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Research article

Spectral analysis assisted photoacoustic imaging for lipid composition differentiation

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ABSTRACT

Recent advances in atherosclerotic plaque detection have shown that not only does lipid core size and depth play important roles in plaque rupture and thrombi formation, but lipid composition, especially cholesterol deposition, is equally important in determining lesion vulnerability. Here, we demonstrate a spectral analysis assisted photoacoustic imaging approach to differentiate and map lipid compositions within an artery wall. The approach is based on the classification of spectral curves obtained from the sliding windows along time-of-flight photoacoustic signals via a numerical *k*-means clustering method. The evaluation result on a vessel-mimicking phantom containing cholesterol and olive oil shows accuracy and efficiency of this method, suggesting the potential to apply this approach in assessment of atherosclerotic plaques.

1. Introduction

Cardiovascular disease has been the leading cause of death over the past century in developed countries [1]. Atherosclerosis is recognized as the pathologic basis of cardiovascular disease, in which lipids accumulate in an artery wall leading to plaque growth and subsequent obstructive lumen narrowing or rupture [2]. The vulnerable, or rupture-prone plaque is typically characterized by a thin fibrous cap, a large lipid-rich necrotic core, and inflammatory infiltrate [3,4]. Of these hallmarks, lipid accumulation has been shown to be the most frequently observed precondition of a plaque rupture [5]. The majority of lipids present in an atherosclerotic plaque are free cholesterol and its esterified form [6]. Cholesterol found in early atherosclerotic lesions participates the formation of macrophage foam cells, while crystalline cholesterol is thought to induce plaque rupture by physical disruption of the fibrous cap [7]. Therefore, cholesterol crystals have been identified as an important hallmark of inflammation and atherosclerotic lesions [8,9]. Cholesteryl esters mainly accumulate in cytoplasmic droplets [2] and constitute a major fraction of lipid-rich necrotic core [10]. Their abundance is highly associated with plaque rupture and thrombi formation [8]. Therefore, the differentiation of crystalline cholesterol from its esterified form in an intact artery is of particular importance for the histopathological classification of advanced atherosclerotic lesions, as well as early diagnostics of the burden of an artery [11].

The widely used methods for studying the cholesterol deposition in atherosclerotic plaques are histological stains. However, none of these methods can label both crystalline cholesterol and cholesteryl ester. Microscopic imaging modalities such as confocal laser reflection microscopy [9] and micro-optical coherence tomography [12] have been successfully applied to visualize reflective components, but neither can provide chemical information of the target. Spontaneous [13] and coherent Raman scattering [14] have been implemented for cholesterol imaging with high performance. However, these approaches are limited as they are not compatible for *in vivo* imaging of an intact artery.

Catheter-based imaging modalities, such as intravascular ultrasound (IVUS) [15], optical coherence tomography (OCT) [16], and near-infrared spectroscopy (NIRS) [17], have been developed to interrogate the artery structure from inside the vessel. IVUS can show the artery morphology with high quality [17]. By combining with virtual histology, it can map the composition distribution within the plaque, thus classify the

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plaque type [17,18]. However, the accuracy of virtual histology IVUS has been challenged, especially for the accurate quantification of necrotic core size. [19,20]. OCT provides excellent spatial resolution of the artery, but it can neither penetrate sufficient depth nor provide compositional information. NIRS is only able to differentiate arterial composition in a very thin layer; thus, not providing depth resolution.

Intravascular photoacoustic (IVPA) imaging is an emerging technique converting optical absorption into ultrasonic wave and detecting it with a sensitive transducer integrated in the same catheter probe through intravascular implementation [21,22]. This imaging technique and catheter design allows for concurrent co-registered IVPA/US imaging. In IVPA systems, excitation wavelengths of 1.2 μm [23] or 1.7 μm [24,25], corresponding to the second and first vibrational overtone absorptions of C—H bond [26,27], were usually utilized to selectively map lipid deposition in atherosclerotic plaque. Although current systems can provide important information of the lipid core size, they lack the ability to separate crystalline cholesterol from cholesteryl ester, thus limiting the capability for accurate identification of the plaque vulnerability and inflammation. Spectroscopic IVPA imaging allows excellent differentiation of different lipid compositions [28]. However, this approach requires multiple wavelength scanning, which considerably sacrifices the imaging speed making it impractical for clinical applications.

Spectral parameters (e.g., slope, y-intercept and mid-band fit) of photoacoustic signals can be utilized to characterize and quantify different tissue types based on their microstructural and mechanical properties [29–31]. In this work, we applied spectral analysis of photoacoustic signals to IVPA imaging to differentiate and map lipid compositions. Different from existing approaches of spectral analysis in both ultrasound and photoacoustic imaging that are based on a set of spectral parameters, here we obtained the spectral curves for each sliding window along A-lines via fast Fourier transform. After calibration and normalization, the spectral curve sections within the effective frequency band of the transducer were selected for *k*-means clustering [32] to classify the chemical components. This method was demonstrated with imaging of a vessel-mimicking phantom composed by cholesterol and olive oil to validate its accuracy and feasibility in differentiating lipid compositions.

2. Methods

2.1. IVPA imaging system

The IVPA imaging system used in this work is described in Fig. 1. A lab-built optical parametric oscillator (OPO) emitting at 1.7 μm with 500 Hz repetition rate [24] was used as the excitation source for photoacoustic generation. A section of multimode fiber coupled the light output from OPO to the IVPA catheter via a self-designed fiber-optic rotary joint (FORJ). The imaging catheter with a collinear optical/acoustic design and a diameter of 1 mm offers highly sensitive and co-registered ultrasound and photoacoustic imaging (Fig. 1, inset) [33]. The imbedded ultrasound transducer has a center frequency of 42 MHz and bandwidth of 50% (Blatek, Inc.). The FORJ, driven by a computer-controlled motor (SM17205D, Moog Inc.), was employed with a linear pullback stage to perform 3-D intravascular imaging. The photoacoustic signal was detected by an installed transducer in the probe and sent to a pulser/receiver (5073PR, Olympus, Inc.) with a 39-dB amplification factor through an electrical slip ring. Within a single optical pulse, a delayed ultrasound wave was sent and echo received by the same transducer via an OPO-triggered delay generator (9500+, Quantum Composers, Inc.) and pulser/receiver. A 16-bit digitizer (ATS9462, AlazerTech, Canada) with 180 MS/s sampling rate was used to collect the detected signals for further imaging reconstruction and analysis. The whole process was controlled by a LabView-based

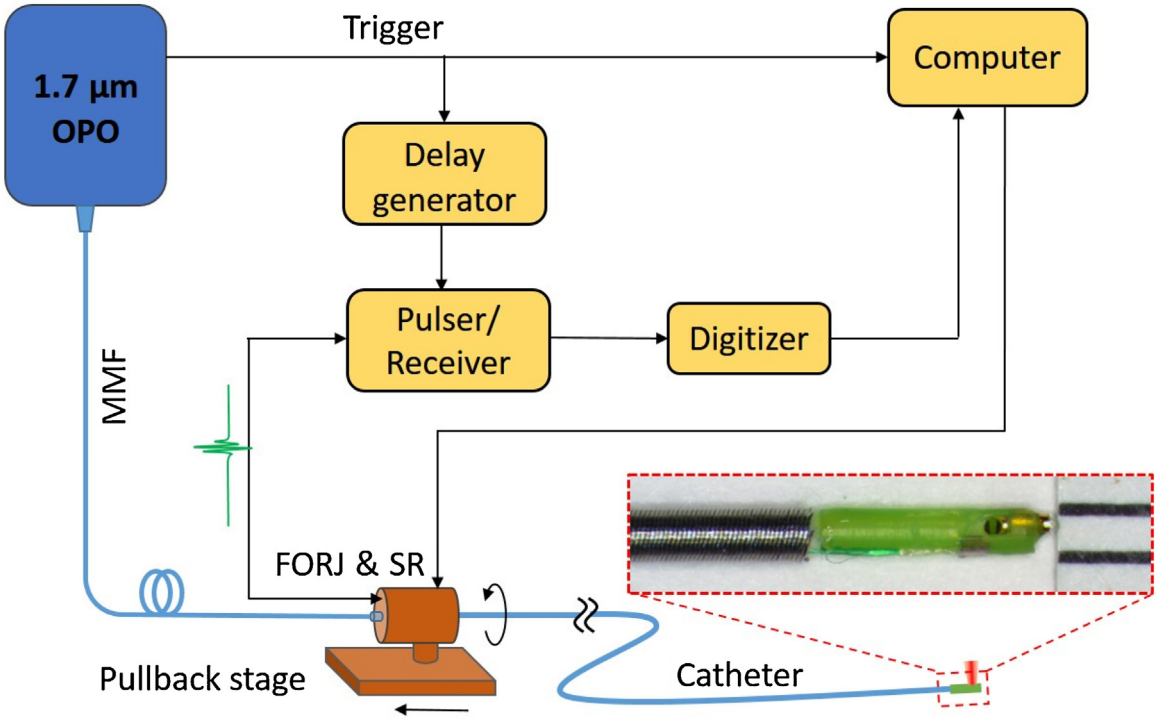

Fig. 1. Schematic of the IVPA imaging system. The inset shows the photograph of IVPA catheter probe. OPO: optical parametric oscillator; MMF, multimode fiber; FORJ, fiber-optic rotary joint; SR, slip ring.