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13. ABSTRACT ***(Maximum 200 Words)***

We propose using a patented multi-pulse contrast imaging technique to improve breast ultrasound imaging. This technique applies two acoustic fields one for bubble excitation and the other for imaging. The excitation field will momentarily increase bubble sizes resulting in an increase in the number of bubbles with a size close to the resonance size corresponding to the (second) imaging field. If the imaging field is applied simultaneously with (or slightly after) the excitation field, acoustic scattering from bubbles around resonance size becomes markedly stronger than without the excitation field.

Significant enhancements of 12 dB in the fundamental mode and 18 dB in the harmonic mode were achieved with excitation enhanced imaging *in vitro*. Moreover, a high frequency (25 MHz), active acoustic detector was employed to unequivocally prove that bubble growth occurs in excitation enhanced contrast imaging. An NIH/SBIR grant using these results as preliminary data was funded. *In vivo* enhancement of 4 dB in the aorta and 1.3 dB within the VX-2 tumors were seen in harmonic mode.

In conclusion, excitation enhanced imaging produces marked enhancement in both fundamental and harmonic mode. This unique modality may significantly improve the sensitivity of currently known contrast imaging modalities.

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4. INTRODUCTION

The goal of any breast imaging modality is to improve the early detection of tumors and to improve the differentiation between benign and malignant lesions. While x-ray mammography is efficacious in diagnosing a high percentage of breast masses, it also produces a high rate of false positives [1]. The percentage of breast biopsies that are actually malignant vary between 10 % and 35 %. Thus, a technique that reliably differentiates between malignant and benign masses would improve the diagnosis of breast cancer and should, therefore, reduce the number of negative biopsies as well as the trauma of the patients. This proposal will attempt to establish such a technique through the novel and innovative use of multi-pulse ultrasound contrast imaging.

Ultrasound imaging is currently an auxiliary modality in breast imaging. It is mainly used to differentiate between cystic and solid lesions [2]. Investigations into the possibility of breast cancer diagnosis based on Doppler ultrasound flow detection have produced mixed results, due to overlap between flow measurements in benign and malignant tumors [3-4]. One problem may be the lack of sensitivity in flow detection in small tumor vessels using ultrasound. This hypothesis is supported by reports in the pathology literature describing angiogenic vascular morphology as an independent predictor of metastatic disease [5].

Ultrasound contrast agents produce increases of 15 to 25 dB in the echo intensities of blood flow signals; especially when combined with new contrast-specific imaging modalities such as harmonic imaging [6-7]. However, harmonic imaging has been found to suffer from reduced blood-to-tissue contrast resulting from second harmonic generation and accumulation in tissue. . Instead, we propose using a patented multi-pulse contrast imaging technique [8]. This technique applies two acoustic fields one for bubble excitation and the other for imaging. The excitation field will momentarily increase bubble sizes resulting in an increase in the number of bubbles with a size close to the resonance size corresponding to the (second) imaging field. If the imaging field is applied simultaneously with (or slightly after) the excitation field, acoustic scattering from bubbles around resonance size becomes markedly stronger than without the excitation field. This project will optimize the performance of ultrasound systems for use in breast imaging with contrast agents, in conventional as well as harmonic imaging modes, by developing multi-pulse contrast specific imaging based on Thomas Jefferson University's patented technology. The improved signal-to-noise ratio will enable clinicians to better depict breast tumor neovascularity and, thus, to better diagnose cancer.

Consequently, this project will examine approaches to and efficacy of inducing instantaneous bubble growth to momentarily enhance the backscattering from contrast microbubbles. This in turn should improve image contrast markedly and enable physicians to improve the diagnosis of breast cancer.

5. BODY

The central hypothesis of this project is that the differentiation between benign and malignant breast lesions can be improved by using ultrasound contrast agents and excitation enhanced

imaging. To investigate this hypothesis excitation enhanced imaging will be investigated in vitro and then in vivo in rabbits with VX-2 tumors.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

5.1 Methods

In Vitro experiments

To measure the shift in size of the microbubble population and, thus, the changes in backscattering of contrast microbubbles suspensions the experimental setup shown in Figure 1 in block diagram form was constructed. This system was based on the design of an active acoustic detector used by Roy and Apfel [9] for detecting microparticles with three transducers placed confocally in the same horizontally plane and immersed in a distilled water bath. The positioning of the transducers was guided with a 0.2 mm miniature needle hydrophone (Precision Acoustics Ltd, Dorchester, England).

The transducer within the excitation block produced a high-intensity (0.4, 0.8 and 1.2 MPa; average of peak positive and negative pressures) focused excitation ultrasound field. The acoustic pressure amplitude was calibrated using a PVDF needle hydrophone specifically designed to measure intensive ultrasound pressures (Imotec Messlehnik, Warendorf, Germany). The transducer was driven by a programmable arbitrary function generator (Model LW420, LeCroy Corporation, Chestnut Ridge, NY) through a 500W power amplifier (Model A-500, ENI, Rochester, NY). The frequency of the excitation transducer was chosen as either 0.528, 1.12 or 2.12 MHz to enable bubble growth [10], but at the same time to be within the bandwidth of current imaging transducers. Pulse lengths of 2, 16 and 128 cycles and pulse repetition frequencies (PRF's) of 2 and 20 Hz were investigated. A time modulus was used to synchronize the delay between the imaging and the excitation ultrasound pulses (varied from 10 – 500 μs). The key part of the modulus is a general-purpose lab pulse-delay generator (model AV-1023-C, Avtech Electosystems Ltd., Ogdensberg, NY).

A programmable function generator (Model 8116A; Hewlett Packard, Santa Clara, CA) produced pulses for transmission within the imaging block (Fig. 1). The transmit signals were first amplified in a broadband 50 dB RF power amplifier (Model 325LA; ENI, Rochester, NY) and then supplied to an acoustic transmit transducer. Signals scattered from contrast microbubbles were sensed by a receive transducer and amplified with a low noise RF amplifier (Model 5052 PR; Parametrics, Waltham, MA). The amplified signals were acquired at a sampling frequency of 25 MHz using a digital oscilloscope equipped with mathematical functions (Model 9350AM; LeCroy, Chestnut Ridge, NY). The command delivery to the function generator and the data transfer from the digital oscilloscope were controlled by LabView® (National Instruments, Austin, TX). The microbubble suspensions were diluted in Isoton® II (Coulter Corporation, Miami, FL) to around 0.02 μl of agent/ml of water and a magnetic stirrer was used to maintain mixture in a small waterbath instead (volume: 4.2 l).

Additionally, an 8mm vessel embedded in a tissue-mimicking flow phantom (ATS laboratories, Bridgeport, CT) was imaged at a low MI of 0.2 in both fundamental (3.0 MHz) and harmonic (2.5/5.0 MHz) grayscale modes with a curvilinear transducer connected to a PowerVision 7000

scanner (Toshiba America Medical Systems Inc, Tustin, CA). Excitation pulses (0.5-2.0 MHz, 2-16 cycles and 0.4-2.0 MPa) were produced by a single-element large-aperture transducer positioned at a 45° angle intersecting the imaging plan and the vessel. Diluted suspensions of ultrasound contrast agents were pumped through the flow system. Images were digitized before and after transmission of the excitation pulses for video intensity measurements using ImagePro Plus software (Media Cybernetics, Silver Spring, MD), which provides direct intensity values.

In Vivo experiments

Four New Zealand white rabbits were studied *in vivo*. Hepatic tumor implantation was performed, as a sterile surgical procedure, in 2 animals. These rabbits were injected percutaneously with 0.5 ml of VX-2 tumor cells (approximately 3 million; Bogden Laboratories, Worcester, MA) into the liver. Following injection, a localized, avascular carcinoma-like mass developed over 10 to 15 days at the site of injection [11]. All rabbits was sedated with 0.65 mg/kg of a mixture of Xylazine hydrochloride (Gemini, Rugby Laboratory, Rockville Centre, NY) and Ketamine hydrochloride (Ketaset, Aveco, Fort Dodge, IA) administered intramuscularly under the supervision of a veterinary technician. The rabbits were maintained under anesthesia with 15 to 20 mg/kg/hr of 1 % Propofol (Diprivan®, Zeneca Pharmaceuticals, Wilmington, DE) as needed for the entire procedure.

The rabbits had their aorta and VX-2 tumors (if present) imaged using a similar setup as for the *in vitro* flow measurements. A tissue harmonic grayscale mode (2.5/5.0 MHz) was employed with a curvilinear transducer (3.5C40H) connected to an Elegra scanner (Siemens Medical Systems Inc, Issaquah, WA). Excitation pulses (1.0 MHz, 16 cycles and 1.0 MPa) were produced by a single-element, large-aperture (2.54 cm) transducer positioned at a 45° angle intersecting the imaging plan. Images acquired before and after transmission of the excitation pulses were digitized for video intensity measurements as described above.

5.2 Results and Discussion

As originally proposed different types of contrast agents were studied. Time constraints and agent availability limited the study to two agents:

a) Sonazoid® (Nycomed-Amersham, Oslo, Norway), which is an encapsulated contrast agent containing a PFC gas.
b) Sonavist® (Schering AG, Berlin, Germany), which consists of air microbubbles with a biodegradable shell composed of polybutyl-2-cyanoacrylate.

Initially, the enhancement obtained with excitation enhanced imaging relative to standard contrast imaging (i.e., the change in signal strength before and after the excitation pulse expressed in dB) was established as a function of the acoustic pressure employed in the excitation field. The PRF was either 2 Hz as in intermittent imaging or 20 Hz equivalent to real time. The excitation pulse length was 128 cycles and the imaging frequency and pressure were 3 MHz and 0.1 MPa, respectively. Marked improvements (approximately 10 dB) over regular contrast imaging was seen in intermittent mode, while the beneficial effects were less pronounced at 20 Hz PRF (on the order of 4 dB). This reduction in the efficacy of excitation enhanced imaging at the higher PRF is most likely due to the bubble destruction associated with real time imaging [12].

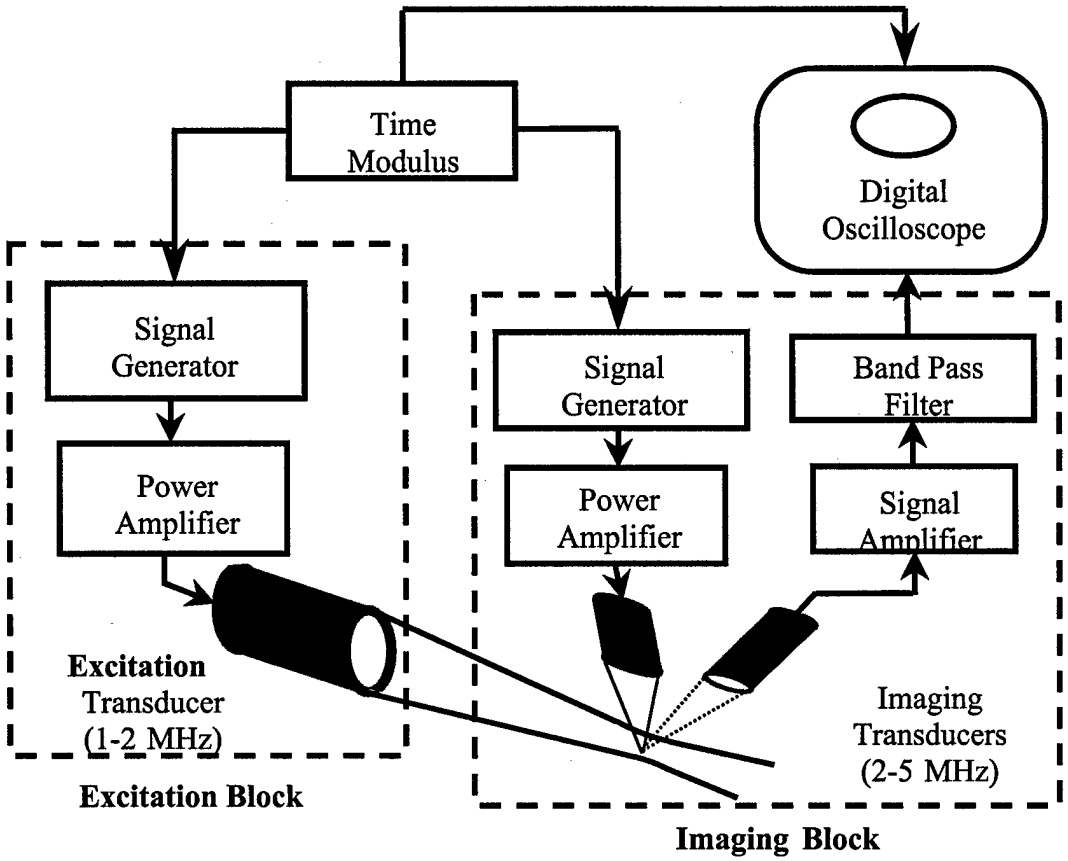

Figure 1. *Block diagram of the experimental in vitro measurement system constructed.*

We also investigated whether the 10 dB enhancement seen at a 2 Hz PRF could be caused by bubble destruction from the excitation pulse itself. If that was the case, it should be possible to duplicate the results by increasing the acoustic pressure of the imaging pulse (i.e., as in intermittent imaging). Table 1 presents the results of increasing the imaging pulse pressure from 0.1 to 0.8 MPa at a 2 Hz PRF. Higher pressures were not possible without damaging the imaging transducer. The excitation pulse used in conjunction with the imaging had a 1.12 MHz center frequency and a pulse length of 16 cycles. The imaging frequency was 3.3 MHz. Clearly, even for a 0.8 MPa imaging pulse excitation enhanced imaging provided an additional 4 dB of enhancement. This proves that the enhancement observed with excitation enhanced contrast imaging is caused not merely by bubble destruction but, as predicted, by bubble growth.

To further substantiate the occurrence of bubble growth in excitation enhanced imaging we employed a high frequency (25 MHz), active acoustic detector developed by our group. Essentially this is the setup of Figure 1 with the two confocally placed imaging probes replaced by 25 MHz transducers (one for transmitting and one for receiving, as shown in the Imaging Block in Fig.1). The resolution of the active acoustic cavitation detector combined with the contrast microbubble concentration allows individual bubbles to be studied [13]. Figure 2a depicts ten sequences received with the 25 MHz transducer without any excitation pulse (pulse

repetition interval 50 μs). Clearly, this signal and thus, the bubble, remained unchanged over the measurement time 450 μs (corresponding to the inter-pulse delays investigated). Conversely, Figure 2b shows the effect of firing a 2.5-MHz excitation pulse during the second sequence (notice the 2.5 MHz modulation from the excitation pulse superimposed on the 25 MHz detection pulse). The bubble size (indicated by the signal amplitude in sequences 3 – 10) is markedly larger following the excitation pulse (compare sequences 3 – 10 to sequence 1). This unequivocally proves that bubble growth occurs in excitation enhanced contrast imaging.

The effect of pulse length on the efficacy of excitation enhanced contrast imaging was limited. Even though the excitation pulse was varied from 2 to 128 cycles in length the enhancement did not change (given the approximately 2 dB standard deviation of the measurements). Likewise, we found that the effect of changing the delay between the excitation pulse and the imaging pulse from 10 to 500 μs was minimal.

Table 1. *Enhancement in dB produced by excitation enhanced contrast imaging with Sonazoid for different imaging as well as excitation pulse pressures.*

Excitation pulse Pressure [MPa]	Imaging pulse pressure [MPa]		
	0.1	0.4	0.8
0.4	5.0	2.5	
0.8	8.0	5.0	4.0
1.2	8.5	4.5	4.0

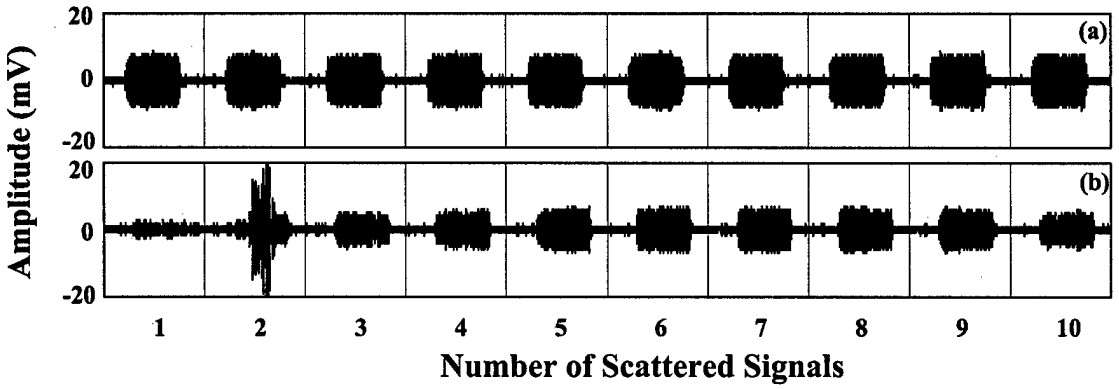

Figure 2. *Sonazoid bubble (a) detected over 450 μs without excitation (and without bubble growth) and (b) demonstrating growth following a 2.5 MHz, 16 cycle 1.8 MPa excitation pulse. The imaging pulse pressure was less than 0.1 MPa in both cases. Notice, the difference in the initial bubble size (sequence 1) in the two cases.*

Experiments were conducted, as mentioned, with the contrast agents Sonazoid and Sonavist, which produced an average enhancement following the excitation pulse on the order of 8 to 10 dB (for the optimal imaging parameters). These results all pertain to fundamental imaging. The same experiments were conducted for second harmonic imaging, where we found similar trends. The best results were obtained with Sonavist, which produced second harmonic enhancements up to 16 dB with an average of 13.4 dB (range 10 – 16 dB). An example of the change induced in Sonavist microbubbles by a 1.06-MHz excitation pulse is shown in Figure 3. Notice, there is even 8 dB of enhancement at the third harmonic (at 7.5 MHz). Sonazoid performed achieved enhancements at the second harmonic frequency ranging from 0 dB (in one case only) to 8 dB with an average of 4 dB. These results were presented at the annual meeting of the American Institute of Ultrasound in Medicine (AIUM) in 2001 [14].

These *in vitro* results were sufficiently encouraging that an NIH/SBIR grant entitled "System for Excitation Enhanced Ultrasound Contrast Imaging" was submitted using these results as preliminary data. We recently learned that this grant is being funded.

While video intensities of scattered signals from the surrounding tissue were unchanged, video intensities of echoes from contrast within the vessel were markedly enhanced in both fundamental and harmonic mode. Optimal enhancements of 12 dB in the fundamental mode and 18 dB in the harmonic mode were achieved *in vitro* using 16-cycle excitation pulses with a center frequency of 0.53 MHz (Figure 4). *In vivo* enhancement of 4 dB in the aorta and 1.3 dB within the VX-2 tumors were seen in harmonic mode following a 1 MHz, 16-cycle excitation pulse (Figure 5). The enhancement within the VX-2 tumors is within the measurement uncertainty and clearly the *in vivo* results are markedly lower than what was obtained *in vitro,* most likely due to difficulties in aligning the excitation transducer and the imaging transducer correctly. These results were presented in part at the annual meeting of the AIUM in 2002 [15] and will be presented in completeness at the upcoming annual meeting of the Radiological Society of North America (RSNA) [16]. We expect to submit full journal papers detailing this work in the near future.

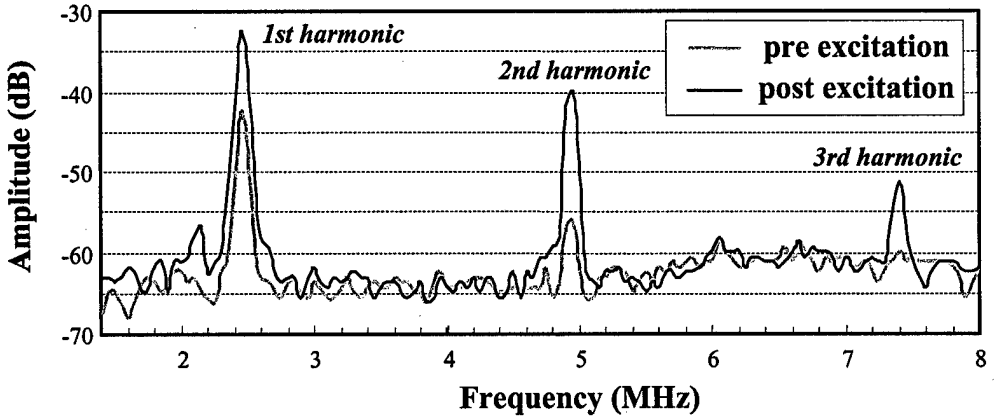

Figure 3. *Sonavist spectra recorded with a 2.5 MHz low amplitude pulse (0.1 MPa) before and after a 1.06 MHz, 1.2 MPa, 16 cycle excitation pulse.*

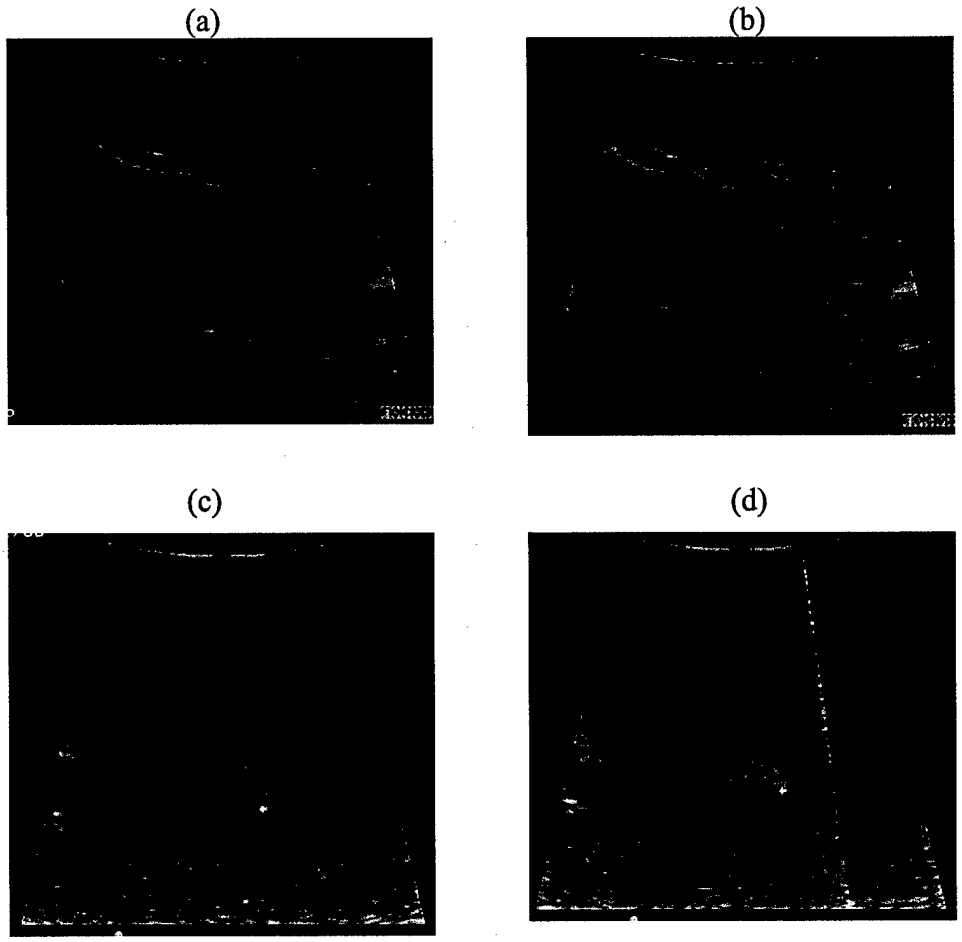

Figure 4. *Imaging of 8 mm vessel in vitro in fundamental mode before (a) and after (b) a 0.53 MHz, 16 cycle excitation pulse. The enhancement is 12 dB. The same vessel seen in harmonic mode before (c) and after (d) the same excitation pulse – the enhancement was 18 dB.*

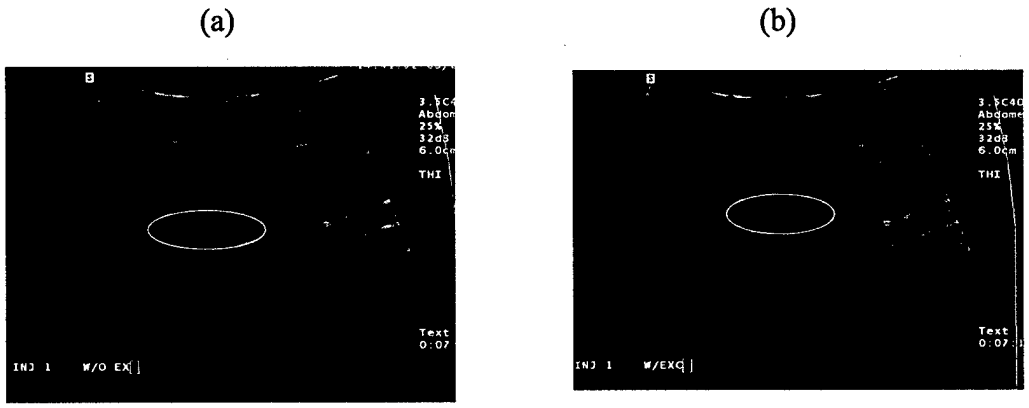

Figure 5. *Harmonic imaging in vivo of the aorta (circled) of a rabbit before (a) and after (b) a 1.06 MHz, 16 cycle excitation pulse. The enhancement is 4 dB.*

6. KEY RESEARCH ACCOMPLISHMENTS

- An *in vitro* system was built to evaluate excitation enhanced imaging.
- Initial experiments were conducted with Sonazoid and Sonavist.
- Optimal acoustical imaging parameters for excitation enhanced imaging were explored.
- Enhancement up to 10 dB and 16 dB was measured in fundamental and harmonic mode, respectively.
- The SNR improvement in an *in vitro* flow system was 12 dB and 18 dB, respectively, in fundamental and harmonic excitation imaging mode.
- *In vivo* evaluation of excitation enhanced imaging was performed in 4 rabbits (2 with hepatic VX-2 tumors).
- Enhancement of up to 4 dB was measured *in vivo*.

7. REPORTABLE OUTCOMES

Manuscripts, abstracts, presentations

Shi WT, Bautista R, Forsberg F, Vecchio C, Bernardi R, Goldberg BB. Evaluation of excitation enhanced ultrasound contrast imaging. *J Ultrasond Med*, 20, S12, 2001

Forsberg F, Shi WT, Merton DA, Liu JB, Vecchio C, Bernardi R, Goldberg BB. In vitro and in vivo multi pulse ultrasound contrast imaging. *J Ultrasound Med*, 21, S21, 2002.

Forsberg F, Shi WT, Merton DA, Liu JB, Vecchio C, Bernardi R, Goldberg BB. Excitation enhanced imaging for improved breast cancer detection. *Proc Era of Hope, DoD Breast Cancer Research Meet,* ??-??, 2002.

Forsberg F, Shi WT, Merton DA, Liu JB, Vecchio C, Bernardi R, Goldberg BB. Excitation enhanced US contrast imaging in vitro and in vivo. Accepted for publication in *Radiology*, 2002.

March 11 - 14, 2001. The 45th Annual Convention of the American Institute of Ultrasound in Medicine, Orlando, FL, USA.

- Evaluation of excitation enhanced ultrasound contrast imaging.

March 10 - 13, 2002. The 46th Annual Convention of the American Institute of Ultrasound in Medicine, Nashville, TN, USA.

- In vitro and in vivo multi pulse ultrasound contrast imaging.

September 25 – 28, 2002. Era of Hope, Dept. of Defense Breast Cancer Research Meeting, Orlando, FL, USA.

- Excitation enhanced imaging for improved breast cancer detection (poster).

Degrees and Grant submissions

NIH (SBIR), grant no R44 HL62830-02A1; System for Excitation Enhanced Ultrasound Contrast Imaging (subcontract to Spectrasonics Inc).

Raymond Ro has been employed on this project and is working towards his PhD degree in Biomedical Engineering (to be obtained from Drexel University) with F. Forsberg (the PI) as his supervisor.

8. CONCLUSIONS

Significant enhancements of 12 dB in the fundamental mode and 18 dB in the harmonic mode were achieved with excitation enhanced imaging *in vitro*. Moreover, a high frequency (25 MHz), active acoustic detector was employed to unequivocally prove that bubble growth occurs in excitation enhanced contrast imaging.

These *in vitro* results were sufficiently encouraging that an NIH/SBIR grant entitled "System for Excitation Enhanced Ultrasound Contrast Imaging" using these results as preliminary data was funded.

In vivo enhancement of 4 dB in the aorta and 1.3 dB within the VX-2 tumors were seen in harmonic mode. Clearly, the *in vivo* results are markedly lower than what was obtained *in vitro,* most likely due to difficulties in aligning the excitation transducer and the imaging transducer correctly.

In conclusion, excitation enhanced imaging produces marked enhancement in both fundamental and harmonic mode. This unique modality may significantly improve the sensitivity of currently known contrast imaging modalities.

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