Observed cumulative time delay between second harmonic and fundamental component of pressure wave fields propagating through ultrasound contrast agentss

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1aBA11. Observed cumulative time delay between second harmonic and fundamental component of pressure wave fields propagating through ultrasound contrast agents

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Several studies on the propagation velocity of pressure wave fields through ultrasound contrast agents (UCAs) have been reported in the literature. However, the variation of propagation velocity between the fundamental and the second harmonic component generated during the propagation of ultrasound through UCAs has, to our knowledge, not been studied yet. To this scope, dedicated transmission and backscattering measurements of pressure wave fields propagating through SonoVue and Definity contrast agents, are analyzed. Results show the occurrence of a cumulative delay between the time signals related to the second harmonic and fundamental component, suggesting a smaller propagation velocity for the second harmonic as compared to the fundamental component. Moreover, this time delay increases with increasing UCA concentration and propagation path length of ultrasound trough microbubbles, depends on mechanical index and frequency, and, most importantly, is not observed in the absence of UCAs. These results may be relevant to contrast-enhanced ultrasonography, opening up to new possibilities to increase contrast-to-tissue ratios and to quantify UCA concentrations.

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INTRODUCTION

Nowadays, dynamic contrast-enhanced ultrasound (DCE-US) imaging is widely used in the clinic as a diagnostic tool. Ultrasound contrast agents (UCAs) are intravascular contrast agents with size comparable to red blood cells and made of gas-filled microbubbles encapsulated in a lipid shell. Thanks to their high echogenicity, UCAs can be utilized in combination with ultrasound imaging to quantify perfusion [1], blood flow [2], and to localize cancer [3, 4, 5]. To better discriminate UCAs from tissue, the nonlinear response of microbubbles to ultrasound may be exploited. In fact, once appropriately hit by ultrasound, microbubbles resonate not just at the ultrasound wave field insonating frequency, but also at its multiples, e.g., at the second harmonic frequency [6], as well as at sub-harmonic, ultra-harmonic, and super-harmonic frequencies [7, 8]. Various techniques have been developed which use the harmonic components, generated from ultrasound-UCAs interaction, to isolate bubble response from tissue [9]. These techniques employ a low mechanical index (MI), required to avoid bubble disruption and minimize the effect of tissue nonlinearity, and allow quantitative analysis of microbubble concentration. However, tissue itself generates harmonic components due to nonlinear propagation of ultrasound [10], de facto deteriorating the contrast to tissue ratio. Moreover, common dynamic contrast enhanced ultrasound imaging techniques, i.e. harmonic imaging, pulse inversion, and amplitude modulation, suffer from artifacts due to nonlinear propagation of ultrasound through microbubbles [11, 12, 13], resulting in possible misinterpretation of bubble concentrations and affecting the contrast to tissue ratio (CTR). Despite several approaches having been presented, e.g., counter propagation imaging [14] and bi-spectral analysis [15], a definitive solution to this problem has not been found yet.

Improving our understanding of the interaction between ultrasound and UCAs may provide new ideas and help in the development on new techniques. To our knowledge, despite several studies on the propagation velocity of pressure wave fields through UCAs have been conducted [16], the variation of propagation velocity between the fundamental and second harmonic component generated during the propagation of ultrasound through UCAs has not been observed yet. For this purpose, transmission and backscattering measurements of pressure wave fields generated with a single element transducer and propagating through gelatin phantoms containing cylindrical cavities of different diameter, filled with different concentrations of SonoVue® contrast agent, are conducted. Different frequencies and MIs are investigated. Furthermore, previous measurements obtained with Definity® contrast agent are also analyzed.

Results show the occurrence of a cumulative delay between the time signals related to the second harmonic and fundamental component. This delay increases with increasing microbubble concentration and diameter of the cavity, it depends on MI and frequency, and, most importantly, it is not observed in the absence of UCAs.

METHODOLOGY

A single element transducer (V306 Panametrics, Waltham, MA) with diameter, center frequency, and focal length equal to 13 mm, 2.25 MHz, and 80 mm, respectively, and a hydrophone (HGL-0400 Onda, Sunnyvale, CA) with a bandwidth of 250 kHz to 20 MHz, were submerged in water and adopted to obtain transmission and backscattering measurements of pressure wave fields propagating through gelatin phantoms containing cylindrical cavities of different diameter d_c [0.5 cm and 1.5 cm]. Different concentrations [0 to 240 μL/L] of SonoVue® contrast agent were utilized to fill the cavity. The center axis of the cavity was manually placed at the transducer focus. To perform backscattering measurements, needles were inserted in the gelatin phantom, before and after the cavity. Figure 1, illustrates the set-up. Measurements were performed employing Hanning windowed pulses designed using Labview® (National Instruments, Austin, TX) and generated with an arbitrary waveform generator (PCI-5412 National Instruments, Austin, TX). Before being transmitted to the transducer, pulses were amplified using a RF power amplifier (240 L ENI, Rochester, NY). Different center frequencies f_0 [2, 2.5 and 3 MHz], and effective MIs [0.05, 0.1 and 0.2] were investigated. The effective MI was determined as the ratio of the peak negative pressure in MPa (as measured with the hydrophone at the transducer focus in water) and the square root of the center frequency f_0 in MHz. The hydrophone was connected to a preamplifier (AH-2010-025 Onda, Sunnyvale, CA) with a bandwidth of 50 kHz to 25 MHz, in its turn connected to a 100 MHz A/D converter (PCI-5406 National Instruments, Austin, TX). Dedicated Labview® software was implemented and used for data acquisition.

Once a pulse is collected with the hydrophone and digitally converted, either after being transmitted through the cavity or backscattered, a FFT is performed, band-pass filters are applied to distinguish fundamental and second harmonic component (-12 dB bandwidth are considered), and an IFFT is performed to obtain the fundamental and

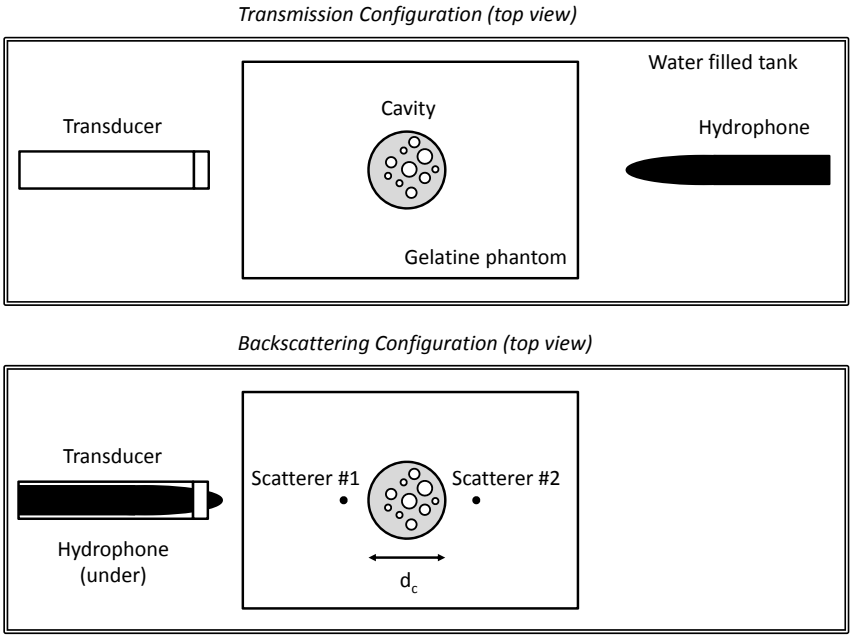

FIGURE 1. Illustrative representation of adopted set-up configurations.

second harmonic time traces, respectively. The envelopes are extracted, and the time delay Δt between the maxima of the second harmonic and fundamental envelope is calculated. Delay is positive in case of delayed second harmonic time trace, as compared to the fundamental. Figure 2 illustrates the procedure in case of a transmission measurement performed with f_0, effective MI, d_c, and SonoVue® concentration equal to 3 MHz, 0.2, 1.5 cm, and 240 μL/L, respectively. F0 and 2H refer to the fundamental and second harmonic component, respectively.

RESULTS

Figure 3 shows results as obtained from transmission measurements when varying d_c, effective MI, UCAs concentration and pulse center frequency f_0. Each plot shows the estimated time delay (maximum, minimum, and mean values as obtained after 5 repeated measurements) between second harmonic and fundamental envelopes, expressed as $\Delta t\, f_0$. Time delay increases with increasing diameter of the cavity, microbubble concentration, and employed effective MI, and depends on pulse center frequency. In particular, a maximum is shown for $f_0 = 2.5$ MHz. Moreover, a saturation phenomenon seems to be present for increasing effective MI.

Figure 4 shows results as obtained from backscattering measurements when varying UCA concentration. For these measurements, d_c, effective MI, and f_0 equal 0.5 cm, 0.2, and 2.5 MHz, respectively. The time delays estimated from the pulse measured from scatterer #1 and #2 are shown, respectively. A negative or smaller than 0.1 cycles time delay (see threshold in Fig. 4) is always observed for scatterer #1, and in case of absent UCAs in the cavity (0 $\mu L/L$) for scatterer #2. Moreover, results confirm increasing time delay with increasing UCA concentration.

Figure 5 shows results as obtained applying the described analysis to transmission measurements performed with Definity® contrast agent. [12] For these measurements, d_c and effective MI equal 2.2 cm and 0.1, respectively. Results for different concentrations and modulated center frequency f_0 are shown. Results are obtained after 3 repeated measurement. Definity® measurements confirm the occurrence of a time delay between second harmonic and fundamental component of ultrasound fields propagating trough UCAs.

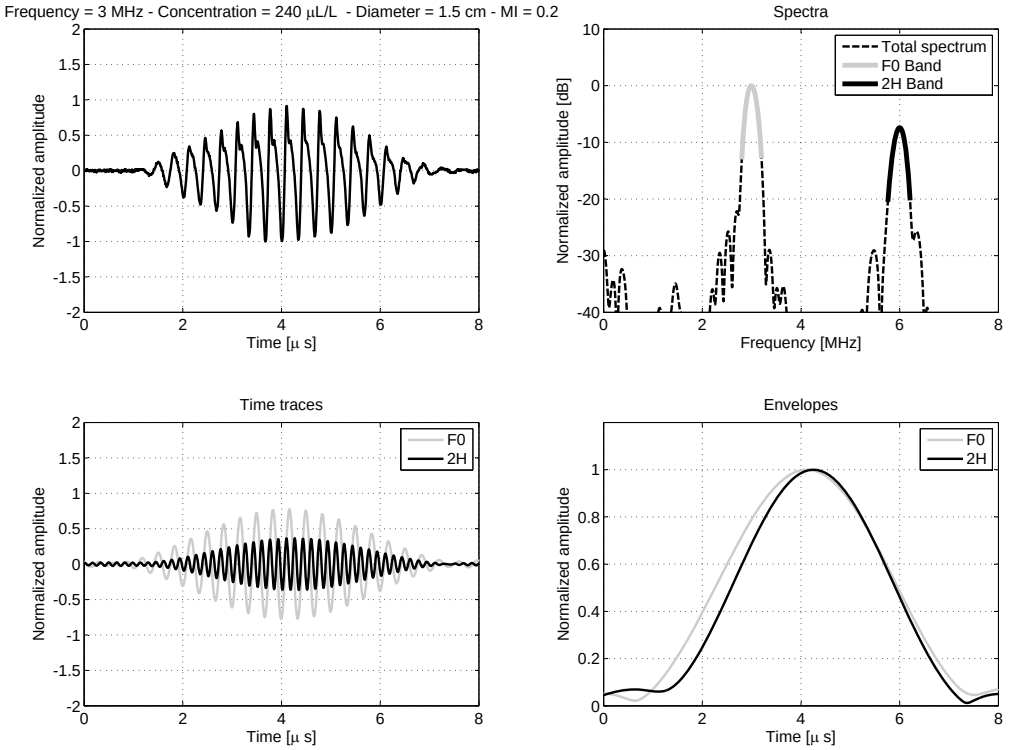

FIGURE 2. Time pulse (top-left), frequency spectra (top-right), time traces (bottom-left) and envelopes (bottom-right) as obtained after a transmission measurement performed with f_0, effective MI, d_c, and SonoVue® concentration equal to 3 MHz, 0.2, 1.5 cm, and 240 μL/L, respectively. F0 and 2H refer to the fundamental and second harmonic component, respectively

DISCUSSION

In this paper, delay estimation is simply performed by locating the maxima of the second harmonic and fundamental time trace envelopes. Although this approach suffices for the presented in vitro measurements (in case of transmission measurements or backscattering measurements involving isolated point scatterers), it is not suitable for in vivo applications. The delay measured in the absence of UCAs may be simply explained by the wave-form steepening due to tissue nonlinearity. The ultimate nature of the cumulative delay observed in the presence of UCAs, even though being consistently observed throughout all the measurements performed, and across different UCAs, is yet to be explained. To address this question, a logical step that will be pursued in future work is the exploitation of numerical models for the interpretation of the observed phenomena, e.g., using forward scattering models based on the modified Rayleigh-Plesset Noltingk Neppiras and Poritsky (RPNNP) equation to approximate the nonlinear behavior of UCAs. [12, 17]

CONCLUSION

Dedicated measurements aimed at investigating nonlinear propagation of ultrasound pressure wave fields through different concentrations of UCAs were performed. Gelatine phantoms were utilized to control the size of the cavity to be filled with SonoVue® UCAs, ultimately controlling the extent of ultrasound propagation path length through UCAs. Different frequencies and MIs were investigated. Transmission and backscattering measurements were performed. Results show the occurrence of a cumulative delay between the time signals related to the second harmonic and fundamental component, respectively, suggesting a smaller propagation velocity for the second harmonic as compared to the

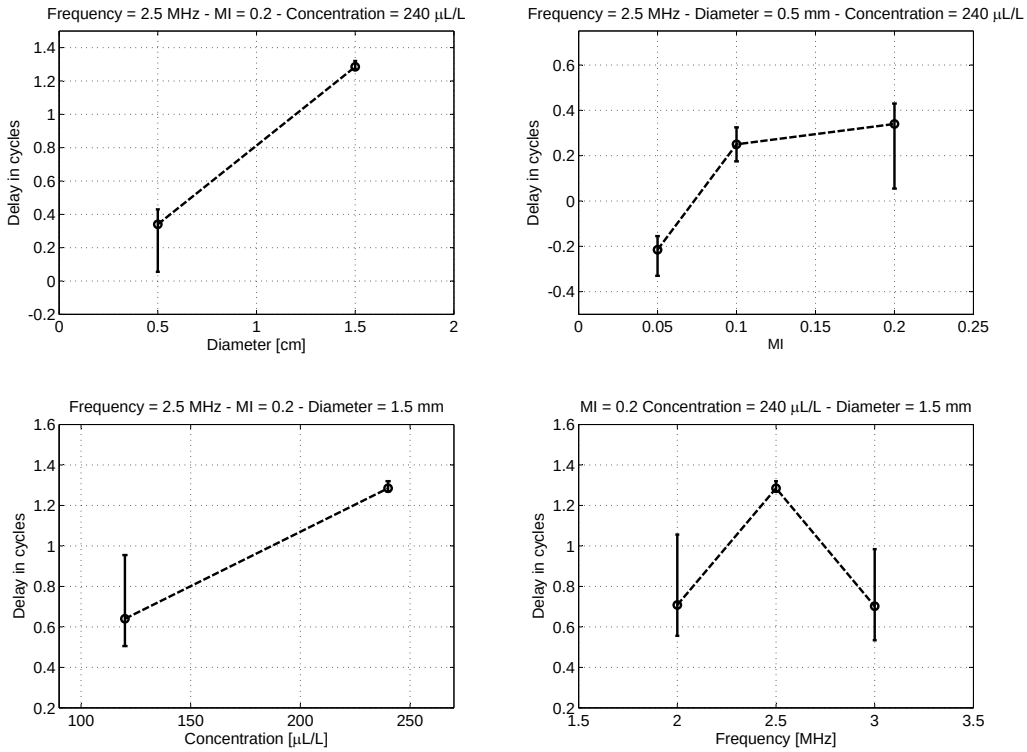

FIGURE 3. Results as obtained from transmission measurements with SonoVue® when varying d_c (top-left), effective MI (top-right), UCA concentrations (bottom-left), and f_0 (bottom-right).

fundamental component. This delay increases with increasing microbubble concentration and ultrasound propagation path length through UCAs, depends on MI and insonating frequency, and most importantly it is not observed in the absence of UCAs. These results may be relevant to contrast-enhanced ultrasonography, providing new possibilities to increase contrast-to-tissue ratios and to quantify UCA concentrations.

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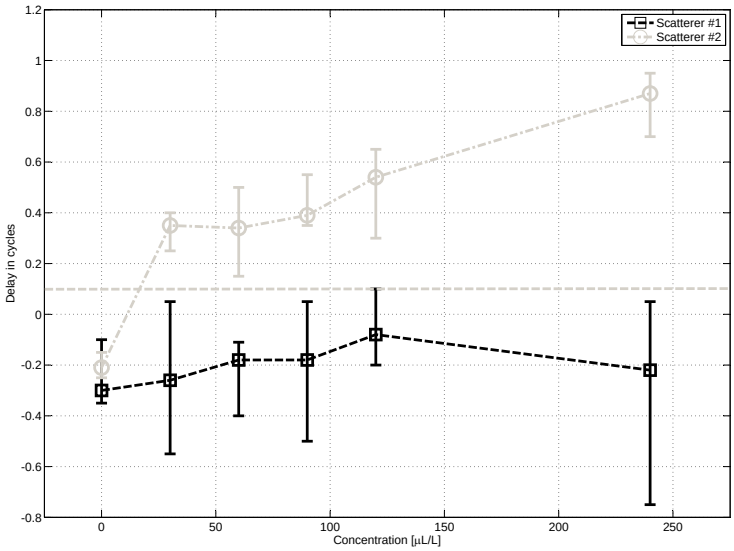

FIGURE 4. Results as obtained from backscattering measurements with SonoVue® when varying UCA concentrations. d_c, effective MI, and f_0 equal 0.5 cm, 0.2, and 2.5 MHz, respectively. The time delays estimated from the pulse measured from scatterer #1 and #2 are shown, respectively. The horizontal gray dashed line marks the threshold exceeded which the presence of UCAs in the cavity could be detected by simply looking at the second harmonic to fundamental time delay.

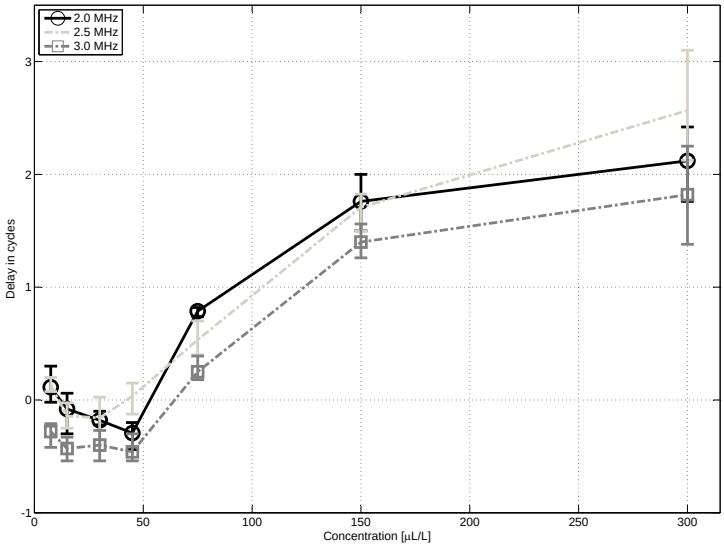

FIGURE 5. Results as obtained from transmission measurements with Definity® contrast agent. d_c and effective MI equal 2.2 cm and 0.1, respectively. Results for different concentrations and modulated center frequency f_0 are shown.

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