ICA 2013 Montreal
Montreal, Canada
2 - 7 June 2013

Biomedical Acoustics
Session 4pBA: High-Frequency Ultrasound (20-80 MHz)

4pBA2. High-frame-rate retrospective imaging of mouse-embryo cardiac function using annular array and Doppler-derived gating

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A high-frequency (HF) imaging system based on a custom 5-element, 40-MHz annular array was used to study cardiovascular development of mouse embryos. High-frame-rate imaging of the heart dynamics was achieved using a retrospective reconstruction method based on electrocardiogram (ECG) waveforms and respiratory gating. The ECG signals were obtained by measuring blood-flow velocities in major arteries of in-vivo mouse embryos using a custom, HF Doppler apparatus made from two, 20-MHz, single-element, PZT transducers. Co-registered M-mode data were acquired from the annular array excited with a 5-channel pulser/receiver. A synthetic-focusing algorithm was used to improve spatial resolution (< 100 μm), depth-of-field (> 10 mm) and signal-to-noise ratio (> 45 dB). This technique was used on embryos aged from 11.5 to 14.5 days and provided high-resolution, morphologically correct B-mode cine-loops of the heart chamber dynamics at frame rates of 1 kHz. The ultra-fine temporal resolution (1 ms) permitted precise quantification of the mean cardiac cycle length and detailed visualization of fast events such as opening and closing of the mitral valve. The speckle characteristics of the high-resolution images could be used to assess blood flow and to quantify myocardial strain at each developmental stage of the embryonic heart.

Published by the Acoustical Society of America through the American Institute of Physics

©2013 Acoustical Society of America [DOI: 10.1121/1.4800362]
Received 22 Jan 2013; published 2 Jun 2013
INTRODUCTION

Electrocardiogram (ECG)-gated imaging methods have allowed for fine temporal-resolution measurements at effective frame rates (> 1 kHz) that exceed the capability of linear-array systems. Retrospective ECG gating with high-frequency ultrasound (HFU, > 20 MHz) has been applied to the adult mouse for applications such as myocardial elastography [1] and high-frame-rate echocardiography using single-element transducers [2] or annular-array transducers [3]. However, these techniques do not translate directly to the mouse embryo because of the difficulty to obtain in utero ECG readings from the embryo. We previously demonstrated an ECG-equivalent gating signal could be derived from the mouse embryo heart by employing a separate HFU Doppler subsystem [4], but we only obtained data with a single-element transducer. Here we improve upon the initial Doppler subsystem and we extend the technique to a 40-MHz annular array excited by a custom, five-channel pulser/receiver. With this approach, we can obtain array-focused image data in a single pass rather than the multiple passes required when we employed just a single-channel excitation source.

METHODS

Data-acquisition System

A custom imaging system was employed based on the 40-MHz annular array developed by Ketterling et al., [3, 5, 6]. The imaging transducer had five elements, a center frequency of 40 MHz, a total aperture of 6 mm, and a 12 mm focal length. The experimental system consisted of digitization, motion, and pulsing subsystems that were integrated into a chassis-based system (PXI-1042, National Instruments [NI], Austin, TX) under PC control. A high-speed linear actuator (LAS35, SMAC, Carlsbad, CA) with 23 mm of total travel was used as the primary imaging axis and an automated cross-axis stage was also available. A custom-made, five-channel, HFU pulser/receiver (Daxsonics, Halifax, Canada) was employed to excite the annular array and the pulser was linked to the annular array and 8-bit digitizers (PXI-5154, NI). The system was fully automated to incorporate all imaging modes including real-time imaging for initial field-of-view orientation before initiating the retrospective imaging modes.

Doppler Subsystem

The continuous-wave (CW) Doppler subsystem was assembled from two 3-mm diameter focused transducers fabricated using a pad-printing technique [7]. The transducers were wired to BNC cables and mounted on a custom-machined aluminium holder with an angle of 30° between the transmit and receive ultrasound beams. The transducers had a central frequency ($f_0$) near 20 MHz, a geometric focus of 15 mm, and 6 dB lateral beamwidth of roughly 0.5 mm. The CW Doppler transducers were connected to a custom-built, quadrature demodulation circuit to detect the Doppler frequency shifts $f_d$ [8]. The in-phase (I) and quadrature (Q) Doppler signals were continuously digitized at a sampling frequency of 24 kHz using a data-acquisition card (PXI-6229, NI) and saved with the M-mode ultrasound data. The ECG gating was handled in post-processing.

Retrospective M-mode Data

M-mode data were acquired at each spatial location of the final reconstructed images. A rapid sequence of five excitations was used such that the five array elements were individually excited, in turn, with enough delay between excitations to avoid an overlap from consecutive firings. With this method, we were able to obtain all 25 transmit-to-receive element combinations.
for later synthetic focusing [6]. A fully non-invasive, \textit{in utero} approach was used to visualize individual mouse embryos spanning embryonic days (E) 12.5 to 14.5, where E0.5 was defined as noon of the day a vaginal plug was found after overnight mating [9]. The body temperature of the mother was monitored by a rectal thermometer and a physiologic temperature of 37°C was maintained. All mice used in these studies were cared for under protocols approved by the Institutional Animal Care and Use Committee of the New York University School of Medicine. Respiratory artifacts were minimized by using a pneumatic pillow placed underneath the chest of the mouse and a differential pressure transducer (TSD160A, BIOPAC Systems, Goleta, CA) provided a respiratory-gating signal.

A typical data set was obtained at 101 evenly spaced lateral locations. At each location, 25 M-mode sequences were obtained corresponding to the 25 transmit-to-receive combinations. Each M-mode set contained 800 A-lines acquired at a pulse repetition frequency (PRF) of 1 kHz. The lateral spacing between image lines was 50 μm and data were digitized at a 250 MHz sampling rate. The total time to acquire all data was roughly 3 min. After the full set of data were acquired, the M-mode data were converted to B-mode images by first identifying the ECG gate time from the acquired IQ Doppler data and then combining the A-lines at the same relative delay from the ECG gate in each M-mode data set. Finally, a synthetic-focusing algorithm was applied to each collection of 25 transmit-to-receive data sets to yield a final, focused image. The resulting B-mode image sequence then had a frame rate of 1000 fps (i.e., 1/PRF).

\section*{RESULTS}

Spectrograms derived from the IQ Doppler waveforms showed periodic, highly pulsatile blood flow. There was a slight time shift of a few milliseconds between the Doppler waveforms and the actual contractions of the heart chambers because the Doppler volume was directed on the aorta rather than on the heart itself. A typical cardiac cycle duration was \(\approx 300\pm10\) ms or 200 beats/min. The blood flow from the descending aorta was detected and the systole and diastole phases of the cardiac cycle were clearly identified with high temporal precision (Fig. 1). Blood flow was visualized as a series of shifting speckle patterns moving in and out of the heart chambers and along the major arteries. Some motion artifacts were visible in the final reconstructed images due to the relatively long time duration (3 min) to acquire all of the data and the unavoidable subtle shifting of the embryo over that time.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Reconstructed B-mode images of the beating heart of an \textit{in utero} E13.5 mouse embryo. The images show the heart a) 44 ms and b) 188 ms after an arbitrary reference gate. The images represent the minimum contraction (a) and maximum expansion (b) of the ventricles \(v\) giving a roughly 208 beat-per-minute heart rate. A 35 dB dynamic range is pictured [u: uterus].}
\end{figure}
CONCLUSIONS

A retrospective-imaging approach using a HFU annular array was developed for imaging the hearts of in vivo mouse embryos. An ECG signal from the embryo heart was derived from a CW Doppler subsystem and the acquired IQ data were used in post-processing to gate, relative to a fixed point in the cardiac cycle, the digitized HFU M-mode data. With this approach, it is possible to generate image sequences with effective frame rates of 1 kHz or beyond. The ultimate limit is the round trip time of the acoustic signal. However, retrospective-imaging approaches can only be used for cyclical processes like cardiac function because the method assumes the dynamic process being imaged consistently repeats itself for time periods of 10 minutes or more.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institutes of Health (EB008606).

REFERENCES


