250-FP-0036 August 2026

Simple Evaluation of Quantum Yield Using the Relative Method

Introduction

Quantum yield is one of the key parameters used to evaluate the photophysical properties of luminescent materials. It is defined as the ratio of the number of photons emitted as fluorescence or phosphorescence to the number of excitation photons absorbed by the sample. Two methods are commonly used to determine quantum yield: the relative method and the absolute method. The absolute method requires an integrating sphere to collect all photons emitted from the sample. In contrast, the relative method enables quantum yield to be determined more easily by comparing the fluorescence intensity of an unknown sample with that of a reference standard of known quantum yield.
In this application note, the fluorescence quantum yields of perylene and rhodamine 6G were measured by the relative method using anthracene as the reference standard.

Overview of Quantum Yield Calculation Using the Relative Method

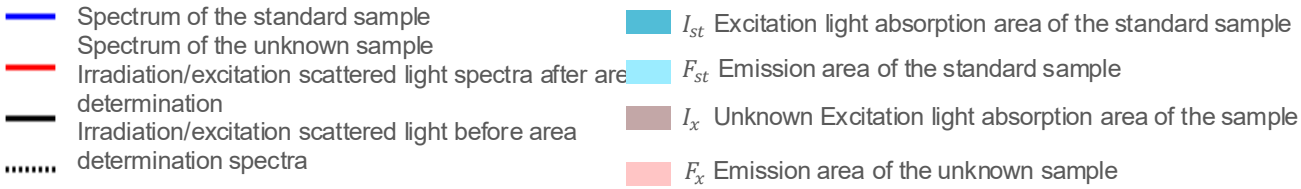
To determine the relative quantum yield, the following parameters are required:
1. The quantum yield of the reference standard.
2. The absorbance at the excitation wavelength for both the reference standard and the unknown sample.
3. The integrated area of the spectrally corrected emission spectrum for both the reference standard and the unknown sample.

In addition, the following parameters may be required depending on the measurement conditions:
i. The average refractive index of the solvent over the wavelength range used for emission spectrum integration, when the solvents used for the reference standard and the unknown sample are different.
ii. The dilution factor, when the sample used for emission spectrum measurement has been diluted relative to the sample used for absorbance measurement.

Table 1. *Parameters Required for Relative Quantum Yield Calculation*

	Subject	Sample	Term
1	Quantum yield	Standard	φ_{st}
2	The absorbance at the excitation wavelength	Standard Unknown	A_{st} A_x
3	Emission area	Standard Unknown	F_{st} F_x
4	Average refractive index of the solvent	Standard Unknown	n_{st} n_x
5	Dilution ratio	Standard Unknown	D_{st} D_x

The following outlines the calculation procedure for determining the quantum yield of an unknown sample using the standard sample as a reference.



Step 1. Calculate the excitation light absorption area of the standard sample

Divide the fluorescence spectral area of the standard sample F_{st} by the known quantum yield φ_{st} to calculate the excitation light absorption area I_{st} .

$$I_{st} = F_{st}/\varphi_{st}$$

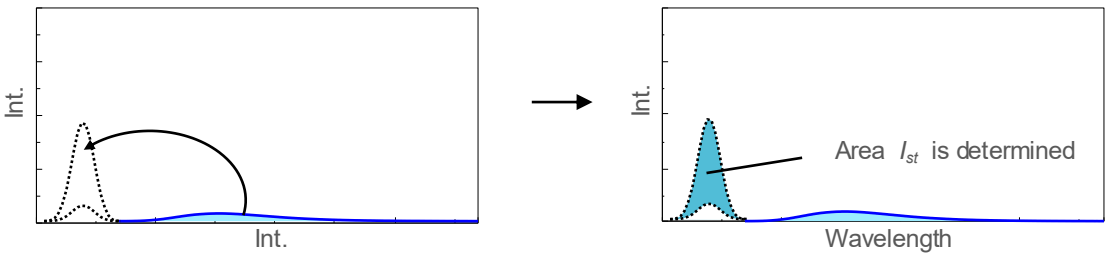


Fig. 1-1 *Flowchart for Calculating Quantum Yield Using the Relative Method (Step 1)*

Step 2. Calculate the area of the incident light spectrum

Divide the excitation light absorption area of the standard sample I_{st} by the absorption coefficient derived from the absorbance A_{st} to determine the area of the incident light spectrum I. Since fluorescence measurements of solutions are generally performed under conditions where absorbance is low, the [Relative Quantum Yield Calculation] program assumes that the absorption coefficient is linearly approximated based on the absorbance.

$$I = I_{st}/(1 - 10^{-A_{st}}) \approx I_{st}/(2.303A_{st})$$

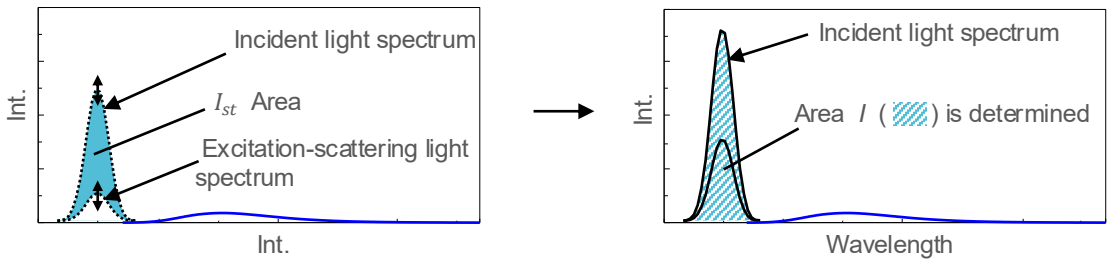


Fig. 1-2 *Flowchart for Calculating Quantum Yield Using the Relative Method (Step 2)*

Step 3. Calculate the excitation light absorption area of the unknown sample

Multiply the area of the incident light spectrum determined in Step 2, I, by the absorption coefficient, A_x, to calculate the excitation light absorption area of the unknown sample, I_x.

$$I_x = I \times (1 - 10^{-A_x}) \approx I \times 2.303 A_x$$

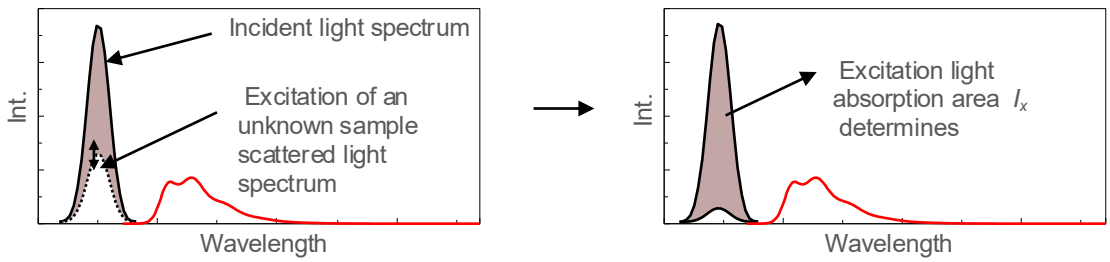


Fig. 1-3 Flowchart for Calculating Quantum Yield Using the Relative Method (Step 3)

Step 4. Calculate the quantum yield of an unknown sample

Divide the fluorescence area of the unknown sample, F_x, by the excitation light absorption area determined in Step 3, I_x, to calculate the quantum yield, φ_x. Furthermore, the procedures in Steps 1 through 3 can be expressed mathematically as follows.

$$\varphi_x = F_x / I_x = \varphi_{st} \cdot \left(\frac{F_x}{F_{st}}\right) \cdot \left(\frac{A_{st}}{A_x}\right)$$

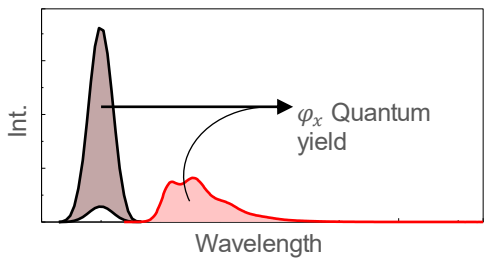


Fig. 1-4 Flowchart for Calculating Quantum Yield Using the Relative Method (Step 4)

If the unknown sample and the standard sample use different solvents, or if the sample used for the absorbance measurement was diluted before the fluorescence spectrum measurement, the equations are as follows.

$$\varphi_x = F_x / I_x = \varphi_{st} \cdot \left(\frac{F_x}{F_{st}}\right) \cdot \left(\frac{A_{st}}{A_x}\right) \cdot \left(\frac{n_x}{n_{st}}\right)^2 \cdot \left(\frac{D_x}{D_{st}}\right)$$

Keywords
Quantum yield, relative quantum yield, spectrofluorometer

Experimental
The literature value[1)] of anthracene was used as a reference to calculate the quantum yields of other fluorescent substances.

Sample

Anthracene solution (standard sample, solvent: cyclohexane)
Perylene solution (solvent: cyclohexane)
Rhodamine 6G solution (solvent: ethanol)

System

Fluorescence Spectroscopy

Equipment:	FP-8550 Spectrofluorometer
Accessories:	ESC-142 Light Source for Spectral Correction (WI)

Absorbance Measurement

Instrument: V-750 UV-Vis Spectrophotometer
*Measurements can also be performed using the FUV-803 absorbance measurement cell block (accessory for the FP-8050 series).

Parameters

Fluorescence Spectroscopy

Excitation Bandwidth:	5 nm	Emission Bandwidth:	5 nm
Sensitivity:	240 V	Excitation Wavelength:	see Table 2
Response:	0.5 sec	Scan Speed:	500 nm/min

Absorbance Measurement

Bandwidth:	2 nm	Response:	0.24 sec
Scan Speed:	1000 nm/min		

Use a U-330 filter for UV-excited samples

Results and Discussion
The measured fluorescence spectra and calculated relative quantum yields are shown below.

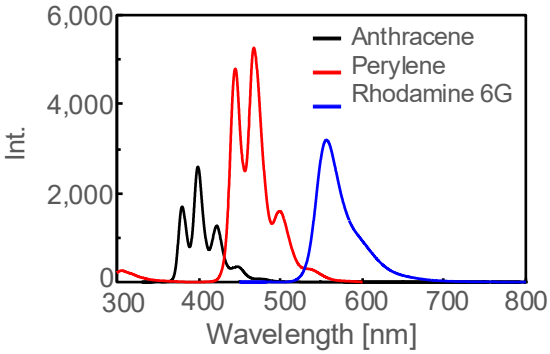


Fig. 2 *Measured Spectra*

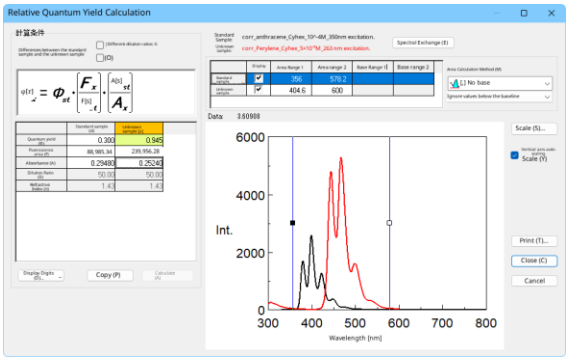


Fig. 3 *[Relative Quantum Yield Calculation] Program Screen*

Table 2. *Quantum Yield Calculation Results Using the Relative Method*

Compound	Solvent Refractive Index	Excitation Wavelength [nm]	Absorbance	Quantum yield	
				Calculation	References
Anthracene	1.43	350	0.2948	-※	0.3[1)]
Perylene	1.43	263	0.2524	0.945	0.94[2)]
Rhodamine 6G	1.36	488	0.1524	0.941	0.94[2)]

*In the relative method, the excitation light absorption area is calculated in reverse from the fluorescence area and quantum yield of the reference substance (in this case, anthracene); therefore, the quantum yield cannot be calculated.

The quantum yields of perylene and rhodamine 6G were calculated using a relative method based on the measurement results for anthracene. Although these three compounds have different excitation wavelengths, appropriate spectral correction allowed us to obtain quantum yields that agree well with literature values, as shown in Table 2.

Conclusion

In the calculation of quantum yields using the relative method, results equivalent to those in the literature were obtained even for samples with different excitation wavelengths or solvents. By selecting appropriate standard samples and setting appropriate measurement conditions, quantum yields can be evaluated with high reliability even using the relative method.
Nippon Spectroscopy's spectrofluorometers come standard with a dedicated program (Fig. 3) that assists in calculating relative quantum yields; by entering the sample's absorbance data, the quantum yield can be easily calculated.

References

1. M. Montalti, A. Credi, L. Prodi, M. T. Gandolfi: *Handbook of Photochemistry,* 3rd ed., CRC Press, Taylor and Francis Group, 2006. DOI:10.1201/9781420015195.
2. A. M. Brouwer: *Pure Appl. Chem.* **83** , 2213 (2011). DOI: 10.1351/PAC-REP-10-09-31.

Note
If the quantum yield varies depending on the reference, the quantum yield calculated using the relative method will vary in proportion to the value in the reference.