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● *Original Contribution*

FAST, LOW-FREQUENCY PLANE-WAVE IMAGING FOR ULTRASOUND CONTRAST IMAGING

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Abstract—Plane-wave ultrasound contrast imaging offers a faster, less destructive means for imaging microbubbles compared with traditional ultrasound imaging. Even though many of the most acoustically responsive microbubbles have resonant frequencies in the lower-megahertz range, higher frequencies (>3 MHz) have typically been employed to achieve high spatial resolution. In this work we implement and optimize low-frequency (1.5-4 MHz) plane-wave pulse inversion imaging on a commercial, phased-array imaging transducer *in vitro* and illustrate its use *in vivo* by imaging a mouse xenograft model. We found that the 1.8-MHz contrast signal was about four times that acquired at 3.1 MHz on matched probes and nine times greater than echoes received on a higher-frequency probe. Low-frequency imaging was also much more resilient to motion. *In vivo,* we could identify sub-millimeter vasculature inside a xenograft tumor model and easily assess microbubble half-life. Our results indicate that low-frequency imaging can provide better signal-to-noise because it generates stronger non-linear responses. Combined with high-speed plane-wave imaging, this method could open the door to super-resolution imaging at depth, while high power pulses could be used for image-guided therapeutics.
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Key Words: **Ultrasound, Microbubble, Contrast imaging, Pulse inversion, Low frequency.**

INTRODUCTION

Plane-wave ultrasound (US) imaging is becoming increasingly common because of improvements in computational power and availability of programmable ultrasound systems, opening new doors in US imaging and therapeutics. In conventional US imaging, select elements are activated to generate a focused beam (scan line), which is then steered across the imaging plane, acquiring data at each location in a line-by-line manner. The full image is then reconstructed from the entire series of acquisitions. In plane-wave (as well as diverging-wave) imaging, all elements are recruited to produce a coherent plane wave, illuminating the entire imaging field, and the echoes are detected and parallel beamformed by all elements (Montaldo et al. 2009). Whereas conventional imaging can take up to hundreds of acquisitions to generate a single image, limiting the frame rate to tens of hertz, plane-wave imaging can produce an image from a single acquisition, allowing for frame rates in the kilohertz range (Couture et al. 2009). Plane-wave (as well as diverging-wave) imaging has been implemented in applications with high-temporal-resolution demands such as echocardiography (Hasegawa and Kanai 2011; Papadacci et al. 2014). The unfocused plane wave generates fewer pulses and lower peak negative pressures (PNPs), making it well suited for contrast imaging because reduced PNP minimizes microbubble (MB) disruption (Couture et al. 2012).

Microbubbles are echogenic gas-filled vesicles of 1–10 μm clinically approved for enhancing endocardial borders (Crouse et al. 1993) and increasingly explored and translated for improving cancer diagnosis *via* perfusion estimations (Kasoji et al. 2016) or targeted imaging (Willmann et al. 2017). On insonation, MBs produce unique backscatter echoes that consist of the fundamental frequency as well as harmonic and sub-harmonic frequencies, which allows the MB signal to be isolated from tissue signal (De Jong et al. 1994). Pulse inversion (PI) is a selective contrast imaging technique that exploits the difference in MB compression and

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rarefaction (Simpson et al. 1999). When a dominantly linear responder such as tissue is insonified with a pulse pair of inverse polarity, the resulting echoes will be of inverse reflections to each other, and summing these two echoes will cancel each other out, resulting in no signal. When a non-linear responder such as a MB is insonified with inverted pulses, the resulting echoes will not be perfect inverse reflections to each other, and the sum of these echoes will result in a non-zero signal (contrast image).

Combining plane-wave/diverging-wave and contrast imaging is a logical next step and has been implemented in various methods including contrast pulse sequence (Couture et al. 2012), pulse inversion (Couture et al. 2012; Leow et al. 2015; Toulemonde et al. 2017) and amplitude modulation (Tremblay-Darveau et al. 2016; Viti et al. 2016). Plane-wave imaging suffers from reduced lateral resolution compared with line-by-line acquisition, but this can be ameliorated with coherent image compounding (Montaldo et al. 2009). As image resolution is closely tied to the transmit frequency, many of the plane-wave contrast pulses tested thus far operate at higher frequencies (>3 MHz). However, imaging penetration depth decreases with increasing frequency, and the higher-frequency imaging does not fully leverage the harmonic content of the most acoustically active MBs, which is one of the MBs' strongest mechanisms for contrast. MBs exhibit a size- and stiffness-dependent resonance frequency, where US backscatter is maximized (De Jong et al. 2002b; Medwin 1977), and experimental work by Gorce et al. (2000) indicates that for SonoVue, MBs of 3−9 μm, despite accounting for only 20% of the MB population, are responsible for 80% of the acoustic efficiency, with the overall attenuation coefficient peaking at 1.5−2 MHz. Toulemonde et al. (2017) successfully implemented ultra-fast diverging-wave contrast echocardiography using low-frequency (1.5 MHz) pulse inversion *in vivo* in a goat. By imaging at frequencies closer to the resonant frequency of the most acoustically active MBs, a low-frequency plane-wave contrast imaging method may be a useful tool because of its ability to image MBs with higher contrast and at greater depths.

In this study we implemented plane-wave pulse-inversion imaging on a programmable US imaging platform using a commercial, low-frequency, phased array transducer (P4-1). Contrast images were acquired and optimized *in vitro* over transmit frequencies of 1.6−4 MHz, where we found low frequency (<2 MHz) to be optimal. We compare contrast images acquired with transducers of different central frequency to illustrate that the enhancement is not due solely to bandwidth limitation. We determined the capability of the low-frequency pulse sequence by characterizing MB half-life and assessing tumor vasculature *in vivo*. Our study is the first to optimize pulse inversion at a low frequency (<2 MHz) for plane-wave imaging and apply the optimized parameters to an *in vivo* cancer model. We also contribute our optimized code for pulse-inversion imaging for other researchers to utilize (Supplementary File S1, online only).

METHODS

Flash-angle pulse inversion contrast imaging

Plane-wave PI imaging was programmed on the Vantage system (Verasonics, Kirkland, WA, USA) using MATLAB (The MathWorks, Natick, MA, USA) for the P4-1 and L7-4 imaging probes (Philips, Amsterdam, Netherlands). We implemented PI in favor of amplitude modulation schemes because of the millisecond-scale delay required to change pulse amplitude while still using all elements to maintain a consistent beam. Flash Angle (FA), a multi-angle plane-wave imaging sequence available on the Vantage system, was modified to perform flash-angle pulse-inversion (FAPI). In FA imaging, an image is reconstructed from the sum of echoes from multiple delay-modulated plane waves directed at various angles (−30° to 30°). In the case of FAPI, a positive pulse and a negative pulse are collected at each angle, summed and reconstructed. Images were reconstructed using the Verasonics native code for multi-angle plane-wave imaging, after acquisition that includes an 11-tap FIR low-pass filter and a 21-tap FIR bandpass filter. Transmit (Tx) frequency, PNP, compounding angle numbers (Na) and pulse cycle duration in cycles (N) for the P4-1 were experimentally optimized on an *in vitro* flow phantom described below. An inter-pulse time of 200 μs was chosen to minimize artifacts caused by motion, while also allowing time for sound waves from previous pulses to attenuate. Images were acquired at a sampling frequency of 25 MHz.

FAPI in vitro *flow phantom characterization*

Flash-angle pulse-inversion parameters were optimized *in vitro* by flowing MBs through a tissue-mimicking flow phantom and acquiring contrast images, in the manner illustrated in Figure 1A. Contrast and phantom signal were characterized by drawing regions of interest (ROIs) over the area of MB flow and the area directly adjacent (Fig. 1B), and their respective mean pixel intensities were calculated. Image analysis was carried out on images that underwent the Verasonics standard square-root compression. Contrast-to-tissue ratio (CTR) was calculated by dividing the mean pixel intensity of the contrast signal ROI by the mean pixel intensity of the phantom ROI.

Flash angle and FAPI images of the *in vitro* flow phantom were acquired and optimized for transmit