Adjustable photoacoustic tomography probe improves light delivery and image quality

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Abstract

One cause for suboptimal photoacoustic tomography (PAT) penetration depth is attenuation of incident light by soft tissue. To better understand this problem, we investigated the effects of illumination fiber optic bundle geometry on PAT penetration depth and signal-to-noise ratio. An adjustable, motorized PAT probe was used to reduce probe-skin reflection artifacts and improve light distribution in the image acquisition plane by tuning fiber orientation. We validated our motorized PAT probe through Monte Carlo simulations and ex vivo imaging of a tissue mimicking phantom, and in vivo imaging of murine periaortic fat. Overall, our ex vivo results showed a several millimeter improvement in penetration depth and in vivo results showed a > 62% increase in lipid signal-to-noise ratio. Our PAT probe also utilized a 7-μm aluminum filter to block in vivo probe-skin reflection artifacts. Together, these findings showed the importance of optimizing illumination geometry to enhance PAT image quality.

1. Introduction

Photoacoustic Tomography (PAT) has been shown to provide real-time compositional information of tissue without the need for exogenous contrast agents and with superior penetration depth compared to conventional optical techniques [1–3]. These optical barriers are overcome since PAT does not rely on conventional ballistic photons, but rather detects acoustic waves that are thermoelastically produced by photon-tissue interactions [1–3]. Therefore, PAT can provide useful compositional information that complements current clinical imaging modalities, thus emphasizing the capability of this imaging approach to improve medical care. These characteristics highlight the potential of the technology to be used for a variety of biomedical applications including atherosclerosis [4–9], cancer [10–12], and nerve imaging [13].

While PAT has shown great potential, there are still certain biological barriers that have limited its use. For example, applications for high-resolution noninvasive lipid-based imaging are limited to roughly 3 mm due to subcutaneous fat absorbers, as well as the intrinsic light attenuation due to optical properties of tissue [4,14]. Therefore, there is still a need for further PAT optimization to fully utilize its capabilities. Previous works have utilized image processing and instrumentation engineering to improve image quality and eliminate PAT-specific artifacts. Light catching mechanisms have been particularly useful for redirecting reflected light back into tissue to increase photon density, thus improving signal intensity [15–17]. While effective, the combination of this approach and manually tuning the angle of the fiber optic bundles to improve photon density at various depths may be a superior technique for improving image quality. This is further supported by previous works that have shown that tissue light distribution is effected by illumination geometry [18,19]. Therefore, we hypothesized that by manipulating fiber-ultrasound orientation we can optimize light penetration into tissue, thus improving penetration depth and signal-to-noise ratio (SNR). Light tuning is dependent on fundamental photoacoustic principles where an initial photoacoustic pressure rise ($p_o$) results from light-induced thermoelastic expansion as characterized by Eq. (1).

$$p_o = \Gamma \mu F$$ (1)

Here the pressure rise $p_o$ is dependent upon the Grüneisen parameter ($\Gamma$), absorption coefficient ($\mu$), and optical fluence ($F$) if we assume that all of the absorbed light is converted to heat energy [1,20]. The Grüneisen parameter is further defined by Eq. (2), where $\alpha$ is the isobaric volume thermal expansion coefficient, $\kappa$ is the isothermal compressibility, $\rho$ is the density of the sample, and $C_p$ is the specific-heat capacity.

$$\Gamma = \alpha / (\kappa \rho C_p)$$ (2)

These parameters, including $\mu$ and $\Gamma$, are dependent on the innate tissue properties of the sample. For example, the absorption coefficient, $\mu$, depends on the optical properties of the tissue, such as pigmentation and vascular content, and can be modified by the choice of illumination wavelength. The Grüneisen parameter, $\Gamma$, is a material property that reflects the dependence of the pressure on the volume change of the tissue, and can be influenced by factors such as tissue elasticity and fluid content. The density of the tissue, $\rho$, is another important parameter that affects the pressure rise, as it determines the amount of energy stored in the tissue during the expansion process.

These findings showed the importance of optimizing illumination geometry to enhance PAT image quality.
properties; therefore we can assume that increasing photon density in the tissue can increase the PAT signal amplitude. Taken together, we aim to design tunable fiber optic PAT probes that can enhance image quality for a wide variety of applications.

Digital image processing techniques have played a tremendous role in minimizing in-plane artifacts and out-of-plane clutter [21,22], however, in some cases artifact prevention strategies may be a more appropriate solution to improve image quality. PAT reflection artifacts is one such example where light reflects off of the skin surface that causes a photoacoustic (PA) effect at the probe face rather than within the sample [23]. This PA ultrasound wave then travels and reflects off of the skin surface, registering in the ultrasound system as originating at a distance two times the probe to skin spacing (Fig. 5A). Singh et al. has previously developed a simple yet effective PAFUSion technique to remove PAT reflection artifacts without the need of additional transducers or algorithms [24,25]. This approach uses the ultrasound transducer to acquire two images where one image is focused on the optical absorber and the other is focused on the acoustic reflector induced artifact. A weighted addition is then performed and used to recreate a corrected image without the reflection artifact. While this approach is effective, we explore an alternative solution by which an aluminum filter is used to decouple the optical absorbance by the ultrasound transducer to eliminate the reflection artifact. We quantitatively evaluate the use of a light reflecting material over our PA probe to prevent optical absorption by the transducer and adequately remove this probe-skin interaction artifact.

Here, we propose to use a method whereby the fiber optic bundles are tuned to increase photon density in the image acquisition plane. This method can be coupled with other light manipulation techniques, such as the light catching mechanism, to further improve penetration depth and SNR. We introduce our methods to design and build a PAT holder that allows tuning of the fiber optic bundle orientation. We also investigate the effect of fiber optic bundle orientation on tissue light distribution using Monte Carlo multilayer (MCML) modeling. We then compared these results to ex vivo and in vivo studies were used focal length to quantify changes in image quality, as we believe this is a more intuitive experimental metric compared to fiber optic bundle angle. We defined focal length as the distance between the bifurcated fiber optic bundles and where the two incident light beams converge. This should not be confused with the transducer focus, which is defined as the depth with the narrowest acoustic beam width. We also present a straightforward and effective method to remove reflection artifacts that can be used on virtually all PAT imaging systems. Overall, the work described here suggests that PAT illumination geometry should be optimized for different biological tissues due to varying optical tissue heterogeneity and to minimize in-plane artifacts and out-of-plane clutter.

2. Methods
2.1. Photoacoustic system design

The PAT system utilized in this study consists of a high-frequency small animal ultrasound system (Vevo2100, FUJIFILM Visual Sonics) and an Nd:YAG pulsed optical parametric oscillator (OPO) laser (Surelite EX, Continuum). Ultrasound system was equipped with a 40 MHz center frequency transducer (MS550D) that allowed the user to acquire images with an axial resolution of 40 μm. The Nd:YAG laser was capable of producing 5 ns pulses at 10 Hz ranging from 670 to 2500 nm. Pulsed light was delivered from the laser to the sample through a 2 m fiber optic bundle with a opening diameter of 1.0 cm and rectangular terminals of 18 mm × 2 mm. This allowed us to produce an optical fluence of 40 mJ/cm², which is below the American National Standards Institute (ANSI) safety standards [26]. A pulse generator (9200, Quantum Composers) synchronized laser excitation with ultrasound and PAT image acquisition by sending 1) appropriately timed 10 Hz, 5 V inverted signals to the laser q-switch and flash lamp and 2) a normal 10 Hz, 5 V pulse signal to the ultrasound system. Finally, to prevent acoustic focus induced changes in SNR the transducer focus was set to 7 mm for ex vivo validation imaging and 5 mm for in vivo validation imaging.

2.2. Photoacoustic tomography fiber-adjusting apparatus design

The PAT fiber-tuning apparatus was first designed using Autodesk Inventor Professional Student Edition (Fig. 1A and B) and built using both 3D printed and fabricated 6061-T6 aluminum parts, as well as commercially available hardware (Fig. 1C). The 3D printed parts were printed from Acrylonitrile Butadiene Styrene plastic using a Stratasys Fortus 400mc 3D Production System. The arms that hold fiber cables were made of 16-gauge-carbon steel, while the remaining plates that mount the stepper motors are made from 0.25 in. 6061-T6 aluminum. A 12 V Nema 17 external linear stepper motor (17LS13-0404E-100H, StepperOnline), a 5.4 V Nema 17 bipolar stepper motor (17HM15-0904S, StepperOnline), and a Stepoko 3-axis controller (ROB-13899, SparkFun) were used to adjust the translation of the ultrasound transducer and rotation of the fiber optic bundles. The external linear
stepper motor specifically controlled the ultrasound transducer height by allowing a step angle of 1.8°, thus producing a movement length of 0.02 mm per step. The bipolar stepper motors, equipped with a 16 tooth 32 pitch motor pinion gear, was able to produce a fiber optic bundle step angle of 0.11° to control each fiber optic cable. These specifications were chosen to give us the appropriate strength, accuracy, and precise control of the fiber optic bundles and ultrasound transducer. These stepper motors were controlled by the Stepoko 3-axis controller using GRBL software to allow us to finely tune the ultrasound and fiber optic bundle position. This apparatus was designed to have a 3 cm offset between the transducer face and motors to make sure that the hardware did not interfere with the sample being imaged. Moreover, the translation of the ultrasound transducer allowed us to adjust the pivot point around which the fiber optic bundles rotated, thus allowing us to tune the focal length of our PAT probe.

2.3. Monte Carlo multilayer simulation study

MCML simulations were performed to assess near-infrared light interaction with 20% polyvinyl alcohol (PVA) at various fiber optic bundle orientations. PVA was specifically chosen due to its well-characterized tissue mimicking optical properties (Table 1). Ray tracing software (TracePro, Lambda Research Corporation) was used to design the tissue-mimicking phantom geometry that was later used for ex vivo validation studies. Tissue-light interaction was simulated through two 18 mm × 2 mm fiber optic bundles that delivered 100 randomly arranged rays with a radiant flux of 0.15 W at 1064 nm. These fiber optic bundles were placed 5 mm away from each other to account for the ultrasound transducer width at orientations of 0°, 15°, 30°, 45°, and 60° with respect to the ultrasound transducer. To further improve the accuracy of our simulation the fiber optic bundles were submerged in a water to account for light attenuation due to acoustic-coupling agents with a distance of 3 mm from the PVA. This MCML simulation model and example output is shown in Fig. 2A and B, respectively. The relevant optical properties and geometries for the water and PVA are summarized in Table 1. Briefly, water scattering anisotropy and refractive index were obtained from previous works [27,28], while absorption coefficient at 1064 nm was interpolated from literature values [29]. We were unable to find scattering coefficient at 1064 nm; therefore, scattering coefficient for 800 nm light was used [30], as light attenuation due to scattering is negligible in the infrared region [31]. PVA absorption, scattering, and refractive index values were obtained from the literature [32,33]. Scattering anisotropy (g) was calculated using reduced scattering (\(\mu_s\)) [34] and scattering coefficients (\(\mu_s\)) [33] as shown in Eq. (3).

\[ \mu_s = \mu_s (1-g) \]

(3)

Two MCML simulations were performed to understand the effect of fiber optic bundle orientation on light penetration. The first simulation profiled the depth dependent changes in radiant flux to quantify the light distribution throughout the PVA. The second simulation assessed the radiant flux in the long-axis cross-sectional plane to quantify the energy delivered in the image acquisition plane. Five measurements were taken at 0.04825 mm step sizes below the center of the PAT probe to account for the 0.193 mm elevational resolution of our transducer. Together, these experiments allow us to profile how fiber optic bundle orientation impacts the radiant flux throughout the PVA (Fig. 2).

2.4. Ex vivo validation study

A depth-profiling phantom was designed to evaluate the performance of our PAT holder. This phantom consisted of 20% PVA by weight with six polyethylene-50 (PE-50) tubes running through it at depths of 1 mm (Fig. 3B). Phantom mold was designed and optimized in Inventor and then 3D printed (Statasys Fortus 400mc). We used 20% PVA due to its tissue-mimicking acoustic and optical properties [33], and PE-50 tubes as they produce PA signal when exposed to 1210 nm light. PVA was prepared using a modified method as described by Kharine et al. [33]. Briefly, 20% PVA (Sigma Aldrich, St. Louis, MO) was prepared by slowly dissolving PVA crystals in a secondary heat bath for 6 h. Temperature of solution was cycled between 60 °C and 90 °C to promote dissolving of PVA crystals. Once dissolved, the 20% PVA solution was centrifuged to remove air bubbles. PVA was then poured into a mold with PE-50 tubes (Braintree Scientific Inc., Braintree, MA) and undergone seven freeze thaw cycles [33]. We defined one freeze thaw cycle as placing the mold in a –20 °C refrigerator for 12 h, followed by thawing the mold at room temperature for 12 h. Once prepared, we imaged our phantom using combined ultrasound and PAT imaging at three different locations. Short-axis B-Mode imaging was first used to identify all six PE-50 tubes, followed by PAT images using 1210 nm light to obtain PE-50 specific contrast. PAT holder was used to acquire ultrasound and PAT images with focal length of 2, 3, 5, and 7 mm. SNR was then calculated using FIJI, where the 16-pixel region of interest was chosen to measure the average PAT signal (\(A_{signal}\)) and background noise (\(A_{noise}\)) for all PE-50 tubes (Eq. (4)). We chose to use 16-pixel region of interest as this allowed us to maximize the measurement area without the incorporation of background noise.

\[ SNR = \frac{A_{signal}}{A_{noise}} \]

(4)

2.5. In vivo validation study

We performed ultrasound and PAT imaging on the infrarenal aorta of apolipoprotein E-deficient (apoE\(^{-/-}\)) mice (\(n = 3\)) to assess the performance of our PAT holder in vivo. Six week old apoE\(^{-/-}\) male mice were obtained from Jackson Laboratory (Bar Harbor, ME), fed a standard chow diet, and weighed 24.7 ± 4.9 g at the time of imaging. A small animal anesthesia system (SomnoSuite, Kent Scientific) was used to anesthetize the animals using 2–3% isoflurane and 225 mL/min room air [35]. Eye lubricate was applied to the eyes of the mouse to prevent corneal desiccation. Animal vital signs were closely monitored to ensure a consistent anesthetic plane. Mice were placed on a heated stage to maintain body temperature at approximately 34–36 °C, which was monitored via rectal probe. Heart rate and respiration were monitored using electrodes built into heated stage and maintained at 500–600 beats/min and 40–80 breaths/min, respectively. Infrarenal aortas was found via long-axis B-mode ultrasound imaging and imaging location was kept consistent between animals by identifying the left renal vein and tail artery bifurcation. The aorta was then imaged using 1210 nm light to target lipids and 1400 nm as an off-resonance control. Similar to our ex vivo studies, we then tuned the focal length of our PAT probe to 2, 3, 5, and 7 mm to assess the effect of illumination geometry.

| Table 1 | MCML Optical Properties and Geometry. |
|-----------------|----------------------------------------|
| Absorption Coefficient (mm\(^{-1}\)) | Scattering Coefficient (mm\(^{-1}\)) | Scattering Anisotropy | Refractive Index | Length × Width × Height (mm) |
| Water | 0.0154 [29] | 3 × 10\(^{-7}\) [30] | 0.890 [28] | 1.32 [27] | 78 × 58 × 10 |
| Polyvinyl Alcohol | 0.035 [33] | 6.90 [33] | 0.930\(^{e}\) | 1.47 [32] | 78 × 58 × 20 |

Summary of optical properties and geometry of the simulated water and PVA. \(\tau\) denotes calculated value.
on image quality. Images were analyzed in FIJI to calculate SNRs using a region of interest of 16 pixels due to small-scale thickness of the periaortic fat.

2.6. Photoacoustic reflection artifact removal

The engineering design specification for materials that have the potential to reduce the PAT reflection artifact include 1) complete reflection of light, 2) minimal attenuation of ultrasound and PAT signal, and 3) an inability to generate a PAT signal. These design criteria were chosen to prevent optical absorption by the ultrasound probe to eliminate reflection artifacts, while allowing easy registration of ultrasound and PAT signals. We chose to experiment with aluminum filter with thickness of 4, 7, and 16 μm as aluminum is cost effective and its material properties allow reflection of light with no generation of PAT signal. We first tested the aluminum filter's ability to prevent light transmission by delivering pulsed laser light to the aluminum filter, with a power meter on the opposite side of a laser to measure light transmission. This was done to validate that the thin aluminum sheets did not have any major defects that would prevent complete blockage.
of light. We then tested the aluminum filter’s ability to remove PAT reflection artifact in vivo by obtaining PAT images of periaortic fat on the infrarenal aorta of apoE−/− mice. The same animal care and imaging protocol was used as the in vivo PAT holder validation studies above. Both sides of the aluminum foil sheet were coated with ultrasound gel to act as an acoustic coupling agent between both the transducer and the foil, and the foil and the mouse. The aluminum foil was mounted on the transducer face prior to fixing the ultrasound probe to the PAT fiber-tuning apparatus, thus allowing both artifact elimination and light delivery through the fiber optic bundles. The transducer was placed on the abdomen of the animal to obtain long-axis ultrasound and PAT images of the infrarenal aorta of the internal aorta of the left renal vein and tail artery bifurcation. We then imaged the mouse with and without the aluminum filter to assess the ability to remove reflection artifact. Images were exported to FIJI and SNR was calculated using region of interest of 16 pixels along the periaortic fat to quantify filter induced signal attenuation.

2.7. Statistical analysis

All MCML radiant flux and ex vivo and in vivo SNR ratio measurements are reported as mean ± standard deviation. Additionally, an ANOVA with a Tukey post-hoc statistical test was used to determine significance between experimental groups (p < 0.05).

3. Results

3.1. Monte Carlo simulation results

MCML simulations were performed to assess depth-dependent and long-axis cross-sectional changes in radiant flux with varying fiber optic bundle orientations. Our depth-dependent simulation showed an exponential decrease in radiant flux from 0.45 ± 0.01 W at the surface of the PVA to 0.05 ± 0.01 W at 10 mm into the PVA (Fig. 2C). We also observed that fiber optic bundles orientation of 0°, 15°, and 30° have greater radiant flux compared to angles of 45° and 60° up to depths of 6 mm. In fact, the average power amongst 0°, 15°, and 30° orientation at 5 mm produced a 21% increase in radiant flux compared to the average between 45° and 60°. While our simulations showed that fiber optic bundle angles play a role in increasing optical influence in our sample, we also wanted to assess the impact of fiber optic bundle orientation on light delivery in our image acquisition plane (Fig. 2D). To do this we designed a simulation to measure radiant flux in five locations along the image acquisition plane. We observed a Gaussian distribution with the greatest radiant flux at 30° (0.28 ± 0.06 W), and the lowest flux at 0° (0.12 ± 0.02 W) and 60° (0.08 ± 0.01 W).

3.2. Ex vivo validation results

Fig. 3 summarizes the results for the ex vivo validation experiments. Using short-axis B-mode ultrasound imaging we were able to resolve all six PE-50 tubes in our PVA phantom. Our PAT results, however, showed the SNR and penetration depths were dependent on the focal length of our probe. Qualitative assessment shows that as we increase the focal length of our PAT probe from 2 mm to 7 mm we can resolve deeper PE-50 tubes that result in a penetration depth improvement of 2 mm (Fig. 3A). This improvement in image quality is supported by our quantitative SNR results (Fig. 3C), which show a focal length of 2 mm resolves the PE-50 tubes up to 3.00 mm depth with SNR above 4.7, but resolves the PE-50 tubes at depths of 3.75 mm and 4.50 mm with a sharp drop-off in SNR of 3.2 ± 0.26 and 2.0 ± 0.53, and no contrast for the PE-50 tube at depth 6.00 mm. As we increase the focal length to 7 mm we see that we can resolve contrast for the PE-50 tubes with depths of 3.75 mm, 4.50 mm, and 6.00 mm with SNR of 3.71 ± 0.30, 2.71 ± 0.42, and 2.12 ± 0.32, respectively. This increase in penetration depth, however, comes at a cost as shown by the decrease in SNR ratio at depths of 1.5 mm (4.37 ± 0.16 to 3.73 ± 0.25), 2.5 mm (4.74 ± 0.31 to 4.34 ± 0.40), and 3.00 mm (4.74 ± 0.28 to 4.26 ± 0.23).

3.3. In vivo validation results

Fig. 4 summarizes the qualitative and quantitative in vivo assessment of our PAT holder using focal lengths of 2, 3, 5, and 7 mm.
Qualitative assessment shows focal length dependent increased signal intensity from subcutaneous and periaortic fat when imaging apoE−/− mice using 1210 nm light (Fig. 4A). This was supported by our quantitative assessment, which shows steady increase of subcutaneous and perivascular fat SNR when focal length is tuned from 7 mm to 2 mm. Specifically, we found subcutaneous fat SNR of 4.7 ± 1.1 at 2 mm focal length, 4.0 ± 0.41 at 3 mm focal length, 3.6 ± 0.29 at 5 mm focal length, and 2.9 ± 0.36 at 7 mm focal length (Fig. 4B). Additionally, we observed a periaortic fat SNR of 2.3 ± 0.37 at 2 mm focal length, 2.1 ± 0.25 at 3 mm focal length, 1.9 ± 0.59 at 5 mm focal length, and 1.3 ± 0.13 at 7 mm focal length (Fig. 4C). Our results also showed a statistically significant increase (p < 0.05) in both subcutaneous and periaortic SNR between focal lengths of 2 and 7 mm. Overall, we observed a 77% increase in subcutaneous SNR and 62% increase in periaortic SNR.

3.4. PAT reflection artifact removal results

We quantitatively assessed both the ability of aluminum filters to completely reflect incoming light and also prevent PAT reflection artifacts. Through our light transmission experiment we found that 4, 7, and 16 μm aluminum filters were all able to completely block light transmission, thus showing potential to prevent PAT-related reflection artifacts through decoupling of light-probe interactions. Moreover, when we applied our aluminum filter to our PAT probe and performed in vivo imaging of periaortic fat on the infrarenal aorta we found that the 4 and 7 μm aluminum filters prevented the PAT reflection artifact (Fig. 5B). The 16 μm aluminum filter, however, did not allow acquisition of both ultrasound and PAT images, potentially because the aluminum was too thick to allow penetration of acoustic waves. Quantitative analysis showed that baseline average periaortic SNR to be 2.37 ± 0.16, while the average periaortic SNR slightly decreased to 2.03 ± 0.34 with the 4 μm aluminum filter and 2.03 ± 0.38 with the 7 μm aluminum filter (Fig. 5C).

4. Discussion

We have developed an adjustable, motorized PAT holder that improves optical fluence at various layers of tissue to increase penetration depth and SNR. This holder consisted of an external stepper motor that allows vertical translation of the ultrasound transducer to adjust the pivot point around which the fiber optic bundles rotate, and two bipolar stepper motors that allow the user to tune the fiber optic bundle angle with respect to the ultrasound transducer. These stepper motors were controlled by a Steppoko 3-axis controller that used GRBL software, allowing for 0.02 mm translation of the ultrasound transducer and 0.11° rotation of the fiber optic bundles. Our rationale for pursuing this light manipulation mechanism is that illumination geometry can impact photon density within the tissue, which will then affect PAT signal generation. By tuning our fiber optic bundle orientation, we can optimize light fluence in our image acquisition plane and improve our PAT penetration depth and SNR.

The MCML simulations revealed that the largest spot size at 30° allowed maximum light penetration and flux in the image acquisition plane, while orientations at 0° and 60° resulted in suboptimal tissue light fluence due to the momentum of the incident light being directed laterally and parallel to the image acquisition plane. These results, however, will vary depending on the ultrasound transducer and fiber optic bundle geometry, as well as distance between PAT probe and tissue surface. For example, in our simulation 30° produced the largest spot size due to the PAT probe offset of 3 mm from the surface of the PVA. If this offset is increased or decreased the fiber optic bundle angle of 30° would not create the largest spot size, producing suboptimal radiant flux. Collectively, these results show that there is an optimal fiber optic bundle configuration that allows maximum fluence throughout the sample of interest, as well as in the image acquisition plane. These configurations, however, will vary depending on the overall PAT imaging geometry.

The ex vivo PAT imaging of PVA embedded with six PE-50 tubes matched well with the MCML simulations. Further the ex vivo images confirmed that tuning fiber angle can alter the SNR at various depths and can lead to improvement in penetration depths. A focal length of 5 mm produced the smallest theoretical spot size and allowed us to resolve the first five PE-50 tubes. When we increased our focal length to 7 mm we also increased our spot size that led to a 2 mm improvement in penetration depth, but with a cost to decreased SNR for more superficial PE-50 tubes. Focal lengths of 2 and 3 mm, however, produced incident light momentum that caused light to move laterally from the image acquisition plane, thus decreasing penetration depth. It is important to note that the improvements in penetration depth and SNR can vary depending on the optical properties of the tissue and the transducer frequency. In this case, our phantom broadly mimicked the acoustic and optical properties of breast tissue [33,36]. Imaging depth is also dependent on application, where breast tumor margin imaging is focused on the first 2 mm of tissue, suggesting a 2 mm improvement can be suitable for shallow imaging [10]. Taken together, our ex vivo validation shows that the focal length of the PAT probe can be tuned to improve the radiant flux in the image acquisition plane to improve penetration depth and SNR.

The in vivo murine images revealed the importance of utilizing a focal length tuning apparatus to obtain improved image quality of subcutaneous and periaortic fat accumulation in apoE−/− mice. We

Fig. 5. Schematic of reflection artifact (A) showing how light reflects off of the optical reflector and induces a photoacoustic effect at the PAT probe face. This PAT signal then travels distance d down towards the acoustic reflector and travels another distance d up towards the ultrasound transducer. The PAT system, therefore, registers the acoustic wave as traveled 2d, creating an artifact that can obscure image quality. The reflection artifact is effectively eliminated when a light-blocking aluminum filter is applied to the transducer face (B) as shown by the ultrasound (top left), PAT without filter (top right), and PAT with 4 μm (bottom left) and 7 μm (bottom right) filter images. Quantitative SNR assessment shows slight decrease in SNR when utilizing aluminum filters (C). The infrarenal aorta is highlighted by the red dotted lines, the periaortic signal by the orange arrows, and the reflection artifact by the red arrow.
observed a 77% increase in subcutaneous fat SNR and a 62% increase in periaortic fat SNR by decreasing the focal length from 7 mm to 2 mm. Additionally, by tuning the focal length we resolved periaortic fat accumulation on both anterior and posterior wall of the aorta, a critical improvement that opens up the possibility of characterizing plaques in small animal models [4]. We did observe, however, that while the ultrasound probe is placed 3 mm from the skin surface, the probe with focal length of 2 mm produced the greatest SNR. We attribute this to the heterogeneity in the absorption and scattering of the tissues that are being imaged (e.g. skin, muscle, intestines) that may cause complex scattering regimes that are not simulated in our homogenous MCML simulation. Another consideration is that the focal length was measured in air and not in water, thus the focal length in water may vary due to slight scattering effects. Nevertheless, the results of our MCML and ex vivo studies suggest that there is little deviation in focal length when light travels through water. These in vivo results, therefore, emphasize the need for PAT holder that can allow the user to optimize their tissue illumination to improve image quality. This highlights the preclinical and clinical utility of an adjustable PAT probe, as the illumination geometry should be carefully considered depending on the tissues being imaged.

Finally, we present an alternative and effective method to remove PAT reflection artifacts that arise due to light interaction with the ultrasound probe face through the use of 4 μm and 7 μm thick aluminum foil. This reflection artifact arises due to light reflecting off of the tissue surface and interacting with the PAT probe face to induce a PA signal. This signal is then reflected off of the skin surface and registered by the ultrasound system, thus producing an artifact that matches the geometry of the tissue surface but at twice the skin depth. One caveat for utilizing the 4 μm aluminum foil, however, is that it is very fragile and easily torn when applied with shear forces. Still, these experimental results show that 4 and 7 μm filters are thick enough to completely reflect light, yet thin enough to allow penetration of acoustic waves. This allowed us to reflect light away from the probe face while also acquiring both PAT and ultrasound signal, thus making aluminum an effective filter to prevent probe-specific reflection artifacts. It is important to differentiate when to use artifact prevention strategies (i.e. foil filter) and post-image processing techniques to remove in-plane artifacts and out-of-plane clutter. In-plane artifacts typically result from high PAT signals that reflect off of acoustically dense structures capable of obscuring regions of interest. On the other hand, photoacoustic transients that originate outside of the image acquisition plane can cause out-of-plane clutter that leads to misinterpretation of PAT images. Examples of reflection and motor-induced interference can be seen in Fig. 3B, with artifact signal increasing up to a focal length of 5 mm, suggesting that illumination geometry may independently affect both PAT signal and artifact intensity. Therefore, it is important to emphasize that an aluminum filter may be effective in removing artifacts due to optical absorption at the probe face, but other sophisticated methods should be used for more troublesome in- and out-of-plane artifacts. Moreover, artifacts due to optical absorption by the ultrasound transducer may be avoided by utilizing a probe with a white surface rather than gray acoustic lenses, as found in many commercially available systems.

There are several specific design criteria that can significantly alter the effectiveness of this PAT probe. First, it is critical to minimize the bandwidth of the fiber optic bundle and ultrasound transducer face, such as the metal casing that typically house the terminal end of the fiber bundle. This is because if there is too much material between the ultrasound transducer and fiber optic bundle face, the users ability to control where the light is focused within the tissue is reduced. Second, the current dimensions of our motorized PAT probe are 6.5 x 6 x 19 cm, meaning that design optimization is required to miniaturize this fiber tuning mechanism for clinical use. Our probe is specifically limited due to large size of the motors that control translation of the ultrasound transducer and rotation of the fiber optic bundles.

As the size of transducers vary, the pivot point in which the fibers must rotate can also vary between transducers. One potential solution is to create an optimal ultrasound transducer that requires only one pivot point to achieve a range of desired focal lengths. Finally, special consideration should be made when determining the length of the fiber optic bundle bifurcation. A bifurcation that is too short will limit the attainable focal lengths, as there is not enough cord length to allow for the proper rotation of the fiber bundles. Alternatively, a bifurcation that is too long could make the probe hard to manage, especially for in vivo clinical applications. Sub-optimal bifurcation length can increase the chances of damage to the fiber optic bundle due to increased stress applied when the fiber is pushed past its bending radius. These design criteria specifically apply to PAT probes that utilize bifurcated fiber optic bundles, as well as laser diodes, and light-emitted diodes. While this adjustable holder is certainly versatile, other photoacoustic microscopy techniques and configurations (e.g. diffuser light source, scanning lens, and catheter probes) will require different design criteria based on application and need [37].

5. Conclusion

In conclusion, we have shown through MCML, ex vivo, and in vivo studies that penetration depth and SNR can be improved by adjusting the fiber optic bundle orientation with respect to the ultrasound transducer. This mechanism improves image quality by increasing photon density in the image acquisition plane. Our results also suggest that illumination geometry should be optimized depending on the optical heterogeneity of the tissue, as well as to maximize SNR and minimize artifacts. With the combination of other light manipulation techniques, such as a light catching mechanism, an advanced PAT probe can be created for high-resolution deep tissue compositional imaging. Further optimization of a tunable handheld PAT probe has the potential to improve image quality for various pre-clinical and clinical applications.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

[1] L.V. Wang, S. Hu, Photoacoustic tomography: in vivo imaging from organelles to organs, Science 335 (6075) (2012) 1458–1462.
[2] X.L. Deán-Ben, S. Gottschalk, B. Mc Laney, S. Shoham, D. Razansky, Advanced photoacoustic methods for multiscale imaging of in vivo dynamics, Chem. Soc. Rev. 46 (8) (2017) 2158–2198.
[3] P. Beard, Biomedical photoacoustic imaging, Interface Focus (2011) rfs20110028.
[4] G.S. Sangha, E.H. Phillips, C.J. Goergen, In vivo photoacoustic lipid imaging in mice using the second near-infrared window, Biomed. Opt. Express 8 (2) (2017) 736–742.
[5] B. Wang, A. Karpionk, D. Veager, J. Amiran, S. Litovsky, R. Smalling, S. Emelianov, Intravascular photoacoustic imaging of lipid in atherosclerotic plaques, Opt. Express 16 (5) (2008) 3362–3367.
[6] K. Jansen, A.F.W. Van Der Steen, H.M.M. Van Beusekom, J.W. Oosterba, G. van Soest, Intravascular photoacoustic imaging of human coronary atherosclerosis, Opt. Lett. 36 (5) (2011) 597–599.
[7] B. Wang, A. Karpionk, D. Veager, J. Amiran, S. Litovsky, R. Smalling, S. Emelianov,
In vivo intravascular ultrasound-guided photoacoustic imaging of lipid plaques using an animal model of atherosclerosis, Ultrasound Med. Biol. 38 (12) (2012) 2098–2103.

[9] B. Wang, J.L. Su, A.B. Karpiouk, K.V. Sokolov, R.W. Smalling, S.Y. Emelianov, Intravascular photoacoustic imaging, IEEE J. Sel. Top. Quantum Electron. 16 (3) (2010) 588–599.

[10] R. Li, P. Wang, L. Lan, F.P. Lloyd, C.J. Goergen, S. Chen, J.-X. Cheng, Assessing breast tumor margin by multispectral photoacoustic tomography, Biomed. Opt. Express 6 (4) (2015) 1273–1281.

[11] S. Manohar, S.E. Vaartjes, J.C.G. van Haspen, J.M. Klase, F.M. van den Engh, W. Steenbergen, T.G. Van Leeuwen, Initial results of in vivo non-invasive cancer imaging in the human breast using near-infrared photoacoustics, Opt. Express 15 (19) (2007) 12277–12285.

[12] M. Toi, Y. Aso, Y. Matsumoto, H. Sekiguchi, A. Yoshikawa, M. Takada, M. Kataoka, T. Endo, N. Kawaguchi-Sakita, M. Kawashima, Visualization of tumor-related blood vessels in human breast by photoacoustic imaging system with a hemispherical detector array, Sci. Rep. 7 (2017) 1–11.

[13] R. Li, E. Phillips, P. Wang, C.J. Goergen, J.X. Cheng, Label-free in vivo imaging of peripheral nerve by multispectral photoacoustic tomography, J. Biophotonics 9 (1–2) (2016) 124–128.

[14] G.S. Sangha, C.J. Goergen, Photoacoustic tomography: applications for atherosclerosis imaging, J. Opt. A. (2016) 084005.

[15] Z. Wang, S. Ha, K. Kim, A new design of light illumination scheme for deep tissue photoacoustic imaging, Opt. Express 20 (20) (2012) 22649–22659.

[16] J. Yu, J.S. Schuman, J.-K. Lee, S.G. Lee, J.H. Chang, K. Kim, A light illumination enhancement device for photoacoustic imaging: in vivo animal study, IEEE Trans. Ultrasound. Ferroelectr. Freq. Control 64 (2017) 1205–1211.

[17] L. Nie, X. Cai, K. Maslov, A. Garcia-Espejo, M.A. Anastasio, L.V. Wang, Photoacoustic tomography through a whole adult human skull with a photon recycler, J. Biomed. Opt. 17 (11) (2012) 110506–110506.

[18] C. Ash, M. Dubec, K. Donne, T. Bashford, Effect of wavelength and beam width on penetration in light-tissue interaction using computational methods, Lasers Med. Sci. 32 (8) (2017) 1909–1918.

[19] G. Held, S. Preisser, H.G. Akarçay, S. Peeters, M. Frenz, M. Jaeger, Effect of irradiation distance on image contrast in epi-optoacoustic imaging of human volunteers, Biomed. Opt. Express 5 (11) (2014) 3760–3780.

[20] M. Xu, L.V. Wang, Photoacoustic imaging in biomedicine, Rev. Sci. Instrum. 77 (4) (2006) 041101.

[21] M. Jaeger, L. Siegenthaler, M. Kitz, M. Frenz, Reduction of background in optoacoustic image sequences obtained under tissue deformation, J. Biomed. Opt. 14 (5) (2009) 054011–054011–10.

[22] M. Jaeger, J.C. Bamberg, M. Frenz, Clutter elimination for deep clinical optoacoustic imaging using localized vibration tagging (LOVIT), Photoacoustics 1 (2) (2013) 19–29.

[23] M.K.A. Singh, M. Jaeger, M. Frenz, W. Steenbergen, Photoacoustic reflection artifact reduction using photoacoustic-guided focused ultrasound: comparison between plane-wave and element-by-element synthetic backpropagation approach, Biomed. Opt. Express 8 (4) (2017) 2245–2260.

[24] M.K.A. Singh, W. Steenbergen, Photoacoustic-guided focused ultrasound (PAFUSion) for identifying reflection artifacts in photoacoustic imaging, Photoacoustics 3 (4) (2015) 123–131.

[25] M.K.A. Singh, M. Jaeger, M. Frenz, W. Steenbergen, In vivo demonstration of reflection artifact reduction in photoacoustic imaging using synthetic aperture photoacoustic-guided focused ultrasound (PAFUSion), Biomed. Opt. Express 7 (8) (2016) 2955–2972.

[26] L.E.O. America, American National Standard for Safe Use of Lasers, (2014).

[27] S. Kedenburg, M. Vieweg, T. Gissibl, H. Giessen, Linear refractive index and absorption measurements of nonlinear optical liquids in the visible and near-infrared spectral region, Opt. Mater. Express 2 (11) (2012) 1588–1611.

[28] M. Jonasz, G. Fournier, Light Scattering by Particles in Water: Theoretical and Experimental Foundations, Elsevier, 2011.

[29] M.J. Weber, Handbook of Optical Materials, CRC Press, 2002.

[30] H. Buivides, J.H.M. Hakkert, M. Donze, Optical Properties of Pure Water, Ocean Optics XII, International Society for Optics and Photonics, 1994, pp. 174–184.

[31] E. Kaniusas, Biomedical Signals and Sensors II, Springer, 2015.