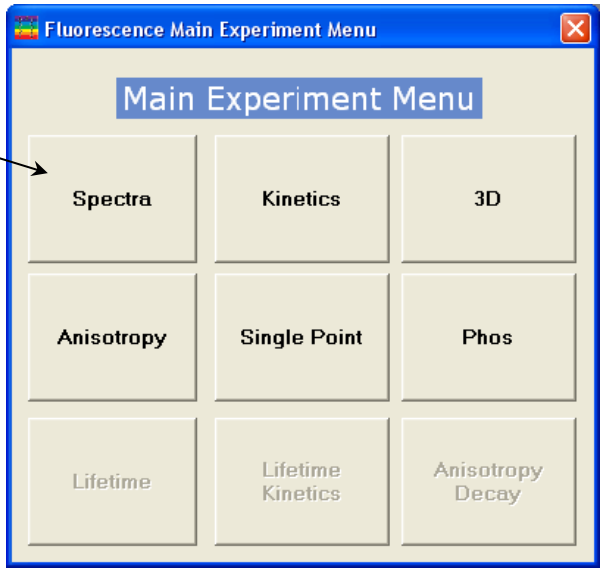
This calibration check verifies the wavelength calibration of your excitation monochromator, using the reference photodiode located before the sample compartment. It is an excitation scan of the xenon lamp's output and should be the first check performed.

1. Close the lid of the sample compartment.
2. On the main FluorEssence toolbar, select the Experiment Menu button  :

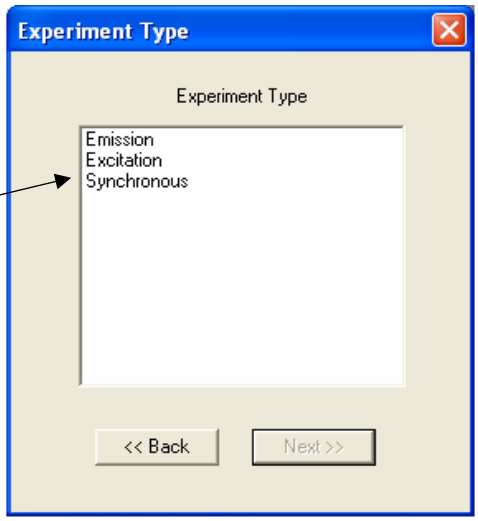


The Fluorescence Main Experiment Menu appears:

3. Choose Spectra.

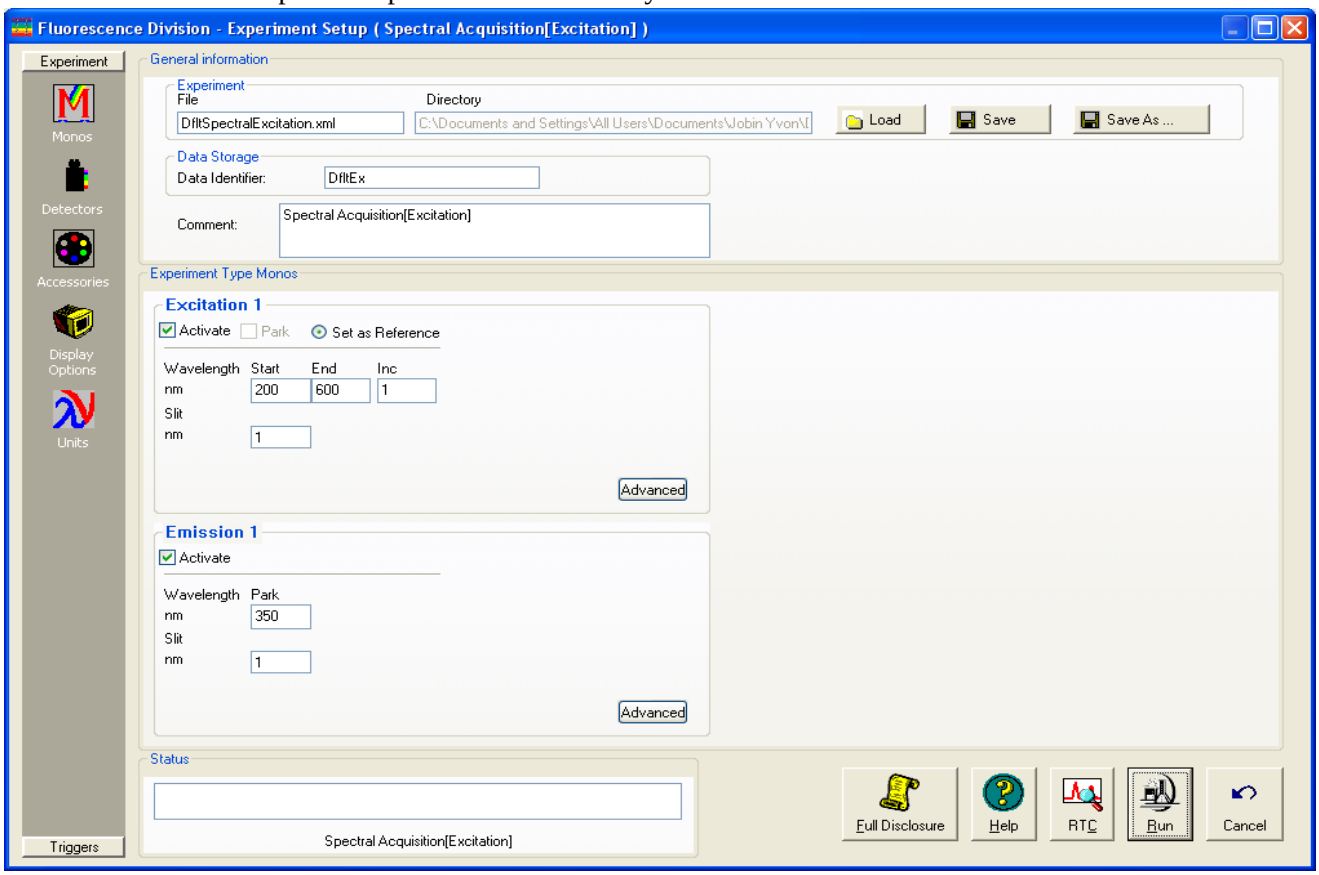


The Experiment Type window appears:



4. Choose Excitation.

The xenon-lamp scan experiment automatically loads:



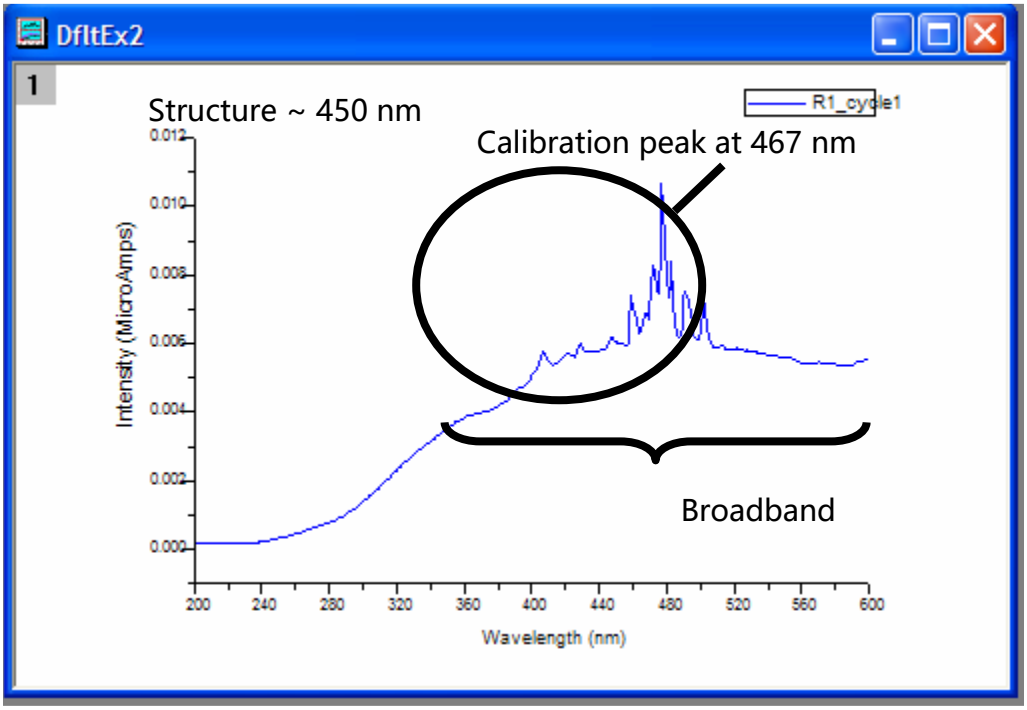
5. Use the default parameters or adjust them.

DEFAULT MONOCHROMATOR PARAMETERS FOR THE XENON-LAMP SCAN				
Monochromator (1200 grooves/mm)	Initial wavelength	Final wavelength	Increment	Slits (bandpass)
Excitation	200 nm	600 nm	1 nm	1 nm
Emission	350 nm	--	--	1 nm

DEFAULT DETECTOR PARAMETERS FOR THE XENON-LAMP SCAN		
Detector (Signal)	Integration time	Units
Reference (R1)	0.1 s	mA

6. Click the Run button. 

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

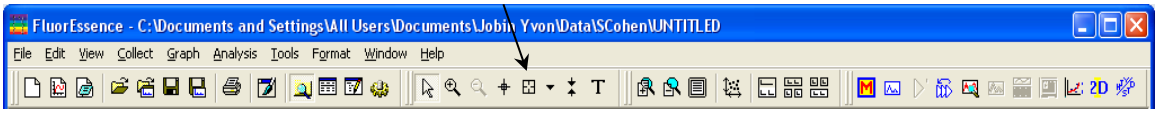


This is an uncalibrated FluoroMax Plus® lamp scan. The main peak ought to be at 467 nm, but here appears near 480 nm.

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

7. Calibrate the excitation monochromator, if required.
    a. With the Graph tab active, double-click the plot to make the plot active.
    b. Expand the plot by clicking the Expand button.

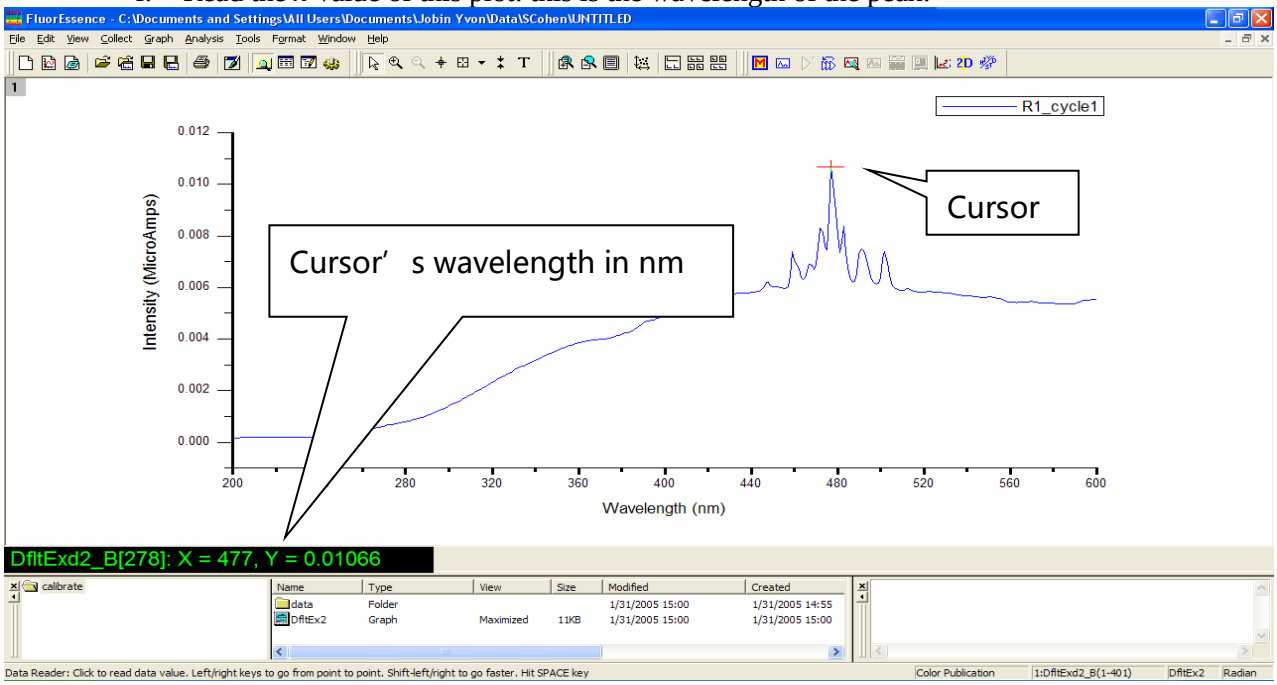
c. Click the cursor button  to start the Cursor function.



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

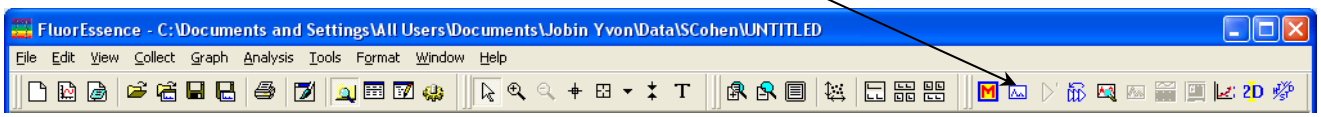
e. Using the left and right arrows on the keyboard, move the cursor to the top of the peak.

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

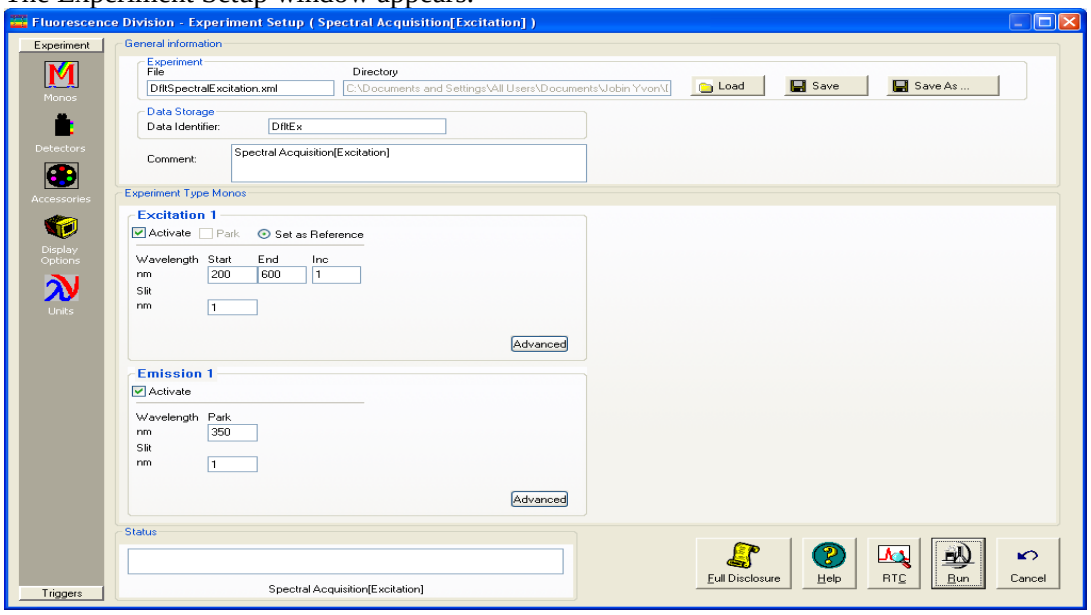


This example shows the peak actually at 477 nm, which is 10 nm too high. Therefore, we must recalibrate the monochromator.

g. Click the Previous Experiment button  .

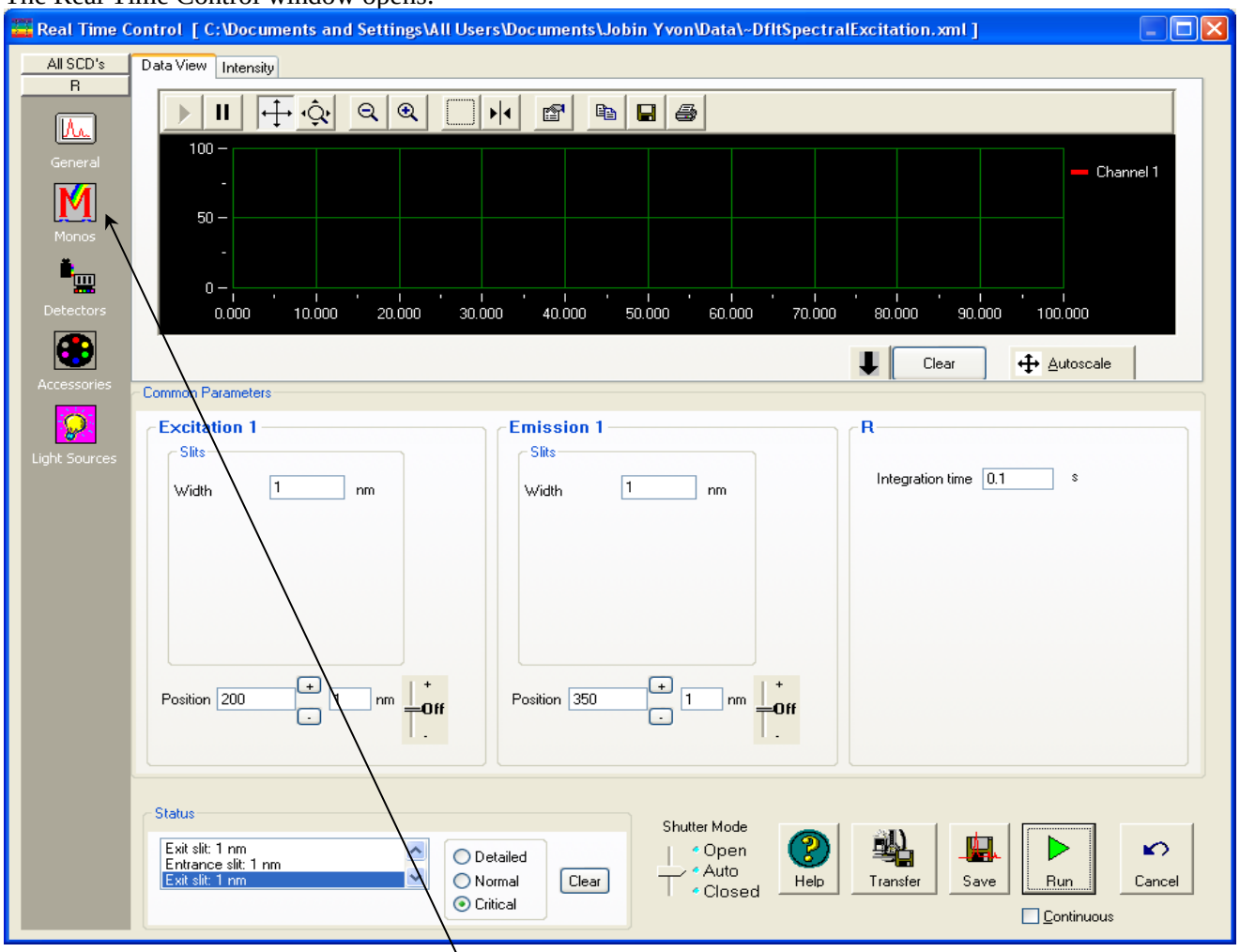


The Experiment Setup window appears.



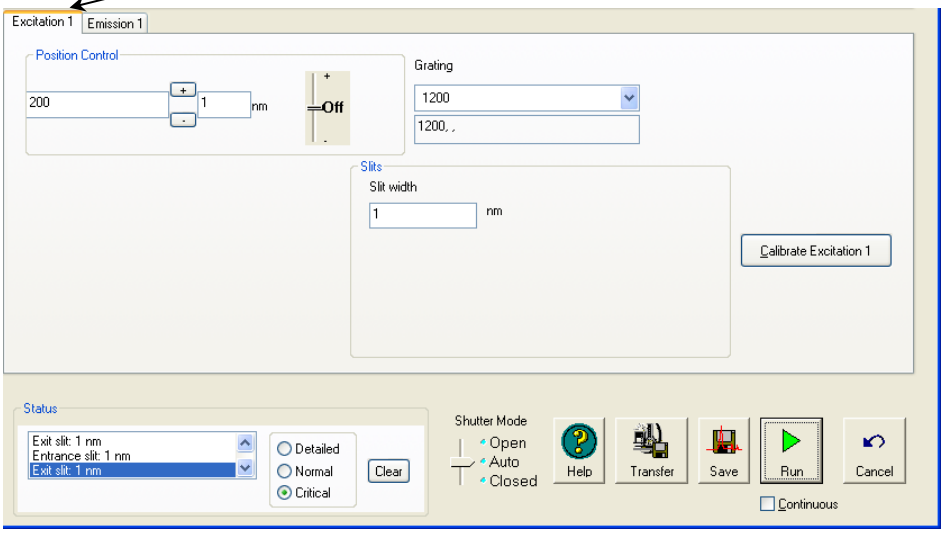
h. Click the RTC button on the lower right.

The Real Time Control window opens:

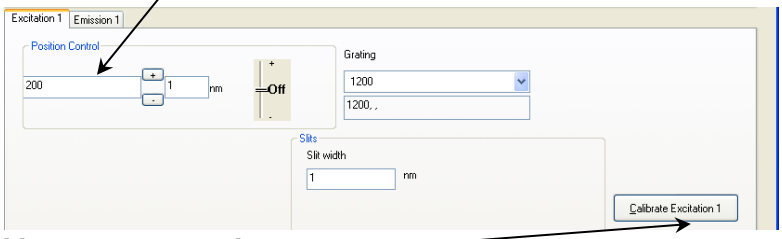


i. Click the Monos icon 

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



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



k. Click the Calibrate Excitation 1 button.

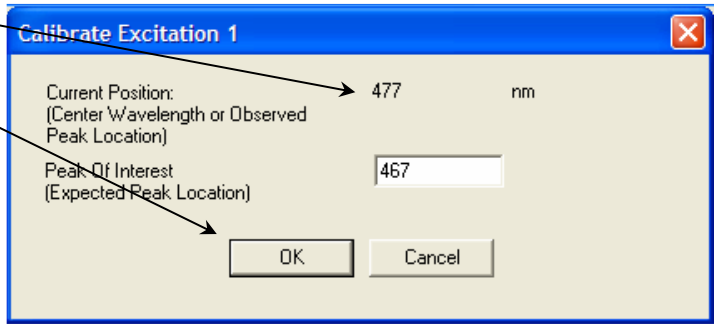
The Calibrate window opens:

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

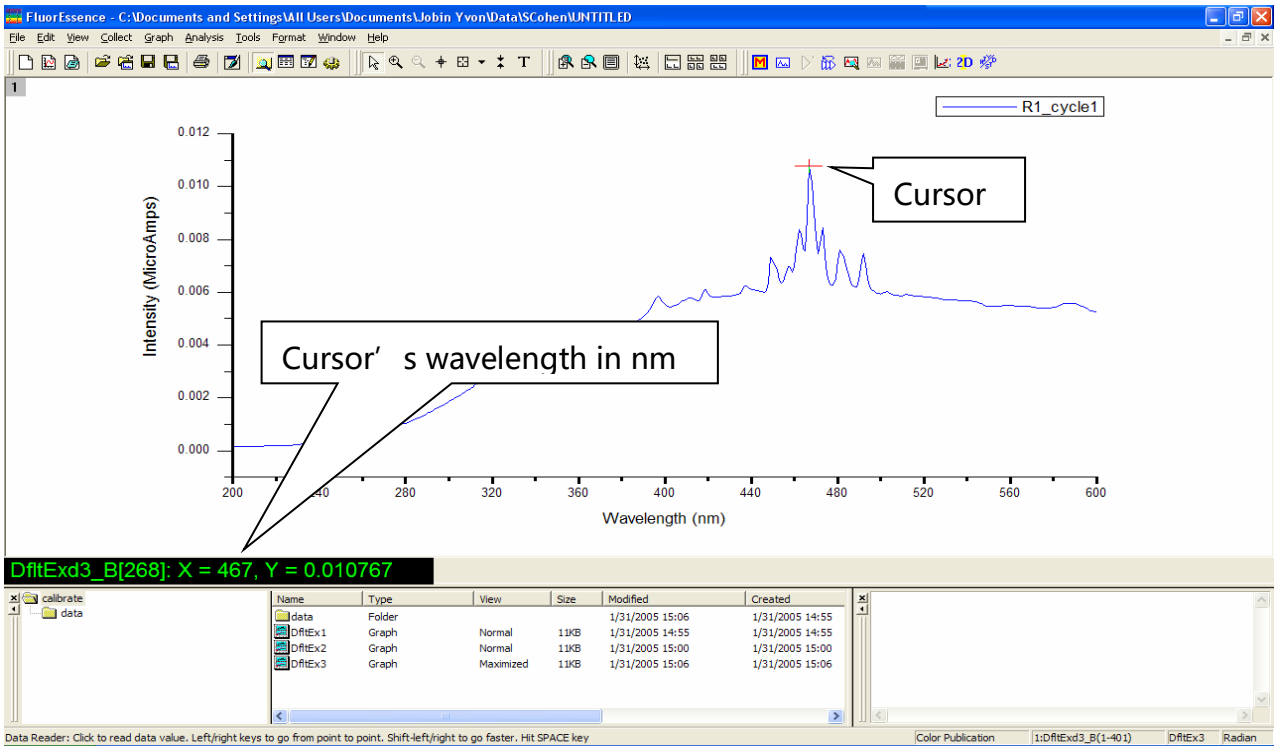
m. Click the OK button.

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

o. In the Experiment window, click the Run button to confirm the correct peak position.



A correct scan is shown below (peak is at 467 nm):



3.4.3 Emission-Monochromator Calibration Check

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.

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

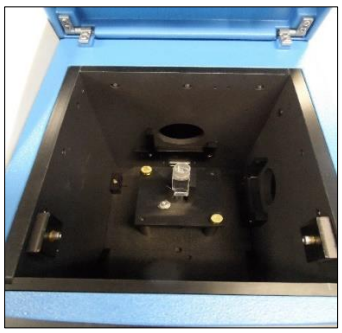
The water sample should be research-quality, triple-distilled water. HPLC-grade (18-MΩ spec.) or equivalent water is suggested for the Raman scan. Impure samples of water will cause elevated background levels as well as distorted spectra with (perhaps) some unwelcome peaks.

Use a 4-mL quartz cuvette.

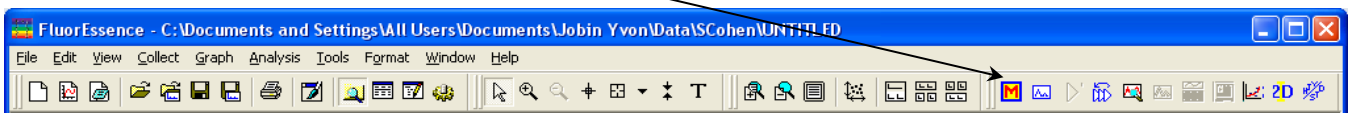
Note: Avoid glass or acrylic cuvettes: they may exhibit UV fluorescence or filtering effects.

1. Turn on the instrument and the FluorEssence software if not already on.

2. Insert the water Raman cuvette into the sample compartment holder and close the lid.



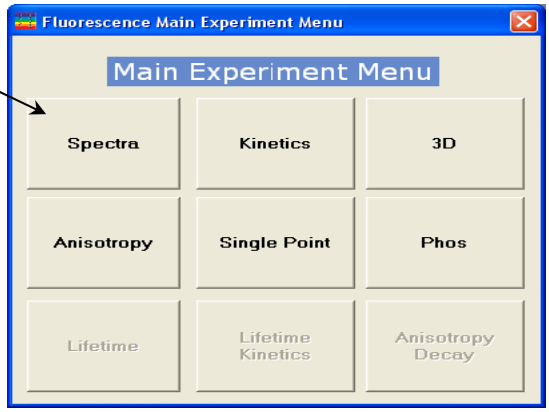
3. Click the Experiment Menu button on the toolbar to initialize the system



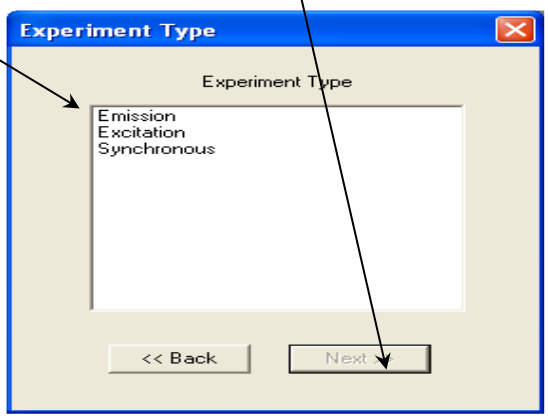
The Fluorescence Main Experiment Menu opens.

4. Click the "Spectra" box.

The Experiment Type window appears:

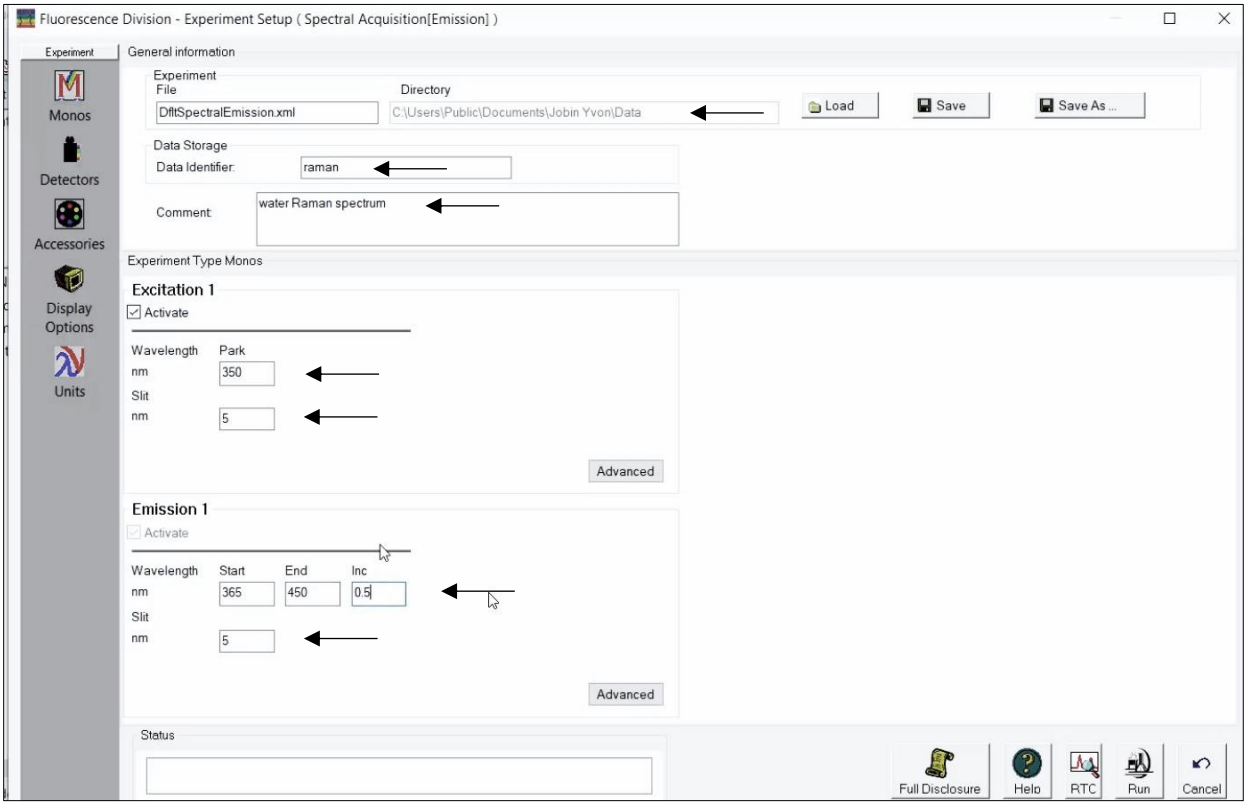


5. Choose Emission, then click the Next >> button.



The water-Raman experiment automatically loads.

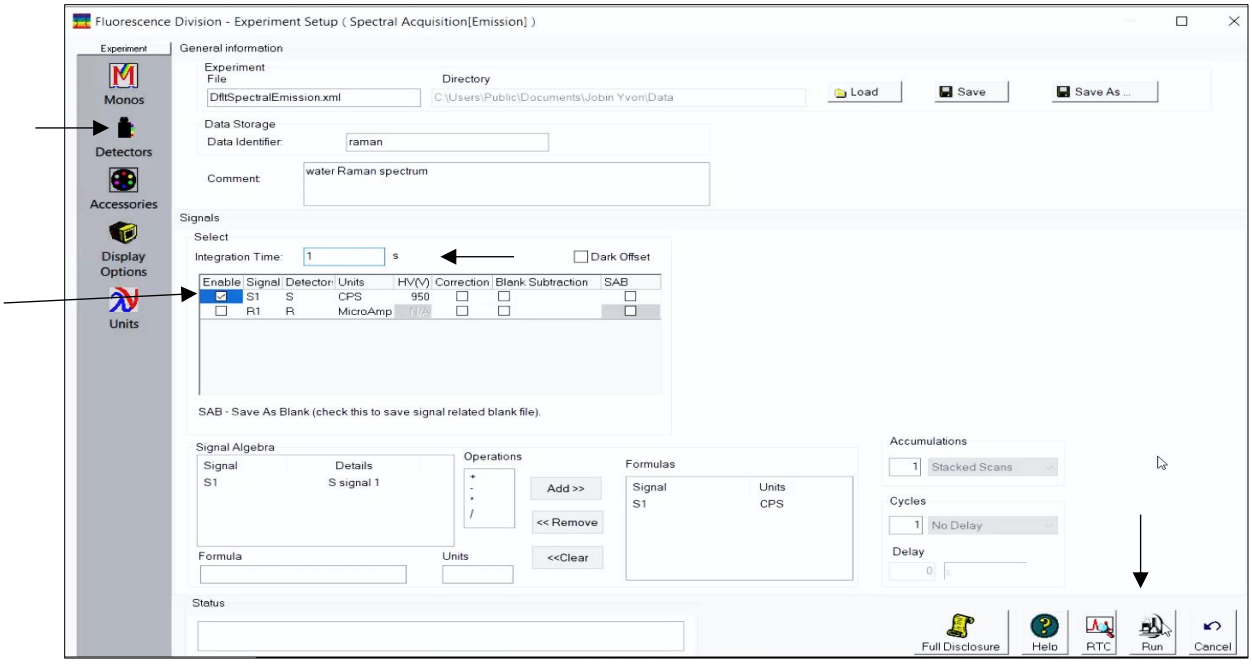
6. Enter the parameters shown below. Also, use the "Data Identifier" field and the "Comment" box to enter further identification for the test to be performed and the "Directory" box to select where the file is to be saved.



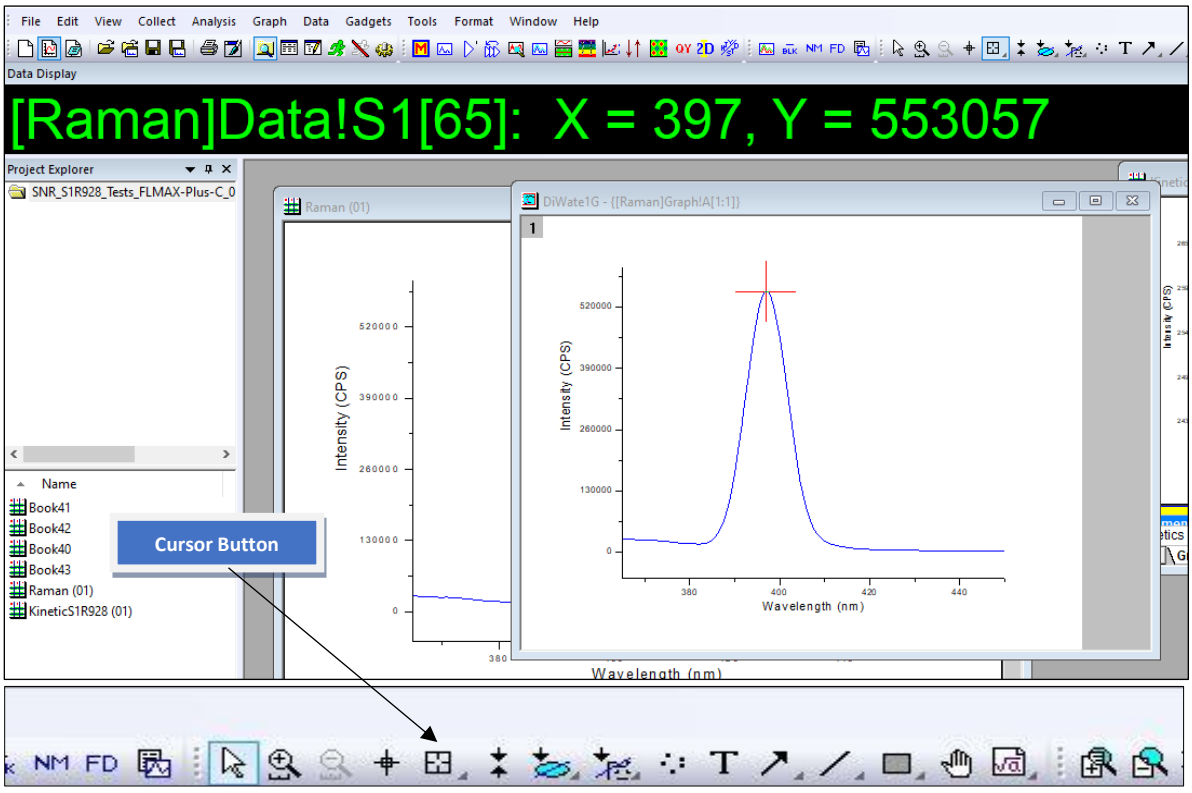
MONOCHROMATOR PARAMETERS FOR THE WATER-RAMAN SCAN				
Monochromator (1200 grooves/mm)	Initial wavelength	Final wavelength	Increment	Slits (bandpass)
Excitation	350 nm	--	--	5 nm
Emission	365 nm	450 nm	0.5 nm	5 nm

7. Click the Detectors icon and change the Integration Time to 1 second while making sure the S1 signal box is checked. Then, click the Run button 

in the lower-right corner. The intermediate display opens and the water-Raman scan runs.



8. When the graph appears, double click it to analyze it. Then, click the Cursor button and place the cursor at the peak to read the wavelength and intensity. The peak should be at 397 ± 1nm.



9. The FluoroMax®Plus SNR is specified for a R928P photomultiplier tube at room temperature.

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.

10. If the peak is at the right wavelength, click the "Save" icon on the toolbar to save the results.

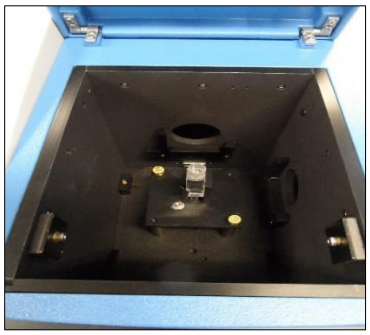
Note: Make sure the test is carried out with the actual detector used. All HORIBA Scientific systems' parameters are specified with a R928P photomultiplier tube at room temperature.

3.4.4 Kinetic Scan

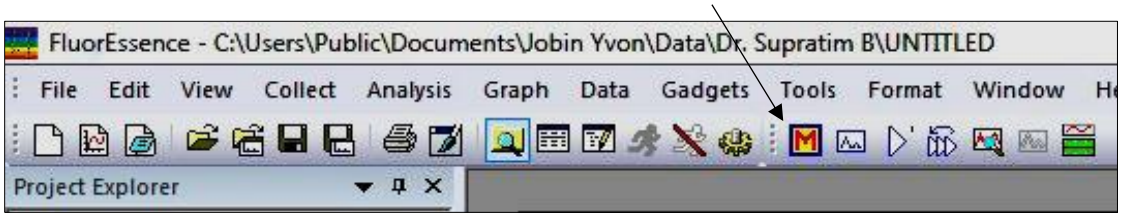
The procedure for performing a kinetic scan is shown below. This test also requires the use of a water sample.

1. Turn on the instrument and open the FluorEssence software, if not already open.

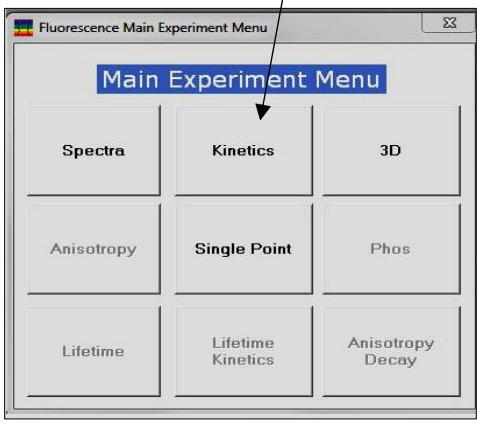
2. Insert the water Raman cuvette into the sample compartment holder, if not already in there, and close the lid.



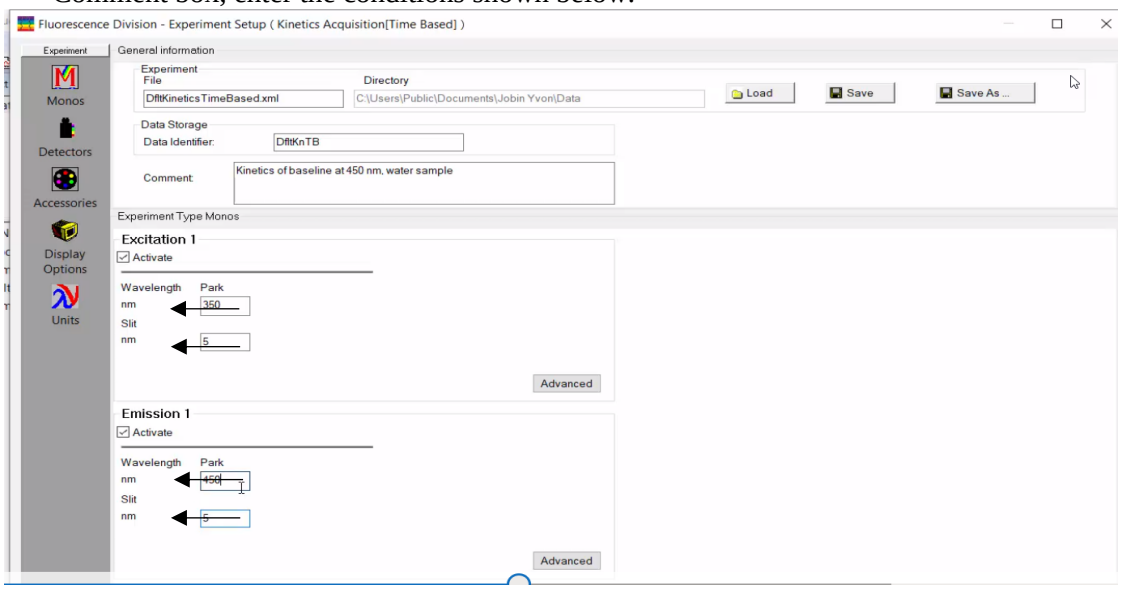
3. Click the experiment menu M icon on the software to initialize the system.



4. Click the Kinetics box.



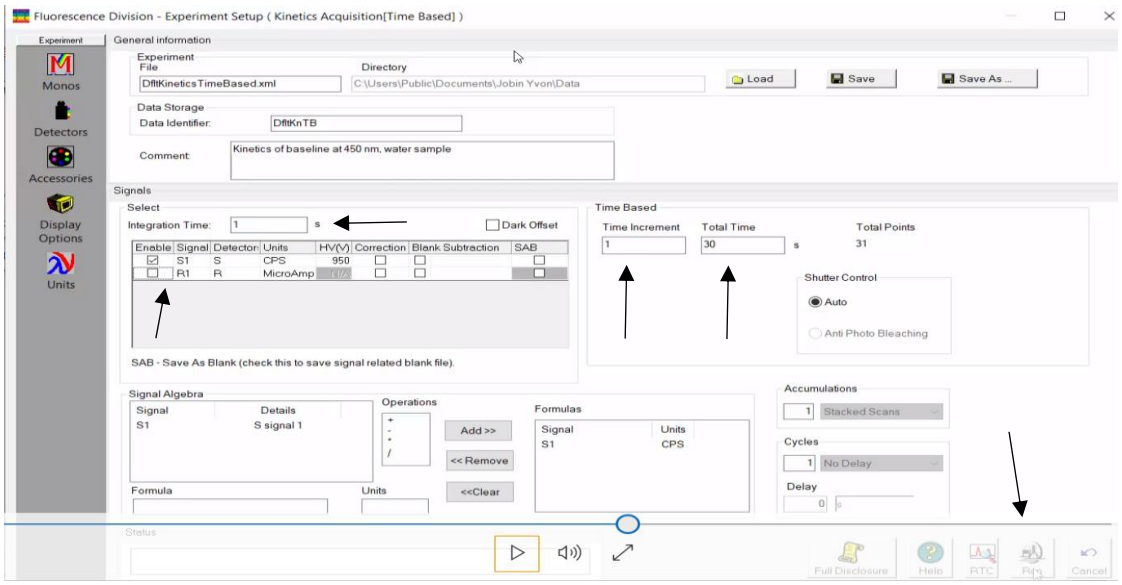
5. After adding the desired name for the test on the Data Identifier field and a description on the Comment box, enter the conditions shown below.



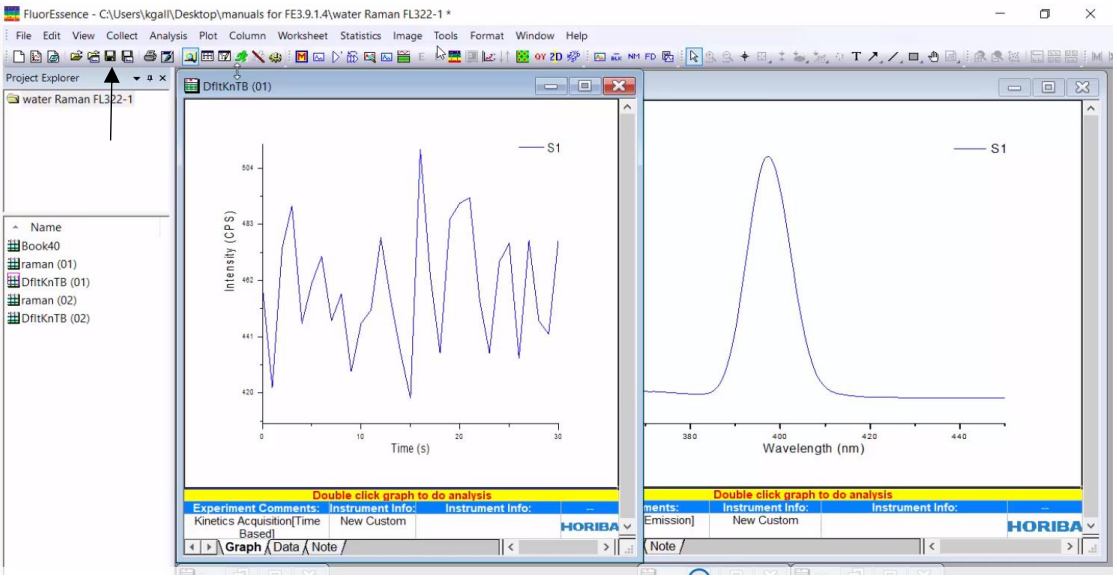
MONOCHROMATOR PARAMETERS FOR A KINETIC SCAN		
Monochromator (1200 grooves/mm)	Initial wavelength	Slits (bandpass)
Excitation	350 nm	5 nm
Emission	450 nm	5 nm

6. Click the Detectors icon. On the Time Based section change the parameter shown below. Then, click the Run icon.

PARAMETERS	
Time Increment:	Change to 1
Total Time:	Change to 30
Integration Time:	Change to 1
Enable R1 box:	Uncheck the box



7. When the results appear, click the Save button on the toolbar.



3.4.5 Calculation of Water-Raman Signal-to-Noise Ratio

3.4.5.1 Introduction

Fluorescence is a highly sensitive analytical technique, so one of the primary specifications to consider when selecting a spectrofluorometer is its sensitivity. The overall sensitivity of a spectrofluorometer is determined by many factors including, but certainly not limited to, the optical design, coupling optics, intensity of delivered excitation light, efficiency of fluorescence collection, spectrometer design, detector technology and much more. For example, a system that has a higher wattage lamp does not guarantee that it offers better overall fluorescence sensitivity.

Given that there are so many variables that go into making a sensitive fluorometer, what is needed is a standard reference test that can be performed by any user to properly compare one fluorometer with another.

In the past, some commercial manufacturers of fluorometers used detection limits for specific fluorescent molecules, such as quinine sulfate or fluorescein to demonstrate sensitivity. However, today, the highest

sensitivity fluorometers are able to detect at such low concentrations of fluorophores, that the ability to accurately perform a serial dilution down to these detection limits becomes questionable. As such, the water Raman test has become an industry standard as a good measure of the relative sensitivity between different instruments. The water Raman test is also preferable because ultrapure water is readily available around the world, the sample is stable, the signal is relatively weak, and the Raman band of water can be measured over the entire wavelength range of the instrument allowing for more robust comparisons, than is possible with a single fluorescent probe. This sensitivity specification is derived from an emission spectrum of the Raman vibrational band for pure water. Typically, it is acquired with the excitation wavelength selected at 350 nm, with an emission scan from 365 to 450 nm. In general, the sensitivity of the fluorometer is expressed in a signal-to-noise ratio (SNR) that is a comparison of a signal value in the presence of a signal, with a value for system noise, in the absence of signal.

Unfortunately, not all manufacturers use the same experimental conditions to acquire this data set, and they also do not use the same formulas for calculating the SNR of the acquired data. There is no right or wrong way to collect data, or analyze it, but it is clear that different methods and analysis can give quite different numbers. Therefore, it is important not only to know how the water Raman spectrum was acquired, but also how the data were treated. If you can ensure the data is acquired and analyzed in the same way, you can be certain to make a fair comparison between two different fluorometers.

In this technical note we point out the different factors influencing the signal-to-noise ratio, and articulate the HORIBA method, to allow investigators to have the necessary tools to make a proper comparison.

3.4.5.2 Formulas for Calculating Signal to Noise Ratio

FSD (or SQRT) Method

For decades now, HORIBA Scientific has defined the SNR as the difference of Peak signal minus Background signal, divided by the square root of the Background signal. We call this the FSD method (First Standard Deviation). It is also referred to as the square root (SQRT) method.

The FSD signal to noise ratio formula:

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

The peak signal is measured at the water Raman peak intensity at 397 nm (for 350 nm excitation) and the noise in a region where no Raman signal is present (450 nm). For a perfect optical system there would be no signal at 450 nm since there is no Raman emission there, however, all electro-optical systems have some levels of stray light and noise, which will contribute to a signal at 450 nm. The above formula assumes that the noise is governed by Poisson statistics and, therefore, can be calculated as the square root of the baseline signal counts at 450 nm. It is only applicable to photon counting detection, so for comparison purposes it should only be used when comparing two photon counting spectrofluorometers.

RMS Method

Another commonly used method is to divide the difference of Peak signal – Background signal, by the Root Mean Square (RMS) value of the noise on the background signal. This second method is used by a number of manufacturers and is the best approach for spectrofluorometers that use analog detectors, where the intensity units will vary from one manufacturer to another.

The RMS signal to noise ratio formula is shown below.

$$\frac{S}{N} = \frac{S_{397\,\mathrm{nm}} - S_{450\,\mathrm{nm}}}{N_{\mathrm{rms,\,background}}}$$

To correctly measure the RMS noise value for the denominator, a second experiment is performed where the fluorometer excites at 350 nm and the kinetics is measured as a function of time at 450 nm emission.

The RMS formula is given by,

$$N_{rms} = \sqrt{\frac{\sum_i^n (S_i - \bar{S})^2}{n}}$$

Where the time-based background signal is measured n times and S is the average intensity value across the kinetic scan.

Not all companies use the RMS formula above. Some use the peak-to-peak noise of the data off peak, say from 420 to 450 nm, and still others use an RMS estimate, either from the off-peak portion of the spectrum, or from a secondary kinetics scan of the peak. In the end, the method for determining the RMS noise is not as critical as applying the exact same formula to any comparisons that one would make.

In conclusion, there is no best way to calculate the signal noise ratio for the Raman band of water, and different companies do it in different ways. The FSD method is valid only for comparing photon counting spectrofluorometers. When comparing one or multiple systems that use analog detection, then RMS, or some RMS estimate method, should be used.

As long as the different data sets are calculating sensitivity in the same way, any particular method will provide a relative measure of the SNR of one spectrum compared to another.

3.4.5.3 Experimental Method

Just as the formula used to calculate the signal to noise ratio can have a dramatic effect on the apparent sensitivity of any particular spectral dataset, the hardware configuration of the instrument, and the experimental parameters of the data acquisition, also have a dramatic effect on the quality of the spectrum acquired.

There are many hardware parameters, settings, and options that all have an effect on the measured sensitivity of a spectrofluorometer. This can make it extremely difficult to absolutely compare the relative sensitivity of two different instruments if they are not used in a virtually identical way. Below we discuss each of these factors and the impact they have on the resulting data.

3.4.5.4 Applicable to All Scanning Fluorometers

Excitation Wavelength: The excitation wavelength should be identical for all systems being compared. The HORIBA method uses 350 nm excitation for the Raman band of water, as do most other manufacturers. When exciting at 350 nm, the Raman emission band for water has a peak at 397 nm.

It is fortunate that most manufactures have standardized this excitation wavelength, as it allows for better comparison. However, it is perfectly valid to move the excitation wavelength to any other value as a way to test sensitivity in a different wavelength range (e.g., the NIR).

Emission Scanning Range: The HORIBA method scans the emission monochromator from 365 to 450 nm, with 0.5 nm increments, so as to collect the entire Raman peak at 397 nm and also the background at 450 nm.

Bandwidth (Slit Size): The HORIBA method uses 5 nm bandpass slits on both the excitation and the emission spectrometers. Some manufacturers specify 10 nm slits which has the effect of increasing the sensitivity compared to 5 nm. It has been reported that doubling the physical slit size at the entrance and exit of a monochromator can quadruple the intensity of excitation and the emission detection throughput since the throughput goes as the square of the size increase, but this a simplistic estimate that should be

measured empirically. HORIBA has measured the factor difference with the HORIBA FluoroMax, and observed that for FluoroMax, doubling the slits size from 5 to 10 nm increases the overall signal to noise ratio for the Raman band of water by a factor of more than 3 times. However, this will be different for all fluorometers, so please be sure to compare with identical band passes.

Integration Time (or Response Time): This refers to how long the detector is allowed to collect a signal at a given wavelength step position. It also plays a significant role in the overall sensitivity measured for a fluorometer. The HORIBA method uses a 1 second integration time at each wavelength point, similar to other manufacturers. However, some manufacturers specify a 2 second response time which increases the overall signal to noise ratio by almost a factor of two. Be sure to use the same integration (response) time when comparing.

PMT Type: Most spectrofluorometers use a photomultiplier Tube (PMT) as their sole detector of fluorescence emission, without any option for changing the detector housing. This is true for most bench-top analytical fluorometers. Some of these bench-top systems allow for the selection of different individual PMT's with different wavelength ranges and specifications. PMTs that do not detect as far into the NIR as other PMTs will have a lower dark count, so that they will provide a better signal to noise ratio in the 350 to 400 nm range, however they may not be useable in the entire emission wavelength range desired for a particular lab. HORIBA's standard PMT used in the FluoroMax Plus, Fluorolog3 and FluoroLog-QM series of fluorometers, is the Hamamatsu R928P PMT, which is considered the industry standard for fluorometry. In these cases, be sure that each fluorometer is using the same PMT, where possible.

Optical Filters: An optical filter can be added to the optical path of a fluorometer, either on the excitation side or emission side of the sample. These can be manually placed into a filter holder inside the sample compartment, or they can be part of a filter wheel that can automatically place different filters into the optical path when different experimental protocols are selected. Optical filters have the effect of improving the stray light rejection at given wavelengths, and they can dramatically improve the signal to noise ratio of a fluorometer. HORIBA does not use any optical filters, other than the scanning spectrometers themselves, when specifying the SNR for water Raman with the FluoroMax, Fluorolog-3 or FluoroLog-QM series specifications. When comparing a HORIBA fluorometer, with a fluorometer that uses automated filters, please do not use a filter, or if it is automatic, confirm what make and type of filters are used, and where they are employed, to replicate a similar experimental method with a HORIBA fluorometer.

3.4.5.5 Applicable to Modular Research Fluorometers

Detector Type: Modular research fluorometers typically include a PMT housing as standard but allow for many different types of single channel detectors to extend the wavelength range, or fluorescence lifetime range of an instrument. Alternative detectors include cooled PMT housings, various solid-state detectors such as InGaAs, MCP PMT's and so on. These different types of detectors will have dramatic effects on the signal to noise ratio of any particular sample measurement, so here again, when trying to compare the sensitivity of one fluorometer against another, be sure that the same detector type is used to collect data on both systems.

Detector Temperature: Most commercial spectrofluorometers use PMT housings that are not cooled, and in fact many instruments do not even offer a cooled detector option. A cooled PMT housing can improve the sensitivity of an instrument by reducing the dark counts (background) compared to the same exact

PMT in an ambient housing. HORIBA's standard PMT housings in the FluoroMax Plus, FluoroLog3 and FluoroLog-QM are ambient PMT housings, however the Fluorolog3 and FluoroLog-QM series do offer optional cooled PMT housings to improve sensitivity and NIR detection. When comparing modular research fluorometers, be sure to compare data collected with the same type of PMT housing (ambient or cooled), and if cooled, then also cooled to the same temperature.

Single versus Double Monochromator: Modular research fluorometers allow a researcher to select single or double monochromators on either the excitation or the emission optical path. Here the term double monochromator refers to two dispersive grating stages, one after the other, with an entrance slit, intermediate slit, and exit slit. A double monochromator can be configured either in the additive or dispersive mode, but in either case the throughput and stray light characteristics of a single versus a double monochromator are vastly different, and will have a big impact on the SNR of a water Raman scan, even if the bandwidths, integration times, and wavelengths are all held constant.

Groove Density of Grating: The groove density of a grating will also affect the throughput, and hence sensitivity of a spectrofluorometer. For most spectrofluorometers this is not too much of an issue because the systems are manufactured with only one particular grating. In this case the most important thing is to ensure the band passes are selected to be the same. However, for modular fluorometers, you can configure the monochromators with different gratings, or multiple gratings. For these systems, you must be very careful to keep things as similar as possible. For example, when you have two instruments that have similar focal length spectrometers, changing the groove density of the grating will increase or decrease the sensitivity for the same 5 nm bandpass setting. The HORIBA method uses gratings with a grove density of 1,200 grooves per millimeter.

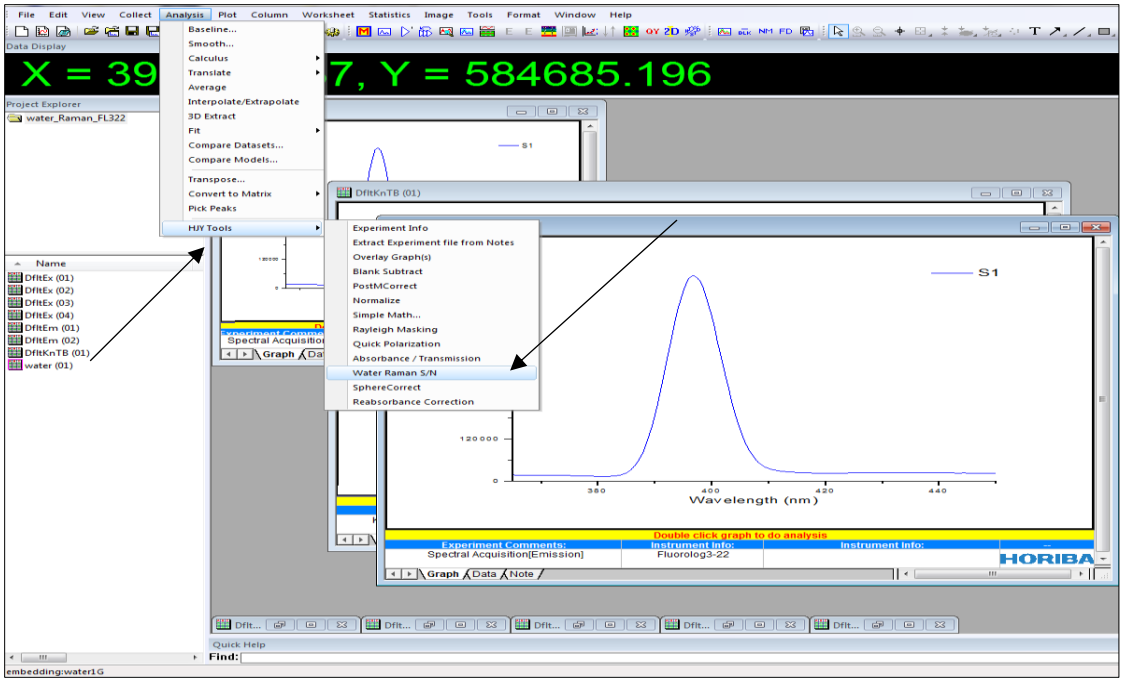
Blaze Angle of Grating: The gratings selected for an excitation or emission monochromator provide optimum throughput at a particular wavelength band, referred to as the blaze angle since this is determined by the angle of grating etching imparted on the grating surface. As such an excitation monochromator with a 350 nm blazed excitation monochromator, and a 400 nm emission monochromator, would be optimal choices to achieve the best water Raman sensitivity when exciting at 350 nm. Since most fluorometers do not allow you to adjust the grating, this variable is not a factor, but for those that do allow you to choose gratings, be sure to choose gratings with the same, or very similar, blaze angle to make a valid comparison.

3.4.5.6 SNR Calculator Tool

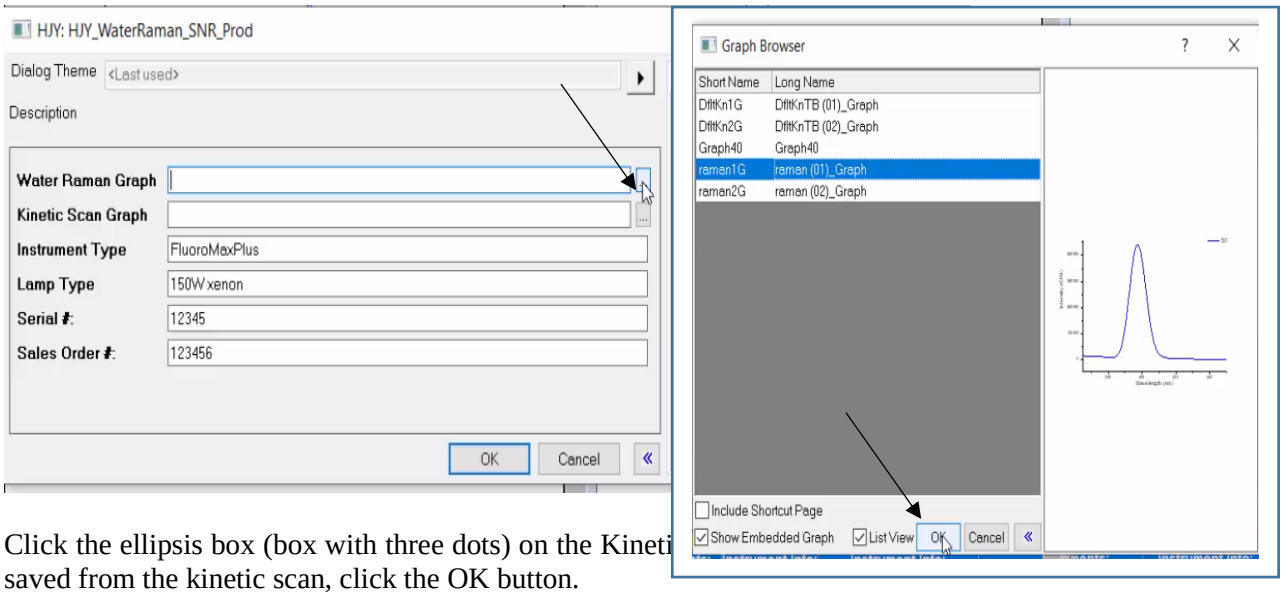
FluorEssence software is equipped with an SNR calculator capable of determining the signal to noise ratio by the First Standard Deviation (FSD) method.

The procedure for using the SNR calculator is as follows:

1. On the toolbar, click the Analysis tab and select the HJY Tools option followed by the Water Raman S/N option.

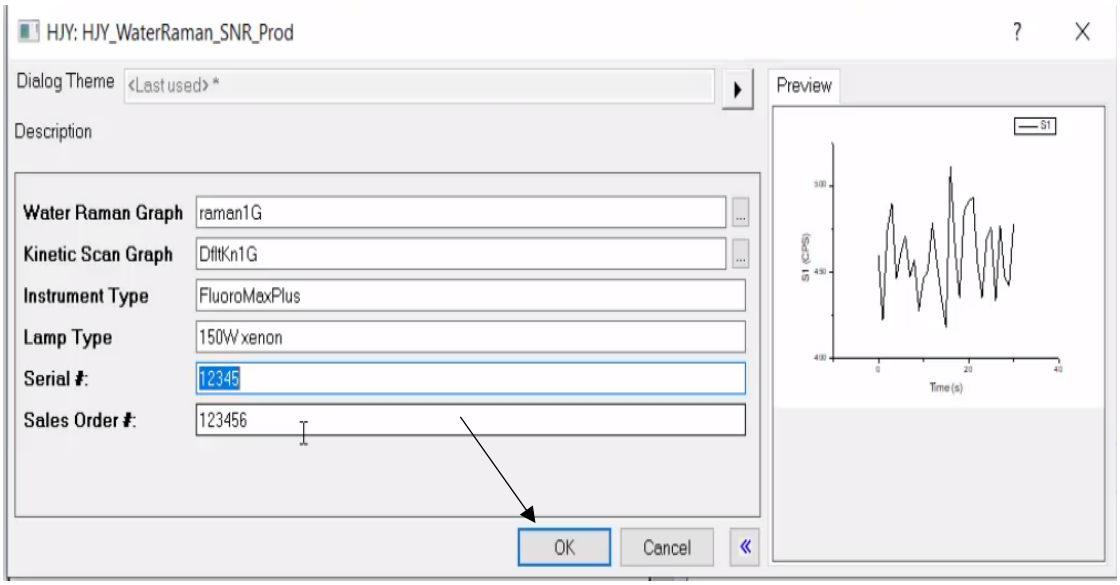


2. Click the ellipsis box (box with 3 dots) on the Water Raman Graph field. Then, select the graph saved from the water Raman test and click the OK button.

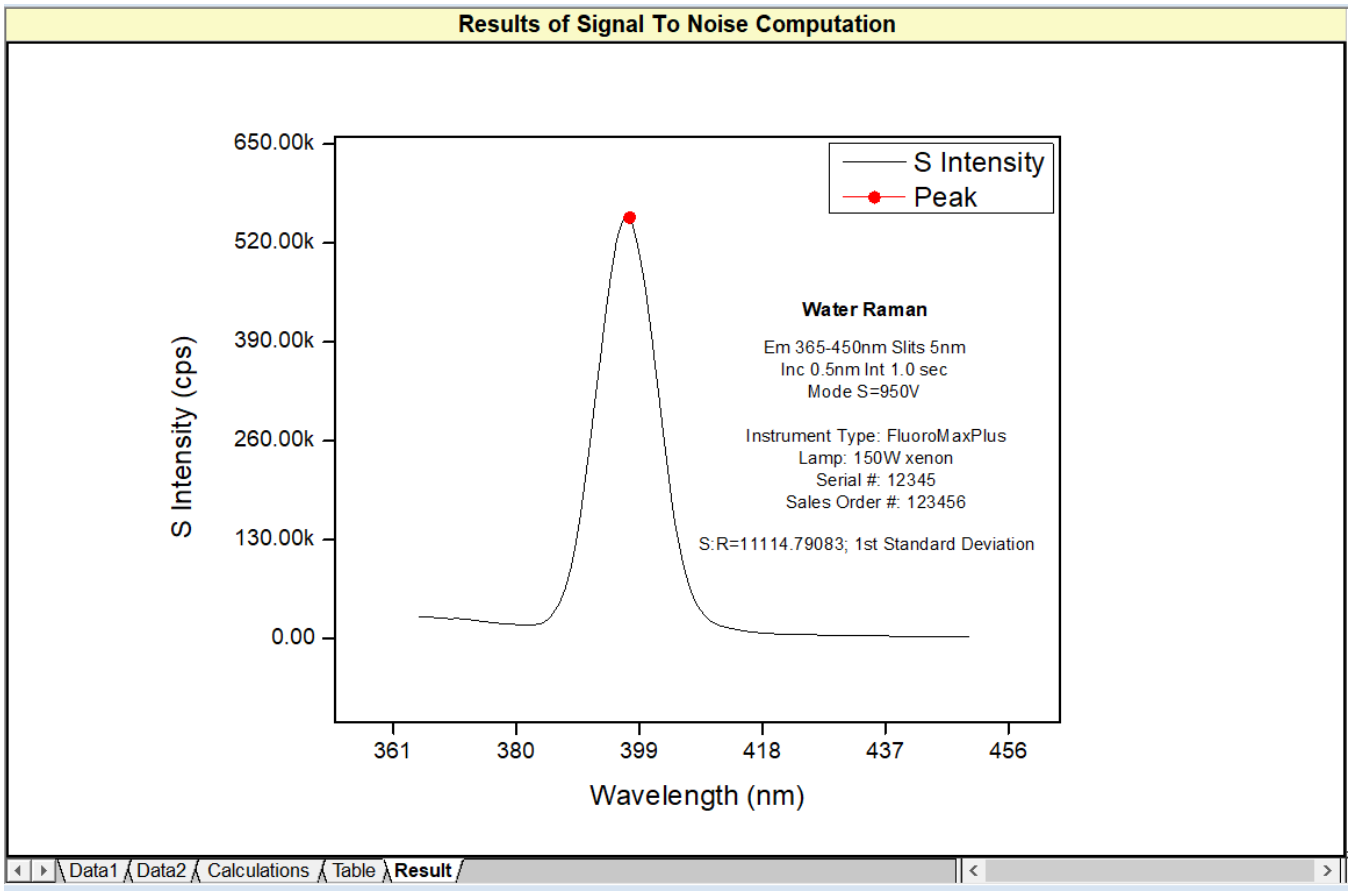


3. Click the ellipsis box (box with three dots) on the Kineti
saved from the kinetic scan, click the OK button.

4. Enter the remaining information: instrument type, lamp type, serial number, and sales order. Then, click the OK button.



5. A graph will appear containing the signal-to-noise ratio calculated by the FSD method. An example of the FluoroMax Plus is given below.



6. Check that the SNR results are within acceptable value. The signal-tonoise ratio (FSD) for the water Raman on the FluoroMax®Plus with R928P detector using this method should be above 10,000:1. For specifications on other detector types, contact local HORIBA representative.

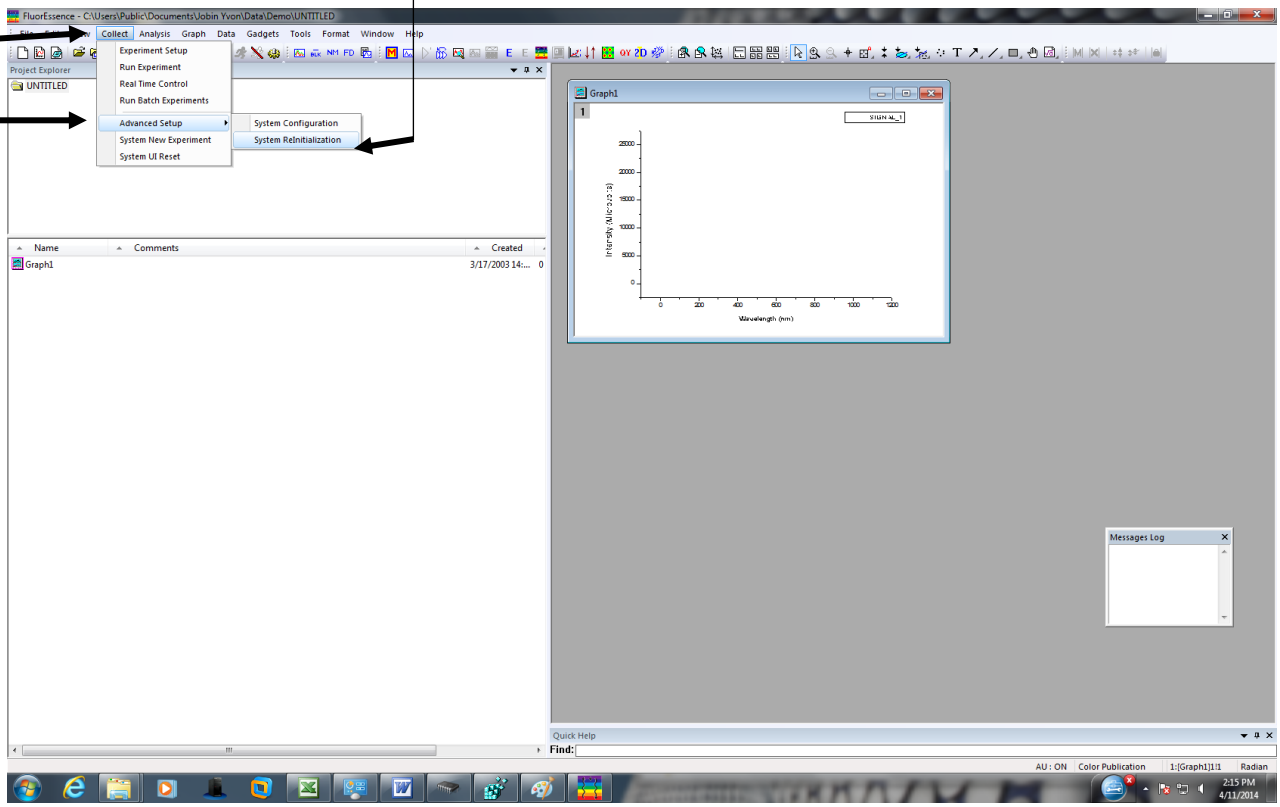
3.5 Switching to Another Detector

To use the second, external detector, you must reinitialize the system with a FluoroMax® Plus configuration.

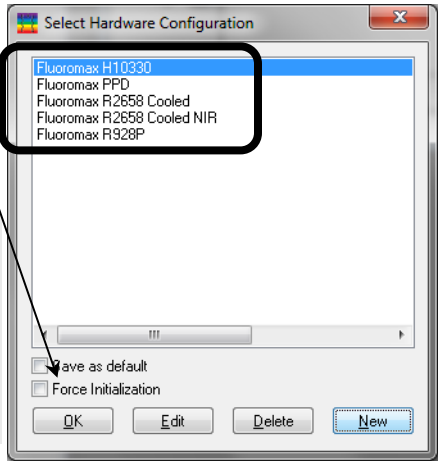
1. In the toolbar, choose the Collect tab.

   A drop-down menu appears.
2. Choose Advanced Setup.

   Another drop-down menu appears.
3. Choose System Re-Initialization.



   The Select Hardware Configuration window appears.
4. Choose the desired detector from the list, then click the OK button.

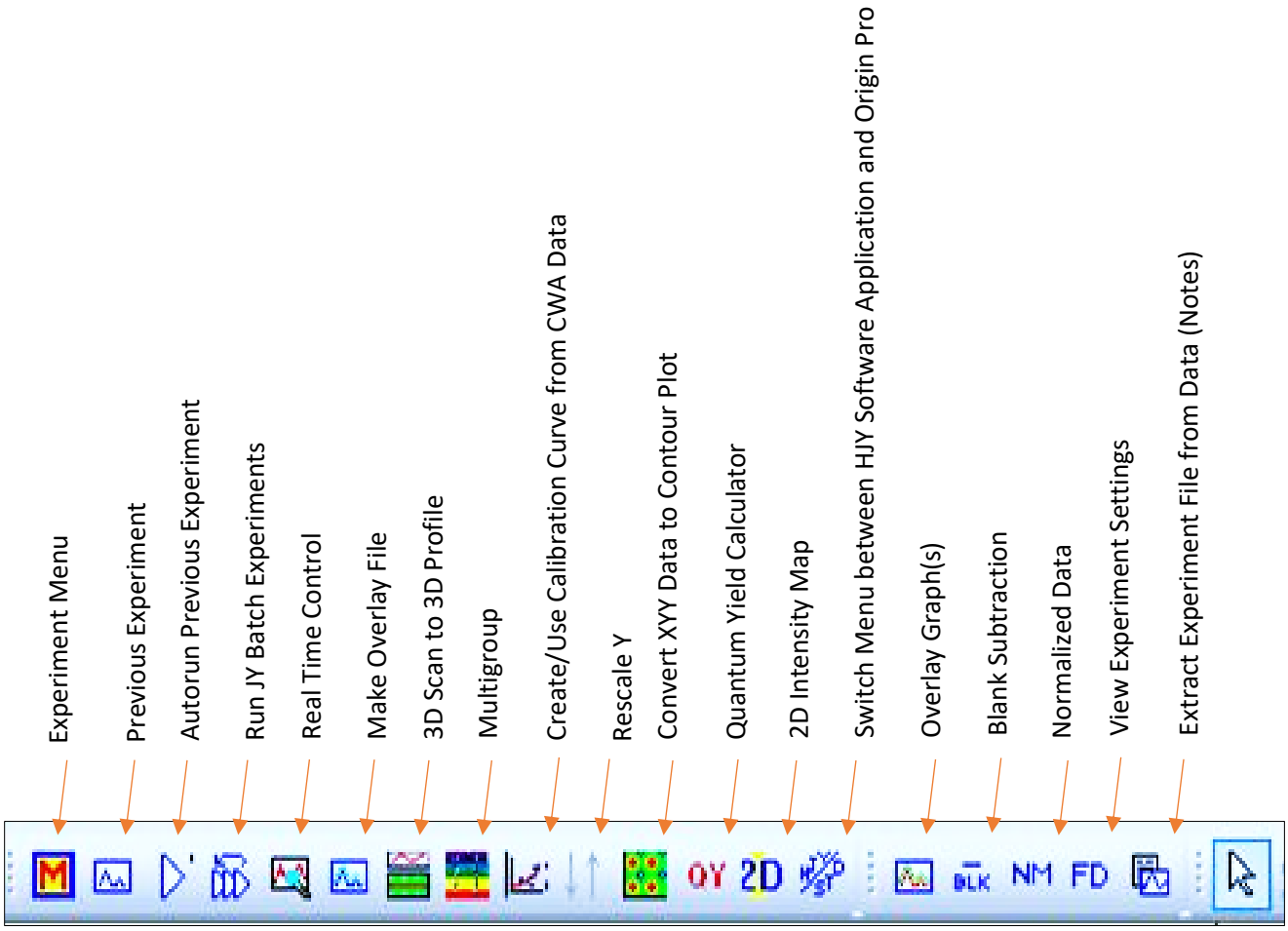


Note: The default internal detector is the R928P. Your external detector will be one of these others also listed.

4 DATA ACQUISITION

4.1 Introduction to FluorEssence™ and Multigroup

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



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

A second program, called Multigroup, offers repeated and sequential fluorescence experiments. Functions such as delays, temperature ramps, ratiometric probes such as Fura-2, and multiple samples and wavelength-groups are allowed within Multigroup. You can start Multigroup from within FluorEssence™, or from a Windows® desktop icon.

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

4.2 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® Plus use default parameters:

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

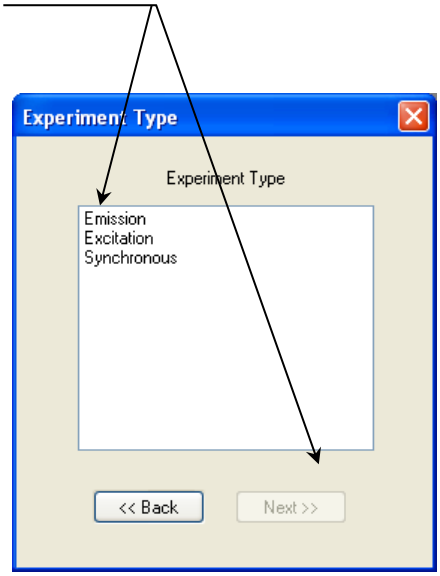
1. To choose an experiment type, click the Experiment Menu button :

   The Fluorescence Main Experiment Menu appears:
2. Choose an experiment type.

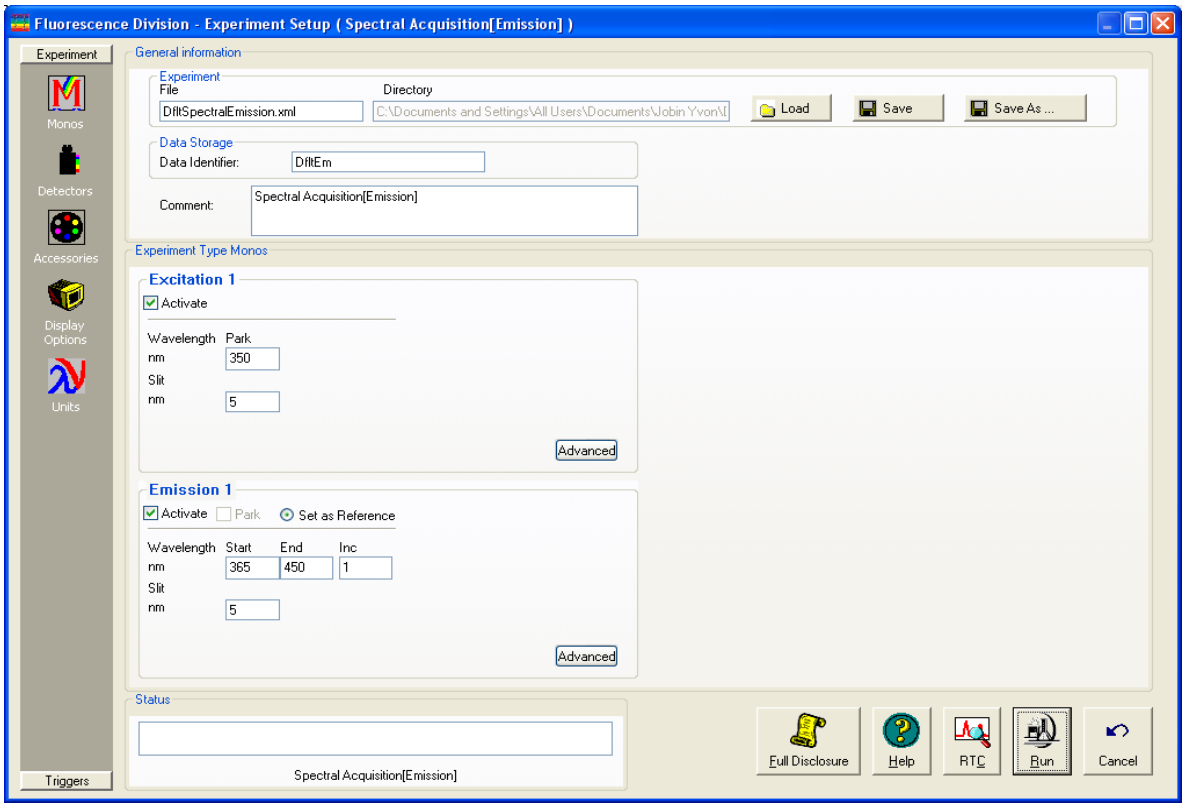
Note: Certain scan types are not allowed with the FluoroMax® Plus, and are grayed out.

3. The Experiment Type window appears.
4. Choose a subtype of experiment, and click the Next >> button.

Note: Some experiment types (for example, Kinetics) do not have subtypes, so no Experiment Type window appears.



The Experiment Setup window appears:



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.

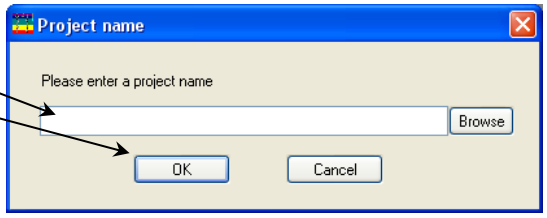
8. Click the Run button 

.

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

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

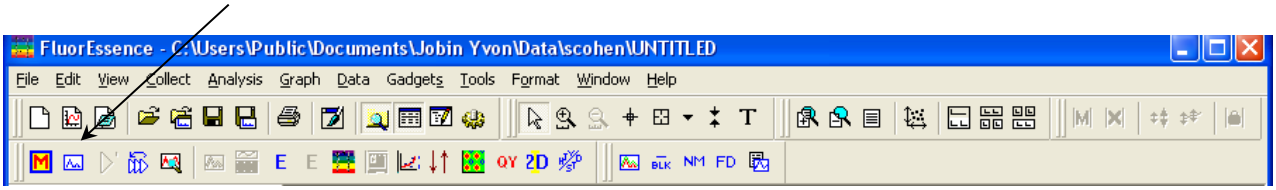
   All data are moved to Origin®'s graph window for post-processing.



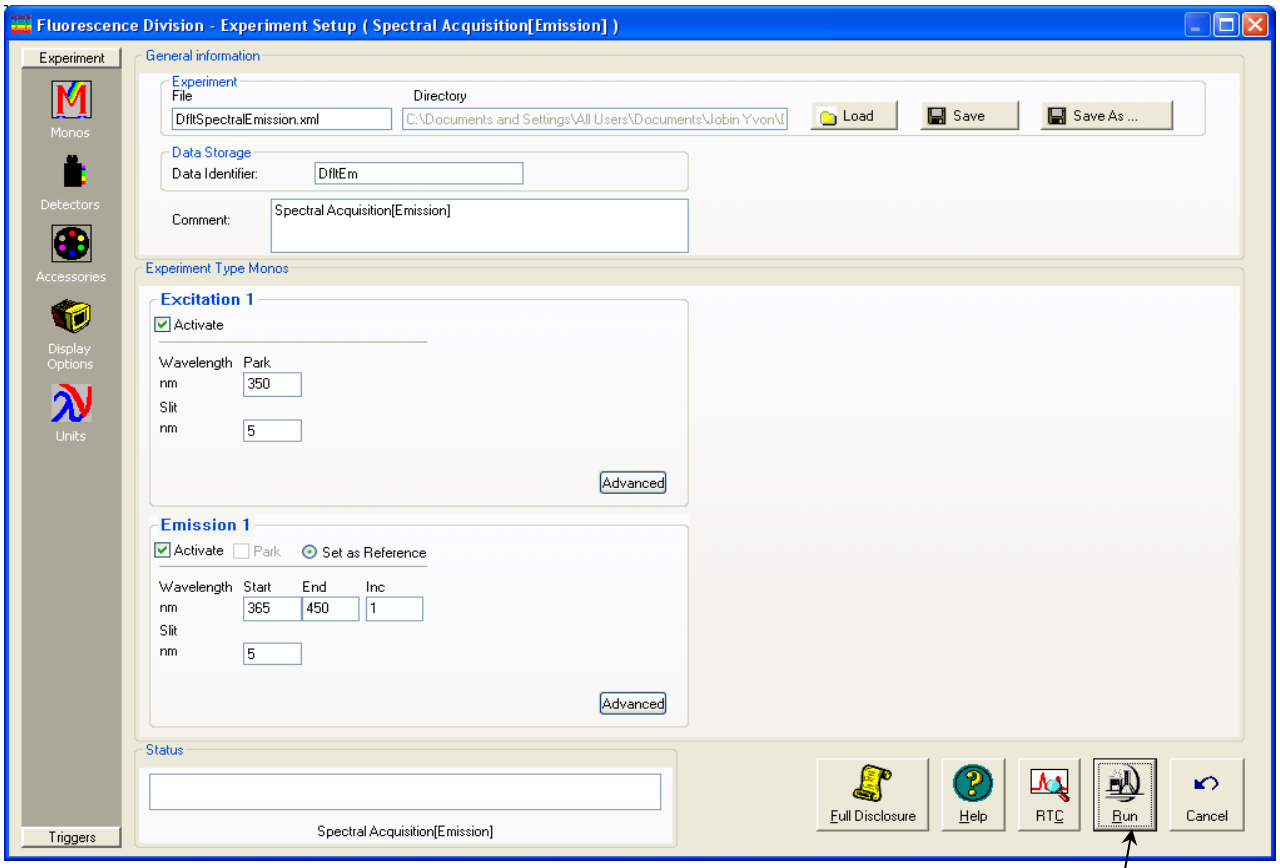
4.3 Previous Experiment Setup Button

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

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



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



2. Modify the experiment's parameters as required.

3. Click the Run button 

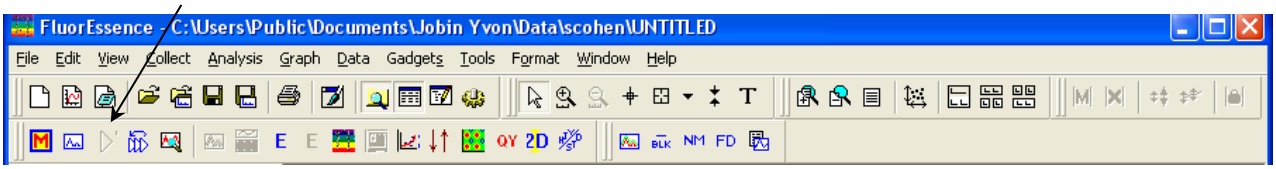
to run the experiment.

4.4 Auto Run Previous Experiment Button 

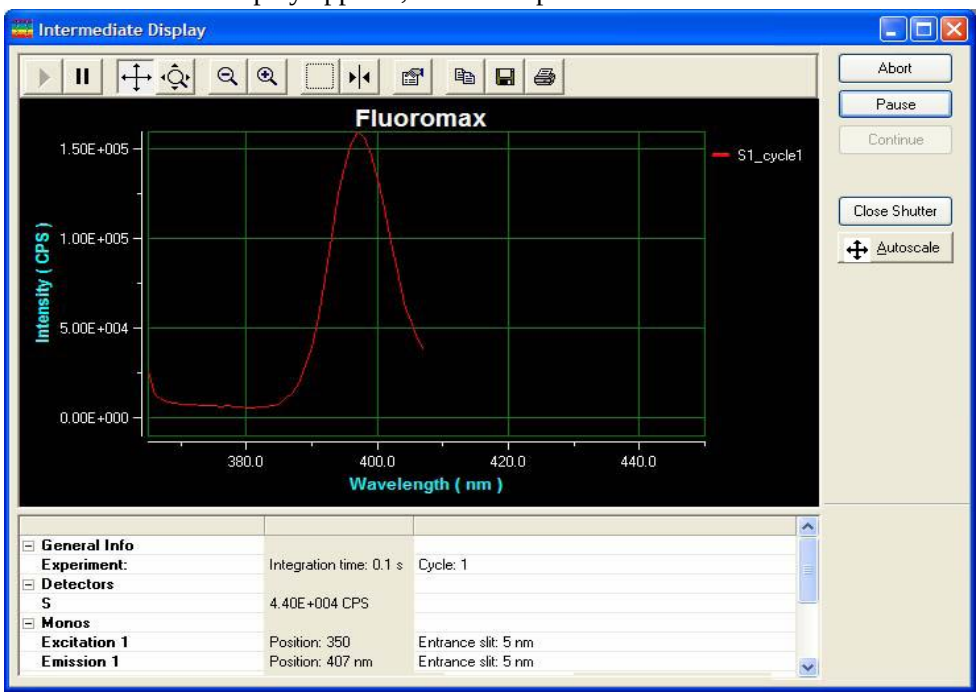
The Auto Run Previous Experiment button reruns the last experiment loaded without modifications.

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

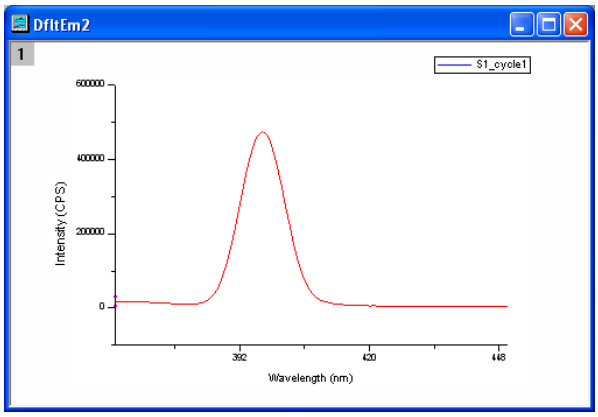
1. Click the Auto Run Previous Experiment button  .



The Intermediate Display appears, and the experiment starts:



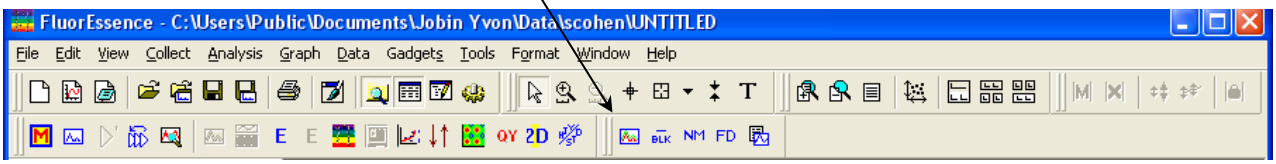
When the experiment is complete, the data are moved into a new Origin® graph window:



4.5 Make Overlay File Button 

The Make Overlay File button creates an *.SPC file for use as an overlay file. An overlay file can be used to compare data in real time. Inclusion of an overlay file is controlled in the Display Options icon in the Experiment Setup window. The overlay file appears in the Intermediate Display as data are acquired. Upon completion of the acquisition, both sets of data appear on the plot.

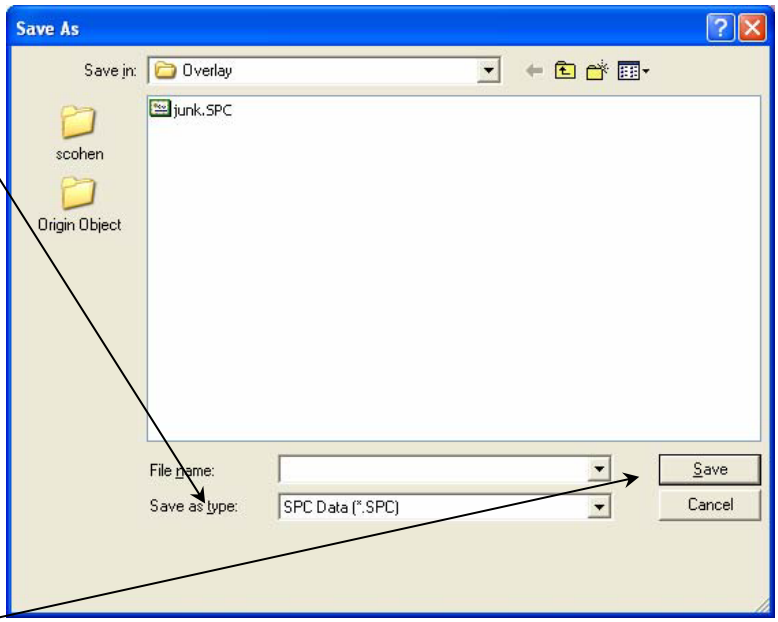
1. Click the Make Overlay File button in the toolbar



   The Save As window appears.

2. Enter a file name in the File Name field
3. Click the Save button.

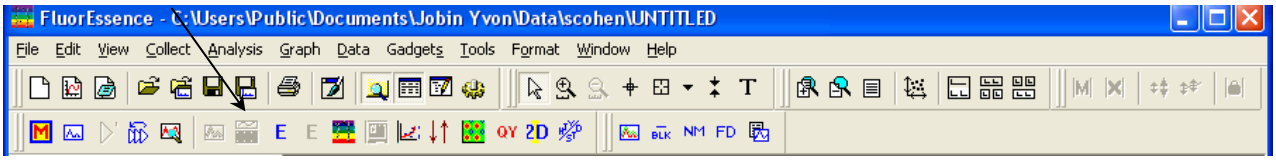
   The overlay file is saved.

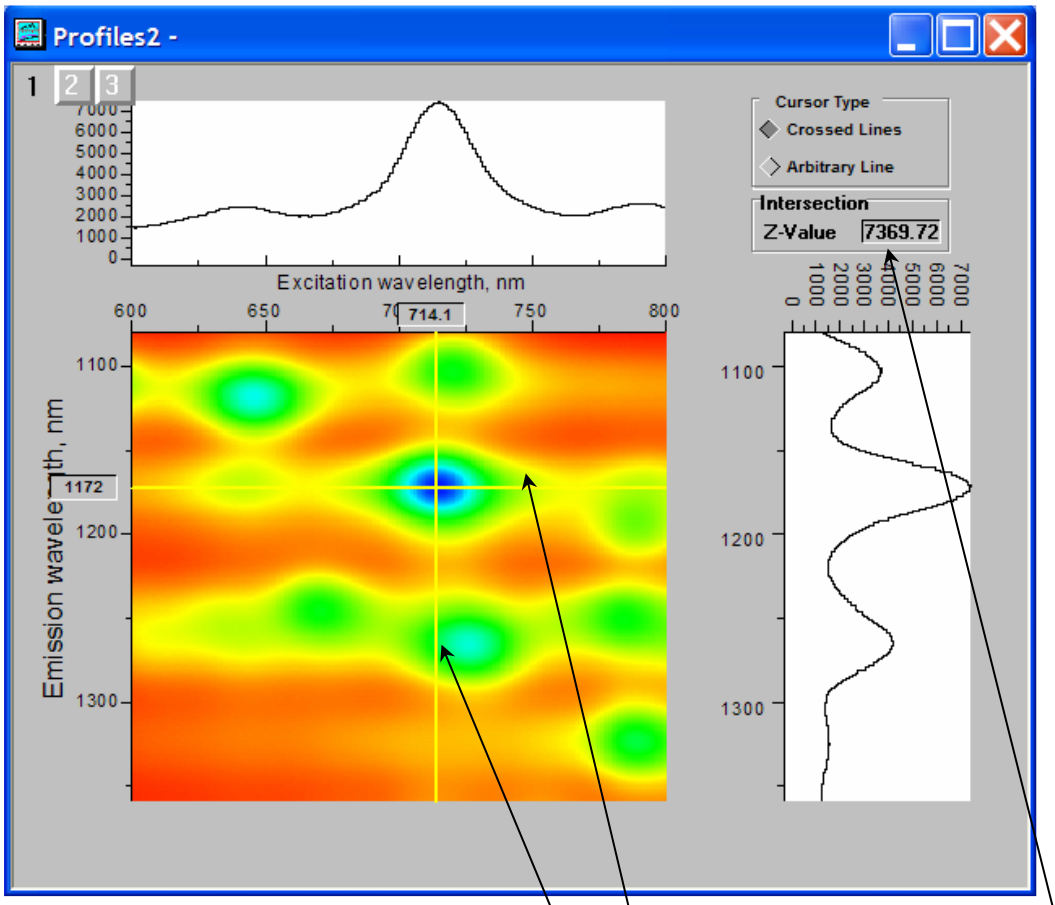


4.6 3D Scan to 3D Profile Button

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

1. Open excitation-emission matrix data.
2. Click the 3D Scan to 3D Profile button in the toolbar.



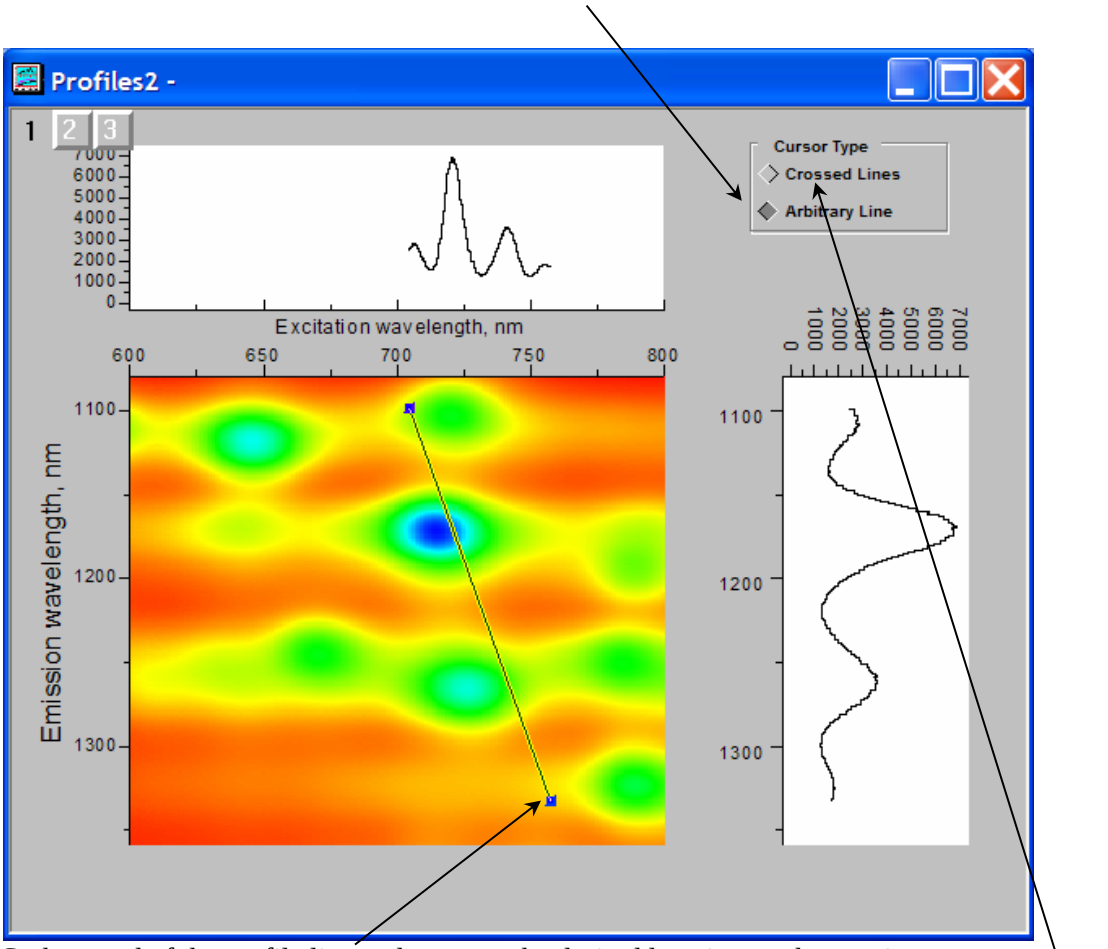


The Profiles window appears.

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.



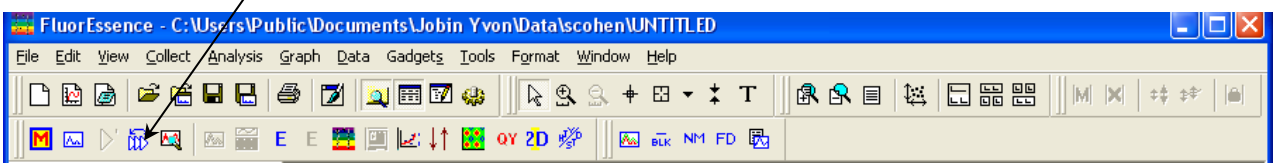
Grab an end of the profile line and move to the desired location on the matrix. The profiles are updated.

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

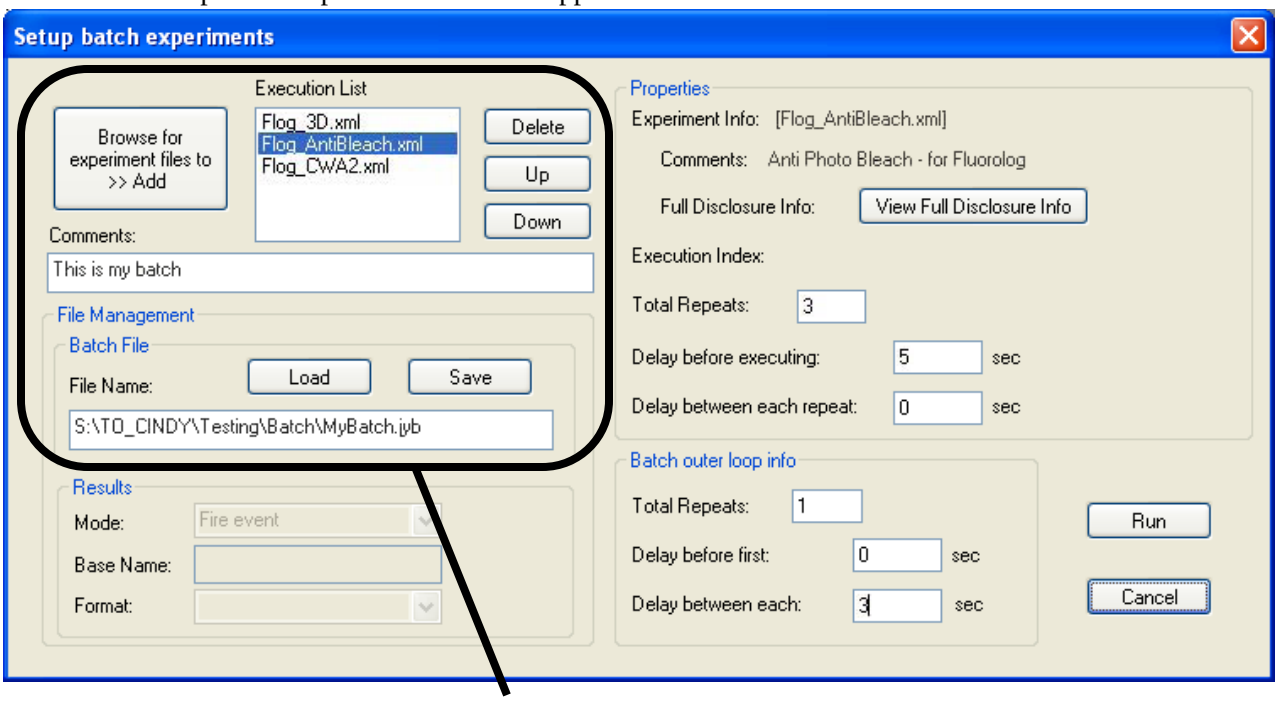
4.7 Run JY Batch Experiments 

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

1. Click the Run JY Batch Experiments button  .



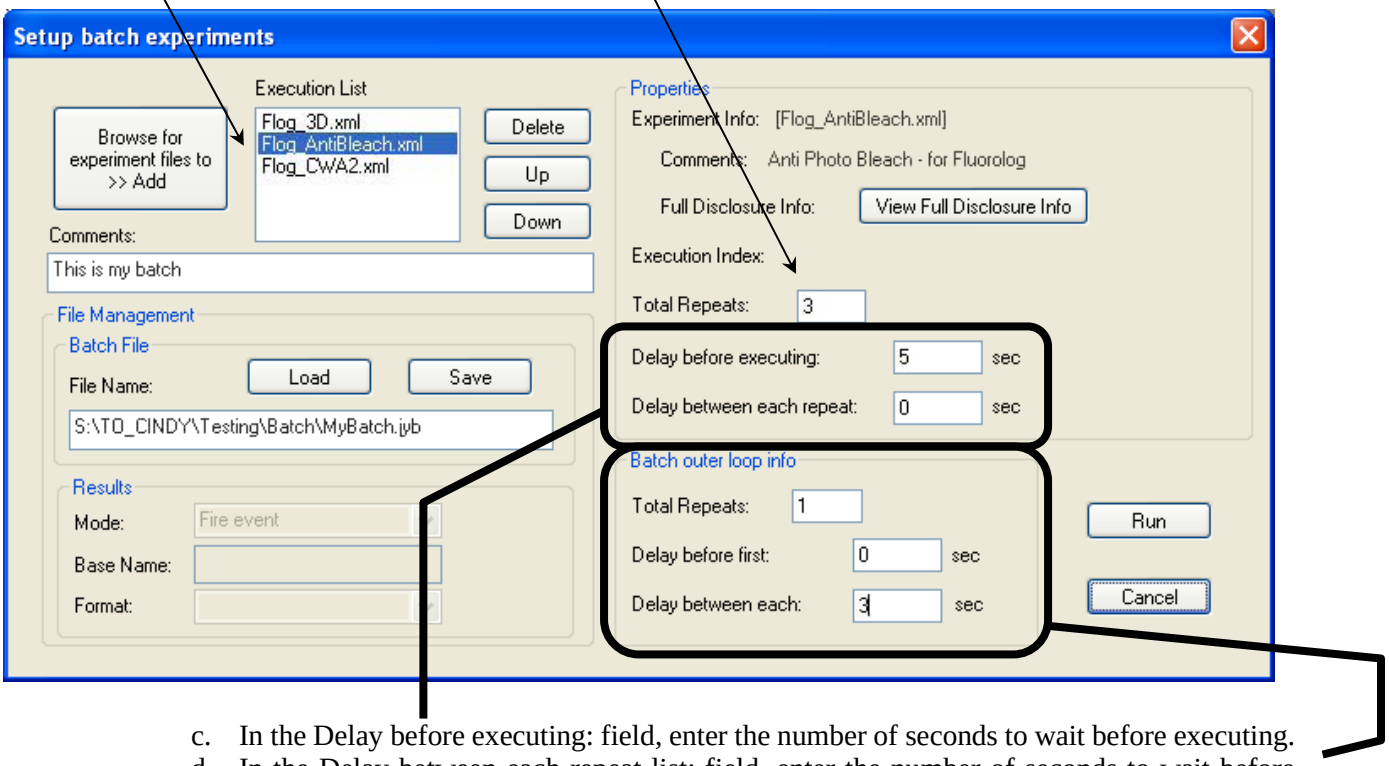
The Setup batch experiments window appears.



2. Get the experiment files to create a batch job, or load a previous batch job.
    a. Load a previously created batch job using the Load button, or browse for experiment files (.xml format) using the Browse for experiment files to >> Add button.
    b. Add each desired experiment file to the Execution List
    c. Reorder or remove the files as necessary using the Delete button, the Up button, and the Down button.
    d. Add comments about the batch file in the Comments: field.
    e. Save the new batch job in the correct path, in the File Name: field, and click the Save button. The file is saved in a .jyb format.

3. Set up each experiment in the batch job.

   a. Select an experiment from the Execution List.
   b. In the Total Repeats: field, enter the number of times that experiment should be repeated.



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

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

   a. In the Total Repeats: field, enter the number of times to run the batch job.
   b. In the Delay before first: field, enter the number of seconds to wait before starting the batch job.
   c. In the Delay between each: field, enter the number of seconds to wait before rerunning the batch job.

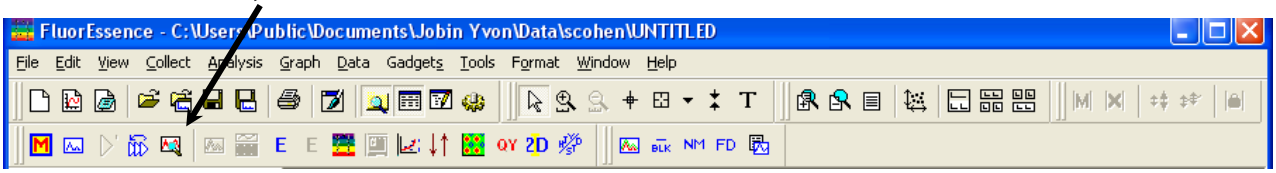
5. Click the Run button to start the batch job.

   The batch job executes.

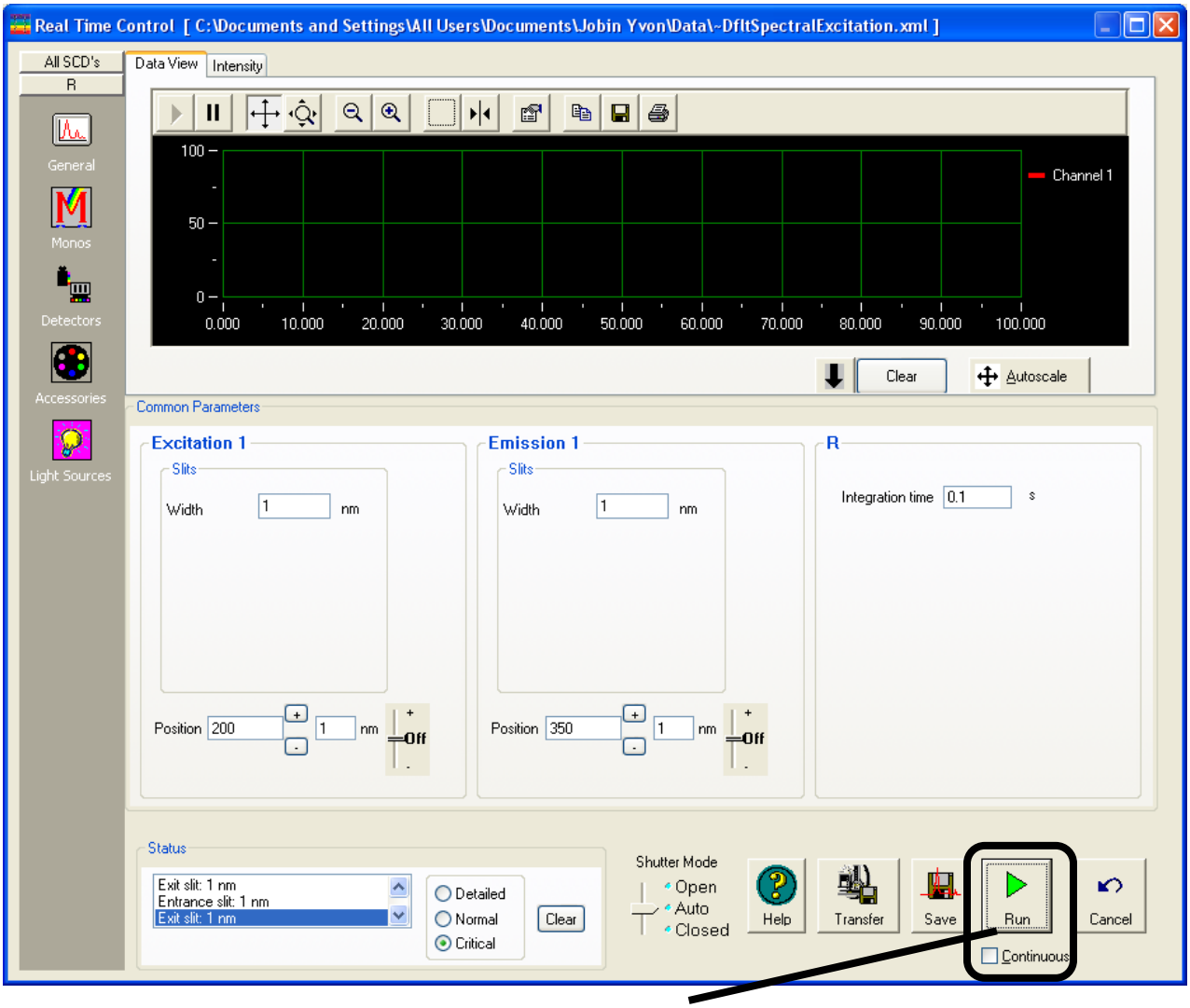
4.8 Real Time Control 

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

1. In the toolbar, click the Real Time Control button  .



The Real Time Control window appears:



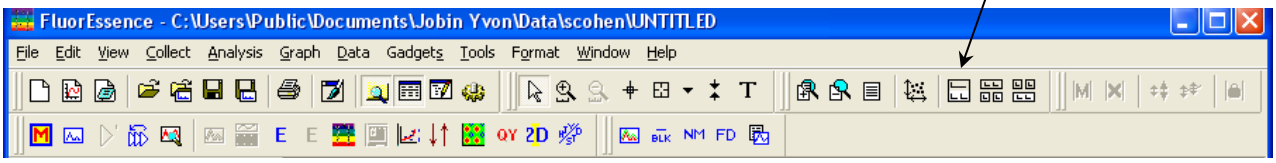
2. Activate the Continuous checkbox, then click the Run button, to monitor the signal.
3. Adjust instrumental parameters as necessary.

4.9 Create/Use Calibration Curve from CWA Data

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

Note: This button only operates if CWA data exist.

1. Click the Experiment Menu button :

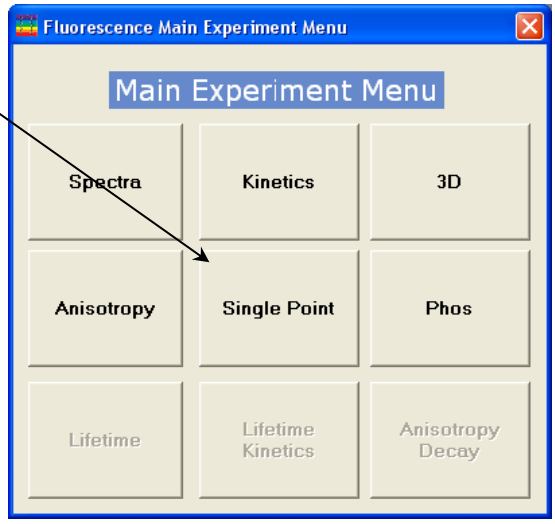


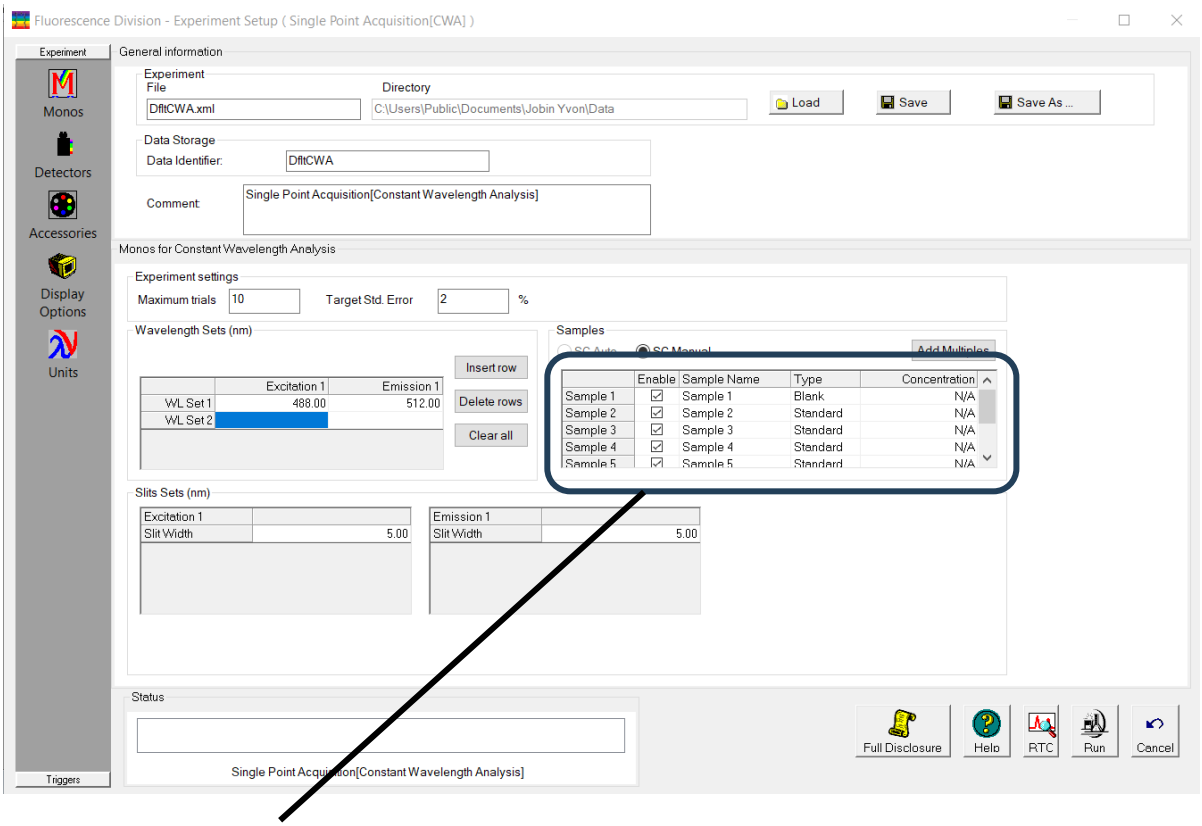
The Fluorescence Main Experiment Menu appears:

Note: Those scan types not allowed with the instrument configuration are grayed out.

2. Choose the Single Point button.

Experiment Setup window appears:





3. Set up the standards and unknown(s). For the standard curve tool to work correctly, one sample must be set to blank.

    a. If you have an automatic sample changer, you can change the samples automatically with the **SC Auto** radio button. For manual use with an automatic sample changer, choose **SC Manual** radio button, and the software prompts you to change the sample.

This allows more than the default number of samples in the sample changer (i.e., more than 2 for the dual sample-changer or more than 4 for the four-sample-changer).

    b. To add more samples (if manually changed), click the **Add Multiples** button.

A new sample row appears below the last sample row.

    c. Under the Type column, choose the type of sample from the drop-down menu: Standard, Unknown, Blank, or Empty.

Note: Note: To do a calibration curve, you must include a blank as the first sample.

    d. Enter the concentrations of the standards in the Concentration column.The unknowns retain the default "N/A" concentration.
    e. Click the **Enable** checkbox of all the samples to be measured.

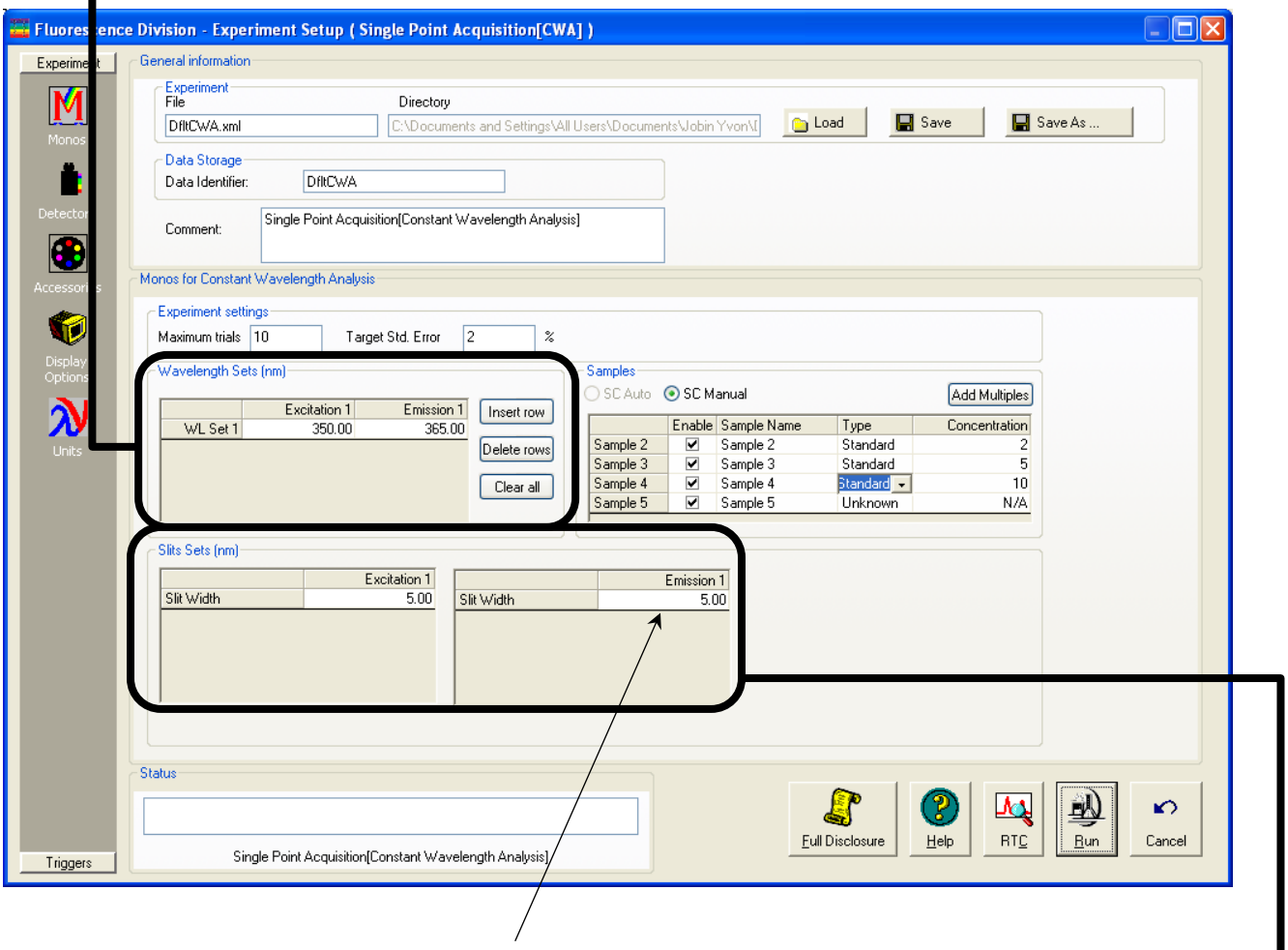
4. Set up the excitation and emission wavelength(s)

   a. Enter the appropriate excitation and emission wavelength set(s), one per row, in the Wavelength Sets area.

   b. To add a wavelength set, click the Insert row button.

   c. To delete a wavelength set, select the rows, and click the Delete rows button.

   d. To clear all wavelength sets, click the Clear all button.



5. Enter the excitation and emission slit-width(s) in the Slit Set area

6. Click the Run button 

.

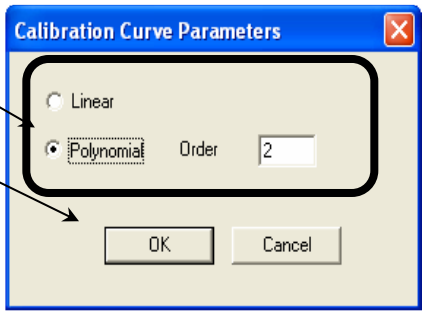
   The experiment starts. FluorEssence™ prompts you to insert the sample if you chose to change the samples manually. The dataset is recorded and appears on the screen.

7. Create the calibration curve.

   a. Click the Create/Use Calibration Curve from CWA Data button  in the toolbar.

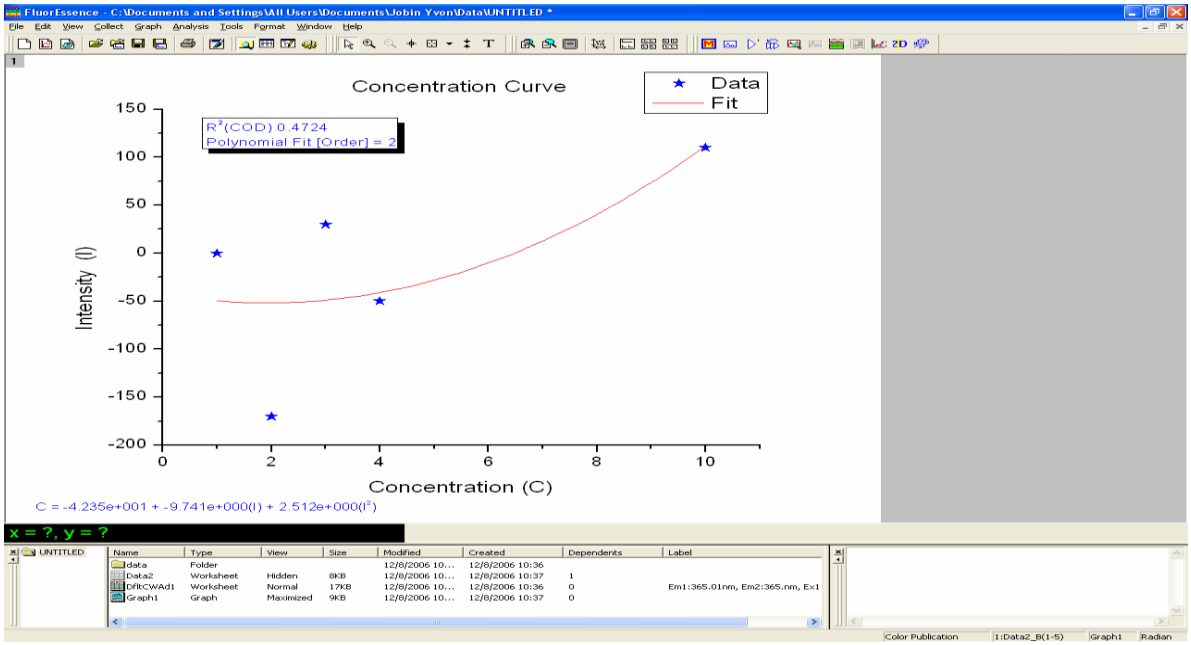
The Calibration Curve Parameters window appears.

   b. Choose a Linear fit, or a Polynomial fit with Order of the polynomial, and then the OK button.



The Save Curve window asks if you wish to save the curve.

   c. Answer yes or no.The calibration curve appears.



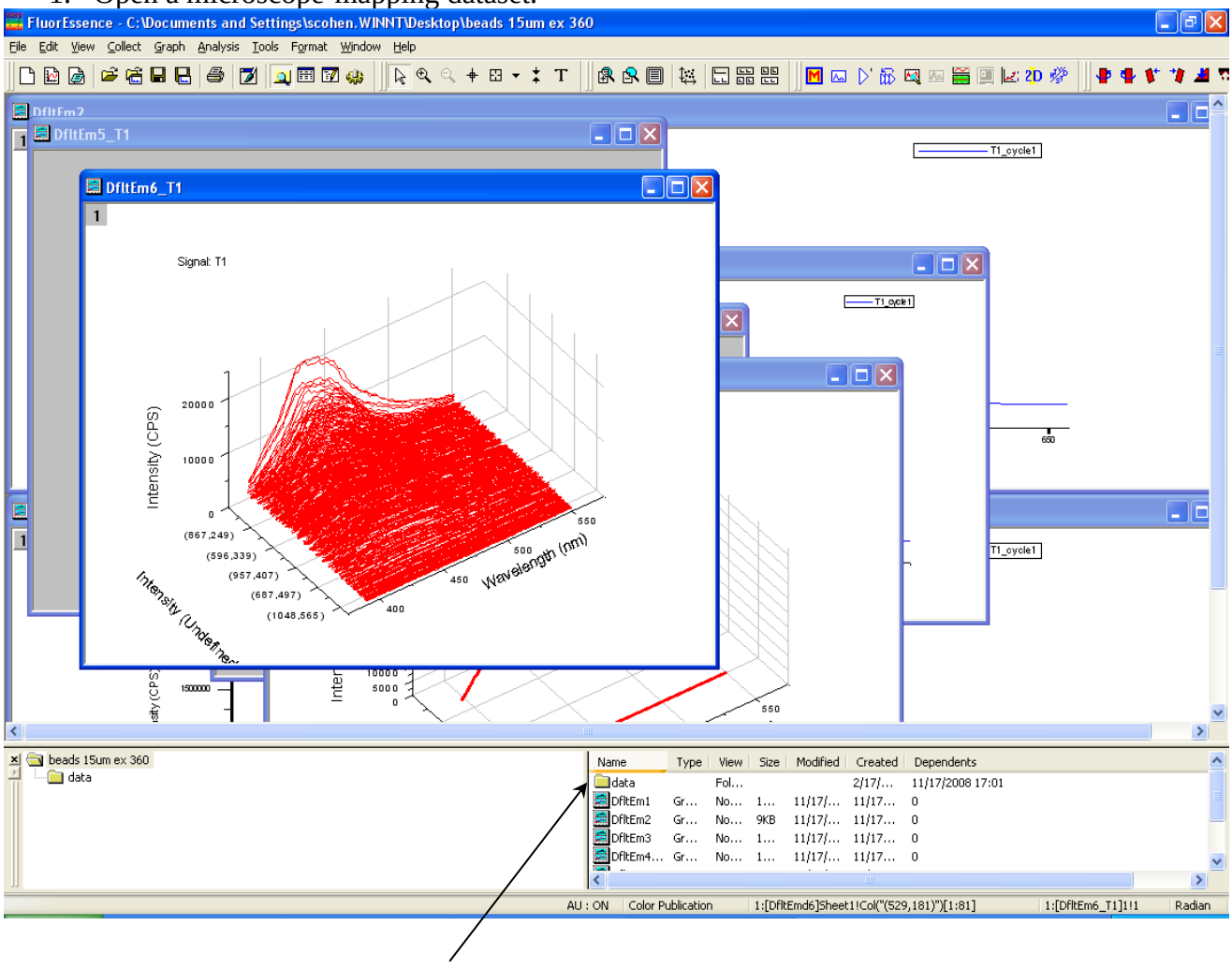
Stars indicate the standards. The unknowns are not plotted, but their calculated concentrations are filled in on the dataset. The fit equation and correlation are displayed on the plot. With more than one detector signal (e.g., S1 or S1c), the software prompts you to choose which signal.

4.10 2D Intensity Map

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

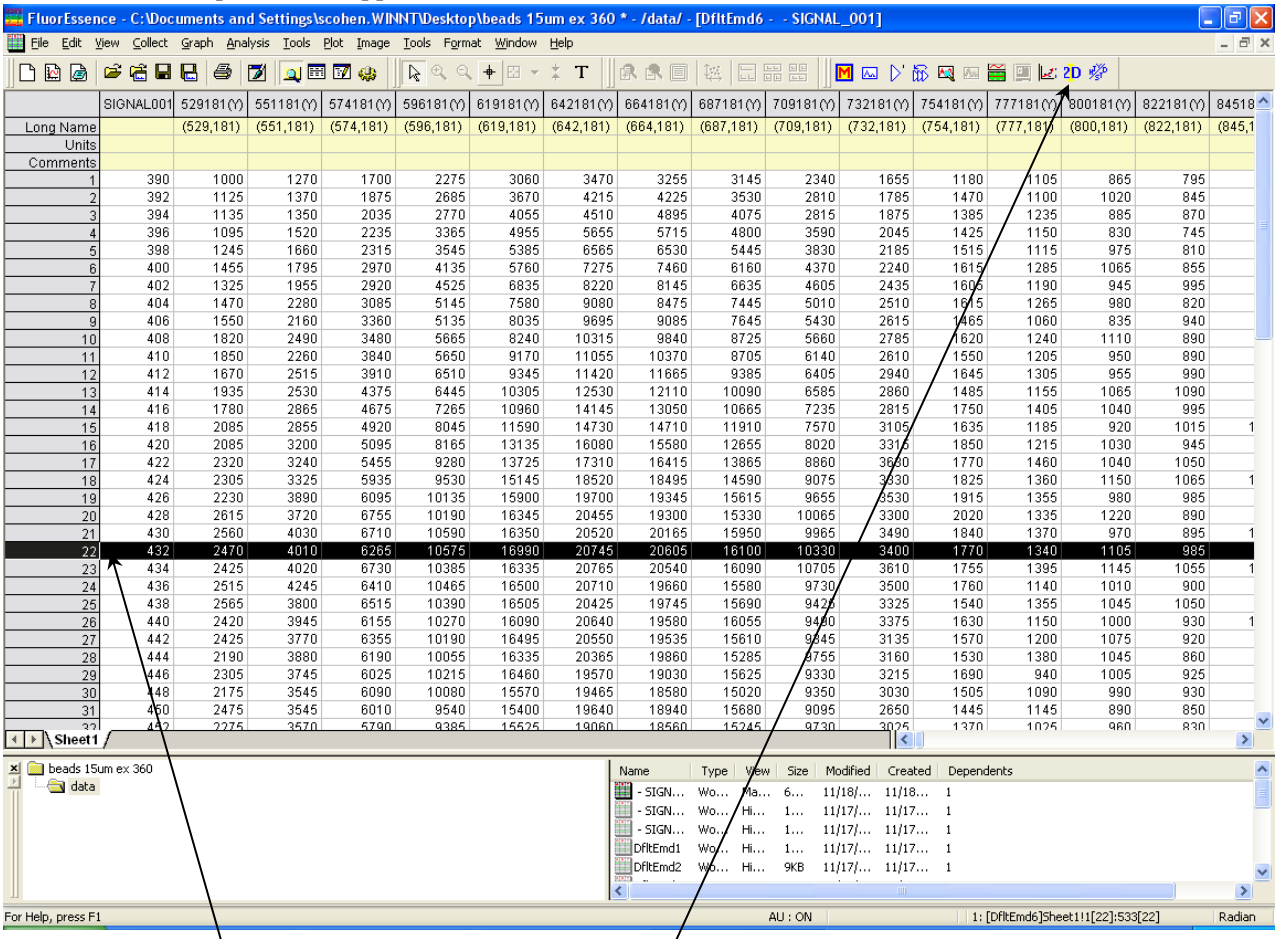
Note: This button only operates if data are displayed, and is used for microscope mapping purposes.

1. Open a microscope-mapping dataset.



2. Click the data folder in bottom area.

The data spreadsheet appears:



	SIGNAL001	529181(Y)	551181(Y)	574181(Y)	596181(Y)	619181(Y)	642181(Y)	664181(Y)	687181(Y)	709181(Y)	732181(Y)	754181(Y)	777181(Y)	800181(Y)	822181(Y)	84518
Long Name		(529,181)	(551,181)	(574,181)	(596,181)	(619,181)	(642,181)	(664,181)	(687,181)	(709,181)	(732,181)	(754,181)	(777,181)	(800,181)	(822,181)	(845,1
Units																
Comments																
1	390	1000	1270	1700	2275	3060	3470	3255	3145	2340	1655	1180	1105	865	795	
2	392	1125	1370	1875	2685	3670	4215	4225	3530	2810	1785	1470	1100	1020	845	
3	394	1135	1350	2035	2770	4055	4510	4895	4075	2815	1875	1385	1235	885	870	
4	396	1095	1520	2235	3365	4955	5655	5715	4800	3590	2045	1425	1150	830	745	
5	398	1245	1660	2315	3545	5385	6565	6530	5445	3830	2185	1515	1115	975	810	
6	400	1455	1795	2970	4135	5760	7275	7460	6160	4370	2240	1615	1285	1065	855	
7	402	1325	1955	2920	4525	6835	8220	8145	6635	4605	2435	1605	1190	945	995	
8	404	1470	2280	3085	5145	7580	9080	8475	7445	5010	2510	1615	1265	980	820	
9	406	1550	2160	3360	5135	8035	9695	9085	7645	5430	2615	1465	1060	835	940	
10	408	1820	2490	3480	5665	8240	10315	9840	8725	5660	2785	1620	1240	1110	890	
11	410	1850	2260	3840	5650	9170	11055	10370	8705	6140	2610	1550	1205	950	890	
12	412	1670	2515	3910	6510	9345	11420	11665	9385	6405	2940	1645	1305	955	990	
13	414	1935	2530	4375	6445	10305	12530	12110	10090	6585	2860	1485	1155	1065	1090	
14	416	1780	2865	4675	7265	10960	14145	13050	10665	7235	2815	1750	1405	1040	995	
15	418	2085	2855	4920	8045	11590	14730	14710	11910	7570	3105	1635	1185	920	1015	1
16	420	2085	3200	5095	8165	13135	16080	15580	12655	8020	3315	1850	1215	1030	945	
17	422	2320	3240	5455	9280	13725	17310	16415	13865	8860	3690	1770	1460	1040	1050	
18	424	2305	3325	5935	9530	15145	18520	18495	14590	9075	3930	1825	1360	1150	1065	1
19	426	2230	3890	6095	10135	15900	19700	19345	15615	9655	3530	1915	1355	980	985	
20	428	2615	3720	6755	10190	16345	20455	19300	15330	10065	3300	2020	1335	1220	890	
21	430	2560	4030	6710	10590	16350	20520	20165	15950	9965	3490	1840	1370	970	895	1
22	432	2470	4010	6265	10575	16990	20745	20605	16100	10330	3400	1770	1340	1105	985	
23	434	2425	4020	6730	10385	16335	20765	20540	16090	10705	3610	1755	1395	1145	1055	1
24	436	2515	4245	6410	10465	16500	20710	19660	15580	9730	3500	1760	1140	1010	900	
25	438	2565	3800	6515	10390	16505	20425	19745	15690	9425	3325	1540	1355	1045	1050	
26	440	2420	3945	6155	10270	16090	20640	19580	16055	9480	3375	1630	1150	1000	930	1
27	442	2425	3770	6355	10190	16495	20550	19535	15610	9845	3135	1570	1200	1075	920	
28	444	2190	3880	6190	10055	16335	20365	19860	15285	9755	3160	1530	1380	1045	860	
29	446	2305	3745	6025	10215	16460	19570	19030	15625	9330	3215	1690	940	1005	925	
30	448	2175	3545	6090	10080	15570	19465	18580	15020	9350	3030	1505	1090	990	930	
31	450	2475	3545	6010	9540	15400	19640	18940	15680	9095	2650	1445	1145	890	850	
32	452	2275	3570	5790	9385	15525	19060	18560	15245	9730	3025	1370	1025	960	830	

3. Choose an excitation wavelength (row) to examine.

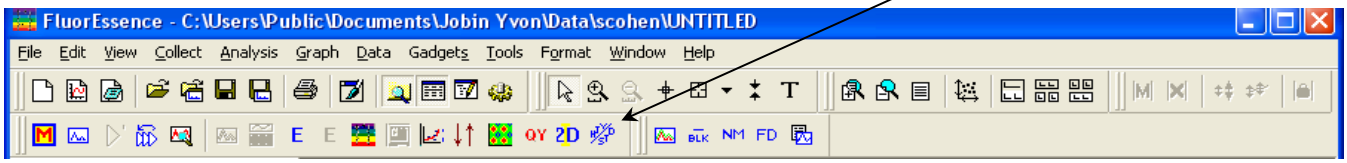
   Here we have chosen 432 nm excitation.

4. Click the 2D Intensity Map button  .

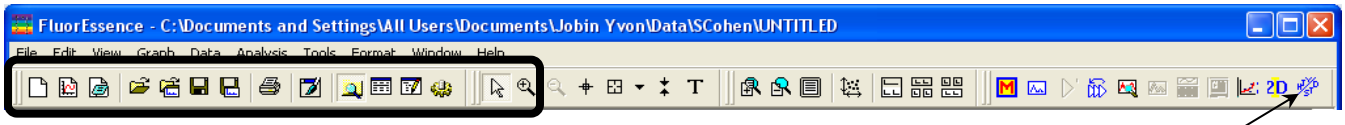
4.11 Switch Menu between HJY Software Application and Origin Pro

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

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



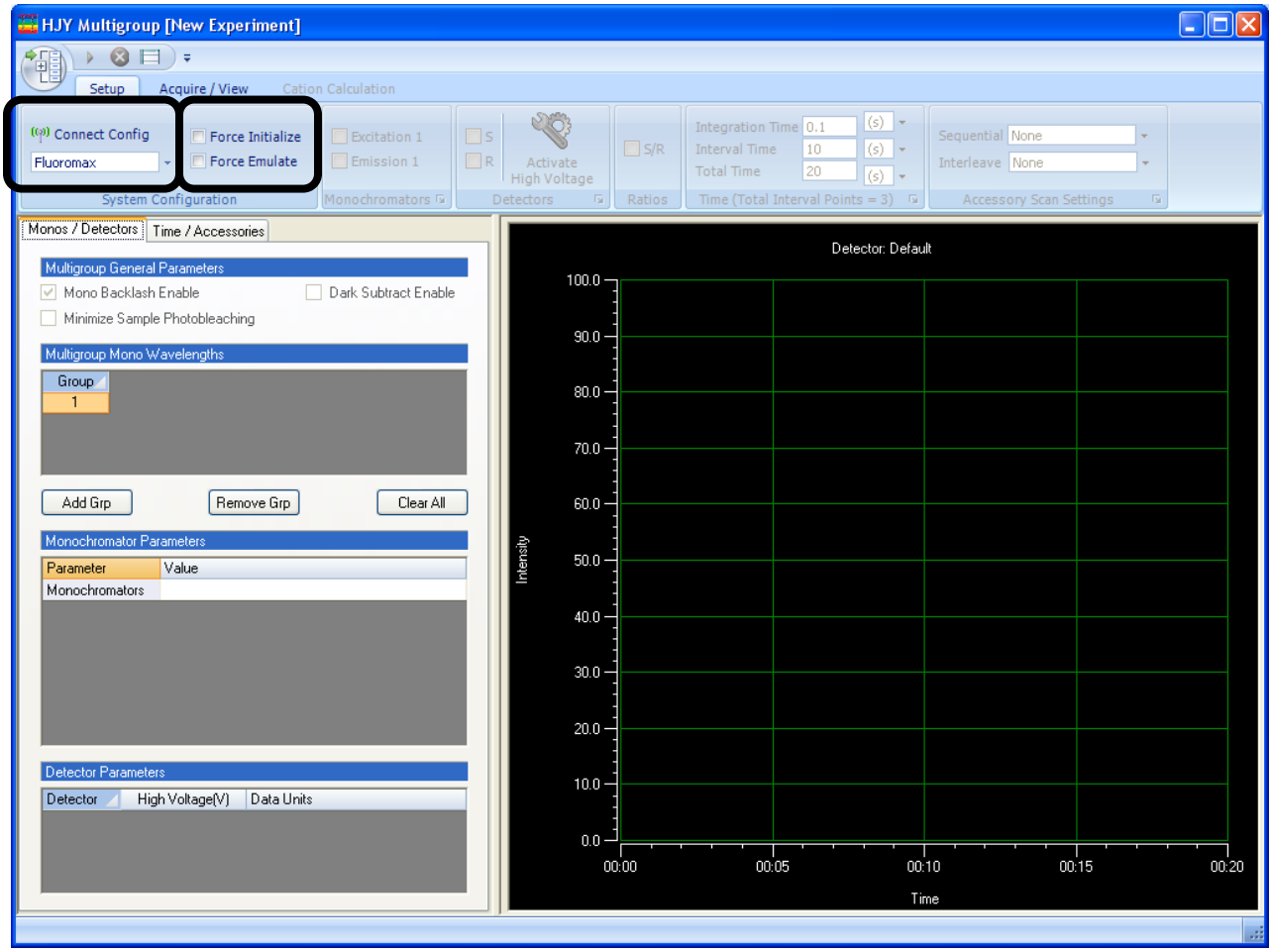
The menus at the top of the FluorEssence window change:



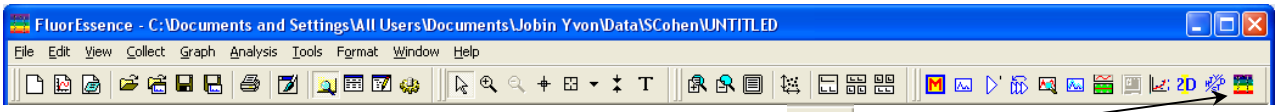
1. Click the Switch menu between HJY Software Application and Origin Pro button  again to return to the original menu functions.

4.12 Multigroup

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



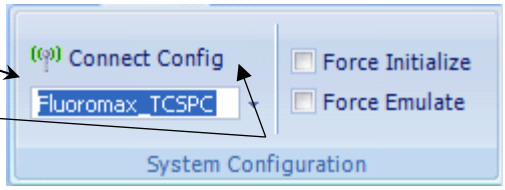
Basic Steps to Run Multigroup



1. In the FluorEssence™ toolbar, click the Multigroup button  ; or on the desktop, click the Multigroup icon.

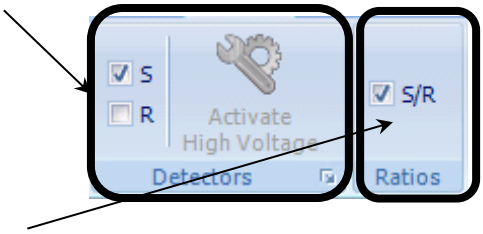
   If FluorEssence™ was active, it closes. The Multigroup window appears.

2. Connect to the desired instrument configuration.
    a. In the System Configuration area, select the configuration from the drop-down menu.
    b. Click the Connect Config button to initialize the instrument.



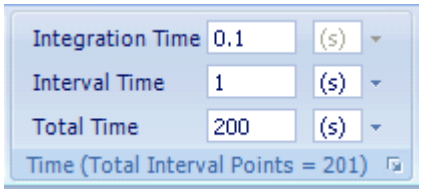
3. Activate the excitation and emission monochromators' checkboxes.

4. Activate the detector checkbox(es) and switch on high voltage if necessary, in the Detectors area.

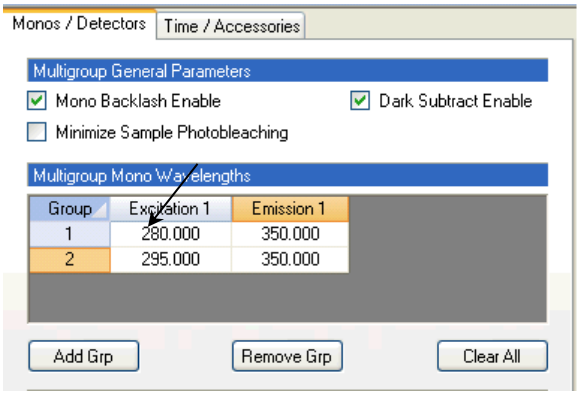


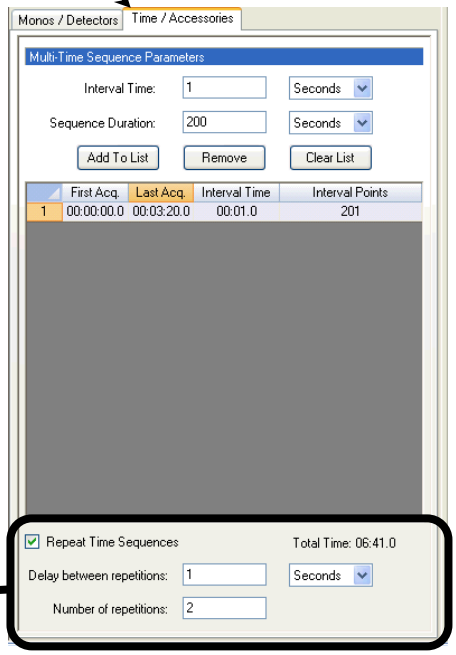
5. If desired, in the Ratios area, activate the ratio S/R checkbox to obtain corrected output.
6. In the Time area, choose various wavelength-groups and integration time.

    a. For multiple integration times, use the Time/ Accessories tab to add multiple time intervals.



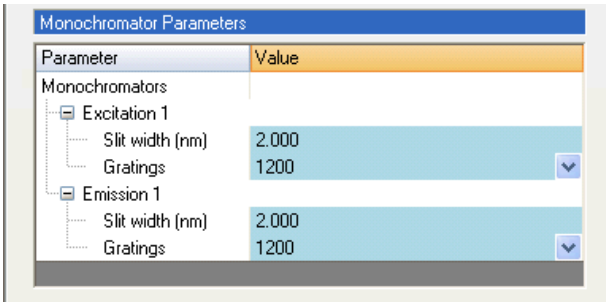
    b. Choose the wavelength pair or multiple wavelength sets in the Monos / Detectors tab.



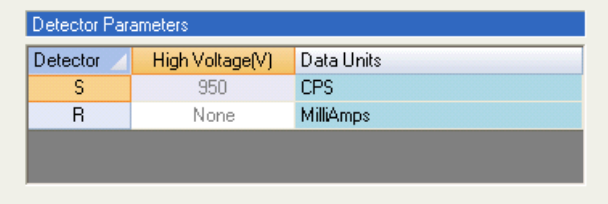


7. Select the accessories, looping, and delays.

8. Choose the slit-width parameters

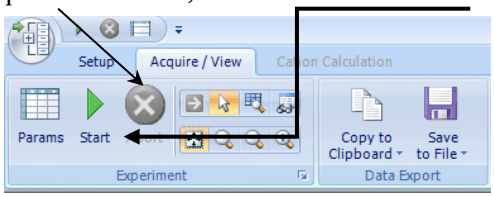


9. Choose the detector parameters



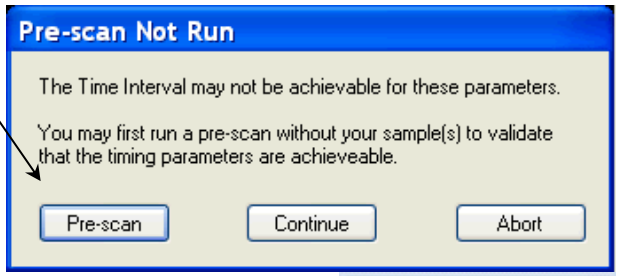
10. In the Acquire / View tab, click the Start button 

to run the experiment.



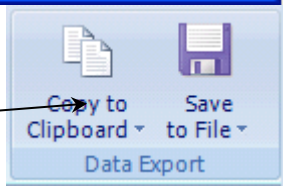
11. Run a "pre-scan" to make sure that the timing of the experiment can be achieved within the hardware limits.

    Click the Pre-scan button, and remove any samples from the sample chamber.



12. Save the data when the scan is finished.

    In the Data Export area, choose either the Copy to Clipboard button or the Save to File button. More details on Multigroup are provided in the *FluorEssence™ User's Guide*.



4.13 Running an Unknown Sample

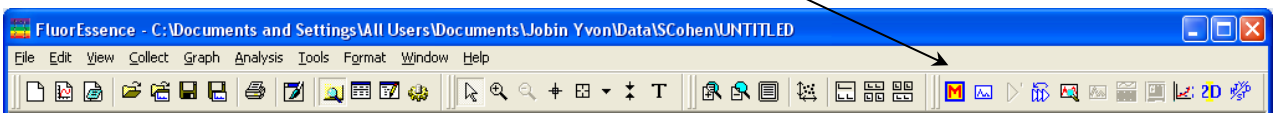
Often a researcher will scan a sample whose spectral characteristics are unknown. For optimal spectra, the optimal excitation and emission wavelengths must be found.

The 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.

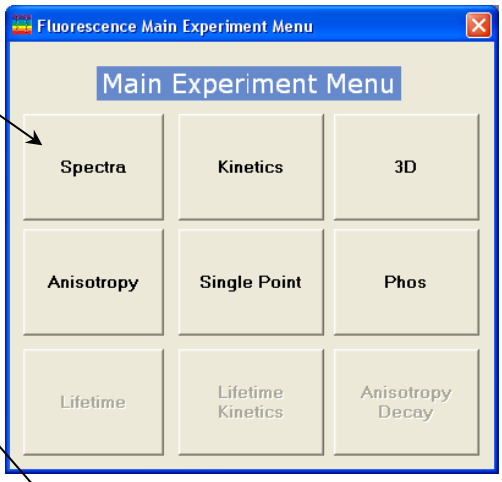
a. Be sure all system components are on, and the FluoroMax® Plus is calibrated as explained in Chapter 3.

b. Click the **Experiment Menu** button  :



The Fluorescence Main Experiment Menu appears:

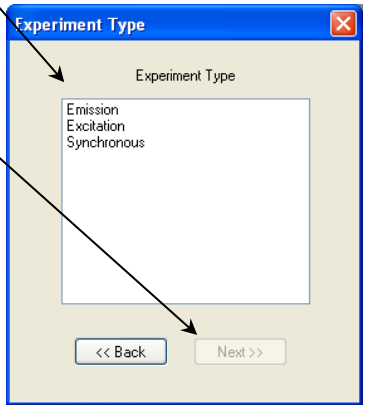
c. Choose the **Spectra** button.



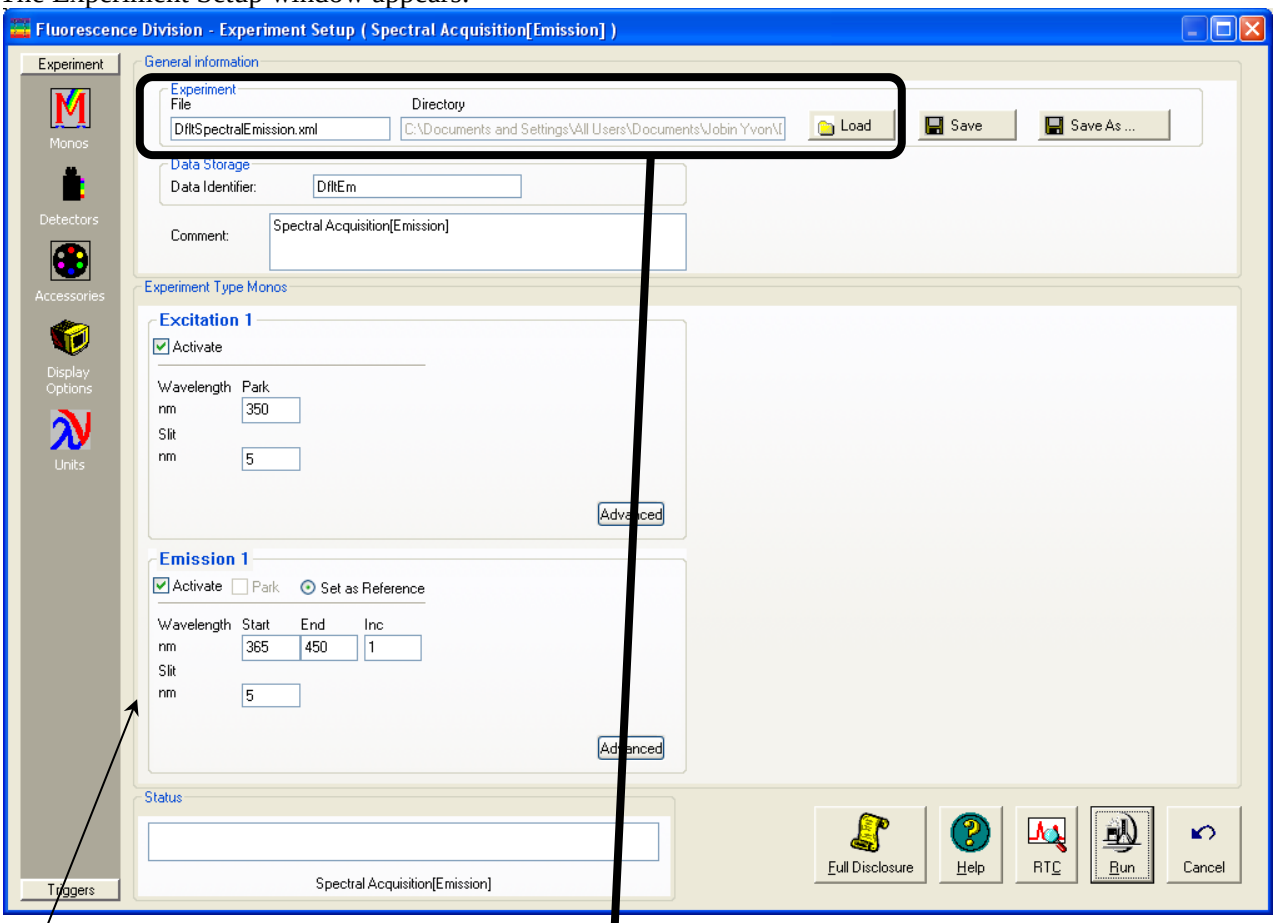
The Experiment Type window appears:

d. Choose Emission.

e. Click the Next >> button.



The Experiment Setup window appears:



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

g. Verify that experimental parameters are correct.

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

h. 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 Scientific 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. Do not forget the 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.

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

j. Click the **Run** button 

.

The scan starts.

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

If the signal exceeds 2×10^6 counts s^{-1}, 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.

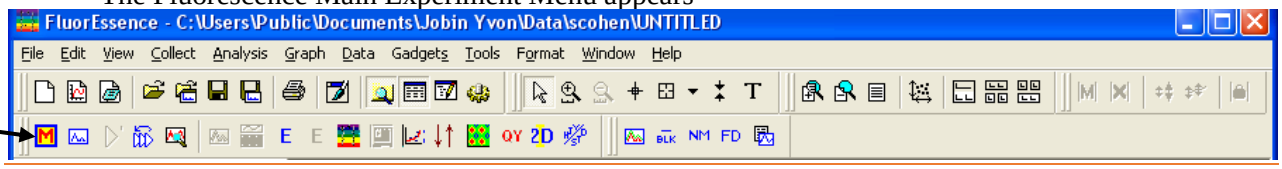
l. With an acceptable emission peak, record its wavelength.

This is the emission maximum. Otherwise, repeat steps i through l until an obvious emission peak appears.

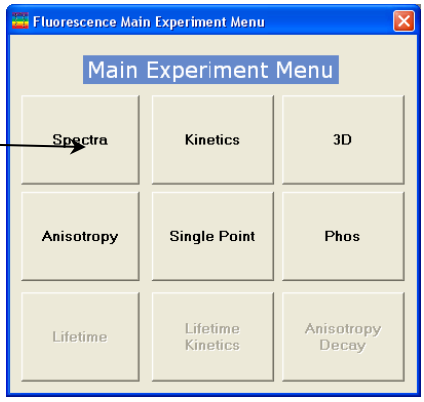
2. Find the optimal excitation wavelength.
   This procedure uses the emission maximum to determine the optimum excitation wavelength, and is similar to step 1.

   a. In the toolbar, click the **Experiment Menu** button  :

   The Fluorescence Main Experiment Menu appears

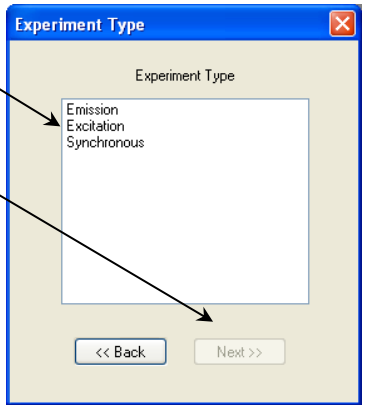


   b. Choose the Spectra button.

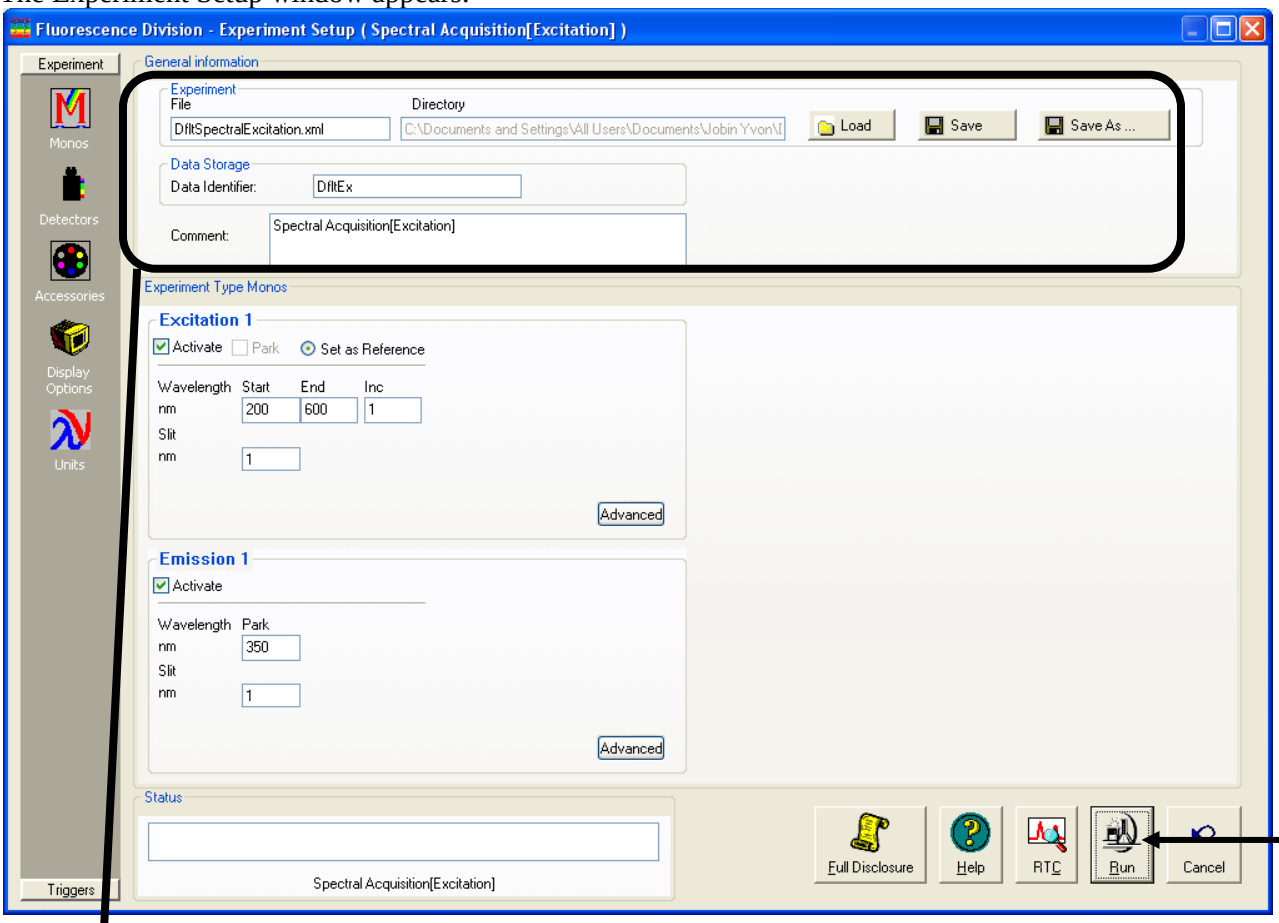


The Experiment Type window appears.

   c. Choose Excitation.
   d. Click the Next >> button.



The Experiment Setup window appears:



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 ratios 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 2×10^6 counts s^{-1} in the S signal detector.

g. Enter the Experiment File name.

h. Click the Run button 

.

The scan starts.

The resulting spectrum shows maximum excitation wavelength. If the raw S-channel signal > 2×10^6 counts s^{-1}, reduce slits, and rescan.

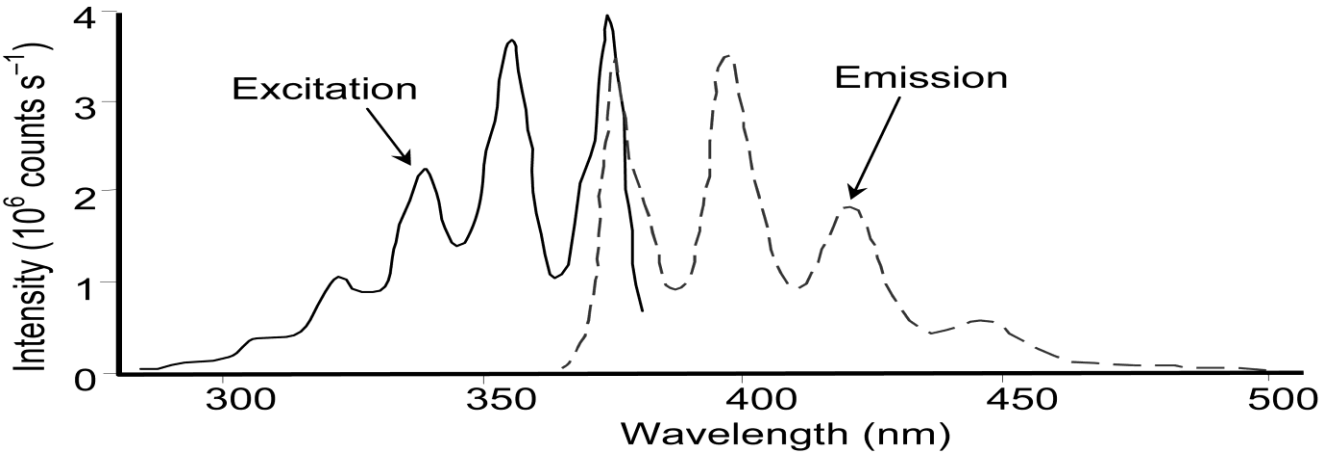
i. Note the excitation peak.

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 is 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.

4.14 Using Corrected Signals in FluorEssence™

4.14.1 Introduction

Subtracting blanks, removing dark noise, and correcting for inhomogeneities in the instrument or detector response give more accurate spectra. Take special precautions to incorporate these functions properly into a FluorEssence™ experiment.

4.14.2 Method

Any corrected signal (with a lower-case "c") or algebraic use of corrected signals must explicitly include all desired corrected signals in the Formulas list. Corrected signals include:

- Dark Offset
- Blank Subtraction
- Correction-factor file

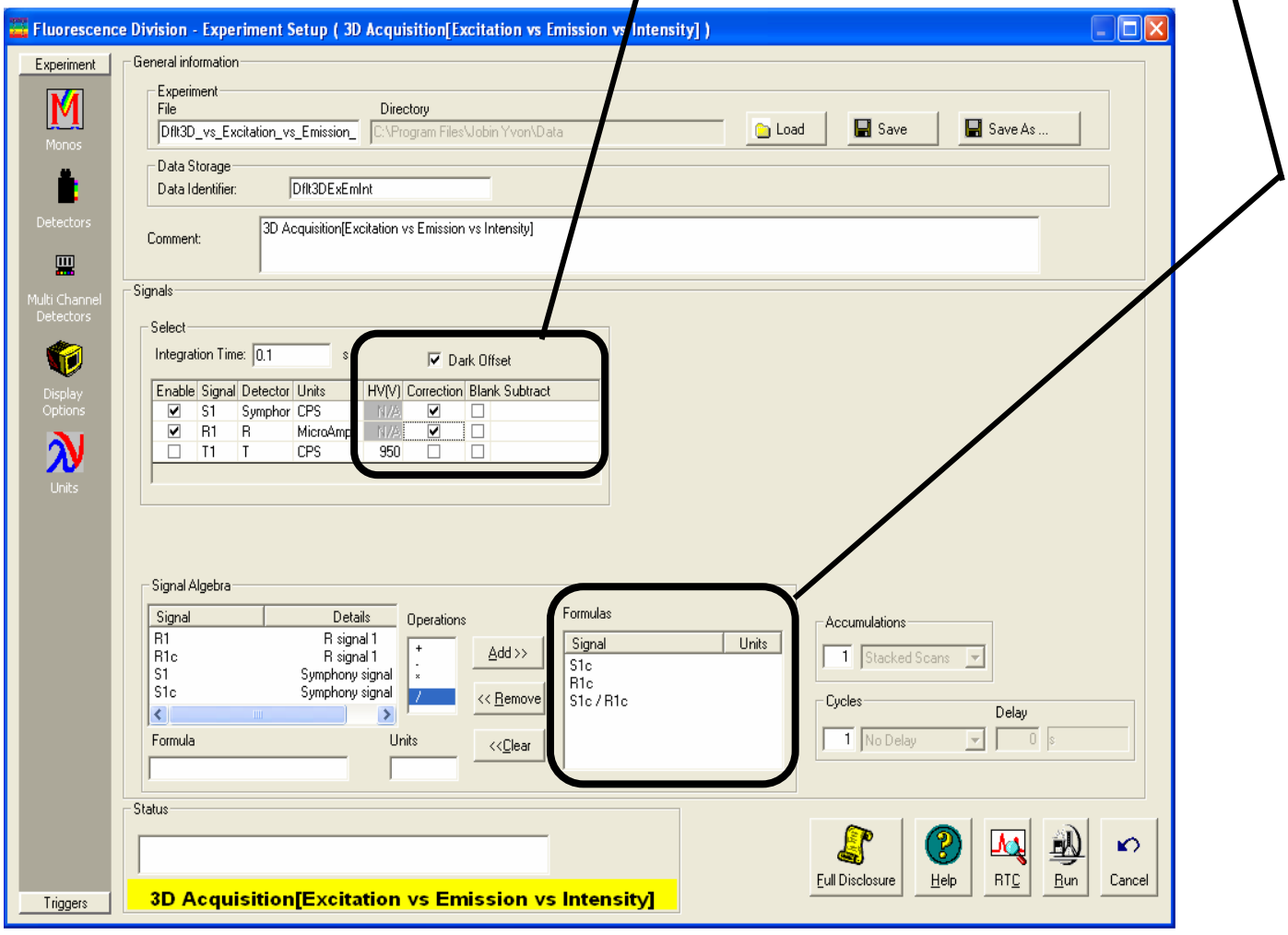
Note: To use a correction-factor file, you must also activate Dark Offset to eliminate generation of a baseline artifact. This artifact is caused by inappropriate multiplication of dark counts not related to the spectral response.

An example is provided on the next page.

4.14.3 Example

Note how the corrected signal, **S1c**, and corrected reference, **R1c**, along with their ratio, S1c/R1c, all must be included in the Formulas list in the **Signal Algebra** area.

Note: Be sure all desired corrections are activated in their respective checkboxes.



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.

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

5.1 Cuvette Preparation

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 clean cotton swab if needed.

   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.
7. Rinse them with de-ionized water before use.

Caution: Soaking the cuvettes for a long period in alkaline detergents such as Alconox® causes etching of the cuvette surface, which results in light-scattering from the cuvettes.

5.2 Sample Preparation

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

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

5.2.1 Small-Volume Samples

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

5.2.2 Solid Samples

Solid samples usually are mounted in the J1933 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.

Note: Avoid thick coverslips, because the excitation beam may not hit the sample directly with a thick coverslip.

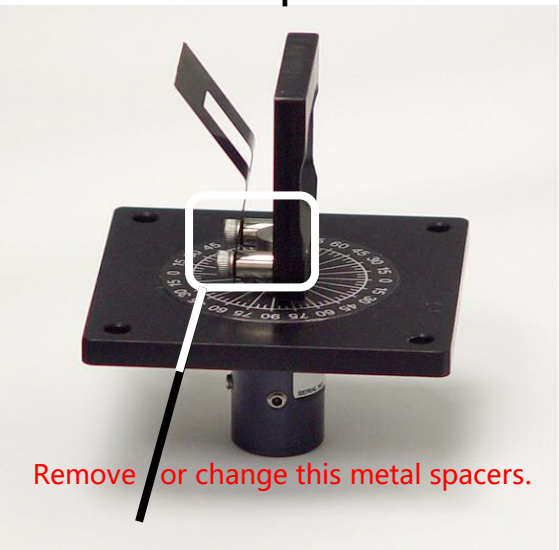
Microscope coverslips are useful, except that they are not quartz, and do not transmit UV light.

- 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.

5.2.3 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.

Solid Sample Holder



5.2.4 Biological Samples

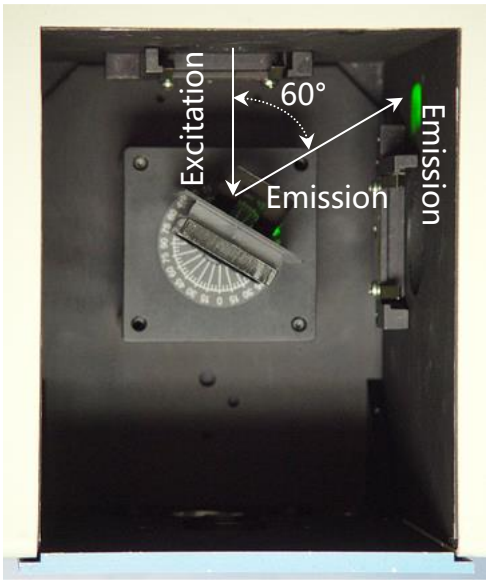
For reproducible results, some samples may require additional treatment. For example, proteins, cell membranes, and cells in solution need constant stirring to prevent settling. Other samples are temperature-sensitive and must be heated or cooled to ensure reproducibility in emission signals.

5.3 Running a Scan on a Sample

5.3.1 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.

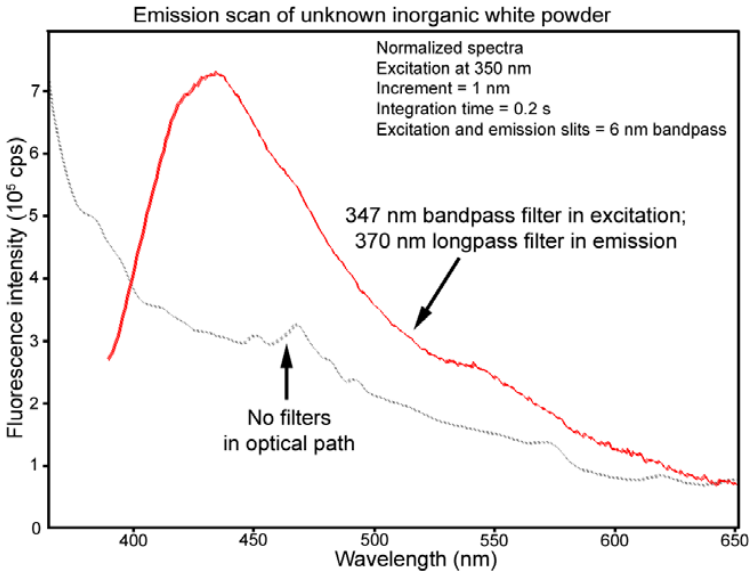
Excitation monochromator



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

5.3.2 Use filters in the optical path.

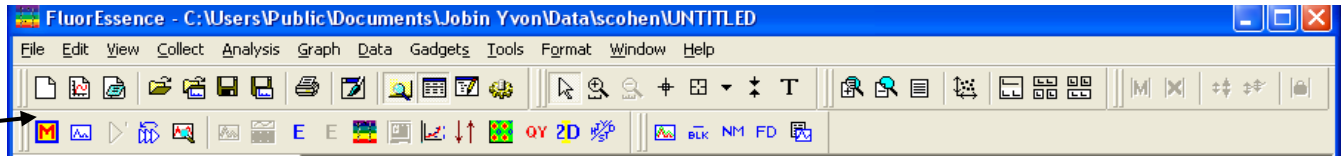
Stray light from the excitation beam can interfere with the emission from the sample. To reduce the deleterious effects of stray light, place a filter that removes excitation wavelengths from the emission beam in the emission optical path. Here is an example of scans with and without filters on a FluoroMax® Plus, 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.



5.4 Measuring the *G* Factor

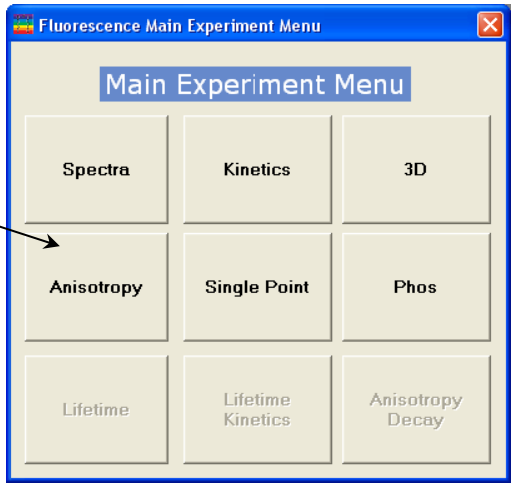
Include the grating factor, or *G* factor, whenever polarization measurements are taken. The *G* factor corrects for variations in polarization wavelength-response for the emission optics and detectors. A pre-calculated *G* factor may be used when all other experimental parameters are constant. In other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



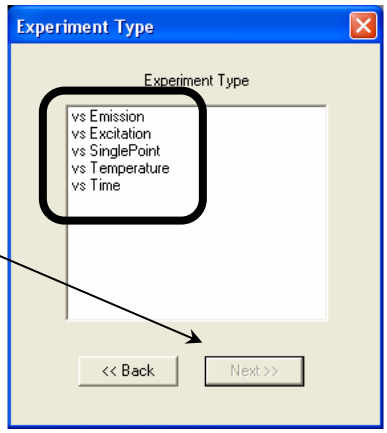
The Fluorescence Main Experiment Menu appears.

2. Click the Anisotropy button.

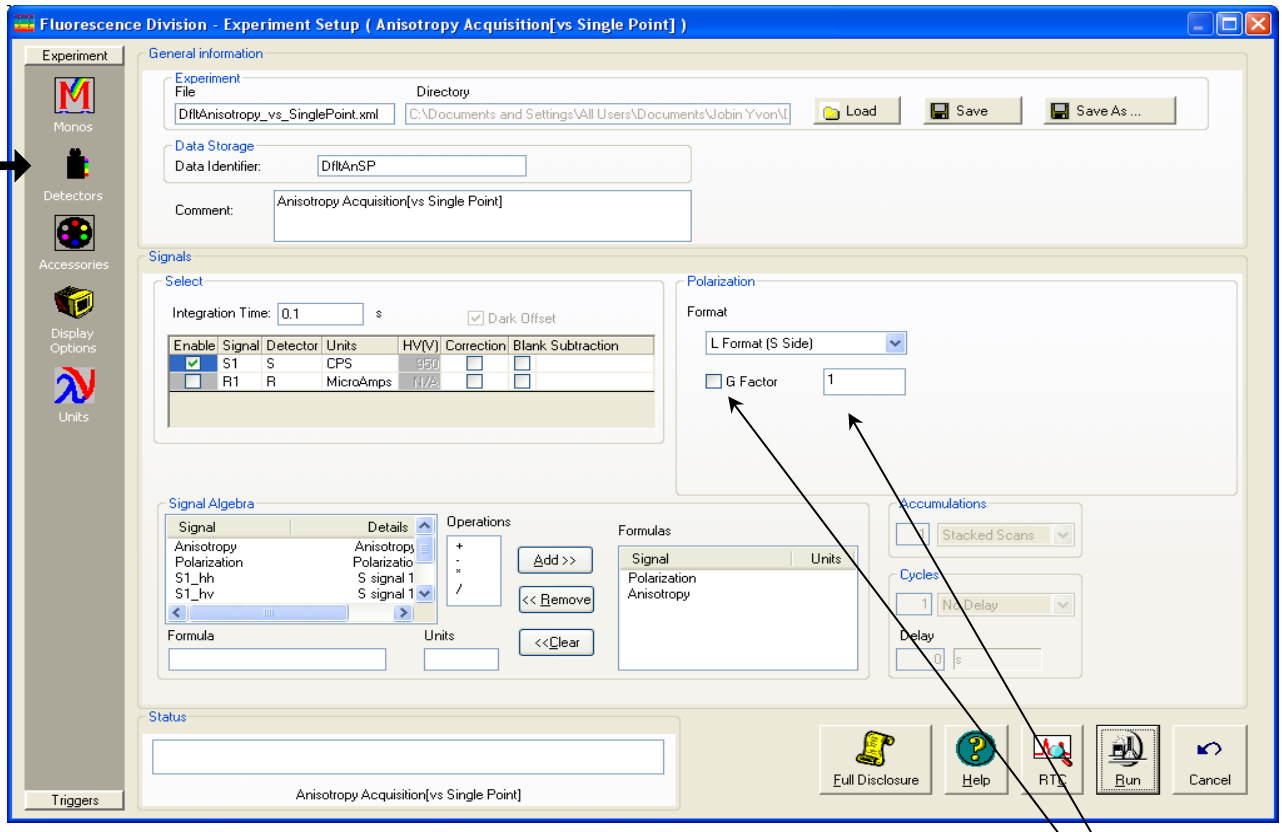


The Experiment Type window appears.

3. Choose the type of Anisotropy experiment, then click the Next >> button.



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



4. Click the Detectors icon.

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

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

   Activate the checkbox ONLY if you know the *G* factor. De-activate the checkbox if you want to measure the *G* factor.

6. Enter a value for the *G* factor in the field if you know and want to use a pre-determined value.

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.

5.5 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 band pass 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.

5.5.1 Determining the Optimum Integration Time

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

For an integration time of 1 second,

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

For an integration time of 2 seconds,

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

or approximately 42%.

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

5.5.1.1 To discover the appropriate integration time:

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

SIGNAL INTENSITY (COUNTS PER SECOND)	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,*

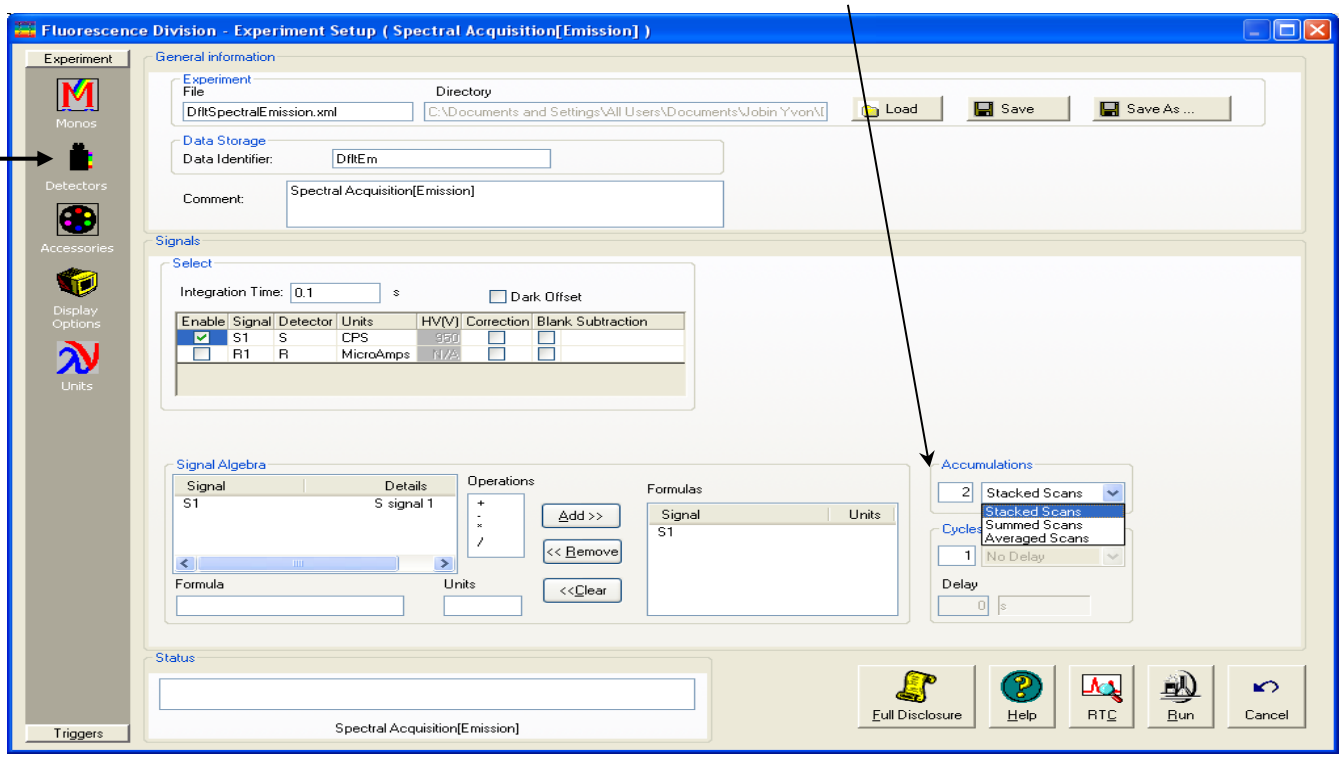
Set integration time through **Experiment Setup** for a specific experiment, or **Real Time Control** to view the effects of different integration times on a spectrum. See the FluorEssence™ on-line help to learn more about setting the integration time.

5.5.2 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.

5.5.2.1 To scan a sample multiple times

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



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

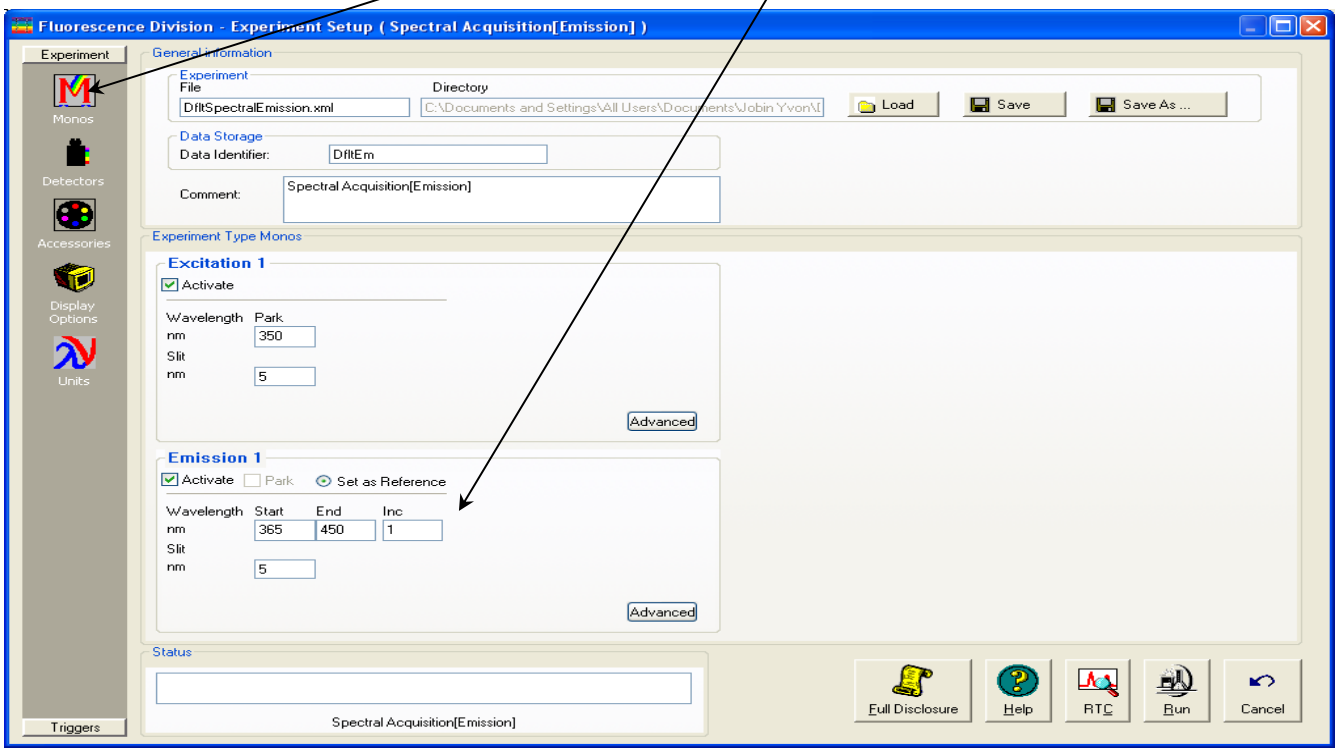
5.5.3 Using the Appropriate Wavelength Increment

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

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

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

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



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

5.5.4 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®+, 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 photobleached, try narrowing the excitation slits and opening the emission slits wider.

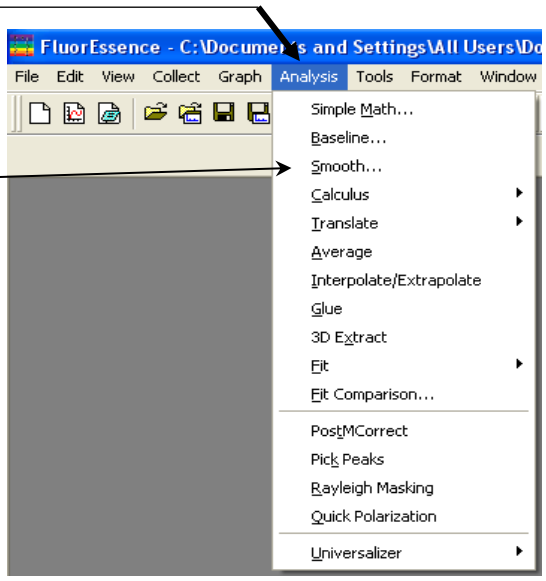
5.5.5 Smoothing Data

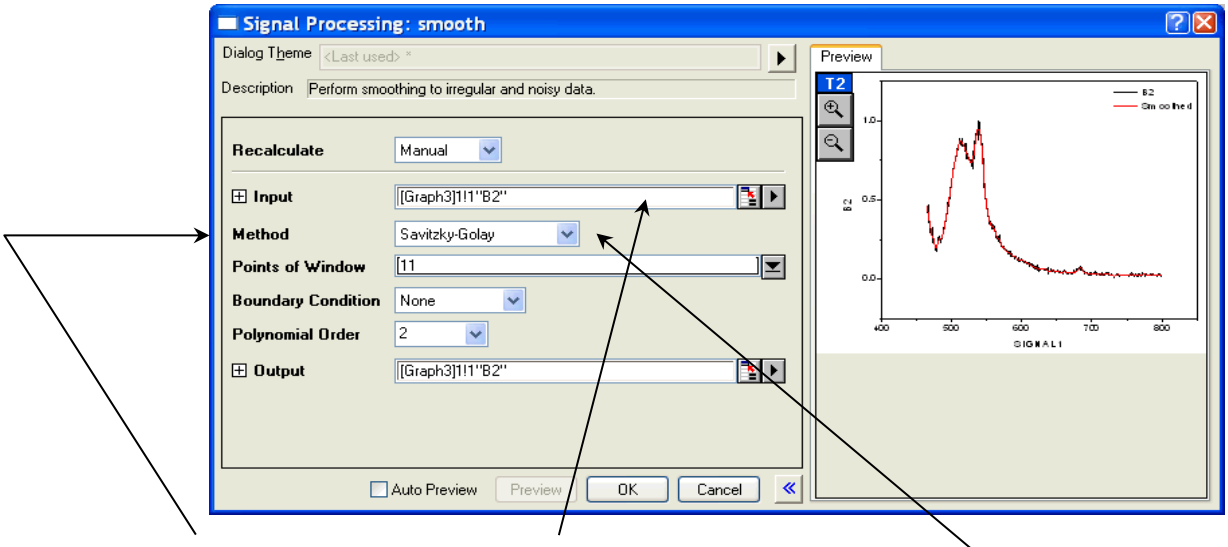
Smoothing the data improves the appearance of the spectrum. Smoothing, as are most post-processing features, is handled by Origin®.

1. Click the Analysis tab on the main FluorEssence™ toolbar.

   A drop-down menu appears.
2. Choose Smooth....

   The Smooth window opens.





3. In the Input drop-down menu, choose the appropriate file to smooth.
4. In the Method drop-down menu, choose FFT Filtering (fast-Fourier transform), Adjacent Averaging, or Savitzky-Golay smoothing.
5. Click the OK button to smooth the data.

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

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

5.6 Correcting Data

5.6.1 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 remove 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 Chapter 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.

5.6.2 During Acquisition

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

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

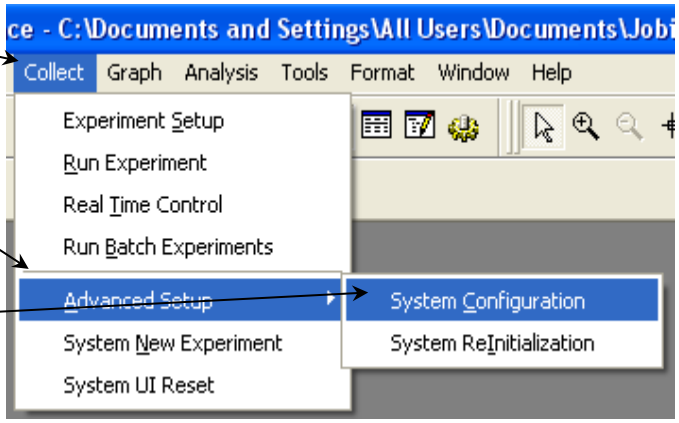
Caution: The mcorrect and xcorrect files are custom generated at the factory for each particular instrument, and cannot be swapped.

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

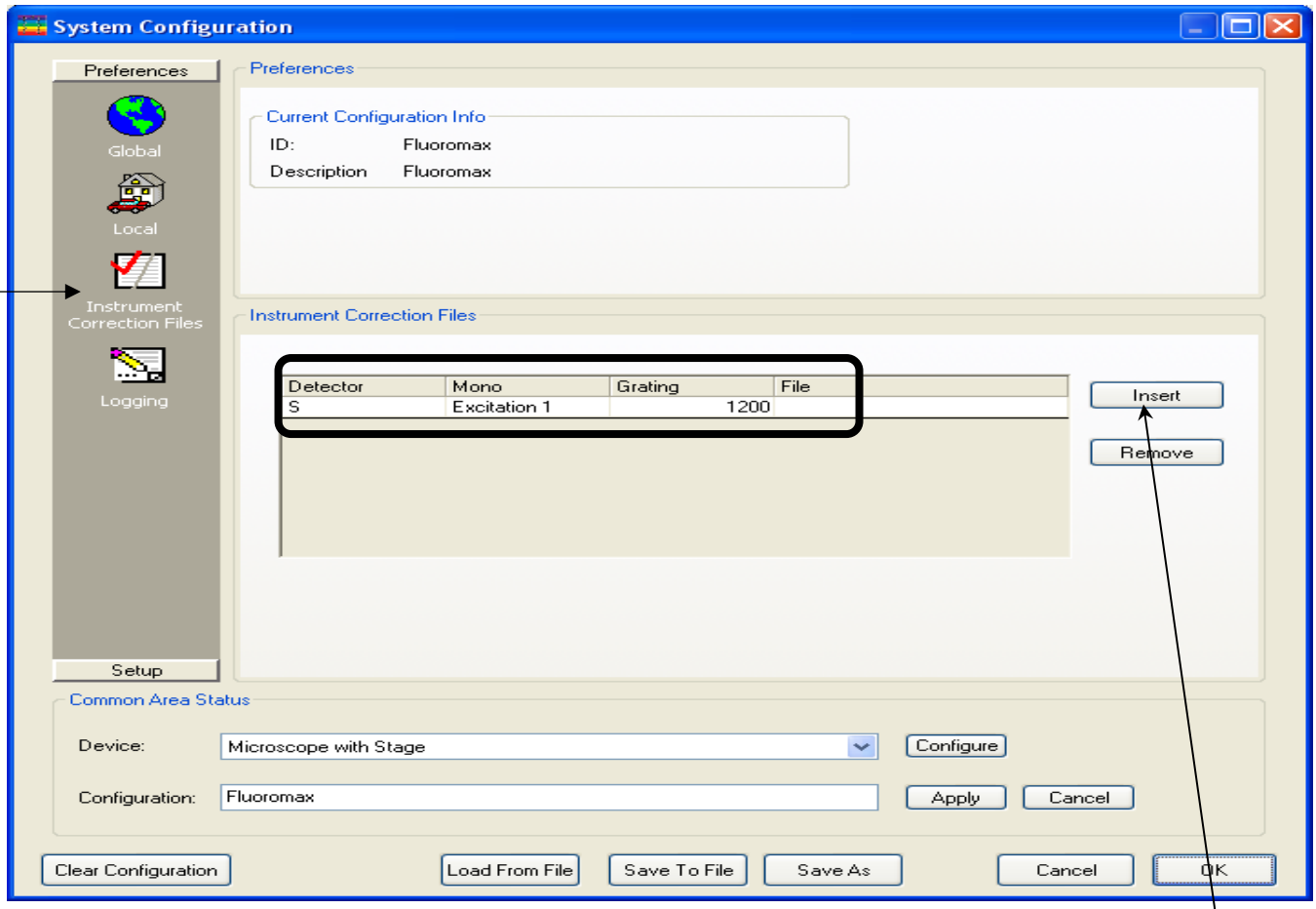
   a. Choose the Collect tab in the toolbar.

A drop-down menu appears.

   b. Choose Advanced Setup, then System Configuration.



The System Configuration window appears:



c. Choose Preferences, then the Instrument Correction Files icon.

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

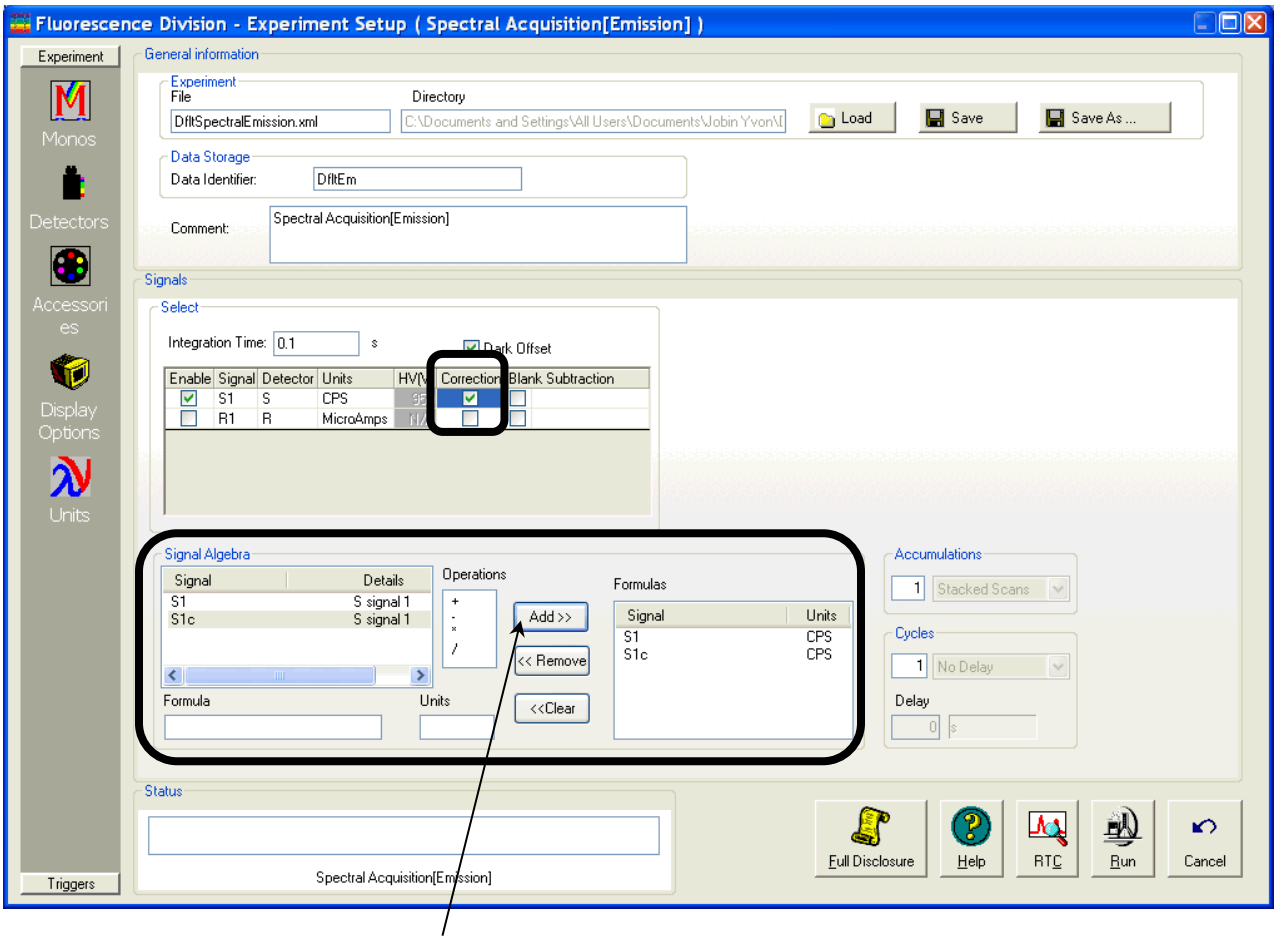
DETECTOR	CORRECTION-FACTOR FILE NAME
S	mcorrect
R	xcorrect

2. Choose correction when setting up the experiment.

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

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

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



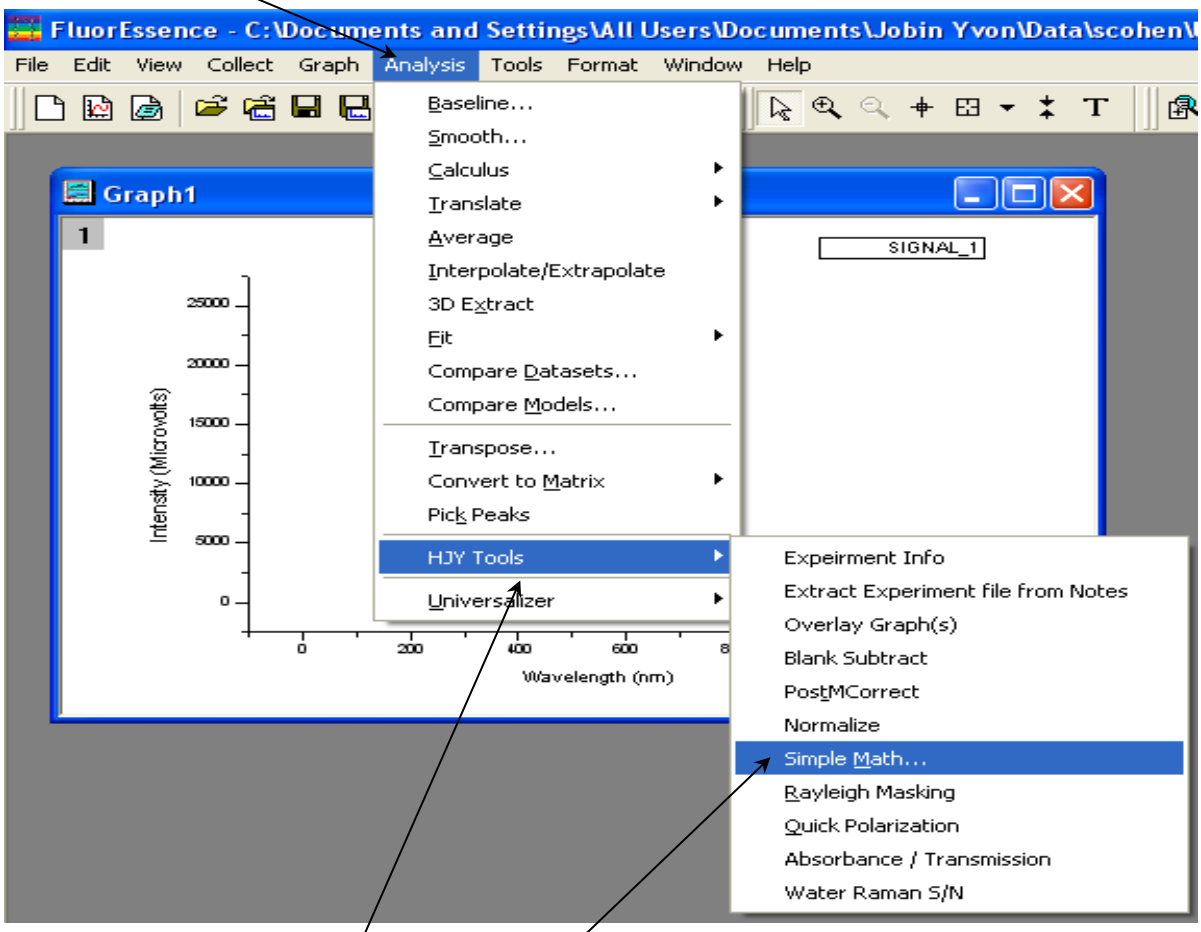
c. Click the Add >> button to add the corrected signal to the Formulas table. The corrected signal appears in the Formulas table.

d. Run the experiment with the corrected signal.

5.6.3 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 the trace to be corrected is active in the main FluorEssence window.
2. Click the Analysis tab.



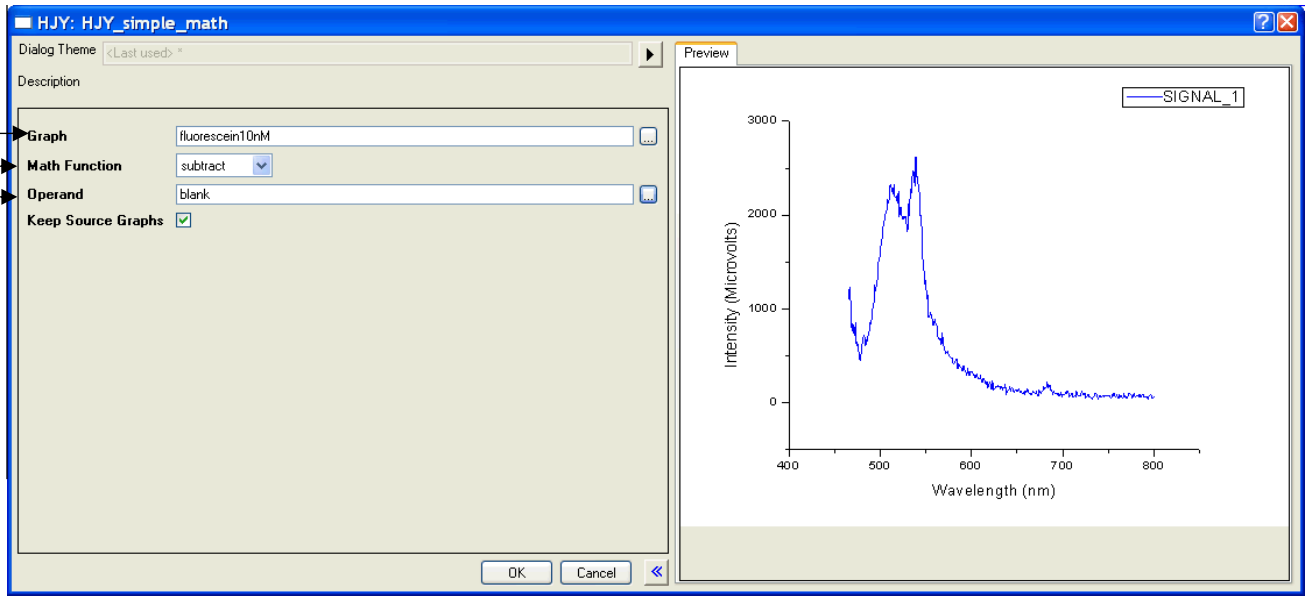
A drop-down menu appears.

3. Select HJY Tools.

Another drop-down menu appears.

4. Select Simple Math....

The HJY_simple_math window opens:



The name of the chosen trace should appear in the Graph field. If not, browse for it with the drop-down menu.

5. From Math Function, select multiply from the drop-down menu.
6. Browse for the appropriate correction-factor file in the Operand field (`mcorrect` or `xcorrect`).

   You may also enter a numerical constant if you choose.
7. If you want to create a new graph, activate the Keep Source Graphs checkbox.

   If the Keep Source Graphs checkbox is inactive, the data in the current graph are overwritten.
8. Click the OK button.

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

6 MAINTENANCE

6.1 Introduction

The FluoroMax® Plus 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.

6.2 Lamp Replacement

6.2.1 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 400 000 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 Scientific suggests leaving the lamp on during brief periods of inactivity.

6.2.2 Parts and Tools Required

6.2.2.1 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® Plus.

Phillips screwdriver

3/32" Allen key

7/64" Allen key

1/8" Allen key

9/64" Allen key

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

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:

6.2.3 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 the 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.

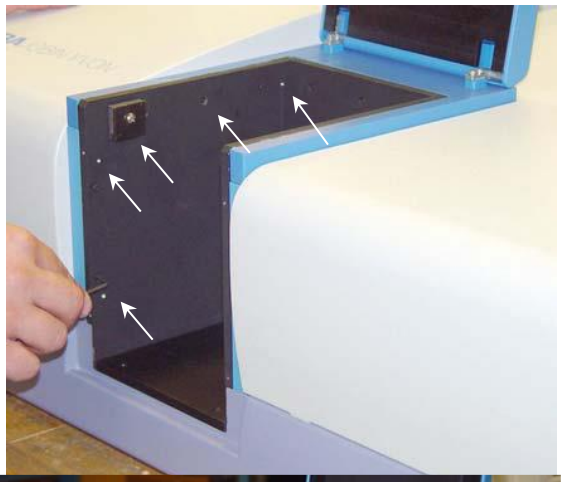
6.2.4 Changing the Lamp

1. Switch off and prepare the FluoroMax® Plus.
    a. Be sure that the FluoroMax®+ and the host computer are turned off.
    b. Remove the AC (mains) power cord from the FluoroMax® Plus.
    c. Disconnect the USB cable, optional trigger-box cable, power cord, and any other cables attached to the spectrofluorometer.
2. Gently remove the sample mount from the front of the FluoroMax® Plus.

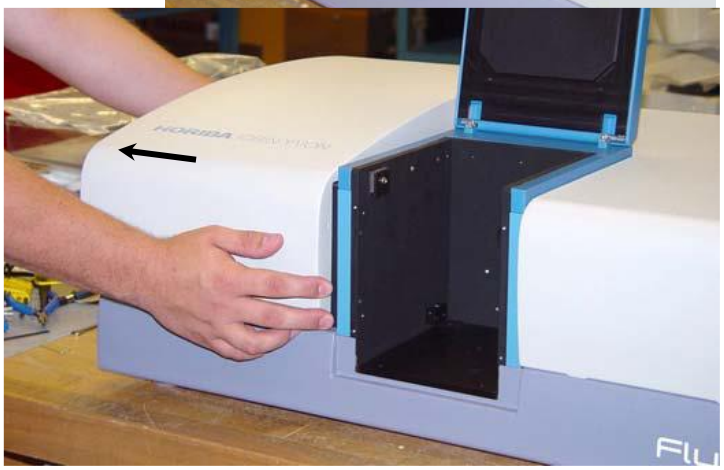
The standard FluoroMax® Plus front is held via a friction fit, with no screws to remove. Some accessories require removal of four screws. Some sample mounts also have a 15-pin connector at the inside end for automated accessories.

3. Remove the lamp cover.

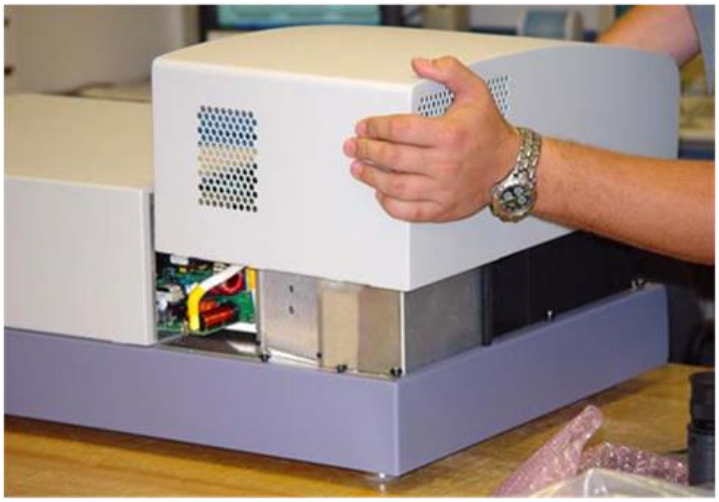
   a. With an Allen key, remove the five screws from inside the left wall of the sample compartment.



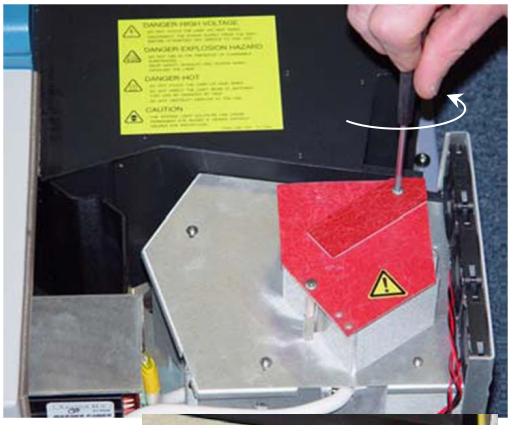
   b. Pull the lamp cover to the left about 2 cm.



   c. Lift the cover vertically off the instrument.

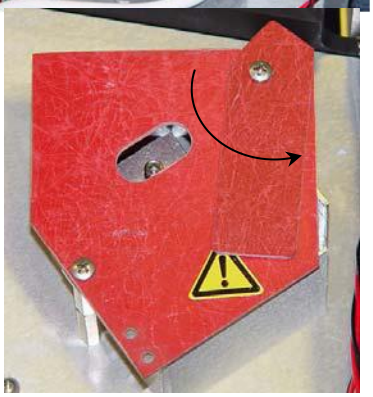


4. Remove the lamp-housing cover at the rear of the instrument.

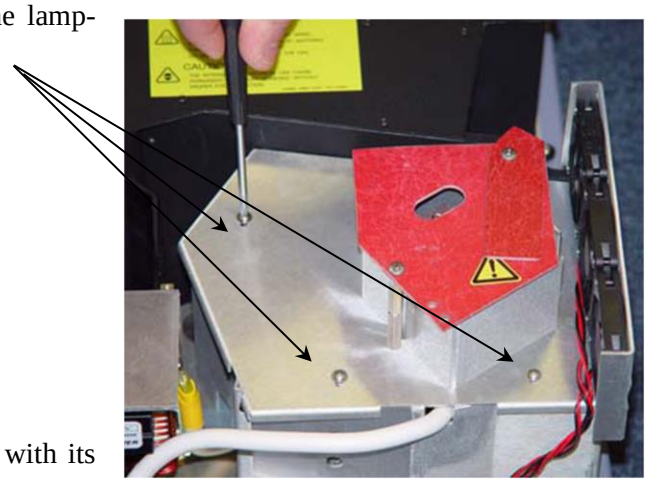


   a. With a Phillips screwdriver, loosen the safety cover's screw.

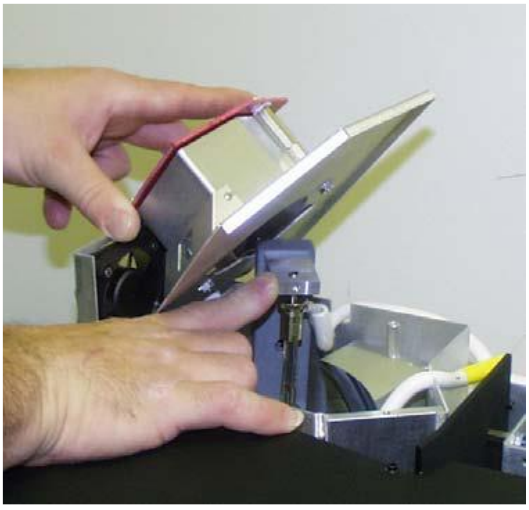
   b. Swing the safety cover out of the way.



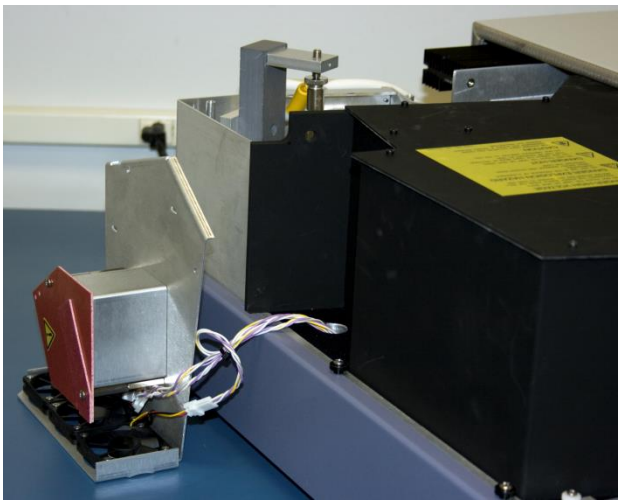
   c. Remove the three cap screws from the lamp-housing cover.



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



e. Rotate the cover backwards, and set it to the side of 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 exposed.
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.

9. Attach connections to the new lamp.

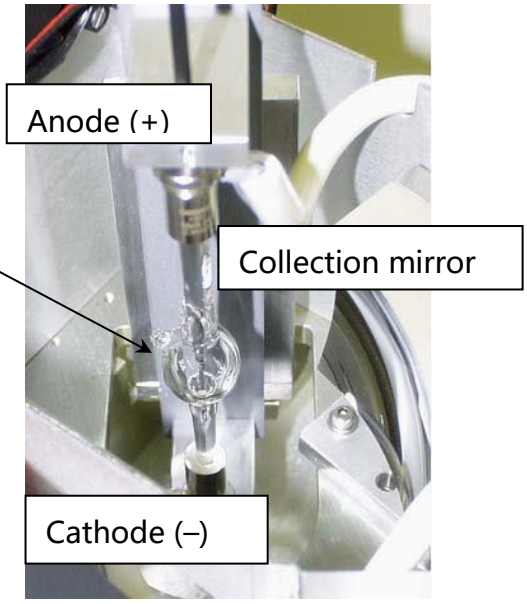
a. Attach the cathode connection to the new lamp, and secure the connection with the new lamp's thumbscrew.

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

b. Attach the anode connection to the new lamp, and secure the connection with the new lamp's thumbscrew.
c. 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 safety cover and lamp-housing cover.

12. Replace the three screws on the lamp-housing cover.
13. Reconnect all cables (power, accessories, etc.) to the FluoroMax® Plus.

Note: Do not replace the FluoroMax® Plus cover until the lamp is correctly adjusted.

6.2.5 Adjusting the New Xenon Lamp

6.2.5.1 Choices

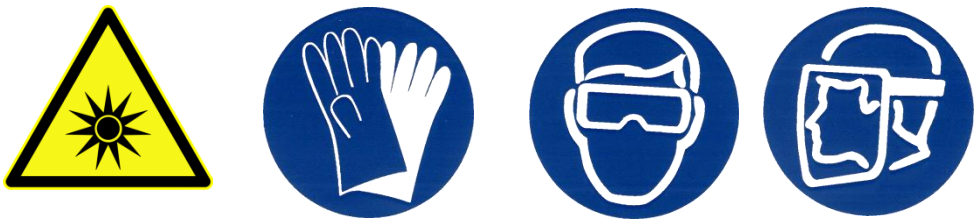
There are two choices after lamp installation:

- 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.

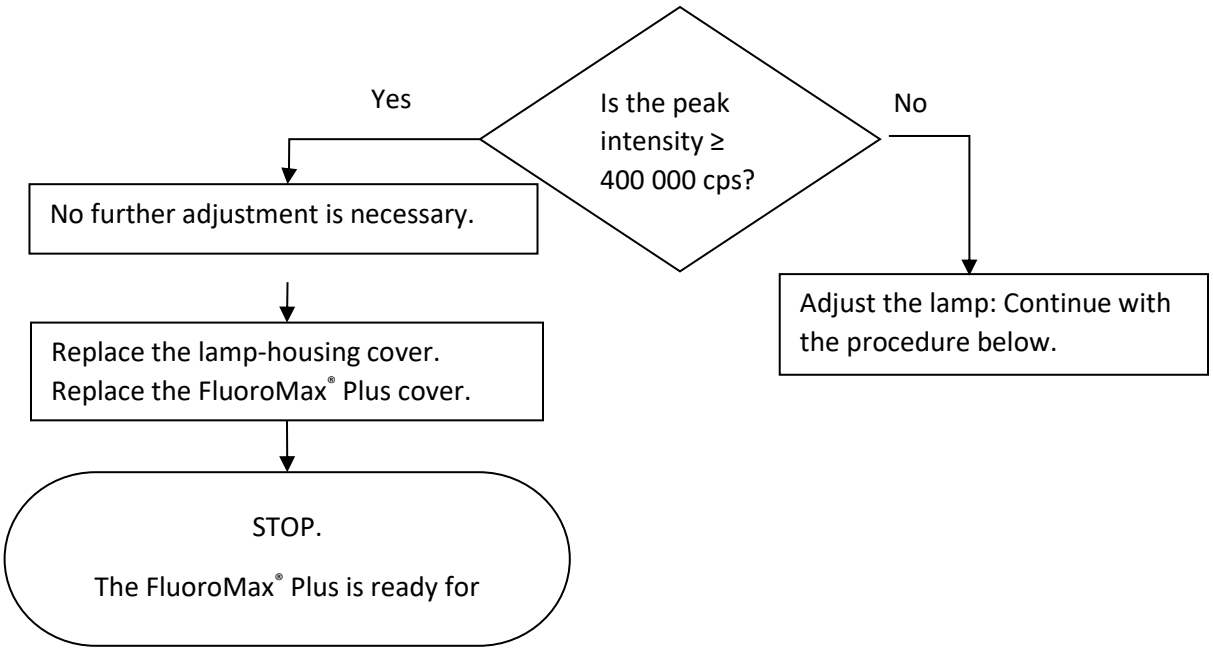
6.2.5.2 Procedure

1. With the lamp cover still removed, turn on the FluoroMax® Plus.



Caution: Intense ultraviolet, visible, or infrared light may be present when the lamp cover is open, so wear eye- and skin-protection, such as light-protective 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.

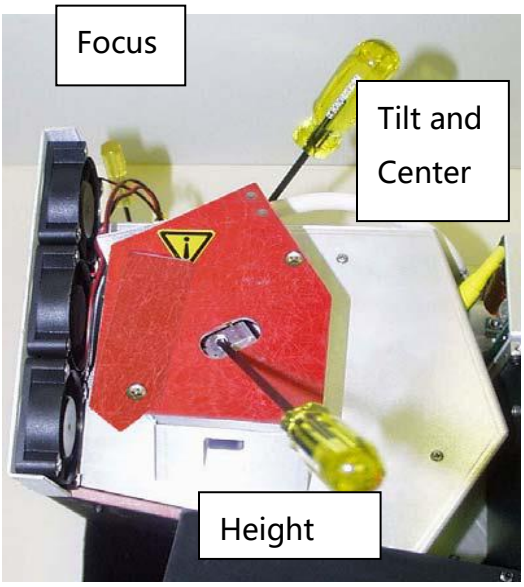


This should confirm that the instrument is roughly calibrated.

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″

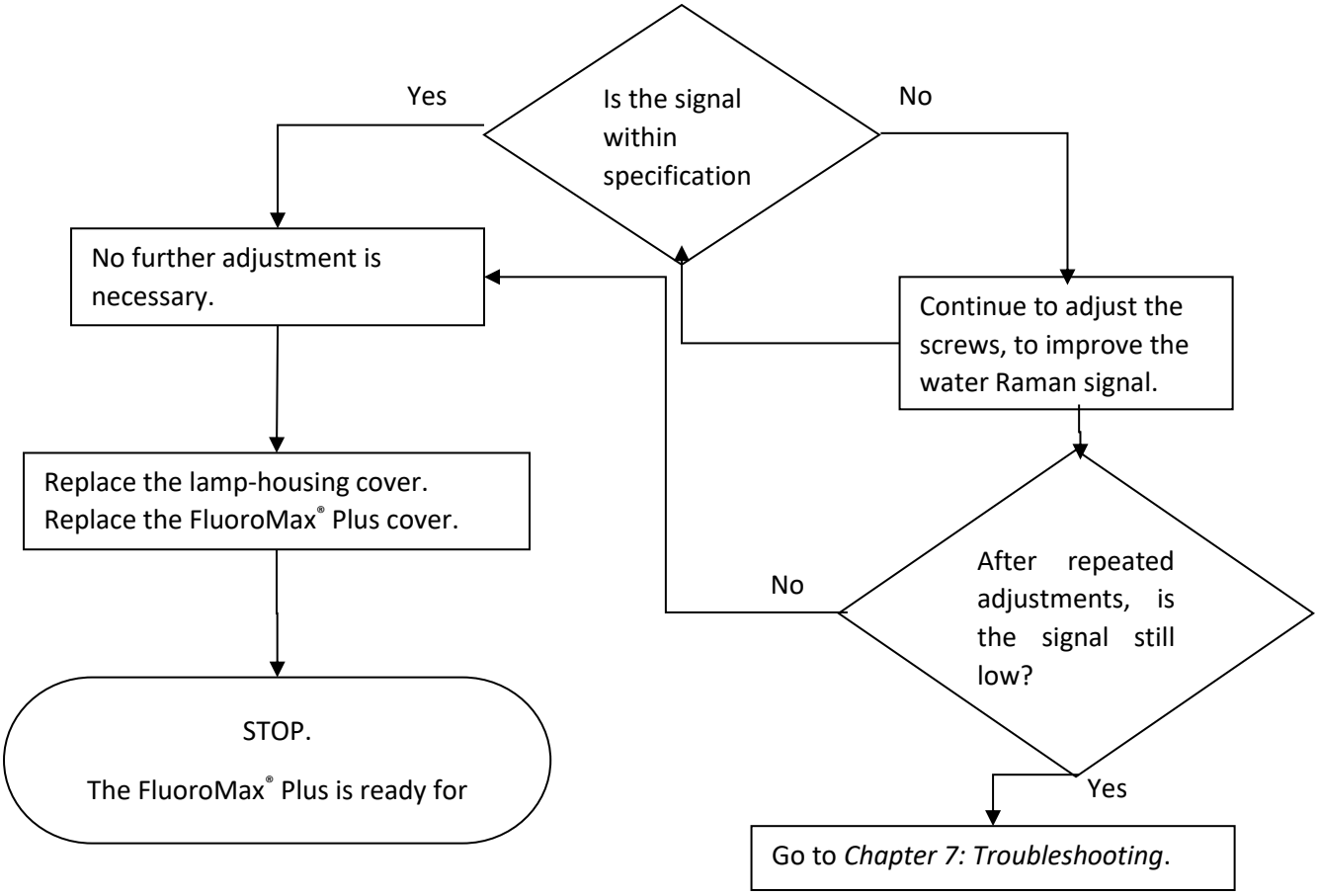


    a. Slowly work each adjustment to optimize the signal.

    At maximum signal (> 400 000 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



6.3 Electronics

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

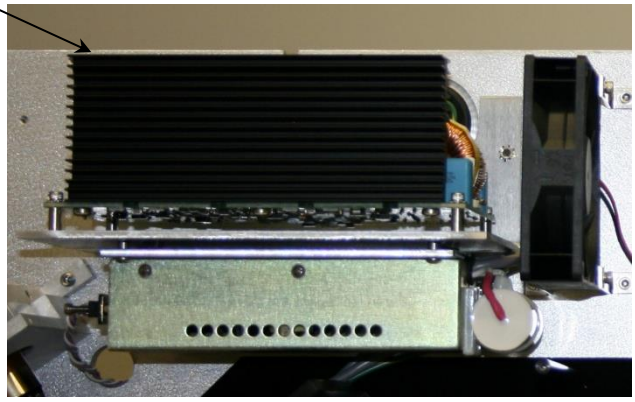
Caution: The information in this section does not constitute permission to adjust, manipulate, or remove any electrical or other components in the FluoroMax® Plus. This information is provided for reference purposes only. Contact the Service Department in case of system failure or suspected failure, before attempting repairs or testing with meters.

DO NOT ATTEMPT ANY REPAIRS OR INSTRUMENT EVALUATION WITHOUT THE EXPRESS PERMISSION OF THE SERVICE DEPARTMENT.

6.3.1 Xenon-Lamp Power Supply

The xenon lamp's power supply has a capacity of 180 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 startup of the lamp—several thousand volts—this contains specialized line filters.

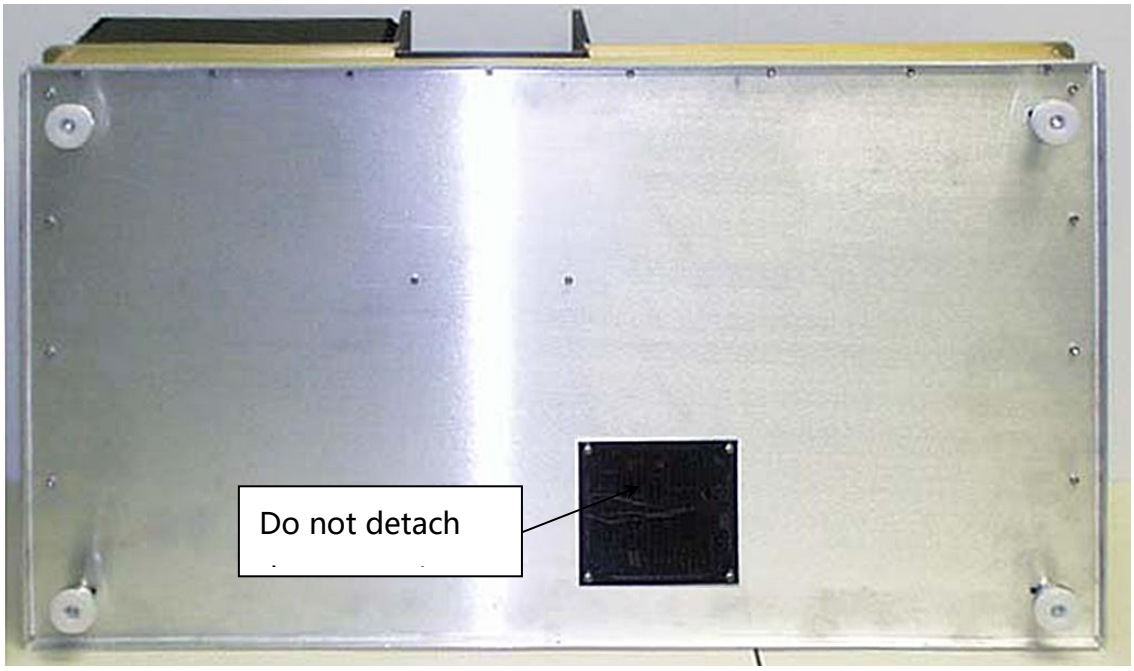
The power supply is preset at the factory for the correct current and voltage to supply the xenon lamp with 150 W. Poor lamp performance or difficulty maintaining a stable lamp arc may be the result of an improperly set power supply. Contact Fluorescence Service for more details.



6.3.2 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 is a J400906 control board underneath the instrument, which controls the drives, slits, shutter, and automated accessories in the monochromators. This board also controls options such as a sample changer.

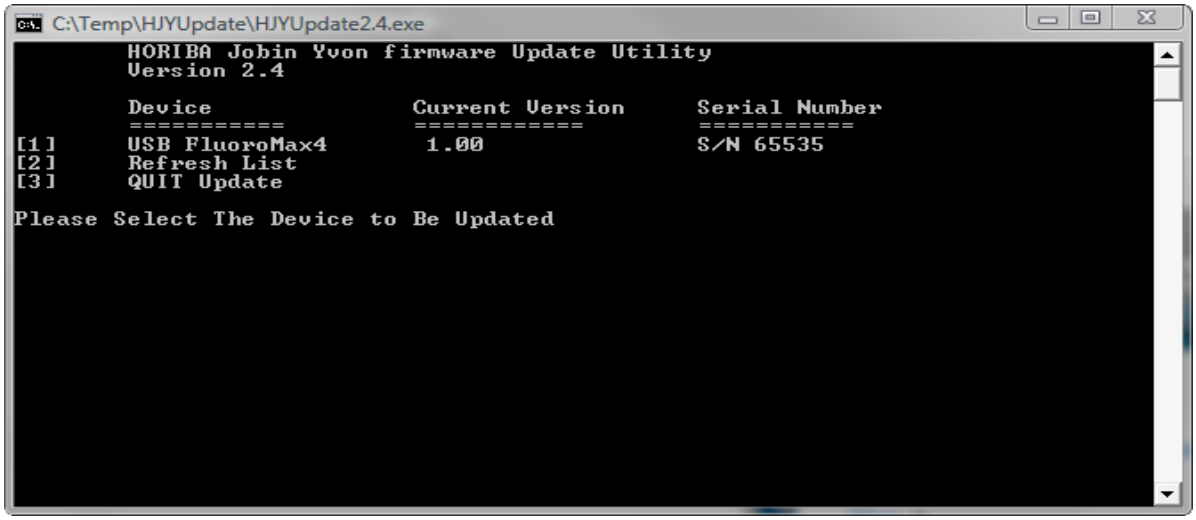
Caution: The information in this section does not constitute permission to adjust, manipulate, or remove any electrical or other components in the FluoroMax® Plus. This information is provided for reference purposes only. Contact the Service Department in case of system failure or suspected failure, before attempting repairs or testing with meters.

DO NOT ATTEMPT ANY REPAIRS OR INSTRUMENT EVALUATION WITHOUT THE EXPRESS PERMISSION OF THE SERVICE DEPARTMENT.

6.4 Updating the FluoroMax® Plus Firmware

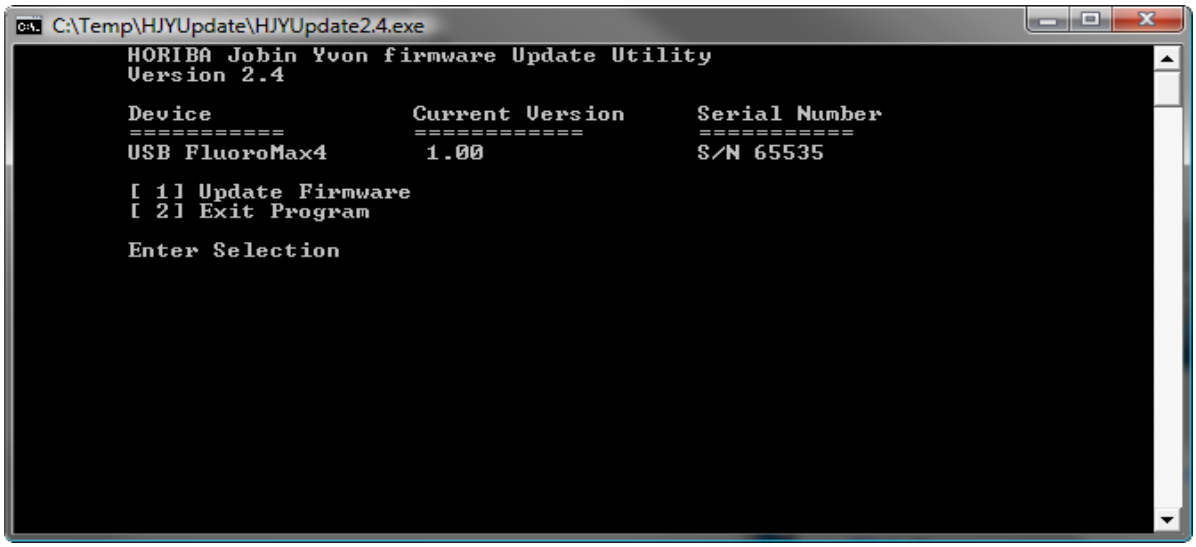
1. Turn on the host PC
2. Insert the installation CD into the CD or DVD drive in the host PC.
3. Using Windows® Explorer, find the Utilities directory in the installation CD. Inside the Utilities directory, find the HJYUpdate subdirectory.
4. In the HJYUpdate subdirectory, double-click on `HJYUpdate2.4.exe` to start the Firmware update.

The console window appears, asking you to choose which device:



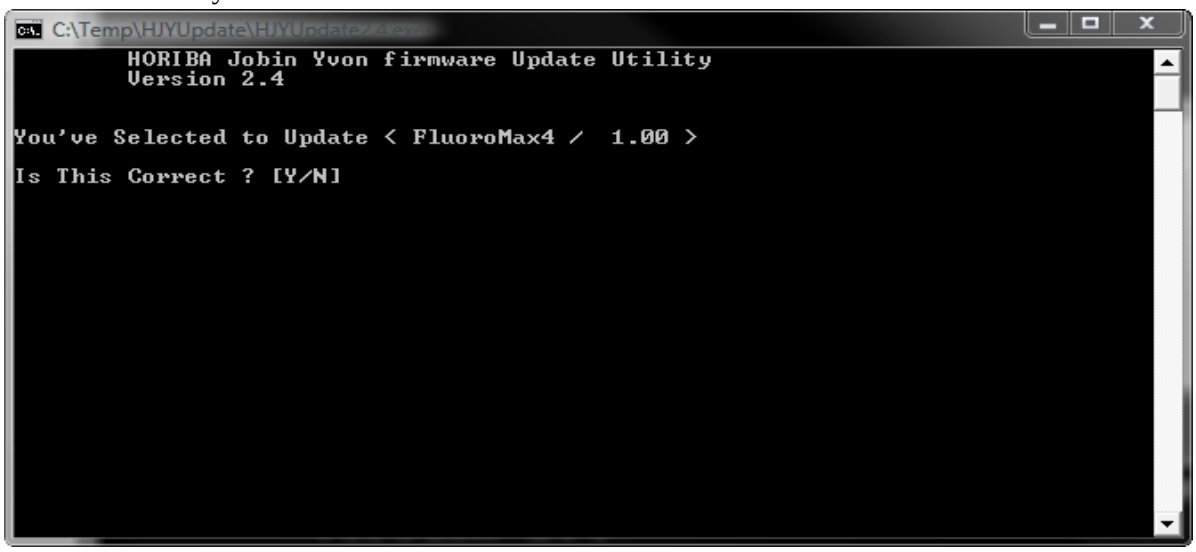
5. Choose option `1` to update the FluoroMax® Plus with USB port and current Firmware version 1.00.

The console asks you which choice: (1) update Firmware, or (2) exit:



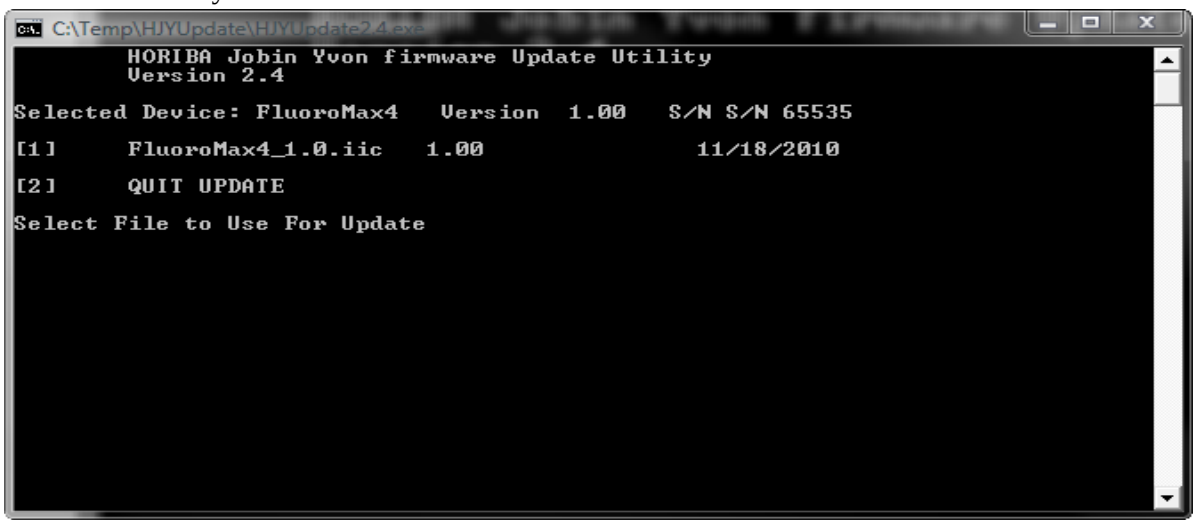
6. Choose `1` to update the Firmware.

The console asks you to confirm this choice:



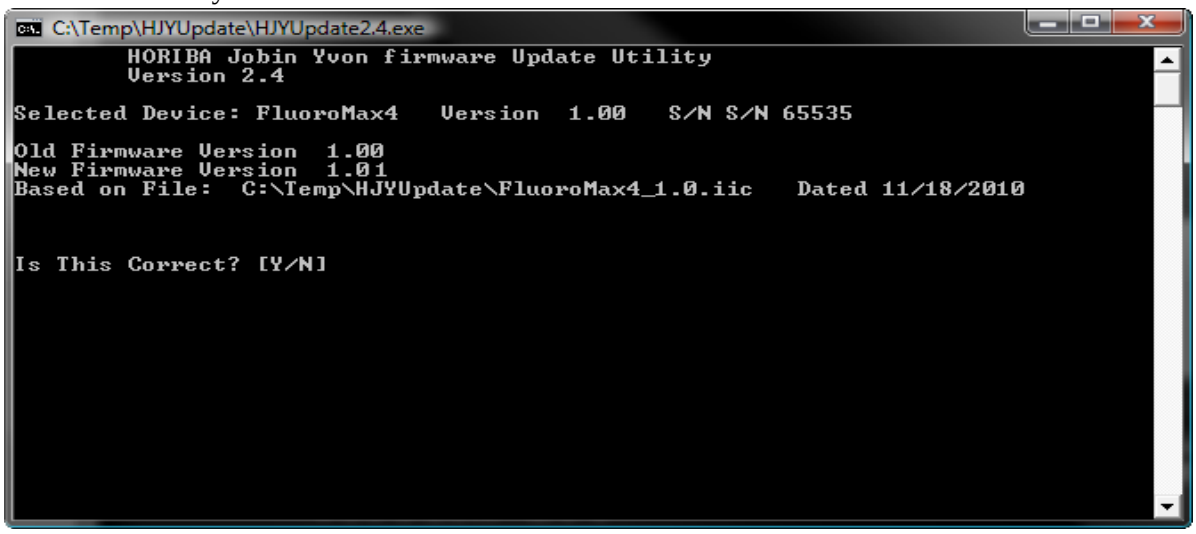
7. Enter `Y` to confirm updating the Firmware.

The console asks you to choose the Firmware:



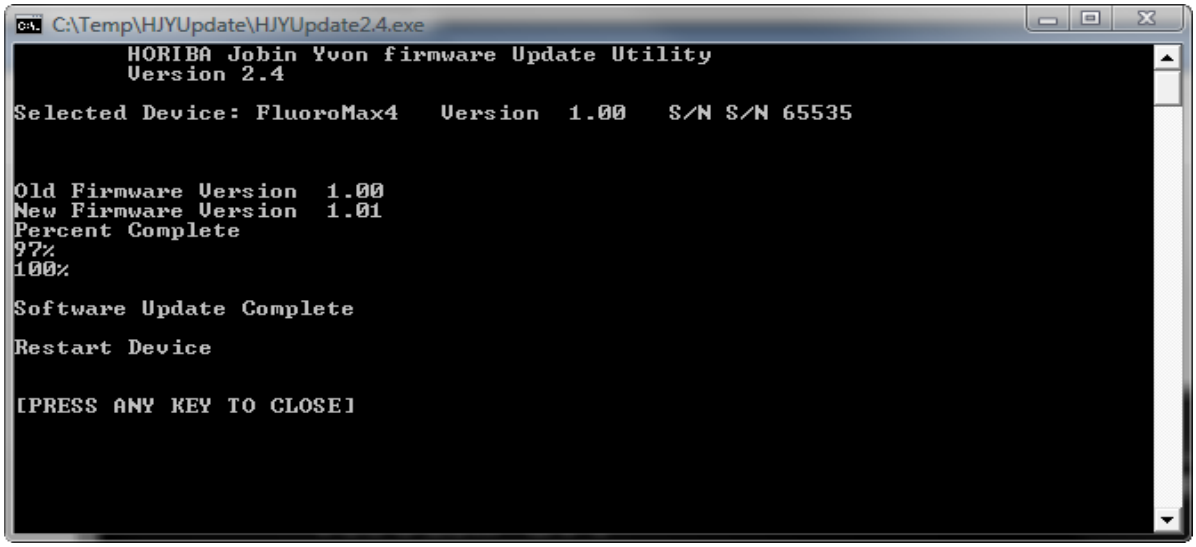
8. Enter `1` for Firmware `FluoroMax4_1.0.iic`.

The console asks you to confirm that the new Firmware version is 1.01:



9. Enter `Y` to confirm this.

The software updates the Firmware. When complete, the console prompts you to press any key to close the program:



10. Switch off the FluoroMax® Plus for about 10 seconds, and then switch it back on to load the Firmware into the instrument.

7 TROUBLESHOOTING

The FluoroMax® Plus 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.

Chart

PROBLEM	POSSIBLE CAUSE	REMEDY
Light not Reaching the Sample.	Excitation shutter closed. Slits are not open to the proper width. FluoroMax® Plus is in phosphorimeter layout. Monochromator is mis calibrated. Sample turret is not in correct position.	Using the software, open the shutter. Adjust the slits. Change to a steady-state layout. Check and recalibrate monochromator. Using FluorEssence™, set the position and open the cover to verify the position.
Low Signal Intensity	CW lamp is not aligned. Slits are improperly set. Shutter(s) is(are) not completely open. Lamp power-supply is set to the wrong current rating. Lamp is too old. Shutter(s) closed.	Align the lamp. Adjust the slit widths. Open the shutter(s) in Real Time Control. Call the Service Department. Replace lamp. (150-W lamp has lamp lifetime 1200–1500 h.) Open all shutters in Real Time Control.
Signal Intensity 10 Times Lower Than Normal	Polarizer is in the light path. Monochromators are set to wrong wavelength. Detectors are saturated. Optical density effects and self-absorption.	In Real Time Control, move the polarizer out of the light path. Select appropriate wavelength based on excitation and emission of sample. Adjust slits. (Signal detector is linear to 2 × 10^6 cps in photon-counting mode. Reference detector saturates at 200 µA.) Sample is too concentrated. Dilute sample by a factor of 10 or 100 and retry experiment.
No Change in Signal Intensity	Detectors are saturated.	Reduce slit settings.
No Signal	Lamp is not on. Detectors are saturated.	Bad lamp: change xenon lamp. Reduce slit settings.
Erratic Signal.	Lamp unstable. Light leaks. Sample has particles that scatter light irregularly.	Let lamp warm up 20 min before use. Check dark value to determine. 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.

PROBLEM	POSSIBLE CAUSE	REMEDY
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. Dirty cuvette. Solid-sample holder in sample compartment.	Place bandpass filters in excitation light path. Decrease emission-spectrometer slit widths. Clean the cuvette as described in Chapter 5. 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. Stirring bar is too large; light beam is striking it.	Use slower stirring speed. Use a smaller stirring bar (available from Bel-Art Products, Pequannock, NJ).
Communication Problem between Computer and Instrument.	USB cable is improperly connected.	Check USB cable's connection.
Hardware Init. Error	Broken IR sensor in monochromator.	Replace IR sensor: Call the Service Department.
Sample Turret not Operating.	Software is not enabled. USB cable is connected improperly.	Check status. Check USB cable's connection.
FluoroMax® Plus 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	User is not logged into Windows® as administrator or power user	Log into Windows® as administrator and or power user, and restart FluorEssence™

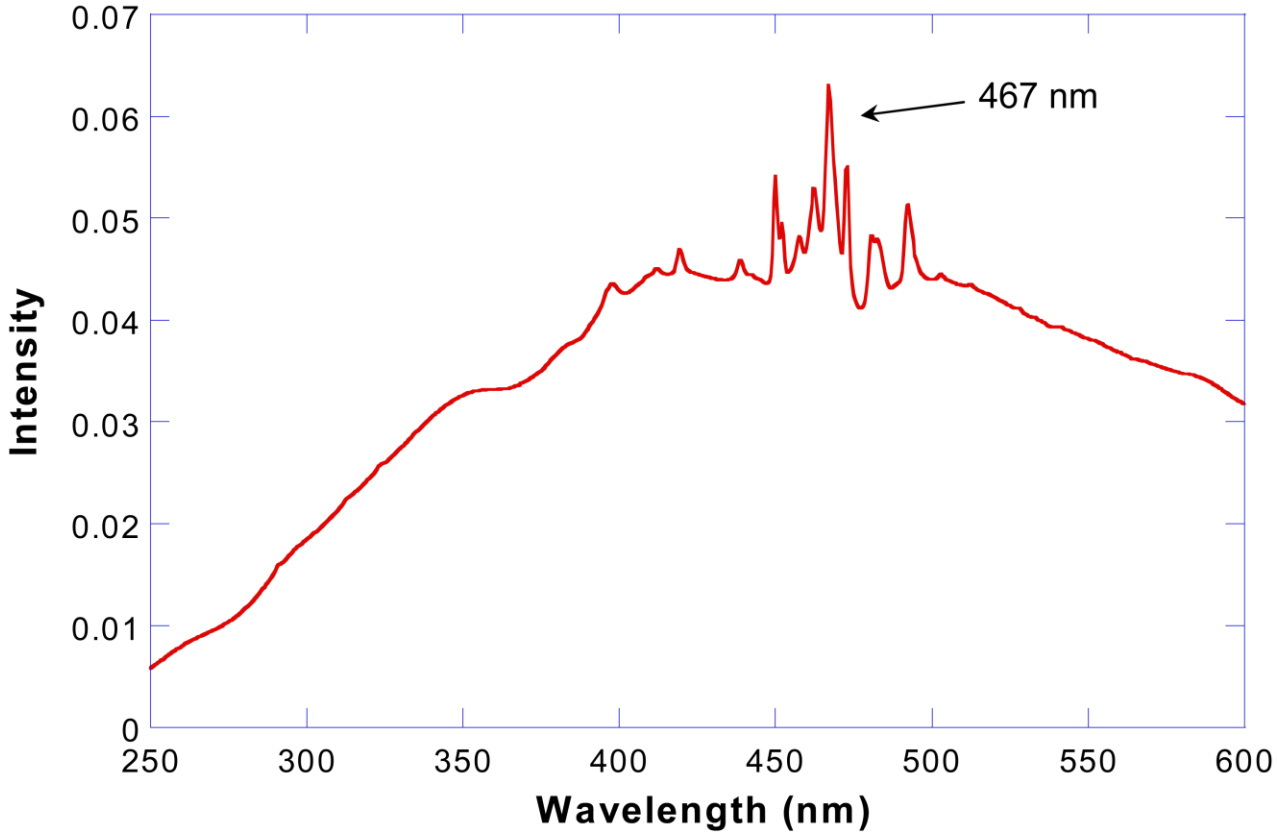
7.1 Using Diagnostic Spectra

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.

7.1.1 Lamp Scan

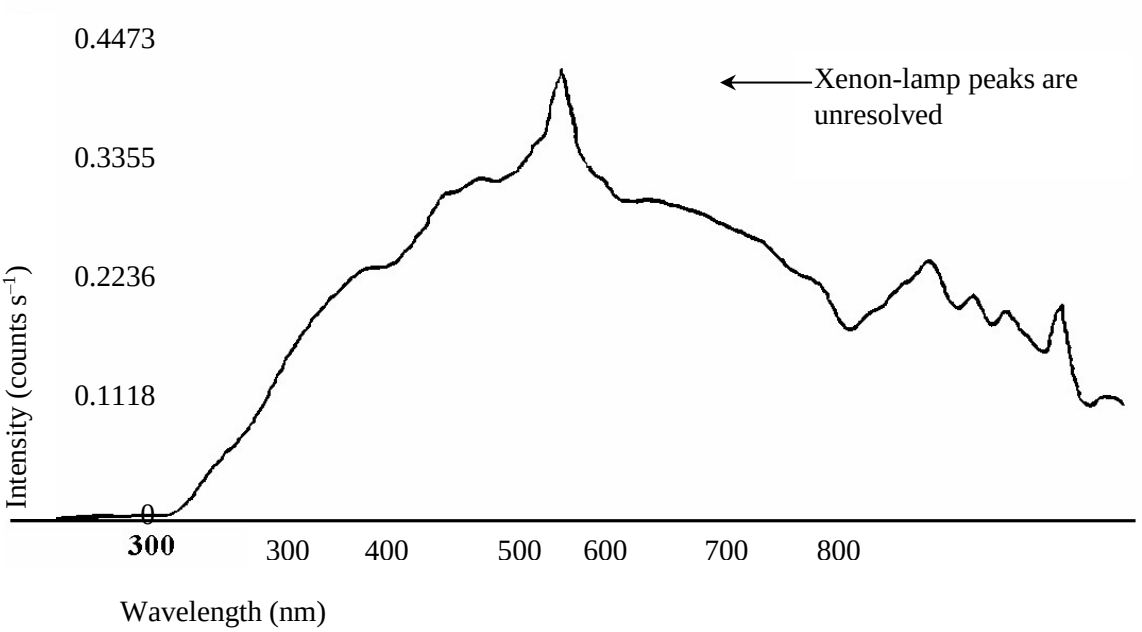
Running a lamp scan verifies system integrity and indicates whether the correct parameters for the best possible trace are being used. The following spectrum shows the trace resulting from a lamp scan run with a known good lamp.

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



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

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



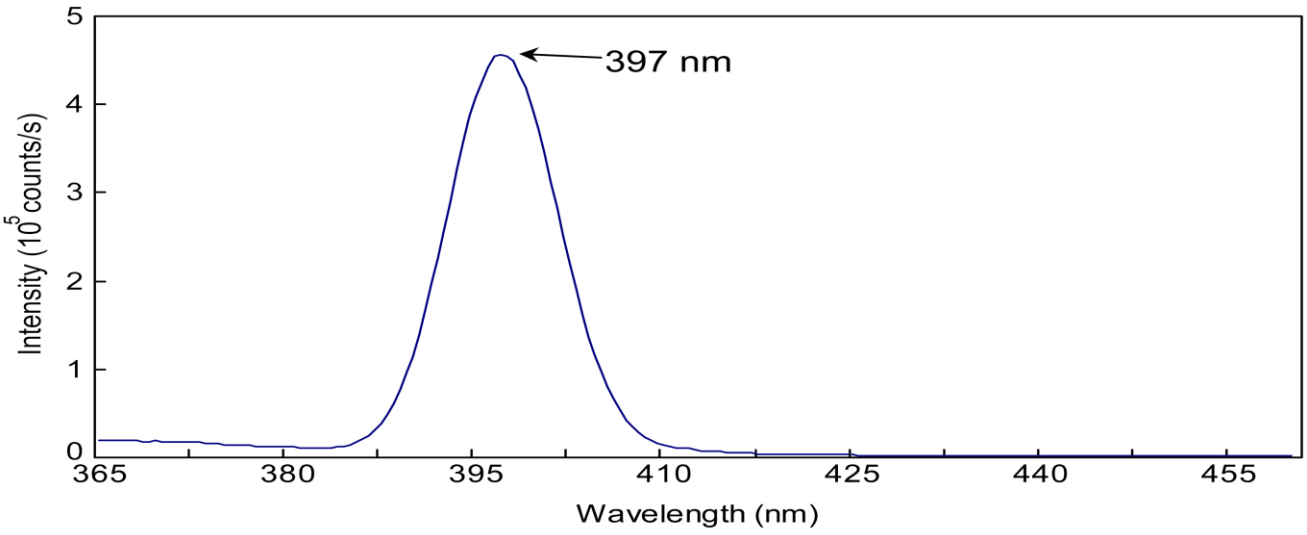
Poor lamp scan of 150-W Xe lamp. Note low resolution in the area near the 467-nm peak.

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

7.1.2 Water Raman Spectra

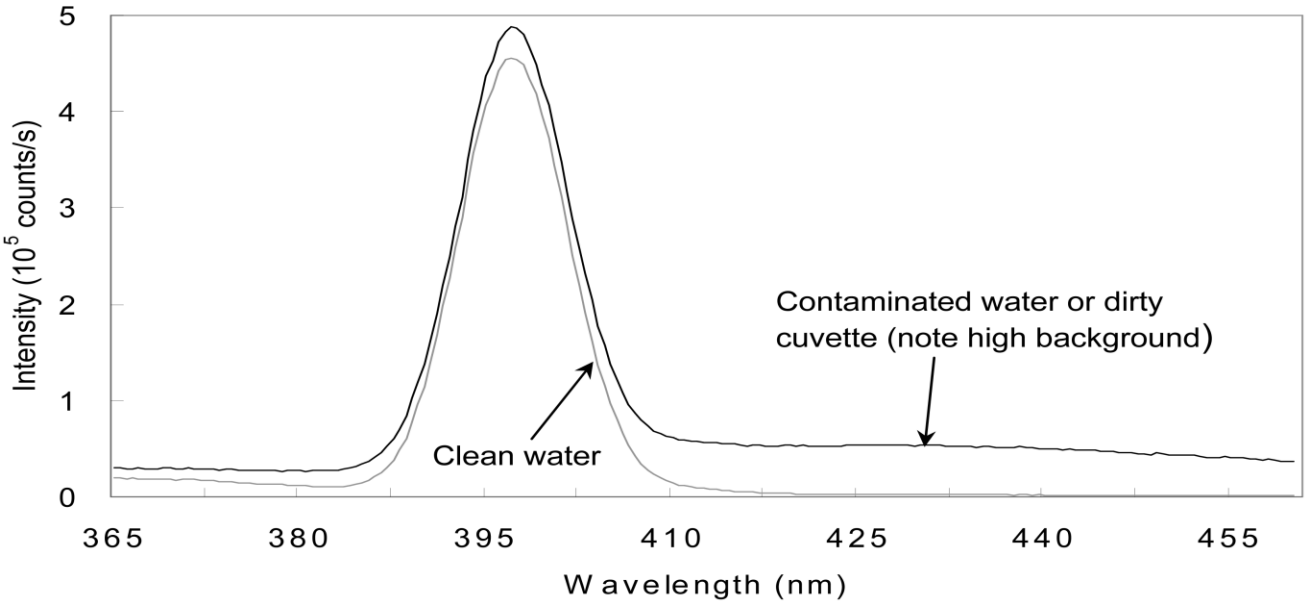
7.1.2.1 Contaminated Water

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.



Contaminated water in a water Raman scan.

In this instance, the water was contaminated, resulting in a high background.

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.

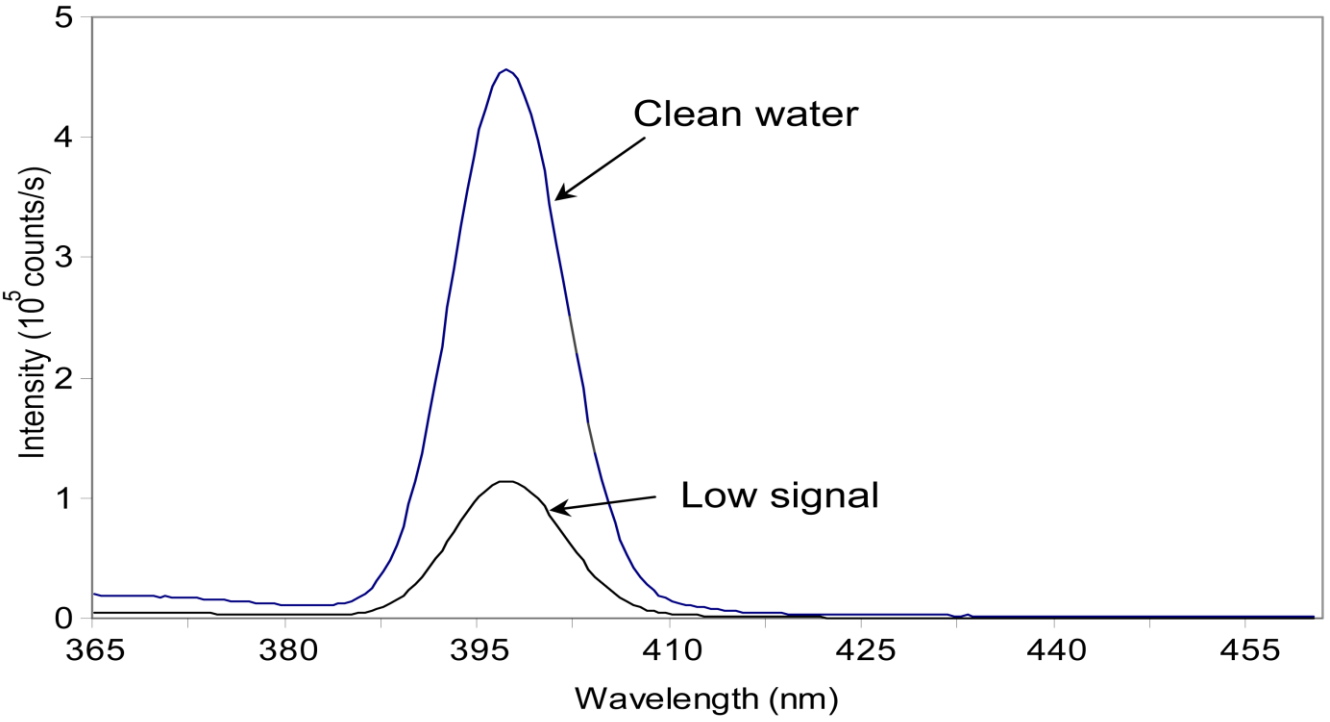
Or

2. Clean the cuvette.
3. Fill with fresh, double-distilled, de-ionized water.

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

7.1.2.2 Light not Striking Cuvette

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



Low intensity during a Raman scan.

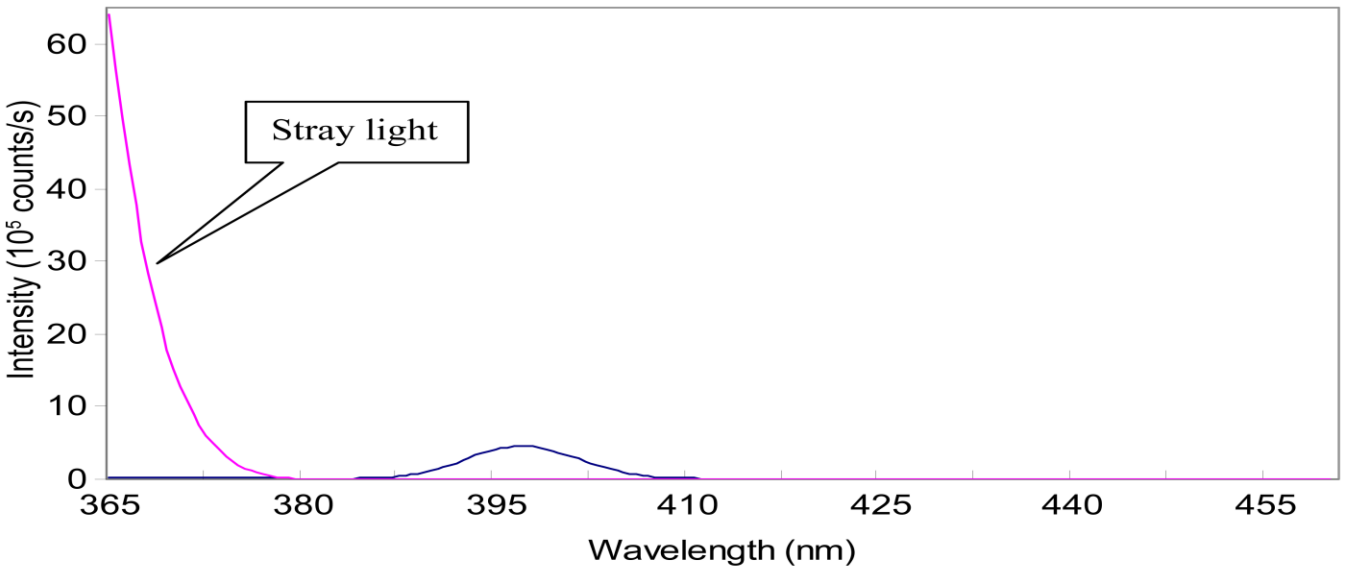
Here the problem is low intensity of the water signal when compared with the superimposed typical water Raman scan. To resolve this problem:

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.

7.1.2.3 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,

a. Inspect the cuvette surface for fingerprints and scratches.
b. Clean the cuvette or use a new one.
c. Verify the excitation and emission slits are set correctly for a water-Raman scan.
d. Verify the excitation monochromator is at the correct position.

7.2 Further Assistance...

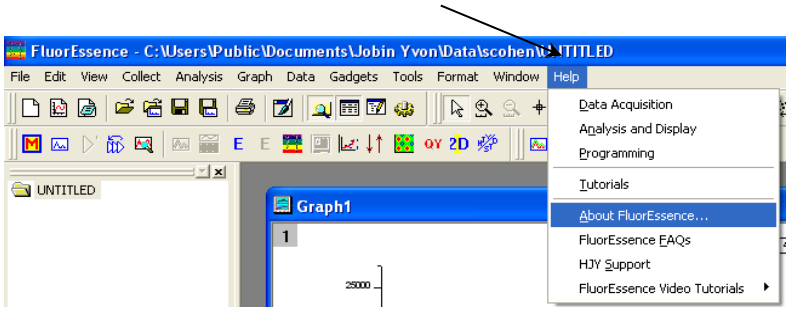
Read all software and accessory manuals before contacting the Service Department. Often the manuals show the problem's cause and a method of solution. Technical support is available for both hardware and software troubleshooting. Before contacting the Service Department, however, complete the following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

1. If this is the first time the problem has occurred, try turning off the system and accessories. After a cool-down period, turn everything back on.
2. Make sure all accessories are properly configured, and turned on as needed.
3. Following the instructions in Chapter 3, System Operation, run a lamp scan and a water Raman scan to make sure the system is properly calibrated.
4. Print the spectrum for each and note the peak intensities.
5. Check this chapter to see if the problem is discussed.
6. 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 problem, a service visit may not be required.
7. If an error dialog box appears in FluorEssence™, write down the exact error displayed.

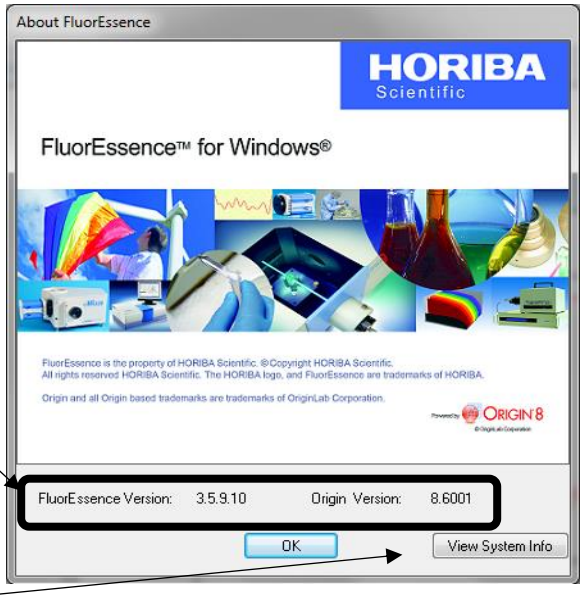
8. In FluorEssence™, in the FluorEssence main window's toolbar, click the Help tab

   A drop-down menu appears.



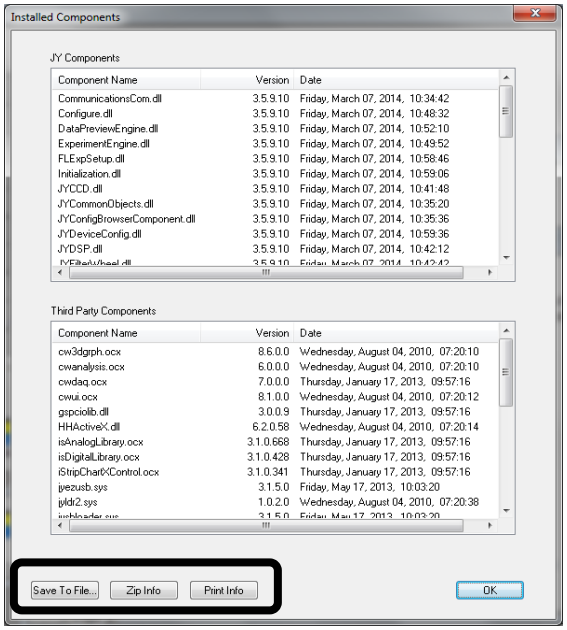
9. Under Help, choose About FluorEssence....

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



10. Click the View System Info button.

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



11. Record the information by clicking the:

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

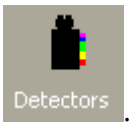
12. Click the OK button to close the Installed Components window.

13. Click the OK button to close the About FluorEssence window.

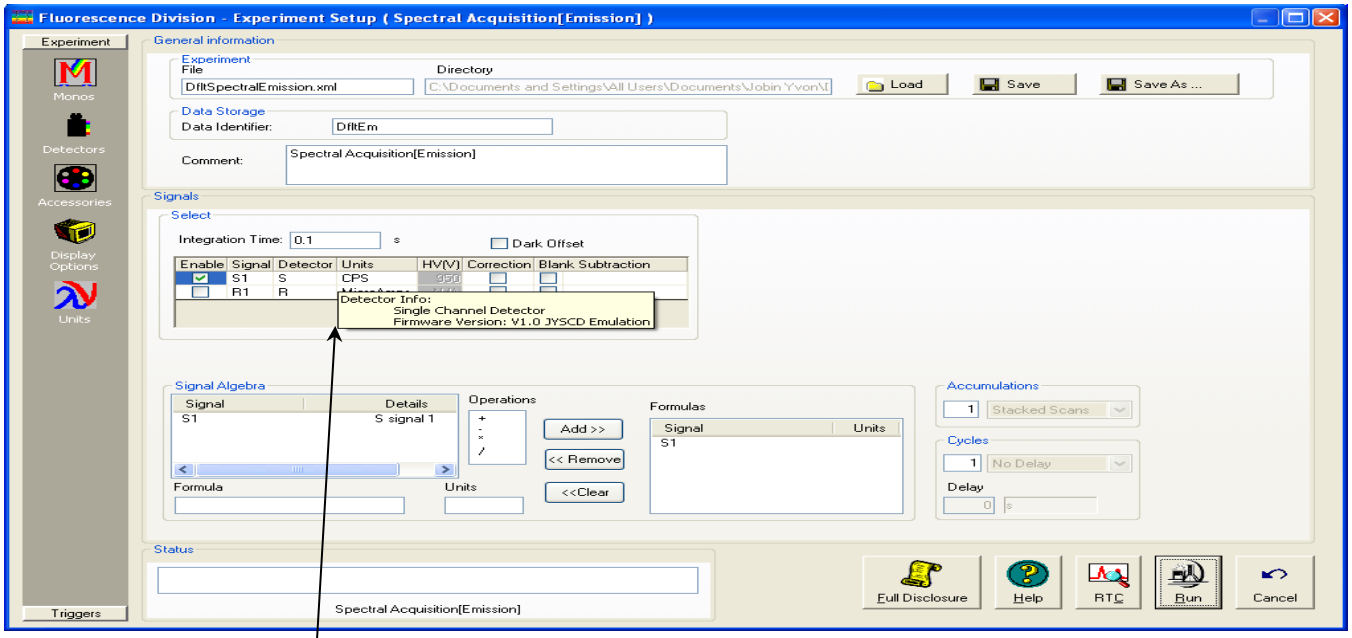
14. Write down the software's version numbers, along with the purchase dates, model numbers, system configuration, and serial numbers of the instrument and its accessories.

15. Determine the SpectrAcq Firmware version:

    a. Open the Experiment Setup window:

    b. Click the Detectors icon 

.



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

If the problem persists or is unlisted, call the Service Department at 1-877-546-7422. Outside the United States, call your local distributor.
You may also reach us by e-mail at fluorescence-service.us@horiba.com.

7.3 Service Information

7.3.1 Service Policy

The equipment is intended to be returned to the manufacturer for servicing and its maintenance to be carried out by qualifying personnel.

For assistance in resolving a problem with the instrument, you may:

- Contact the Customer Service department directly. Often, it is possible to correct, reduce, or localize the problem through discussion with one of our Customer Service engineers.

- If residing outside the United States, contact our representative or affiliate covering your location as indicated below.

- If the problem relates to software, verify the computer's operation by running any diagnostic routines that were provided with it. Also, refer to the software documentation for troubleshooting procedures. If calling Technical Support for assistance, be ready to provide the system's serial number as well as the software version and Firmware version of any controller or interface options in the system. The software version can be determined by selecting the software name at the right end of the menu bar and clicking the "About" option.

IN THE USA	IN FRANCE	WORLDWIDE
HORIBA Instruments Incorporated	HORIBA France SAS	China, Beijing: +86(0)10 21 8567 9966
20 Knightsbridge Road	16-18 rue du Canal	Germany, Dresden: +49 (0) 6251 8475 77
Piscataway, NJ 08854	91165 Longjumeau Cedex	Italy: +39 651 59 22 1
USA	France	Japan, Kyoto:+81 75 321 7184
Tel: 1-877-546-7422	Tel: +33(0) 1 69 74 72 02	UK, Northampton:+44 (0) 1604 542 500
Fax: 732-494-8796	Fax: +33 (0)1 69 09 07 21	
Email: fluorescence-service.us@horiba.com	Email: services.hfr@horiba.com	

If an instrument or component must be returned, the method described below should be followed to expedite servicing and reduce downtime.

7.3.2 Return Authorization

All instruments and components returned to the factory must be accompanied by a Return Authorization Number issued by our Customer Service department.

In order to obtain a Return Authorization number (RMA) from the Customer Service department, the following information must be provided:

- Model and serial number of the instrument
- List of items and/or components to be returned
- A description of the problem, including operating settings
- The instrument user's name, mailing address, telephone, and fax numbers
- Return address
- Purchase order number and billing information for non-warranty services
- Original sales order, if known
- Customer account number, if known
- Special instructions

7.3.3 Warranty

The terms are as follows:

- For any item sold by Seller to Buyer or any repair or service, Seller agrees to repair or replace without charge to Buyer for labor or materials or workmanship of which Seller is notified in writing before the end of the applicable period set forth below.

  - New equipment, product, and laboratory apparatus: 1-year with the following exceptions:
    - Computer and Peripherals

- Glassware and Glass Products

- Repairs, replacement, or parts: The greater of 30 days and the remaining original warranty period for the item that was repaired or replaced.

- Installation Services: 90 days

- The above warranties do not cover components manufactured by others and which are separately warranted by the manufacturer. Seller shall cooperate with Buyer in obtaining the benefits of warranties by manufacturers of such items but assumes no obligations with respect thereto.

- All defective items replaced pursuant to the above warranty become the property of the seller.

- This warranty shall not apply to any HORIBA Instruments Incorporated manufactured components that have been repaired, altered, or installed by anyone not authorized by HORIBA Instruments Incorporated in writing.

- This warranty shall not apply to any components subjected to misuse due to common negligence, adverse environmental conditions, accident, or any components not operated in accordance with the printed instructions in the operations manual. Labor, materials, and expenses shall be billed to the Buyer at current rates for any repairs or replacements not covered by this warranty.

- The limitation on consequential damages set forth is intended to apply to all aspects of this contract including without limitation Seller's obligations under these standard terms.

The terms above shall constitute complete fulfillment of all liabilities of Seller, and Seller shall not be liable under any circumstances for special or consequential damages, including without limitation loss of profits or time or personal injury caused.

THE ABOVE WARRANTIES AND ANY OTHER WARRANTIES SET FORTH IN WRITING HEREIN ARE IN LIEU OF ALL OTHER WARRANTIES OR GUARANTEES EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR PURPOSE OR TIME OR PERSONAL INJURY CAUSED.

8 PRODUCING CORRECTION FACTORS

8.1 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® Plus 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 240–850 nm, and the correction range for emission spectra is from 290–850 nm.

8.2 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.

8.2.1 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 Service Department. To arrange for a visit and a fee estimate, call the Service Department. To update the correction factors without a service visit, call the 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 light-scattering assembly. HORIBA Scientific offers the F-3026 Standard Lamp Accessory.

The F-3026 Standard Lamp Assembly is a complete correction-factor kit, which includes the following items:

- Calibrated lamp
- Irradiance values for the calibrated lamp
- Power supply
- Neutral-density filter
- Sample-compartment cover

8.3 Calculating Emission Correction Factors

8.3.1 Introduction

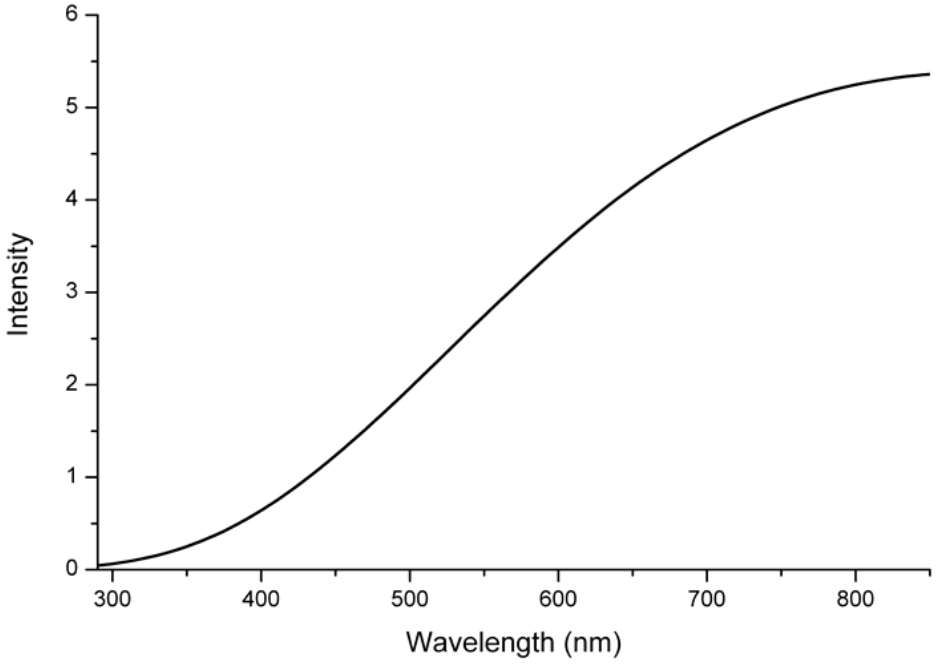
For more information about the theory and application of radiometric correction, consult *Accuracy in Spectrophotometry and Luminescence Measurements*, Mavrodineau, Schultz, and Menis, NBS Spec. Publ. 378 (1973), especially p. 137, "Absolute Spectro fluorometry," by W.H. Melhuish.

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®+ 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.

Load the irradiance file supplied by HORIBA Scientific by opening the FluorEssence™ software, choose File in the toolbar, choose Import, and then choose SPC file.

    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®+.

2. Calculate the correction factors.

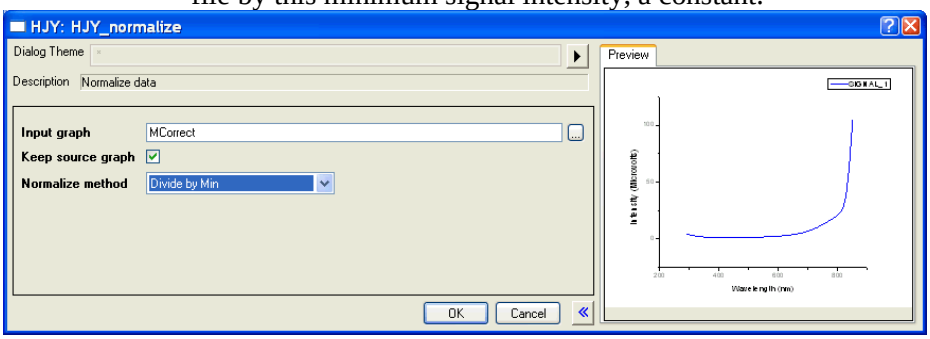
    Using the Simple Math Tool window under the Analysis/HJY Tools menu, divide IRR by stdlamp2, and name the resulting file mcorrect:

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

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

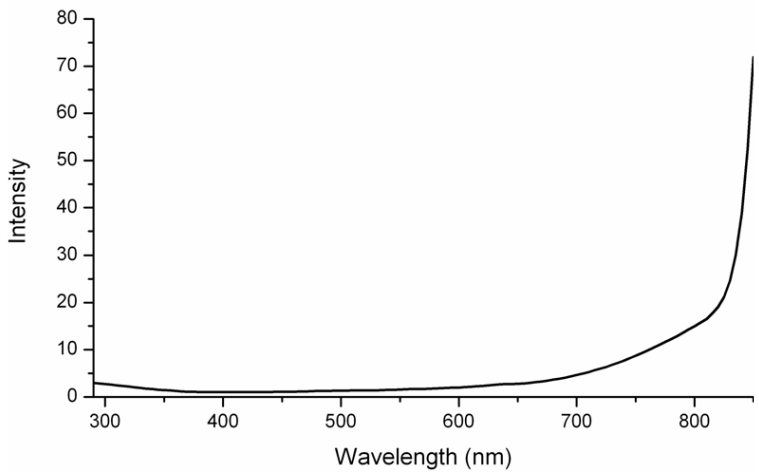
3. Normalize the new mcorrect file.

    a. Using the HJY_normalize window under the Analysis/HJY Tools menu, divide the mcorrect file by this minimum signal intensity, a constant.



    b. 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:



Once the emission correction factors are 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.

8.3.2 Emission Correction Factor

1. Remove the Sample Drawer from the sample compartment.

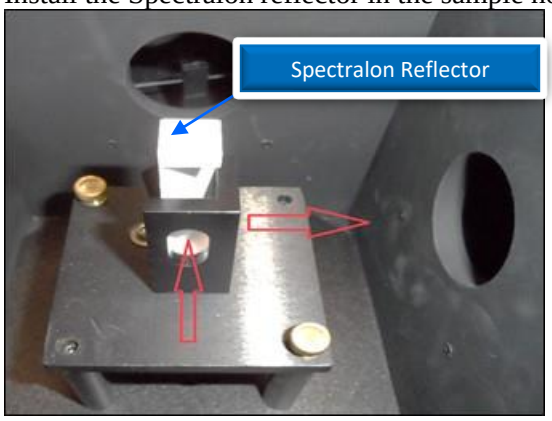
   It is not necessary to close the software or turn off the instrument to do this step.
2. Gather the lamp-housing drawer reserved for this purpose.

3. Mount the calibrated tungsten lamp onto the lamp-housing drawer.

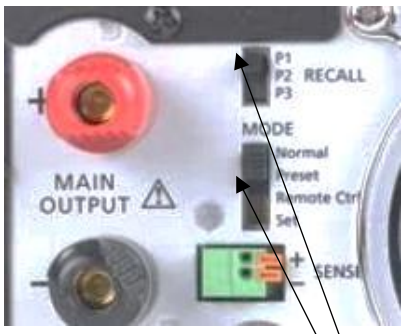
4. Install the drawer and secure in place.

   Fix the drawer firmly to the sample compartment with screws through the front panel.

5. Install the Spectralon reflector in the sample holder.



6. Install the Delrin pinhole on the emission entrance.

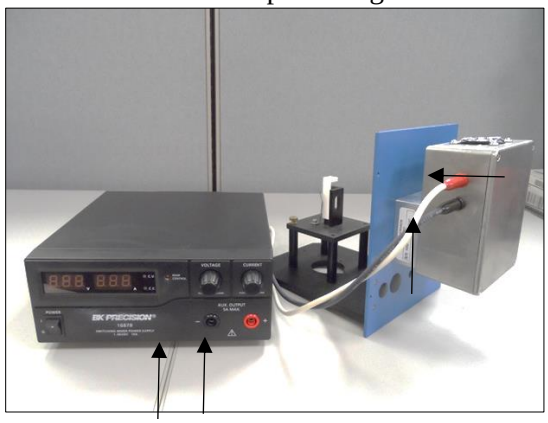


7. Verify switches on the back of the power supply are set correctly before connecting it to an electrical outlet.

   The switches on the back must be set as follows:
   Recall = P1
   Mode = Preset

   In the preset mode, the front panel current and voltage knobs are locked out to prevent a sudden increase in voltage. Any other settings can damage the lamp.

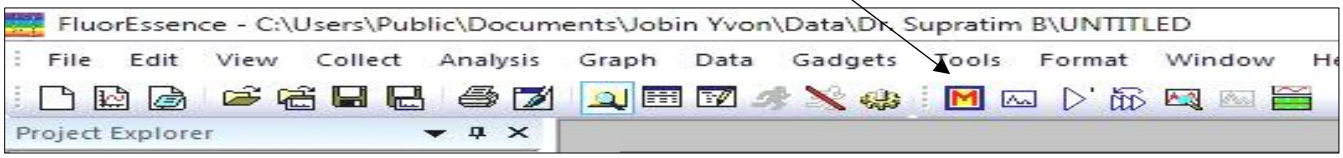
8. Use the color coded cables to connect the power supply (front panel bottom right) to the connectors on the side of the lamp-housing drawer.



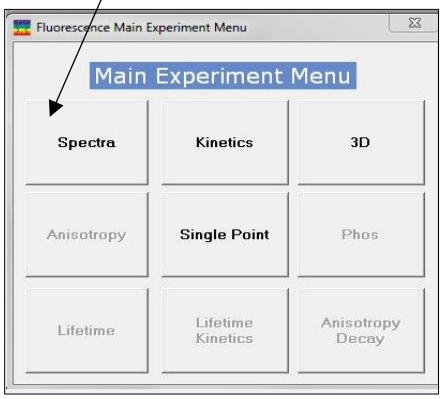
9. Turn on the power supply. Wait for 5 minutes for the temperature in the lamp-housing to stabilize before starting a scan.

8.3.3 Run an Emission Scan.

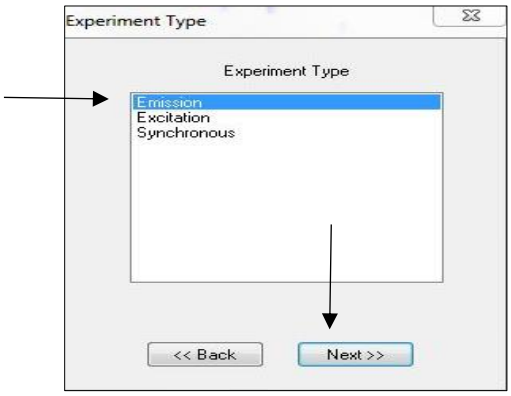
1. Start FluorEssence™ and click the M icon to initialize the system.



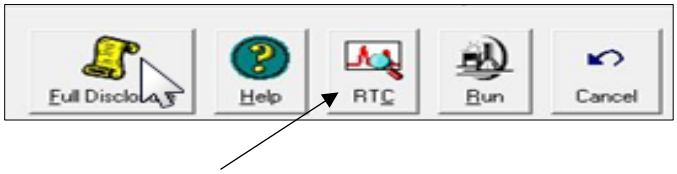
2. Click the “Spectra” box.



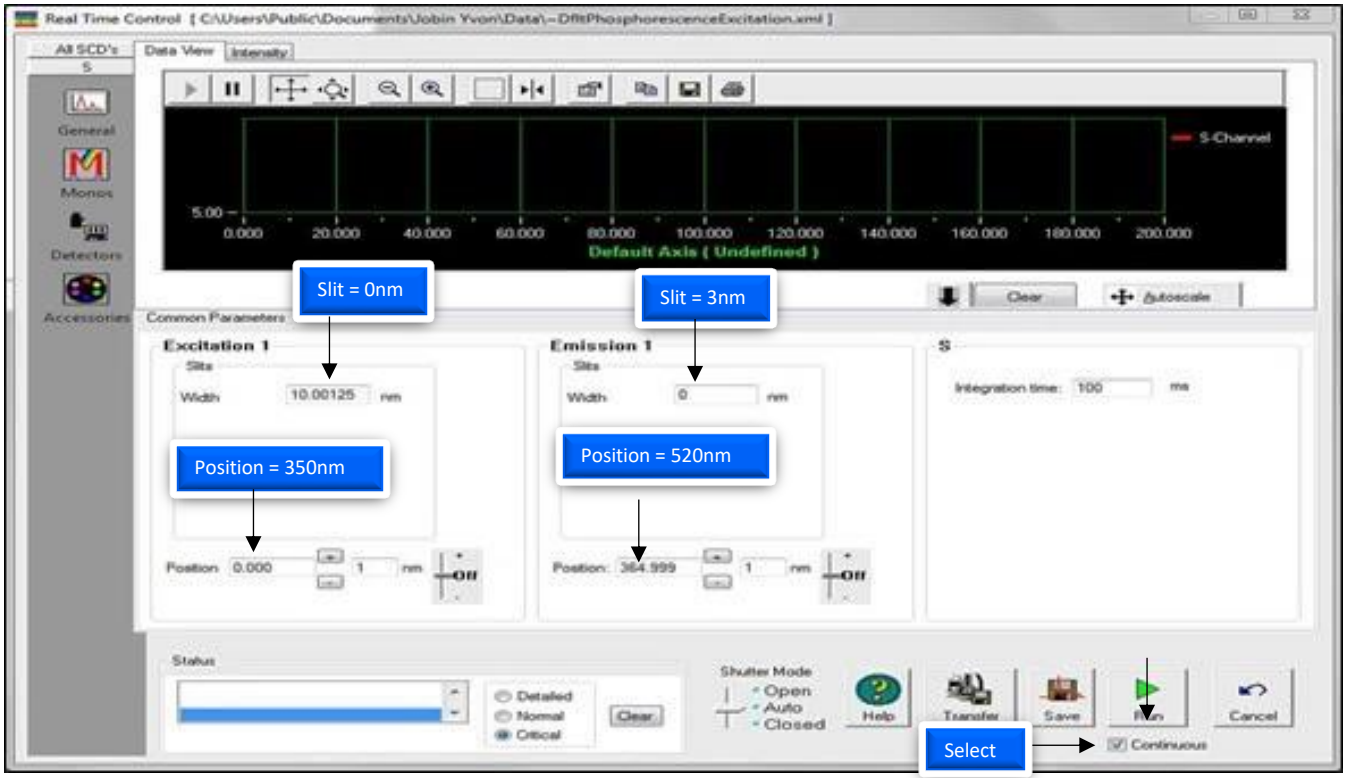
3. Select the emission option and click the Next button.



4. Click the RTC icon at the bottom of the screen.

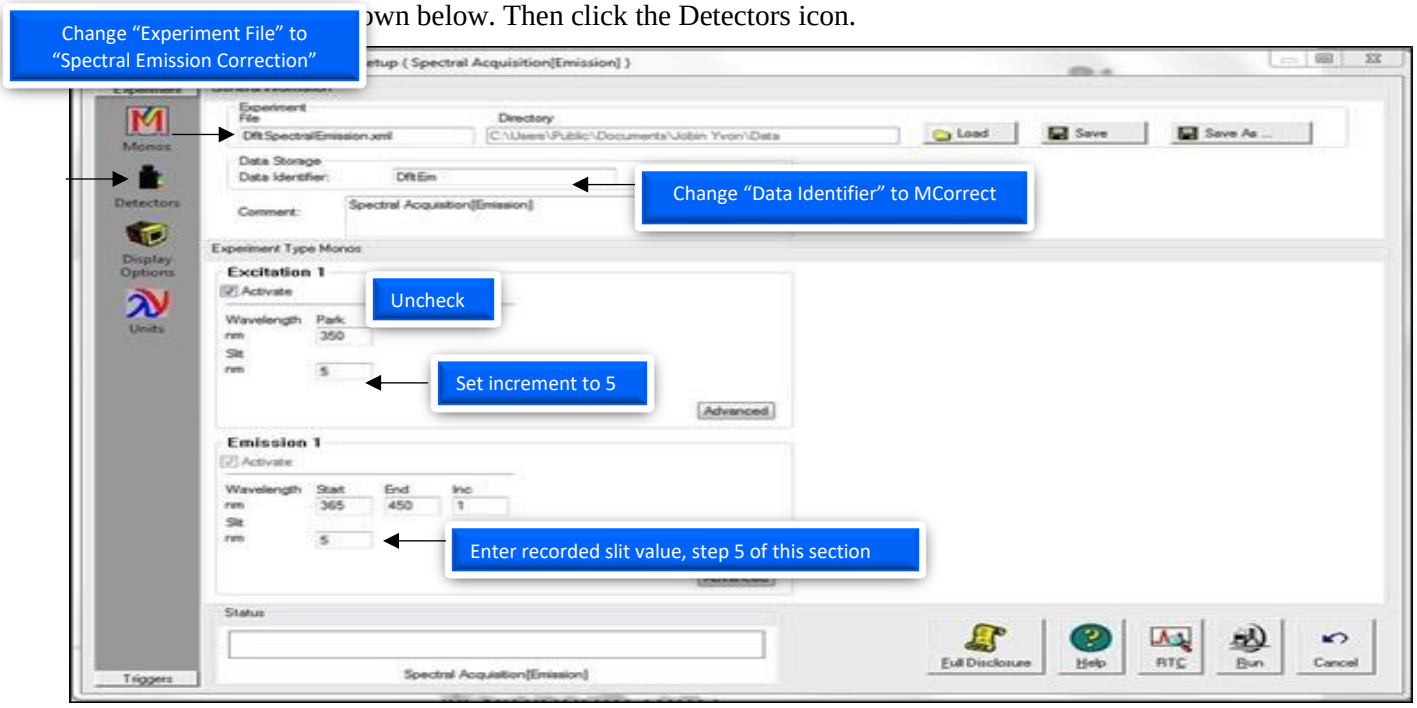


5. Set the excitation and emission parameters as shown below. Click the Continuous box and click the Run icon. Change the emission slit size and repeat the experiment until an intensity of one million cps is achieved. Record the slit value.

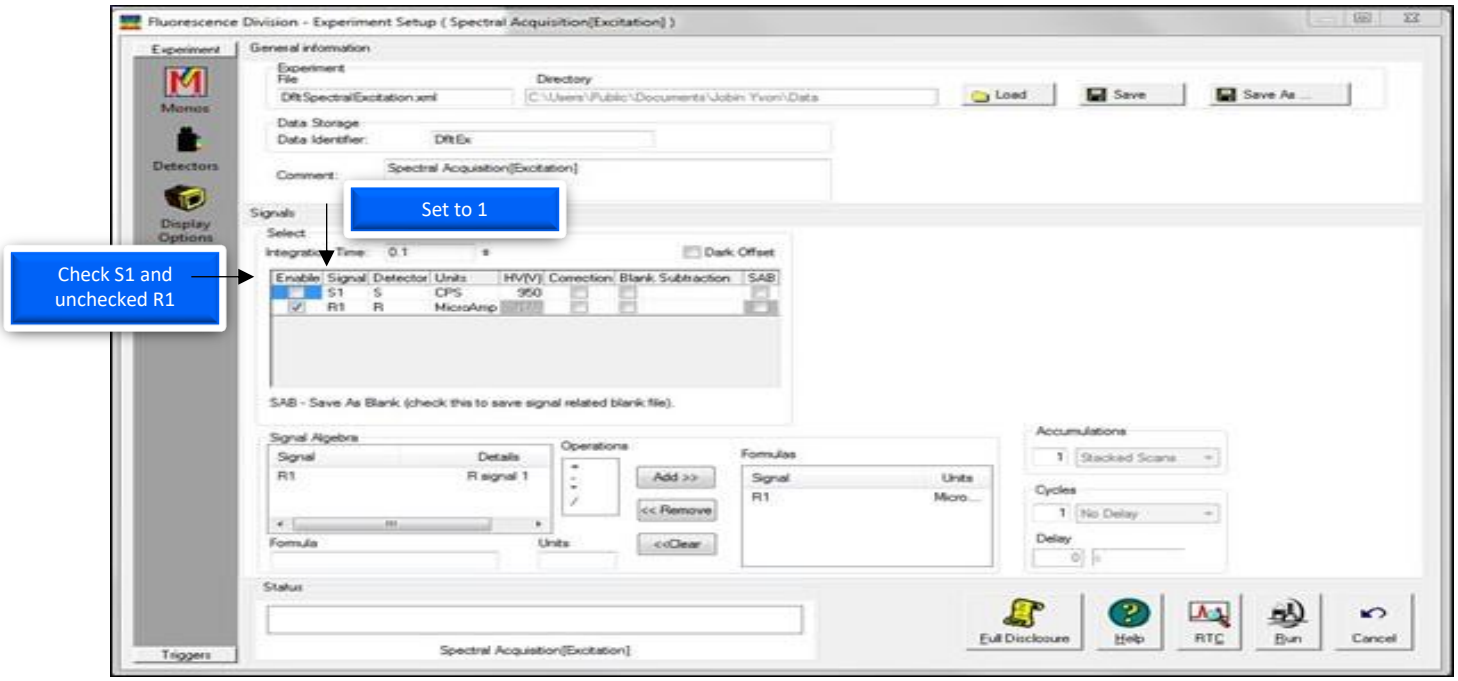


6. Click the experiment window, M icon.

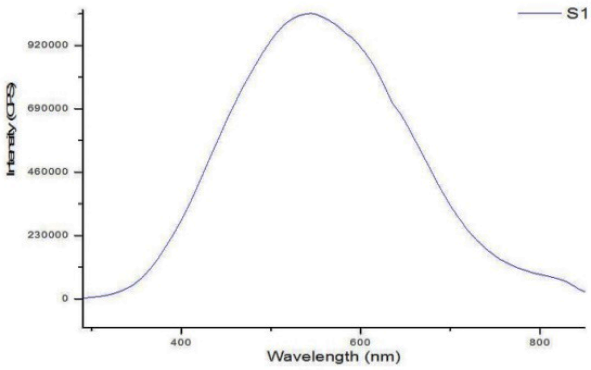
7. Choose the starting/ending wavelength position based on the range of the detector and set the own below. Then click the Detectors icon.



8. Change the integration time to 1.0, check the S1 to enable it, and uncheck the R1. Then, click the Run icon.



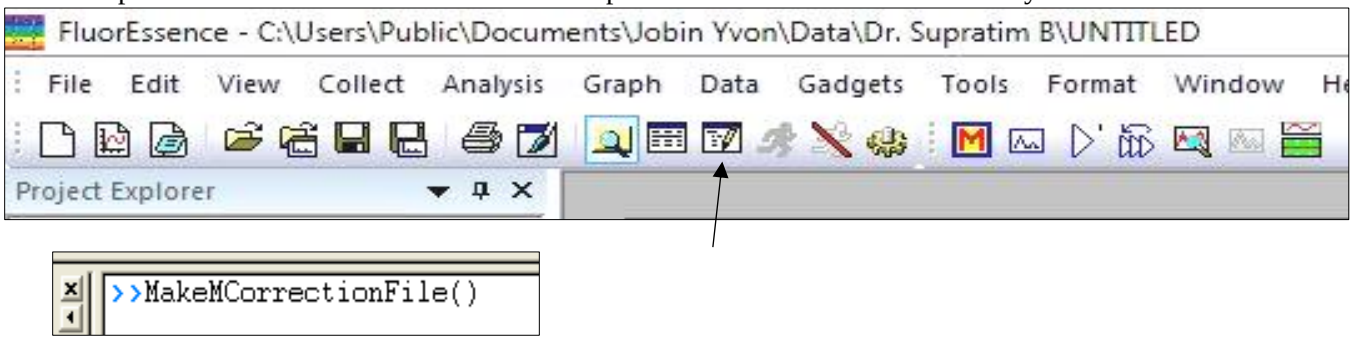
9. The following graph appears.



Example of FluoroMax Emission Correction Scan

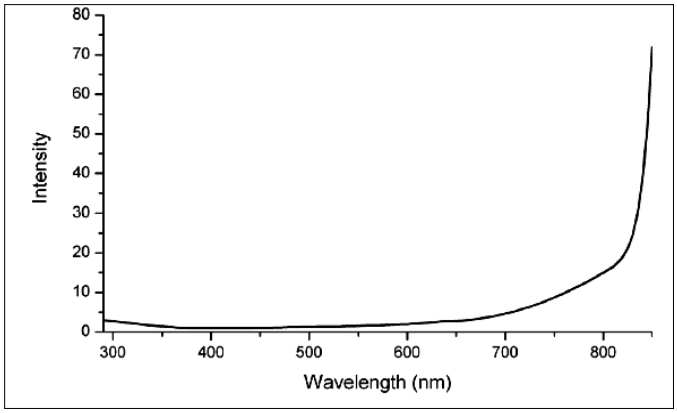
8.3.4 Analyze the Data

1. Open the Command window. Enter the script shown below and hit the enter key.



2. Select the file from the USB stick that corresponds to the lamp being used (ensure file is in Quanta units). For example, EX>SN2677-1009.SPC

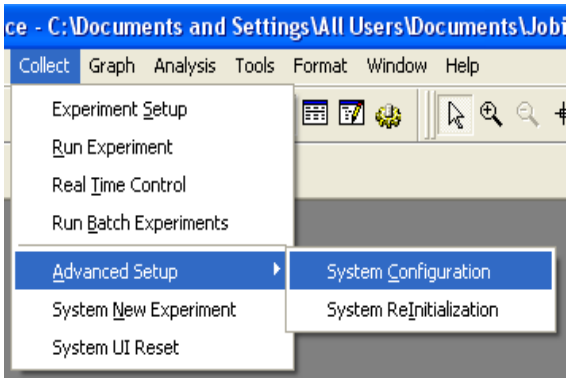
3. Calculations are made and the graph shown in step 9 is converted to the graph shown below.



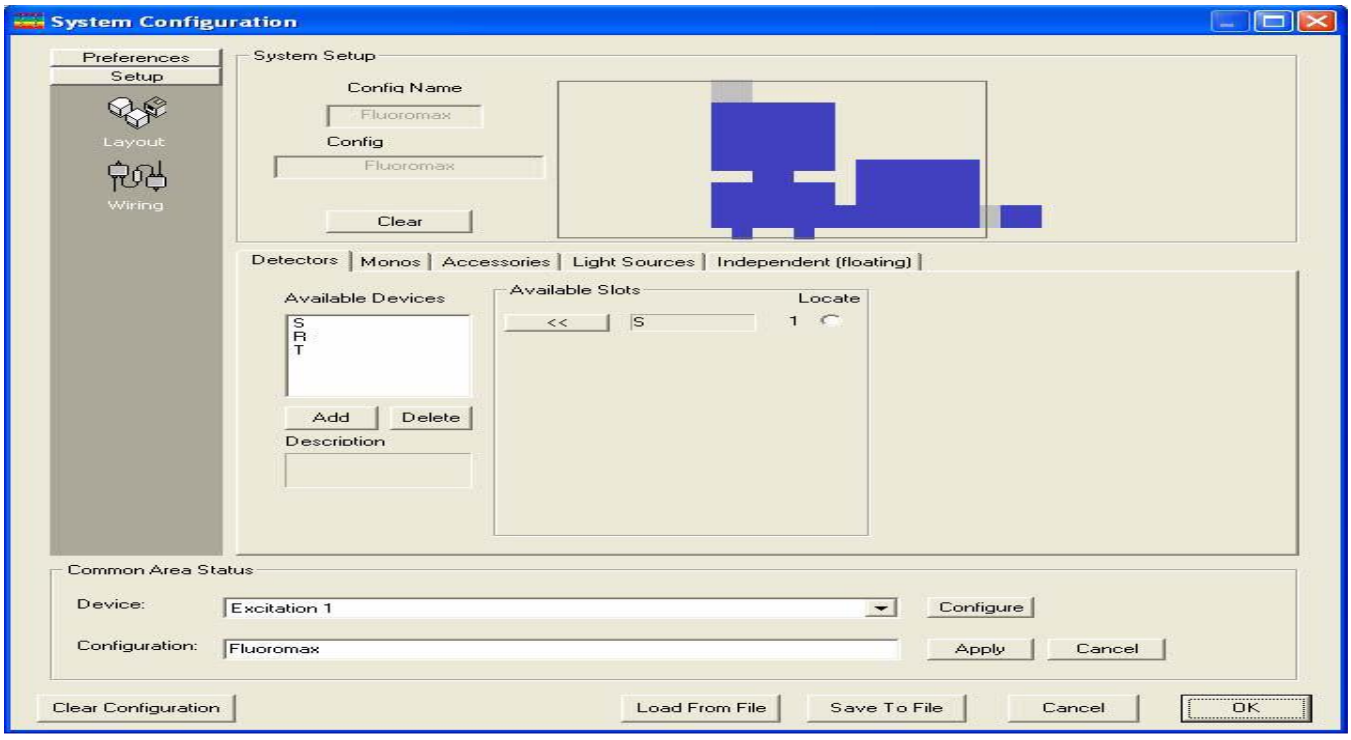
Example of FluoroMax Emission Correction Scan

8.3.5 Add New Correction File to Existing System Configuration

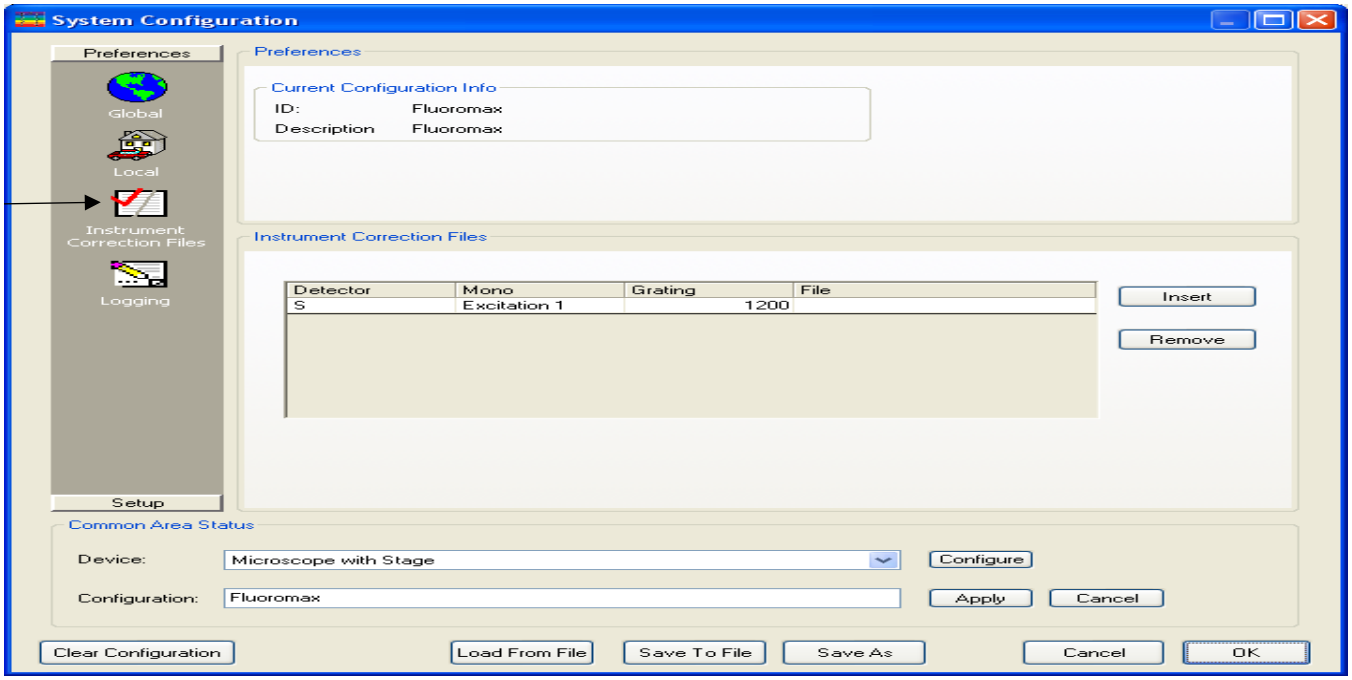
1. In the FluorEssence toolbar, click the Collect tab and select Advanced Setup followed by the System Configuration option.



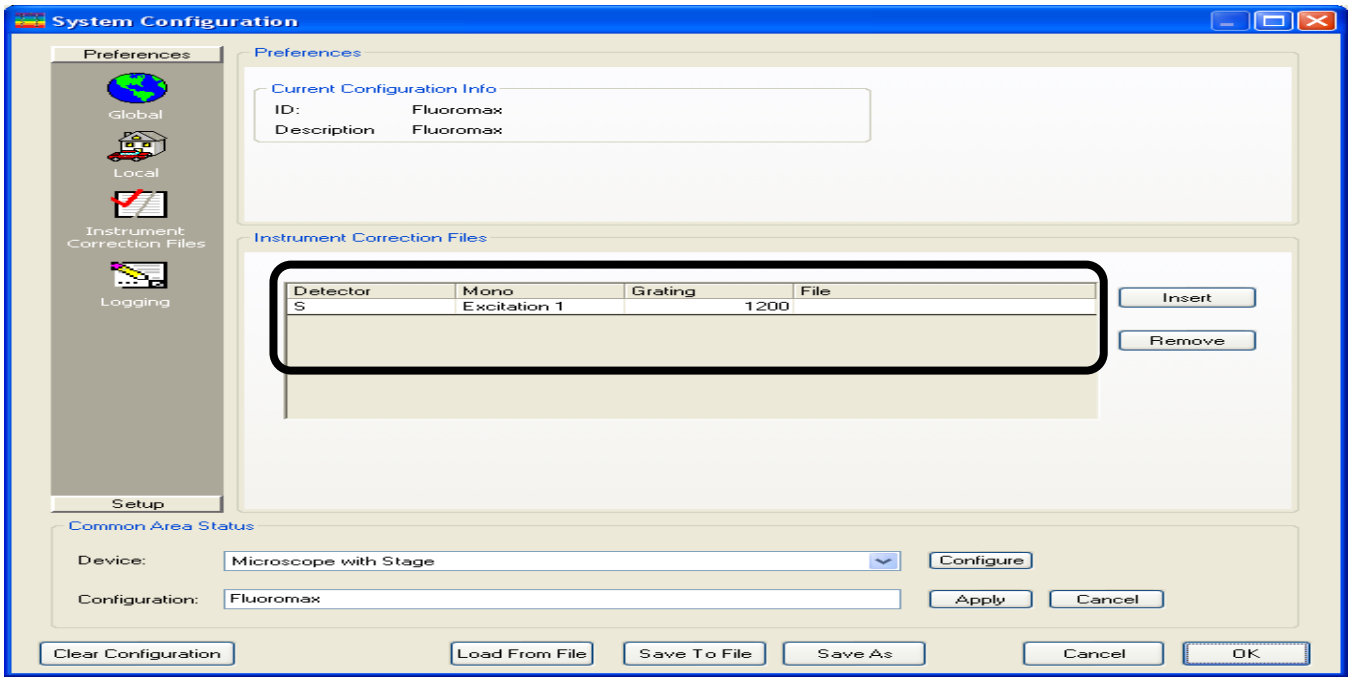
2. Click the Preferences button.



3. Click the Instrument Correction Files icon.

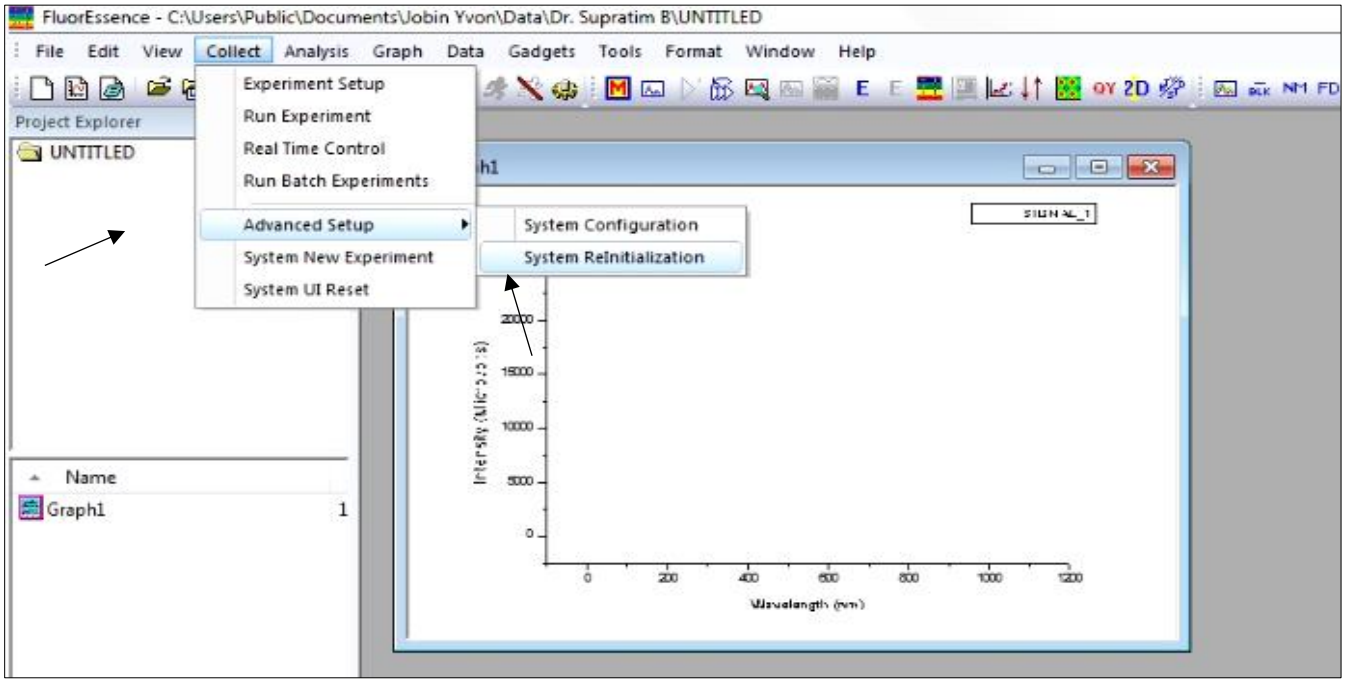


4. Replace the old MCorrect file by clicking the Browse button under the file column. Find the MCorrect.SPC saved before. Then click the OK button.

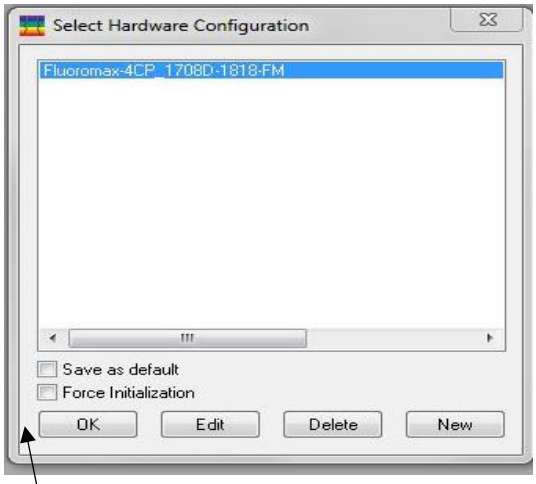


8.3.6 Reinitialize the System

1. On the FluorEssence toolbar, click the Collect tab and select the Advanced Setup followed by the System Configuration option.



2. Click the Force Initialization box and click the OK button.



3. Turn off the BK power supply.

4. Disconnect the color coded cables from the lamp housing drawer and power supply.

5. Remove the Lamp Housing and reinstall the single-cell drawer.

8.4 Excitation Correction Factors

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

9 FLUOROMAX®PLUS PHOSPHORIMETER OPERATION

9.1 Introduction

The FluoroMax® Plus-P includes an optional 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® Plus-P is identical in operation in all other respects to the FluoroMax® Plus.

9.2 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.

9.2.1 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:

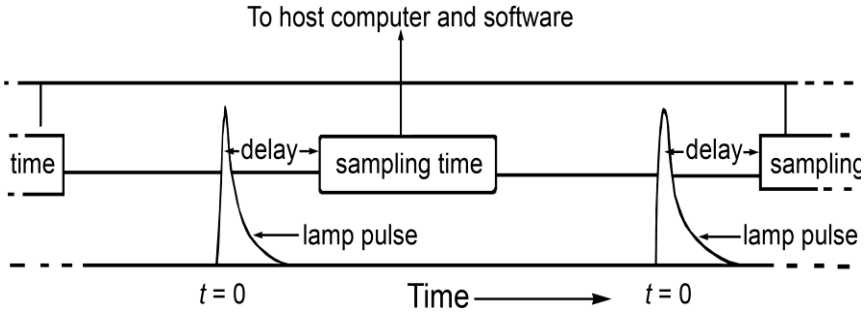
INTEGRATION TIME	MAXIMUM SIGNAL (COUNTS) PER FLASH
100 µs	200
1 ms	2000
10 ms	20 000
100 ms	200 000
1 s	2 000 000

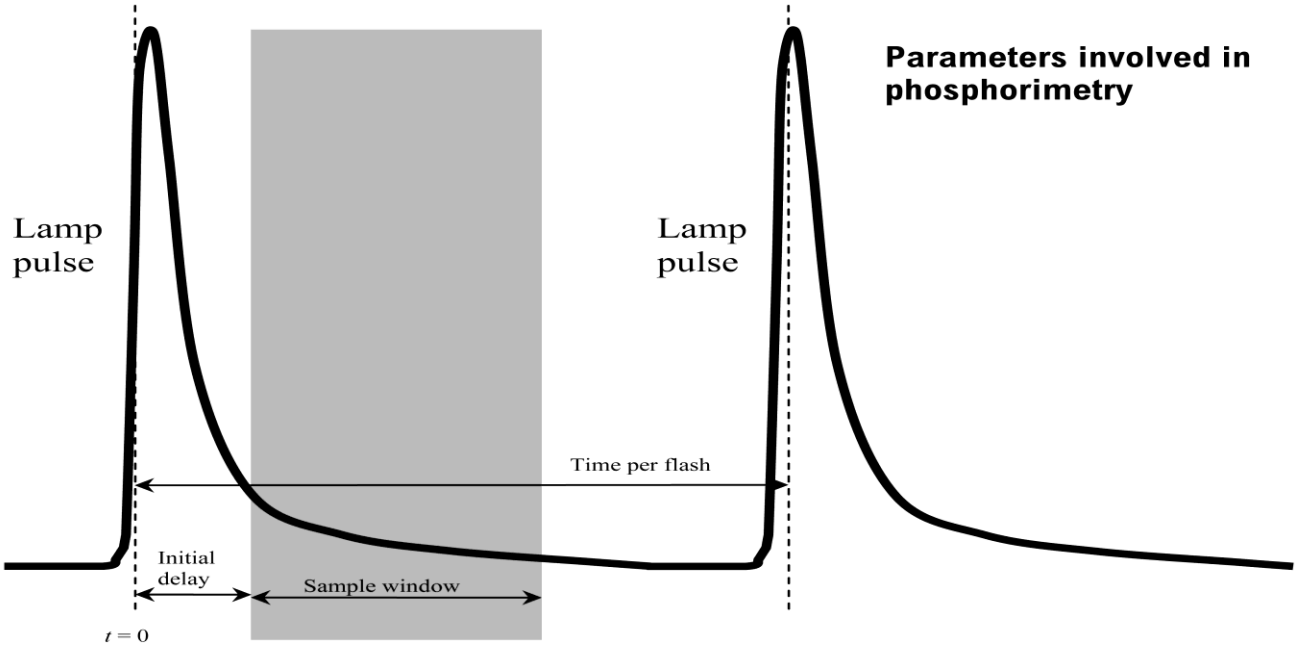
With, for example, the counts integrated over 10 ms, the maximum detectable signal is:

(1 second/integration time) × (counts/flash) = total counts

(1 second/0.01 second) × (20 000 counts/flash) = 2 000 000 counts total

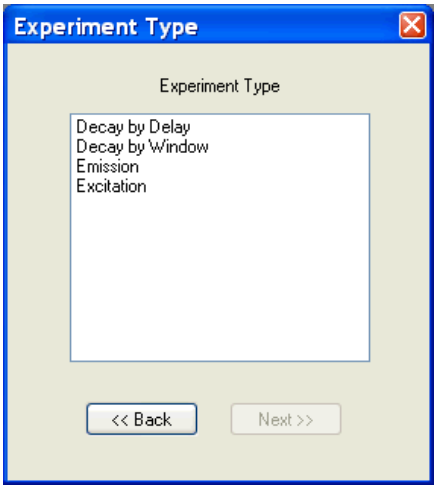
A typical sequence of data-acquisition (see below) starts with a flash from the pulsed lamp, sensed by the control module as time *t* = 0. The light enters the excitation monochromator, where it is dispersed. Monochromatic light from the monochromator excites the sample. Luminescence emission from the 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 sampling time (the 0). Any signal arriving before or after the gating is ignored.





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:

- Decay by Delay
- Decay by Window
- Emission
- Excitation



9.2.2 Phosphorimeter Parameters

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

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 missing. 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.

In a Decay by Delay type of scan, the Time per flash is governed by

Time per Flash ≥ Maximum Delay + Sample Window + 20 ms

In a Decay by Window type of scan, the Time per Flash is governed by

Time per Flash ≥ Initial Delay + Maximum Sample Window + 20 ms

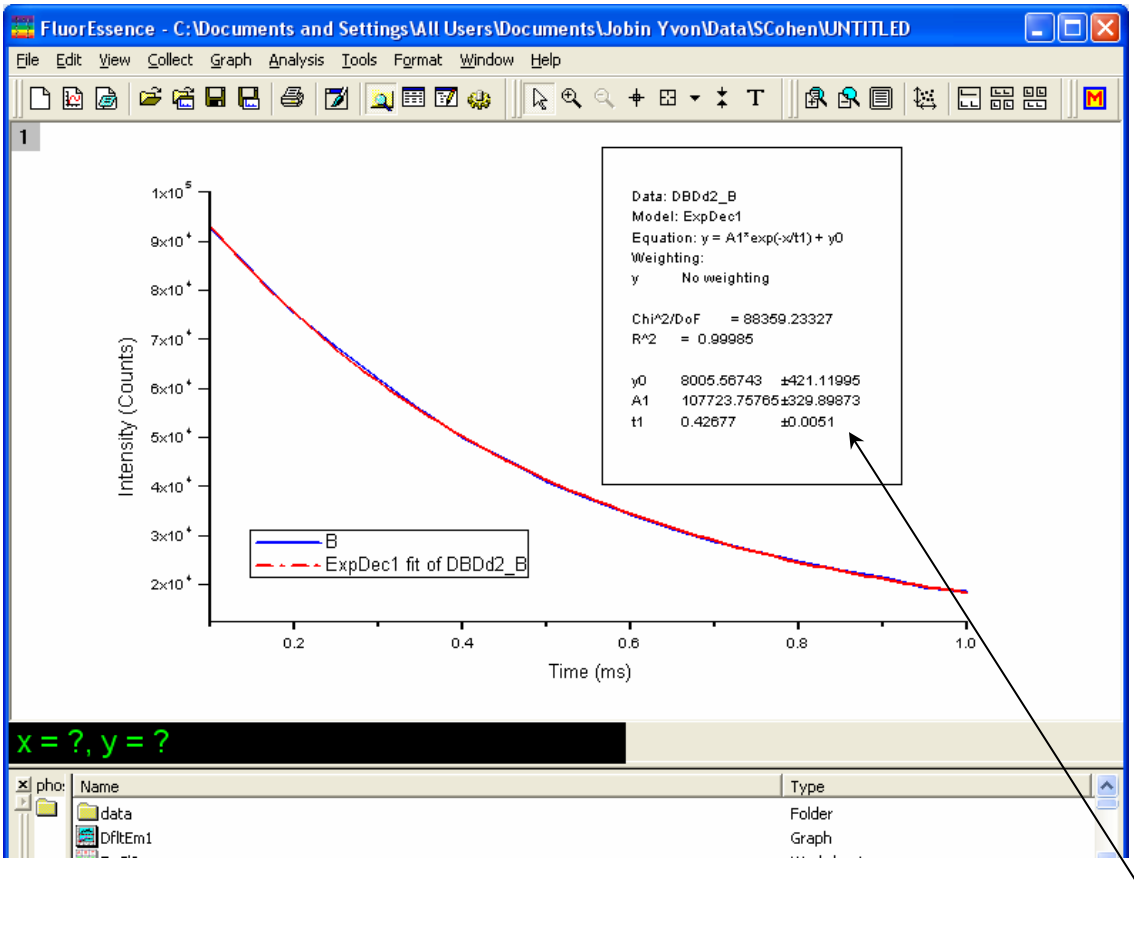
Flash count

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.

9.3 Applications for the Phosphorimeter

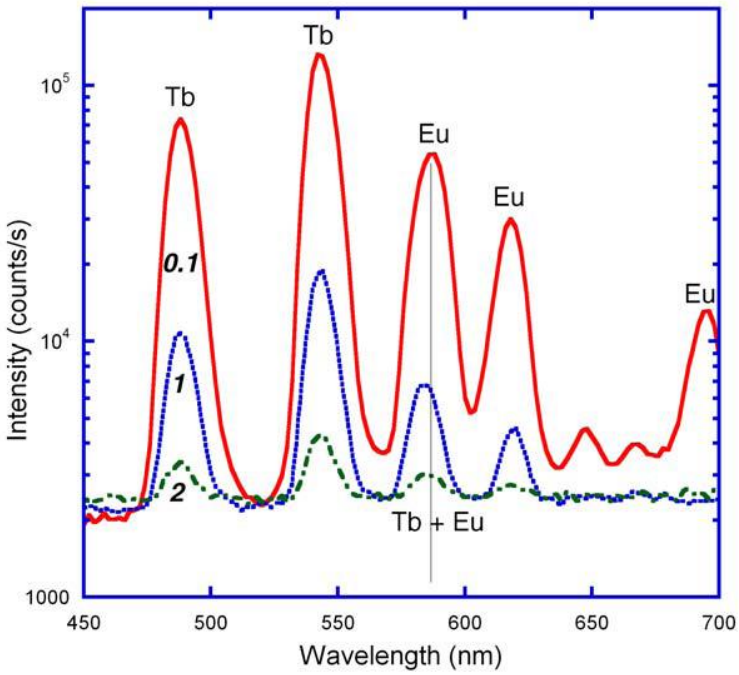
9.3.1 Phosphorescence Decay Curve



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.

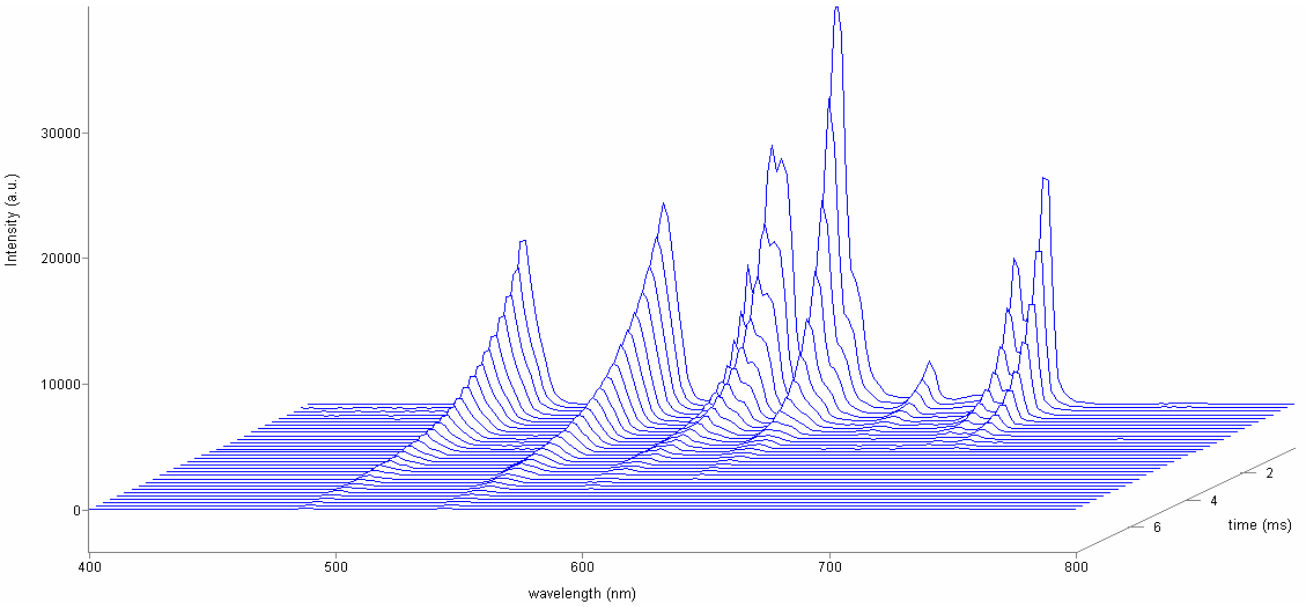
9.3.2 Isolate Components in a Mixture Based on Lifetimes

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.



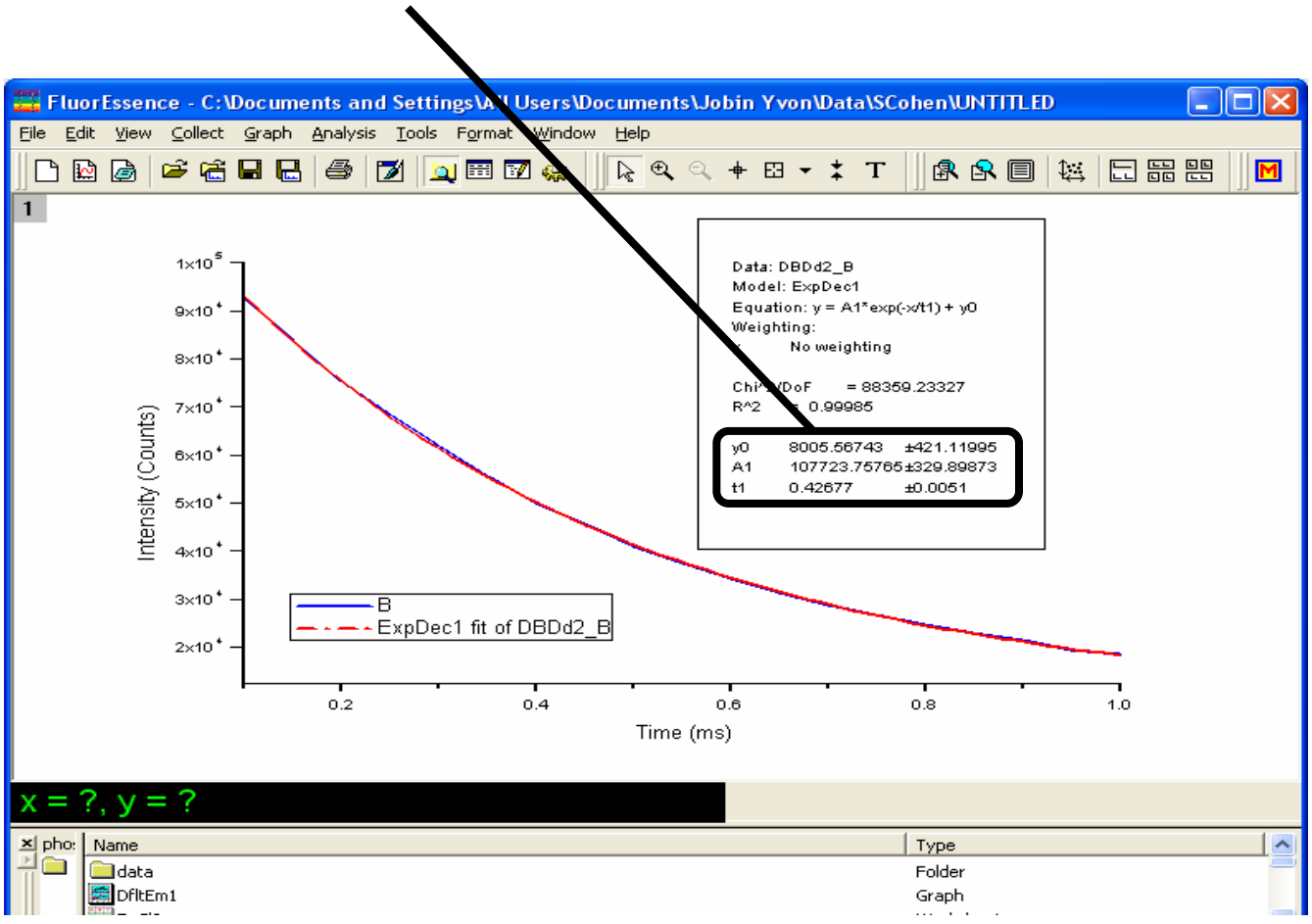
9.3.3 Three-Dimensional Plots

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.



9.3.4 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.



9.4 Operation of the Phosphorimeter

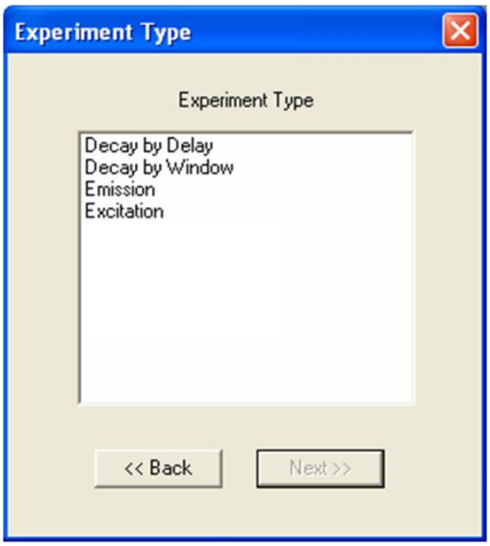
9.4.1 Start-up

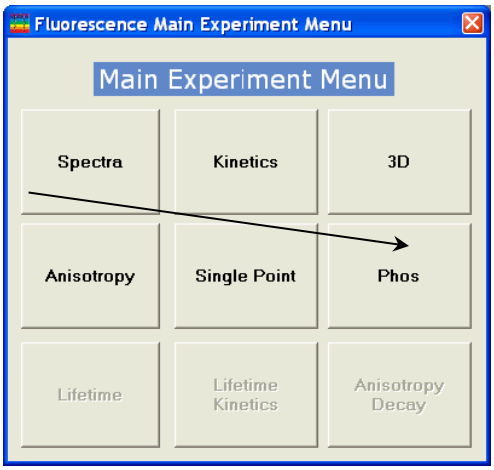
Load an appropriate instrument configuration that includes the phosphorimeter.

9.4.2 FluorEssence™ Features

9.4.2.1 Main Experiment Menu

This is where you choose to scan with the phosphorimeter, via a Phos scan:



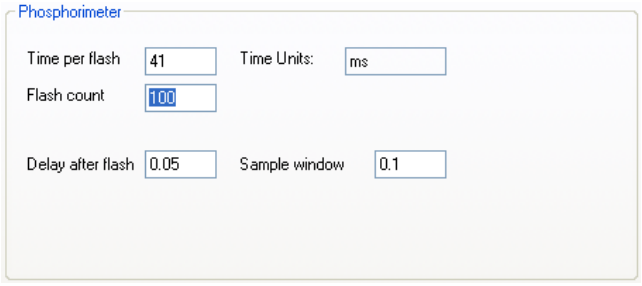


9.4.2.2 Experiment Type Window

After choosing the Phos experiment type in the Fluorescence Main Experiment Menu, four experiment types are available for the phosphorimeter in the Experiment Type window:

Excitation
Emission

These are similar to the standard excitation, and emission 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 (extracted from the Experiment Setup window).

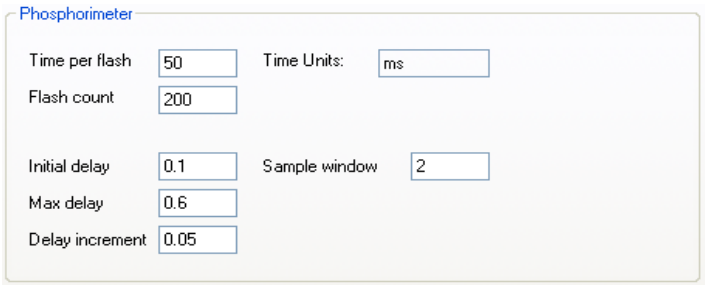


Above is a Phosphorimeter area with a typical set of parameters for the emission of $EuCl_3$. Use an excitation monochromator set to 393 nm; the emission monochromator should start at 570 nm, end at 750 nm, with an increment of 1 nm.

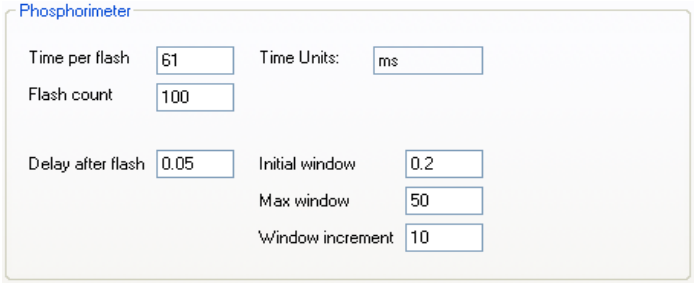
Decay by Delay

These produce a decay of phosphorescence over time. Decay by Delay varies the Delay after flash in order to construct the decay curve:

Decay by Window



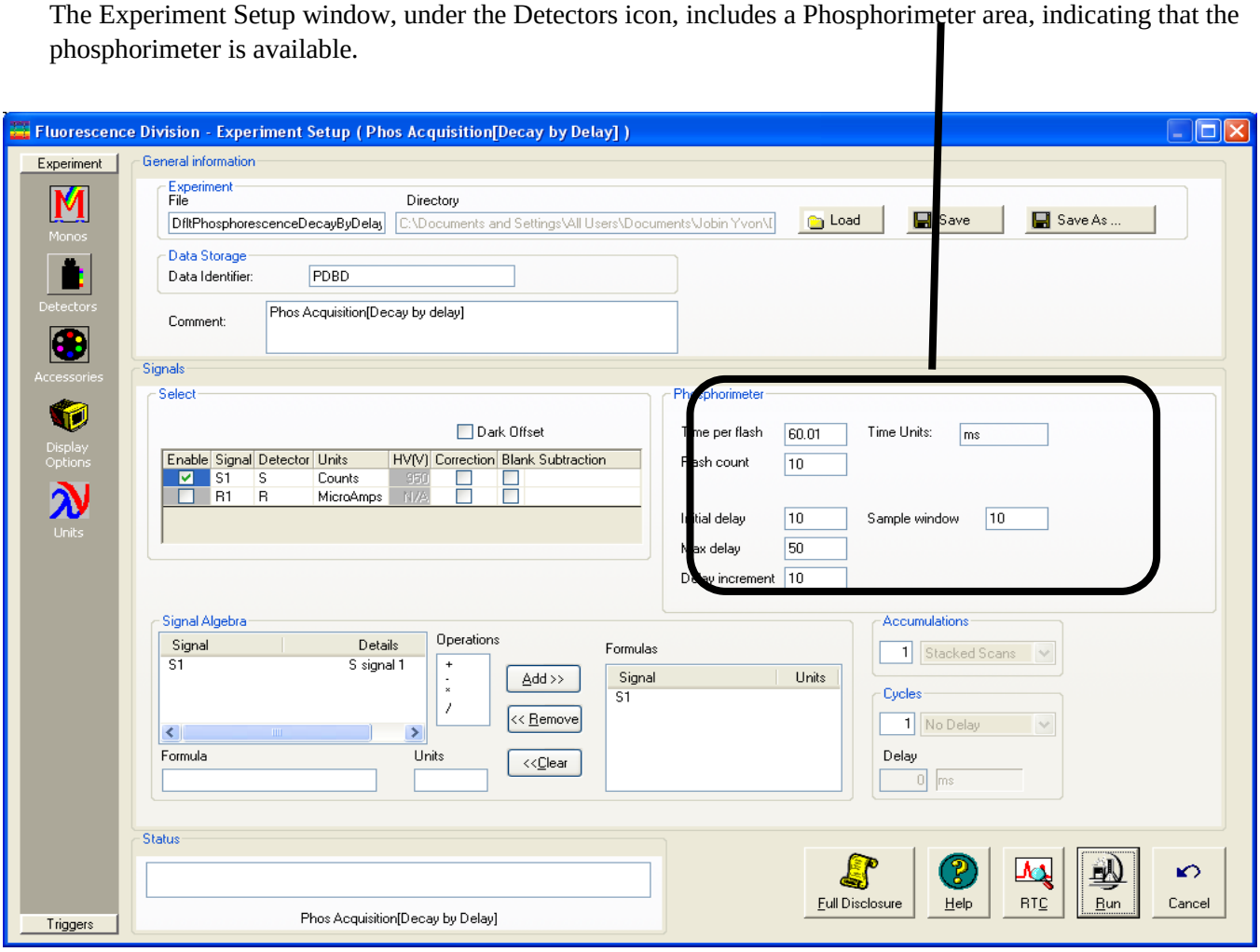
Decay by Window varies the length of the Window increment with constant Delay after flash while taking data, to construct the decay curve:



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.

9.4.2.3 Experiment Setup Window

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



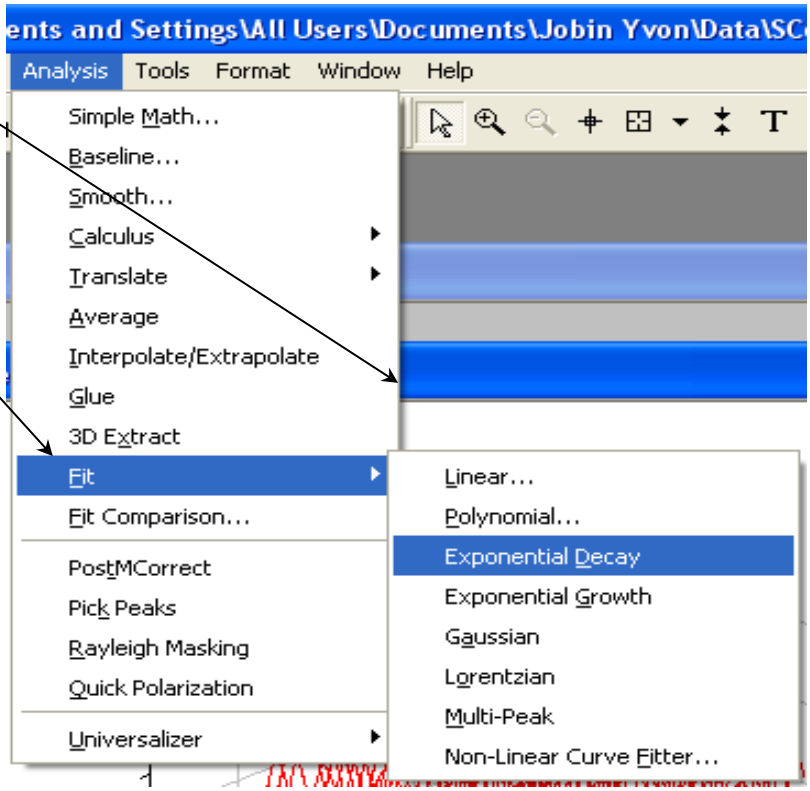
9.5 Processing Phosphorimeter Data

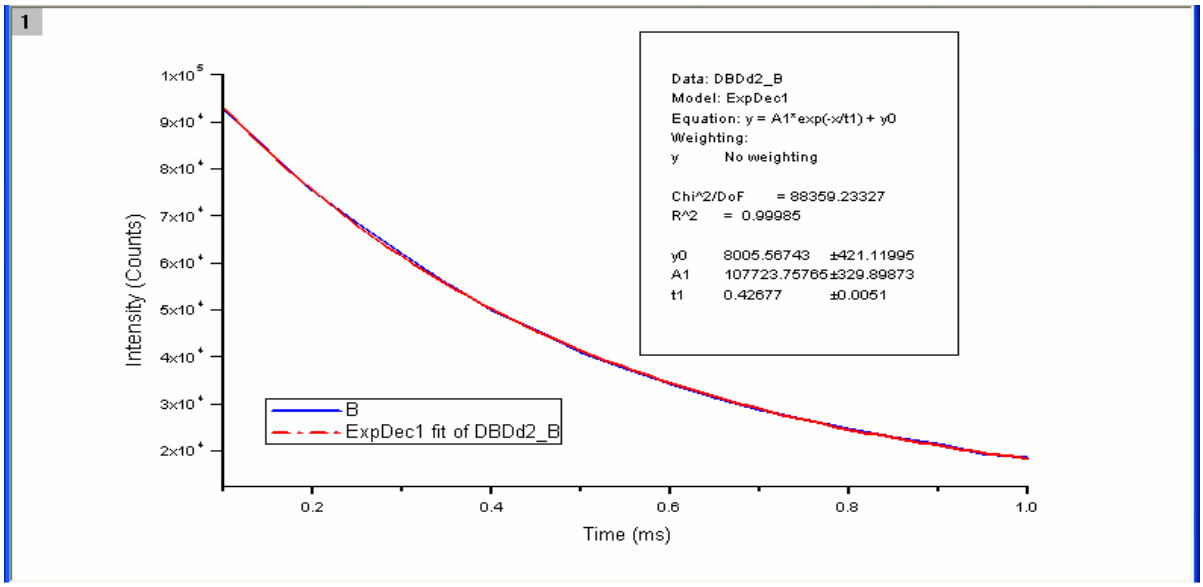
1. Open the graph to be processed.
2. Click on the data points to be processed.
3. In the toolbar, choose Analysis.

A drop-down menu appears.

4. Choose Fit.
5. From the submenu, 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.





9.6 Lamp Replacement

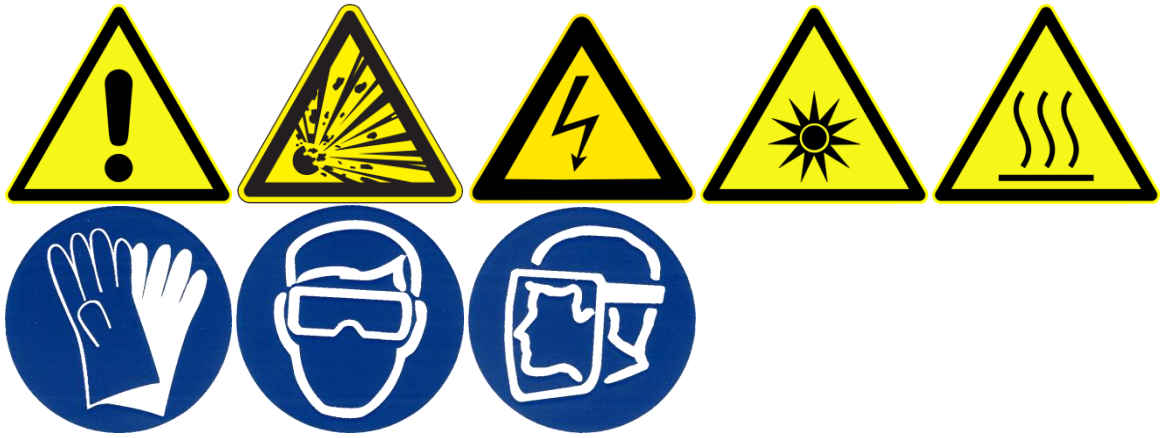
The xenon flash lamp typically has a half-intensity life of at least 10 million flashes. Follow the procedure below for replacement and alignment.

Warning: High voltage exists within the FluoroMax® Plus-P. To avoid fatal shocks, before removing the lamp cover, unplug the FluoroMax® Plus-P's power cord, and wait at least 5 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:

9.6.1 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 the 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.

9.6.2 Method

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

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

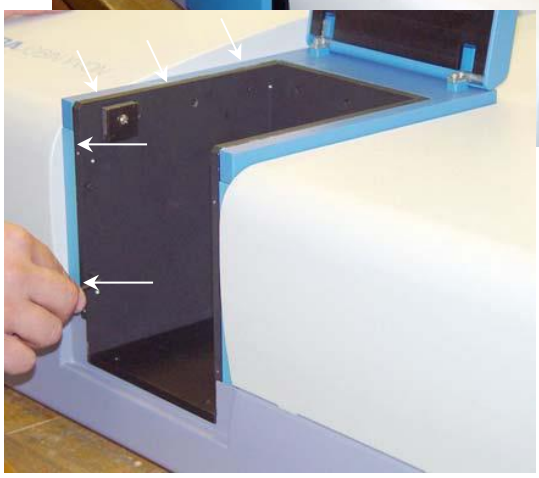
Caution: Never rotate the flip mirror inside the lamp housing manually. This can strip the gears in the gearbox.

2. Switch off and prepare the FluoroMax® Plus-P.
    a. Be sure that the FluoroMax® Plus-P and the host computer are turned off.
    b. Remove the AC (mains) power cord from the FluoroMax® Plus-P.
    c. Disconnect the RS-232 cable, power cord, and any other cables attached to the spectrofluorometer.

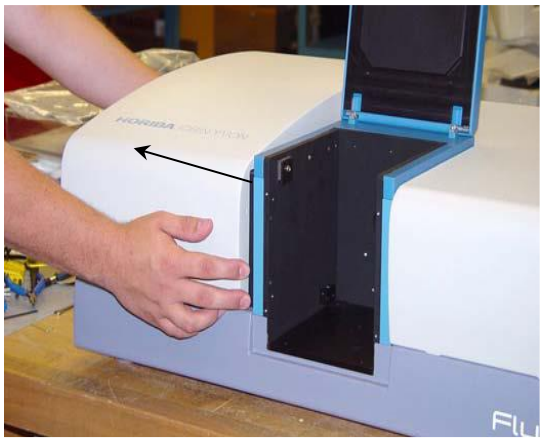
3. Gently remove the sample mount from the front of the FluoroMax® Plus-P.

    The standard FluoroMax® Plus-P front is held via a friction fit, with no screws to remove. Some accessories require removal of 4 screws. Some sample mounts also have a 15-pin connector at the inside end for automated accessories.

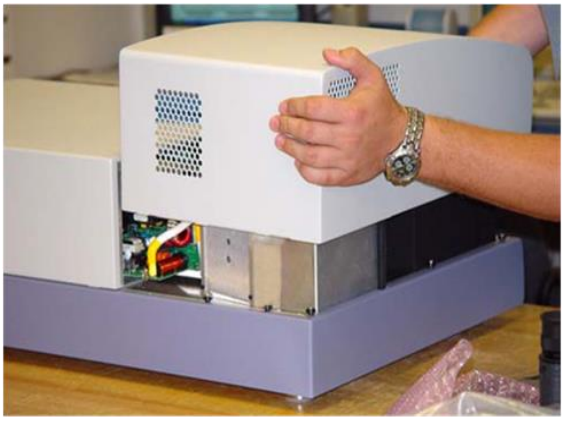
4. Remove the lamp cover.
    a. With an Allen key, remove the five screws from inside the left wall of the sample compartment.



    b. Pull the lamp cover to the left about 2 cm.

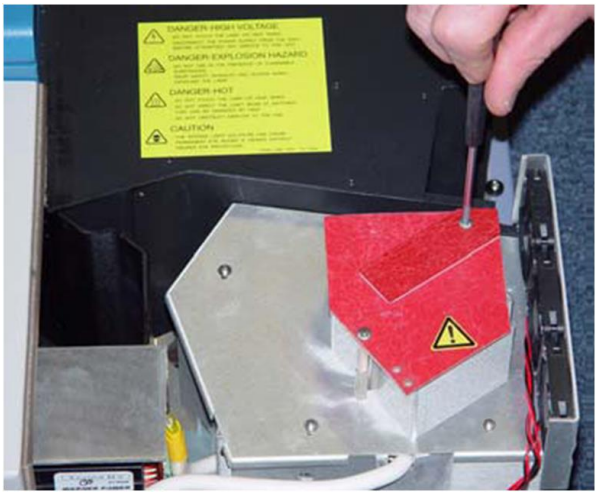


c. Lift the cover vertically off the instrument.

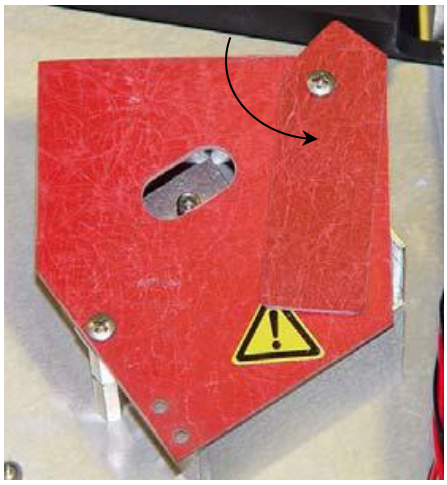


5. Remove the lamp-housing cover at the rear of the instrument.

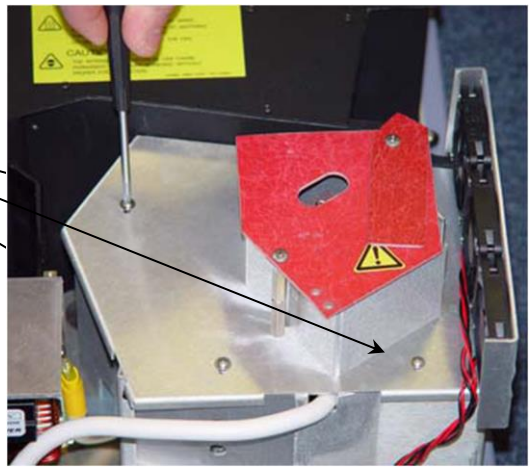
a. With a Phillips screwdriver, loosen the safety cover's screw.



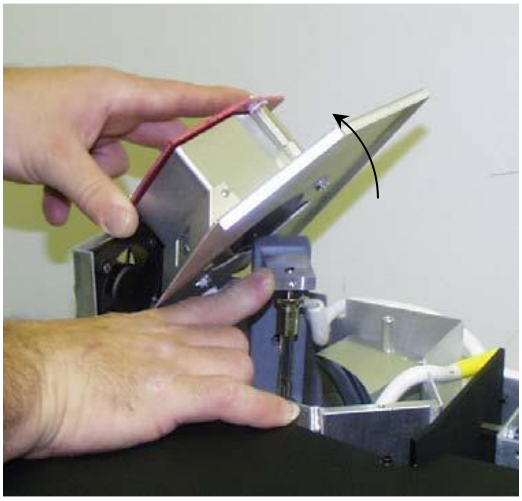
b. Swing the safety cover out of the way.



c. Remove the three Phillips screws from the lamp-housing cover.

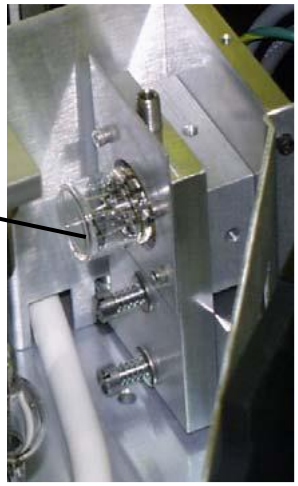


d. Gently rotate the lamp-housing cover with its cooling fans attached.



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 safety cover and lamp-housing cover.
9. Secure the three screws on the lamp-housing cover.
10. Replace the instrument cover, and reseat the five screws.
11. Reconnect all cables (power, accessories, etc.) to the FluoroMax® Plus-P.

10 AUTOMATED POLARIZERS

10.1 Introduction

10.1.1 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 photo selection. Vertically polarized light typically is used to excite a population of molecules whose absorption dipole is oriented in the vertical direction. For photos election, vertically polarized exciting light usually is produced using a polarizer in the excitation path. A laser whose emission is V-oriented also may be used.

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

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

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

Ideally, polarization (P) and anisotropy ($\langle r \rangle$) are measured using only the vertically polarized excitation with the horizontal and vertical emission components. These measurements are designated I_{VV} and I_{VH}, respectively, where the first subscript indicates the position of the excitation polarizer, and the second, the emission polarizer. Vertically oriented polarizers (V) are said to be at 0° with respect to normal, and horizontally oriented polarizers (H) are said to be at 90°. Polarization and anisotropy are expressed as follows:

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

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

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

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

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

Polarization in a spectrofluorometer is defined as:

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

Anisotropy in a spectrofluorometer is defined as:

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

Polarization and anisotropy can be interconverted using these two equations:

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

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

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 $\langle r \rangle$ and P are:

Parameter	α = 0°	α = 90°
P	+0.5	–0.333
$\langle r \rangle$	+0.4	–0.2

Values of $\langle r \rangle > 0.4$ indicate scattered light is present in the measurement of $\langle r \rangle$. Values of $\langle r \rangle < 0.2$ indicate the rotation correlation time to be faster than the luminescence lifetime of the sample. 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 $\langle r \rangle$ 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.

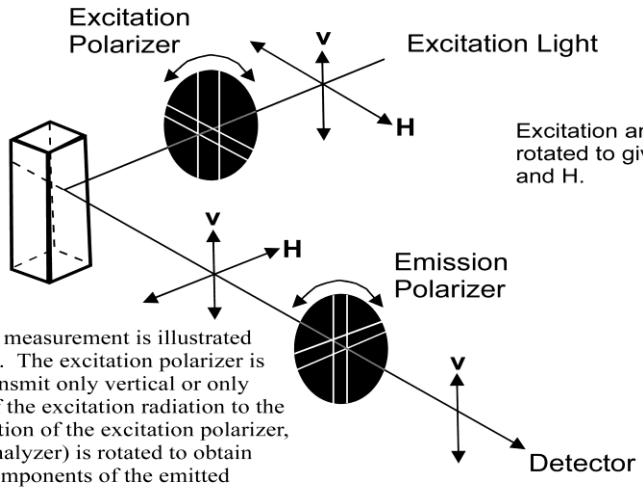
10.1.2 Polarization Geometries

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® Plus 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:



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.

10.1.3 Magic-Angle Conditions

Some fluorescent compounds exhibit molecular rotations on the same timescale 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 photo selection 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: The majority of samples do not exhibit an appreciable change in their spectrum when they are measured under magic-angle conditions. Thus, magic angles need not be used for most samples.

10.2 Installation

HORIBA Scientific's polarizers are pre-installed in the FluoroMax® Plus spectrofluorometer, if ordered as an option. 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.

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

10.3 Alignment

10.3.1 Introduction

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. The Ludox® we use as the reference is Aldrich 420859-1L, Ludox® TMA Colloidal Silica, 34 wt. % suspension in water, de-ionized.

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

The alignment test may be a measurement of the polarization or anisotropy within the software using the Anisotropy scan-type, or use of the Remeasure Anisotropy Only utility (click Advanced..., and the Polarizer Alignment window opens). The test also may be performed manually using the Real Time Control application. One measures the polarization, anisotropy, or the polarization ratio of scattered light (typically, the excitation and emission monochromators are both set to 370 nm for the measurement). To calculate the *polarization ratio*, use the definition:

$$\text{polarizati on ratio} = \frac{I_{VV} * I_{HH}}{I_{VH} * I_{HV}} \quad (9)$$

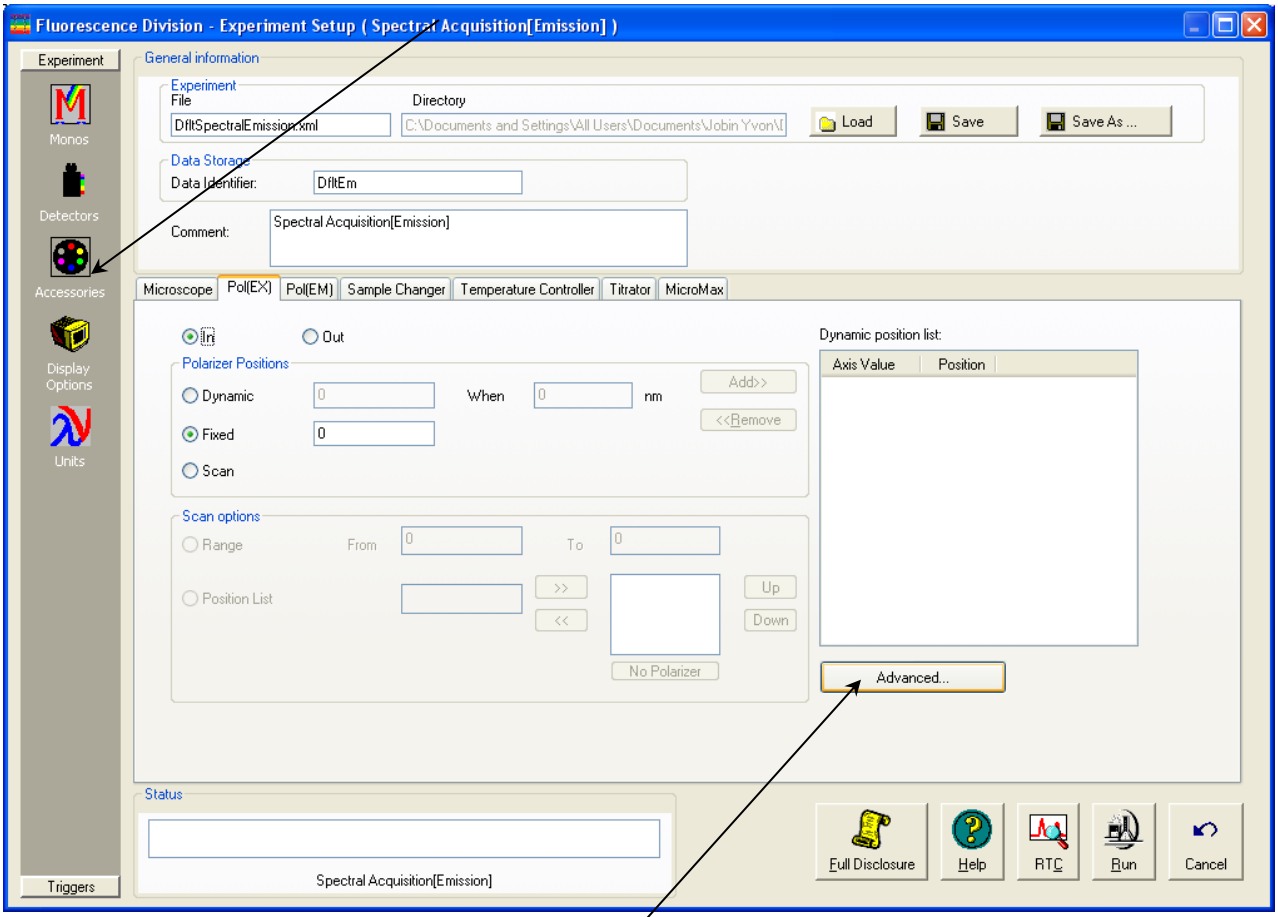
Alignment is satisfactory when the polarization ratio ≥ 100, or $P \geq 0.98$, or $\langle r \rangle \geq 0.97$. The check below assumes a sample of Ludox® or glycogen is used.

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

10.3.2 Method

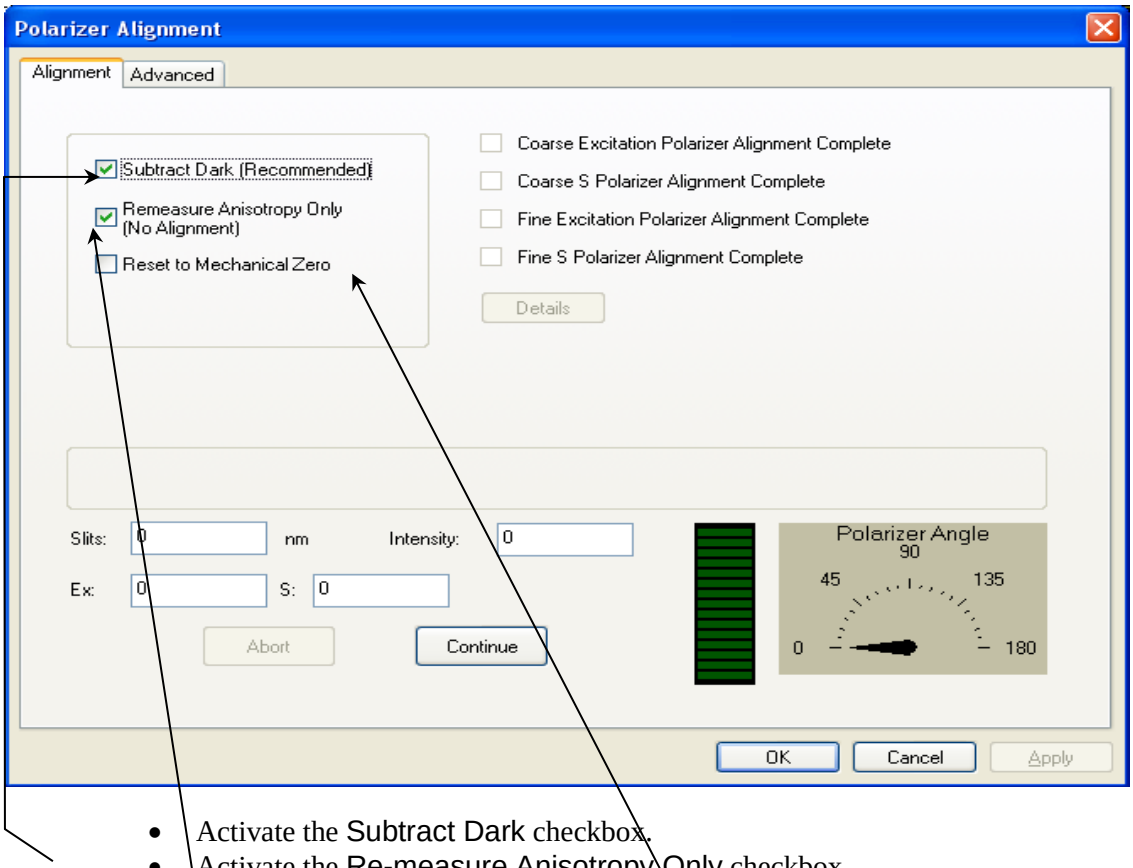
1. Start FluorEssence™.
2. Open the Experiment Setup window.

   You may use any instrument configuration with polarizers.
3. Click the Accessories icon.



4. Click the Advanced... button.

This opens the Polarizer Alignment window:



- Activate the Subtract Dark checkbox.
- Activate the Re-measure Anisotropy Only checkbox.
- Activate Reset to Mechanical Zero only if the polarizers are definitely mis calibrated. This deletes the previous calibration.

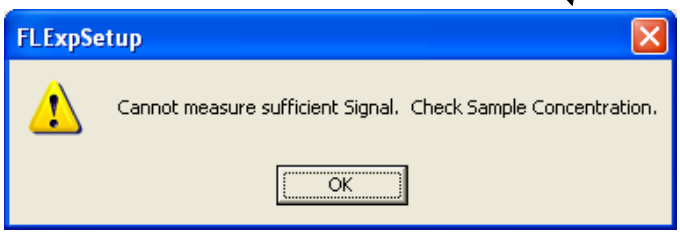
5. Place the Ludox® or glycogen in the sample holder.

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

6. Click the Continue button.

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

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



7. Approve or retry the measurement based on satisfaction with the result.
8. To quit, hit the Cancel button at any time during the procedure.

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

10.3.3 Re-alignment of Polarizers

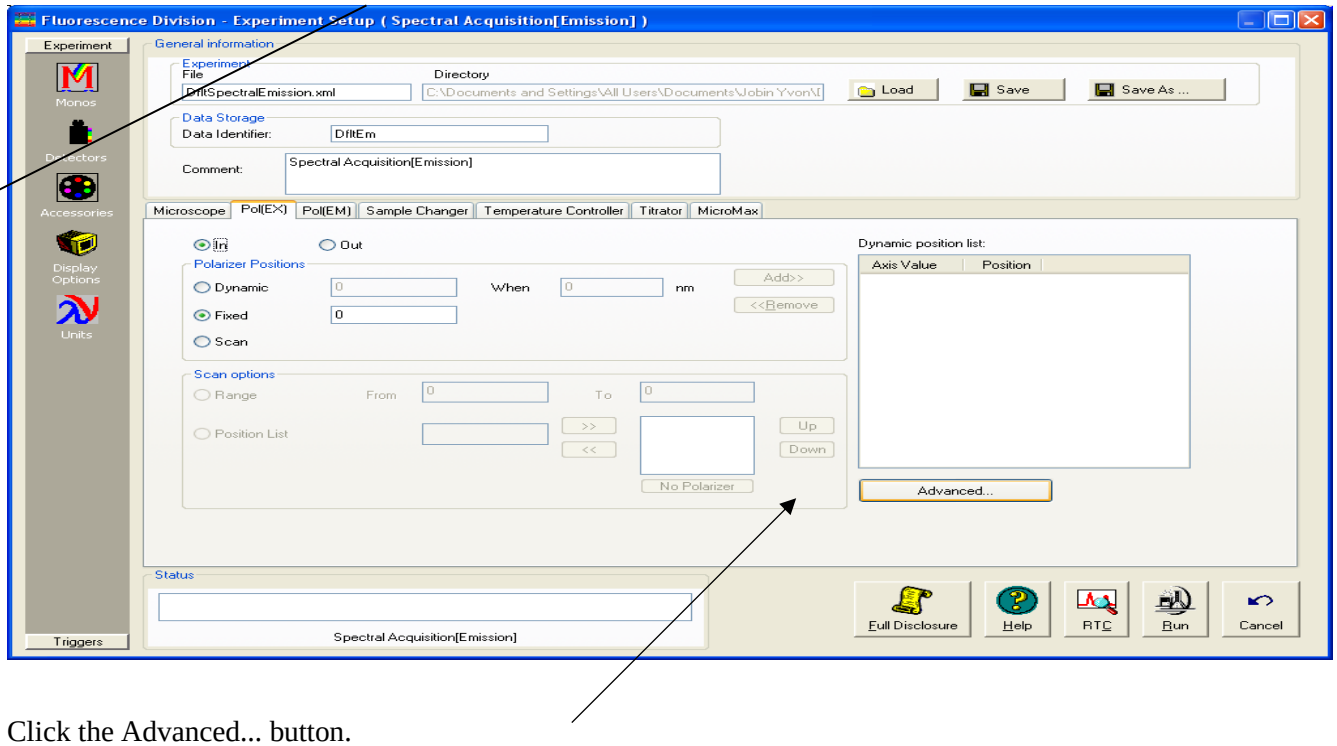
FluoroMax® Plus auto polarizers may be aligned using a software routine called Polarizer Alignment in Experiment Setup.

Note: Polarizers are aligned at the factory, and should not need realignment.

10.3.3.1 Using Polarizer Alignment

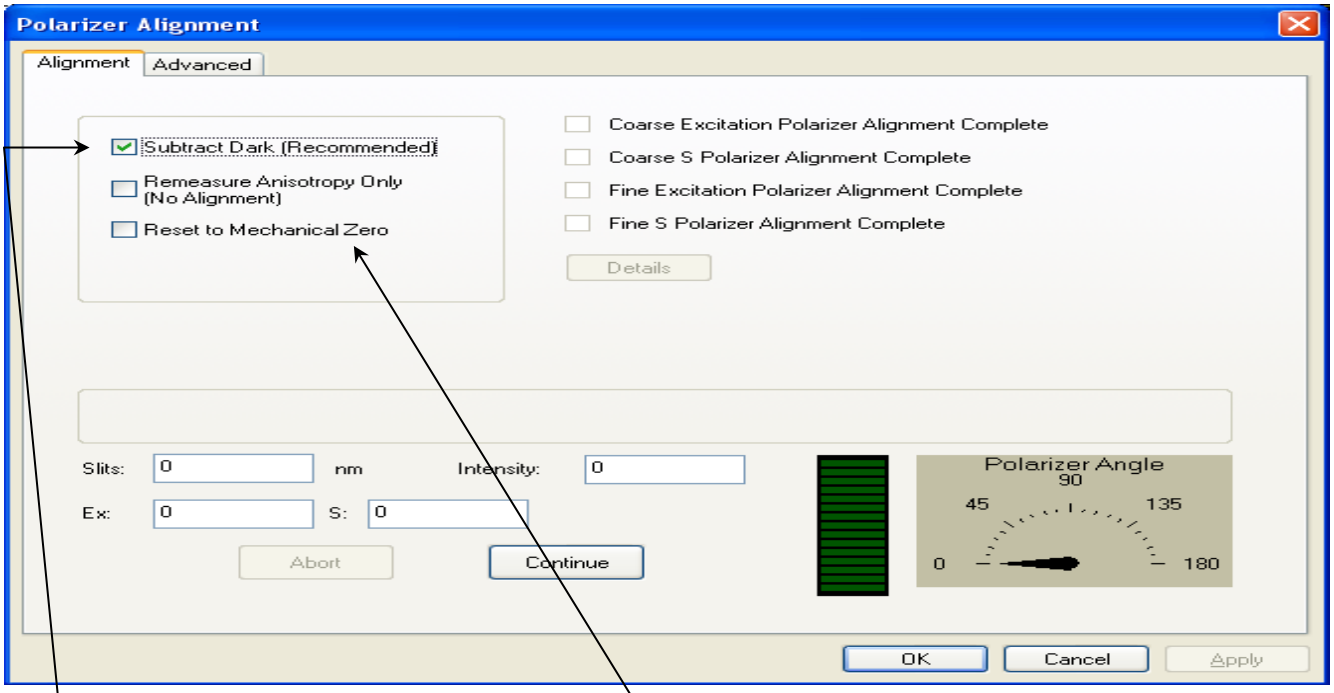
This routine automatically calibrates auto polarizers. Use a sample of Ludox® or glycogen to run the alignment routine. The software rotates the polarizers in 1° increments and locates the optimal positions for each auto polarizer. After completion, the anisotropy for the scattering solution is measured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

1. Start FluorEssence™.
2. Open the Experiment Setup window.
3. Click the Accessories icon.



4. Click the Advanced... button.

   This opens the Polarizer Alignment window:



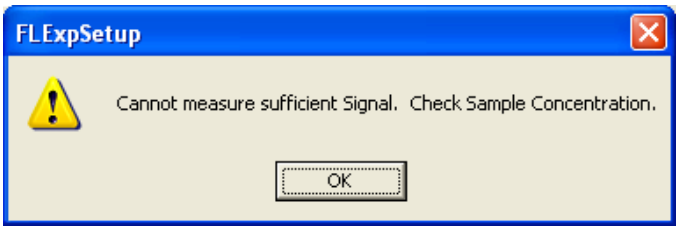
- Subtract Dark (recommended)
- Reset to Mechanical Zero—only if the polarizers are definitely mis calibrated. This deletes the previous calibration.

Note: Do not check the Remeasure Anisotropy Only checkbox.

5. Place the Ludox® or glycogen in the sample holder.
6. Click the Continue button.

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

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



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

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

   Acceptable values are anisotropy ≥ 0.97 and polarization ratio ≥ 100.

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

10.4 Using Automated Polarizers

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

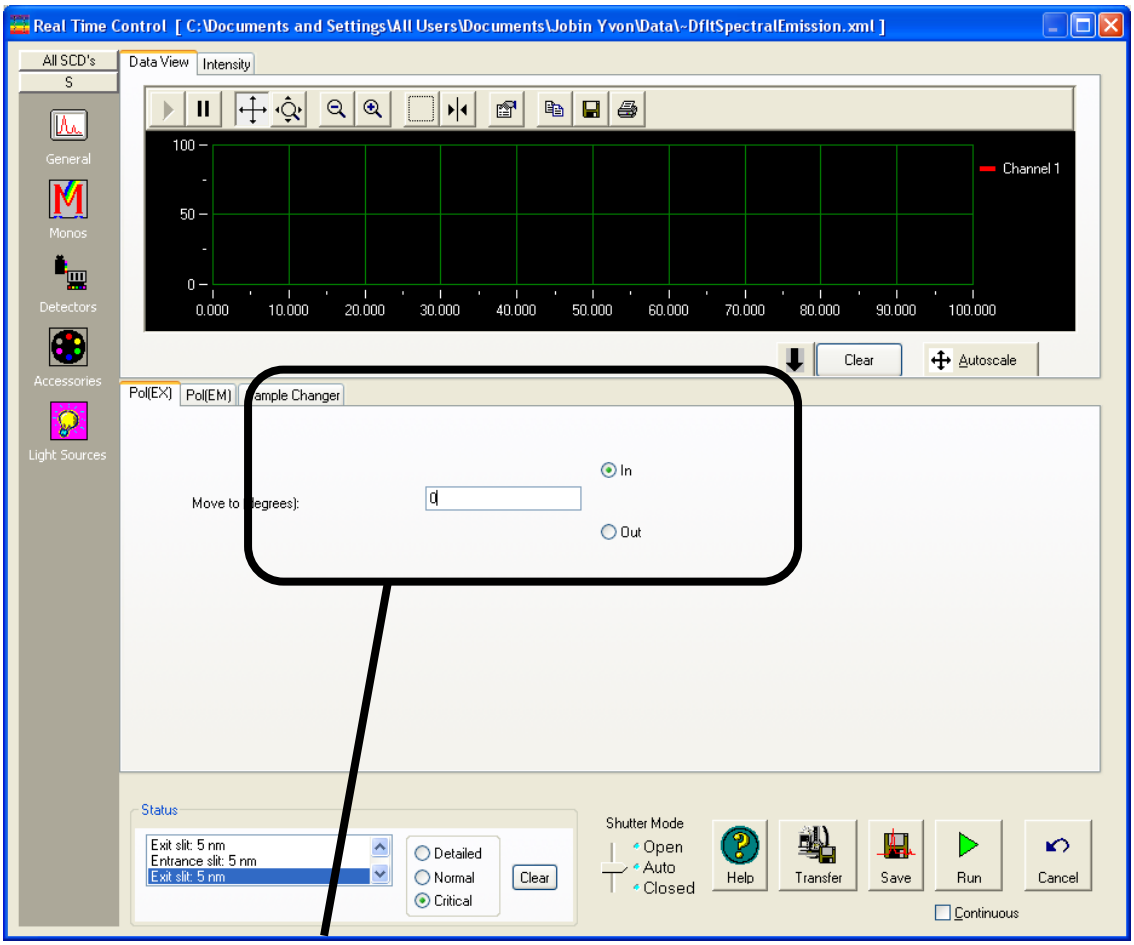
10.4.1 Applications for Polarizers

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

10.4.2 Using FluorEssence™

To use the auto polarizers, load an instrument configuration with auto polarizers.

10.4.2.1 Real Time Control

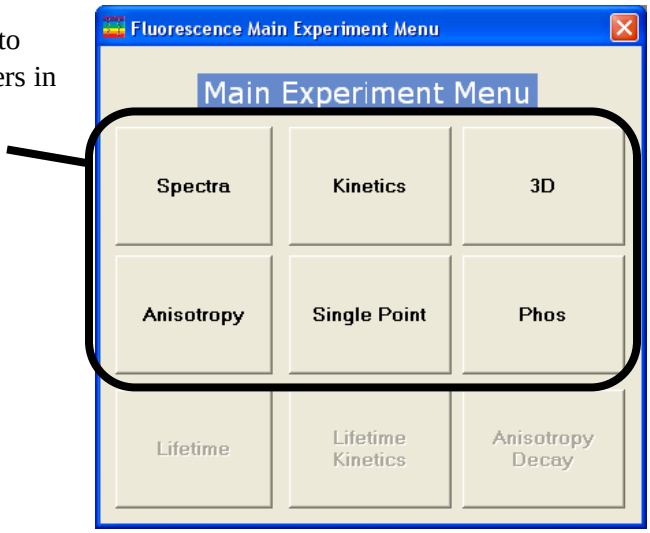


Real Time Control manipulates the polarizers and other instrument settings, to observe and optimize the spectrofluorometer in real time. Under the Accessories icon, each polarizer may be set independently into or out of the optical path under its own index-card tab. A custom angle may be set from 0–180°,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

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

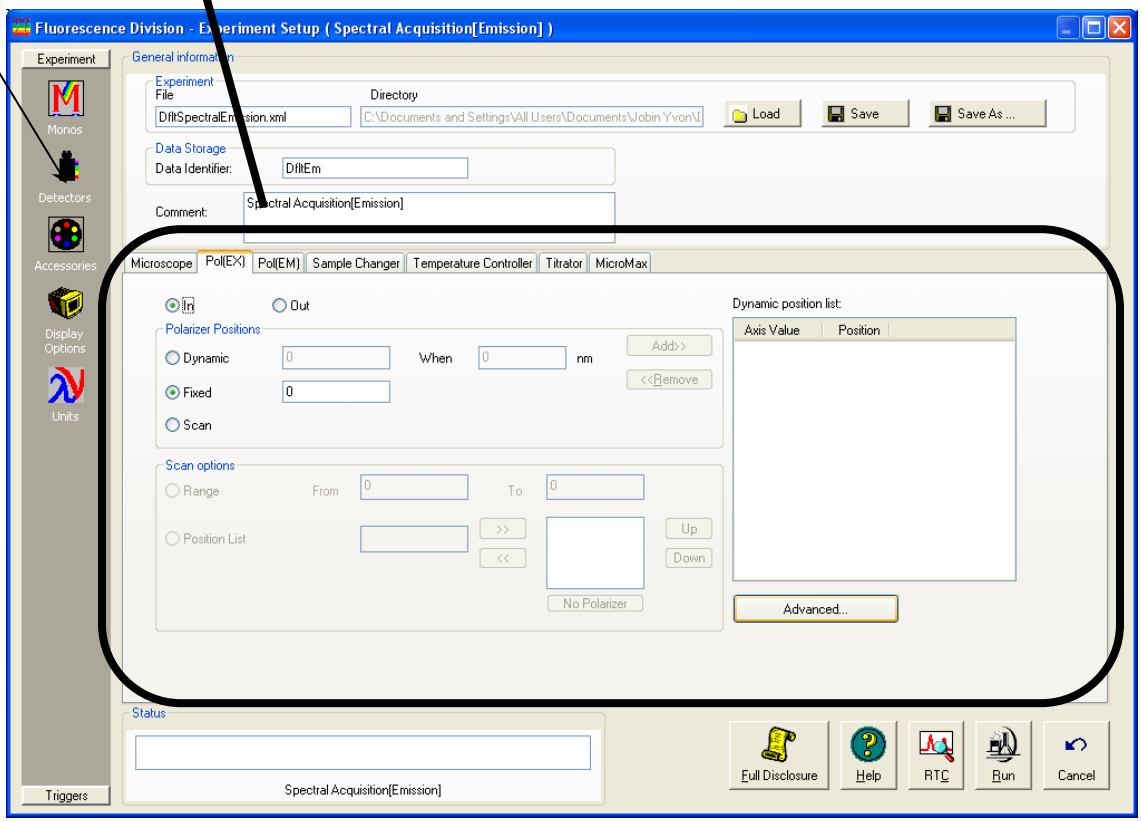
10.4.2.2 Experiment Setup

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



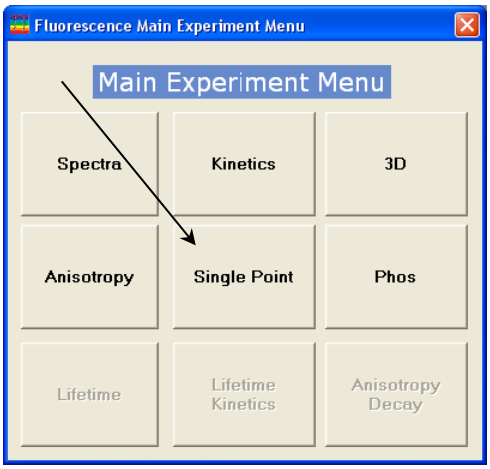
The Experiment Setup window appears.

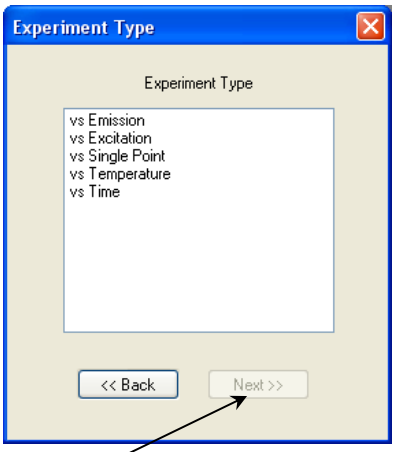
Adjust polarizer parameters under the Accessories icon. One index-card tab appears for each polarizer.



10.4.2.3 Constant Wavelength Analysis

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

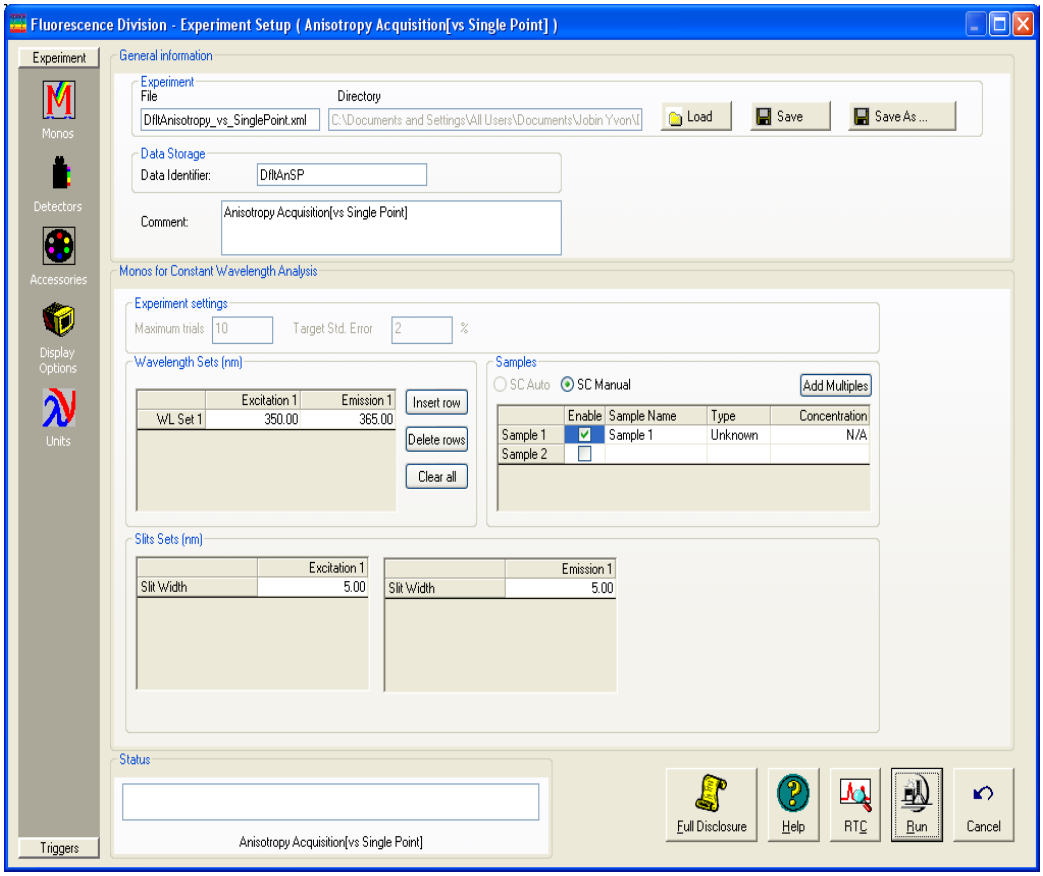




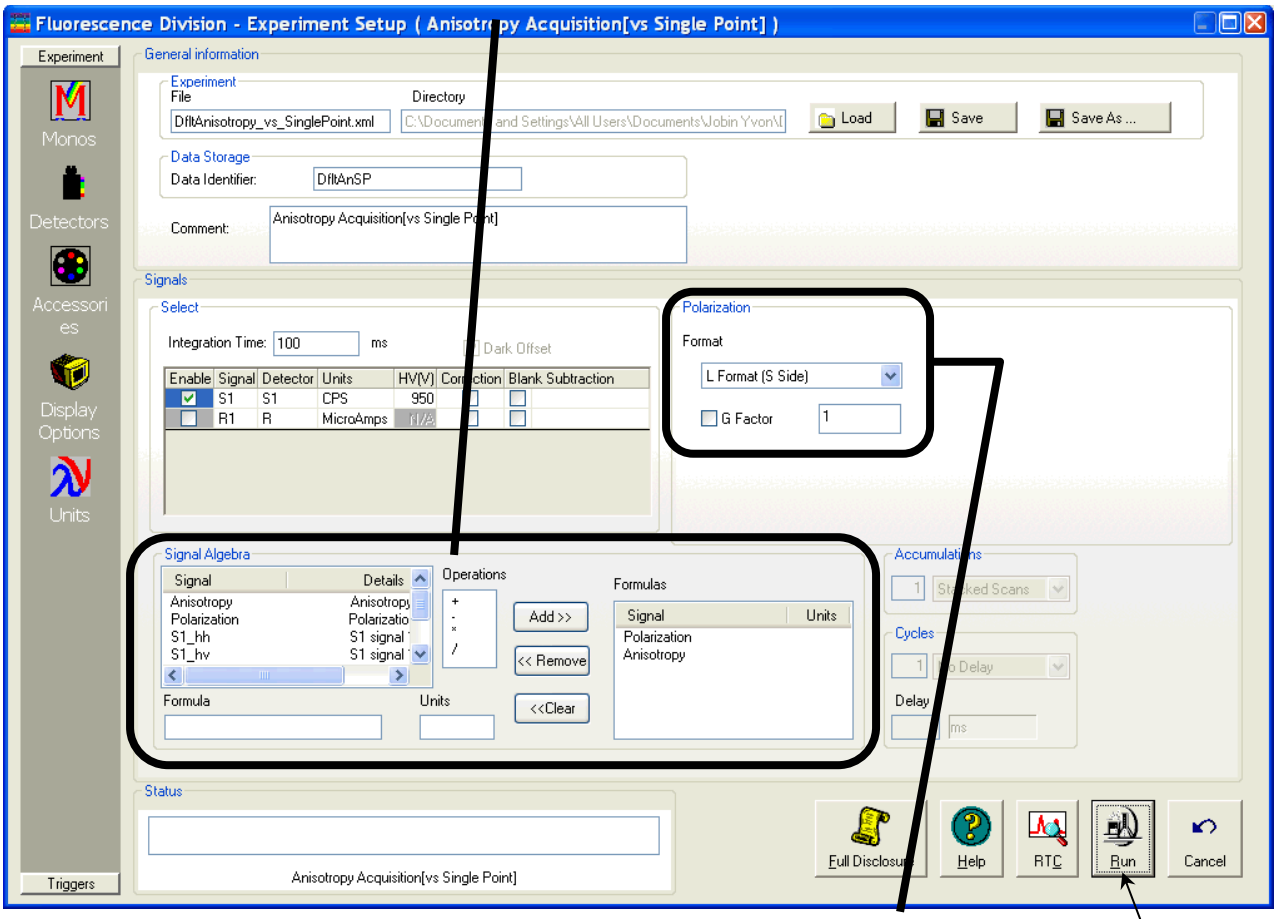
The Experiment Type window opens.

Choose vs SinglePoint, then click the Next >> button.

The Experiment Setup window appears.



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



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

Click the Run button when ready.

10.5 Maintenance

Like all optics, polarizers should be handled with care and stored properly. With proper care, a polarizer should last for many years. Should the polarizer windows need cleaning, apply a mild solution of methanol, and blow it dry.

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. To do this anisotropy test, use a very dilute solution of scattering particles such as Ludox®, with a bandpass of 2–3 nm on both excitation and emission, to give a signal of 1×10^6 counts per second with polarizers rotated to VV. Under these conditions, you should get a $P \geq 0.985$.

Caution: Refer to your Material Safety Data Sheets (MSDS) for hazards regarding the use of methanol and Ludox® or other scatterer.

10.6 Troubleshooting

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

1. Note the problem and record any error messages.
2. See if the problem is listed on the following pages.

   If so, try the suggested solutions. Be sure to note carefully the steps taken to remedy the problem and the result. Refer to the appropriate section of this manual (and the software manuals, if necessary).
3. If the problem persists, or is not listed,

   Call the Service Department by phone at 1-877-546-7422, or fax at 732-494-8796. Outside the United States, call your local distributor.
   You may also reach us by e-mail at fluorescence-service.us@horiba.com.

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

PROBLEM	CAUSE	POSSIBLE REMEDY
Poor Polarization Data	Improper sample concentration Photomultiplier saturated; slits improperly set Dirty cuvette Polarizer misaligned	Adjust sample concentration. Check that sample signals are in linear region (< 2×10^6 cps on S, < 10 µA on R). Reset slits. Clean the cuvette. Check polarizer alignment.
	Using plastic or cylindrical cuvette System misaligned	Use rectangular, glass or quartz cuvette. 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.
Auto polarizers Do Not Initialize (Do Not Move during Initialization).	Wrong instrument configuration is loaded	Check that a configuration with auto polarizers is loaded.
Software Failure Initializing Auto polarizers	Wrong instrument configuration is loaded	Check that a configuration with auto polarizers is loaded.
	Bad cable connections	With the system power off, recheck cable connections.
	Computer hang-up	Exit the software, and reboot the system and host computer.

11 TECHNICAL SPECIFICATIONS

11.1 Introduction

Each FluoroMax® Plus system consists of:

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

Optional polarizers and phosphorimeter add

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

Each system is controlled by a Windows 10-compatible computer.

The details and specifications for each component of the FluoroMax® Plus spectrometer follow.

11.2 Spectrofluorometer System

11.2.1 FluoroMax® Plus

The FluoroMax® Plus spectrofluorometer consists of components controlled by specialized software. The basic (standard) FluoroMax® Plus spectrofluorometer system contains of the following components:

Excitation Source	150 W xenon, continuous output, ozone-free lamp
Optics	All-reflective, for focusing on 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 80 nm s^{-1} Accuracy ±0.5 nm Step Size 0.0625–100 nm Range 0–1150 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 240–1000 nm. • Emission detector is an R928P for high sensitivity in photon-counting mode (200–850 nm). High voltage = 950 V, linearity to 2 × 10^6 counts s^{-1}, < 1000 dark counts s^{-1}. • Second emission detector as per customer choice
Sensitivity	Double-distilled, de-ionized, ICP-grade water-Raman scan 10000:1 signal-to-noise ratio at 397 nm, 5-nm bandpass, 1-s integration time, background noise first standard deviation at 450 nm.
Excitation Shutter	Computer-controlled
Integration Time	0.001–160 s
Slit Width	0–29.4 nm bandpass, continuously adjustable via host computer
Dimensions (Instrument)	32⅝″ wide × 11⅛″ high × 19¾″ deep 82.9 cm wide × 28.3 cm high × 50.2 cm long Height needed to open sample-compartment lid: 20½″; 52.1 cm
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	Universal AC single-phase input power; 85–250 V AC; line frequency 50–60 Hz.
Fuses	Two 5 × 20 mm IEC approved, 4.0 A, 250 V, Time Delay fuses (Cooper Bussman part number GDC-4A or equivalent)

11.2.2 Phosphorimeter Option

The following components and specifications also apply to the FluoroMax® Plus with FM-2005 upgrade.

Source	UV xenon flash tube
Flash rate	0.05–25 Hz
Lifetime range	Down to 10 µs
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.

11.2.3 TCSPC Option (DeltaTime Upgrade)

The following components and specifications also apply to FluoroMax® Plus with standard PMT and DeltaTime upgrade.

Source	Interchangeable DeltaDiodes or NanoLEDs; peak wavelengths 250-980 nm
Lifetime range	200 ps–0.1 ms
Minimum channel resolution	26 ps/channel
Detection method	Time-correlated single-photon counting (TCSPC)

11.3 Minimum Host-Computer Requirements

Microprocessor	Intel i3 or higher recommended
Operating system	Windows® 7, 10, or 11
Hard disk	10 GB of free storage
DVD-ROM drive	Required
Memory	4 GB RAM or higher
Video display	Video resolution of at least 1024 × 768
Keyboard	A 104-key keyboard, plus USB or PS/2 mouse
Available port	4 (FMaxPlus, USB license dongle, keyboard, and mouse)

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

Note: Administrator rights are required on the computer that the software is installed on, and failure to properly assign user rights will result in instrument performance errors.

11.3.1 host Rights

The user of HJY Application Software (FluorEssence or SynerJY) should have "**local administrative rights**", not IT-privileged administrative rights.

IT should give all users of the instrument full control of the following:

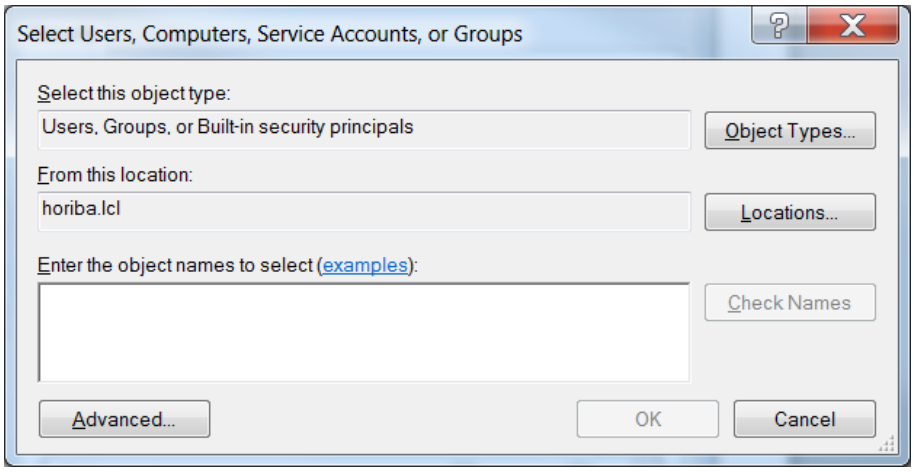
a. the directory and all subdirectories of "C:\Program Files (x86)\Jobin Yvon" on 64-bit systems or "C:\Program Files\Jobin Yvon" on 32-bit systems.
b. the directory and all subdirectories of "C:\Users\Public\Documents\Jobin Yvon\Data"
c. the registry key and all subkeys of:
    - "HKEY_LOCAL_MACHINE\SOFTWARE\Wow6432Node\Jobin Yvon" on 64-bit systems or
    - "HKEY_LOCAL_MACHINE\SOFTWARE\Jobin Yvon" on 32-bit systems.

If the customer has FluorEssence TCSPC lifetime package (with DataStation), then IT should give all users of the instrument full control of the following as well:

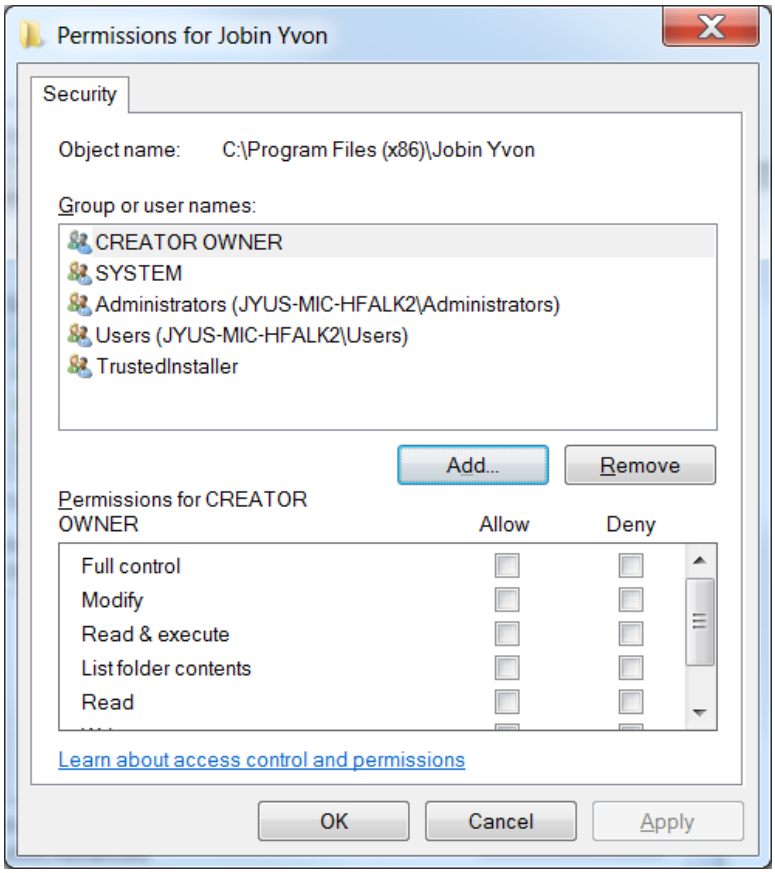
a. the directory and all subdirectories of "C:\Program Files (x86)\HORIBA Scientific" on 64-bit systems or "C:\Program Files\ HORIBA Scientific " on 32-bit systems.
b. the registry key and all subkeys of
    - "HKEY_LOCAL_MACHINE\SOFTWARE\Wow6432Node\HORIBA Jobin Yvon IBH" on 64-bit systems or
    - "HKEY_LOCAL_MACHINE\SOFTWARE\ HORIBA Jobin Yvon IBH " on 32-bit systems.

To give the full permission for directory and subdirectory in Windows Explorer:

1. Select the directory. Right-click and select “Properties.” Select “Security” tab.
2. Select “Edit” button
3. Select “Add” button
4. Enter the proper user domain name or type a part of the name in the box “Enter the object names to select” and click “Check names”



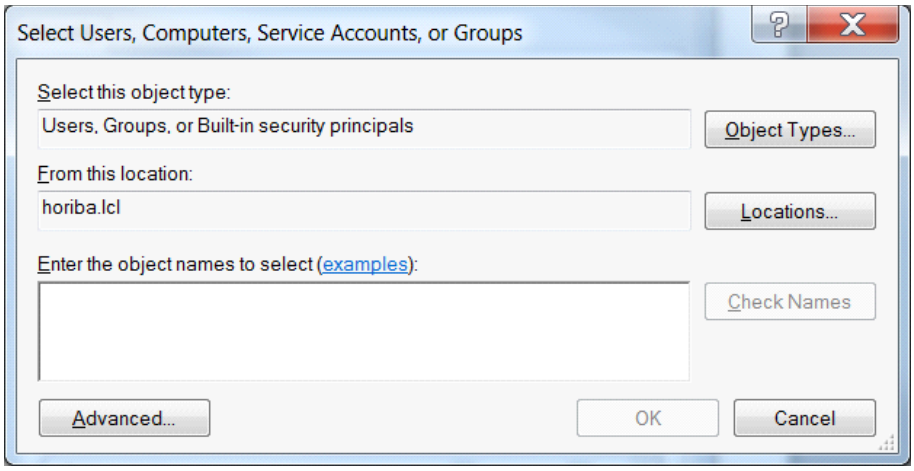
5. Once the username is properly recognized, select “OK”
6. Be sure that the username is selected, select “Full Control” permissions


Permissions for Jobin Yvon
Security
Object name: C:\Program Files (x86)\Jobin Yvon
Group or user names:
CREATOR OWNER
SYSTEM
Administrators (JYUS-MIC-HFALK2\Administrators)
Users (JYUS-MIC-HFALK2\Users)
TrustedInstaller
Add...
Remove
Permissions for CREATOR OWNER
Allow
Deny
Full control
Modify
Read & execute
List folder contents
Read
Learn about access control and permissions
OK
Cancel
Apply

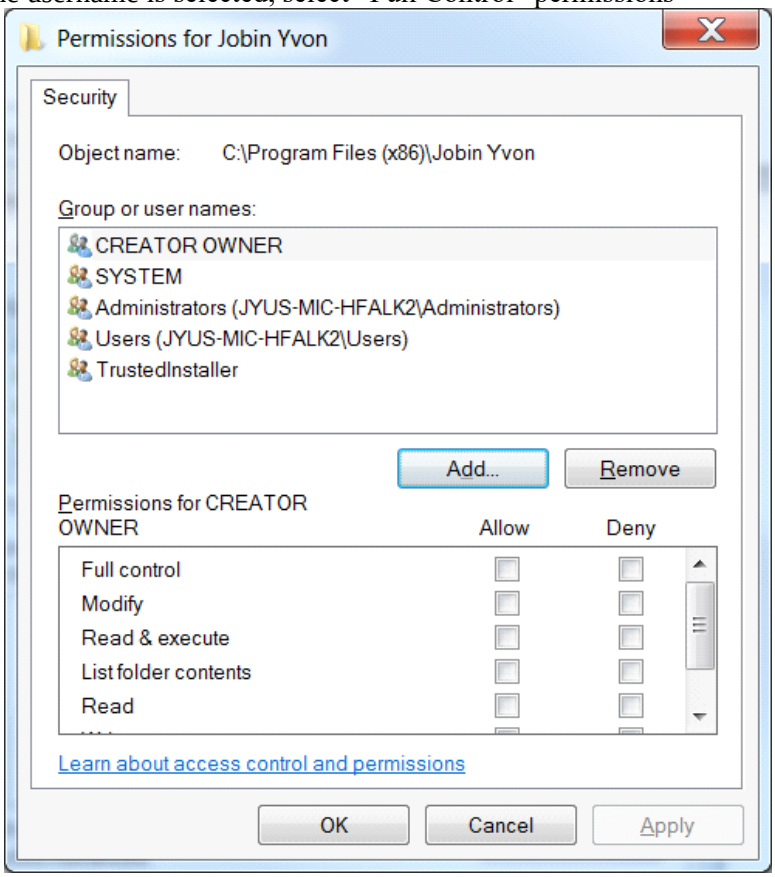
7. Select “OK.”

To give the full permission at the registry level:

1. Select the directory. Right-click and select “Permissions”
2. Select “Add” button
3. Enter the proper user domain name or type a part of the name in the box “Enter the object names to select” and click “Check names”



4. Once the username is properly recognized, select “OK”
5. Be sure that the username is selected, select “Full Control” permissions



Select “OK”

11.4 Software

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

12 COMPONENTS & ACCESSORIES

Accessories for the FluoroMax® Plus 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® Plus 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.

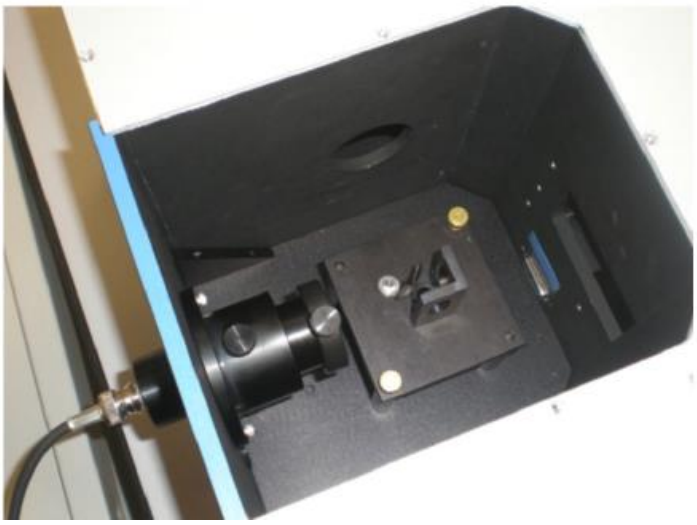
For additional information or product literature on any of these items, contact your local Sales Representative.

12.1 Itemized List of FluoroMax® Plus Accessories

ITEM	PART #
Absorption/Transmission Accessories	5500003031
	5570820061
Assembly, Liquid-Nitrogen Dewar	5500450066
Cell Holder, Dual-Position, Thermostatted	5500450437
Cell Holder, Four-Position, Thermostatted	5500450438
Cell Holder, Four-Position, Temp. Controlled for Fluorolog-QM, FluoroMax Plus, or Nanolog (F4POS-QNW)	5700005137
Cell Holder, Single-Position, Thermostatted	5500450433
Cell HPLC Flow	5500001955
Cell, Micro, Adapter 1cm Path Length 250 microliter	5500000008
Cell, Micro, 250microL, 3mm SQ, Quartz	5500000019
Cell, Microsense	5500001924
Cell, Reduced Volume	5500650518
Cell with Teflon® Stopper	5500001925
Cell with Teflon® Cap	5500001920
Cryostat, Janis	5500000153
DeltaTime TCSPC-MCS Kit for DeltaDiode Sources on FluoroMax Systems (DT-DD-FM)	5700010066
DeltaTime TCSPC-MCS Kit for DeltaDiode Sources on FluoroMax Systems (DT-DD-FM-Temp)	5700010070
DeltaTime TCSPC-MCS Kit for NanoLED Sources on FluoroMax Systems (DT-NL-FM)	5700010067
DeltaTime TCSPC-MCS Kit for NanoLED Sources on FluoroMax Systems (DT-NL-FM-Temp)	5700010071
DeltaTime Kit for SpectraLED Sources on FluoroMax Systems (DT-SL-FM)	5700010068
DeltaTime Kit for SpectraLED Sources on FluoroMax Systems (DT-SL-FM-Temp)	5700010072
DeltaTime Kit for 980nm Pulsed Laser on FluoroMax Systems (DT-980-FM)	5700010069
Front Mounted Laser	Various (Refer to Section **15.6**)
Emission Correction Factor Kit (F-3026)	5700014790
Excitation Correction Factor Kit (F-3028)	5500355954
Fiber Optic Mount (5500000022) and Fiber Optic Bundles (5500000117)	
Filter, Cut-On	5500001939
Filter Holder, Cut-on	5500450202
Injector Autotitration	5500081050
Injector Port	5500450432
Lamp, 150W, Xenon	5500000398
MicroMax 384, Microwell Plate Reader	5500352117
Polarizer, L-Format	5500450499
Quanta-ϕ Photoluminescence Quantum Yield (PLQY) Accessory	5700011752
Quartz Windows for the Sample Compartment	5500450497
Rapid Peltier Temperature Controlled Single Sample Holder for TCSPC Equipped System (F1PosTCSPC-QNW)	5700003355
Shutter Accessory	5500450185
Solid Sample Holder	5500001933
Stopped-Flow Accessory	5500003025
Temperature Bath	5500450520
Trigger Cable	5500400981

12.2 5500003031 & 5570820061 Absorption/Transmission Accessories

The 5500003031-transmission accessory allows the collection of absorbance (and transmittance) spectra of fluorescence samples. Such spectra can be used to verify if the sample concentrations are appropriate or even correct for inner-filter effects. It comes assembled as a compete gap-bed, ready to insert into the instrument. The spectral range is 220-1000nm. The 5500003031 includes a 5500034001 electronics module and required cables. When used, it is configured as an "A" (auxiliary) detector in the instrument configuration.



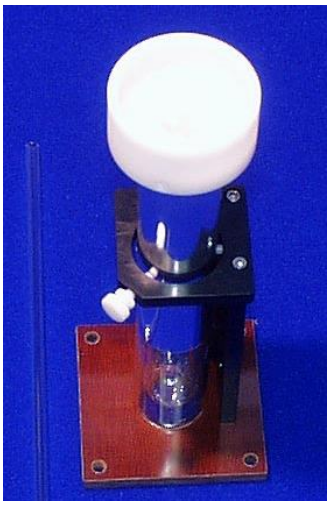
The 55570820061 accessory uses a reflector (barium sulfate, 250-2500nm) to reflect the excitation light across the sample and in the direction of the emission channel. The accessory fits into the standard cuvette, and it includes a side-mounted 10x10mm cuvette holder.

ABS-ACC cuvette absorbance transmission accessory

12.3 5500450066 Assembly Liquid Nitrogen Dewar

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

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



5500450066 Liquid Nitrogen Dewar Assembly.

Included in the 5500450066 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.*

12.4 5500450437 Cell Holder, Dual-Position, Thermostatted

The 5500450437 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.

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

12.4.1 Installation

1 Remove the present holder from the posts.

2 Replace with the 5500450437.

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.*

12.4.2 Use

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

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

2 Place a cuvette in each holder.

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

3 Allow the sample to reach the desired temperature.

4 Turn on the magnetic stirrer.

5 Select the appropriate speed.

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

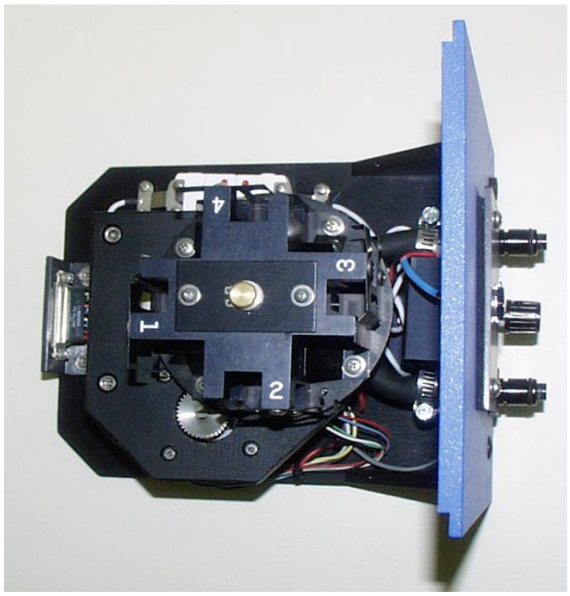
Note: *Selecting too high a speed may create a vortex, which could affect the reproducibility of the measurement.*

7 Run your experiment as usual.

12.5 5500450438 Cell Holder, Four-Position, Thermostatted

This thermostatted software-controlled four-position cell holder uses standard cuvettes (12.5 x 12.5mm external) for the temperature control of the samples from -20oC to 80ºC. It also includes an adjustable magnetic stirring with a stirring bar and filter holders for each position for 1x2 inch filters. The cell holder requires but it does NOT include a water bath (Tbath.Tbath220 for compatibility with FluorEssence software.

5500450438 Four-Position Thermostatted Cell Holder.



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

12.5.1 Installation

1 Remove the sample compartment gap-bed.

2 Position the 5500450438 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.*

12.5.2 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.

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

2 Place a cuvette in each holder.

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.

6 Run your experiment as usual.

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

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.

12.6 5700005137 Cell Holder, Four-Position, Temp. Controlled for Fluorolog-QM, FluoroMax Plus, or Nanolog (F4POS-QNW)

The cell holder includes magnetic stirring for all four positions, dry gas purge to reduce condensation at low temperature operation, temperature controller, 4 stir bars and an opaque cap. The holder is controlled directly from the FluorEssence software, and it includes a self-contained water circulator to remove heat. No plumbing required.

For rapid control of the sample's temperature in the FluoroMax®Plus sample compartment, choose the 1-position [5700005135] or 4 position [5700003355] Peltier-controlled thermostatted cuvette holder trays. The Peltier device heats and cools the sample thermoelectrically and fast without overshooting the target the way a

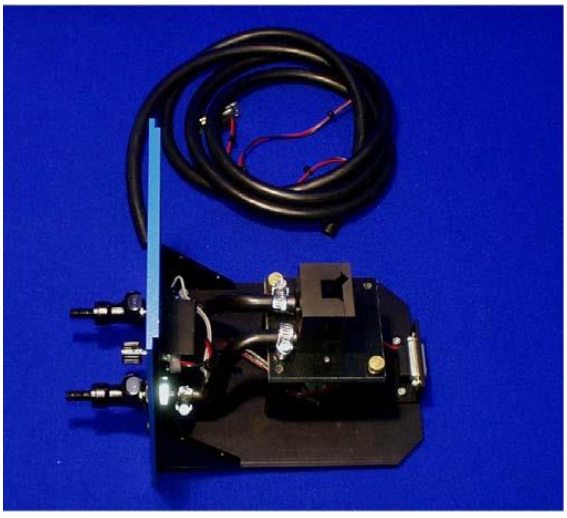
circulating refrigerated controller does. The temperature range is -10°C to +120°C. To prevent condensation of moisture on chilled cuvettes, an injection port for dry nitrogen gas is provided. The temperature and turret (if applicable) are controlled directly from FluorEssence software. It includes a self-contained circulator to remove heat. No plumbing required. For use with pulsed light sources as part of TCSOC or MCS experiments, the F1PosTCSPC-QNW holds DeltaDiodes, NanoLEDs or SpectraLED pulsed sources with a single-cuvette holder and the same temperature control available.

5700005135

5700003355

12.7 5500450433 Cell Holder, Single-Position, Thermostatted

The 5500450433 Single-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.



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

12.7.1 Installation

1 Remove the present holder from the posts.

2 Replace with the 5500450433.

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.*

12.7.2 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 the cuvette in the holder.

3 Allow the sample to reach the desired temperature.

4 Turn on the magnetic stirrer.

5 Select the appropriate speed.

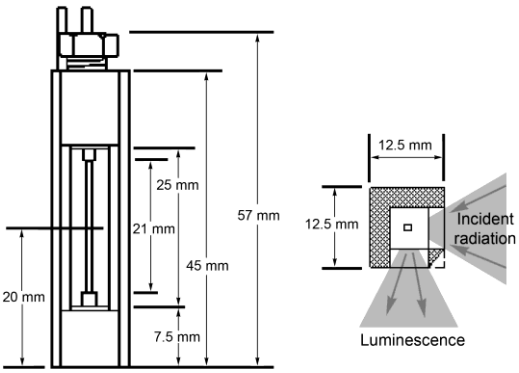
The speed at which the sample should be mixed depends on the viscosity of the sample.

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

6 Run your experiment as usual.

12.8 5500001955 Cell HPLC Flow

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.



12.9 5500000008 Cell, Micro, Adapter 1cm Path Length, 250microL.

This is the adapter to hold the 5500000019 Micro Cell in the Sample Holder.

12.10 5500000019 Cell, Micro, 250microL, 3mm SQ, Quartz

This non-fluorescing fused silica cylindrical cell holds 250 µL. A magnetic stirrer cannot be used with this cell.

12.11 5500001924 Cell, Microsense

To measure fluorescence of ultra-small samples with volumes less than 10 µL, HORIBA Scientific offers the Microsense. This microliter sample cell allows you to add your sample via a pipette, without dilution for easy recovery. Microsense provides accurate, repeatable fluorescence measurements.

12.12 5500650518 Cell, Reduced Volume

This non-fluorescing fused silica cell is selected for samples with a volume of 500 µL. The square cross-section of the sample cavity is 5 mm. The precise imaging capability of the excitation light focused onto the sample allows for high sensitivity. The adapter (5500650519) and a “flea” magnetic stirrer are included.

12.13 5500001925 Cell with Teflon® Stopper

This cell measures 10mm x 10mm in cross-section and comes with a Teflon a stopper to contain volatile liquids.

12.14 5500001920 Cell with Teflon® Cap

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

12.15 5500000153 Cryostat, Janis

The 5700006782 Janis cryostat VNF-100 is a liquid-nitrogen variable-temperature cryostat with the sample located in flowing vapor. Ideal for experiments with samples that are difficult to thermally anchor, e.g., liquids or powders, the 5700006782 features a top-loading sample chamber for rapid sample-exchange, and four-way $f = 1.0$ optical access to the sample chamber. Among the cryostat's features are:

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

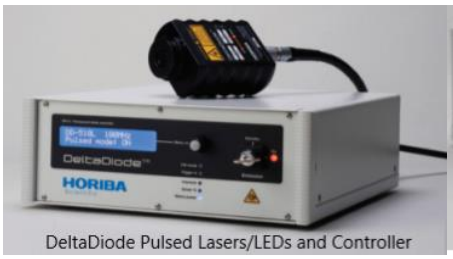
Caution: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of cryogenic materials such as liquid nitrogen.*

12.16 5700010066 DeltaTime TCSPC-MCS Kit for DeltaDiode Sources on FluoroMax Systems (DT-DD-FM)

The kit includes DeltaHub TCSPC-MCS (Time-Correlated Single Photon Counting and Multichannel Scaling) timing electronics (lifetime measurable from 25ps to 1s, subject to light source and detector), DeltaDiode controller (operates at repetition rates up to 100MHz, subject to attached head), delay box, sample compartment interface with pulsed-source holder, software, all cables, and connections. Note: FluoroMax systems with "P" phosphorescence option require additional cables for MCS:FM-PhosCBL, not included.

12.17 5700010070 DeltaTime TCSPC-MCS Kit for DeltaDiode Sources on FluoroMax Systems (DT-DD-FM-Temp)

The kit includes DeltaHub TCSPC-MCS timing electronics, DeltaDiode controller, delay box, sample compartment interface (FM4-NSCTherm) with pulse-source holder compatible with circulating bath, software, all cables, and connections.



DeltaDiode Pulsed Lasers/LEDs and Controller

DeltaHub Timing Electronics

12.18 5700010067 DeltaTime TCSPC-MCS Kit for NanoLED Sources on FluoroMax Systems (DT-NL-FM)

The kit includes DeltaHub TCSPC-MCS timing electronics NanoLED controller, sample compartment interface with pulse-source holder, software, all cables, and connections.

12.19 5700010071 DeltaTime TCSPC-MCS Kit for NanoLED Sources on FluoroMax Systems (DT-NL-FM-Temp)

The kit includes DeltaHub TCSPC-MCS timing electronics, NanoLED controller, sample compartment interface (FM4-NSCTherm) with pulsed-source holder compatible with circulating bath for the temperature control of the samples, software, all cables, and connections.

12.20 5700010068 DeltaTime Kit for SpectraLED Sources on FluoroMax Systems (DT-SL-FM)

The kit includes DeltaHub timing electronics for the use of the SpectraLED in MCS mode, sample compartment interface with pulsed-source holder, software, all cables, and connections.

12.21 5700010072 DeltaTime Kit for SpectraLED Sources on FluoroMax Systems (DT-SL-FM-Temp)

The kit includes DeltaHub timing electronics for the use of the SpetraLED in MCS mode, sample compartment interface (FM4-NSCTherm) with pulsed-source holder compatible with circulating bath for the temperature control of the samples, software, all cables, and connections.

12.22 5700010069 DeltaTime Kit for 980nm Pulsed Laser on FluoroMax Systems

The kit includes DeltaHub timing electronics for the use of the laser in MCS mode software, all cables, and connections.

12.23 Front Mounted Laser

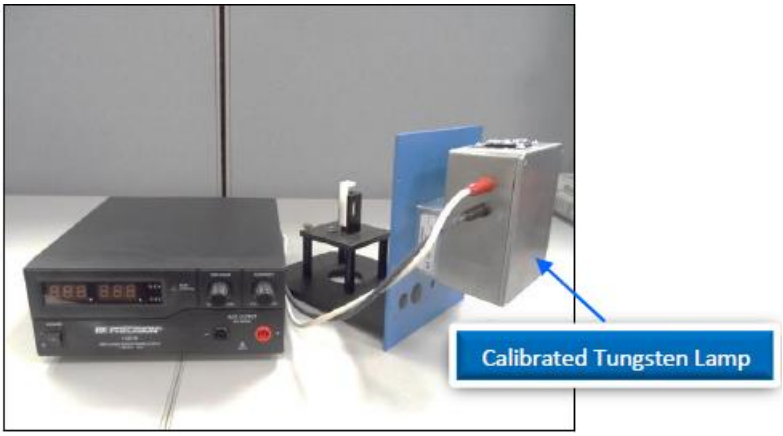
Caution: Lasers emit intense UV, visible or near-infrared light and heat and require the use of laser safety precautions, including the use of laser safety goggles. Understand all safety precautions before handling or using lasers.

The FluoroMax can be customized to include a removable laser system. The laser system can be added as an excitation source mounted to the front of the sample compartment (sample drawer). Refer to section **15.6** for approved laser list. For more information refer to Addendum 1 (Section: **15** of this manual) or contact your HORIBA Sales representative to discuss your specific application requirements.

FluoroMax Plus with Front Laser System

12.24 5700014790 Emission Correction Factor Kit (F-3026)

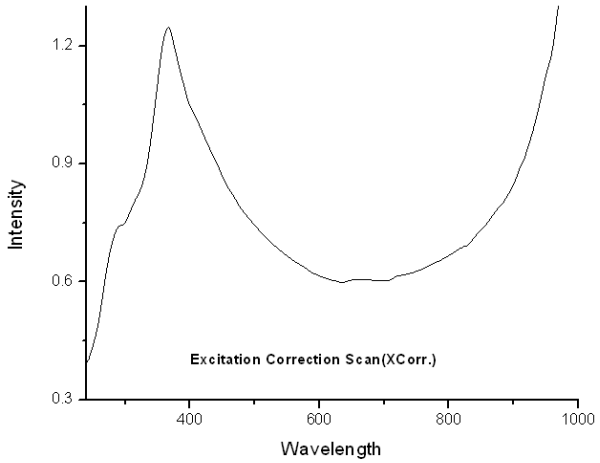
This kit includes a calibrated tungsten lamp mounted to the sample tray, fixed power supply for the lamp, reflector, and a calibrated irradiance file for the lamp. With the output of the lamp known, the spectrum of the tungsten lamp through the emission optics and detector can be measured and a spectral efficiency curve for the emission optics and detector can be created. These "Correction Factors" can be applied to fluorescence data in the software to account for differences in efficiency across the wavelength range of each detector/grating set. The tungsten lamp is calibrated from 240-2100 nm, so this kit is useful for both UV-visible correction and NIR correction as well.



12.25 5700355954 Excitation Correction Factor Kit (F-3028)

Caution: This lamp emits intense light and heat, and requires the use of safety goggles. Understand all safety precautions before handling or using this tungsten lamp.

This kit is a calibrated silicon photodiode mounted on the sample tray. All cables and connections are included. The xenon lamp is measured across 240-1000nm and the measured response of the ExCorr reference photodiode is measured in comparison to the corrected spectrum measured on the photodiode of this kit. The ratio of the corrected calibrated photodiode to the measured ExCorr response is used to get the response of the ExCorr reference photodiode. The correction factor response can then be loaded into the software for application to any fluorescence measurement using the derived ExCorr reference photodiode signal for accurate correction of the xenon lamp source on the final data.

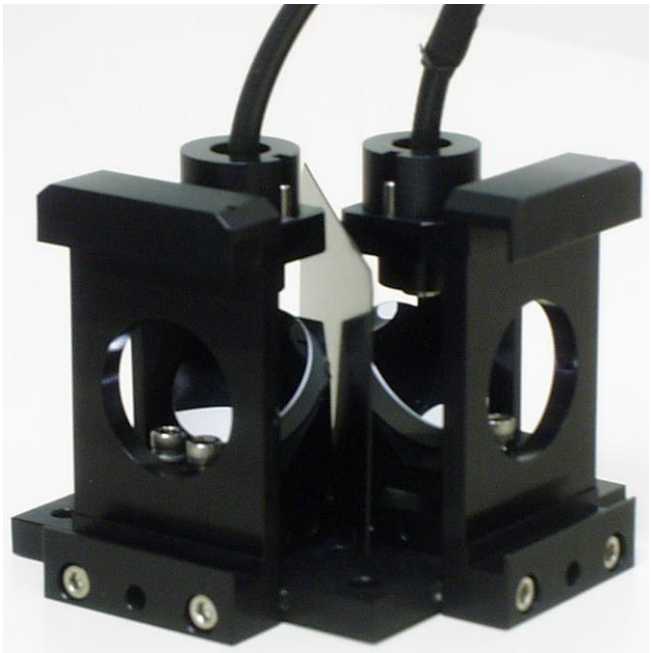


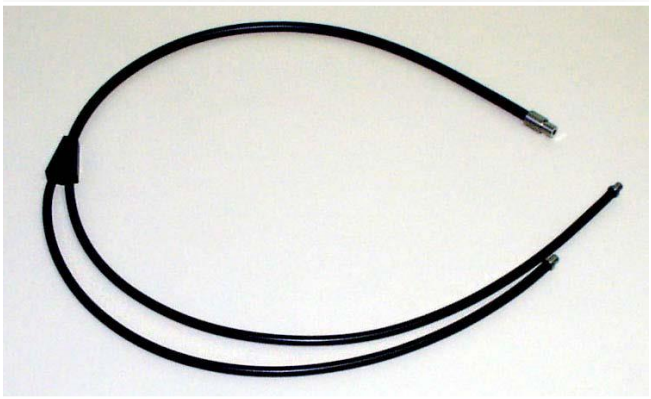
Example of Excitation Correction Factor

12.26 5500000022 Fiber Optic Mount and 5500000117 Fiber Optic Bundles

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 5500000022 Fiber Optic Mount (plus fiber-optic bundles) allows remote sensing of fluorescence. The 5500000022 couples to the sample compartment; light is focused from the excitation monochromator 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 (5500000117) ranging in length from 1 meter to 5 meters are available. Contact the local Sales Representative for details.

5500000022 Fiber Optic Mount (above) and 5500000117 fiber-optic bundle (below).

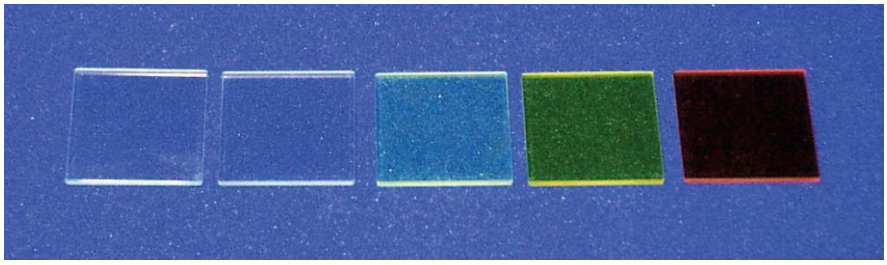




Caution: *Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*

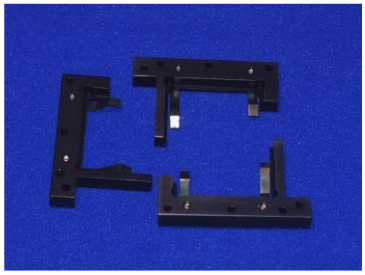
12.27 5500001939 Filter, Cut-On

The model 5500001939 Cut-On Filter set consists of five 2″ × 2″ filters with cut-on wavelengths of 370 nm, 399 nm, 450 nm, 500 nm, and 550 nm. To position the filter properly, the 5500450202 Filter Holder is required. The sample compartment has slots to hold the Model 5500450202 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.



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

12.28 5500450202 Filter Holder, Cut-On



Cut-on filters eliminate second-order effects of the gratings. The sample compartment has three slots that can hold the 5500450202 Filter Holder. Refer to the 5500001939 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.

12.29 5500081050 Injector, Autotitration

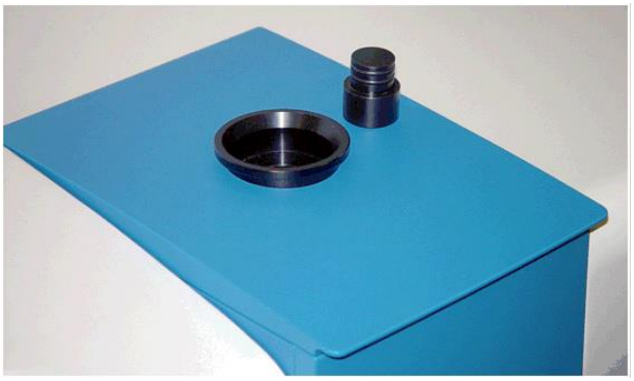
For controlled, automatic injection of aliquots into the sample of your choice, the 5500081050 Autotitration Injector is just the thing, available in both 110-V and 220-models. The 5500081050 comes with dual syringes, for complete control over dispensing and aspirating volumes of liquids into and out 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.

5500081050 AutoTitrator Injector

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

12.30 5500450432 Injector Port

For the study of reaction kinetics, such as Ca^{2+} measurements, the 5500450432 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, preventing both light and air from reaching the sample.



The Injector Port will accommodate most pipettes and syringes, with an injection-hole diameter of 0.125″ (3.2 mm). A cap is included to cover the port when not in use.

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

12.31 5500000398 Lamp,150W Xenon

The 5500000398 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.*

12.32 5500352117 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-Based
- Multigroup

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

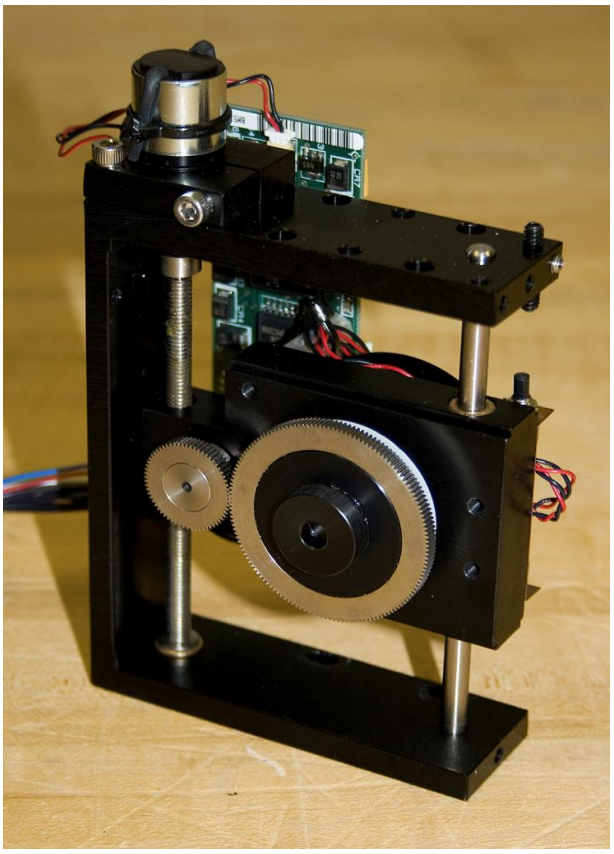
***Caution**: Intense ultraviolet, visible, or infrared light may be present when the sample compartment is open. Do not aim fiber-optic bundles onto the skin or eyes. Use extreme caution with the fiber-optic probes.*

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

12.33 5500450499 Polarizer, L-Format

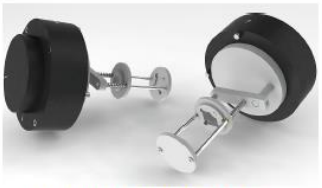
For L-format spectrofluorometers such as the FluoroMax®Plus, the 5500450499 dual polarizer is ideal. The kit includes two polarizers, placed at the optical entrance and exit of the sample compartment. The polarizers are fully automated, and are adjustable to within 1° rotation. Insertion and removal of the polarizers from the optical path is controlled by the host computer.

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

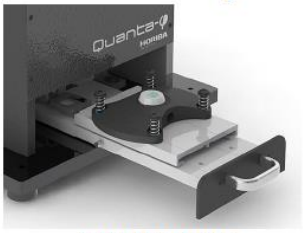


12.34 5500011752 Quanta-φ Photoluminescence Quantum Yield (PLQY) Accessory

This accessory uses a 6-inch (152mm) diameter external sphere (Spectralon, 250-2500nm) connected to the instrument using optical fibers to measure luminesce quantum yields and chromaticity of various solids, liquids, powders, thin films, and small light sources. The accessory also includes 1 x QO-01 powder cup with 5 x QP-02 cover slips, 1 x QP-03 spectralon plug, 1 x center-mount cuvette-holder, 1 x top-mounted excitation fiber holder, 1xQP-05 uncalibrated reflector, and the FM4-fiber coupler interface. Neutral density filters required for PLQY but not included. It requires FluorEssence software.



Two views of the cuvette holder for liquids.



Sample tray for solid samples.

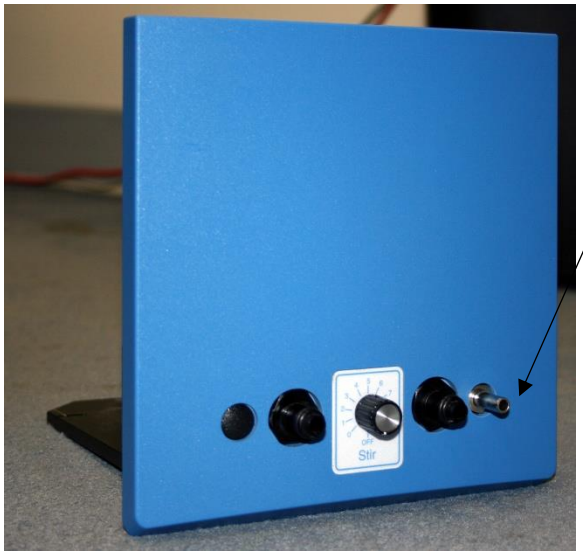
Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

Integrating Sphere (5700005605): This internal integrating sphere [3.2-inch (81mm), barium sulfate for PLQY measurement in the 380-1500nm region] is mounted in a sample tray. No fiber optics are required. Its easily removable top enables changing samples in seconds. It also accepts regular 10 x 10mm cuvettes. Slide holder and powder holder included. Neutral density filters required for PLQY are not included.

12.35 5500450497 Quartz Windows for Sample Compartment

This accessory is for use with experiments requiring special atmospheres, dry nitrogen for example. The windows provide for a clear optical path while keeping the sample in the gas environment introduced into the sample compartment through the purge port on the front of the sample tray. The windows also keep harmful gases or vapors from reaching the optics inside the FluoroMax®Plus monochromators. In addition, a purge port allows free escape of dry nitrogen from the sample chamber.

Purge port



Purge port mounted on the front of the FluoroMax®Plus' sample compartment

12.36 5700003355 Rapid Peltier Temperature Controlled Single Sample Holder for TCSPC Equipped System (F1PosTCSPC-QNM)

It has a temperature range from -15 degree C to +105 degree C with stir and stir bar, water circulator, and software driver.

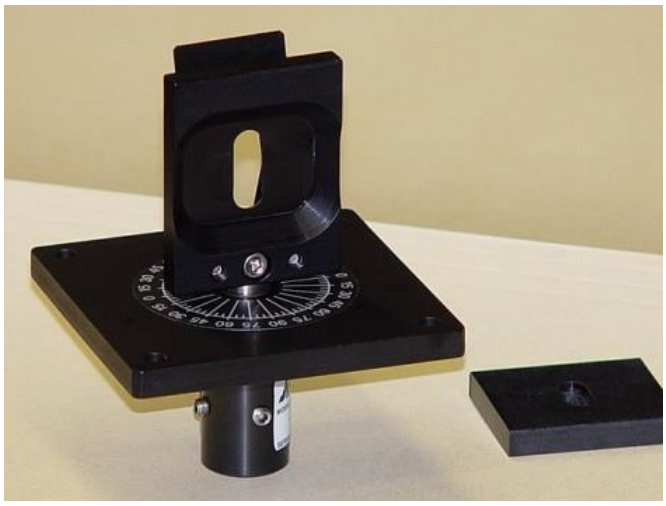
12.37 5500450185 Shutter Accessory

The 5500450185 Shutter accessory is entirely controlled by your software. As an added feature, it includes a microswitch on the sample-compartment lid to activate the shutter when the sample compartment is opened. This protects the detector from unwanted excess light.

12.38 5500001933 Solid Sample Holder

12.38.1 Introduction

The 5500001933 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.



5500001933 Solid Sample Holder (with sample block nearby).

12.38.2 Installation

1 Remove the present holder.

2 Position the base on the posts.

3 Tighten the two thumbscrews.

12.38.3 For pellets, crystals, creams, gels, powders, and similar materials:

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

1 Fill the well of the block.

2 Place a quartz coverslip or Teflon® film over the well.
This holds the sample in place when vertically positioned.

3 Carefully insert the block between the bracket and spring clip, so that the sample is angled approximately 60° to the excitation light.

4 This prevents reflections from entering the emission monochromator, and lets the fluorescence emission to be measured with minimal interference from scattered light.

12.38.4 Use with samples such as thin films, microscope slides, fibers, or other materials:

1 Place the material on the block on the side opposite that of the well.

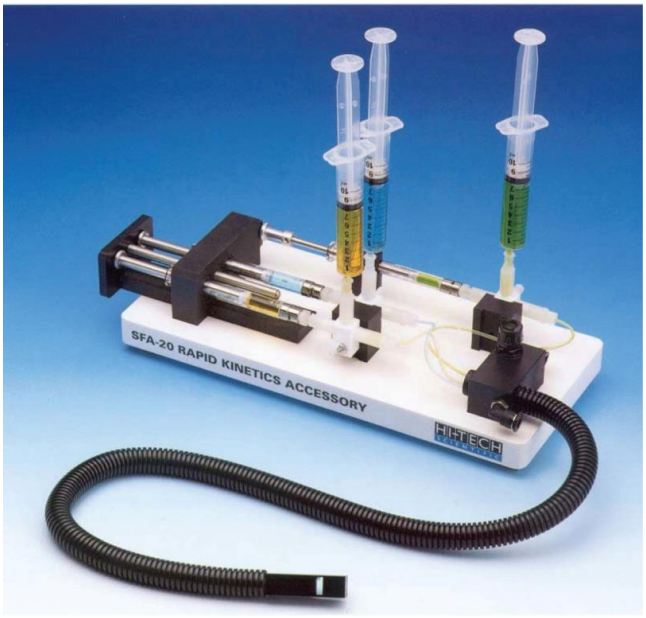
Caution: *Always read the Material Safety Data Sheet (MSDS) to understand the hazards of handling your sample.*

2 Carefully insert the block between the bracket and spring clip, so that the sample is angled approximately 60° to the excitation light.

This prevents reflections from entering the emission monochromator, and lets the fluorescence emission to be measured with minimal interference from scattered light.

12.39 5500003025 Stopped-Flow Accessory

The stopped-flow rapid-kinetics accessories 5500003025 and 5500004461 offer 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 improving sample economy.

The 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 accessory 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.

Model 5500003025 includes 600 mm of tubing, while 5500004461 has 300 mm of tubing.

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

12.40 5500450520 Temperature Bath

For studies of samples whose properties are temperature-dependent, use the 5500450520 temperature bath. The controller circulates fluids externally, with tubes leading to the sample chamber. The temperature range is from –25°C to +80°C. Sensor and all cables are included with the 5500450520 and 5500000264. The Temperature Bath is available in a 110 V (5500450520) and 220 V (5500000264) version.

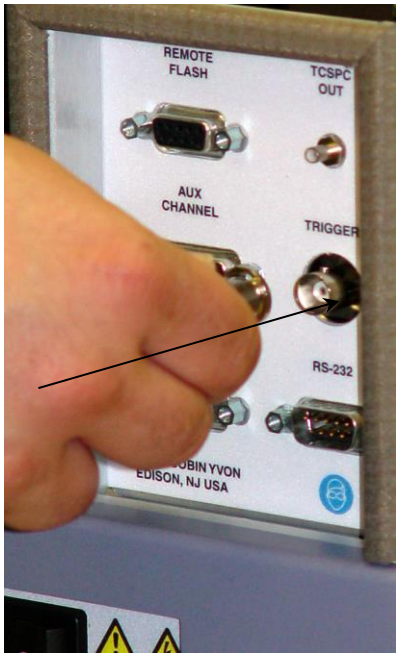
Warning: *Refer to your Material Safety Data Sheet (MSDS) for information on the hazards of an ethylene-glycol–water mixture.*

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

12.41 5500400981 Trigger Cable

The 5500409811 Trigger Cable permits the fluorescence system to be triggered by hand.

5500400981 Trigger Cable.



The Trigger Cable is attached to the FluoroMax® via the BNC TRIGGER jack on the side of the instrument. Simply press the button on the end of the cable to start the scan.

13 GLOSSARY

Absorption	Transition, when a photon enters a molecule, from the ground state to the excited singlet state. This process typically occurs in ~10^{-15} s.
Absorbance	The extent of light absorption by a substance. Absorbance, $A = -\log T$, where T is the transmittance of the sample. Absorbance is also synonymous with optical density, OD. Absorbance can be calculated using the Beer-Lambert Law: $A = \varepsilon c l = \text{OD} = -\log T$ ε = extinction coefficient (M^{-1} cm^{-1}) C = sample concentration (M) l = path length (cm).
Acquisition Modes (R, S Channels)	The logical input channels are 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® Plus 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. Auto polarizers on the FluoroMax® Plus 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 wavelength range of light passing through the excitation and emission spectrometers. The wider the bandpass, the higher the signal intensity.
Bandpass Filter	Optical element that selectively transmits a narrow range of optical wavelengths.
Bioluminescence	Emission of light originating from a chemical reaction in a living organism.
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 luminescence 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	Wavelength at which a grating is optimized for efficiency. Generally, the gratings are efficient to ⅔ before the blaze wavelength to twice the blaze wavelength. The excitation and emission gratings are blazed in the UV and visible, respectively.
Chemiluminescence	Emission of light originating from a chemical reaction.
Color Effect for Pulse Technique	Time-dependent wavelength distribution of the lamp pulse.
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	Compensates for the wavelength-dependent components of the system, like the xenon lamp, gratings, and signal detector. Emission and excitation correction-factor files are included with the software and are titled xcorrect.spc and mcorrect.spc.
CTI Card	This acquisition and control card, located in the rear of the FluoroMax® Plus, 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® Plus.
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.
Cut-on Filter	Optical component that passes light of a higher wavelength.
Cut-off Filter	Optical component that passes light of a lower wavelength.
Dark Counts	Inherent background signal 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® Plus system has dark counts < 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.
Data file	A file used to store spectral data, constant-wavelength analysis data, or other recorded data. In FluorEssence™, the most common data file 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.).
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 is 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® Plus is a 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 monochromator to measure the fluorescence emission.
Emission Scan	Shows the spectral distribution of light emitted by the sample. During an emission scan, the excitation monochromator remains at a fixed wavelength while the emission monochromator scans a selected region.
Energy Transfer	The transfer of 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: Total Luminescence Spectroscopy
Excitation Monochromator	The monochromator, located between the xenon lamp and the sample compartment, is 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® Plus is a 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	Shows the spectral distribution of light absorbed by the sample. To acquire an excitation scan, the excitation monochromator scans a selected spectral region while the emission monochromator remains at a fixed wavelength.
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 Fluorescence Experiment Menu has its own default experiment file (e.g.,

	DfltEm1.xml is the default emission-scan definition). Use experiment files to archive scan settings for acquisitions that are performed routinely.
Extrinsic Fluorescence	Inherent fluorescence of probes used to study non-fluorescent molecules.
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 lamp that provides pulsed-light output to excite a sample. Can be either "free running" or "gated."
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 ~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 usually is selected for samples such as powders, thin films, pellets, cells on a cover-slip, and solids.
Grating	Optical element in the monochromator, consisting of finely scribed grooves that disperse white light into a spectrum.
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-path-length 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 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.
Jablonski (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® Plus, 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 is excited from a variety of processes, resulting in an electronic transition within the molecule to a lower energy state. See also Bioluminescence, Chemiluminescence, and Fluorescence.
MCD Shutter	Multi-channel device shutter. The Uniblitz® shutter is used for its rapid cycle time.
Mercury Lamp	Light source that emits discrete, narrow lines as opposed to a continuum. A mercury lamp can be used to check the monochromator's calibration.
Mirror-image Rule	When the emission profile appears to be the mirror image of the absorption spectrum.

Molar Extinction Coefficient (ε)	The absorptivity of a particular substance, in M^{-1} cm^{-1}.
Monochromator	The component in a spectrofluorometer that is scanned to provide the excitation and emission spectra. Monochromators are chosen for stray-light rejection, resolution, and throughput.
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 a .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	This special software 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 spectrofluorometers will cycle through each pair, integrating for the specified time, before moving on to the next point. Use Multigroup software 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	A synonym of *absorbance*. See 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 as well as the apparent intensities. 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 occurs 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 photobleached, 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 nonlinearity 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 = mP × 1000.
Pulse-sampling Method	A technique for measuring fluorescence lifetimes, in which an initial population of fluorophores is excited by infinitely short pulses of light. An advantage of this technique is the direct recording of time-resolved emission spectra.
Quantum Yield (Fluorescence Quantum Yield)	The efficiency of the absorption of a photon to be emitted (fluoresced). Quantum yields typically are expressed as percent. 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 $Ru(BPY)_3$.
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 is 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 4^{th} 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 is used to monitor the output of the xenon lamp. A silicon photodiode with enhanced-UV response is used for the FluoroMax® Plus, and is connected to input channel R. Use S/R to correct for the xenon-lamp response during an excitation scan.
Resolution	The ability to separate two closely spaced peaks. Resolution can be improved by decreasing the bandpass and the increment (step size).
Right-angle Detection	Collection of fluorescence at 90° to the incident radiation. Right-angle detection typically is selected for dilute and clear solutions.
Sample Changer (Automated)	An automated accessory that automatically positions up to four cuvettes 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 many 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® Plus saturates at 1×10^7 cps.
Scatter	A combination of Raman, Rayleigh, and Rayleigh-Tyndall scattering, which can distort fluorescence spectra with respect to intensities and wavelengths.
Signal Channel	See: Acquisition modes.
Signal Photomultiplier	A 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 is 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 represented 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® Plus 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 HORIBA Scientific 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 that remains. Spectral correction is accomplished with a variety of options on HORIBA Scientific 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	All 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, a 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	Generally, 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).
TCSPC	**T**ime-**c**orrelated **s**ingle-**p**hoton **c**ounting. Technique in which the sample is excited by a pulsed source, and the sample's fluorescence is collected over the course of many pulses. The arriving photons are timed after the excitation. Gradually a decay curve is built up and the sample's fluorescent lifetime is calculated.
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. HORIBA Scientific spectrofluorometers offer various methods for such correction, including spectral correction, dark offset, blank subtraction, and others.
Temperature Scan	A FluorEssence™ Kinetics scan-definition 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 thermostatted 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 resulting from 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	Lamp that produces a continuum of light from the ultraviolet to the near infrared for sample excitation.
Xenon-lamp Scan	A profile of the lamp output as a function of wavelength. The lamp scan is acquired 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 Letter

15 ADDENDUM 1

This addendum provides the guidelines of removable laser systems. There are many laser options including various manufacturers, wavelengths and power levels (up to and including a class 4 laser system). In all instances, the laser head is connected directly to the front of the sample compartment, used in conjunction with the Xenon arc lamp or as the primary light source. The laser can be direct coupled, or fiber coupled.

It is critically important for the user (purchaser) to read and comply with the guidelines (and laser specification) provided herein as well provided by the laser manufacturer. For information specific to the laser, please refer to the provided laser system manual.

15.1 Laser Specification

FluoroMax with a laser accessory is a class 1 laser product that is intended to incorporate up to a class 4 laser system. The user shall choose from the list of available lasers provided in section **15.6**. Contact Horiba Sales (or Service) for compatibility with lasers not listed in section **15.6.** The removable laser specifications are as follows:

- Certified laser system in accordance with FDA requirements defined in 21CFR 1040.10 and 1040.11.
- Certified (and tested) according to EN61010-1:2010 (including EN60825-1:2014).
- Mechanical Interface: Securely connected using appropriate tools to the beam tube, ensuring proper alignment to prevent user access. Access to the laser beam shall be prevented by a protective enclosure consisting of:
    - Laser mounting flange shall be designed to mate the laser to HORIBA laser assembly drawer such that there is no exposed beam. The beam shall be completely contained within the sample area.
    - The following components are provided by HORIBA sample compartment design and construction.
        - Sample Compartment: light tight aluminum walls, floor and cover.
        - HORIBA laser assembly sample drawer is constructed of aluminum.
        - HORIBA sample drawer lid: Opaque plastic material.
        - Two Interlocks on the sample lid / drawer (Refer to Section:**15.3.2)**
        - Mounting screws (requiring the use of special tools) to attach the laser sample drawer to the front of the sample drawer. Refer to the image below:

FluoroMax with front facing Class 4 Laser system

- Electrical Interface: It is essential to connect the safety interlock to the remote interlock connector of the removable laser system.
    - Front face installation requires purchase of the HORIBA front face laser assembly sample drawer.
    - Connect the interlock cable from the LEMO connector on the front of the sample drawer to the remote interlock connector of the removable laser system. The remote interlock connector type may vary. Contact HORIBA service to define the proper cable for compliance.
      Refer to Section: **15.3.2** for more details on interlocks.

15.2 Additional Risks of the Laser Beams

Warning: Laser beams are dangerous. Please read the following precautions.

Refer to Section: **0.3** for general safety symbols and safety summary. Refer to laser manual for additional safety symbols located on laser system. The dangers associated with class 3B, or class 4 laser include:

- Skin burns caused by contact with high powered laser beams.
- Permanent retina damage with direct exposure to laser beams.
- Fire ignited by intense laser beams.
- Interaction of other nearby chemicals with intense ultraviolet, visible, or infrared radiation.
- High-voltage components in powerful lasers can pose an electrocution risk if safety precautions are not followed.
- Some lasers use chemical dyes or gases as their lasing medium. These chemicals can be hazardous if not handled properly.

⚠ **Caution: Use of controls or adjustments, or performance of procedure other than those contained in the operation, maintenance or any other manual provided with this instrument may result in hazardous radiation exposure.**

Do not remove the laser from the laser product.

Contact HORIBA Service to complete laser installations or service.

Never look directly OR indirectly into the laser light.

Do not use the laser in the places marked "No Smoking" or "Flammable and Explosive", which may cause danger.

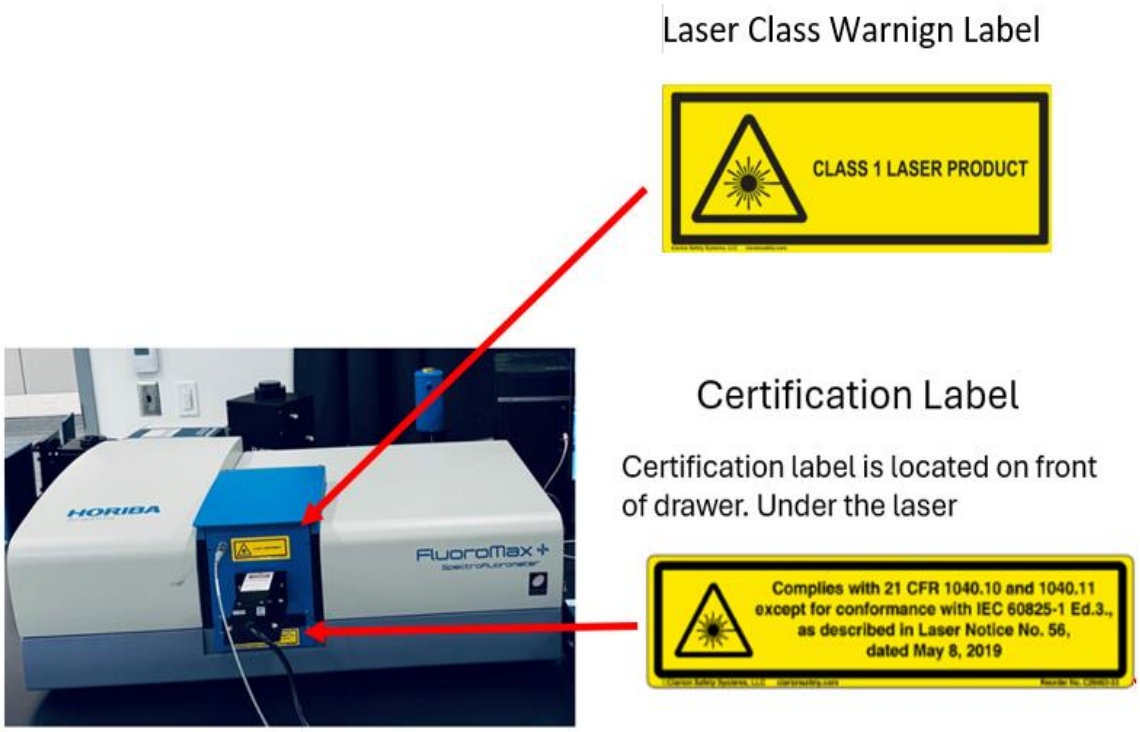
Laser choices exist in both the visible and invisible spectra for this instrument.

When safety procedures are followed, no hazards are found.

This instrument is not designed and manufactured for any medical diagnosis purpose.

15.2.1 Safety and Certification Labels

Clear and legible symbols are placed on the system. Label position and size may vary depending upon customer requirements. The FluoroMax with laser accessory: Appropriate warning labels, FDA certification label, Laser class label and Identification label. Refer to the images below:



FluoroMax Plus With Front Laser System Labels

FLMAX With Front Facing Lase System Identification Label

15.3 System Operation

Refer to Section:**1** for general FluoroMax Plus system operation.

For laser operation, refer to laser manual.

15.3.1 Installation of a Laser Accessory

Disclaimer: Customer is required to contact HORIBA Service department for proper integration of a laser system with the FluoroMax. It is critical for the safety of the user that the mounting hardware, protective housing and the safety interlocks are designed and installed by qualified personnel to maintain the reliability and instrument integrity. Contact HORIBA Sales (or Service) to discuss your application requirements.

UNDER NO CIRCUMSTANCE shall attempts be made to operate the laser system with safety interlocks bypassed or portions of the protective housing removed. Further, DO NOT turn the laser system on until the laser system is properly mounted, installed AND interlocked.

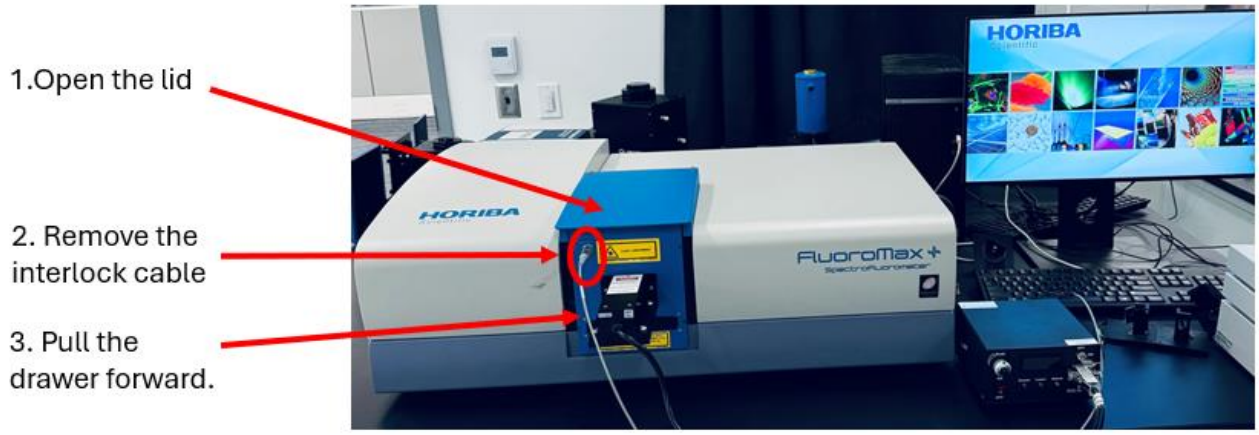
There are many laser options compatible with the FluoroMax. It is common for a customer to require different wavelength lasers. Based on your specific laser requirements, there exists a diverse array of laser types to select from. Each type offers unique characteristics and operational parameters, such as wavelength, power output, beam quality, and modulation capabilities, etc. These variations cater to different applications. If the designed laser does not appear in the section **15.6**, please contact HORIBA Sales.

These guidelines below are furnished to facilitate the replacement of the front face laser system. Adherence to the outlined steps will ensure the successful and safe installation of the new system.

1. Remove and save the (4) screws from the front of the laser sample drawer as shown in the image below:

2. Disconnect the interlock cable from laser sample compartment.

3. Open the lid and remove the laser sample drawer.



4. Install the new laser sample drawer in the same location, close the lid and install the screws saved from step 1 in the front of the new laser sample drawer.
5. Reconnect the interlock cable.

Caution: BEFORE TURNING ON THE INSTRUMENT AND LASER CONTROLLER, CHECK THE FOLLOWING:

1.THE SAMPLE COMPARTMENT LID SHOULD BE CLOSED.

2.THE POWER CONTROL KNOB ON THE LASER CONTROLLER SHOULD BE TURNED DOWN TO ZERO.

6. Test the interlock
    a. Verify the sample compartment lid is closed and the drawer is fully inserted into the FluoroMax.
    b. Using the laser controller, turn the laser power down to zero. Refer to the laser manual for laser operating instructions.
    c. Turn on the FluoroMax, laser controller, and computer in that order.
    d. Turn the key on the laser controller to the ON position, and adjust the laser power by turning the knob to the desired level.

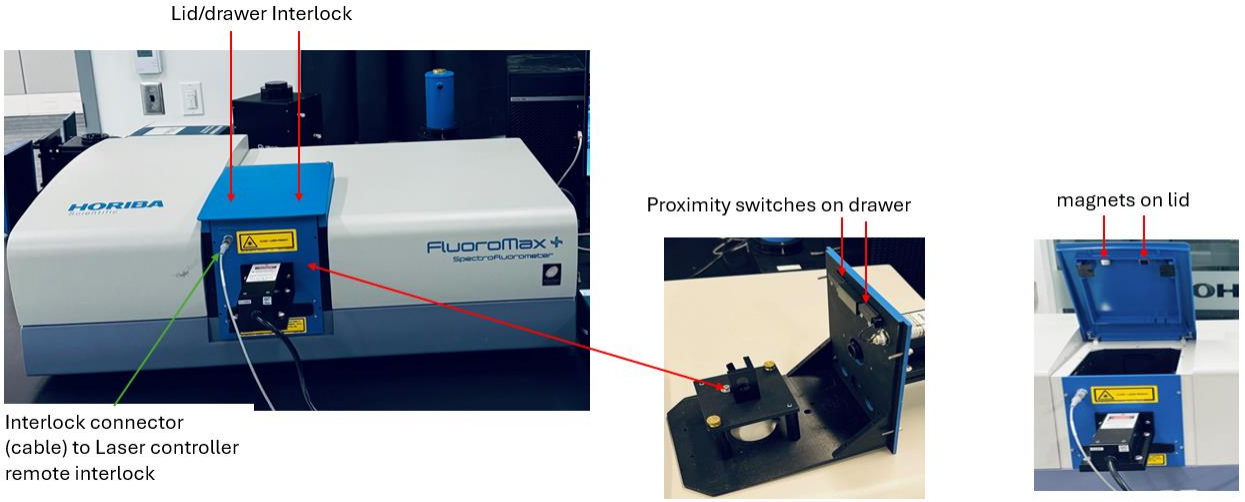
e. Open the sample compartment lid and check that the laser turns off and the controller goes down to zero. This will validate the laser interlock is functioning properly.

15.3.2 Interlocks

The FluoroMax is a class 1 laser product. The FluoroMax with laser system incorporates a protective enclosure and interlocks to protect the user from (accidental) exposure to class 4 laser radiation during operation. The safety interlock circuit utilizes the remote interlock of the removable laser system.

Interlock description:

- The sample compartment drawer includes two magnetic proximity switches (wired in series) to the front panel LEMO connector. The sample lid includes two magnets. When the lid closes, the magnetic force will close the proximity switches creating a short circuit. The LEMO connector is connected (via interlock cable) to the remote interlock of a removable laser. When the lid is closed the proximity switches close creating a short circuit enabling the laser to operate. If the lid is open OR if the drawer is removed the interlock is an open circuit, shutting off the laser. For more information on the remote interlock of the removable laser, please refer to the laser manual provided with this purchase.



- For custom systems, contact HORIBA Service to customize the interlock cable to connect to remote interlock on removable laser controller.

15.3.3 Laser Power Supply

Refer to laser manual for laser power supply information.

15.4 System Operation

Refer to Section: **3** for general system operation.

For laser operation, refer to laser manual.

15.5 Software Operation

For a complete discussion of the almost limitless power provided by FluorEssence™, refer to the FluorEssence™ User's Guide and the on-line help for Origin® and FluorEssence™, which accompany the system.

15.6 Approved Lasers

HORIBA Model Number	HORIBA PN	Manufacture	Manufactures PN	Laser Class
FL-LAS-808	5700016745	Changchun New Industries Optoelectronics Tech. Co., LTD.	LLC MDL-III-808	Class IV
FL-LAS-432	5700041341	Changchun New Industries Optoelectronics Tech. Co., LTD.	MDL-III-438	Class IV
FL-LAS-532	5700003577	Changchun New Industries Optoelectronics Tech. Co., LTD.	MGL-111-532 W/ 1% POWER STABILITY	Class IIIB
FL-LAS-460	5700034986	Changchun New Industries Optoelectronics Tech. Co., LTD.	LLC MDL-111-460	Class IV
FL-LAS-660	5700035403	Changchun New Industries Optoelectronics Tech. Co., LTD.	MDL-III-660D	Class IV
FL-LAS-852	5700035453	Changchun New Industries Optoelectronics Tech. Co., LTD.	MDL-III-852	Class IV
FL-LAS-980	5500990162	Changchun New Industries Optoelectronics Tech. Co., LTD.	MDL-III-980	Class IV
Las-980 Add-on Kit	5500990162	Changchun New Industries Optoelectronics Tech. Co., LTD.	MDL-III-980	Class IV
Las-808 Add-on Kit	5700016745	Changchun New Industries Optoelectronics Tech. Co., LTD.	LLC MDL-III-808	Class IV
Las-532 Add-on Kit	5700035377	Changchun New Industries Optoelectronics Tech. Co., LTD.	MGL-111-532 W/ 1% POWER STABILITY	Class IIIB
Las-460 Add-on Kit	5700034986	Changchun New Industries Optoelectronics Tech. Co., LTD.	LLC MDL-111-460	Class IV
FL-QM-OPO	5700031144	Changchun New Industries Optoelectronics Tech. Co., LTD.	OPLTE UX10230	Class IV
FL-QM-OPO-UV	5700040613	Changchun New Industries Optoelectronics Tech. Co., LTD.	OPLTE UX10230U.11.230.00.0200.RRR00	Class IV
NL-FIU-6	5700010237	NKT PHOTONICS, INC.	S483-065-000	Class IV
NL-FIU-6-PP	5700017003	NKT PHOTONICS, INC.	S483-065-010	Class IV
FL-QM-FIU-6	5700010237	NKT PHOTONICS, INC.	S483-065-000	Class IV
FL-QM-FIU-6-PP	5700017003	NKT PHOTONICS, INC.	S483-065-010	Class IV
QM-FIU-15-PP	5700021553	NKT PHOTONICS, INC.	S473-155-010	Class IV
QM-FIU-15-PP-FD	5700021553	NKT PHOTONICS, INC.	S473-155-010	Class IV
DeltaDiode-DC	5700014166	HORIBA Jobin Yvon IBH Limited	5700014166	Class IIIB LED

DeltaDiode-670L	5500000670	HORIBA Jobin Yvon IBH Limited	DELTADIODE-670L	Class IIIA / 3R
DeltaDiode-730L	5500000730	HORIBA Jobin Yvon IBH Limited	DELTADIODE-730L	Class IIIB
DeltaDiode-980L	5500000980	HORIBA Jobin Yvon IBH Limited	DELTADIODE-980L	Class IIIB
DeltaDiode-1060L	5500001060	HORIBA Jobin Yvon IBH Limited	DELTADIODE-1060L	Class IIIB
DeltaDiode-1310L	5500003517	HORIBA Jobin Yvon IBH Limited	DELTADIODE-1310L	Class IIIB
DeltaDiode-340	5500003527	HORIBA Jobin Yvon IBH Limited	DELTADIODE-340	Class IIIB LED
DeltaDiode-360	5500003529	HORIBA Jobin Yvon IBH Limited	DELTADIODE-360	Class IIIB LED
DeltaDiode-370	5500003530	HORIBA Jobin Yvon IBH Limited	DELTADIODE-370	Class IIIB LED
DeltaDiode-375L	5500003531	HORIBA Jobin Yvon IBH Limited	DELTADIODE-375L	Class IIIB
DeltaDiode-395L	5500003533	HORIBA Jobin Yvon IBH Limited	DELTADIODE-395L	Class IIIB
DeltaDiode-405L	5500003534	HORIBA Jobin Yvon IBH Limited	DELTADIODE-405L	Class IIIA / 3R
DeltaDiode-425L	5500003536	HORIBA Jobin Yvon IBH Limited	DELTADIODE-425L	Class IIIA / 3R
DeltaDiode-440L	5500003537	HORIBA Jobin Yvon IBH Limited	DELTADIODE-440L	Class IIIA / 3R
DeltaDiode-450L	5500003539	HORIBA Jobin Yvon IBH Limited	DELTADIODE-450L	Class IIIA / 3R
DeltaDiode-470L	5500003541	HORIBA Jobin Yvon IBH Limited	DELTADIODE-470L	Class IIIA / 3R
DeltaDiode-485L	5500003543	HORIBA Jobin Yvon IBH Limited	DELTADIODE-485L	Class IIIA / 3R
DeltaDiode-510L	5500003546	HORIBA Jobin Yvon IBH Limited	DELTADIODE-510L	Class IIIA / 3R
DeltaDiode-635L	5500003548	HORIBA Jobin Yvon IBH Limited	DELTADIODE-635L	Class IIIA / 3R
DeltaDiode-785L	5500003553	HORIBA Jobin Yvon IBH Limited	DELTADIODE-785L	Class IIIB
DeltaDiode-830L	5500003554	HORIBA Jobin Yvon IBH Limited	DELTADIODE-830L	Class IIIB
DeltaDiode-390	5700002227	HORIBA Jobin Yvon IBH Limited	DELTADIODE-390	Class IIIB LED
DeltaDiode-560LN	5700002620	HORIBA Jobin Yvon IBH Limited	DELTADIODE-560LN	Class I LED
DeltaDiode-532LN	5700003078	HORIBA Jobin Yvon IBH Limited	DELTADIODE-532L	Class IIIA / 3R
DeltaDiode-650L	5700006442	HORIBA Jobin Yvon IBH Limited	DELTADIODE-650L	Class IIIA / 3R
DeltaDiode-310	5700010190	HORIBA Jobin Yvon IBH Limited	DELTADIODE-310	Class IIIB LED
DeltaDiode-415L	5700010191	HORIBA Jobin Yvon IBH Limited	DELTADIODE-415L	Class IIIA / 3R
DeltaDiode-395L-CW	5700010198	HORIBA Jobin Yvon IBH Limited	DELTADIODE-395L-CW	Class IIIB
DeltaDiode-405L-CW	5700010199	HORIBA Jobin Yvon IBH Limited	DELTADIODE-405L CW PULSED	Class IIIA / 3R
DeltaDiode-415L-CW	5700010200	HORIBA Jobin Yvon IBH Limited	DELTADIODE-415L-CW	Class IIIA / 3R

DeltaDiode-485L-CW	5700010205	HORIBA Jobin Yvon IBH Limited	DELTADIODE-485L-CW	Class IIIA / 3R
DeltaDiode-635L-CW	5700010206	HORIBA Jobin Yvon IBH Limited	DELTADIODE-635L-CW	Class IIIA / 3R
DeltaDiode-650L-CW	5700010207	HORIBA Jobin Yvon IBH Limited	DELTADIODE-650L-CW PULSED	Class IIIA / 3R
DeltaDiode-670L-CW	5700010208	HORIBA Jobin Yvon IBH Limited	DELTADIODE-670L-CW PULSED	Class IIIA / 3R
DeltaDiode-730L-CW	5700010209	HORIBA Jobin Yvon IBH Limited	DELTADIODE-730L-CW PULSED	Class IIIA / 3R
DeltaDiode-495L	5700017001	HORIBA Jobin Yvon IBH Limited	DELTADIODE-495L	Class IIIA / 3R
DeltaDiode-265	5700035495	HORIBA Jobin Yvon IBH Limited	DELTADIODE-265	Class IIIB LED
DeltaDiode-285	5700035496	HORIBA Jobin Yvon IBH Limited	DELTADIODE-285	Class IIIB LED
DeltaDiode-325	5700035497	HORIBA Jobin Yvon IBH Limited	DELTADIODE-325	Class IIIB LED
DeltaDiode-375LB	5700035498	HORIBA Jobin Yvon IBH Limited	DELTADIODE-375LB	Class IIIB LED
DeltaDiode-395LB	5700035500	HORIBA Jobin Yvon IBH Limited	DELTADIODE-395LB	Class IIIB LED
DeltaDiode-405LB	5700035501	HORIBA Jobin Yvon IBH Limited	DELTADIODE-405LB	Class IIIB LED
DeltaDiode-420LB	5700035502	HORIBA Jobin Yvon IBH Limited	DELTADIODE-420LB	Class IIIB LED
DeltaDiode-450LB	5700035503	HORIBA Jobin Yvon IBH Limited	DELTADIODE-450LB	Class IIIB LED
DeltaDiode-470LB	5700035504	HORIBA Jobin Yvon IBH Limited	DELTADIODE-470LB	Class IIIB LED
DeltaDiode-485LB	5700035505	HORIBA Jobin Yvon IBH Limited	DELTADIODE-485LB	Class IIIB LED
DeltaDiode-510LB	5700035506	HORIBA Jobin Yvon IBH Limited	DELTADIODE-510LB	Class IIIB LED
DeltaDiode-440LB	5700039159	HORIBA Jobin Yvon IBH Limited	DELTADIODE-440LB	Class IIIB LED
DeltaDiode-635LB	5700039190	HORIBA Jobin Yvon IBH Limited	DELTADIODE-635LB	Class IIIB LED
DeltaDiode-650LB	5700039191	HORIBA Jobin Yvon IBH Limited	DELTADIODE-650LB	Class IIIB LED
DeltaDiode-670LB	5700039192	HORIBA Jobin Yvon IBH Limited	DELTADIODE-670LB	Class IIIB LED
DeltaDiode-730LB	5700039193	HORIBA Jobin Yvon IBH Limited	DELTADIODE-730LB	Class IIIB LED
DeltaDiode-785LB	5700039194	HORIBA Jobin Yvon IBH Limited	DELTADIODE-785LB	Class IIIB LED
DeltaDiode-830LB	5700039195	HORIBA Jobin Yvon IBH Limited	DELTADIODE-830LB	Class IIIB LED
DeltaDiode-980LB	5700039196	HORIBA Jobin Yvon IBH Limited	DELTADIODE-980LB	Class IIIB LED
DeltaDiode-1060LB	5700039197	HORIBA Jobin Yvon IBH Limited	DELTADIODE-1060LB	Class IIIB LED
DeltaDiode-1310LB	5700039198	HORIBA Jobin Yvon IBH Limited	DELTADIODE-1310LB	Class IIIB LED

15.7 Maintenance, Troubleshooting, Warranty and Service Information

Refer to Section: **7** for detailed information. For further assistance, contact HORIBA service department.

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[Design Concept]
The HORIBA Group application imaged is collaged in the overall design.
Beginning from a nano size element, the scale of the story develops all the way to the Earth with a gentle flow of the water.


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