

Chapter 14

Principles and Applications of Circularly Polarized Luminescence Spectrophotometer



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Abstract Materials that exhibit circularly polarized light characteristics are finding an increasing range of applications, such as liquid crystal display backlights, three-dimensional displays, holographic displays, plant growth control illumination, security systems for optical communications, and printing. In this chapter, we will describe the basic principles of circularly polarized luminescence (CPL) spectroscopy, the instruments used to measure CPL signals, including optical systems and signal acquisition methods in commercially available CPL instruments, and also methods for calibrating such equipment. Example measurements are presented for camphor and camphorquinone, which are long-established CPL samples, in addition to lanthanoid complexes that are promising CPL luminescent materials, biopolymers that are highly sensitive to circularly polarized light, and solid samples that have been attracting attention in recent years.

14.1 Introduction

Circular dichroism (CD) spectroscopy is widely used in the study of optically active substances. However, in recent years, circularly polarized luminescence (CPL) spectroscopy has also attracted a great deal of attention. The two methods are complimentary to each other. Materials that exhibit circularly polarized luminescence are currently being actively investigated for applications in fields such as liquid crystal display backlights, three-dimensional (3D) displays, holographic displays, light sources for controlling plant growth, and security systems for optical communications and printing. It is therefore important to identify molecules that emit one-handed circularly polarized light (large g_{lum}) with a high quantum yield. Consequently, there is a need for the development of highly sensitive CPL measuring instruments.

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In this report, we introduce the basic principles of CPL measurements, which include the meaning of the signals obtained in CPL spectroscopy, the type of equipment used, the calibration methods, and measurement examples.

14.2 Introduction to CPL

CPL can be best described by comparing it to CD, which involves a difference in the amount of absorption of left- and right-handed circularly polarized light by an optically active substance, as shown in Fig. 14.1a. In contrast, as shown in Fig. 14.1b, CPL spectroscopy measures the difference in the intensity of left- and right-handed circularly polarized fluorescence during optical excitation of a sample.

The magnitude of the CD and CPL signals is expressed by Eqs. (14.1) and (14.2), respectively.

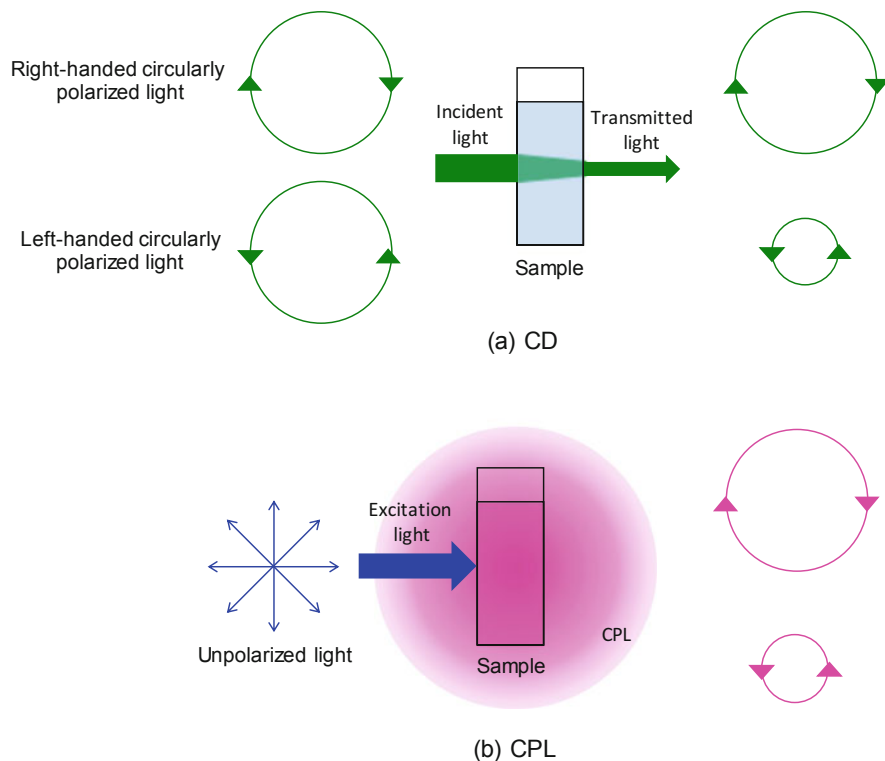


Fig. 14.1 Comparison of CD (a) and CPL (b)

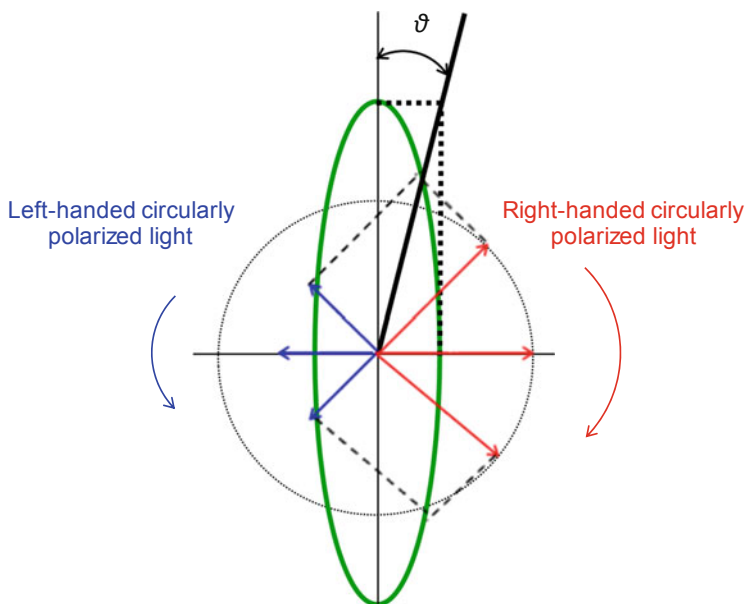


Fig. 14.2 Ellipse angle (θ) for CD and CPL signals

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R \quad (14.1)$$

$$\Delta I = I_L - I_R \quad (14.2)$$

ε extinction coefficient for left- (L) and right-handed (R) circularly polarized light, I fluorescence intensity of left- (L) and right-handed (R) circularly polarized light

CD and CPL signals are generally normalized by the averaged absorbance and the averaged fluorescence intensity for a sample, and are expressed in terms of g_{abs} and g_{lum} values:

$$\text{CD} : g_{\text{abs}} = \frac{2(\varepsilon_L - \varepsilon_R)}{\varepsilon_L + \varepsilon_R} \quad (14.3)$$

$$\text{CPL} : g_{\text{lum}} = \frac{2(I_L - I_R)}{I_L + I_R} \quad (14.4)$$

Both g_{abs} and g_{lum} take values between -2 and 2 . When only left-handed circularly polarized light is absorbed or emitted, the g value is $+2$, while when only right-handed circularly polarized light is absorbed or emitted, it is -2 .

The vertical axis in a CD or CPL spectrum is traditionally the ellipse angle θ [mdeg]. Figure 14.2 shows the overall polarization trajectory for a mixture of left- and right-handed circularly polarized light. If there is a difference in the intensity of the left- and right-handed polarized light, the overall trajectory is ellipse.

14.3 Instrumentation

14.3.1 *Optical Layout in JASCO CD and CPL Spectrometer*

Figure 14.3 shows schematics of the optics for CD and CPL spectroscopy. In a CD spectrometer, as illustrated in Fig. 14.3a, light from a light source is dispersed by a monochromator, and is linearly polarized by a polarizer. In contrast, a commercially available CD spectrometer (e.g., JASCO J-1000 series) is capable of not only monochromating light but also producing linearly polarized light using a quartz prism. This linearly polarized light is modulated at a frequency of 50 kHz using a photo-elastic modulator (PEM) to produce left- and right-handed circularly polarized light. This circularly polarized light is introduced to a sample, and the difference in the transmitted left- and right-handed circularly polarized light is measured using a detector. Figure 14.3b shows a schematic diagram of optics layout in CPL measurements. Light from a light source is first passed through a monochromator, and the sample is then irradiated by nonpolarized light. The left- and right-handed circularly polarized fluorescence emitted from the sample is alternately linearly polarized light at a frequency of 50 kHz using a PEM. A polarizer is placed at the back of the PEM, and the polarized fluorescence is transmitted synchronously with the modulation of the PEM, and is passed through a monochromator and then detected by a detector. The CPL signal corresponds to the difference in fluorescence intensity between left- and right-handed circularly polarized light.

14.3.2 *Phase-Sensitive Detection of CD and CPL With Photo-Elastic Modulator (PEM)*

As shown in Fig. 14.4a, a PEM is an optical device consisting of an isotropic optical material such as quartz, and a piezo actuator. The polarization of the incoming light

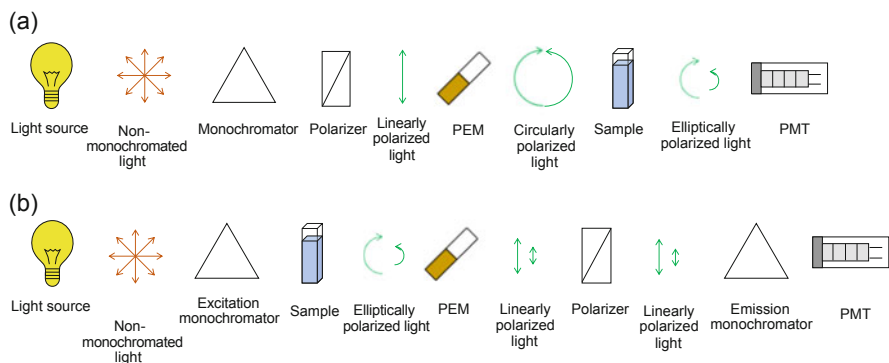


Fig. 14.3 Schematic diagram of optics layouts in CD (a) and CPL (b) measurement principles

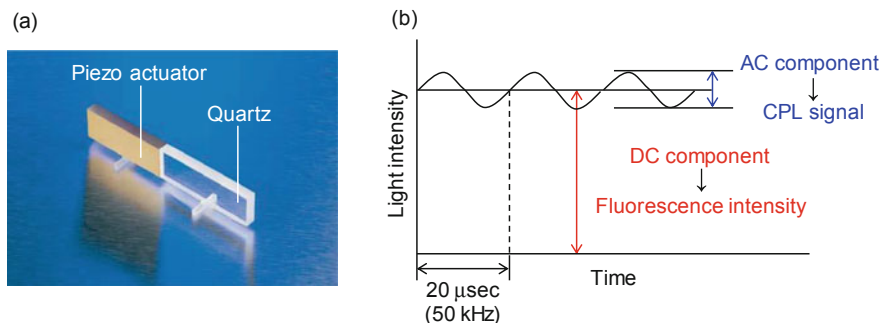


Fig. 14.4 Phase-sensitive detection of CPL using PEM. (a) Appearance of PEM. (b) Time-domain signal of CPL and fluorescence

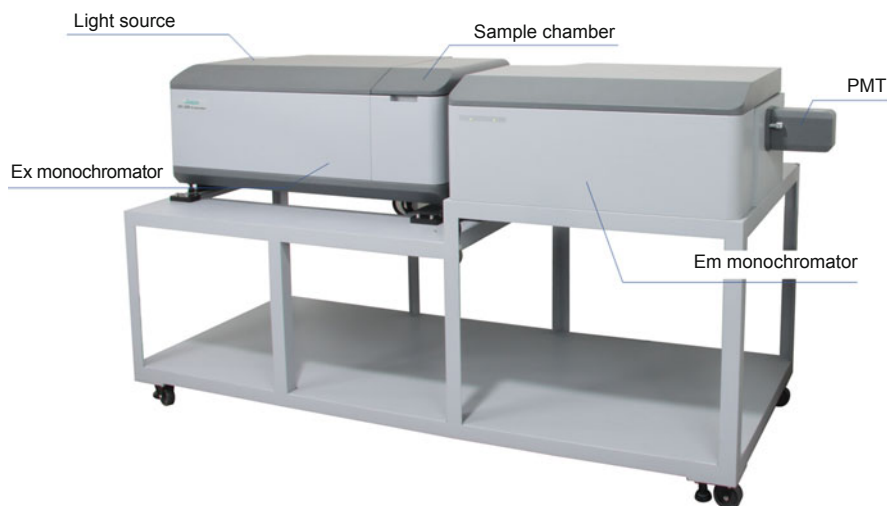


Fig. 14.5 External view of CPL-300

can be changed by expanding and contracting the piezo actuator at a particular frequency, thereby applying a periodic stress to the quartz to produce birefringence. During CPL measurements, left- and right-handed circularly polarized fluorescence emitted by the sample is converted to linearly polarized light synchronously with the PEM frequency. This is transmitted through the polarizer and then detected. When a sample exhibits CPL, an alternating current (AC) signal is produced as shown in Fig. 14.4b, and its amplitude corresponds to the CPL signal intensity. The direct current (DC) component corresponds to the normal fluorescence spectrum.

We next describe the JASCO CPL-300 circularly polarized luminescence spectrophotometer (Fig. 14.5), as an example.

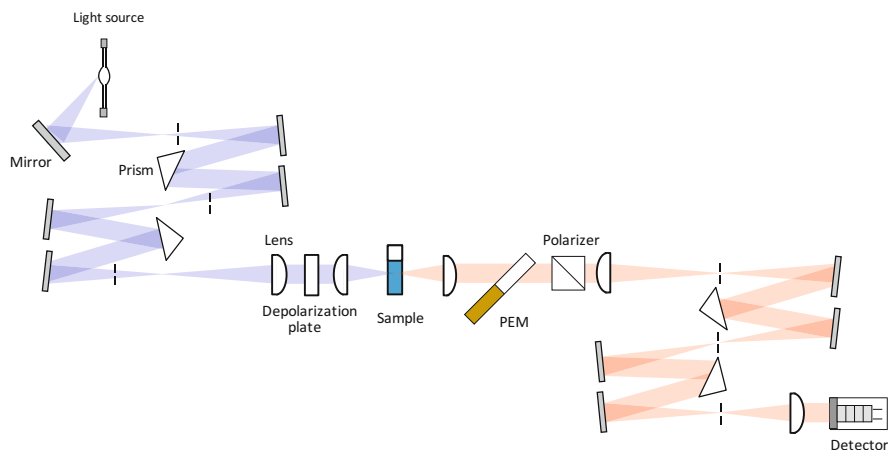


Fig. 14.6 Optical system for CPL-300

Figure 14.6 shows a schematic of the optical system used in the CPL-300. The light source for CPL measurements can be a laser, a xenon or mercury xenon lamp, or a light-emitting diode (LED). In the CPL-300, a high-brightness xenon light source is installed by default and the excitation wavelength can be varied depending on the sample. The light is first passed through a monochromator that uses a prism or a diffraction grating as the dispersive element. This light is then depolarized by a quartz depolarizer and irradiates the sample. Since CPL signals are generally very weak, the instrument must be capable of high-sensitivity measurements that are free from artifacts. The CPL-300 uses a double-prism monochromator in order to achieve low stray light levels, avoid higher-order light due to a diffraction grating and light polarization due to Woods anomalies, and minimize the distortion of the CPL spectrum. Circularly polarized fluorescence emitted from the sample should be collected by the photo-detector placed in the angle of 90° or 180° toward the extinction direction. The light from the sample first passes through a PEM and polarizer, and is then passed through a monochromator and detected by a detector. In the 90° arrangement, although the detected light is less likely to be affected by excited scattered light, in the case of highly viscous or solid samples, there is a possibility that the CPL spectrum will be distorted by fluorescence anisotropy. To avoid this, the CPL-300 employs the 180° arrangement. A highly sensitive photomultiplier tube is used for the photo-detector.

14.4 Calibration of CPL Instrument

The most important aspect of CPL measurements is the accuracy of the spectrum. It is therefore important to have a reliable calibration method. In ultraviolet and visible spectrophotometers, fluorescence spectrophotometers, and circular dichroism

spectrometers, the most common method of calibrating the wavelength is to use a bright line spectrum. In the CPL-300, a low-pressure mercury lamp is built into both the excitation and emission monochromators, and the wavelength is calibrated at the wavelength of 546.1 nm of a bright emission line. This is done first for the emission monochromator and then for the excitation monochromator.

A (1S)-(+)-10-ammonium camphorsulfonate aqueous solution is used to calibrate the CD intensity. The CD value at 290.5 nm for a 0.06% (w/v) aqueous solution of this substance is known to be +190.4 mdeg for an optical path length of 10 mm, and this is used for the scale standard for CD measuring instruments [1]. In the CPL-300, this solution is placed in the sample compartment, and the wavelengths of both the excitation and emission monochromators are set at 290.5 nm. Since the difference in the transmitted light intensity for left- and right-handed circularly polarized light is accurately known, this is used to calibrate the instrument, as described in detail below.

The CD value for (1S)-(+)-10-ammonium camphorsulfonate aqueous solution is mentioned above, indicating that absorption of left-handed circularly polarized light is larger than that of right-handed circularly polarized light, as shown in Eq. (14.1). This difference of transmitted left- and right-handed polarized light will be the clear standard of the intensity for CPL spectrometer. When both excitation and emission wavelengths of CPL spectrometer are then set to 290.5 nm, the sign of the signal is negative and the intensity is -190.4 mdeg according to Eq. (14.2).

14.5 Measurement Examples

Substrates that show CPL are widely authorized including organic compounds, metal complexes, fluorescent proteins, and colloidal molecules. The following sections describe examples involving camphor, camphorquinone, lanthanoid complex, and green fluorescent protein (GFP). Finally, CPL measurements of solid samples are discussed, since such applications have recently been attracting attention.

14.5.1 *Camphor and Camphorquinone*

These compounds have been the subject of CPL measurements for a long time. Figure 14.7a, b show CD, CPL, absorption, and fluorescence spectra of camphor and camphorquinone, respectively.

Because there are two carbonyl groups in camphorquinone, compared to just one in camphor, the structural difference between the ground and excited states for

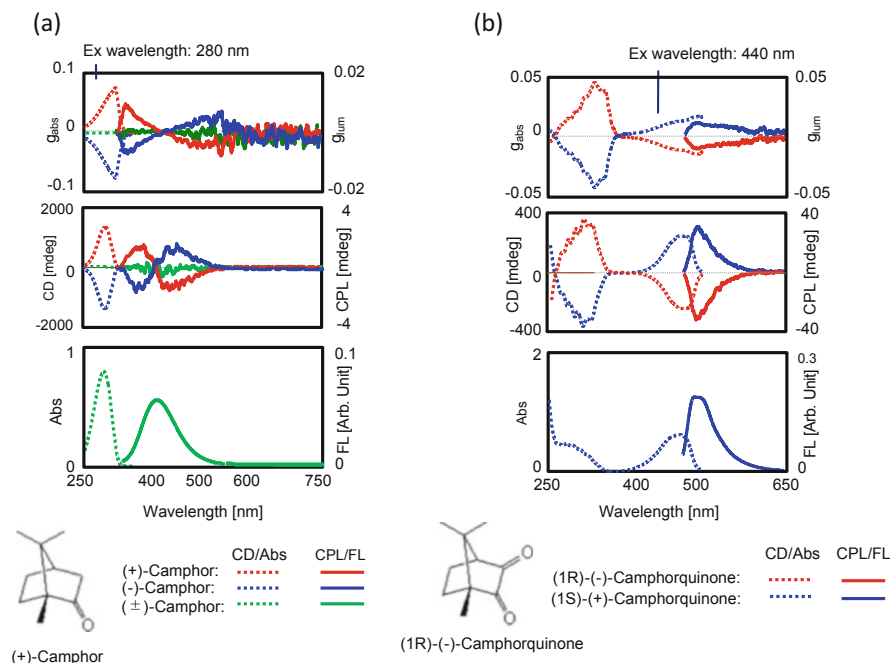


Fig. 14.7 CD, CPL, absorption, and fluorescence spectra of camphor (a) and camphorquinone (b)

camphorquinone is predicted to be smaller because the molecule has a rigid structure. This is supported by the fact that although CD and CPL spectra associated with $n-\pi^*$ transitions in carbonyl groups have been observed for both camphor and camphorquinone, g_{abs} and g_{lum} for camphorquinone are closer than those for camphor [2].

14.5.2 Lanthanoid Complexes

Figure 14.8 shows CPL and fluorescence spectra of the lanthanoid complex (3-(trifluoromethylhydroxymethylene)-(+)-camphorate: $\text{Eu}(\text{facam})_3$). Such complexes are widely used as LED materials because of their strong sharp emissions. They also potentially possess an ability in applications such as 3D displays and security markers. The synthesis and evaluation of lanthanoid complexes exhibiting CPL are underway. It is generally known that forbidden transitions such as $n-\pi^*$, $d-d$, and $f-f$ produce a larger g_{lum} than allowed transitions such as $\pi-\pi^*$ [3]. Muller et al. reported europium complexes with a large g_{lum} of +1.38 [4]. In Fig. 14.8, it can be seen that $\text{Eu}(\text{facam})_3$ also exhibits a large g_{lum} . It should be noted that

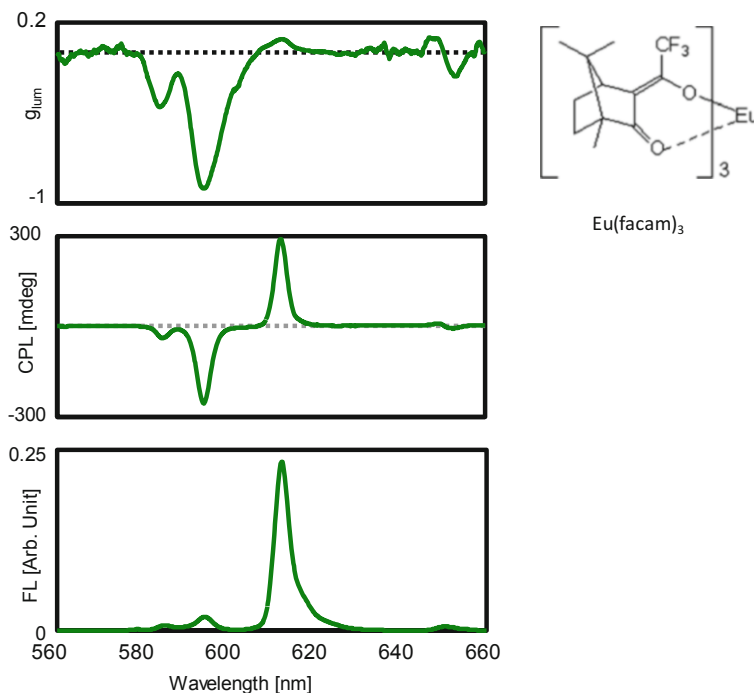


Fig. 14.8 CPL and fluorescence spectra of 5.5-mM $\text{Eu}(\text{facam})_3$ in dimethyl sulfoxide (DMSO) at room temperature

measurements of such sharp CPL spectra require careful setting of bandwidth for the quantitative evaluation of CPL intensity.

Figure 14.9a shows the change in the CPL spectrum of $\text{Eu}(\text{facam})_3$ with fluorescence bandwidth. As the fluorescence bandwidth is increased, the CPL spectrum becomes broader, and g_{lum} in the vicinity of 595 nm decreases slightly. Therefore, for compounds showing a very sharp CPL spectrum like $\text{Eu}(\text{facam})_3$, it is necessary to use a small fluorescence bandwidth to perform high-resolution measurements. Figure 14.9b shows a comparison of the fluorescence spectrum obtained using the CPL-300 with a fluorescence bandwidth of 3 nm and that obtained using the general-purpose FP-8300 fluorescence spectrophotometer with a fluorescence bandwidth of 2.5 nm. The good agreement between these spectra reveals that the CPL-300 is capable of high-resolution measurements. Since a trade-off relation exists between the bandwidth of the instrument and the signal-to-noise ratio (S/N), when measuring faint CPL signals at high resolution, cumulative measurements should be performed.

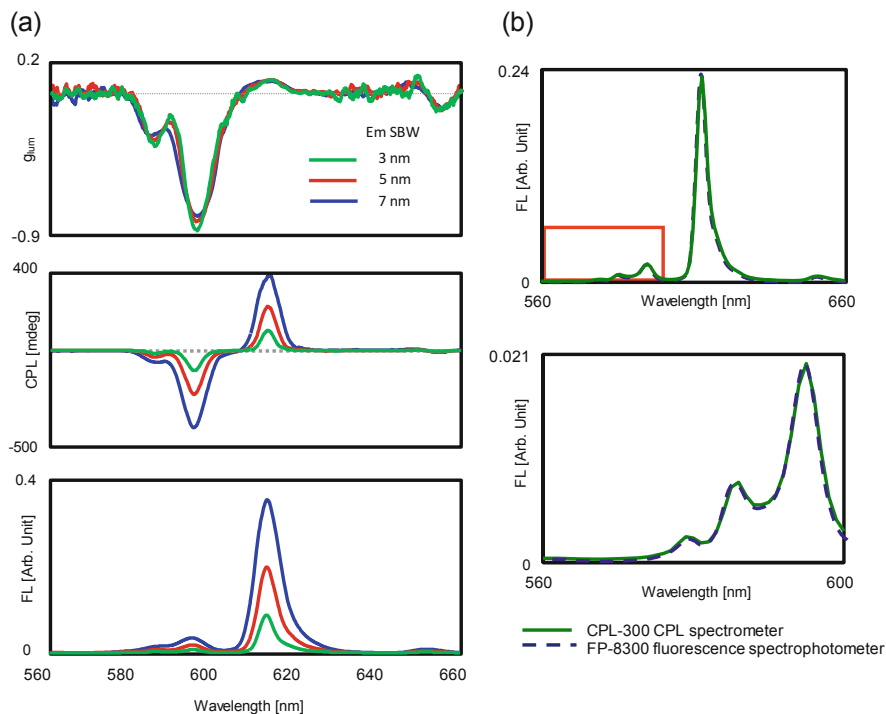


Fig. 14.9 High-resolution CPL spectra. **(a)** CPL spectra in various bandwidth settings. **(b)** Comparison of CPL-300 (Em SBW = 3 nm) and general purpose fluorescence spectrophotometer (Em SBW = 2.5 nm)

14.5.3 Green Fluorescent Proteins

Many biological organisms respond to circularly polarized light. For example, the crustacean *Odontodactylus scyllarus* can detect circularly polarized light [5], and the growth rate of seaweed is promoted by right-handed circularly polarized light but is disturbed by left-handed circularly polarized light [6]. The reason why circularly polarized light affects biological communication of creatures and plant growth is considered to be the optical activity of the organism itself. We therefore performed measurements on green fluorescent protein (GFP) derived from a biological organism.

Figure 14.10a shows the CPL spectrum of wild-type GFP. Although the sample concentration was only 30 $\mu\text{g}/\text{mL}$ and the optical path length was only 10 mm, the CPL signal could be clearly observed by increasing the number of accumulations. Although the S/N could be further improved by using a larger number of accumulations, proteins can become photodegraded under prolonged illumination. The occurrence of photodegradation of this GFP was therefore investigated using a small excitation bandwidth in order to decrease the excitation light intensity, and

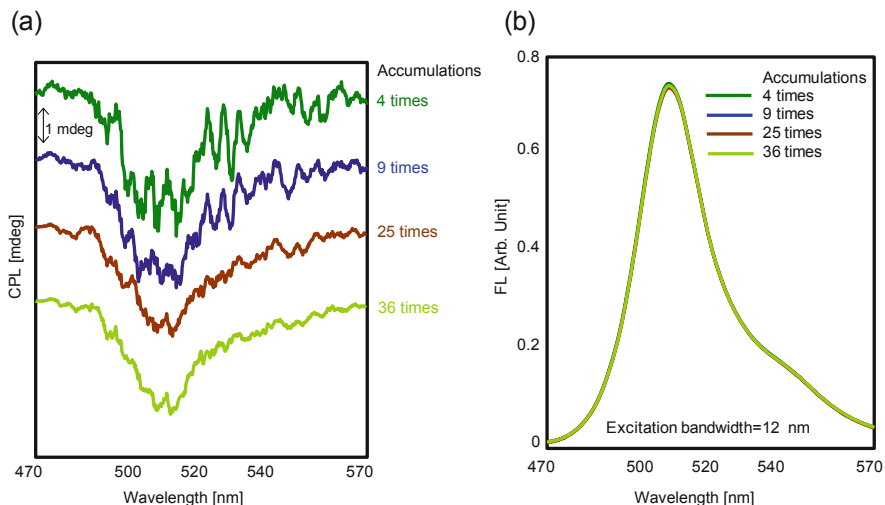


Fig. 14.10 CPL and fluorescence spectra of wild-type GFP. (a) Accumulation dependence on CPL spectra of GFP. (b) Sample stability check during CPL spectra accumulation

the temporal change in the fluorescence spectrum was monitored. Figure 14.10b shows the 1st, 9th, 25th, and 36th fluorescence spectrum obtained during the CPL measurements. It can be seen that even after 36 accumulations, no change in the spectral shape occurred.

Figure 14.11 shows CD and CPL spectra of this GFP. The CD and absorption spectra were obtained with 30 $\mu\text{g}/\text{mL}$ of the sample concentration and 20-mm path length. The CD originated from aromatic amino acid side chains and was observed in the near-ultraviolet region, whereas the optical absorption and CPL originating from functional groups emitting green fluorescence appear in the visible region. Since g_{abs} and g_{lum} are similar to each other, structural differences between the ground and excited states of the fluorescent functional groups are considered to be very small.

Figure 14.12 shows CD and CPL spectra of enhanced GFP, which offers higher-intensity emission with respect to wild-type GFP. In this case also, g_{abs} and g_{lum} are small and similar to each other, indicating that structural differences between the ground and excitation states are very small.

14.5.4 CPL Measurements of Solid Sample

Finally, we introduce a method for measuring CPL for a solid sample. When circularly polarized light-emitting materials are used for displays and illumination, these materials are expected to be not liquids but solids. Therefore, it is necessary to

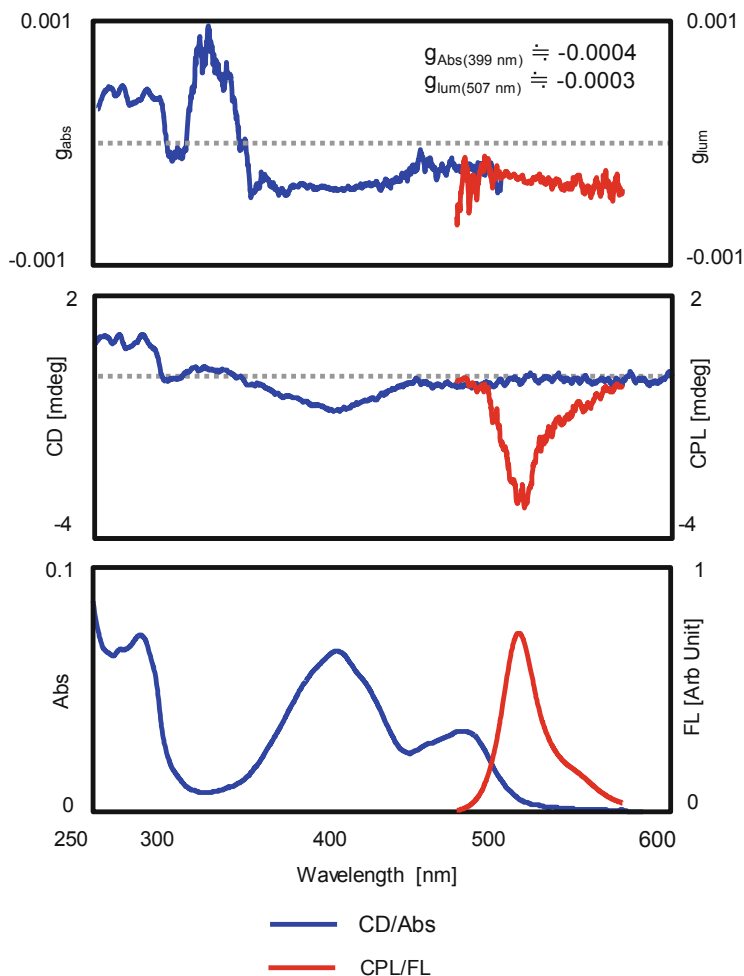


Fig. 14.11 CD, CPL, absorption, and fluorescence spectra of wild-type GFP

have a means of evaluating CPL in materials in solid form like powder and film. We herein introduce a method for measuring powder samples.

In the Nujol mull method, a powder sample is coated on a quartz plate, and the KBr pellet method, commonly utilized in infrared spectroscopy, can be used. The CPL-300 is designed to detect transmitted fluorescence through samples. Thus, it can detect fluorescence emitted from the back of a pellet while suppressing the effect of fluorescence anisotropy (Fig. 14.13). In the case of a solid sample containing a powder, the spectrum may be affected by artifacts due to birefringence. To determine whether such artifacts are present, it is necessary to measure enantiomers to obtain symmetrical spectra. Furthermore, the confirmation of the isotropic CPL spectra by the sample rotation to the optical axis of a light polarizer is necessary. Such measurements can be

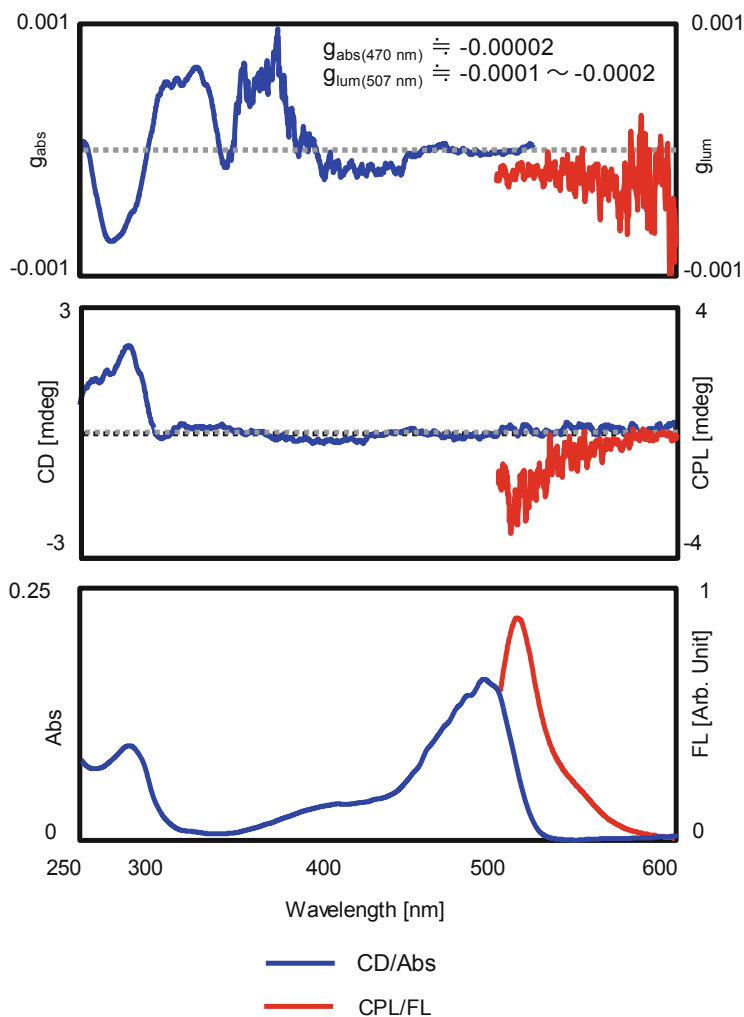


Fig. 14.12 CD, CPL, absorption, and fluorescence spectra of enhanced GFP

performed using the pellet holder shown in Fig. 14.14a, which has a scale indicating the rotation angle. Figure 14.14b shows a KBr pellet installed in the holder.

Figure 14.15 shows spectra of powdered $\text{Eu}(\text{facam})_3$ in a KBr pellet measured using this pellet holder. The spectra were obtained at rotation angles of 0° , 45° , and 90° . There is no significant change in spectral shape with rotation angle, indicating that there is little effect of anisotropy.

Since g_{lum} for europium complexes is known to be strongly affected by the type of solvent used, it was expected that g_{lum} determined using the KBr pellet method would be different to that for a solution. Figure 14.16 shows CPL spectra of $\text{Eu}(\text{facam})_3$ in different solvents and in a KBr pellet. g_{lum} values obtained using the KBr

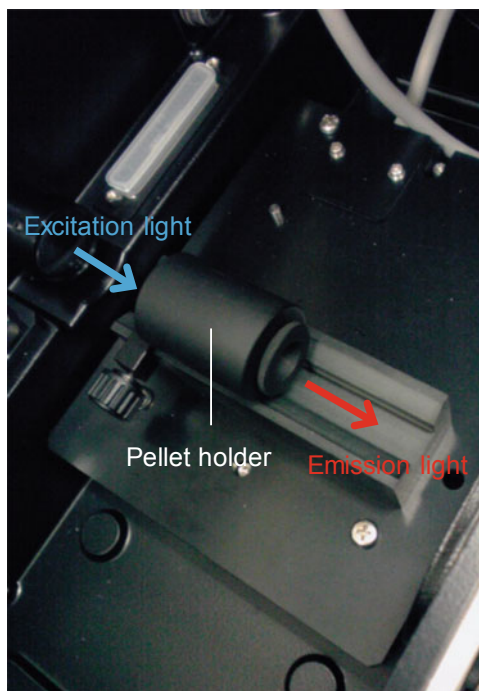


Fig. 14.13 Sample compartment of CPL-300 with installed pellet holder

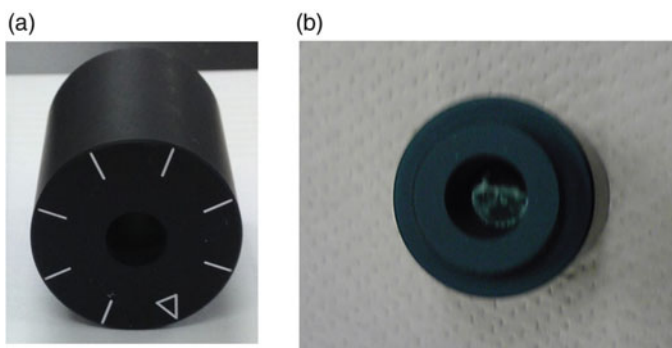


Fig. 14.14 External view of pellet holder. (a) Front view of pellet folder. (b) KBr pellet installed in pellet holder

pellet method are much smaller than those obtained using dimethyl sulfoxide (DMSO) and *N,N*-dimethylformamide (DMF) as a solvent, and the value around 590 nm is positive.

In addition to inorganic and organic molecules, including lanthanoid complexes, there is a great deal of research into materials that exhibit the CPL characteristics of supramolecular assemblies, and polymers and colloids that are aggregates of

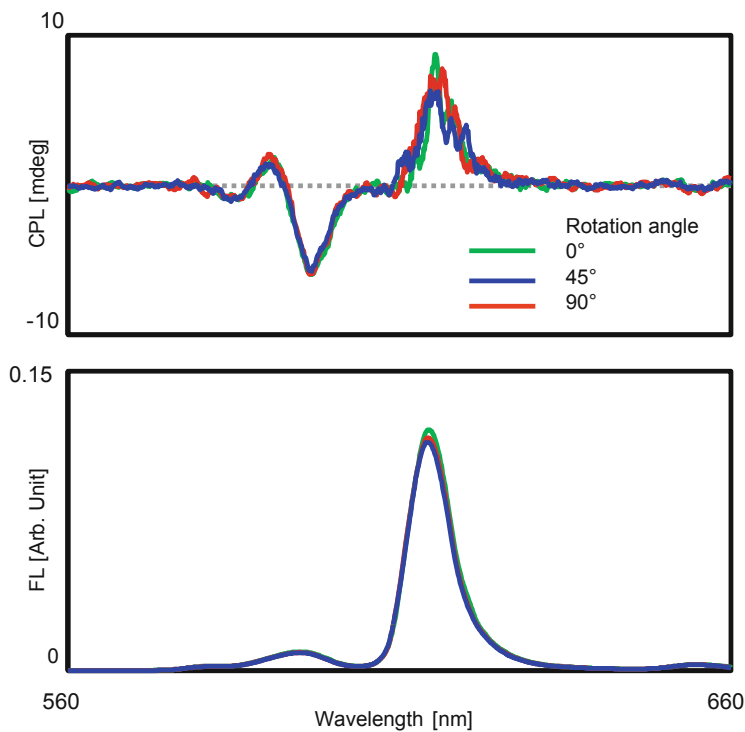


Fig. 14.15 CPL and fluorescence spectra of $\text{Eu}(\text{facam})_3$ in KBr pellet

molecules. This latter group of materials offers advantages in that characteristics such as the emission wavelength and sign of the g value can be easily tailored. In addition, aggregation-induced emission (AIE) [7], first discovered by Tang et al. in 2001, has applications to materials that are capable of light emission in aggregate form, and can be used in organic LEDs (OLEDs) and cell dyeing agents. These materials are often measured in thin-film form. However, it is necessary to ensure that CPL measurements of solid sample are not affected by the artifacts described above.

Furthermore, recent results concerning the design of CPL materials based on quantum chemistry calculations, and the subsequent synthesis of such a material [8], highlight the possibility of a method for developing novel CPL materials that does not require an empirical approach. Given the wide range of potential applications of circularly polarized light-emitting materials, the ability to perform CPL measurements in the solid state is highly significant.

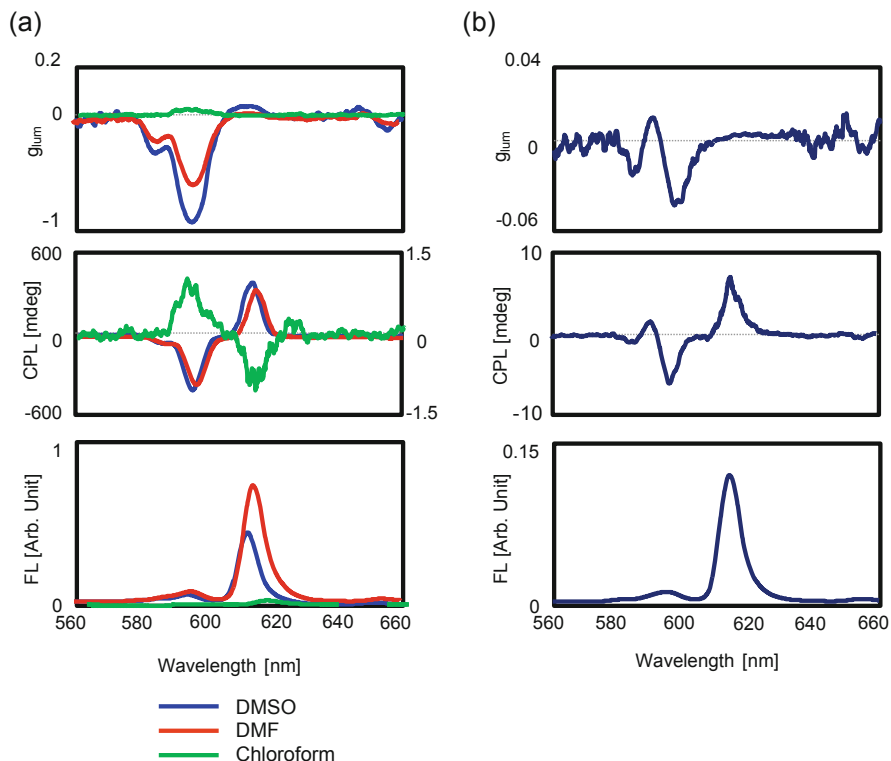


Fig. 14.16 CD, CPL, absorption, and fluorescent spectra of $\text{Eu}(\text{facam})_3$. (a) $\text{Eu}(\text{facam})_3$ in various solvents. (b) $\text{Eu}(\text{facam})_3$ in KBr pellet

14.6 Summary

In this chapter, we introduced the principles of CPL measurement instruments, calibration methods, and measurement examples. We expect that research into CPL materials will accelerate, and that these materials will find a wide range of practical applications such as 3D displays and security ink.

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