Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2020 May ; 67(5): 934–942. doi:10.1109/TUFFC.2019.2960211.

High-Frequency Multipulse, Plane-Wave Acoustic Contrast Imaging

Jeffrey A. Ketterling [Senior Member, IEEE],

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

Ronald H. Silverman [Senior Member, IEEE]

Department of Ophthalmology, Harkness Eye Institute, Columbia University Irving Medical Center, New York, NY 10032 USA.

Abstract

Multipulse (MP) ultrasound contrast agent (UCA) imaging is a method to increase the contrast-to-background (CBR) ratio in regions of blood flow. Plane-wave imaging allows high frame rates, and with high-frequency ultrasound, fine-spatial and temporal resolution. MP and plane-wave imaging have not been applied to high-frequency ultrasound. Here, an 18-MHz linear array was employed to implement the MP methods of pulse inversion (PI) and amplitude modulation (AM) using high-speed, multiangle, compound plane-wave imaging. A flow of the UCA DEFINITY[®] at a dilution ratio of 2000:1 circulating through a 2-mm-diameter flow channel in a tissue-mimicking phantom was used to characterize CBR and compared with cases of standard, multiangle compound plane-wave imaging. The relative improvement of PI and AM versus standard plane-wave imaging ranged from 5 to 10 dB. The CBR was observed to be stable over a 60-min time duration for a 2000:1 dilution ratio and a 2000:1 dilution ratio provided an optimal CBR.

Keywords

High-frequency ultrasound; microbubbles; multipulse imaging; plane-wave imaging

I. Introduction

An ultrasound transmission in tissue generates minimal higher order harmonics, whereas gas-filled, shell-encapsulated ultrasound contrast agents (UCAs) can be excited to generate subharmonics and higher order harmonics. The nonlinear response of UCAs have been utilized successfully to improve contrast between flowing UCAs and tissue [1], [2]. At the simplest level, the backscatter signal can be filtered in the band of the subharmonic or second harmonic [3], [4]. The subharmonic has the advantage of solely deriving from the UCA backscatter, but the reduction in frequency results in a decrease in resolution. The higher order harmonics have the advantage of improved resolution, but harmonics develop from nonlinear propagation and acoustic attenuation is increased, which results in a reduced

penetration depth. As the transmitted signal is often broadband, there is a fair amount of overlap between the transmission band and the harmonic content making it difficult to use a simple high-pass filter to detect solely the harmonic content.

Multipulse (MP) transmission approaches have been employed, which take advantage of the pressure- or phase-dependent response of UCAs [5] and have been used to improve the contrast-to-tissue ratio. Broadly, the methods are categorized as pulse inversion (PI) [6] or amplitude modulation (AM) [7]. A simple PI sequence would consist of two transmissions of opposite polarity (+1, -1). When the backscattered waveform pair are summed from linear scatterers (i.e., tissue), the residual signal approaches zero with some inherent weak nonlinear propagation that shifts energy to higher frequency components. When the backscatter is from UCAs at sufficient acoustic drive pressure, the fundamental component is reduced, but the nonlinear even harmonics will remain because the responses from the PI pulse pairs are not symmetric. A simple AM sequence would consist of a transmission of amplitude 1 and a transmission of amplitude 1/2. The 1/2 amplitude backscatter component is doubled and then subtracted from the +1 amplitude transmission. This approach retains the even and odd harmonics.

Nonlinear imaging approaches ideally require a transducer with sufficient bandwidth to detect energy at the desired frequencies or a compromise can be made by shifting the transmit frequency toward one edge of the bandwidth. When using single-element transducers [4] or pairs of transducers [8], [9], the bandwidth limitation can be more readily overcome. In practice, linear and phased arrays are the most common transducers employed for clinical imaging in the low megahertz range. Nonlinear imaging methods are routinely performed with these transducers [10], but their sound fields are more complicated than single-element transducers and the available bandwidth can potentially be an issue, particularly above the second harmonic. However, bandwidth limitations do not rule out nonlinear imaging approaches [11].

Frame-rate limitations imposed by MP nonlinear imaging modes can be overcome by using plane-wave approaches [11]–[13]. The unfocused nature of coherent wavefront transmissions reduces peak pressures relative to traditional line-by-line image formation, but nonlinear imaging generally requires lower pressures in order to avoid UCA destruction. UCAs are usually optimized for imaging in the low megahertz frequencies where most clinical imaging is performed with the peak UCA size distribution in the 1–3- μm -diameter range. However, UCAs typically have a wide size distribution and some fraction of agents will be smaller and will respond to frequencies above 10 MHz [14], [15]. These frequencies are of interest for small-animal and small-parts imaging [16]. MP, high-speed plane-wave imaging approaches at high frequencies have yet to be explored.

Various transmission approaches have been utilized to detect and localize UCAs at high frequencies. Goertz *et al.* [4] used a single-element transducer to transmit at 20 and 30 MHz and perform harmonic imaging. Needles *et al.* [17] used a 22-MHz linear array with PI and AM methods. Deng *et al.* [18] used a 10-MHz single-element transducer to detect changes in UCA backscatter when modulated at 0.5 MHz and this method, termed radial modulation imaging [19], was later utilized with a 20-MHz intravascular catheter [20]. A similar dual

transducer approach, termed acoustic angiography [21], has been used to detect the harmonic backscatter content with a low-frequency excitation and high-frequency receive, which has the effect of suppressing the tissue backscatter. Errico *et al.* [22] used high-speed plane-wave imaging and singular value decomposition to localize individual UCAs at a sparse concentration at 15 MHz.

Here, we evaluated the MP techniques of PI and AM using an 18-MHz linear array with high-frequency, high-speed, multiangle plane-wave excitation in order to assess the level of tissue suppression relative to a flow of the UCA DEFINITY[®] (Lantheus Medical Imaging, Inc., N. Billerica, MA, USA). A circular flow of diluted UCA was established in a wall-less phantom with a 2-mm-diameter channel. The contrast-to-background ratio (CBR) of the flow relative to the tissue-mimicking background material of the phantom was quantified. The transmission parameters of drive pressure, pulse repetition frequency (PRF), and drive frequency were varied. The CBR as a function of experiment time duration and UCA concentration was also characterized. Acoustic pressures were measured with a hydrophone, and a wire target was used to characterize the pulse-echo response and to validate the phase and amplitude of the transmitted waves.

II. Methods

A. Ultrasound System

Data were acquired with a Vantage 128 (Verasonics, Redmond, WA, USA) and an 18-MHz, 128-element linear array with an 8-mm elevation focus (Verasonics L22-14v). The array had a 100- μm pitch, an 80- μm element width, and a 1.5-mm element length. The transducer was attached to an automated three-axis motion system controlled via a LabVIEW interface (National Instruments [NI], Austin, TX, USA). The motion system permitted precise control of the transducer position relative to the flow phantom and assisted with acoustic field characterization.

A MATLAB (The MathWorks Inc., Natick, MA, USA) script was created that permitted a sweep through parameters of interest, such as PRF, transmit frequency, transmit voltage, or transmit angles of a plane-wave batch. At each parameter combination, a predetermined number of plane waves were transmitted and the backscattered, raw radio frequency (RF) data were saved to the hard drive of the control computer. Changes to the method of MP transmission or the number of plane waves in a batch required the script to be restarted. Data were acquired at a sampling rate of 62.5 MHz and a 1–31-MHz digital bandpass filter was used on receive. The receive signal underwent a depth-dependent gain.

Three modes of operation were used for imaging: standard multiangle plane-wave imaging with batches of transmissions sweeping a defined angle range, PI with a +1 and –1 amplitude transmission pair at each angle, and AM with a +1 and +1/2 amplitude transmission at each angle. The 1/2 amplitude was achieved using an apodization weighting of 0.5 on transmit. The apodization approach permitted an arbitrary PRF to be used, whereas directly adjusting the transmit voltage limits the PRF to ≈ 1 kHz or lower in order to achieve a stable voltage. The scaling apodization was accomplished with pulsewidth modulation, and this was not expected to be a perfect 1/2 replica of the +1 amplitude waveform. For the AM

case, the $+1/2$ transmission was doubled in amplitude and inverted on receive (e.g., converted to -1 equivalent) in order to simplify the postprocessing beamforming.

The total angle sweep of transmissions was chosen to be 20° . For the multiangle compound plane-wave cases, three $[-10 \ 1 \ 10]$ and six angles $[-10 \ -6 \ -2 \ 2 \ 6 \ 10]$ were utilized. For the AM and PI cases, the transmissions were at $[-10 \ 1 \ 10]$ degrees with a transmission pair at each angle. A zero-degree transmission was not employed because the particular transducer we used had a floating ground plane that resulted in a significantly reduced transmit pressure when all the elements were excited in phase.

B. Contrast Agent

Experiments were performed with the perflutren lipid encapsulated UCA DEFINITY[®]. The agent was prepared as recommended by the manufacturer using an agitator (Vialmix, Lantheus Medical Imaging, Inc., North Billerica, MA, USA) for a 45-s duration. UCA was drawn just after preparation from the vial with an 18-gauge needle with a second needle inserted in the rubber stopper of the vial to avoid creating a partial vacuum while drawing a sample. Size distribution has been reported to be primarily in the $1\text{--}3\text{-}\mu\text{m}$ range with 1.6×10^{10} bubbles/mL [23]. Prior to an experiment, 0.1 mL of DEFINITY[®] was drawn into a syringe and then diluted into a 200-mL reservoir of filtered water (2000:1 ratio). The water was degassed to just below full atmospheric saturation to avoid over saturation that could result in gas content nucleating on surfaces and to avoid any potential gas diffusion out of the UCAs that can occur in fully degassed water.

C. Acoustic-Field Characterization

The sound field of the linear array was characterized with a calibrated $40\text{-}\mu\text{m}$ needle hydrophone (Precision Acoustics Ltd., Dorset, U.K.). The calibration range was $1\text{--}40$ MHz and signals were sampled at 250 MHz. The three-axis positioning system was used to place the hydrophone at the center of the array at an 8-mm axial distance in the location of the peak elevation pressure. An output trigger synchronized to the transmitted plane waves was used to trigger an acquisition on an external digitizer (PXI-5152, NI). Transmit/receive waveforms were acquired from $25\text{-}\mu\text{m}$ -diameter wire placed in the center of the linear-array acoustic field at an 8-mm axial distance. The pressure versus drive voltage, acoustic-field dimensions, and sensitivity versus drive frequency were quantified.

D. Flow Phantom

A custom wall-less flow phantom with five linear channels ($1\text{--}4$ mm diameters) was used to simulate flow in a vessel (CIRS, Norfolk, VA, USA). The tops of the channels were situated 2.75 mm below the phantom surface. Only the 2-mm channel was employed for these studies. The phantom material was a proprietary urethane rubber compound with $30\text{-}\mu\text{m}$ glass particles. The speed of sound was 1460 m/s and, at 15 MHz, attenuation was estimated to be $1.8\text{--}2.5$ dB/cm/MHz [24]. This material has a nonlinear relation between attenuation (α) and frequency (f): $\alpha = 0.18 * f^{1.83}$ [24]. Before entering the flow channel, the acoustic pressure was attenuated by $7\text{--}10$ dB at 15 MHz.

A reservoir of fluid was placed on a stir plate and a recirculation flow through the channel was established with a micropump (REGLO-Z Digital ISM901B, Ismatec, Glattbrugg, Switzerland) and suction flow head (GA Series, Micropump, Inc., Vancouver, WA, USA). The flow phantom contained a reservoir above the top surface and this was filled with degassed water. The phantom was placed at a slight upward tilt in the downstream flow direction to reduce the likelihood of UCA building up at the top surface of the flow channel. The elevation focus of the linear array (8 mm) was placed at the center of the channel and the flow passed through the image plane in the lateral direction. A single flow rate of 6 mL/min was used, which corresponded to an average flow velocity of 3.2 cm/s. Because the flow channel was within the image plane, data were acquired with the phantom shifted such that only the phantom material was in the image plane. The backscatter from the flow region was referenced against a region in background material at the same depth to calculate a CBR.

The flow phantom was also used to characterize the residual-to-noise ratios (RNRs) of MP approaches versus standard multiangle, plane-wave, compound imaging. For these measurements, the 2-mm flow channel passed through the image plane of the transducer. The channel was filled with water to provide an anechoic inclusion within the image plane. Here, a lower RNR is sought for MP methods, which indicates that the linear background signal approaches noise. Finally, the phantom was used to characterize the residual signal as a function of drive voltage for PI and AM by processing a region of the background material at the flow depth.

E. Data Processing

Raw RF data were postprocessed with a graphic processing unit (GPU)-based (RTX-2080Ti, NVIDIA Corporation, Santa Clara, CA, USA) algorithm that generated beamformed data [25]. A GPU codec was compiled as a MATLAB executable (MEX) file. Beamformed RF data were generated to maintain the sampling rate of the original data (62.5 MHz) and 256 RF lines were formed (50- μ m spacing between the lines).

After beamforming, rectangular regions of interest (ROIs) (0.7 mm $\times$ 4.8 mm, 5280 pts) were defined within the center of flow region and at the same depth within the background material. The root mean square (rms) of the RF data in the ROI was calculated. Absolute magnitudes were compared and the ratio of flow-to-background provided a CBR.

ANOVA and post-hoc Tukey tests (OriginLab 2019, Northampton, MA, USA) were used to determine whether significance existed between the CBR values for MP and standard multiangle, plane-wave imaging cases. Significance was defined as $p < 0.05$. A total of $N = 15$ separate experimental runs were collected. Each experimental run for a collection of standard MP plane wave, PI, and AM data took approximately 5 min.

RNR in the water-filled flow channel was calculated using the same method as would be utilized to characterize contrast-to-noise ratio, namely

$$\text{RNR} = \frac{|\mu_b - \mu_s|}{\sqrt{\sigma_b^2 + \sigma_s^2}} \quad (1)$$

where μ_b and μ_s were the rms values of the RF envelope within an ROI in the background material and flow channel, respectively, at the same depth. σ_b and σ_s were the corresponding standard deviations within the ROIs. A value of zero would mean that the residual is equal to noise.

III. Results

A. Acoustic Field

The acoustic pressure as a function of drive voltage, transmission angle, and polarity of transmission is shown in Fig. 1. The positive (+1) transmissions at a collection of angles are shown as lines, while the inverted (−1) transmissions are shown as symbols with matching color. The drive voltages were 2, 5, 10, 15, 20, and 25 V. Only a single location was measured at the center of the transducer at a distance of 8 mm. This was not the maximum pressure in the axial direction but was the location of the elevation focus. The peak negative pressures of the +1 cases matched closely to the −1 cases up to 15-V drive, and then, the −1 cases were more negative, on average, by 3%.

The transmit-to-receive frequency response of the array for defined transmission frequencies between 8 and 24 MHz was measured using a 25- μm -diameter wire as a target (see Fig. 2). These data were acquired with a bandpass of 1–31 MHz. The peak transmit/receive echo amplitude occurred with a transmit frequency of 14 MHz and then decreased on either end of the bandwidth. At 8 and 24 MHz, the transmit/receive sensitivity was down by 13 dB from that at 14 MHz. The peak amplitude receive frequency and the −6-dB bandwidth receive center frequency are also plotted as a function of transmit frequency as is a solid line representing a one-to-one relationship between the defined transmit frequency and the receive center frequency. The peak-amplitude frequency component and the −6-dB center frequency on receive were a very close match across the transmit frequency range, which indicates that the spectrum was symmetric around the center frequency.

Raw time-domain receive waveforms from a 25- μm wire target for a PI and AM transmission are shown in Figs. 3 and 4. The PI waveforms (see Fig. 3) show the +1 (solid line) and −1 (dashed line) amplitude transmission for a -10° and 10 V (0.85 MPa) transmission. The waveforms, after inverting the receive signal, show a close match. The difference between the two waveforms reveals the residual (dotted line). A small residual remains as a result of some nonlinear propagation in water and small shifts in the time-domain signal from vibration, sample clock jitter, and on-perfect matching of the transmit waveforms.

Fig. 4 shows the transmission pair for an AM scheme with a -10° transmission and 10-V excitation. The transmissions were at 1 (solid line) and 1/2 (dashed line) amplitude, but the 1/2 amplitude was received with a gain of −2 in order to simplify postprocessing. As in the PI case, the pair of signals shows the inverted relation after the −2 gain and the 1/2

amplitude was, as intended, one-half of the +1 case. The residual (dotted line) shows significant cancellation overall, but not as good as the PI case. Comparing the +1 cases from the AM and PI transmissions reveals that they are not exact replicates, which provides another source for increased residual because of small variations in pulse-to-pulse waveforms.

B. Residual Characterization

Fig. 5 quantifies the RNR as a function of transmit frequency. The values represent an average of five measurements. The standard six-angle and three-angle transmission cases had a uniform RNR across the transmit frequencies with the six-angle case ≈ 0.16 higher, on average, because the three additional transmissions reduced the noise amplitude in the anechoic region. The PI case had low RNR above 10 MHz revealing good cancellation of the linear background material. The AM case retained a larger portion of the fundamental and had an RNR lower than the standard imaging cases but well above PI beyond 10 MHz.

Fig. 6 characterizes the residual signal as a function of drive voltage for PI and AM at three transmission angles. An ROI in the background material at the same depth of the flow was processed to find the mean of the rms of the individual PI and AM pulse pairs and the rms of the residual. A value of 0 dB would indicate that the residual signal peak amplitude was the same as the mean of the individual pulse pairs. For all drive voltages, PI and AM led to a decrease in intensity. The residual signal decreased as drive voltage increased and the SNR of the individual transmit signals improved. As was seen in Fig. 5, PI suppressed the linear signal component more effectively than AM.

C. Contrast Flow Enhancement

Fig. 7 shows the representative B-mode images of the contrast flow for the three transmission cases: standard multiangle compounding, PI, and AM. The standard case with three transmissions angles [see Fig. 7(a)] had a similar image intensity for the flow and the background material. The PI case [see Fig. 7(b)] showed increased contrast between flow and background with the background noticeably dropping in intensity relative to flow. The AM case [see Fig. 7(c)] also had an increase in contrast between the background and the flow, but the change was less pronounced than for the PI case, which is consistent with the results in Figs. 5 and 6.

The changes in contrast for the multiangle, PI, and AM transmission cases over the range of acoustic drive pressures are summarized in Fig. 8. The intensity of the background signal was seen to increase with acoustic drive pressure as would be expected [see Fig. 8(a)]. The standard imaging case had the highest intensity and provided the zero dB reference for the absolute intensity scales. Above 10-V excitation (0.32-MPa derated), the PI case was ≈ 23 dB below the standard case and the AM case was ≈ 16 dB below the standard.

The set of curves for the intensity in the flow region for UCA and water flow is shown in Fig. 8(b). The region of flow was visible against the phantom background at all acoustic drive pressures. As in the background case, the standard multiangle plane-wave sequence had the highest intensity and the intensity increased with drive pressure. At the lowest drive pressure where the nonlinear UCA response was minimal, the MP residual for UCA flow

was slightly above the water flow. Above 2 V (0.05-MPa derated), the AM case was ≈ 7 dB below the standard case and the PI case was 13 dB below the standard case. The PI intensity was about 6 dB below the AM intensity, indicating that, for our particular experimental system, the PI approach was more effective at canceling the linear signal components. This was consistent with some past reports that showed the AM residual retained a greater portion of the fundamental than the PI residual [9].

Fig. 8 contains a second pressure scale bar that accounts for the 2.75 mm of attenuation in the tissue-mimicking material. The derated scale bar represents an 8.6-dB decrease in acoustic pressure (factor of 2.7) and is an estimate of the attenuated pressure that was incident on the near edge of the UCA flow.

Of particular interest is the CBR [see Fig. 8(c)] as this represents the contrast to improve localization of *in vivo* contrast flow. The standard multiangle plane-wave imaging case maintained a constant CBR of -3 dB. The CBRs of PI and AM with water flowing in the channel were below those with UCA flow at all pressures except for the lowest pressure of 0.05-MPa derated. At the lowest drive pressures, the nonlinear response for UCA flow was weak and the CBRs were similar to the standard multiangle case. The PI and AM cases showed a 5–10-dB enhancement of the flow region relative to background for derated pressures above 0.3 MPa. The ANOVA and Tukey test analysis of the three means revealed that the means of the PI and AM cases were statistically different from the three-angle transmission case for derated pressures above 0.3 MPa ($p < 10^{-4}$).

In order to simulate a variation in flow velocity, the absolute transmission PRF was varied from 0.3 to 18 kHz. Fig. 9 shows the CBR as a function of the absolute PRF (PI with six transmissions at an absolute PRF of 6 kHz has a 1-kHz effective frame rate after beamforming). A decreasing PRF simulates an increasing flow velocity. The CBR was observed to be flat for the case of standard multiangle plane-wave transmission. As in Fig. 8(c), the 10-V PI and AM cases had similar CBRs. However, below 1.5 kHz, CBR increased for the AM and PI cases. As the background was the same for each case, the change was due to the flow region. As the PRF decreased, the transmission-to-transmission correlation reduced such that the fundamental signal components no longer canceled efficiently. As the PRF increased, the correlation reached a steady state and the CBR remained constant.

D. Spectra

Examples of the backscatter spectra for a low and high PRF are shown in Fig. 10 where the spectra were normalized to the number of transmissions and 0 dB was the maximum value of the standard imaging sequence. A 1.5-cycle, 16-MHz, 10-V (0.32 MPa derated) transmission was used. At a low PRF of 750 Hz [see Fig. 10(a)], the spectra for PI and AM nearly overlapped and the fundamental frequency decreased by 7 dB relative to the standard imaging sequence. At an absolute PRF of 6 kHz, the PI spectrum was 21 dB below the standard transmission at the fundamental frequency and the AM spectrum was 15 dB below the standard three-angle plane-wave transmission spectrum. The increased correlation between transmissions at high PRF reduced the fundamental component in the PI and AM transmissions.

IV. Discussion

The frequency response of the linear array used in these studies did not allow an ideal broadband frequency range that permitted a second harmonic or subharmonic to be readily detected. As was shown in Fig. 2, the -6 -dB bandwidth was $\approx 67\%$. At $\approx$ Transmit/receive frequency the upper end of the bandwidth peaked around 18 MHz. Transmitting at a frequency below 10 MHz would result in a reduced energy and second-harmonic sensitivity would be low.

The results reported here can be compared against past experimental work in phantoms at a range of frequencies although it is important to stress that results may not represent the maximum contrast attainable because experimental systems often cannot generate perfectly matched waveforms. We are not aware of any plane-wave MP excitation results in the 15-MHz range. Kusunose and Caskey [13] used in-house UCAs with 2-MHz plane waves and saw a CBR of 10 dB with PI, while the standard plane-wave imaging had a CBR around 4 dB for a relative change of 6 dB. Viti *et al.* [11] used Bracco BR-14 (Bracco Research S.A., Geneva, Switzerland) with 3.5-MHz plane waves and saw a CBR of 25 dB using AM but did not use a PI scheme. Couture *et al.* [26] used Bracco NA3507 experimental agent with 7.5-MHz plane waves and saw up to a 16-dB CBR using a combined PI and AM approach. Needles *et al.* [17] utilized a linear array with focused transmits using the UCA MicroMarker (FUJIFILM VisualSonics, Inc., Toronto, ON, Canada) and saw a CBR in the 18-MHz fundamental band of 12 dB with standard imaging, 15 dB with PI, and 24 dB with AM. For a 21-MHz transmission and standard imaging, the CBR was 10 dB in the 21-MHz fundamental band, 15 dB with PI, and 20 dB with AM. Other reports of CBR with 2 MHz single-element transducer showed a 4-dB CBR increase of AM relative to PI [9].

We utilized in-plane flow for our experiments and CBR was calculated using a background region at the same depth as the flow. Our CBR values above a derated pressure of 0.3 MPa were between 8 and 13 dB. This represented a relative improvement versus a standard three-angle plane-wave transmission case of between 5 and 10 dB. These results were very close to the 6-dB relative improvement. Kusunose and Caskey [13] observed for PI also using a Verasonics system with plane-wave imaging, but no AM results were reported. The relative change in CBR between standard imaging and an MP approach helps normalize the results because phantoms and experimental parameters vary across reports in the literature. Although there are differences in exposure parameters, phantoms, and measurement methods, the results reported here are consistent with past experimental work [11], [13], [17], [26].

While the AM results showed an improved CBR relative to the three-angle, compound plane-wave transmission cases, AM did not outperform PI as is frequently reported in the literature [9], [17]. As noted in Fig. 4, the AM transmission waveforms were not ideally matched to minimize the residual, whereas the PI pulse pair (Fig. 3) was a close match. The impact on background suppression was observed in Figs. 5 and 6. The AM cases did not remove as much fundamental signal as theoretically possible and the results presented here do not represent the optimal performance possible with AM at high frequency. As noted earlier, the high PRF of these experiments imposed limitations on how the $1/2$ amplitude

AM transmissions were generated, resulting in a good match in the 1/2 peak amplitude but not the overall time signal.

The results show that MP techniques can still be effective even when the transmit waveforms are not ideal, the transducer is not ideal or when the transmit frequency is much higher than what the UCAs were designed for. Due to the wide size distribution, the backscattered signal has a rich harmonic and fundamental content throughout the bandwidth and it is not a requirement to filter and detect just the subharmonic or higher harmonic content. Filtering in the bandwidth of the transducer still revealed nonlinear residuals in the PI and AM receive signals. Viti *et al.* [11] reached a similar conclusion using a 4.6-MHz linear array with 104% bandwidth as have others [13], [27].

Fig. 9 shows that the transmission rate has an impact on the CBR. For high PRFs, the equivalent of slow flow, transmission-to-transmission correlation becomes strong and the linear signal components canceled efficiently [see Fig. 10(b)]. When the PRF was too low relative to the flow, the backscatter decorrelates, with less linear signal cancellation [see Fig. 10(a)] which has the effect of increasing CBR. Couture *et al.* [26] noted a similar increase in CBR after decorrelation from UCA destruction induced by a linear array with increasing drive pressure, and Lin *et al.* [28] reported an increased CBR with a low PRF using a second-harmonic PI approach with a linear array at 1.7 MHz.

While this may appear to reduce the rationale for MP techniques, real-world imaging takes place *in vivo* and the tissue component of the signal experiences some decorrelation unlike the benchtop setup used here where the background material was static. In addition, MP techniques are often implemented in Doppler imaging schemes [6], and this requires transmission rates sufficiently high to avoid aliasing. One means to reduce the chance of decorrelation is to use two PRFs: one for interpulse transmission and the other for the effective frame rate. The interpulse transmission PRF can be chosen to be just above the round-trip propagation time that represents the minimum time between the transmissions.

As was noted in [26], UCA bubble destruction can increase the apparent CBR. We did not observe any bubble destruction or radiation force effects as drive pressure increased to a maximum peak-negative pressure of 0.87 MPa (derated). Bubble destruction was evaluated by acquiring data over a 50-ms time duration and analyzing the rms backscatter as a function time. For all drive pressures, the rms backscatter was constant over the acquisition time duration. If UCA were destroyed, the signal would have decreased over time. Because we had in-plane flow, we would have also observed speckle movement in the direction of acoustic propagation if there had been radiation force effects. The lack of observed UCA destruction was consistent with the inertial cavitation thresholds reported in the literature. King *et al.* [29] observed an inertial cavitation for DEFINITY[®] of 0.68 MPa at 2.8 MPa, 1.63 MPa at 4.6 MHz, and 2.1 MPa at 7.1 MHz; Chatterjee *et al.* [30] found a threshold of 1.2 MPa at 5 MHz. We would expect at 16 MHz a threshold of >2 MPa, well above the pressures we utilized.

Another potential source of uncertainty was the time duration of an experiment with a single UCA mixture. To assess any impact the time duration of an experiment and the UCA

concentration had on an experiment, data were acquired at a concentration of 2000:1 over a 1-h period and at dilutions ratios from 1000:1 (1/2 $\times$) to 32 000:1 (16 $\times$).

Fig. 11(a) shows the CBR as a function of time for standard multiangle plane-wave imaging, PI, and AM excitations with a 15-V excitation (0.6-MPa derated). Over the 60 min, there was not an obvious decreasing trend for the curves. Fig. 11(b) shows the CBR as a function of dilution ratio for a 15-V excitation. From the 1 $\times$ to 4 $\times$ dilution ratios, there was no obvious change in CBR. As the dilution increased to 16 $\times$, the CBR began to show a decreasing trend. Increased attenuation and the resulting decrease in CBR was seen for the 1/2 $\times$ dilution case. These results were consistent with those from Moran *et al.* [31] who showed about a 6 dB decrease in backscatter for 10 $\times$ decrease in concentration and minimal change in backscatter over a 60-min period for a fixed concentration. We can conclude that the experimental results remain within the expected error bars for a 30–60-minute interval as gradual changes in concentration and size distribution occur.

The material of our flow phantom was designed for low megahertz where attenuation is 0.5 dB/MHz/cm. The nonlinear nature of the material results in a much higher attenuation at 15 MHz (1.8–2.5 dB/cm/MHz [24]) and resulted in a large signal loss ($\approx$ 8 dB) between the phantom surface and the edge of flow channel. However, the CBR of PI and AM were relatively flat at the higher range of pressures used here, which would indicate increased acoustic pressure would not necessarily improve CBR.

V. Conclusion

With the emergence of high-frequency ultrasound and plane-wave imaging for small-animal and human-subject applications, there is a growing interest in combining these methods with UCAs. MP imaging approaches provide a means to improve CBR to better localize blood flow for perfusion, contrast or Doppler imaging. Here, a linear array with a nominal 18-MHz center frequency was used to compare standard multiangle plane-wave compounding to PI and AM MP approaches. The UCA used in these studies was DEFINITY[®] which, although designed for 2–5 MHz use, had a substantial population in the submicrometer size range and has been shown to be well suited to high-frequency applications [4], [32]. We found that MP methods resulted in a 5–10-dB improvement in CBR relative to standard plane-wave imaging. PI and AM performed similarly except at higher pressures where PI had a CBR of $\approx$ 3 dB higher than AM. The experimental results were found to be relatively insensitive to the time duration of the experiments and dilution changes on the order of 4 $\times$ did not impact the results.

The experiments conducted here were benchtop and highly controlled. While the results point to the potential to implement MP, high-frequency, plane-wave UCA imaging *in vivo*, further experiments would be needed to fully validate the approach and, for the AM case, to improve the matching of the 1 and 1/2 amplitude transmissions. Needles *et al.* [17] demonstrated MP techniques could be implemented with traditional linear-array beamforming so the approach is likely to be effective with plane-wave imaging. The PI and AM methods implemented here were simple in that they only involved transmission pairs of (+1, -1) or (1, 1/2). Combinations of the approaches (i.e., PIAM) have been shown to further

improve CBR and provide an additional path to explore for high-frequency, MP, plane-wave UCA imaging [9], [17].

Acknowledgment

The authors would like to thank J. Mamou for abundant and fruitful discussions. They would also like to thank B. Yiu and A. Yu for providing the GPU beamforming codec.

This work was supported in part by the National Institutes of Health under Grant HD097485, Grant EB022950, Grant EY028550, Grant EY025215, and Grant P30 EY019007, in part by the Investigator Sponsored Trial Grant from Lantheus Medical Imaging, Inc., and in part by an unrestricted grant to the Department of Ophthalmology, Columbia University, from Research to Prevent Blindness.

Biographies

Jeffrey A. Ketterling (Senior Member, IEEE) was born in Seattle, WA, USA. He received the B.S. degree in electrical engineering from the University of Washington, Seattle, in 1994, and the Ph.D. degree in mechanical engineering from Yale University, New Haven, CT, USA, in 1999. His thesis focused on experimental studies of phase-space stability in single bubble sonoluminescence.

He joined Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY, USA, in 1999, where he is currently the Associate Research Director. He serves as a Principal Investigator for programs supported by the National Institutes of Health that deal with high-frequency annular arrays for small-animal and ophthalmic imaging, acoustic contrast agents for microcirculation imaging, vector-flow imaging of blood-flow patterns in animal models, high-speed plane-wave imaging, and hydrophone arrays for characterizing the instantaneous acoustic fields of lithotripters.

Dr. Ketterling was the Technical Chair of the Biomedical Acoustics Committee of the Acoustical Society of America from 2008 to 2011. He is currently the Chair of the Medical Ultrasonics Technical Program Committee of the IEEE International Ultrasonics Symposium.

Ronald H. Silverman (Senior Member, IEEE) was born in New York, NY, USA. He received the B.S. degree in chemistry from Brooklyn College, New York, in 1971, and the M.S. degree in bio-engineering and the Ph.D. degree in computer science from Polytechnic University in Brooklyn, Brooklyn, NY, USA, in 1979 and 1990, respectively. His doctoral

thesis focused on the use of neural networks for pattern recognition and classification in biomedical ultrasound images.

He worked at the Department of Ophthalmology, Cornell University Medical Center, New York, from 1982 to 2010. He worked closely with D. Jackson Coleman, MD, and in collaboration with F. Lizzi of the Riverside Research Institute, New York, on ultrasound imaging and therapeutic applications to the eye. Since 2010, he has been with the Department of Ophthalmology, Columbia University Irving Medical Center, New York, where he is currently a tenured Professor of ophthalmic science. His work continues to focus on ophthalmic ultrasound applications, especially plane-wave imaging of blood flow in humans and preclinical models.

References

- [1]. Phillips PJ, "Contrast pulse sequences (CPS): Imaging nonlinear microbubbles," in Proc. IEEE Ultrason. Symp, 10 2001, pp. 1739–1745.
- [2]. Dave JK, Donald MEM, Mehrotra P, Kohut AR, Eisenbrey JR, and Forsberg F, "Recent technological advancements in cardiac ultrasound imaging," *Ultrasonics*, vol. 84, pp. 329–340, 3 2018 [Online]. Available: <http://www.sciencedirect.com/science/article/pii/S0041624X1730344X> [PubMed: 29223692]
- [3]. Miller D, "Ultrasonic detection of resonant cavitation bubbles in a flow tube by their second-harmonic emissions," *Ultrasonics*, vol. 19, no. 5, pp. 217–224, 1981.
- [4]. Goertz DE et al., "High frequency nonlinear B-scan imaging of microbubble contrast agents," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 52, no. 1, pp. 65–79, 1 2005. [PubMed: 15742563]
- [5]. Morgan KE, Allen JS, Dayton PA, Chomas JE, Klibaov AL, and Ferrara KW, "Experimental and theoretical evaluation of microbubble behavior: Effect of transmitted phase and bubble size," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 47, no. 6, pp. 1494–1509, 11 2000. [PubMed: 18238696]
- [6]. Simpson DH, Chin CT, and Burns PN, "Pulse inversion Doppler: A new method for detecting nonlinear echoes from microbubble contrast agents," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 46, no. 2, pp. 372–382, 3 2002.
- [7]. Brock-Fisher GA, Poland MD, and Rafter PG, "Means for increasing sensitivity in non-linear ultrasound imaging systems," U.S. Patent 5577505 A, 11 26, 1996.
- [8]. Goertz DE, Wong SWS, Chin CT, Cherin E, Burns PN, and Foster FS, "Non-linear scattering from microbubble contrast agents in the 14–40 MHz range," in Proc. IEEE Ultrason. Symp, 10 2001, pp. 1747–1750.
- [9]. Eckersley RJ, Chin CT, and Burns PN, "Optimising phase and amplitude modulation schemes for imaging microbubble contrast agents at low acoustic power," *Ultrasound Med. Biol.*, vol. 31, pp. 213–219, 2 2005. [PubMed: 15708461]
- [10]. Seol S-H and Lindner JR, "A primer on the methods and applications for contrast echocardiography in clinical imaging," *J. Cardiovascular Ultrasound*, vol. 22, pp. 101–110, 9 2014.
- [11]. Viti J, Vos HJ, de Jong N, Guidi F, and Tortoli P, "Detection of contrast agents: Plane wave versus focused transmission," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 63, no. 2, pp. 203–211, 2 2016. [PubMed: 26642451]
- [12]. Couture O, Bannouf S, Montaldo G, Aubry J-F, Fink M, and Tanter M, "Ultrafast imaging of ultrasound contrast agents," *Ultrasound Med. Biol.*, vol. 35, no. 11, pp. 1908–1916, 11 2009, doi: 10.1016/j.ultrasmedbio.2009.05.020. [PubMed: 19699026]
- [13]. Kusunose J and Caskey CF, "Fast, low-frequency plane-wave imaging for ultrasound contrast imaging," *Ultrasound Med. Biol.*, vol. 44, pp. 2131–2142, 10 2018. [PubMed: 30057134]

- [14]. Goertz DE, de Jong N, and van der Steen AF, "Attenuation and size distribution measurements of Definity and manipulated Definity populations," *Ultrasound Med. Biol.*, vol. 33, pp. 1376–1388, 9 2007. [PubMed: 17521801]
- [15]. Ketterling JA, Mamou J, Allen JS, Aristizábal O, Williamson RG, and Turnbull DH, "Excitation of polymer-shelled contrast agents with high-frequency ultrasound," *J. Acoust. Soc. Amer.*, vol. 121, pp. EL48–EL53, 1 2007. [PubMed: 17297826]
- [16]. Aristizábal O, Mamou J, Ketterling JA, and Turnbull DH, "High-throughput, high-frequency 3D ultrasound for in utero analysis of embryonic mouse brain development," *Ultrasound Med. Biol.*, vol. 39, pp. 2321–2332, 12 2013. [PubMed: 24035625]
- [17]. Needles A et al., "Nonlinear contrast imaging with an array-based micro-ultrasound system," *Ultrasound Med. Biol.*, vol. 36, no. 12, pp. 2097–2106, 12 2010, doi: 10.1016/j.ultrasmedbio.2010.08.012. [PubMed: 21092832]
- [18]. Deng CX, Lizzi FL, Kalisz A, Rosado A, Silverman RH, and Coleman DJ, "Study of ultrasonic contrast agents using a dual-frequency band technique," *Ultrasound Med. Biol.*, vol. 26, pp. 819–831, 6 2000. [PubMed: 10942829]
- [19]. Hansen R and Angelsen BAJ, "SURF imaging for contrast agent detection," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 56, no. 2, pp. 280–290, 2 2009. [PubMed: 19251515]
- [20]. Yu FTH, Villanueva FS, and Chen X, "Radial modulation contrast imaging using a 20-MHz single-element intravascular ultrasound catheter," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 61, no. 5, pp. 779–791, 5 2014. [PubMed: 24803134]
- [21]. Gessner RC, Frederick CB, Foster FS, and Dayton PA, "Acoustic angiography: A new imaging modality for assessing microvasculature architecture," *Int. J. Biomed. Imag.*, vol. 2013, 2013, Art. no. 936593, doi: 10.1155/2013/936593.
- [22]. Errico C et al., "Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging," *Nature*, vol. 527, no. 7579, p. 499, 2015. [PubMed: 26607546]
- [23]. Stapleton S et al., "Acoustic and kinetic behaviour of Definity in mice exposed to high frequency ultrasound," *Ultrasound Med. Biol.*, vol. 35, pp. 296–307, 2 2009. [PubMed: 18950930]
- [24]. Browne JE, Ramnarine KV, Watson AJ, and Hoskins PR, "Assessment of the acoustic properties of common tissue-mimicking test phantoms," *Ultrasound Med. Biol.*, vol. 29, pp. 1053–1060, 7 2003. [PubMed: 12878252]
- [25]. Yiu BYS, Tsang IKH, and Yu ACH, "GPU-based beamformer: Fast realization of plane wave compounding and synthetic aperture imaging," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 58, no. 8, pp. 1698–1705, 8 2011, doi: 10.1109/TUFFC.2011.1999. [PubMed: 21859591]
- [26]. Couture O, Fink M, and Tanter M, "Ultrasound contrast plane wave imaging," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 59, no. 12, pp. 2676–2683, 12 2012. [PubMed: 23221216]
- [27]. Shen C-C and Li P-C, "Pulse-inversion-based fundamental imaging for contrast detection," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 50, no. 9, pp. 1124–1133, 9 2003. [PubMed: 14561029]
- [28]. Lin F, Cachard C, Mori R, Varray F, Guidi F, and Basset O, "Ultrasound contrast imaging: Influence of scatterer motion in multipulse techniques," *IEEE Trans. Ultrason., Ferroelectr., Freq. Control*, vol. 60, no. 10, pp. 2065–2078, 10 2013. [PubMed: 24081255]
- [29]. King DA, Malloy MJ, Roberts AC, Haak A, Yoder CC, and O'Brien WD, "Determination of postexcitation thresholds for single ultrasound contrast agent microbubbles using double passive cavitation detection," *J. Acoust. Soc. Amer.*, vol. 127, pp. 3449–3455, 6 2010. [PubMed: 20550244]
- [30]. Chatterjee D, Jain P, and Sarkar K, "Ultrasound-mediated destruction of contrast microbubbles used for medical imaging and drug delivery," *Phys. Fluids*, vol. 17, no. 10, 2005, Art. no. 100603.
- [31]. Moran CM, Watson RJ, Fox KAA, and McDicken WN, "*In vitro* acoustic characterisation of four intravenous ultrasonic contrast agents at 30 MHz," *Ultrasound Med. Biol.*, vol. 28, pp. 785–791, 6 2002. [PubMed: 12113791]

- [32]. Goertz DE et al., "Subharmonic contrast intravascular ultrasound for vasa vasorum imaging," *Ultrasound Med. Biol.*, vol. 33, no. 12, pp. 1859–1872, 2007. [PubMed: 17683850]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1.

Peak negative acoustic pressure in water at a distance of 8 mm as a function of drive voltage and transmission angle for a 16-MHz, 1.5 cycle transmission. The +1 cases (lines) represent normal transmission phase and the -1 cases (symbols) are with the waveform inverted.

Fig. 2.

Transmit/receive sensitivity (diamond), peak receive frequency (square), and -6 -dB center frequency (circle) on receive and as a function of transmit frequency using a $25\text{-}\mu\text{m}$ diameter wire as a target. The target was placed at the center of the linear array at a depth of 8 mm. The transmit waveform was 1.5 cycles with a 2-V transmission (0.13 MPa). The straight line represents a reference for a one-to-one relation between the transmit frequency and the receive frequency. A 1–31-MHz bandpass filter was utilized.

Fig. 3.

Raw time-domain waveforms on a central array element from a 25- μm wire target with a 16-MHz, 1.5 cycle, 10 V (0.84 MPa), and -10° transmission using a PI scheme. The +1 (solid line) and -1 (dashed line) transmissions are shown as is the residual (dotted) after taking the difference of the two waveforms. The -1 case was inverted for display.

Fig. 4.

Raw time-domain waveforms on a central array element from a 25- μm wire target with a 16-MHz, 1.5-cycle, 10 V (0.85 MPa), and -10° transmission angle using an AM scheme. The +1 (solid line) and +1/2 (dashed line) transmissions are shown as is the residual (dotted line) after taking the difference of the two waveforms. The +1/2 waveform was doubled in amplitude upon receive and displayed as such.

Fig. 5.

RNR at an 8-mm depth in the 2-mm water, filled-flow phantom versus transmit frequency for a 6-kHz absolute PRF, 1.5 cycle, 15-V transmission (0.47-MPa derated). The standard three-angle transmission (squares), six-angle transmission (open circles), PI (solid circle), and AM (triangle) are represented. The PI has a low RNR relative to the other cases above 10 MHz indicating good suppression of the linear background signal.

Fig. 6.

Residual for PI and AM at three transmission angles $[-10 \ 1 \ 10]$ as a function of drive voltage for a 1.5 cycle, 16-MHz transmission. 0 dB represents a residual peak amplitude equal to the mean of the individual pulse-pair peak amplitudes.

Fig. 7.

Representative flow images with a 6-kHz absolute PRF, 15-V drive (0.47-MPa derated), 1.5 cycle 16-MHz excitation. (a) Standard three-angle transmission. (b) PI. (c) AM. 0 dB is referenced to the maximum of each plot.

Fig. 8.

Absolute rms values from (a) background, (b) flow, and (c) CBR as drive pressure increased. A 6-kHz absolute PRF, 1.5 cycle, 16-MHz transmission was used. In (b) and (c), reference curves are shown for a water flow. The intensities of (a) and (b) were greatest for the standard imaging case and the PI case had the lowest intensity. The standard imaging case had a CBR near 3 dB, which indicates that the backscatter intensity from the UCA flow was slightly above the background. 0 dB is referenced to the maximum amplitude of (a). The rms intensity data were normalized to the number of transmissions. A pressure scale representing the derated incident pressures after 2.75 mm of attenuation in the phantom is also shown.

Fig. 9.

CBR as a function of absolute transmission PRF for a 16-MHz, 1.5 cycle, 10-V (0.32-MPa derated) transmission. At low PRFs, the equivalent to fast flow, decorrelation led to less fundamental cancellation in the MP flow cases and an increased CBR. Above a PRF of 4 kHz, the CBR remained unchanged.

Fig. 10.

Power spectra from the flow region, after beamforming and compounding, for a 16-MHz, 1.5-cycle, 10-V (0.32-MPa derated) transmission for a standard three-angle transmission case (solid line), PI (dashed line), and AM (dotted line) for (a) 750-Hz and (b) 6-kHz absolute frame rate.

Fig. 11.

CBR as function of (a) time and (b) UCA dilution for a 16 MHz, 1.5 cycle, 15-V (0.47-MPa derated) excitation. A 1 $\times$ concentration represents a 2000:1 ratio. The CBR displayed minimal change over a 1-h period and a gradual change as the UCA was diluted from 1 $\times$ to 16 $\times$ dilution. From 1 $\times$ to 4 $\times$ dilution, there was minimal change in CBR. At 1/2 $\times$, the attenuation significantly reduced the rms backscatter in the ROI leading to a drop in CBR.