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Dual-frequency insonation of single microbubbles

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Radial modulation imaging is a new medical imaging technique based on dual-frequency insonation of ultrasound contrast agents. The difference in echo between a high frequency 'imaging' pulse transmitted at either the compression or rarefaction phase of a low frequency 'modulating' pulse is detected by regular correlation techniques. Little is however known about the contrast agent microbubble dynamics in a dual-frequency ultrasound field, which were investigated in this study. Using a high-speed camera system, the radial excursions of single phospholipid-coated microbubbles were recorded. The microbubbles were simultaneously insonified with a four-cycle pulse at 0.5 MHz and 30 kPa and a 33-cycle pulse at 3.75 MHz and 80 kPa. The microbubbles studied had diameters ranging from 1.1 to 5.2 μm. Microbubbles with a size smaller than 1.4 μm diameter frequently showed shrinkage. Microbubbles larger than 2.6 μm showed low (< 5 dB) or no amplitude modulation of the high frequency radial excursion. Microbubbles with diameters between 1.4 and 2.6 μm showed high amplitude modulation (up to 22 dB) and strong compression-only oscillation, which both may be explained by nonlinear shell properties. The observed behaviour may be beneficial for the detection of contrast agents.

1 Introduction

Ultrasound contrast agents consist of fluids containing coated microbubbles. They are used to enhance the scattering of blood in echographies. Various strategies have been developed to improve the detectability of the contrast agents, such as pulse inversion [1] and power modulation [2], which take advantage of the nonlinear properties of the encapsulated microbubbles. Radial modulation imaging is a new medical imaging technique based on dual-frequency insonation of ultrasound contrast agents [3-6]. The microbubbles are insonified with compound pulses, composed of a low frequency (LF) signal, which acts as a modulator signal and a high frequency (HF) signal, which is used as an imaging signal. Two compound pulses with opposite polarities of the LF pulse are transmitted and the backscattered HF signals are combined to suppress the signal scattered from the tissue and to extract the signal scattered by the microbubbles. A general Doppler processing technique can be used to detect decorrelation of the HF signals induced by the radial modulation of the microbubbles. Decorrelation may occur due to amplitude or phase differences.

In comparison with free gas microbubbles, the coating influences the responses of the contrast agent microbubbles in an ultrasound field. For example in a previous study, we have observed "compression-only behavior" of phospholipid-coated microbubbles [7]. In [7], phospholipid-coated microbubbles were insonified using sine wave bursts with center frequencies varying from 1 to 4 MHz and acoustic peak pressures from 50 to 200 kPa. Part of the responses of these microbubbles showed a limited expansion amplitude compared to the compression amplitude, which in case of a factor two difference (expansion ≤ 0.5·compression) was defined as compression-only behavior.

Previous studies have focused on different aspects of dual frequency insonation with respect to radial modulation as an imaging technique [3-6]. Dual frequency insonation may however also reveal frequency-dependent coated microbubble behavior [8]. To study this, optical measurements are useful. Bouakaz et al. have shown one example of a radially modulated microbubble using a high-speed camera system [4]. In the current study, the same high-speed camera system was used to investigate the influence of microbubble size on the behavior of single phospholipid-coated microbubbles in a dual-frequency ultrasound field.

2 Methods

The Brandaris-128 high-speed camera system [9] was used to optically record single microbubbles. Fig. 1 schematically shows the applied set-up. Two ultrasound transducers were mounted in a water tank at an angle of 90°. The center frequencies of these transducers were 0.5 MHz (V389, Panametrics-NDT^{TM}, Olympus NDT, Waltham, MA, USA) and 3.5 MHz (V380, Olympus NDT). They were both focused on a cellulose Cuprophan® capillary tube (inner diameter 160 μm and outer diameter 200 μm, Akzo Nobel Faser AG, Wuppertal, Germany). The transducers were controlled by a two-channel waveform generator (8026, Tabor Electronics Ltd., Tel Hanan, Israel) and two power amplifiers (LF: 150A100B, AR, Souderton, PA, USA and HF: A-500, ENI, Rochester, NY, USA). The objective of a customized BXFM microscope (Olympus Nederland BV, Zoeterwoude, The Netherlands) was positioned above the capillary tube and projected the microbubbles with 240x magnification (LUMPlan 60x water immersion objective and 2x2 magnifiers) onto the high-speed camera system.

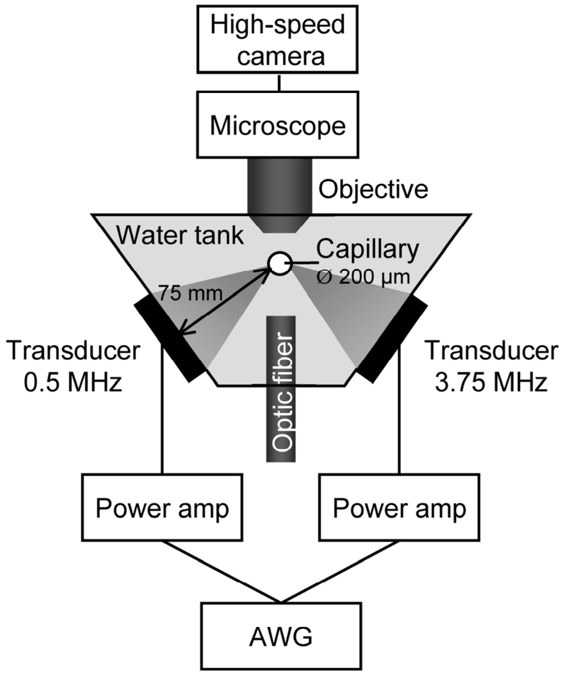

Fig.1 Schematic representation of the experimental set-up, where AWG is arbitrary waveform generator and Power Amp is power amplifier.

The phospholipid-coated contrast agent SonoVue® was used, which was prepared as prescribed by the manufacturer (Bracco Research SA, Geneva, Switzerland) and diluted such that after injection in the capillary tube only a few microbubbles (preferentially one) were present in the image frame of 31x42 μm. The insonified microbubbles were recorded in a sequence of 128 image frames at a frame rate of 12 million frames per second. The microbubbles were insonified by both the LF and HF pulses. The LF pulse was produced by the 0.5 MHz transducer and consisted of a gated four-cycle-sine wave burst at 0.5 MHz center frequency and a peak negative pressure of 30 kPa. The HF pulse was produced by the 3.5 MHz transducer, which transmitted a gated 33-cycle-sine wave burst at 3.75 MHz centre frequency and a peak negative pressure of 80 kPa. In a separate experiment, a calibrated 0.2-mm PVDF hydrophone (Precision Acoustics Ltd., Dorchester, UK) was used to verify the acoustic pressures. The results are shown in Fig. 2.

For the processing procedure, single microbubbles in the focus of the microscope were selected. In each image frame, the diameters of the selected microbubbles were measured with a semiautomatic procedure using a minimal cost algorithm [10], which resulted in the microbubble diameter as a function of time, *D(t)*.

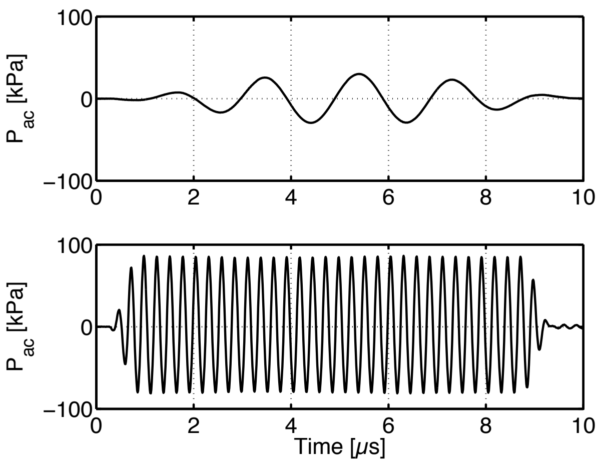

Fig.2 Results from hydrophone measurements, the acoustic pressure (P_{ac}) as a function of time.

3 Results

Fig. 3 shows four examples of the microbubble responses observed in a dual frequency ultrasound field. The frequency spectra were normalised with respect to the HF response. For the biggest microbubble (diameter: 5.0 μm), the LF response dominated. Small HF oscillations on top of the LF response were observed. The LF response shows more compression than expansion. The compression phase (C) had an amplitude of 0.9 μm and the expansion phase (E) 0.5 μm, which almost fulfils the definition for compression-only behaviour (E/C < 0.5). A small response at 4.25 MHz is visible, which may also be the case for 3.25 MHz. These responses are an indication of nonlinear mixing of LF and HF.

In comparison with the 5.0 μm diameter microbubble, for the 3.6 μm diameter microbubble, the HF response was more apparent on top of the LF response. Also for this microbubble, a preference for compression compared to expansion in the LF response was observed. In the frequency spectrum, for this microbubble the sidebands at 3.25 and 4.25 MHz are clear and show a nonlinear mixing of LF and HF, which can only occur in a nonlinear system.

The microbubble with a size of 2.0 μm diameter showed even more nonlinear behaviour. The LF response did not show any expansion, only compression was observed. Moreover the HF response was most apparent in the compression phase of the microbubble. In the frequency spectrum, this behaviour was observed as a HF response that was larger than the LF response and the HF response had significant sidebands. This behaviour was also partly observed for the microbubble of 1.7 μm diameter, but after two cycles of the LF pulse, this microbubble had shrunk 13% in diameter and showed no more response.

4 Discussion

Optical recordings were used to study phospholipid-coated microbubbles in a dual-frequency ultrasound field. Responses at LF and HF were both observed, whereby the larger microbubbles showed relatively more LF response and the smaller microbubbles relatively more HF response. Moreover the LF and HF responses mixed nonlinearly. In the frequency spectra, sidebands were observed at HF ± N·LF, where N is an integer, $N \leq 2$.

The nonlinear responses of the microbubbles depended on their resting size. For the largest microbubble with a size of 5.0 μm diameter, small sidebands were observed (see Fig. 3). For the microbubbles with a size of 3.6 μm diameter, the sideband peaks were more evident, but for the smallest microbubbles with sizes of 2.0 and 1.7 μm diameter respectively, the sidebands had the relative highest values. In the corresponding diameter-time curves of these microbubbles, the HF response was only present in the compression phase of the LF response, showing a large amplitude modulation of the HF response.

This large amplitude modulation was related to the compression-only behaviour of these microbubbles, which was introduced by the LF pulses. In Fig. 3 we observed for all microbubbles, a preference for compression compared to expansion, but for the smallest microbubbles of 1.7 and 2.0 μm diameter, the compression-only behaviour had the strongest effects. During the LF pulse expansion phase, the HF oscillations were dampened significantly. The mechanisms explaining this behaviour are not known. In straightforward terms, the LF response influenced the boundary conditions of the HF response. It is more complicated to include the possible influence of the behaviour of the phospholipids in the microbubble coating. Following the paper of Marmottant et al. [11], we hypothesize that during the LF compression phase, the microbubble coating was in the buckled state. In this state, the surface tension is very low. When the microbubble is compressed, the coating will buckle. Probably, a buckled microbubble is able to oscillate at a secondary (higher) frequency. During the LF pulse expansion phase, the microbubble was in the elastic state. In this state, the elasticity of the coating is much higher compared to the buckled state. The forces between the phospholipid-molecules in the coating were high enough to oppose expansion. In this state of increased tension within the

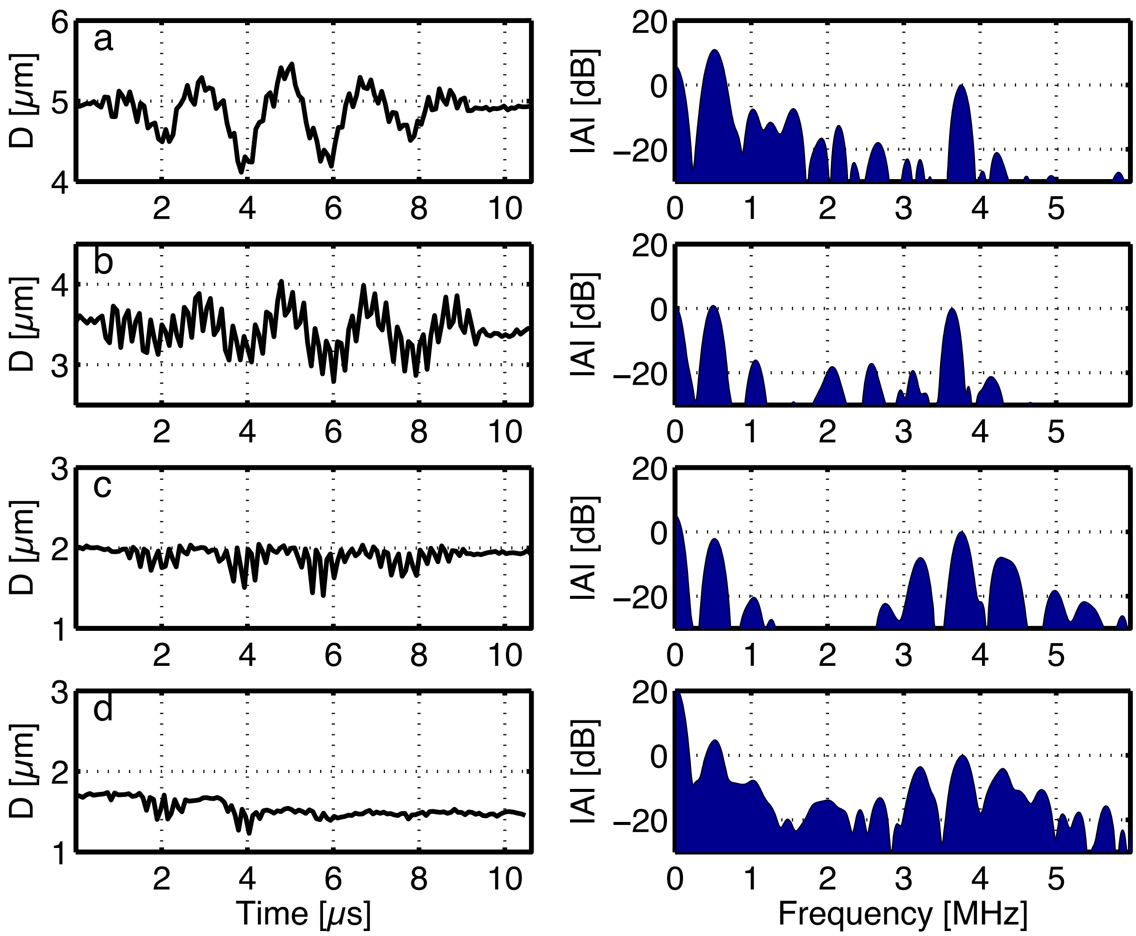

Fig.3 Examples of dual frequency insonation responses, diameter-time curves (left column) and corresponding frequency spectra (right column) for four microbubbles with different resting sizes: a) D_0 = 5.0 μm, b) D_0 = 3.6 μm, c) D_0 = 2.0 μm, and d) D_0 = 1.7 μm.

coating, it could have been that the microbubble was less susceptible for the HF pulse. This behaviour may resemble earlier observed threshold behaviour [12]. Compression-only behaviour was not observed by Bouakaz et al. [4], whereby it must be noted that they measured a 4 μm diameter microbubble and applied higher acoustic pressures. Our results show that microbubble size and shell effects largely influence the responses of single microbubbles in a dual frequency ultrasound field, which is important for an imaging technique such as radial modulation imaging.

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