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Application News

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Spectrophotometric Analysis

Fluorescence Spectrum Measurement of Fluorescence Dye Indocyanine Green in Long Wavelength Region

In the medical and biochemical fields, the use of fluorescent dye for labeling specific molecules is a commonly used technique for the observation of tissue lesions. Indocyanine Green fluorescent dye displays fluorescence in the near infrared region in the vicinity of 810 nm, and because light of this wavelength easily passes through the living body, this dye is often used for such observation.
Measurement in wavelength regions greater than 750 nm has been difficult using conventional spectrofluorophotometers, but the newly-developed Shimadzu RF-6000 spectrofluorophotometer permits measurement over a wide wavelength range from 200 to 900 nm. Furthermore, the automatic spectral correction feature of this instrument makes it possible to automatically obtain comparable data from different instruments. Here, we introduce an example of measurement of Indocyanine Green using the RF-6000.

Instrument and Three-Dimensional Spectrum Measurement

Fig. 1 shows a photograph of the RF-6000. The RF-6000 features automatic spectrum correction, which permits the acquisition of spectra (fluorescence/excitation) in which instrument-specific characteristics are eliminated automatically. This elimination of instrument-related data anomalies is conventionally conducted as a data-processing function, but the RF-6000 eliminates the need for such a post-acquisition correction. Such corrected spectral data can thus be compared among different instruments.
Indocyanine Green powder was dissolved in pure water to prepare a 1.3 mg/L aqueous solution. First, to determine the optimal excitation wavelength, the 3D Spectrum feature of the LabSolutions RF software was used to conduct a three-dimensional spectrum measurement. A three-dimensional spectrum is a spectrum that is displayed as a mapping image which is generated by conducting fluorescence spectrum measurements while sequentially changing the excitation wavelength. The measurement results are shown in Fig. 2, and the analytical conditions that were used are shown in Table 1. The horizontal axis is used to plot the fluorescence wavelength Em, and the vertical axis is used to plot the excitation wavelength Ex. This three-dimensional spectrum is generated using the corrected spectrum.
Fluorescence is observed in the region of the peak shown in Fig. 2. The peak position is defined by the emission and excitation coordinates (Em 808 nm, Ex 780 nm), indicating that the greatest degree of fluorescence associated with this sample occurs at the excitation wavelength of 780 nm. It is possible to extract either the fluorescence or excitation spectrum for isolated viewing at any of the excitation or emission coordinates of the three-dimensional spectrum. Each of the spectra acquired at the peak position is shown in Fig. 3. The peak associated with the excitation line A is the generated fluorescence spectrum, and that associated with emission line B is the extracted excitation spectrum. As is evident from these results, the fluorescence peak wavelength is 808 nm, and the optimal excitation wavelength is 780 nm.

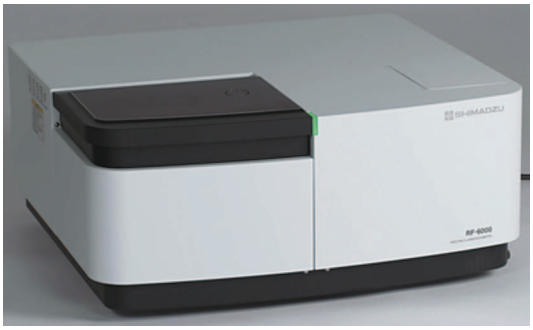

Fig. 1 RF-6000 Spectrofluorophotometer

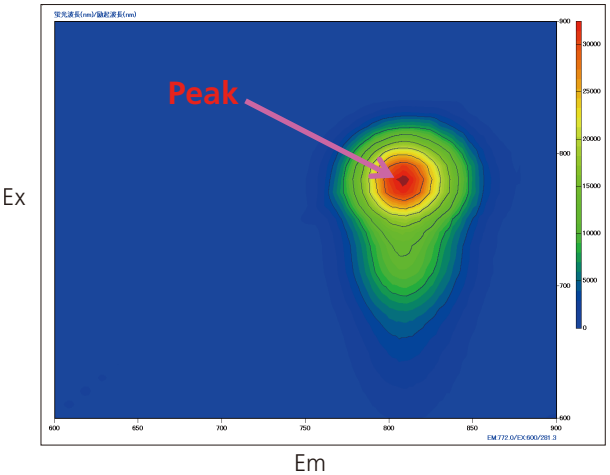

Fig. 2 Three-Dimensional Spectrum for Indocyanine Green

Table 1 Analytical Conditions Used for Three-Dimensional Spectrum

Instrument	: RF-6000 spectrofluorophotometer
Spectrum Type	: 3-dimensional spectrum
Measurement	
Wavelength Range	: Em: 600 - 900 nm, Ex: 600 - 900 nm
Scan Speed	: 2000 nm/min
Wavelength Interval	: Ex 10 nm, Em 2 nm
Bandwidth	: Ex 5 nm, Em 5 nm
Sensitivity	: High

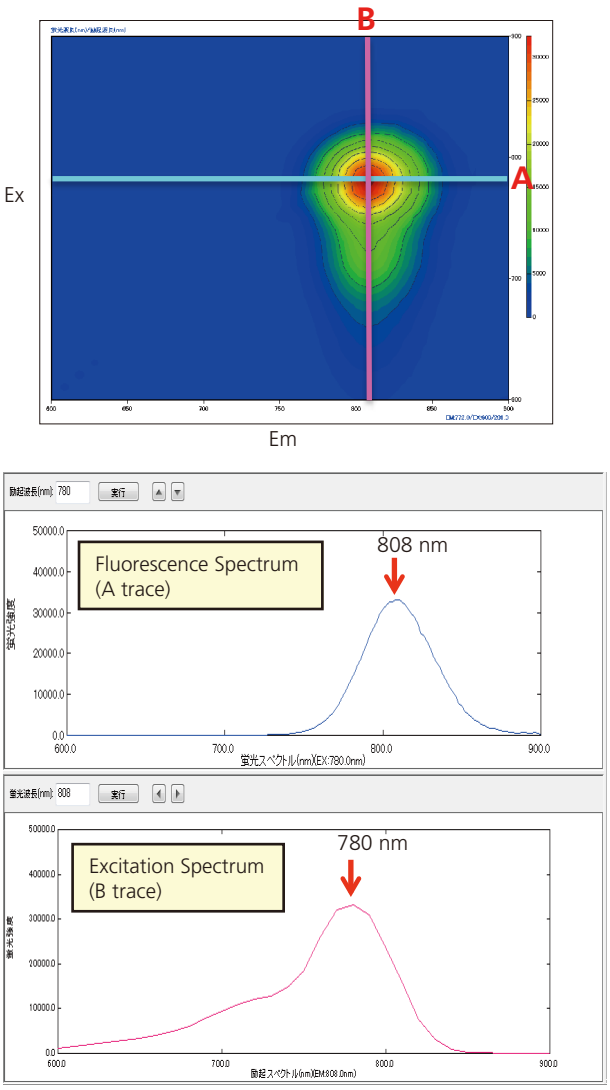

Fig. 3 Fluorescence Spectrum and Excitation Spectrum Extracted from Three-Dimensional Spectrum

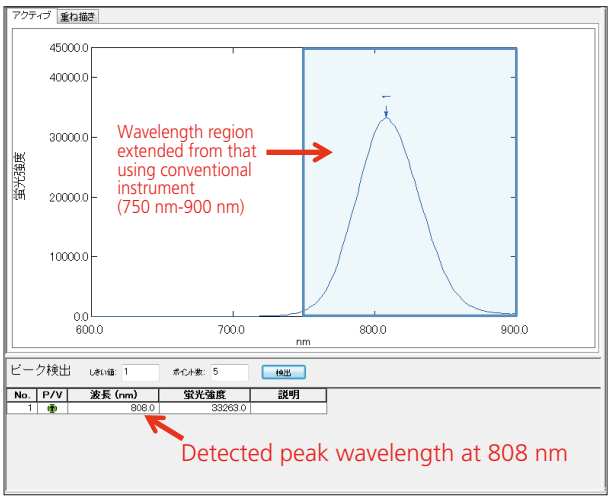

Fig. 4 Fluorescence Peak Detection

Table 2 Analytical Conditions for Fluorescence Spectrum Measurement

Instrument	: RF-6000 spectrofluorophotometer
Spectrum Type	: Fluorescence spectrum
Excitation Wavelength	: 780 nm
Measurement	
Wavelength Range	: 600 - 900 nm
Data Interval	: 2.0 nm
Scan Speed	: 200 nm/min
Bandwidth	: Ex 5 nm, Em 5 nm
Sensitivity	: High

Fluorescence Spectrum Measurement

Using the Spectrum function of the LabSolutions RF software, the results obtained from measurement of the fluorescence spectrum with a scan speed of 200 nm/min together with the peak detection results are shown in Fig. 4. The measurement conditions are shown in Table 2. Measurement with good sensitivity is clearly obtained up to the long wavelength region. The light blue region in Fig. 4 is the wavelength region (750 nm to 900 nm) in which measurement has now become possible.

Conclusion

The RF-6000 is an instrument that permits measurement over a wide wavelength range, from 200 nm to 900 nm. Using the RF-6000, we were able to conduct measurement of Indocyanine Green with its fluorescence peak in the vicinity of 810 nm with good sensitivity, a type of measurement that has been difficult with conventional instruments. Further, the newly included automatic spectrum correction function makes it possible to obtain corrected spectra in real time. The RF-6000 with its extended measurement range will permit its use in a wider range of new applications.

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Shimadzu Corporation
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