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High-frequency Ultrasound Doppler System for Biomedical Applications with a 30 MHz Linear Array

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Abstract

In this paper, we report the development of the first high-frequency (HF) pulsed-wave Doppler system using a 30 MHz linear array transducer to assess the cardiovascular functions in small animal. This array based pulsed-wave Doppler system included a 16-channel HF analog beamformer, a HF pulsed-wave Doppler module, timing circuits, HF bipolar pulsers, and analog front-ends. The beamformed echoes acquired by the 16 channel analog beamformer, were directly fed to the HF pulsed-wave Doppler module. Then the in-phase and quadrature-phase (IQ) audio Doppler signals were digitized by either a sound card or a Gage digitizer and stored in a PC. The Doppler spectrogram was displayed on a PC in real time. The two-way beam-widths were determined to be 160 μm to 320 μm when the array was electronically focused at different focal points at depths from 5–10 mm. A micro flow phantom, consisting of a polyimide tube with inner diameter of 127 μm , and the wire phantom were used to evaluate and calibrate the system. The results show that the system is capable of detecting motion velocity of the wire phantom as low as 0.1 mm/s, and detecting blood-mimicking flow velocity in the 127 μm tube lower than 7 mm/s. The system was subsequently used to measure the blood flow *in vivo* in two mouse abdominal superficial vessels with diameters of approximately 200 μm , and a mouse aorta close to the heart. These results demonstrated that this system may become an indispensable part of the current HF array based imaging systems for small animal studies.

Keywords

high-frequency array; high-frequency Doppler; beamformer

INTRODUCTION

Small animals such as mice and rats are preferred animal models in current evaluations of gene and drug therapies. Due to the small size of these animals, micro-computed tomography (Micro-CT), micro-magnetic resonance imaging (Micro-MRI) and micro-positron emission tomography (Micro-PET) have been developed to assess structure and functionality (Hwang et al. 2002). Ultrasound is another useful imaging modality because it can be used to achieve high resolution images and in the meantime is capable of visualizing blood flow at a high frame rate. Therefore we believe that ultrasound will also be proven to be invaluable tool for small animal research.

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High-frequency (HF) ultrasound imaging, capable of achieving good spatial resolution at an affordable price, has been shown to be useful for imaging small animals in biological and pharmaceutical research (Christopher et al. 1997; Foster et al. 2000; Hozumi et al. 2004; Lockwood et al. 1996). The frequency range of HF ultrasound has been extended to over 100 MHz. To date, HF ultrasound imaging is almost exclusively performed with single element transducer based ultrasound backscatter microscopes (UBMs). Commercial UBMs used in small animal research provide high-resolution real-time imaging with Doppler features (Foster et al. 2002; Gan et al. 2007). They have been utilized in preclinical cancer and cardiovascular research (Perrino et al. 2006; Visualsonics 2007). With special scanheads, the latest system can perform high frame rate (>240 frame/s) B-scan imaging in a limited field of view (<1 mm) (Visualsonics 2007). In addition, a frame rate of greater than 1000 frame/s can be realized, using certain off-line reconstruction algorithms (Cherin et al. 2006; Liu et al. 2006). The mechanical nature and fixed focal point of single element transducer, however, limit the further improvement of image quality and frame rate (>200 frame/s) in a wider field of view such as those frequently encountered in small animal cardiac studies (Li et al. 2005; Xu et al. 2005). As with clinical ultrasound systems, a better solution would be a system that utilizes HF ultrasonic linear arrays (>30 MHz), which have been successfully developed recently (Brown et al. 2006; Cannata et al. 2006; Lukacs et al. 2006; Ritter et al. 2002).

Preliminary studies of B-scan imaging with HF linear arrays have been reported (Hu et al. 2006; Xu et al. 2005). Mouse heart movements have been captured at 100 frame/s using the system (Xu et al. 2005). Cardiovascular and tumor research utilizing mice requires not only B-scan imaging, but also ultrasound Doppler to evaluate blood flow (Goertz et al. 2002; Reddy et al. 2005a,b). The peak velocity, mean velocity, and peak velocity intervals of blood flow, are often used to identify hemodynamic diseases (Reddy et al. 2005b). To the best knowledge of the authors, the development of Doppler systems with HF linear arrays has not yet been reported.

Considering the limitations of large sample volume in a continuous-wave Doppler, and the design complexities of color Doppler (Jensen 1996), pulsed-wave Doppler appears to be a more viable approach in an initial attempt for detecting blood flow in individual vessels with HF arrays. In this paper, a HF pulsed-wave Doppler system utilizing a 30 MHz linear array was successfully implemented and tested by combining an analog beamformer with a Doppler module. This system has the capability of measuring both high blood velocities in the mouse heart (>400 cm/s) (Reddy et al. 2005b), and low blood velocities in microcirculation (<0.5 mm/s) (Christopher et al. 1997; Ferrara et al. 1996).

MATERIALS AND METHODS

Fig. 1 illustrates the block diagram of the pulsed-wave Doppler system with HF arrays. The system included two separated modules: a 16-channel analog beamformer module in the dash-dot line box, and a HF directional pulsed-wave Doppler module in the dash line box. The main clock of the system was generated by a programmable clock generator ECS-P83/P85 (ECS, Inc. International, Kansas City, KS, USA). The two modules were synchronized by clocks which were distributed by a clock driver CD328 (Texas Instruments, Dallas, TX, USA). The 16-channel imaging system consisted of a 30MHz array, timing circuits, 16-channel transmit beamformer, N-cycle bipolar pulsers, analog front-ends, and a 16-channel programmable analog receive beamformer. The beamformed echoes were directly fed to the HF directional pulsed-wave Doppler module, which included an in-phase and quadrature (IQ) demodulator, low-pass filters, sample-and-hold circuits, pulse repetition frequency (PRF) filters, wall filters, and audio amplifiers. The array characteristics and electronic circuits are discussed in detail in the following sections.

Array Characteristics

A 30 MHz piezo-composite ultrasound array was used. It was fabricated using the same mechanical dicing method described by Cannata et al. (2006). The characteristics of the array are listed in Table 1. Element 1 to Element 16 were used in the experiments described in the following sections.

Timing Circuits and Bipolar Pulsers

The timing circuits provided a PRF clock (114 Hz–117 kHz), a sample gate (0.1–2 μ s) at a specific sample depth (0–50 μ s), and an N-cycle (N=1–255) transmit trigger signal. The above N-cycle trigger signal was duplicated to 16 identical triggers. These 16 triggers were then delayed by the 16-channel transmit beamformer which consisted of programmable digital delay lines PDU16F-1 (Data Delay Device Inc., Clifton, NJ, USA) and the microprocessor 89S8252 (Atmel Corp., San Jose, CA, USA). The delay time of PDU16F-1 could be programmable from 0 ns to 63 ns with an increment of 1 ns. The appropriate delay time for each channel, which was calculated before experiments, was stored in the microprocessor. Each delayed trigger signal drove a bipolar pulser (Xu et al. 2007). Typical timing sequences are shown in Fig. 2 and a 30 MHz 7-cycle bipolar pulse with amplitude of 60 V_{pp} in Fig. 3. The output amplitude variation across the 16-channel bipolar pulsers was less than 0.3 dB at 30 MHz. The 16 bipolar pulsers were connected to Element 1 to Element 16 of the 30 MHz array.

In a Doppler system, the lateral length of its sample volume is determined by the lateral resolution of its transducer; the axial length of its sample volume is determined by its sample gate length and transmit pulse length (Jensen 1996). In our system, we adjusted the sample gate length same as the transmit pulse length.

Analog Front-ends

After each bipolar pulser, a limiter including a pair of back-to-back diodes (Lockwood et al. 1991) was used to prevent high-voltage pulses from damaging the following analog front-end channel. Each channel included a variable gain amplifier AD8331 (Analog Devices, Norwood, MA, USA), an optional band-pass filter PBP-35W with –3 dB bandwidth of 88% (Mini-Circuits Inc., Brooklyn, NY, USA), and an optional second amplifier MAX4107 (Maxim Integrated Products, Sunnyvale, CA, USA). The total achievable amplification was 70 dB. The measured minimum detectable signal can be as low as 20 μ V_{pp}, which is close to the weakest received signals 10 μ V_{pp} at transducers (Brunner 2002).

Analog Beamformer

The analog receiving beamformer included sixteen 4-bit programmable analog delay circuits and an analog adder. Each amplified RF signal was delayed by the programmable analog delay circuit, which was a combination of four fixed analog delay lines FDC1005-FDD15005 (ELMEC Technology of America Inc., San Mateo, CA, USA), and four 2-to-1 multiplexers AD8182 (Analog Devices, Norwood, MA, USA) in cascade as shown in Fig. 4. By setting the 2-to-1 multiplexer, each delay line can have two possible states: the delay line is used, or the delay line is bypassed. The programmable analog delay circuit can achieve a maximum delay time of 60 ns (four 15 ns delay lines used), and a minimum increment of 1 ns (at least one 1 ns delay line used). By adding more delay lines and multiplexers in each analog delay circuit, the 6-bit or 8-bit programmable analog delay circuit can be implemented. Subsequently the sixteen delayed signals were summed to form the ultrasound beam, using the analog adder made of AD8009 (Analog Devices, Norwood, MA, USA).

The theoretical and measured delays of each channel at differential focal points are shown in Fig. 5. The –6 dB bandwidth of the delay circuit was measured to be over 90 MHz, by sweeping

the input continuous sinusoid signal from 10 MHz to 100 MHz. This bandwidth, which is higher than the one of the digital beamformer reported by Hu (2006), is sufficient for the current 30–35 MHz arrays, and may be suitable for use with future arrays with frequencies as high as 90 MHz.

HF Pulsed-wave Doppler Module

The implementation of HF directional pulsed-wave Doppler was based on in-phase and quadrature demodulation, and sample-and-hold circuits (Jensen 1996). The beamformed echoes were first filtered by a 30 MHz band-pass filter with a –3 dB bandwidth of 30% (BBP-30, Mini-Circuits, Brooklyn, NY, USA), and the echoes were fed to an in-phase and quadrature (IQ) demodulator (MIQC-60WD, Mini-Circuits, Brooklyn, NY, USA). The reference clock of the IQ demodulator was generated from one output clock of the clock driver CD328, after a low-pass filter with a –3 dB cut-off frequency of 35 MHz (PLP-30, Mini-Circuits, Brooklyn, NY, USA), and an attenuation network. The demodulated intermediate frequency (IF) signals I_{IF} and Q_{IF} were low-pass filtered to remove harmonics and noise by two low-pass filters with a –3 dB cut-off frequency of 14 MHz (BLP-10.7, Mini-Circuits, Brooklyn, NY, USA). Then the I_F and Q_F were sampled and held by two AD783s (Analog Devices, Norwood, MA, USA). Followed by 2-channel 8th-order PRF filters, which consisted of switch-capacitor filters (MAX291, Maxim Integrated Products, Sunnyvale, CA, USA), the I_{SH} and Q_{SH} signals were cleaned by removing sample-and-hold harmonics. Before the I_A and Q_A were amplified by the stereo amplifiers (LM386, National Semiconductor Corp., Santa Clara, CA, USA), optional 4th-order wall filters MAX7490 (Maxim Integrated Products, Sunnyvale, CA, USA) were used to remove low frequency clutter signals. The cut-off frequencies of both the PRF filters and wall filters were adjusted by their clocks from the timing circuits. Doppler audio signals could be played by stereo speakers HK-206 (Dell Inc., Round Rock, Texas, USA). Alternatively, the Doppler audio signals were digitized by either an integrated sound card, or a Gage A/D card CS14200 (Gage Applied Technologies Inc., Montreal, Quebec, Canada), depending on the bandwidth of the audio signals. The digitized signals were processed and converted into a directional spectrogram by Labview-based software (National Instruments Corp., Austin, TX, USA) in real time. Further off-line analysis was conducted using Matlab (The MathWorks, Inc., Natick, MA, USA) based software. The above circuits, including sample-and-hold circuits, PRF filters, and wall filters, were well balanced in order to achieve good direction isolation. The measured direction isolation was better than 30 dB.

Flow Velocity Estimation

The flow velocity v can be calculated by the following formula (Jensen 1996):

$$v = \frac{f_D}{2f_0 \cos \theta} c \quad (1)$$

where f_D is the Doppler shift frequency, f_0 is the center frequency of the transducer, c is the sound velocity in a medium, and θ is the angle between acoustic beam and flow. Flow velocities and directions were obtained by processing the acquired Doppler signals I_A and Q_A with complex fast Fourier transform (cFFT) (Aydin et al. 1994).

Eq. (1) indicates that the flow velocity is proportional to Doppler shift frequency. The signal bandwidth of the integrated sound card on the PC motherboard is approximately 20 Hz–20 kHz, and its sampling frequency is up to 44.1 kHz. These sound card specifications meet the requirements of acquiring the Q_A and I_A data in most cases. However, in order to detect extremely low flow velocities, or extremely high flow velocities, neither the signal bandwidth of the sound card is low enough, nor is its sampling frequency high enough. Therefore, the Gage A/D card was used to detect extremely low or high velocities. The Gage card can be used

to digitized DC signals, and its sampling frequency can range from 50 kHz to 200 MHz with 14-bit resolution.

As noted in (Christopher et al. 1997), proper temporal and velocity resolution can be determined when each cardiac cycle contains at least 25 discrete time intervals, and the peak-to-peak velocity range also contains similar numbers of velocity intervals. In our experiments, the cFFT was performed on each segment of the digitized audio signals. The length of each segment corresponded to the temporal resolution, and the point number of cFFT and the sampling frequency determined the velocity resolution. The appropriate temporal and velocity resolutions were selected in each experiment.

System Performance Evaluation

The performance of the array-based Doppler system was tested by evaluating the lateral two-way beamwidth of the array with the beamformer, the minimum detectable velocity, the sensitivity, and the *in vivo* flow measurements.

First, the lateral two-way beamwidth of the array with 16 channel transmit and receive beamformer was measured using a wire phantom with five 20 μm tungsten wires arranged diagonally as the target (California Fine Wire, Grove Beach, CA, USA) (Cannata 2004; Huang and Shung 2005). The array and the wire phantom were mounted on a 3-axis positioning system (Burleigh Inc., Fishers, NY, USA), and placed in a water tank filled with deionized water. The wire phantom was moved laterally in 50 μm steps. The one channel beamformed RF data was digitized by the Gage A/D card with a sampling frequency of 200 MHz. The beam profile was plotted in Matlab-based software. The measured lateral two-way -6 dB beamwidths were compared to those estimated by $\lambda \times f_{\text{number}}$, where λ is the wavelength in water and f_{number} is the ratio between the transducer aperture and focal distance (Lockwood et al. 1996).

The minimum detectable velocity of the system was evaluated to demonstrate the systems capability in detecting slow velocities in the microcirculation. The same wire phantom, used in the lateral beamwidth measurements, was used to calibrate and evaluate the Doppler system with the 30 MHz array. Both the array and the wire phantom were placed in a tank filled with deionized water. The distance between the tungsten wire and the array was adjusted to be equal to the sample depth 8 mm. For this experiment the array was mounted on a step motor (Model RFT-57-102-B11, Parker Hannifin Corp., Rohnert Park, CA, USA) which traveled at a constant speed. Due to the expected low Doppler shift frequencies, the audio Doppler signals I_A and Q_A were digitized by the Gage A/D card at its lowest sampling frequency of 50 kHz. The signals obtained were down sampled and processed by Matlab.

The sensitivity of the system was evaluated in a micro-flow phantom experiment. The micro-flow phantom was used to simulate small vessels and low backscattered signals from blood in microcirculation. It consisted of a polyimide tube with an inner diameter of 127 μm and wall thickness of 20 μm (5707K11, McMaster-Carr Supply Company, Atlanta, GA, USA). The blood-mimicking fluid, which was prepared by diluting 1–5 μm spheriglass solid spheres (10004E, Potters Industries Inc., Carlstadt, NJ, USA) in deionized water, passed through the tube. The flow velocity in the tube can be adjusted by gravity (i.e. adjusting the height of the blood-mimicking fluid container). The system was then tested to determine its capability in measuring the flow velocities of the blood-mimicking fluids in the tube.

Finally, *in vivo* animal experiments were performed using approved protocols by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern California. Balb/c mice (Simonsen Laboratories, Gilroy, CA, USA) at an age of 4 weeks were anesthetized with pentobarbital sodium diluted in saline to 4 mg/ml given intraperitoneally at a dose of 10 $\mu\text{l/g}$ body weight. After being anesthetized, animal chest areas were shaved and

further cleaned with chemical hair remover to minimize ultrasound energy loss. Water was used as a coupling medium. A 50 MHz sector scanner developed at the Resource Center was used to produce real-time B-mode images for locating vessels, and thus position the array (Sun et al. 2007). The blood flow in two mouse abdominal superficial vessels with diameters of approximately 200 μm and a mouse aorta close to the heart was measured. The duration of the experiments were less than two hours. During the experiments, a heat lamp was used to maintain the body temperature of the mouse in order to ensure normal physiological activities. Following the procedure the animals were euthanized by placing them in a chamber with a carbon dioxide concentration of 60–70% for at least 10 minutes.

In all experiments, the sample gate was located at the depth of 8.0 mm and its sample volume was 7 cycles. An oscilloscope LeCroy 9350AL (LeCroy Corp., Chestnut Ridge, NY, USA) was used to monitor the sample gate and the beamformed echoes, ensuring that the sample gate was located at the correct positions of the wire, tube, or blood vessel.

RESULTS

Lateral Beamwidth Measurements

The lateral beam profile of 16 elements of the array at different depths was measured and illustrated in Table 2 and Fig. 6. The 16 channel transmit and receive beamformers were electronically focused at 4.8 mm, 6.4 mm, 8.0 mm and 9.6 mm respectively at each step. The lateral two-way -6 dB array beam-widths were measured from 160 μm to 320 μm at depths from 5–10 mm. The axial beam profile of the array is determined by the transmitted pulse length. 3–16 cycle pulses were used to obtain an adequate signal-to-noise ratio (SNR) in our experiments.

Minimum Detectable Velocity Measurement

The minimum detectable velocity of the Doppler system was determined to be 100 $\mu\text{m/s}$. The Doppler angle θ was 0° , the PRF was 228 Hz, and no wall filters were used during this measurement. As shown in Fig. 7, the measured Doppler shift frequency was approximately ± 4 Hz, and its corresponding velocity was ± 100 $\mu\text{m/s}$ which was the preset motor speed. The positive and negative velocities indicated that the array traveled either toward, or away from the wire phantom, respectively. The temporal resolution in Fig. 7 was 100 ms with a temporal overlap of 80%, and the velocity resolution was 5 $\mu\text{m/s}$. The measured up and down traveling velocities were slightly different. This difference may be caused by gravity and the mounting structure between the array and the step motor. The 100 $\mu\text{m/s}$ system minimum detectable velocity should be sufficient for most microcirculation studies (Christopher et al. 1997).

Micro-flow Phantom Tests

The 127 μm tube was submerged in water, and the system was used to measure the flow velocities in the tube. The PRF was set to 7.3 kHz, the Doppler angle θ was estimated as 45° , and the sound card was used. A software high-pass filter with a cut-off frequency of 5 Hz was applied to the digitized Doppler signals. As shown in Fig. 8, a velocity lower than 7 mm/s was measured from the tube, and its corresponding Doppler shift frequency was 200 Hz. The temporal resolution was 25 ms with a temporal overlap of 50%. The velocity resolution was 190 $\mu\text{m/s}$. This figure also shows strong Doppler signals which were caused by air bubbles in the solution.

In vivo Studies

Fig. 9(a) shows a B-mode image obtained by the 40 MHz sector scanner (Sun et al. 2007) as guidance to the array Doppler measurement. Two abdominal superficial vessels with diameters

about 200 μm were identified in that image. Fig. 9(b) shows the spectrograms of the detected Doppler signals in those vessels. The positive velocity corresponded to the Doppler signals from an artery due to its pulsatile profile, and the negative one corresponded to the Doppler signals from a vein because of the relatively constant velocity. The heart rate of the mouse was also estimated at approximately 300 beat/min based on the intervals between two velocity peaks. Fig. 10 shows the detected Doppler signals from an aorta close to the heart. Its peak velocity was over 1 m/s.

DISCUSSION AND SUMMARY

As an initial attempt, a HF pulsed-wave Doppler system with a 30 MHz linear array was implemented. The experimental results demonstrate the capability of the system in measuring blood flow *in vivo*.

However, the achieved lateral resolution of 160 μm may still be inadequate for a number of imaging applications. Either increasing the beamformer channel count, or increasing the center frequency of arrays would alleviate this problem. Both 64-channel beamformers and 128 element linear array with center frequency higher than 30 MHz are currently being developed. Further improving the SNR of the system is also critical both for obtaining superior B-scan images and blood flow measurements. Increasing the transmit voltage is a way to increase SNR, but it is not always feasible due to transducer heating issues. Decreasing the receiver's noise would be a more applicable solution. Carefully designed matched filters for RF signals, determined by both bandwidths of transducers and transmitted pulses, can optimize the SNR of the system (Jensen 1996). Grounding the entire system properly would also increase the SNR (Kester and Bryant 1996; Ott 1988). In addition, the duplex image mode (i.e. displaying B-mode images and Doppler spectrograms simultaneously) and color Doppler mode may be realized after updating the current hardware and software. Finally, further studies on developing improved high-frequency digital beamformers and digital signal processors (DSPs) will be pursued.

This paper reports the development of a HF pulsed-wave Doppler system to in conjunction with a 30 MHz linear array. In the *in vitro* and *in vivo* experiments, the system successfully detected the velocities as low as 0.1 mm/s, and as high as 1 m/s. The detection of blood flow in small vessels with diameters less than 200 μm shows a promising future of this system in small animal and human research.

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Fig. 1.
Block diagram HF ultrasound Doppler with array.

Fig. 2. Timing sequences of signals in the timing circuits and the bipolar pulser. A is the pulse repetition frequency, B is the sample gate indicating an appropriate sample depth and sample volume, C is a N-cycle trigger signal, D is a high-voltage pulse without delay, and E is a high-voltage pulse with delay.

Fig. 3.
Measured 7-cycle 30 MHz bipolar pulse (solid line) and its spectrum (dashed line).

Fig. 4.
Block diagram of HF programmable analog delay circuits in the analog beamformer.

Fig. 5. Calculated (lines) and measured (solid triangle) delay times of each channel in the analog beamformer.

Fig. 6. Measured two-way beam-widths of the 30 MHz array with 16-channel transmit and receive beamformers at the depths of 5.0 mm, 6.6 mm, 8.0 mm, and 9.5 mm.

Fig. 7.
Doppler spectrogram showing minimum detectable velocity of 0.1 mm/s using the wire phantom.

Fig. 8. Doppler spectrogram showing detectable velocity lower than 7 mm/s in a 127 μm tube filled with blood-mimicking fluid.

Fig. 9. B-mode image of two abdominal superficial vessels with diameters about $200\ \mu\text{m}$ obtained by the 50 MHz sector scanner (a), and the detected Doppler signals from the vessels (b).

Fig. 10.
Detected Doppler signals from an aorta closed to the heart.

TABLE 1

Measured Characteristics of the 30 MHz Array.

Characteristic	Value
Number of elements	64
Pitch	100 μm
Element dimensions	2 mm $\times$ 85 μm
Average center frequency*	29.8 MHz
Highest/lowest center frequency*	30.6/28.8 MHz
Average bandwidth*	56 %
Highest/lowest bandwidth*	61%/52%
Average sensitivity*	1.51 V
Highest/lowest sensitivity*	1.63/1.35 V
-20 dB pulse length*	92 ns (typical element)
Insertion Loss	16 dB (typical element)

* Panametrics 5900PR (Olympus NDT Inc., Waltham, MA) with the setup of 1 μJ energy and 20 dB amplification were used in the measurements.

TABLE 2

The Two-way Array -6 dB Beam-widths produced using the 16 Channel Beamformer.

Focal Distance	Expected ($\lambda \times f_{number}$)	Measured
5.0 mm	154 μm	160 μm
6.6 mm	204 μm	220 μm
8.0 mm	247 μm	275 μm
9.5 mm	293 μm	310 μm

The value of c was assumed as 1485 m/s in water and blood-mimicking fluid (Christopher et al. 1997).