



Sensitivity of the Fluorolog[®] and FluoroMax[®] Spectrofluorometers

Introduction

Many companies claim high sensitivity for their spectrofluorometers. In their specifications, instrument vendors often cite excitation of de-ionized water at 350 nm, resulting in Raman emission at 397 nm (an energy loss of 3380 cm^{-1}). This Raman-emission band of water is used because the signal is red-shifted and weak, which mimics weak fluorescence. De-ionized water is easy to prepare, so sample-to-sample reproducibility is straightforward.

Other wavelengths, however, also must be considered, because a spectrofluorometer is used across a wide spectral range. Optical design of less-sensitive instruments usually involves lenses and concave gratings, which lose focus at wavelengths other than the design wavelength, degrading instrument performance away from the optimum wavelength.

Spectrofluorometers from HORIBA Jobin Yvon, however, are free from such artifacts, because their design uses only mirrors and plane gratings, which remain in focus at all wavelengths, to maintain high performance from the deep-UV to the near-IR. The Raman scatter of water becomes less intense as the excitation wavelength increases. Measurement of a series of Raman spectra across the instrument's usable wavelength range is much more relevant as a figure of merit of instrument sensitivity, as shown here.

Experimental method

Measurements were performed on a Fluorolog[®]-3 with double-grating

monochromators, 450-W xenon lamp, and a cooled R928 photomultiplier tube run at 950 V in photon-counting mode. Bandpass was 5 nm on excitation and emission monochromators. The same grating (330-nm blaze, optimized for 500 nm) was used for all scans; only integration time varied (Table 1). The sample was de-ionized water purified in a Millipore Simplicity[®] 185. Excitation of the sample was from 250–600 nm, with 50-nm increments. Each emission scan used a step size of 1 nm. Scans were taken under ambient room conditions.

λ_{exc} (nm)	Integration time (s)
250	1
300	1
350	1
400	2
450	5
500	5
550	10
600	60

Table 1. Excitation wavelengths and their respective integration times.

Results and discussion

The largest water Raman emissions are the 335 nm, 397 nm, and 463 nm peaks (Fig. 1), which also have the best signal-to-noise ratio, S/N (Table 2). As the incident wavelength increases, the Raman peaks are displaced farther from the incident wavelength, but the energy loss is a constant 3380 cm^{-1} . Raman peaks also occur at 275 nm, 531 nm, and 693 nm. Emission spectra are rescaled to reveal the Raman bands more clearly at 667 nm and 753 nm (Fig. 2). These results demonstrate the high sensitivity of the

Fluorolog[®] even at these long wavelengths.

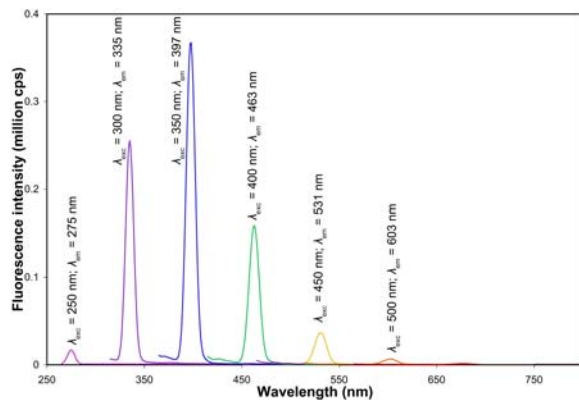


Fig. 1. Raman emission from water for 250–800 nm at excitation wavelengths 250, 300, 350, 400, 450, and 500 nm. See text for scan conditions.

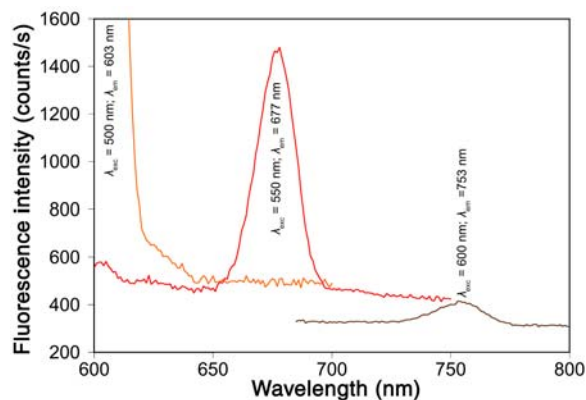


Fig. 2. Expanded section of Fig. 1: Raman emission of water from 600–800 nm at excitation wavelengths 500, 550, and 600 nm. See text for scan conditions.

Comparing sensitivity values

Be sure that sensitivity calculations are identical for a valid comparison. HORIBA Jobin Yvon conservatively quotes a minimum S/N of 3000:1 for the FluoroMax[®]. In 2001, the average FluoroMax[®] S/N was closer to 5000:1. Compare this to other manufacturers who quote 500:1.

Let us compare experimental

conditions. We calculate S/N via a water-Raman test with 5-nm bandpass. Other companies use a 10-nm bandpass on both emission and excitation monochromators. This lets twice the light into each monochromator and twice the light out, a factor of four per monochromator, and 16 for the entire instrument. Thus, the other companies' data are inflated by more than an order of magnitude. The S/N varies as the square root of the signal. Therefore, under similar conditions, the seemingly respectable number of 500:1 is actually closer to 100:1!

λ_{exc} (nm)	λ_{em} (nm)	$\lambda_{baseline}$ (nm)	S/N
250	275	400	580
300	335	450	8100
350	397	450	10 750
400	463	520	8700
450	531	595	3500
500	603	650	660
550	677	725	225
600	753	800	190

Table 2. Excitation and emission wavelengths, wavelength used for baseline noise measurements, and corresponding S/N . The S/N is the emission peak counts divided by the square-root of the baseline counts.

So, what does this mean for you?

Certainly the main indicator of sensitivity is the ability to examine lower concentrations. For expensive samples, less sample or probe is needed. Yet there are two other advantages of sensitivity: *accuracy* and *measurement time*.

- **Measurement time:** When comparing data, recall that the square root of the measured number of photons determines S/N . If two instruments have a S/N difference of a factor of two, one actually takes four times longer than the other to measure the same signal.

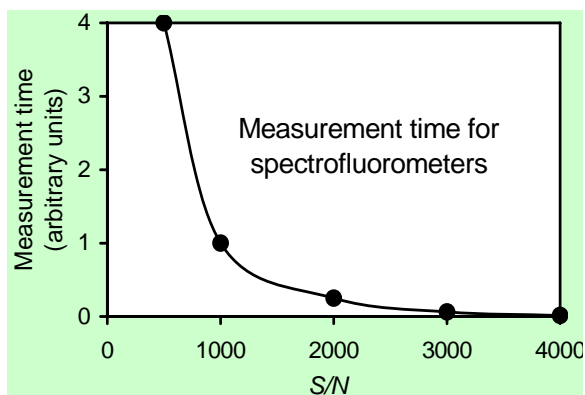


Fig. 3. Plot of how the measurement time varies with the signal-to-noise ratio.

- **Accuracy:** A final measurement is only as good as the amount of data put in. To count photons with 1% accuracy, at least 10 000 photons are needed. We assume random error and Gaussian statistics, so the first standard deviation equals the square root of the measured number,¹

$$\sigma = \sqrt{\frac{\sum_i (x_i - \bar{x})^2}{n - 1}}$$

Quality of electronics does not matter: without 10 000 photons, the accuracy cannot reach 1%. (Analog detection has other problems, e.g., hidden noise in the signal, and response times that skew the data.) Hence, an instrument with twice the *S/N* is twice as accurate under the same conditions.

Conclusions

Water-Raman transitions are detectable across a large spectral range. Therefore, the Fluorolog[®] demonstrates high sensitivity to fluorescence signals throughout the UV-

visible region; see if your system can handle this task. Kinematically mounted gratings allow optimization of the spectrofluorometer for custom applications at various wavelengths. Gratings, blazed at different wavelengths and optimized for different spectral regions, are changed easily with instant alignment. The Fluorolog[®] reaches the statistical noise limit: this means that we provide the best system possible. Water-Raman experiments prove that the bench-top FluoroMax[®] is superior in every respect.

If everyone needs sensitivity, why doesn't everyone have it? It's a question of economics and quality. Photon-counting electronics, reflective (mirror-based) rather than refractive (lens-based) optics, planar instead of concave gratings, full instrument automation, and all the other high-quality components of a HORIBA Jobin Yvon spectrofluorometer are more expensive. Thus, an accurate, time-saving, sensitive instrument is worth more than one that is not.

How much is your research worth?

¹ Strobel, H.A. and Heineman, W.R., *Chemical Instrumentation: A Systematic Approach*, 3rd ed., Wiley, New York, 1989, p. 345.

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