Ultrasonic Pulse-Inversion Fundamental Imaging with Liposome Microbubbles at 25-50 MHz

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Abstract - Pulse inversion based fundamental imaging was proposed for the enhancement of contrast detection in a previous study. Performance of the imaging method was tested with a commercial contrast agent (Levovist®) at 1.5-3 MHz. In this study, we applied pulse inversion fundamental imaging at 25-50 MHz with liposome microbubbles that were produced in-house. The pulse inversion technique involves two firings with inverted waveforms. Because the reaction of the bubbles under compression is different from that under rarefaction, the signal in the fundamental band is used to enhance the contrast-to-tissue ratio. Phantom experiments were performed. Liposome microbubbles were made in-house with a recipe developed in our lab. Compared to conventional fundamental imaging, the contrast-to-tissue ratio was enhanced by 7~18 dB when the transmit signal was at 25~50MHz. Compared to the conventional second harmonic imaging, the pulse inversion fundamental imaging provides an additional advantage. That is, the bandwidth of the transmit signal is not limited to accommodate both the fundamental and harmonic bands within the passband of the transducer. With this technique, the microbubbles can be combined with ligands for small animal molecular imaging.

I. INTRODUCTION

Small animal models have been used extensively in disease research, genomics research, drug development, and developmental biology. A non-invasive, high frequency ultrasound imaging system with high spatial resolution is beneficial to the above-mentioned research [1]. Compared to conventional ultrasound imaging system at lower frequencies (<10MHz), the signal-to-noise ratio (SNR) in high frequency ultrasound is generally insufficient for weak flow detection. Micro-bubble based ultrasonic contrast agents have been widely used to enhance the detection of blood [2]. For high frequency imaging, however, the clinically available contrast agents are not suitable for nonlinear contrast imaging because the ultrasound frequency does not correspond to the resonance frequency of the microbubbles. Therefore, one of the purposes of this study is to develop in-house microbubbles for high frequency (25-50MHz) applications.

Another purpose of this study is to test the performance of pulse inversion fundamental imaging that we proposed previously for lower frequencies [3]. Pulse inversion fundamental imaging was proposed to increase the contrast-to-noise ratio (CTR) by detecting the fundamental signals from bubbles. The hypothesis is based on the fact that with pulse inversion imaging, the fundamental tissue signal is cancelled out whereas the fundamental contrast signal is not completely cancelled because the bubble's reaction under compression is different from that under rarefaction. In this study, we investigate performance of pulse inversion fundamental imaging using the in-house microbubbles at high frequencies. The microbubbles that we used in this study are liposome based. [4]

II. ACOUSTIC CHARACTERISTICS OF MICROBUBBLES

Ultrasonic microbubbles produce strong backscattered signal. In addition, the microbubbles oscillate when the impinging sound wave is near the resonance frequency of the bubbles. Oscillation of microbubbles produce scattered energy in frequency ranges that are integer multiples of transmit frequency, as well as into sub-harmonic and ultra-harmonic frequency ranges. The resonant frequency (f_0) of a free air bubble is represented as:

$$f_0 = \frac{1}{2\pi r}\sqrt{\frac{3\gamma P_0}{\rho_0}} \qquad (1)$$

where r is the radius of the bubble. When an air bubble is encapsulated by the shell. The above equation becomes:

$$f_0 = \frac{1}{2\pi r}\sqrt{\frac{3\gamma}{\rho_0}\left(P_0 + \frac{\pi}{3}\frac{S_e}{\gamma r}\right)} \qquad (2)$$

where Se is elasticity coefficient of shell, P_0 is the pressure of surrounding medium, ρ_0 is the density of surrounding medium, and γ is the ideal gas constant. The presence of a shell increases the resonance frequency of a bubble according to (2).

The measured shell properties of the in-house liposome bubbles were measured according to the principles described in [5] and are listed as follows:

depth	parameter	average
3cm	**Shear modulus (MPa)**	**61.4±10.1**
	Shear viscosity (Ns/m^2)	**3.91±0.39**

III. PULSE INVERSION IMAGING

Various techniques have been proposed to detect the microbubbles. For example, harmonic imaging was proposed to increase the CTR by taking advantages of the nonlinear response of the contrast agent. However, since the surrounding tissue also produces significant harmonic echoes, the contrast improvement is often limited. An improved method over conventional harmonic imaging is harmonic power Doppler. Though the CTR is significantly improved with the aid of Doppler technique, the frame rate is reduced since the Doppler estimation must be performed with multiple transmissions. In addition, the flash artifact associated with color Doppler imaging may also be a problem. To increase the contrast without using Doppler techniques, Krishnan et al proposed that the CTR in contrast harmonic imaging can be increased by nulling the tissue harmonic signal with a prebiased transmit pulse [5]. On the other hand, Kirkhorn et al suggested that the release burst can be applied to rupture the microbubbles, and the microbubbles can be distinguished from the background tissue by measuring the decorrelation between the echo signals before and after the release burst [6]. Detection of contrast agents can also be improved by imaging the subharmonic or superharmonic signal [7], which is unique for the contrast agent but not for the tissue. Another approach is based on the microbubble's nonlinear response to pulses with different phases and amplitudes. In [3], it was proposed and verified that pulse inversion technique can be used to increase the CTR by detecting the fundamental signals from bubbles. The hypothesis is based on the fact that with pulse inversion imaging, the fundamental tissue signal is cancelled out whereas the fundamental contrast signal is not completely cancelled because the bubble's reaction under compression is different from that under rarefaction. In this paper, the approach proposed in [3] is tested at 25-50 MHz.

The pulse inversion technique uses a pair of amplitude inverted transmit pulses, with summing of the corresponding received echoes. for tissue, the received echo $y(t)$ can be modeled as a combination of power series of the fundamental signal so that the harmonic response due to tissue nonlinearity is included:

$$y(t) = a_1 x(t) + a_2 x^2(t) + a_3 x^3(t) + \ldots \qquad (3)$$

where $x(t)$ is the linear backscattered signal of transmit pulse, and $x^2(t)$ and $x^3(t)$ are the second- and third-order nonlinear responses, respectively. The higher order (>3) terms are omitted in (3). When the positive and negative echoes are summed, only even-order harmonics remain. Thus, the fundamental signal of tissue is completely cancelled in ideal situation.

On the other hand, the response when the bubble is compressed during positive pressure is different from the response when it is expanded during negative pressure. So the fundamental signal of bubbles is still present. Based on the above discussion, it is suggest that the fundamental signal can be used for contrast detection [3].

In harmonic imaging, the bandwidth of the transmit signal is limited in order to accommodate both the fundamental and harmonic bands within the passband of the transducer. In pulse inversion fundamental imaging, however, the passband of the transducer can be fully utilized, allowing the use of broadband transmit pulses for better axial resolution.

IV. EXPERIMENTAL RESULTS

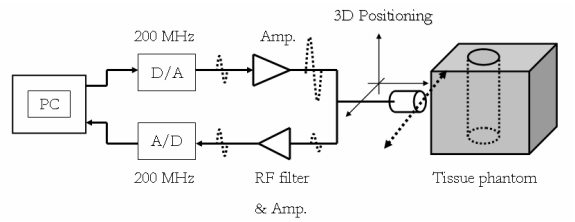

Fig 1 : Schematic diagram of experimental setup.

Fig. 1 shows the experimental setup. This system allows the use of arbitrary transmit waveforms. The transmit waveforms pass through an RF power amplifier, and into a single-element, fixed-focused transducer. This lithium niobium (LiNbO3) transducer has a central frequency 45MHz with an 55% fractional bandwidth. The received signal is amplified and filtered by the pulser/receiver and then digitized at a 200 MHz sampling rate. To form an image, the single crystal transducer is mounted on a computer-controlled positioning stage and is mechanically scanned. The tissue mimicking phantom has a cylindrical hole in the middle for holding the microbubbles. After injecting the liposome contrast agent in the hole, lateral cross-sectional scanning was performed.

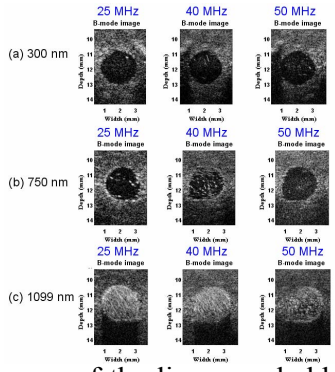

Fig. 2: Images of the liposome bubbles with a 300 nm (top), 750 nm (middle), 1099 nm (bottom) diameter. The transmit frequency is 25 (left), 40 (middle), 50 MHz (right) , respectively.

Several batches of liposome microbubbles with different sizes were produced and the corresponding B-mode images at different frequencies are shown as Fig2. The 1 μm liposome microbubbles produced strongest backscattered signals in 25~50MHz, and were used for subsequent experiments.

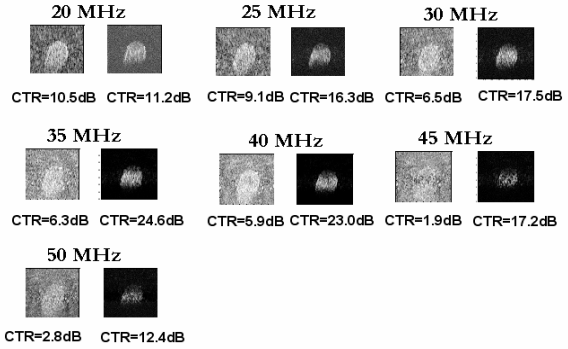

Fig. 3: Comparison of linear imaging (left) and pulse inversion fundamental imaging (right).

The pulse inversion fundamental imagese are shown in Fig. 3 and compared to linear imaging results. The results indicate that the tissue signal was suppressed successfully. Compared to linear imaging, the CTR was enhanced by 7~18 dB. Fig. 4 summarizes the CTR improvement at various frequencies and transmit pulse lengths. The improvements are clearly demonstrated.

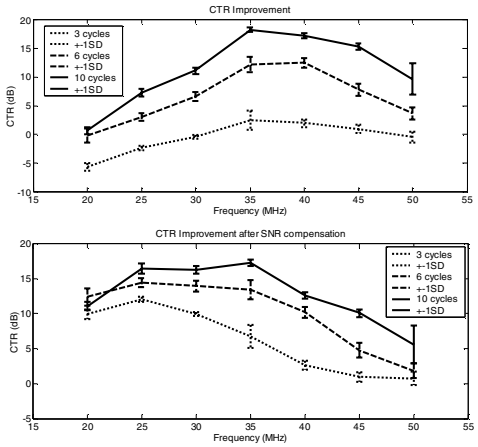

Fig. 4: The CTR improvements with different transmit cycles and different transmit frequencies. The upper and lower portions are before and after SNR compensation separately.

$$CTR \text{ improvement} = CTR_{PI} - CTR_{F} \quad \text{(4-a)}$$

$$= (I_{PI-bubbles} - noise) - (I_{F-bubbles} - I_{F-tissue}) \quad \text{(4-b)}$$

$$= (I_{F-tissue} - noise) - (I_{F-bubbles} - I_{PI-bubbles}) \quad \text{(4-c)}$$

The CTR improvement can be represented as equation (4-a)`(4-b)`and (4-c), where $I_{PI-bubbles}$ is the signal intensity from bubbles in pulse-inversion fundamental imaging. The other parameters ($I_{F-bubbles}$ ` $I_{F-tissue}$) are

similarly defined. The first term in (4-c) is determined by the SNR of the measurement system, and the second term is according to nonlinearity of the microbubbles.

The second harmonic images are shown in Fig. 5 when the transmit frequency is 20 and 25MHz. Compared to second harmonic imaging, the pulse inversion fundamental imaging has approximately 6 and 11dB CTR improvement at 20MHz and 25MHz, respectively.

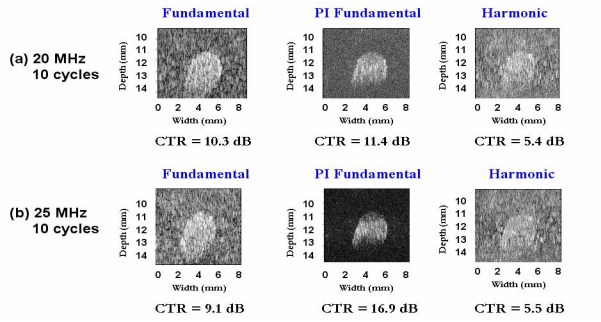

Fig. 5: Comparisons of linear imaging, pulse inversion fundamental imaging and second harmonic imaging.

V. CONCLUSIONS

In this paper, we investigated pulse inversion fundamental imaging in 20~50MHz ultrasounds with in-house liposome microbubbles. This technique helps to improve flow detection in high frequency small animal imaging. Future work will focus on in vivo experiment and small animal molecular imaging.

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