Linearization of the SPEX-3D® Spectrofluorometer

Introduction

The SPEX-3D® is capable of acquiring a full excitation and emission matrix (EEM) spectrum from a homogenous sample in less than a second. Initially, the excitation/emission matrix spectrum is captured by a two-dimensional-array CCD detector in pixel units. The pixel unit in this matrix has to be calibrated to the correct wavelength in order for the user to analyze the EEM spectrum. This calibration is complicated, because the optical image is not linearly dispersed onto the CCD's active area. A new method has been developed for a precise calibration of CCD pixels to wavelength, fully compensating for the distortion of the original image.

Calibration of Emission Pixels

A low-pressure mercury-lamp (Hg) emission spectrum was first measured on the SPEX-3D® with the xenon lamp off (see Figure 1).

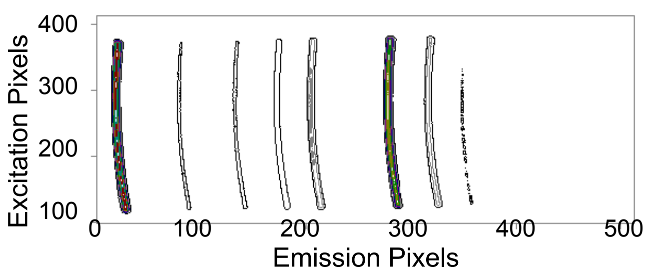

Figure 1. Hg emisson spectrum.

A software routine then was applied to extract an emission spectrum at every excitation pixel position, for a total of 312 spectra. Peak tables were also created for every extracted emission spectrum. Each pixel position at the maximum Hg-emission peak was assigned to its corresponding wavelength, in nm. The Hg-emission wavelength was plotted versus its pixel position and a second-order polynomial ($ax^2 + bx + c$) curve fit was applied. At different excitation pixel positions, the constants a, b, and c in the fit can be different because of the distortion of the image (see Figure 1). The distortion of the 3D image results from the design constraints of the optical path. Therefore, each horizontal row of pixels on the CCD chip was converted to the corresponding wavelength by using the appropriate second-order polynomial equation generated as above. The constants for every horizontal row of pixels on the chip were stored for later use. Figure 2 shows the Hg-emission spectrum after calibration. The distortion of the image was completely corrected by the calibration routine.

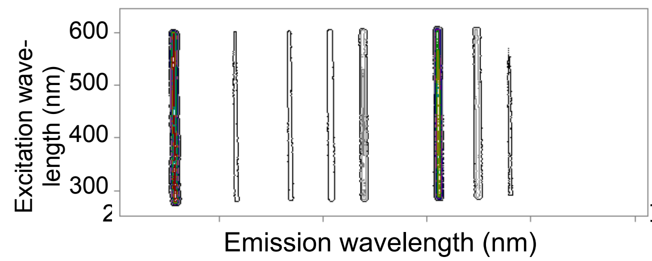

Figure 2. Calibrated Hg emission spectrum.

Calibration of Excitation Pixels

A Rayleigh-light-scattering spectrum of Ludox®*, a scattering medium of colloidal silica suspension in water, was measured with the 3D spectrometer. As seen in Figure 3, the units are pixels, and must be converted to units of length (e.g., nanometers) to be useful.

Because of the nature of the scattering, the excitation wavelength must be equal to its emission wavelength at each scattering peak. A software routine was applied to identify emission and excitation pixel positions at about 20 scattering peaks along the Ludox® spectrum. The wavelength corresponding to the emission pixel can be calculated using the above second-order polynomial equations and plotted versus the corresponding excitation pixel. The curve fits another second-order polynomial equation ($a'x^2 + b'x + c'$), which is used to calibrate the excitation pixel in the entire range to its correct wavelength. The image distortion was small along the vertical direction (i.e., excitation pixels) on the CCD chip. The constants of the polynomial equations for every emission pixel are the same. Figure 4 shows the Ludox® spectrum after the calibration.

* Ludox® is a registered trademark of E.I. duPont de Nemours and Co., Inc.

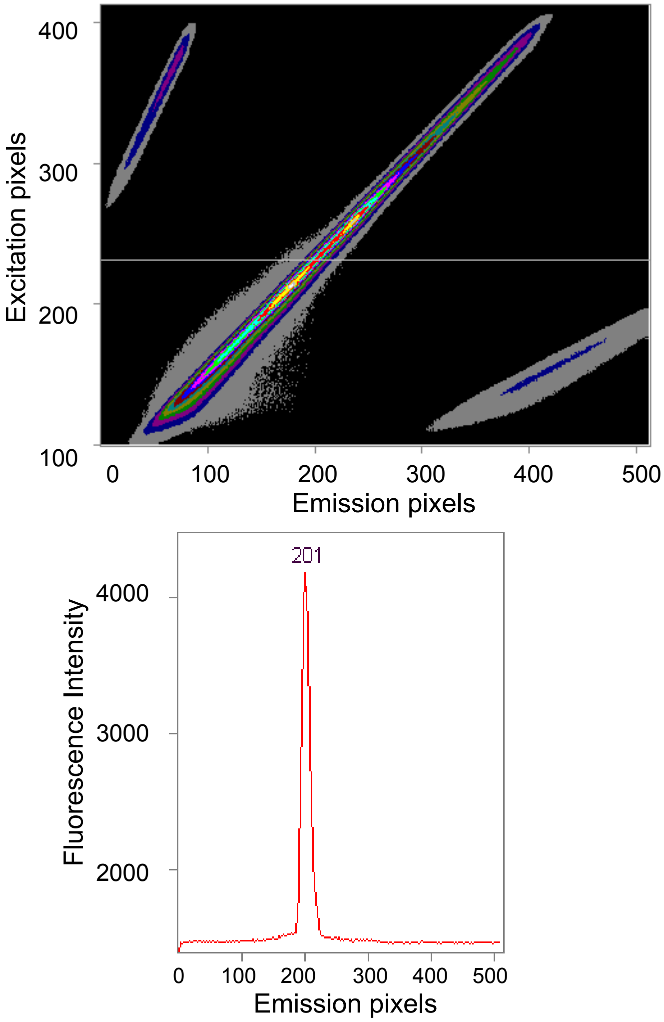

Figure 3. Uncorrected Ludox® spectrum. The lower plot is an emission spectrum of the horizontal gray line (pixel 231) in the upper plot.

At the scattering peak, the emission wavelength is equal to the excitation wavelength after the calibration routine was applied.

Conclusions

Implementation of this calibration algorithm makes it possible to analyze the full excitation emission spectrum collected from a SPEX-3D® system.

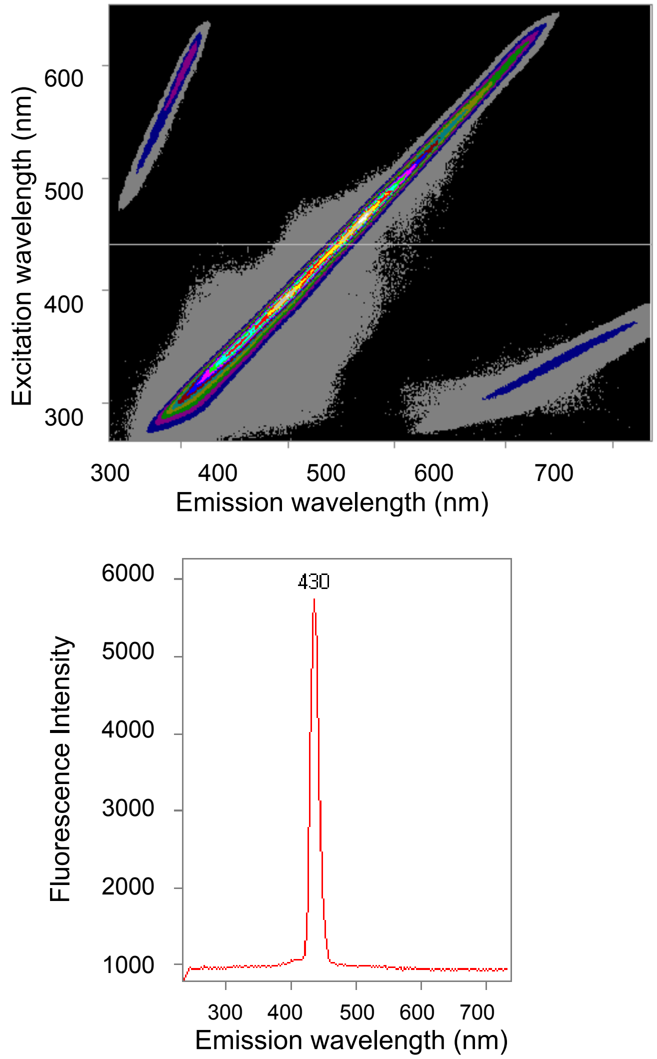

Figure 4. Corrected Ludox® spectrum. The lower plot is an emission spectrum of the horizontal gray line (excitation = 430.0197 nm) in the upper plot.

This method can be also generalized for any CCD detection system. The calibration of emission pixels can be simplified into one polynomial equation to convert entire emission pixels to corresponding wavelengths, if optical image distortion does not occur.

CCD Linearization software is now available for Jobin Yvon instrumentation.