NIST Develops Fluorescence Standards with the Fluorolog®

Introduction

An accurate map of the wavelength-distribution of fluorescence signal in excitation and emission spectra is critical to determine overlaps in these spectra that cause energy-transfer, as well as accurately measure quantum efficiency. Scientists know, though, that measurements affect results. Fortunately, there are straightforward ways to correct for wavelength-dependent responses of high-performance systems like the Fluorolog®.

The National Institute of Standards and Technologist (NIST) was seeking a high-performance spectrofluorometer to develop new, valuable standard reference materials (SRMs) for fluorescence-intensity correction and validation.[1] These SRMs include UV (2942), blue (2943), green (2941) and orange (2940). The modular, research-grade Fluorolog® provides these key features:

- Low stray-light interference
- No chromatic aberration
- High optical throughput.

To develop SRMs, NIST needed to evaluate and correct the spectral response of the Fluorolog®'s optical path. Correcting the instrument response is essential for all spectrofluorometers because their raw spectra are influenced by the wavelength-dependence of optics and electronic detection components. To record a true fluorescence spectrum, the optics and detection components can be corrected using NIST-traceable calibrated light-sources, reflectors, and detectors; or certified SRMs.

Fig. 1. *Fluorolog® spectrofluorometer.*

Experiment

Excitation spectral correction compensates for the source's varying intensity as a function of wavelength—crucial in excitation scans. A correction factor is found by collecting the signal from the reference detector and comparing it to a calibrated detector placed in the sample position. The correction factor is independent of the lamp's spectrum through the monochromator, but is influenced by beam splitters and the response of the uncalibrated photodiode.

Emission spectral correction removes the instrument's spectral response from the sample to the emission detector(s), including gratings, monochromators and detector(s). Emission correction ratios a calibrated light source's known spectrum at the sample position (or a standard sample's emission spectrum) to the

[1] P.C. DeRose, E.A. Early, and G.W. Kramer, "Qualification of a fluorescence spectrometer for measuring true fluorescence spectra", *Rev. Sci. Inst.* **78** (2007), 033107.

emission detection system's spectral response. This calibration is influenced by the spectral bandpass and—with multi-channel detectors—choice of center wavelength.

A Fluorolog®-3-22 (double-grating monochromators with 1200 grooves/mm gratings blazed at 330 and 500 nm for excitation and emission, respectively) was used. The signal detector was an R928P photomultiplier tube for emission detection (voltage = 950 V). A 450 W CW Xe lamp was the excitation scan's excitation source. A calibrated tungsten lamp was the emission scan's source. A Si-PIN photodiode was the reference detector. A cuvette (path length = 1 cm) was the sample holder. Calibration setups are shown in Fig. 2.

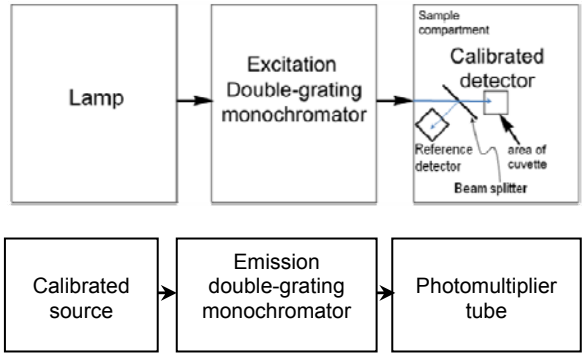

Fig. 2. *Experimental setups for calibration. Top: excitation; bottom: emission.*

Results

The excitation beam's intensity was measured at the cuvette position in the Fluorolog®'s sample compartment (Fig. 2), with results in Fig. 3. Because samples fluoresce in proportion to the source's intensity, all spectra must be corrected to account for variations in the detector and lamp with wavelength. NIST's emission correction data are presented in Fig. 4.

An actual comparison of excitation- and emission-corrected versus an uncorrected spectrum of aqueous tryptophan, an excitation-emission matrix (EEM), is presented in Fig. 5. Note how the general shape of the contours changes and maxima in the plots shift as more corrections are applied.

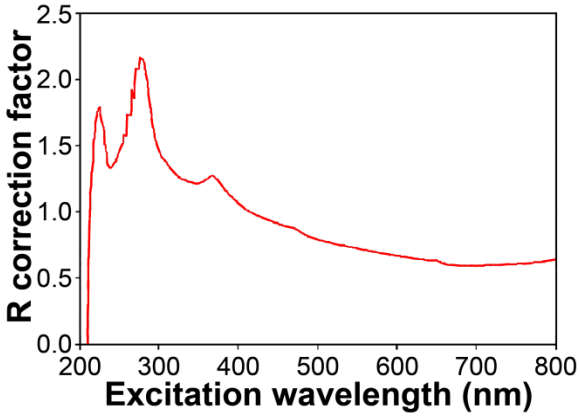

Fig. 3. *Correction factor vs. wavelength for the reference photodiode detector, R.*

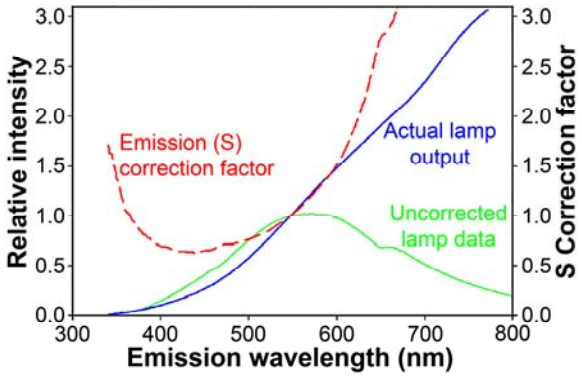

Fig. 4. *Outputs of (blue) actual lamp and (green) uncorrected lamp, as recorded by the instrument. Red: emission (S) correction factor. The y-axis is normalized to 547 nm for both uncorrected data and emission correction factor.*

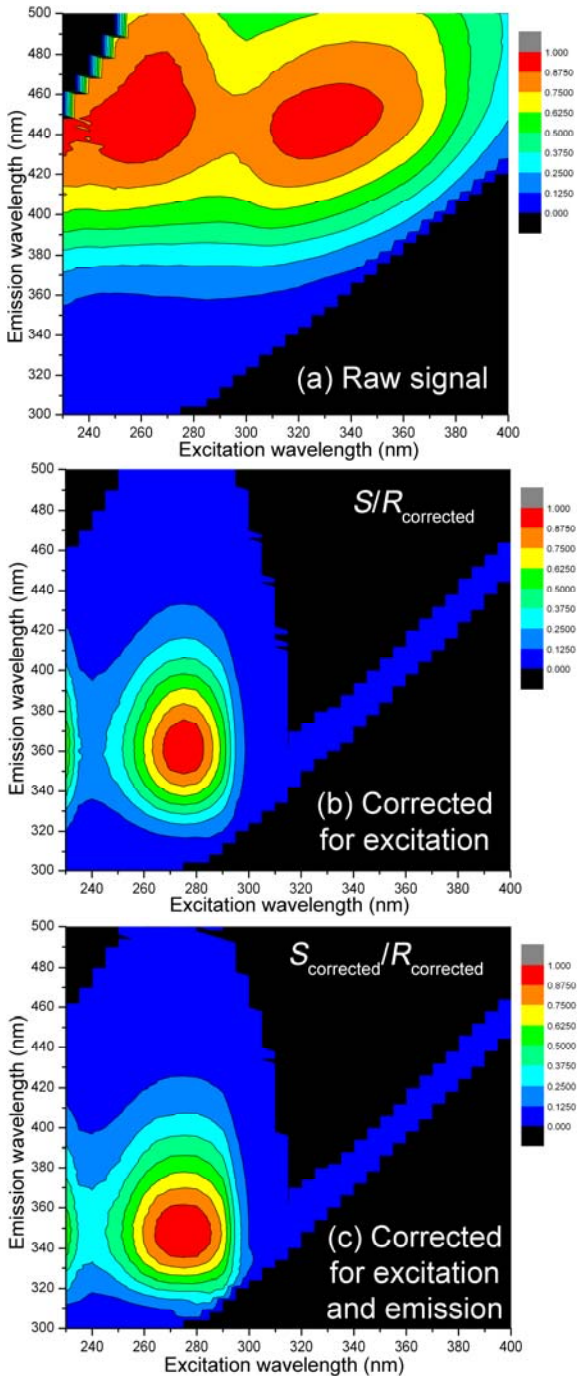

Fig. 5. *Aqueous tryptophan EEMs of (a) raw signal; (b) raw signal corrected for excitation; and (c) raw signal corrected for excitation and emission. Dual maxima shift from (λ_{ex}, λ_{em}) = (265 nm, 452 nm) and (330 nm, 446 nm) for the uncorrected plot (a), to a single maximum in the excitation-corrected version (b) at (275 nm, 358 nm), to another maximum for the excitation- and emission-corrected scan at (275 nm, 346 nm).*

Conclusions

NIST used HORIBA Scientific's Fluorolog® to qualify SRMs to generate fluorescence correction factors. The method includes the entire spectral region—320–800 nm—of most UV-Visible fluorometer systems. The Fluorolog®'s optical characteristics fulfilled the NIST's stringent requirements. Choose the Fluorolog® for all your precision fluorescence research needs.

Acknowledgements

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