Development of a Vibrational Circular Dichroism Spectrometer using a Quantum Cascade Laser

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Abstract

Vibrational circular dichroism spectrometer based on Fourier transform infrared optical system (FT-VCD) is used to determine the absolute configuration of chiral compounds, because it provides three-dimensional structural information about vibrational transitions in the infrared region. However, since the intensity (ΔAbs) in the vibrational circular dichroism (VCD) spectrum can be as low as 10^{-4} to $10^{-5,}$ it is necessary to increase the sensitivity of the measurement system. To achieve this, we have newly developed a vibrational circular dichroism spectrometer using a quantum cascade laser (QCL-VCD) as a monochromatic light source instead of a Fourier transform infrared spectrometer (FT-IR). The QCL-VCD (wavenumber range: 1740 to 1500 $cm^{-1)}$ was combined with the FT-VCD (wavenumber range: 4000 to 850 cm^{-1}) to realize a system (MultiD-VCD-MIRAI -2020) that achieves both high sensitivity and a wide measurement range.

Compared to the light sources used in conventional FT-VCD, quantum cascade lasers produce a higher-intensity output, so in addition to increasing the sensitivity of the measurement system, it is also possible to measure samples exhibiting strong absorption. The QCL-VCD can be applied to measurements of highly concentrated samples, in addition to aqueous solutions that have been difficult to measure in the past. Furthermore, by taking advantage of the high sensitivity of this system, it is expected that it can be used to measure extremely small regions of a sample, and for in-plane mapping measurements. In this report, we describe the effectiveness of the newly developed QCL-VCD by providing application examples.

Keywords: Vibrational Circular Dichroism, Quantum Cascade Laser, Aqueous Solutions, Glycyl-leucine, Mapping, Alanine

1. Introduction

Vibrational circular dichroism spectroscopy (VCD) is used for the analysis of chiral compounds. Since many absorption bands arising from the vibrational transitions of molecules are observed in the infrared region, the absolute configuration of a sample can be determined by analyzing its VCD spectra [1, 2]. However, the signal intensity (ΔAbs) of VCD spectra is as weak as 10^{-4} to 10^{-5}, and it often makes it difficult to measure samples. To develop VCD as an analytical method for a wider range of samples, the sensitivity of the system needs to be improved further.

To solve this problem of low sensitivity, we have developed a new vibrational circular dichroism spectrometer (MultiD-VCD-MIRAI-2020) using a quantum cascade laser (QCL, wavenumber range: 1740 to 1500 cm^{-1}) as a light source. Compared to the vibrational circular dichroism spectrometer (FV-VCD) based on the conventional Fourier transform infrared spectrometer (FT-IR), the system using QCL has a higher sensitivity due to its high brightness light source and enables us to measure samples with high absorption intensities. Therefore, the system using QCL can be applied to the measurement of highly concentrated samples and aqueous solutions, which have been considered difficult to measure in the past. In addition, since QCL is a monochromatic light source, it is possible to obtain results in a shorter time than FT-VCD in measuring specific wavenumbers. These characteristics of high speed and high sensitivity are expected to allow one to apply the system to the measurement of microregions and to mapping measurement, which can analyze the in-plane distribution of chiral compounds within the sample. In this paper, we report the effectiveness of the VCD measurement system using a quantum cascade laser.

2. MultiD-VCD-MIRAI-2020

2-1 System

Fig. 1 shows the external view of MultiD-VCD-MIRAI-2020, and Fig. 2 shows the optical system around its sample chamber. MultiD-VCD-MIRAI-2020 is a vibrational circular dichroism spectrometer whose light source can be selected between the FT-VCD (FT-IR) and the QCL-VCD (QCL) which share an optical system around the sample chamber. The section of FT-VCD uses FVS-6000 VCD spectrometer (JASCO Corporation, Japan). The light source can be switched between the FT-VCD and the QCL-VCD to measure the same sample by witching mirror in front of the sample chamber.

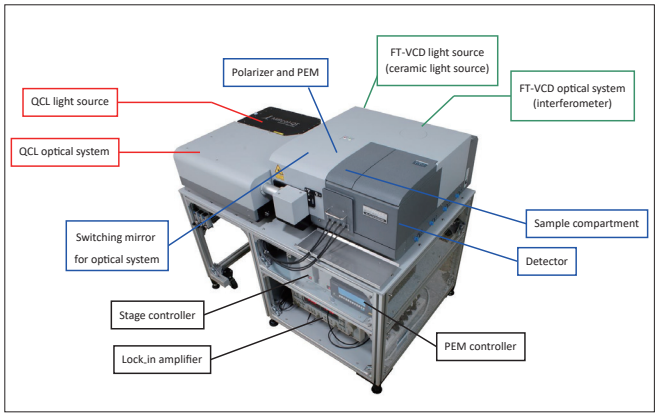

Figure 1 Photograph of MultiD-VCD-MIRAI-2020

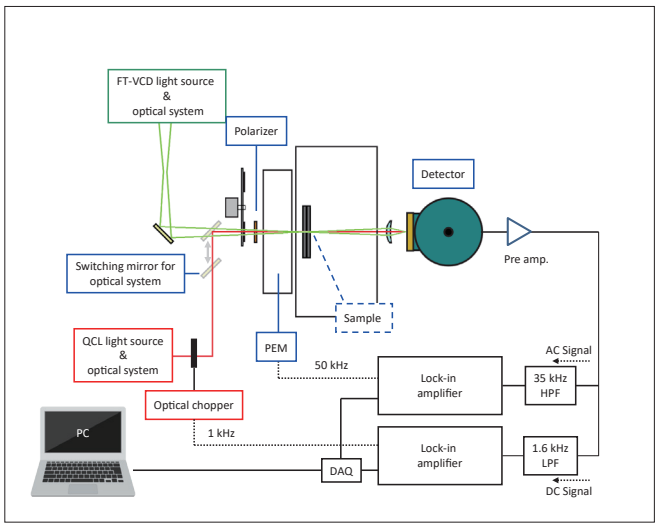

Figure 2 MultiD-VCD-MIRAI-2020 optical system

Infrared monochromatic light emitted from the QCL-VCD is introduced into the QCL optical system and modulated by a chopper at a frequency of 1 kHz. The light is then linearly polarized by a polarizer and modulated into left and right circularly polarized light at 50 kHz by a photoelastic modulator (PEM). The sample is irradiated with circularly polarized light, and the transmitted infrared light is observed by a mercury cadmium telluride (MCT) detector cooled with liquid nitrogen. The detected signal is a superposition of a DC signal, which is the intensity of infrared monochromatic light corresponding to the modulation frequency of the chopper, and an AC signal, which is the difference in intensity between the transmitted light with left circular polarization and that with right circular polarization corresponding to the modulation frequency of the PEM. The DC signal is separated from the high-frequency noise signal by a low-pass filter that eliminates signals above 1.6 kHz, and the AC signal is separated from the low frequency noise signal by a high-pass filter that eliminates signals below 35 kHz, and then the signal components synchronized with the modulation frequency are acquired by a lock-in amplifier. The ratio of the DC signal to the one without the sample is output as the IR spectrum, and the ratio of the AC signal to the DC signal (AC/DC) as the VCD spectrum.

The sample stage (Fig. 3) is installed in the sample chamber, and mapping measurement is realized by moving the sample position.

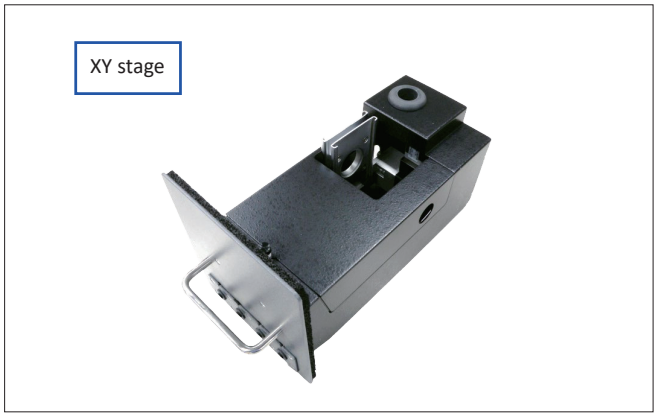

Figure 3 Photograph of XY-Stage

2-2 QCL Light Source

The MIRcat-QT mid-infrared laser manufactured by DAYLIGHT SOLUTIONS was adopted as the QCL light source. The oscillation mode of the "MIRcat-QT mid-infrared laser" is CW (continuous wave), and infrared light of 1740 ~ 1500 cm^{-1} is emitted. The maximum output power is 300 mW, and the wavenumber accuracy is within 1 cm^{-1}. The MIRcat-QT can be equipped with up to four laser heads with different oscillation bandwidths, so that the wavenumber range for measurement can be extended in the future.

Before integrating the QCL into the system, we compared the output power of the QCL with that of the high-intensity infrared light source initially installed in the FT-VCD to confirm its performance as a light source. Fig. 4 shows the output spectra of these light sources measured using FT-IR. The left figure in Fig. 4 is the spectrum measured by scanning the oscillation wavenumber of the QCL around 1600 cm^{-1}, and the right figure the spectrum measured by the infrared light source in the FT-VCD. The intensity at 1600 cm^{-1} was 250,626 for the QCL and 4.4 for the infrared light source in the FT-VCD, showing that the output of the QCL is more than 55,000 times larger than that of the infrared light source. In addition, light from the QCL is not led to the FT-IR in this system, so that the power energy loss by the Michelson interferometer can be avoided.

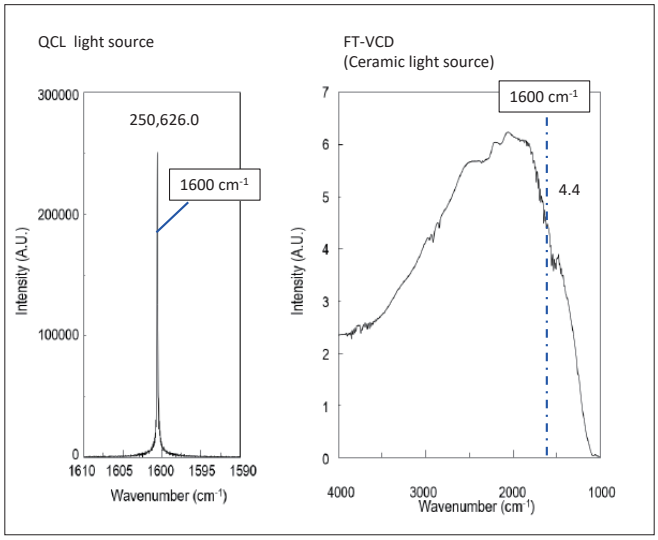

Figure 4 Comparison of light source brightness for QCL-VCD and FT-VCD

2-3 Characteristics

Using a QCL as a high brightness light source in the system is expected to realize "a high system sensitivity," "the measurement of samples with strong absorption intensity," and "its use for mapping measurement."

In addition to the above, it is also a characteristic that MultiD-VCD-MIRAI-2020 can be used with its light source selected between the FT-VCD and the QCL-VCD. While the measurement range of the QCL-VCD is limited to 1740 ~ 1500 cm^{-1}, the QCL was selected so that it covered the absorption peaks of mainly amide I and amide II in measuring them. In contrast to the QCL-VCD, the FT-VCD can measure from 4000 to 850 cm^{-1} and obtain information of various functional groups. The system allows us to switch between the FT-VCD and the QCL-VCD depending on the purpose of measurement.

3. Measurement

3-1 Measurement of High Absorbance Samples

We studied the measurement of high absorbance samples, which was expected to be one of the characteristics of the QCL. Before conducting the high absorbance measurement of VCD, we examined how accurately the system could measure IR absorption intensity. As samples for this, we used polystyrene films of different thicknesses for different absorptions. We also used DLATGS (Deuterated L-Alanine Triglycine Sulphate), which operates at room temperature, as the detector. Fig. 5 shows the IR spectrum of polystyrene. Focusing on the absorption intensity at 1601 cm^{-1}, we examined the relationship between the thickness of the sample and the absorbance at 1601 cm^{-1} of the polystyrene films. The left figure in Fig. 6 shows the result of QCL-IR, and the right figure the result of FT-IR. They show that while the linearity of absorption for QCL-IR was maintained up to around an absorbance of 5, the linearity for FT-IR was broken above an absorbance of 3.

We then studied the VCD measurement of high absorbance samples. In general, the signal intensity of VCD increases proportionally with the increase in the signal intensity of IR. When IR absorption is large, however, the infrared light observed by the detector becomes weak in a logarithmically proportional manner, and the influence of noise rapidly increases. If the influence of noise becomes larger than the signal intensity of VCD, the accurate VCD spectral shape cannot be maintained. It was expected that using a QCL with high brightness as a light source would secure the intensity of infrared light transmitted through the sample and reduce the influence of noise, which would allow us to accurately measure the VCD spectra of samples with high absorbance. The samples used were NEAT liquids of (1R)-(+)-α-Pinene and (1S)-(-)-α-Pinene, and the IR absorbance at 1658 cm^{-1} was adjusted to about 2 using a cell with an optical path length of 500 μm. The results of QCL-VCD are shown on the left in Fig. 7, and those of FT-VCD on the right. As for the VCD spectrum, FT-VCD shows large VCD noises, failing to produce symmetric VCD signals. On the other hand, QCL-VCD produced symmetric VCD signals. Therefore, QCL-VCD was shown to be effective in measuring samples with strong absorption.

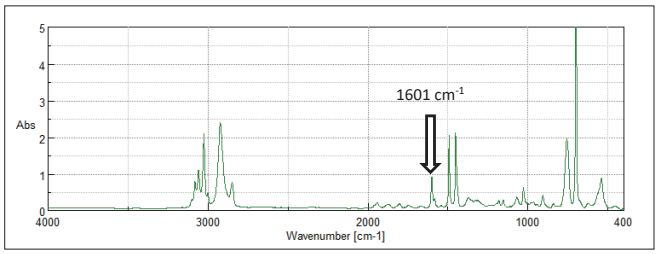

Figure 5 Polystyrene IR spectrum

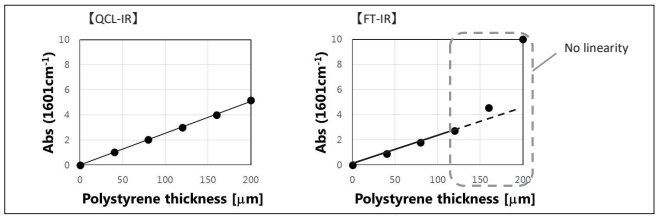

Figure 6 Relationship between poystyrene thikness and bsorbance at 1601 cm^{-1}
Left: QCL-IR, Right: FT-IR

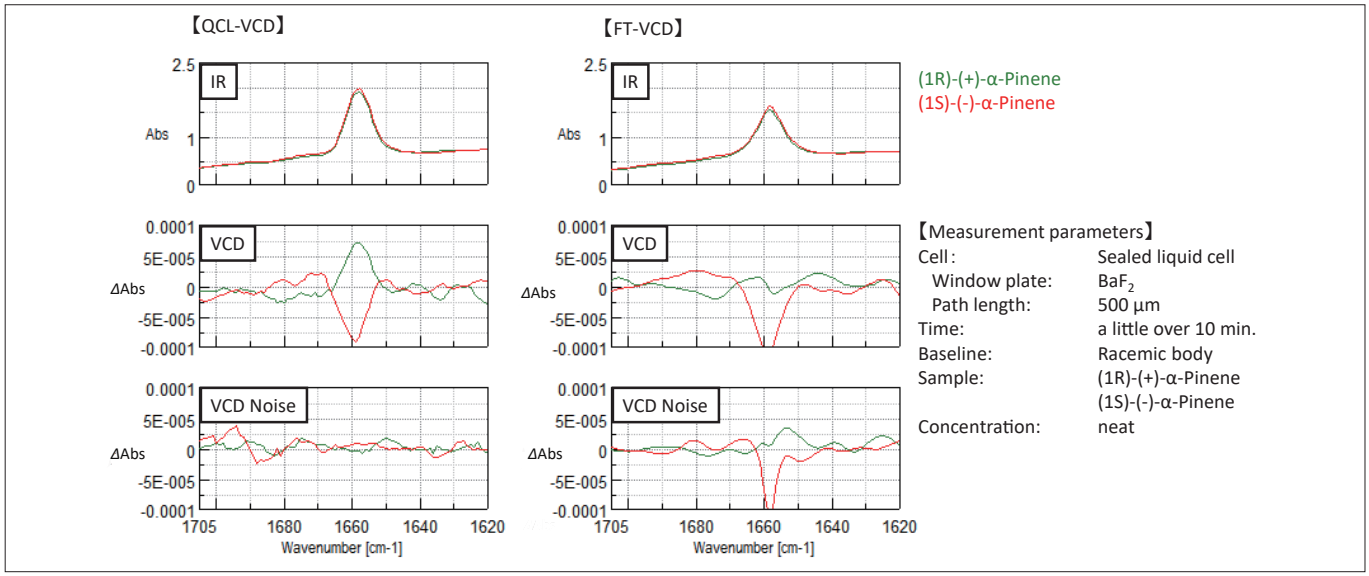

Figure 7 VCD results of high absorbance sample
Left: QCL-IR, Right: FT-IR

3-2 Measurement of Aqueous Solutions

One of the applications of VCD high absorbance measurement is the measurement of aqueous solutions. Aqueous solutions are studied in various fields, and their measurement is useful especially for the biological sample. However, the infrared absorption of water is very strong, and the path length of sample cells generally used is several μm. Fig. 8 shows the IR spectrum of water measured using a spacer of thickness 4 μm. The absorption due to the bending vibration of OH of water is seen at around 1640 cm^{-1}, and it is a strong signal of absorbance about 0.8. As described in "3-1 Measurement of High Absorbance Samples" above, the measurement of high absorbance samples is difficult in ordinary FT-VCD measurement, and thus it is difficult to measure aqueous solutions, which have strong absorption. In addition, if the optical path length is short, the absorption intensity of a sample dissolved in water becomes small, and the resulting VCD signal also becomes weak. A short cell path length also makes sampling difficult. Furthermore, the sampling of a sample with high concentration into a cell of a short optical path tends to cause the clogging or contamination of the cell. Using QCL-VCD for measuring aqueous solutions possibly solve these problems.

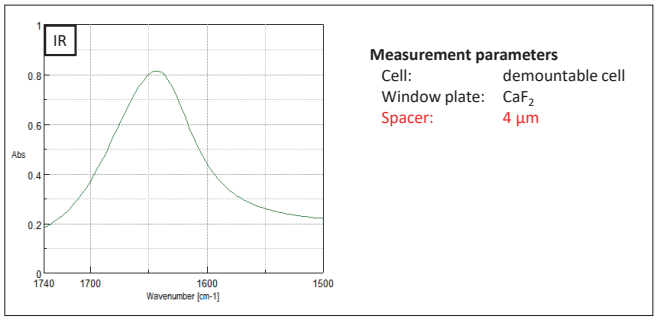

Figure 8 IR spectrum of water

We thus measured the VCD spectra of aqueous solutions, using the QCL-VCD system.

We used the D-isomer and the L-isomer of Glycyl-leucine (Gly-Leu) (concentration: 0.3 M) as aqueous samples. We also used a spacer of thickness 25 μm, which made the optical path length longer than the ordinary path length. Fig. 9 shows the results of the measurement. The figure on the left in Fig. 9 is the IR spectra of the Gly-Leu solutions and water, and the figures on the right the IR, VCD, and VCD noise spectra of Gly-Leu solutions after subtracting the water spectrum. The figure on the left shows that absorption by water is dominant among the IR spectra, while the amide II absorption of the samples can be seen at around 1580 cm^{-1}. The absorbance of this amide II absorption as well as that of water is very large, being about 1 ~ 2. Nevertheless, the VCD signal of the amide II absorption band at 1580 cm^{-1} was clearly observed in the figure on the right in Fig. 9. This clearly shows that QCL-VCD is very effective in the measurement of aqueous solutions, which have been difficult in the past.

3-3 Mapping Measurement

The QCL-VCD system can reduce the scanning time because of its high sensitivity and also allows one to obtain spectra in a shorter time than FT-VCD by measuring only the wavenumber range in focus. As a result, the system is expected to be applied to mapping measurement, in which multiple VCD spectra are measured by moving the sample. We thus investigated mapping measurement, which is one of the characteristics of the system. Fig. 10 shows images of the sample used. To confirm the validity of the system, we prepared a tablet of KBr containing D-Alanine in the right half and

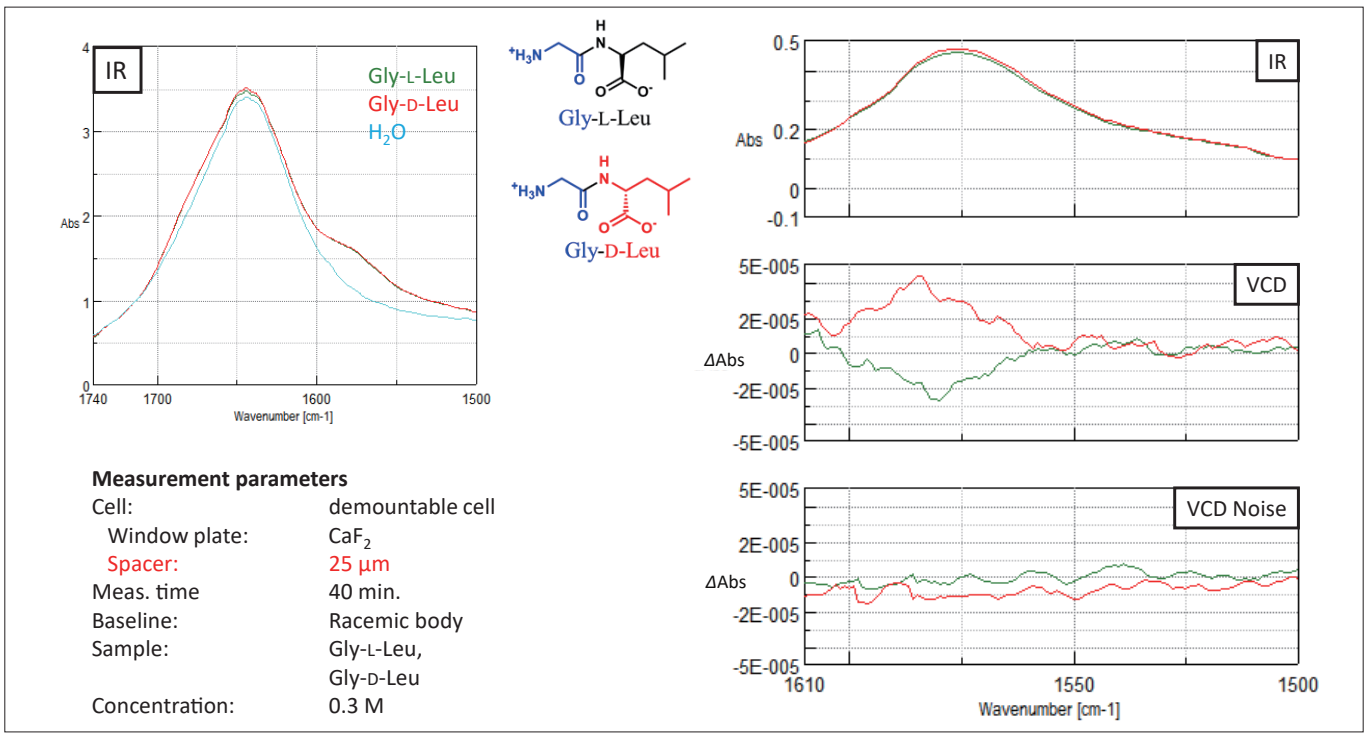

Figure 9 Measurement results of Gly-Leu aqueous solution
Left: Gly-Leu IR spectrum
Right: IR, VCD, VCD noise spectra of Gly-Leu (after subtracting water spectrum)

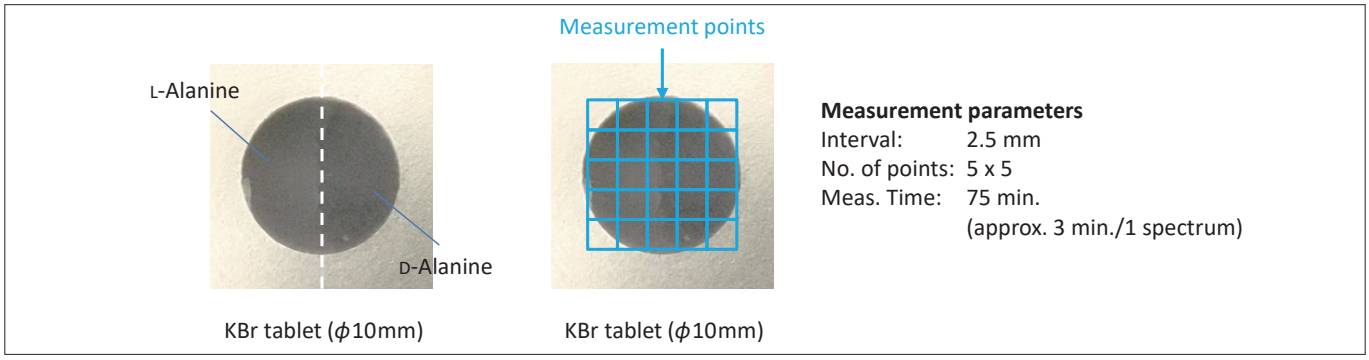

Figure 10 Sample of mapping measurement

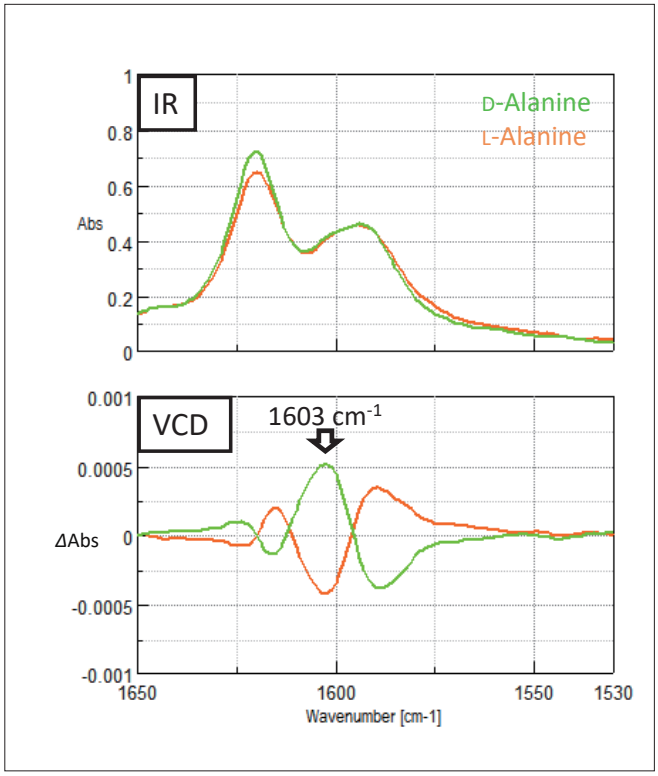

Figure 11 Spectrum of pure d-alanine and pure l-alanine

L-Alanine in the left half as a sample with known distributions of chiral compounds. In preparing the sample, each of D-Alanine and L-Alanine was mixed with KBr at a ratio of 0.15 wt% and ground in an agate mortar. These portions were spatially separated in a hydraulic press for tableting, and a tablet of diameter 10 mm and thickness 0.58 mm was produced. Mapping measurement was performed at 25 points (5 x 5) disposed at intervals of 2.5 mm on the tablet to obtain the distribution of chiral compounds. The diameter of the beam irradiated on the sample was 10 mm (the spatial resolution being 5.0 mm [3]). The time of VCD spectrum measurement was about 3 minutes for each point, and the total time for the mapping measurement was about 75 minutes.

Before discussing the results of the measurement, we show the IR and VCD spectra of D-Alanine and L-Alanine in Fig. 11 as a reference. It shows that while the IR spectra have a similar shape, the VCD spectra have symmetric shapes. The peak at 1603 cm^{-1} in the VCD spectrum is positive for D-Alanine and negative for L-Alanine. Therefore, D-Alanine and L-Alanine can be distinguished by identifying the sign of this peak.

Fig. 12 shows the results of the mapping measurement.

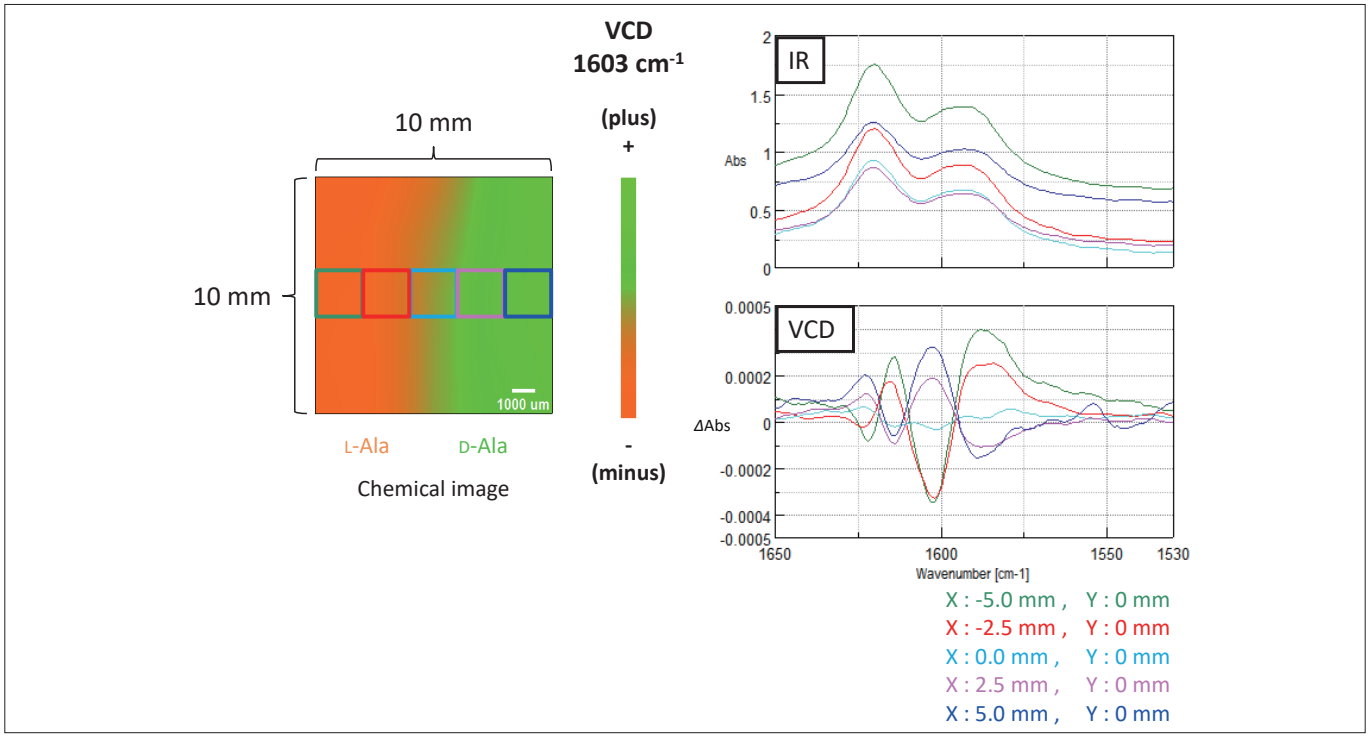

Figure 12 Mapping results
Left: Chemical image, Right: IR and VCD spectra

The figure on the left shows an image of the measured points distinguished by color based on the VCD peak intensity at 1603 cm^{-1}, where yellow-green is used if the VCD peak at 1603 cm^{-1} was positive (D-Alanine), and orange if it was negative (L-Alanine). As we expected, it shows the existence of D-Alanine in the right half of the tablet and that of L-Alanine in the left half. In addition, the spectra obtained by mapping measurement (on the right in Fig. 12) show that the right side of the sample (X = 2.5, 5.0 mm) produced the spectra of D-Alanine, and the left side of the sample (X = -2.5, -5.0 mm) the spectra of L-Alanine. These results show that QCL-VCD can be applied to mapping measurement to examine the distribution of chiral compounds.

4. Summary

We developed MultiD-VCD-MIRAI-2020, which combines QCL-VCD and FT-VCD. QCL-VCD is effectivein measuring aqueous solutions, as an application of high absorbance measurement. It can be also applied to mapping measurement, by which the distribution of chiral compounds in a sample can be visualized. We hope that the developed system will provide more occasions to gain new insights into measurements and samples that are difficult to apply conventional FT-VCD.

It is noted here for your reference that while we did not describe the microscopic measurement system for measuring microregions in this report, it was reported in reference [4, 5].

5. Acknowledgements

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