Wideband Sensitivity of the FLUOROLOG®-3

Introduction

Many companies claim high sensitivity for their spectrofluorometers. In their specifications, instrument vendors often cite excitation of de-ionized water at 350 nm, which results 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, thus mimicking weak fluorescence. De-ionized water is easy to prepare, making sample-to-sample reproducibility straightforward.

Other wavelengths, however, also should be considered, because a spectrofluorometer is used across a wide range of wavelengths. 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.

SPEX® spectrofluorometers, however, are free from these artifacts, because their design uses only mirrors and plane gratings. Mirrors and plane gratings remain in focus at all wavelengths, maintaining 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 the following experiment demonstrates.

Experiment

Measurements were performed on a FLUOROLOG®-3 spectrofluorometer with double-grating monochromators, a 450-W xenon lamp, and a cooled R928 photomultiplier operated at 950 V in the photon-counting mode. The bandpass was set to 5.0 nm on both excitation and emission spectrometers. The same grating (330-nm blaze, optimized for 500 nm) was used for all scans. Only the integration time varied (see Table 1). The sample was de-ionized water purified in a Millipore Simplicity 185. Excitation of the sample occurred from 250–600 nm, in 50-nm increments. Each emission scan was recorded with a step size of 1 nm. The scans were taken under ambient room conditions.

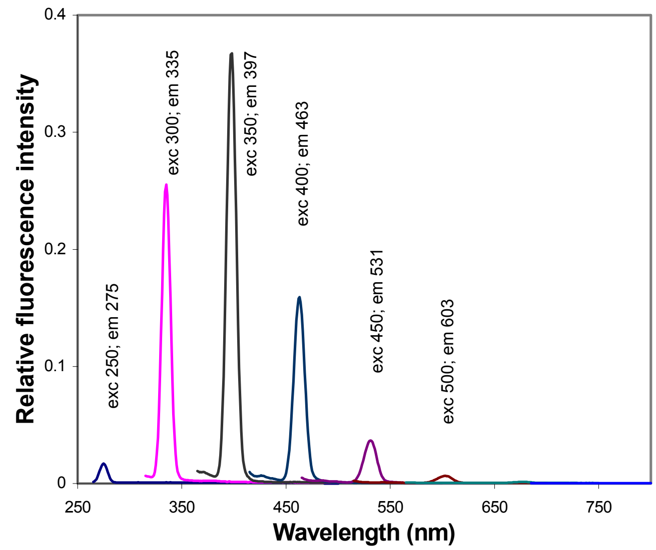

Figure 1. Raman emission from water for 250–800 nm at excitation wavelengths 250, 300, 350, 400, 450, and 500 nm. See text for scan conditions.

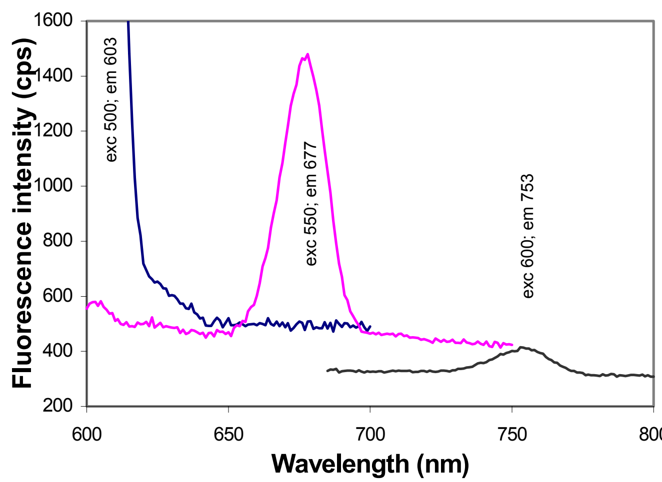

Figure 2. Raman emission of water from 600–800 nm at excitation wavelengths 500, 550, and 600 nm. See text for scan conditions.

Results and Discussion

The largest water Raman emissions are the 335-nm, 397-nm, and 463-nm peaks (see Figure 1), which also have the best signal-to-noise ratio, *S*/*N* (see

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 are also visible at 275 nm, 531 nm, and 693 nm. The emission spectra are rescaled to reveal the Raman bands more clearly at 667 nm and 753 nm (see Figure 2). These results demonstrate the high sensitivity of the SPEX® FLUOROLOG®-3 even at these long wavelengths.

Conclusions

Water-Raman transitions are detectable across a large spectral range. Therefore, the SPEX® FLUOROLOG®-3 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 SPEX® FLUOROLOG®-3 reaches the statistical noise limit: this means that SPEX® gives you the best system possible.

Excitation λ (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.

Excitation λ (nm)	Emission λ (nm)	Baseline λ (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 signal-to-noise ratio, *S*/*N*. The *S*/*N* is the emission peak counts divided by the square-root of the baseline counts.

In the USA:
Jobin Yvon Inc.
3880 Park Avenue
Edison, NJ 08820
Tel: 1-732-494-8660
Fax: 1-732-549-5157
E-Mail: fluorescence@jyhoriba.com
1-800-533-5946

In France:
Jobin Yvon S.A.
16-18, rue du Canal
91165 Longjumeau cedex
Tel: (33) 1/64.54.13.00
Fax: (33) 1/69.09.93.19

Germany: 89/46.23.17-0
Italy: 2/57.60.30.50
U.K.: 020/204.81.42