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Prospective ECG-gated Mouse Cardiac Imaging with a 34-MHz Annular Array Transducer

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Abstract

Prospective imaging with electrocardiogram (ECG) and respiratory gating presents an imaging application that leverages the improved image quality of high-frequency (> 20 MHz) annular arrays without the need for rapid mechanical motion. The limitation of prospective imaging is that the object being imaged must have a periodically stable motion. The present study investigated the implementation of prospective imaging with a 34-MHz annular-array scan system in order to image the mouse heart at high effective frame rates (> 200 frames/s, fps). M-mode data for all transmit-to-receive pairs were acquired at a series of spatial locations using ECG and respiratory gating and the data were then synthetically focused in post-processing. The pulse-repetition frequency of the M-mode data determined the effective frame rate of the final B-mode image sequence. The hearts of adult mice were prospectively imaged and compared to retrospective data acquired with a commercial ultrasonic biomicroscope (UBM). The annular-array data were acquired at an effective frame rate of 500 fps spanning 0.5 s and the UBM data were acquired at 1000 fps spanning 0.15 s. The resulting images showed that multiple heart cycles could be clearly resolved using prospective imaging and that synthetic focusing improved image resolution and SNR of the right ventricle, interventricular septum, posterior edge of the left ventricle (LV), and papillary muscles of the LV versus fixed-focused imaging and the retrospective imaging of the UBM machine.

I. INTRODUCTION

High-frequency ultrasound (HFU, > 20 MHz) has proven to be very effective for imaging small-scale structures that are situated within ≈ 2 cm from an external tissue boundary. Because of this limitation on penetration depth, HFU has been primarily utilized in ophthalmology [1], [2], small-animal imaging [3], [4], [5], and intravascular ultrasound (IVUS) [6].

The transducers used for these imaging applications are typically mechanically scanned, single-element, focused transducers. These devices have excellent resolution but a limited depth of field (DOF). Frame rates with mechanically scanned transducers have upper bounds that relate to the maximum sustainable pulse-repetition frequency (PRF), the linear distance of the scan, and the velocity of the motor. Commercial systems are able to reach frame rates on the order of 100 fps over an ≈ 8 -mm scan length [7]. For many anatomical and functional imaging applications, these frame rates are sufficient. The eventual introduction of linear arrays will improve the DOF and frame rates versus single-element systems [8], [9]. However, even the higher frame rates possible with linear arrays will not be sufficient to perform adult mouse

cardiac imaging where frame rates on the order of 1 kHz are necessary to capture events that occur over millisecond time durations such as valve openings or local motion of cardiovascular tissue [10].

Cardiac imaging in mice has traditionally been implemented in magnetic resonance imaging (MRI) and X-ray computed tomography (CT) using electrocardiogram (ECG)-gated prospective and retrospective techniques [11], [12], [13], [14]. In retrospective imaging, image data and physiologic waveforms (ECG and respiratory) are continuously acquired and collated in post-processing to produce cine loops of the cardiac cycle at high temporal resolution. In prospective imaging, the ECG signal is used to trigger the data acquisition. Unlike retrospective imaging, no post-processing is necessary because the digitized data are already synchronized relative to the period of the cardiac cycle.

In addition to ECG-gating, prospective and retrospective imaging approaches often employ a respiratory gate to reduce the impact of chest motion. The gate can derive from radio-frequency (RF) data that track chest movement or from commercially available hardware which measures the changes in electrical bioimpedance of the mouse's skin as the chest expands and contracts [15]. The purpose of the respiratory gate is to delay the ECG-gate relative to some reference point in the respiration cycle where chest motion is fairly minimal. Chest motion leads to significant axial displacement with some out of plane motion also possible, and, if unchecked, will seriously degrade image quality. Even with respiratory gating, noticeable respiratory motion is unavoidable if the duration of a single M-mode is greater than the breath cycle. However, if properly gated, the respiration will simply appear as an overall displacement in the image.

Recently, HFU adult-mouse-cardiac-high-frame-rate imaging was implemented with a single-element transducer using an ECG-based, retrospective imaging approach [10], [16], [15], [17], and end-diastolic (EDS) and end-systolic (ESS) measurements were performed. In HFU retrospective imaging, a set of M-modes and an ECG waveform are acquired at each lateral position of the image. The M-mode data sets are then reassembled into a set of B-mode images with the time between each B-mode image equal to the inverse of the PRF of the M-modes. The PRF can be as high as the inverse of the round-trip time of the pulse-echo signal and the total number of B-mode frames can be at least as high as the total memory depth of the digitizer. The total acquisition time is a function of the number of M-mode data sets, the PRF of the M-modes, and the number of RF lines acquired. A typical acquisition requires ≈ 2 s per M-mode at each spatial location and about 400 s would be necessary to generate final B-mode images with 200 RF lines.

The long-total-time duration for retrospective and prospective imaging means that the techniques are only practical when imaging objects, such as a mouse heart, that have periodic motion at a rate beyond what can be captured with current single-element technology. Normally, mechanically-scanned B-mode frame rates are limited by the length of the scan, the depth of the image, and a transducer velocity that permits the return echo to be captured near the transmission location. These restrictions are not an issue for retrospective or prospective imaging because the transducer only moves a small distance between M-mode acquisitions. The lack of rapid mechanical scanning makes gated imaging with an annular array an attractive approach to achieve high effective frame rates with an improved DOF versus single-element transducers. However, prospective imaging has not yet been implemented with an array-based imaging approach.

We demonstrated that HFU annular arrays improve DOF, and lateral resolution over that DOF, when compared to single-element HFU transducers [18], [19], [20]. We currently acquire data for all transmit-to-receive element combinations by pulsing one ring and simultaneously

digitizing the return echo on all the rings. To form a real-time image, this approach requires multiple passes across the target or multiple transmit pulses roughly at the same spatial position. Either way, the frame rates are effectively limited to r/N , where N is the number of elements and r is the frame rate that could be achieved with a single-element transducer. While real-time frame rates > 10 fps will eventually be possible with annular arrays, implementing either prospective or retrospective imaging within our current data-acquisition system presents an excellent means to achieve frame rates equivalent to single-element retrospective systems while improving the DOF and image-resolution versus single-element transducers.

In this paper, we present a prospective ECG- and respiratory-gated imaging approach implemented with a 34-MHz annular array. The annular array permits an improved DOF versus single-element transducers and prospective imaging permits effective frame rates higher than can now be achieved with traditional scanning methods. To the best of our knowledge, our work represents the first time HFU prospective imaging has been used with an array transducer and the first time prospective, rather than retrospective, imaging has been implemented with a HFU system. The transducer and data acquisition system were presented in detail in previous publications and will only be briefly summarized. The ECG-gating component of the system will then be demonstrated using a rigid target rotating at 10 revs/s and the respiratory-gating method will be demonstrated with representative data from an adult mouse. Finally, we will demonstrate the full prospective-imaging technique by imaging an adult mouse heart at an effective frame rate of 500 fps.

II. MATERIAL AND METHODS

A. Annular Array

Fabrication of the annular array used in these studies was described in a previous publication [21] and will be briefly summarized here. The transducer was fabricated by creating an array pattern on a copper-clad polyimide (CCP) film and then bonding a piezopolymer film to the CCP with a non-conductive epoxy. In our earlier array work, we utilized a 9- μm polyvinylidene fluoride (PVDF) membrane. Here, we employed a 9- μm poly(vinylidene fluoride-tetrafluoroethylene) [P(VDF-TrFE)] membrane, frequently referred to as copolymer, because of its improved coupling coefficient versus PVDF ($k_t = 0.3$ vs. 0.15) [22]. The use of copolymer led to an improvement in insertion loss (IL) by ≈ 8 to 12 dB versus our original PVDF-based array [21]. The final array consisted of five, equal-area rings with a total aperture of 6 mm, a radius of curvature of 12 mm, and 100- μm spacing between elements. Table I summarizes the key performance properties of center frequency, f_c , relative sensitivity, $\text{dB}@f_c$, -6-dB bandwidth, $\text{BW}_{6\text{dB}}$, minimum IL, and the frequency of the minimum insertion loss, f_{IL} . These measurements were taken using a pulser/receiver (5900, Panametrics, Waltham, MA) with a quartz plate positioned at the geometric focus of the transducer [21].

B. Data Acquisition

The data acquisition system differed slightly from the one described previously [18]. The system consisted of three main components: motion control, acoustic excitation, and digital signal acquisition. The motion-control component of the system employed three motorized translation stages with 1- μm resolution and was controlled via a motion-control card (PXI-7534, National Instruments, Austin, TX). In addition, a fourth axis was configured to control a linear actuator (LAS35, SMAC, Carlsbad, CA) with 25 mm of total available travel. The acoustic-excitation component consisted of a mono-cycle pulser (Avtech AVB2-TA-C-CVA, Ottawa, Ontario, Canada) which linked to each element of the transducer via a multiplexor (CXL/8X8 Cytec, Penfield, NY). In our previous experiments, we used the Cytec unit in a cross-point-switch configuration in which the driving channel of the array had a transmit/receive circuit between the switch module and the pulser, and the remaining channels

were directly digitized. Here, each channel of the array had its own transmit/receive circuit. By changing the switch configuration, we were able to reduce the lengths of our transmission lines which led to a 2 dB increase in the signal-to-noise ratio (SNR) of the system when using the mono-cycle pulser and a quartz flat positioned at the geometric focus.

The digital-signal-acquisition component of the experimental system permitted simultaneous data acquisition from the five array elements. Two PCI-based digitizers were used to acquire the data and each channel was dedicated to a single array element. This differed slightly from our previous system in which the receive channels varied depending on which array element was being excited. One channel was digitized within the control PC with a single-channel digitizer (DP110 Acqiris, Monroe, NY) and the remaining four channels were digitized with a crate-based, four-channel digitizer (DC271A Acqiris). Both digitizers had 8-bit resolution, up to 1-GHz sampling rates, 2 MB of memory per channel, and signals were amplified by 46 dB (AU-1313, Miteq, Hauppauge, NY) before sampling.

The overall experimental system permitted automated scanning and data collection under the control of custom software (LabVIEW, National Instruments). Annular-array data were acquired using an approach that permitted us to digitize all possible transmit-to-receive element pairs and then to implement synthetic focusing in post-processing to form a final image [18], [19], [20]. This approach required five transmit events at each spatial location in order to acquire all 25 transmit-to-receive M-mode data sets. Synthetic focusing consisted of applying appropriate digital delays to each transmit-to-receive pair in order to focus to an axial-depth d [23] and then summing the 25 pairs of data to form a single RF line. The process was repeated at a series of axial focal zones and the focused data from each focal zone were merged into a final composite image. If data were summed with no digital delays, the resulting RF data simulated a single-element transducer with the same total aperture and geometric focus as the annular array. By employing this technique, we were able to fully realize the DOF and lateral resolution improvements that the annular array affords. However, as noted earlier, prospective imaging does not require rapid mechanical motion and, therefore, the need to remain at one position for a relatively long time does not have a negative impact.

C. Assembly of Prospective Images

The method of data collection for gated acquisition consisted of a series of M-mode data sets acquired at a fixed PRF over a set of equally spaced lateral locations. Each M-mode represented a set of A-lines at one spatial location, and consecutive A-lines had a fixed time delay ($1/\text{PRF}$) [Fig. 1(a)]. Each M-mode was gated in the sense that the M-mode spanned a fixed time duration and that the start of the M-mode was synchronized to a start trigger representing a fixed phase of some periodically moving event. For system validation, the periodic event was a rotating rigid target and the reference trigger occurred once each rotation. For mouse-heart imaging, the periodic event was heart motion and the reference trigger was an ECG signal.

Once a full set of M-mode data were acquired, the data could be reassembled into a series of B-mode data sets with a fixed time interval between B-mode images [Fig. 1(b)]. Let jM_k represent an RF line k from the M-mode set j where the total number of M-modes is $j \in [1, \dots, s]$ and the number of RF lines in each M-mode set is $k \in [1, \dots, r]$. The B-mode, B , data sets are then assembled into a cine loop from the M-mode data via the relation $jM_k = kB_j$ which yields r B-mode frames each having s RF lines. For instance, frame k would consist of the k^{th} RF line from each M-mode data set such that $kB = [1M_k, 2M_k, \dots, sM_k]$.

D. Gated Acquisition

With the annular array, gating was implemented as described above except that M-mode data sets were acquired at each spatial location for all of the array transmit-to-receive pairs. Because

we transmitted on a single array element and then received the echo signal on all five array elements, we had to perform gating five times at each spatial location in order to digitize the 25 transmit-to-receive element pairs. While this adds some time to data acquisition compared to an experiment with a single-element transducer, the underlying assumption of periodic and stable motion of the object being imaged means that the added acquisition time has no effect on the final high-speed-image reconstruction. In principle, the final reconstructed B-mode-image planes would be stable whether the acquisition times between M-modes were 1 s or 10 s. However, it is always better to minimize the total acquisition time because the heart rate will eventually start to increase as the effects of the injectable anesthetic wear off.

To implement gated acquisition in our experimental system, a counter/timer on a multipurpose data-acquisition card (PCI-6024, National Instruments) was configured to create a defined PRF upon receiving a gate trigger. The gate trigger could derive from any trigger related to the object being imaged. The output of the counter/timer triggered the pulser and digitizers, and essentially acted as a delay and synchronization device within our existing software. As detailed below, we were also able to include additional trigger conditions, such as a respiratory trigger.

A respiratory gate was also included in order to reduce motion artifacts caused by movement of the mouse chest during experiments. Representative M-mode data that show how the mouse chest moved during respiration is shown in Fig. 2. Breathing occurred at a rate of ≈ 2 Hz, with the chest remaining fairly stationary for most of the respiratory cycle except for a brief period where air was exchanged. The aim of the respiratory trigger was to delay the ECG-gate trigger until just after chest motion occurred. The peak movement of the respiratory motion occurred at 0.20, 0.66, 1.11, and 1.54 s.

Rather than add a new layer of hardware into the system, we used the active transmit array element to acquire A-line RF data at a sampling frequency of 250 MHz and at a low PRF (100 Hz) prior to activating the counter/timer ECG gate. These A-line data were analyzed in real-time in order to track the motion of the chest. The tracking algorithm consisted of computing the RF envelope; passing the envelope through a median filter of rank 40; computing a running average of the median filtered RF lines; thresholding the running average to obtain an index representing the depth of the acoustic reflection from the chest; computing a two-point backward derivative of the index values; and, finally, performing a median filter of length 10 on the derivative. A respiratory trigger was defined as the first minus-to-plus zero crossing of the smoothed derivative.

Figure 3 shows an example of the tracking algorithm using the data in Fig. 2. Figure 3(a) represents the threshold index of the chest motion relative to the transducer. A more positive value indicates movement of the chest away from the transducer. Although the plot gives the appearance of sinusoidal motion, the actual respiratory motion tends to be a sudden movement followed by a period of minimal displacement. The derivative of Fig. 3(a) and the median filtered derivative (dashed and solid curves, respectively) are shown in Fig. 3(b). The time points (0.27, 0.73, 1.19, and 1.61 s) that met the definition of a respiratory trigger are indicated by vertical bars and represent the time just after breathing caused sudden movement of the chest towards the transducer.

During an actual experiment, data are acquired point by point in real time and tracking would stop at 0.27 s and the prospective data collection would commence on the next ECG-gate trigger. Therefore, M-mode gated data could be acquired as often as once per breath cycle provided that the time delay between the respiratory gate and ECG-gate plus the time duration of the M-mode data acquisition was less than one breath cycle.

E. Experiments

1) Rigid Target Validation—Initial system testing was performed with a single-element transducer rather than the full annular array for several reasons. First, the transducer needed to be submersed because of the geometry of the validation experiment, and the annular array was not designed for full submersion. Second, the validation experiment was testing the addition of gated imaging to our data acquisition system, not the performance of the annular array, and, therefore, any submersible transducer could be used. Finally, once the gated-acquisition was validated, including the annular array was simply a matter of performing the equivalent of five single-element gated acquisitions, one for each element of the annular array.

Validation tests were performed with a 40-MHz transducer (PI 50-2, Panametrics) having a 12-mm focal length and a 6-mm aperture. For these tests, the transducer was fixed to the XYZ motion stages and driven with a pulser/receiver (5900, Panametrics). The transducer was fully submersed in water such that the acoustic propagation was in the horizontal direction and directed at the hexagonally-shaped head of a nylon screw (Fig. 4). Each face of the screw head was 11 mm wide and the height of the head was 8 mm. The screw was placed in water with the threaded shaft in a vertical orientation, attached to a DC servo motor, and the motor was controlled as the fourth axis of the motion controller. The motor rotated at a constant rate of 10 revs/s and a spatial trigger that acted as the ECG-gate substitute was generated once each rotation. For these experiments, 121 M-mode data sets with 500 RF lines and 3000 RF points/line were acquired at a PRF of 1 kHz. The lateral spacing between M-modes was 180 μm and total data acquisition took $\approx$ one minute. Respiratory gating was not necessary for these experiments.

2) Mouse Experiments—In these experiments, imprinting-control-region (ICR) adult mice were used and maintained under protocols approved by the Institutional Animal Care and Use Committee of the New York University School of Medicine. The mice were anesthetized with an intraperitoneal injection of Nembutal with a dose of 6 g/100 g of body mass and their chests were shaved to eliminate acoustic scattering from hair and trapped air bubbles. The mice were placed in a supine position on an imaging stage and each limb was taped to a conductive pad after applying standard ECG gel to the pad. The stage was interfaced to a physiological monitoring system (THM100, Indus Instruments, Webster, TX) that detected the ECG signal and the rectal temperature. In order to obtain data at physiologic conditions, a custom warming pad was placed underneath the mouse and the current adjusted from a power supply until the mouse rectal temperature was stabilized to 37° C. A digital output from the THM100 synchronized to the R-wave of the ECG trace was used to trigger the array data acquisition.

Experiments were performed with the annular array placed above the mouse and attached to the linear actuator. Acoustic propagation was in the vertical direction. A saline-filled plastic Petri dish with a 25-mm circular cutout was placed against the mouse chest to facilitate acoustic coupling between the array and mouse. Because of the convex shape of the rib cage, a thin membrane cut from an ultrasound gel pad (Parker Labs, Fairfield, NJ) was placed between the Petri dish and mouse. The gel pad minimized saline leakage while maintaining the acoustic path between the transducer and the mouse. When conducting an experiment, the annular array was placed several mm into the saline bath and any gas trapped on the transducer surface was removed by suction. Prior to acquiring gated data, the center element of the array was excited and an eight-fps-real-time-imaging mode was employed to find an appropriate parasternal short-axis imaging plane. After obtaining the desired view, prospective imaging with ECG and respiratory gating was performed.

The acquired prospective M-mode data sets had 250 scan lines; were sampled at 250 MHz; contained 3200 points/line; and were acquired at a PRF of 500 Hz. The lateral spacing between M-mode sets was 75 μm and 121 M-mode sets were acquired. The time between M-mode

acquisitions typically ranged from 1 to 1.5 s and total acquisition time for the complete experiment was ≈ 12 minutes. After data collection, the M-mode data were parsed into B-mode images corresponding to each transmit-to-receive pair and the resulting B-mode data were synthetically focused, log compressed, and then converted into an image cine loop. Before final image analysis, the cine loops were imported into ImageJ [24] and the frames were processed using a five-frame running average. Each cine loop represented 0.5 s of time (250 frames) and each heart cycle lasted ≈ 0.15 s (75 frames).

Mice were also imaged using a Vevo 770 scanner (VisualSonics, Toronto, Ontario, Canada) in order to compare the annular-array data to a “state of the art” ultrasonic biomicroscope (UBM) system capable of generating equivalent kilohertz frame rates. These experiments utilized a 40-MHz transducer with a 6-mm focal length and a 3-mm aperture (RMV704). Because this transducer is self contained within a fluid chamber, acoustic coupling between the transducer and mouse was achieved using ultrasound coupling gel (Aquasonics 100, Parker Labs). After finding an appropriate short-axis view using a real-time imaging mode, cine loops were acquired using the Vevo’s ECG-based Kilohertz Visualization (EKV) presentation mode [16]. In this mode, A-lines were acquired at 1 kHz over several heart beats while at the same time the ECG signal was digitized. B-mode images were then retrospectively assembled in such a way that the images were gated with respect to the ECG data and data corrupted by respiratory motion were discarded. The respiratory cycle was ≈ 0.5 s or 120 breaths per minute [Fig. 2(a)] and this rate of breathing was consistent for the annular array and the Vevo imaging sessions.

III. EXPERIMENTAL RESULTS

A. Rigid Target Validation

A B-scan of the stationary hexagonal screw head is shown in Fig. 5(a). Each side of the nylon screw head was 11 mm wide and 8 mm high. This scan represents the view we expect after reconstructing the gated-M-mode data into B-mode images. With the screw rotating at 10 revs/s, a B-mode image highlights the limited use of a low-frame-rate mechanical scan when the target is rapidly moving [Fig. 5(b)]. For this case, the geometry of the screw head was impossible to visualize.

After the initial reference scans, gated data were acquired using a spatial trigger that occurred once each revolution (10 Hz). The M-mode data sets had 500 lines; were sampled at 200 MHz; contained 3000 points/line; and were acquired at a PRF of 1 kHz. The lateral spacing between M-mode sets was 180 μm and 121 M-mode sets were acquired. Examples of two M-modes that corresponded to lateral positions of 4 and 11 mm in Fig. 5(a) are shown in Fig. 5(c) and (d), respectively. Within an M-mode image, each side of the screw head was visualized as a sideways parabola. Over the 0.5 s duration of the acquired M-mode data, 30 screw sides were visible, which agreed with the expected number based on the 6 surfaces and 10 rev/s rotation rate. The time for one screw-head face to pass (leftmost edges of a parabola) corresponded to 16 ms, in close agreement with the 0.1 s duration of one full rotation.

Examples of reconstructed B-mode images are shown in Fig. 6. The figures represent times 0, 8, and 400 ms relative to Fig. 5(c) and (d) and they show quantitative agreement with the geometry of the screw head. Each B-mode image consisted of 121 scan lines with 180 μm between lines. The images represent the initial orientation [Fig. 6(a)] at the spatial-trigger time, a position representing 1/12 of rotation after the initial trigger [Fig. 6(b)], and then four full rotations after the initial trigger [Fig. 6(c)]. Note that Fig. 6(a) and (c) look identical because they represent the same view of the screw head but separated by four rotations (i.e., 400 ms). An image sequence (Multimedia-Movie 1) of the total set of B-mode images from Fig. 6 shows 0.5 s of motion at a frame rate of 1000 fps. The image sequence clearly shows the five full

rotations of the screw head and demonstrates how prospective imaging can achieve high effective frame rates of rapidly moving objects that have periodic motion.

B. Mouse-heart Experiments

Examples of the cardiac anatomy for the cases of fixed-focus array imaging, synthetically-focused array imaging, and Vevo EKV-retrospective imaging are shown in Fig. 7. The dorsoventral axis in the images is defined from top (ventral) to bottom (dorsal). The fixed-focus and synthetic-focus cases were derived from the same set of data but processed using different focusing techniques. The Vevo data were acquired with a different mouse on a separate day. With the geometric focus (black arrows) placed above the left ventricle, cine loops were produced for the fixed-focus array [Fig. 7(a) and (b)] and synthetically-focused array [Fig. 7(d) and (e)] cases. The columns labeled systole and diastole represent the maximum ventricular contraction and the maximum ventricular relaxation of the cardiac cycle.

In the fixed-focus array images [Fig. 7(a) and (b)], the right ventricle (RV), interventricular septum (IVS), and the ventral edge of the left ventricle (LV) were visualized, although with poor resolution and poor boundary demarcation. The corresponding cine loop (Multimedia-Movie 2) showed the outline of the LV, but it was out of focus. In contrast, the synthetically-focused array images (d and e) showed the RV, IVS, and LV in more detail, with finer resolution and with improved delineation of the boundaries. In addition, the papillary muscles of the LV endocardium were visible. The corresponding cine loop of the synthetically-focused data (Multimedia-Movie 3) clearly showed that the ECG-gated data were synchronized to the heart cycle; without accurate respiratory and ECG gating, the focusing algorithm would not be effective because a particular feature would be in a random axial position at the start of each M-mode. However, both cine loops did show some residual gating artifacts as manifested by image lines that appeared shifted. These artifacts were most visible along chest boundary and became more pronounced as the number of effective heart cycles increased due to subtle shifts in the duration of each heart beat. However, even with these slight degradations in image quality, it is remarkable that the synthetic focusing of data acquired over 12 minutes can be utilized to create a high-speed cine loop representing 0.5 s of cardiac motion that spans nearly four heart beats.

The Vevo EKV-imaging-mode retrospective data are shown in Fig. 7(g) and (h). These data were acquired with a different mouse on a different day and we attempted to image a short-axis view that was equivalent to the above array data. The Vevo data were acquired with a 40-MHz probe and the final cine loop contained 192 RF lines and 148 frames with 1 ms between frames (Multimedia-Movie 4). For these data, the transducer focus (black arrow) was centered within the LV. In the systole and diastole images (g) and (h, respectively), both papillary muscles were visible (white arrows) but only about half of the LV epicardium outline was discernible and the IVS and RV were not visible at all. Some of the missing outline of the left ventricle almost became visible in the end-diastolic frame [Fig. 7(g)]. These results highlight the advantage of the improved DOF of the annular array and the resulting ability to resolve the entire heart in a single image without the need to carefully position the location of the geometric focus.

The high-frame-rate cine loops were imported as time-series data into the Amira 4.1 software package (Visage Imaging, Carlsbad, CA). Using the StandardView module, cursors could be placed anywhere on the B-mode image to display anatomical M-mode (AMM) [25] images in the axial direction of the original data or, virtually, in the lateral direction. With M-mode lines positioned in the axial direction as shown in Fig. 7(b), (e) and (h), the corresponding AMM images [Fig. 7(c), (f) and (i)] showed periodic variation in the thickness of the RV, IVS and LV. The AMM images generated from the retrospective Vevo cine loop revealed the ventral LV wall with a faint trace of the dorsal LV wall. The advantage of the array focusing can be

appreciated by the fact that the dorsal wall of the LV was clearly resolved only in the array-focused AMM image.

The AMM images of Fig. 7(c) and (f) showed a motion artifact during the second heart beat that was primarily visible as a loss of signal strength and an apparent discontinuity at the ventral wall of the LV. Because the majority of the AMM image was stable in terms of the ventral LV wall reconstruction, we suspect that out-of-plane respiratory motion caused a rib or a valve to enter the field of view either individually or in combination. The vertical banding in Fig. 7(c) and (f) in which the background noise seemed to drop and the signal strength weakened could be caused by a rib, while the discontinuity in the trace of the LV ventral wall could be caused by valve movement.

With the chest wall lying 4 to 5 mm above the geometric focus, the speckle pattern in the UBM AMM image [Fig. 7(i)] traced out a layered set of horizontal lines indicating a near perfect cancellation of the respiratory motion. In contrast, the AMM images from the fixed- and array-focus cases revealed a low-amplitude residual periodic motion of the chest. This motion was not observed in the commercial UBM images partly because the acoustic window of the probe was in contact with the chest, thus limiting axial motion, but mostly because retrospective imaging permits greater ease in aligning adjacent spatial locations in post-processing as a result of the acquired data spanning multiple respiratory cycles.

IV. DISCUSSION

Retrospective HFU imaging achieves equivalent kilohertz frame rates without the necessity of rapidly scanning a transducer. We demonstrated this imaging approach in a prospective fashion where the mouse's ECG was used to trigger the data-acquisition system and a respiratory gate was used in order to minimize motion artifacts. Our implementation of respiratory gating is analogous to navigator-triggered-prospective-acquisition-correction techniques used in clinical MRI [26] where respiratory motion is tracked without the need to add an additional layer of hardware.

The example of prospective imaging that we showed in Fig. 7 represented the distillation of over 3000 heart beats into an effective real-time representation of four heartbeats. The ability to achieve these results highlights the remarkable stability of heart function over long time scales and also the elegance of ECG-gating techniques. Our results are the first implementation of prospective imaging with a high-frequency array and show that ECG-gated imaging can easily be extended to HFU array devices provided that appropriate respiratory gating is utilized. Our data show that the multiple ECG-gates at each spatial location, as well as the unavoidable minor axial and out-of-plane motions, are not obstacles to achieving results that are essentially equivalent to single-element data but at an improved DOF and resolution. However, in order to utilize prospective imaging to perform quantitative analysis of normal and abnormal heart function, further studies are necessary in order to understand how aperiodicity and beam forming impact measurements such as local tissue velocity, segmented tissue volume, etc.

Most indices that quantify cardiac function rely on manual delineation of endocardial and epicardial borders of the LV over a cardiac cycle. HFU adult-mouse cardiac imaging is almost exclusively performed with probes having a 30-MHz center frequency because it allows the myocardium to be imaged while still maintaining a reasonable resolution. Even at these frequencies, single-element probes have an ≈ 1.6 -mm DOF which is approximately a factor of two smaller than the systolic LV diameter at maximal cross-section. We showed that 40-MHz, fixed-focus, UBM images lack the SNR, penetration and DOF to yield good contrast in images of cardiac anatomy. With the increase in SNR, DOF and resolution of the prospective annular-array images, contrast of the endocardial and epicardial borders of the LV improved versus

fixed-focus and retrospective images. The improved DOF with the annular array also produced high-quality delineations of the IVS and RV and opens up the opportunity to perform comparative measurements between the RV, IVS and LV. Because of the circular geometry of the LV, the lateral diameter of the myocardium will be oriented almost perpendicularly to the ultrasound beam, and at these points, contrast gets reduced significantly. To remedy this loss of signal, we can implement coded excitation without much change to the instrumentation of the array system. We recently demonstrated a marked increase in SNR and penetration depth by using coded excitation for *in-utero* mouse-embryo imaging using our 40-MHz annular-array system [27], [28].

Given that precise 2D segmentation of the myocardium is the first crucial step for cardiac analysis, we will need to address the motion artifacts that degraded the left ventral borders of the myocardium in our prospective data. A first step will be to modify our gating algorithm so that we can reliably identify and reject data that are not properly synchronized with respiratory motion and, thus, reduce residual motion artifacts. With the built-in flexibility of the data-acquisition work flow in our annular-array system, we can also derive respiratory motion from alternate sources and feed them into the respiratory gating part of the algorithm. One such possibility would be to use a continuous-wave (CW) or pulsed-wave (PW) Doppler system [29], and put the Doppler sample volume over the chest to track the motion of the chest cavity and generate the proper gate. By using the range gating of a PW Doppler system with unfocused Doppler transducers [30] the sample volume could be positioned over the diaphragm. Gating based on diaphragm motion is commonly used in cardiac MRI because the motion of the organ to be imaged is occasionally not synchronized to motion of the abdominal wall [26].

Compared to MRI or CT, retrospective and prospective B-mode imaging increase, by a factor of 10 or more, the number of image frames acquired per cardiac cycle [11], [12], [13]. These high frame rates facilitate visualization of high temporal resolution events in the cardiovascular system such as cardiac dyssynchrony [17] and regional contractile dysfunction [31]. At even higher frame rates, one can perform elastography of the mouse LV [32] and evaluate mechanical and electromechanical waves as they travel through the cardiac tissue [10]. The data processing in the above techniques typically begins by manually segmenting the 2D myocardial boundaries or by implementing a speckle tracking algorithm. The improved image resolution and SNR achieved with our annular-array imaging approach will greatly increase the accuracy of these algorithms and will permit the visualization of subtle changes in abnormal mouse cardiac models.

Due to respiration artifacts, our previous array-imaging work with mouse embryos was performed immediately after sacrificing the mother [20]. With the robust respiratory-gating method developed in this study, we are now ready to tackle full *in vivo* imaging of mouse embryos. Because of the inherent flexibility of our triggering method, we are now in a position to perform prospective or retrospective embryonic heart imaging *in vivo*. For embryonic cardiac imaging, there will still be a need for cardiac-triggered-image acquisition because the heart rates in the mouse embryo lie within the range of 200–300 beats per minute [33]. Although acquiring an ECG signal from the developing embryonic heart will be difficult, particularly *in utero*, Doppler signals from the embryonic cardiovascular system could potentially be used to derive a cardiac trigger. Such methods have been previously performed for adult and fetal echocardiography [34], [35].

V. CONCLUSION

By implementing ECG-gated, respiratory-triggered, prospective imaging into our annular-array system, we demonstrated effective high frame rate *in vivo* synthetically-focused imaging of the adult mouse heart. This prospective-imaging approach was easily incorporated into our

existing annular-array-scan system because there was no requirement for high-speed mechanical scanning of the array. In the future, we could include retrospective imaging into our system by digitizing the physiologic waveforms simultaneously with the ECG-gated data. Some work has been done in comparing MRI [12] and CT [14] prospective and retrospective imaging, and this comparison can be extended to HFU imaging. One future direction of this research will address the differences between prospective and retrospective array-focused cardiac imaging in terms of image quality and cardiac functional indices. One other natural extension will be to implement coded excitation with HFU in order to increase the SNR and the penetration depth. Coded excitation has the potential to image actual blood flow within the mouse heart and further improve feature delineation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Algorithm for collating M-mode data sets into a high-temporal resolution B-mode cine loop.

a) Collection of s M-mode data sets indexed by the label jM where $j \in [1, \dots, s]$. Each of the r RF lines (dashed lines) are separated in time by $1/\text{PRF}$. jM_k labels the k^{th} RF line in the j^{th} M-mode. b) B-mode frame kB , where $k \in [1, \dots, r]$, is assembled from RF line k of M-modes 1 through s . The assembly process is described by $kB_j = jM_k$.

Fig. 2. Mouse chest M-mode data from Ring 1 (center element) of the annular array taken at a PRF of 100 Hz. Breathing caused a fairly rapid movement of the chest towards the transducer at a rate of ≈ 2 Hz.

Fig. 3.

Chest tracking algorithm applied to the M-mode data in Fig. 2. The index tracking the chest motion (a) shows sinusoidal-like movement. The derivative of the index curve (b, dash) was smoothed with median filtering (b, solid). The respiratory gate was defined as a minus-to-plus crossing of the smoothed derivative as marked with the four vertical bars.

Fig. 4.

Experimental setup used to validate the gated-imaging mode of the data acquisition system. A screw with a hexagonal head was attached to a motor, rotated at 10 revs/s, and a spatial trigger was generated once each rotation. A single-element transducer was then scanned in the lateral direction and M-mode data sets were acquired and referenced to the spatial trigger.

Fig. 5.

a) B-mode image showing the geometry of the stationary nylon screw head. b) A B-scan of the rotating screw showed little about the geometry. M-mode data sets c) 22M and d) 60M referenced to a spatial trigger acquired, in relation to (a), at lateral positions 4 and 11 mm, respectively. Image data were log-compressed and scaled to 40 dB. Note that the signal was heavily saturated for these data.

Fig. 6.

Three B-mode frames of the rotating screw head reconstructed from the M-mode data. The frames represent times a) 0 (0B), b) 8 (8B), and c) 400 ms (400B). Multimedia-Movie 1. Image data were log-compressed and scaled to 40 dB. Note that the signal was heavily saturated for these data.

Fig. 7.

Short-axis view at two points in the cardiac cycle of an adult mouse heart from fixed-focus (a and b) and array-focus (d and e) prospective imaging, and from fixed-focus retrospective imaging from a commercial UBM (g and h). The transducer was positioned at the top of the images. Relative to the start of gated acquisition, the images represent (a and d) 158 ms (79B), (b and e) 96 ms (48B), (g) 0 ms (0B), and (h) 74 ms (74B). The array-focused images (d and e) clearly visualized the RV, IVS, lumen of the LV, and both papillary muscles (white arrows). The papillary muscles (white arrows) were also visible in the UBM images (g and h). Anatomical M-mode images (c, f and i), notated by 101M, corresponding to the line of sight indicated by the dashed white lines running from top (ventral) to bottom (dorsal). Black arrows indicate the geometric focus of each transducer; annular-array image data were log-compressed and scaled to 45 dB; B-mode images are 6 mm × 10 mm; and M-mode images represent 10-mm depth.

TABLE I

34-MHz Annular Array Performance

Ring	f_c (MHz)	$\text{dB}@f_c$	$\text{BW}_{6\text{dB}}$ (%)	IL (dB)	f_{IL} (MHz)
1	33.0	0	35	-20	37
2	34.5	-4	30	-26	37
3	34.0	-6	35	-31	38
4	33.5	-7	32	-29	37
5	34.0	-8	41	-28	38