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FLS980 Series Reference Guide

Integrating Sphere for Measurements of Fluorescence
Quantum Yields and Spectral Reflectance



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Contents

1.	INTRODUCTION	3
2.	PREPARING FOR MEASUREMENTS	6
2.1.	Preparing the Instrument	6
2.2.	Preparing the Integrating Sphere for Liquid Samples Measurements	6
2.3.	Preparing the Integrating Sphere for Solid Samples Measurements	7
2.4.	Preparing the Samples.....	8
3.	THE PRINCIPLE OF ABSOLUTE QUANTUM YIELD MEASUREMENTS	9
3.1.	“Direct Excitation” Method	9
3.2.	“Direct & Indirect Excitation” Method	11
4.	ABSOLUTE FLUORESCENCE QUANTUM YIELD MEASUREMENTS	13
5.	ABSOLUTE FLUORESCENCE QUANTUM YIELD CALCULATIONS	15
6.	CORRECTIONS FOR TRUE ABSOLUTE FLUORESCENCE QUANTUM YIELDS.....	17
7.	SPECIAL CASES OF ABSOLUTE FLUORESCENCE QUANTUM YIELD MEASUREMENTS	19
7.1.	Samples with Low Fluorescence Quantum Yield	19
7.2.	Scatter and Emission Measured with Different Detectors	19
8.	REFLECTION MEASUREMENTS	21
9.	ABSORPTION MEASUREMENTS	22

1. Introduction

This Reference Guide helps you to use the Integrating Sphere Assembly, F-M01, which is an accessory to the FLS980 spectrometer. The integrating sphere has been designed for the measurement of the absolute fluorescence quantum yield of liquid samples, powders, films and bulk samples. The module can also be used for the measurement of spectral reflectance curves.

The FLS980 is a flexible, research grade fluorescence spectrometer. The integrating sphere accessory for the measurement of fluorescence quantum yields is one of its many accessories available. Using the FLS980 and the integrating sphere for fluorescence quantum yields offers great flexibility, in particular for measurements of absolute quantum yields in the near infrared spectral range or for measurements of samples with very low quantum yield or low absorbance. However, this also has the consequence that a large variety of measurement parameters of the FLS980 must be optimised for the absolute quantum yield measurements. This requires practice, experience, careful experiment planning and often patience, as measurements can be time consuming.



Figure 1: Integrating Sphere accessory for FLS980 spectrometer

The integrating sphere consists of a 120 mm inside diameter spherical cavity, which is machined from a PTFE like material with a reflectance >99% over the spectral range of 400 nm – 1500 nm and with >95% reflectance within 250 nm – 2500 nm. The delicate reflecting material is surrounded by an aluminium shell for handling and protection. The sphere module splits in half horizontally to allow easy access to the samples under test. The top handle is to facilitate the splitting of the sphere for sample placement and should never be used to carry the sphere.

The integrating sphere has two ports 90° apart, one with a lens to focus the excitation beam into the sample, the other being an open aperture through which the emission or scatter can be monitored by the FLS980 emission arm. There is a baffle near the exit aperture which guarantees that only diffusely scattered radiation can exit the sphere.

The sphere has an internal mirror directing the incident light either to the side for measuring liquid samples or toward the bottom for the measurement of solid samples, films, or powders. Mirror and samples are positioned so that directly reflected light from the sample cannot exit the sphere.

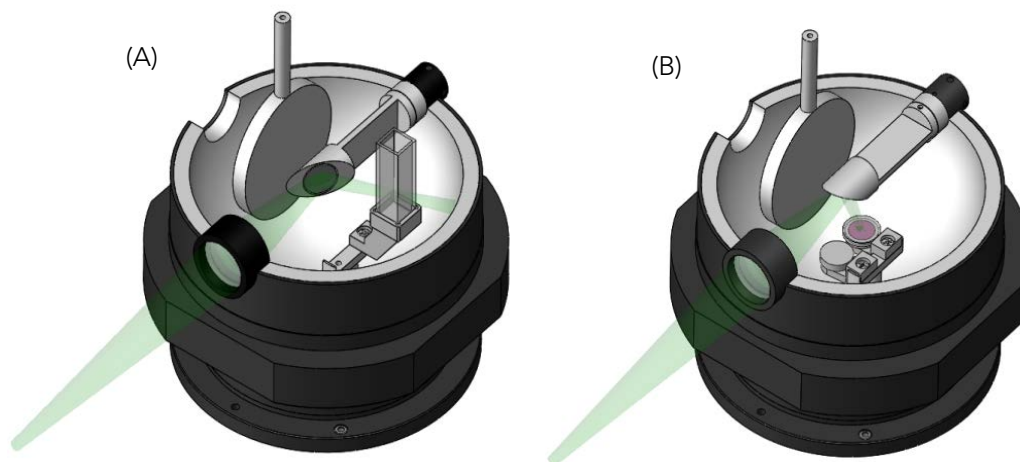


Figure 2. Beam path, mirror and sample position for measurements of liquid samples (A) and bulk, powder, film samples (B)

The integrating sphere is supplied with two quartz cuvettes, polished on 5 sides and with stopper, and with the different sample holders as shown in Figure 3. Cuvette holder, powder tray holder and powder trays are made from PTFE, the blanking plug is made from the same material that is used for the sphere.

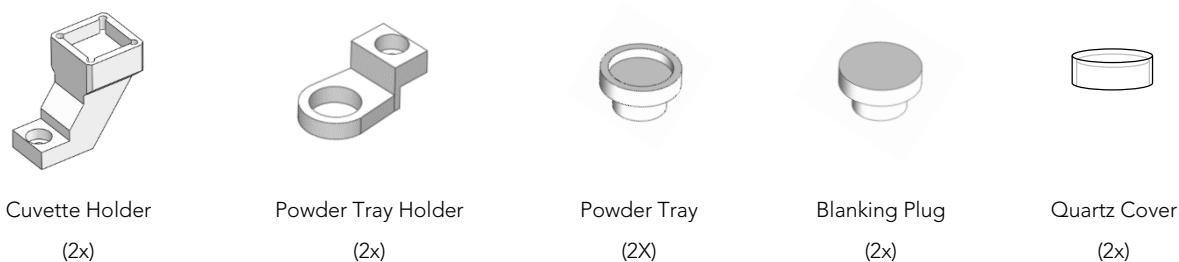


Figure 3. Sphere Accessories

Good practice when working with the Integrating Sphere assembly:

- ✓ Use powder-free gloves. Do not to touch the inner surface with bare hands.
- ✓ Take extra care when placing samples inside the sphere. Any sample spillage will contaminate the sphere
- ✓ Seal cuvettes with stoppers.
- ✓ Take extra care when handling powder samples.

Cleaning of the white reflecting material:

- ✓ To clean the sphere from rough dust and non-dissoluble powders it is recommended to blow it off with either an inert gas or air under pressure.
- ✓ It is much more difficult to clean the sphere from liquid spillage because the sphere material is very absorbent for anything with low surface tension. Water runs off, but organic solvents are rapidly absorbed. One might try dripping dichloromethane onto the contaminated area and holding a tube connected to a vacuum pump to one side, so that the solvent goes in, picks up the contamination and is sucked back out again.

Instrumental factors that can have dramatic effects on the measured quantum yields:

- ✓ Correction File

Ensure that the correct spectral correction file that is valid for the sphere, grating and detector is used for spectral correction of your raw (sphere) data. Using the wrong correction file is probably the biggest source of errors in absolute quantum yield measurements. It causes systematic errors with resulting quantum yields being either too big or too small.

By selecting the correct "light path" of your FLS980 spectrometer the correction file is automatically selected.

- ✓ Detector Linearity

The number of recorded signal photons must be linear with the number of "available" photons. With the standard photomultiplier your system should be linear to about 2 million photons per second. However, to be on the safe side we recommend to use an upper limit of about 1 million photons per second.

2. Preparing for Measurements

2.1. Preparing the Instrument

- Remove the sample holder.
Also remove the lenses in the excitation and emission beam paths assemblies, but before doing this take note of the original positions of these lenses so that they can be replaced in their original positions after measurements with the integrating sphere have been completed.

2.2. Preparing the Integrating Sphere for Liquid Samples Measurements

- Set a cuvette holder in the bottom part of the sphere as shown in Figure 4. A sample in this position is directly excited by incident light.

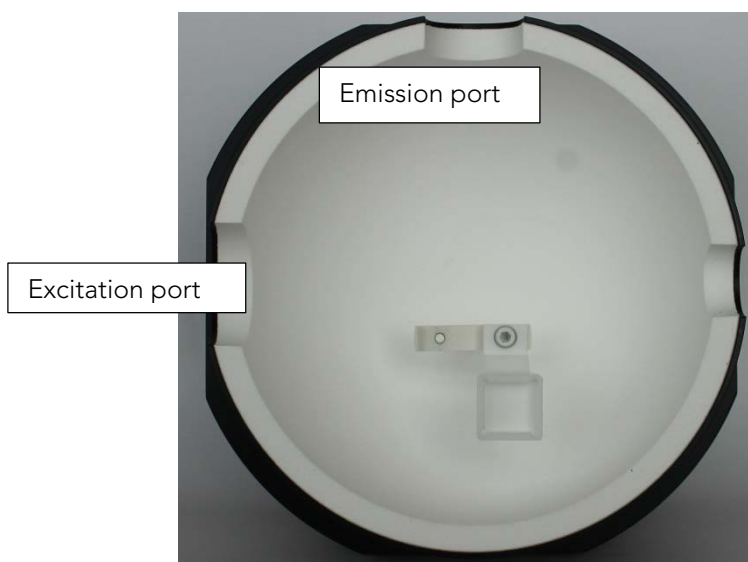


Figure 4. Top view of the bottom part of the sphere set for liquid samples measurements
Note the correct position of the cuvette holder on the right hand side

- Place the bottom part of the sphere inside the sample chamber taking care that the excitation and emission ports are directed towards the correct lenses.
- Set the rotating fold mirror on the upper part of the sphere in the 'Cuvette' position.



Figure 5. Two position mirror dial set for liquid samples measurements

Ensure that the mirror is pointing to the side as shown in Figure 6:



Figure 6. View into the upper half of the sphere half; setup for liquid samples measurements

2.3. Preparing the Integrating Sphere for Solid Samples Measurements

- Set two solid samples holders in the bottom part of the sphere as shown in Figure 7. A sample in position (1) is directly excited by incident light, while position (2) is designed for indirect sample excitation.

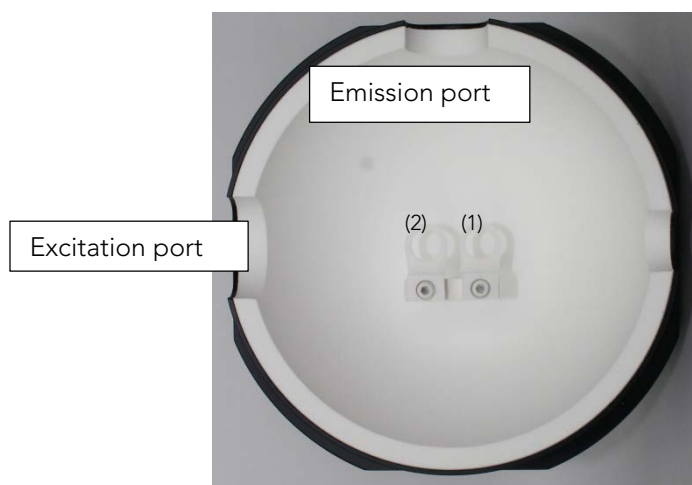


Figure 7. Top view of the bottom part of the sphere set for solid samples measurements.
Note the two positions for powder trays

- Place the bottom part of the sphere inside the sample chamber taking care that the excitation and emission ports are directed towards the correct lenses.
- Set the rotating fold mirror on the upper part of the sphere in the 'Powder' position.



Figure 8. Two position mirror dial set for solid samples measurements

The mirror now points to the sample on the bottom of the sphere. The top half of the sphere is shown in Figure 9:



Figure 9. View into the upper half of the sphere; setup for solid samples measurements

2.4. Preparing the Samples

Liquid Samples:

Liquid samples are measured in standard (10 mm x 10 mm) quartz cuvettes with PTFE (white) stopper. Use a stopper or cap to prevent spillage and to avoid evaporation. Ideally you will require 2 identical cuvettes, one containing the sample, the other containing the solvent only (blank). When preparing the cuvettes ensure that both sample and blank have the same filling height. 3 ml of sample volume is ideal. Do not use less than 2 ml.

You also need an absorption (UV-VIS) spectrometer or the absorption accessory of the FLS980 to set up absorbance (OD) values of your sample. Prepare a sample with an optical density of 0.1 at the excitation wavelength. Quantum yield measurements with different optical densities may also be carried out, e.g. to investigate the effect of the sample concentration on the quantum yield. However, these experiments should be left until you have gained sufficient experience.

Solid Samples:

Powders and bulk samples are measured on 10 mm diameter trays that are part of the integrating sphere assembly. Film samples can either be measured on the same trays, or they are positioned directly on top of the ring-like holder for the trays. When working with powders extra care must be taken to avoid spillage into the sphere. It is also important to fill the powder trays with the same amount of powders for consistency.

For powder samples, a reference sample could be either a supplied reference plug or BaSO₄ sample. The best reference for a thin film sample is a substrate of exactly the same size as the sample; if this is not available the reference plug should be used.

3. The Principle of Absolute Quantum Yield Measurements

The absolute fluorescence quantum yield, η , is, by definition, the ratio of the number of photons emitted to the number of photons absorbed:

$$\eta = \frac{N^{em}}{N^{abs}} \quad (1)$$

There are two different methods for the measurement of the absolute fluorescence quantum yield: “**Direct Excitation**” measurements and “**Direct & Indirect Excitation**” measurements.

With “Direct Excitation” measurements one records the scatter and the emission of the sample being directly excited by the radiation from the excitation monochromator only, whereas with “Direct and Indirect Excitation” one also records the emission of the sample while it is in a position where it is only indirectly excited by excitation radiation bouncing within the sphere.

The “Direct Excitation” method is the most common method applied to liquid samples because liquid samples are likely to be isotropic (uniform in all orientations) in their emissions and the quantum yields upon direct and indirect excitation are likely to be identical.

“Direct & Indirect Excitation” method is often used for powders, bulk and film samples that are more likely to be anisotropic in their emissions and the quantum yields of direct excitation and indirect excitation may well be different.

3.1. “Direct Excitation” Method

This method only requires two experimental setups, see figure 10.

Note that with the “Direct Excitation” method the emission measurement actually contains the information of both direct and indirect excitation, as photons that pass the sample in the direct excitation beam may still be absorbed after scattering in the sphere.



Figure 10. Two different measurement configurations required for Direct Excitation measurements:
 (A) reference sample (solvent only) in sample position (1);
 (B) test sample in position 1
 (position 2 remains empty for both measurements.)

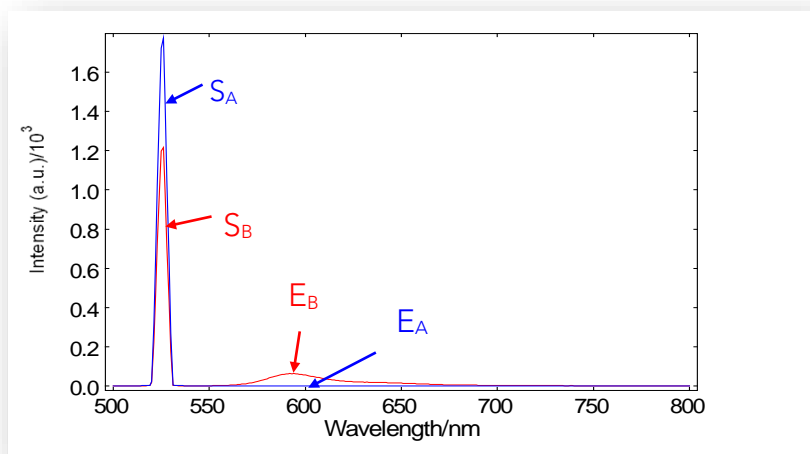


Figure 11. Spectral scans of the excitation scatter region or **S-region** (peaks on the left) and the emission region (**E-region**) of the sample and the solvent. The indices "A" and "B" refer to the experimental setup illustrated in Figure 10. Note that the quantities S_A , S_B , E_A , and E_B refer to the integral of the scans

The absolute fluorescence quantum yield, calculated with the "Direct Excitation" method is calculated as follows:

$$\eta_{DExc} = \frac{E_B - E_A}{S_A - S_B} \quad (2)$$

$E_A(\lambda)$ and $S_A(\lambda)$, as well as $E_B(\lambda)$ and $S_B(\lambda)$ may be measured in four individual scans. However, it is often convenient to measure these spectra in two scans only. For the calculation of the integrals, the selection of the integral regions, and the final calculation of η_{DExc} use the quantum yield wizard that is supplied with the F980 software.

If the sphere background, $E_A(\lambda)$, is sufficiently low the measurement of this region may be omitted to save measurement time. In this case the equation degrades to:

$$\eta_{DExc} = \frac{E_B}{S_A - S_B} \quad (3)$$

3.2. "Direct & Indirect Excitation" Method

This method includes a third setup in addition to the above two: measurements of the emission of the sample in a position where it is not directly excited by the excitation beam, instead the sample is only excited indirectly, i.e. by photons that are scattered at the sphere surface before entering the sample (Figure 12, C)

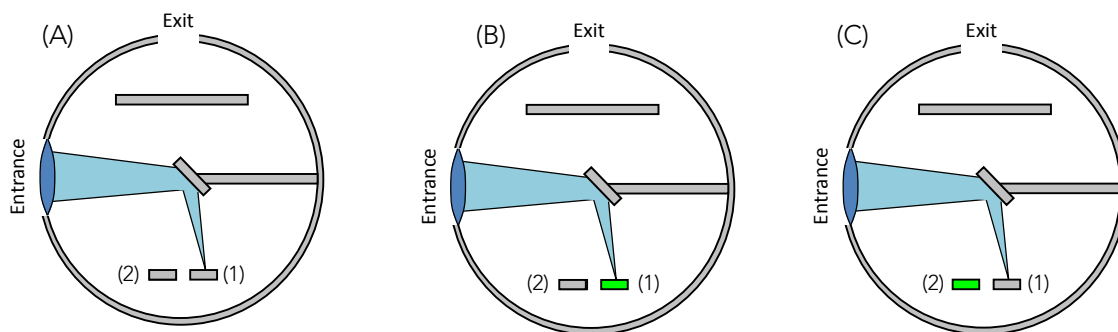


Figure 12. Three different measurement configurations required for Direct and Indirect Excitation measurements:

- (A) reference samples (blanking plug) in both sample positions 1 and 2;
- (B) test sample in position 1 reference sample (blanking plug) in position 2
- (C) reference sample (blanking plug) in position 1 and test sample in position 2

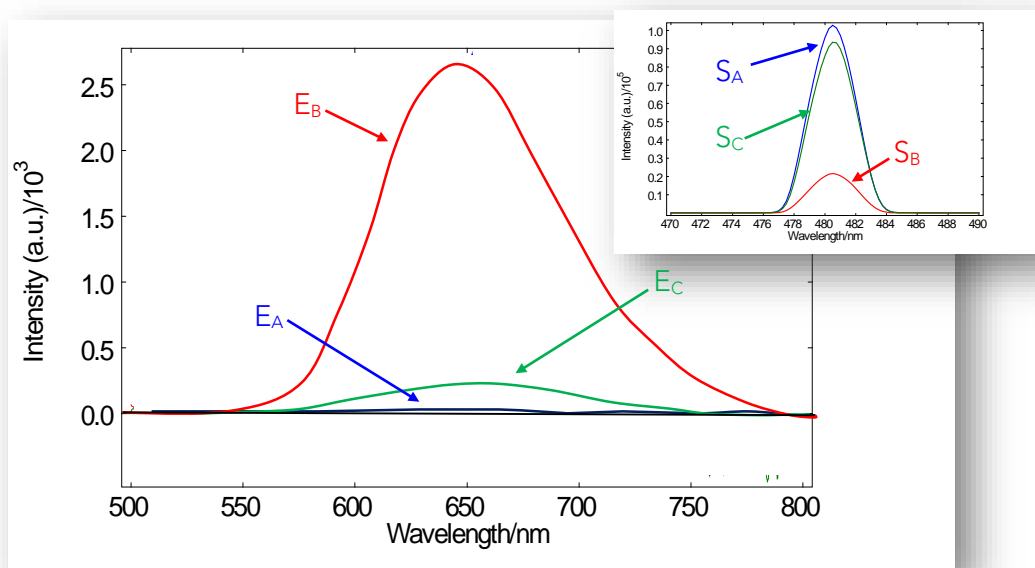


Figure 13. Spectral scans of the excitation region (peaks in the inset) and of the emission region of the sample and the reference plug. The indices "A", "B" and "C" refer to the experimental setup illustrated in Figure 12. S_A , S_B , S_C , E_A , E_B and E_C refer to the integrals of the scans.

The absolute fluorescence quantum yield, calculated with the "Direct and Indirect Measurement" method is calculated as follows:

$$\eta_{DIExc} = \frac{S_B(E_C - E_A) - S_C(E_B - E_A)}{(S_B - S_C)S_A} \quad (4)$$

$E_A(\lambda)$ and $S_A(\lambda)$, as well as $E_B(\lambda)$ and $S_B(\lambda)$ and $E_C(\lambda)$ and $S_C(\lambda)$ may be measured in six individual scans. However, it is often convenient to measure these spectra in three scans only. For the calculation of the integrals, the selection of the integral regions, and the final calculation of η_{DIExc} use the quantum yield wizard that is supplied with the F980 software.

If the sphere background, $E_A(\lambda)$, is sufficiently low the measurement of this region may be omitted to save measurement time. In this case the equation degrades to

$$\eta_{DIExc} = \frac{S_B E_C - S_C E_B}{(S_B - S_C)S_A} \quad (5)$$

4. Absolute Fluorescence Quantum Yield Measurements

It is essential that you have a basic understanding of the fluorescence properties of your samples. For determining the quantum yield you need to choose an appropriate excitation wavelength that is sufficiently low so that the scattered excitation light does not interfere with the emission spectrum. This is likely not to be the peak of the absorption.

The scattering of the excitation light (at λ_{exc}) of the reference sample will produce the highest signal, this is normally why the signal level is adjusted with a reference sample.

Procedure 1. "Direct Excitation" Method

Measurement of the reference

- 1-1 Insert the reference sample into the sample holder. Confirm that the mirror in the upper half of the sphere points to the sample and carefully replace the upper half of the sphere.
- 1-2 Open the *Signal Rate* screen. Set both excitation and emission wavelengths to the chosen λ_{exc} . Set initially a narrow bandwidth in the emission path $\Delta\lambda_{\text{em}} = 0.1$ nm.
- 1-3 Set the excitation bandwidth $\Delta\lambda_{\text{exc}} = 3$ nm – 10 nm. Observe the detector signal rate and ensure the signal is not excessively high. Using a wide spectral band pass in the excitation path is recommended for producing accurate QY results as the area under the scattering peak is used in the quantum yield calculation.
- 1-4 Leave $\Delta\lambda_{\text{exc}}$ fixed and use $\Delta\lambda_{\text{em}}$ to adjust the signal level to about 1×10^6 cps.
- 1-5 Close the signal rate screen and open the dialogue for an emission scan. Set scan limits from 20 nm below the excitation wavelength to the longest wavelength of the expected emission spectrum. Set step size depending on the width of the excitation slit: the narrower the slit is the smaller the step should be. For instance, use a wavelength step size of 1 nm for 10 nm excitation slit, but 0.1 nm for 3 nm slit. Set integration time between 0.2 s and 1 s. Use excitation correction for highest repeatability.
- 1-6 When the measurement is complete it is useful to rename the scan as "blank" (or "Ref").

Measurement of the sample

- 1-7 Replace the reference with the sample.
- 1-8 Repeat steps 1-5 of the procedure above using exactly the same measurement parameters.
- 1-9 Combine both sample and reference measurements in the same measurement window.

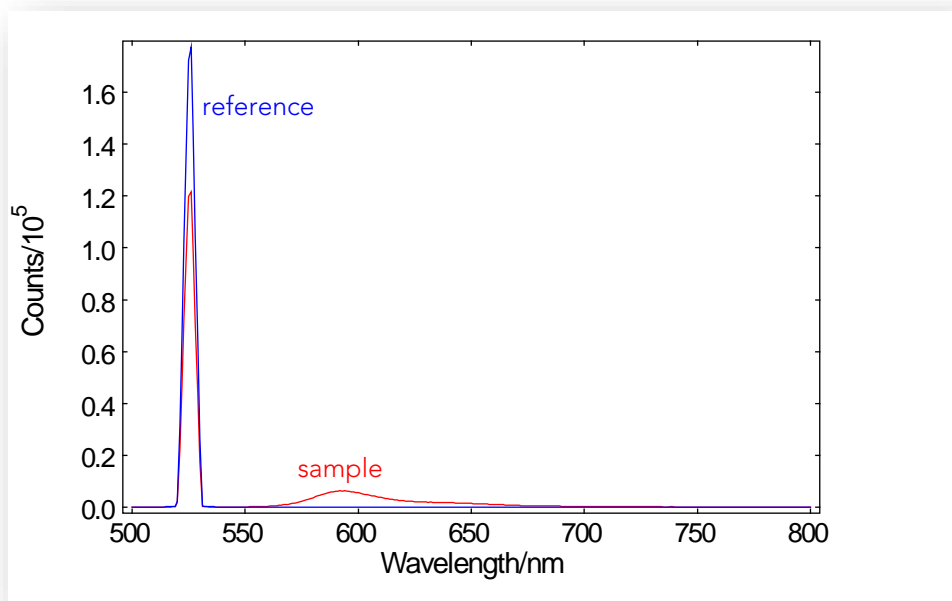


Figure 14: Blank spectrum and sample spectrum. Sample: Rhodamine 101 in ethanol.

Procedure 2. "Direct & Indirect Excitation" Method

Measurement of the reference

- 2-1 Insert the reference samples into both sample holders.
- 2-2 Proceed according to steps 1-5 of the procedure above.


Measurement of the sample under direct excitation

- 2-3 Replace the reference with the sample.
- 2-4 Repeat steps 1-5 of the procedure above using exactly the same measurement parameters.

Measurement of the sample under indirect excitation

- 2-5 Swap positions of the reference and the sample (Figure 12, C).

5. Absolute Fluorescence Quantum Yield Calculations

Combine the two scans into one graph () and use the Quantum Yield Wizard to calculate the quantum yield QY^{obs} (*Analysis >> Quantum Yield*). The quantum yield wizard will automatically show the scans in a semi logarithmic scale.

- Load the scans as shown in Figure 15. Note that if no *Ref. Emission* is loaded the calculation will be performed without background subtraction.

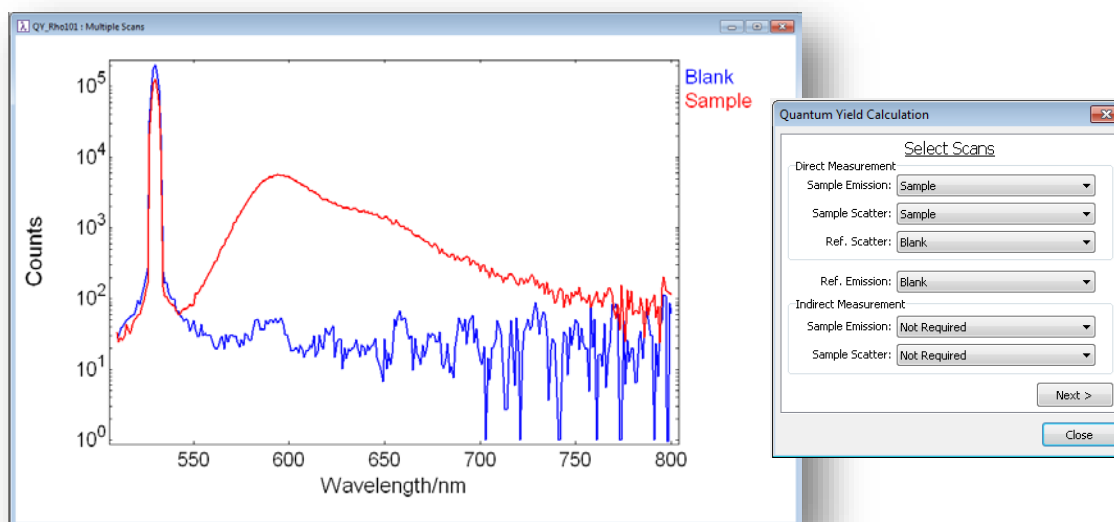


Figure 15. First step in QY calculation wizard

- Select the scattering region (500 nm - 540 nm in the rhodamine example) and the fluorescence region (540 nm - 800 nm) by dragging the mouse over the graph.

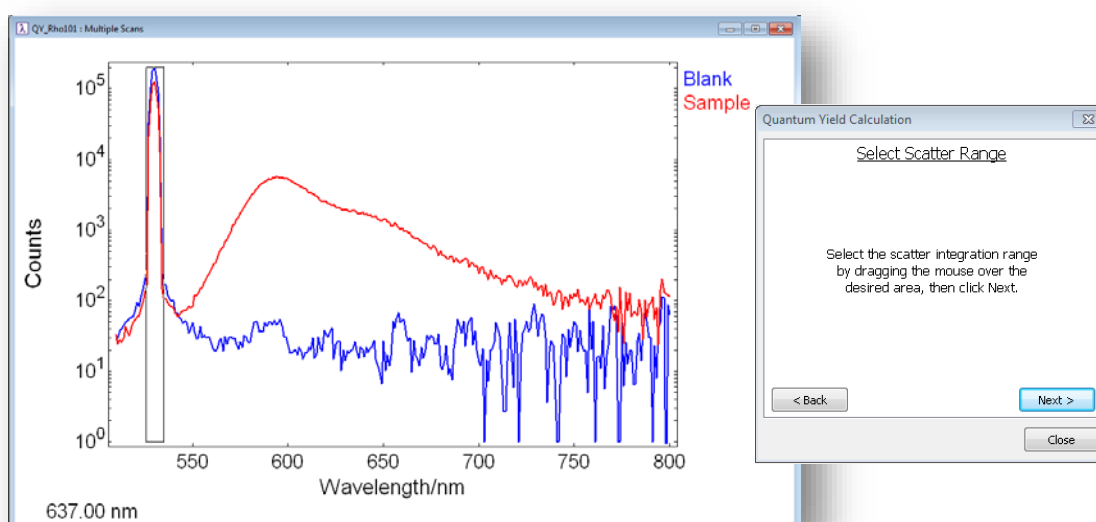


Figure 16. Second step in QY calculation wizard

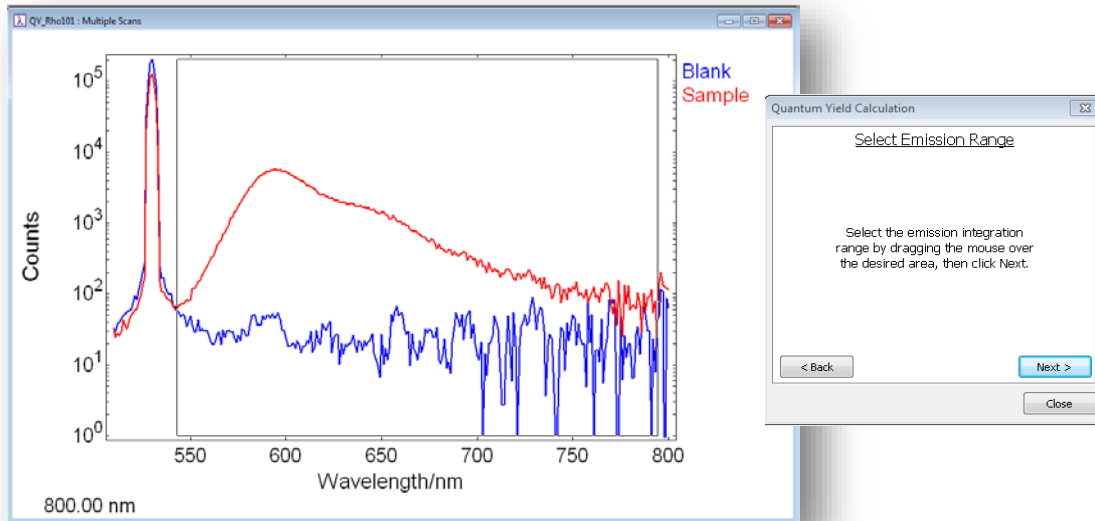


Figure 17. Third step in QY calculation wizard

- At the final step the wizard will present the calculated quantum yield

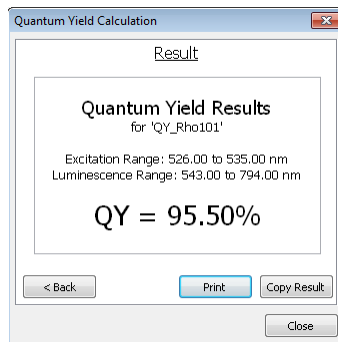


Figure 18. Results window in QY calculation wizard.

6. Corrections for True Absolute Fluorescence Quantum Yields

The procedures in section 4 describe how to measure the **observed** fluorescence quantum yield. However, the **true** fluorescence quantum yield of organic samples with a strong overlap of excitation and emission can be substantially higher than the observed quantum yield.

The difference between true and observed quantum yield is caused by re-absorption. When liquids are measured in an integrating sphere the reabsorption effects are much stronger than with normal measurements. This is because the effective path length of the beam through the liquid is much longer.

Procedure 3: To produce the true fluorescence quantum yield of organic samples

- 3-1 Prepare a very low concentration of your sample (optical density < 0.1 at the peak of the excitaton) and accurately measure its emission spectrum in the standard system configuration using the standard sample cuvette holder. This measurement gives you the “true” emission spectrum, F^{true} , that is free of re-absorption
- 3-2 Compare this emission spectrum with the emission spectrum measured in the integrating sphere (F^{obs}). Scale one against the other using the emission at the long wavelength end of the spectra to produce the “tail matched” spectra as shown in Figure 19. The difference in the two areas, a , is the fraction reduced by re-absorption. Integrate the two areas (**Data >> Integrate**) and calculate the fraction, a , of the re-absorbed area. To obtain an accurate value ‘ a ’, accurate tail matching is required.

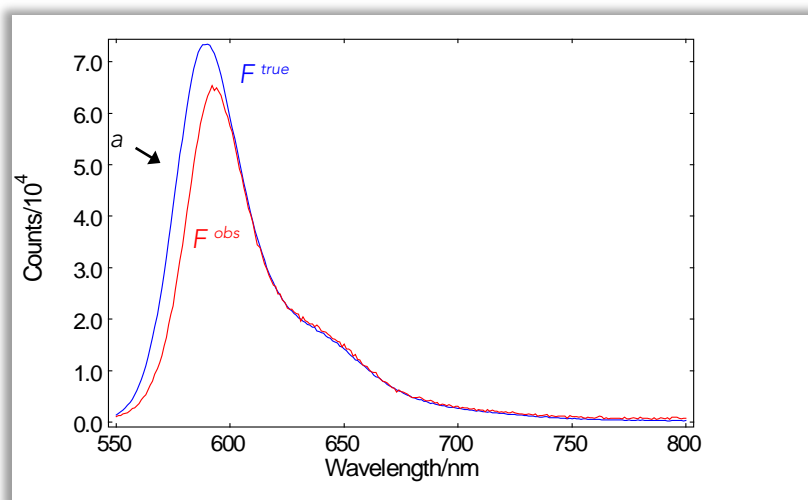


Figure 19. True emission spectrum of Rhodamine 101 in ethanol, excited at 525 nm, compared with the fluorescence emission spectrum measured in the sphere in step 3 (F^{obs}).

- 3-3 Use the following formula to calculate the true fluorescence quantum yield QY^{true} .

$$QY^{true} = \frac{QY^{obs}}{1 - a + a \cdot QY^{obs} / 100} \quad (6)$$

For the rhodamine measurement in the above graph $a = 0.118$, $QY^{obs} = 94.9\%$, and $QY^{true} = 95.5\%$.

Note also that for high quantum yield samples (i.e. $QY^{obs}/100 \sim 1$) the re-absorption correction of the observed quantum yield is small, even if re-absorption is significant (see the rhodamine example). However, for samples of lower quantum yields the re-absorption correction procedure can result in significant corrections.

7. Special Cases of Absolute Fluorescence Quantum Yield Measurements

7.1. Samples with Low Fluorescence Quantum Yield

Samples with low fluorescence quantum yield (below 5%) are best measured with the help of neutral density filters.

Low quantum yield samples require accurate measurements over an extreme dynamic range. To get sufficient luminescence signal in the **E-region** (refer to Figure 11) one is tempted to further open the monochromator slits. However, this would cause the scatter peaks in the **S-region** to enter the range of detector (counting) saturation. To avoid this problem one measures the **S-region** with appropriate neutral density filters.

Procedure 4. To do QY measurements using a neutral density filter

- 4-1 Adjust slit width and select a filter based on the criteria:
 - a. Signal at scattering peak for a reference sample is c.a. 10^6 cps when a neutral density filter is set on excitation
 - b. Signal at emission peak for a sample is higher than 10^4 cps
- 4-2 Measure a reference sample :
 - a. Put a filter into filter slot on the excitation arm and measure signal across scattering range
 - b. Remove the filter and measure signal across emission range. Note, measurement parameters such as excitation and emission slits and integration time should be the same as previously. Step size could be different because later on the QY wizard will take this difference into account.
- 4-3 Repeat (4-2) for a sample under study.
- 4-4 Scale scattering peaks according to transmittance value of the used neutral density filter. To increase accuracy it is recommended to measure true transmittance on your FLS980 system using Synchronous scan option.
- 4-5 Join all the measurements into one graph and calculate QY using Quantum Yield wizard of the F980 software.

7.2. Scatter and Emission Measured with Different Detectors

When a sample emits in NIR range upon excitation in the UV-VIS range it is likely that two different detectors must be used. Typically these detectors are standard PMT and NIR-PMT. In this case the difference in detectors sensitivity must be taken into account and the formula (2) can be written as:

$$\eta = \frac{E_B - E_A}{S_A - S_B} k \quad (7)$$

where ' k ' is the ratio of detectors sensitivities.

Procedure 5. To do QY measurements using a two detectors

- 5-1 Firstly, measure an emission spectrum of a sample placed in the integrating sphere with an NIR-PMT detector.

- 5-2** Next, measure the scattering peaks of the reference sample and the sample under study with a standard PMT detector. It should be expected that signal for scattering peak is too high to be measured directly, so a neutral density filter could be needed on the **excitation** light path.
- 5-3** To determine the ratio of sensitivity between two detectors the same signal should be measured with both detectors in the range of overlap of their sensitivities and the ratio between emission-corrected signals should be calculated. The simplest way is to measure a scattering peak for any wavelength within the range of 700 nm - 800 nm using two detectors. A ratio between these measurements will give *k*-value:

$$k_{\text{NIR-PMT/standard PMT}} = \text{Signal}_{\text{NIR-PMT}} / \text{Signal}_{\text{standard PMT}}$$

To increase accuracy *k*-value could be evaluated as averaged ratio between two synchronous scans over the above range (i.e. emission and excitation wavelengths change simultaneously with zero offset).

- 5-4** Scale scattering peaks by a neutral density filter factor and by $k_{\text{NIR-PMT/standard PMT}}$. Join all the measurements into one graph and calculate QY using Quantum Yield wizard of the F980 software.

8. Reflection Measurements

Reflection measurements are often converted into absorption measurements (see section 9 below).

Procedure 6: To measure the reflection spectrum of a sample

- 6-1 Use the Signal Rate dialogue box to assess the signal intensity, in particular in those regions where the xenon lamp produces strong peaks (468 nm). Use the same wavelength for excitation and emission, but be aware of signal saturation. **Avoid detector overload!** Use a large spectral band pass in the excitation (e.g. $\Delta\lambda_{\text{exc}}=3$ nm) and use the spectral band pass of emission to adjust for a suitable signal intensity, which must be below 2×10^6 cps.
- 6-2 Using identical conditions, do one Synchronous scan for the reference or blank sample, and another for the sample. Join both scans in one window.

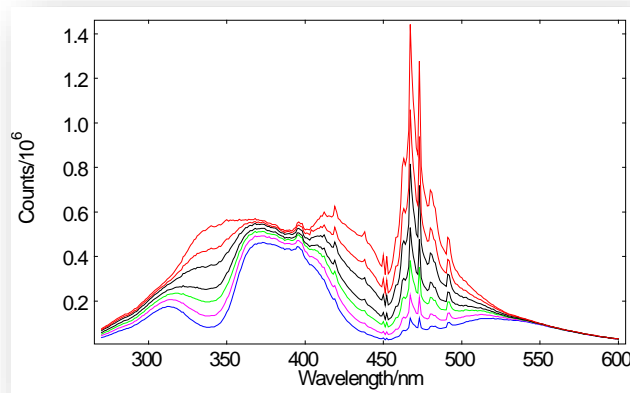


Figure 20. Synchronous scans of BaSO_4 (upper red curve) and a series of YAG:Ce powder, diluted with BaSO_4 to concentrations from 100% down to 20%.

- 6-3 Go to **Analysis >> Reflectance**, select the reference or blank measurement and click OK. This will produce a window with the reflectance measurements.

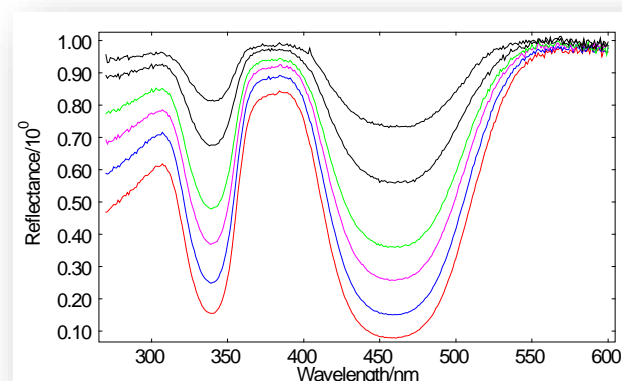


Figure 21. Reflectance of a series of YAG:Ce powder, diluted with BaSO_4 to concentrations from 100% down to 20%.

9. Absorption Measurements

Procedure 7: To measure the reflection spectrum of a sample

- 7-1 Repeat steps 6-1 and 6-2 described in section 8.
- 7-2 Go to *Analysis >> Absorption*, select the reference or blank measurement and click OK. This will produce a window with the absorbance measurements.

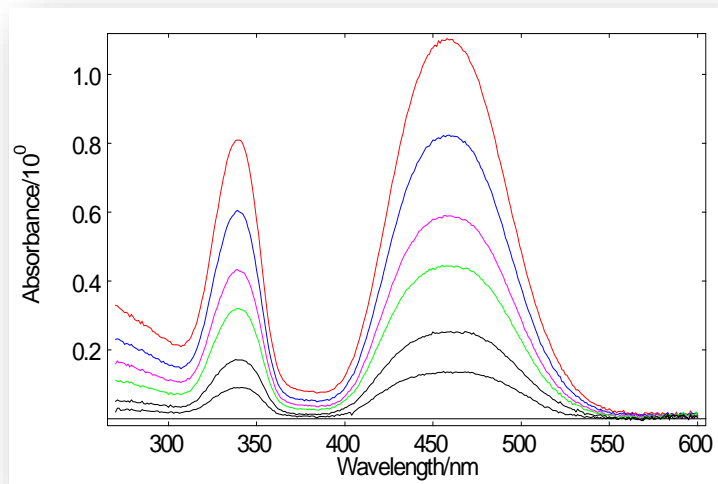


Figure 22. Absorbance of a series of YAG:Ce powder, diluted with BaSO₄ to concentrations from 100% down to 20%.

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