Linear array-based real-time photoacoustic imaging system with a compact coaxial excitation handheld probe for noninvasive sentinel lymph node mapping

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Abstract: We developed a linear ultrasound array-based real-time photoacoustic imaging system with a compact coaxial excitation handheld photoacoustic imaging probe for guiding sentinel lymph node (SLN) needle biopsy. Compared with previous studies, our system and probe have the following advantages: (1) the imaging probe is quite compact and user-friendly; (2) laser illumination and ultrasonic detection are achieved coaxially, enabling high signal-to-noise ratio; and (3) GPU-based image reconstruction enables real-time imaging and displaying at a frame rate of 20 Hz. With the system and probe, clear visualization of the SLN at the depth of 2 cm (~human SLN depth) was demonstrated on a living rat. A fine needle was pushed towards the SLN based on the guidance of real-time photoacoustic imaging. The proposed photoacoustic imaging system and probe was shown to have great potential to be used in clinics for guiding SLN needle biopsy, which may reduce the high morbidity rate related to the current gold standard clinical SLN biopsy procedure.

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OCIS codes: (110.5120) Photoacoustic imaging; (170.5120) Photoacoustic imaging.

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1. Introduction

Sentinel lymph node biopsy (SLNB) is now a standard clinical procedure for breast cancer staging in patients [1,2]. The sentinel lymph node (SLN) is defined as the first lymph node receiving drainage from the tumor. Thus, the SLN is most likely to host metastatic tumor cells. During the SLNB procedure, surgeons generally first inject a radioactive substance and blue dye in the tissue site near the breast tumor. A handheld gamma probe-based radioactive device is then used to locate the SLN from outside of the body. Next, the breast is surgically opened, and the SLN can be precisely identified macroscopically due to the blue dye staining. Once the accurate position of the SLN is identified, the SLN is removed and examined histologically to determine whether metastatic tumor cells are present. If the SLN is negative for metastatic tumor cells, it is highly unlikely that other nodes are affected [3,4]. Although the current gold standard in clinics, conventional SLNB still has a few adverse effects. The tracer is radioactive, which is associated with radiation safety concerns. Open surgery and lymph node removal are necessary during this procedure, which is associated with potential postoperative complications, such as seroma formation, lymphedema, and sensory nerve injury [5].

Ultrasound-guided fine needle aspiration biopsy has been utilized for axillary lymph node biopsy and breast cancer staging [6,7]. During this procedure, a biopsy needle is inserted into the lymph node with ultrasound imaging guidance to extract partial tissue samples for further histological examination. Unlike SLNB, needle biopsy is non-ionizing and minimally invasive, leading to shorter recovery and fewer sequela [8]. However, despite the fact that certain morphological information about lymph nodes can be visualized by ultrasound imaging, its capability for precise SLN identification is very limited as ultrasound imaging is incapable of confirming whether the lymph nodes are SLNs. Another technology that has been explored for SLN identification is fluorescence imaging [9], but due to the strong optical scattering of biological tissue, the imaging resolution of this technology for deep SLN identification is very limited. In addition, depth-resolved information is unavailable with fluorescence imaging currently used for SLN identification, and thus an open surgery is still needed to remove the SLN for histological examination.

As a hybrid biomedical imaging modality, photoacoustic imaging has been evolving quickly during the past two decades. This technology combines the high contrast of optical imaging with the depth-resolved high resolution of ultrasound imaging at large tissue depths [10–12]. Moreover, optical dyes (such as blue dye) are excellent photoacoustic imaging contrast agents. Therefore, photoacoustic image-guided fine needle aspiration biopsy is a promising technology for precise SLN identification noninvasively and breast cancer staging in clinics, potentially eliminating the harm of SLNB as well as the high false-negative rate of SLN identification based on ultrasound-guided fine needle aspiration biopsy [13–15].

For clinical SLN imaging applications, high imaging speed is highly desirable. Therefore, photoacoustic tomography imaging systems based on an array ultrasound scanner are greatly preferred over conventional raster scanning-based single-element detector systems. A number of groups have worked towards building such photoacoustic tomography systems or photoacoustic/ultrasound dual-modality imaging systems by adding optical excitation to the array ultrasound scanners [13,16–26]. The key difference among these systems is primarily the design of the photoacoustic probes, i.e., the integration of the excitation light with the ultrasound detection scanners. Figure 1(a) shows the design adopted by most research groups, which accommodates the light illumination by the side of the ultrasound scanner for optical excitation of the photoacoustic signals. Because no optical excitation occurs on the sample surface directly below the ultrasound scanner (ultrasound detection area in Fig. 1(c)), this design is called the dark-field illumination design. Although it is convenient, the light delivery efficiency to the region of interest (i.e., light delivered to the sample volume below the ultrasound scanner) is relatively low [13–15,27]. Moreover, oblique incidence of light to the imaging plane of the ultrasound scanner confines the excitation optical fluence to a
specific depth range of the sample, which is not optimal when imaging the entire region of interest below the ultrasound scanner, especially the layer close to the surface of the sample. Figure 1(b) shows a different light illumination strategy for the photoacoustic probe, called the bright-field illumination design, in which the light is guided to the sample surface directly below the ultrasound scanner. As this design enables perfect overlapping of optical excitation and acoustic detection, this design is also called the optical and acoustic coaxial design. Compared with the dark-field illumination design, a higher signal-to-noise ratio and increased imaging depth range (starting from the surface of the sample) can be acquired with the bright-field illumination design. Montilla et al. first reported this type of design by utilizing an optically transparent acoustic reflector [16]. However, as the optical delivery module is configured perpendicularly to the ultrasound scanner, the probe is physically bulky, impairing its operational adaptability for clinical application.

In this study, we developed and engineered a handheld probe-based photoacoustic imaging system that was built on an open-platform ultrasound imaging system with a linear-array ultrasound scanner. A new design was implemented for enabling the photoacoustic probe to achieve bright-field optical and acoustic coaxial illumination. Moreover, the size of the probe remained physically compact, making it very user-friendly and highly applicable for potential clinical translation. First, we performed a Monte Carlo simulation of photon propagation in biological tissues to validate the higher optical delivery efficiency of our probe design compared with the conventional dark-field illumination probe design. Then, we fabricated the probe and built the photoacoustic imaging system to evaluate its imaging performance. In vivo SLN mapping in Sprague Dawley (SD) rat was subsequently performed to test the feasibility of the probe and system for SLN imaging. To further verify the potential
of the probe and system for clinical application in human SLN imaging, a layered 1.5-cm-thick chicken breast was added to the surface of the rat’s skin, and we then performed in vivo photoacoustic imaging of rat SLN as well as image-guided needle insertion towards the SLN.

2. Materials and methods

2.1 Simulation

To validate the advantages of the optical and acoustic coaxial bright-field illumination design (Fig. 1(b)) compared with the conventional dark-field illumination design (Fig. 1(a)) for optical energy delivery, we performed a simulation of photon propagation in a scattering medium mimicking biological tissue for both designs using Tracepro software (Lambda Research Corporation, Littleton, MA, USA). Figure 1(c) and Fig. 1(d) show the light patterns for both designs at the tissue sample surface. For the dark-field illumination design, the size of the laser beams at both sides of the ultrasound scanner was set to be 38 × 0.4 mm, and the incident angle was 30 degrees. The distance between the center lines of two laser beams was 15.4 mm. For the optical and acoustic coaxial bright-field illumination design, the laser beam at the tissue surface was set to be 38 × 5 mm, and the incident angle was 0 degrees (i.e., the laser beam enters the biological tissue vertically). All the parameters for the simulation were based on the actual size of the ultrasound scanner and the true optical excitation condition of the photoacoustic probes. The optical absorption coefficient, optical scattering coefficient, anisotropic factor, and refractive index of the tissue sample were set to 0.1 cm⁻¹, 200 cm⁻¹, 0.9 and 1.37, respectively, which approximate the real conditions of biological tissue [28].

2.2 Probe design and fabrication

Figure 2(a) and 2(b) show the detailed configuration of our probe design from two different angles. Figure 2(c) shows the photo of the fabricated probe. The probe is composed of an optical fiber with a 1.5-mm core diameter, two cylindrical lenses (Plano-convex and Plano-concave cylindrical lens, AYL1210-B and LK1006L1-B, Thorlabs, Newton, NJ, United States), a custom-made optical/acoustic coupler, a 128-element linear array ultrasound scanner (7 MHz, LA0303, S-sharp, Taiwan), and a custom-designed 3D-printed holder shell. The transmission efficiency of the optical fiber is maintained above 85%. The two cylindrical lenses are utilized to shape the excitation light coming out of the optical fiber into a narrow rectangular pattern with a size of 38 × 5 mm. The optical/acoustic coupler mounted beneath the ultrasound array is composed of two parts, each with a rhomboid and trapezoid shape. Both parts are made of polymethyl methacrylate (PMMA), and the rhomboid part is galvanized with silver film on both oblique surfaces. The excitation light is reflected at both galvanized surfaces of the rhomboid part and eventually to the sample (directly below the ultrasound scanner) for optical and acoustic coaxial purposes. Because the ultrasound impedance of PMMA material is not much different from the ultrasound scanner or biological tissues, the excited photoacoustic signals from the sample can penetrate through the optical/acoustic coupler to be detected by the ultrasound scanner.
The shell holder houses all the optical and acoustic components and provides easy handling of the probe, which is composed of three parts (Fig. 2(c)): two symmetrically identical parts at each side and one pedestal part at the bottom. The three parts can be sealed together with eight screws. All the optical and acoustic components are mounted and assembled inside the shell. Ultrasonic gel fills the space between the ultrasound scanner and PMMA module (i.e., the optical/acoustic coupler) to provide coupling for photoacoustic signal propagation. Sealing rings keep the gel from leaking out of the shell. We utilized SolidWorks (Dassault Systèmes SolidWorks, Concord, MA) software to design the holder, which allows for quick manufacturing of the holder with resin material using a 3D printer (SL660, Zrapid, Billerica, MA). Because the wall thickness of the holder is only 2 mm, the overall size of the original ultrasound scanner did not increase much. The dimensions of the photoacoustic probe were $5.5 \times 4 \times 10$ mm. Thus, the probe is highly compact and user-friendly during clinical operation in the future.

2.3 Photoacoustic imaging reconstruction

The conventional Back-Projection (BP) reconstruction algorithm of photoacoustic imaging assumes that the speed of sound is constant in the sample [29] and coupling medium. However, for our photoacoustic probe design, the BP algorithm performed poorly in the reconstruction of photoacoustic images due to the presence of the PMMA module mounted beneath the probe. The sound velocity of PMMA is 2484 m/s, which is different from the surrounding medium, e.g., ultrasound gel or soft tissue (~1540 m/s). This heterogeneity causes phase aberration and bending of the ultrasonic rays at both the upper and bottom surfaces of the PMMA module, leading to photoacoustic signal refraction.

To correct this phase aberration, we applied a fast marching method (FMM) based non-iterative BP algorithm [30]. This algorithm calculates the route and arrival time of the acoustic rays from each pixel of the photoacoustic image to each element of the ultrasound scanner in the acoustic heterogeneous medium, provided that the speed of sound at each point of the medium is known. As a result, the phase delay can be directly included in the BP algorithm [31,32]. The modified BP based on the fast marching method can be expressed as:
\[
P(r_j) = \sum_{i \text{-receiver}} \left[ p_i(t_{i,j}) - t_{i,j} \frac{\partial p_i(t_{i,j})}{\partial t} \right]
\]

\(P(r_j)\) is the magnitude of the photoacoustic signal at the \(j\)th pixel location in the reconstructed image. \(p_i(t_{i,j})\) is the PA signal received by the \(i\)th element of the ultrasound scanner. \(t_{i,j}\) is the arrival time of the wave front from the \(j\)th pixel location to the \(i\)th element of the ultrasound scanner, which is calculated from FMM. Equation (1) is the approximation of the conventional BP algorithm.

2.4 Photoacoustic imaging system construction and probe performance validation

To validate the performance of the photoacoustic probe, a photoacoustic imaging system was built based on an open-platform commercial ultrasound imaging system (Prodigy, S-sharp, Taiwan). Figure 3 shows the schematic of the system. For photoacoustic signal excitation, we utilized a pulsed OPO (optical parameter oscillator) laser source (Spitlight, Innolas, Germany). The excitation laser was first attenuated by a neutral density filter and then focused by a convex lens before coupling into a 1500 \(\mu\)m multimode optical fiber via a fiber coupler. The pulse duration and pulse repetition rate of the laser source were 8 ns and 20 Hz, respectively. The laser beam coming out of the fiber was first reshaped and reflected by a series of optical components and then reflected by the PMMA optical/acoustic coupler before finally being delivered beneath the ultrasound scanner to excite photoacoustic signals (see part 2.2 for detailed information). The photoacoustic signals detected by the ultrasound scanner were transferred to the 128-channel data acquisition board integrated inside the ultrasound imaging platform for further post-processing and image reconstruction. The trigger out signal of the laser source was used to trigger the ultrasound platform for signal acquisition synchronization. Following the firing of each laser pulse, photoacoustic signals were acquired, and a frame of the B-scan image was reconstructed, enabling a frame rate of 20 Hz, which is limited by the pulse repetition rate of the OPO laser source. To achieve this high imaging frame rate, raw photoacoustic data collected by the ultrasound imaging platform were processed on a GPU card (GeForce GTX 650Ti, NVIDIA) with the Fast-Marching-based BP reconstruction algorithm written by Visual Studio (Microsoft, Redmond, WA). The implementation time for one frame of photoacoustic image reconstruction is approximately 30 ms, including raw data transferring, reconstruction algorithm computing, and display on the GPU card.
We performed phantom studies to validate the performance of the photoacoustic probe and system. For spatial resolution measurement, several human hairs with a cross-sectional diameter of \( \approx 100 \mu m \) were horizontally placed in water medium at different depths under the photoacoustic probe to obtain B-scan images of the hairs. To evaluate the capability of the system and probe for deep imaging in the scattering medium, a black tape stripe (2mm*30mm) was placed in a 2% fat emulsion solution at different depths to acquire photoacoustic signals. The photoacoustic images of the stripe at different depths were obtained and combined together. The light energy emitting out of the photoacoustic probe is approximately 17 mJ measured at the bottom of the probe, which corresponds to a laser fluence of 8 mJ/cm\(^2\) at the surface of the sample.

### 2.5 Animal handling and in vivo SLN imaging

All animal handling and in vivo studies were performed according to the protocol approved by the Animal Study Committee of Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

Healthy SD rat was used for in vivo SLN imaging. The hair in the left axillary region was gently depilated with hair-removal lotion before photoacoustic imaging. Indocyanine green (ICG) diluted in water was injected hypodermically into the left forepaw pad with a total volume of 75 \( \mu L \) and a concentration of approximately 3 mg/mL. Before ICG injection, we acquired a control photoacoustic image of the region of interest (where the SLN was located). Immediately after ICG injection, we performed photoacoustic imaging of the same region. The imaging duration was 7 sec to capture the ICG accumulation dynamics in the SLN. Later, photoacoustic imaging was also conducted at several different time points until 20 min post-ICG injection. To demonstrate the clinical application potential of the photoacoustic probe and system for imaging-guided SLN needle biopsy, we added a layer of chicken breast tissue on top of the rat’s skin surface and inserted a needle into the chicken breast and pushed the needle towards the rat SLN under the guidance of real-time photoacoustic imaging.
Moreover, to acquire the 3D information of SLN, we positioned the imaging probe above the rat and scanned the probe along the elevational direction to cover the entire area around SLN. The 780-nm wavelength, close to the peak optical absorption of ICG, was utilized for photoacoustic signal excitation throughout the experiment. The laser fluence on the rat skin or chicken breast surface was approximately 8 mJ/cm², well below the ANSI (American National Standards Institute) safety standard (30 mJ/cm²) [33]. The distance between the bottom of the ultrasound scanner and the skin surface of the rat was 30 mm. The rat remained fully anesthetized throughout the imaging experiment using 1.5% isoflurane gas (Euthanex, Palmer, Pa) mixed with oxygen. At the end of the imaging process, the rat was sacrificed, and necropsies were performed to verify the SLN position via white light imaging.

3. Results

The laser fluence and absorbance in the scattering medium mimicking biological tissues for both the bright-field and dark-field optical illumination designs described in this study is shown in Fig. 4. Figures 4(a)-4(f) show the laser fluence of dark-field illumination at depths of 0.1 mm, 5 mm, 10 mm, 15 mm, 20 mm and 25 mm below the sample surface, respectively. Figures 4(g)-4(l) show the laser fluence of bright-field illumination at the same depths. The white rectangle in each image indicates the ultrasonic detection area at the corresponding depth, which varies in each image due to the elevational focus of the array probe. To quantitatively compare the light delivery efficiency of the two optical illumination schemes, we calculated the laser fluence in the ultrasonic detection area at each depth; these values are displayed in Fig. 4(m). The solid lines in the figure are the fitting curves of the calculation. Compared with dark-field illumination, the fluence of bright-field illumination in the ultrasound scanner detection area is significantly higher when the depth is less than 15 mm, despite the exponential decrease from 0.1 mm to 15 mm. At 15 mm, the fluence of the bright-field illumination is still about two times that of the dark-field illumination, indicating a two-fold increase in the photoacoustic signal excitation efficiency. Figure 4(n) is the zoomed-in view of the green dashed rectangle area in Fig. 4(m) in which the fluence of the bright-field illumination is similar to the dark-field illumination after the 25-mm depth, indicating comparable photoacoustic signal-to-noise ratios of the two illumination schemes. Figure 4(o) and 4(p) show the absorbance map for the bright-field (4o) and dark-field (4p) optical illumination when embedding 6 optical absorbers (μa = 10 cm⁻¹) at different depths (2, 5, 10, 15, 20, 25 mm) in the scattering medium below the center of the ultrasound scanner.
Fig. 4. Simulation results of laser fluence and absorbance at different depths in tissue mimicking scattering medium for the bright-field (a-f) and dark-field (g-l) optical illumination schemes. The white rectangles represent the detection area of the ultrasound scanner at the corresponding depth. (m) Quantitative comparison of laser fluence in the ultrasonic detection area at different depths for the two optical illumination schemes. Red line: bright-field illumination. Black line: dark-field illumination. (n) Zoomed-in view of the green rectangle in Fig. m. (o) The absorbance map of the bright-field optical illumination. (p) The absorbance map of the dark-field optical illumination.

Figure 5 shows the reconstructed cross-sectional photoacoustic images (B-scan images) of human hairs with the conventional BP reconstruction algorithm (Fig. 5(a)) and FMM-based BP reconstruction algorithm (Fig. 5(b)). Compared with Fig. 5(a), significant enhancement of both spatial resolution and signal-to-noise ratio (45.43 dB vs. 35.94 dB) can be observed in Fig. 5(b), indicating the advanced reconstruction capability of the FMM-based algorithm. Moreover, the position shift of the targets in the axial direction in Fig. 5(a) compared with
their real positions (due to the bending of photoacoustic signals at the PMMA module surfaces) has also been corrected by the FMM-based algorithm.

![Fig. 5. Reconstructed images of human hair using two algorithms. (a) Reconstruction image based on the conventional BP algorithm. (b) Reconstruction image based on the FMM-improved BP algorithm.](image)

To quantify the performance of the proposed photoacoustic probe and system, we calculated the axial and lateral FWHM (full width at half maximum) of the human hair signal in the B-scan image at approximately 42-mm depth (green dashed rectangle in Fig. 5(b)), and the results are shown in Fig. 6(a) and 6(b). The axial resolution is 285 μm, which is similar to the theoretical value of 235.7 μm. The lateral resolution is 532.5 μm, which is approximately 60% larger than the theoretical value of 336.3 μm, presumably due to the existence of the PMMA coupling module. We also calculated the axial and lateral resolutions at other imaging depths, which varied slightly within 20 μm for axial resolutions and 50 μm for lateral resolutions, respectively, corresponding to the varying focus of the ultrasound scanner at different depths.
Figure 6(c) is the photoacoustic image of the black tape stripe at different imaging depths in optically turbid medium (2% fat emulsion in water). The image was obtained by combining cropped images at each depth. At the depth of 60 mm, the imaging target can still be visualized with decent image quality, indicating an imaging depth of larger than 60 mm in tissue mimicking scattering medium. Figure 6(d) shows the fitting curve of the calculated signal-to-noise ratios for the targets in Fig. 6(c), which increases gradually with the imaging depths for the first 35 mm and decreases almost linearly afterwards, presumably due to the laser fluence differences and the varying sensitivity of the ultrasound scanner at different depths. The higher SNR at 35 mm compared to the shallower depths is mainly due to the higher acoustic detection sensitivity, as more elements of the ultrasound scanner can be included for image reconstruction at this depth compared to the shallower depths due to the limited view angle of each element.

The feasibility of the handheld photoacoustic probe and system for in vivo SLN mapping application was demonstrated on a living rat using ICG as a photoacoustic contrast agent. Figure 7(a) shows the control photoacoustic image of the SLN region obtained before ICG injection, while Fig. 7(b) shows an image of the same site 5 min after injection. Although superficial blood-vessel signals can be visualized in Fig. 7(a), no photoacoustic signals were detected from the SLN. However, after ICG injection, the location of the SLN can be clearly recognized from the photoacoustic image as shown in Fig. 7(b). To demonstrate the real-time imaging capability of the system, ICG accumulation dynamics in the SLN were also obtained and shown in supplementary Visualization 1, which shows the photoacoustic imaging frames for the first 7 sec after ICG injection. The slight movement of the SLN between different frames of the visualization corresponds to the respiratory action of the rat. The size of the SLN in the B-scan cross-sectional image is approximately 1.5 × 1.2 mm, and the location of the SLN is approximately 3 mm beneath the skin surface. The signal-to-noise ratio of the SLN region was 44.25 dB without any frame averaging. Figure 7(c) shows the co-registered...
photoacoustic and ultrasound image of the SLN region. The ultrasound image is primarily used to illustrate the structural features of the imaging area, such as to visualize the boundary of the skin surface. Figure 7(d) is the maximum amplitude projection (MAP) image of the SLN, which was obtained by scanning the photoacoustic probe along the elevational direction. Figure 7(e) is the autopsy photograph of the rat axillary region after all imaging experiments were completed, and the ICG stained SLN was visually identified. The size of the stained SLN measured 3.5 × 1.5 mm in cross-section and 2-3 mm in depth, which matched well with the photoacoustic cross-sectional imaging results and the MAP results.

To validate the clinical application potential of the photoacoustic probe and system for human SLN imaging, a 15-mm-thick chicken breast layer was added on top of the rat skin surface to simulate the condition of a human SLN, which is typically around 20 mm below the skin surface. As shown in Fig. 7(f), the SLN can be clearly visualized at a depth of approximately 18 mm in biological tissues. The signal-to-noise ratio of the SLN region was calculated to be 30.16 dB without any frame averaging. Moreover, to demonstrate the clinical application potential of the photoacoustic probe and system for imaging-guided SLN needle biopsy, we inserted a 0.35-mm-diameter (28 gauge) needle into the chicken breast tissue. With the guidance of real-time photoacoustic imaging, we pushed the needle towards the rat SLN to simulate the PA-guided needle aspiration biopsy process (supplementary Visualization 2). Figure 8(a) shows the photoacoustic imaging result of one transient moment of the needle insertion process. Both the SLN and needle can be visualized with high contrast in the image. Figure 8(b) shows the corresponding ultrasound imaging result in which the needle and SLN can hardly be detected. Thus, the photoacoustic imaging has a significantly higher contrast compared with ultrasound imaging for SLN detection and needle tracking. Figure 8(c) is the co-registered image of both photoacoustic and ultrasound imaging results.

Fig. 7. In vivo photoacoustic imaging results of rat SLN. (a) Photoacoustic imaging of SLN before ICG injection. (b) Photoacoustic imaging of SLN after ICG injection. (c) Co-registered photoacoustic and ultrasound images of the SLN region obtained 1 hour after ICG injection. (d) Photoacoustic maximum amplitude projection image of SLN. (e) Autopsy photograph of rat axillary region; the green colored staining area in the white circle indicates the SLN. (f) Co-registered photoacoustic and ultrasound images of the SLN region obtained 2 hours after ICG injection with a 15-mm-thick chicken breast layer added on top of the skin surface.
Fig. 8. *In vivo* photoacoustic image-guided SLN needle biopsy. (a) Photoacoustic image of the needle and SLN. (b) Ultrasound image of the needle and SLN. (c) Co-registered photoacoustic and ultrasound images of the needle and SLN.

4. Discussion

The laser fluence on the rat skin surface in this study was well below the ANSI safety standard. Therefore, a significantly improved signal-to-noise ratio of photoacoustic imaging can be expected given more laser energy being delivered to the sample. The current laser fluence is primarily limited by the laser energy that the optical fiber can deliver as well as the optical reflection efficiency of the coated membrane at PMMA surfaces (both of which can be potentially improved). Furthermore, materials with better acoustic properties and similar optical performances can replace the PMMA for the optical/acoustic coupling module, which will further enhance the signal-to-noise ratio of the probe as the photoacoustic signals can penetrate through the optical/acoustic coupling module more efficiently for detection by the ultrasound scanner. Benefitting from the compact configuration and deep-imaging capability, the probe also has the potential to be utilized in other clinical applications in addition to guiding SLN needle biopsy procedures, such as intra-operative navigation. Based on endogenous contrast, such as oxygen saturation or with the help of exogenous contrast agents, tumor margins may be distinguished from background normal tissues in real-time with the probe and system, providing surgeons with key information for determining whether the tumor has been excised thoroughly. Other potential applications of the probe and system include drug metabolism monitoring, the real-time guidance of ablation therapy, and postoperative treatment evaluation.

5. Conclusion

In this study, we developed an innovative handheld ultrasound linear array-based photoacoustic imaging probe and system. The probe and system possess the following unique features: (1) the probe is physically compact, making it very convenient and user-friendly for potential clinical applications; (2) the probe is based on a design to achieve co-axial laser illumination and ultrasonic detection, resulting in high delivery efficiency of laser energy into the ultrasound detection volume, high signal-to-noise ratio, and large imaging depth; (3) GPU-based data processing acceleration is applied to the system to achieve 20 Hz frame rate real-time image reconstruction and display, limited by the pulse repetition rate of the laser source; and (4) the photoacoustic imaging system modified on the commercial ultrasound platform retains the inherent ultrasound imaging of the platform, enabling photoacoustic and ultrasound dual modality imaging capability. The performance of the photoacoustic imaging probe and system in this study was validated both *in vitro* and *in vivo* using human hairs and black tape stripes placed at different imaging depths as well as the ICG-injected rat SLN. *In vivo* SLN imaging at a large depth in biological tissues was also demonstrated by placing chicken breast on top of the rat skin surface. To mimic the SLN needle biopsy process, a fine needle was inserted into the biological tissue and pushed towards the SLN based on the guidance of real-time photoacoustic imaging. The proposed photoacoustic imaging probe and
system have a high potential for becoming a vital imaging tool for guiding the SLN needle biopsy procedure, providing a minimally invasive solution for SLN biopsy to reduce the severe pain of patients and high morbidity rate related to the current gold standard clinical SLN biopsy procedure.

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**Disclosures**

The authors declare that there are no conflicts of interest related to this article