High Frequency Nonlinear Scattering and Imaging of a Submicron Contrast Agent

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Abstract— We investigate high frequency nonlinear scattering and imaging of a contrast agent comprised of submicron bubbles. Agent characterization experiments conducted at 20 and 30 MHz transmit frequencies with a broadband PVDF transducer confirm the production of substantial amounts of energy in the subharmonic and second harmonic regions. Nonlinear contrast imaging with intravascular ultrasound (IVUS) is then explored with a prototype mechanically scanned system. Pulse-inversion techniques were employed with a 20 MHz transmit frequency (F20) for second harmonic imaging (H40), and with a 40 MHz transmit frequency (F40) for subharmonic imaging (SH20). H40 was found to produce improvements in contrast to tissue signal ratios (CTR) for low transmit amplitudes (<0.3 MPa). SH20 was demonstrated at a range of pressures (0.2 to 2.2 MPa). These results show the feasibility of using a submicron agent for high frequency (>15 MHz) nonlinear contrast imaging and suggest the potential application of these techniques in IVUS.

I. Introduction

The extension of contrast imaging techniques to frequencies above 15 MHz has the potential to yield applications in the areas of ophthalmology, dermatology, small animal, and cardiac imaging. Initial studies at high frequencies were conducted examining or assuming linear acoustic properties for existing contrast agents [1], [2]. More recently, nonlinear microbubble scattering and imaging has been demonstrated at transmit frequencies (f_{Trans}) of up to 30 MHz [3], [4]. The results of the latter studies indicated the feasibility of subharmonic (SHI) and ultraharmonic imaging (UHI). Second harmonic imaging (HI) was ineffective at improving contrast to tissue ratios (CTR) due to the presence of tissue propagation harmonics under the investigated experimental conditions. This work was conducted with modifications to existing ultrasound biomicroscopy instrumentation, employing broadband focused PVDF transducers and analog filtering to isolate nonlinear signals. The agent used was Definity™, which is not optimized for use at high frequencies.

A reasonable hypothesis is that the nonlinear scattering observed for higher transmit frequencies is associated with the stimulation of resonant behavior of a subpopulation of smaller bubbles present in current agents. Though not yet validated, the application of existing encapsulated bubble models suggests that bubbles of diameters below 1-2 microns may be active nonlinearly for transmit frequencies above 15 MHz [5]. This is supported by mechanical filtration experiments that isolated subpopulations of bubbles below 1 and 2 microns in diameter and found that nonlinear signal ratios were improved at 20 and 30 MHz transmit frequencies [5]. It was also found that decantation is a viable method of improving the harmonic ratios of an agent designed for use at lower frequencies by preferentially skewing the bubble size distribution towards smaller bubbles. Unfortunately this approach was not effective, at least under the conditions evaluated, in isolating submicron bubbles. An alternative approach is to employ agents specifically produced to be comprised of submicron bubbles.

The first objective of this paper is to investigate nonlinear scattering and imaging of a submicron agent at transmit frequencies in the 20 to 40 MHz range. A second objective is to explore the feasibility of conducting nonlinear microbubble imaging with intravascular IVUS. A primary application considered for IVUS contrast is the detection and imaging of microvessels (vasa vasorum) in atherosclerotic coronary arteries. Vasa vasorum are increasingly recognized to be associated with plaque development, and potential therapeutic targets.

This paper begins with a description of agent characterization experiments using a PVDF transducer, then presents a prototype nonlinear IVUS imaging system, and finally shows the results of phantom validation experiments.

II. Agent Characterization

Methods: The contrast agent employed in this study was BG2423 (Bracco Research, Geneva), an experimental lipid encapsulated formulation comprised of bubbles below 1 micron in diameter. Agent characterization experiments were conducted using a flow cell apparatus and a broadband focused PVDF transducer (f#1.6; aperture 8 mm; center frequency 19 MHz) center frequency as described in [5]. This approach was used as a first step since it permitted knowledge of pressure in a well defined focal zone and bandwidths that were large relative to IVUS transducers.

Experiments were conducted as a function of bandwidth (5-50% 6 dB input bandwidths) and pressure using gaussian enveloped pulses with center frequencies of 20 or 30 MHz. Pressures (peak negative pressures specified) were measured with water tank hydrophone experiments and were in the range of 200 kPa to 6 MPa. One type of experiment performed was to measure the average (60 traces) first pulse response of agent, which was achieved while agent was flowing. A second type of experiment was to bring a group of bubbles within the focal zone, wait until the agent was stationary, and then send a sequence of 1000 pulses at 2 kHz as a test for bubble destruction. The agent was diluted by a factor of 1000 times relative to that in the vial.

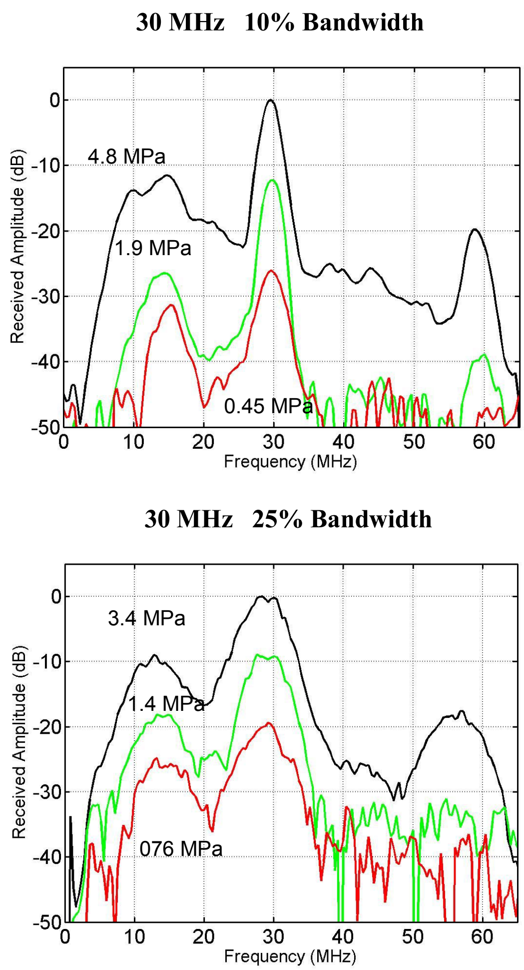

Figure 1. Received spectra for a 30 MHz transmit pulses as a function of pressure. (top) for a 10% bandwidth pulse and (bottom) for a 25% bandwidth pulse. In both cases, pronounced peaks are evident at the fundamental frequency as well as the subharmonic and second harmonic frequencies.

Results: Example results for a transmit frequency of 30 MHz are shown in Fig. 1. For a 10% transmit bandwidth, a peak is observed at the transmit frequency at all pressures. A peak is also observed at the order one-half subharmonic frequency (15 MHz) and, for higher pressures, at the second harmonic frequency. Results have not been corrected for the transducer frequency response on receive. The broadband signal at the high pressure levels is associated with bubble disruption, as determined with the destruction experiments (not shown here). A qualitatively similar pattern is observed for the 25% bandwidth results.

III. IVUS System Description

A prototype nonlinear IVUS system was developed in our laboratory for the purposes of tissue harmonic and contrast agent imaging. The configuration employed in tissue harmonic imaging is described in [6], and an overview of the arrangement for contrast agent imaging is shown in Fig. 2. An arbitrary waveform generator (AWG 520 Tekronix Beaverton, OR) was used to generate pulse sequences, which were amplified with a 60-dB gated linear power amplifier (LPI-10, ENI, Rochester, NY). Signals were then bandpass filtered and were sent through a rotational motor unit to a commercial single element IVUS catheter (Du-Med, Rotterdam, Netherlands). On receive, the RF signals were amplified, bandpassed (12-60 MHz) and digitized at 200 MS/s with a PC based 12-bit acquisition board (DP310 Acqiris, Geneva, Switzerland). Data were then processed off-line.

In this study we assess the feasibility of using the second harmonic signal (H40) of a 20 MHz transmit (F20; 50% -6 dB gaussian enveloped input pulse), and the subharmonic signal (SH20) of a 40 MHz transmit (F40; 25% -6 dB gaussian enveloped input pulse). In both cases, a pulse-inversion (PI) approach was employed. The IVUS catheter was nominally centered at 30 MHz, but had reasonable sensitivity at 20 and 40 MHz [6]. Pressure measurements were performed in a water tank, and we quote the peak negative on-axis values.

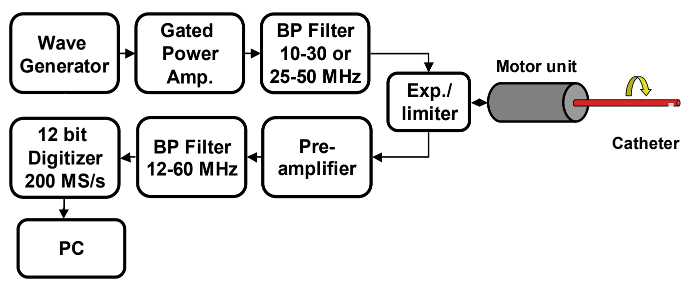

Figure 2. Schematic overview of the prototype nonlinear IVUS system employed in this study.

IV. Phantom Experiments : Methods

Phantom experiments were performed to determine the feasibility of producing and detecting nonlinear signals with IVUS, and to form images based on nonlinear signals during mechanical scanning

Methods: As shown in Fig. 3, an IVUS transducer was situated at the boundary between a suspension of agent and a block of tissue mimicking phantom (agar/gelatin/silica). A single rotation of the catheter was sufficient to permit an analysis of the received signals from both agent and tissue. The transducer was rotated at 5 Hz, which, using a PRF of 12.5 kHz, resulted in the acquisition of approximately 2500 pulses per rotation. This pulse density was selected as a conservative estimate of the maximum number of pulses per rotation that can be achieved at a 30 Hz frame rate assuming a maximum penetration depth of 10 mm. Processing was done on 8 pulse ensembles with 50% overlap between adjacent pulse groups.

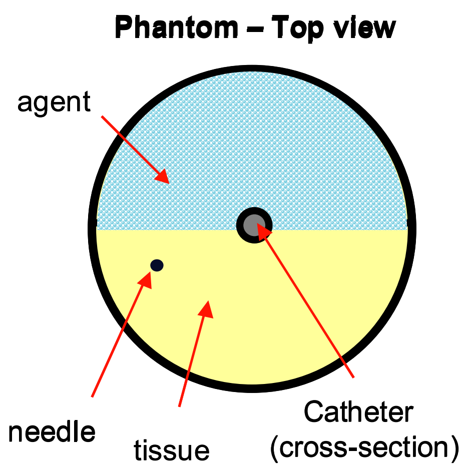

Figure 3. Top view of the phantom used to acquire agent and tissue spectra, and to test feasibility of image formation. The catheter rotational axis is out of the page.

V. Phantom Experiments: Results

Average spectra from tissue (phantom) and contrast regions (between 2-3 mm offset from transducer face) for the 20 MHz transmit case (0.3 MPa) are shown in Fig. 4. In tissue, there is a pronounced peak just above 20 MHz (shifted higher due to frequency response of transducer). With the application of pulse-inversion, this is reduced by approximately 22 dB. In the contrast region, there is also a peak towards 20 MHz, but the energy is distributed more broadly. With PI, the remaining energy, due to nonlinear second harmonic scattering from the agent, is preferentially retained.

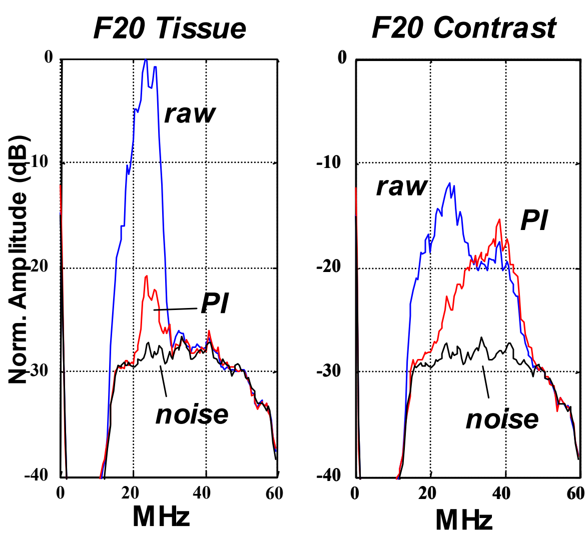

Figure 4. Raw and pulse-inversion spectra from the tissue (left) and contrast agent (right) regions for a 20 MHz transmit pulse (50% input bandwidth; 0.3 MPa). Second harmonic scattering is evident in the contrast agent, and is preferentially retained with pulse-inversion methods during a rotational scan.

Average spectra from tissue and contrast regions (between 2-3 mm offset from transducer face) for the 40 MHz transmit case (0.9 MPa) are shown in Fig. 5. In tissue, there is a pronounced peak just below 40 MHz (shifted lower due to transducer frequency response). With the application of PI, this is reduced by approximately 25 dB. In the contrast region, there is also a peak towards 40 MHz, but a lower peak towards the subharmonic region is also evident. Upon application of PI, the subharmonic peak becomes the dominant portion of the remaining energy.

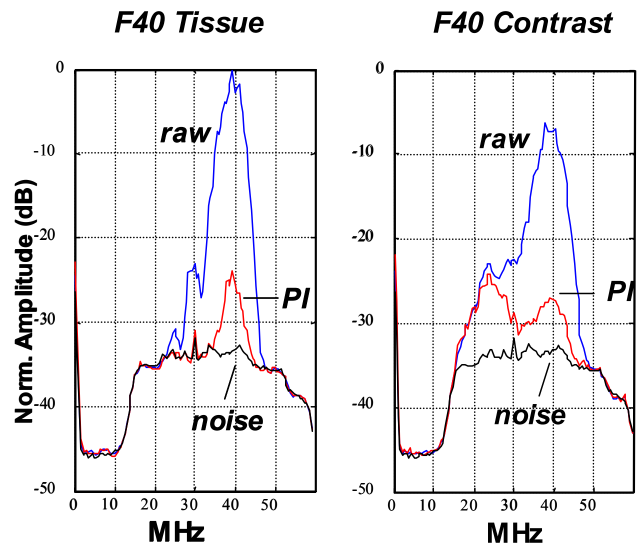

Figure 5. Raw and pulse-inversion spectra from the tissue (left) and contrast agent (right) regions for a 40 MHz transmit pulse (40% input bandwidth; 0.9 MPa). Subharmonic scattering is evident in the contrast agent, and is preferentially retained with pulse-inversion methods during a rotational scan.

Example results of F20 and H40 image formation are shown in Fig. 6 (top) for the 0.3 MPa transmit case. In F20 mode, the agent signal is substantially weaker than the tissue signals. With digital bandpass filtering (28-50 MHz), the agent signal becomes dominant, but residual tissue signals are present, primarily due to fundamental frequency leakage. With the additional application of PI, the H40 agent signal is retained and the remaining fund frequency signals are below the noise floor. As pressure is increased, tissue propagation harmonic degrades CTR (not shown here).

The results for F40 and SH20 image formation are shown in Fig 6. (bottom) for the 0.9 MPa transmit case. In F40 mode, the agent signal is again substantially weaker than the tissue signals. With digital bandpass filtering (13-30 MHz), the agent signal becomes prominent, but a residual tissue signal is still present, due to F40 energy leakage. When PI is applied, the subharmonic agent signal remains, while the F40 tissue signal leakage is suppressed to below the noise floor.

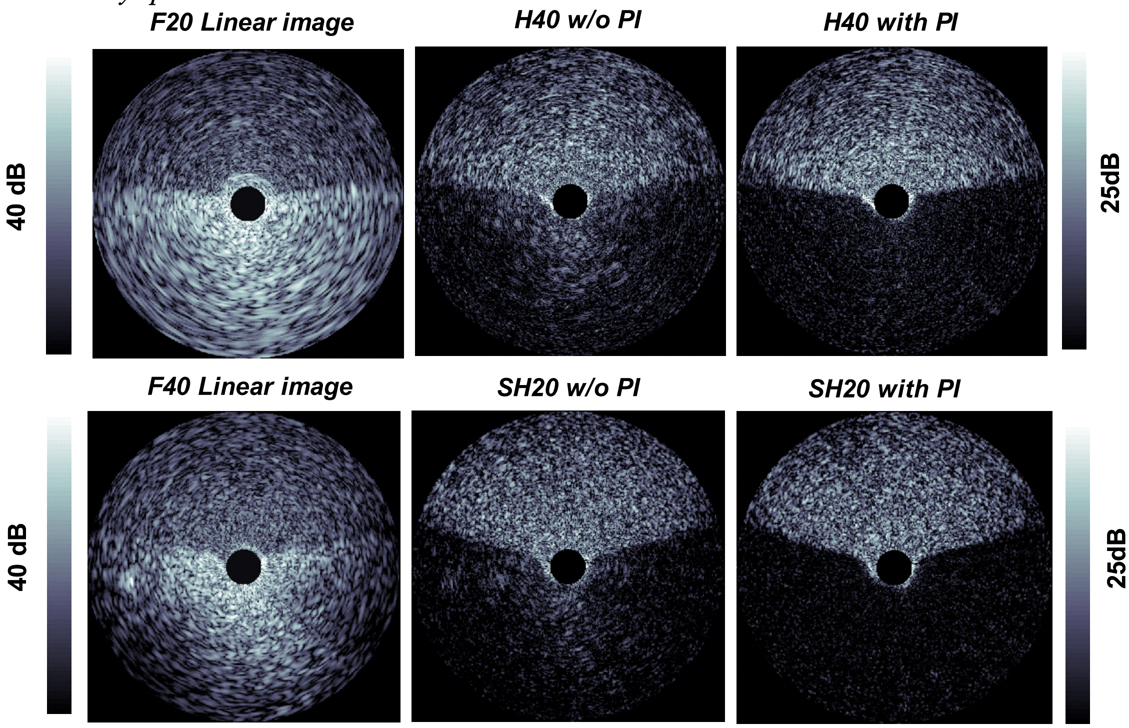

Figure 6. (top left) Phantom image constructed in F20 mode (50% transmit BW; 0.3 MPa) indicates contrast signals lower than tissue signals; (top middle) H40 mode (no PI) results in substantial suppression of tissue signals; (top right) H40 mode with PI suppresses tissue signals to below noise floor. (bottom left) Phantom image constructed in F40 mode (25% transmit BW; 0.9 MPa) indicates contrast signals lower than tissue signals; (top middle) SH20 mode (no PI) results in substantial suppression of tissue signals; (top right) SH20 mode with PI suppresses tissue signals to below noise floor. Images are 12 mm in diameter.

VI. Discussion

This study has demonstrated that nonlinear scattering is possible for transmit frequencies in the 20 to 40 MHz range using an agent comprised mainly of submicron bubbles. The pressure levels used to achieve detectible nonlinear scattering are lower than previously reported with Definity™ [3], [4], and bandwidths are higher. While these results are encouraging, it should be noted that it has not been determined if the agent used in this study is optimal for nonlinear scattering in this frequency range, and further improvements may be possible.

Previous imaging studies [3], [4] employed analog receive filtering, which imposed bandwidth and therefore resolution limitations. It has been shown that PI extraction of both subharmonic and second harmonic signals is possible in this frequency range. Substantial suppression of the tissue signals was achieved during mechanical scanning, with a pulse density that is compatible with maintaining high frame rates (i.e. decorrelation due to transducer translation is sufficiently low).

In [3], no improvement of CTR was found by using H40 mode relative to F20. The results of the current study show that improvements of CTR in H40 mode are possible at lower pressures. This may be due to a number of factors that favor the production of a detectible second harmonic bubble signals at pressure levels that do not result in significant propagation harmonic. These include not only the use of a more effective contrast agent at high frequencies, but may also relate to transducer efficiency and beam geometry differences, as well as the use of pulse averaging.

Apart from nonlinear signal generation, submicron bubbles may also be advantageous from the perspective of imaging microvessels at high spatial resolution. For a given volume fraction of bubbles, a smaller mean size corresponds to a larger number of bubbles, and thereby increases the likelihood of a bubble being present within in a particular microvessel.

A key issue influencing the feasibility of implementing these methods *in vivo* will be the concentration of agent that can be realized. For IVUS detection of vasa vasorum in coronary arteries, it will be possible to locally inject agent through a catheter, which may enable the attainment of high local concentrations of agent. This is the subject of more realistic phantom and *in vivo* experiments.

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