

# Highly efficient CD/CPL systems for accurate simultaneous CD/Abs and CPL/FL measurements

Satoko Suzuki, Akio Kaneta, and Ken-ichi Akao

JASCO Corporation, Tokyo, Japan, <https://www.jasco.co.jp/jpn/home>

satoko.suzuki@jasco.co.jp

## Introduction

CD and CPL spectrometers are widely used for evaluation of the structure of chiral compounds, secondary structure estimation (SSE) for proteins, and CPL characterization of synthetic compounds. Most CD applications require not only the CD spectrum, but also the absorption (Abs) spectrum obtained using a UV/Vis spectrophotometer. For phosphors with CPL properties, the fluorescence (FL) spectrum of the sample is recorded using a spectrofluorometer, and the CPL and DC spectra are measured using a CPL spectrometer to obtain the  $g_{lum}$  value. Here, we describe example measurements using a JASCO J-1500 CD spectrometer, which can simultaneously record highly accurate CD and Abs spectra. The method for obtaining the FL spectrum using a CPL-300 CPL spectrometer and the accuracy of the spectral shape are discussed. An example of simultaneous measurement of CPL and FL spectra with the correct spectral shape for fluorescent substances such as Eu (facam)<sub>3</sub> and camphor is presented.

## Experiment

### Measurement System



#### J-1500 CD Spectrometer

- Simultaneous measurement of CD, Abs, and FL spectra
- High S/N data can be acquired

#### V-760 UV/Vis Spectrophotometer

- Excellent photometric and wavelength accuracy
- Measurable up to Abs 6

#### CPL-300 CPL Spectrometer

- 180° sample configuration to reduce artifacts
- Low stray light and no Wood's anomalies
- Highly sensitive detection by lock-in amplifier

#### FP-8550 Spectrofluorometer

- Best-in-class sensitivity
- User-friendly operation
- Instrument maintenance functions

### Procedure

#### 1. Evaluation of Abs Accuracy of J-1500

The Abs value for the standard NIST SRM 930 sample for determining the photometric accuracy of spectrophotometers was obtained using the HT-Abs formula in advance, and the accuracy was calculated. In addition, the absorption spectra of IgG aqueous solutions with different concentrations were measured using the J-1500 and V-760 and the results were compared.

#### 2. Comparison of SSE using Abs value determined using J-1500 and V-760

CD and absorption spectra of the proteins were measured using the J-1500, and absorption spectra were also measured using the V-760. The concentration of each sample was calculated from the measured Abs<sub>214</sub> using the  $\epsilon_{214}$  obtained from the amino acid sequence. The vertical axis was converted to mean residue ellipticity (MRE), and SSE was performed using BestSel.

#### 1. Evaluation of Accuracy of FL Spectral Shape

The DC spectrum of a sample with a known FL spectrum was measured using the CPL-300. Spectral correction was performed, and the shapes of the corrected and reference spectra were compared.

#### 2. Simultaneous Measurements of CPL and FL spectra

CPL and DC spectra of typical CPL samples were measured using the CPL-300, and FL spectra of the samples were obtained using the FP-8550. Spectral correction was performed for each, and the shapes of the corrected spectra were compared.

## Samples

### CD Measurements

#### 1. Evaluation of Abs Accuracy

- NIST SRM 930
- 0.1, 0.21, 0.43, 0.64, 0.88 mg/L IgG from human serum in water

#### 2. SSE using Abs measured by J-1500 and V-760

- 0.16 mg/mL Herceptin® in water
- 0.1 mg/L IgG from human serum in water

### CPL Measurements

#### 1. Evaluation of FL Spectral Shape

- NIST SRM 2943
- 0.77 mg/L tryptophan in water, 0.16 mg/L tetraphenylbutadiene (TPB) in cyclohexane, 0.16 mg/L coumarin 153 in methanol, 0.04 mg/L 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM) in methanol

#### 2. Measurement of CPL and FL spectra

- 5g/L Eu(facam)<sub>3</sub> in DMSO, 4 g/L (+)-camphor, (-)-camphor, (±)-camphor in ethanol

## SSE



<http://bestsel.elte.hu/index.php>

- Online Web server used worldwide.
- Superior SSE performance

- 1) A. Micsonai, F. Wien, L. Keryna, Y.H. Lee, Y. Goto, M. Refregiers, J. Kardos, PNAS, **112** (2015) 3095-3103.
- 2) A. Micsonai, F. Wien, E. Bulyaki, J. Kun, E. Moussong, Y.H. Lee, Y. Goto, M. Refregiers, J. Kardos, Nucleic Acids Res, **46** (2018) 315-322.

## Spectral Correction

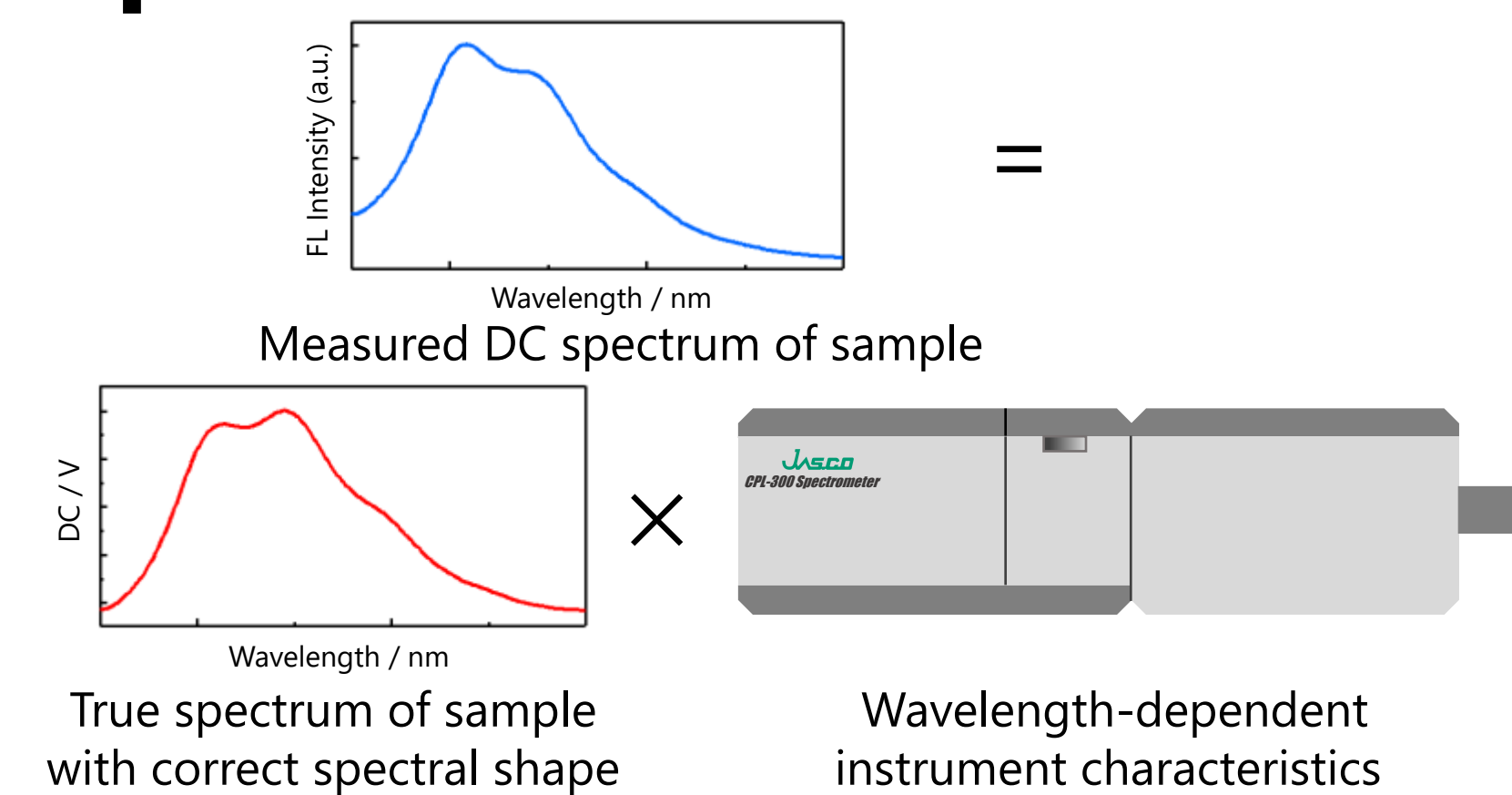


Figure 5. Need for spectral correction



### Light Source for Spectral Correction

- Halogen and deuterium lamps with emission spectra linked to national measurement standards.

## Results and Discussion

### Accuracy of Abs value for J-1500

- The results for the NIST SRM 930 sample using the J-1500 gave a photometric accuracy of  $\pm 0.01$  or less (Table 1), indicating that the J-1500 has excellent measurement accuracy for a single-beam spectrophotometer.

Table 1. Abs accuracy for J-1500

Criterion	0.5577	0.7297	1.033
Average	0.5614	0.7327	1.0391
Deviation	0.004	0.003	0.006

### Comparison of Abs Between J-1500 and V-760

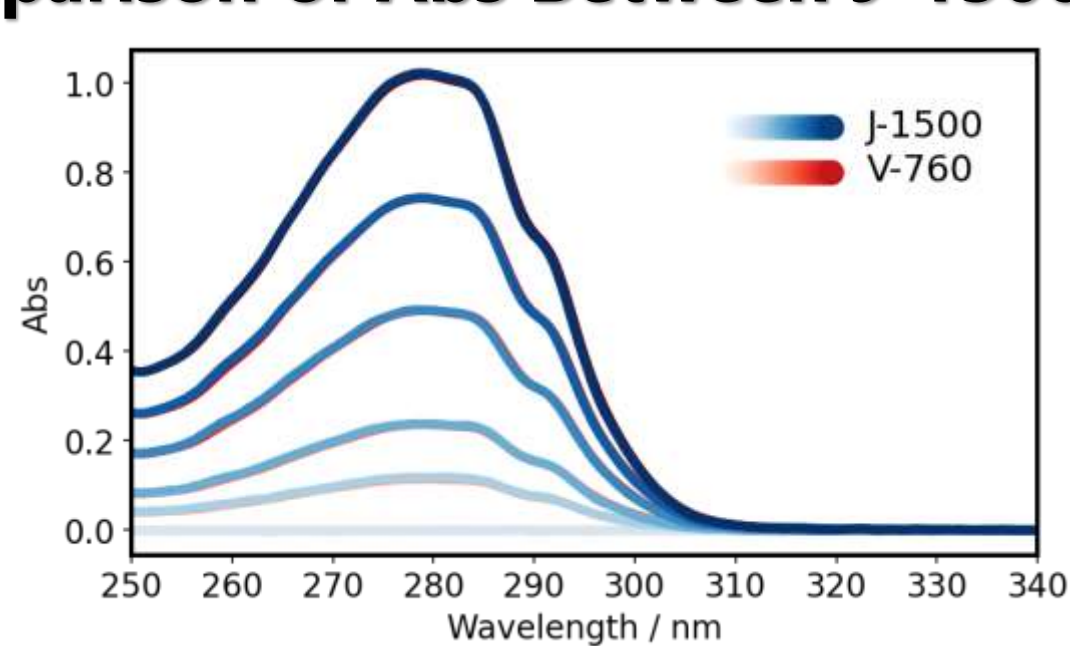


Figure 1. Abs spectra of IgG

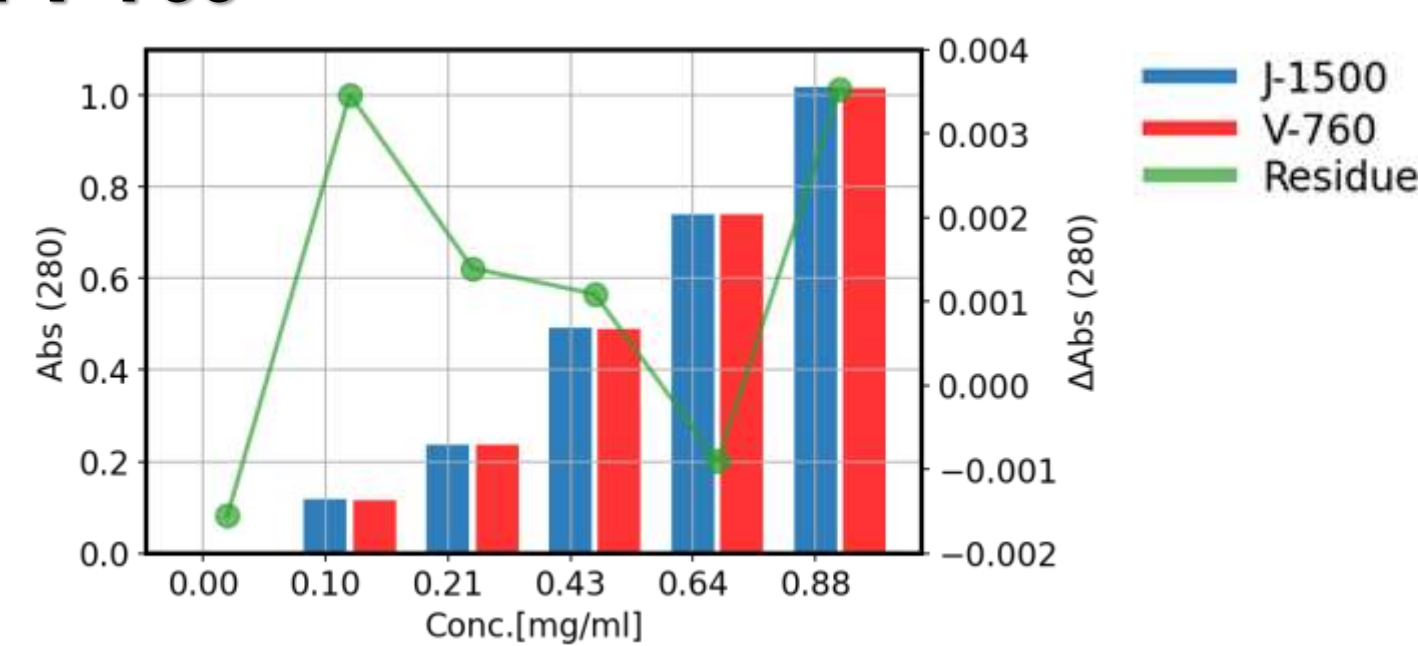


Figure 2. Abs and difference for IgG at 280 nm

- The results revealed that the difference between the J-1500 and V-760 at 280 nm was  $\pm 0.005$  Abs or less, and the difference was very small (Fig. 2).

### CD, Abs, and MRE Spectra

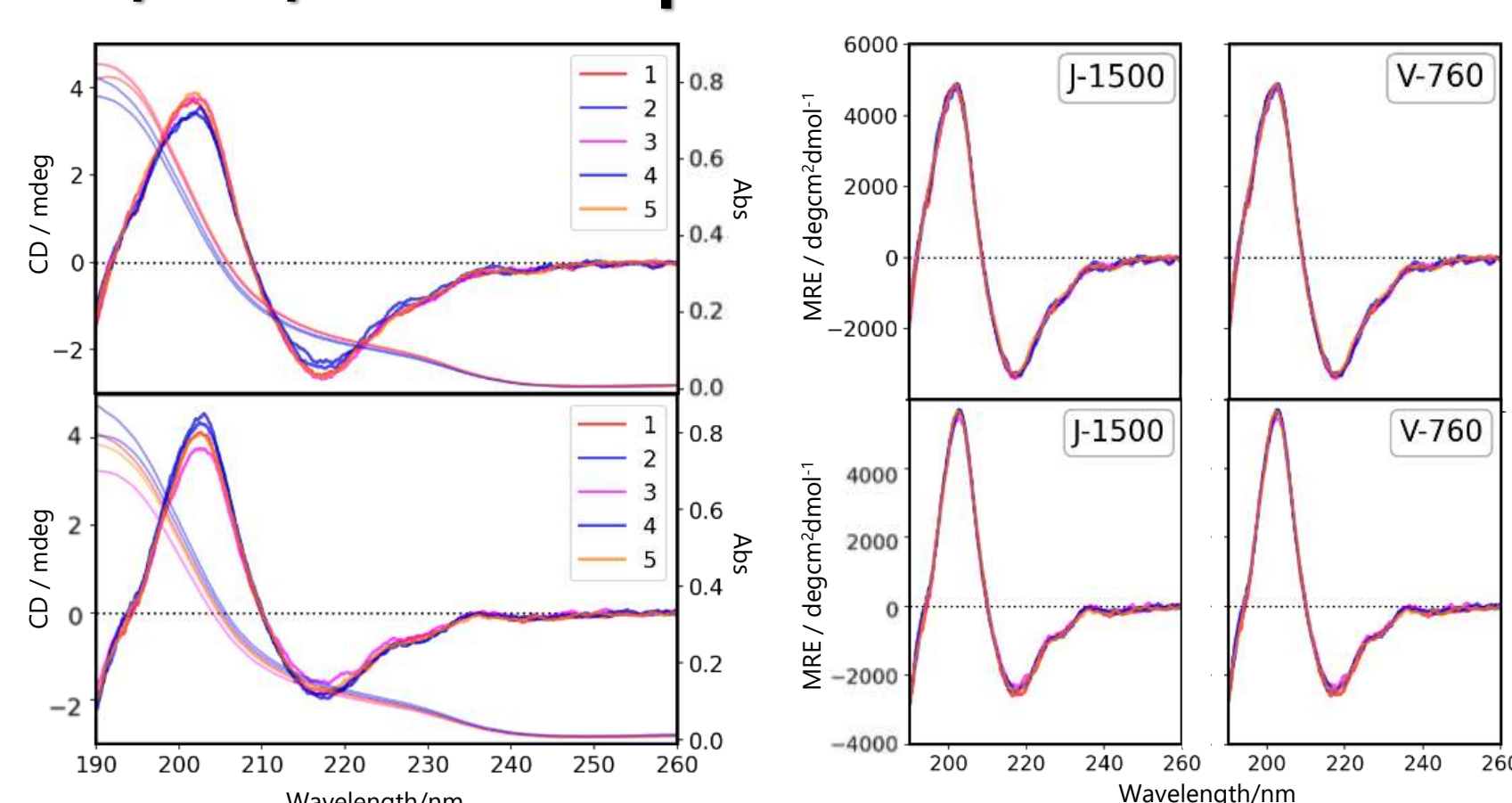


Figure 3. (a) CD and Abs spectra (Top panel: IgG, bottom panel: Herceptin)

- No significant difference was found in the MRE calculated using the absorbance obtained with either instrument.

### Results of SSE

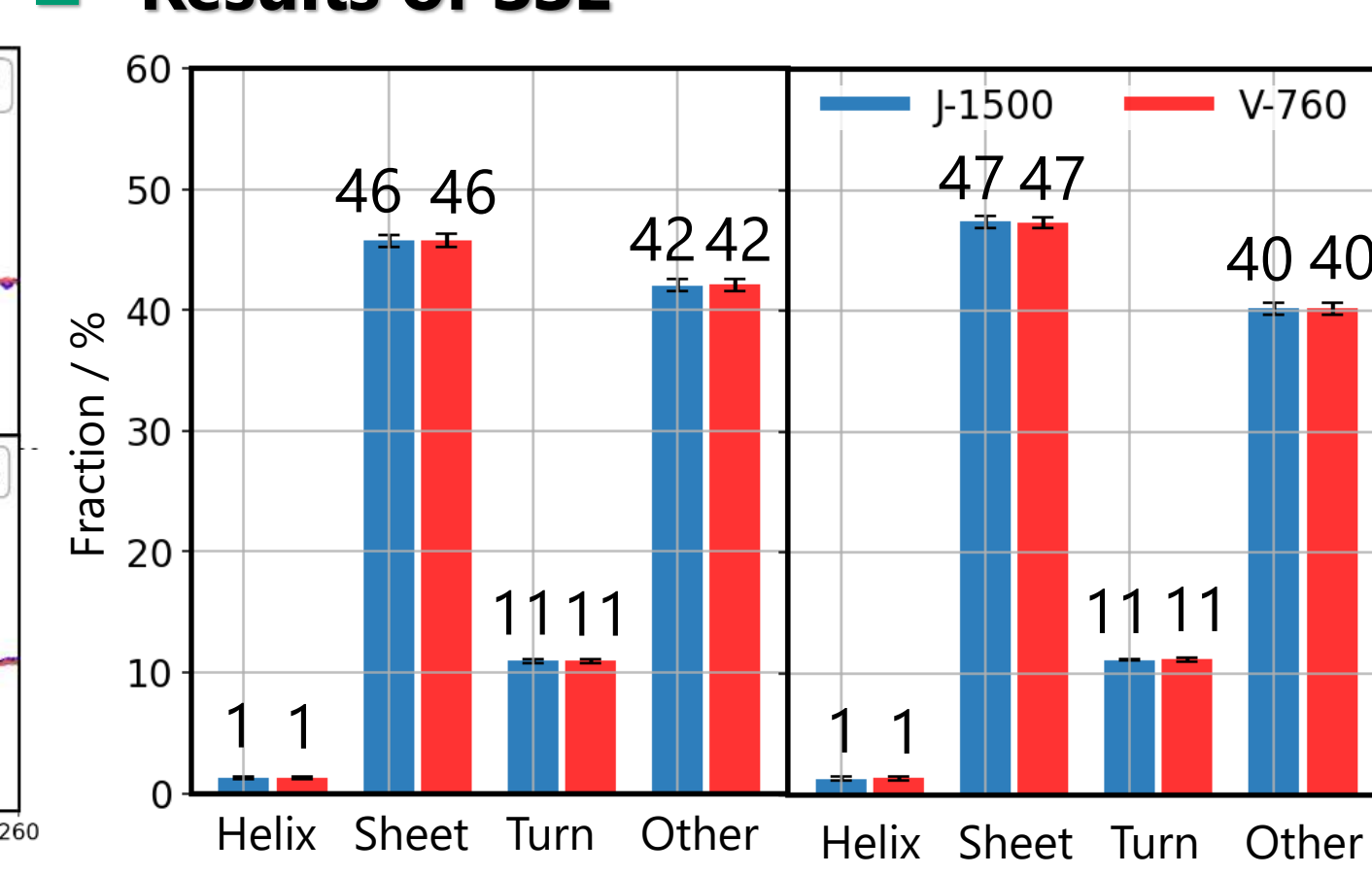


Figure 4. Results of SSE (Left: IgG, Right: Herceptin)

- The absorbance difference between the J-1500 and V-760 does not affect the results of SSE.

## Conclusion

### CD/Abs Measurements using CD Spectrometer

- The J-1500 CD spectrometer exhibited excellent photometric accuracy for a single-beam instrument.
- The absorbance difference between the J-1500 and V-760 did not affect the results of SSE. Therefore, it can be concluded that the J-1500 has sufficient absorbance measurement accuracy for performing detailed assessments.

### CPL/FL Measurements using CPL Spectrometer

- It was demonstrated that fluorescence spectra with the correct shape can be obtained over a wide wavelength range by performing spectral correction of the DC signal measured by the CPL-300.
- It may be useful to perform spectral correction not only for the DC spectrum but also for the CPL spectrum to compare the spectral shapes between instruments.

### Accuracy of FL Spectral Shape for CPL-300

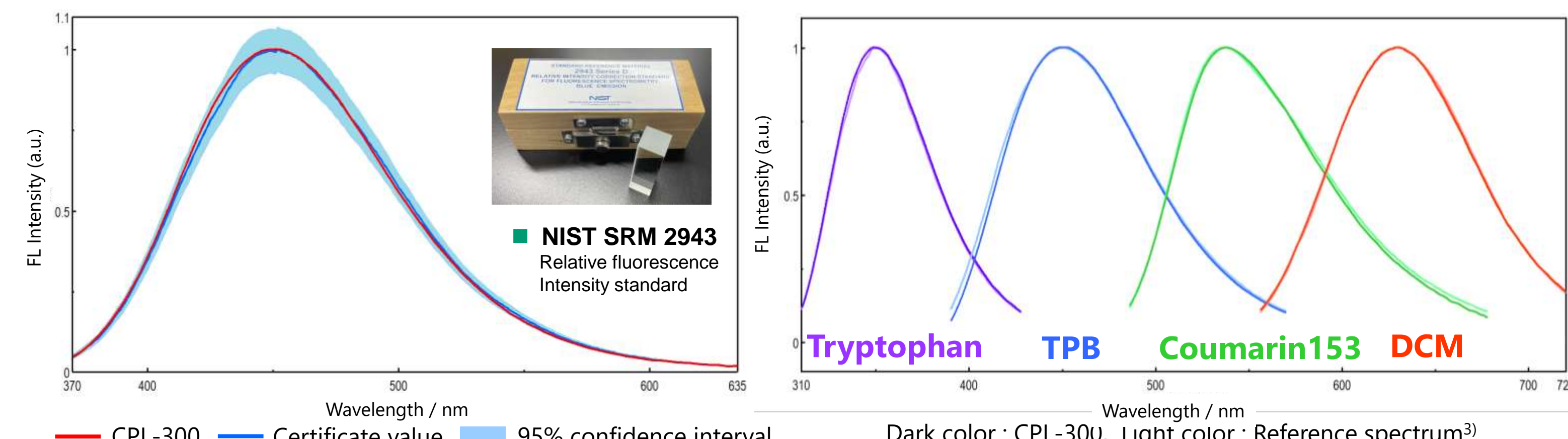


Figure 6. FL spectra of NIST SRM 2943

Figure 7. FL spectra of secondary FL standard solutions

- The FL spectrum can be obtained with high accuracy using the CPL-300.
- FL spectra with the correct shape can be obtained over a wide wavelength range.

3) J. A. Gardecki, M. Maroncelli, Appl. Spectrosc., **52** (1998) 1179-1189.

### Simultaneous Measurements of CPL and FL spectra

#### Eu(facam)<sub>3</sub> in DMSO

#### Camphor in Methanol

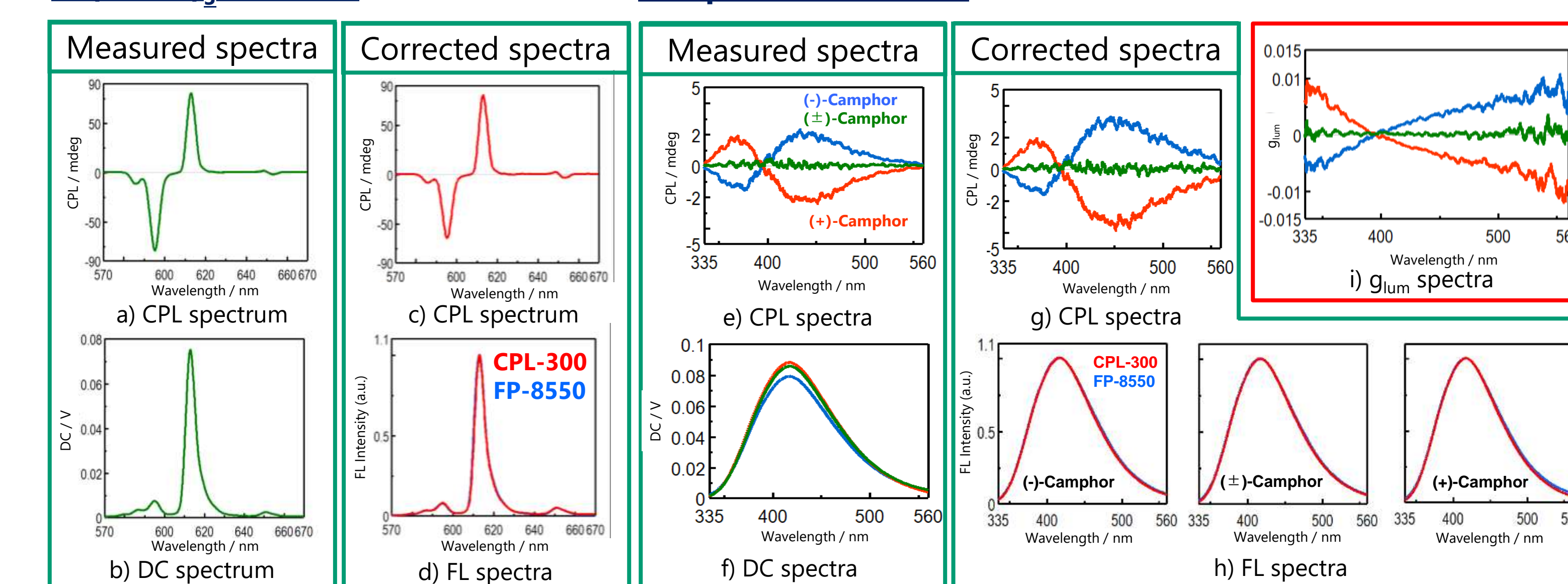


Figure 8. CPL and FL spectra before and after spectral correction, and  $g_{lum}$  spectra

- FL spectra obtained by CPL-300 and FP-8550 showed good agreement.