

Measurement of Fluorescence Quantum Yields on ISS Instrumentation Using Vinci

Yevgen Povrozin and Ewald Terpetschnig
ISS Inc.

Introduction

The luminescence quantum yield is given as the ratio of the number of photons emitted to the number of photons absorbed by the sample:

$$Q = \frac{\text{photons}_{em}}{\text{photons}_{abs}}$$

The quantum yield Q can also be described by the relative rates of the radiative and non-radiative pathways, which deactivate the excited state:

$$Q = \frac{k_r}{k_r + \sum k_{nr}}$$

where k_r and k_{nr} correspond to radiative and non-radiative processes, respectively.

In this equation, $\sum k_{nr}$ describes the sum of the rate constants for the various processes that compete with the emission process. These processes include photochemical, and dissociative processes including other, less well characterized changes that result in a return to the ground state with simultaneous dissipation of the energy of the excited state into heat. These latter processes are collectively called non-radiative transitions and two types have been clearly recognized: intersystem crossing and internal conversion. Intersystem crossing related to the radiationless spin inversion of a singlet state (S_1) in the excited state into a triplet state (T_1).

While measurements of the “absolute” quantum yield do require more sophisticated instrumentation [1], it is easier to determine the “relative” quantum yield of a fluorophore by comparing it to a standard with a known quantum yield. Some of the most common standards are listed in table below:

Quantum Yield [Q.Y.] Standards	Q.Y. [%]	Conditions for Q.Y. Measurements	Excitation [nm]	Ref.
Cy3	4	PBS	540	2
Cy5	27	PBS	620	2
Cresyl Violet	53	Methanol	580	3

Quantum Yield [Q.Y.] Standards	Q.Y. [%]	Conditions for Q.Y. Measurements	Excitation [nm]	Ref.
Fluorescein	95	0.1 M NaOH, 22°C	496	3
POPOP	97	Cyclohexane	300	3
Quinine sulfate	58	0.1 M H ₂ SO ₄ , 22°C	350	3
Rhodamine 101	100	Ethanol	450	4
Rhodamine 6G	95	Water	488	4
Rhodamine B	31	Water	514	4
Tryptophan	13	Water, 20°C	280	3
L-Tyrosine	14	Water	275	3

Table 1. List of common standards for fluorescence quantum yield measurements.

The quantum yields of these compounds are mostly independent of the excitation wavelength, allowing these standards to be used wherever they display useful absorption. This is what Vavilov discovered already in 1927 [5]. Exceptions to Vavilov's Law can occur [6].

Determination of the relative quantum yield is generally accomplished by a comparison of the wavelength-integrated intensity of the unknown sample with the standard. There are two approaches for relative quantum yield measurements: The "one point" method, where the quantum yield is calculated, using only one emission value for the sample and one for the standard is fast, but not always reliable.

The second approach is the comparative method described by Williams et al. [7]. It is more time consuming yet providing higher accuracy by comparing the integrated fluorescence intensity and the absorption for sample and reference.

Instrumentation

The fluorescence intensity measurements were performed on PC1, the photon-counting fluorescence spectrometer from ISS.

The PC1 is a sensitive, compact, computer-controlled photon-counting spectrofluorometer designed for applications in physical chemistry, biochemistry, physiology, neurochemistry,

molecular biology, environmental analysis, and immunoassay research. Vinci, a comprehensive and flexible fluorescence spectroscopy analysis software package, enables instrument control and data acquisition directly from the PC computer.

The PC1 can be fully upgraded to the K2™ Multifrequency Phase Fluorometer for fluorescence and phosphorescence lifetime measurements with picosecond resolution. A large variety of light sources and accessories are available for a wide range of applications.



PC1™ Photon Counting Spectrofluorometer

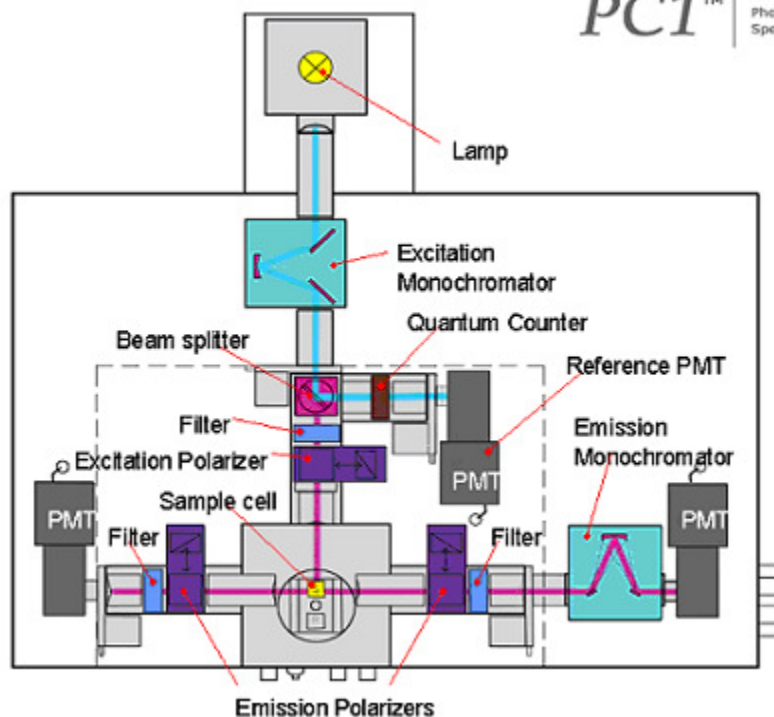


Figure 1. Schematic drawing of PC1, the photon-counting spectrofluorometer from ISS.

Experimental Conditions

Quantum yield standards should be chosen to ensure maximum overlap of the absorption and emission between sample and reference.

Instruments Setup. Once the spectrofluorometer parameters are adjusted and set, they should be kept unchanged during the entire measurement.

Cuvettes. In order to minimize the error for measured absorption values, it is advisable to use cuvettes with optical path lengths of 20 mm - 50 mm. However, 10 mm cuvettes are acceptable.

Optical density. To avoid inner filter effects, the optical density must be below 0.1 at the excitation wavelength.

Measurement Procedure

1. Absorption spectrum of the solvent.
2. Absorption spectrum of the sample.
3. Subtract absorption spectrum of the solvent from the absorption spectrum of the sample.

4. Determine the optical density of the sample at the anticipated excitation wavelength.
5. Take the emission spectrum of the solvent.
6. Take the emission spectrum of the sample.
7. Subtract the emission spectrum of solvent from the emission spectrum of sample. Use the corrected emission spectrum and calculate the area under the curve.
8. Repeat the same routine for the quantum yield standard.

How to improve the precision of quantum yield measurements

Use cuvettes with extended optical path lengths (20 mm - 50 mm), which allow more accurate measurement of lower concentrations.

Prepare solutions with optical densities ranging from 0.1 to 0.01, and calculate the quantum yield using the "gradient method" described below.

Calculation

The relative quantum yield is generally determined by comparing the wavelength-integrated intensity of an unknown sample to that of a standard. The quantum yield of the unknown sample is calculated using:

$$Q = Q_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2}$$

where Q is the quantum yield, I is the integrated intensity, n is the refractive index, and OD is the optical density. The subscript R refers to the reference fluorophore of known quantum yield.

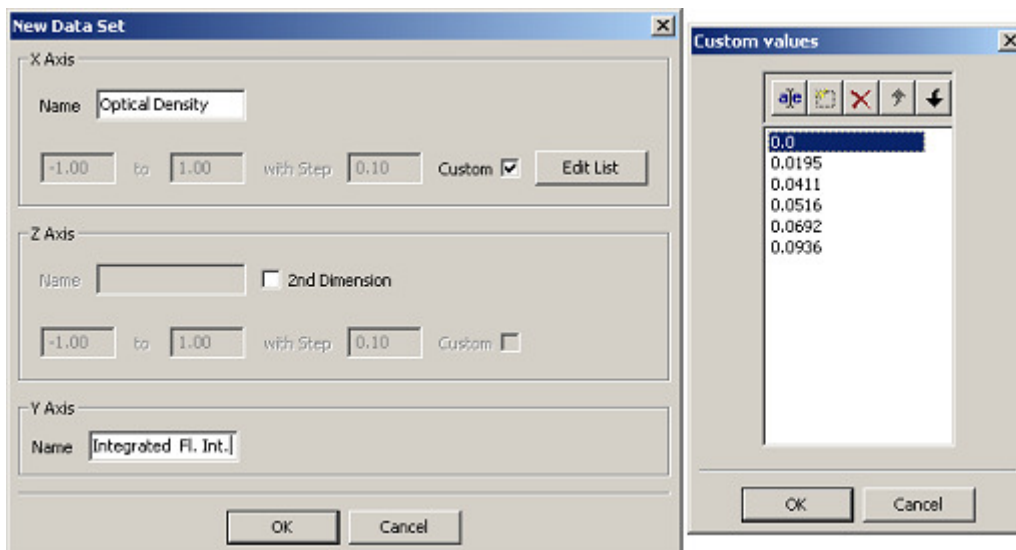
However, a more accurate method to determine quantum yields is to prepare solutions with optical densities between 0.1 to 0.01, and to calculate the quantum yield using the gradients determined for the sample and the reference. In this case quantum yield can be calculated using:

$$Q = Q_R \left(\frac{Grad}{Grad_R} \right) \left(\frac{n^2}{n_R^2} \right)$$

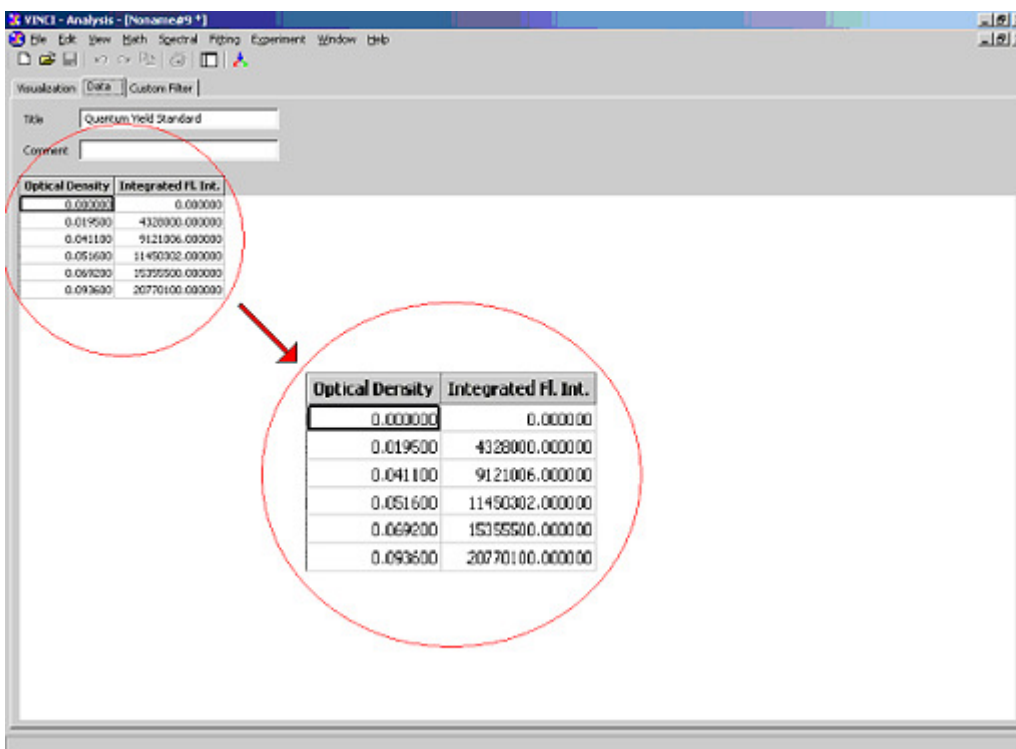
where $Grad$ is the gradient obtained from the plot of the integrated fluorescence intensity vs. optical density (see below). **Vinci** analysis software enables to carry out these procedures and to calculate the quantum yields using the gradient method.

Measurement of Quantum Yields Using Vinci

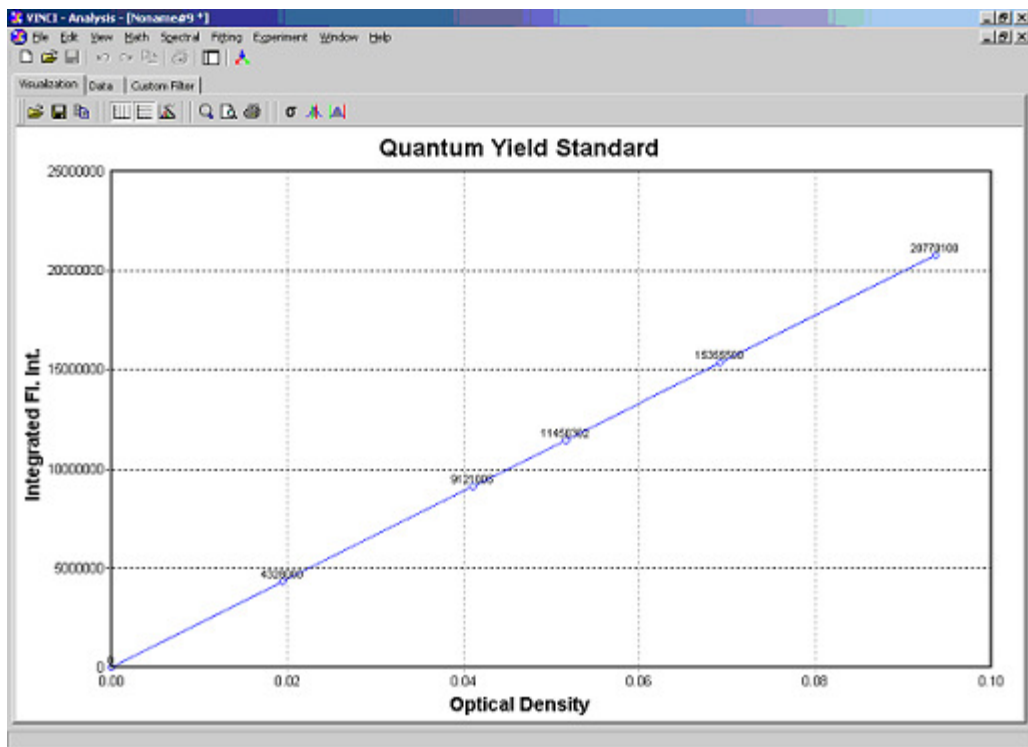
Upon completion of the recording of the emission spectra with **Vinci** - Instrument and Experiment Control switch to **Vinci** Analysis. Click on **File -> New** and the following **New Data Set** dialog box will appear; here the X and Y-axes are defined; choose **Custom** and press **Edit List**. This will allow you to fill in the X values (Optical Density).



After all X values are filled in, press **OK** and another **New Data Set** dialog box will come up. The next step is to input the Y data (**Integrated Fluorescence Intensity**): click on **Data** and proceed with adding the Y-data values.



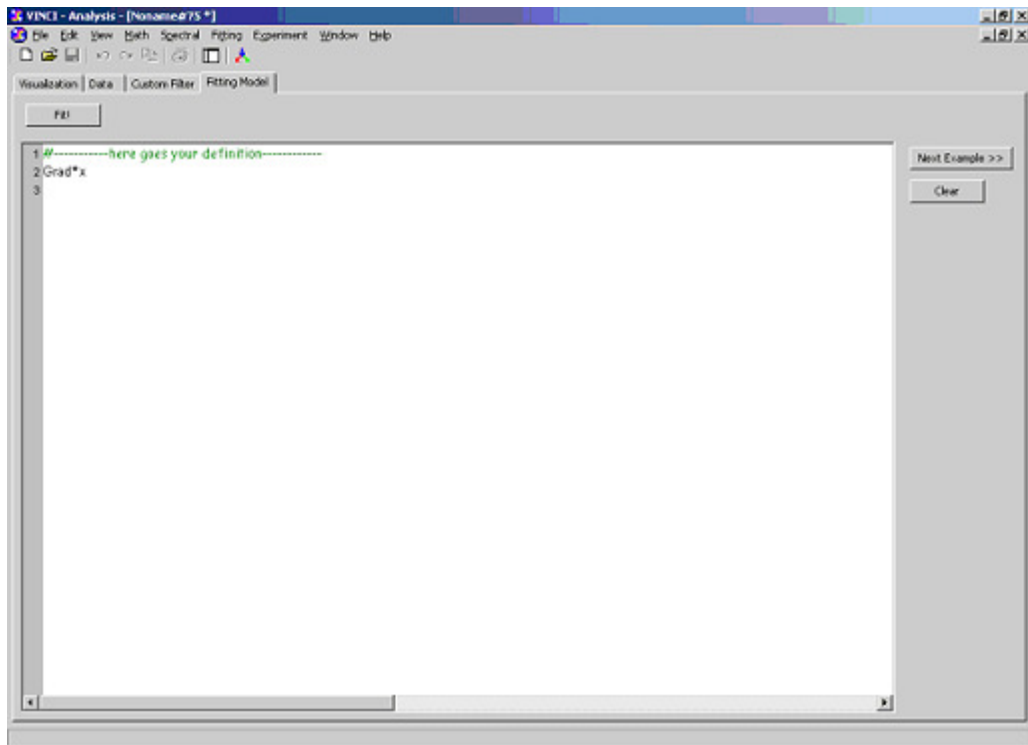
After this step, switch to **Visualization** and you will obtain the following screen:



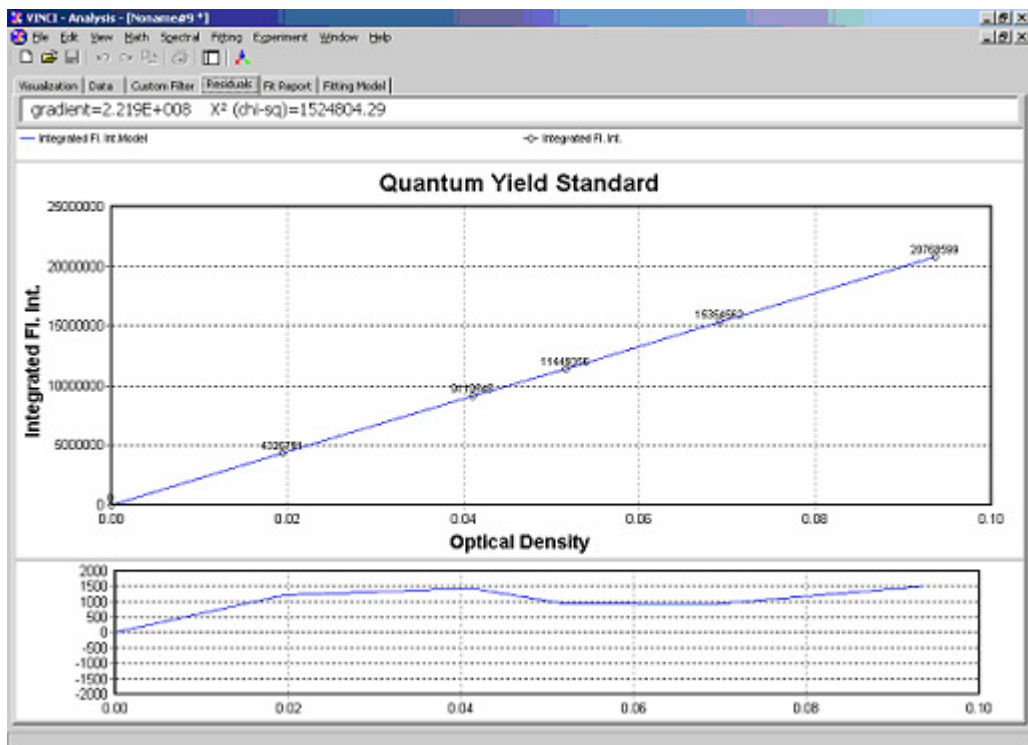
The above **Integrated Fluorescence Intensity vs. Optical Density** graph was obtained from the experimental data. To obtain the **quantum yield** of the sample it is necessary to determine the gradient of this curve: Press **Fitting -> Custom model**, then click on **Fitting model** and enter the following equation

$$\text{Grad} * x$$

in line 2 below the #----- here goes your definition ----- line and press **Fit!**

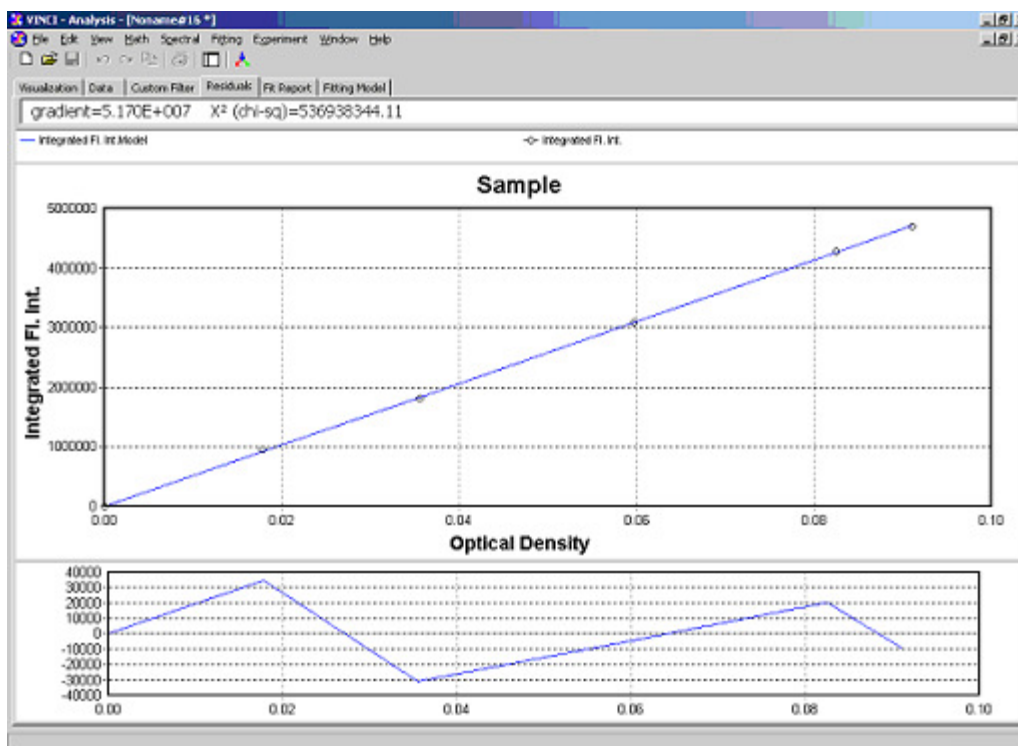


This will produce the fit with the numerical value for the gradient:



The numerical value of the gradient of the **quantum yield standard** - $Grad_R = 2.219 \times 10^8$ - is then used for the calculation of the quantum yield of the sample.

The same steps need to be repeated to obtain the sample(s) gradient(s):



$$\text{Grad} = 5.170 \times 10^7 \text{ (for the sample)}$$

If we assume that the quantum yield of the Reference (Q_R) is 1, and the refractive index of sample and reference to be the same, which is true for measurements in the same solvent system, then the quantum yield of the sample (Q) is:

$$Q = 1 * (5.170 * 10^7 / 2.219 * 10^8) = 0.23 \text{ (23\%)}$$

References

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