

Measurement of the Amount and Number of Pollen Particles of *Cryptomeria japonica* (*Taxodiaceae*) by Imaging with a Photoacoustic Microscope

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Abstract—A photoacoustic microscope (PAM), which includes a condenser microphone and a pair of linear-motor-driven pulse stages, was specially designed for spectroscopic applications. The PAM was applied to measure the amount and number of pollen particles of *Cryptomeria japonica* (CJ), which is known for its allergic function against eyes and nose. The advantage of photoacoustic (PA) imaging is both its high sensitivity and its counting ability up to high concentrations of the specimen. The CJ pollen particles were fixed on a piece of adhesive tape or on albumen (egg white) on a glass slide set in a PA cell. The PA image showed the ability of this method to count CJ pollen from the several-hundred-milligram region to even a single particle. The PA signal obtained was integrated over the specimen surface. The dependence of the PA signal on the amount or number of the pollen particles was measured. The resulting coefficients of correlation of the calibration curves for the amount and the number of pollen particles were 0.94 and 0.97, respectively.

I. INTRODUCTION

CRYPTOMERIA JAPONICA (CJ) is an evergreen needle-leaved tree and a member of the indigenous race of *Taxodiaceae*, which is distributed throughout far-east Asia, especially in China, Korea, and Japan. It is known for its allergic function against eyes and nose, similar to that caused by hogweed [1], [2]. Horiguchi and Saito first described the allergy to CJ pollen in 1962 [3]. It has since increased rapidly [4], [5] and now affects approximately 10% of the Japanese population [6]. So, the counting of the amount of its pollen is important in the environmental science [7]. For the determination of the amount of CJ pollen, the Durham method [8] based on eye inspection has been the predominant approach until now.

In order to achieve an automated pollen count, various methods including laser scattering [9]–[11] have been introduced. However, it is difficult to achieve both high sensitivity and a wide dynamic range of detection. On the other hand, photoacoustic (PA) detection has both high sensitivity and a wide dynamic range, because even in the

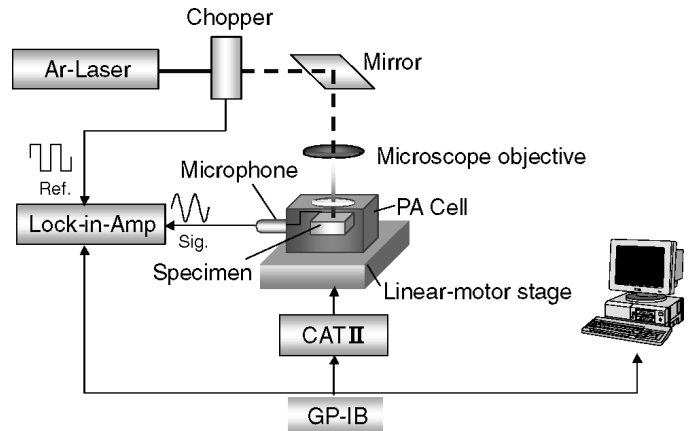


Fig. 1. The basic experimental setup of the PAM.

regime of multiple scattering of light, the absorption of the scattered light by the total absorber can be summed up in the PA detection scheme.

In this paper, a photoacoustic microscope (PAM), in which the modulated light energy absorbed by the sample is converted into acoustic or elastic energy via the conversion of the light into thermal energy, was applied to measure the amount and the number of CJ pollen particles [12]–[14]. The advantage of PA imaging is both its high sensitivity and its counting ability up to high concentrations. The PA signal was integrated over the specimen surface. The PA signal dependence on the amount of the pollen was measured and the results were compared with the amount of CJ pollen in order to investigate the ability of this method to measure the pollen amount. This method is also applied to pollen number counting at low concentrations.

II. EXPERIMENTAL APPARATUS

The PAM is based on the photothermal effect, in which the light energy absorbed by the sample is converted into heat and, especially for modulated light, into acoustic or elastic energy. The basic experimental setup of the PAM is similar to that presented in previous publications [15], [16] and shown in Fig. 1. The apparatus fundamentally consists of five divisions: optics, mechanical drivers, detec-

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tor, signal processor, and computer. The optical division includes an Ar-laser (model 2017, Spectra Physics K.K., Tokyo, Japan) as an optical source, a mechanical chopper (model CH-353, NF Circuit Block Co., Ltd., Yokohama, Japan), and an optical microscope (Optiphot, Nikon Instech Co., Ltd., Kawasaki, Kanagawa, Japan). Microscope objectives of $\times 10$ and $\times 40$ magnification with a long focal length are used. The device has a spatial resolution of $30\text{ }\mu\text{m}$, which was measured by the knife-edge method [17] and ascertained using a laser-beam profiler.

The mechanical division consists of a two-axis pulse stage driven by a pair of single-axis stepper linear motors (LMSS, ALD-105-H1L, Chuo Seiki K.K., Tokyo, Japan), their drivers (LD-P2), and a controller for them (CAT-II). The spatial resolution and drive speed of the LMSS were $0.1\text{ }\mu\text{m/s}$, and 4 mm/s , respectively.

The advantages of using slide stages controlled by linear motors are the quietness for condenser microphone detection and the high spatial resolution. Concerning the first advantage, sound noise was reduced from 83 dB to 52 dB when linear-motor slide stages were used instead of pulse-motor slide stages with high-speed mode. The spatial resolution for a single step was also improved from $2\text{ }\mu\text{m}$ to $0.1\text{ }\mu\text{m}$.

The detection division consists of a PA cell and a highly sensitive condenser microphone (Type 4166, Bruel & Kjaer). The PA cell is made of brass and is composed of a glass window, a specimen space, and an acoustic guide that connects the specimen chamber with a condenser microphone. The specimen chamber, 20 mm in diameter by 4 mm deep, is carefully designed to reduce the inside space as much as possible. The signal processing division includes a lock-in amplifier (model LI-5610B, NF Circuit Block), and the computer division includes a personal computer (IBM compatible machine) and a GP-IB bus to control all apparatus.

PA signal amplitude and phase signals simultaneously obtained by phase-sensitive detection with the reference signal are reconstructed as amplitude and phase images, and displayed and recorded on the computer. All control software, working on windows, is made with a graphical-interface language, LABVIEW (National Instruments Corp., Austin, TX).

III. SPECIMENS

The collected pollen particles of CJ were chosen to be specimens. The size of a single grain of CJ pollen is about $30\text{ }\mu\text{m}$ in diameter. Photographs of a single CJ pollen particle and multiple particles are shown in Fig. 2(a) and (b), respectively. The reflection spectrum of the CJ pollen shown in the Appendix [18] suggested that they absorbed light energy in an Ar laser line (488 nm) [12].

A. The Pollen Mass Measurement

In this study, visualization of the CJ pollen was done as shown in Fig. 2(b). The pollen was collected and fixed

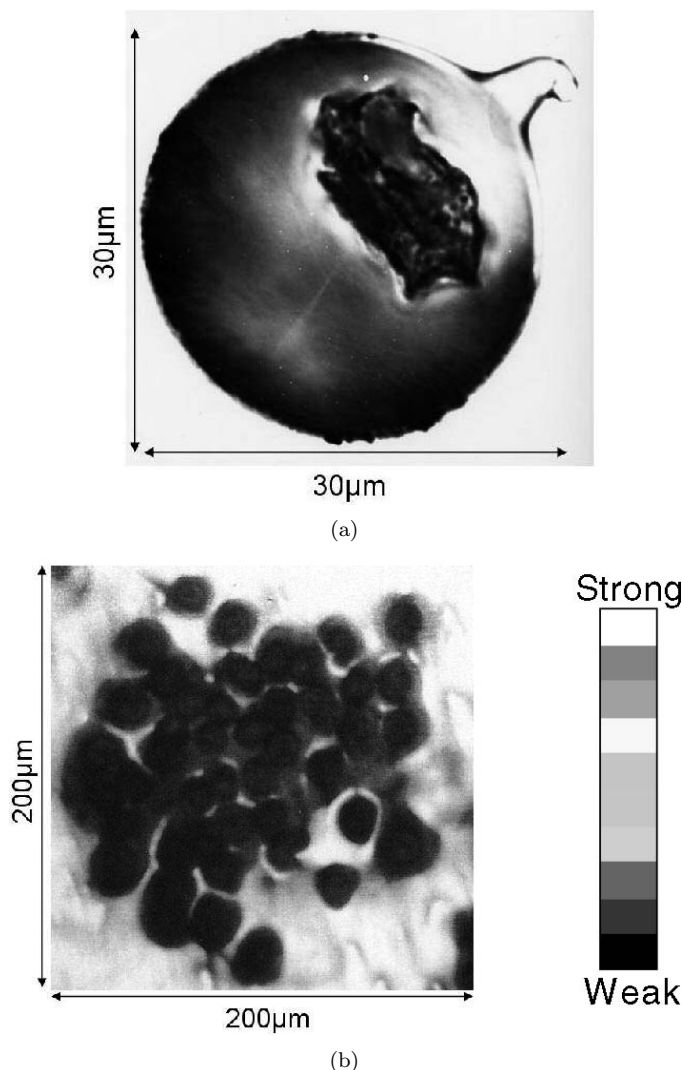


Fig. 2. (a) Photograph of a CJ pollen particle. (b) Photograph of about 50 CJ pollen particles.

on a piece of adhesive tape on a glass slide set in a PA cell. Specimens of the CJ pollen of various weights were prepared, and the PA measurement was carried out.

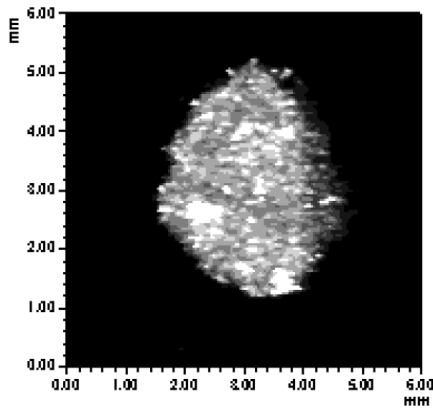
B. The Pollen Number Measurement

For number measurement, the pollen of from a single to several tens of particles were collected and fixed on protein albumen (egg white) spread on a glass slide set in a PA cell. As in the case of the mass measurement, specimens of various numbers were made and measured.

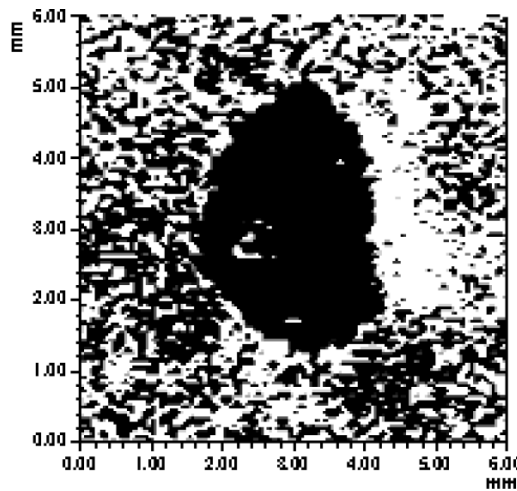
IV. EXPERIMENTAL RESULTS AND DISCUSSION

The resolution and definition of the PA image can be easily set at the front panel of the initial setting menu in the graphical interface of the control software. The data obtained are recorded in ASCII form so that they can be exported to spread sheet-handling software, and can be processed as 2D or 3D images or contour map, etc.

Weak  Strong

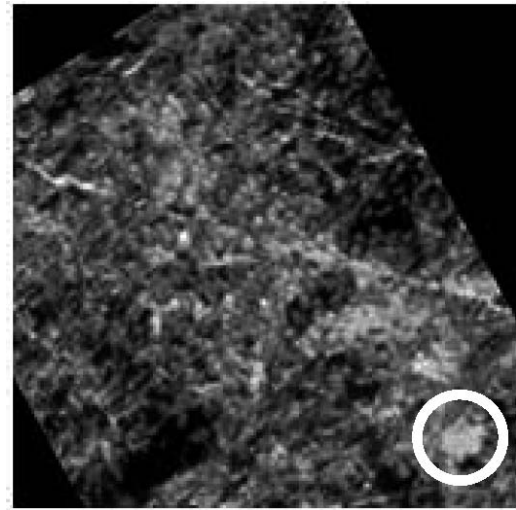


(a)



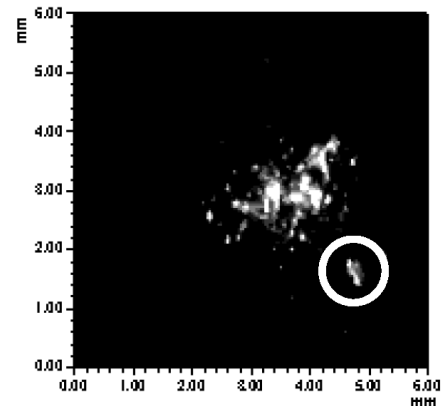
(b)

Fig. 3. (a) PA amplitude image and (b) PA phase image of 0.45 mg of CJ pollen.



(a)

Weak  Strong



(b)

Fig. 4. (a) Photograph and (b) PA amplitude image of 0.03 mg of CJ pollen.

A. The Pollen Mass Measurement

The PA amplitude and phase images, shown in Fig. 3(a) and (b), respectively, were obtained at a modulation frequency of 90 Hz. The laser power was 10 mW. The amount of the CJ pollen corresponding to this image was 0.45 mg. The size of the scanned areas was 6×6 mm, and the resolution was 100×100 pixels. In the case of the amplitude image, the color of the image changes from black to white with increasing intensity, whereas in the phase image its color changes in a relative manner as the phase advances.

In Fig. 3(a), the gray region of the amplitude image is interpreted as the CJ pollen. This is because the pollen region absorbs more light energy than the background (adhesive tape) so that the generated heat in this region is greater than in the other region of the image. The CJ pollen can also be detected in the phase image in Fig. 3(b). However, the image contrast between the phase and amplitude images is different.

Next, the photograph and the amplitude PA image of the CJ pollen corresponding to a weight of 0.03 mg are shown in Fig. 4(a) and (b), respectively. The lump shown in the lower right area and the central scattered pollen group in the photograph can be clearly visualized in the corresponding images in the PA amplitude image. Furthermore, amplitude images of the pollen for weights of 0.10, 0.23, 0.34, and 0.40 mg are respectively shown in Fig. 5(a), (b), (c), and (d). The integrated PA signal increases with increasing the pollen weight.

The above measurements were carried out for various pollen weights: 0.03, 0.05, 0.08, 0.12, 0.23, 0.33, 0.40, 0.46, 0.51, 0.62, and 0.70 mg. The measured PA amplitude in each case was integrated individually over the specimen surface, and the result was plotted as a function of pollen weight. The results are summarized in Fig. 6. (The measured data corresponding to the masses of 0.05, 0.08, 0.12, and 0.46 mg are shown with error bars, since a sheet with a hole was used as a mold for them and they have mass

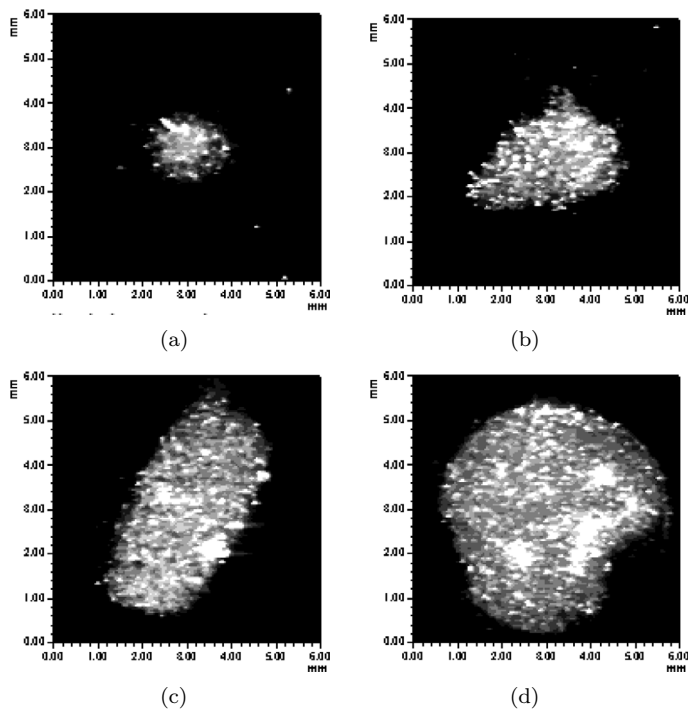


Fig. 5. PA amplitude images of (a) 0.10 mg, (b) 0.23 mg, (c) 0.34 mg, and (d) 0.40 mg of CJ pollen.

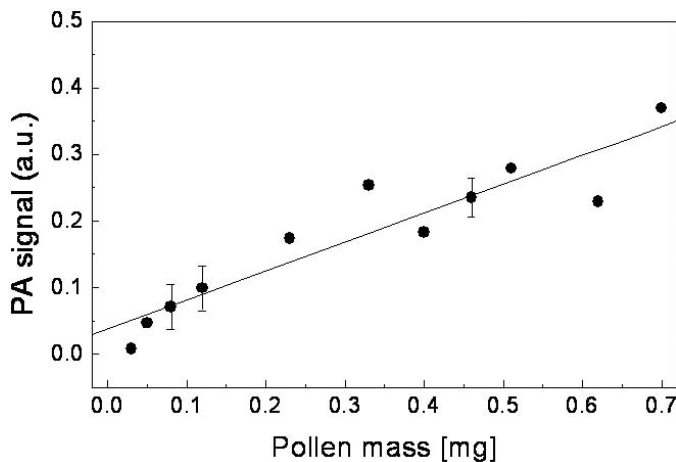


Fig. 6. The calibration curve of the PA signal as a function of the mass of CJ pollen.

reproducibility.) The correlation coefficient of Fig. 6 was about 0.94. Therefore, it was concluded that the integrated PA signal is proportional to the amount of pollen.

B. The Pollen Number Measurement

Amplitude and phase PA images and a corresponding photograph of ten CJ pollen particles are shown in Fig. 7(a), (b), and (c), respectively. Those corresponding to a single pollen particle are also shown in Fig. 8(a), (b), and (c), respectively. In both cases, experimental conditions were as follows: modulation frequency of 90 Hz, scanned area of 1×1 mm, resolution of 100×100 pixels, laser power

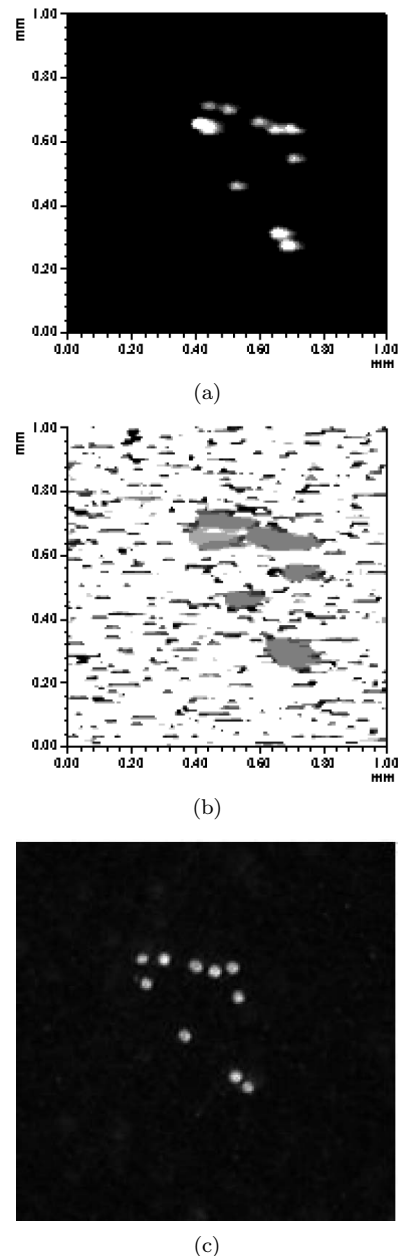


Fig. 7. (a) PA amplitude image, (b) PA phase image, and (c) photograph of ten CJ pollen particles fixed by albumen.

of 10 mW, measurement time <90 minutes, and laser spot size of $30 \mu\text{m}$. These PA images clearly show that even a single particle can be resolved in PA images. In this case, the pollen was collected and fixed on the albumen expanded on a glass slide set in a PA cell to reduce the background due to the optical absorption. As a result, we could achieve background reduction comparable to that obtained in the previous method using adhesive tape. PA amplitude signal integrated over the specimen surface was plotted as a function of the pollen number. The results are summarized in Fig. 9. The correlation coefficient for the calibration curve shown in Fig. 7 was calculated to be about 0.97. Therefore, it was shown that linearity exists between the integrated PA signal and the CJ pollen number.

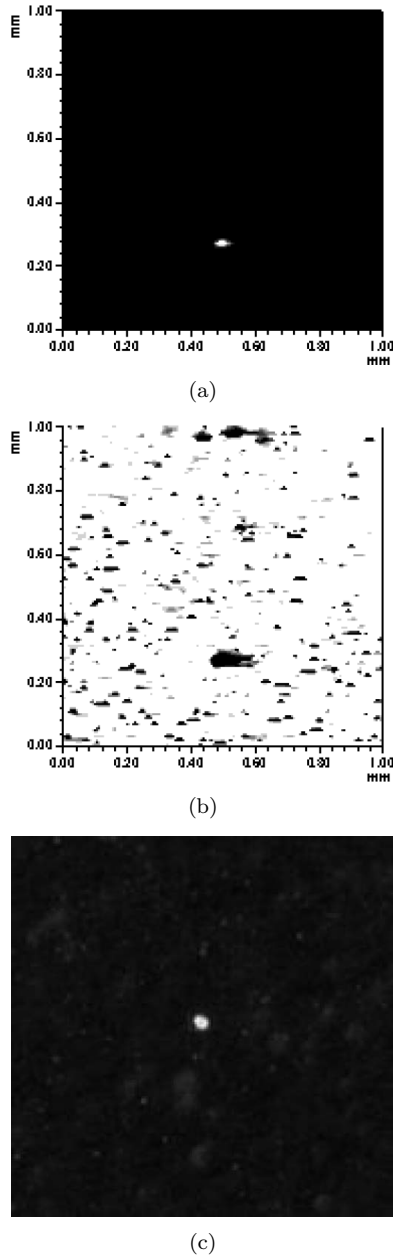


Fig. 8. (a) PA amplitude image, (b) PA phase image, and (c) photograph of a single CJ pollen particle fixed by albumen.

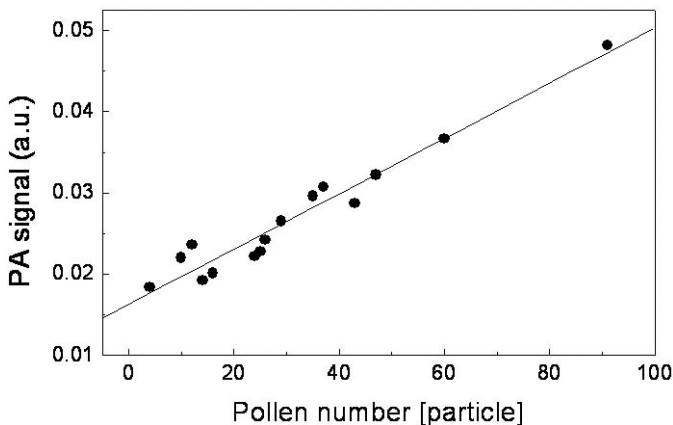


Fig. 9. The calibration curve of the PA signal as a function of the number of CJ pollen particles.

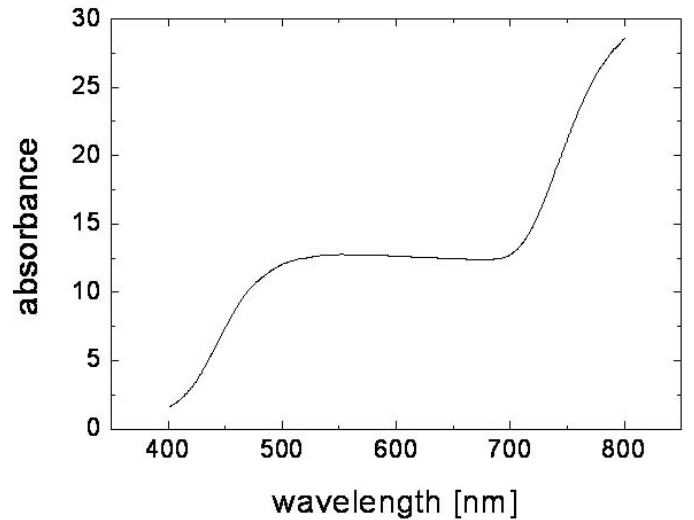


Fig. A1. Absorption spectrum of CJ pollen.

V. CONCLUSION

In this study, the PA amplitude and phase images of the CJ pollen were obtained with a photoacoustic microscope. The amplitude PA image was integrated over the specimen surface. The integrated amplitude PA image was plotted as a function of pollen amount. This technique was also applied to measure the PA signal dependence on CJ pollen number. The visualization of the CJ pollen individual particle, even a single particle, was also achieved. As a result, the integrated PA amplitude images showed good linearity with respect to the mass and numbers of the CJ pollen particles. The PA signal dependence on pollen mass and numbers showed correlation coefficients of about 0.94 and 0.97, respectively. Thus, it was shown that CJ pollen measurement using the photoacoustic microscope can be applied to concentration measurement with a large dynamic range from low to high concentrations. Furthermore, it was suggested that the automated measurement of the CJ pollen number using a photoacoustic microscope would be possible in the near future.

APPENDIX A

ABSORPTION SPECTRUM OF THE CJ POLLEN

The absorption spectrum of the CJ pollen was measured by the reflection spectrum with an integration sphere method [19].¹ The absorption spectrum was obtained from the reflection spectrum by the standard Kramers-Kronig analysis. The result is shown in Fig. A-1. It has a broad absorption band over the visible spectrum [18].

If we try to apply the differential absorption technique for the actual measurement in the urban area, two optical sources must be chosen, for example, at 488 nm and in the infrared region.

¹See homepages of optical instrument manufacturers, for example, <http://www.perkinelmer.com/>.

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