HORIBA Scientific

Quanta-φ
F-3029 Integrating Sphere

Operation Manual
Part number J81089 rev. C

Quanta-φ

F-3029 Integrating Sphere

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Part Number J81089

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0 : Introduction

About the Quanta-φ integrating sphere

This manual explains the theoretical and practical issues of operating and maintaining the Quanta-φ integrating sphere. The Quanta-φ is used with the Fluorolog®-3, FluoroMax®-3 and -4, and Fluorolog®-Tau-3 spectrofluorometers to study fluorescence from solid, powder, thin-film and liquid samples. The main purpose of the Quanta-φ is the measurement of photoluminescence quantum yields of such materials.

The Quanta-φ integrating sphere accessory is external to the spectrofluorometer. Light from the sample compartment is directed into the sphere via a fiber-optic cable and the F-3000 Fiber-Optic Adapter, and returned to the sample compartment (and then to the emission monochromator) via a second fiber-optic cable and the F-3000.

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

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Caution:

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Caution:

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

Wear protective gloves.

Note:

General information is given concerning operation of the equipment.

Risks of ultraviolet exposure

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

Overexposure to ultraviolet rays threatens human health by causing:

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

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

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

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

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

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

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

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

Training

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

Additional risks of xenon lamps

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

Among the dangers associated with xenon lamps are:

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

Visible radiation

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

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

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

Infrared radiation

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

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

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

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

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

1 : Theory of Operation

Introduction

An ideal integrating sphere is designed to integrate light for collection over all emission angles from the sample. No integrating sphere is ideal, and so there are various approximations required.

Spherical enclosures and radiance

When light hits a diffuse surface, such as the interior of an integrating sphere, radiation exchange occurs. Imagine an area dA_1 that reflects light to another area dA_2. We can write the exchange factor dF, the fraction of energy leaving dA_1, traveling a distance S, and going to dA_2.

$$dF = \frac{\cos\theta_1 \cos\theta_2}{\pi S^2} dA_2 \tag{1}$$

For a spherical enclosure, the area dA_1 actually exchanges light with an area A_2 of definite size. With geometrical considerations, we can integrate the differentials to get

$$F = \frac{A_2}{4\pi r^2} = \frac{A_2}{A_{sphere}} \tag{2}$$

Equation (2) tells us that the fraction of light F that A_2 receives is just A_2's fraction of the sphere's total surface area. The parameter F is independent of the viewing angle, is the same when measured from anywhere within the integrating sphere, and is proportional to the area of the sphere.

Radiance, L, is the flux density of light emanating per unit solid angle. For a diffuse surface receiving an incident flux Φ_i,

$$L = \frac{\Phi_i \rho}{\pi A} \tag{3}$$

where A is the area under illumination, ρ is the reflectance, and π is the solid angle from the surface. The main issue here is the reflectance for an integrating sphere. The reflectance can be rewritten as a simplification of a power series from multiple reflections within the sphere. Thus we rewrite the radiance as

$$L = \frac{\Phi_i}{\pi A} \cdot M \tag{4}$$

where M is a factor called the sphere multiplier. The sphere multiplier takes into account the total fractional area f that the entrance and exit ports occupy (and thus reduce the reflectance), plus multiple reflections:

$$M = \frac{\rho}{1-\rho(1-f)} \tag{5}$$

For a typical integrating sphere whose $\rho \sim 0.95$ and $f \sim 0.03$, M is between 10 and 30.

Time decay of signal

An incoming signal (such as a rapid fluorescence-decay) can be stretched temporally because of the multiple diffuse reflections inside an integrating sphere. This can be important for fluorescence lifetime determinations. The impulse response of an integrating sphere takes the form

$$e^{\frac{-t}{\tau}}$$

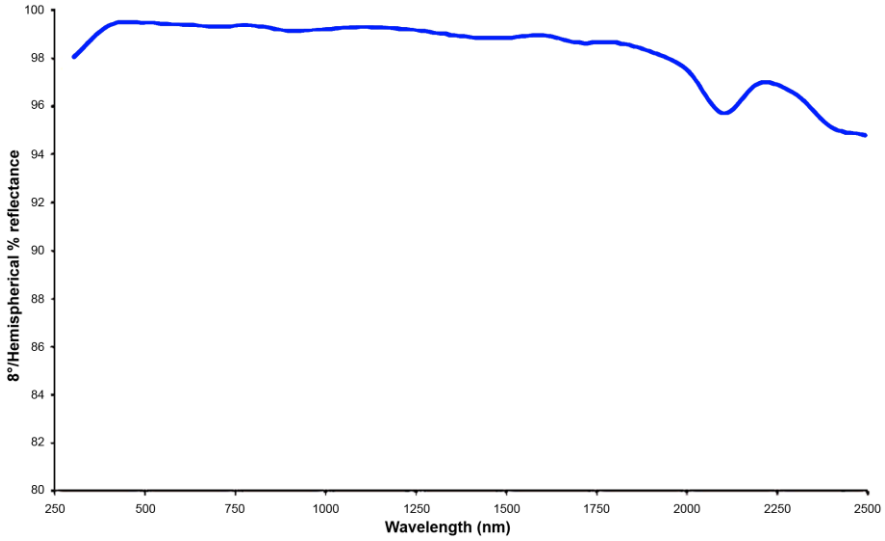
where τ is the time constant of the integrating sphere, and is

$$\tau = -\frac{2}{3} \cdot \frac{d_{sphere}}{c} \cdot \frac{1}{\ln \bar{\rho}} \tag{6}$$

Equation (6) considers also the diameter of the integrating sphere, d_{sphere}, the average reflectance, $\bar{\rho}$, and the speed of light, c. A typical τ might range from several ns to several dozen ns.

Coating of an integrating sphere

The interior reflective coating of an integrating sphere affects its overall performance. The interior of the HORIBA Scientific integrating sphere is made from a proprietary material known as Spectralon®, which has a very wide, flat reflectance of over 95% from 250 nm to 2.5 µm (see graph below). Thus this integrating sphere is useful throughout the spectrofluorometer's scanning range, from the UV through the near-IR.



Photoluminescence quantum yield

An important use of the integrating sphere in conjunction with your spectrofluorometer is the determination of a sample's photoluminescence quantum yield. The photoluminescence quantum yield for a particular molecular species is determined by the first-order rate processes that compete for the excitation energy. The three main processes can be defined with first-order rate constants, such as k_F, the rate constant of fluorescence decay (in units of s^{-1}), k_{NR}, the rate constant of nonradiative decay, and k_T, the rate constant of photochemical energy transfer. Using these three rate constants, the fluorescence quantum yield φ is defined as

$$\varphi = \frac{k_f}{k_f + k_{NR} + k_T}$$

The fluorescence quantum yield is directly related to the fluorescence lifetime τ:

$$\tau = \frac{1}{k_f + k_{NR} + k_T}$$

Any increase in the nonradiative decay processes (k_{NR}) related to events such as quenching and thermal dissipation or energy-transfer (k_T) processes—including Förster Resonance Energy Transfer (FRET)—will decrease both the quantum yield φ and fluorescence lifetime τ. Likewise any decrease in k_{NR} or k_T will increase φ and τ.

The multiple randomized, diffused reflections in the integrating sphere eliminate any isotropic (directional) features of the sample emission from the excited molecular species. Hence, measurement of polarization is not possible within an integrating sphere.

Following is a brief description of the theory and recommended procedure for measuring quantum yield using the Quanta-φ integrating sphere.

General approach

For a general approach, not requiring an integrating sphere, see Joseph R. Lakowicz's book, *Principles of Fluorescence Spectroscopy*.[1]

Theory

The sample is placed in the integrating sphere, and excited with a monochromatic source of wavelength λ. The film absorbance, A, is

$$A = \frac{L_b - L_c}{L_b} \qquad (7)$$

[1] Joseph R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., New York, Springer, 2006, pp. 8–10, 54–55.

where L_b is the integrated excitation profile when the sample is diffusely illuminated by the integrating sphere's surface; and L_c is the integrated excitation profile when the sample is directly excited by the incident beam.

The quantum yield, φ, is, by definition, photons emitted to photons absorbed:

$$\varphi = \frac{E_c - (1-A)\cdot E_b}{L_a \cdot A} = \frac{E_c - E_a}{L_a - L_c} \qquad (8)$$

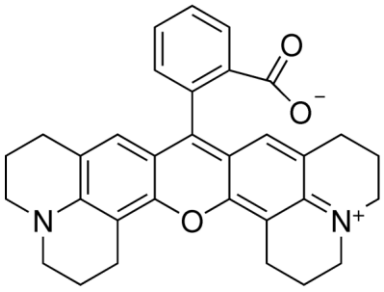
where E_c is the integrated luminescence of the film caused by direct excitation, and E_b is the integrated luminescence of the film caused by indirect illumination from the sphere. The term L_a is the integrated excitation profile from an empty integrating sphere (without the sample, only a blank). Here E_a is the integrated luminescence from an empty integrating sphere (only a blank).

For integration of function L over the wavelength, λ, the integration limits can be from 10 nm below the excitation wavelength to 10 nm above the excitation wavelength.

The spectra recorded must be background corrected, using a blank sample holder, and corrected for wavelength dependence of the spectrofluorometer, sampling optics, and integrating sphere.

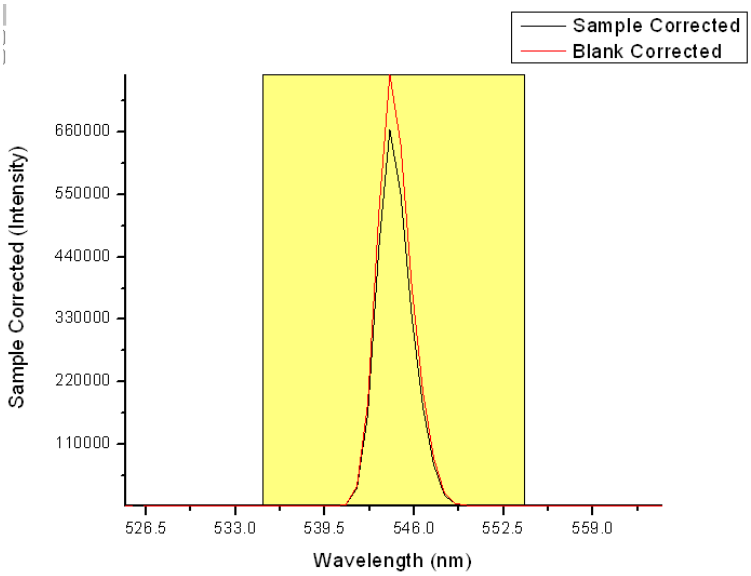
Example

An example is given for rhodamine 101 (see right) in anhydrous ethanol (with a peak absorbance value of OD = 0.063 at 563 nm) excited using 545-nm light (OD = 0.035) using 1 nm bandpass for both excitation and emission.



Caution: *Always attenuate or adjust the signal intensities to prevent saturation of the detector. Saturated signals can seriously deteriorate the measurement precision and accuracy, and possibly damage the detection electronics.*

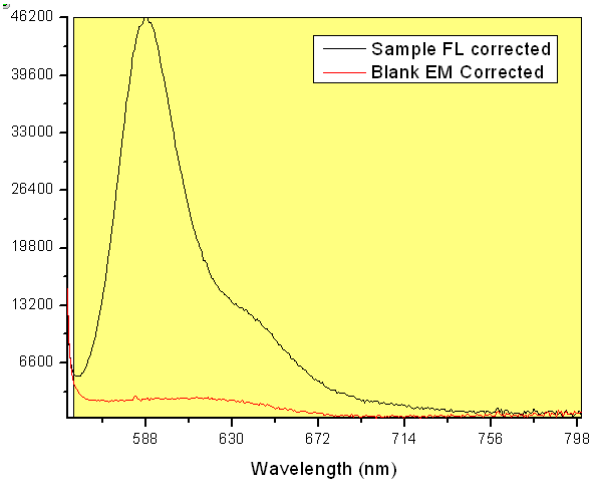
Using a CCD detector, the scattered 545 nm excitation spectra were recorded for both the blank cuvette containing only ethanol (red, L_a), and the sample cuvette (black, L_c). Integration of the instrument-corrected incident beam's signal intensities I was performed from 535 to 553 nm for 0.5 s (20 summed accumulations).



$$L_a = \int_{535}^{553} I(\lambda)d\lambda = 2.27954\times10^6 \qquad (9)$$

$$L_c = \int_{535}^{553} I(\lambda)d\lambda = 1.9856\times10^6 \qquad (10)$$

Next, the sample and blank's fluorescence spectral region intensities were measured using a 4-second integration time under otherwise the same instrument conditions as the L_a and L_c. Integration of the emission-signal region E_a (blank) and E_c (sample) was from 553 to 800 nm.



$$E_a = \int_{553}^{800} I(\lambda)d\lambda = 3.29376\times10^5 \qquad (11)$$

$$E_c = \int_{553}^{800} I(\lambda)d\lambda = 2.51648\times10^6 \qquad (12)$$

Because the integration time constant used to collect E_a and E_c was 8 times longer than for L_a and L_c, we need to divide the difference between E_a and E_c by 8 to calculate the quantum yield, φ, below using the four parameters E_c, E_a, L_a, and L_c,.

$$\begin{aligned}\varphi &= 100\% \times \frac{[(E_c - E_a)/8]}{L_a - L_c} \\ &= 100\% \times \frac{[(2.51648\times10^6 - 3.29376\times10^5)/8]}{2.27954\times10^6 - 1.9856\times10^6} \\ &= 93.01\%\end{aligned}$$

This φ is slightly lower compared to some literature reports for rhodamine 101 in acidifed ethanol (~ 96–99%) primarily due to the anhydrous ethanol used as the solvent.

Error-propagation analysis

In addition to the calculation of φ, the Quanta-φ software performs an error-propagation analysis to help evaluate when signal levels are not properly balanced. This may occur with dilute concentrations of the sample, or when samples exhibit very low quantum efficiency. The error propagation is based on the Poissonian statistics of photon-counting, where the standard deviation σ is equal to the square-root of the photon-count value, *i.e.*,

$$\sigma = \sqrt{photon\ count}$$

The error propagation is performed in a stepwise manner.

First, find the standard deviations for E_a, E_c, L_a, and L_c, respectively as $\sigma_{E_a} = {E_a}^{0.5}$, $\sigma_{E_c} = {E_c}^{0.5}$, $\sigma_{L_c} = {L_a}^{0.5}$ and $\sigma_{L_c} = {L_c}^{0.5}$.

Second, evaluate the standard deviation of the numerator *num* = $[(E_c - E_a)/8]$ of the φ equation (considering the constant factor of 8 in the integration time) as

$$\sigma_{num} = \sqrt{\left(\frac{\sigma_{E_a}}{8}\right)^2 + \left(\frac{\sigma_{E_c}}{8}\right)^2}$$

Then evaluate the standard deviation of the denominator *den* = $(L_a - L_c)$, as

$$\sigma_{den} = \sqrt{{\sigma_{L_c}}^2 + {\sigma_{L_a}}^2}$$

Propagation of the respective relative (σ_{rel}) and absolute errors (σ_{abs}) of φ is as follows:

$$\sigma_{rel} = \sqrt{\left(\frac{\sigma_{den}}{den}\right)^2 + \left(\frac{\sigma_{num}}{num}\right)^2}$$

$$\sigma_{abs} = \sigma_{rel} \cdot \varphi$$

For the example given above for rhodamine 101 (φ = 93.01%), σ_{rel} = 0.007 and σ_{abs} = 0.654 %.

Consider that:

- These values can be used to diagnose trends in the precision and accuracy of φ, especially when evaluated under conditions where the sample concentration or quantum efficiency is being systematically varied. An example is with a quenching experiment or when titrating the sample concentration to minimize reabsorption (inner filter) effects.

- The error-propagation routine is normalized to the L_a and L_c integration conditions as a general rule. This is because the E_a and E_c values (which are typically less intense) are normally collected under conditions of longer integration time or higher excitation power so that these values must be divided by a constant factor in the equation for φ.

- The largest absolute source of error in the φ measurement is likely to be in the larger L_a and L_c values. Therefore HORIBA Scientific recommends that you carefully consider the required integration time and excitation-power conditions for the E_a and E_c values to prevent the larger noise-levels in L_a and L_c from "swamping out" the smaller fluorescence area signal value. **This is a particularly important problem with the ratiometric nature of the φ equation, because the larger source of error is in the denominator.**

Self-absorption and inner-filter effects

The measurement of φ, as an absolute value, is strongly influenced by the sample concentration. Relative changes and observed values of φ can be easily complicated by concentration-related artifacts. Because φ is determined by the number of photons absorbed, the concentration must be measured under the "Beer-Lambert" criterion of a linear relationship between OD and sample concentration.

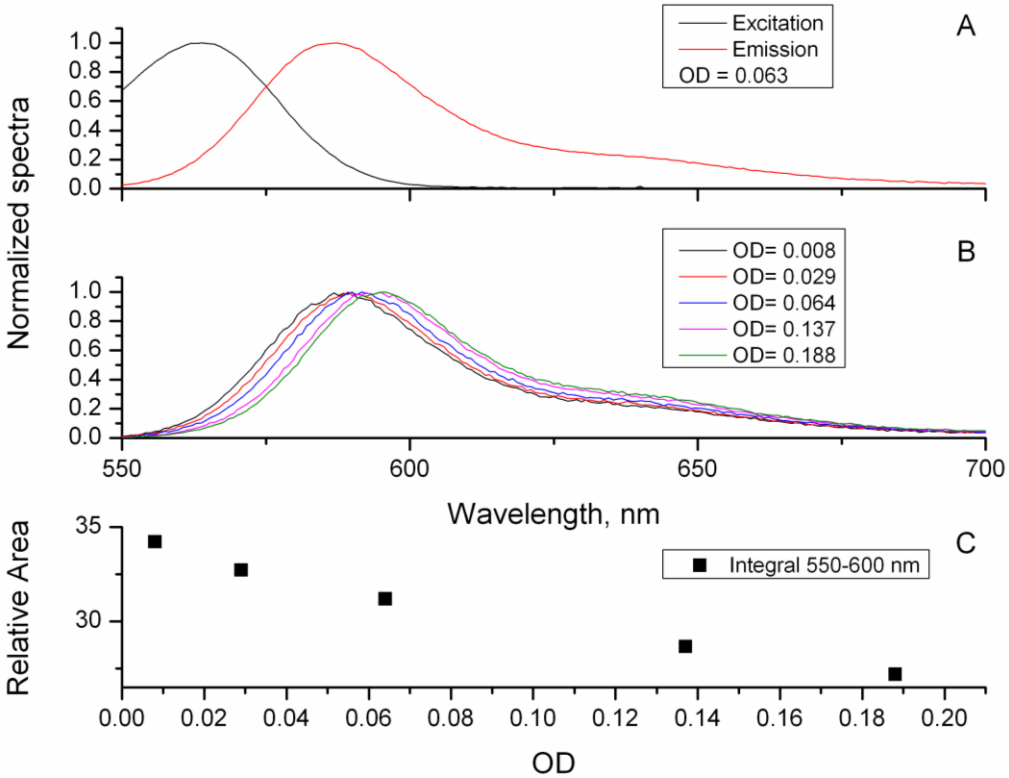
One important effect of increasing sample concentration is "self-absorption", a depression in the bluer edge of the emission spectral region, where it overlaps with the redder edge of the excitation or absorbance spectrum.

HORIBA Scientific recommends the following practical considerations to avoid self-absorption:

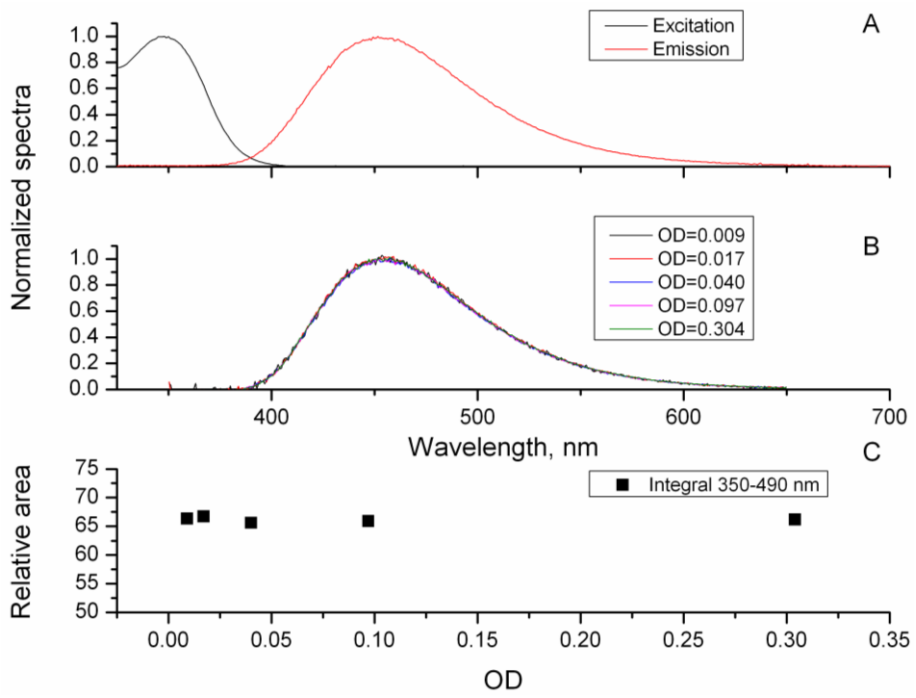
- Adjust the sample concentration so that, when plotting absorption (A) or optical density (OD) against concentration, a linear plot appears. Generally this occurs at the λ_{peak} when OD < 0.1, and better around 0.05.
- When possible, make multiple measurements while varying the sample concentration (OD), to determine if (and when) observed φ and E_c area fall in a linear region when plotted against sample concentration or OD.
- Plot the "normalized" E_c spectral areas, and evaluate their integrated areas relative to the sample OD, to determine if the blue edges are depressed in a manner systematically related to the OD. For compounds with φ < 100%, and narrow Stokes shifts between the excitation spectrum and emission spectrum, the relative areas

will be directly related to the measured φ values. For samples with high quantum yields (like rhodamine 101), however, or with large Stokes shifts between excitation and emission spectra (like quinine sulfate), re-absorbance effects are significantly smaller.

The following figures provide examples how increasing concentration and absorbance affect emission spectra of rhodamine 101 in anhydrous ethanol, and quinine sulfate in 0.1 *N* H_2SO_4, as measured in the Quanta-φ. The top plots (A) for both figures exhibit the normalized excitation (black) and emission (red) spectra to illustrate the overlapping regions responsible for self-absorption. Clearly the overlap is much larger in rhodamine 101 than in quinine sulfate. The middle plots (B) show the normalized emission spectra at particular ODs, revealing self-absorption. Again, the rhodamine 101 spectra are systematically red-shifted as OD increases (from self-absorption). Qunine sulfate, however, shows little or no change in the emission spectrum as the OD changes. The lower plots (C) reinforce the visual observations in plots A and B, by showing the relative area. Integrals decrease as OD rises for rhodamine 101, but there is no change in quinine sulfate.

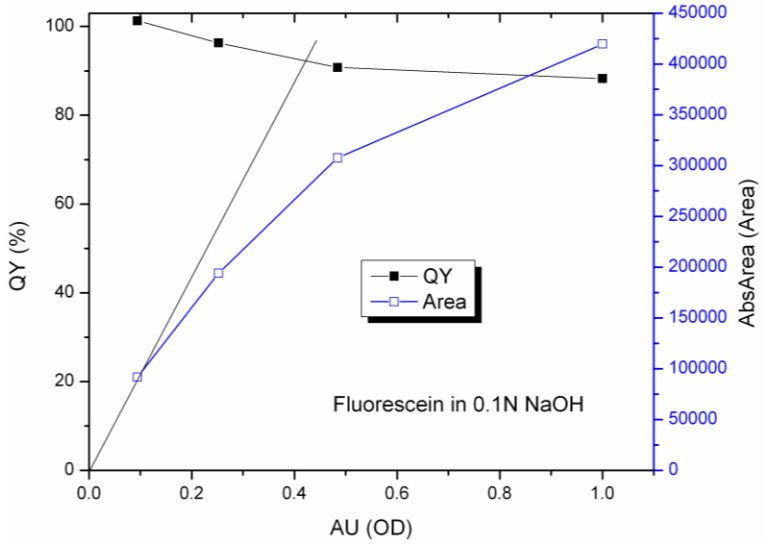


Influence of OD on fluorescence emission of rhodamine 101 dissolved in anhydrous ethanol.



Influence of OD on fluorescence emission of quinine sulfate dissolved in 0.1 *N* H_2SO_4.

In both compounds there is no correlation of φ with concentration because rhodamine 101 has a $\varphi \approx 93\%$ and quinine sulfate ($\varphi \approx 55\%$) shows no re-absorption. Yet when the sample's intrinsic φ is $<< 100$ % and self-absorption is strong, the measured φ will be influenced in proportion to the absorbance (OD) of the sample.



In contrast to rhodamine 101 and quinine sulfate, the figure to the right depicts the observed relationships between the integrated fluoresence area, the absorbance, and quantum yield (%) of fluorescein in 0.1 *N* NaOH. Clearly as the OD increases beyond 0.1 units, the relationship between the fluorescence area (hollow symbols) and quantum yield (filled symbols) becomes increasingly non-linear. Likewise the observed quantum yield becomes depressed with increasing OD, consistent with the inner-filter effects.

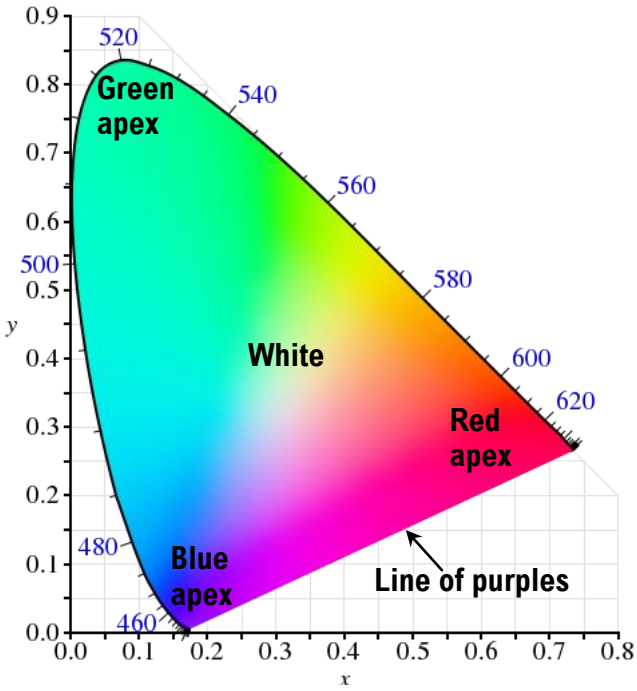
Similar to the fluorescein data above, self-absorption is also a problem with many solid samples and powders whose ODs cannot easily be determined. As with liquid samples, in solid and powder samples, when possible, HORIBA Scientific advises you to vary

the main chromophore's concentration systematically to evaluate the self-absorbance properties of the emission. In powder samples, for instance, pulverize and homogenize the chromophoric sample with a non-luminescent powder such as barium sulfate, in order to dilute the solid. The principle is similar to that for liquids, in that the sample is diluted to vary and minimize self-absorption at the surface. The solid dilution also serves to dilute the chromophoric sample to the point where its light-scattering properties are primarily determined by the surrounding non-luminescent powder. This is important because non-luminescent powder—when similarly pulverized and homogenized—may be used as the blank sample. In powder measurements, homogenization of the particle-size and light-scattering properties (that is, matching the sample and blank) is vital to acquiring precise and accurate values for φ.

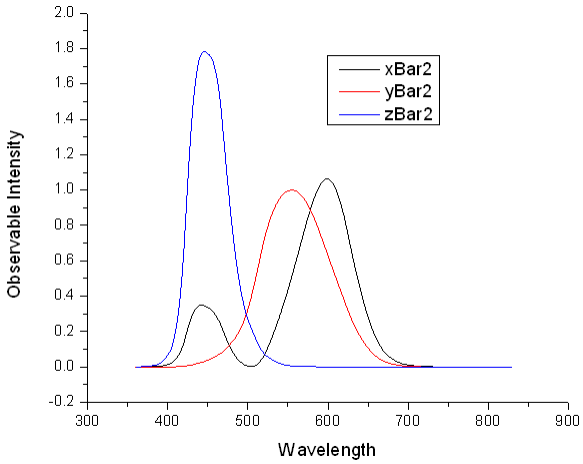
Calculation and evaluation of chromaticity

Theory

The Quanta-φ software automatically calculates and presents the numerical and graphical coordinate information for the spectral region ($E_c - E_a$) dealing with the chromaticity observable indices CIE 1931 and CIE 1976. The chromaticity indices provide a means for comparing the observed color (or tint) of the luminescence relative to the human visual perception of color. As displayed in the figure on the right, relative to the CIE 1931 index plot, the boundary area outlines all potential hues visible to the human eye.



The chromaticity indices are based on the overlapping regions of the normalized luminescence emission spectrum, with each of the three spectral profiles depicted in the figure on the right.



The regions of spectral overlap for the luminescence emission spectrum is evaluated by calculating the sum-product of the emission spectrum with each of three observable profiles, namely, xBar, yBar and zBar. The observable profiles are related to the three visual sensory-cell responses in the human eye, namely, the red (xBar), green (yBar) and blue (zBar). Note that the red profile also includes a portion of the blue spectral region.

Calculations

The Quanta-φ calculations for CIE 1931 and CIE 1976 proceed as follows.

- First the emission spectral regions from 360 nm–800 nm for E_c and E_a are interpolated to 1-nm intervals, and the spectral curve of the blank (E_a) is subtracted from the sample (E_c).
- Then the resultant blank-subtracted sample spectral profile is converted from photon units to power units, by division of each intensity point by the wavelength value in nm at which it was measured.

- The resultant sample power spectrum is then used to calculate three sum-product values, namely, CIE_TX, CIE_TY and CIE_TZ, from the observable intensity spectra xBar, yBar and zBar, respectively.

For the CIE 1931 index, the following two coordinates are calculated,

$$x = \frac{CIE\_TX}{CIE\_TX + CIE\_TY + CIE\_TZ}$$

$$y = \frac{CIE\_TY}{CIE\_TX + CIE\_TY + CIE\_TZ}$$

The coordinates *x* and *y* are plotted graphically inside the CIE 1931 boundary area, which is defined numerically for the *x* coordinate at each wavelength value, λ,

$$\text{x BA}_\lambda = \frac{\text{xBar}_\lambda}{\text{xBar}_\lambda + \text{yBar}_\lambda + \text{zBar}_\lambda}$$

and for the *y* coordinate at each wavelength value, λ,

$$\text{y BA}_\lambda = \frac{\text{yBar}_\lambda}{\text{xBar}_\lambda + \text{yBar}_\lambda + \text{zBar}_\lambda}$$

where xBar_λ, yBar_λ and zBar_λ are the intensity values from the three observable profiles at wavelength λ. The corresponding xBA_λ and yBA_λ values are plotted against each other to generate the semielliptical two-dimensional area-boundary plot for CIE 1931 (blue, left) as shown in the sample plot for rhodamine 101 in anhydrous ethanol in the screenshot on the next page.

For the CIE 1976 index, two coordinates are calculated as follows:

$$u' = \frac{4 \times CIE\_TX}{CIE\_TX + 15 \times CIE\_TY + 3 \times CIE\_TZ}$$

$$v' = \frac{9 \times CIE\_TY}{CIE\_TX + 15 \times CIE\_TY + 3 \times CIE\_TZ}$$

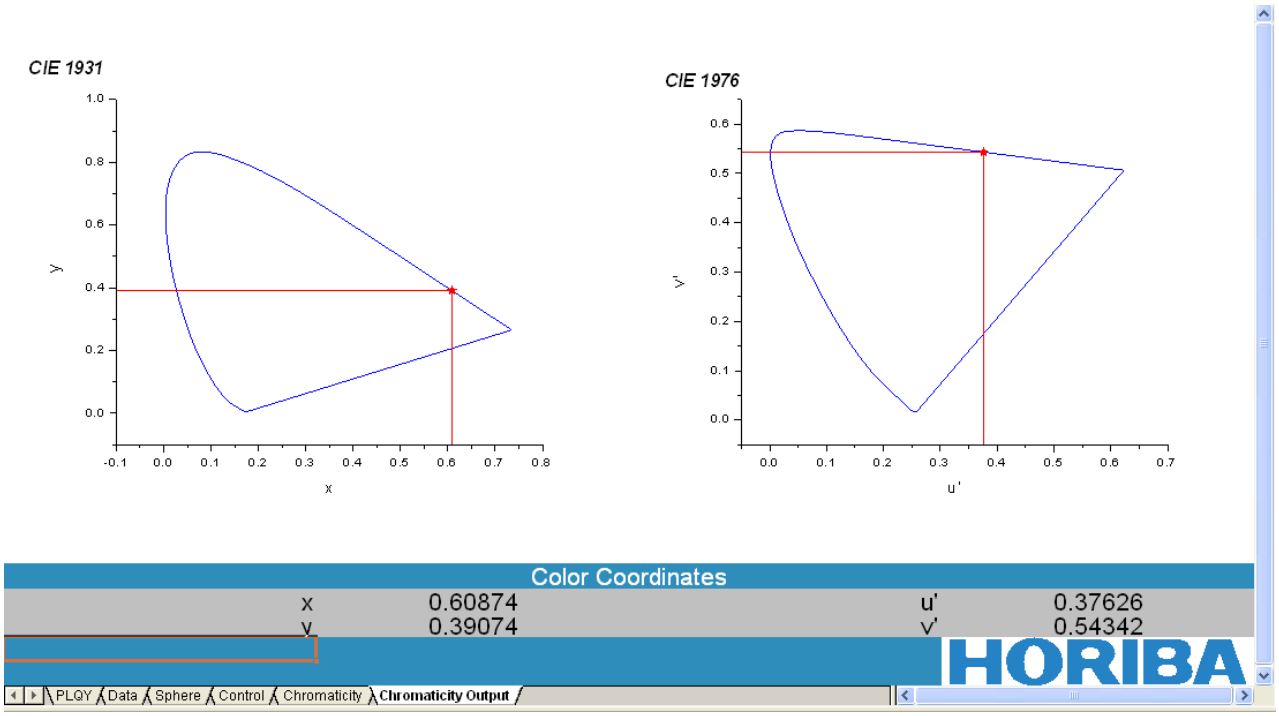
The coordinates u' and v' are plotted graphically inside the CIE 1976 boundary area, which is calculated from the xBA_λ and yBA_λ values for the u' coordinate at each wavelength value, λ,

$$u'\ \text{BA}_\lambda = \frac{4 \times \text{xBA}_\lambda}{(-2 \times \text{xBA}_\lambda + 12 \times \text{yBA}_\lambda + 3)}$$

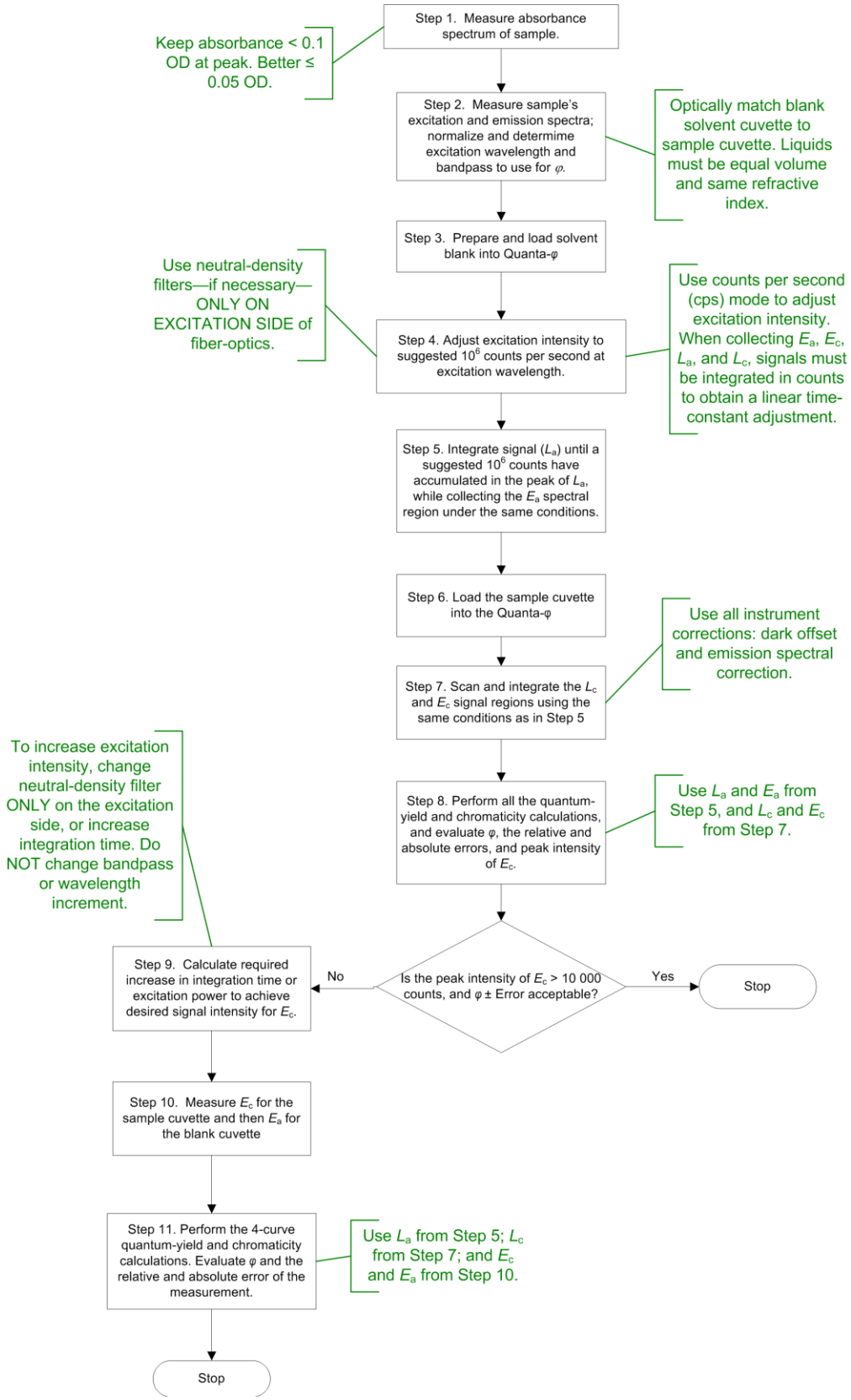
and for the v' coordinate at each wavelength value, λ,

$$v' \mathrm{BA}_{\lambda} = \frac{9 \times \mathrm{yBA}_{\lambda}}{(-2 \times \mathrm{xBA}_{\lambda} + 12 \times \mathrm{yBA}_{\lambda} + 3)}$$

The corresponding $u'\mathrm{BA}_{\lambda}$ and $v'\mathrm{BA}_{\lambda}$ values are plotted against each other to generate the closed, semielliptical two-dimensional area-boundary plot for CIE 1976 (blue, right) as shown in the sample plot for rhodamine 101 in anhydrous ethanol in the screenshot below.



Flowchart of method for data-acquisition



Additional references:

"An improved experimental determination of external photoluminescence quantum efficiency", J.C. deMello, H.F. Wittmann, and R.H. Friend, *Adv. Mater.* **9**, 230 (1997).

"Measurement of Solid-State Photoluminescence Quantum Yields of Films using a Fluorimeter", L.-O. Pålsson, A.P. Monkman, *Adv. Mater.* **14**(10), 2002, 757–758.

"Absolute Measurements of Photoluminescence Quantum Yields of Solutions Using an Integrating Sphere", L. Porrès, A. Holland, L.-O. Pålsson, A.P. Monkman, C. Kemp, and A. Beeby, *J. Fluorescence*, **16**, 2006.

2 : Requirements & Unpacking

Environmental requirements

- Temperature 59–86°F (15–30°C)
- Maximum temperature fluctuation ± 2°C
- Ambient relative humidity < 75%
- Low dust levels
- No special ventilation

Software requirements

- FluorEssence™ version 3.5 or higher with valid USB key
- Excel® 2000 or later software

Unpacking

Introduction

The integrating sphere is delivered in a single packing carton. All hardware is included with the delivery.

Examine the shipping box 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, unpack the equipment 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 Scientific assumes no responsibility for damage occurring during transit, the company will make every effort to aid and advise.*

Standard integrating-sphere carton contents

Quantity	Item	Part number
1	Integrating sphere assembly	
3	Plugs for top and fiber-optic-bundle ports	
1	Toolkit	
1	Liquid-sample holder	
1	Top-mounting fiber-optic-bundle holder	
5	Powder cups for solid samples	F-3029-01
5	Quartz coverslips	F-3029-02
1	FL3-3000 (for Fluorolog®-3) or FM4-3000 (for FluoroMax®-4) sample-compartment drawer	
2	Fiber-optic cables	
1	Optional Calibrated Cuvette Reflector	
1	Optional Uncalibrated Cuvette Reflector	
1	Optional Calibrated Powder-Holder Reflector	
1	Optional Cuvette-holder rack	
1	CD-ROM including *F-3000 Operation Manual*, *F-3029 Integrating Sphere Operation Manual*, sphere-correction files, and AVI video tutorials	

Unpacking the Integrating Sphere

Caution: *The Integrating Sphere is delicate. Mishandling may seriously damage its interior reflective surface.*

1. Carefully open the shipping carton.
2. Remove the foam-injected top piece and any other shipping restraints in the carton.
3. Carefully lift the integrating sphere from the carton, and rest it on the laboratory bench.
4. Inspect for previously hidden damage.
   Notify the carrier and HORIBA Scientific if any is found.
5. Check the packing list to verify that all components are present.

3 : Installation & Use

Caution: *Do not touch the surfaces of the Integrating Sphere's sample-holder. This can degrade its highly reflective surface. Wear clean protective gloves when handling the interior of the sphere.*

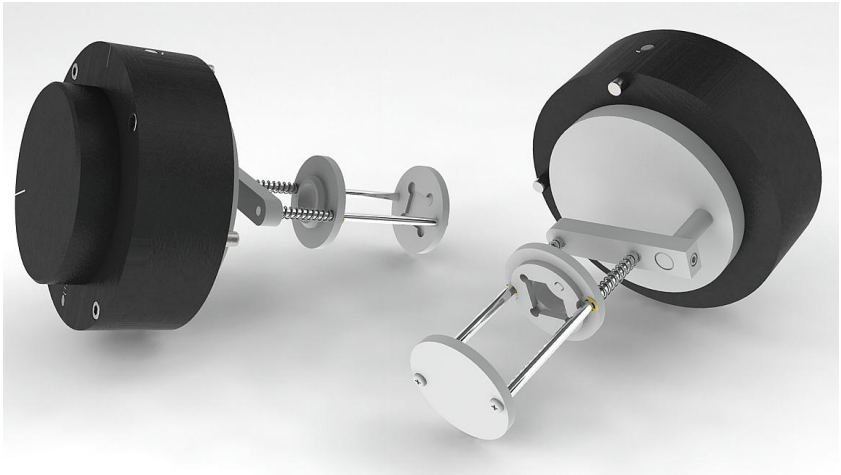
Note: *Be sure to verify calibration for the instrument, including Xe lamp-scan and water-Raman signal, before using the Integrating Sphere accessory.*

Method

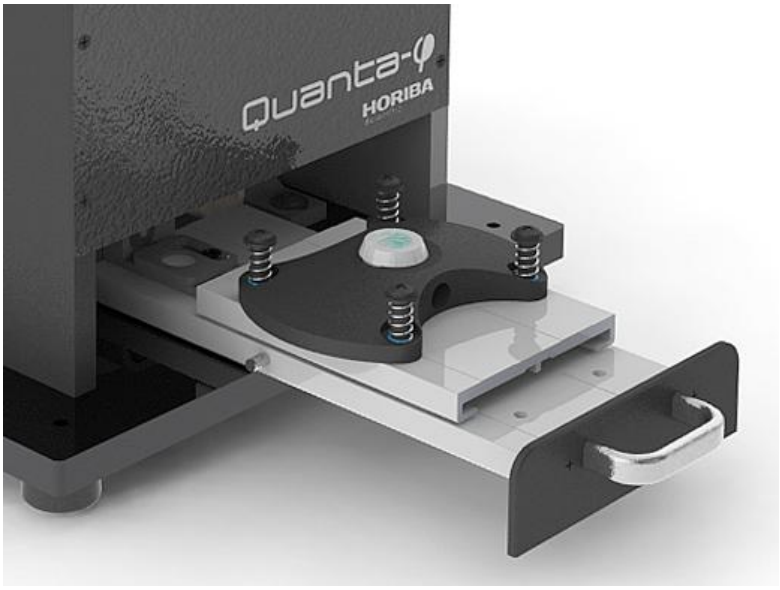
1. Remove the cover of the integrating sphere.
2. Choose the sample holder.

Note the two types of sample-holder:

- Liquid samples in a stoppered, quartz cuvette with 1 cm path-length.
- Solid samples under a quartz coverslip.



Two views of the cuvette holder for liquids.



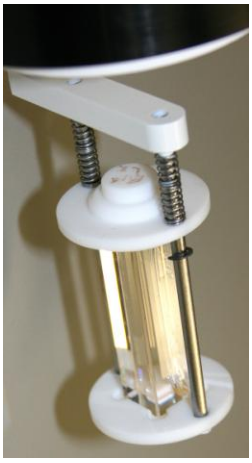
Sample tray for solid samples.

3 For liquid samples once the absorbance and excitation and emission properties are understood, follow this procedure:

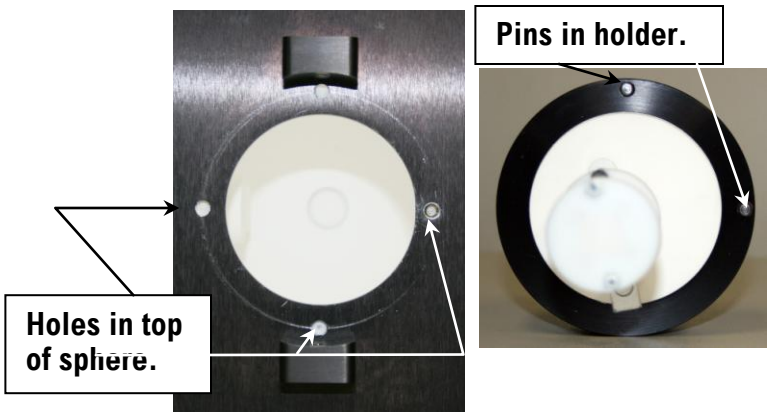
a Place the appropriate blank solvent in a firmly stoppered quartz cuvette with 12 mm × 12 mm outer dimensions (the same dimensions as a standard 4 mL, 1 × 1 cm path-length cell, HORIBA part number FL-1925). Also prepare the dissolved sample (in the same solvent), using the same volume of liquid in another, optically matched, quartz, stoppered cuvette.

Caution: *Never tilt the cuvette for insertion into the holder such that the liquid comes in contact with the top seal. Always use a stopper to prevent spills or contamination of the cuvette-holder in case of an accidental tipping. Before using the cuvette, verify that it has a clean stopper, and there is no contamination of the seal between cuvette and stopper.*

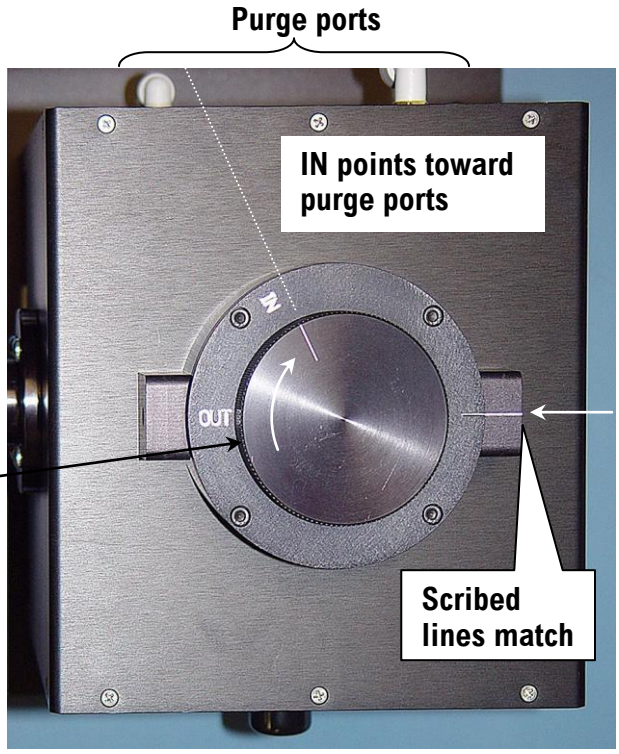
b Place the stoppered cuvette with the blank solvent into the liquid sample-holder. The sample-holder is spring-loaded. Be sure the cuvette's cap is correctly seated in the mobile, spring-loaded disk; the square base of the cuvette should be in the square depression at the bottom of the holder.



c Lower the liquid sample-holder into the sphere gently.

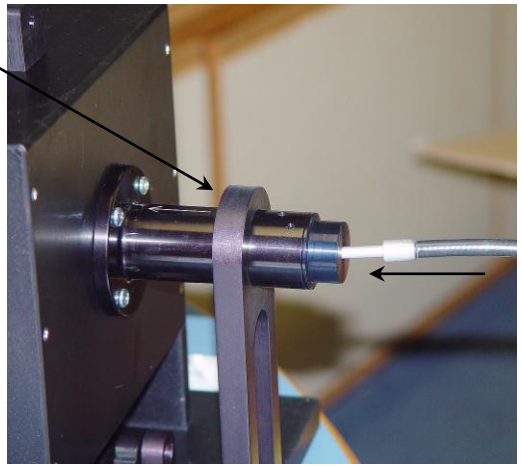


As you seat the holder in the sphere, align the holes around the sample-holder port with the pins on the sample-holder. Be sure the label IN is in the direction of the N_2 purge ports, and the scribed line on the lid matches the scribed line on the sphere.

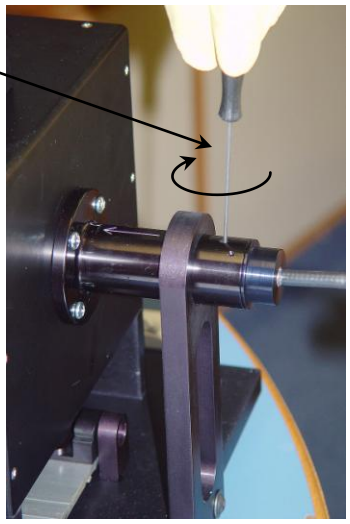


d Rotate the liquid-sample-holder top so that the pointer points to IN.

e Remove the protective cap from the excitation port. Connect the excitation fiber-optic bundle to the excitation port (inward arrow).

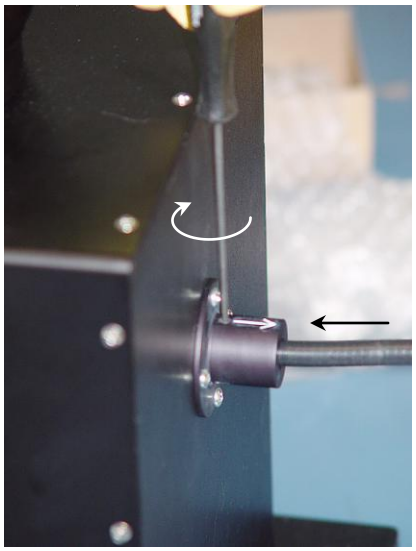


f Tighten the set screw with the Allen key.



g Remove the protective cap from the emission port (outward arrow). Connect the emission fiber-optic bundle to the emission port.

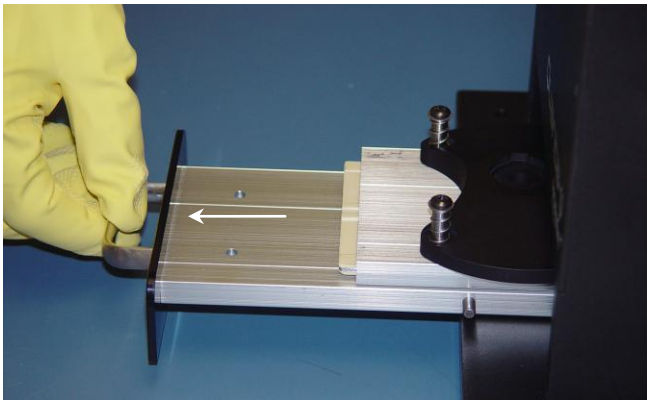
h Tighten the set screw to fix the emission bundle in place.



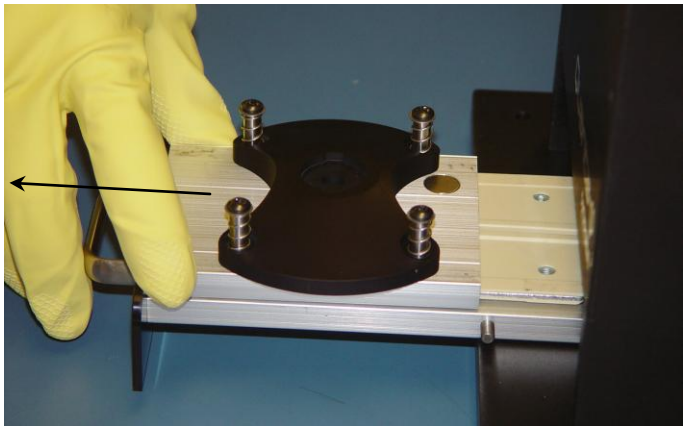
4 For solid samples, follow this procedure.

Note: *Only films with a maximum diameter of 12 mm can be mounted in the sample holder. For other sizes or shapes, contact HORIBA Scientific.*

a Pull sample-drawer handle out.

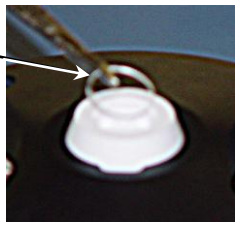


b Pull solid-sample support itself out.

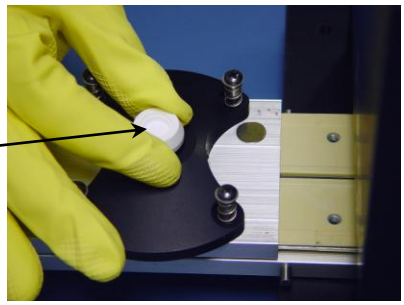


With the Spectra-lon® sample holder located safely outside and away from the sample-compartment drawer, add the solid sample into the sample cup.

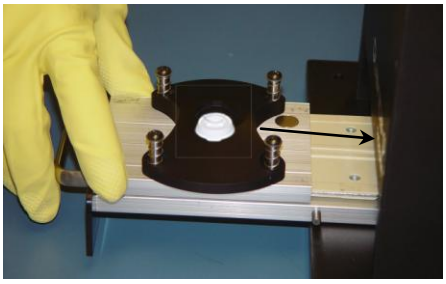
c Drop the coverslip on top of the sample.



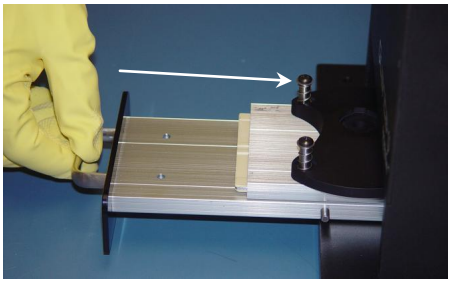
d To avoid possible contamination, be certain all traces of the sample material are removed from the outside of the sample holder, then insert the sample holder into the support.



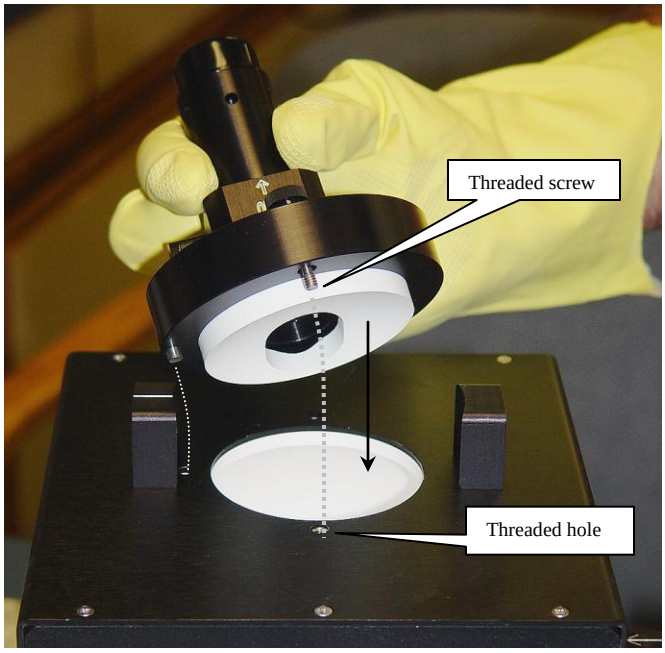
e Slide sample holder back in.



f Push sample drawer in, so that the sample is raised into the integrating sphere.

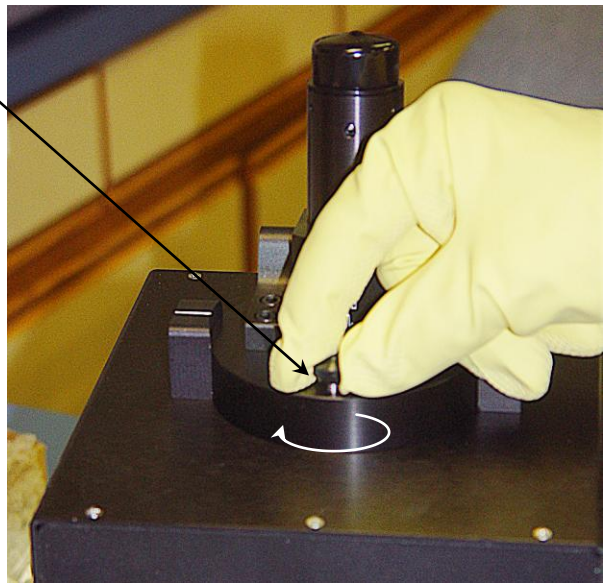


g Place the solid-sample cover on the integrating sphere.

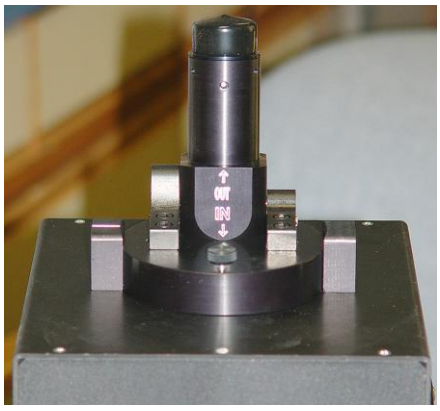


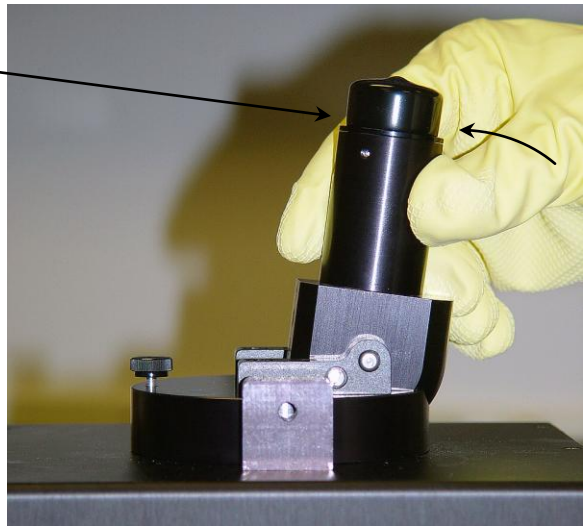
Note: *Match the threaded thumbscrew on the lid to the threaded hole on the sphere.*

h Tighten the thumbscrew to fix the lid in place.

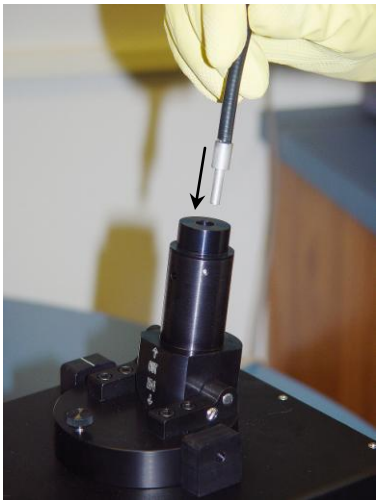


i Raise the excitation port to the IN position.

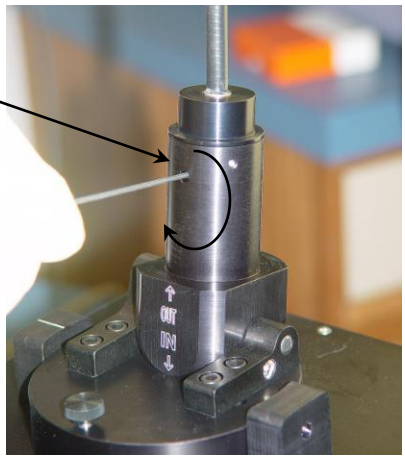




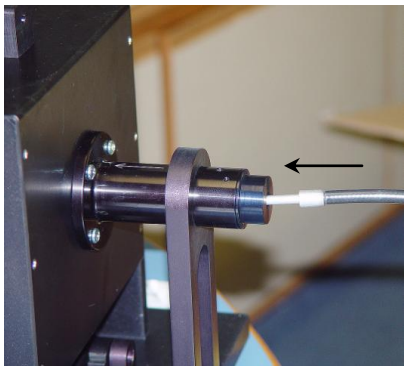
j Remove the protective cap from the excitation port. Connect a fiber-optic bundle to the integrating sphere emission port.



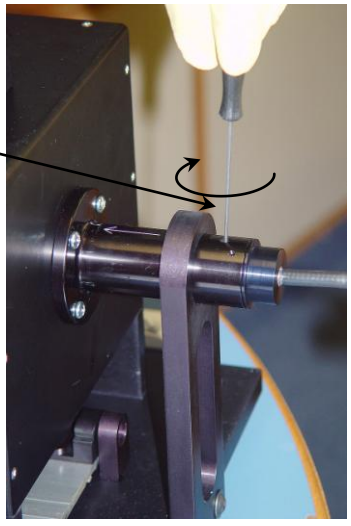
k Tighten the set-screw with an Allen key.



l Remove the protective cap from the emission port. Connect a fiber-optic bundle to the integrating sphere emission (outward arrow) port.



m Tighten the set-screw with an Allen key.

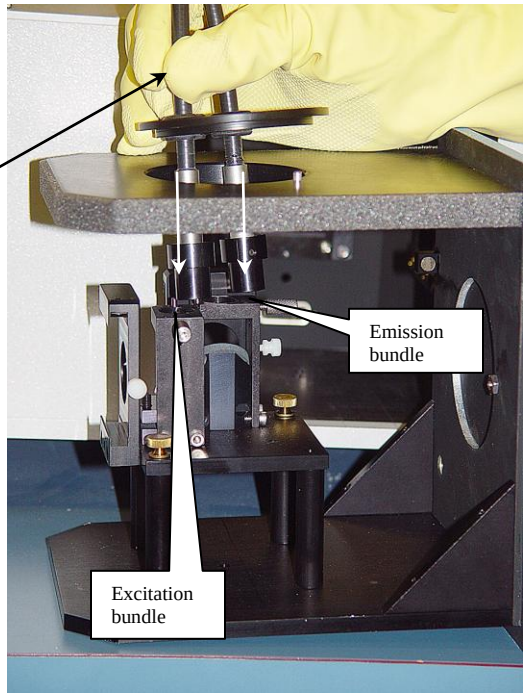


5 Set up the F-3000 Fiber-Optic Adapter drawer.

Note: *Follow detailed instructions in the Fiber-Optic Adapter manual for insertion and alignment of the fiber-optic bundles.*

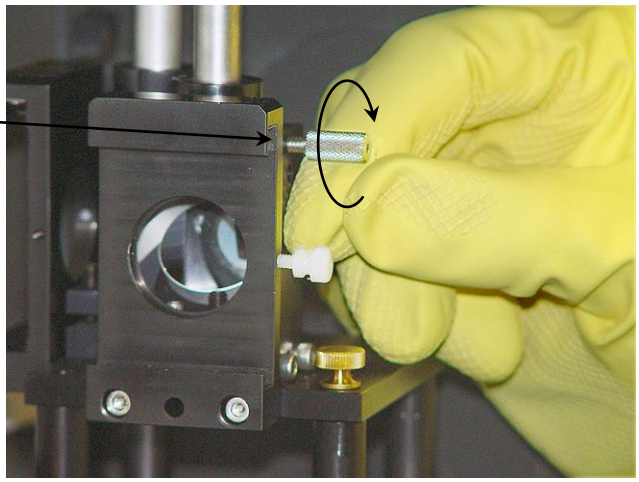
a Remove the standard sample drawer from the instrument's sample compartment.

b Insert the excitation and emission fiber-optic bundles into the Adapter.

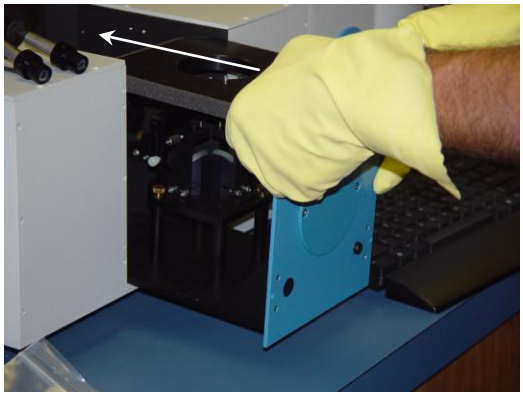


Note: *Carefully note the arrangement of excitation and emission bundles.*

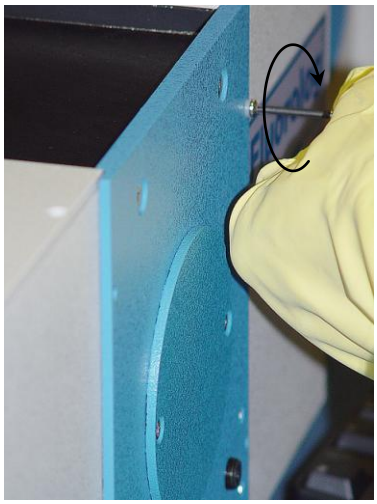
c Tighten the thumbscrews on both fiber-optic bundle-holders to lock the bundles into place.



d Place the Fiber-Optic Adapter drawer in the sample compartment.



e Tighten the screws to fix the drawer solidly to the sample compartment.



The sample-compartment drawer should look like this when installed:

6 Start FluorEssence™.

Continue here if your detector is a CCD.

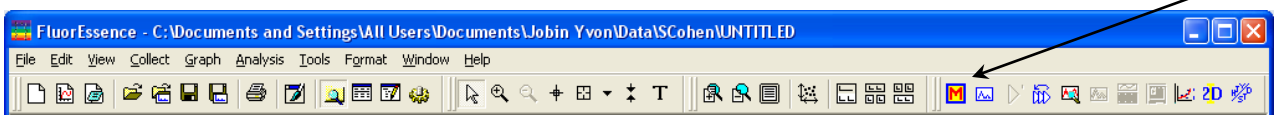
7 Adjust the Rayleigh excitation peak for the blank (solvent or solid) to the desired count rate (suggested value ≈ 10^6 counts/s).

Note: *See the section later in this chapter if using a photomultiplier tube as the detector.*

We are trying to maximize but not saturate the excitation Rayleigh peak, to obtain an optimal luminescence signal-to-noise ratio.

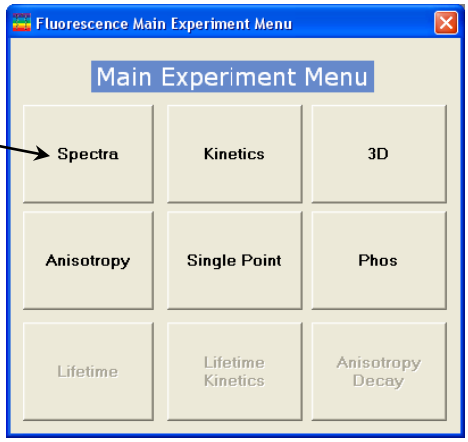
a Place the solvent blank cuvette (or blank solid material) into the Integrating Sphere.

b In the FluorEssence™ toolbar, click the Experiment Menu button .



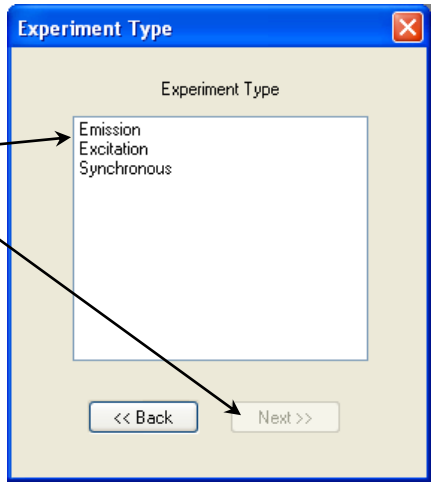
The **Fluorescence Main Experiment Menu** appears:

c Click the Spectra button.

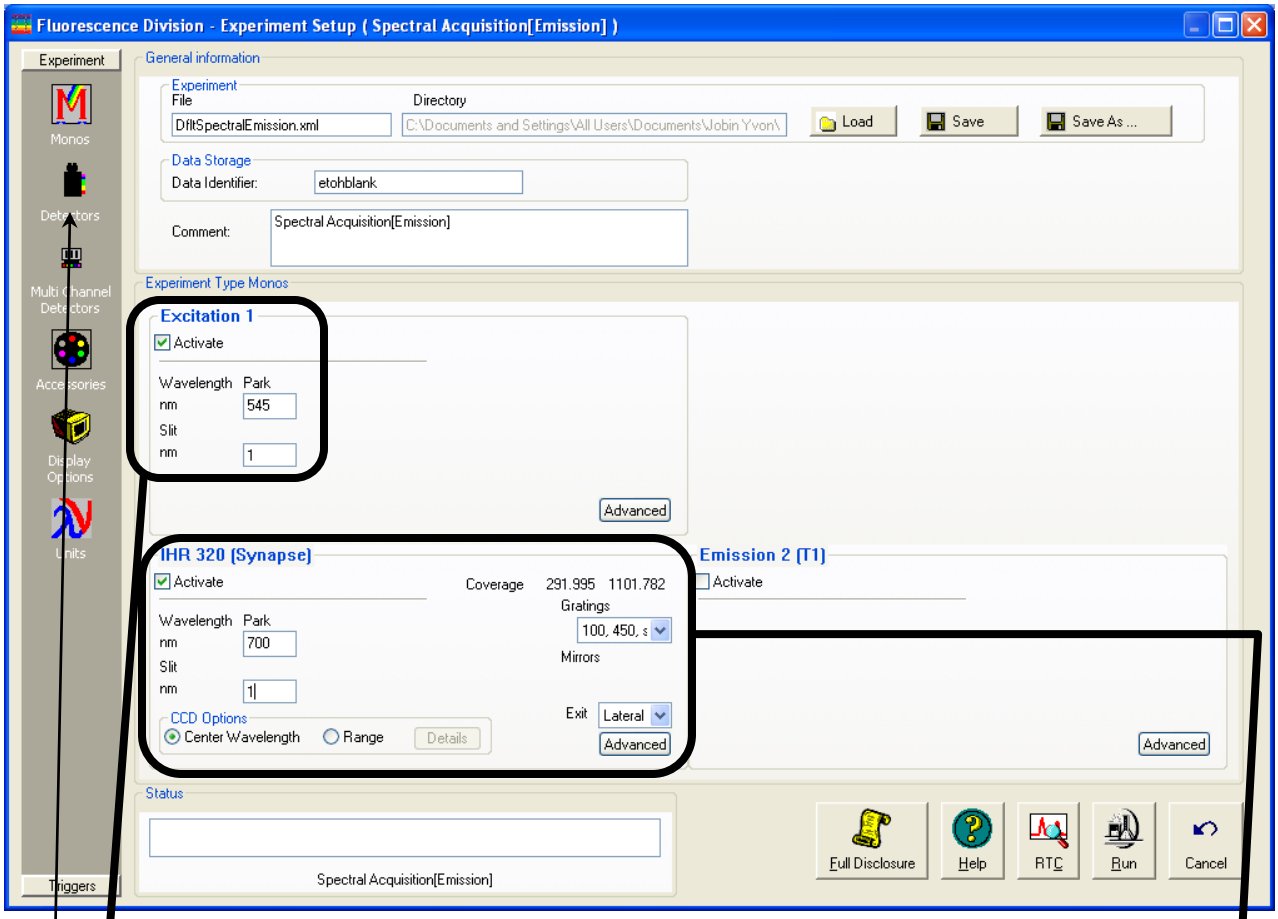


The **Experiment Type** window appears.

d Choose the Emission subtype of experiment, and click the Next >> button.



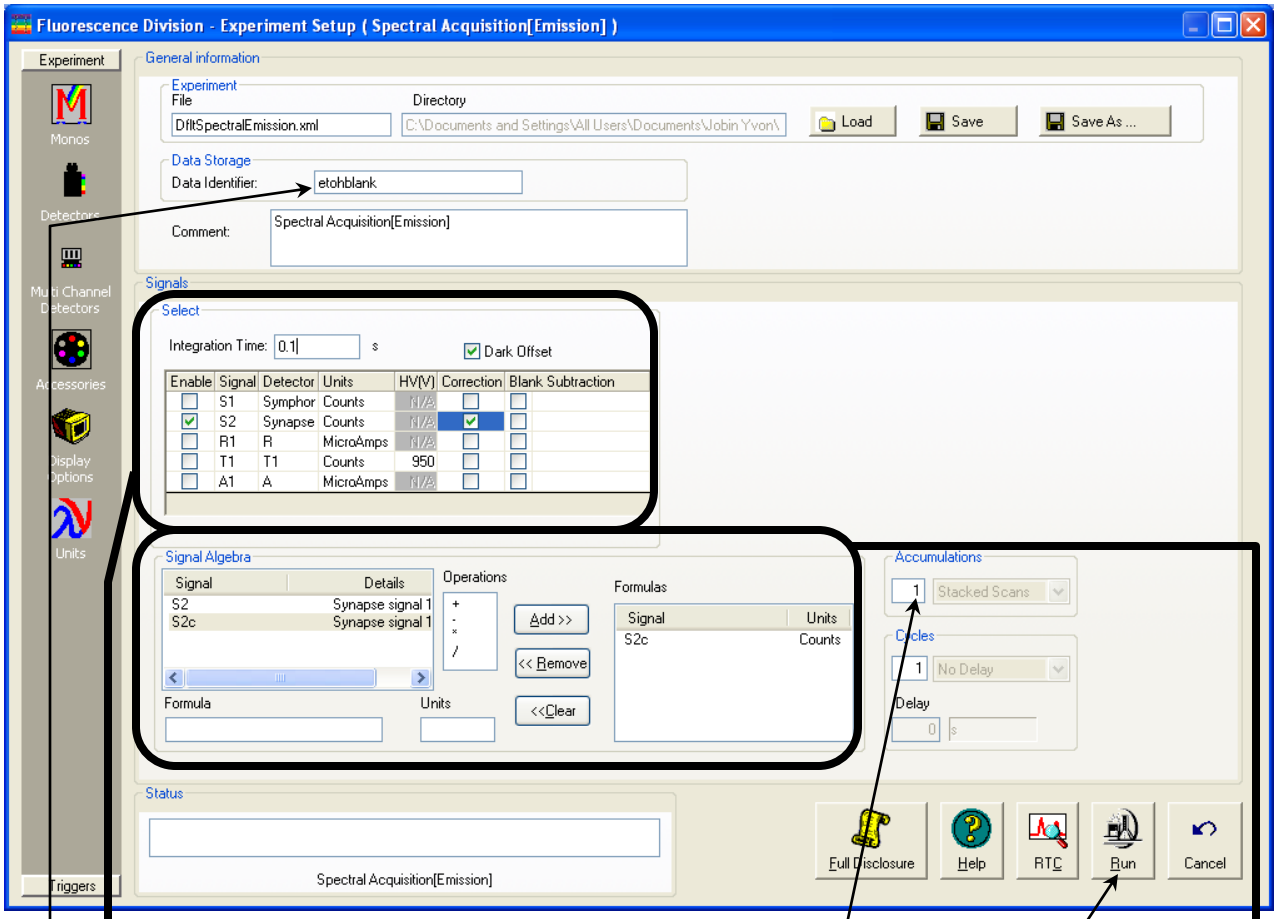
The **Experiment Setup** window appears:



e Set the excitation wavelength.
In this example, for rhodamine 101 in ethanol, we Park the excitation monochromator at 545 nm, with a bandpass of 1 nm.

f Set the emission wavelength range to include both excitation light and the likely or expected range of sample luminescence.
In this case, because the CCD correction factor was collected using the center wavelength of 700 nm, we measure the range of 290 nm (to include excitation at 545 nm) to 1100 nm, based on the Center Wavelength of the CCD used in the iHR detector. (A photomultiplier tube detector has slightly different parameters to set.)

g Click the Detectors tab to reveal the Detectors page:



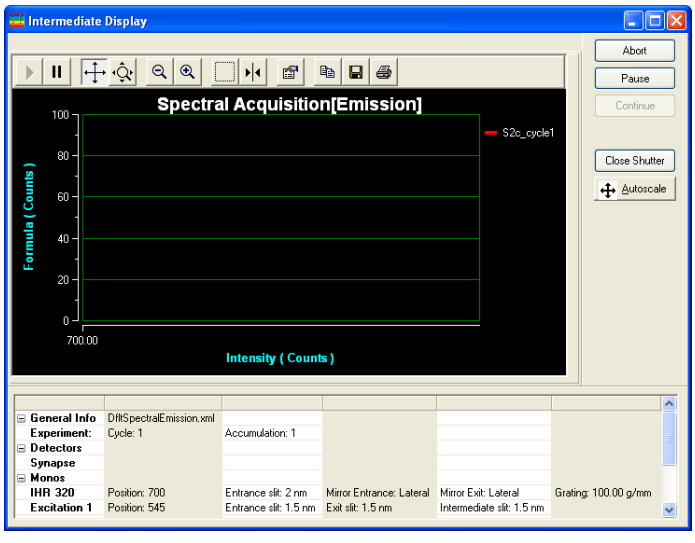
h Enable the emission detector, the Correction checkbox, and the Dark Offset checkbox. Set the Integration Time to 0.1 s.

i Add >> the corrected signal (with c subscript) to the Formulas table.

j Give this file a Data Identifier.

k Choose 1 Accumulations.
If this is too noisy, you will increase the accumulations later.

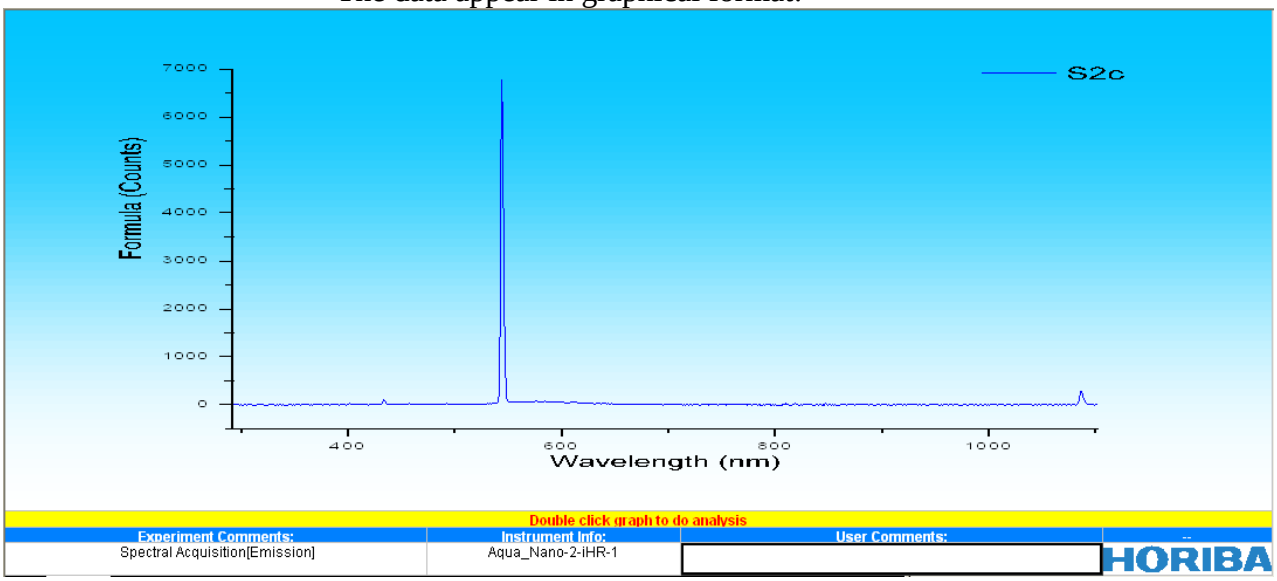
l Click the Run button.
The **Intermediate Display** appears.



m A **Warning** window may appear that the length range cludes values outside of the correction-factor file's range.

Click the OK button if this message appears.
When the scan is finished, the **Project Name** window appears.

n Give the project a name, and click the OK button.
The data appear in graphical format:



8 Maximize the Rayleigh peak without saturating it.

a Double-click in the graph to open it.

b The same graph appears in a new window.

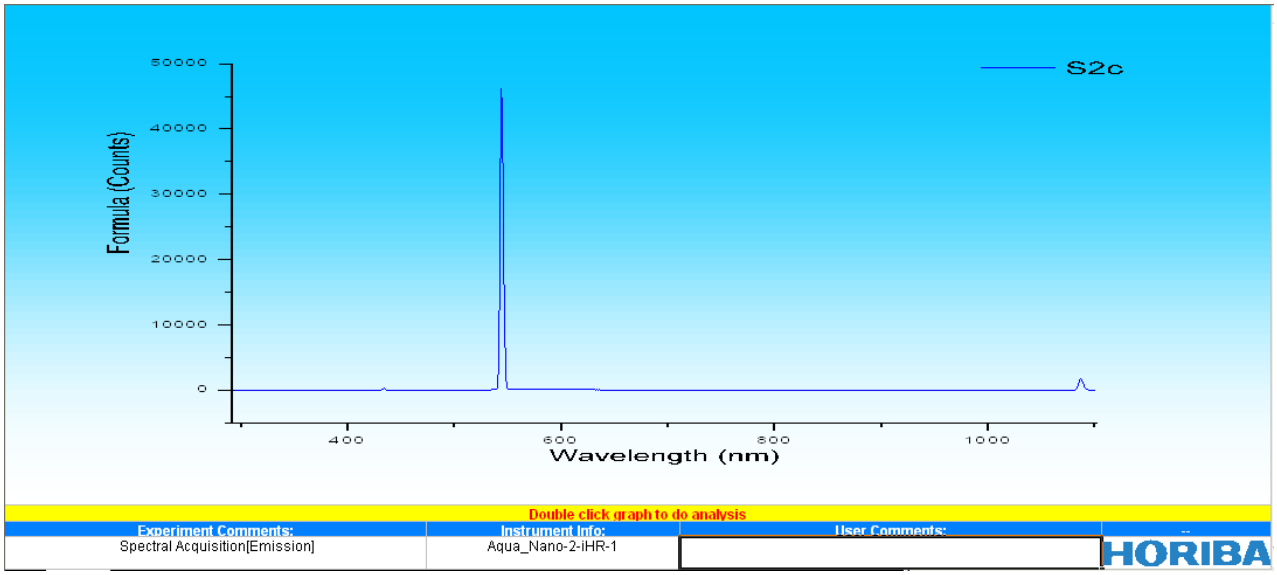
c Zoom with the Scale In button  and select the luminescence to see its strength.

d Rescale and examine the Rayleigh peak:
In this case, the Rayleigh excitation peak is not saturated, so it is acceptable. But the luminescence was low (only 7000 counts), so you may:

- Widen the Slit values (bandpass)
- Decrease attenuation from neutral-density filter
- Increase the Integration Time
- Increase the number of Accumulations

Note: *When using a photomultiplier tube, keep the signal-level within the linear range (<1.5 × 10^6 counts/s). For CCDs, keep the counts to ~ 45 000 per accumulation.*

After adjusting our experimental parameters to 1.5 nm excitation bandpass and 2 nm emission bandpass, we attain the following number of counts:

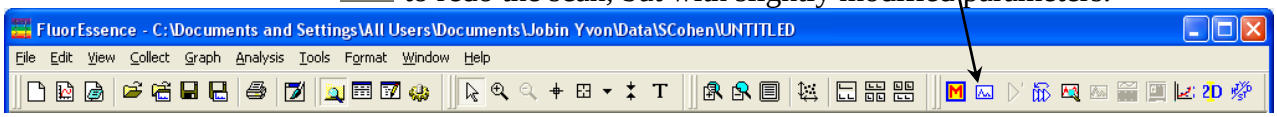


Here we have around 47 000 counts, which is close to ideal.

9 Run the experiment.

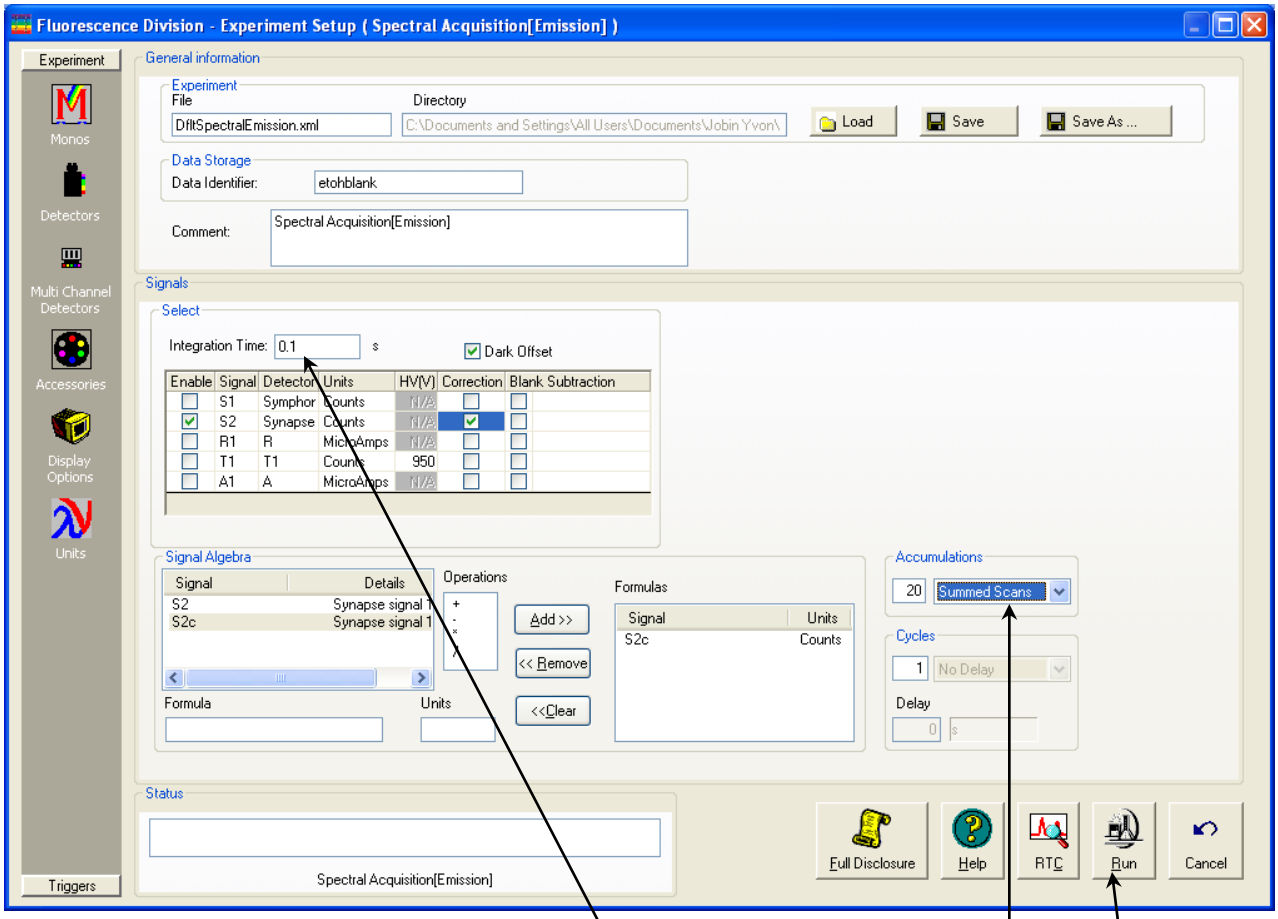
In our case, we will increase the Integration Time and Accumulations so that we have enough signal (~10^6 counts/s) for analysis.

a In the FluorEssence™ toolbar, click the Previous Experiment button  to redo the scan, but with slightly modified parameters.



The **Experiment Setup** window appears.

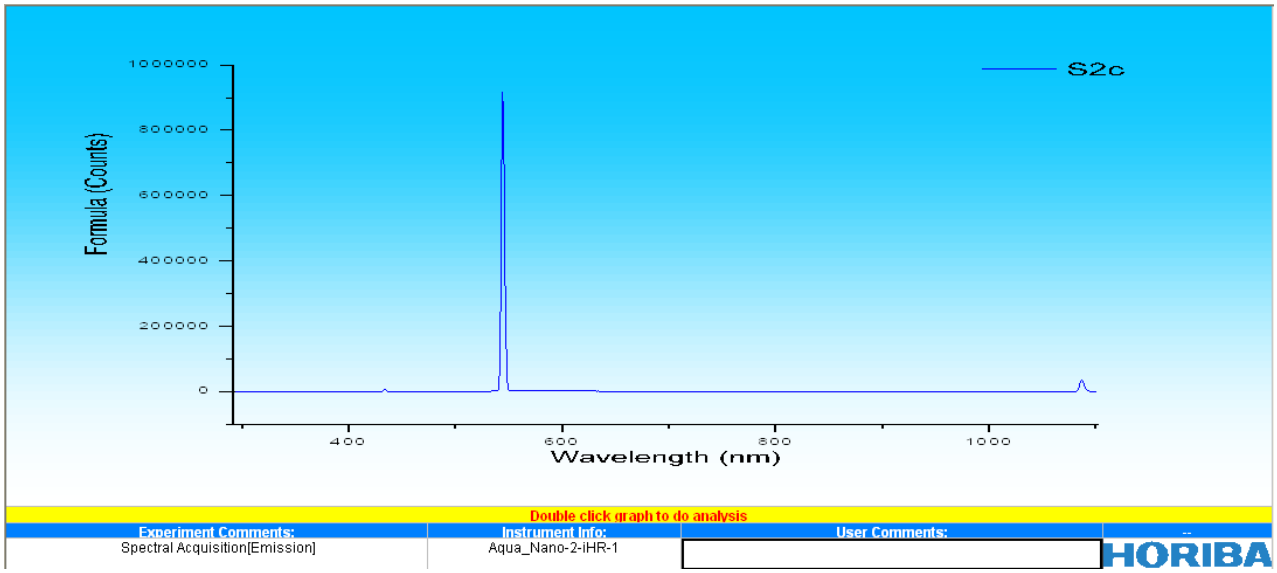
b Click the Detectors icon.



c Change Accumulations to `20` Summed Scans, and Integration Time to `0.1` s.

d Click the Run button.
The **Intermediate Display** appears.

e A **Warning** window may appear that the wavelength range includes values outside of the correction-factor file's range. Click the OK button if this message appears.
As before, the graph appears. There are more than 9×10^5 counts/s, which is acceptable:

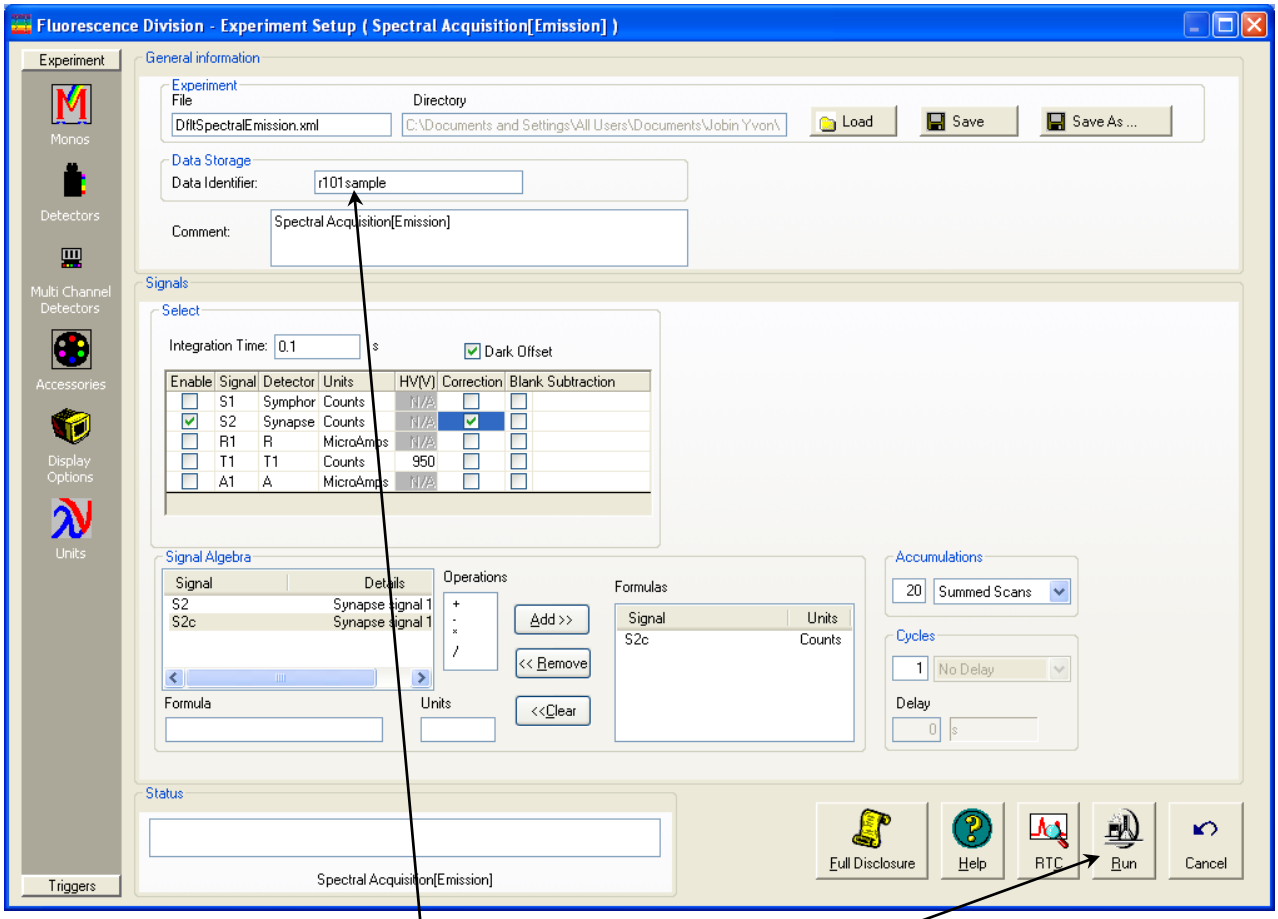


f Carefully remove the blank from the Integrating Sphere.

10 Run the scan on the sample.

a Insert the sample cuvette into the liquid-sample holder, and replace the liquid-sample holder into the integrating sphere. (Or place the sample in the solid-sample holder, and insert the drawer into the sphere.)

b Click the Previous Experiment button  to redo the scan:



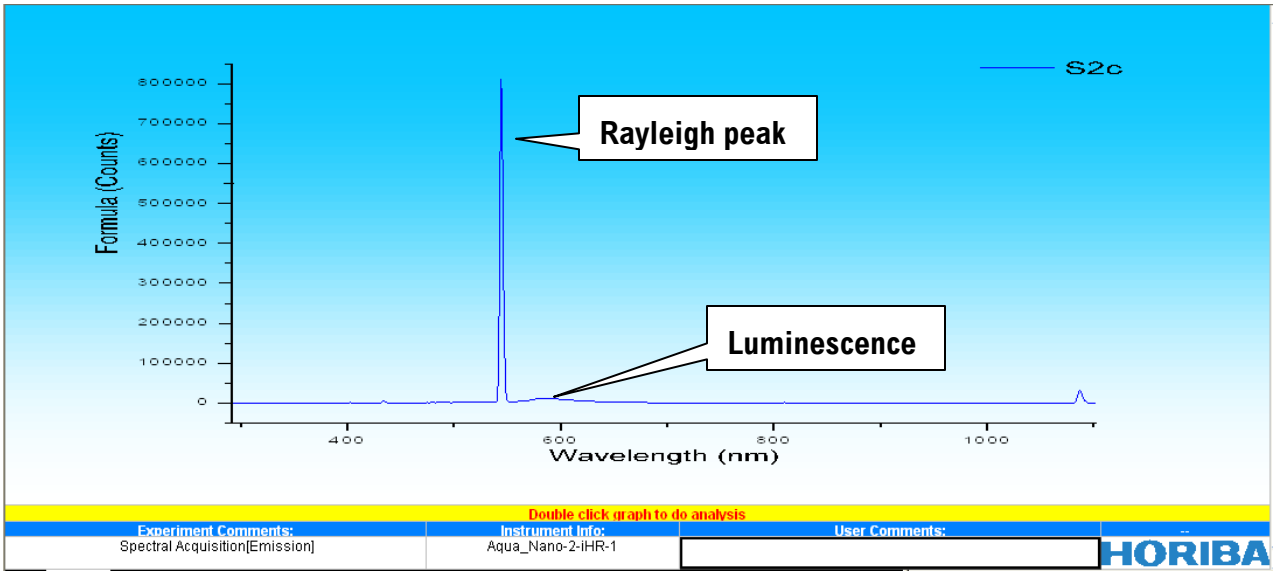
c Change the Data Identifier field to acknowledge that the sample is now in the sphere.

d Click the Run button to rerun the scan with exactly the same parameters as in step 10.

Note: *The blank's Rayleigh peak will always be larger than the sample's Rayleigh peak, because the sample absorbs more photons.*

e When the scan is completed, check the sample's luminescence spectrum, to be sure there are no anomalies. You can double-click on the plot and zoom in to examine the graph.

Note: *Placing the sample and blank scans side by side helps.*

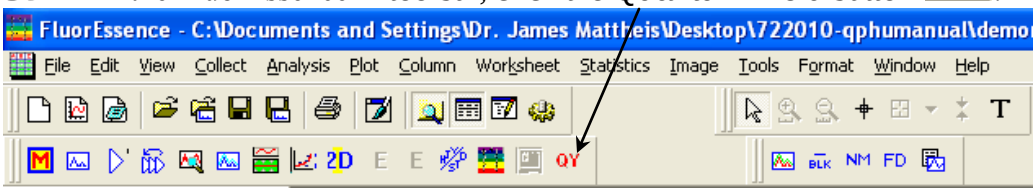


11 Set up the analysis of the data.

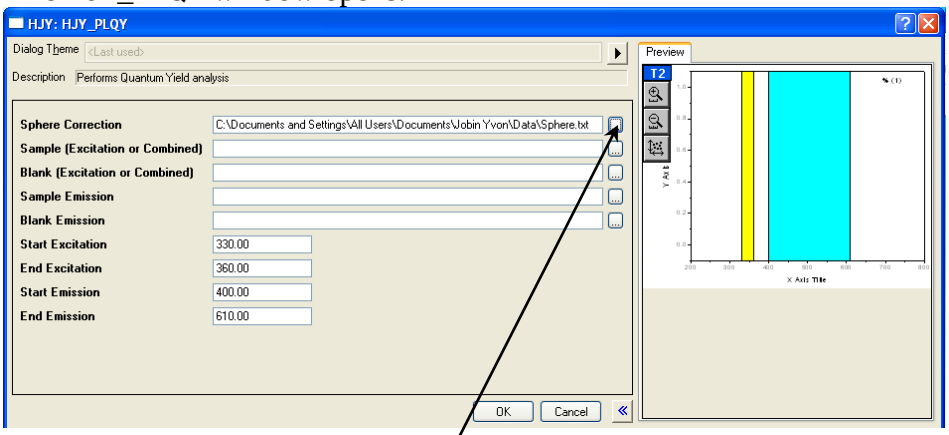
There are two methods of analyzing the data: 2-curve analysis and 4-curve analysis. In the 2-curve analysis, the Rayleigh line and luminescence band are included together in the sample's and blank's data. Thus only two curves are used for analysis. In the 4-curve method, the Rayleigh lines and luminescence bands are in separate datasets for the sample and blank; therefore there are four curves used for analysis. Both methods are demonstrated below.

a In the FluorEssence™ toolbar, click the Quantum Yield button 

.



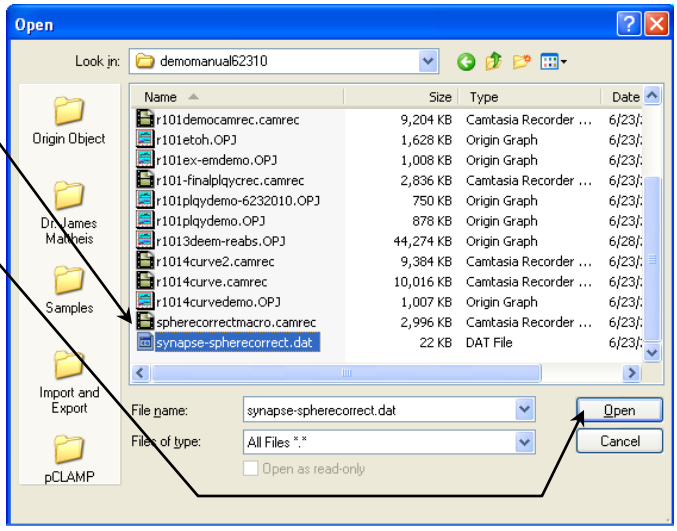
The **HJY_PLQY** window opens.



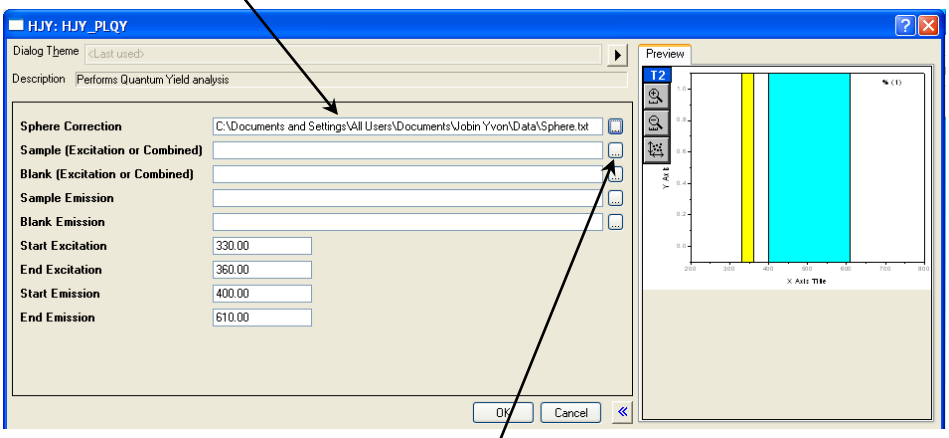
b Browse with the Browse button  to find the Sphere Correction file. The **Open** window appears:

c

Select the correct sphere-correction file, and click the Open button.



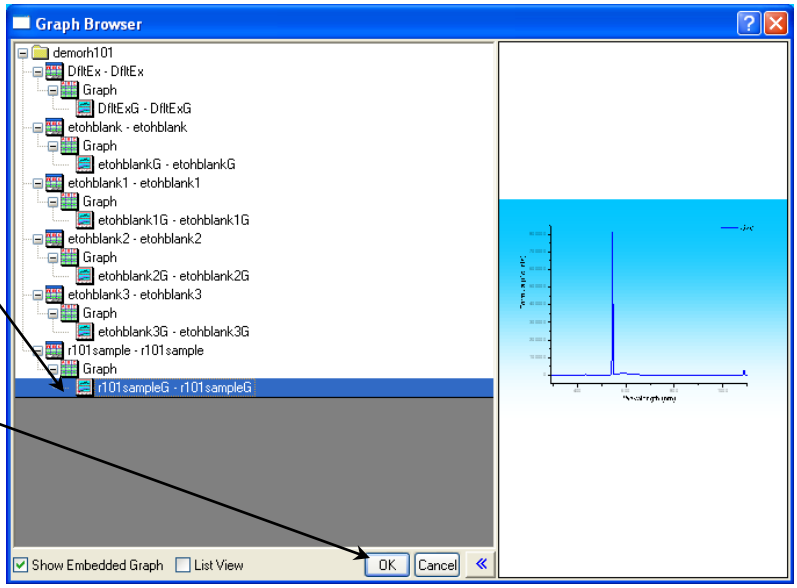
The sphere-correction file name appears in the Sphere Correction field:



To run a 2-curve analysis, see the method below:

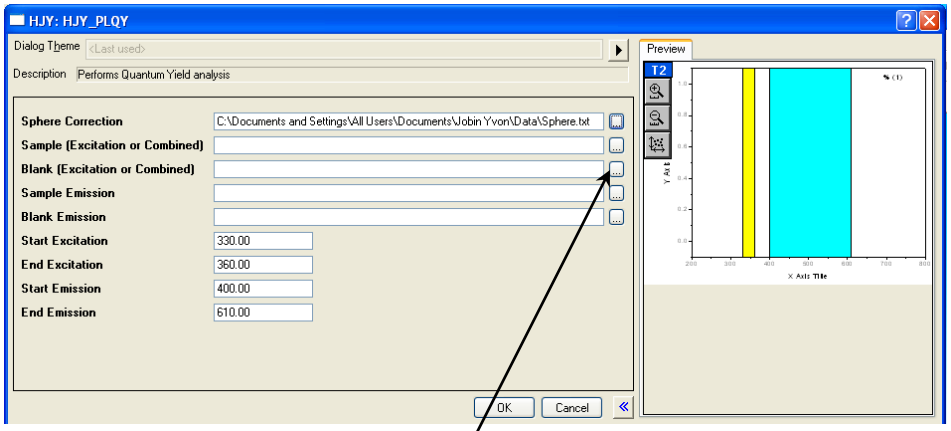
d

Browse with the Browse button  for the combined sample data file. The **Graph Browser** window opens. A preview of the data appears on the right half of the **Graph Browser** window.



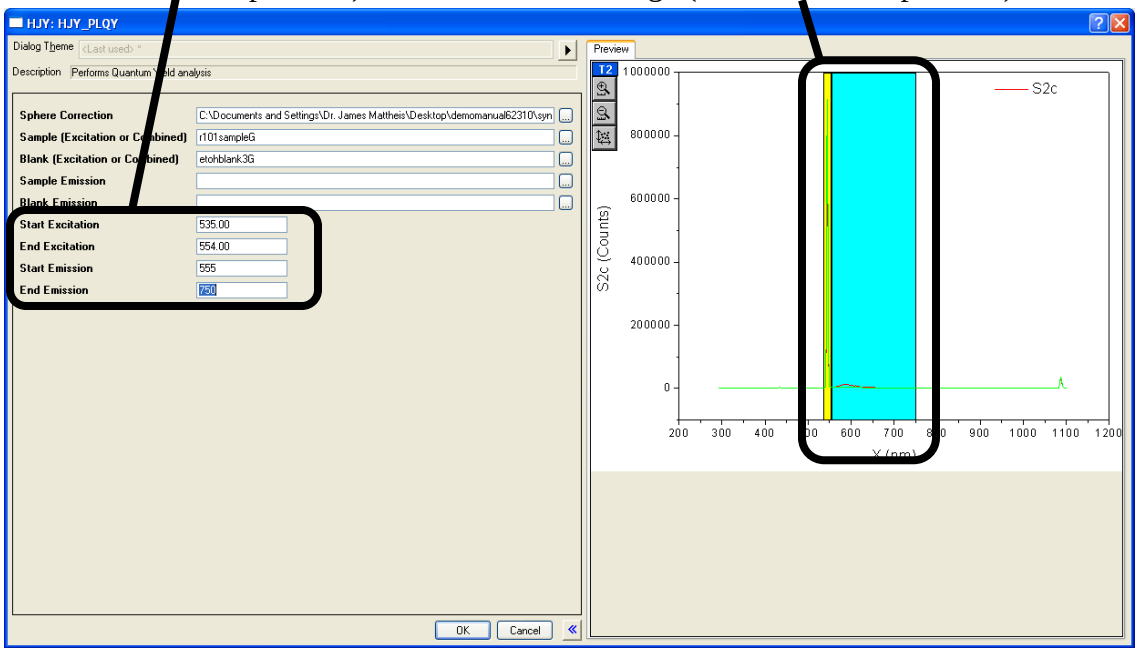
e

Choose the sample data file, and click the OK button.



f Likewise, browse with the Browse button for the combined blank data file.

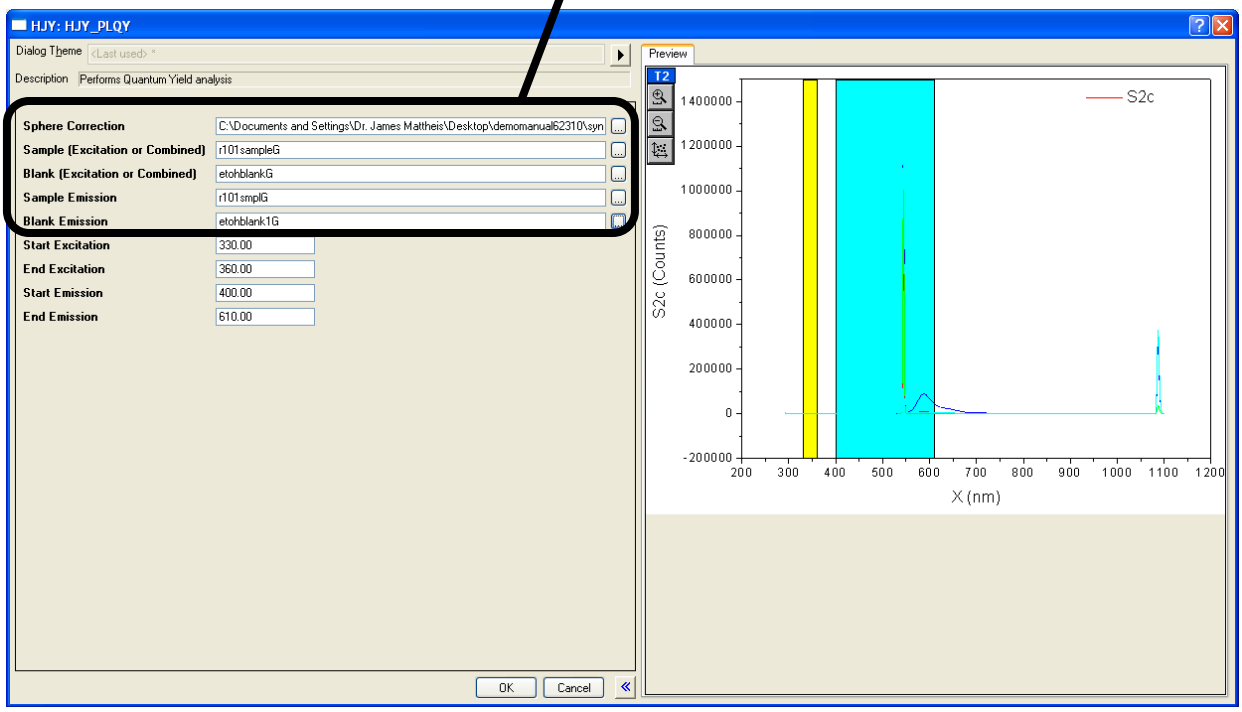
g Enter the Start Excitation, End Excitation, Start Emission, and End Emission values to find the correct Rayleigh-line range (yellow area in the preview) and luminescence range (blue area in the preview):



h Zoom in to view the Rayleigh scattering (yellow area) and luminescence (blue area), in order to refine the wavelength ranges.

To run a 4-curve analysis, see the method below:

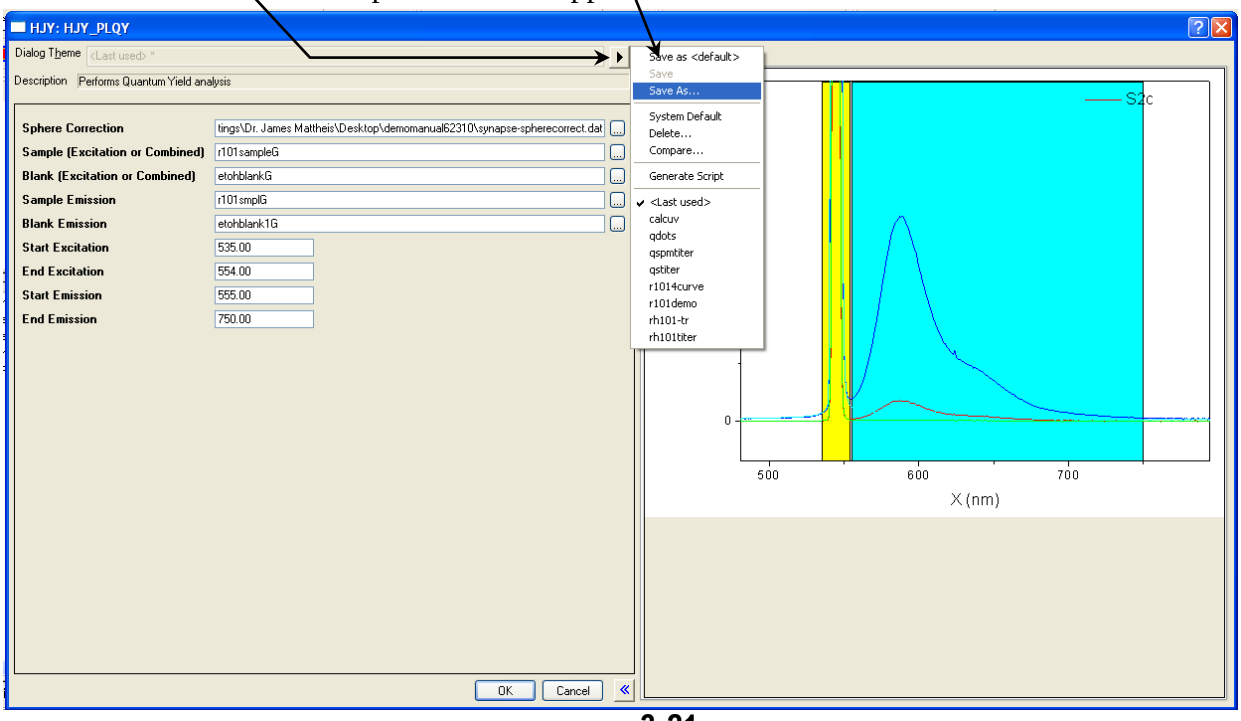
Follow the 2-curve analysis, except choose the four different datasets (sample Rayleigh, blank Rayleigh, sample emission, and blank emission), and enter them into the **HJY_PLQY** window's fields, as shown below:

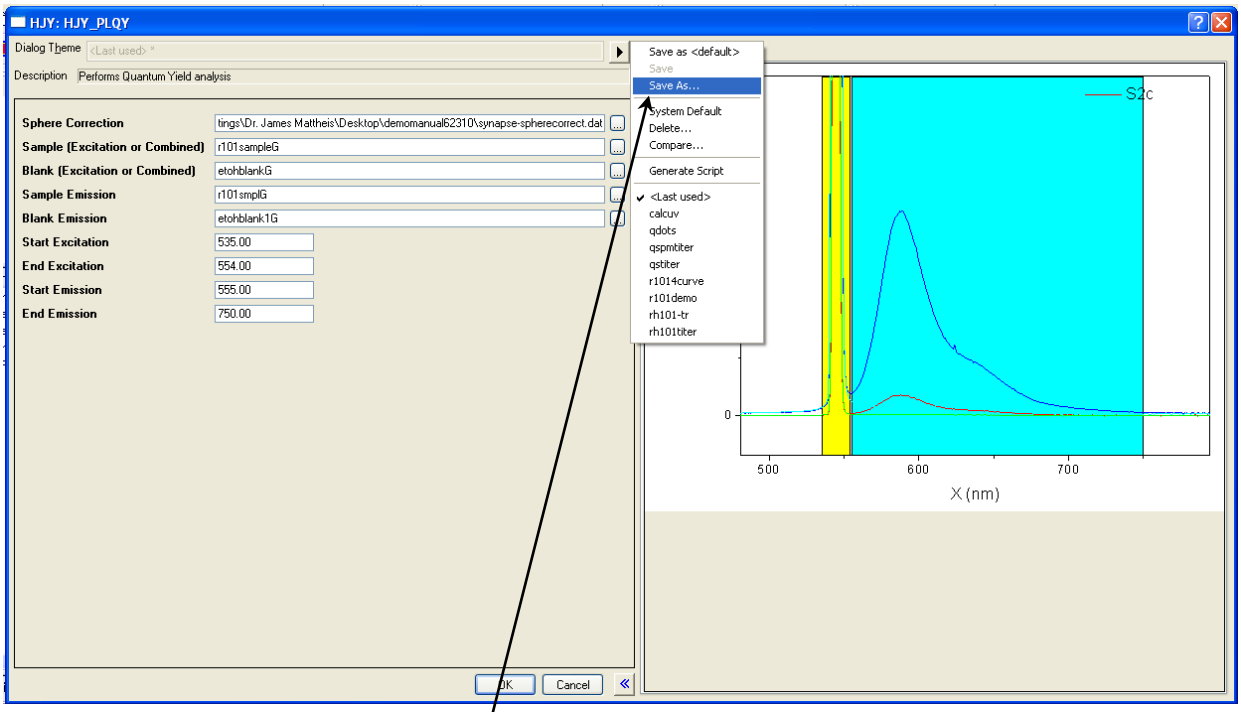


In all cases continue here.

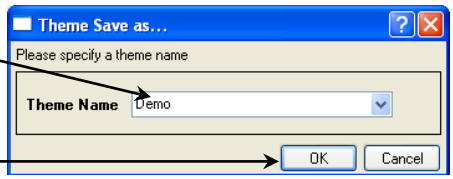
12 Save the theme.

a Click the Right Arrow button ▶ near the top of the window. A drop-down menu appears:



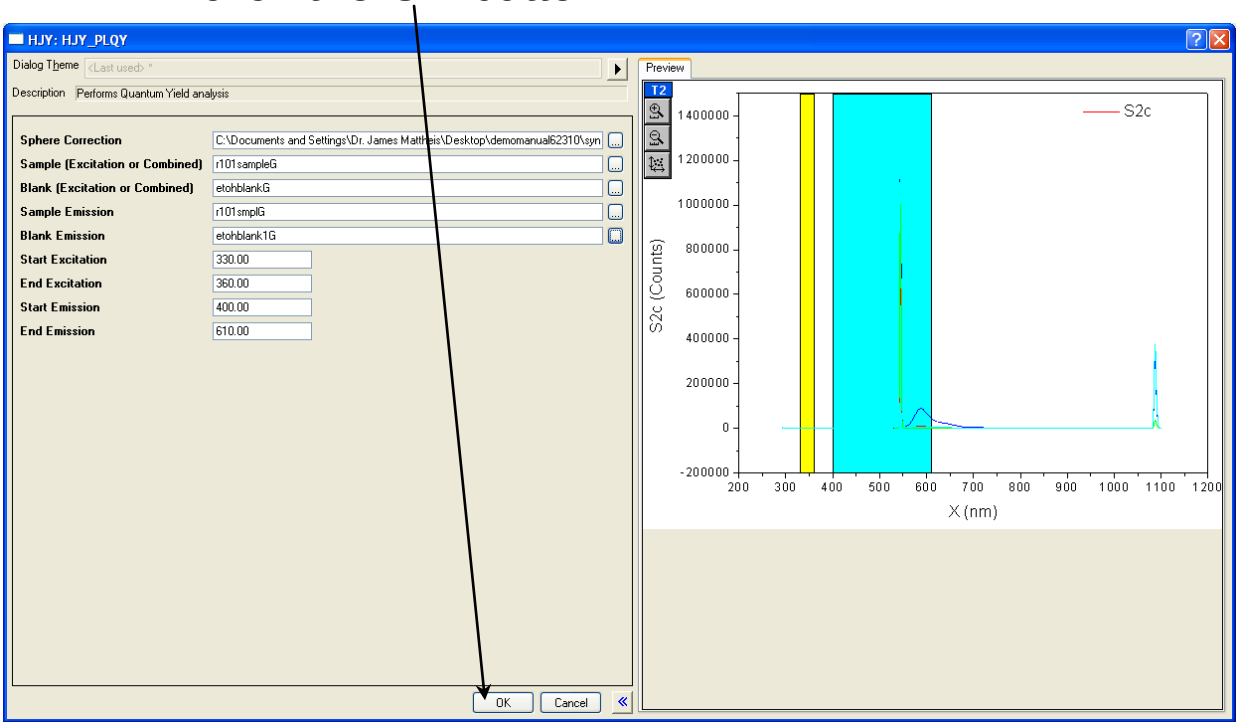


b Choose Save As.... The **Theme Save as…** window appears.



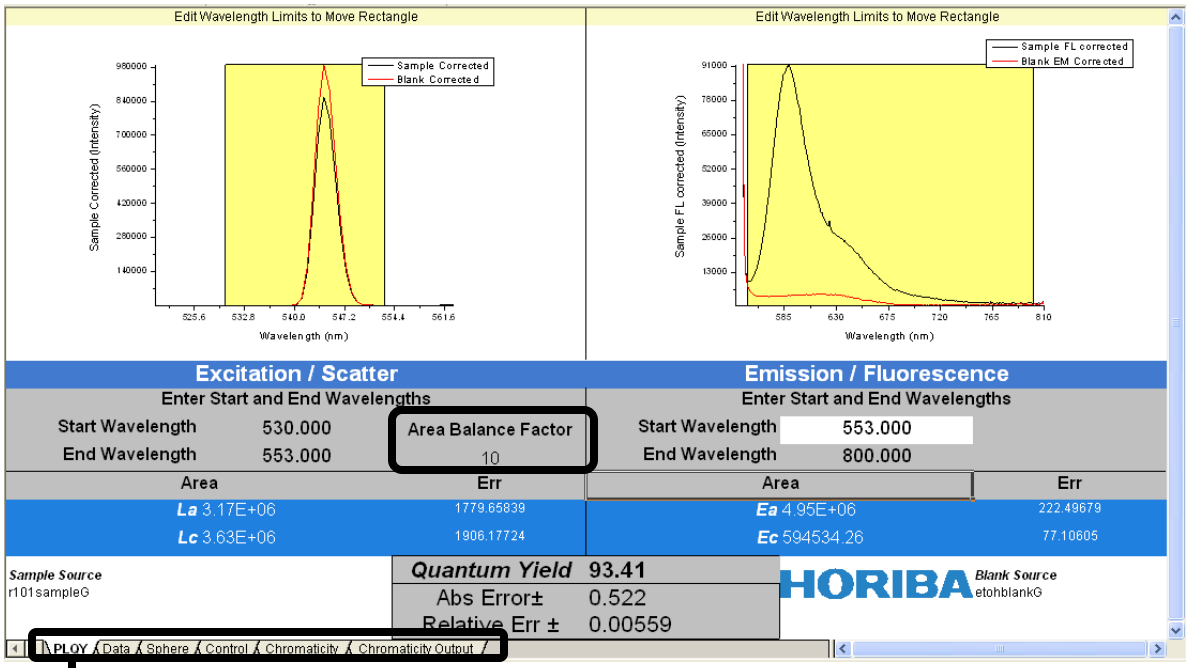
c Enter a Theme Name, and click the OK button.

13 When you have selected the desired ranges, click the OK button.



Results appear in the spreadsheet.

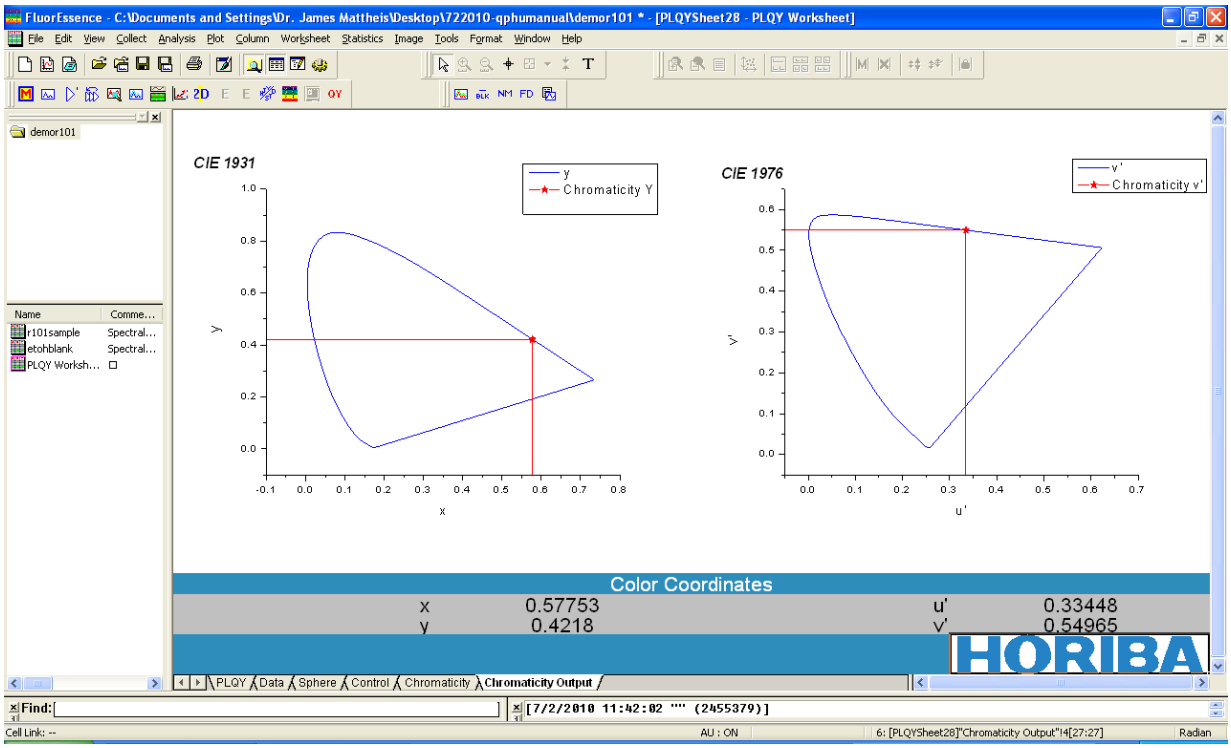
In the 4-curve method, for the sample and blank luminescence, we increased the integration time from 0.1 s to 1 s (a factor of 10), to account for weak emission in comparison to the strong Rayleigh scattering. After pressing the OK button to run the analysis, the software automatically accounts for this difference in integration time: the Area Balance Factor = 10:



To change the wavelength range for calculations, enter new values in the Start Wavelength and End Wavelength cells. The software automatically recalculates values and replots.

You can view the integrated areas L_a and L_c. Tabs show

- Data
- Sphere correction
- Control, which are the parameters used for calculations
- Chromaticity
- Chromaticity Output, which is a chromaticity graph:

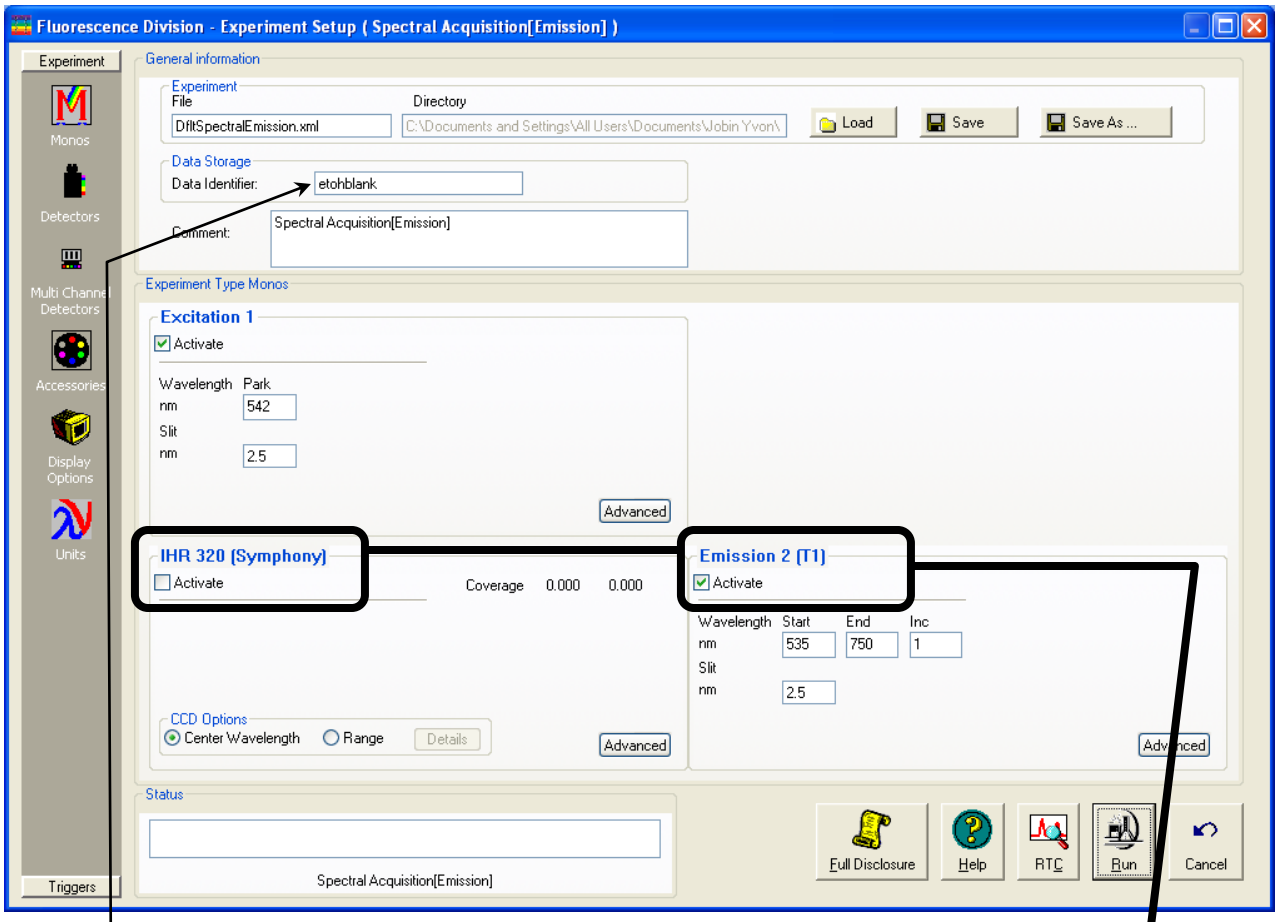


Using a photomultiplier tube as the detector

Instead of a CCD detector, a photomultiplier tube (PMT) may be used as the detector. Data-acquisition is slightly different in this case.

For a 2-curve analysis:

1. Place the blank into the integrating sphere, and properly close the sphere.
2. Start FluorEssence™; run an emission scan.
3. In the **Experiment Setup** window, set the parameters as shown below:



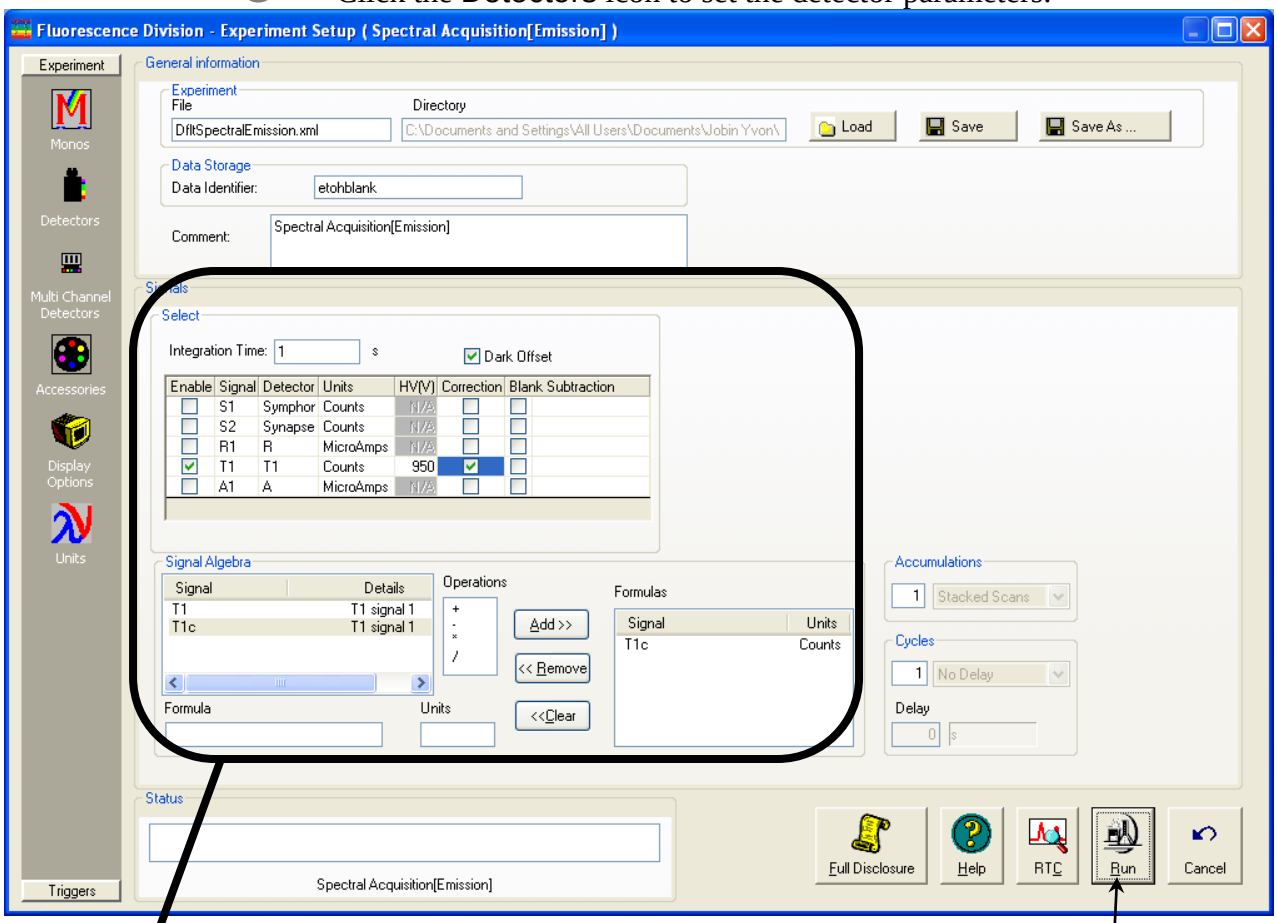
a Enter a Data Identifier to indicate a blank scan.

b In the above case, our PMT is on the T-side, so we de-activate the S-detector (Symphony iHR) checkbox, and activate the Emission 2 (T1) checkbox. Your system may be different.

c Park the excitation monochromator, and set the Slit bandpass to 2.5 nm.

d Set the emission monochromator Start, End, and Inc, plus set the Slit bandpass to encompass the Rayleigh and luminescence regions.

e Click the Detectors icon to set the detector parameters:



f Enable the PMT detector checkbox (here it is T1)

g Set units to cps.

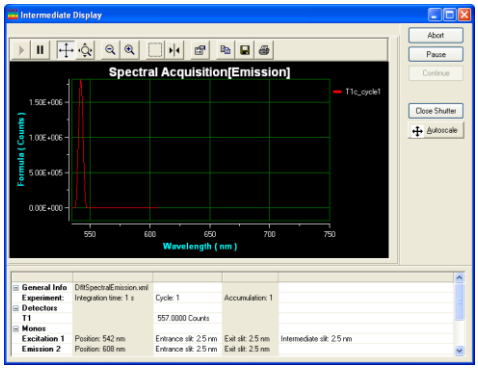
h Enable Correction and Dark Offset checkboxes.

i Add >> the corrected PMT signal to the Formulas list.

j Set the Integration Time to 0.1 s.

4 Click the Run button to start the scan.

The **Intermediate Display** appears:
The **Project Name** window may appear, then the data appear.



5 Analyze the scan for the counts in the Rayleigh peak.

If the peak is ~10^6 counts, this is acceptable.

6 Rerun the scan if the peak is not ~10^6 counts.

a Click the Previous Experiment button  .

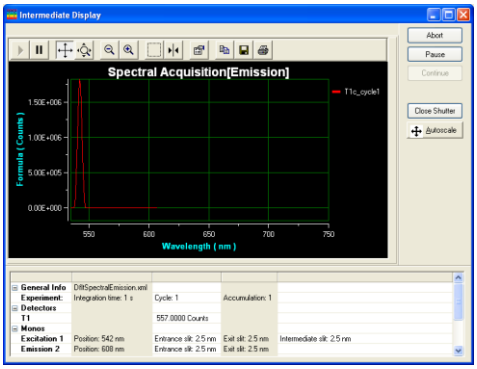
b Click the Detectors icon to see the detectors page.

c Set the units to Counts.

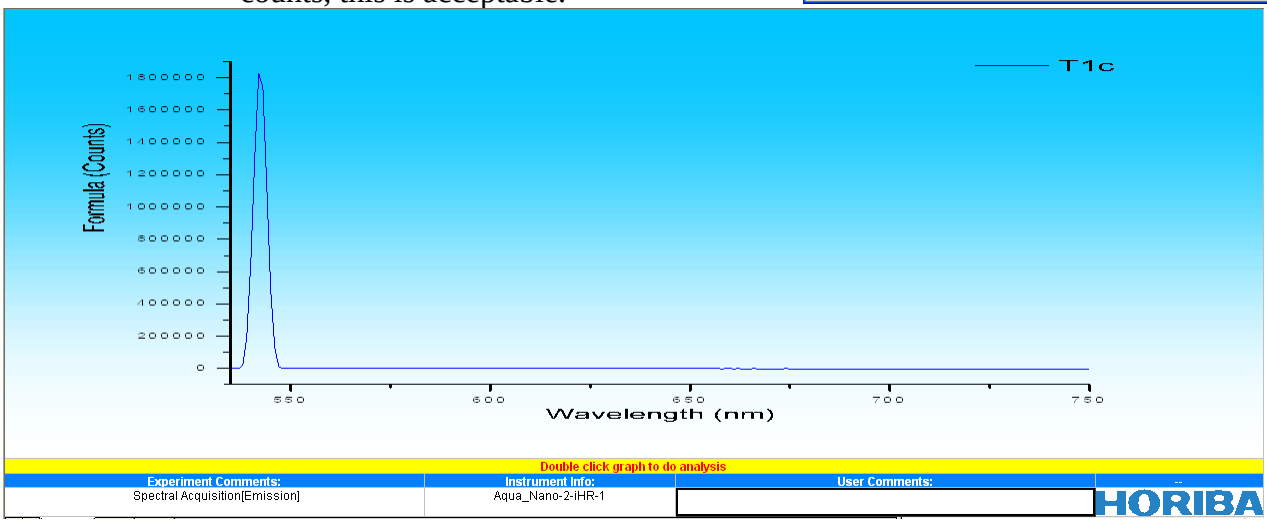
d Set the Integration Time to yield 10^6 counts in the Rayleigh peak.

7 Click the Run button to start the scan.

The **Intermediate Display** appears:

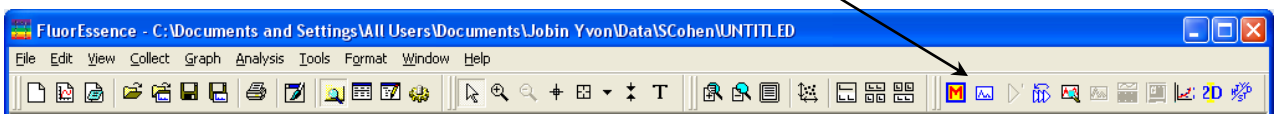


The **Project Name** window may appear, then the data appear. If the peak is ~10^6 counts, this is acceptable.

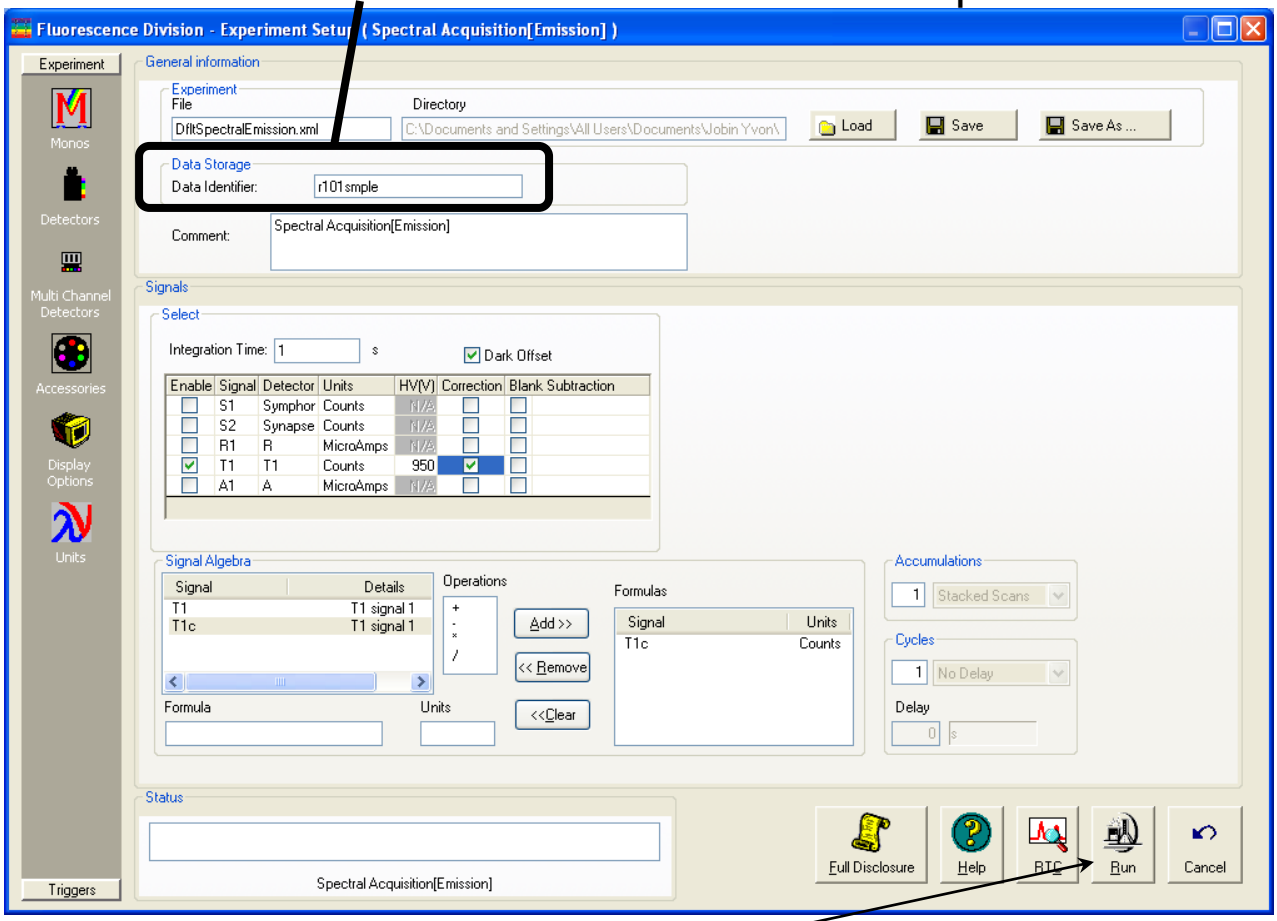


8 Remove the blank, and place the sample inside the integrating sphere.

9 In the toolbar, click the Previous Experiment button  to rerun the scan with the same parameters.

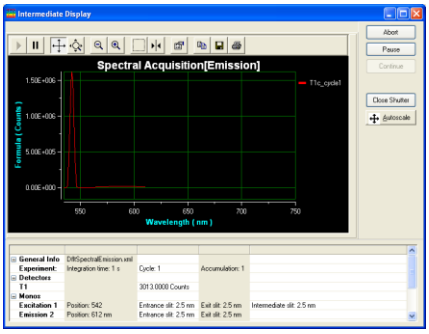


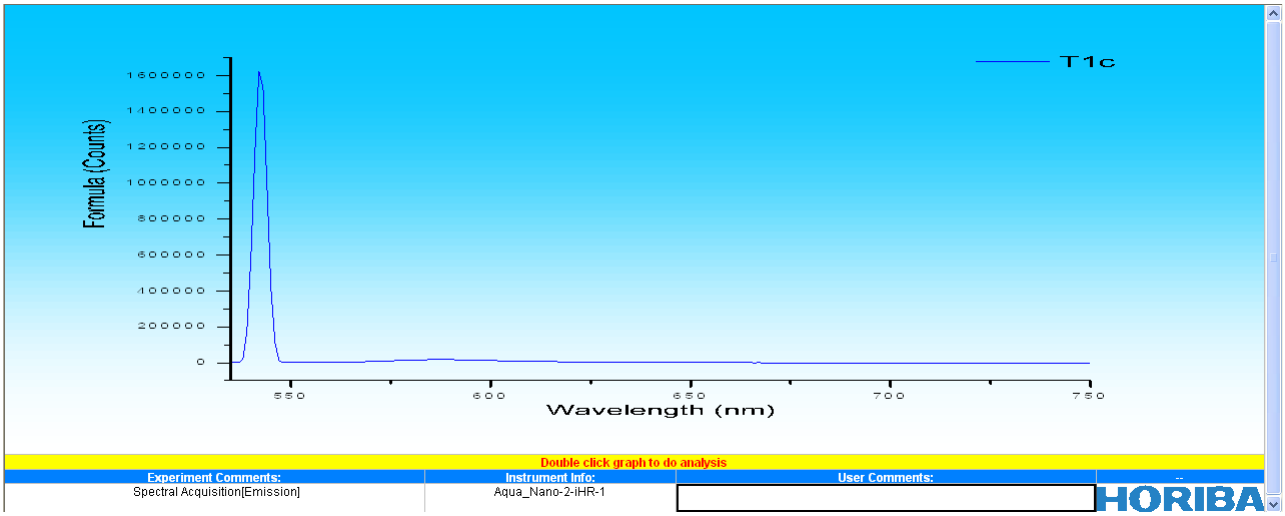
10 In the **Experiment Setup** window, change the Data Identifier field to indicate a sample scan:



11 Click the Run button to start the scan.

The **Intermediate Display** appears, then the data:





12 Continue with the previous section, to set up and run the quantum-yield calculations.

For a 4-curve analysis,

Follow the 2-curve analysis method, but you may only adjust neutral-density attenuation and integration time to bring peak signals into the correct range. You may scan only the luminescence region to obtain a higher signal-to-noise ratio.

Note: *If you change the neutral-density filter, then you must change the Area Balance factor in the final spreadsheet analysis to account for the change.*

- You many measure the transmittance of a neutral-density filter with a spectrophotometer.
- You may buy a calibrated neutral-density filter.
- You may measure the attenuation of the Rayleigh signal (unsaturated peak) with a spectrophotometer.

4 : Maintenance

Handling

Caution: *The integrating sphere is delicate. Mishandling may seriously damage its interior reflective surface.*

Wear clean gloves when handling the Integrating-Sphere halves or the optional calibrated reflector, in order to prevent the skin's natural oils from contaminating the Spectralon® surface.

Cleaning

Light soil

If the interior of the Integrating Sphere or the surface of the optional calibrated reflector becomes lightly soiled, blow a jet of clean, dry air or nitrogen gas on the surface.

Caution: *Never use Freon® or any other organic solvent to clean the Spectralon® surfaces. Always read the Material Safety Data Sheets before using dry nitrogen or other pressurized gases.*

Heavy soil

Contact HORIBA Scientific for advice.

5 : Generating Correction Files

Introduction

Just as gratings, filters, detectors, and other spectrometer components have response characteristics that are functions of wavelength so do the optical components of the Quanta-φ integrating sphere. 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, optical fiber, filter- and spectrometer-response characteristics must be corrected. Corrections are made for each of these potential problems by generating and using radiometric correction factors for the Quanta-φ.

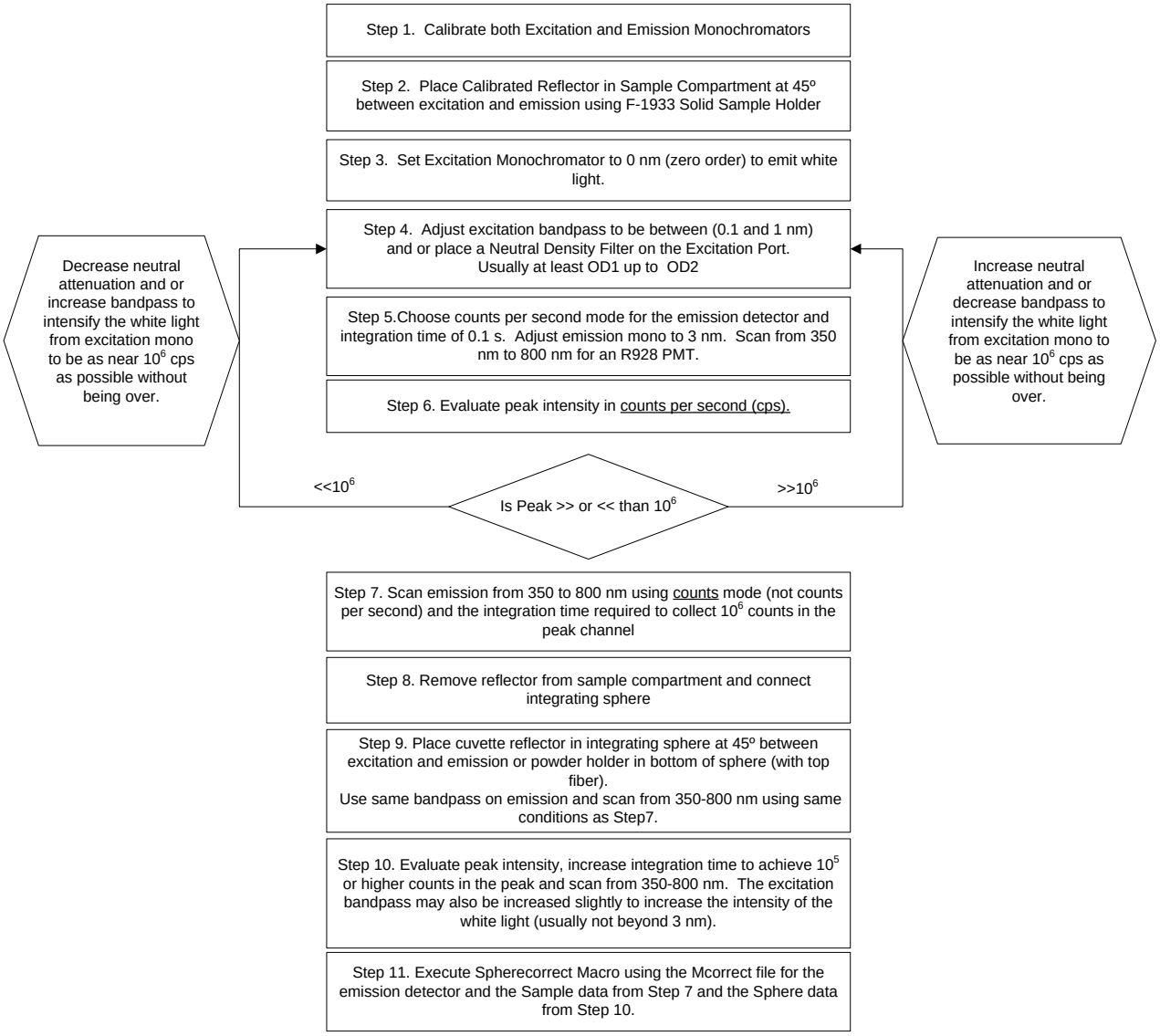
For Quanta-φ integrating spheres accompanying new instruments, HORIBA Scientific's policy is to generate sphere-correction factors in the factory for each for the corresponding detector-grating combinations specified for the system. All correction-factor files are contained on the CD-ROM accompanying the accessory. You may, however, generate your own Quanta-φ correction files using an optional calibrating reflector accessory, with the following procedure.

Note: *The CD-ROM's number must match the number on the Integrating Sphere for the calibration to be valid. If the numbers do not match, contact HORIBA Scientific.*

Below are HORIBA sphere-correction ranges created in the factory for common detector-grating combinations:

Emission detector	Grating	Correction-file wavelength-range (nm)
R928P	1200 grooves/mm × 500 nm blaze	350–800
R928P	1200 grooves/mm × 330 nm blaze	300–800
R2658P	1200 grooves/mm × 500 nm blaze	350–1000
Synapse OE CCD	100 grooves/mm × 450 nm blaze	300–1000

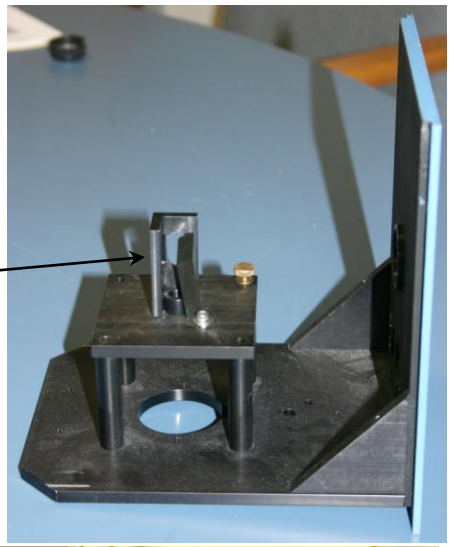
Flowchart for generating Quanta-φ correction-factor files



Methods for generating Quanta-φ correction-factor files

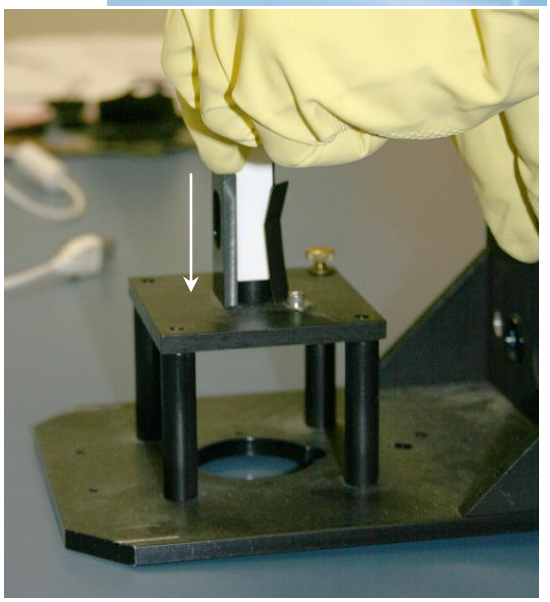
Generating correction-factor files for the cuvette holder:

1 Scan the reflectance spectrum in the sample compartment.

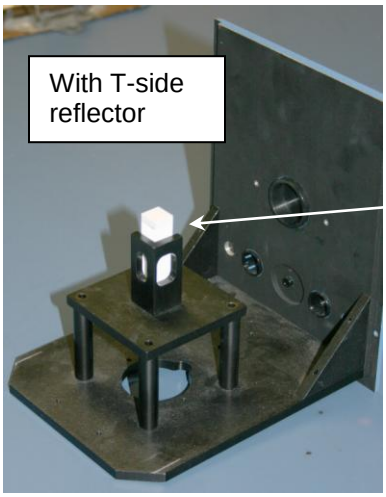


a On the standard sample-compartment drawer, attach the single-cell sample-holder.

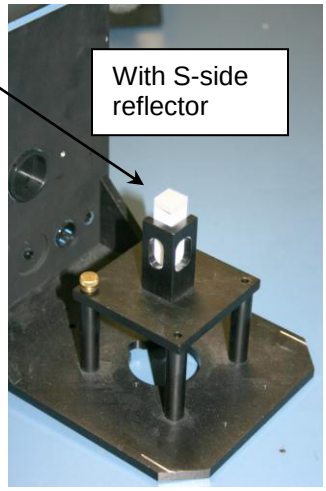
b Place the uncalibrated reflector into the single-cell holder.



Note: *Be sure that the 45° angle is between the excitation and emission slits in the sample compartment.*

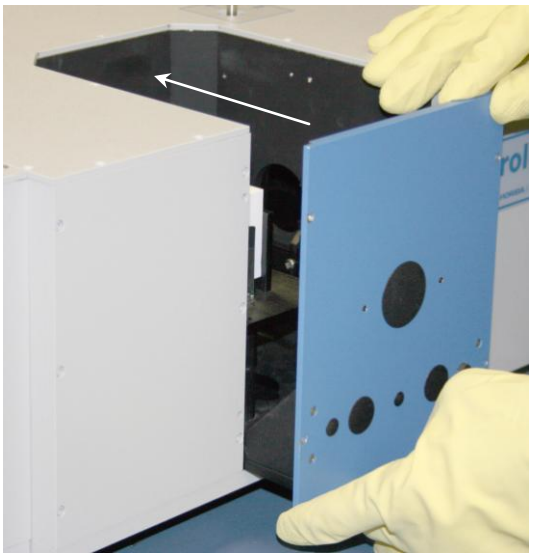


With an S-side detector, the reflector looks like this (see right); for a T-side reflector, see the left photo.



c Place standard sample-compartment drawer into the sample compartment.

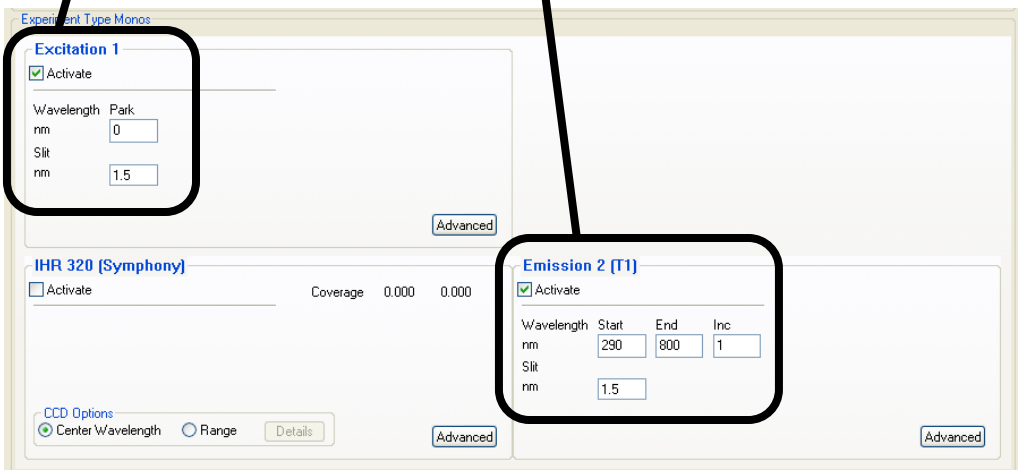
d Attach the four screws to hold the drawer to the sample compartment.



e Place the sample-compartment cover onto the sample compartment.

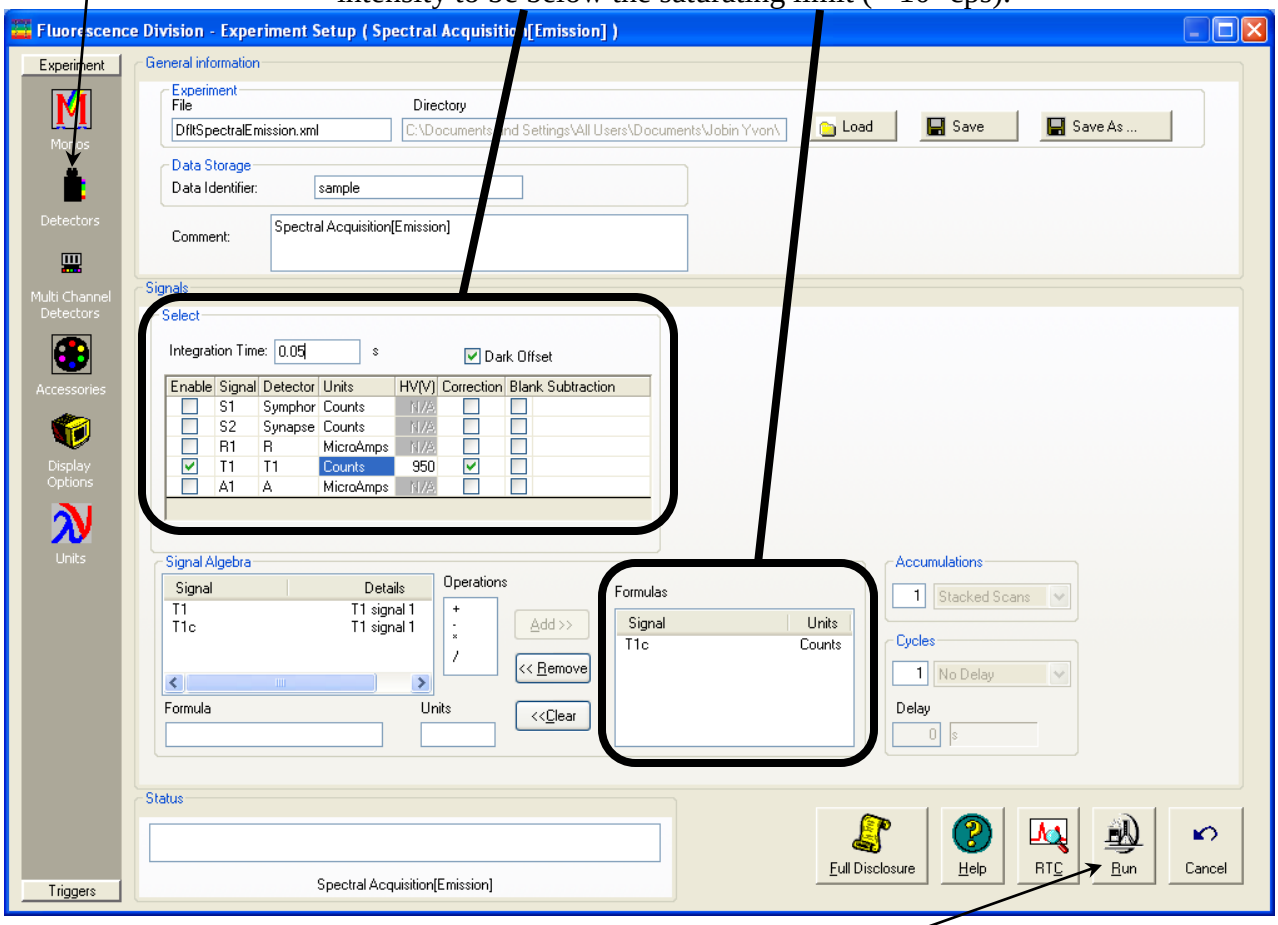
f In FluorEssence™, choose an emission experiment, and click the Monos icon.

g Set the excitation monochromator to 0 nm (zero order) for white-light transmission, the emission scanning range to match the corrected region (see table on p. 5-1), and bandpass of 1 to 3 nm on the excitation and emission monochromators.



h Click the Detectors icon.

i Choose the corrected detector signal (c subscript) and apply all instrument corrections (activate Dark Offset and Correction checkboxes). Use counts per seconds (cps) units to adjust the peak intensity to be below the saturating limit (< 10^6 cps).



j Click the Run button to start the scan.
The **Intermediate Display** appears, then the data.

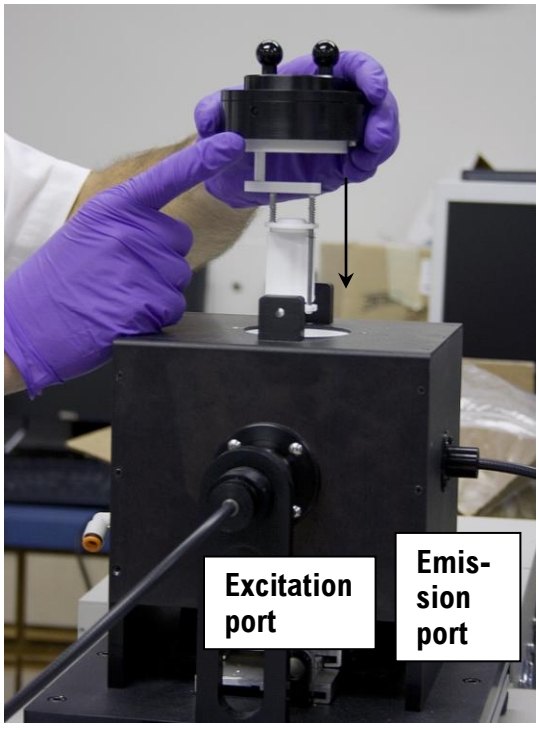
k Evaluate the peak intensity in cps units, change the units to counts, and adjust the integration time and neutral-density attenuation to acquire as close to 10^6 counts as possible without more. Then rerun the scan.

i Insert the uncalibrated reflector into the cuvette sample-holder. Be sure the square top and bottom of the reflector are seated properly into the square depressions in the sample holder.

Caution: *Do not touch the surfaces of the Integrating Sphere's sample-holder or the reflector accessory. This can degrade its highly reflective surface. Wear clean protective gloves when handling the interior of the sphere and reflector.*

2 Scan the reflectance spectrum in the integrating sphere.

a Insert the sample holder into the integrating sphere. Be sure the plane of the reflector forms a 45° angle between the excitation and emission ports.



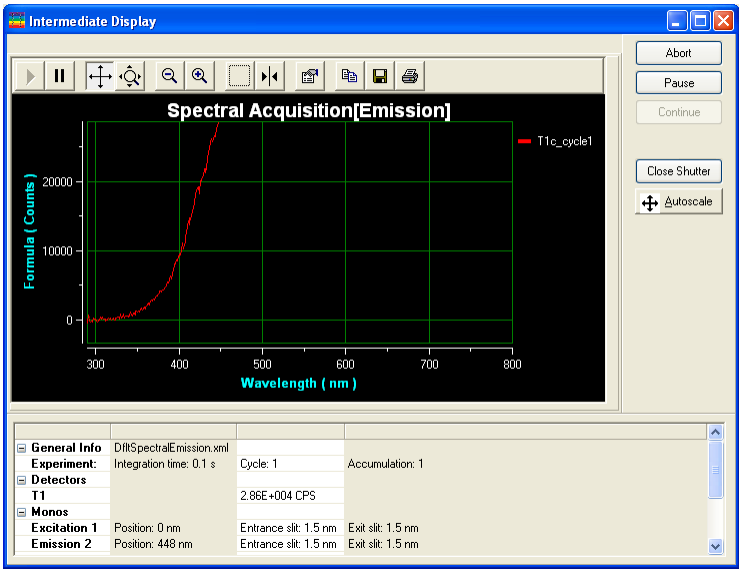
b In the FluorEssence™ **Experiment Setup** window, scan the reflectance spectrum as an emission scan using the same experimental conditions as in Step i above.

Note: *A neutral-density filter affects the correction file for the sphere AND sample compartment.*

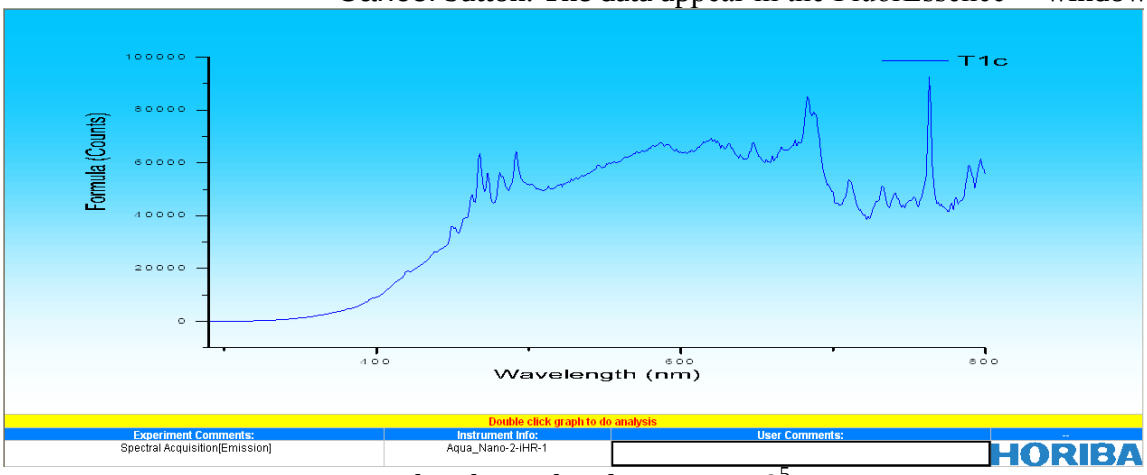
Note: *The signal will be decreased by inclusion of the sphere and optics by 1–2 orders of magnitude.*

c Evaluate the peak intensity.
Increase the integration time or ***slightly*** change excitation bandpass to get at least 10^5 counts in the peak.

d Click the Run button. The **Intermediate Display** appears:

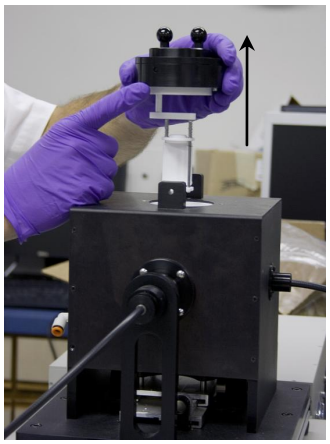


When the scan is complete, the **Project Name** window appears. You may enter a name or click the Cancel button. The data appear in the FluorEssence™ window:



Note that the peak value is near 10^5 counts.

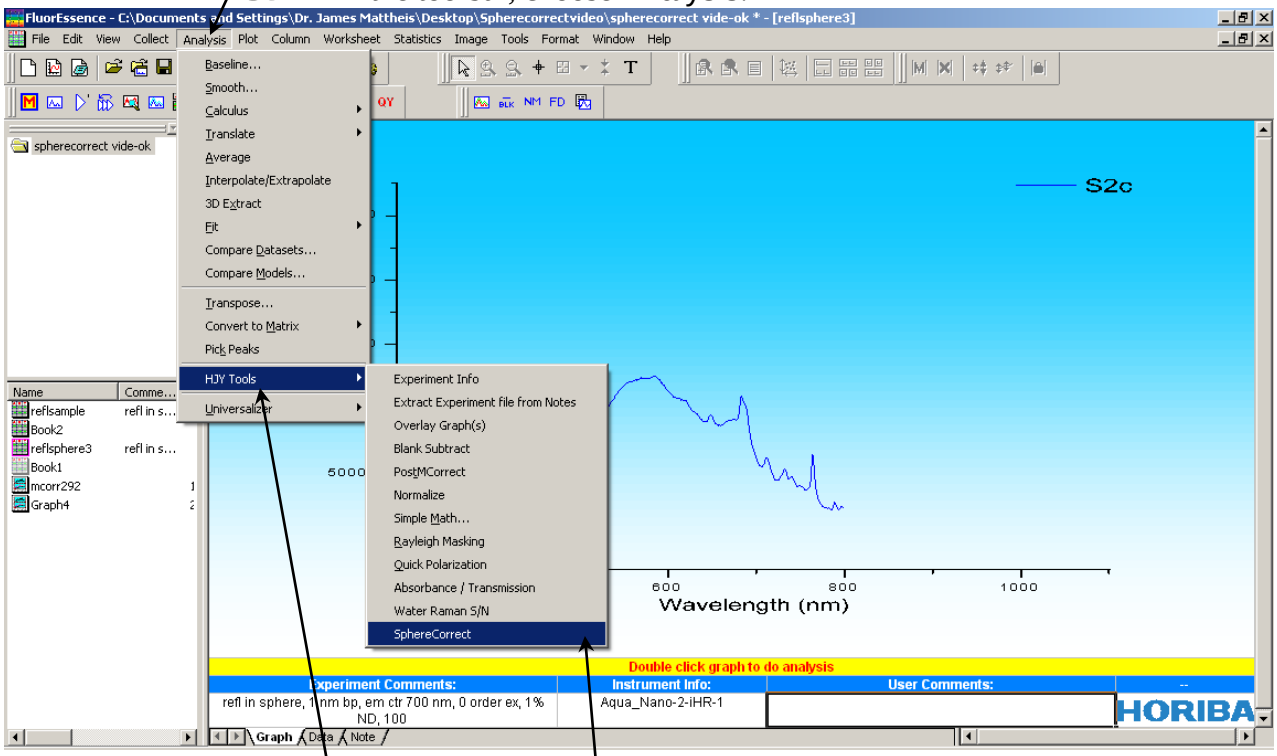
e Remove the cuvette holder from the integrating sphere.



f Remove the calibrated reflector from the cuvette holder.

3 Calculate the sphere correction-factor from the ratio of the reflectance in the sphere to the sample compartment alone.

a In the toolbar, choose Analysis.

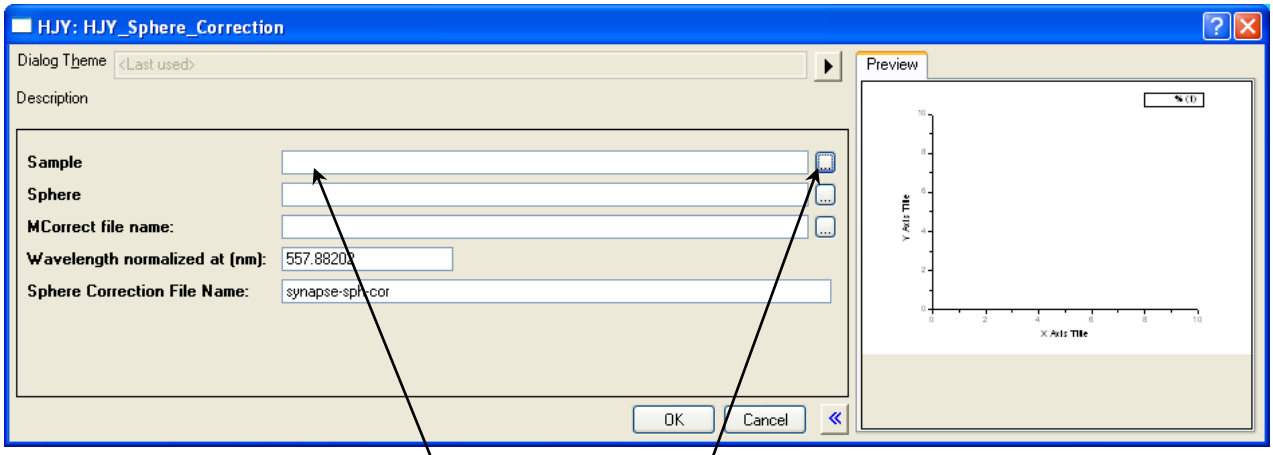


A drop-down menu appears.

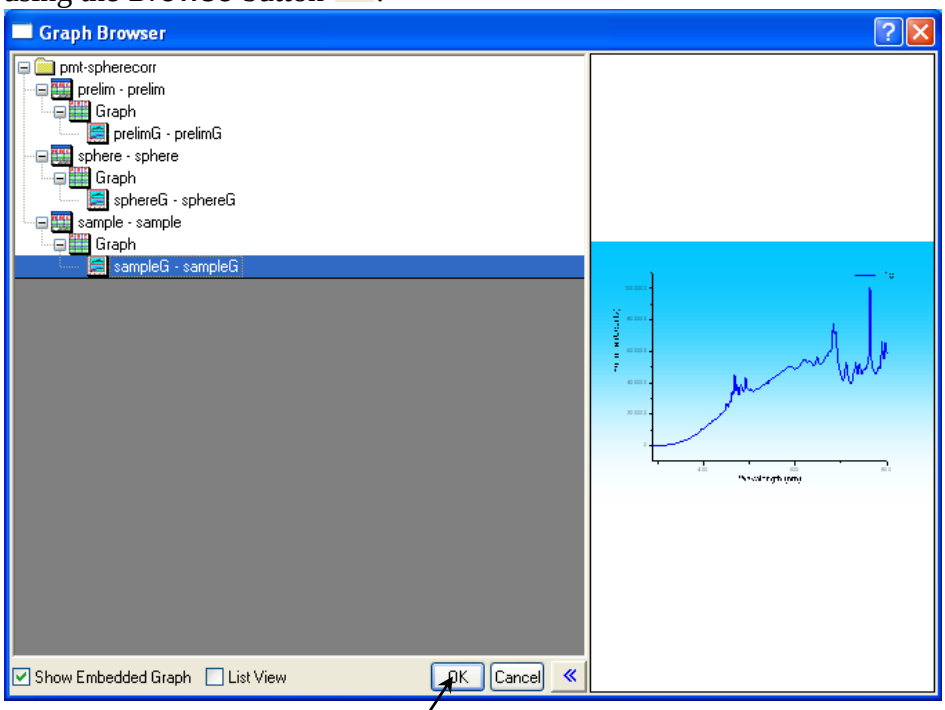
b Choose HJY Tools.
Another drop-down menu appears.

c Choose SphereCorrect.

d The **HJY_Sphere_Correction** window appears:

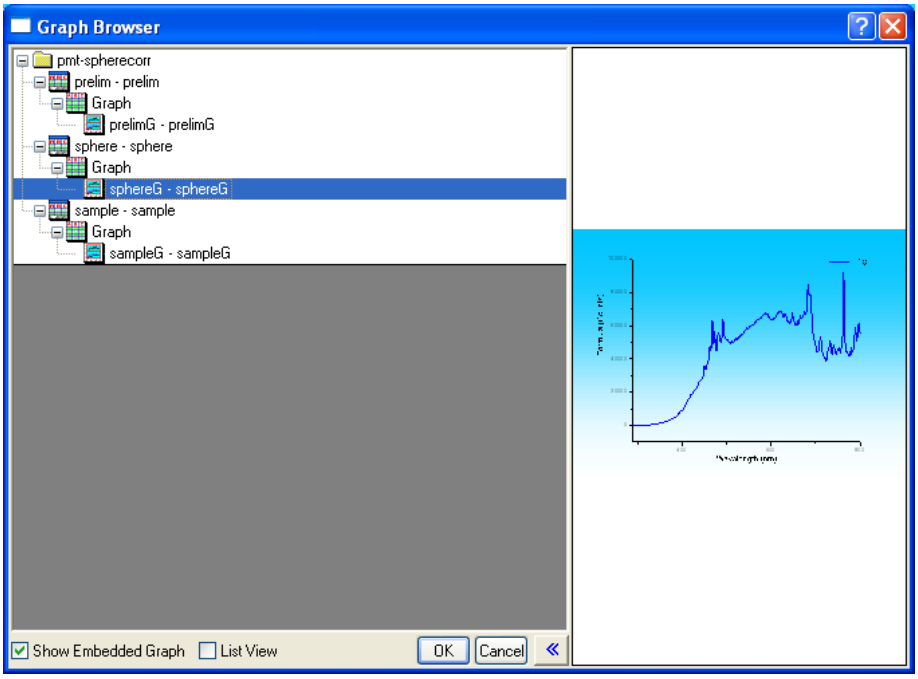


e In the Sample field, browse for the sample data (the last data collected) using the Browse button  :

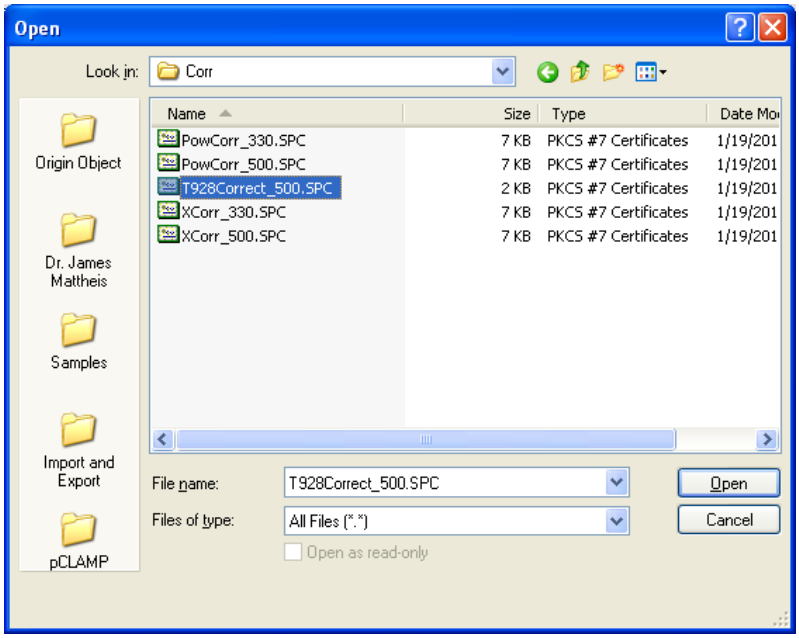


f Click the OK button when found.

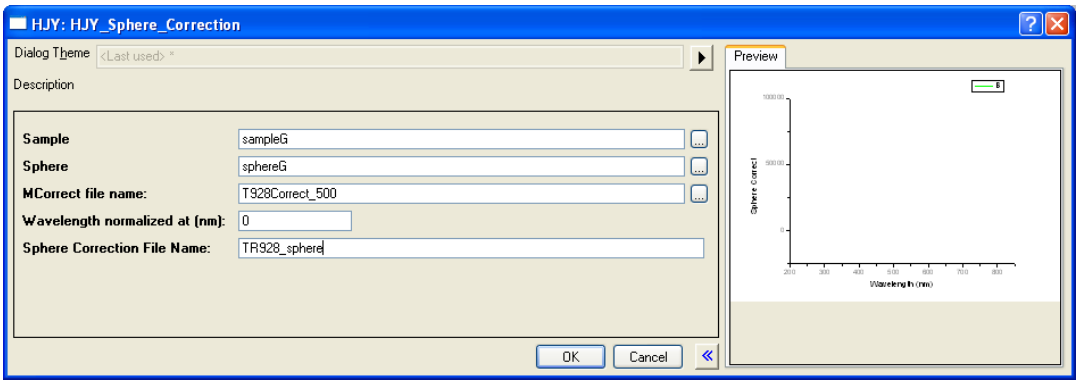
g Similarly, find the sphere data using the Browse button  in the Sphere field:



h Browse for the MCorrect file name using the Browse button  :

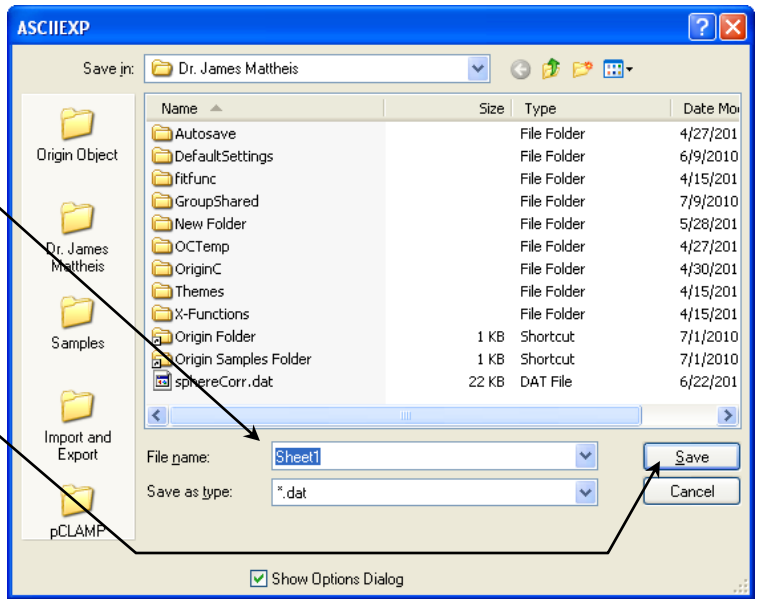


The completed **HJY_Sphere_Correction** window looks something like this:

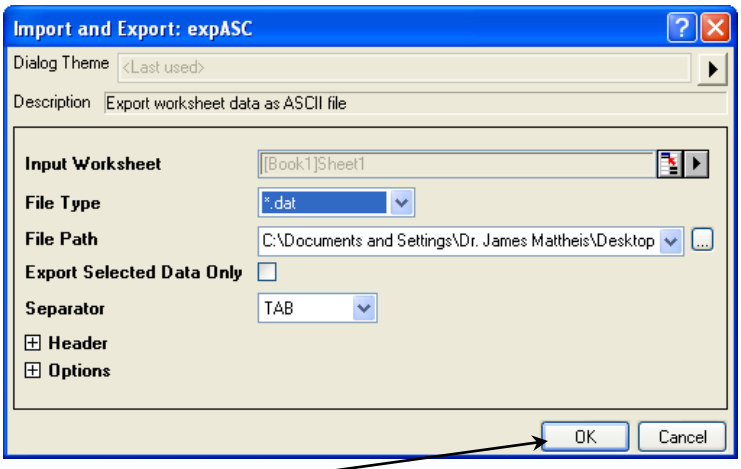


i Click the OK button when ready.
The **ASCIIEXP** window opens:

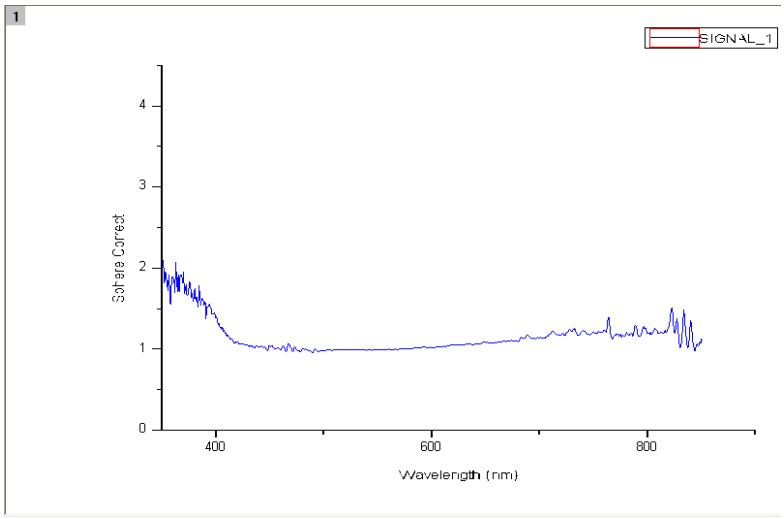
j Enter a name for the final ratio file in the File name field, and click the Save button.



The **Import and Export** window appears:

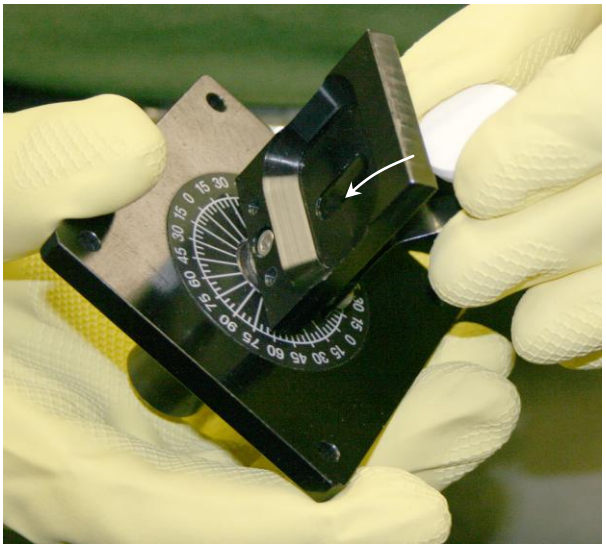


k Click the OK button.
The final correction-file appears:



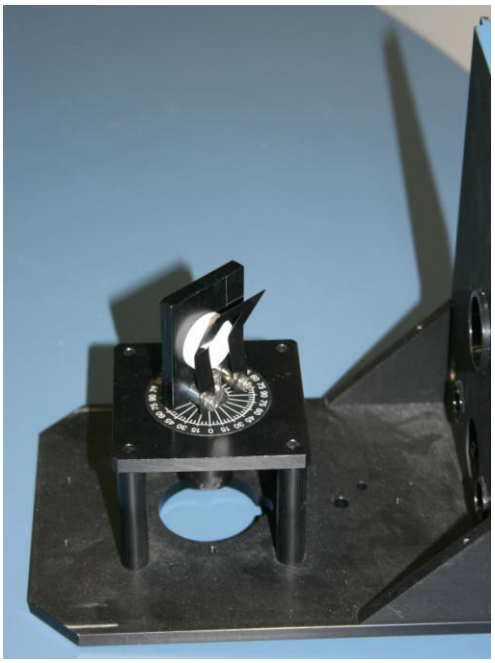
Generating correction-factor files for the powder/film holder:

1 Scan the reflectance spectrum in the sample compartment.



a Insert the powder reflector disk into the 1933 Solid-Sample Holder.

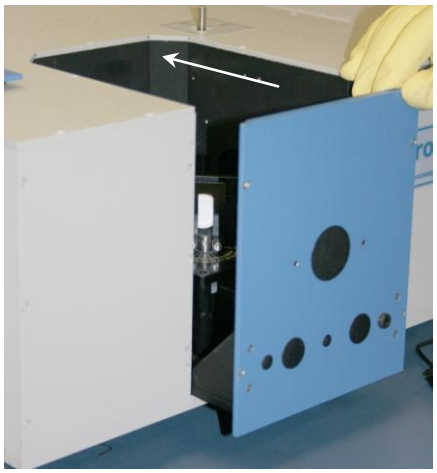
b Place the Sample-Holder on the sample drawer.
Keep a 45°-angle between excitation and emission slits. The photo depicts an S-detector configuration.



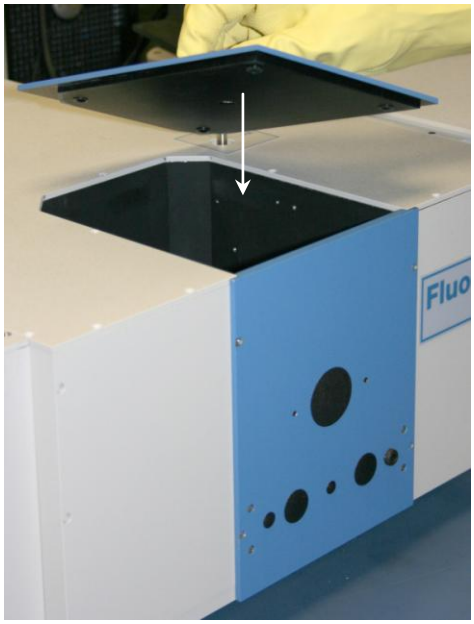
Note: *Rotate the Sample Holder 90° for a T-detector setup.*

c Insert the sample drawer into the sample compartment.

d Attach the four screws to hold the drawer to the sample compartment.

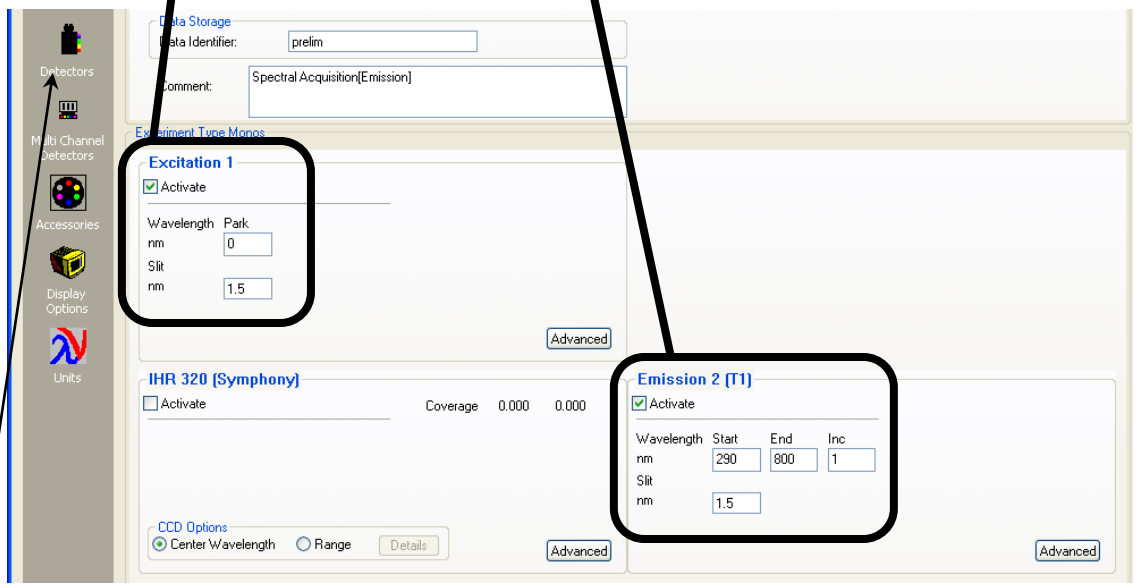


e Place the sample-compartment cover onto the sample compartment.



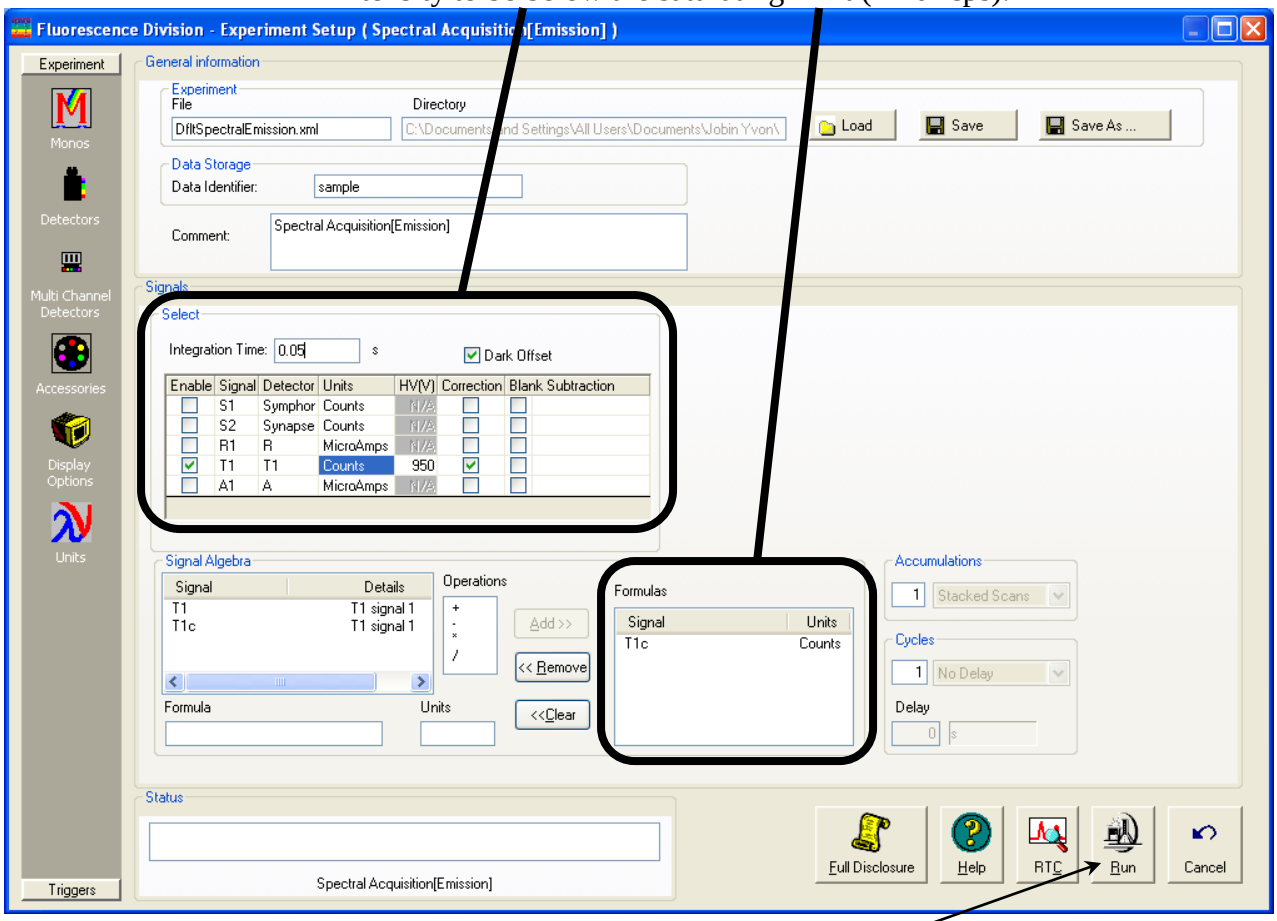
f In FluorEssence™, choose an emission experiment, and click the Monos icon.

g Set the excitation monochromator to 0 nm (zero order) for white-light transmission, the emission scanning range to match the corrected region (see table on p. 5-1), and bandpass of 1 to 3 nm on the excitation and emission monochromators.



h Click the Detectors icon.

i Choose the corrected detector signal (c subscript) and apply all instrument corrections (activate Dark Offset and Correction checkboxes). Use counts per seconds (cps) units to adjust the peak intensity to be below the saturating limit (< 10^6 cps).

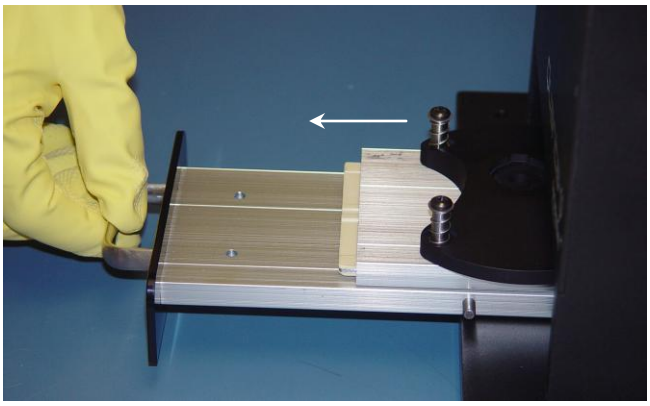


j Click the Run button to start the scan.
The **Intermediate Display** appears, then the data.

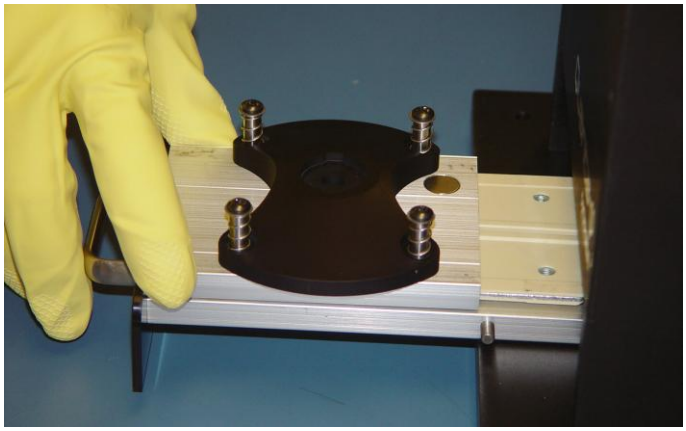
k Evaluate the peak intensity in cps units, change the units to counts, and adjust the integration time and neutral-density attenuation to acquire as close to 10^6 counts as possible without more. Then rerun the scan.

2 Scan the reflectance spectrum in the integrating sphere.

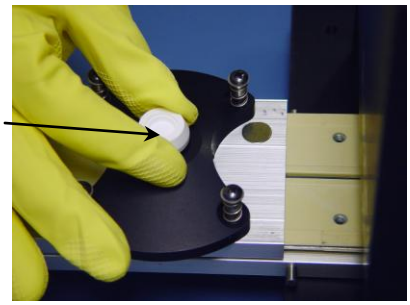
a Pull sample-drawer handle out.



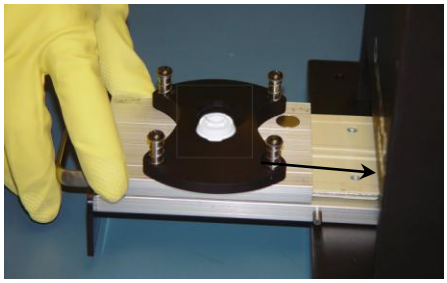
b Pull solid-sample support itself out.



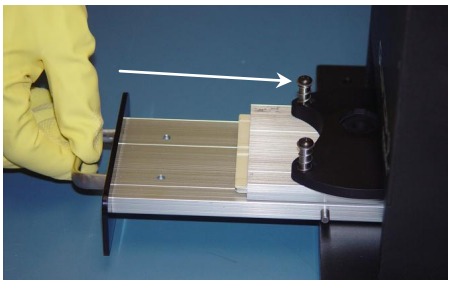
c Insert the uncalibrated powder reflector into the support.



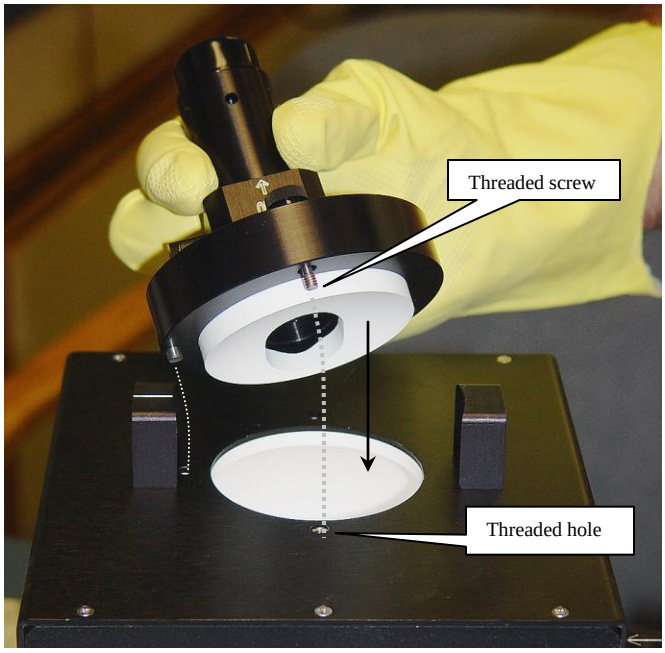
d Slide sample holder back in.



e Push sample drawer in, so that the sample is raised into the integrating sphere.

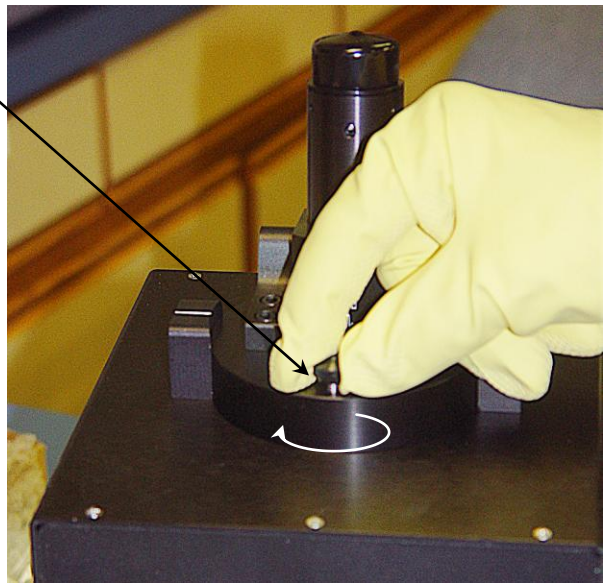


f Place the solid-sample cover on the integrating sphere.

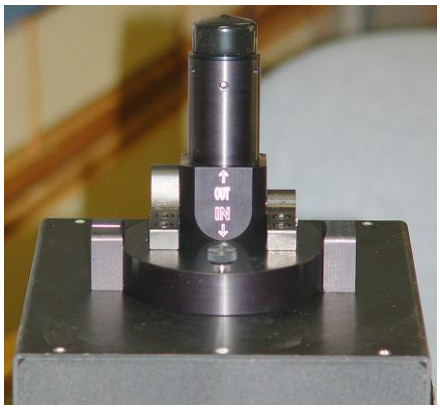


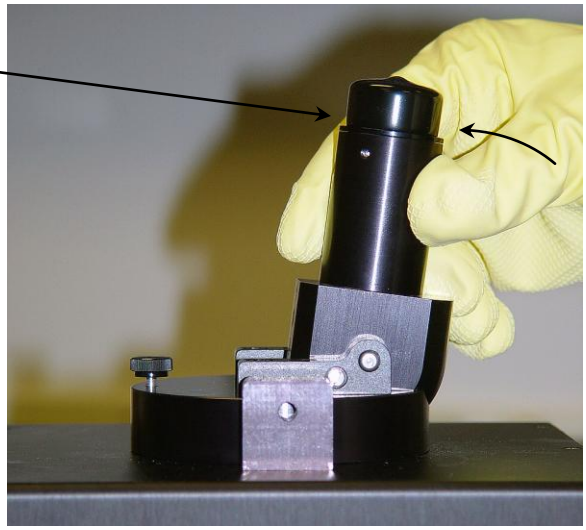
Note: *Match the threaded thumbscrew on the lid to the threaded hole on the sphere.*

g Tighten the thumbscrew to fix the lid in place.

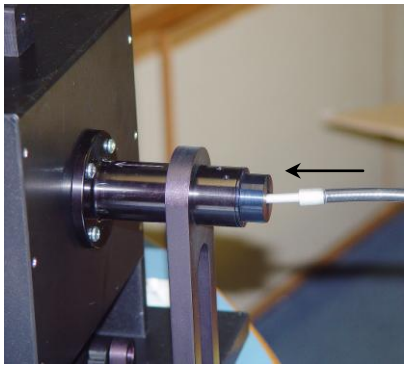


h Raise the excitation port to the IN position.

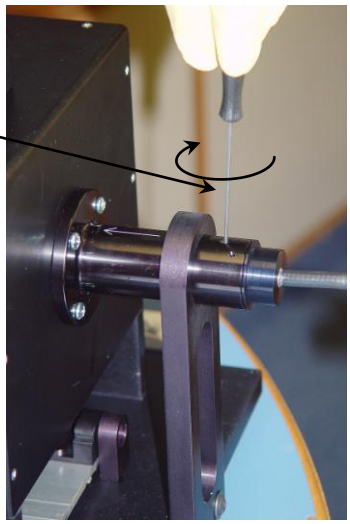




i Remove the protective cap from the emission port. Connect a fiber-optic bundle to the integrating sphere emission (outward arrow) port.



j Tighten the set-screw with an Allen key.



3 Calculate the sphere correction-factor from the ratio of the reflectance in the sphere to the sample compartment alone.

Follow the procedure in the previous section, “Generating correction-factor files for the cuvette holder”.

6 : Troubleshooting

The Quanta-φ accessory has been designed to operate reliably and predictably. Should a problem occur, examine the chart below.

Symptom	Possible Problem	Proposed Solution(s)
Spikes in emission spectrum and/or high background	Light leak in or around integrating sphere	Verify that the bottom sample drawer's plug has lifted into the sphere
		Check that cuvette holder is properly set in place
		Check that fiber optics are mounted correctly
		Check that the purge ports are properly screwed in and sealed.
Excitation signal missing or too low	Lamp is not illuminated	Turn on lamp and allow it to warm up for 30 min.
	Detector is not receiving power, or is connected improperly	Check detector power, cables, and SW settings.
	Bandpass is too narrow	Increase bandpass.
	Neutral-density or other optical filter or obstruction is in optical path	Remove unneeded filters and obstructions.
	Wavelength settings are improperly calibrated or selected	Correct calibration of excitation and emission monochromators, and select appropriate grating.
	Wrong detector or mirror selected from signal list or instrument configuration.	Select proper detector and mirror settings in **Experiment Setup** window.
	Fiber optics positioned or oriented wrongly for T or S format	Correctly place fiber optics to T and or S format.
Emission signal is low	Fiber optics are in wrong relative positions for excitation and emission ports	Properly connect fiber-optics to correct ports.
	Cuvette holder or upper fiber-optic holder is not in IN position	Move to IN Position.
	F-3000 is not correctly aligned for fiber optics	Verify F-3000 alignment as per *F-3000 Operation Manual*.
	Unneeded neutral-density or other optical filter is placed in sample compartment	Remove sample compartment and check for filters or obstructions.
	No sample in sphere	Place sample in sphere.
	Sample concentration or quantum yield is too low	Check that OD is sufficient, and increase integration time or excitation power appropriately.

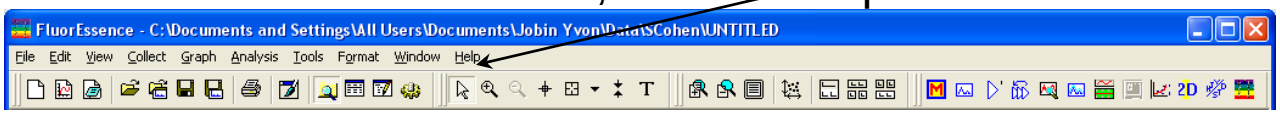
	Insufficient integration time	Increase integration time.
Quantum yield is unreasonably large or small, or its error values are too large	Area-balance factor is not properly adjusted to account for changes in integration time or neutral-density filter attenuation	Correctly enter area-balance factor to account for combined (integration time × neutral attenuation) factor
	Either excitation or luminescence signal areas are not integrated to reach a sufficient signal-to-noise ratio	Integrate longer or increase excitation power.
	Sample preparation is not consistent with literature conditions	Check chemical preparation, age, oxidation state and solvent conditions, pH, polarity, refractive index, as well as chemical purity. Consider recrystallizing standards, chemicals, and filtering solvents.
	Incorrect application of `Mcorrect.spc` file in data acquisition (Signal List or Instrument Configuration)	Enter correct emission correction factor in instrument configuration and **Experiment Setup** window.
	Incorrect application `Spherecorrection.dat` file in PLQY tool	Enter correct Sphere correction factor.
	Incorrect selection of blank and sample graphs and data in PLQY tool	Select correct blank and sample graphs.
	Use of inappropriate theme in PLQY tool	Select appropriate theme for PLQY experimental conditions.
	Sample and blank cuvette or powder holders are not optically matched. Uneven volumes, scattering or scratches, mismatched refractive indices, etc.	Carefully balance volumes and solvents for liquids, avoid scratched cuvettes, fingerprints, or suspended particles on cuvettes. For powders or solids, ensure blank substrate is representative of sample. Consider solid-dilution method to homogenize and equilibrate the surface properties.
	Counts per second (cps) units were used instead of required Counts units for data acquisition	Always use Counts units when collecting data for the PLQY tool. The integration-time constant will otherwise not be correct.
	Different wavelength or bandpass settings were used for sample and blank	Always match bandpass and wavelength settings for sample and blank. Only change integration time or excitation power.
Emission spectrum of sphere and blank sample(s) or with empty cuvette holder or empty blank powder-holder indicates contamination.	Possible contamination of cuvette or powder holder or bottom plug or blank sample(s).	Check for and remove obvious contamination, tape, powder, liquid droplets. **Always be sure cuvette is stoppered and cleaned of any external liquid.**
	Possible contamination of bottom sample plug	Replace and check with new plug.

	Possible contamination of inner sphere	**Internal contamination voids the sphere warranty.** The sphere interior may need to be replaced because the porous Spectralon® is very difficult to decontaminate.
CCD signal > 65 000 counts per accumulation cycle for a blank excitation signal (> 10^6 cps).	Excessive count rate (CPS) or integration time	CCD count-rate for the blank excitation band should be adjusted to between 500 000 and 1 million counts per second (i.e., 50 000 counts between 0.05 and 0.1 s).
	Bandpass too wide	Bandpass should be set for complete resolution of the spectral features of the excitation bands and fluorescence signal areas. Usually from 1 to 5 nm is sufficient.
	Insufficient neutral-density attenuation	Use neutral attenuation on excitation side if the suggested count rate (500 000–1 000 000 cps) is exceeded when the bandpass is between 1 and 5 nm.
PMT signal > 1 million cps (rate) for blank excitation signal	Insufficient neutral-density attenuation	Neutral attenuation should be used on excitation if the suggested count rate (500 000–1 000 000 cps) is exceeded when the bandpass is between 1 and 5 nm.
	Bandpass is too wide	Bandpass should be set for complete resolution of the spectral features of the excitation bands and fluorescence signal areas. Usually from 1 to 5 nm is sufficient.

Further assistance...

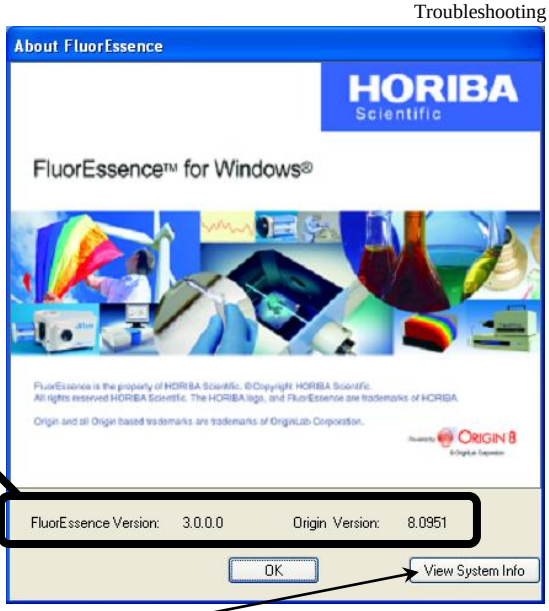
Read all software and accessory manuals before contacting the Service Department. Often the manuals show the problem's cause and a method of solution. Technical support is available for both hardware and software troubleshooting. Before contacting the service department, however, complete the following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

1. If this is the first time the problem has occurred, try turning off the system and accessories.
   After a cool-down period, turn everything back on.
2. Make sure all accessories are properly configured, and turned on as needed.
3. Following the instructions in *System Operation*, run a xenon-lamp scan to make sure the system is properly calibrated.
   Print the spectrum for each and note the peak intensities.
4. Check this chapter to see if the problem is discussed.
5. Try to duplicate the problem and write down the steps required to do so.
   The service engineers will try to do the same with a test system. Depending on the the problem, a service visit may not be required.
6. If an error dialog box appears in FluorEssence™, write down the exact error displayed.
7. In FluorEssence™, in the FluorEssence main window's toolbar, choose Help:



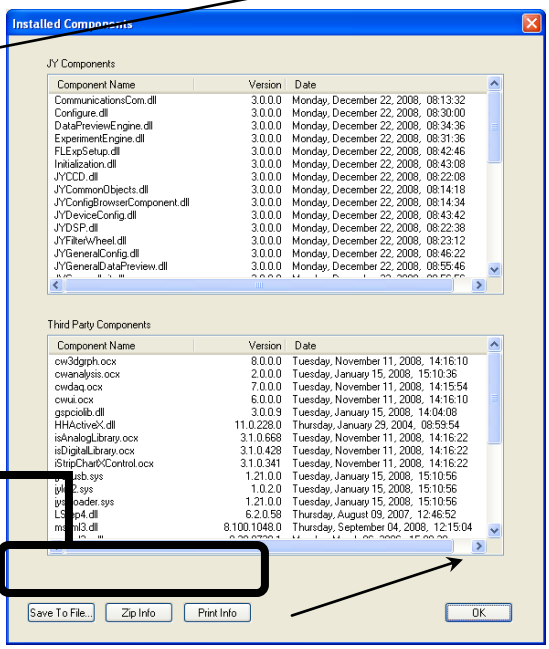
   A drop-down menu appears.
8. Under Help, choose About FluorEssence....
   This opens the **About FluorEssence** window:

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



9 Click the View System Info button.

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



10 Record the information by clicking the:

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

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

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

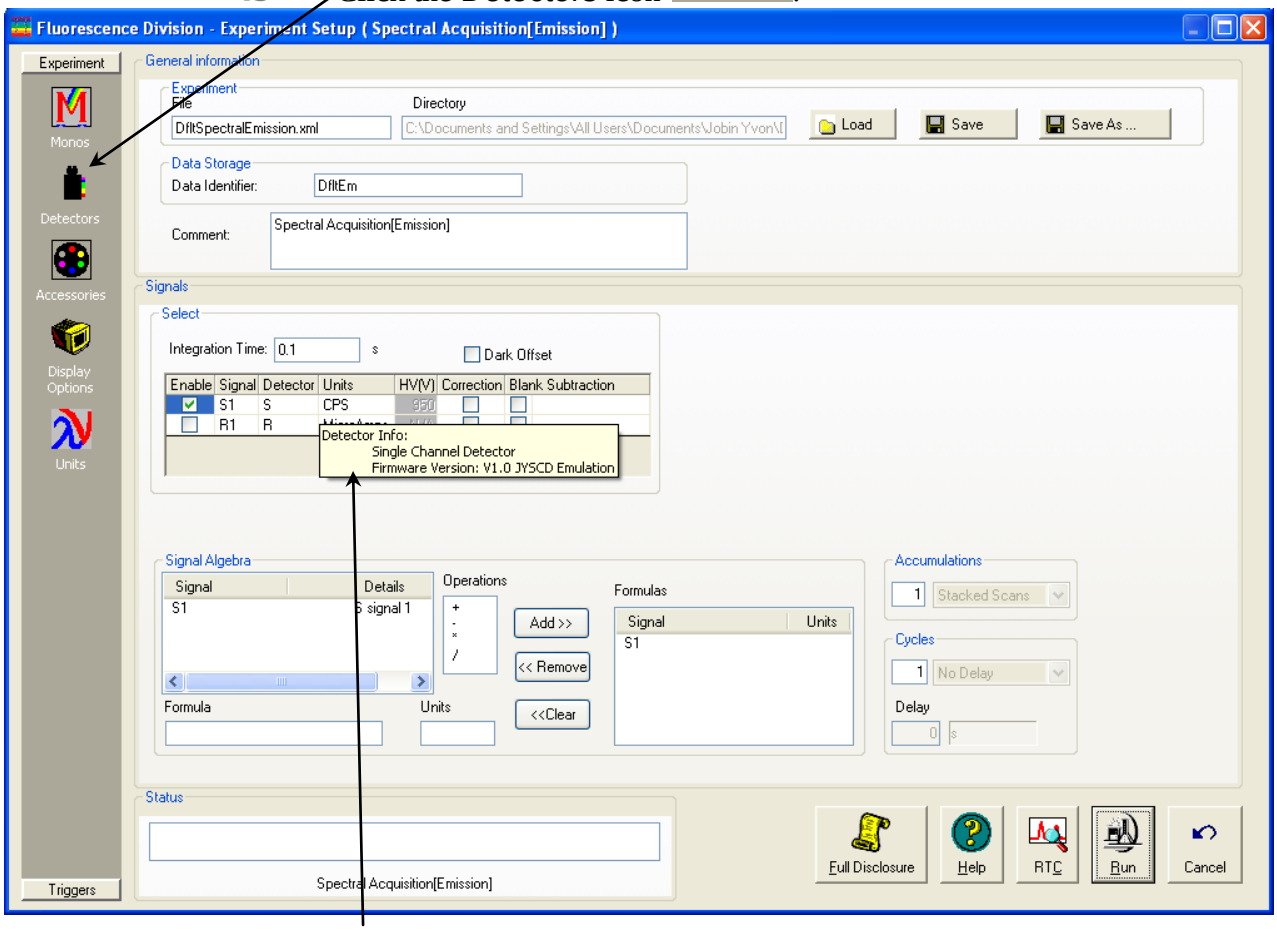
13 Write down the software's version numbers, along with the purchase dates, model numbers, system configuration, and serial numbers of the instrument and its accessories.

14 Determine the SpectrAcq firmware version:

a Open the **Experiment Setup** window:

b Click the Detectors icon 

.



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

If the problem persists or is unlisted, call the Service Department at (732) 494-8660 × 160, or fax at (732) 549-5125. Outside the United States, call the local distributor. You may also reach us at our web page:

http://www.HORIBA.com/scientific

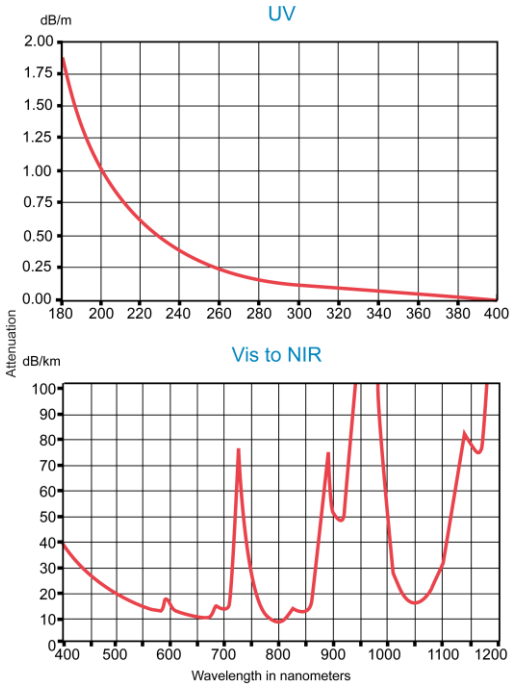
When you contact the Service Department, have the purchase date, serial number, system configuration, and software version available. Be prepared to describe the malfunction and the attempts, if any, to correct it. Note any error messages observed and have any relevant spectra (sample, polarization ratio,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

7 : Technical Specifications

Hardware

Dimensions (including mounting platform for sample drawer)	10″ × 13″ × 12¼″ (width × length × height) 25 cm × 33 cm × 31.1 cm (width × length × height)
Weight	7.47 lbs. (3.39 kg) without sample drawer 9.47 lbs. (4.29 kg) with sample drawer
Integrating-sphere diameter	152 mm (6″)
Solid-sample entrance-port diameter	20 mm
Top entrance port diameter	66.68 mm (2.625″)
Rear input-port diameter	25.4 mm (1.00″)
Side fiber-hole diameter	5.08 mm (0.200″)
Port fraction in cuvette mode	1.043%
Port fraction in solid-sample mode	2.103%
Optics-head field-of-view diameter	25.4 mm (1.00″)
Integrating-sphere material	Spectralon®
Reflectivity	> 95% from 250–2500 nm
Purge ports	Compatible with ¼″ O.D. SMC tubing available from Allied Electronics
Sample	Thin films, 12 mm dia. or smaller Powders Liquids (Other types of samples need custom holders.)
Fiber-optic bundle	Slit-round configuration, 180 fibers. Slit-end termination 10 mm O.D. x 50 mm long. Round-end termination FR-274. Sheath is PVC monocoil. Length is 1.5 m.
Fiber-optic numerical aperture	0.22

Transmission curve of fiber-optic bundles



Software

FluorEssence™ version 3.5 or higher with valid USB key
Excel® 2000 or later software

8: Index

Key to the entries:

Times New Roman font.....subject or keyword
Arial font.....command, menu choice, or data-entry field
Arial Condensed Bold font.....dialog box
`Courier New font`.....file name or extension

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[Design Concept]

The HORIBA Group application images are collaged in the overall design.
Beginning from a nano size element, the scale of the story develops all the way to the Earth with a gentle flow of the water.