Comparison of FMO and CCD spectrometer

Introduction

Fluorescence spectra can be acquired using a CD spectrophotometer by placing an additional detector at 90 degrees to the excitation light. There are currently two systems on the market for fluorescence detection using a CD spectrometer: a PMT-based spectrometer and a CCD-based multi-channel spectrometer. In PMT-based spectrometers, a spectrum is obtained as a grating scans through a specified wavelength range. In CCD-based spectrometers, an entire spectrum is captured in a single image, which requires less time and signal processing. For these reasons, CCD-based spectrometers are generally considered as highly sensitive, high speed detection systems.

This article will compare the fluorescence spectra of the two add on fluorescence detection options to determine which configuration provides the best S/N.

Experimental

Using the FMO-522 or the PMT-based spectrometer, the fluorescence from the sample cell is directly introduced into the single monochromator and detected by the PMT. The CCD-based spectrometer collects the fluorescence signal through a fiber optic cable which is placed at the back wall of the sample cell. The spectral bandwidth of both FMO-522 and CCD based spectrometer were set to be 5 nm. The bandwidth of the CCD-based spectrometer was set by selecting the slit at the entrance of CCD-based spectrometer. A dark measurement was obtained prior to the sample measurement. The CCD-based spectrometer's dark spectrum acquisition time was 30 seconds, while the FMO-522 dark measurement was acquired in ~ 1 second. The sample measurement time for both the CCD-based spectrometer and FMO-522 were 30 seconds.

The obtained spectra for 1 ppm quinine sulfate and 0.1 mg/mL lysozyme in a 1 mm pathlength cell are shown in Figures 1 and 2, respectively.

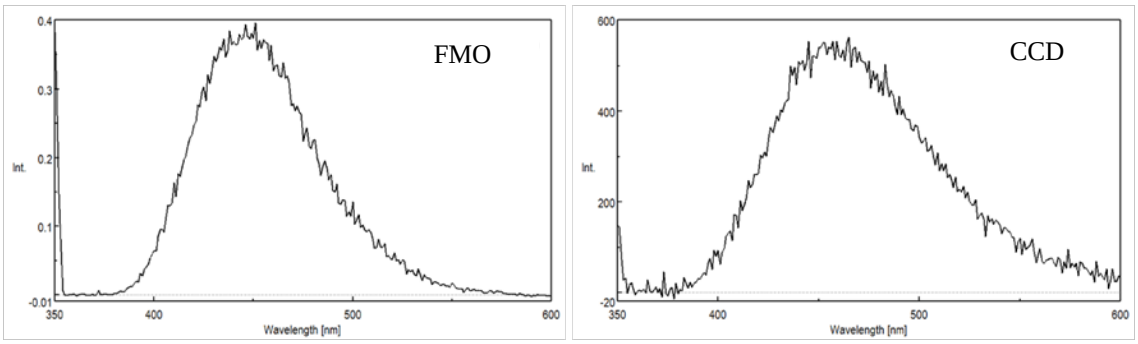

Figure 1 Comparison data of FMO (left) and CCD-based spectrometer (right) of 1 ppm quinine sulfate.

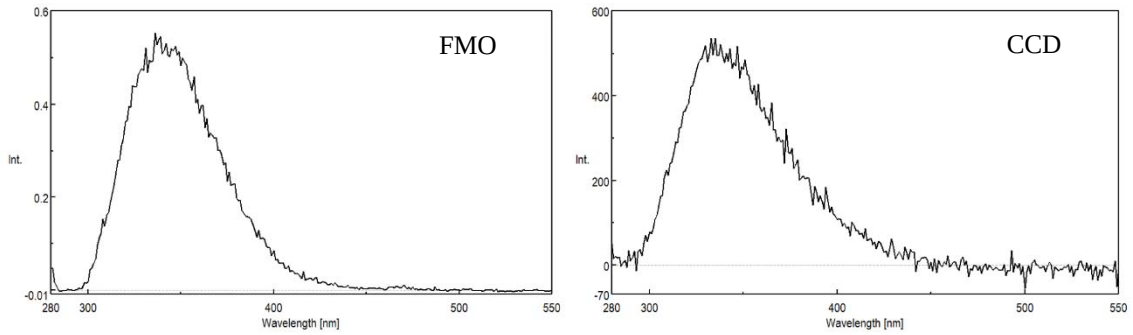

Figure 2 Comparison data of FMO (left) and CCD-based spectrometer (right) of 0.1 mg/mL lysozyme.

The quinine sulfate and lysozyme spectrums obtained with the FMO-522 demonstrated better S/N than the spectrums obtained using the CCD-based spectrometer. This result was also acquired with the FMO in half the total measurement time of the CCD-based spectrometer. The f-number of the FMO system is f/2, while the usually available CCD-based spectrometer claims a f-number of 4. The f-number is the ratio of the focal length to the aperture diameter. The smaller the f-number, the greater the light throughput to the detector. Therefore, the FMO system has the ability to collect more signal intensity from the sample cell. Additionally, the FMO only has one reflective optical element while the usually available CCD-based spectrometer has three. Since some light energy is lost each time it is reflected off a surface, the FMO optical design is more effective at collecting light from the CD's monochromator.

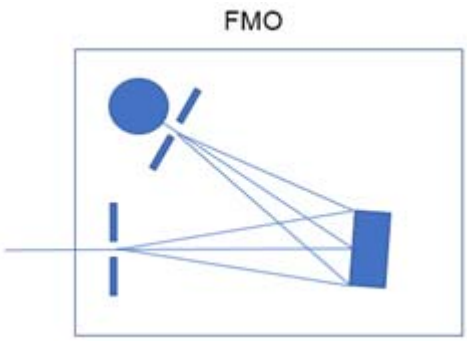

Figure 3 Optical layout of the FMO

Conclusion

While CCD spectrometers are known for their high sensitivity and high speed measurements, the JASCO FMO-based system can acquire fluorescence data with better S/N and faster than the CCD-based spectrometer.