TECHNICAL NOTE

Guide for the Measurements of Absolute Quantum Yields of Liquid Samples

TN_P21; September 2012

EDINBURGH INSTRUMENTS

Introduction

Body copy This Guide is designed to help you to determine the Fluorescence Quantum Yields (QY) of liquid samples using your FLS920 spectrometer and the Integrating Sphere accessory.

Fluorescence Quantum Yields can be measured using the relative method by comparing fluorescence parameters to a published quantum yield standard, or they can be determined by the absolute method in which the number of absorbed photons of a sample and the number of consequently emitted photons are measured. This Guide explains how to use the sphere by means of the Absolute Quantum Yield method.

Specific measurement parameters are required to accurately and reliably perform quantum yield measurements. In instruments specifically designed with the sole purpose of QY measurement these parameters are invariably fixed. The FLS920 is a flexible, research grade fluorescence spectrometer where the integrating sphere accessory for the measurement of fluorescence quantum yields is only one of its many applications. It is therefore very important that the measurement parameters of the FLS920 are fixed and consistent when QY measurements are being made.

Accurate and reliable QY measurements require practice. They also can be quite time consuming. It is not only a question of having the proper instrumentation and having it correctly setup, but also a question of following the correct procedure.

There are two instrumental factors that can have dramatic effects on the measured quantum yields:

1.) 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 error in absolute quantum yield measurements. It causes systematic errors with resulting quantum yields being either too big or too small.

2.) 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 here to use an upper limit of about 1 million photons per second.

Liquid Samples are measured in standard (10mm x 10mm) quartz cuvettes with PTFE (white) stopper. You need ideally 2 identical cuvettes, one containing the sample, the other containing the solvent only (to be used as a blank). When preparing the cuvettes ensure that both sample and blank have the same filling height. 3ml of sample volume is ideal. Do not use less than 2ml.

You also need an absorption (UV-VIS) spectrometer (or the EI absorption accessory) to set up absorbance (OD) values of your sample.

Step 1: Measurement of the Emission Spectrum

Get to know the absorption and emission spectra of your sample.

Choose an appropriate excitation wavelength that is sufficiently low not to interfere with the emission spectrum. This is likely not to be the peak of the absorption.

Adjust the concentration of the sample so that the optical density is set to OD=0.1 at the wavelength of excitation.

Use the FLS920 spectrometer to produce an emission spectrum. Use the standard liquid sample holder for this. Use either manual or automated spectral correction, using the correction file that is valid for the standard liquid sample holder. Use the measurement to discover the wavelength range required to scan from baseline to baseline i.e from just after the excitation to the background level on the long wavelength side.

This might well be several 100 nanometres beyond the peak of the emission. Viewing the graph in logarithmic scale can be helpful. Figure 1 shows an example of the emission spectrum.

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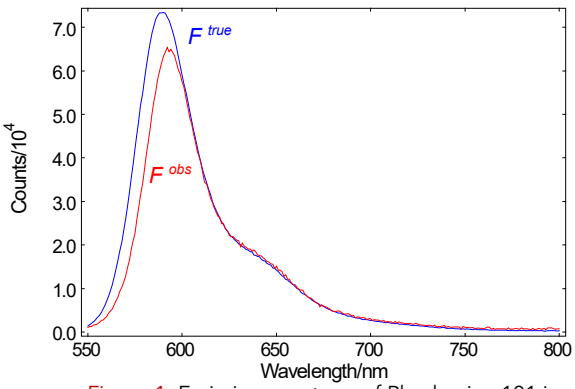

Figure 1: Emission spectrum of Rhodamine 101 in ethanol, excited at 525nm

Step 2: Measurement of the Blank

The sphere must now be mounted in the FLS920 sample chamber, do this remove the standard sample holder and all the lens optics and place the sphere in the centre of the sample chamber with the entrance containing the lens facing the excitation monochromator.

Now use the sphere to produce the measurement of the blank sample. The scattering of the blank sample will produce the highest signal (at λ_{exc}), it is recommended therefore to measure the blank sample first, or at least use the blank sample to set up the measurement parameters (to ensure you are using the detector within its linear range).

Accurately position the blank sample in the sphere and check that the knob on the back of the sphere is in the "cuvette" position.

Use the signal rate screen to determine the parameters: Set both excitation wavelength and emission wavelength to the wavelength chosen in step 1 above (λ_{exc}). Use a wide band width in the excitation path ($\Delta\lambda_{exc}$ = 12nm) and a narrow band width in the emission path ($\Delta\lambda_{em}$ ~ 0.1nm).. Using a wide spectral band pass in the excitation path is essential for producing accurate QY results as the area under the scattering spike is used in the quantum yield calculation. From now on leave $\Delta\lambda_{exc}$ = 12nm fixed and use $\Delta\lambda_{em}$ to adjust the signal level to ~ 1M cps.

Run an emission spectrum that extends from 25nm below the wavelength of excitation right up to the longest end of the expected emission spectrum as determined in step 1. Use a wavelength step size of 1nm and an integration time of 1s (= 0.2 s dwell time x 5 repeats).

You may use automated spectral correction or manual spectral correction, however it is very important that this time the correction file for the sphere is used. Once the measurement is complete it is useful to rename the file Ref.

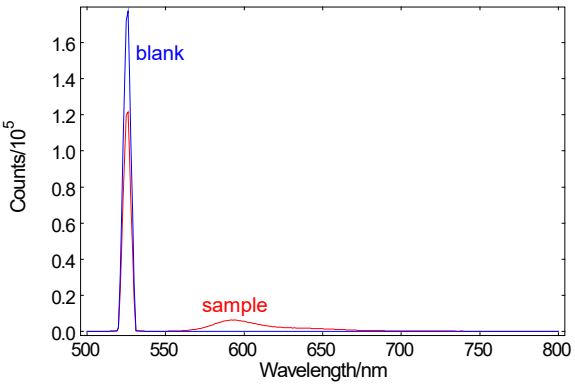

Fig. 2: Blank spectrum (cuvette filled with the solvent ethanol that is used for Rhodamine 101) shown in comparison to the sample spectrum that will be measured in the next step.

N.B. For basic calculations of the QY scanning the blank measurement over the whole emission range (550nm-800nm in figure 2) is not required. However the QY wizard used later will have the option to correct for a background level, so the full blank measurement can be used for this.

Step 3: Measurement of the Sample

In the sphere replace the blank by the sample (as prepared in step 1) and use exactly the same scan parameters to measure the sample scan.

A sample with the optical density of 0.1 at the wavelength of excitation will result in a measurement that shows a scattering peak at about 70% of that of the blank sample, refer to figure 2. [Note that the "absorbed area" (= blank scatter – sample scatter) is not linear with OD, as OD is measured on exactly 10mm path length and the scatter is measured in the sphere where the effective path length is longer.]

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The sample measurement should also show the emission, refer to figure 2. The amplitude of the emission is directly related to the quantum yield.

As with the blank measurement above, the sample spectrum must be spectrally corrected using the correction file that is valid for the sphere.

The ratio of "absorbed area" (light absorbed by the sample) and "emitted area" (integrated emission) is the observed fluorescence quantum yield, QY^{obs}. A visible inspection of the two areas is easily possible by subtracting the blank scan from the sample scan.

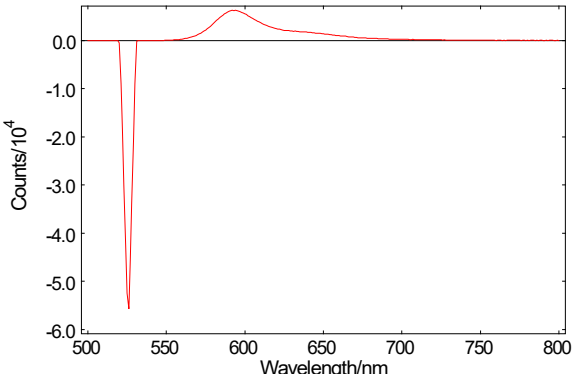

Fig. 3: Visualisation of the two areas used in the quantum yield wizard for calculating the fluorescence quantum yield QY^{obs}.

Step 4: Calculation of QY^{obs}

Use the graph shown in figure 2 and the Quantum Yield Wizard of the F900 to calculate the quantum yield QY^{obs} (*Data >> Quantum Yield ...*)

Load the scans as shown in figure 4, then select the scattering region (500nm-540nm in the example) then select the fluorescence region (540nm-800nm in the example). The quantum yield wizard will automatically show the scans in a semi logarithmic scale. This enables you to select the regions of scatter and fluorescence more accurately.

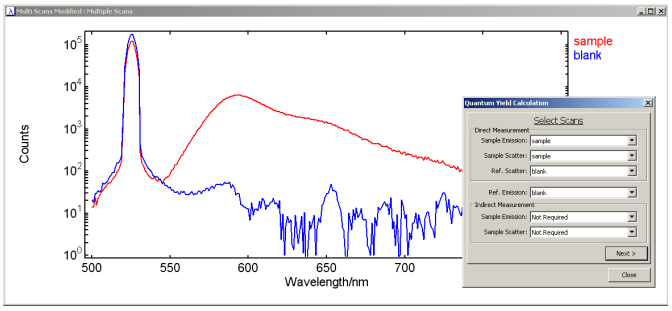

Fig. 4: Using the F900 quantum yield wizard

Note that if no Ref. Emission is loaded (see figure 4) the calculation will be performed without background subtraction. Also note that quantum yield calculation for liquid samples only works for direct measurements.

The last step of the wizard will present the calculated quantum yield (figure 5).

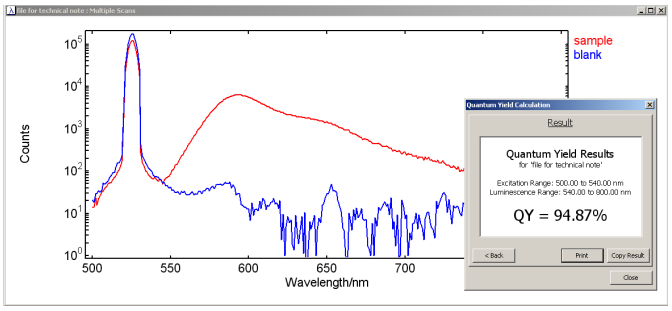

Fig. 5: Final step of the F900 quantum yield wizard.

Step 5: Measurement of the "True" Emission Spectrum

Adjust the concentration of the sample so that the optical density at the peak of the absorption is less than 0.1.

Use the FLS920 spectrometer to produce an accurate emission spectrum. Use the standard liquid sample holder for this. Set a narrow band width in the emission path ($\Delta\lambda_{em} \leq 0.5$nm), use the signal attenuator (iris) and $\Delta\lambda_{exc}$ to adjust an appropriate signal level. Use a wavelength step size of 1nm and an integration time of 1s (= 0.2 s dwell time x 5 repeats). Ensure that you acquire the spectrum from baseline to baseline.

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Use either manual or automated spectral correction, using the correction file that is valid for the standard liquid sample holder.

This measurement gives you the "true" emission spectrum, F^{true}, that is free of re-absorption. You will need this spectrum in step 6 to produce the corrected Quantum Yield.

The graph below (figure 6) shows the difference between the true emission spectra (F^{true}) and the emission spectra observed in the sphere (F^{obs}) – as measured in step 3.

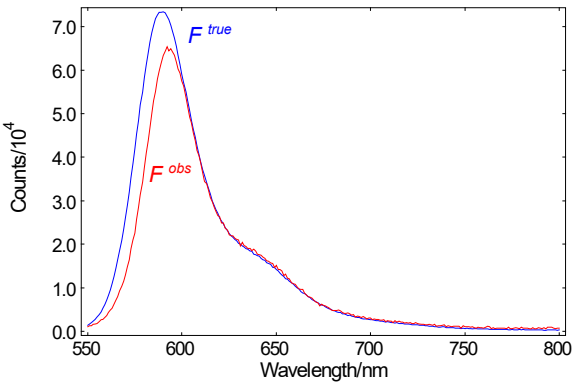

Fig. 6: True emission spectrum of Rhodamine 101 in ethanol, excited at 525nm, compared with the fluorescence emission spectrum measured in the sphere in step 3 (F^{obs})

Step 6: Calculation of QYtrue

It is clear from figure 6 that the fluorescence spectrum measured in step 3 is different from that measured in step 5. The reason is re-absorption, i.e. photons that are emitted from the sample, scattered inside the sphere with a probability that they will re-enter the sample, some of these photons are then re-absorbed by the sample again. The effect of re-absorption depends on the sample itself, and it depends of the reflectance of the sphere.

Samples with a strong overlap between absorption and emission (such as the sample rhodamine 101 used in this example) show strong re-absorption. A high reflectance sphere will also increase re-absorption.

Ideally one would measure the fluorescence quantum yield at very low sample concentration, when re-absorption is negligible. However, this is not possible as the quantum yield accuracy is reduced when the sample concentration is low.

The solution to this problem is to correct for the re-absorption effect.

Take the two fluorescence spectra measured in steps 3 and 5. Scale one against the other (using the emission at the red end of the spectra) to produce the "tail matched" spectra as shown in figure 1. Integrate the two areas and calculate the fraction, ***a***, of the re-absorbed area. In the example of figure 6 this fraction is a=0.118.

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

$$QY^{true} = \frac{QY^{obs}}{1 - a + a \cdot QY^{obs}}$$

For the Rhodamine 101 example we obtain QY^{true} = 95.45%

Error Estimation:

We assume there is no error in the sample preparation and the purity of the sample.

Under these conditions we have an error of the Possionian photon noise for both the area of fluorescence emission and sample/blank scatter. For high quantum yield samples these errors are about the same and negligible.

The dominating error comes with the accuracy of sample replacement and the accurate replacement of the upper half of the sphere after changing the sample. These two factors particularly affect the accurate determination of the "absorbed area". With a good and careful routine we can estimate an absolute error of ±2%. Thus for the Rhodamine sample used in this Guide we obtain QY^{true} = (95.5 ± 2)%.

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