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● *Original Contribution*

FREQUENCY ANALYSIS OF THE PHOTOACOUSTIC SIGNAL GENERATED BY CORONARY ATHEROSCLEROTIC PLAQUE

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Abstract—The identification of unstable atherosclerotic plaques in the coronary arteries is emerging as an important tool for guiding percutaneous coronary interventions and may enable preventive treatment of such plaques in the future. Assessment of plaque stability requires imaging of both structure and composition. Spectroscopic photoacoustic (sPA) imaging can visualize atherosclerotic plaque composition on the basis of the optical absorption contrast. It is an established fact that the frequency content of the photoacoustic (PA) signal is correlated with structural tissue properties. As PA signals can be weak, it is important to match the transducer bandwidth to the signal frequency content for *in vivo* imaging. In this *ex vivo* study on human coronary arteries, we combined sPA imaging and analysis of frequency content of the PA signals. Using a broadband transducer (−3-dB one-way bandwidth of 10–35 MHz) and a 1-mm needle hydrophone (calibrated for 1–20 MHz), we covered a large frequency range of 1–35 MHz for receiving the PA signals. Spectroscopic PA imaging was performed at wavelengths ranging from 1125 to 1275 nm with a step of 2 nm, allowing discrimination between plaque lipids and adventitial tissue. Under sPA imaging guidance, the frequency content of the PA signals from the plaque lipids was quantified. Our data indicate that more than 80% of the PA energy of the coronary plaque lipids lies in the frequency band below 8 MHz. This frequency information can guide the choice of the transducer element used for PA catheter fabrication. (E-mail: v.daeichin@erasmusmc.nl) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Photoacoustic, Broadband receiver, Acoustic spectra, Frequency content, Hydrophone, Atherosclerosis, Lipid.**

INTRODUCTION

Cardiovascular disease is a major cause of death worldwide (World Health Organization 2015). A substantial proportion of cardiac deaths are due to acute coronary syndromes. The majority of these fatal acute coronary syndromes are caused by ruptures of vulnerable atherosclerotic plaques and thrombosis (Davies and Thomas 1985; Schaar et al. 2004, Virmani et al. 2000). The identification of unstable atherosclerotic plaques in the coronary artery is emerging as an important tool for guiding percutaneous coronary interventions. The structure and composition of the plaque are significant determinants of its stability (Schaar et al. 2006). Imaging modalities have provided a greater understanding of factors involved in the atherosclerosis disease process and have improved therapeutic interventions (Puri et al. 2013). In particular, intra-vascular imaging approaches can characterize the burden, composition and functionality of atherosclerotic plaque, neointimal hyperplasia and allograft vasculopathy that develop within coronary arteries (Puri et al. 2013).

Currently available intra-vascular imaging modalities, however, have limitations: Intra-vascular ultrasound (IVUS) imaging is unable to distinguish plaque constituents other than calcium (Allen et al. 2012; Choudhury et al. 2004). Intra-vascular optical coherence tomography has better soft tissue contrast, but its penetration depth is limited to 1–3 mm, and it requires flushing blood from the lumen of the vessel (Niccoli et al. 2014), a limitation that also applies to angioscopy. The intra-vascular chemical sensing techniques near infrared spectroscopy, Raman spectroscopy and intra-vascular fluorescence imaging

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cannot provide depth-resolved information (Jansen et al. 2014a, 2014b; Puri et al. 2011).

Intra-vascular photoacoustic (IVPA) imaging, particularly spectroscopic IVPA (sIVPA) imaging, aims to fill this gap by imaging plaque components in a vessel wall with reasonably large imaging depth (Jansen et al. 2011; Wang et al. 2011, 2012a, 2012b, 2013a, 2013b). This imaging technique, which applies pulsed light excitation, benefits from the optical absorption properties of tissue composition as contrast. Typically, photoacoustic (PA) imaging uses the signal envelope of the PA signal to reconstruct macroscopic optical absorbers in an image (Wang and Hu 2012). Theoretical solutions of the wave equation for simple geometries predict that the PA pressure amplitude is proportional to the radius of the absorber squared, whereas the main frequency of the PA signal is inversely related to the size of the absorber (Diebold et al. 1990): compared with smaller sources, larger sources will emit PA pressure waves with higher amplitude and lower frequencies. As PA signals can be weak for *in vivo* imaging applications, it is important to match the transducer bandwidth to the major frequency contents of the PA signal. The frequency content of PA signals has recently been investigated to estimate the microscopic properties of absorbers in phantoms (Xu et al. 2015; Yang et al. 2012) and in more complex biological tissues (Hysi et al. 2013; Kumon et al. 2011; Patterson et al. 2011, 2014; Saha and Kolios 2011; Strohm et al. 2013; Xu et al. 2014). The frequency content of plaque lipid PA signal has not been investigated to date, and may be distinct from, for example, the adipose tissues surrounding the vessel. Although this peri-adventitial fat has a homogeneous structure, there is variability in size, shape and concentration of the lipid deposits, acting as PA sources, in plaque. This contrast in spatial structure affects the acoustic frequencies emitted. The small device size and stringent optical power limits of endoscopic applications mean that the signal-to-noise ratio (SNR) cannot be arbitrarily increased by using a larger sensor surface or increasing excitation power, so optimization of transducer sensitivity is a key step toward a reliable, tolerable and usable IVPA technology.

Generally, to obtain co-registered IVUS and IVPA images, transducers with central frequencies of 30–85 MHz have been used, which guarantees decent IVUS images. However, in the absence of any data on the typical frequencies generated by PA imaging in lipid-rich atherosclerosis, it is unknown whether the frequency range >30 MHz is a suitable choice for plaque lipid detection. To investigate this question, we performed both a simulation and a statistical analysis of the frequency content in the range 1–35 MHz of PA signal obtained from human coronary artery plaques *ex vivo*.

METHODS

Experiment

Setup. An overall schematic of the imaging setup is provided in Figure 1(a). A tunable laser (Vibrant B/355-II, OPOTEK, Santa Clara, CA, USA) served as an optical excitation source for PA imaging (pulse width: 5 ns, repetition rate: 10 Hz, energy per pulse at 1200-nm wavelength: 2 mJ). A tapered multimode optical fiber (Oxford Electronics, Four Marks, UK; input diameter: 1 mm, output diameter: 360 μm) coupled the laser light to a custom-built forward-looking optical probe for light delivery (fiber core diameter: 400 μm). A needle hydrophone (Hydrophone 1875, Precision Acoustics, Dorset, UK) calibrated from 1 to 20 MHz and a polyvinylidene difluoride focused transducer calibrated from 10 to 35 MHz received the PA signals. Combining these two broadband receivers allowed a large frequency range (1–35 MHz) to be covered. We refer to the hydrophone as the low-frequency (LF) probe, and the polyvinylidene difluoride transducer, as the high-frequency (HF) probe in the remainder of this article. A holder with a window size of 5 × 5 mm held the artery samples. The received signal level was optimized by the relative positioning of the tissue sample and the ultrasound receivers: the tissue sample was positioned in the focus of the HF probe and approximately 5 mm from the LF probe, respectively. The sample holder was connected to a motorized translation stage (MP 63-25-DC, Steinmeyer, Albstadt, Germany) to scan the samples. The PA signals were amplified by a 43-dB amplifier (AU1263, Miteq, Hauppauge, NY, USA) and digitized at sample frequency of 350 MHz with a 12-bit data acquisition card (Acqiris DP310, Agilent, Santa Clara, CA, USA).

Phantom measurement. Spectroscopic PA imaging on a phantom was performed to study the PA radiofrequency spectra of intra-plaque lipids at different frequency bands. A polyvinyl alcohol phantom (10% wt polyvinyl alcohol crystals in demineralized water, three freeze/thaw cycles of at least 2 h) was prepared with a lumen size of 3 mm and a 1.5-mm-round × 5-mm-deep cylindrical cavity at 500 μm from the lumen. The cavity was filled with cholesterol, cholesterol oleate and cholesterol linoleate (Sigma-Aldrich C8667, C9253 and C0289, respectively), which are the predominant components of plaque lipid (Lundberg 1985), and a piece of human coronary adipose tissue. The cavity was emptied and cleaned before placing a new sample. Spectroscopic PA imaging was performed from wavelengths of 1125 to 1275 nm in 2-nm steps (in water at room temperature). The PA signals were 32 times averaged to improve the SNR. Reference sPA signals were acquired using a transducer with the central frequency of 40 MHz (referred to as the very