

Fluorescence Spectroscopy

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Absolute Fluorescence Quantum Yields Using the FL 6500 Fluorescence Spectrometer

Introduction

Fluorescence quantum yield (Φ_F) is a characteristic property of a fluorescent species and

is denoted as the ratio of the number of photons emitted through fluorescence, to the number of photons absorbed by the fluorophore (Equation 1). Φ_F ultimately relates to the efficiency of the pathways leading to emission of fluorescence (Figure 1), providing the probability of the excited state being deactivated by fluorescence rather than by other competing relaxation processes. The magnitude of Φ_F is directly related to the intensity of the observed fluorescence.¹

$$\Phi_F = \frac{\text{Number of photons emitted through fluorescence}}{\text{Number of photons absorbed}} \quad (1)$$

Fluorescence quantum yield can be measured using two methods: the relative method and the absolute method. In comparison with relative quantum yield measurements, the absolute method is applicable to both solid and solution phase measurements. The Φ_F can be determined in a single measurement without the need for a reference standard. It is especially useful for samples which absorb and emit in wavelength regions for which there are no reliable quantum yield standards available.²

The importance of Φ_F has been shown in several industries, including the research, development and evaluation of audio visual equipment, electroluminescent materials (OLED/LED) and fluorescent probes for biological assays.³ This application demonstrates the use of the PerkinElmer FL 6500 Fluorescence Spectrometer with integrating sphere accessory and Spectrum FL software for the rapid determination of absolute fluorescence quantum yield. This software provides an inbuilt method for measuring the Φ_F of samples with the option of relative or absolute measurement. The absolute quantum yield methods provide the user with the choice of; Simplification or de Mello approach. In the case of this application, the Simplification method was used with rhodamine 6G and fluorescein, both of which have well documented quantum yields.

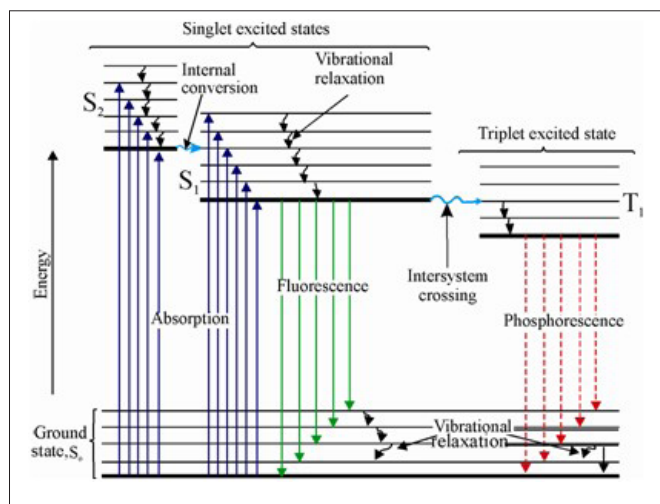


Figure 1. Jablonski diagram illustrating the main primary deactivation processes following excitation from the ground electronic singlet state (S_0) to excited electronic singlet states (S_1 and S_2).⁴

Relative and Absolute Fluorescence Quantum Yields

Two methods are available for determining Φ_F using fluorescence spectroscopy; the relative method and the absolute method.

The relative Φ_F method relies on the use of fluorescence standards, with optical properties closely matching the sample of interest, and uses a conventional fluorescence spectrophotometer which only detects a fraction of the light emitted. This fraction depends on a wide range of factors including the refractive index of the solvent, scattering of the sample, and the emission wavelength. The method compares the fluorescence intensity of a sample of known Φ_F , generally referred to as the reference, against a sample of unknown Φ_F to calculate the Φ_F value of the unknown. This approach requires knowledge of the refractive index of the solvent, absorbance of both reference and sample, and therefore requires further measurements and as such it is only applicable to samples which can go into solution.⁵

The absolute method, on the other hand, is applicable to both solid and solution phase measurements and the Φ_F can be determined in a single measurement without the need for a

standard. It is especially useful for samples which absorb and emit in wavelength regions for which there are no reliable quantum yield standards available. This method requires an integrating sphere which allows the instrument to collect all of the photons emitted from the sample. By simply comparing the total number of emitted photons with the total number of absorbed photons, Spectrum FL is able to automatically calculate the absolute fluorescence quantum yield of the sample. The number of absorbed photons is determined from the decrease in the incident excitation light intensity following sample absorption, in comparison with the blank.^{5,6}

As the sample is irradiated in the sphere by the excitation source, some of the light is absorbed. Any light which is unabsorbed or scattered is diffusely reflected by the surface of the sphere, which can cause secondary excitation of the sample. The sphere has a baffle located in front of the exit aperture to ensure only diffused light (both fluorescence emission and exciting light) reaches the detector. Therefore, as opposed to the relative method, the absolute method requires no correction for solvent refractive index.⁷ The use of the integrating sphere is especially critical when measuring the Φ_F of solid/thin film samples which display some angular dependence of the emission and excitation.²

Simplification vs. de Mello Absolute Quantum Yield Methods

Spectrum FL software allows absolute quantum yield to be determined by using either the 'Simplification' method or the 'de Mello' method, with the choice of method being dependent on the user requirements. The Simplification method allows for higher sample throughput whilst de Mello allows greater accuracy and a more robust method.

When using the Simplification method, the analysis relies on measuring the scatter and emission of the sample only when it is directly excited by the radiation from the excitation monochromator. The sample is thus directly facing the excitation beam. This direct method is commonly applied to liquid samples because they are likely to exhibit isotropy in their fluorescence emission (equal intensities in different directions), resulting in identical quantum yields when measured using direct and indirect excitation.⁵ The Quantum Yield for the Simplification method is calculated using Equation 2 and uses 'Empty/solvent blank' and 'Direct' spectra, much like the spectra shown in Figure 2 for fluorescein.

$$\text{Quantum Yield} = \frac{E_c}{(L_a - L_c)} \quad (2)$$

Where:

- E_c = Total number of photons emitted in the emission wavelength region
- L_a = Total number of incident photons
- L_c = Total number of photons not absorbed by the fluorescent species under direct excitation

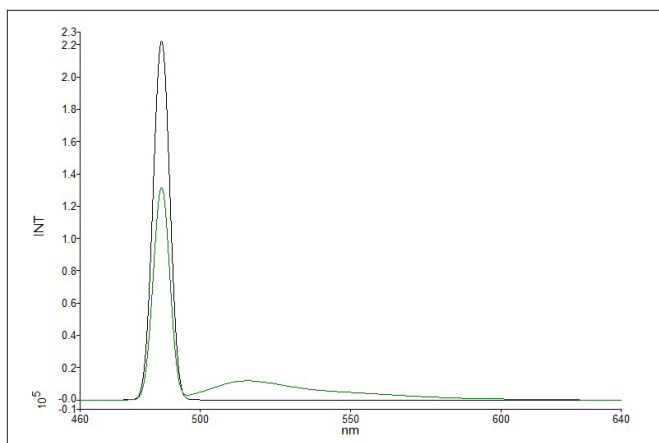


Figure 2. Simplification method for absolute quantum yield determination of fluorescein. Empty measurement using solvent as the blank (black) and direct measurement of fluorescein (green).

The de Mello method, on the other hand, measures the sample using both direct and indirect excitation. This method corrects the direct quantum yield calculation because the sample will be subject to secondary excitation within the integrating sphere. The indirect excitation is measured by placing the sample in a position where it is only indirectly excited by reflected sample fluorescence within the sphere. Powders and film samples are often analysed using the de Mello method as they are more likely to exhibit fluorescence anisotropy, resulting in different quantum yields when measured using direct and indirect excitation.^{5,6} The Quantum Yield for the de Mello method is calculated using Equation 3 and uses 'Empty/solvent blank', 'Indirect' and 'Direct' spectra, much like the spectra shown in Figure 3 for fluorescein.⁷

$$\text{Quantum Yield} = \frac{E_c[(1-A) \cdot E_b]}{L_a \cdot A} \quad (3)$$

$$A = 1 - \left(\frac{L_c}{L_b} \right)$$

Where:

- E_b = Total number of photons emitted by reflected light
- L_b = Total number of photons after absorption of reflected light
- A = Sample absorbance in integrating sphere

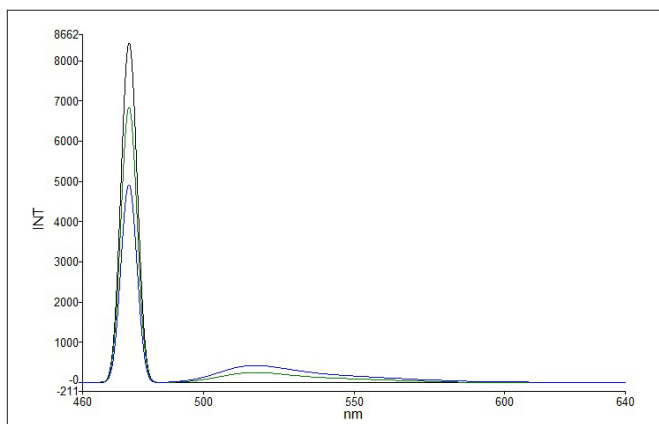


Figure 3. De Mello method for absolute quantum yield determination of fluorescein. Empty measurement using solvent as the blank (black), indirect measurement of fluorescein (green), and direct measurement of fluorescein (blue).

Method

Quantum yield measurements require the absorbance (optical density) of the solution at the excitation wavelength to be ≤ 0.1 , thus appropriate sample concentrations must be prepared. Fluorescence grade rhodamine 6G, fluorescence grade fluorescein, 200-proof anhydrous ethanol ($\geq 99.5\%$), and anhydrous sodium hydroxide pellets ($\geq 98\%$) were obtained from Merck (previously Sigma-Aldrich). Fluorescein (0.5 mg/L) was prepared in 0.1 N sodium hydroxide and rhodamine 6G (0.5 mg/L) was prepared in ethanol. The absorbance of each solution was determined using a PerkinElmer LAMBDA 265 UV/Vis Spectrophotometer. In the absence of a separate UV-Vis instrument, the PerkinElmer FL 6500 or FL 8500 Fluorescence Spectrometers absorbance module accessory can be used.

All samples were measured using the PerkinElmer FL 6500 Fluorescence Spectrometer (Figure 4) with the integrating sphere accessory (Figure 5, P/N: N4201017). The PerkinElmer FL 8500 Fluorescence Spectrometer may also be used. Quartz fluorescence cuvettes (10 x 10 mm) were used with a white PTFE stopper to prevent evaporation and contamination in the sphere. Absolute quantum yield measurements require emission spectra to be collected using a solvent blank, in order to correct for any emission arising from the solvent, and a cuvette containing sample. It is necessary that both the blank and sample cuvettes are filled to similar heights (at least 3 ml). Spectrum® FL software was used with the instrument settings specified in Table 1.



Figure 4. PerkinElmer FL 6500 Fluorescence Spectrometer.

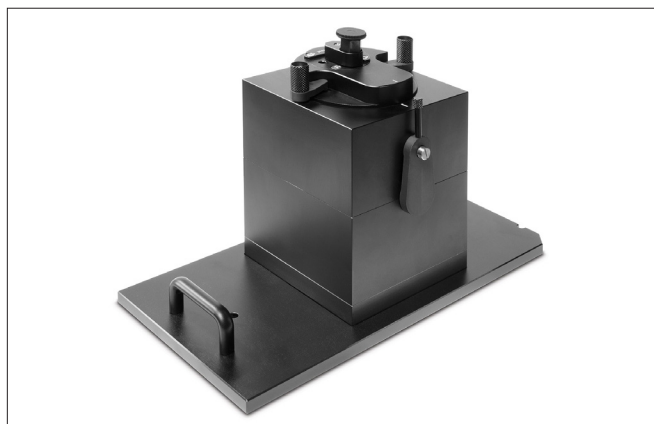


Figure 5. PerkinElmer Integrating Sphere Accessory (P/N: N4201017).

Table 1. PerkinElmer FL 6500 scan parameters for the determination of rhodamine 6G and fluorescein absolute quantum yields using the simplification method.

		Fluorophore	
		Rhodamine 6G	Fluorescein
Source	Excitation correction	On	On
	Initial dark	On	On
	Power (kW)	80	80
	Frequency (Hz)	100	100
Excitation	Excitation wavelength (nm)	530	488
	Slit width (nm)	5	5
Emission	Wavelength range (nm)	460 – 700	460 – 650
	Slit width (nm)	5	5
	Scan speed (nm/min)	240	240
Acquisition	Voltage (V)	300	300
	Response width (nm)	0.5	1
	Emission correction	On	On
	Gain	Auto	Auto

Results

Fluorescence quantum yields of fluorescein in 0.1 N NaOH and rhodamine 6G in ethanol have been well-studied. The work of Magde *et al.* demonstrated quantum yields of 0.925 ± 0.015 and 0.950 ± 0.015 for fluorescein and rhodamine 6G respectively, using the same solvents.^{8,9} The fluorescence spectra of these two materials are shown in Figure 6. Spectrum FL software calculates the variables required for the method selected, and then calculates the quantum yield, allowing rapid analysis and high sample throughput. Peak calculation points (peak start, peak end and peak bases) can be manually refined. Table 2 shows the calculated variables and quantum yield obtained for each sample. The absolute quantum yields for fluorescein and rhodamine 6G using the Simplification method were 0.924 and 0.954 respectively, which were in agreement with the published literature values.^{8,9}

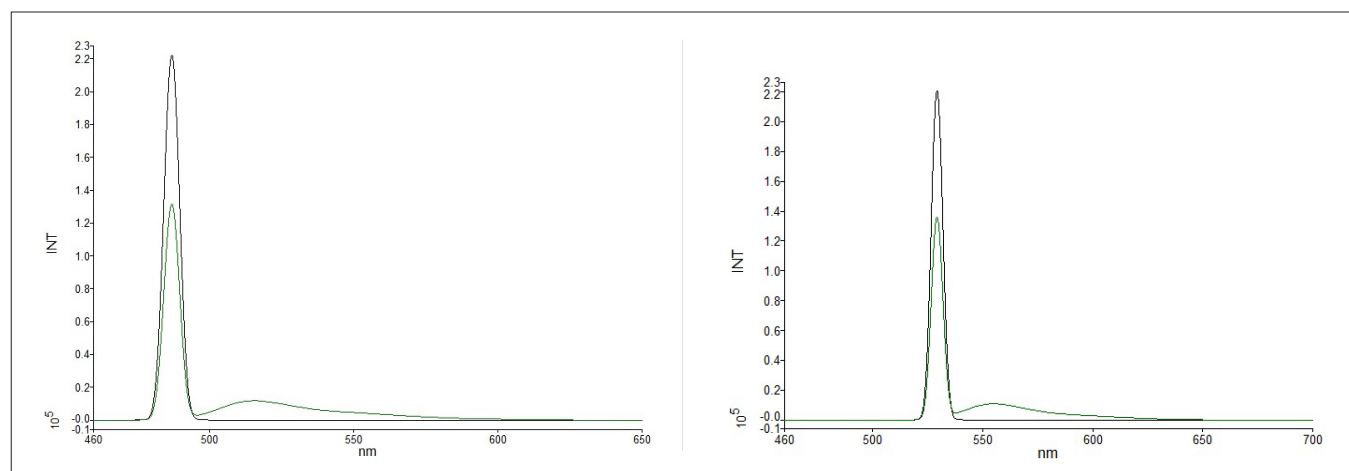


Figure 6. Fluorescence emission spectra of fluorescein (left) and rhodamine 6G (right) where black spectra were obtained using the 'empty/solvent blank' configuration, containing only solvent, and the green spectra were obtained using the 'direct' sampling configuration.

Table 2. Calculated variables and quantum yields for fluorescein and rhodamine 6G using Spectrum FL software.

	L_a	L_c	E_c	Φ_F
Fluorescein	1471866.75	880128.64	546843.91	0.9241
Rhodamine 6G	1424030.47	897322.87	502346.55	0.9537

Conclusion

This application demonstrated the use of the PerkinElmer FL 6500 with integrating sphere accessory for the determination of absolute fluorescence quantum yield, using fluorescein in 0.1 N NaOH and rhodamine 6G in ethanol as examples. The quantum yields of fluorescein and rhodamine 6G were determined to be 0.92 and 0.95 respectively, demonstrating good agreement with the literature values. The ease of use of the FL 6500 and Spectrum FL software makes it highly suitable for determination of absolute quantum yield in academia and research laboratories. It is especially useful for samples which absorb and emit in wavelength regions for which there are no reliable quantum yield standards available. Spectrum FL software provides a step-by-step approach which makes the absolute quantum yield easily determinable by using either the 'Simplification' method or the 'de Mello' method, with the choice of method being dependent on the user requirements.

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Appendix – Example Screen Captures of Quantum Yield Method for Fluorescein

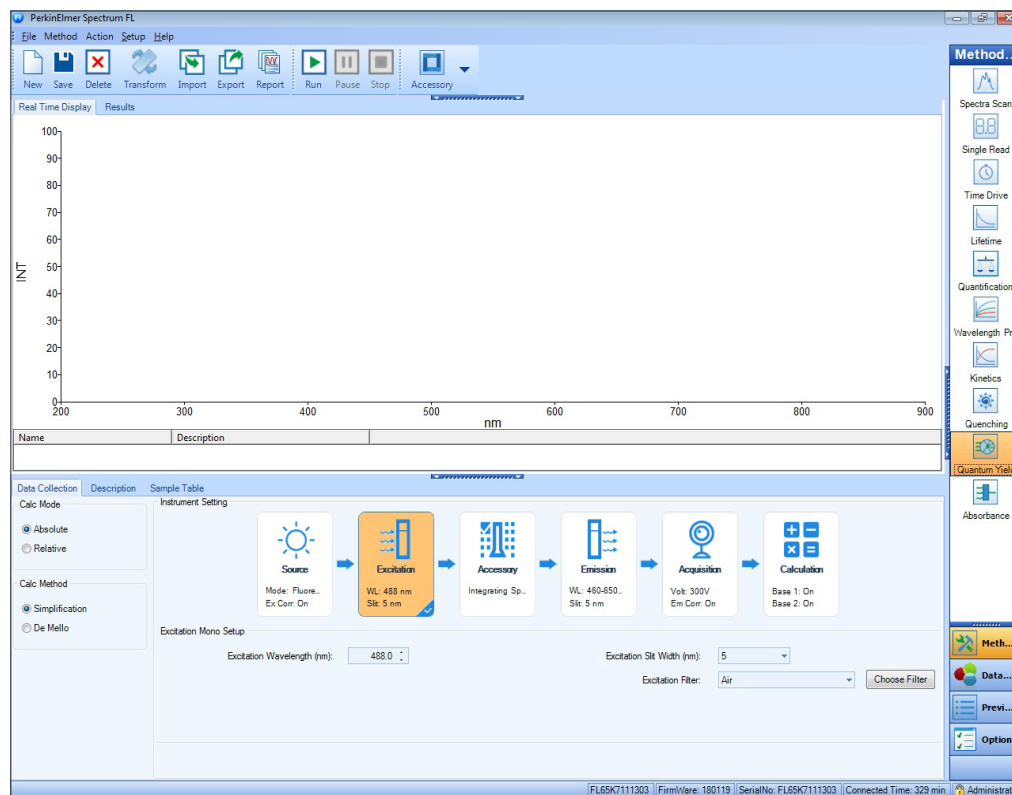


Figure 7. Spectrum FL quantum yield method setup module.

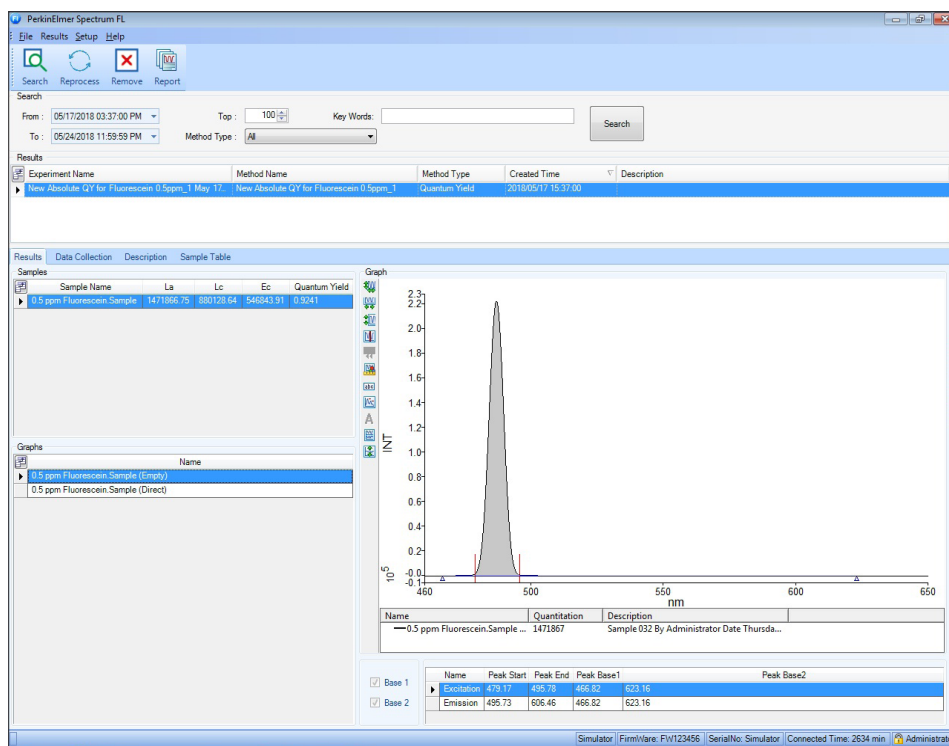


Figure 8. Spectrum FL software demonstrating the fluorescein absolute quantum yield calculation parameters for an 'empty/solvent blank' spectrum.

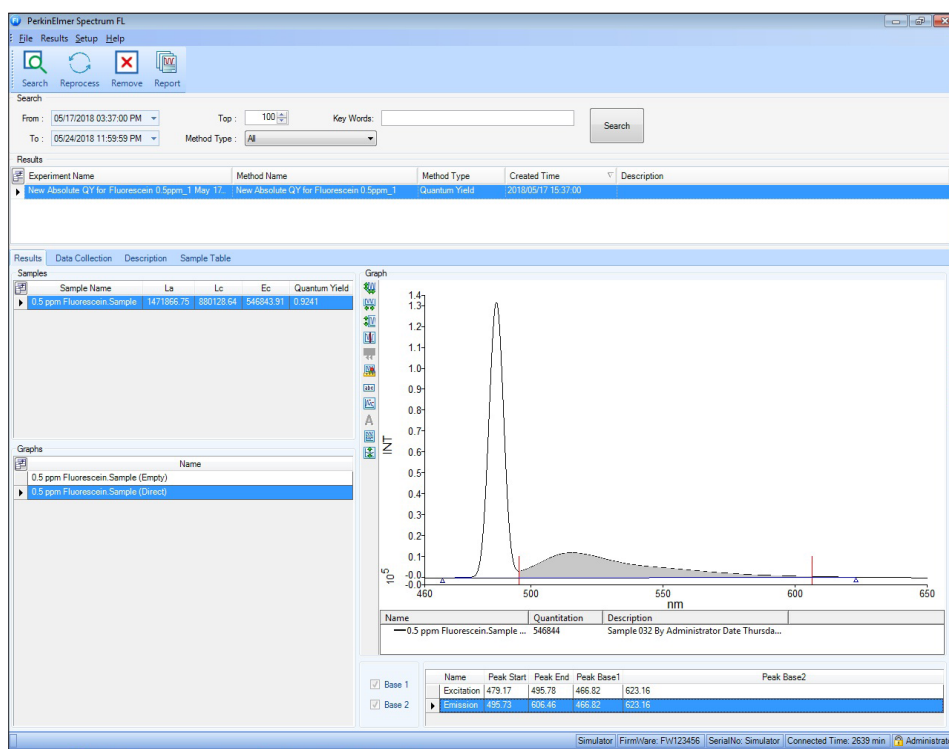


Figure 9. Spectrum FL software demonstrating the fluorescein absolute quantum yield calculation parameters for a 'direct' spectrum.

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