

PTI Technical Note

Signal to Noise Determination for PTI QuantaMaster™ Fluorometer

Introduction

Fluorescence spectroscopy is one of the most sensitive optical techniques. It is used in many different hardware representations, i.e. as classical cuvette-based spectrofluorometers, microscope photometry and imaging instruments, plate readers, fluorescence chromatography, fluorescence correlation spectroscopy and others. Assessment of sensitivity, i.e. the signal to noise (S/N) ratio is the most important characteristic of the fluorescence instrument, as it ultimately determines the limit of its usefulness.

The sensitivity is determined by 3 main factors: the light source, the detector and the overall optical design. Most of the high-end conventional fluorometers utilize a photomultiplier operating in the photon counting mode. Photomultipliers are capable of detecting a single photon originating from a single molecule, so if properly selected they will not limit the sensitivity of the instrument. The light sources (typically xenon arc lamps) on the other hand are offered at different power levels, somewhere from 60W to 1000W and, in principle, can have a profound effect on sensitivity. However, it's not enough to simply select a higher power lamp to ensure a higher sensitivity level, e.g. a 450W 'monster' lamp will not necessarily outshine a 75W lamp. Optical performance will depend on how efficiently the light is collected and delivered to the sample. For example, a specially designed ellipsoidal reflector for the 75W xenon lamp in PTI QuantaMaster™ 40 offers sensitivity superior to instruments utilizing 300W and 450W lamps. Fluorometers with inferior optical design tend to use excessively high-powered arc lamps. Another, equally important aspect of a good optical design is how well stray light is controlled. Excessive stray light will increase the background level and will lower the S/N ratio.

S/N Determination from Water Raman Spectrum Measurement

The Raman spectrum of water has been widely accepted as the industry 'standard' to quantify the S/N ratio of steady state fluorometers. The premise is that high purity water is readily available and there is no chemical degradation or photobleaching compared with organic fluorophores. In practical terms, purity of water is the main difficulty in getting the Raman peak that is truly representative of the instrument performance. Even minute traces of fluorescence impurities may significantly increase the background level and thus adversely affect the S/N. Therefore, the highest purity water should be used. The glassware and especially the cuvette need to be very clean and should never be used for holding fluorescence samples.

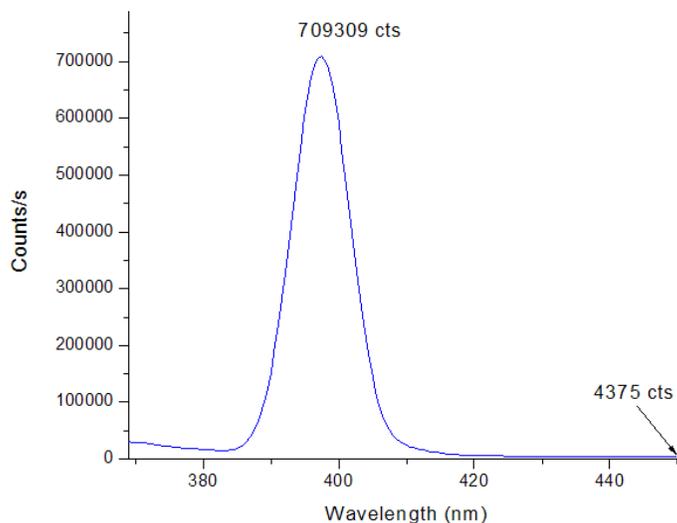


Fig. 1. Water Raman spectrum measured with the QM-40 system

PTI and most of fluorometers manufacturers record the water Raman under the following conditions, while operating in the photon counting mode:

- Exc wavelength = 350 nm
- integration time = 1 s
- Exc and em slits = 5 nm

A typical Raman spectrum obtained with PTI QuantaMaster™ 40 spectrofluorometer using the above parameters is shown in Fig. 1. The water Raman peak is always red-shifted by 3382 cm^{-1} from the excitation, so with the 350 nm excitation the Raman peak maximum is at 397 nm.

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S/N Calculation

Method 1

While everybody agrees how to measure the Raman peak, not everybody does the actual calculation the same way. What to use as the signal value is quite obvious: it's the photon count at the peak, $N_p(397)$ minus the background. However, what to use as the background value is somewhat arbitrary. Some manufacturers use the photon count at 450 nm, $N_b(450)$, as background assuming that at that wavelength the Raman intensity is already absent. If the noise is caused only by statistical fluctuations of photons, which are governed by Poisson distribution, the noise can be calculated as $\sqrt{N_b(450)}$. The S/N ratio can be then expressed as:

$$S/N = \frac{N_p(397) - N_b(450)}{\sqrt{N_b(450)}} \quad (1)$$

Using the water Raman data obtained with the QuantaMaster™ 40 in Fig. 1 one gets

$$S/N = \frac{709309 - 4375}{\sqrt{4375}} = 10658$$

This is an acceptable S/N ratio value for the QuantaMaster™ 40 fluorometers, which are rated at S/N = 10,000:1 or higher.

Method 2

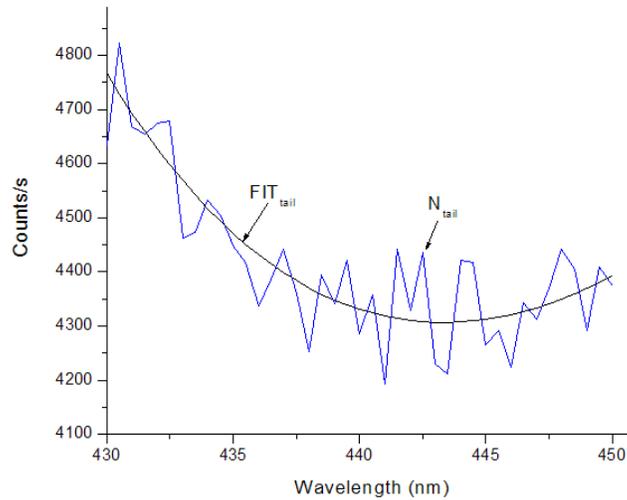


Fig. 2. Tail portion of Raman spectrum and a fit

PTI uses a somewhat different calculation. Method 1 does not take into account any other potential noise sources, such as caused by detector electronics or RF pickup, in addition to photon fluctuations. Therefore, we prefer to explicitly calculate the RMS error from the tail portion of the water Raman spectrum (Fig. 2). This way we can account for all sources of noise even though it may produce a lower S/N ratio than in Method 1. In order to calculate the S/N ratio, the tail portion of the Raman spectrum $N_{tail}(\lambda)$ is fitted by a polynomial or multiexponential function and the resulting fit $FIT_{tail}(\lambda)$ is subtracted from $N_{tail}(\lambda)$ producing a flat noise distribution function $Noise(\lambda)$ shown in Fig. 3:

$$Noise(\lambda) = N_{tail}(\lambda) - FIT_{tail}(\lambda) \quad (2)$$

The RMS is then calculated from the usual formula:

$$RMS = \sqrt{\frac{\sum_{i=1}^n Noise_i(\lambda)^2}{n}} \quad (3)$$

where n is the number of data points.

The S/N ratio is then calculated as

$$S/N = \frac{N_p(397) - N_b(450)}{RMS} \quad (4)$$

Using the data from Fig. 1 through 3

$$S/N = \frac{709309 - 4375}{69.6} = 10128$$

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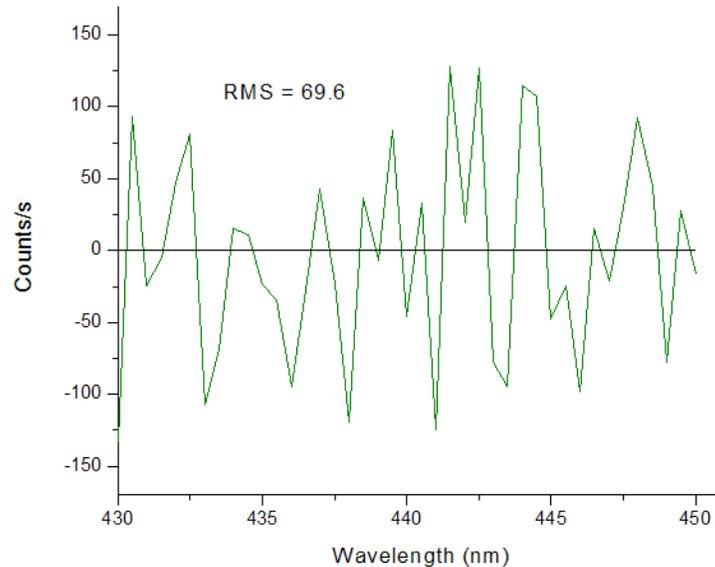


Fig. 3. Noise distribution function based on water Raman tail

Conclusion

Two S/N ratio calculation methods applied to the water Raman spectrum obtained with the QuantaMaster™ 40 system produce practically the same result. The method used by PTI, based on RMS calculation, is more general, as it does not require an assumption of Poisson noise statistic in the data and will result in lower and more realistic S/N ratio than Method 1 if other sources of noise are present in the data.

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