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● *Original Contribution*

# IMAGING MICROVASCULATURE WITH CONTRAST-ENHANCED ULTRAHARMONIC ULTRASOUND

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**Abstract—Atherosclerotic plaque neovascularization was shown to be one of the strongest predictors of future cardiovascular events. Yet, the clinical tools for coronary wall microvasculature detection *in vivo* are lacking. Here we report an ultrasound pulse sequence capable of detecting microvasculature invisible in conventional intracoronary imaging. The method combines intravascular ultrasound with an ultrasound contrast agent, *i.e.,* a suspension of microscopic vascular acoustic resonators that are small enough to penetrate the capillary bed after intravenous administration. The pulse sequence relies on brief chirp excitations to extract ultraharmonic echoes specific to the ultrasound contrast agent. We implemented the pulse sequence on an intravascular ultrasound probe and successfully imaged the microvasculature of a 6 days old chicken embryo respiratory organ. The feasibility of microvasculature imaging with intravascular ultrasound sets the stage for a translation of the method to studies of intra-plaque neovascularization detection in humans. (E-mail: david.maresca@gmail.com)** © **2014 World Federation for Ultrasound in Medicine & Biology.**

***Key Words:*** **Intravascular ultrasound, Ultrasound contrast agent, Contrast-enhanced ultrasound, Ultraharmonic, Pulse inversion, Microvasculature, Vasa vasorum.**

## INTRODUCTION

The progression of human coronary atherosclerosis is associated with increased arterial wall microvasculature density (Barger et al. 1984; Gössl et al. 2010). This network of microvasculature, the vasa vasorum, originates from the adventitial layer of large blood vessels to supply the deeper media and intima layers with nutrients and oxygen (Mulligan-Kehoe 2010). In atherosclerotic arteries, erratic vasa vasorum neovascularization can occur (Fig. 1), leading to intra-plaque hemorrhage and ultimately plaque rupture (Hellings et al. 2010; Virmani et al. 2005). It is hypothesized that atherosclerotic plaque neovascularization density could serve as surrogate marker of plaque vulnerability (Hellings et al. 2010). However, existing clinical tools are unable to detect coronary vasa vasorum *in vivo*. Intravascular ultrasound (IVUS) in combination with an ultrasound contrast agent has demonstrated potential for vasa vasorum imaging (Goertz et al. 2006, 2007a) but fails to reach clinical practice to date because of IVUS transducer limitations in frequency bandwidth and sensitivity (Maresca et al. 2013). To overcome these limitations, we developed ultraharmonic IVUS imaging, a narrow frequency bandwidth contrast detection method able to visualize sub-resolution microvascular networks without the blood flow velocity limitations of Doppler ultrasound (Mace et al. 2011).

Ultrasound contrast agents are suspensions of high molecular weight gas microbubbles encapsulated by thin lipid shells (Frinking et al. 2000). Bubbles are typically designed to be of a size (1–10 $\mu$m) that passes through capillary beds and are acoustically active at diagnostic ultrasound frequencies. The primary advantages of using ultrasound contrast agents are the improvement of the signal strength from blood, which becomes bright in echographic images (a parallel can be drawn with bright blood pulse sequences in magnetic resonance imaging), and the detection of slow micro-vascular flow in the presence of tissue motion (Burns 2002). Contrast ultrasound

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imaging techniques rely upon the stimulation of resonant microbubble behaviours to generate and detect harmonic ultrasound contrast agents' backscattered signals (Frinking et al. 2000) that differ from tissue backscattered signals. Current harmonic contrast ultrasound imaging methods are limited to second harmonic (echo at twice the transmit frequency) or subharmonic (echo at half the transmit frequency) imaging. However, second harmonic echoes are not ultrasound contrast agent specific, while the use of subharmonic echoes lowers image resolution (Goertz et al. 2005). Furthermore, detecting second or subharmonic echoes demands a transducer frequency bandwidth of 67%, which exceeds clinical IVUS transducers specifications (Maresca et al. 2013). Several contrast echocardiography studies relied on second order ultraharmonics at 2.5 times the transmit frequency (Kuersten et al. 2001; Shiogai et al. 2004). But this strategy requires very wideband systems (86% frequency bandwidth) and sensitive detection of these low amplitude harmonics is further limited by the frequency-dependent ultrasound attenuation in cardiac tissue (0.5 dB/cm/MHz).

Our approach consisted in imaging contrast microbubbles using first order ultraharmonics that arise at 1.5 times the transmit frequency (Frinking et al. 2000). These signals are specific to ultrasound contrast agents and their proximity to the transmit frequency makes their capture possible with a transducer frequency bandwidth of 40% (*Materials and Methods*). Hence, ultraharmonic imaging allows for the emission and reception of ultrasound pulses within the sensitive frequency range of commercially available IVUS transducers. Furthermore, relying on contrast-specific ultraharmonic echoes prevents misclassifying tissue artefacts as microbubbles (ten Kate et al. 2012), as in the case of second harmonic imaging. For a given probe having a given bandwidth, it also provides an enhanced lateral image resolution compared to subharmonic imaging (Goertz et al. 2005). In practice, the ultrasound echoes backscattered by ultrasound contrast agents are dominated by signal at the transmit frequency. In order to isolate contrast agent ultraharmonics, we transmitted pairs of phase inverted chirp excitations, summed the backscattered echoes and filtered the residual signal in the ultraharmonic frequency range (*Materials and Methods*). This approach, known as pulse-inversion (Simpson et al. 1999), suppresses transmit frequency signals while preserving most of harmonic signals.

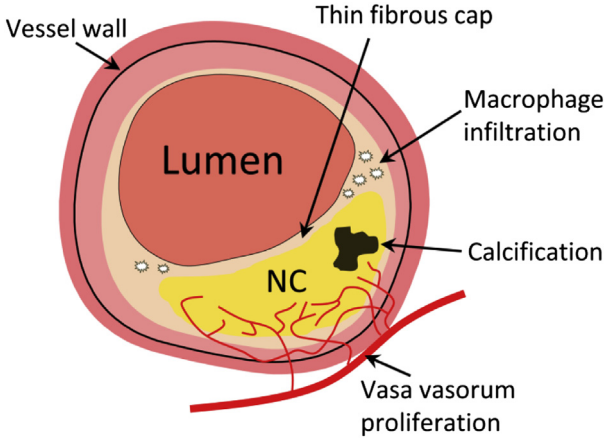


Fig. 1. Schematic cross section of a vulnerable atherosclerotic plaque, illustrating an outside-in proliferation of the vasa vasorum into the atherosclerotic lesion. Other makers of atherosclerotic plaque vulnerability are represented *e.g.,* large necrotic core, thin fibrous cap, calcification and macrophage infiltration.

## MATERIALS AND METHODS

### *Frequency bandwidth of harmonic contrast ultrasound imaging methods*

The frequency bandwidth $BW$ required for harmonic contrast ultrasound imaging is defined as the difference between the transmit ultrasound pulse frequency $f_T$ and its harmonic echo frequency $f_H$ over the center frequency $f_c = (f_T + f_H)/2$,

$$BW = \frac{|f_T - f_H|}{f_C} = \frac{2|f_T - f_H|}{f_T + f_H} \quad (1)$$

For second harmonic ($f_H = 2\,f_T$) and subharmonic imaging ($f_H = f_T/2$), the frequency bandwidth requirement is $BW = 2/3$ or 67%. For ultraharmonic imaging ($f_H = 3\,f_T/2$), the frequency bandwidth requirement is $BW = 2/5$ or 40%. The feasibility of a given harmonic contrast ultrasound imaging method depends on the frequency bandwidth of the ultrasound imaging probe utilized.

### *Laboratory intravascular ultrasound imaging system*

We conducted this study with a miniaturized unfocused IVUS transducer (Zhou et al. 2007) (34 MHz center frequency, 56% frequency bandwidth) assembled into a side-looking IVUS probe and actuated with a rotary stage (Steinmeyer DT105, Albstadt, Germany) (Fig. 2). The transmission circuit of the IVUS imaging system comprised an arbitrary waveform generator (Tabor Electronics WW2571 A, Tel Hanan, Israel), a custom-made expander and the IVUS probe. The receive circuit consisted of a custom-made limiter, a 43 dB low noise amplifier (Miteq AU1263, Hauppauge, NY, USA) and a 12 bit digitizer (Acqiris DP310, Geneva, Switzerland) (Fig. 3). The imaging system functioned as follows: short ultrasound pulses were coded and transmitted as 16 V peak-peak electric signals by the arbitrary waveform generator. The expander stopped low voltage noise while letting the pulses go to the transducer. The transducer converted