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Photoacoustic Imaging for Lymphatic Vein Anastomosis – Examination using small animals

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Abstract— **The diameters and shapes of lymphatic vessels and the blood vessels which surround them provide important information used in determining the degree of progression of lymphedema and the subsequent planning of lymphatic vein anastomosis. The aim of this project is to display this information in three dimensions using photoacoustic imaging (PAI). For this study, we used a hemispherical sensor array PAI system that provides both a high resolution and a wide field of view and displays a 3D image.**

We used indocyanine green (ICG) to detect lymphatics using PAI. Because oxyhemoglobin, deoxyhemoglobin, and ICG have different optical absorption spectra, we were able to distinguish between lymphatic vessels and blood vessels by using two wavelengths (800 nm and 850 nm). Our PAI system performed 3D reconstruction by performing 2D scanning with a hemispherical sensor array. We injected ICG into mouse and rat fingers and imaged the lymphatic and blood vessels of the entire foot. After performing photoacoustic measurements, we confirmed the diameter and shape of lymphatic and blood vessels using dissection and ICG fluorescence imaging.

For measurements of rats, lymphatic vessels containing ICG are visible on both sides of the blood vessel. In PAI of smaller mice, lymph nodes are displayed along with lymphatic vessels. It is easy to distinguish lymphatic vessels and blood vessels using PAI, and we were able to demonstrate lymphatic and blood vessels in three dimensions.

Keywords—photoacoustic imaging, lymphatic vessels, hemispherical sensor array

I. INTRODUCTION

The lymphatic system is an essential part of all living bodies. The diameters and shapes of lymphatic vessels and the blood vessels surrounding them contain important information used to determine the degree of progression of lymphedema and for the subsequent planning of lymphatic vein anastomosis. Fluorescent imaging is currently in widespread clinical use for the imaging of lymphatic vessels, but this technology presents only 2D images and cannot depict both lymphatic vessels and blood vessels simultaneously. Our aim is to display lymphatic and vascular information in three dimensions using photoacoustic imaging (PAI).

Photoacoustic microscopy has conventionally been used for lymphatic imaging of small animals using PAI [1, 2]. This technique has high resolution (several micrometers) but provides a narrow field of view and low measurement depth (only a few millimeters). Hence, measurement using a handheld PAI system is mainly used for human lymphatic vessel measurement [3]. However, a handheld PAI system provides only 2D information and does not have a sufficiently high resolution for anastomosis planning. Therefore, for this study, we used a hemispherical sensor array PAI system that has both a high resolution and a wide field of view and offers a 3D image [4].

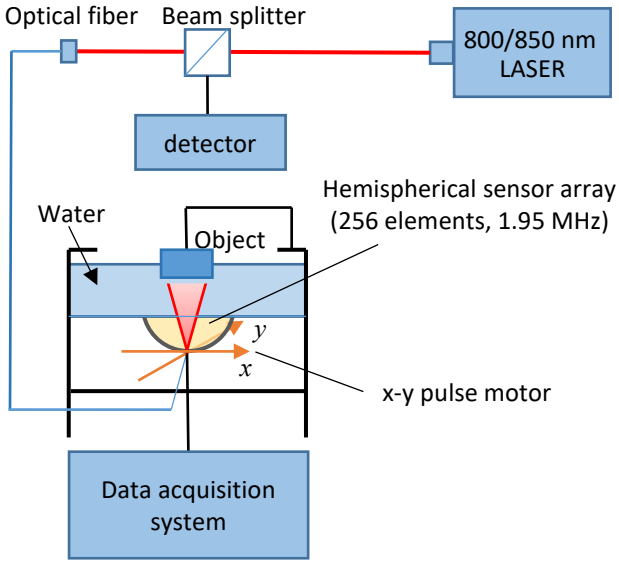

Fig. 1. Schematic of experimental setup

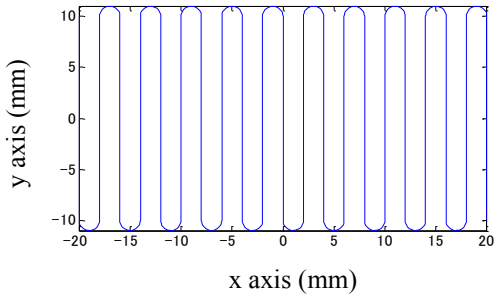

Fig. 2 Motion of the hemispherical sensor array during 2D scanning

II. METHODS

A. Experimental system

Figure 1 depicts a schematic of our overall experimental setup. A hemispherical sensor array (with a 10-cm diameter, 256 elements, 1.95-MHz center frequency) located on a mechanical stage was connected to a research ultrasound system (Vantage 256; Verasonics Inc.). Laser pulses (800 nm and 850 nm wavelength, 30 Hz PRF) guided by an optical fiber were irradiated upward along the vertical axis of the hemispherical sensor array. Photoacoustic (PA) signals were generated from objects in deaerated water and received by all elements on the hemispherical sensor array; these were then stored in the data acquisition system. Our PAI system reconstructed a 3D PA image with a wide field of view by horizontally scanning a hemispherical sensor array, as shown in Fig. 2. Our system had a field of view of up to 150 mm × 150 mm × 40 mm.

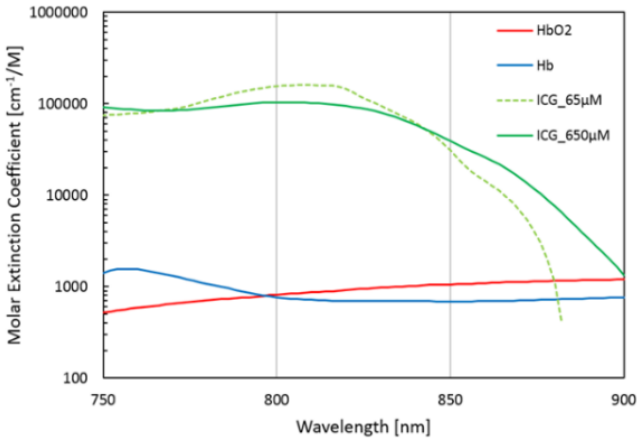

Fig. 4. Optical absorption spectra

B. Evaluation of resolution

We calculated the spatial resolution by scanning a cross section of a hair (53 μm diameter) with the hemispherical sensor array at 800 nm. We then evaluated the full width at half maximum (FWHM) in the depth direction and horizontal direction of a given image (see Fig. 3(a)). Figures 3(b) and 3(c) show profiles of the depth direction and horizontal direction respectively. These results indicate that the resolution of our system in depth is approximately 0.44 mm and horizontally is approximately 0.42 mm.

C. Imaging of small animals

We injected modest doses of ICG (5 mg/ml) into mouse and

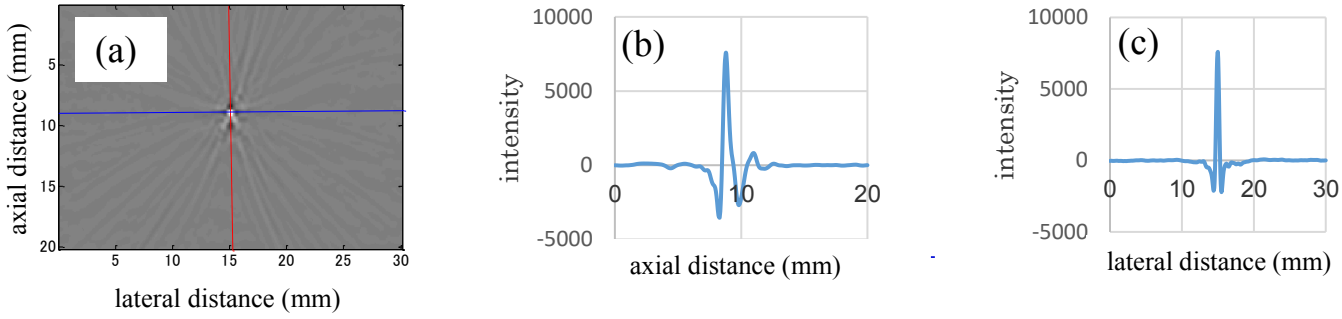

Fig. 3. Evaluation of resolution
(a) Photoacoustic image of a hair, (b) Profile of depth direction, (c) Profile of horizontal direction

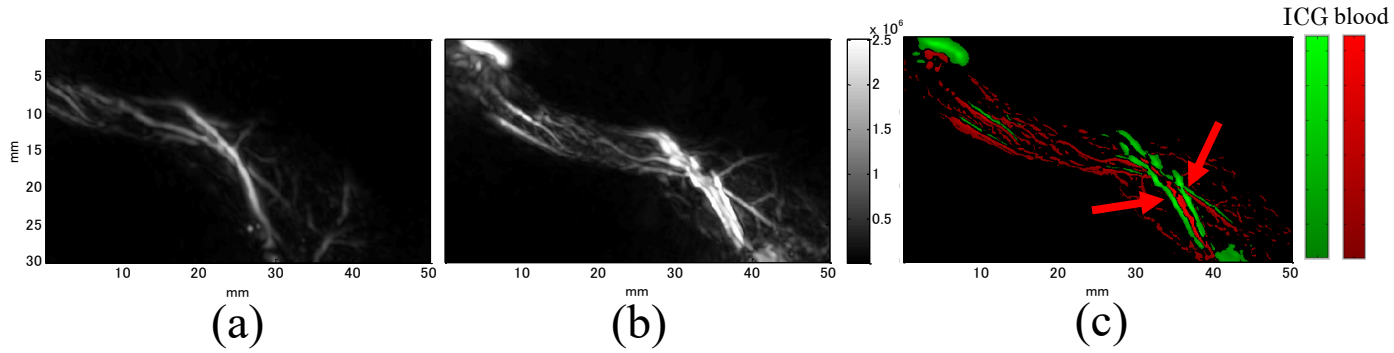

Fig. 5. PA Images of rat
(a) Maximum intensity projections (MIP) of legs before injection of ICG at 800 nm, (b) MIP of legs after injection of ICG at 800 nm, (c) Image extracts lymphatic vessels (red arrows) and blood vessels (red parts)

rat, specifically into their feet. We then used our system to image the lymphatic vessels (ICG) and blood vessels of the foot.

D. Distinguishing between lymph (ICG) and blood vessels

Because blood and ICG have different optical absorption spectra (Fig. 4), we can distinguish between lymphatic vessels (ICG) and blood vessels by using two wavelengths; they are 800 nm and 850 nm respectively. In the following formula, the amplitude of the photoacoustic signal of the wavelength λ as normalized by the optical fluence is PA (λ). By calculating the ratio of PA (850 nm) and PA (800 nm), we can distinguish lymphatic vessels (ICG) from blood vessels. That is, we can define a vessel to be lymphatic (ICG) when this ratio is less than 1 and to be blood when this ratio is larger than 1.

$$\begin{aligned} &\text{PA (850 nm) / PA (800 nm)} < 1 \rightarrow \text{ICG} \\ &\text{PA (850 nm) / PA (800 nm)} > 1 \rightarrow \text{blood} \end{aligned} \qquad (1)$$

III. RESULTS AND DISCUSSION

A. Imaging of a rat

Figures 5(a) and (b) represent the results obtained before and after ICG injection into rats. We distinguished between ICG and blood using the ratio of PA (850 nm) to PA (800 nm), and then colored the ICG and blood green and red respectively, as shown in Fig. 5(c). Lymphatic vessels containing ICG are clearly visible on both sides of the blood vessel in this figure. We also confirmed the position of lymphatic vessels by dissection and ICG fluorescence imaging. The actual diameter of the lymphatic vessel was approximately 0.1 mm. This proves that our system can present lymph vessels of sub-resolution diameters. Due to technical limitations, the lymphatic vessel diameter in the photoacoustic image is displayed as thicker than the actual diameter.

B. Imaging of a mouse

Figures 6(a) and 6(b) depict the before and after views of ICG injected into a mouse. As before, we distinguished between

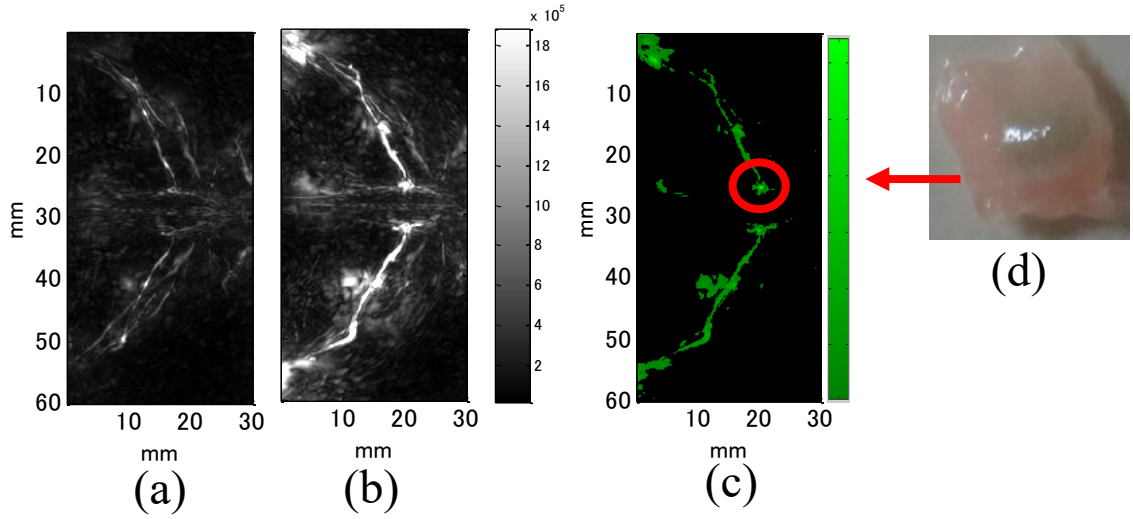

Fig. 6. PA Images of mouse
(a) Maximum intensity projections (MIP) of legs before injection of ICG at 800 nm, (b) MIP of legs after injection of ICG at 800 nm, (c) Image extracts lymphatic vessels (yellow arrow) and lymph nodes (red circle), (d) Image of a lymph node

ICG and blood by using the ratio of PA (850 nm) to PA (800 nm), but this time, we abstracted only the green lymph part (ICG), as shown in Fig. 6(c). In this image, lymph nodes are also presented along with lymphatic vessels. We also confirmed the position of lymph nodes by dissection (Fig. 6(d)).

IV. Conclusion

We have shown that the differences between lymphatic and blood vessels can clearly be distinguished using PAI. Moreover, we have also demonstrated that PAI has the capability of displaying lymphatic and blood vessels in three dimensions. These conclusions are expected to contribute to more accurate operations in lymphatic vein anastomosis.

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