Performance comparison of cell phantoms developed for optical-resolution photoacoustic microscopy

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1. Introduction

Photoacoustic imaging (PAI) is an imaging method based on the photoacoustic effect that can selectively image cells and tissue. Among various PAI modalities[1)], optical-resolution photoacoustic microscopy (OR-PAM) has a high lateral resolution superior to the other PAI modalities and has achieved a sub-micron imaging capability with an optimal combination of the optical and acoustic elements[2)]. This spatial resolvability is considered to be useful for imaging individual cells and intracellular structures. Furthermore, using PA contrast agents (e.g., metal nanoparticles) is expected to extend the ability to image various cells with low optical absorbers. However, there has been a lack of experimental tools to simulate and characterize photoacoustic signals from the cells. Here, we report a cell phantom for testing a single cellular OR-PAM system using simple material and fabrication protocol. In addition, we evaluated the efficacy of the devised phantom as a cellular phantom for the OR-PAM system compared with an agar-gel phantom.

2. Methods

2.1 High-resolution OR-PAM

In this study, we utilized a high-resolution OR-PAM system[3)]. A green pulse laser (wavelength: 532 nm, pulse reputation frequency: 10 kHz, pulse width: 6ns) was focused with optical elements and irradiated to imaging targets that induce the photoacoustic (PA) waves from the targets. The PA waves were acquired with the ultrasound transducer (Center freq. 50 MHz) at a sampling frequency of 5 GS/s and recorded as a three-dimensional (X, Y, and time) data set. PA "A-line" signal at each pixel was averaged over 100 PA wave acquisitions.

The recorded PA signals were transformed into analytics signal through a bandpass filter (passband: 20-100 MHz) and the Hilbert transformation, and the 2D C-mode images were generated with Maximum Intensity Projection (MIP).

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2.2 The cell phantom for OR-PAM system

To develop a cell phantom, we considered a simple fabrication protocol utilizing simple and low-cost materials. Fig. 1 shows a schematic of the developed cell phantom. This phantom was made of echo jelly and cling film. First, an object mimicking an intracellular optical absorber was placed on the bottom of a glass base dish (35 mm Glass Base Dish: Glass diameter: 12 mm; IWAKI). Later, the optical absorber was covered with echo jelly (SONO JELLY, Canon Medtech Supply Corp.) and a cling film. The echo jelly decreased the attenuation of acoustic propagation and mimic the intracellular environment. In addition, the cling film kept an imaging environment and protected the ultrasound transducer. When imaging is performed, the coupling medium (i.e. pure water) was filled in the void space of the dish (Fig. 1 (a)). To store the phantom, the coupling

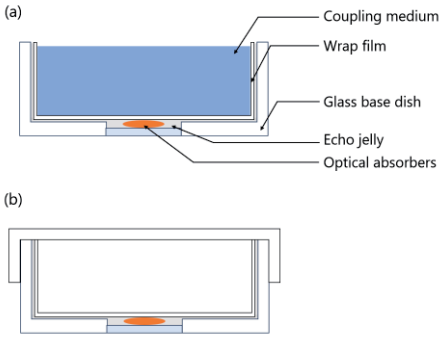

Fig. 1 The structure of the cell phantom developed in this study. (a) For imaging, (b) For storing

medium was discarded and the lid was set on the dish (Fig. 1 (b)).

2.3 Comparison with conventional phantom

We compared the developed phantom with the water and the agar phantom (Fig. 2). we choose black tape as an imaging target and put it on the glass base dish. Fig. 2 (a) was the water phantom, and this phantom was filled with pure water. Fig. 2 (b) was developed cell phantom type. The black tape was covered with the echo jelly and wrap film. Fig. 2 (c) was the agar phantom[4),5)]. The Agar was prepared by dissolving agar powder in hot water to a

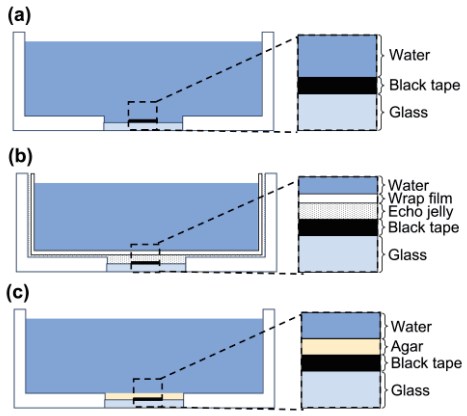

Fig.2 (a) Water phantom, (b) developed cell phantom, (c) Agar phantom (2% w/v agar)

concentration of 2% w/v and stirring until it became homogeneous. After that, the black tape was covered with the agar gel and left at room temperature for 1 hour to solidify.

PA images of each phantom were acquired. PAI parameters were set as the range of 50 µm x 50 µm at 500 nm step (100,000 A-lines) and a laser power of 9 nJ/pulse, respectively. After the acquisition the maximum intensity of the signal was compared. In addition, the averaged A-lines and spectrums of the averaged A-lines were compared among the phantoms.

3. Results and discussion

Fig. 3 (a) shows the maximum intensity of each phantom. The value of the water phantom was highest because agar and cell phantoms have some boundaries that have acoustic properties changed. The difference value between the agar phantom and cell phantom 8.0 x 10^{-5} [a.u.].

Fig. 3 (b) and (c) show the average A-lines and spectrum of the averaged A-lines for each phantom. The spectrum was normalized with water phantom. Compared with the water phantom and the cell phantom, the frequency properties were similar in the range of the ultrasound transducer's bandwidth (<100 MHz).

Therefore, we could confirm that the PA signal characteristic of the developed new style phantom was comparable to one of the agar phantom. The developed cell phantom was expected to be a suitable phantom for cellular imaging OR-PAM.

4. Conclusion

In this study, we developed a new cell phantom with the echo jelly and cling wrap film for OR-PAM system. Experiments showed that the acquired PA signal characteristics were found to be similar to that of the agar phantom. Therefore, the developed cell phantom can be suitable for cellular imaging OR-PAM.

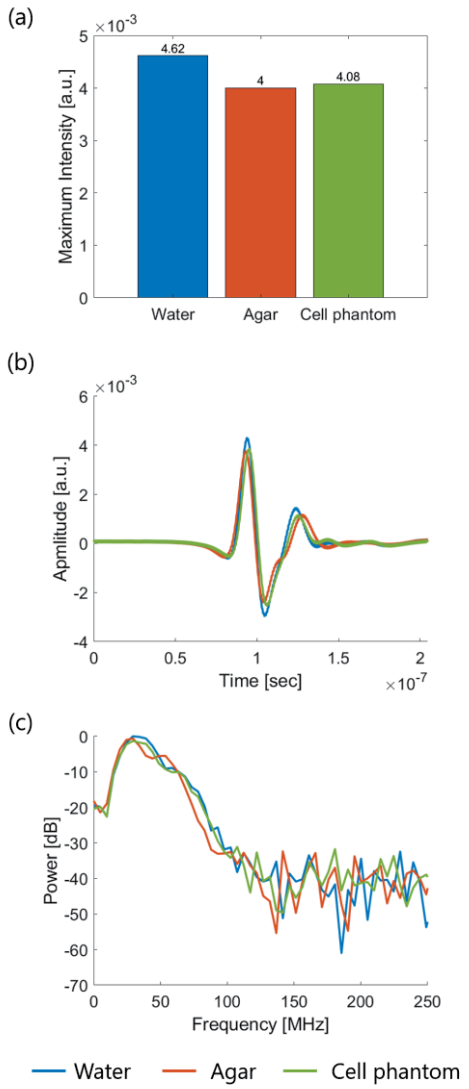

Fig. 3 (a) Maximum intensity of each phantom, (b) average A-line, (c) spectrum of average A-line (Normalized by water phantom criteria)

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