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Application News

No.A490

Spectrophotometric Analysis

Three-Dimensional Spectra Measurement of Fluorescent Probes used for DNA Detection

DNA probes labeled with fluorescent dye (below referred to as fluorescent probes) are used extensively to detect and identify specific DNA when conducting life science studies. The mechanism involves the selective binding of the probe to specific DNA, thereby permitting the detection of that DNA. However, due to the wide variety of fluorescent dyes, it is important to know the exact wavelength at which the probe fluoresces to ensure DNA detection.
Here, using the three-dimensional spectral measurement feature of the RF-6000 Spectrofluorophotometer, we introduce examples of fluorescence measurement of two types of fluorescent probes.

Instrument, Measurement Method and Results

An external view of the RF-6000 is shown in Fig. 1. Utilizing the three-dimensional spectral measurement and automatic spectrum correction features of the RF-6000 enables quick measurement over a wide wavelength range. The automatic spectrum correction feature eliminates any instrument-related effects, ensuring that accurate fluorescence and excitation spectra are obtained automatically.
Here, fluorescent probes A and B, labelled with different fluorescent pigments, were used as samples. Three-dimensional spectral measurement of each of the probes was then conducted. Here, as the fluorescence spectra were measured, mapped data images were acquired while sequentially changing the excitation wavelength. The horizontal axis represents the fluorescence spectrum (Em), while the vertical axis corresponds to the excitation spectrum (Ex).
The measurement results for the fluorescent probe A are shown in Fig. 2, and the results for fluorescent probe B are shown in Fig. 3. These are the three-dimensional spectra generated from the corrected spectra. The measurement time for each sample was about 3 minutes. As indicated with the arrows in Figs. 2 and 3, respectively, fluorescence peaks 1 and 2 were generated using fluorescent probe A, and fluorescence peak 1 was generated with fluorescent probe B.
Peak 1, corresponding to fluorescent probe A (Fig. 2), indicates fluorescence at approximately 600 nm, and excitation at 550 nm, while peak 2 indicates fluorescence at approximately 530 nm, and excitation at 510 nm. Peak 1, generated using fluorescent probe B (Fig. 3), displays fluorescence at 600 nm, and excitation at 550 nm.

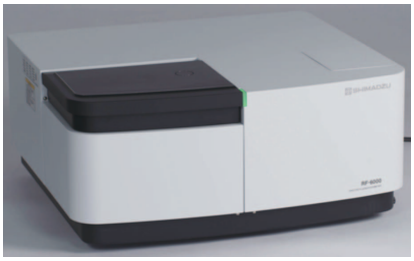

Fig. 1 RF-6000 Spectrofluorophotometer

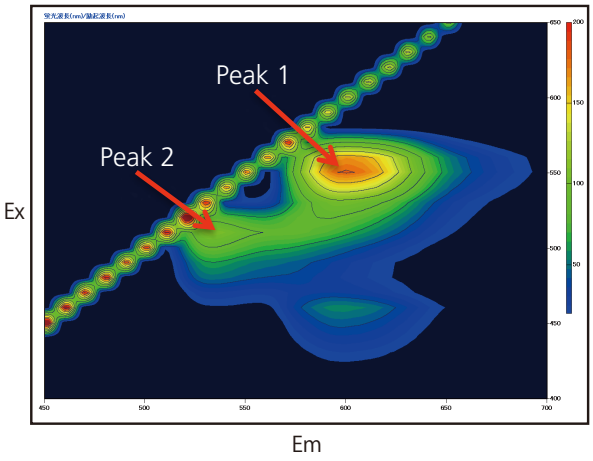

Fig. 2 Three-Dimensional Spectrum Using Fluorescent Probe A

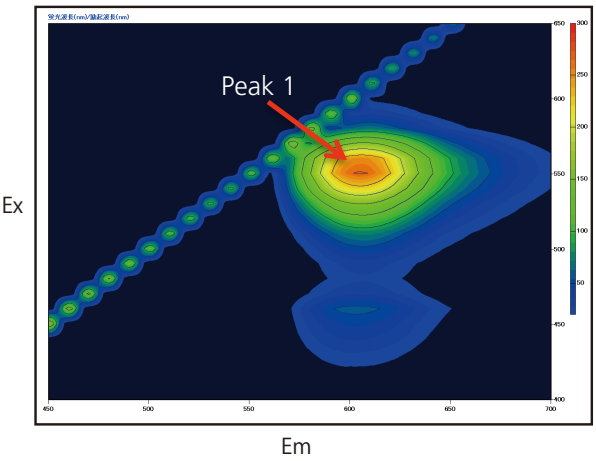

Fig. 3 Three-dimensional Spectrum Using Fluorescent Probe B

Table 1 Analytical Conditions

Instrument	: RF-6000 spectrofluorophotometer
Spectrum type	: Three-dimensional spectrum
Measurement wavelength range	: Ex 400 nm - 650 nm, Em 450 nm - 700 nm
Scan speed	: 2000 nm/min
Wavelength interval	: Ex 10 nm, Em 2 nm
Bandwidth	: Ex 5 nm, Em 5 nm
Sensitivity	: High

Measurement of Spectrum

It is possible to switch between measurement of the fluorescence spectrum and excitation spectrum at arbitrary coordinates using a three-dimensional spectrograph. In that case, the spectrum cut in the horizontal direction corresponds to the fluorescence emission spectrum, and that cut in the vertical direction corresponds to the excitation spectrum.
Here, we acquired peaks 1 and 2 of the fluorescence emission spectrum generated using fluorescent probe A (Fig. 2). The results are shown in Fig. 4 and Fig. 5. The fluorescence emission peak wavelength of peak 1 was determined to be 598 nm, and that of peak 2 was determined to be 532 nm.
As for fluorescent probe B (Fig. 3), both the fluorescence spectrum and excitation spectrum corresponding to peak 1 were acquired. The fluorescence spectrum is shown in Fig. 6, and the excitation spectrum, in Fig. 7. The fluorescence peak wavelength and optimum excitation wavelength were determined to be 604 nm and 550 nm, respectively.
The obtained three-dimensional spectra clearly demonstrate that a corrected spectrum unaffected by instrument-related attributes can be obtained, thereby permitting identification of the correct wavelength position of the fluorescence peak.

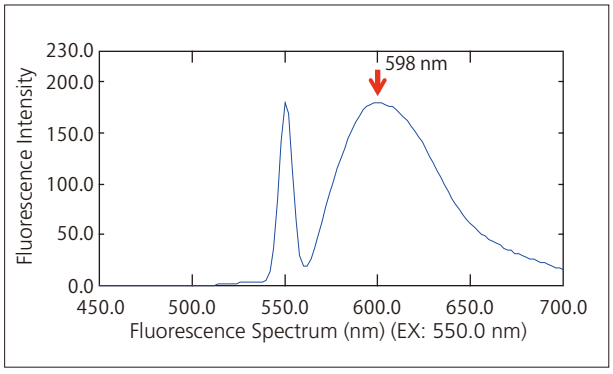

Fig. 4 Fluorescence Spectrum of Fluorescent Probe A (Ex 550 nm, Fig. 2 Peak 1)

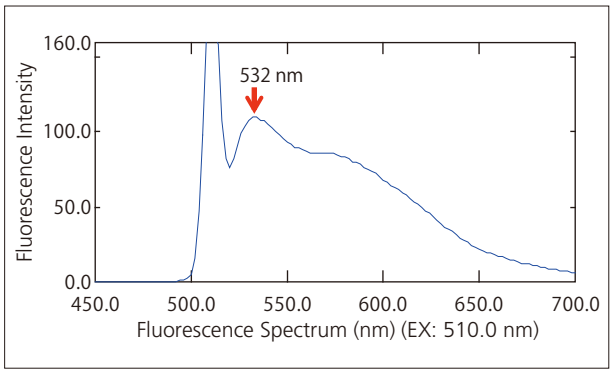

Fig. 5 Fluorescence Spectrum of Fluorescent Probe A (Ex 510 nm, Fig. 2 Peak 2)

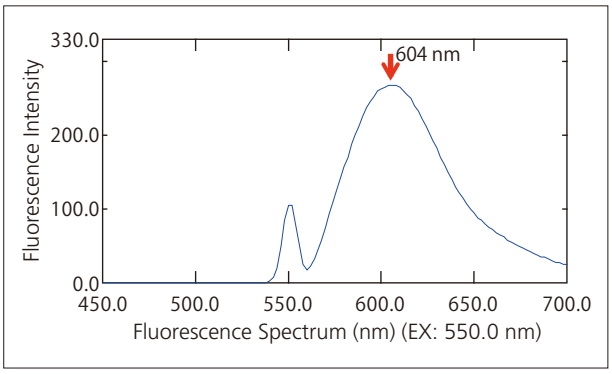

Fig. 6 Fluorescence Spectrum of Fluorescent Probe B (Ex 550 nm, Fig. 3 Peak 1)

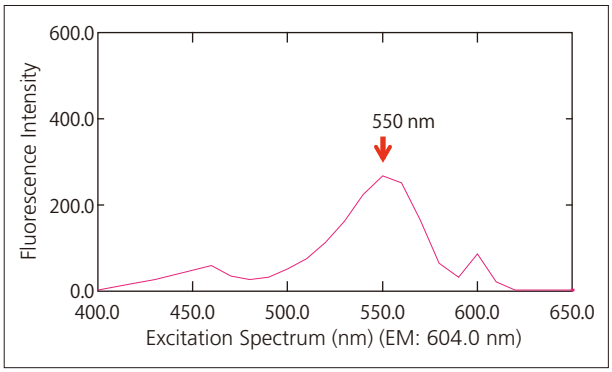

Fig. 7 Excitation Spectrum of Fluorescent Probe B (Em 604 nm, Fig. 3 Peak 1)

Conclusion

We conducted three-dimensional spectral measurement of fluorescent probes and were able to obtain corrected spectra automatically. Up to now, manual data processing was required to obtain corrected spectra, but with the RF-6000, accurate and efficient fluorescence measurement can be performed automatically.

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