The response of phospholipid-encapsulated microbubbles to chirp-coded excitation: Implications for high-frequency nonlinear imaging

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The current excitation strategy for harmonic and subharmonic imaging (HI and SHI) uses short sine-bursts. However, alternate pulsing strategies may be useful for enhancing nonlinear emissions from ultrasound contrast agents. The goal of this study was to corroborate the hypothesis that chirp-coded excitation can improve the performance of high-frequency HI and SHI. A secondary goal was to understand the mechanisms that govern the response of ultrasound contrast agents to chirp-coded and sine-burst excitation schemes. Numerical simulations and acoustic measurements were conducted to evaluate the response of a commercial contrast agent (Targestar-P[®]) to chirp-coded and sine-burst excitation (10 MHz frequency, peak pressures 290 kPa). The results of the acoustic measurements revealed an improvement in signal-to-noise ratio by 4 to 14 dB, and a two- to threefold reduction in the subharmonic threshold with chirp-coded excitation. Simulations conducted with the Marmottant model suggest that an increase in expansion-dominated radial excursion of microbubbles was the mechanism responsible for the stronger nonlinear response. Additionally, chirp-coded excitation detected the nonlinear response for a wider range of agent concentrations than sine-bursts. Therefore, chirp-coded excitation could be a viable approach for enhancing the performance of HI and SHI. © 2013 Acoustical Society of America. [http://dx.doi.org/10.1121/1.4798677]

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I. INTRODUCTION

The rupture of atherosclerotic plaques triggers a majority of deaths due to stroke and cardiovascular disease.¹ The abnormal growth of the *vasa vasorum* is believed to accelerate plaque rupture.^{2,3} Therefore, an imaging modality capable of characterizing the *vasa vasorum* could be used to screen the general public for life-threatening atherosclerotic plaques.⁴ Researchers have proposed contrast-enhanced ultrasound imaging techniques to visualize the *vasa vasorum* in the carotid artery.^{5,6} However, introducing *vasa vasorum* imaging in routine clinical practice will be dependent on its ability to detect ultrasound contrast agents (UCAs) with high sensitivity and specificity.⁴

Nonlinear imaging techniques such as harmonic and subharmonic imaging (HI and SHI) are suitable for *vasa vasorum* imaging as they offer improved UCA detection over linear modes.^{7,8} However, most commercial UCAs are designed for cardiac imaging (1 to 5 MHz),⁹ which results in a weak nonlinear response when they are excited at higher frequencies.¹⁰ Therefore several groups, including ours, are developing techniques to enhance the high-frequency (HF) nonlinear response of UCAs.^{11–14} In particular, there is interest in characterizing UCAs in the frequency range of 7 to 12 MHz for noninvasive assessment of atherosclerosis in the carotid artery.^{11,13,15,16}

One approach to improve the nonlinear response of UCAs is to design contrast agents for high-frequencies. For example, modifying the size distribution of the agent has

been shown to improve its HF nonlinear response.¹⁷ Additionally, the nonlinear response of UCAs is known to be affected by the excitation pulse. Therefore, alternate pulsing strategies may be useful for HF imaging. Chirp-coded excitation is one such technique that has been widely investigated for contrast-enhanced ultrasound imaging.^{14,18–27}

In a theoretical study, Barlow and colleagues reported an analytical solution to the Keller-Herring model, to predict the response of microbubbles to chirp-coded excitation.¹⁸ A classical perturbative analysis was employed²⁸ to gain insight into the impact of excitation parameters on the nonlinear response. Chetty *et al.* reported a simulation study based on a modified Rayleigh-Plesset model to investigate the acoustic response of UCAs to phase and amplitude modulated chirp-encoded sequences.¹⁹ It was demonstrated that the chirp length can be increased and its bandwidth reduced to improve the signal-to-noise ratio (SNR), though the resolution was sacrificed. Borsboom and colleagues assessed the impact of chirp-coded excitation on HI.^{20–22} The feasibility of enhancing the subharmonic response of UCA with chirp-coded excitation was demonstrated by Zhang and co-workers.²³ Shen and colleagues incorporated chirp-coding into dual frequency difference excitation imaging to excite UCAs close to their resonance frequency.²⁴ They reported improvements in the SNR, at the expense of a reduced agent-to-tissue contrast. It was also demonstrated that chirp-coded excitation improved the SNR of $3f_0$ transmit phasing techniques;²⁵ however, the agent-to-tissue contrast was reduced. In another study, the contrast agent response to increasing and decreasing chirp-coded pulses was observed optically and acoustically.²⁶ The received echoes were not time-reversed replicas, which inspired a multi-pulse imaging technique called “chirp-reversal.”^{26,27}

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The preceding citations described studies that were conducted at low frequencies (<5 MHz), motivated by traditional diagnostic applications. Imaging at high frequencies is typically more challenging due to (1) a weaker nonlinear response of UCAs, and (2) nonlinear propagation artifacts observed in tissue. Further, studies using ultrafast imaging have demonstrated that the dynamics of UCAs at high frequencies are different from that observed at low frequencies, both qualitatively and quantitatively.¹¹ Therefore, an important question is whether the performance observed at low frequencies with chirp-coded excitation in previous studies will extend to high frequencies. Very few studies have investigated the non-linear response of lipid-encapsulated agents to chirp coded excitation at frequencies higher than a conventional diagnostic range (1 to 7 MHz).

Sun and colleagues investigated the response of lipid-encapsulated microbubbles to chirp and sine-burst excitation at 10 MHz, using high-speed optical photography and acoustic measurements.²⁹ Their primary motivation was to understand the mechanisms responsible for UCA destruction, therefore, higher pressures were used than those employed in imaging.³⁰ Our group has previously reported a comparative study of the response of narrowband sine-bursts and wideband chirp-coded pulses. Both excitation schemes were designed to have the same peak-pressures and pulse duration.¹⁴ The results revealed that: (1) Chirp-coded excitation improved the subharmonic response relative to narrowband sine-bursts, which suggests that wideband chirp-coded pulses excite a wider population of microbubbles in subharmonic mode. (2) No difference was observed in the pressure threshold for inciting subharmonic behavior between chirp-coded and sine-burst excitation strategies. This observation indicates that the threshold is independent of the bandwidth of excitation, as long as the excitation pulses have the same energy. However, this study compared narrowband sine-bursts (5% fractional bandwidth) against chirp-coded excitation pulses with a wider bandwidth. With the advent of multi-pulse techniques such as pulse-inversion, nonlinear imaging can be conducted with pulses that have a relatively wider bandwidth. Therefore, it is important to gain insight into the performance of chirp-coded excitation when compared to sine-burst pulses with equal bandwidth. It is likely that different mechanisms will contribute to the nonlinear response of UCAs when pulses with the same bandwidth are used, instead of those with the same energy. Additionally, our previous work was limited to SHI, while the goal of the present study was to investigate both subharmonic and harmonic modes.

We hypothesized that chirp-coded excitation can enhance HF HI and SHI by (1) improving the harmonic and subharmonic SNR, (2) reducing the thresholds for subharmonic oscillations, and (3) improving the detection of HI and SHI modes. To corroborate this hypothesis, we performed numerical simulations with the Marmottant model, and experimentally measured the acoustic response of a lipid-encapsulated contrast agent (Targestar-P®, Targeson Inc., San Diego, CA). A parametric study was conducted to understand how transmit pulse parameters and the concentration of the agent would impact the performance of HI and SHI. Further, we conducted additional simulations to gain insight into the possible

mechanisms driving the phenomena reported in this study. To our knowledge, this is the first reported study that investigates both the subharmonic and harmonic response of UCAs to chirp-coded excitation. The studies reported in this paper were conducted at 10 MHz, keeping in mind the range of frequencies typically used in noninvasive screening of atherosclerosis (7 to 12 MHz).

II. PHYSICAL BACKGROUND

HI exploits the UCA response at twice the transmit frequency, in order to improve agent-to-tissue contrast over linear imaging.^{31,32} High transmit pressures are used when HI is conducted at high frequencies, to overcome frequency dependent attenuation. However, a strong tissue harmonic signal is generated at high pressures, which limits the agent-to-tissue contrast of HI.^{33,34} Although HI can be conducted at low transmit pressures to improve agent-to-tissue contrast,³⁴ such a strategy will compromise the SNR and limit imaging depth.

SHI overcomes this limitation by visualizing agent echoes at half the transmit frequency.^{33,35,36} Although the SHI has a lower spatial resolution, it is highly specific because subharmonic signals are not generated in tissues. The main limitation of SHI is that it is a threshold phenomenon—subharmonic signals are generated only when the excitation pressure exceeds a certain threshold.^{34,36} This phenomenon reduces imaging sensitivity, especially at high frequencies where increased encapsulation damping produces high pressure thresholds. Additionally, imaging with high transmit pressures can disrupt agent microbubbles and reduce their *in vivo* persistence.³⁰

The nonlinear response of UCAs depends not only on the peak pressure, but also on the characteristics of the excitation pulse, such as its pulse duration and bandwidth. The current pulsing scheme (sine-burst excitation) imposes a trade-off between the SNR and the axial resolution of imaging.³⁷ For example, long excitation pulses can reduce the threshold for SHI, but at the expense of reducing the axial resolution, which may hamper its clinical utility. Further, there is evidence that long sine-burst pulses may excite a narrower size-range of microbubbles in nonlinear modes than wideband pulses—a phenomenon which could reduce imaging sensitivity.¹⁴ However, chirp-coded excitation can achieve a high SNR without degrading the axial resolution.³⁷ Chirp coding shifts the phase of the frequencies in the transmitted pulse with respect to the starting frequency, which creates a pulse with a time-bandwidth product greater than unity. The axial resolution can be recovered by matched filtering, which compresses the received pulse temporally by a factor of its time-bandwidth-product.³⁷ Since the time-bandwidth-product of sine-bursts is unity, matched filtering does not improve the axial resolution of sine-bursts.³⁷

III. METHODS

A. Modeling the dynamics of UCAs: Theory and implementation

The dynamic behavior of encapsulated microbubbles has been studied previously using modified forms of the Rayleigh Plesset equation.^{28,38,39} Such models typically assume a linear

viscoelastic shell with properties that remain constant during the radial oscillations of UCA—an assumption that is reasonable only for small amplitude oscillations. However, phospholipid-encapsulated microbubbles demonstrate large amplitude oscillations at relatively low pressures.^{40,41} Recent studies using ultrafast microscopy revealed asymmetric oscillations, compression only behavior, and low subharmonic thresholds, which cannot be explained by assuming constant coating properties for UCA.⁴⁰ We have previously observed this discrepancy—the thresholds for subharmonic oscillations predicted by the modified Rayleigh Plesset model were an order of magnitude higher than those observed

experimentally.¹⁴ The Marmottant model overcomes these limitations by incorporating buckling and rupture regimes governed by the “effective surface tension,” which accounts for shell elasticity.⁴² This assumption is based on the finding that the surface tension of the microbubble shell depends on the surface concentration of phospholipid molecules.⁴² The change in the effective surface tension during microbubble oscillations causes rapid changes in shell elasticity, which results in a strong nonlinear response at low pressures—a trend that is consistent with experiments.⁴⁰

The Marmottant model assumes that the time dependent radial oscillation of a single microbubble is given by

$$\rho \left(R(t) \ddot{R}(t) + \frac{3}{2} \dot{R}(t)^2 \right) = \left(P_0 + \frac{2\sigma(R_0)}{R_0} \right) \left(\frac{R_0}{R} \right)^{3\kappa} \left(1 - 3 \frac{\kappa}{c} \dot{R}(t) \right) - P_0 - \frac{2\sigma(R(t))}{R(t)} - 4\mu \frac{\dot{R}(t)}{R(t)} - 4\kappa_s \frac{\dot{R}(t)}{R(t)^2} - P(t), \quad (1)$$

where $R(t)$ represents the instantaneous microbubble radius, R_0 represents the initial radius, ρ represents density, μ represents shear viscosity, and c represents the speed of sound. The polytropic index of the encapsulated gas is denoted by κ , and κ_s denotes the surface dilational viscosity. P_0 and $P(t)$ represent the ambient and the excitation acoustic pressures, respectively.

The term $\sigma(R(t))$ denotes the effective surface tension of the lipid shell, which is assumed to vary in three distinct regimes (i.e., buckling, linear elastic, and rupture) as a function of the microbubble area, as given below

$$\sigma(R(t)) = \begin{cases} 0, & R(t) < R_b \\ \chi \left(\frac{R(t)^2}{R_b^2} - 1 \right), & R_b \leq R(t) \leq R_r \\ \sigma_w, & R(t) > R_r. \end{cases} \quad (2)$$

The buckling and rupture behavior of the shell were modeled using three parameters—a buckling radius R_b , the elastic compression modulus (χ), and a rupture surface tension (σ_r). The surface tension term was assumed to vanish when the instantaneous radius of the microbubble was lower than R_b . When the microbubble radius was between R_b and R_r , an elastic regime was assumed, which was characterized by an elastic compression modulus χ . Within this range, the surface tension varied linearly, as a function of the surface area of the microbubble. The rupture surface tension σ_r determined the radius at which the microbubble shell ruptures (R_r). This radius was given by

$$R_r = R_b(1 + \sigma_r/\chi)^{1/2}. \quad (3)$$

When the instantaneous radius increased above R_r , the gas core of the microbubble was assumed to be in direct contact with water, and the surface tension term was equal to that of water (σ_w).

The backscattered pressure P_s of an oscillating bubble was computed from the time dependent radial response as follows:²⁸

$$P_s(t) = \rho \frac{R(t)}{d} (2\dot{R}(t)^2 + R(t)\ddot{R}(t)), \quad (4)$$

where d represents the radial distance. The response of the individual microbubbles was computed for microbubbles with radii between 0.35 and 10 μm , under initially elastic and initially buckled states for different excitation schemes.

Since UCAs typically have a wide size distribution, a weighting scheme was used to synthesize the overall acoustic response from a population of microbubbles.⁴³ The total scattered pressure $P_{sc}(t)$ was given by

$$P_{sc}(t) = \sum_{k=i}^j P_s(R_{0k}, t) w_k, \quad (5)$$

where $P_s(R_{0k}, t)$ was the scattered pressure emanating from a single microbubble with resting radius R_{0k} . The symbols i and j represent the minimum and maximum radii that were measured by the Casy counter—0.35 and 10 μm , respectively. The quantity w_k denotes the number-fraction of microbubbles, which have a resting radius R_{0k} .

The Marmottant model was implemented in MATLAB[®] (version 2011a, the Mathworks Inc., Natick, MA) and solved with a variable order Runge-Kutta method with initial conditions of $R = R_0$ and $\dot{R} = 0$.

B. Simulation study

We simulated the acoustic response of UCAs to sine-burst and chirp-coded excitation (center frequency: 10 MHz, pressure amplitudes: 100 to 290 kPa, fractional bandwidth: 10% to 40%). The fractional bandwidth of excitation denotes the full-width-half-maximum (FWHM) bandwidth of the pulse divided by the nominal center frequency of the

TABLE I. Transmit pulse parameters.

FWHM bandwidth	Pulse-duration (sine-burst) (μs)	Pulse-duration (chirp) (μs)
1 MHz (10%)	0.48	2.4
2 MHz (20%)	0.65	3.25
3 MHz (30%)	0.95	4.75
4 MHz (40%)	1.8	9

transmitting transducer. The fractional bandwidth and time duration of the excitation pulses used in this study are listed in Table I.

We computed the acoustic response of the microbubbles at a distance $d = 50$ mm—the focal length of the transducer used in the subsequent experiments. The model parameters and physical constants were as follows:^{13,42} elastic compression modulus $\chi = 2.5$ N/m, surface dilatational viscosity $\kappa_s = 1 \times 10^{-9}$, surface tension of water $\sigma_w = 0.073$ N/m, density of water $\rho = 1000$ kg/m³, viscosity of water $\mu = 1 \times 10^{-3}$ Ns/m², hydrostatic pressure $P_0 = 1.013 \times 10^5$ kPa, polytropic exponent $\kappa = 1.07$, speed of sound $c = 1480$ m/s, $R_r = 1.0018 R_0$. Initially elastic and buckled states were simulated by setting the buckling radius R_b as $0.98 R_0$, and as R_0 , respectively. The size distribution of the simulated UCA was based that of Targestar-P, which was measured using a CasyTM Cell Counter (Model TTC, Roche Innovatis AG, Rotkreuz, Switzerland) as described in Sec. III C.

C. Experimental study

We conducted an experimental study to corroborate the predictions of the simulation study, and to assess the detectability of nonlinear agent response in the presence of electronic noise. More specifically, the goals of the experimental study were to assess (1) the effect of transmit pulse parameters, and (2) the effect of agent concentration on the measured nonlinear response.

The nonlinear response of the agent to sine-burst and chirp-coded excitation was investigated as the fractional bandwidth was varied from 10%–40%. These experiments were conducted for a fixed agent concentration of 8×10^6 microbubbles/ml—a concentration which is used typically in the context of preclinical studies. The concentration and size distribution of Targestar-P was estimated prior to this study, using a CasyTM Cell Counter (Model TTC, Roche Innovatis AG, Rotkreuz, Switzerland).¹⁴

To assess the detectability of the nonlinear response with sine-burst and chirp-coded excitations, we varied the agent concentration—suspensions with 3×10^6 , 1.5×10^6 , 0.75×10^6 , and 0.38×10^6 microbubbles/ml were investigated. These measurements were conducted with 10% fractional bandwidth excitation pulses.

1. Experimental setup and data acquisition

The same experimental setup was used for both studies (see Fig. 1). The acoustic measurements were conducted at room temperature and pressure in a large water tank

FIG. 1. (Color online) The experimental setup used for acoustic measurements with single element transducers in a confocal mode.

(dimensions: 80 cm $\times$ 50 cm $\times$ 15 cm). The large size of the tank prevented reflections from walls from being included in the measurements. A pair of single element transducers were used—a 10-MHz transmitting transducer (60% fractional bandwidth, Model A 312S, Olympus NDT, Waltham, MA), and a 15-MHz broadband receiving transducer (85% fractional bandwidth Model V 317, Olympus NDT, Waltham, MA). Both transducers had focal lengths and aperture diameters of 50 and 6.5 mm, respectively. We characterized the frequency response of these transducers at their focus, with a calibrated broadband hydrophone (Model HGL-0085, Onda Corporation, Sunnyvale, CA).¹⁴ The transducers were arranged confocally at right angles, as described in previous studies.³⁹

Gaussian-windowed sine-bursts and chirp-coded pulses were created in MATLAB[®], and uploaded to an arbitrary waveform generator (Model 81150 A, Agilent, Santa Clara, CA) via a GPIB interface. The excitation pulses were designed to have the same bandwidth; however, the pulse duration of chirp-coded excitation was 5 times that of sine-bursts—a 7 dB increase in transmit energy. The signal from the arbitrary waveform generator was boosted by 40 dB with a linear power amplifier (Model 411LA, Electronic Navigation Industries, Rochester, NY) and used to excite the transmitting transducer. The pressure amplitude of the transmit pulses was less than 300 kPa (Mechanical Index < 0.17) to reduce (a) agent disruption and (b) nonlinear propagation effects.³⁴ A suspension with a known concentration was created by diluting the agent in IsotoneTM (Beckman Coulter Inc., Chino, CA) solution in a Plexiglas chamber (20 mm diameter $\times$ 70 mm height) with an acoustically transparent SaranTM membrane (S.C. Johnson, Racine, WI) window. The Plexiglas chamber was placed in the confocal zone of the transducers.

We minimized specular reflection from the SaranTM window by adjusting the grazing angle. The contrast agent was gently stirred before each measurement and replenished after the completion of each set of measurements. The back-scattered echoes were received with the broadband transducer, boosted by 30 dB using a preamplifier (DPR 300, JSR Electronics, Pittsford, NY), digitized to 12 bits at 500 MHz with an oscilloscope (Lecroy Wave Runner 44Mxi, Lecroy

FIG. 2. Number and volume weighted size distribution of Targestar-P that was measured using a CasyTM counter.

Inc., Chester Ridge, NY), and stored to a disk for offline analysis in MATLAB[®].

The spectrum obtained from individual radio frequency (RF) lines had a poor SNR, thus the nonlinear response was not apparent in the computed power spectra. In order to improve spectral estimation, we collected five groups of statistically independent waveforms (40 RF waveforms per group) for each transmitted pressure, and averaged the frequency spectra of each group. Each group of RF lines was collected after nearly 5 s had elapsed since the previous measurement, and the agent suspension was gently agitated during this interval. We assumed that the resolution cell was

TABLE II. Size distribution estimates of Targestar-P.

Number of microbubbles/ml	1.73×10^9
Mean diameter (number weighted)	$1.71 \pm 0.051 \mu\text{m}$
Median diameter (number weighted)	$1.44 \pm 0.04 \mu\text{m}$
Mean diameter (volume weighted)	$4.1 \pm 0.12 \mu\text{m}$
Median diameter (volume weighted)	$3.23 \pm 0.097 \mu\text{m}$

replenished with “fresh” microbubbles (those not insonated previously), before every measurement. To quantify the nonlinear response, we computed the peaks in the agent spectrum that were localized in 0.5, 1, and 2 MHz frequency bands centered at the subharmonic, fundamental, and harmonic frequency, respectively. We assessed detectability by computing the peak nonlinear signal amplitude relative to the noise floor. The error bars represented ± 1 standard deviation and were used to quantify the variability in the acoustic response of the agent. An unpaired *t*-test was used to assess if the observed differences in the nonlinear response were statistically significant.

IV. RESULTS

A. Effect of transmit pulse parameters on agent response

The number and volume-weighted size distributions of the agent are shown in Fig. 2. It can be observed that volume-weighted size distribution emphasizes the contribution of larger bubbles in the agent. The mean and median diameters of the agent and its concentration are listed in Table II.

FIG. 3. (Color online) The predicted agent response to 10% to 40% bandwidth sine-bursts [(a)–(d), top row], and chirp-coded excitation [(e)–(h), bottom row] when the excitation pressure was progressively increased from 100 to 290 kPa.

FIG. 4. (Color online) The predicted fundamental (F), subharmonic (SH), and harmonic (H) peak levels attained with sine-bursts and chirp-coded excitation when the bandwidth was varied between 10% and 40%.

Figure 3 shows the power spectra of the simulated agent response to sine-burst and chirp-coded excitation. The power spectra were normalized to the peak fundamental signal obtained with chirp-coded excitation. The simulation predicted nonlinear oscillations with both excitation strategies. Strong nonlinear response (response at $1.5f_0$) was apparent with chirp-coded excitation [see Figs. 3(e)–3(g)], especially when high pressures and narrowband pulses were used. Distinct subharmonic and ultraharmonic peaks were absent from the agent spectra when sine-burst excitation was used. This observation indicates that chirp-coded excitation can excite a stronger nonlinear response from the agent. Figure 4 compares the spectral peaks of the simulated fundamental, harmonic, and subharmonic signal for sine-burst and chirp-coded excitation strategies. For the range of pressures employed, the fundamental, harmonic, and subharmonic signals had higher spectral peaks when chirp-coded excitation was employed, because chirp-coded pulses had a higher energy.

Figure 5 shows the power spectra of the experimentally measured agent response to sine-burst and chirp-coded excitation. The trends were similar to those observed in simulations. Chirp-coded excitation demonstrated stronger nonlinear response. Specifically, stronger subharmonic peaks were observed, especially when high pressures and lower bandwidths were employed. In an isolated case (10% bandwidth), an ultraharmonic response was also observed when chirp-coded excitation was used. Figure 6 compares the spectral

peaks of the experimentally measured fundamental, harmonic, and subharmonic signal for both excitation strategies. The thresholds predicted by simulations were higher than those observed experimentally (Figs. 5 and 6), which could be due to the choice of parameters used in the Marmottant model. The simulation also predicted strong ultraharmonic response for 10% and 20% bandwidth chirp excitation, but such a response was observed experimentally only for 10% bandwidth excitation (Figs. 3 and 5). The ultraharmonic response was not apparent in experimental results because of limited SNR and the insufficient sensitivity of the receiving transducer. Nonetheless, the general trends predicted were consistent with the experiments. The noise floors attained with chirp-coded and sine-burst excitation were similar (see Fig. 5). This observation was expected, since electronic noise is proportional to the bandwidth of excitation, which was equal for both excitation schemes.

B. Effect of agent concentration on the detectability of the nonlinear response

Figure 7 shows the spectral response of the agent to 10% bandwidth excitation, when the UCA concentrations were 3×10^6 , 1.5×10^6 , 0.75×10^6 , and 0.38×10^6 microbubbles/ml. It is apparent that the nonlinear spectral amplitude decreased with decreasing concentration. However, the nonlinear response was observable with chirp-coded excitation

FIG. 5. (Color online) The experimentally measured spectral response of the agent to 10% to 40% bandwidth sine-bursts [(a)–(d), top row] and chirp-coded excitation [(e)–(h), bottom row].

for all concentrations investigated. The differences in the measured signal are assessed more quantitatively in Fig. 8. It is apparent that the subharmonic and harmonic signal decreased more sharply with a decrease in agent concentration than the fundamental signal. For example, while the fundamental signal decreased by 6 dB as the concentration was reduced from 3×10^6 to 0.38×10^6 microbubbles/ml, the harmonic and subharmonic signal decreased by about 10 dB. However, the nonlinear signal was detected for a wider range of concentrations when chirp-coded excitation was used.

C. Mechanistic investigation: Simulations with a single microbubble

Figure 9 shows the subharmonic-to-fundamental ratio plotted as a function of microbubble radii. This plot shows the subharmonic-to-fundamental ratios only for microbubbles that demonstrated a distinct subharmonic response when excited with 10% chirp-coded and sine-burst pulses (peak pressure: 290 kPa). These bubbles were assumed to be in the elastic state initially ($R_b = 0.98R_0$). Chirp-coded excitation excited a subharmonic response from a wider size range of microbubbles than when sine-bursts were employed. Further, higher subharmonic-to-fundamental ratios were observed with chirp-coded excitation.

Figures 10–12 show the response of a single microbubble with radius $R_0 = 0.78 \mu\text{m}$, to chirp-coded and sine-burst excitation under different conditions. In Fig. 10, the microbubble was assumed to be initially in an elastic state ($R_b = 0.98R_0$). Larger radial oscillations were observed with

chirp-coded excitation than sine-burst excitation [see Figs. 10(a) and 10(b)], which produced a stronger subharmonic response [see Figs. 10(c) and 10(d)].

Figure 11 shows the response of the microbubble when an initially buckled state was assumed ($R_b = R_0$). In this configuration, the radial oscillations in response to chirp-coded and sine-burst excitation were very similar [see Figs. 11(a) and 11(b)]. A weak subharmonic response was observed with both excitation schemes [see Figs. 11(c) and 11(d)], relative to that observed for an initial elastic state.

Figure 12 shows the response of the microbubble to low pressure excitation (40 kPa).

In this configuration, the radial oscillations were similar [see Figs. 12(a) and 12(b)], and a prominent subharmonic was observed with both excitation schemes [see Figs. 12(c) and 12(d)].

V. DISCUSSION

We reported the results of a study conducted with sine-burst and chirp-coded excitation to assess how transmit pulse parameters impact the performance of HI and SHI at high frequencies. We also performed studies to assess how the concentration of the contrast agent impacts the harmonic and subharmonic response. Simulation studies were conducted to understand the mechanisms that govern the observed behavior of UCAs in response to sine-burst and chirp-coded excitation.

The primary findings of this study can be summarized as follows: (1) Lower pressure thresholds (two- to threefold)

FIG. 6. (Color online) Experimentally measured fundamental (F), subharmonic (SH), and harmonic (H) peak levels attained with 10% to 40% fractional bandwidth sine-bursts and chirp-coded excitation.

were needed to excite the subharmonic signal when chirp-coded excitation was used, (2) chirp-coded excitation improved the SNR of harmonic, subharmonic, and fundamental signals, (3) the subharmonic and harmonic signals were detectable for a wider range of agent concentrations and transmit pressures with chirp-coded excitation, and (4) expansion-dominated oscillations of microbubbles initially in an elastic state was the dominant mechanism behind the observed improvements.

A. Effect of transmit parameters on agent response

Figures 3 and 4 show that chirp-coded excitation can enhance the nonlinear SNR. In particular, the subharmonic response was enhanced when chirp-coded excitation was used. The heights of the spectral peaks were reduced as the bandwidth of excitation was increased, due to the reduced pulse energy. The simulation results were consistent with the experimental observations (see Figs. 5 and 6), but were dependent on the choice of model parameters. For example, it has been previously reported that increasing the shear viscosity of the agent increases the threshold for subharmonic oscillations.⁴⁴ Changing the shell stiffness enhances the resonance frequency of the agent, and alters the range of sizes of bubbles that demonstrate the strongest nonlinear oscillations.⁴⁴ Two other parameters—the buckling-radius and break-up radius—are reported to strongly impact the predicted nonlinear response of the agent.^{13,45} Sun and colleagues have shown previously that choosing buckling and rupture radii close to the resting radius of the microbubble results in a stronger

nonlinear response.⁴⁵ In this study, we were more concerned with comparing the performance of chirp-coded and sine-burst excitation schemes than investigating the impact of model parameters on the simulation results. A detailed investigation of the impact of model parameters on the agent response is currently underway.

Figure 5 demonstrated that the spectral peaks of the fundamental, harmonic, and subharmonic signal were higher when chirp-coded excitation was used. Additionally, when 40% bandwidth excitation was used, the subharmonic signal and the fundamental signal became indistinguishable. Although wider bandwidth excitation can improve the axial resolution,^{14,23} it reduces the spectral separation between linear and non-linear imaging modes. Therefore, techniques such as pulse-inversion⁸ should be used in combination with digital filtering if bandwidths higher than 30% are to be employed for SHI.

Figure 6 demonstrates that chirp-coded excitation improved the fundamental, harmonic, and subharmonic SNR ($p < 0.01$) by 6 to 8 dB, 4 to 6 dB, and 5 to 14 dB, respectively. The thresholds for subharmonic generation observed with chirp-coded excitation were almost two- to threefold lower. For example, for 10% bandwidth excitation, while subharmonics were first observed at 146 kPa for sine-burst excitation, the threshold was only 50 kPa for chirp-coded excitation. The reduction in subharmonic threshold with chirp coded excitation should improve the sensitivity of SHI.

Chirp-coded excitation improved the fundamental and subharmonic signal to a greater extent than the harmonic

FIG. 7. (Color online) Experimentally measured spectral response of the agent to 10% fractional bandwidth sine-bursts [(a)–(d), top row] and chirp-coded excitation [(e)–(h), bottom row], when the concentration was decreased progressively from 3×10^6 to 0.38×10^6 microbubbles/ml.

signal. The transfer of energy to non-integral harmonics could be a possible reason for this observation.⁴⁶ Therefore, the harmonic response of UCAs may not be as sensitive to pulse energy as the subharmonic response. These results suggest that a trade-off exists between SNR improvement and the strength of the harmonic signal relative to the fundamental. Therefore, if equal transmit pressures were employed for clinical imaging with sine-burst and chirp-coded excitation, the agent-to-tissue contrast of the latter would be slightly reduced. However, chirp-coded excitation will yield a higher SNR. The increased SNR could be exploited to conduct HI at lower transmit pressures than that used for sine-burst excitation, which should reduce tissue harmonics and improve imaging specificity.

B. Effect of agent concentration on the detectability of the nonlinear response

Figures 7 and 8 demonstrate that reducing the agent concentration reduced the magnitude of the harmonic and subharmonic peaks. As the concentration was reduced, the SNR decreased; therefore, the harmonic emissions obtained with sine-burst excitation were not apparent in the spectra. The thresholds for subharmonic generation were different in the study conducted to assess the impact of agent concentration on the agent response than that reported in Figs. 5 and 6. The subharmonic response was observed only with chirp-coded

excitation (Fig. 7). This difference could be due to the variation in the acoustic response of the agent from one batch to another. More specifically, the results reported in Fig. 5 were obtained using a different batch of UCAs. The size distribution of different batches of Targestar-P is consistent; therefore, a variation in UCA shell properties may be responsible for the observed differences in the subharmonic threshold. Specifically, the surface concentration of phospholipids on microbubble shells may vary from batch to batch. A variation in the subharmonic thresholds between different batches of phospholipid-encapsulated contrast agents has been previously observed by Sijl and colleagues.⁴⁷ Consequently, we are currently investigating the subharmonic thresholds of different batches of Targestar-P.

Figure 8 demonstrates that the nonlinear response was detectable for a wider range of pressures when chirp-coded excitation was used—a trend which was expected, due to the higher SNR of chirp coded excitation. The fundamental response decreased only by 6 dB in response to an eightfold decrease in agent concentration. This observation seems to have occurred because the concentrations investigated were in the range that is typically used for imaging. Stride and Saffari have previously reported that multiple scattering can occur at concentrations as low as 10^6 microbubbles/ml; therefore, the fundamental response of the agent may not scale linearly with the concentration.⁴⁸ Nonetheless, these results demonstrate that chirp-coded excitation improved the detectability of the

FIG. 8. (Color online) Experimentally measured fundamental (F), subharmonic (SH), and harmonic (H) peak, relative to noise floor, when the agent concentration was decreased progressively from 3×10^6 to 0.38×10^6 microbubbles/ml.

nonlinear response of the agent. In our study, chirp-coded excitation was able to detect both the subharmonic and the harmonic response for concentrations up to 0.38×10^6 microbubbles/ml. Such an improvement in detection sensitivity could enhance the visualization of microvessels such as the *vasa vasorum*.

Other researchers have previously reported enhancement in the subharmonic response of phospholipid coated UCAs by chirp-coded excitation, which is consistent with our results.^{23,29} These studies were conducted with relatively high-pressure excitation pulses; therefore, the threshold for

subharmonic emission was not assessed. To our knowledge, the present study is the first to demonstrate that the thresholds for subharmonic emission can be reduced by employing chirp-coded excitation. Our experiments support the hypothesis that the higher energy of chirp-coded excitation was responsible for the reduced subharmonic threshold.

The simulations and experimental studies reported in this paper did not reveal an improvement in the harmonic-to-fundamental ratio when chirp-coded excitation was employed. In fact, the improvement in harmonic SNR was lower than the improvement in fundamental SNR, which suggests a slight degradation in the harmonic-to-fundamental ratio (see Fig. 6). Our results are in agreement with those reported previously by Borsboom and colleagues at 2 MHz excitation frequency.^{21,22} In their study, chirp-coded excitation was found to improve harmonic SNR; however, the experimentally observed harmonic-to-fundamental ratio and contrast-to-tissue ratio were lower than those observed with sine-bursts.^{21,22} The increase in harmonic SNR was consistent with the increased energy of chirp-coded pulses.

C. Mechanistic investigation: Simulations with a single microbubble

We observed a higher enhancement in the subharmonic SNR than that observed in the fundamental, when chirp-coded excitation was employed. Consequently, chirp-coded excitation improved the subharmonic-to-fundamental ratio, and produced lower thresholds for subharmonic emissions. Simulations conducted with single microbubbles with a

FIG. 9. The subharmonic-to-fundamental ratio attained with chirp-coded and sine-burst excitation schemes, for the range of microbubble sizes that demonstrate a noticeable subharmonic response.

FIG. 10. The response of a micro-bubble initially in the elastic state, to chirp-coded and sine-burst excitation at 290 kPa. The radial oscillation relative to resting radius is shown for (a) chirp-coded and (b) sine-burst excitation. The power spectra for chirp-coded and sine-burst excitation are shown in (c) and (d), respectively.

range of sizes and initial shell surface tension provided insight into the possible mechanisms for the observed phenomenon. Note that the initial surface tension of the micro-bubble shell determines whether it is in a buckling or

elastic state at equilibrium.⁴² Figure 9 demonstrates that the subharmonic response was observed from a wider size distribution of microbubbles when chirp-coded excitation was used. While a subharmonic response was observed with

FIG. 11. The response of a micro-bubble initially in the buckled state, to chirp-coded and sine-burst excitation at 290 kPa. The radial oscillation relative to resting radius is shown for (a) chirp-coded and (b) sine-burst excitation. The power spectra for chirp-coded and sine-burst excitation are shown in (c) and (d), respectively.

FIG. 12. The response of a microbubble initially in the buckled state, to chirp-coded and sine-burst excitation at 40 kPa. The radial oscillation relative to resting radius is shown for (a) chirp-coded and (b) sine-burst excitation. The power spectra for chirp-coded and sine-burst excitation are shown in (c) and (d), respectively.

microbubbles of radii between 0.74 and 0.86 μm with sine-burst excitation, chirp-coded excitation produced a subharmonic response from microbubbles with radii ranging from 0.66 to 0.97 μm . Furthermore, the subharmonic-to-fundamental ratio increased by 5 to 19 dB when chirp-coded excitation was employed. Therefore, the higher energy of chirp-coded excitation incited a stronger subharmonic response due to (1) a wider range of microbubble sizes contributing to the overall signal, and (2) the stronger subharmonic response of individual microbubbles.

Figure 10 demonstrates that expansion-dominated oscillations were present from bubbles (initially) in an elastic state, in response to both excitation schemes [see Figs. 10(a) and 10(b)]. However the normalized radial oscillation was greater when chirp-coded excitation was used, which produced a stronger nonlinear response than sine-burst excitation [see Figs. 10(c) and 10(d)]. Although subharmonic and ultraharmonic peaks were enhanced when chirp-coded excitation was employed, the harmonic response was similar to that observed with sine-burst excitation. Expansion-dominated oscillations, such as those shown in Figs. 10(a) and 10(b) have been previously reported by Sun and colleagues.⁴⁵

Intuitively, one would expect the increased energy of chirp-coded excitation to produce larger radial oscillations in all configurations and range of pressures. However, our results suggest that this is not necessarily true. For example, Fig. 11 demonstrates that when the microbubble was in a buckling state, the radial excursions of sine-burst and chirp-coded excitation are equal. In this configuration, the subharmonic response was weaker for both excitation schemes, compared

to that observed when an initial elastic state was assumed. Figure 12 demonstrates that a strong subharmonic response was observed at low pressures (40 kPa) with both excitation schemes when the bubble was in the buckling state. Such strong subharmonics predicted by the Marmottant model at pressures <50 kPa have been previously reported by Frinking and colleagues.⁴¹ However, we found that the radial excursion was similar with both excitation schemes in this configuration; therefore, chirp-coded excitation did not increase the subharmonic response noticeably.

The simulations presented in our paper suggest that at low pressures the microbubbles that are in the bucked state are responsible for a subharmonic signal. However, the expansion dominated oscillation of bubbles initially in the elastic state is the dominant contributor to the subharmonic signal at higher pressures. The increased radial oscillation of the microbubbles in response to chirp-coded excitation appears to be the mechanism responsible for enhancement in the subharmonic response reported in this work.

D. Study limitations

A limitation of the present study was that the shell parameters used in the model were those reported for another commercial UCA (BR14, Bracco Research, Geneva, Switzerland).¹³ We assumed that the shell properties to Tagestar-P[®] were similar to BR14, based on their physico-chemical properties—they are both composed of a perfluorocarbon core encapsulated by a phospholipid monolayer. Nonetheless, we do not expect the differences in shell properties to impact the observed trends.

Another limitation of this study was that the simulations employed a size integration technique to estimate the acoustic response of a population of microbubbles, which ignores multiple scattering, disruption, gas diffusion, and the non-uniformity of encapsulation properties within the population. Despite this limitation, the general trends predicted by the model were consistent with the experimental study.

We reported an enhancement in the nonlinear SNR and the subharmonic threshold in this study, which is encouraging. However, the actual improvement in nonlinear imaging sensitivity should be evaluated under physiological conditions. Therefore, we plan to conduct an *in vivo* imaging study with a modified ultrasound scanner to compare the performance of chirp-coded HI and SHI.

VI. CONCLUSIONS

In this paper, we demonstrated that chirp-coded excitation can enhance the nonlinear response of UCAs—a finding that was consistent with the higher energy of chirp-coded pulses. Simulations conducted with single microbubbles indicated that strong expansion-dominated oscillations enhanced the subharmonic response when chirp-coded excitation was employed. These results suggest that chirp-coded excitation can improve the performance of HF HI and SHI. More specifically, HI could be enabled at low transmit pressures with chirp-coded excitation, which will reduce tissue harmonics and improve the specificity of imaging. Further, chirp-coded excitation resulted in lower thresholds for subharmonic generation, which should enhance the sensitivity of imaging. Therefore, chirp-coded excitation may be a promising strategy for *vasa vasorum* imaging.

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