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High-Frequency Chirp Ultrasound Imaging with an Annular-array for Ophthalmologic and Small-Animal Imaging

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Abstract

High-frequency ultrasound (HFU, > 20 MHz) is an attractive means of obtaining fine-resolution images of biological tissues for ophthalmologic, dermatological, and small-animal imaging applications. Even with current improvements in circuit designs and high-frequency equipment, HFU suffers from two inherent limitations. First, HFU images have a limited depth of field (DOF) because of the short wavelength and the low fixed F-number of conventional HFU transducers. Second, HFU is usually limited to shallow imaging because of the significant attenuation in most tissues. In a previous study, a five-element annular array with a 17-MHz center frequency was excited using chirp-coded signals, and a synthetic-focusing algorithm was used to extend the DOF and increase penetration depth. In the present study, a similar approach with two different five-element annular arrays operating near a center frequency of 35-MHz is implemented and validated. Following validation studies, the chirp-imaging methods were applied to imaging vitreous-hemorrhage mimicking phantoms and mouse embryos. Images of the vitreous phantom showed increased sensitivity using the chirp method compared to a standard monocycle imaging method, and blood droplets could be visualized 4 mm deeper into the phantom. Three-dimensional datasets of 12.5-day-old, mouse-embryo heads were acquired *in utero* using chirp and conventional excitations. Images were formed and brains ventricles were segmented and reconstructed in three dimensions. The brain-ventricle volumes for the monocycle excitation exhibited artifacts that were not apparent on the chirp-based dataset reconstruction.

Keywords

High-frequency; coded-signals; small-animal

INTRODUCTION

High-frequency (i.e., $f > 20$ MHz) ultrasound (HFU) is under considerable investigation because the short wavelengths (e.g., $\approx 75 \mu\text{m}$ at 20 MHz) and small focal-zone beam diameters of HFU provide fine-resolution images. Studies have demonstrated the unique ability of HFU systems to image shallow or low-attenuation tissues for biomedical applications. For example, HFU has been successful for small-animal (Turnbull et al, 1995; Aristizábal et al, 1998;

Turnbull and Foster, 2002), ocular (Silverman et al, 1995a, 2008), intravascular (de Korte et al, 2000; Saijo et al, 2004), and dermatological imaging (Vogt and Ermert, 2007; Huang et al, 2007). However, HFU penetration depth is reduced because of frequency-dependent attenuation. Furthermore, most current HFU images have a small depth-of-field (DOF) because low F-number transducers are employed to improve cross-range resolution. Therefore, typical HFU images have fine resolution only at shallow depths and over a limited DOF.

In a previous study conducted using a 17-MHz, 5-element annular array, we implemented and validated an approach intended to mitigate the limitations of HFU imaging (Mamou et al, 2008). Our approach consisted of combining synthetic-focusing to increase DOF (Ketterling et al, 2005, 2006) and chirp-coded excitations (i.e., engineered excitation pulses) to increase the effective penetration depth by improving the signal-to-noise ratio (SNR) (O'Donnell, 1992; Chiao and Hao, 2005; Misaridis and Jensen, 2005a). The results of our study demonstrated that we could successfully increase DOF and penetration depth while maintaining satisfactory resolution (Mamou et al, 2008).

In the present study, similar imaging methods were used with 35-MHz-center-frequency 5-element annular arrays. Chirp-coded excitation signals were used to excite the annular-array to increase penetration depth and synthetic focusing was employed to increase DOF.

These methods are presented and reviewed only briefly here because of their similarity to the methods of the previously described study (Mamou et al, 2008). The emphasis of the present paper is on the validation of the chirp-imaging approach at 35 MHz and implementation of the approach in two specific biomedical applications.

The first biomedical application involved imaging a low-attenuation phantom containing low-contrast scatterers (i.e., rabbit blood droplets in agar); this phantom serves as a model for vitreous hemorrhage. Organized or clotted blood in the vitreous compartment of the eye is readily imaged ultrasonically at high frequencies (Hewick et al, 2004). However, a diffuse vitreous hemorrhage produces very-low-amplitude echoes that are difficult to detect in a conventional examination performed with a broadband HFU transducer. Although optical coherence tomography (OCT) has become an established tool for visualization of the retina, light absorption and scattering in a vitreous hemorrhage prevent assessment of the hemorrhage and the underlying retina (Restori, 2008). Over a decade ago, Silverman et al (1995b) showed that narrowing the excitation-signal bandwidth (i.e., effectively emitting a longer signal) improved sensitivity in detecting vitreous pathologies, but sacrificed resolution. One of our hypothesis is that chirp imaging will have greater sensitivity to hemorrhage than a broadband pulse because its time duration is greater (e.g., microseconds compared to hundreds of nanoseconds at 35 MHz) while retaining satisfactory image resolution by appropriate chirp compression on receive.

The second biomedical application evaluated the use of chirp imaging for developmental studies in mouse embryos. Early embryonic-mouse brain development is a complex three-dimensional (3D) dynamic process in which the ventricles undergo rapid volumetric changes (Zhang et al, 2003). A reliable method for visualizing these changes *in vivo* in 3D over several days would be a valuable tool for quantifying normal and abnormal brain development. Such assessments are not easily accomplished with traditional, destructive tools such as histological examination. The embryonic ventricular system is composed of fluid-filled cavities surrounded by proliferative neuronal cells. Therefore, the ventricular system is well suited for ultrasound imaging because these cavities provide significant acoustic impedance contrast under ultrasound interrogation. A previous study showed the utility of imaging mouse embryos using a high-frequency annular-array (Aristizábal et al, 2006). In that study, a semi-invasive procedure was used to externalize the embryos to allow more-direct access for imaging and

limiting attenuation and phase-aberration artifacts by eliminating the skin and fat layers along the sound path. Results indicated that the extended DOF of the annular array allowed identifying structures a few millimeters away from the geometric focus by using synthetic focusing, enabling improved segmentation and 3D visualization of the brain-ventricle system. In order to extend this time window, non-invasive *in utero* imaging will be essential, and methods have been developed in which mouse embryos can be predictably identified and imaged over a period of several days (Ji and Phoon, 2005). However, *in utero* imaging would challenge the semi-automatic segmentation algorithm because of the decrease in contrast between brain tissue and brain cavities attributed to attenuation and phase-aberration artifacts caused by intervening tissue layers. In the present study, we evaluated the effect of the increase in SNR and penetration depth on the accuracy and robustness of brain-ventricle segmentation in 12.5-day old mouse embryos imaged *in utero*.

MATERIALS AND METHODS

Annular Array and synthetic focusing

Two annular arrays, AA1 and AA2, were utilized during the course of these studies. The transducers were fabricated with identical methods as described previously (Ketterling et al, 2005). Transducer AA1 was fabricated with a PVDF membrane (Ketterling et al, 2005) and transducer AA2 was fabricated with a P(VDF-TrFE) membrane. Each array was assembled from a 9- μm -thick membrane (Ktech Corp., Albuquerque, NM) bonded to a single-sided, copper-clad-polyimide (CCP) film (RFlex 1000L810, Rogers Corp., Chandler, AZ). An array pattern was etched onto the CCP using standard printed-circuit-board techniques. Both arrays had five, equal-area annuli, a radius of curvature of 12 mm, and a total aperture of 6 mm. Table 1 summarizes the performance characteristics of the two annular arrays. Over all, the properties of both annular arrays were very similar, but AA1 elements had slightly higher center frequencies than those of AA2 (i.e., average of 36.0 and 33.8 MHz for AA1 and AA2, respectively). The difference in center frequencies was most likely due to the slightly different electrical impedances of PVDF and P(VDF-TrFE). The most-significant difference between the two arrays was in their respective insertion losses (IL). The superior performance of AA2 was due primarily to the improved coupling coefficient of P(VDF-TrFE) compared to PVDF respectively ($k_t = 0.30$ compared to $k_t = 0.15$)

A synthetic-focusing algorithm previously was developed to permit the focus of the annular array to be axially shifted by post-processing acquired data (Mamou et al, 2008; Ketterling et al, 2006). To focus the array to a depth d on transmit, the time delay t_n required for ring n of the array is $t_n = \left[a_n^2 (1/R - 1/d) \right] / 2c$ where R is the geometric focus and a_n is the average radius of ring n (Arditi et al, 1982). The total round-trip delay, t^{tot} , is then the sum of the transmit, t^T , and receive, t^R , delays; $t^{tot} = t^T + t^R$. To focus at a depth d , t^{tot} is calculated for all 25 transmit-receive pairs. The delays then are applied to the A-lines and the resulting signals are summed. In order to increase the DOF over a fixed axial span, d is shifted in intervals of Δd . Then, an overall image is formed by windowing the data at each focal depth and assembling the windowed data into a final composite image. In all of our experiments, Δd is chosen to be 0.2 mm. (If $t^{tot} = 0$ is utilized for all of the delays, then the array performs as if it were a 12-mm-fixed-focus single element-transducer with a total aperture of 6 mm.)

Chirp-coded Excitation

In the present study, only linear, chirp-coded excitations were used. A linear chirp is a coded signal that linearly spans a frequency band $B = f_2 - f_1$, where f_1 and f_2 are the starting and ending frequencies, respectively. If the chirp sweeps from f_1 to f_2 over a time, T , then the chirp-coded excitation is described by

$$s(t) = w(t) \cos\left(2\pi f_1 t + \pi \frac{B}{T} t^2\right). \quad (1)$$

After several iterations between, simulations and quartz-plate reflections, we developed a set of optimal chirp parameters for our applications. We chose $f_1 = 15$ MHz, $f_2 = 65$ MHz and $T = 4 \mu\text{s}$. Finally, $w(t)$, the tapering window, was a 9%-Tukey window. In all of our experiments, the compression filter consisted of the time-reversed excitation chirp weighted by a Chebyshev window with a prescribed sidelobe level of -80 dB. The same chirp and compression filters were used for AA1 and AA2.

The time-bandwidth product, TB , of a chirp, using a rectangular window, $w(t)$, is equal to the expected SNR improvement compared to an impulse excitation (Misaridis and Jensen, 2005a). With our values for T and B , the expected SNR gain would be 26 dB. However, because we used a Tukey window to limit compression sidelobes (Misaridis and Jensen, 2005b), which can drastically degrade image quality, we anticipate a smaller SNR gain.

Data Acquisition

Data acquisition was performed using minor modifications to the previously described equipment and methods (Mamou et al, 2008). Briefly, image data were acquired with the annular array by making five scan passes across the test object. On each pass, one of the array elements was excited and the receive echoes were digitized at 400 MHz on all five channels. In this way, we were able to acquire the full set of 25 transmit-to-receive combinations. A cross-point-switch unit (CXL/8X8 Cytec, Penfield, NY) was configured to act as a multiplexer to automate the selection of the excited element. Each array channel had its own transmit/receive circuit which included a pre-amp with 46 dB of gain (AU-1313, Miteq, Hauppauge, NY). After saving the data, the digitized signals were linearly filtered by the compression filter. The compressed data then were synthetically focused, envelope detected, and log compressed for B-mode display. In addition to the chirp-encoded experiments, we acquired data using a mono-cycle pulser (Avtech AVB2-TA-C-CVA, Ottawa, Ontario, Canada) and a "gold standard" pulser-receiver unit (Panametrics 5900, Olympus NDT, Waltham, MA). The scanning method and image formation for the mono-cycle excitation (i.e., Avtech or Panametrics) were the same as for the chirp-imaging method, except that no compression was performed. For chirp-coded-excitation imaging, the pulser-receiver unit was replaced by an arbitrary-waveform generator (WW1281, Tabor Electronics, Haifa, Israel) and a broadband power amplifier with a gain of 50 dB (ENI 350L, Rochester, NY). To design and download coded waveforms into the arbitrary-waveform generator, custom software was designed in Matlab (The MathWorks, Natick, MA) and LabVIEW (National Instruments, Austin, TX).

Experiments

The method-validation experiments (i.e., resolution and penetration depth) were performed with both AA1 and AA2. The blood-phantom were conducted with AA1 and mouse-embryo experiments were conducted with AA2. (The excitation chirp and compression filters were kept the same independent of which annular array was used.)

To provide a fair comparison between the different imaging methods, the received peak voltage obtained with chirp excitation was equalized to that obtained with Avtech excitation. This was accomplished by appropriately adjusting the peak-to-peak voltage setting of the arbitrary-waveform generator. The highest-output voltage setting of the Avtech was used to maximize signal strength and penetration into tissue. The Panametrics pulser was used only in the resolution study because it could not achieve a received-voltage level equivalent to the Avtech. For the resolution study, the Panametrics pulser was used with its lowest energy setting (i.e.,

1 μ J). This energy setting provided the largest output bandwidth (and best resolution) and was chosen to challenge the chirp and Avtech- imaging methods.

Resolution and penetration-depth experiments

To validate the coded-excitation methods, two experimental studies were performed to quantify and compare image resolution and penetration depth between conventional and chirp imaging. Again, these studies were similar to those used to validate our methods with the 17-MHz annular array (Mamou et al, 2008). The resolution studies compared the -6-dB axial and lateral resolutions using the Avtech pulser, the Panametrics pulser, and chirp excitation. For each imaging method, we scanned across 12- μ m diameter tungsten wires placed at depths ranging from 8 to 18 mm.

The penetration-depth study consisted in imaging a tissue-mimicking phantom (ATS Laboratories, Bridgeport, CT) containing 10- μ m diameter glass beads (8×10^6 beads/cm³). The phantom was scanned using chirp and Avtech excitation. The phantom had an attenuation of 0.5 dB/cm/MHz near 6 MHz. Following imaging, the envelopes of the backscattered signals were used to estimate SNR and penetration depth for each imaging method. The scan consisted of 100 A-lines spaced 20 μ m apart. Each A-line was acquired and averaged 200 times because the phantom was too attenuating to obtain any significant data without averaging.

Blood-phantom experiments

We fabricated a phantom in which fresh rabbit blood was suspended in agar to model a vitreous hemorrhage. To make the phantom, 15 g of agar was diluted in 1 L of normal saline solution. After stirring, the solution was heated in a microwave oven until the agar was dissolved, which took approximately one minute. About 2 cc of blood was withdrawn from the ear vein of a rabbit using a 23-gauge needle and placed in a tube with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Then, the solution was divided into 10-mL containers, and droplets of the fresh rabbit blood were injected with a needle into each container while the agar was still warm ($> 100^\circ$ C) and not fully cured and solidified. Figure 1 displays a picture of the freshly injected rabbit blood after the agar began to solidify. Animal experiments were performed in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Research and under a protocol approved by the Institutional Animal Care and Use Committee of the Weill Cornell Medical College. The agar phantom had low attenuation and its acoustic properties were similar to those of water or vitreous. Also, the blood droplets constituted a low-contrast (i.e., weak-backscatter) target because they were small and had an acoustic impedance value similar to (i.e., within 5% of) that of agar. During the data experiment, the front surface of the phantom was placed 8 mm away from the transducer surface (i.e., 4 mm before the natural focal depth of the transducer), because we anticipated that the phantom would have low attenuation and that we would be able to image deep into it. Each B-scan was composed of 225 A-lines, and adjacent A-lines were 18 μ m apart.

Mouse-embryo experiments

In utero imaging of mouse embryos provided an opportunity to evaluate the effectiveness (increased penetration depth and SNR) of the chirp-coded excitation compared to monocycle (e.g., Avtech or Panametrics pulsers) excitation in a realistic imaging environment. Mouse embryos were imaged with the AA2 transducer at embryonic day (E) 12.5, where E0.5 was defined as noon of the day a vaginal plug was found after overnight mating. All mice used in these studies were maintained under protocols approved by the Institutional Animal Care and Use Committee of the New York School of Medicine. In these studies the pregnant mouse was euthanized humanely by cervical dislocation immediately before image acquisition to eliminate breathing motion during the long data acquisition times of our prototype scanner.

With the mouse placed in a supine position and its four limbs taped to an imaging stage, the stomach was shaved and a Petri dish with a circularly cut 25-mm imaging window was placed over the stomach, filled with isotonic saline, and secured. The annular-array transducer was lowered into the saline-filled Petri dish, and the center array element was used for real-time, fixed-focus imaging at 3 frames per second. This imaging mode was used to quickly locate an embryo, to position the image planes close to one of the standard slice orientations, and to set up the imaging parameters for a full 3D study. With the geometric focus placed superficial to the embryo, a full 3D dataset was acquired for standard mono-cycle and chirp-coded excitation, as described above, and stored for offline signal processing and image analysis. To image a complete embryo in 3D, we acquired 120 adjacent B-scans. The spacing between adjacent B-scan planes and adjacent A-lines in each B-scan plane was 50 μm . Each B-scan was approximately 7.5 mm wide (i.e., 151 A-lines) and 9 mm deep (i.e., 5000 RF points per scan line). Acquiring and saving a 3D dataset required approximately 10 minutes.

3D Mouse-embryo image analysis

After compression filtering and synthetic focusing, the 3D stacks of B-mode images were imported into Amira v4.1.1 (Mercury Computer Systems, San Diego, Ca) visualization software for volumetric segmentation, visualization and analysis. Before segmentation, data were processed with a 3D Gaussian filter to smooth the speckle. Unbiased, semiautomatic segmentation of the cerebral ventricles was accomplished using an active contour segmentation tool. After the operator placed seeds in the ventricles throughout the stack, a 3D contour was computed based on the initial image and edge sensitivity parameters, and, under software control, the contour evolved until the edges were detected. These parameters were fixed to their default values for each mono-cycle and chirp-coded excitation dataset. Because of the high contrast between the mouse proper and the surrounding amniotic liquid, surface rendering of the embryonic head was accomplished using threshold segmentation. 3D spatial orientation of the brain ventricles was visualized by co-registration with surface rendering of the embryonic head.

RESULTS

Resolution and penetration-depth results

Figure 2 displays the -6-dB axial and lateral resolutions measured using three imaging methods for the two arrays. The AA1 axial-resolution results revealed that the chirp imaging performs nearly as well as the Panametrics, and better than the Avtech method. The Panametrics axial resolution was $\approx 9\%$ better than the chirp axial resolution at depths near the geometric focus of the transducer (i.e., depths in the range of 10.5 to 13.5 mm). However, the chirp method outperformed the Avtech method by more than 18% over depths in the range of 9 to 16 mm. The AA2 axial resolution results were better than those for AA1 because, independent of the imaging method, the axial resolution was fairly constant and equal to 50 μm except near the geometric focus where it was equal to 40 μm . Also, the chirp axial resolution degraded more rapidly at depths less than the focal distance (i.e., depths smaller than 9.5 mm). The resolution measurements for AA2 were conducted after the minor circuit improvements outlined in the previous section, which may explain why the AA2 axial-resolution results were nearly the same for all three methods.

As expected, the lateral resolution results were approximately the same for all three methods and for both annular arrays. The chirp processing was conducted individually for each A-line and therefore did not alter the lateral resolution. In theory, lateral resolution is proportional to the product of the F-number and the wavelength (Kino, 1987) and these quantities are nearly equal for each annulus of the arrays (Table 1). For all three imaging methods and both annular

arrays, the resolution worsens with increasing depth because the synthetic-focusing algorithm simulates transducers with increasing F-numbers.

Table 2 displays the SNRs, penetration depths, and penetrations into phantom for the four imaging methods and for both annular arrays. The penetration depth was estimated as the depth at which the absolute value of the gradient of the average backscatter signal dropped below a certain small threshold. Details regarding how penetration depth and SNR were estimated were previously reported (Mamou et al, 2008). The surface of the phantom was placed 10.4 mm away from the transducer (i.e., 1.6 mm before the geometric focal depth of the transducer). Therefore, the actual penetration into the highly-attenuating phantom was obtained by subtracting 10.4 mm from the obtained penetration depth.

Results obtained using AA1 indicated that the SNR improved with synthetic focusing and coded excitation. This trend was expected because synthetic focusing increases signal strength and chirp compression decreases noise levels. The results also demonstrated that synthetic focusing and chirp coding independently contribute to increasing the effective penetration depth. The maximum effective penetration depth (i.e., 17.6 mm) and SNR (i.e., 73.0 dB) were obtained when both methods were combined. The chirp method (with synthetic focusing) allowed an increase in penetration depth of 1.8 mm (i.e., provided a 25% increase within the phantom) and in SNR of 12.7 dB when compared to the Avtech imaging method (with synthetic focusing). These improvements are significant and demonstrate the potential for achieving deeper HFU imaging with synthetically-focusing and chirp-coded signals.

Table 2 also present the results obtained using AA2. These results followed the same trend as those obtained with AA1: SNRs and penetration depths increased with chirp and synthetic focusing and the best results were obtained when chirp and synthetic focusing were combined. However, the AA2 SNR and penetration depth values were better than those obtained with AA1 for the four imaging methods. The better performance of AA2 can be explained in part by the better insertion loss of AA2 (Table 1) and also by the improved circuit design since the AA1 experiments were conducted. The results obtained with AA2 also revealed that the synthetically-focused Avtech improvement are greater than the synthetically-focused chirp improvements. For example, the SNR increased by 13.2 dB and 6.5 dB for the synthetically-focused Avtech and synthetically-focused chirp methods, respectively. One possible explanation is that the new improved transducer, new circuit design, and the signal averaging, the optimal SNR and penetration depth have been obtained with the Avtech (leaving limited room for improvement using the chirp-imaging method).

The results of the resolution and penetration depth studies confirm the validity of our synthetically-focused chirp-imaging methods for HFU: the resolutions were not degraded and the SNR and penetration depth were greatly improved. Furthermore, AA2 seems to outperform AA1 in the SNR and penetration-depth study while performing nearly the same in the resolution study: AA2 is now the transducer routinely used for ongoing and future studies in this area.

Blood-phantom results

Figures 3a and 3b display images of a blood phantom using Avtech and chirp imaging, respectively. For comparison, both images were synthetically focused and displayed with 45-dB dynamic range. The Avtech excitation case (Fig. 3a) had modest SNR (i.e., 33.2 dB) because the blood droplets were small (i.e., < a few wavelengths at 35 MHz) and were weak reflectors of sound; their acoustic impedance was similar to that of agar (Shung and Thieme, 1993). At depths ranging from 10 to 12 mm, the blood was easily visible, but beyond 12 mm, it was more difficult to visualize the blood droplets confidently because of the noisy background and of the weak backscatter. Figure 2 also revealed that the resolution of our imaging method remained

satisfactory at depths up to 16 mm. Therefore, at depths greater than 15 mm, the lack of contrast cannot be attributed to poor image resolution.

The chirp excitation case (Fig. 3b) has a greatly improved SNR (i.e., 49.0 dB). The SNR increase of 15.8 dB relative to the Avtech image was comparable to the 12.7 dB SNR increase obtained in the penetration study (AA1 results in Table 2). This improved SNR led to an image with greater contrast, and distinct blood droplets were visualized significantly deeper (i.e., > 16 mm) into the phantom (Fig. 3b).

This dramatic improvement could be critical in the diagnosis of vitreous hemorrhage because it would allow visualization of blood and other faintly reflective structures (cellular debris, vitreous membranes) with greater sensitivity than any currently available imaging techniques.

In utero mouse-embryo-imaging and brain-ventricle-segmentation results

Three-dimensional data sets were acquired with the geometric focus of the annular array placed superficial to an embryo (Fig. 4a, yellow arrows) to simulate the most challenging situation to visualize brain ventricles. In this configuration, the ventricles are far from the natural focus of the annular array and deep (≈ 6 mm) into attenuating tissue. Figures 4a, b, d, and e show the same representative slice from the stack of reconstructed images using the four imaging methods (i.e., chirp or Avtech excitation, with or without synthetic focusing). In the four images, the superficial skin (denoted by S on Fig 4e) was the brightest feature and, the increased penetration depth achieved by using chirp excitation was locally visible in Fig. 4d because the complete outline of the embryo was visualized including a hint of the brain ventricles, which lay about 5 mm deeper than the geometric focus.

Synthetic focusing increased the DOF enough to resolve the entire contour of the embryonic head as shown in the Avtech image (Fig. 4b) and chirp image (Fig. 4e). The increase in SNR allowed by the chirp-imaging method permitted resolution of the complete two-dimensional extent of the brain ventricles (denoted by 1v and 3v in Fig. 4e). Furthermore, at the geometric focus, both chirp images nicely resolved the amnion (indicated by the white arrow in Fig. 4e), but only the synthetically-focused-chirp image resolved the entire extent of the amnion surrounding the embryo. Nevertheless, the region of the images near the skin boundary appeared to be better resolved on the Avtech image (Fig. 4b) than on the chirp image (Fig. 4e). This was caused by minor sidelobe artifacts that appeared after pulse compression because the received chirp signals were partially saturated. Note that because we maintained the same digitizer scale and peak-received amplitudes between the chirp and the Avtech datasets, the received Avtech signals were also saturated but did not lead to artifacts. We decided to saturate the skin-echo signals in order to obtain good images and because the sidelobe artifacts would not interfere with the important parts of the image (i.e., the embryo's head).

Using a semi-automatic segmentation procedure, surface renderings of the embryonic brain ventricles were visualized from synthetically-focused datasets for Avtech (Fig. 5a) and chirp (Fig. 5b) excitation. The images reconstructed from the Avtech dataset showed significant artifacts (indicated by the arrows in Fig. 5a). However, all four ventricles were clearly visible without obvious artifacts in the Chirp-based reconstruction in Fig. 5b. Following segmentation and rendering, total brain cavity volumes were estimated. The Avtech-based volume estimate (11.4 mm^3) was 80% larger than the chirp-based volume estimate (6.2 mm^3).

Threshold segmentation of the embryonic head showed several anatomical features such as the ear, eye, and limb bud (denoted by e, i, and l on Fig. 5d). Co-registration of the embryonic head with the brain ventricle volumes allowed visualizing the spatial extent and orientation of the ventricles with respect to the head. In particular, co-registration of these volumes revealed errors made by the semi-automatic segmentation algorithm because of the lack of contrast

between the brain cavity and the surrounding tissue. One of the errors resulted in the placing of the location of brain cavities outside the head of the embryo, as shown by the arrows in Fig. 5c and 5d. These images also revealed that the greatest errors were made by the Avtech-based ventricle reconstruction. These segmentation errors are visible in the cross-sectional plane that cuts across the magenta line for the Avtech reconstruction (Fig. 5c) and the yellow line for the chirp reconstruction (Fig. 5d). The Avtech (i.e., magenta) and chirp (i.e., yellow) cross-sectional planes were displayed on Figs. 4c and 4f, respectively. In these images, the contour for the leftmost lateral ventricle extends into the chorionic cavity and crosses the embryonic head contour. This error was drastically more severe on the Avtech cross-section than on the chirp cross-section.

The increased SNR, DOF, and penetration depth allowed by chirp-coded excitation permitted the *in utero* visualization of the brain-ventricle system with satisfactory contrast. Thus, 3D segmentation of the brain-ventricle system from the chirp dataset was possible with minor errors whereas significant errors were present in the Avtech-based segmentation. The superior robustness of the chirp-imaging method will enable translational studies of normal and abnormal development of the embryonic nervous system.

DISCUSSION

In this study tackled tchirp coded-excitation imaging methods were developed and tested at 35 MHz. The two annular arrays used in this study were custom-made and proved to be powerful tools for HFU because they allowed the DOF to be significantly improved through appropriate synthetic-focusing algorithms. Both annular arrays had total apertures similar to typical single-element HFU transducers (e.g., 5-20 mm) and their axial symmetry led to a high-quality radiation pattern using fewer elements than a typical linear or phased array employs. However, annular arrays need to be mechanically scanned to obtain a 2D image.

In the present study, the extended DOF of the annular arrays was critical to exploit the increased penetration depth and SNR allowed by the chirp-imaging method. While nothing prevents from using a chirp-imaging method successfully with a spherically-focused, single-element transducer, the increased penetration depth and SNR could fall into a region where the sound field is unfocused. Figure 4d illustrates this point, the SNR of the chirp image was better than that of the Avtech image (Fig. 4a), but no tissue structures were better resolved on the chirp image far from the geometric focus because of the modest DOF achieved without synthetic focusing.

Resolution, penetration depth, and contrast are critical to HFU image quality. Under conventional excitation (i.e., impulse or short tone bursts), these parameters present a tradeoff. A longer signal propagates deeper into an attenuating tissue, but leads to an image with poor axial resolution. In the case of a low-attenuation medium, such as water or vitreous, a longer signal permits the detection of low-contrast scatterers, but also at the expense of axial resolution. Our results demonstrated that chirp-coded excitation permitted a significant increase in SNR while maintaining satisfactory resolution through appropriate pulse compression on receive. In particular, we were able to translate the SNR increase successfully into either improved penetration depth or improved contrast. In the ATS phantom study, the synthetically-focused-chirp image had a greater penetration depth than the synthetically-focused Avtech image; the increase was 1.8 mm (i.e., $\approx 42\lambda$), using AA1 and 0.8 mm (i.e., $\approx 19\lambda$) using AA2. Moreover, the ATS phantom was made to mimic tissue attenuation at conventional frequencies and its attenuation at high frequencies appears to be much greater than seen in most tissues.

Following the resolution and penetration-depth studies, the chirp-imaging methods were tested for two specific biomedical applications. The first application investigated whether chirp imaging could detect hemorrhage in the vitreous. In the blood phantom study, the synthetically-focused-chirp image had much better contrast and SNR than the synthetically-focused-Avtech image. This increased contrast allowed visualizing blood droplets several millimeters deeper into the phantom. Based on the results of our blood-phantom studies, we anticipate that the chirp-imaging method could become valuable for clinical ultrasound imaging examinations (such as evaluation of the vitreous) for which the visualization of weak scatterers is important. The healthy young vitreous is a homogeneous sonolucent gel. With aging, the vitreous condenses and separates into solid and liquid components. If the solid vitreous maintains a tractional attachment to the retina, a risk of retinal tearing and detachment exists, especially when other disease processes, such as diabetes or retinopathy, are present. The improvement in sensitivity provided by chirp imaging would facilitate detection of vitreous inhomogeneities at an earlier stage than currently possible with single-element probes that are excited by conventional methods. Similarly, diffuse uncoagulated hemorrhages or the cellular debris sometimes associated with macular holes are difficult to visualize with current technology. Enhanced sensitivity offers the potential of early detection and improved patient management because cellular organization and proliferation pose further risks to the retina and the eye.

The increase in penetration depth and SNR while maintaining good axial and lateral resolution offered by annular-array-chirp imaging also can be very beneficial for *in utero* imaging of mouse embryos where intervening skin and fat layers can greatly attenuate the available signal and where the 3D extent of the embryos can be greater than the typical DOF of fixed-focus HFU transducers. Our results show the utility of this imaging technique when trying to visualize the developing nervous system in the mouse embryo. Mouse embryos (up to ten in a litter) can be oriented in many directions and at varying depths. Furthermore for volumetric analysis on mutants, the fraction of embryos that have the prescribed phenotype can be as low as 25%. Thus, imaging as many embryos as possible within a litter is imperative for efficient analysis. The developing cardiovascular system is also an extended 3D system that is of great importance, and its normal and abnormal development can be assessed only with tools such as those described here.

Because few studies have addressed volumetric HFU imaging of the embryonic-mouse central-nervous system (CNS) (Turnbull et al, 1995; Aristizábal et al, 2006), our results are best compared with those in micro MRI (μ MRI), which offers high-contrast between different tissue types. The 3D brain ventricle system reconstructed from our chirp dataset was compared with one generated from μ MRI datasets of fixed embryos (Zhang et al, 2003). This comparison indicates that the estimated volumes from both methods are morphologically equivalent. To better compare the chirp and MRI volumes of the brain-ventricle system, 3D μ MRI datasets were downloaded from the Caltech μ MRI Atlas of Mouse Development (<http://mouseatlas.caltech.edu>) for Theiler Stage (TS) 20 (E12-E12.5) and TS 21 (E12.5-E13), which corresponds to the development stage used in our study. Figures 6a and 6b display the semi-automatic segmentation of the brain ventricles from developmental stages TS 20 and TS 21, respectively. Comparing Figs. 6a and 6b with Figure 5b reveals that the morphology of the E12.5 chirp brain-ventricle system is more similar to that from μ MRI at stage TS 21 than from that at stage TS 20.

Moreover, the volume calculated from the for the μ MRI TS 21 brain-ventricle system, 7.5 mm^3 , is similar to the volume calculated from the chirp-ultrasound dataset (6.2 mm^3); the volume computed for the TS 20 stage is only 2.9 mm^3 . The μ MRI volume from Fig. 6b reveals a thin, tube-like connection between the roof of the third ventricle and the lateral ventricles only after manual segmentation guided by the Kaufman mouse embryo atlas (Kaufman, 1995). The semi-automatic segmentation of the chirp dataset fails to find this tube-like

connection, probably due to the lack of contrast and the small cross-sectional area of this segment and the fact that this part of the cavity lies too far (i.e., ≈ 4 mm) from the annular-array geometric focus. *In utero* MRI imaging of mouse embryos has been difficult because of poor SNR and excessive physiologic motion. Recent work using manganese-enhanced MRI with respiratory gating has yielded high-quality data that allowed successful segmentation the developing CNS from E12.5 to E17.5 (Deans et al, 2008). However, segmentation of the early-development-stages of the CNS was possible only with high doses of manganese. The high toxicity of the manganese compromises the viability of the embryo, and therefore limits manganese-enhanced MRI use for translational studies. The morphology of the CNS closely follows the morphology of the brain ventricles for early stage embryos (Zhang et al, 2003); therefore, the methods described in this paper will complement μ MRI for investigating early-stage embryos. Finally, we currently are extending this work to conduct *in vivo* studies on genetically-engineered mice that develop disruptions in the neuroarchitecture of developing embryos.

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Figure 1.
Making of the blood phantom. Fresh rabbit blood is injected while the agar cures.

Figure 2. Axial (a) and lateral (b) -6-dB resolutions obtained as a function of depth using chirp, Avtech, and Panametrics excitation.

Figure 3. Synthetically-focused images of a blood phantom obtained using the Avtech pulser (a) and the chirp-imaging method (b). Dynamic range of images is 45 dB.

Figure 4.

Representative Avtech (a and b) and chirp images (d and e) *in utero* mouse-embryo images from 3D ultrasound datasets with (b and e) and without (a and d) synthetic focusing. Geometric focus (yellow arrows in a and d) is placed superficial to the embryo. Maternal skin (S), uterus (U), and amnion (white arrow) are easily identifiable (e). Representative semi-automatic brain-ventricle segmentation results from synthetically-focused datasets for Avtech (magenta contour, c) and chirp (yellow contour, f). The segmentation of the embryonic head is also shown (pink contour, c and f).

Avtech Chirp

Figure 5.

Semi-automatic surface segmentation of embryonic brain ventricles from the synthetically-focused Avtech (a) and chirp dataset (b). The chirp dataset shows the two lateral ventricles (lv) and the third (3v) and fourth (4v) ventricles. The reduced SNR in the synthetically-focused Avtech dataset produces obvious reconstruction errors (arrows, a). The surface rendering of the embryonic head visualizes anatomical features such as the ear (e), limb bud (l), and the eye (i). The spatial orientation of the brain ventricles with respect to the embryonic head are visualized in (c) and (d). Registration with the embryonic head reveals errors in the volume reconstruction of the brain ventricles for both data sets (arrows in c and d).

TS 20**TS 21** **Figure 6.**

Semi-automatic surface reconstructions of the brain ventricles from MRI data of fixed mice embryos at (a) Theiler stage 20 and (b) Theiler stage 21. Scale bars equal 1 mm.

Table 1

Summary of Transducer Performance

Ring	AA1					AA2				
	f_c (MHz)	dB@ f_c	BW _{6dB} (%)	IL (dB)	f_{TL} (MHz)	f_c (MHz)	dB@ f_c	BW _{6dB} (%)	IL (dB)	f_{TL} (MHz)
1	35.0	0	36	-28	38	33.0	0	35	-20	37
2	36.0	-5	31	-33	38	34.5	-4	30	-26	37
3	36.0	-6	33	-35	40	34.0	-6	35	-31	38
4	36.0	-8	35	-38	42	33.5	-7	32	-29	37
5	37.0	-8	39	-36	41	34.0	-8	41	-28	38

Table 2

SNR and penetration-depth results obtained with both annular arrays. (The surface of the tissue-mimicking phantom was at a depth of 10.4 mm.) For AA2, the values between parentheses are the difference with the results obtained with AA1 for the same method

AA1				
Excitation signal	Synthetic Focusing	SNR (dB)	Penetration depth (mm)	Penetration into phantom (mm)
Avtech	No	46.5	14.1	3.7
Avtech	Yes	59.3	15.8	5.4
Chirp	No	58.3	15.2	4.8
Chirp	Yes	73.0	17.6	7.2
AA2				
Avtech	No	56.2 (+9.7)	15.5	5.1 (+0.4)
Avtech	Yes	72.5 (+13.2)	16.9	6.5 (+1.1)
Chirp	No	68.7 (+10.4)	16.5	6.1 (+1.3)
Chirp	Yes	79.5 (+6.5)	17.7	7.3 (+0.1)