Improving the sensitivity of high-frequency subharmonic imaging with coded excitation: A feasibility study

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Purpose: Subharmonic intravascular ultrasound imaging (S-IVUS) could visualize the adventitial *vasa vasorum*, but the high pressure threshold required to incite subharmonic behavior in an ultrasound contrast agent will compromise sensitivity—a trait that has hampered the clinical use of S-IVUS. The purpose of this study was to assess the feasibility of using coded-chirp excitations to improve the sensitivity and axial resolution of S-IVUS.

Methods: The subharmonic response of Targestar-pTM, a commercial microbubble ultrasound contrast agent (UCA), to coded-chirp (5%–20% fractional bandwidth) pulses and narrowband sine-burst (4% fractional bandwidth) pulses was assessed, first using computer simulations and then experimentally. Rectangular windowed excitation pulses with pulse durations ranging from 0.25 to 3 μ s were used in all studies. All experimental studies were performed with a pair of transducers (20 MHz/10 MHz), both with diameter of 6.35 mm and focal length of 50 mm. The size distribution of the UCA was measured with a CasyTM Cell counter.

Results: The simulation predicted a pressure threshold that was an order of magnitude higher than that determined experimentally. However, all other predictions were consistent with the experimental observations. It was predicted that: (1) exciting the agent with chirps would produce stronger subharmonic response relative to those produced by sine-bursts; (2) increasing the fractional bandwidth of coded-chirp excitation would increase the sensitivity of subharmonic imaging; and (3) coded-chirp would increase axial resolution. The experimental results revealed that subharmonic-to-fundamental ratios obtained with chirps were 5.7 dB higher than those produced with sine-bursts of similar duration. The axial resolution achieved with 20% fractional bandwidth chirps was approximately twice that achieved with 4% fractional bandwidth sine-bursts.

Conclusions: The coded-chirp method is a suitable excitation strategy for subharmonic IVUS imaging. At the 20 MHz transmission frequency and 20% fractional bandwidth, coded-chirp excitation appears to represent the ideal tradeoff between subharmonic strength and axial resolution. © 2012 American Association of Physicists in Medicine. [http://dx.doi.org/10.1118/1.3694101]

Key words: coronary artery disease, ultrasound contrast agents, *vasa vasorum*, subharmonic imaging, coded excitation

I. INTRODUCTION

Coronary artery disease (CAD) kills more Americans than all cancers combined.¹ These deaths may occur when a life-threatening plaque ruptures in the advanced stages of the disease.^{2,3} Life-threatening plaques usually have a thin fibrous cap (<200 μ m), a large lipid pool, and chronic inflammation⁴; they are difficult to detect because (1) atherosclerosis is asymptomatic, and (2) coronary angiography, the current “gold standard” used to assess the severity of CAD in symptomatic patients, can only visualize the lumen.⁵ However, the adventitial *vasa vasorum* grows abnormally in life-threatening plaques.^{6–9} An imaging modality that could visualize the adventitial *vasa vasorum* would allow cardiologists to detect life-threatening plaques more easily. Intravascular ultrasound (IVUS) and intravascular magnetic resonance imaging (IMRI) have been used to manage patients with CAD, but they cannot visualize the adventitial *vasa vasorum*.¹⁰

Subharmonic IVUS (S-IVUS) is an emerging imaging technique that could visualize the adventitial *vasa vasorum*.^{10,11} S-IVUS uses the nonlinear acoustic response of ultrasound contrast agents^{7–9} to discriminate between blood vessels and surrounding tissues, but the narrow bandwidth of commercial IVUS systems makes subharmonic imaging challenging. Recently, researchers overcame this limitation using a clinical prototype subharmonic IVUS system equipped with a dual-frequency imaging catheter, with peak responses at 15 MHz (subharmonic frequency) and 30 MHz (fundamental frequency).^{11,12} This system demonstrated the feasibility of using S-IVUS to visualize the adventitial *vasa vasorum* in an atherosclerotic rabbit model. In this study, sine-burst excitation pulses with Gaussian windowing and 25% fractional (–6 dB) bandwidth were used to excite DefinityTM, a commercial ultrasound contrast agent. The excitation pressure was varied between 120 and 780 kPa, and subharmonic oscillations occurred for pressure thresholds between 250 and 360 kPa. Although subharmonic signals

were observed in the adventitial *vasa vasorum*, coded excitation could incite stronger subharmonic responses in the contrast agent at lower pressure thresholds.¹³

De Jong and colleagues were one of the first research groups to use coded-chirp excitation in harmonic imaging.^{14–16} They demonstrated that the signal-to-noise ratio (SNR) of harmonic signals obtained with long-duration coded-chirp pulses was noticeably higher than those obtained with short sine-burst pulses, but to the best of our knowledge this group has not studied the benefits of using coded-chirp excitation in subharmonic imaging—the primary emphasis of the work reported in this paper. Zhang and colleagues studied the performance of subharmonic imaging at low transmission frequencies (5 MHz). They observed that subharmonic emission increased by 20 dB when the contrast agent was excited with chirps, relative to when it was excited with short sine-bursts.¹³ Higher pressures were required to generate subharmonic signals with short excitation at higher frequencies. Williams and colleagues demonstrated that ultrasound contrast agents emitted stronger fundamental, second harmonic, subharmonic, and ultraharmonic signals when the agents were insonated with high-frequency coded-chirp signals.¹⁷ To our knowledge, this is the only study that reported the use of coded-chirp excitation at frequencies greater than 15 MHz.

The overall goal of the work reported in this paper is to determine the optimum excitation parameters when subharmonic imaging is performed at high transmission frequencies (>15 MHz). We hypothesized that coded-chirp excitation would incite a subharmonic response from more microbubbles than sine-burst excitation of the same pulse duration, which should improve subharmonic emission and the sensitivity of S-IVUS. To corroborate this hypothesis, we performed simulated and experimental studies at 20 MHz on Targestar-TM, a commercial ultrasound contrast agent.

II. PREVIOUS RELATED WORK

Microbubble ultrasound contrast agents undergo volumetric oscillations in response to ultrasonic excitation, emitting energy in linear and nonlinear modes.^{18–23} Second harmonic and subharmonic oscillations of the contrast agent have been exploited to improve imaging specificity of blood-filled structures.^{24–27} In harmonic imaging (HI), ultrasound signals are transmitted at the fundamental frequency (f_o) and echo signals are received at the second harmonic ($2f_o$), at which the ultrasound contrast agent produces a distinct signal.¹⁹ However, human tissue also produces second harmonic signals²⁸ that have reduced specificity.²² Subharmonic imaging (SHI) overcomes this limitation by transmitting at the fundamental frequency (f_o) and receiving at the subharmonic ($f_o/2$) frequency^{22,29,30}—in human tissues, subharmonic signals are weak for the range of acoustic pressures used in diagnostic imaging. The main challenge in subharmonic imaging is that it is a threshold phenomenon,²² and such phenomena are known to affect imaging sensitivity, especially at high transmit frequencies (>15 MHz), where pronounced damping effects

produce higher pressure thresholds.³¹ More specifically, subharmonic oscillation occurs only when the exciting acoustic signal exceeds a certain threshold level.³² Nevertheless, it is envisaged that SHI should offer better lateral resolution than HI. Consequently, several research groups, including that ours, are investigating the potential of subharmonic imaging.^{11,21,22,26,33–35} Initial results with *in vitro* and *in vivo* high-frequency SHI (transmitting/receiving at 20/10 MHz or at 30/15 MHz) have demonstrated the feasibility of using DefinityTM, an FDA-approved microbubble contrast agent for nonlinear imaging, at these frequencies.^{11,35}

Excitations with high acoustic pressures have improved the sensitivity of subharmonic imaging; however, bubble disruption limits the pressures that can be used.^{36–38} Long-duration excitations have been used to reduce the threshold pressures required to generate subharmonics and to induce strong yet stable subharmonic oscillations in the agent.^{27,31} However, this approach would degrade the imaging's axial resolution; in commercial IVUS systems, short 2–4 cycle sine-bursts are used to attain high-resolution images. While the axial resolution is not critical for imaging of large organs, it is important when conducting IVUS imaging of the *vasa vasorum*, as the axial resolution allows visualization of image-localized spatial branching and perfusion, which may be of clinical significance.¹¹

III. MATERIALS AND METHODS

III.A. Computer simulations

Radio-frequency (RF) echo signals were synthesized by summing the weighted acoustic responses of a population of microbubbles at known radial distances, as described in Zheng *et al.*³⁹ The mechanical response (radial oscillations) of the simulated microbubble (gas filled, lipid coated) to a known ultrasound excitation was predicted using a modified Rayleigh–Plesset model, given by

$$\begin{aligned} \rho_L \left[R_1 \ddot{R}_1 + \frac{3}{2} \dot{R}_1^2 \right] = & p_0 \left[\left(\frac{R_{10}}{R_1} \right)^{3\kappa} - 1 \right] + p_i(t) - 4\mu_L \dot{R}_1 \left[\frac{\dot{R}_1}{R_1} \right] \\ & - 12\mu_s \left[\frac{d_s R_{10}^2 \dot{R}_1}{R_{10}^4} \right] \\ & - 12G_s \left[\frac{d_s R_{10}^2}{R_{10}^3} \left(1 - \frac{R_{10}}{R_1} \right) \right], \end{aligned} \quad (1)$$

where R_1 and R_{10} represent the instantaneous and equilibrium inner bubble radii, respectively; ρ_L and μ_L represent the equilibrium density and the shear viscosity of the surrounding liquid, respectively; and p_0 and κ represent the atmospheric pressure and the polytropic index of the gas, respectively. In this model, the surrounding medium was assumed to be infinite, and the pressure at infinity was assumed to be due to the excitation pulse $p_i(t)$. The bubble shell was assumed to be viscous and incompressible; and shell properties, i.e., thickness, shear modulus, and shear viscosity, were represented by d_s , G_s , and μ_s , respectively. The outer (R_2) and inner radii (R_1) of the bubble were related as follows:⁴⁰

$$R_2^3 = R_{20}^3 - R_{10}^3 + R_1^3, \quad (2)$$

where R_{20} represents the equilibrium outer radius. The pressure at the gas-shell interface (P_g) was governed by changes in the bubble radius and was computed as follows:⁴⁰

$$P_g(R_1, t) = p_0 \left(\frac{R_{10}}{R_1} \right)^{3\kappa}. \quad (3)$$

The backscattered pressure (P_{sc}) of an oscillating microbubble was computed as follows:³²

$$P_{sc}(r, t) = \rho_L \frac{R_2(t)}{r} (2\dot{R}_2^2(t) + R_2(t) \ddot{R}_2(t)), \quad (4)$$

where r represents the radial distance from the microbubble where the pressure was computed.

III.B. Simulation study

The goal of the simulation study was to gain insight into how different excitation schemes and pulse parameters, such as the fractional bandwidth and time duration, might impact the performance when subharmonic imaging was conducted at high frequencies—in our case, 20 MHz. To predict the subharmonic behavior of microbubble contrast agents in response to (a) 20 MHz sine-bursts (4% fractional bandwidth) and (b) 20 MHz chirps, with -6 dB bandwidths ranging from 1 to 4 MHz (5%–20% fractional bandwidth), we solved Eq. (1) with a variable order Runge–Kutta method. All computations were performed with initial conditions of $R_1 = R_{10}$ and $\dot{R}_1 = 0$. The numeric values of the parameters used in the simulation are given in Table I. The peak-negative pressure of the simulated excitation pulses was varied from 850 kPa (the threshold for the onset of subharmonic behavior) to 1450 kPa, and computations were performed in MATLABTM (The Mathworks, Inc., Natick, MA, USA) on a Xenon-based computer server operating at 3 GHz under the CentOS operating system (version 5.6). The pressure emanating from the oscillating bubble was computed at the focus of the receiving transducer. The focus of the simulated transducer was chosen to be 50.8 mm, to represent the Olympus V317 and A531S transducers (Olympus NDT, Waltham, MA, USA) that were used in the experimental studies. The pressure response of a polydisperse population of microbubbles to the acoustic excitation was computed by summing their weighted responses. To visualize the spectral response of the microbubble population, we computed the power spectrum of the RF signal. The subharmonic-to-fundamental

ratio of the agents' response to the excitation pulse was used as a metric for imaging sensitivity. To quantify the resolution, we used a two-step process to extract the subharmonic signal from the agent response: (1) the fundamental frequency and noise components were removed from the simulated RF waveforms using a 12th order Butterworth bandpass filter, and (2) the resulting RF waveform was match-filtered by correlating with a 10 MHz signal (10 MHz sine and chirp pulses, when the bubble was excited with a sine-burst and linear modulated chirp, respectively) that had the same duration and half the bandwidth of the excitation pulse.^{13,15} Coded-chirp excitation employs long-duration frequency-modulated bursts. However, unlike sine-burst excitation, in which the phase of all the frequency components is the same, in chirps, every frequency component is phase-shifted with respect to the starting frequency. Therefore, a match-filter was used to adjust the phase of the RF chirp, which shortened the pulse length and improved axial resolution.^{13,41} Match-filtering was performed on both sine-burst and coded-chirp pulses; however, pulse compression did not improve the axial resolution of sine-bursts. The axial resolution was computed from the full-width-half-maximum (-3 dB) of the intensity envelope of the match-filtered pulse.¹³

III.C. Experimental study

To corroborate the computer simulations' predictions, we measured (1) the size distribution and the frequency-dependent attenuation and (2) the subharmonic response of a commercial contrast agent (Targestar-pTM, Targeson, Inc., La Jolla, CA, USA) to a 20 MHz sine-burst and to 20 MHz coded-chirp pulses. The number of microbubbles/ml and size distribution of Targestar-pTM was measured with a CasyTM Cell Counter (Model TTC, Roche Innovatis AG, Switzerland).

III.C.1. Attenuation measurements

To assess the potential of Targestar-pTM for subharmonic generation at high transmit frequencies (>15 MHz), we estimated its frequency-dependent attenuation characteristics in the range of 5–25 MHz. Two single-element transducers (Models V310 and V317, Olympus NDT, Waltham, MA, USA) were used to generate signals between 5–10 MHz and 11–25 MHz, respectively. All measurements were made with a calibrated broadband hydrophone (Model HGL-0085, Onda Corporation, Sunnyvale, CA, USA), in a 60 cm (width) $\times$ 50 cm (length) $\times$ 10 cm (depth) water tank at room temperature, as shown schematically in Fig. 1(a). A narrowband through-transmission substitution technique^{42,43} was used, in which the baseline signal and the signal through the agent were recorded, and the attenuation was estimated by subtracting the peak amplitude of the frequency spectra (in decibels) at each frequency.¹¹ The chamber used to hold the agent had two acoustically transparent saran-membrane windows, which had negligible losses for frequencies up to 25 MHz. The narrowband transmit pulses used in this study consisted of 30 cycle rectangular windowed sine-bursts for all frequencies between 5 and 25 MHz. To minimize multiple scattering effects¹¹ during the attenuation measurements,

TABLE I. List of parameters used in the modified Rayleigh–Plesset model.

d_s	Equilibrium shell thickness (nm)	1.5
R_{10}	Equilibrium inner radius (μm)	0.35–2
R_{20}	Equilibrium outer radius (μm)	$R_{10} + d_s$
r	Radial distance along axis (cm)	5.08
ρ_l	Density of blood (kg/m^3)	1.05×10^3
μ_s	Shear viscosity of shell ($\text{kg}^{-1} \text{s}^{-1}$)	0.07
μ_l	Shear viscosity of blood ($\text{kg}^{-1} \text{s}^{-1}$)	0.03
G_s	Shear modulus of lipid shell (MPa)	190

FIG. 1. Experimental setup used in (a) attenuation measurements and (b) subharmonic generation studies.

we diluted the contrast agent to a concentration of 0.4×10^6 particles/ml.

The measurement procedure used can be summarized into the following steps:

1. Use the computer-controlled mechanical manipulation system (Velmex, Inc., Bloomfield, NY, USA) to align the hydrophone 75 mm in front of the transducer (Model V310, Olympus NDT, Waltham, MA, USA).

2. Place 150 ml of IsotoneTM (Coulter Electronics, Luton, UK) solution in the acoustically transparent sample chamber (50 mm diameter $\times$ 70 mm height); then place the sample chamber at the focus of the transducer (50.8 mm).

3. Use the arbitrary waveform generator (Model 81150A, Agilent, Santa Clara, CA, USA) to produce a sequence of narrowband pulses consisting of 30 cycles of sine-burst pulses with a pulse repetition frequency (PRF) of 1 kHz and a peak-negative pressure of 90 kPa. Set the center frequency of the narrowband sine-burst to 5 MHz.

4. Amplify the narrowband pulses produced by the waveform generator with the linear power amplifier (Model 411LA, Electronic Navigation Industries, Rochester, NY, USA) and use the amplified signal to excite the transmitting transducer.

5. Use the digital oscilloscope (Model Waverunner 44MXi, Lecroy, Inc., Chester Ridge, NY, USA) to digitize the resulting hydrophone signal to 12 bits at a sampling frequency of 500 MHz. Acquire 100 waveforms and store the signals to disc for offline analysis in MATLABTM (The Mathworks, Inc., Natick, MA, USA).

6. Increase the frequency of the narrowband signal by 1 MHz and continue acquiring RF waveforms for frequencies up to 10 MHz as described in steps 4 and 5. Then, change the transducer to Model V317 (Olympus NDT, Waltham, MA, USA) and repeat steps 4 and 5 for frequencies from 11 to 25 MHz in increments of 1 MHz.

7. Change the transducer back to model V310 and reset the frequency of the function generator to 5 MHz.

8. Add 30 μ l of contrast agent to the sample chamber to produce an agent with a dilution ratio of 1:5000. Use the magnetic stirrer (Model 7X7-ALU, VWR International, West Chester, PA, USA) to keep the agent in suspension during the acoustic measurements.

9. Repeat steps 5–7.

III.C.2. Characterizing the subharmonic response of the agent

The equipment used in this study is shown schematically in Fig. 1(b). All measurements were performed in a 60 cm (width) $\times$ 50 cm (length) $\times$ 10 cm (depth) water tank at room temperature, in a transmission mode^{13,44} study with two confocally aligned transducers—a 20 MHz transmitting transducer (Model V317, Olympus NDT, Waltham, MA, USA) and a 10 MHz receiving transducer (Model A312S, Olympus NDT, Waltham, MA, USA). The diameters and focal lengths of both transducers were 6.35 and 50.8 mm, respectively.

The agent was diluted in IsotoneTM solution by a factor of 1:3750 and kept in suspension by a magnetic stirrer (Model 7X7-ALU, VWR International, West Chester, PA, USA). The transmitting transducer was excited, first with rectangular windowed sine-burst pulses and then with coded-chirp pulses that were generated in MATLABTM (The Mathworks, Inc., Natick, MA, USA), and uploaded to the waveform generator (Model 81150A, Agilent, Santa Clara, CA, USA) via a GPIB interface. We compared the subharmonic-to-fundamental ratios of the agent's spectra obtained with both types of excitation pulses (i.e., rectangular windowed sine-bursts and chirps) at peak-negative pressures ranging from 30 to 380 kPa. We also computed the axial resolution over the same range of pressures when the pulse length was varied from 0.7 to 3 μ s.

The transmit signal was amplified with a 40 dB linear power amplifier (Model 411LA, Electronic Navigation Industries, Rochester, NY, USA). The receiving stage of a pulser-receiver (Model DPR300, JSR Electronics, NY, USA) was used to amplify the signal produced by the receiving transducer by 20 dB. The amplified signal was then digitized to 12 bits at a sampling frequency of 500 MHz by a digital oscilloscope (Model Waverunner 44MXi, Lecroy, Inc., Chester Ridge, NY, USA). Five groups of RF waveforms, each consisting of 20 RF traces, were acquired with each pulse and stored to disc for offline analysis in MATLABTM.

The experimental results were analyzed in a similar manner as the simulated data—the subharmonic response of the agent to sine-bursts and chirps was quantified using the subharmonic-to-fundamental ratio. The subharmonic signal was extracted from the RF and compressed, following which the axial resolution was calculated.

IV. RESULTS

Figures 2(a) and 2(b) show a representative example of the number and volume weighted size distribution acquired from CasyTM counter measurements. The mean and median diameters and the concentration/ml of the agent microbubbles were computed from three independent measurements, as listed in Table II. Figure 2(b) shows a plot of the attenuation vs frequency that was measured using the through-transmission substitution technique. The error bars in Fig. 2(c) represent ± 1 standard deviation computed over 5 statistically independent measurements.

Attenuation in the agent peaked at 10 MHz and decreased when the frequency was increased further. The attenuation characteristics were similar to those reported for DefinityTM (Lantheus Medical Imaging, North Billerica, MA, USA), which was expected, because both DefinityTM and Targestar-pTM are lipid-coated contrast agents filled with perfluorocarbon gas.

Figures 3(a) and 3(b) show the frequency response of the transducers used in the subharmonic generation experiments. Figure 3(c) shows the beam patterns of the transmitting and receiving transducers. The axial and lateral -3 dB focal dimensions were 22 and 1 mm for the transmitting transducer and 50 and 6 mm for the receiving transducer, respectively. Figure 4 shows the frequency response of the calibrated broadband hydrophone.

Figure 5 shows the frequency response of contrast agents to three transmit conditions: (a) 1.5 μ s, 30 kPa chirps, (b) 0.7 μ s, 190 kPa chirps, and (c) 1.5 μ s, 190 kPa chirps. Subharmonics were not apparent at low transmit peak pressure (a) or for short pulse duration (b); however, the fundamental and the subharmonic peaks were visible at 20 and 10 MHz when long pulse duration and high transmit pressure (c) were employed. Figure 6

TABLE II. Size distribution estimates of Targestar-pTM

Size distribution estimates of Targestar-p TM	
Number of microbubbles/ml = 1.97×10^9	
Mean diameter (number weighted) = $1.92 \pm 0.096 \mu\text{m}$	
Median Diameter (number weighted) = $1.67 \pm 0.005 \mu\text{m}$	
Mean diameter (volume weighted) = $4.25 \pm 0.013 \mu\text{m}$	
Median diameter (volume weighted) = $3.56 \pm 0.011 \mu\text{m}$	

demonstrates that Gaussian windowing of excitation pulses sharply decreases the subharmonic emission and raises the threshold required to produce subharmonics. The estimated subharmonic-to-fundamental ratios for sine-burst and coded-chirp excitation with peak-negative pressures ranging from 60 to 380 kPa and 1.5 μ s duration are shown in Fig. 7. Subharmonics were observed at approximately 60 kPa for all excitation pulses. The subharmonic threshold (for constant pulse duration) was independent of chirp bandwidth, but dependent on peak-negative pressure, which is consistent with the results reported at lower transmission frequencies¹³. The subharmonic-to-fundamental ratio first increased with increasing peak-negative pressure up to nearly 190 kPa for all excitation pulses and then decreased with further increase in peak-negative pressure. Figure 8 shows a representative example of the simulated and experimentally acquired frequency spectra and the filtering operation used to extract subharmonic spectra.

Figure 9 shows a representative example of signal produced after Butterworth and match-filtering, which demonstrated the suppression of the fundamental mode signal and the enhancement of axial resolution.

Figure 10 compares the axial resolution of the subharmonic signal obtained with sine-bursts and chirps, when the pulse duration was varied from 1 to 3 μ s. Although the axial resolution achieved with sine-bursts was dependent on pulse duration, the axial resolution of chirps was independent of pulse duration.

Figure 11 demonstrates the axial resolutions achieved using sine-burst and coded-chirp excitations of 1.5 μ s duration for the pressure range of 60–380 kPa.

FIG. 2. (a) The number weighted and (b) volume weighted size distribution of Targestar-pTM measured with a CasyTM cell counter and (c) the frequency-dependent attenuation of Targestar-pTM. The error bars represent ± 1 standard deviation computed over 5 statistically independent measurements.

FIG. 3. Frequency response of (a) the transmitting transducer (20 MHz nominal center frequency) and (b) the receiving transducer (10 MHz nominal frequency). (c) The measured intensity beam-pattern of the transmitting (20 MHz) and receiving (10 MHz) transducers.

V. DISCUSSION

Significant damping effects increase the pressure required to produce subharmonic signals at high frequencies, which may compromise the sensitivity of subharmonic IVUS images.¹¹ The goal of the work reported in this paper was to investigate the feasibility of improving the sensitivity of high-frequency subharmonic imaging by exciting the agent with chirps. More specifically, long-duration chirps were used to incite strong subharmonic signals from the agent, and pulse compression was used to prevent the degradation of axial resolution.

V.A. Attenuation characteristics of the agent

In this study, we used a transmission substitution method wherein the peak pressure was maintained constant and a narrow band of sine-burst pulses was used to sweep the

bandwidth from 5 to 25 MHz. This method facilitated the use of exactly the same pressures for the whole frequency range, eliminating the possibility of pressure-dependent attenuation artifacts.⁴² Further, narrowband sine-burst pulses had high SNR due to longer pulse duration, which facilitated measurements at low acoustic pressures.¹¹

The frequency-dependent attenuation plot in Fig. 2(c) demonstrates the resonant response of the agent in the vicinity of 10 MHz, beyond which it decreases appreciably. The strongest subharmonic response was achieved when the agent was excited at twice its resonant frequency⁴⁵; therefore, the attenuation peak at 10 MHz was consistent with the observed subharmonic generation efficiency at 20 MHz. The sharp drop in attenuation beyond 10 MHz indicates that the size distribution of the agent was not optimum for subharmonic imaging at higher transmit frequencies (30–40 MHz), but increasing the relative number of smaller microbubbles in the agent by filtration may enhance subharmonic emission at higher frequencies.^{10,31,45}

V.B. Subharmonic response of agent to sine-burst and chirp excitation

Figure 5(a) demonstrates that no subharmonic signal was observed with 30 kPa coded-chirp excitation pulses (fractional bandwidths 5%–20%) of 1.5 μ s duration. Figure 5(b) shows that subharmonics were absent for pulse durations of 0.7 μ s, even when a peak-negative pressure of 190 kPa was used. However, distinct subharmonic behavior was observed with 190 kPa excitation pulses of 1.5 μ s duration [Fig. 5(c)].

Figure 7 demonstrates three interesting observations. First, exciting the agent with chirps produced a stronger subharmonic response relative to that produced by sine-bursts. More specifically, subharmonic-to-fundamental ratios obtained with chirps were up to 5.7 dB higher than those

FIG. 4. The frequency response of the broadband membrane hydrophone.

FIG. 5. Experimentally acquired frequency spectra showing the response of contrast agents under three transmission conditions. (a) $1.5 \mu\text{s}$, 30 kPa coded-chirps, (b) $0.7 \mu\text{s}$, 190 kPa coded-chirps, and (c) $1.5 \mu\text{s}$, 190 kPa coded-chirps. Subharmonics were not observed for 30 kPa peak pressures (a) and $0.7 \mu\text{s}$ pulse durations (b); however, the fundamental and the subharmonic peaks at 20 and 10 MHz were clearly discernable for (c).

produced with sine-bursts of similar duration, which suggests that more energy was transferred into subharmonic modes when coded-chirp excitation was used. The increased efficiency of chirps suggests that more of the agent microbubbles oscillated nonlinearly when chirps were employed, the excitation energy being spread across a relatively wider frequency range. Second, the subharmonic-to-fundamental ratio first increased with increasing peak-negative pressure, but decreased when the pressure exceeded 190 kPa. This decrease occurred because the subharmonic signal saturated, while the fundamental signal continued to increase with increasing peak-negative pressure, a trend that has been reported previously.^{13,45} Third, the subharmonic-to-fundamental ratio increased by 5.7 dB with increasing bandwidth between 1 and 3 MHz and then decreased slightly by 0.7 dB for the 4 MHz bandwidth chirp. This behavior typically occurs because the strongest subharmonic response is observed from individual microbubbles when they are excited exactly at twice their resonant frequency.⁴⁵ When the excitation bandwidth was increased significantly, the energy was distributed in a wide frequency band, which reduced its ability to excite individual bubbles in subharmonic mode, weakening the overall subharmonic response. The enhancement in subharmonic-to-fundamental ratio with increasing bandwidth was tested for statistical significance using unpaired t-tests. There was no significant difference between subharmonic-to-fundamental ratios obtained with 4% bandwidth

sine-burst and 5% bandwidth chirps ($p > 0.01$), which is as expected, as they have the same pulse duration and nearly the same fractional bandwidth. However, all the other differences were found to be statistically significant.

Figure 8 demonstrates that the fundamental signal was significantly suppressed (by over 50 dB) when a Butterworth and a match-filter were applied to the received RF signal.

Figure 9 demonstrates that pulse compression by match-filtering improves the axial resolution. The phase of the subharmonic signal was affected by the constructive and destructive interference of the signals resulting from nonlinear oscillations of microbubbles located at different spatial positions. This caused the envelope of the subharmonic signal to modulate [see Fig. 9(f)]. The computational model did not properly account for this phenomenon; therefore, such a modulation is not present in the simulated subharmonic signal.

V.C. Excitation pulse parameters

In this study, we employed 20 MHz transmission frequencies because clinical IVUS scanners of 20 MHz center frequencies are commonly used clinically.⁴⁶ We are currently developing a prototype S-IVUS system with 20 and 40 MHz center frequency catheters. Currently, we have not observed subharmonics at 40 MHz transmit frequencies. However, in the future, we plan to optimize the size distribution and shell

FIG. 6. The effect of tapering of excitation pulses on the subharmonic-to-fundamental ratio and the threshold of subharmonic emission. Figure 6(a) shows that the subharmonic-to-fundamental ratio decreased by 20 dB when the excitation pulse was windowed by 50% with a tapered Gaussian window. Figure 6(b) shows that subharmonics were first observed at higher threshold pressures for tapered excitation pulses relative to the rectangular windowed sine-burst.

parameters of the contrast agent and investigate the performance of S-IVUS at higher frequencies (up to 40 MHz).

Wideband chirps (nearly 100% of transducer bandwidth) have been employed in B-Mode imaging to enhance the axial resolution⁴¹; however, in nonlinear imaging, the bandwidth of chirps employed is relatively narrow.^{13–16} Using wideband chirps such as those employed in B-Mode imaging for subharmonic imaging can cause the overlay of the fundamental and subharmonic spectrum; the energy in the fundamental frequency band may leak into the energy in the subharmonic frequency band.¹⁵ In such cases, it may not be possible to completely eliminate the fundamental energy associated with tissue backscatter, resulting in loss of imaging specificity. It appears that 20% bandwidth chirps represent a reasonable tradeoff between subharmonic signal strength and axial resolution (described in Sec. V D).

Rectangular windowed coded-chirp excitation may result in temporal side-lobes⁴¹; however, in the context of ultrasound contrast imaging, a side-lobe level of -20 dB is considered

Fig. 7. Subharmonic-to-fundamental ratio computed from the agent spectra, when the peak-negative-pressure was increased progressively from 60 to 380 kPa, with pulse duration of $1.5 \mu\text{s}$.

acceptable.^{14,15} In our study, a relatively low threshold of 60 kPa was observed (see Fig. 5) and the temporal side-lobe level in the compressed signal was reasonable; i.e., -30 dB [Fig. 9(h)]. The rectangular windowing of pulses (instead of Gaussian windowing) and the use of long-duration pulses were responsible for the onset of subharmonic behavior at a low peak-negative pressure (60 kPa) than previously reported.^{44,45} Longer duration pulses have higher energy, which have enhanced nonlinear oscillations from contrast agents, resulting in stronger subharmonic oscillations and lower thresholds for subharmonic emission.^{13,47} Furthermore, for fixed transmit frequencies, bubble disruption was more correlated to the peak-negative pressure than to pulse length; therefore, long-duration pulses may lead to stable yet strong subharmonic behavior from the contrast agent.^{36,47}

Figure 6(a) demonstrates that tapering of excitation pulses by Gaussian windowing adversely affected the subharmonic-to-fundamental ratio, and Fig. 6(b) demonstrates that the threshold for subharmonic emission increased as the percentage windowing of the excitation pulses was increased. These results were consistent with the observations made by Biagi and associates,⁴⁴ who postulated that the smoothness of the initial portion of the excitation pulse adversely affected the generation of the subharmonic pulse. In our study, 50% Gaussian windowing of $2 \mu\text{s}$ excitation pulses led to a sharp decrease (over 20 dB) in the subharmonic-to-fundamental level. While subharmonic emission was first observed with 70 kPa for rectangular windowed pulses, the thresholds observed for 20% and 50% Gaussian windowed sine-bursts were higher—105 and 200 kPa, respectively.

Zhang *et al.* reported a study in which subharmonic emission from short tone bursts was enhanced when compared to long-duration chirps of equivalent bandwidth.¹³ Gaussian windowed excitation pulses with 5 MHz center frequency, 4%–20% fractional bandwidth, and long-pulse durations in the range of 10–40 μs (corresponding to 50–200 cycle

FIG. 8. (a) Simulated and (c) experimentally acquired acoustic response of the agent to 4 MHz bandwidth chirp excitation with peak-negative pressure of 190 kPa. The subharmonic spectra extracted using the combined Butterworth and match-filtering operation are shown in (b) and (d).

sine-bursts) were employed. They measured subharmonic thresholds of 0.5 MPa; in our study, notably lower thresholds were observed (60 kPa). Furthermore, our studies employed short pulses compared to those used in their study, which may have reduced the disruption of microbubbles over time.

V.D. Resolution attainable

High sensitivity and axial resolution are desirable in order to visualize and quantify the localized spatial branching and perfusion patterns of the *vasa vasorum*. Figures 10 and 11 demonstrate that axial resolution in this study improved with increasing bandwidth of the chirps. Coded-chirp excitation

employs long-duration frequency-modulated bursts. Unlike sine-burst excitation, in which the phase of all the frequency components was the same, every frequency component in coded-chirp excitation was phase-shifted with respect to the starting frequency.^{13,41} Match-filtering was used to decode the chirp RF time traces backscattered from the contrast agent. Match-filtering adjusts the phase of the RF chirp, resulting in the shortening of spatial pulse length, which improves the axial resolution. To perform match-filtering in this study, the received RF pulse was correlated with an *a priori* estimate of the subharmonic signal, which had the same duration as the excitation pulse but was centered at the subharmonic frequency (10 MHz).¹³ Butterworth filtering was performed prior to match-filtering to suppress the

FIG. 9. Shows the simulated (above) and experimental (below) results obtained with 4 MHz coded-chirp for (b) and (f) subharmonic RF pulse and (c), (g), and (h) axial resolution enhancement by pulse compression.

FIG. 10. The simulated (a) and (b) experimentally estimated axial resolution when pulse duration of sine-burst and coded-chirps was varied from 1 to 3 μs .

unwanted frequency components and noise, which improved the efficacy of the match-filtering.¹³

Coded excitation has been previously used for B-mode ultrasound imaging, in which axial resolution has been enhanced by

the time bandwidth product of the compression chirp.⁴¹ However, since the bandwidth of the chirps employed in subharmonic imaging was lower (Sec. V B), the gain in axial resolution was lower than that typically achieved in B-Mode imaging.⁴¹ In theory, a gain in axial resolution of the order of time-bandwidth-product of the compression chirp may be expected if there is a complete match between the compression chirp and the emitted subharmonic signal. However, we observed that the actual gain in axial resolution was lower, primarily because the phase of the received subharmonic was modulated because of interference among signals emitted from microbubbles located at different spatial positions. Coded-chirp excitation improved the axial resolution over sine-burst excitation (Figs. 10 and 11). These figures demonstrate that the axial resolution improved with increasing bandwidth of the excitation chirp. While the axial resolution of sine-bursts degrades almost linearly with pulse duration, the axial resolution of chirps remained almost the same for pulse durations of 1–3 μs . However, shorter pulses (1.5 μs) may be used to minimize bubble disruption over time. Figure 11(b) shows that the axial resolution achieved with 20% fractional bandwidth chirps after pulse compression improved nearly twofold relative to that of 4% fractional bandwidth sine-bursts. The differences in axial resolution obtained with different pulses were statistically significant ($p < 0.01$) for the different excitation pulses, except for sine-bursts and 4% bandwidth chirps for pressures up to 220 kPa. An axial resolution of approximately 400 μm was achieved with 20% fractional bandwidth chirps of 1.5 μs duration, which is similar to the size of larger microvessels. Therefore, while individual microvessels may not be visualized accurately with 20 MHz transmit frequency and 1.5 μs pulse duration, the axial resolution of 400 μm may be enough to characterize regions of high neovessel density and perfusion.¹¹ Higher transmit frequencies should further improve the axial resolution; therefore, we plan to conduct studies at higher frequencies (40 MHz).

V.E. Study limitations

Studies were performed with a transmission mode experimental setup. Therefore, in the experimentally acquired

FIG. 11. The axial resolution obtained when the agent was excited with sine-bursts and coded-chirps. The results were obtained by processing (a) simulated and (b) experimentally acquired RF echo traces.

spectra, the fundamental mode energy was also contributed directly from the transmitting transducer to the receiving transducer, which increased the fundamental level in the spectra relative to the subharmonic emanating from the agent. However, in the simulated spectra, the fundamental peak represented the linear signal generated from the oscillations of the agent alone. Therefore, the simulation did not adequately model experimental conditions. Second, the simulation did not account for the band-pass transducer response: the sensitivity of the receiving (10 MHz) transducer was 21 dB lower at the fundamental frequency [see Fig. 3(b)], which reduced the fundamental peak. Therefore, in this study, the simulation results were only compared qualitatively with the experimental results, and the subharmonic-to-fundamental ratio represented a differential metric for comparing the subharmonic response of sine-burst and coded-chirp excitations.

The modified Rayleigh–Plesset model used in this study also has intrinsic limitations: first, it does not account for microbubble disruption, bubble liquid nonlinearity, multiple scattering, or attenuation effects; and second, it predicts pressure thresholds for subharmonic generation which are relatively higher than those observed experimentally. This observation may be attributed in part to the simplistic elastic model for the shell. Although alternative models have better matched the experimental results at low frequencies,^{48–50} we chose the simple Rayleigh–Plesset model, as the ability of computational models to predict subharmonic behavior from agent populations at high frequencies is limited.⁵¹ Therefore, we are exploring more appropriate models such as that developed by Marmottant and colleagues.⁵⁰ Although these models should provide better predictions than the Rayleigh–Plesset model, the general trends predicted with different excitation pulse parameters should be similar to those predicted by the Rayleigh–Plesset model.

We also assumed that the shell parameters for Targestar-TM were similar to those reported for DefinityTM, based on the similarity of their attenuation characteristics.³⁶ However, we plan to verify this assumption by estimating the shell parameters of Targestar-TM using a model-based method for our future studies. Further, we employed a weighted summation technique for modeling a polydisperse agent.⁵² While this technique has been demonstrated to be accurate in low-frequency studies with low concentrations, its utility for accurately predicting the response of the agent population for concentrations approaching *in vivo* doses may be suspect.

VI. CONCLUSIONS

It is envisaged that *vasa vasorum* imaging could be used to identify vulnerable, life-threatening coronary plaques. However, methods must be developed to improve the sensitivity and spatial resolution of subharmonic intravascular ultrasound imaging. The results of the theoretical and experimental studies reported in this paper demonstrate that the coded-chirp method of exciting ultrasound contrast agents can improve the sensitivity by almost 6 dB and the

axial resolution by 95%. Therefore, coded-chirp excitation may be an ideal excitation strategy for subharmonic intravascular ultrasound imaging and may enhance clinical ability to identify rupture-prone plaques. At 20 MHz transmit frequency and 20% fractional bandwidth, coded-chirp excitation appears to be the ideal tradeoff between subharmonic strength and axial resolution.

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