High-frequency annular array with coaxial illumination for dual-modality ultrasonic and photoacoustic imaging

Erwan Filoux,^{1,a)} Ashwin Sampathkumar,¹ Parag V. Chitnis,¹ Orlando Aristizábal,^{2,3} and Jeffrey A. Ketterling^{1,b)}

¹*Lizzi Center for Biomedical Engineering, Riverside Research, New York, New York 10038, USA*

²*Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, New York, New York 10016, USA*

³*Department of Radiology, New York University School of Medicine, New York, New York 10016, USA*

(Received 13 November 2012; accepted 29 April 2013; published online 21 May 2013)

This paper presents a combined ultrasound and photoacoustic (PA) imaging (PAI) system used to obtain high-quality, co-registered images of mouse-embryo anatomy and vasculature. High-frequency ultrasound (HFU, >20 MHz) is utilized to obtain high-resolution anatomical images of small animals while PAI provides high-contrast images of the vascular network. The imaging system is based on a 40 MHz, 5-element, 6 mm aperture annular-array transducer with a $800\text{ }\mu\text{m}$ diameter hole through its central element. The transducer was integrated in a cage-plate assembly allowing for a collimated laser beam to pass through the hole so that the optical and acoustic beams were collinear. The assembly was mounted on a two-axis, motorized stage to enable the simultaneous acquisition of co-registered HFU and PA volumetric data. Data were collected from all five elements in receive and a synthetic-focusing algorithm was applied in post-processing to beamform the data and increase the spatial resolution and depth-of-field (DOF) of the HFU and PA images. Phantom measurements showed that the system could achieve high-resolution images (down to $90\text{ }\mu\text{m}$ for HFU and $150\text{ }\mu\text{m}$ for PAI) and a large DOF of >8 mm. Volume renderings of a mouse embryo showed that the scanner allowed for visualizing morphologically precise anatomy of the entire embryo along with corresponding co-registered vasculature. Major head vessels, such as the superior sagittal sinus or rostral vein, were clearly identified as well as limb bud vasculature. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4804636>]

I. INTRODUCTION

Photoacoustic (PA) imaging (PAI), also referred to as optoacoustic imaging, is a hybrid modality that combines optics and acoustics. PAI relies on local absorption of a brief ($\sim\text{ns}$) monochromatic light pulse and subsequent thermoelastic conversion of the optical energy into a broadband ultrasonic (US) pulse.^{1,2} These US waves can then be detected using piezoelectric transducers to produce molecular images of soft tissue.^{2,3} The resolution of PA images typically depends on the receive properties of the US transducer employed to detect the PA signals. Transducer parameters such as center frequency, radius of curvature, number of elements, element size, and aperture size critically influence the utility of a transducer for different applications of PAI.⁴ Therefore, the design of the transducer is an important consideration for optimizing the PA-signal acquisition in various settings.

Linear arrays are commonly used for PAI because they are readily available and are easy to integrate into ultrasound machines to permit electronic focusing along the axial and azimuthal directions.⁴ However, linear arrays have a non-symmetric beam due to fixed elevation focusing that results in an out-of-plane beamwidth greater than the in-plane beamwidth. Linear arrays can also be difficult to fabricate at

higher frequencies due the necessary small element size, and arrays, once packaged, are somewhat limited in terms of light delivery options. In comparison to linear arrays, a mechanically scanned, single-element, spherically focused transducer operating at high frequencies is easier to manufacture and can provide significantly better spatial resolution^{5,6} but only over a narrow depth-of-field (DOF). The limitation in DOF can be overcome by using annular-array transducers, which provide spatial resolution comparable to fixed-focus, single-element transducers, and DOF similar to the linear arrays. In a previous study, we used a 40 MHz, 5-element annular array, and a synthetic-focusing (SF) algorithm to achieve a sixfold increase in DOF compared to an equivalent single-element transducer.^{7,8} Using a similar array, we demonstrated combined high-frequency (HF) ultrasound (HFU) and PAI with broad, uniform illumination from a bifurcated optical-fiber assembly.⁹

One application that is gaining attention is vascular imaging in small animals, such as mice, for studying human disease^{10,11} and mammalian development.¹² Unlike imaging modalities that require exogenous contrast agents such as diagnostic ultrasound or magnetic-resonance imaging (MRI), PAI can produce vascular images based on endogenous contrast by selecting an optical wavelength that is preferentially absorbed by blood.^{13–15} This makes PAI an ideal imaging modality for longitudinal studies of the vascular development of crucial organs such as the heart and the brain in small

^{a)}Present address: VERMON S.A., 37038 Tours, France.

^{b)}Email: jketterling@riversideresearch.org

animals. Because the diameter of blood vessels in small animals typically ranges from 5 to 300 μm , PAI of the embryonic vasculature requires transducers operating at high frequencies (i.e., >20 MHz) to achieve the necessary spatial resolution. A complication associated with operating at high frequencies is the frequency-dependent attenuation of the HFU waves propagating in tissue. This, in conjunction with the optical attenuation (combination of scattering and absorption) in tissue, can severely limit the imaging depth which scales inversely with acoustic frequency.⁴ This is why PAI of the embryonic vasculature has typically been performed *ex vivo* in order to avoid the optical attenuation and noise generated by the surrounding tissues.^{16,17}

When using the PAI system based on our annular array with bifurcated illumination, we obtained co-registered, high-resolution HFU, and PA images of the vasculature from the head of an *in vivo* mouse embryo over a DOF of >5 mm.⁹ One drawback of this configuration was that the radio-frequency (RF) data acquired at each scan location contained spurious signals originating from off-axis locations, which degraded the signal-to-noise ratio (SNR) and the quality of the PA images. In this paper, we have modified our annular-array system to allow for combined coaxial HFU-PA imaging using a collimated laser source. The laser illumination was directed coaxially through the center of the annular-array transducer. The confined optical and acoustic beams were collinear so that the optical energy density was maximized all along the central axis of the transducer. A backscatter configuration was used, as is done in most *in vivo* applications, because it was more practical to have the light source and detector on the same side of the specimen. In this configuration, coaxial illumination optimally delivers incident optical energy along the acoustic axis of the transducer.

The performance of the system was characterized in terms of spatial resolution, DOF, SNR, and penetration depth using hair phantom measurements. The utility and imaging capabilities of the HFU-PA system were demonstrated by producing co-registered, *in utero*, anatomical and vascular images of a mouse embryo. To the best of our knowledge, our approach of using an annular array that provides simultaneous, spatially co-registered, dual-modality images with improved DOF and penetration depth compared to single-element transducers, is unique. Our system is the first that demonstrates *in utero* PA imaging of the neuro-vasculature in an embryonic brain, and would potentially allow longitudinal studies of the development of various organs.

II. MATERIALS AND METHODS

A. Annular array

The annular array used in this study was fabricated through a process that was previously described.¹⁸ Briefly, the transducer was fabricated by bonding a piezopolymer film to an array pattern etched onto a copper-clad polyimide (CCP) film using a non-conductive epoxy. The active element was a 9 μm copolymer film of poly(vinylidene fluoride-tetrafluoroethylene) [P(VDF-TrFE)] which provided a coupling coefficient of $k_t = 0.3$.¹⁹ The pattern was composed of

FIG. 1. Schematic of the annular-array transducer with (a) side laser illumination using light guides and (b) coaxial, collimated illumination through a hole drilled along the central axis of the array. In both cases, light diffuses due to scatterers in the medium. When using a coaxial, collimated laser beam, the illuminated area is smaller and off-axis PA signals are reduced.

five equal-area rings with 100 μm spacing between rings and a total aperture of 6 mm. The final transducers were geometrically focused at 12 mm and non-conductive epoxy was used as a backing.

A similar transducer was used in a previous version of the combined HFU-PA imaging system with bifurcated, side illumination⁹ (Fig. 1(a)), but the broad laser excitation generated strong off-axis signals that degraded SNR. To overcome this limitation, the transducer was modified to accommodate a laser beam that could be aligned coaxially with the acoustic beam of the transducer (Fig. 1(b)) through a 800 μm hole drilled precisely along the central axis of the array. Due to the low Curie temperature of the piezopolymer film (≈ 102 $^{\circ}\text{C}$), water cooling was used to prevent depolarization during the drilling.

B. PA assembly

A picture of the coaxial PA system is shown in Fig. 2. The output beam of the laser was 2 mm in diameter, defined as the distance over which the beam intensity falls below 13.5% ($1/e^2 \approx 0.135$, with e the exponential constant) of the maximum value. The beam was aligned with the central axis of the transducer using a series of mirrors mounted on kinematic mounts. Using collimation lenses with focal distances of 100 (lens 1) and 25.4 mm (lens 2), the beam was reduced and collimated to a spot size of 800 μm so that most of the energy could pass through the hole in the transducer. The components were placed in a periscopic cage assembly mounted on a two-axis motion system allowing for the laser beam to be translated in a coaxial arrangement with the transducer for acquiring B-mode images and 3D volumetric data.

C. Data-acquisition system

The data-acquisition system consisted of four main components: motion control, acoustic excitation, optical excitation, and digital-signal acquisition. The motion-control component of the system employed a 2-axis, motorized translation stage (LAL35, SMAC, Carlsbad, CA) with 1 μm resolution and was driven via a motion-control card (PXI-7534, National Instruments (NI), Austin, TX). Two different single-channel pulsers were used to excite the central element of the annular array (the other elements were not used in transmit because the pulsers could excite only one channel at a time): a

FIG. 2. (a) Picture of the PA system allowing for a coaxial arrangement of the laser beam with the acoustic beam of the annular array. Four mirrors (M1, M2, M3, and M4) were used so that the alignment was maintained during scanning. The whole periscopic assembly was mounted on a 2-axis, linear motorized stage to acquire volumetric HFU and PA data. (b) Detail of the drilled annular-array transducer mounted in a manual, 2-axis stage for precise alignment with the laser beam.

broadband pulser/receiver (Daxsonics, Halifax, Nova Scotia, Canada) and a monocycle pulser (Avtech AVB2-TA-C-CVA, Ottawa, ON, Canada). The broadband pulser/receiver was used to obtain *in vitro* data for system characterization. It provided a 100 V_{pp} impulse and up to 45 dB variable gain in receive. The monocycle pulser was used to acquire *in vivo* data. It delivered a 200 V_{pp} monocycle impulse and low-noise amplifiers (AU-1313, Miteq, Hauppauge, NY) with a fixed 46 dB gain were used in receive. A frequency-doubled Nd:YAG laser (Minilite II, Continuum, Santa Clara, CA) operating at a wavelength of 532 nm with a peak energy of 25 mJ/pulse was employed for the optical excitation. The laser pulse was 5 ns in duration with a repetition rate of 10 Hz. The digital signal-acquisition component of the system permitted simultaneous data acquisition of the five transmit-to-receive (TR) data pairs from the five array elements using three digitizer cards (PXI-5152, NI) that had 8-bit resolution. The acquired data were sampled at 500 MHz and stored for post-processing.

Scanning of the HFU-PA assembly and data collection were automated and controlled using a custom LabVIEW (NI) program. To acquire simultaneous HFU and PA data, the laser pulse and the ultrasound impulse excitation were fired at the same time using a synchronization signal from the laser's

Q-switch. The time for the HFU round-trip echoes to reach the transducer was twice that of the one-way PA signals. Simple time gating could be used to parse HFU and PA data.

D. Synthetic focusing

A SF algorithm was applied to the digitized RF data in order to axially focus the annular-array receive data.⁷ To focus the array on receive at a depth d , the one-way time delay t_n required for ring n of the array is approximately $t_n = [a_n^2(1/F - 1/d)]/2c$, where F is the geometric focus, c is the speed of sound, and a_n is the root mean square of the inner and outer radii of ring n .²⁰ The total delay $t_n^{tot} = t_n^T + t_n^R$, with t_n^T on transmit and t_n^R on receive, was calculated for each of the five elements and applied to the acquired A-lines. In the case of HFU data, the total delays t_n^{tot} correspond to a two-way propagation of the acoustic waves with a transmit delay $t_n^T = t_1^T$ from the central element (ring 1). In the case of the PA data, excitation is considered instantaneous ($t_n^T = 0$) and the delays correspond to a one-way acoustic propagation. In each case, the delayed signals were summed to form an image with a focus at depth d . The focus was shifted to many different depths and windowed data were assembled to form a composite image with multiple focal zones (>50). If no delays are applied ($t_n^{tot} = 0$ for all TR pairs), the result simulates a single-element transducer with the same geometric focus and total aperture as the annular array.

E. Mouse embryo imaging

In order to validate our annular-array system for small-animal imaging, data were obtained from *in vivo* embryos using a semi-invasive imaging protocol.²¹ Animals used in these studies were maintained according to protocols approved by the Institutional Animal Care and Use Committee at New York University Medical Center. Briefly, a pregnant mouse was anesthetized with 1.5% isoflurane and the intact uterus containing a mouse embryo at the twelfth day of gestation (E12.5) was externalized into a fluid-filled Petri dish. The transducer assembly was mechanically scanned in 2D in 50 μm increments to acquire image data representing a 3D volume of $7 \times 6 \times 9 \text{ mm}^3$. A laser-pulse energy of 3 mJ/pulse was used to generate the PA signal and RF lines were acquired during the resting phase of the respiratory cycle.²² A single image plane was obtained in $\approx 15 \text{ s}$ and the acquisition of 3D volumetric data took $\approx 30 \text{ min}$. The scan times were limited by the 10 Hz PRF of the laser source.

The RF data were imported into custom-analysis software (MATLAB, The MathWorks, Inc., Natick, MA) that extracted the HFU-PA echoes from each RF line and applied the SF algorithm to each signal to produce dynamically focused RF lines. B-mode HFU and PA images were generated directly from the envelope-detected, log-compressed, beam-formed RF lines. Volumetric images were generated by loading the 2D image stack into visualization software (Amira v5.2, Mercury Computer Systems, San Diego, CA). The volume data were pre-processed using histogram normalization and a 3D Gaussian filter. The embryonic head was segmented

TABLE I. Annular-array characteristics.^a

Ring	f_c (MHz)	dB@ f_c	BW _{6 dB} (%)
1	43.2	0.0	32.6
2	43.0	-1.5	36.7
3	42.8	-1.9	33.6
4	42.4	-3.5	33.2
5	42.0	-4.0	32.4

^a f_c = -6 dB center frequency; dB@ f_c = relative sensitivity; BW_{6 dB} = -6 dB bandwidth.

out from extra-embryonic tissue by creating a mask after manually delineating the contour of the head on a slice-by-slice basis and arithmetically multiplying the mask with the volume. Because PA and HFU volumes were perfectly co-registered, the same mask was used on the PA volume data set to segment out the head vasculature. The high contrast between the brain ventricles and the surrounding tissue facilitated segmentation of the embryonic brain ventricles and removal of the extra-embryonic tissue (such as the uterine wall).

III. RESULTS

A. Transducer performance

Transducer characteristics (relative sensitivity, spatial resolution, and SNR) were measured using the broadband pulser/receiver. The relative sensitivities of the transducer elements were measured by acquiring pulse-echo measurements from a quartz plate immersed in degassed water and positioned at the transducer focus normal to the acoustic axis.¹⁸ After the 800 μm hole had been drilled, the surface of the central element was reduced by $\approx 10\%$ and a decrease of ≈ 1 –2 dB in sensitivity was observed. The sensitivity of the other elements were unchanged after drilling. The characteristics of each element are summarized in Table I. The typical decrease in sensitivity from the central to the outer element mainly results from acoustic diffraction.

Wire phantoms were used to measure the spatial resolution of the HFU and PAI systems. Ideally, the spatial resolution of both modalities would be assessed simultaneously using a single wire phantom, but it is difficult to find very thin wires (diameters on the order of a few dozen microns for HFU characterization) with sufficient optical absorption at 532 nm. Therefore, the spatial resolutions of the HFU and PAI modalities were measured separately.

The lateral resolution of the transducer was determined by acquiring HFU images of a 13 μm diameter, metal-alloy wire immersed in degassed water. The wire was positioned at depths ranging from 8 to 20 mm (distance from the transducer), in 1 mm intervals, and B-mode data were acquired with a 5 μm lateral spacing between adjacent A-lines. The RF data were synthetically focused and the envelope-detected, log-compressed B-mode images were stitched together to show the evolution of the acoustic beam with depth (Fig. 3(a)). The lateral resolution was measured at each depth by determining the beamwidth at -6 dB (full width at half maximum) and the resulting curve is plotted in Fig. 4. A minimum beamwidth of 90 μm was obtained at a depth of 9 mm

FIG. 3. (a) Composite B-mode HFU image of a wire phantom (13 μm diameter) at different depths in water. (b) Composite PA image of a hair phantom (80 μm diameter) at the same depths in water. HFU and PA data were processed using a SF algorithm to focus the beam at all depths. Each figure is displayed with 35 dB of dynamic range.

and it remained below 200 μm as far as 20 mm from the transducer. The SNR was calculated at each depth and a peak value of 40 dB was observed at 10 mm with a steady decrease out to 20 mm (Fig. 5).

B. Photoacoustic system characterization

An agar-based phantom that contained an 80 μm diameter hair strand was used to characterize the spatial resolution of the PAI modality. Other than the method of acquiring the data, the methods to characterize the PA beam properties were the same as described for the HFU mode. Although the diameter of the hair fiber was over six times that of the 13 μm metal wire, the hair fiber was much smaller than the laser beamwidth of 800 μm . Therefore, relative to the 100 μm acoustic beamwidth, the hair acts as a nominal PA point source and a smaller-diameter hair would lead to a negligible change in the measured PAI beam properties.

A pulse energy of 0.4 mJ/pulse was found to be sufficient to obtain an SNR above 30 dB at the geometric focus.

FIG. 4. Lateral resolution versus depth for the imaging system in HFU and PA modes, measured from SF images of the wire and hair phantoms in water, respectively.

FIG. 5. SNR versus depth measured from SF images of the HFU wire and PA hair phantoms in water.

Figure 3(b) is the composite B-mode images obtained from the PA signals of the hair fiber positioned at different depths. The evolution of the lateral resolution and SNR follow similar trends compared to the HFU case (Fig. 3(a)). One can see in Fig. 4 that the beamwidth in PA mode was about 50% larger than in HFU mode. However, the maximum PA beamwidth ($\approx 350 \mu\text{m}$) remains smaller than the laser spot size ($800 \mu\text{m}$), which means that the lateral resolution of the PAI system was only dependent on the one-way beamwidth of the transducer.

The amplitude of the PA signal around the transducer focus (Fig. 6) shows that the -6 dB duration of the signal generated by the hair phantom was about 40% shorter than the HFU case, and allowed the front and back surfaces of the hair fiber to be resolved. This is mostly due to the broadband nature of the PA pulses emitted during the brief thermoelastic expansion of the hair, resulting in improved axial resolution compared to HFU. Spurious peaks can be observed (around $8.2 \mu\text{s}$) due to the interference of off-axis PA signals created in the elevation direction along the part of the hair strand that was illuminated (i.e., the hair was not an ideal point source).

The SNR as a function of depth (Fig. 5) revealed a peak of 42 dB, similar to the HFU behavior at 10 mm. However, the PA SNR showed a more gradual decrease versus depth com-

FIG. 6. Normalized, log-compressed envelope of pulse-echo HFU and PA signals acquired from the wire and hair phantoms in water, respectively. The phantoms were aligned with the central axis of the transducer and were positioned at the geometric focus (12 mm).

FIG. 7. (a) HFU image of the mid-sagittal plane of the embryonic mouse head with a dynamic range of 45 dB. (b) Co-registered PA image displayed with a dynamic range of 20 dB. (c) Overlaid HFU and PA images that illustrate co-registration. (3V and 4V = third and fourth cerebral ventricles; CP = choroidal plexus; BA = basilar artery; VA = vertebral artery; SGS = superior sagittal sinus; AQ = aqueduct.)

pared to the HFU case, mainly due to the low attenuation and diffusion of the laser beam in transmit. In a diffusive medium, the effective diameter of the laser beam would increase with depth more rapidly than observed for the wire phantom and the SNR would decrease more rapidly due to optical scattering and attenuation.

C. Imaging of embryonic vasculature

Tissue has a large number of smaller vessels and capillaries that can be considered subresolution and, in effect, represent noise relative to larger vessels that can be distinctly resolved. Depending on the imaging application, this background signal from blood-rich areas can either represent true image noise or in some cases, like the mouse embryo, it can help identify distinct tissue regions. By adjusting the dynamic range of the image, it is possible to emphasize the vasculature of the overall tissue.

Synthetically focused, co-registered HFU and PA images of the head of an E12.5 embryo (mid-sagittal planes derived from the 3D volume data) are shown in Fig. 7. The B-mode HFU image (Fig. 7(a)) reveals the 2D morphology of the brain using a 45 dB dynamic range, with the brain ventricles (fluid-filled cavities in a developing brain) easily identifiable. The co-registered PA image (Fig. 7(b)) shows the vasculature of the embryo head. Our system was not capable of resolving the dense network of small vessels and capillaries in tissue. These blood-rich regions generate background PA signals that can help identify different tissues but, in our case, the dynamic range in the PA image is lowered to 20 dB in order to emphasize the major embryonic blood vessels. While the 2D images permitted the visualization of the larger blood vessels, the extended vascular morphology was not readily identifiable.

To better visualize the complex nature of the embryonic anatomy and vasculature, volumetric images of the entire E12.5 embryo head were rendered (Fig. 8). Gross anatomical features could be observed in the HFU, such as the head, eye, tail, and limb bud, along with extra embryonic organs such as the placenta (Fig. 8(a)). Spatially co-registered, color-coded PA data showed that the signals generated by blood mainly originated in the head and torso of the embryo. By rotating the view and adjusting the threshold level and transparency of HFU and PA volumes, major blood vessels could be

FIG. 8. (a) Volumetric PA (color) image of E12.5 embryo overlaid on the HFU (gray scale) image. HFU depicted anatomical features such as the head, eye, tail, and limb. (b) Rotated transparency-adjusted PA image visualized major blood vessels and the vascular plexus. (SGS = superior sagittal sinus; RV = rostral vein; LBV = limb blood vessel.)

identified in the embryonic head, including the superior sagittal sinus (SGS) and rostral vein (RV), as could the limb bud vessels (LBV) (Fig. 8(b)). The HFU volume provided morphological landmarks that greatly simplified the identification of the vasculature in the PA data.

IV. DISCUSSION

MRI is the gold standard technique frequently employed to obtain fine-resolution, high-contrast images of the vasculature of the embryonic mouse, but the protocol typically entails injection of MR contrast agents, fixation of the embryo, and scan times that exceed 2 h. Recently, an *in vivo* MRI protocol was demonstrated to produce vascular maps of relatively good contrast but the scan time lasted approximately 3 h.²³ Aside from their long scan times, MRI systems are cost prohibitive and they require the use of MR-compatible equipment. Our HFU system can acquire single-plane data in <0.2 s and is only limited by the low repetition rate of our laser (10 Hz). A laser capable of firing pulses at a PRF >3 kHz would allow the acquisition of co-registered anatomical and vascular images in under 5 min, accounting for latencies associated with the motion of the transducer. It would also allow the possibility of averaging the PA signals to increase SNR by rapidly acquiring multiple PA A-lines for each HFU A-line. Image reconstruction algorithms, such as those based on acoustic time reversal,²⁴ could be implemented to further improve image quality. Using this reconstruction technique, Laufer *et al.*²⁵ were able to obtain PA volumetric maps of the complete vasculature of mouse embryos *in vivo* with high resolution and acquisition times <10 min, but the all-optical scanner could not acquire HFU data to provide anatomical information about the surrounding tissues.

Here, the HFU and PA images of the embryo *in vivo* were obtained without averaging or using advanced reconstruction techniques, which shows the high potential of the annular-array system for pre-clinical imaging of small animals and superficial tissues. Compared to conventional PA

scanning systems based on a single-element or linear-array transducer, the annular array provides increased DOF and superior spatial resolution away from the fixed-focus region. Systems based on a linear-array transducer typically have a large DOF but the poor out-of-plane resolution usually requires side illumination²⁶ because modifying a linear array for coaxial illumination would be technically challenging and expensive.

By modifying our annular array to allow for co-axial illumination, we showed that, for HFU and PAI, a lateral beamwidth below $200\text{ }\mu\text{m}$ could be obtained over a 6 mm depth range, and below $350\text{ }\mu\text{m}$ over a 12 mm depth range. These properties enabled the acquisition of co-registered HFU and PA images of *in utero* mouse embryos with sufficient resolution to visualize the embryonic vasculature. It should be noted that the HFU images were obtained by exciting only the central element of the annular array. Transmitting with all five elements using a multi-channel pulser would be easy to implement and would improve spatial resolution and SNR.²⁷

Compared to PA scanners with side optical illumination, the collimated, co-axial laser excitation of our system reduces the amount of off-axis PA signals received by the HFU transducer (Fig. 1) and maximizes the amplitude of the PA response, because the optical energy distribution is centered on the acoustic axis of the annular array. In this study, the laser beamwidth was collimated to a diameter larger than the acoustic beamwidth of the transducer, such that the lateral resolution of the PA scanner was dependent on the transducer properties. In the non-diffusive regime, if the laser beamwidth was reduced to below the beamwidth of the transducer, no PA signal would be generated outside the illuminated region and the lateral resolution in the PA images would only depend on the effective diameter of the laser beam. Optical diffusion will make the laser beam expand with depth, but even in strongly diffusive media, it would still be possible to obtain high-resolution PA images with a minimal off-axis PA signal. In applications such as ophthalmology where optical scattering is low, coaxial illumination could permit imaging the retinal vasculature down to a resolution of a few micrometers.²⁸

V. CONCLUSION

This work demonstrated the imaging capabilities of a 40 MHz, 5-element, annular-array-based scanner that was designed for the simultaneous acquisition of co-registered HFU and PA data. The system characteristics were quantified using wire and hair phantoms. By applying a SF algorithm to the HFU and PA data, a large DOF (>8 mm) was achieved and the lateral resolution was optimized at all depths. The co-axial arrangement of the confined laser illumination relative to the transducer acoustic field allowed the visualization of detailed anatomical features in an *in vivo*, *in utero* mouse embryo. The acquired images showed that the system achieved sufficient spatial resolution and contrast to obtain 3D renderings of the developing embryonic head and limbs with the associated vascular network.

ACKNOWLEDGMENTS

This research was supported by the National Institutes of Health (NIH) (Grant No. EB008606). We thank Dan Gross for developing the precision drilling tools used for the coaxial light channel.

- ¹A. A. Oraevsky, S. L. Jacques, R. O. Esenaliev, and F. K. Tittel, in *Advances in optical imaging and photon migration*, edited by R. R. Alfano (OSA Press, Washington, D.C., 1994), Vol. 21, pp. 161–165.
- ²M. Xu and L. V. Wang, *Rev. Sci. Instrum.* **77**, 041101 (2006).
- ³A. A. Oraevsky, S. L. Jacques, and F. K. Tittel, *Proc. SPIE* **1882**, 86 (1993).
- ⁴P. Beard, *Interface Focus* **1**, 602 (2011).
- ⁵K. Maslov, G. Stoica, and L. Wang, *Opt. Lett.* **30**, 625 (2005).
- ⁶H. Zhang, K. Maslov, G. Stoica, and L. Wang, *Nat. Biotechnol.* **24**, 848 (2006).
- ⁷J. A. Ketterling, S. Ramachandran, and O. Aristizábal, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **53**, 623 (2006).
- ⁸E. Filoux, J. Mamou, O. Aristizábal, and J. A. Ketterling, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **58**, 994 (2011).
- ⁹O. Aristizábal, D. H. Turnbull, A. Sampathkumar, J. Mamou, E. Filoux, J. A. Ketterling, and P. Chitnis, *Proc.-IEEE Ultrason. Symp.* 288 (2011).
- ¹⁰M. A. Bedell, N. A. Jenkins, and N. G. Copeland, *Genes Dev.* **11**, 1 (1997).
- ¹¹P. C. Wong, H. Cai, D. R. Borchelt, and D. L. Price, *Nat. Neurosci.* **5**, 633 (2002).
- ¹²R. S. Krauss, *Curr. Top Dev. Biol.* **84**, xi (2008).
- ¹³Y. Lao, D. Xing, S. Yang, and L. Xiang, *Phys. Med. Biol.* **53**, 4203 (2008).
- ¹⁴G. Lungu, M. Li, X. Xie, L. Wang, and G. Stoica, *Int. J. Oncol.* **30**, 45 (2007).
- ¹⁵H. Brecht, R. Su, M. Fronheiser, S. Ermilov, A. Conjusteau, and A. Oraevsky, *J. Biomed. Opt.* **14**, 064007 (2009).
- ¹⁶J. Xia, Z. Guo, K. Maslov, A. Aquirre, Q. Zhu, C. Percival, and L. Wang, *J. Biomed. Opt.* **16**, 090505 (2011).
- ¹⁷J. Laufer, J. Cleary, E. Zhang, M. Lythgoe, and P. Beard, *Proc. SPIE* **7564**, 75641A (2010).
- ¹⁸J. A. Ketterling, O. Aristizábal, D. H. Turnbull, and F. L. Lizzi, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **52**, 672 (2005).
- ¹⁹L. Brown, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **47**, 1377 (2000).
- ²⁰M. Arditi, W. B. Taylor, F. S. Foster, and J. W. Hunt, *Ultrason. Imaging* **4**, 1 (1982).
- ²¹A. Liu, A. L. Joyner, and D. H. Turnbull, *Mech. Dev.* **75**, 107 (1998).
- ²²O. Aristizábal, J. Mamou, D. H. Turnbull, and J. A. Ketterling, *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **2009**, 1987–1990 (2009).
- ²³C. A. Berrios-Otero, Y. Z. Wadghiri, B. J. Nieman, A. L. Joyner, and D. H. Turnbull, *Magn. Reson. Med.* **62**, 1431 (2009).
- ²⁴B. T. Cox, S. Kara, S. Arridge, and P. Beard, *J. Acoust. Soc. Am.* **121**, 3453 (2007).
- ²⁵J. Laufer, F. Norris, J. Cleary, E. Zhang, B. Treeby, B. Cox, P. Johnson, P. Scambler, M. Lythgoe, and P. Beard, *J. Biomed. Opt.* **17**, 061220 (2012).
- ²⁶A. Needles, A. Heinmiller, P. Ephrat, C. Bilan-Tracey, A. Trujillo, C. Theodoropoulos, D. Hirson, and F. Foster, *Proc.-IEEE Ultrason. Symp.* 390 (2010).
- ²⁷J. A. Ketterling and E. Filoux, *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **59**, 1830 (2012).
- ²⁸R. H. Silverman, F. Kong, Y. Chen, H. Lloyd, H. Kim, J. Cannata, K. Shung, and D. Coleman, *Ultrasound Med. Biol.* **36**, 733 (2010).