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Abstract. We propose a compact and easy to use photoacoustic imaging (PAI) probe structure using a single strand of optical fiber and a beam combiner doubly reflecting acoustic waves for convenient detection of lymph nodes and cancers. Conventional PAI probes have difficulty detecting lymph nodes just beneath the skin or simultaneously investigating lymph nodes located in shallow as well as deep regions from skin without any supplementary material because the light and acoustic beams are intersecting obliquely in the probe. To overcome the limitations and improve their convenience, we propose a probe structure in which the illuminated light beam axis coincides with the axis of the ultrasound. The developed PAI probe was able to simultaneously achieve a wide range of images positioned from shallow to deep regions without the use of any supplementary material. Moreover, the proposed probe had low transmission losses for the light and acoustic beams. Therefore, the proposed PAI probe will be useful to easily detect lymph nodes and cancers in real clinical fields. © 2017 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.22.9.091513]

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1 Introduction

Photoacoustic imaging (PAI) can provide *in vivo* functional and molecular imaging by using ultrasonic waves generated by pulsed laser light signals.¹ It has the advantage of being able to easily identify the properties of the target materials such as lymph nodes, cancer cells, capillary blood vessels, and the oxygen saturation of hemoglobin.² In addition, the PAI imaging system can achieve large-depth imaging like diffuse optical tomography³ or high-resolution imaging like optical coherence tomography.⁴ Therefore, PAI has great potential for future diagnostic imaging.⁵

For diagnostic detection of lymph nodes and cancers, the PAI system has to be able to achieve both shallow and deep imaging because human lymph nodes are positioned at various depths.⁶ Additionally, the PAI system needs real-time monitoring and a compact structure in order to be useful in medical imaging diagnosis. Recently, the handheld PAI probe structure and system that can achieve deeply penetrating and real-time imaging has attracted increasing research interest.

Most developed handheld PAI probes employ an optical fiber or optical fiber bundles attached to the side of the ultrasound transducer for light beam delivery.^{7–12} The probes have the merits of low transmission losses of light and sound, because they do not use any combiner so that a probe with the structure can receive the largest photoacoustic signal as a function of the light intensity.¹³ However, the detectable imaging region depends on the angle of incident light and there is an undetectable zone, called dark-field illumination, because the illuminated light

beam is oblique with respect to the acoustic beam, and the image is obtained in the intersecting region between the illuminating light beam and receiving acoustic beam. In order to obtain images at shallow depths, the probe may require a dummy material such as a chicken breast or water. Otherwise, it is hard to simultaneously detect the images in the shallow as well as deep regions without any supplementary or dummy material. The property can result in inconveniences in real *in vivo* clinical applications for investigating lymph nodes spread at various depths.

To eliminate the undetectable range and expand the detectable imaging range, a bright-field PAI probe structure was reported in a previous work¹⁴ as similar to the bright-field photoacoustic microscopic structure.¹⁵ In a bright-field PAI probe, the light beam and the ultrasound beam are combined as shown in Fig. 1(c). The illuminated light beam path coincides with the ultrasound beam path in the skin detection area. Thus, it can obtain PAI in shallow and deep regions at the same time. However, this PAI probe has a wide skin contact area because the ultrasound transducer is perpendicularly positioned to the light beam delivery module, making an L-shape. With such a probe, it would be inconvenient to examine particular areas such as the clavicle and armpit in diagnosis. Moreover, general PAI probes employ optical fiber bundles to transmit high-power laser beams and irradiate wide areas. However, the probe cables composed of the optical fiber bundle and coaxial cable tend to be rigid, which prevents the PAI probes from the practical use in clinic.

This paper proposes a compact and easy to use PAI probe structure providing a bright-field illumination system. First, the proposed probe structure is designed to have a small skin contact

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Fig. 1 Various PAI structures. (a) and (b) Dark-field illumination structures: the previous structure has an undetectable zone in which it cannot detect ultrasound imaging caused by oblique illumination. (a) Difficult to detect shallow region. (b) Difficult to detect deep region. (c) and (d) Bright-field illumination structures: to remove the dead zone and expand the imaging range. (c) Previous bright-field illumination structure that has wide skin contact area. (d) Proposed photoacoustic probe structure that has a small skin contact area.

area as an ultrasound detector is positioned parallel to the light beam delivery module [Fig. 1(d)]. This approach, similar to an optical resolution photoacoustic microscopic probe (I-shape),¹⁶ can provide a compact and handheld structure. Therefore, it becomes easy to obtain PAI in the armpit area. Second, one strand of a multimode optical fiber is used as a high-power laser transmission material instead of optical fiber bundles. This use of a single-fiber strand not only makes the cable lighter, but also increases the efficiency of light transmission compared to the bundles of multiple fibers and it still delivers a high laser power. Because of the efficient high transmission, a deeper image can be achieved with the same energy of pump laser energy. In other words, lower pump laser energy would be needed for the same characteristics; therefore, the size and cost of the light source can be reduced. Finally, the probe is designed to have a line-shaped light beam by using three cylindrical lenses for B-scan PAI and real-time PAI. We here demonstrate the proposed PAI probe and achieved images in shallow as well as deep regions simultaneously. Moreover, it was also possible to detect the lymph node of a nude mouse without any supplementary material. The developed probe was compact and easy to use, and the detection imaging range was expanded. It would be useful to detect lymph nodes spread at various depths and other tissues in the armpit area.

2 Materials and Methods

Figure 2 shows the schematic diagram of the proposed PAI probe, which was composed of a beam combiner, a line-beam generator, and an ultrasound array detector. On the longitudinal view, the dotted box indicates the line-beam generator, which consisted of three cylindrical lenses (order production, Teleoptics Co., Ltd., Korea) with antireflection coatings and one multimode optical fiber with an optical fiber connector. The optical pulse was emitted from the multimode optical fiber with a solid angle by the numerical aperture of the optical fiber. A convex cylindrical lens (lens 1) collimates the optical pulse beam emitted from the optical fiber in the transverse direction and another convex cylindrical lens (lens 2) widely collimates the optical pulse beam passed through lens 1 in the longitudinal

direction. The other convex cylindrical lens (lens 3) focuses the collimated optical pulse beam from lens 2 in the transverse direction. Finally, the output laser beam at the end of the line-beam generator had a line-shape. The line-shaped beam irradiating the tissue after passing through a beam combiner is illustrated in the bottom dotted box of the transverse view of Fig. 2. The ultrasound generated by the line-shaped beam was double-reflected perpendicularly to the two-slide glasses (microscope slides, Marienfeld-Superior) inside the line beam and proceeded into an ultrasound detector.

The beam combiner not only aligned the light and ultrasound beams to the same plane, but also provided a compact one-body structure for easy handling because the line-beam generator was positioned parallel to the ultrasound transducer. The beam combiner was composed of two-slide glasses and was filled with distilled water. Here, filling the beam combiner with water improved the transmission characteristics by light index matching and acoustic impedance matching. The light with a 45-deg incident angle had a theoretical transmission loss of 0.97 dB at slide glass 1 ($n_{\text{glass}} = 1.5$) in the water. Moreover, the ultrasound wave with a 45-deg incident angle was totally perpendicularly reflected on the two-slide glasses in the water because the critical angle for total reflection was estimated to be 15.6 deg, which was calculated using the acoustic impedance values for the slide glass [$Z_{\text{glass}} = 12.3 \times 10^6 \text{ kg}/(\text{m}^2\text{s})$] and water. The transmission loss for the ultrasound wave passing through the water can be negligible because water has an ultrasound attenuation of 0.002 dB/(5 MHz cm).

To prevent water leakage from the beam combiner, two plastic plates (acrylic and low-density clear plastic) were used. The output beam from the line-beam generator passed through the acrylic plate, which was coated for antireflection of the 780-nm incident light wavelength. The low-density clear plastic on the bottom of the probe was used as a probe window, and the estimated acoustic and optical transmission losses from reflection were 0.1 and 0.01 dB, respectively. Here, the low-density clear plastic had an acoustic impedance [$Z_{\text{plastic}} = 1.84 \times 10^6 \text{ kg}/(\text{m}^2\text{s})$] and refractive index ($n_{\text{plastic}} = 1.46$) similar to the water. Inside the low-density clear plastic with a thickness of 1 mm and

Fig. 2 Schematic of the PAI probe that has the line beam generator and the beam combiner.

acoustic attenuation coefficient of 1.1 dB/(MHz cm), the ultrasound attenuation loss was estimated to be 0.55 dB at a frequency of 5 MHz. The total acoustic and optical transmission losses by the beam combiner and the windows were estimated to be 0.65 and 0.98 dB, respectively.

3 System Performance

The casing of the PAI probe had a curved shape for convenience in medical diagnosis. The bottom face size of the PAI probe was $55 \times 25 \text{ mm}^2$ for easy handling of the bright-field illumination PAI probes. Figure 3(a) shows a photograph of the line-shaped beam illumination ($20 \times 5.5 \text{ mm}^2$) emitted from the PAI probe at 780-nm wavelength, and Fig. 3(b) shows a three-dimensional (3-D) intensity profile of the line-shaped beam performance generated at the PAI probe using a beam profiler (Beamon LA, Duma Optronics Ltd.). We used a compact gain-switched Ti:sapphire laser pumped by a frequency-doubled Nd:YAG pulse laser (Ultra, Quantel Laser) operated at an output energy of 37 mJ with a 532-nm wavelength, 10-Hz repetition rate, and 11-ns pulse duration.¹⁷ The pulsed laser was coupled into a connectorized multimode optical fiber cable (#HP-2020-10A3CH-040E, Fiberguide industries) with a 1000- μm core and 0.22 numerical aperture.

The optical fiber cable was connected at the PAI probe as shown in Fig. 2. The emitted laser pulse energy at the end of the probe was 6.3 mJ per pulse, corresponding to a laser fluence of 5.3 mJ/cm^2 . The total optical loss from the laser to the probe output was experimentally measured to be 2.4 dB. Figures 3(c) and 3(d) show the intensity distributions of the line-shaped beam using a beam. The beam sizes in the transverse and longitudinal directions were 5.6 and 21 mm, respectively. The transverse intensity distribution shows that the line-shaped optical beam

was well formed by the three cylindrical lenses. Although the proposed probe utilized a single-strand optical fiber cable, it delivered sufficient optical energy to obtain PAI.

Figure 4(a) shows a schematic diagram of the PAI acquisition system using the proposed PAI probe to confirm the bright-field illumination system. An ultrasound detector (L3-8, Alpinion Medical Systems) with 128 elements and an ultrasonography machine (E-cube 9, Alpinion Medical Systems) was used to acquire the PAI. The ultrasound detector had a center frequency of 5 MHz, focal length of 35 mm, and 6 dB bandwidth of 66.78%.

In order to acquire the photoacoustic signals and test the detection range, we used an indocyanine green (ICG) tube containing 1 mg/ml ICG solution diluted in dimethyl sulfoxide whose absorption peak is 780 nm.¹⁸ The ICG tube was 10-cm in length and 5-mm in diameter and placed between polyvinyl chloride plastisol (PVC) phantom with 0.25 wt. % TiO_2 . The PVC served as a phantom material because it was found to be similar enough to human breast tissues in photoacoustic properties.¹⁹ PAIs from the ICG positioned at various depths were well obtained at the various depths as shown in Fig. 4(b). These results show that our system could achieve PAI in the shallow and deep regions with a single probe and without changing its configuration.

To test the shallow PAI, we obtained images of the lymph nodes positioned immediately below the skin by using an *in vivo* small animal model. In order to dye the lymph nodes, the ICG solution with the concentration of 1 mM (0.78 mg/mL) diluted in the dimethyl sulfoxide solution was injected into a nude mouse. In the following experiments, we used another ultrasound detector (L8-17, Alpinion Medical Systems) with 128 elements. The ultrasound detector had a center frequency of

Fig. 3 Characteristics of the proposed probe using a beam profiler. (a) Two-dimensional and (b) 3-D plots of line-shaped beam intensity showing the performance of the PAI probe; (c) and (d) longitude and transverse profiles of the beam intensity distribution.

10 MHz, focal length of 20 mm, and 6-dB bandwidth of 58.95%.

Figure 5(a) shows the imaging of lymph nodes, which contains the photoacoustic (color) and ultrasound (monochrome) imaging of two dyed brachial and axillary lymph nodes of the mouse. The detected image depths were measured to be 0.5 mm (brachial) and 0.7 mm (axillary) from the mouse skin, respectively, and both sizes of the two lymph nodes were 3.0 mm $\times$ 1.5 mm.

When optical fiber bundles are attached to the side of an ultrasound receiver, such a PAI probe generally requires either imaging in a bath filled with water or being supplemented with chicken breast tissue as a dummy material to enable PAI of substances just beneath the skin. On the contrary, the proposed probe obtained the PAI by using only an ultrasound gel between the PAI probe and mouse without any supplementary material as shown in Fig. 5(b). To check the depth of the brachial lymph nodes, we made a 5- to 8-mm incision as shown in Fig. 5(c).

Fig. 4 (a) Schematic of the proposed system to acquire PAI that can image immediately below the window of the PAI probe and (b) a merged image of each ICG image from the PAI system at various depths in the PVC phantom.

Fig. 5 (a) Imaging of lymph nodes that contain the photoacoustic (color) and ultrasound (monochrome) imaging of two dyed brachial and axillary lymph nodes of the mouse, (b) nude mouse experiment view without any material, (c) dissection imaging, and (d) extracted lymph node.

After the incision, one extracted brachial lymph node was located 1 mm below the skin and the size was $3.2 \text{ mm} \times 1.6 \text{ mm}$ as shown in Fig. 5(d). The depth and size of the lymph node detected by the proposed probe agreed well with the measured result from the dissection. Therefore, we could obtain the PAI of a lymph node existing in the shallow region beneath the skin and demonstrate that the minimum detecting depth was less than 1 mm.

All experimental animal procedures were approved by the Institute Animal Care and Use Committee (IACUC) of Seoul National University Hospital Biomedical Research [13-0219-C3A0(1)], and all experiments of imaging acquisition were performed at the Electronics and Telecommunications Research Institute.

4 Discussion and Conclusion

We have proposed and developed a compact PAI probe that is easy to handle because one strand of optical fiber cable was used instead of fiber bundles. Moreover, the laser output at the probe window had a line-shaped beam shape created by three cylindrical lenses to be suitable for the B-scan PAI, and the illuminated light beam path coincided with the ultrasound beam path. The developed probe obtained PAIs in the shallow as well as deep regions at the same time. However, our PAI system was not optimized for a PAI system and we will improve the PAI system using a synthetic algorithm in the future work.²⁰

In the small animal experiment, the lymph nodes near the skin could be detected without the need for any supplementary material. The proposed compact PAI probe will be convenient and easy to use in medical diagnostics because it does not need any supplementary material. We believe that our proposed PAI probe will have a significant effect on future clinical medical diagnostics.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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