Ultrasound in Med. & Biol., Vol. 34, No. 7, pp. 1139–1151, 2008

0301-5629/08/$–see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2007.12.011

● *Original Contribution*

HIGH-FREQUENCY SUBHARMONIC PULSED-WAVE DOPPLER AND COLOR FLOW IMAGING OF MICROBUBBLE CONTRAST AGENTS

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(*Received* 4 *January* 2007, *revised* 5 *December* 2007, *in final form* 18 *December* 2007)

Abstract—A recent study has shown the feasibility of subharmonic (SH) flow imaging at a transmit frequency of 20 MHz. This paper builds on these results by examining the performance of SH flow imaging as a function of transmit pressure. Further, we also investigate the feasibility of SH pulsed-wave Doppler (PWD) imaging. *In vitro* flow experiments were performed with a 1-mm-diameter wall-less vessel cryogel phantom using the ultrasound contrast agent Definity™ and an imaging frequency of 20 MHz. The phantom results show that there is an identifiable pressure range where accurate flow velocity and power estimates can be made with SH imaging at 10 MHz (SH10), above which velocity estimates are biased by radiation force effects and unstable bubble behavior, and below which velocity and power estimates are degraded by poor SNR. *In vivo* validation of SH PWD was performed in an arteriole of a rabbit ear, and blood velocity estimates compared well with fundamental (F20) mode PWD. The ability to suppress tissue signals using SH signals may enable the use of higher frame rates and improve sensitivity to microvascular flow or slow velocities near large vessel walls by reducing or eliminating the need for clutter filters. (E-mail: aneedles@visualsonics.com)

Key Words: **Microbubbles, Subharmonic, Definity™, Color flow, Power Doppler, Pulsed-wave Doppler, Clutter filter, High-frequency ultrasound.**

INTRODUCTION

High-frequency (HF) ultrasound imaging (>20 MHz) is an established technique for high-resolution imaging of tissue microstructure and has seen growing application in dermatology, ophthalmology and small animal imaging (Foster et al. 2000). High-frequency ultrasound flow imaging (*i.e.*, color flow [CF] and power Doppler) systems have also been developed (Kruse et al. 1998; Goertz et al. 2003a) and have shown the capability to detect slow flow (<1 mm/s) in small vessels (down to 15 to 20 μm). The signal from blood is much larger at high frequencies compared with lower (clinical) frequencies (Foster et al. 1994), implying that the need for linear microbubble imaging strategies is less important at high frequencies from a signal-to-noise-ratio (SNR) perspective. The problem, however, of segmenting flow from tissue signals still exists when imaging at high frequencies. An example of this occurs when imaging the microvasculature, where blood velocities are very slow (mm/s to sub mm/s) and vessels are a fraction of the imaging sample volume and often at perpendicular orientations with respect to the beam axis. Another example of the difficulty of segmenting flow from tissue occurs when imaging slow blood flow near large, quickly moving vessel walls (known as the "wall thump" (Zhang et al. 2004) artifact in Doppler ultrasound). In the presence of very-high-grade stenoses, the accuracy of slow blood flow measurement near the arterial wall is often compromised (Berger and Jou 2000). The accuracy of blood flow measurement may also be critical in many experimental animal models of cardiovascular abnormalities or disease (Zhou et al. 2003). In addition, current HF flow imaging systems use single-element transducers that operate in a swept-scan mode. With the swept-scan approach, successive received signals are modulated by the transducer beam function, and the received (tissue and blood) signals undergo spectral broadening of the Doppler frequency spectrum in proportion to the transducer speed (Magnin 1987; Burckhardt 1989). At high frequencies, this imposes considerable limitations on scanning

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velocities for microvascular imaging because of the potential for overlap of tissue and slow-moving blood signals in the Doppler frequency domain (Goertz et al. 2003a). Thus, current commercial HF imaging systems operate with frame rates on the order of 1 Hz when imaging blood flow, to limit the effects of spectral broadening and maintain sensitivity to slow flow. This clearly limits applications and motivates the development of other techniques, such as interframe filtering (Needles et al. 2007), that are compatible with increased frame rates. Interframe filtering was demonstrated to be effective up to 20 fps; however, this limit could potentially be increased even further with higher line densities and higher-order filters. Although interframe filtering does provide a means for overcoming spectral broadening limitations, it is limited in the clutter velocities that it can suppress, and there will always be a lower velocity threshold because it is a form of clutter filtering.

Nonlinear microbubble detection schemes have led to improved methods for segmenting flow signals from tissue at low frequencies, as tissue suppression is achieved solely on the nonlinear detection without applying clutter filters (Burns et al. 1994; de Jong et al. 2000; Deng and Lizzi 2002; Chang et al. 1995; Hope-Simpson et al. 1999). Nonlinear oscillations of microbubbles transfer scattered energy into frequencies centered about integer ($n > 1$) multiples of the transmitted frequency (f_T) (de Jong et al. 1994), as well as into subharmonic ($f_T/2$) and ultraharmonic ($f_T \cdot (n - [1/2])$) frequency bands (Schrope et al. 1992; Shi and Forsberg 2000). Nonlinear scattering from microbubbles has been demonstrated at high frequencies (Goertz et al. 2001), leading to the development of prototype nonlinear B-mode (Goertz et al. 2005a) and flow (Goertz et al. 2005b) imaging instrumentation. This approach enables the detection of slow microvascular flow signals in the presence of strong tissue clutter. Of particular interest, and consequently the motivation for this study, was that the subharmonic (SH) signal from the commercially available contrast agent Definity™ showed the highest contrast-to-tissue ratio (CTR) of all the nonlinear frequency bands analyzed (SH, ultraharmonic [UH], second harmonic [SecH]) (Goertz et al. 2005a). The majority of the results from this study were based on a 20-MHz transmit frequency and showed that the CTR of the SH was on the order of 20 dB. Although higher transmit frequencies are desirable for many HF applications, flow imaging often suffers from poor penetration depth and for deeper structures within the tissue, particularly in animals larger than mice (*e.g.*, rats, rabbits), lowering the transmit frequency can provide the additional signal gains necessary despite the loss in resolution. The feasibility of SH flow imaging with Definity™ at 20 MHz was then demonstrated both *in vitro* and *in vivo* (Goertz et al. 2005b); however, the performance of the flow imaging system was not evaluated over a range of transmit pressures. This evaluation of the SH flow imaging system is necessary to optimize the sensitivity of contrast agent detection while ensuring accurate velocity estimates and quantifiable flow information.

With regards to transmitted pressure levels, three key considerations for SH flow imaging require investigation. Firstly, because of the threshold nature of SH emissions, transmit pressures must be sufficiently high to initiate detectible SH signals. The lower bound for transmit pressure, therefore, large enough to scatter SH energy from Definity™ and produce sufficient SNR for accurate velocity estimation, needs to be established. Secondly, if the transmit pressure is too large, bubble instability or fragmentation may occur, which will degrade the ability to perform coherent velocity estimation. Qualitative evidence of unstable bubble behavior was observed by Goertz et al. (2005b), which appeared in the form of a chaotic pattern in the CF velocity maps, with forward and reverse flow pixels erroneously indicated. Identifying a threshold for implementing coherent velocity estimation, so that unstable bubble behavior does not occur between successive pulses (for autocorrelator-based velocity estimators (Loupas et al. 1995)) or on the time scale of the pulse ensemble (for PWD). Finally, because of the highly compressible nature of microbubbles, high transmit pressures may also induce an acoustic radiation force. If large enough, this radiation force could alter microbubble velocities, biasing the overall estimation of the blood flow velocity. Because radiation force increases with the square of the transmit pressure (Dayton et al. 1997), identifying a pressure threshold where radiation force begins to become a factor for velocity estimation is of significant importance.

Characterizing the performance of the SH imaging system at different transmit pressures will help to optimize the quality of both SH CF/PD imaging and SH PWD. This characterization will also serve as a demonstration of SH PWD, a velocity estimation technique that has not been reported previously, but could be well-suited for applications in small animal cardiovascular research where slow flow in diseased vessels is masked by heavy tissue motion. To maintain consistency with the previously published report on SH flow imaging (Goertz et al. 2005b), the same contrast agent (Definity™) and transmit frequency (20 MHz) was used in this study. Given this choice of parameters, therefore, the goal of this study was two-fold: Firstly, to identify a transmit pressure range that is suitable for both high-frequency subharmonic (HF SH) flow imaging (*i.e.*, CF and PD) and high-frequency subharmonic pulsed-wave Doppler (HF SH PWD), and secondly, to demonstrate for the first time the feasibility of HF SH PWD both *in vitro* and *in vivo*.