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● *Original Contribution*

NONLINEAR CONTRAST IMAGING WITH AN ARRAY-BASED MICRO-ULTRASOUND SYSTEM

A. Needles,* M. Arditi,† N. G. Rognin,† J. Mehi,* T. Coulthard,* C. Bilan-Tracey,* E. Gaud,† P. Frinking,† D. Hirson,* and F. S. Foster*‡

*VisualSonics Inc., Toronto, Ontario, Canada; †Bracco Suisse SA, Geneva, Switzerland; and ‡Sunnybrook Health Sciences Centre and Dept of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

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Abstract—The main goal of this study was to determine the optimal strategy for a real-time nonlinear contrast mode for small-animal imaging at high frequencies, on a new array-based micro-ultrasound system. Previously reported contrast imaging at frequencies above 15 MHz has primarily relied on subtraction schemes involving B-mode image data. These approaches provide insufficient contrast to tissue ratios under many imaging conditions. In this work, pulse inversion, amplitude modulation and combinations of these were systematically investigated for the detection of nonlinear fundamental and subharmonic signal components to maximize contrast-to-tissue ratio (CTR) in the 18–24 MHz range. From *in vitro* and *in vivo* measurements, nonlinear fundamental detection with amplitude modulation provided optimal results, allowing an improvement in CTR of 13 dB compared with fundamental imaging. Based on this detection scheme, *in vivo* parametric images of murine kidneys were generated using sequences of nonlinear contrast images after intravenous bolus injections of microbubble suspensions. Initial parametric images of peak enhancement (PE), wash-in rate (WiR) and rise time (RT) are presented. The parametric images are indicative of blood perfusion kinetics, which, in the context of preclinical imaging with small animals, are anticipated to provide valuable insights into the progression of human disease models, where blood perfusion plays a critical role in either the diagnosis or treatment of the disease. (E-mail: aneedles@visualsonics.com)

Key Words: **Parametric imaging, Micro-ultrasound, Contrast-enhanced ultrasound, Microbubble, Nonlinear microbubble detection, Blood perfusion, High frequency, Small animal, Mouse.**

INTRODUCTION

Recent developments in linear array technology have pushed traditional ultrasound imaging frequencies higher, into the range of 15–70 MHz (Ritter et al. 2002; Lukacs et al. 2006; Brown et al. 2007). Many of these devices have been fabricated; however, the complete integration of array-based imaging systems including beamforming electronics was often lacking. Recently, an integrated array-based micro-ultrasound imaging system was developed (Foster et al. 2009) and subsequently commercialized. It has a 64-channel, high-frequency beamformer, capable of driving linear arrays in the 15–50 MHz range. Compared with single-element transducers found on previous generation micro-ultrasound systems (Foster et al. 2002), a linear array offers key advantages of improved depth-of-field (DOF), improving overall image quality significantly and allowing more flexibility in pulsing schemes in both Doppler and imaging data paths. The larger DOF allows better contrast agent detection over larger depth ranges, which improves the overall quality and consistency of microbubble contrast images. Microbubble-based ultrasound contrast agents (UCA) have been used in small animal studies by a number of groups using previous generation micro-ultrasound systems (Lyshchik et al. 2007; Rychak et al. 2007; Willmann et al. 2008). In these studies, the UCA was visualized with standard B-mode (gray-scale) imaging, owing to its high echogenicity in response to high-frequency interrogating ultrasound pulses. With postprocessing subtraction algorithms, these UCA echoes could, in certain conditions, be visualized against background tissue echoes. However, the challenge is that for tissue perfusion conditions (*i.e.*, low UCA concentrations in small capillaries), the UCA provides levels of scattered ultrasound below tissue levels, making the separation of

Address correspondence to: Andrew Needles, VisualSonics Inc., 3080 Yonge St., Toronto, Ontario, Canada, M4N 3N1. E-mail: aneedles@visualsonics.com

the UCA contributions difficult by subtracting images or signals after rf demodulation. Image subtraction algorithms also have difficulties in the presence of breathing and cardiac-induced motion, and do not lend themselves to rigorous quantification of perfusion unless the signals are first linearized, to remove effects of log compression. To achieve high sensitivity to UCA echoes, tissue backscatter echoes must somehow be removed from the received ultrasound signals, while preserving signals originating from the UCA. This can preferably be achieved by multi-pulse imaging schemes, where each line of sight is imaged by sequences of pulses, with rf-echo processing designed to cancel linear echo components. In addition, applying such an approach allows the visualization of UCA to occur in real time. Initial attempts to improve UCA detection with higher-frequency systems used nonlinear harmonic imaging (Goertz et al. 2005, 2006; Needles et al. 2007). These systems used single-element, mechanically scanned transducers, which imposed limitations for small animal contrast-enhanced imaging. The fixed focus of these transducers limited the DOF of the ultrasound beam, leading to poor UCA detection above and below the focal zone. The maximum penetration depth of the imaging signal was limited ($<$5 mm), in addition to resulting in a nonhomogeneous brightness over depth. Furthermore, the mechanical motion of the transducer made multi-pulse imaging sequences for nonlinear UCA detection more difficult to implement, while maintaining a sufficiently high frame rate for real-time imaging ($>$20 Hz).

An array transducer can be electronically controlled to create desired transmit and receive beam patterns (the latter focused dynamically for all depth samples in the image). This allows a substantial and valuable improvement in DOF and, consequently, an improvement in UCA sensitivity over image depth. The first goal of this study, therefore, was to determine the optimal imaging strategy for a real-time nonlinear contrast mode, on the new array-based micro-ultrasound system. A secondary goal was to validate the optimal strategy *in vivo* by generating parametric images based on nonlinear contrast detection.

Nonlinear contrast agent detection

Many techniques have been proposed for nonlinear imaging of microbubble-based UCA (Burns et al. 1994; Chang et al. 1995; Hope-Simpson et al. 1999; de Jong et al. 2000; Phillips 2001; Deng and Lizzi 2002). Both microbubbles and tissue will generate linear and nonlinear echoes when exposed to ultrasound. Because of their high compressibility, microbubbles will scatter a large component of nonlinear energy (at low acoustic pressures) relative to tissue. Nonlinear echoes from tissue are typically generated through nonlinear propagation and can be minimized with using a low acoustic pressure. In standard fundamental (*i.e.*, B-mode) imaging, the dominating components from both microbubbles and tissue are linear, and often on a similar order of magnitude, making the separation of microbubble signal from tissue difficult. The challenge of UCA imaging, therefore, has been to develop specific pulse sequences and signal processing that maximize detection efficiency for signals arising from the UCA while suppressing signals arising from tissue. This has generally required the development of pulse sequences in which the phase and amplitude of the insonifying pulses are manipulated to suppress or enhance particular linear and nonlinear components. The most recent techniques send multiple ultrasound pulses down individual image lines and apply signal processing on receive. The two most basic multi-pulse techniques, pulse inversion (PI) and amplitude modulation (AM), form the basis for modern-day nonlinear UCA detection (Eckersley et al. 2005). The goal of these techniques has been to maximize the ratio of detected UCA signal to the residual tissue signal; this is termed the *contrast-to-tissue ratio* (CTR). In the following discussion, comparing the improvement in CTR of a nonlinear imaging technique to fundamental imaging will be denoted $\triangle$CTR.

Pulse inversion

PI was initially proposed by Hope Simpson et al. (1999) and uses two ultrasound pulses that are sent consecutively into tissue, with the second pulse being an inverted copy of the first. Based on the properties of linear systems, summing the echo signals resulting from the two pulses results in cancellation of all linear echo components (including linear scattering from tissue), and UCA signals are retained because of nonlinear oscillations. PI retains even-order harmonics, namely the second harmonic and subharmonic (SH), which are most frequently used for UCA imaging, and cancels odd-order harmonics. It was shown by Eckersley et al. (2005) that PI allows a CTR improvement over fundamental imaging (*i.e.*, $\triangle$CTR) of 14 dB at a transmit frequency of 2 MHz. At higher frequencies, PI was demonstrated by Goertz et al. (2006) with a 30 MHz intravascular ultrasound transducer for both SH and second harmonic imaging. This study used a range of image depths (1 to 6 mm) and transmit pressures (peak negative pressure = 0.1 to 2 MPa), which resulted in $\triangle$CTR ranging between 10–20 dB (second harmonic) and 5–15 dB (SH). It was shown that at high frequencies, particularly for second harmonic imaging, the $\triangle$CTR decreases dramatically as imaging depth and transmit pressure are increased.