

ACOUSTICAL LETTER

Compact test setup for sensitivity evaluation of photoacoustic contrast agent

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1. Introduction

Photoacoustic (PA) tomography has dramatically developed in the past 20 years and is expected to become a common imaging method in hospitals in the next decade [1,2]. The first generation of PA tomography adopted human elements as the target absorber, for example, DNA, hemoglobin, and lipids [3,4]. Recently, the second generation of PA tomography has adopted an exogenous high-absorption contrast agent to improve its contrast, spatial resolution, and functionality. This may extend the imaging depth from genes to the whole human body [5,6]. In this letter, to accelerate the development of photoacoustic contrast agents, we report the design of a compact and cost-effective test machine for the sensitivity evaluation of photoacoustic contrast agents. The system adopts two laser diodes driven by a modulated continuous source in the kilohertz region, and the generated acoustic signal is detected by a small microphone attached on an acoustic resonator. The operation of the proposed setup is examined using ink solutions and an existing contrast agent.

2. Design of photoacoustic evaluation system

2.1. Setup of photoacoustic evaluation system

Figure 1 shows the setup of the proposed evaluation system for a contrast agent. Two laser diodes (LD) operating at 660 and 785 nm are employed as excitation sources (660 nm LD, Mitsubishi ML101J27; 785 nm LD, Thorlabs L785P090), and driven by a 100%-depth modulated voltage using a function generator (Tektronix, AFG310) via an appropriate resistor. The LDs are selected with a switch. The average output optical power is set to approximately 20–40 mW. The spot diameter is collimated to around 5.0 mm using a collimator lens. We chose 660 and 785 nm as the working wavelengths in this experiment, but these should be selected by consideration of the target materials. The two laser paths are combined with a polarization beam splitter (PBS) and led to the sample liquid to generate a PA signal. A one-dimensional PA cell is employed to enhance the PA signal using the acoustic resonance of the cell. The bottom end of the PA cell is in contact with the surface of the sample liquid so that part of the PA signal generated in the sample liquid can be led to the cell. Air is confined in the PA cell. The PA cell is made of copper and resonates in the fixed-fixed mode since the top of the PA cell is sealed with a thin glass plate. A small microphone based on a micro–electro–mechanical systems ($1.45 \times 6.15 \times 3.76\,\mathrm{mm}^3$, Knowles Acoustics, SP0103NC3-3) is attached at the middle of the PA cell to pick up the PA signal. The microphone covers the frequency band from 100 Hz to 10 kHz. The PA signal is processed with a preamplifier (NF Corp., 3611) and a lock-in amplifier (NF Corp., 5560).

2.2. Design of photoacoustic cell

The inner diameter and length of the PA cell are 5 and 86 mm, respectively. The inner surface is polished to minimize the spurious PA signal due to the absorption on the wall. The cell acts as a fixed-fixed acoustic resonator to amplify the PA signal. Resonance modes having a pressure loop at the center can be used since the microphone is attached at the center. The wavelength for the nth resonance is written as

$$\lambda_n = \frac{2L}{n} \quad (n = 2, 4, 6 \ldots\ldots), \tag{1}$$

using the length of the PA cell L (= 86 mm). Then, the nth resonance frequency is

$$f = \frac{nc}{2L} \quad (n = 2, 4, 6 \ldots\ldots). \tag{2}$$

Here, c is the sound speed.

2.3. Considerations for PA signal generation

PA phenomena in a liquid are categorized into six different cases according to the relationship between the sample length, optical absorption length, and thermal diffusion length [7,8].

In our experiment, the thermal diffusion length of the ink solution is similar to that of water (≈ 0.0006 cm) owing to its low concentration (<0.2%). We always keep the depth of the sample solution greater than the thermal diffusion length. A laser ray penetrates through the sample. Thus, we have the following relationship for the experiments reported in this letter:

$$u_\beta > l > u_\mathrm{s}, \tag{3}$$

where l is the depth of the sample, u_β is the absorption length, and u_s is the thermal diffusion length of the sample. The intensity of the PA signal *PAS* and the other parameters satisfy the following relationship according to Ref. [7]:

$$PAS \propto \frac{I_0 \cdot \beta \cdot u_\mathrm{s}}{\omega^{\frac{3}{2}}}. \tag{4}$$

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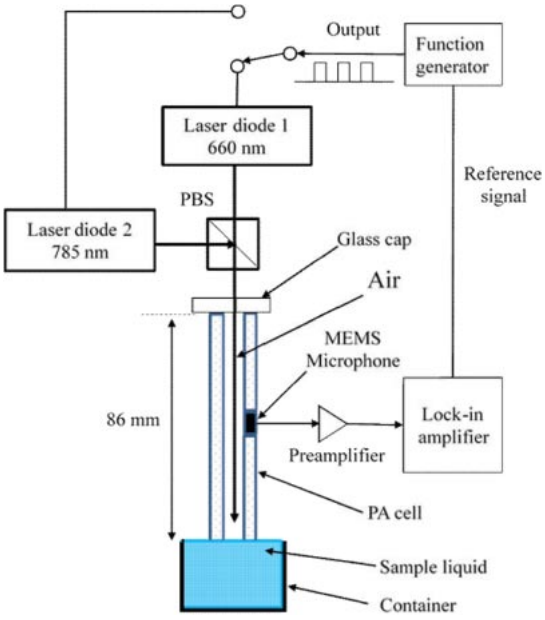

Fig. 1 Structure of photoacoustic evaluation system.

Table 1 Optical absorption coefficients for water and inks in cm^{-1}.

Wavelength (nm)	Water [9]	Black ink	Dye ink
660	0.004	754.71	228.45
785	0.01	203.41	90.47
Ratio (660/785)	0.40	3.7	2.5

Here, I_0 is the incident monochromatic light flux, β is the optical absorption coefficient of the sample, and ω is the modulation frequency. In our experiments, the PA signal is determined by the optical absorption coefficient of the sample. This is the key principle of the proposed PA system.

3. Characteristics of photoacoustic system

3.1. Absorption coefficients of tested samples (inks)

First, we tested solutions of a black ink, a dye ink, and their mixture and a sample contrast agent to verify the proposed system. The absorption coefficients of the inks and the ratios are summarized in Table 1. The absorption coefficient of the black ink is larger than that of the dye ink at both 660 and 785 nm wavelengths, while the absorption coefficients of water are much smaller than those of the inks. The ratios of the absorption coefficient at 660 nm to that at 785 nm were 3.7 and 2.5 for the black ink and dye ink, respectively.

3.2. Resonant characteristics of PA cell

Here, we tested the resonant frequency of the PA system using the black ink solution (45 mg/dl). The 785 nm LD acted as a light source and its modulation frequency was varied from 0.1 to 12.0 kHz with steps of 0.1 or 0.02 kHz. As shown in Fig. 2, the resonant frequencies were found approximately at 4, 8, and 12 kHz, in agreement with Eq. (2). Considering

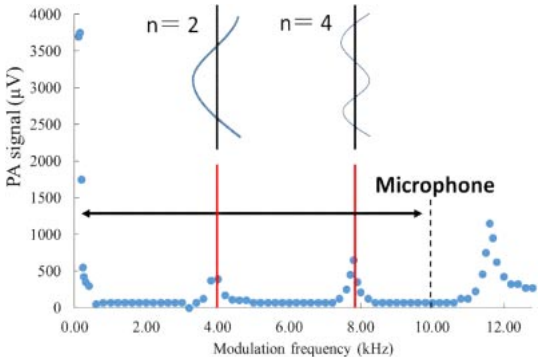

Fig. 2 Resonant frequencies of PA cell measured for black ink solution at 785 nm.

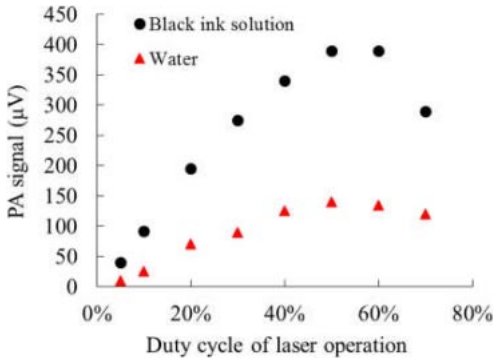

Fig. 3 Duty ratio of the light source and PA output level.

the rated frequency range of the microphone (100 Hz to 10 kHz), we chose the resonance at around 7.78 kHz as the working frequency in this experiment. The working frequency was adjusted to track the resonance in the range from 7.71 to 7.85 kHz since the temperature ranged from 17 to 26°C. The quality factor of the resonance was estimated to be approximately 40. The response observed at very low frequencies is thought to be a spurious signal due to the electronic system.

3.3. Modulation duty ratio and PA signal

The duty ratio of the modulation for the LDs was altered and the PA output was recorded. We carried out the test at 785 nm for water and a black ink solution (45 mg/dl) as samples. The results are shown in Fig. 3. The output signal level increased with increasing duty ratio and showed a maximum at a duty ratio of 50%. This means that the sample is continuously heated and expanding during the on-time of the laser, and the output exhibits a maximum when the heating period is equal to the cooling period. In a future study, we need to verify that the PA sensitivity evaluated by this modulated method is identical to the sensitivity under single-pulse excitation.

4. Evaluation of mixture ratio and concentration of samples

4.1. Concentration of black ink and PA signal

We gradually added black ink to a water sample (height, 78 mm; volume, 2.35 dl) to increase the concentration of the solution in a step of 10 mg/dl. The PA signals at both 660 and

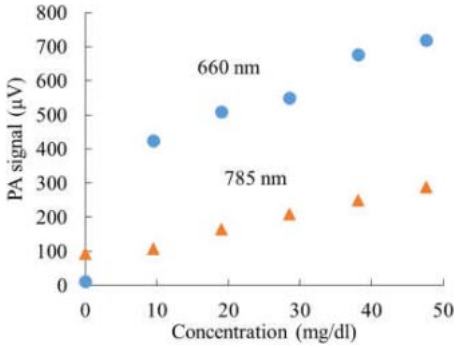

Fig. 4 PA signals at 660 and 785 nm as functions of concentration of black ink solution.

Table 2 Samples with black and dye inks mixed in 0.5 dl water.

Sample group	0	1	2	3	4	5
Black ink (mg)	0	200	150	100	50	0
Dye ink (mg)	0	0	50	100	150	200

785 nm wavelengths increased linearly with the concentration in the range of 10–50 mg/dl as shown in Fig. 4. The average optical power was adjusted to 20 mW. The signal level at 660 nm was always approximately three times that at 785 nm. The results agreed well with the absorption coefficients at 660 and 785 nm and the ratio between them shown in Table 1. When the sample was water (0 mg/dl), the PA signal for 785 nm was higher than that for 660 nm because the absorption coefficient of water at 785 nm is higher than that at 660 nm. However, the PA signal ratio for water at 660 and 785 nm was not exactly the same as the absorption coefficient ratio of water because these low PA signals had already reached the level of system noise, which was observed to be around 80 μV.

4.2. Mixture of black ink and dye ink

In the second experiment, we prepared six groups of samples as listed in Table 2.

The first sample was water and the other samples were ink solutions composed of the black ink and dye ink. The results are summarized in Fig. 5. From group 1 to 5, the quantity of black ink decreased, while that of the dye ink increased. The PA signals at 660 and 785 nm decreased from group 1 to 5 because the absorption coefficient of the dye ink is smaller than that of the black ink at both wavelengths. The PA signals at 660 nm are always higher than those at 785 nm because the absorption coefficients of both inks at 660 nm are higher than those at 785 nm. Then, the ratio of the PA signal at 660 nm to that at 785 nm was calculated and plotted in the figure. The ratio of sample 0 (water) is less than one owing to its absorption coefficient ratio. The ratio decreased from group 1 to 5 in accordance with the mixture ratio since the absorption coefficient ratio of the dye ink at the two wavelengths was smaller than that of the black ink. The mixture ratio of the black ink to the dye ink could be estimated from the signal ratio. Here, the average optical power for both wavelengths was around 20 mW.

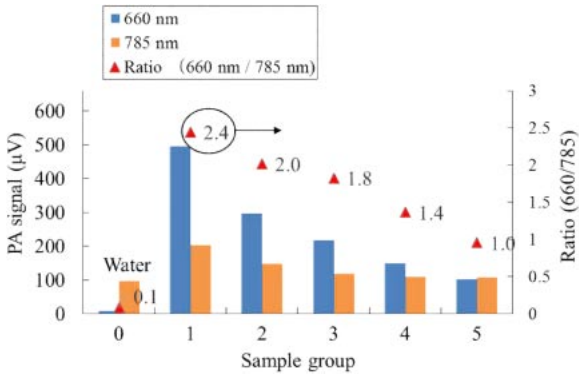

Fig. 5 PA signals for solutions mixed with black and dye inks at 660 and 785 nm.

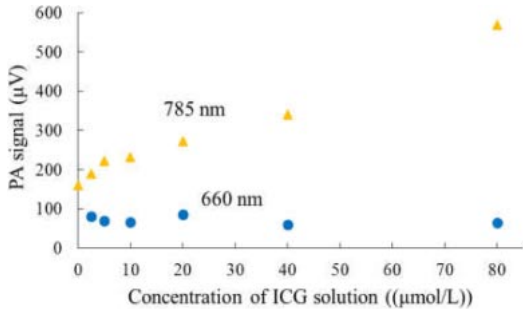

Fig. 6 PA signal level as a function of concentration of ICG solution.

4.3. Test for ICG solution

Indocyanine green (ICG) is used for video angiography in hospitals owing to its fluorescence characteristics. Here, we tested the photoacoustic capability of ICG solution using the proposed evaluation system. ICG was diluted by dimethyl sulfoxide (DMSO), which is a common solvent and has little absorption at these two wavelengths. A 4 ml sample was prepared in a small glass dish of 20 mm diameter and 4 mm depth. The PA signal was recorded as a function of the concentration of the solution using the 660 and 785 nm LDs. The absorption of ICG at 785 and 660 nm was 123,000 and 21,200 $L \cdot cm^{-1} \cdot mol^{-1}$ respectively.

The PA signal increased almost proportionally to the concentration at 785 nm as shown in Fig. 6. However, the responses were not stable at higher concentrations. This might be due to the poor stability of the sample. During the measurement, the average optical power at 785 nm of approximately 40 mW was injected into the sample, where thermal and optical effects excited by the optical power on the sample must be considered. The PA signal at 660 nm remained unchanged at lower levels around the noise level (around 80 μV) of the system because the absorption was much smaller at this wavelength, and the injected optical power was 20 mW, which was half of that at 785 nm.

If ICG is mixed with another material, or injected into a tissue with a strong-absorption chromophore, this PA system with two working wavelengths can determine the mixture ratio of two materials as we demonstrated in the previous

experiment, or can determine the concentration of the chromophore from the concentration of ICG.

5. Conclusions

A compact photoacoustic system using continuous excitation at a low modulation frequency was designed as evaluation equipment for PA contrast agents for medical applications. We confirmed the successful operation of the prototype through measurements of different ink solutions. ICG solutions were also tested using the prototype. The experiments reported in this letter were conducted under uncontrolled room temperature and humidity. Moreover, the laser power was not stabilized. It is necessary to introduce regulated conditions both for the sample and the excitation for practical use. In the near future, we need to compare the results obtained by the proposed system and the quality of PA images obtained by a practical PA imaging machine using various contrast agents. We expect that the proposed simple test setup will accelerate the development of new functional contrast agents and be of help in pioneering a new generation of medical applications of PA imaging.

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