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Signal to Noise Ratio of Spectroscopy Utilizing Spectral Images

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Imaging spectroscopy allows spatial and characteristic information to be included in an image from which the sample characteristics can be derived. In this study, a method to improve the signal-to-noise ratio of a spectral signal obtained by using imaging spectroscopy is proposed and demonstrated experimentally. Imaging spectroscopy method using spatial information was found to have a signal-to-noise ratio compared to the conventional spectroscopy method that obtains an average value by using a single photo-detector. Because the spatial distribution information of the sample is known in imaging spectroscopy, unnecessary spectral signals can be removed, and the signal-to-noise ratio can be improved.

Keywords: Imaging spectroscopy, Signal-to-noise, Spectroscopy

I. INTRODUCTION

Optical spectroscopy can be used to examine the properties of a material by measuring how the sample interacts with light of different wavelengths [1–3]. Normally, this information is obtained nondestructively, such that the material undergoes no alterations during the measurement process. The wavelength absorbed depends on the analyte concentration and elemental constituents of the material. In each of these phenomena, the optical intensity of the sample changes with the wavelength, therefore providing information about the material, Beer-Lambert law [4–8,11]. Thus, the signal quality from the sample must be reliable and have a high signal-to-noise ratio (SNR), where the SNR indicates the signal quality [9,10].

Since classical optical spectroscopy uses a single photo-detector, it measures the average value of the signal passing through or reflected from the sample. If the sample is homogeneous, it does not affect the signal-to-noise ratio, but if the sample is not homogeneous, the spatial distribution characteristic cannot be known with a single photo-detector, so only the average value can be known, and the spatial distribution information and characteristics of the sample cannot be known. For example, if a single photometer is used to measure the analyte concentration of hemoglobin or sugar in a human blood vessel, the signal-to-noise ratio is reduced because the sample is only part of the area through which the light passes and the spatial distribution information of the sample is not known.

In order to improve this problem, image spectroscopy has emerged. In image spectroscopy, two-dimensional (2D) sensors, charge-coupled device (CCD) or complementary metal oxide semiconductor (CMOS), have been applied. Imaging spectroscopy simultaneously records the spectroscopic and spatial information of a sample. With this technique, it is possible to acquire a complete reflectance spectrum for each picture element (pixel) in an image [12,13]. Imaging spectroscopy are used in many areas such as Medicine, Biology, Surveillance and Biometrics. The use of imaging techniques for scene evaluation has become key technology for nondestructive, remote evaluation [14–19].

In this study, the SNR according to the size of the probe beam and the sample size ratio was studied, and the improvement of the SNR was conducted using imaging spectroscopy.

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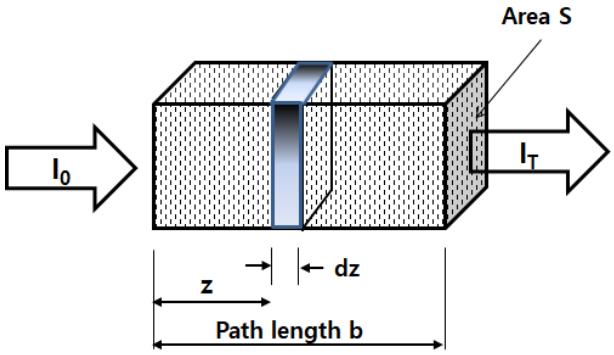

Fig. 1. (Color online) Beer-Lambert law.

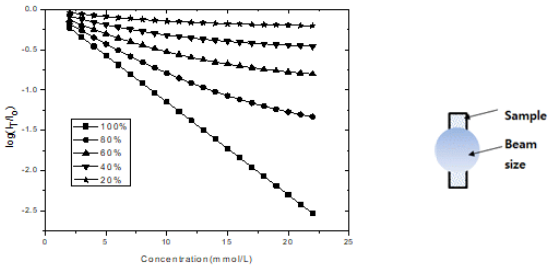

Fig. 2. (Color online) Simulation result of absorbance ($\log \frac{I_T}{I_0}$) as a function of ratio of probe beam size and sample size.

II. SIGNAL TO NOISE RATIO IN SPCTROSCOPY

Ultraviolet/visible (UV/Vis) spectroscopy is routinely used in analytical chemistry for quantitative and qualitative analyses. Spectroscopic analysis is commonly carried out on solutions; however, solids and gases can also be studied. The basic principle of quantitative absorption spectroscopy is to compare the absorption of a sample solution with that of a set of standards under radiation of a selected wavelength, through the application of the Beer–Lambert law. The Beer–Lambert law states that the absorbance of a solution is directly proportional to the path length and analyte concentration of the absorbing species in the solution.

Consider a beam of monochromatic light with intensity I_0 irradiating the sample surface at normal incidence, as shown in Fig. 1. After passing through path length b of the sample, which contains N molecules/cm^3, the intensity of the light is reduced to I_T. The Beer–Lambert law describes the relationship between the attenuation of light as it passes through the substance and the properties of the substance, as given below:

$$-\ln \frac{I_T}{I_0} = \sigma N b \tag{1}$$

In Eq. (1), σ is the cross-sectional area, specifically, the effective area seen by the light wavelength, λ. If λ approaches resonance, σ approaches its maximum value.

In traditional spectroscopy, the sample is usually contained within a cell and the light passes through, or is reflected from, the sample. In this case, the size of the probe beam is similar to smaller than the cell area, thus allowing the characteristics of the sample to be investigated using the Beer–Lambert law. However, in non-invasive spectroscopy, the light irradiates the entire area of the sample. When using a single photo-detector, the SNR is relatively low, as it cannot distinguish the signal from the sample area from the undesired background signal.

The Beer–Lambert law from Eq. (1) shows the linear dependence of absorbance on the analyte concentration when the optical path is constant. Figure 2 represents the simulated absorbance ($\log \frac{I_T}{I_0}$) when the area of the probe beam (A) is larger than the sample area (S). When the ratio of the sample area to the probe beam area is 1 (100%), as indicated by the square (■) in Fig. 2, the absorbance shows a linear dependence with respect to the analyte concentration, as described by Eq. (1). However, if the ratio of S and A is less than 1, then the relationship deviates from linearity and the slope is also reduced. A reduction in the slope can be attributed to a decrease in the measurement sensitivity. When the probe beam area is larger than the sample area, the unabsorbed stray light affects the SNR. In non-invasive spectroscopy, the area of the irradiated beam is generally larger than the sample area, such that the light reflected or transmitted from the sample cannot be easily distinguished from the undesired signal. Therefore, the measurement sensitivity can be improved by acquiring a 2D image with a digital imaging acquiring system and using it to investigate changes in the light intensity of the region of interest in non-invasive spectroscopy.

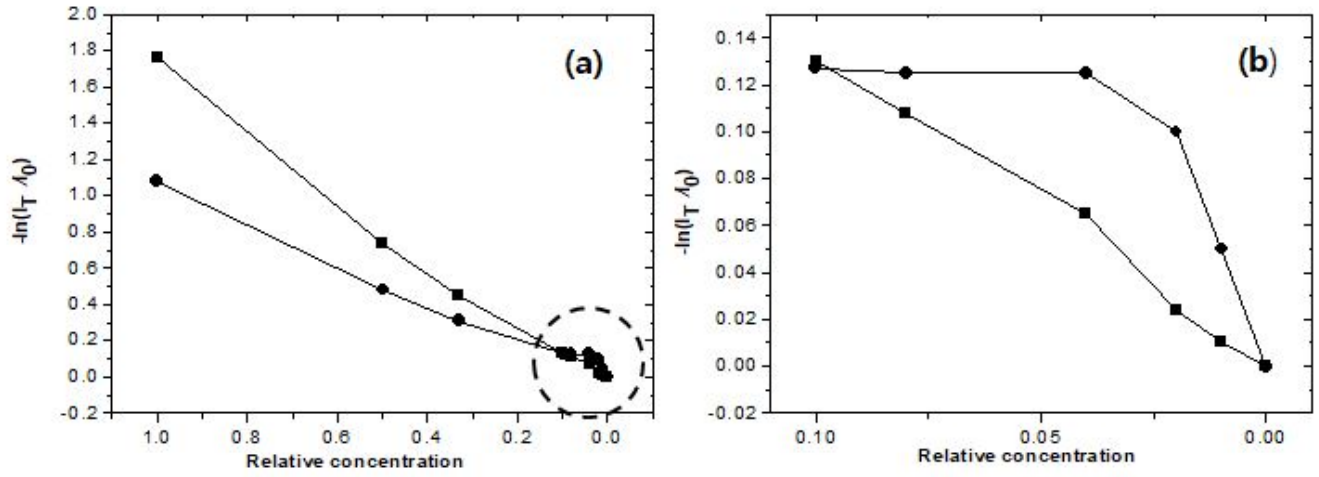

Fig. 3. Absorbance as a function of relative concentration for different probe beam sizes: (a) high and (b) low concentration. Squares and circles represent probe beam diameters of 4 and 20 mm, respectively.

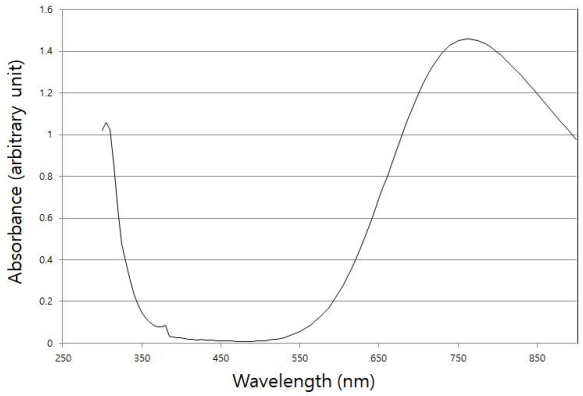

Fig. 4. Absorbance of the sample as a function of wavelength (nm).

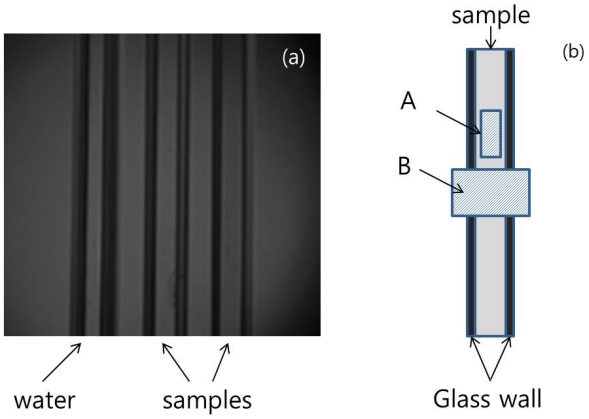

Fig. 5. (Color online) (a) Photograph of samples and the data acquisition areas, and (b) a schematic diagram of the sampling vessel (diameter = 3 mm) and sampling areas (A and B) used in the experiments. Area A includes the sample only; area B includes the sample and the background.

III. EXPERIMENT AND RESULTS

Figure 3 shows absorbance as a function of analyte concentration with respect to the ratio of the probe beam size and sample size. The sample was in a 10 mm × 10 mm square cell, and the sample was prepared at relatively low concentrations by mixing water with the reference concentration of 1.0. Figure 4 shows the absorbance spectrum of the sample used in the experiment. A light-emitting diode (LED) with a wavelength of 635 nm and bandwidth of 20 nm was used. To control the size of the probe beam, a 30-mm-diameter collimated beam was formed using a fiber and a lens. The size of the probe beam was adjusted using an aperture stop. The diameter of the probe beam was reduced to 4 mm (■) or 20 mm (●) using aperture stop. The transmitted light intensity is shown in Fig. 3(a) as a function of concentration. Figure 3(b) shows the low-concentration region as an enlarged part of the dotted circle in Fig. 3(a). In Fig. 3, it can be seen that when the probe beam size is small (■), it is measured linearly from high to low concentration compare to large beam size (●). As expected from the computer simulations (Fig. 2), if the size of the probe beam is smaller than the sample, the absorbance changes linearly from high- to low concentration according to the Beer–Lambert law. Otherwise, the absorbance deviates from linearity. Thus, the measurement sensitivity, i.e.,

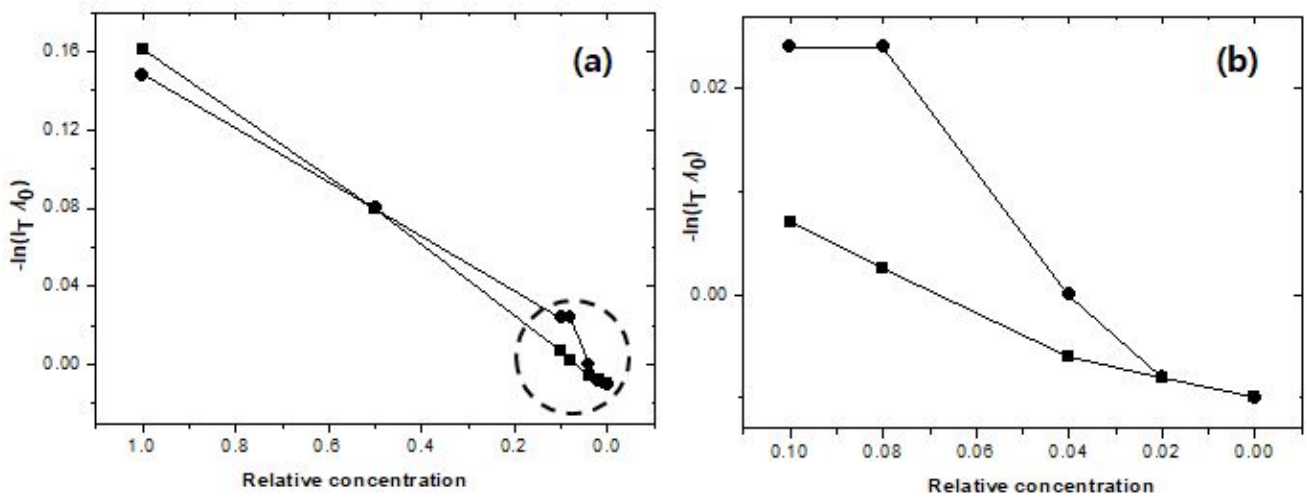

Fig. 6. Absorbance as a function of the relative concentration of different sampling areas: (a) high and (b) low concentration, where squares and circles represent sampling areas A (desired signal area) and B (signal area + background), respectively.

the SNR, is high when the beam size is smaller than, or similar to, the sample size.

Figure 5(a) is a photograph of the sample area taken with a CCD camera; this photograph corresponds to the sampling area shown in Fig. 5(b). Area A is within the sample, and area B includes both the sample and background. Figure 6 shows the absorbance obtained from this CCD image. The squares (■) in Fig. 6(a) correspond to the absorbance obtained based on the intensity data of area A only, as shown in Fig. 5(b); circles (●) represent the absorbance obtained using the intensity data of area B. Figure 6(b) shows the low-concentration area. From Fig. 6, when absorbance is calculated using only the light intensity from the sample [area A, Fig. 5(b)], it shows a linear relationship with the analyte concentration according to the Beer–Lambert law. However, when both the sample and background are included [area B, Fig. 5(b)], the absorbance deviates from linearity with respect to the analyte concentration. In this case, as shown in Fig. 3, data other than the sample affect the absorbance calculation, and the measurement accuracy decreases due to the reduced SNR. Therefore, image spectroscopy with a CCD camera was capable of distinguishing the desired and unwanted signal for an enhanced SNR.

IV. CONCLUSION

In non-invasive optical spectroscopy using a single photo-detector, the SNR is low because the signal from the sample cannot be distinguished from the undesired signal. This was evident in our computer simulation and experimental results, under the assumption that the size ratio of the irradiation beam and the sample affects the SNR. That is, when using a single photo-detector, it was confirmed that the SNR was relatively large when the size of the probe beam was smaller than the sample size. In non-invasive spectroscopy, an imaging spectroscopic system was constructed using a CCD camera to spatially distinguish signal and non-signal regions. Our experimental results showed that the SNR increased when the absorption intensity was calculated by based only on the signal regions in CCD images. This method is expected to be useful in non-invasive spectroscopy applications.

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