The implementation of acoustic angiography for microvascular and angiogenesis imaging

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***Abstract*— Recently, it has been demonstrated that through the use of contrast agents and multi-frequency transducer technology, high resolution and high signal to noise ultrasound images can be obtained which illustrate microvascular structure in unprecedented detail for an ultrasound modality. The enabling technology is ultrasound transducers which are fabricated with elements which can excite microbubble contrast agents near resonance and detect their broadband harmonics at a much higher bandwidth (several times the fundamental frequency). The resulting images contain very little background from tissue scattering and thus provide high contrast, and can have a resolution on the order of 130 microns with an appropriate high frequency receiving element. Because microbubbles are strictly an intravascular agent, this approach enables visualization of microvascular morphology with unique clarity, providing insight into angiogenesis associated with tumor development.**

I. Introduction

Ultrasound imaging is already widely used in clinical and preclinical imaging, with several advantages over competing modalities such as low cost, safety, convenience, high frame rate, and soft tissue contrast. Microbubbles provide additional utility as contrast agents in ultrasound imaging by increasing backscatter from blood flow, which would be otherwise difficult to image in small vessels due to low signal to noise. To date, contrast enhanced ultrasound has shown unique utility in enhancing blood-tissue border definition, such as myocardial border delineation; perfusion imaging, where microbubble flow into an organ can provide quantifiable information about local blood flow; and molecular imaging, where targeted contrast agents can elucidate the presents of molecular markers expressed on the endothelium. However, until recently, contrast enhanced ultrasound could not clearly define microvessel structure without using "persistence" imaging approaches, which track contrast presence over a long (many seconds) time period.[1] This is largely due to the fact that contrast imaging has been primarily used at low frequencies, which have resulted in low resolution, and the fact that current contrast imaging techniques can still suffer from tissue nonlinearities contaminating the contrast signal. Although persistence imaging can provide some useful images depicting vascular morphology, the presence of any tissue motion quickly degrades the image even in 2-D, and thus persistence images are very challenging to acquire in 3-D, which is what is ultimately required for mapping microvascular structure.

In this paper, we describe an approach for using contrast ultrasound to image the microvasculature in 3-D with high resolution and contrast. This approach, which we refer to as "acoustic angiography" because of its similarity in appearance to x-ray angiography, is enabled through the use of ultrasound transducers fabricated with multiple elements to achieve a very broad bandwidth across transmit and receive, enabling detection of broadband superharmonic content from cavitating microbubbles.[2]

II. Methods

A. Transducer Design

The enabling technology for acoustic angiography is the dual-frequency transducer, which provides a low frequency bandwidth for transmitting and a high frequency bandwidth for receiving. The principle behind the imaging is that excited microbubbles produce very broad harmonic content as they oscillate aggressively in an acoustic field. Driving these microbubbles can be performed effectively with transmission frequencies which induce maximal oscillation, which are typically low frequencies (a few MHz). Kruse et al. demonstrated even with 2 MHz excitation, the resulting high frequency energy can be as high as 45 MHz.[3] To date, no commercial imaging transducers have been able to transmit and receive with such a broad bandwidth, so our team has fabricated transducers for this purpose.[4] Our prototypes consist of a modified Visualsonics RMV probe with a 30 MHz center frequency element. Confocally aligned with this element is a low frequency (4 MHz) annulus used as a transmitter. The high frequency element can be used for standard pulse-echo b-mode imaging to obtain anatomical information, or can be used as a receiver only to obtain contrast only information. The transmitter is driven by an RF amplifier and pulse generator synchronized with the Visualsonics Vevo 770 commercial ultrasound system. A 7th order 10 MHz butterworth high-pass filter on the receive line filters out any received tissue echoes.

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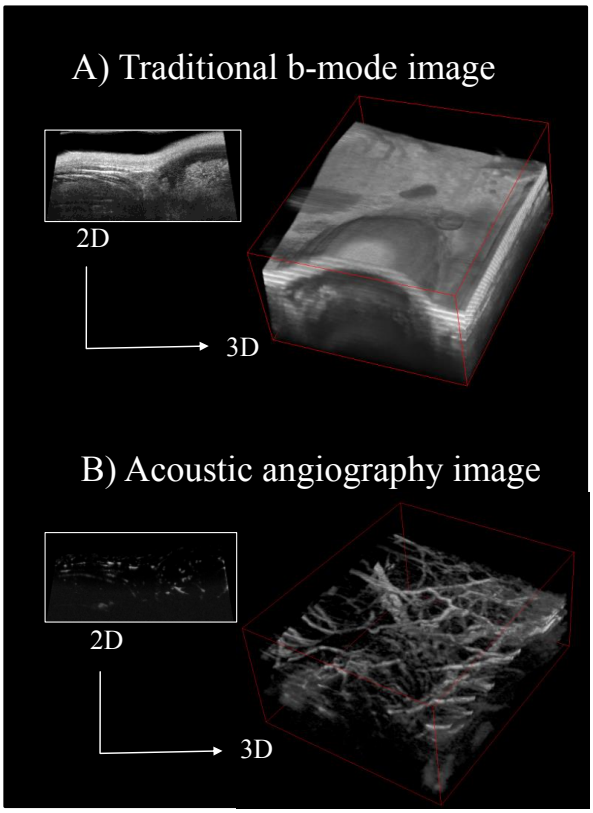

Figure 1. A) 30 MHz b-mode imaging of a subcutaneous FSA tumor in a rat in 2D and 3D. B) acoustic angiography of the same sample volume, illustrating microvasculature within the tissue and tumor region.

B. In Vivo Imaging

All imaging studies were approved by the IACUC at the University of North Carolina at Chapel Hill. In-vivo imaging was performed on healthy mice or rats bearing subcutaneous fibrosarcoma (FSA) tumors, as described previously.[5] Lipid-shelled perfluorocarbon-filled microbubble contrast agents, as previously described,[6] were administered via continuous infusion. A mechanical stage translated the custom transducer probe in the elevational direction to enable 3-D volume reconstruction during imaging.

III. RESULTS

A. Transducer Performance

The custom transmit 4 MHz/receive 30 MHz RMV probe demonstrated a -6dB focal beamwidth for the receiver of approximately 130 microns, suggesting a resolution on this order. Most imaging was performed at a mechanical index of approximately 0.6 (1.2 MPa at 4 MHz) which is less than the recommended energy for Definity imaging in patients,[7] but likely sufficient for contrast agent destruction, although our additional data (not shown here) suggests that some bubbles can survive multiple pulses before complete destruction at this frequency and pressure combination. We utilized low frame rate imaging (several Hertz), which enabled sufficient contrast wash in into the area of interest for imaging with the parameters utilized.

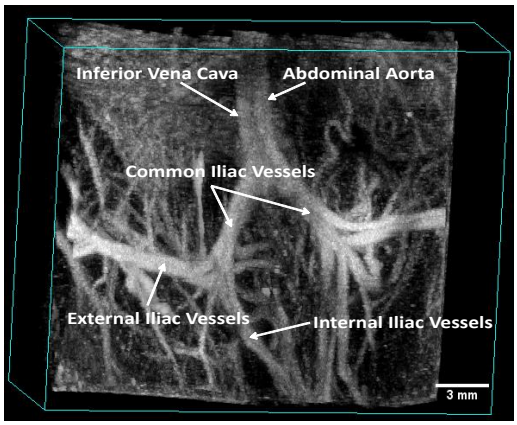

Figure 2. Acoustic angiography of mouse abdomen showing vascular structure.

The dual-frequency probe could be utilized in two separate imaging modes, standard pulse-echo imaging at 30 MHz, which would provide anatomical information (Figure 1A), or transmit 4 MHz/receive 30 MHz mode, which would provide contrast information (Figure 1B). Contrast imaging illustrated microvasculature with high resolution and high signal (acoustic angiography) to noise due to the detection of flowing contrast limited to within the vascular space, and low background from tissue due to the substantial bandwidth separation. Both images could be readily registered to provide simultaneous tissue and microvascular information (not shown). Acoustic angiography of the mouse abdomen provided clear delineation of major and minor vasculature (Figure 2).

B. Angiogenesis Imaging

When applied to subcutaneous tumors, angiogenesis was readily visualized. Figures 3(A-B) show acoustic angiography of subcutaneous tumors in a rat. The angiogenic growth of feeder vessels from left to right into the

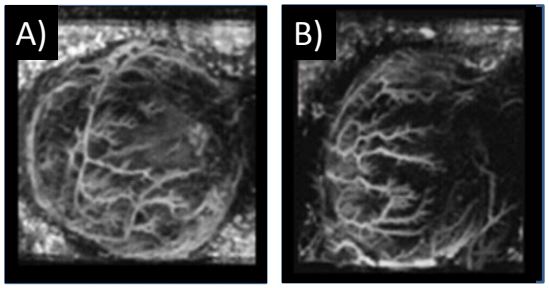

Figure 3. A) and B) exampes of acoustic angiography (maximum intensity projections from 3-D datasets) of angiogenic microvasculature present in subcutaneous FSA tumors in rats.

tumor tissue is readily visualized. Each image frame is approximately 15 mm square.

C. Derived Features of Microvascular Morphology

One of the exciting possibilities with acoustic angiography is the ability to quantify microvascular characteristics due to the high resolution and high contrast in the 3-D images. We have previously shown that these images can be segmented and analyzed. Features such as vessel size and vascular density can be measured,[8] as well as measures of microvascular tortuosity.[5] It is well believed that tortuosity is a characteristic abundantly present in angiogenic vessels and less present in healthy vessels. Our prior studies have demonstrated that through acoustic angiography analysis of rodents, we could distinguish healthy tissue from tumor-bearing tissue based on microvessel morphology alone using measurements of microvascular tortuosity. Similar techniques have been applied to magnetic resonance angiography data,[9-13] although it has not been until the development of acoustic angiography that microvascular morphology analysis has been feasible on ultrasound data.

IV. Conclusion

The development of dual-frequency transducers to take advantage of the high-frequency broadband energy from contrast agent microbubbles has enabled a new contrast imaging approach which produces ultrasound images with unprecedented resolution and contrast. Although this technique has some notable limitations, such as limited depth of penetration due to the high frequency received energy, and the likely need for bubble destruction to obtain the best signal, acoustic angiography will likely provide utility as a tool in serial imaging of angiogenesis and microvessel morphology. For this technique to become more widely useful, multi-frequency array transducers will need to be fabricated and integrated with commercial hardware. One alternate application also in development is micro-scale dual-frequency transducers for intravascular contrast ultrasound imaging, which may play a future role in vasa vasorum imaging.[14]

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