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A New Imaging Strategy Using Wideband Transient Response of Ultrasound Contrast Agents

Dustin E. Kruse [Member, IEEE] and Katherine W. Ferrara [Senior Member, IEEE]

Abstract

High-resolution clinical systems operating near 15 MHz are becoming more available; however, they lack sensitive harmonic imaging modes for ultrasound contrast agent (UCA) detection, primarily due to limited bandwidth. When an UCA is driven to nonlinear oscillation, a very wideband acoustic transient response is produced that extends beyond 15 MHz. We propose a novel strategy using two separate transducers at widely separated frequencies and arranged confocally to simultaneously excite and receive acoustic transients from UCAs. Experiments were performed to demonstrate that this new mode shows similar resolution, higher echo amplitudes, and greatly reduced attenuation compared to transmission at a higher frequency, and superior resolution compared to transmission and reception at a lower frequency. The proposed method is shown to resolve two 200 μm tubes with centers separated by 400 μm . Strong acoustic transients were detected for rarefaction-first 1-cycle pulses with peak-negative pressures above 300 kPa. The results of this work may lead to uses in flow and/or targeted imaging in applications requiring very high sensitivity to contrast agents.

I. Introduction

ALTHOUGH the backscatter from blood increases significantly with frequency, the echoes from blood cells from within minute vessels are difficult to detect even at frequencies above 10 MHz because of the limited dynamic range associated with high-resolution, wideband imaging. Ultrasound contrast agents (UCAs) are shell-encapsulated microbubbles that are 1000 or more times more compressible than blood and thus significantly enhance acoustic backscatter. The UCA shells generally are categorized as being either hard, as in the case of albumin, or soft, as in the case of lipid, oil, surfactant, and elastic polymer shells. The encapsulated gas may be air, or more commonly, a low-solubility, high-molecular weight gas to aid survival time in vivo.

The nonlinear oscillation of UCAs has been the subject of many experimental and theoretical investigations [1]–[3]. Nonlinear oscillations provide the opportunity to distinguish tissue and microbubble echoes based on their center frequency or response to the phase of the transmitted pulse. Imaging modes, including phase-inversion [4], [5] and subharmonic modes [6]–[8], exploit these nonlinear properties; however, they typically require a lower transmission center frequency and, therefore, result in a loss of spatial resolution. Second harmonic imaging offers better resolution compared to subharmonic modes due to reception at twice the transmitted frequency; however, it suffers from poor tissue rejection due to overlap with the second tissue harmonic. Multipulse techniques designed to reject tissue harmonics, based on a memoryless model of tissue echoes [9]–[11], offer good resolution and tissue rejection but require multiple pulses for agent detection, thus limiting the frame rate. This limited frame rate is suboptimal in the presence of substantial tissue motion. High-frequency, subharmonic imaging has been demonstrated by Goertz *et al.* [12] for transmission at 20 MHz and reception at 10 MHz and applied to resolve vessels on the order of 1 mm *in vitro* and 500 μm *in vivo*. From this work,

the excitation of the subharmonic requires pressures in excess of 1 MPa in weakly attenuating media. Transmission at such high frequencies also limits depth of penetration; and, when combined with attenuating media, very high pressures may be required to penetrate even 1 cm of tissue. Although contrast-assisted methods have demonstrated the ability to detect flow in subresolution vessels of the microcirculation [8], [13], [14], to the best of the authors' knowledge, no existing methods that take advantage of nonlinear UCA scattering have been demonstrated to resolve vessels on the scale of the microcirculation.

In this work, we investigate the strongly nonlinear transient response of acoustically-driven UCA microbubbles. The transient response results from the high bubble wall velocity and acceleration achieved when a microbubble collapses. This is readily seen from Rayleigh-Plesset-based models of bubble dynamics that predict the received echoes to be determined by the wall velocity and acceleration of the contrast agent [15]. To illustrate this phenomenon, an example simulation is shown in Fig. 1 for a 2- μ m diameter microbubble driven by two 400 kPa peak-pressure pulses at 2.25 MHz. The two single-cycle driving pulses are shown in Fig. 1(a), in which the dashed line is noninverted and the solid line is inverted. The corresponding radius-time curve and emitted pressure are shown in Fig. 1(b) and (c), respectively. For the inverted pulse, bubble collapse is observed to occur during the compressional phase following the rarefactional phase. In the opposite case of compression followed by rarefaction, the transient response is suppressed as is observed in Fig. 1(c). Fig. 1(d) shows a clipped version of Fig. 1(c) to show the relative magnitude of the transient pressure spikes with respect to the lower frequency components. The effect of the phase of transmission can be exploited in phase inversion imaging and has been confirmed experimentally for UCAs [4], [5]. For albumin-shelled agents, a transient acoustic signature known to accompany UCA disruption following transmission of a high-intensity pulse is the basis of power-enhanced UCA imaging modes [17]. The explanation for the enhancement is still an open question in the literature [3], although many hypothesize that disruption of the hard shell releases the encapsulated gas into the fluid, and that the resulting enhancement is due to stronger oscillation of a free gas bubble compared to the damped oscillation of an encapsulated bubble [17]. Similar destructive transient behavior also is observed in flash echo imaging for transmission at 2 MHz [18], [19] using Levovist (Schering AG, Berlin), which has a very compliant shell consisting of palmitic acid. Agent destruction has been shown to accompany transient echoes for compliant lipid-shelled agents as well. The mechanism(s) of destruction for soft-shelled agents remains an open question. For this paper, it is assumed that destruction or changes in the agent composition can accompany the generation of transient acoustic echoes.

The transient response has been measured experimentally for individual sonoluminescing free air bubbles using a wideband needle hydrophone and a 10 MHz transducer [20]. These measurements show that the transient response (for a free air bubble) has frequency content ranging from near 10 MHz to possibly over 100 MHz. Shi *et al.* [21] performed experiments to study the destruction of contrast agents insonified at 2.25 MHz, and they used a 25 MHz passive-receiving transducer to monitor cavitation activity. Bouakaz *et al.* [22] conducted a study of super harmonic imaging, which involved transmitting two cycles (phase not specified) at 0.8 MHz and receiving frequency components up to the fifth harmonic (~ 4 MHz) using a dual-frequency probe. The study described in [22] did not distinguish between higher order harmonics and the wideband spectrum produced by an acoustic transient (see Fig. 4(A) in [22]). Other recent work by Reddy and Szeri [23] has focused on optimizing the driving pulse to exploit the transient response for pulse-inversion imaging. To the best of our knowledge, no previous studies have considered exploiting and optimizing the reception of transients for the purposes of improving imaging resolution.

This paper is organized as follows. In Section II, a method will be introduced for studying the transient response of single UCA microbubbles, and the transient response will be

demonstrated using this method. In Section III, an imaging mode will be introduced that takes advantage of the transient response, and performance metrics related to resolution and contrast will be quantified as a function of pressure, in comparison to conventional imaging methods. The resolution, attenuation, and signal-to-noise ratio (SNR) performance of the proposed method will be demonstrated using a two microtube flow phantom. Section IV discusses results and potential applications followed by conclusions in Section V.

II. The Transient Response from a Single UCA

In this section, a method is described for studying the acoustic behavior of individual UCA microbubbles, and an example of UCA transient detection is shown by imaging in a through-transmission mode. In subsequent sections, imaging in a pulse-echo mode will be explored.

A. Methods and Experimental System

In order to experimentally determine when acoustic transients occur with respect to the phase of a low-frequency driving wave, two spherically-focused transducers were aligned, using micrometer stages, with an angle of 180 degrees (pointing directly at each other) in a bath of distilled water. The speed of sound was assumed to be constant for both the transmitted waveform and for the forward scattered response of the bubbles within the tube. Consequently, the arrival of both sets of waves at the receive transducer is phase locked. Due to the orientation of the transducers, the received acoustic wave includes a substantial component of the energy transmitted from the low-frequency transducer. To better isolate the microbubble transient response from the transmitted pulse and associated harmonic content due to nonlinear propagation, the transmission center frequency was chosen to be 1 MHz and the receive center frequency was chosen to be 20 MHz. The relevant specifications of the transducers used are shown in Table I (both from GE Panametrics, Waltham, MA).

A 200- μm inner diameter cellulose microtube (Spectrum Laboratories, Inc., Rancho Dominguez, CA) was placed at the focus of both transducers at 45 degrees with respect to each transducer. The angle was required to eliminate reverberation artifacts due to the thin tube walls. The low-frequency waveforms were generated using an arbitrary waveform generator (model AWG2021, Sony-Tektronix, Tokyo, Japan) and driven using a power amplifier (model 3200L, ENI, Rochester, NY). The acoustic pressures generated were measured using a calibrated needle hydrophone (model PZT-Z44-0400, Specialty Engineering Associates, Soquel, CA). The echoes received from the 20 MHz transducer are bandpass filtered limiting the -3 dB bandwidth from 1 to 50 MHz and amplified and/or attenuated using a pulser/receiver (model PR5900, GE Panametrics, Waltham, MA). The amplified radio frequency (RF) signals were sampled at 125 MHz and 12-bits using a computer-based digitizer board (model PDA12A, Signatec, Corona, CA).

The UCA microbubbles used throughout this study have a lipid monolayer shell that encapsulates a high molecular weight gas core of octafluoropropane. The UCA is diluted in distilled water by a factor of 1×10^5 in order to observe individual microbubble events from within the focal volume of the high-frequency transducer. Assuming a maximum undiluted UCA concentration of 4.0×10^9 bubbles per milliliter (upper limit determined by Coulter count), the average number of bubbles per 100 μm length of tubing should be no greater than 0.13. The diluted UCA solution was pumped using a syringe pump to produce a 1 cm/s mean flow velocity and the pulse repetition frequency (PRF) was set to 1 Hz to assure that the sample volume is constantly refreshed because of the high likelihood of agent destruction at the frequencies and pressures required for transient generation.

The received echoes were nonstationary, and thus required time-frequency analysis in order to distinguish the arrival times of the sharp transients relative to the strong interference

produced by directly receiving the transmitted pulse. The S-transform proposed by Stockwell *et al.* [24] is the time-frequency representation used to decompose the signals. This distribution was chosen because it localizes high-frequency components with good time resolution; and, when integrated over time, it yields the Fourier transform. Therefore, a one-to-one correspondence can be made between the S-transform and the Fourier transform.

B. Results

An example of a received waveform for a 1 MHz, 10 cycle pulse is shown in Fig. 2 (dashed, scaled down by 30 dB) with an overlaid waveform that was digitally bandpass filtered from 10 to 45 MHz (solid). The peak-negative pressure (PNP) for this example was 280 kPa and the peak-positive pressure (PPP) was 340 kPa. In the bandpass filtered waveform, the transient spikes were synchronous with the 1 MHz component of the transmitted waveform, and 10 transients were apparent. The transients appear to occur after the onset of the compressional wave and after peak compression (which is consistent with the simulation shown in Fig. 1). It is interesting to note that the transient amplitudes grew during the first few cycles of the driving waveform, even after the driving waveform amplitude leveled off. The low SNR of the bandpass filtered waveform was due to the fixed dynamic range required to capture both responses simultaneously. For the 10 cycle pulse, the threshold for transient production was a PNP in the range of 100 to 200 kPa. The corresponding time-frequency image (bottom Fig. 2) shows that the transients were of very short duration and have frequency content extending from 10 MHz to above 40 MHz.

III. Acoustic Transients for Imaging

The results of the previous section show that the acoustic transients have substantial frequency content above 10 MHz, which would be useful for separating bubble echoes from tissue scatter at the transmission frequency. Therefore, we propose imaging with two transducers, with a low-frequency transducer centered at 2.25 MHz to excite the transient response and a high-frequency transducer centered at 15 MHz to receive the response. This configuration is denoted T2R15. Our choice for transmitter and receiver frequencies is partly based on the availability of clinical arrays that are optimized for operating at these frequencies. Undoubtedly, it is possible to take advantage of the transient response using different choices for both frequencies, e.g., T1R13. In the interest of minimizing the possibility of bioeffects arising from this method, we feel that a higher transmitted frequency is optimal in this regard. Furthermore, imaging at 15 MHz is becoming more available on clinical scanners (e.g., transducer model 15L8, Siemens Medical Solutions USA, Inc., Malvern, PA), and there is a growing need to develop sensitive high-resolution UCA imaging methods at this frequency. To illustrate the potential resolution of this method, experiments were performed using a single 200 μm tube to compare T2R15 to T15R15. The resolving ability of T2R15 was further tested using two 200 μm tubes separated by 400 μm .

A. Methods and Experimental System

The experimental system and methods were similar to those described above. For the following experiments, the dilution of UCA was reduced to a factor of 1.7×10^3 , which yields a maximum of 7.5 bubbles per 100 μm of tube. This concentration also was equivalent to a 3 mL injection of UCA into a 5 L blood volume. For the single tube experiments, the transducers were placed at approximately 90 degrees with respect to one another with their respective foci aligned on the tube. The tube was placed at a 45 degree angle with respect to the plane defined by the two transducers to eliminate reverberation. The tube also was tilted 60 degrees upward in the direction of flow to reduce the rate of UCAs floating to the top of the tube. Acoustical measurements of the transient response were acquired at the 2.25 MHz transmitted center frequency for a range of pressures, using a 1-cycle, rarefaction-first sinusoidal pulse. A one-

cycle pulse was used because multiple cycles would produce multiple transients and reduce spatial resolution, as seen in Fig. 2. Comparison measurements also were recorded for T15R15 where the same transducer was used for both transmission and reception. The transducer specifications are shown in Table II (both obtained from GE Panametrics, Waltham, MA). The mean flow velocity was set to 1 cm/s and the PRF was set to 5 Hz in order to refresh the sample volume between pulses. In the single tube experiments, the following parameters were measured as a function of pressure, which include the power spectra, spectral power at 15 MHz, the -6 dB bandwidth, and the -6 dB axial width of the tube.

For the double tube experiments, two 200 μ m tubes were placed beside one another with centers separated by 400 μ m using thin wires. An image of the tubes is shown in Fig. 3 under 20 $\times$ magnification in order to verify the distance between the tubes.

The transducer configuration was similar to that used for the single tube experiments. Similar experiments were repeated for T2R2 using separate transducers and T15R15 using one transducer. The M-modes were recorded lasting 50 seconds (512 pulses) at a PRF of 10 Hz for a line-of-sight placed through the center of both tubes with a mean flow velocity of 2 cm/s in both tubes. The received echoes for T2R15 were highpass filtered with a cutoff frequency of 10 MHz in order to remove frequency components near the driving center frequency of 2.25 MHz and associated harmonic content.

B. Single Microtube Results

Fig. 4 shows received power spectra averaged over 128 pulses from the single tube for T2 PNPs ranging from 100 kPa to 740 kPa (solid, six pressures) and for T15 PNPs ranging from 300 kPa to 1330 kPa (dashed, four pressures). It should be noted here that a T15 PNP of 300 kPa (the lowest tested) corresponds to a transmitted PNP of 1.7 MPa for 2 cm of tissue at an attenuation of 0.5 dB/cm/MHz, neglecting nonlinear propagation. Attenuation in this case was much less significant for T2 at a PNP of 300 kPa, which would require a transmitted PNP of 390 kPa. For both T2 and T15, the spectral amplitude increased as pressure was increased. At the lowest PNP of 100 kPa, harmonics were visible for T2R15, but there was a rapid transition to a broadband response between 140 and 200 kPa. Overall pressure levels for T15R15, the center frequency and -6 dB bandwidth remained constant at 14 MHz and 10 MHz, respectively. For T2R15, the center frequency and -6 dB bandwidth increased from 3.5 MHz and 3 MHz at 100 kPa to 10 MHz and 13 MHz at 200 kPa, respectively, where they remained approximately constant as PNP increased, due to the limited bandwidth of the 15 MHz receiving transducer.

Fig. 5 summarizes the echo amplitude at 15 MHz as a function of PNP for both T2R15 and T15R15. The T2R15 case produced a stronger echo than T15R15 for PNPs above 200 kPa. Above this pressure level, the power at 15 MHz increases more rapidly as a function of PNP compared to T15R15.

To compare the axial resolutions for both methods, Fig. 6 shows the measured -6 dB single tube diameter as a function of PNP for T2R15 and T15R15. For Fig. 6, the T2R15 echoes were first highpass filtered with a cutoff frequency of 10 MHz. The observed -6 dB width of the tube at PNP > 200 kPa was slightly smaller for T2R15 at approximately 180 μ m than it was for T15R15, where it was approximately 220 μ m. It should be noted that, in both cases, the orientation of the R15 transducer was identical.

C. Double Microtube Results

To illustrate the need for reception at a higher frequency, Fig. 7(a) shows a T2R2 M-mode power Doppler image directed through the center of two tubes with centers separated by 400 μ m (with approximately 170 μ m between inner tube walls) for a PNP of 370 kPa. Fig. 7(b)

shows the mean power as a function of depth from the surface of the transducer averaged over 512 pulses. Clearly, the vessels cannot be resolved in this case.

Fig. 8(a) shows a T2R15 M-mode power Doppler image directed through the same pair of tubes for the same PNP of 370 kPa. Fig. 8(b) shows the mean power as a function of depth averaged over 512 pulses. Note the clear separation between the tubes, which was approximately 15 dB. At a similar PNP of 300 kPa, the tubes were also resolved with T15R15 as is shown in Figs. 7(c) and (d), with slightly better separation of 16 dB.

D. Double Microtube with Simulated Attenuation

Neglecting attenuation in water, the echo amplitude received from contrast agents using T2R15 was approximately 12 dB higher compared with T15R15 (using the same receive transducer, microbubble concentration, and similar transmitted pressures) as can be seen by comparing Figs. 8(b) and (d). For the double tube result, a simple comparison was made between T2R15 and T15R15 regarding the influence of attenuation using the echoes shown in Fig. 8. The following assumptions were made in this analysis. First, it was assumed that the relatively small attenuation of the transmission at 2 MHz (~ 2.25 dB) can be compensated for on transmission by increasing the transmitted pressure. Second, it was assumed that the scattering at 15 MHz is predominantly linear near 300 kPa, which is a reasonable assumption based on theoretical predictions of linear resonance.

To simulate attenuation in both cases, a filter was designed and applied assuming that both vessels were 2-cm deep within an attenuating medium (0.5 dB/cm/MHz), and the attenuation was assumed to be constant over the vessels. In the case of T2R15, the one-way attenuation of the UCA echoes was compared to two-way attenuation of T15R15. After accounting for attenuation by applying filters to both T2R15 and T15R15 for the data shown in Fig. 8, the result shown in Fig. 9 was obtained.

Comparing Figs. 9(b) and (d), the power received from contrast agents using T2R15 was found to be 19 dB higher than for T15R15. Furthermore, the separation between the vessels following attenuation was 10 dB for T15R15 and 12 dB for T2R15, thus indicating marginally better resolution of T2R15 following attenuation. It also should be noted that, for a fixed noise floor at -55 dBm [shown in Fig. 8(a)], the T15R15 echoes shown in Fig. 9(d) were barely above the noise, compared to the T2R15 case in Fig. 9(b). Likewise, the vessel was barely detectable for a fixed 50 dB dynamic range as was shown in Fig. 9(c), whereas the SNR was significantly higher, allowing the vessel to be detected as shown in Fig. 9(a).

IV. Discussion

Fig. 4 shows that T2R15 UCA backscatter has greater bandwidth and higher echo amplitude than T15R15 above a PNP of approximately 200 kPa, for the lipid-shelled agent used in this study. The substantial low-frequency components shown in Fig. 4 for T2R15 are expected due to close proximity of the transmission and resonance frequencies for the UCA used. Assuming that the low-frequency components are removed (e.g., those below 10 MHz), substantial echo amplitude remains for high-resolution imaging of UCAs. Following transmission at 2 MHz and -200 kPa, the backscattered power at 15 MHz is approximately equivalent to what is expected for T15 at -200 kPa (Fig. 5). With sufficient dynamic range, the transient response should be detectable at PNPs above 200 kPa. Furthermore, because the transients need only propagate one-way, high-resolution imaging of UCAs using T2R15 at higher transmitted pressures should be possible at approximately double the penetration depth of T15R15, assuming that a slight increase in transmission pressure compensated for the one-way attenuation of the transmission at 2 MHz. This also translates into much higher SNR over the penetration range of T15R15. It should be noted that the simple attenuation analysis performed

above has limitations when considering the highly nonlinear response shown in Fig. 5 (solid), particularly near -200 kPa at which small changes in pressure result in large changes in backscatter at 15 MHz.

In microbubble second harmonic imaging, the tissue harmonic interference cannot be removed easily because both occupy the same frequency range. Though not tested for this study, if the T2 and R15 transducers are arranged to be confocal (0 degrees), the wide separation between the transmission and reception frequency should allow tissue harmonic interference to be removed out to at least the fourth harmonic. A simple calculation of the expected tissue harmonic content [25] at a depth of 2 cm can be made for a transmitted frequency of 2.25 MHz, peak pressure of 400 kPa, and B/A equal to 8. Neglecting the substantial effects of attenuation, the fourth tissue harmonic centered at 9 MHz is approximately 58 dB below the first harmonic. The same tissue harmonic calculation made at 1 MHz gives a 4 MHz, fourth harmonic at 79 dB below the first harmonic. Therefore, choosing a lower transmission frequency and/or a higher reception frequency only improves the rejection of tissue harmonics using the proposed method.

The proposed T2R15 method can be combined with existing wideband phase-inversion techniques [4], [23] to optimize detection of UCAs. Single-cycle phase-inversion imaging inherently relies upon differences in the transient response between inverted pulses. The demand on phase-inversion to reject tissue echoes is lessened by the proposed method, because tissue echoes and associated harmonics should be significantly attenuated following application of a suitable highpass filter.

The resolution criteria tested in this paper demonstrates axial resolution only. Because the transient response involves focusing on reception, it is expected that the resolution in the lateral and elevation directions for T2R15 will be degraded compared with the resolution obtained for T15R15 due to the lack of focusing on transmission. However, the proposed method offers several benefits for UCA detection, including: higher axial resolution than T15R15, better rejection of tissue echoes and associated harmonics due to nonlinear propagation over second harmonic imaging, and higher sensitivity and depth-of-penetration than T15R15.

Previous studies [26] of similar lipid-shelled UCAs in this frequency and pressure range show that the shell is disrupted, leading to destruction of the UCA, particularly at frequencies below 3 MHz and at high pressures. This is perhaps one of the main drawbacks to using the proposed T2R15 method, because the destruction leads to uncorrelated samples between pulses, thus making it impossible to apply correlation-based color Doppler techniques for velocity estimation. Perhaps future formulations of UCA shell material can be made robust enough to survive expansion and rapid collapse.

For applications that involve simply detecting the presence of UCAs, such as in targeted imaging [27] in which a targeting ligand (such as biotin) is incorporated into the shell, the transient response may play an important role in detecting the location of targeting molecules (such as streptavidin) with very high resolution when combined with a clinical system modified to operate in a T2R15 configuration. The influence of UCA attachment to a cell or vessel wall is not well known in terms of the harmonic and transient responses, and this is a subject of ongoing investigation. Another related application is in the use of UCA microbubbles to disrupt the membranes of endothelial cells to make them more porous and permeable to large drug molecules, also known as sonoporation [28], [29]. At pressures greater than 1 MPa, as required for sonoporation, the UCAs are driven sufficiently to produce a transient response, and thus a method for imaging the location and degree of sonoporation may be useful. A final application involves the use of UCAs combined with high-intensity ultrasound to produce strong cavitation for tissue erosion as a noninvasive surgical tool [30]. In this application, the transient echoes

from collapsing bubbles could be overlaid in false-color on a conventional B-mode image, which would allow clinicians to observe and possibly quantify the degree of tissue erosion.

The transient response is intimately related to bubble collapse and cavitation, which is a controversial subject facing UCA use in medical ultrasound. There are a growing number of studies that have looked at the bioeffects of combining UCAs with the high pressures that clinical ultrasound systems produce. However, the pressures found to produce measurable bioeffects are typically above 1 MPa, with transmission frequencies below 2 MHz, and require much longer pulse lengths than what is required to drive UCA transients. More studies are clearly needed to determine the safe operating parameters for diagnostic ultrasound with the use of UCAs. That being said, the methods presented in this paper are well within all current expository limits dictated by the Food and Drug Administration for medical ultrasound in soft tissues.

V. Conclusions

The transient response was demonstrated for a single microbubble using a new method that allows detection of the transient echoes simultaneously with the transmitted pulse. A method for studying the transient response of UCAs was introduced, and the results show that strong transients are generated for lipid-shelled UCAs under moderate pressures starting near 300 kPa. A new method, T2R15, was proposed for imaging with transients using two transducers at widely separated frequencies. The method was demonstrated by resolving two closely spaced microtubes. Both without and with attenuation, T2R15 produced higher sensitivity and SNR than T15R15. Furthermore, T2R15 was shown to have superior axial resolution compared with T2R2, and (after accounting for attenuation) better resolution than T15R15. The potential uses of this technique include targeted imaging, sonoporation, noninvasive surgical ultrasound, and improved phase inversion.

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Fig. 1.

Simulated response of a 2-μm diameter microbubble in water to 2.25 MHz inverted (solid) and noninverted (dashed) pulses, shown in (a), using a modified Rayleigh-Plesset equation of motion [16]. The radius time curve for both pulses is shown in (b), and the emitted pressure is shown in (c) and (d), where (d) clips the amplitude to better show the weaker low-frequency components. Note the pressure spike or acoustic transient (denoted by the arrow) corresponding to the inverted driving pulse shown in (c), and its correspondence to maximum compression in (b).

Fig. 2.

Top: plot of received 10 cycle pulse (dotted, scaled down by 30 dB) and following application of a bandpass filter from 10 to 45 MHz (solid). The y-axis is voltage in millivolts at the output of the transducer for the filtered echo. Bottom: time-frequency power spectrum of nonfiltered echo showing 30 dB of dynamic range. Vertical scale is in megahertz and horizontal scale is time in microseconds.

Fig. 3. Optical image under 20 $\times$ magnification of two 200 μm cellulose tubes spaced apart by 400 μm , center-to-center. Scale bar at lower left is 200 μm wide.

Fig. 4.

Received power spectra (R15) from contrast agent echoes averaged over 128 pulses from the single tube for T2 PNPs of 100, 140, 200, 370, 520, and 730 kPa (solid) and for T15 PNPs of 300, 540, 940, and 1330 kPa (dashed). Vertical scale is in units of decibels referenced to 1 mW.

Fig. 5. Spectral power at 15 MHz averaged over 128 pulses from the single tube for T2R15 (solid) and T15R15 (dashed). Vertical scale is in units of decibels referenced to 1 mW. Error bars are $\pm$ one standard deviation.

Fig. 6. Measured -6 dB single tube diameter in microns as a function of PNP for T2R15 (solid) and T15R15 (dashed). Each point was averaged over 128 pulses.

Fig. 7.

(a) T2R2 (PNP = 370 kPa) power Doppler M-mode image of two tubes separated by 400 μm .
 (b) Mean power for T2R15 averaged over 512 pulses. The mean flow velocity in each tube was 2 cm/s and the PRF was 10 Hz for both cases. Color scale shows a 50 dB dynamic range.

Fig. 8.

(a) T2R15 (PNP = 370 kPa) power Doppler M-mode image of two tubes separated by 400 μm . (b) Mean power for T2R15 averaged over 512 pulses. (c) T15R15 (PNP = 300 kPa) power Doppler M-mode using the same receive transducer and orientation used for T2R15. (d) Corresponding mean power averaged over the duration of the M-mode. The mean flow velocity in each tube was 2 cm/s, and the PRF was 10 Hz for both cases. The T2R15 echoes were highpass filtered with cutoff at 10 MHz prior to envelope detection. Color scales show a 50 dB dynamic range.

Fig. 9.

Same as Fig. 8 except that one-way attenuation was applied to T2R15 (a) and (b) and two-way attenuation was applied to T15R15 (c) and (d). The attenuation was assumed to be constant across both vessels with a value of 0.5 dB/cm/MHz.

Dustin E. Kruse (S'99-M'04) received a B.A. degree in physics from the State University of New York College at Geneseo in 1996, the M.E. degree in electrical engineering from the University of Virginia in 1999, and the Ph.D. degree in biomedical engineering in 2004, also from the University of Virginia. His graduate work involved the development and application of high frequency ultrasound to image blood flow in the microcirculation.

He is currently a research engineer at the University of California, Davis, Department of Biomedical Engineering, where his research interests are in the areas of velocity estimation, high frequency ultrasound, contrast-assisted imaging, and therapeutic ultrasound. He is a member of Sigma Pi Sigma.

Katherine Whittaker Ferrara (S'82–M'82–S'87–M'89–SM'99) Following the B.S. and M.S. degrees in electrical engineering, Dr. Ferrara worked for Sound Imaging, Inc. Folsom, CA and for General Electric Medical Systems, Rancho Cordova, CA in the areas of magnetic resonance and ultrasound imaging, during 1983–1988. She received her Ph.D. in electrical engineering and computer science in 1989 from the University of California, Davis. From 1989–1993 she was an Associate Professor in the Dept. of Electrical Engineering at California State University, Sacramento. From 1993–1995, she was a principal member of the research staff at the Riverside Research Institute, New York, NY, and an Adjunct Associate Professor at Cornell University Medical School, and from 1995–1998 she was an Associate Professor in the Department of Biomedical Engineering at the University of Virginia, Charlottesville. Since December of 1998, Dr. Ferrara has been Professor and Chair of Biomedical Engineering in the newly created Department of Biomedical Engineering at the University of California, Davis. Dr. Ferrara is an Associate Editor of the IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control. She chaired the technical program for the 1997 IEEE Ultrasonics Symposium. She is a member of Tau Beta Pi and Sigma Xi and a fellow of the Acoustical Society of America. Her research interests are medical imaging and biomedical signal processing and particularly in the areas of ultrasonics and acoustics.

TABLE I

Transducer Specifications.

Transducer	1 MHz	20 MHz
Model	V303	V317
Focal length	20.3 mm	12.7 mm
Aperture diameter	12.7 mm	6.4 mm
−6 dB bandwidth (two-way)	78%	65%
−6 dB beamwidth (two-way)	2.4 mm	0.15 mm

TABLE II

Transducer Specifications.

Transducer	2.25 MHz	15 MHz
Model	V305	V328
Focal length	50.8 mm	15.24 mm
Aperture diameter	19 mm	12.7 mm
−6 dB bandwidth (two-way)	70%	66%
−6 dB beamwidth (two-way)	1.8 mm	0.12 mm