Endovascular optical coherence tomography intensity kurtosis: visualization of vasa vasorum in porcine carotid artery

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Abstract: Application of speckle variance optical coherence tomography (OCT) to endovascular imaging faces difficulty of extensive motion artifacts inherently associated with arterial pulsations in addition to other physiological movements. In this study, we employed a technique involving a fourth order statistical method, kurtosis, operating on the endovascular OCT intensity images to visualize the vasa vasorum of carotid artery in vivo and identify its flow dynamic in a porcine model. The intensity kurtosis technique can distinguish vasa vasorum from the surrounding tissues in the presence of extensive time varying noises and dynamic motions of the arterial wall. Imaging of vasa vasorum and its proliferation, may compliment the growing knowledge of structural endovascular OCT in assessment and treatment of atherosclerosis in coronary and carotid arteries.

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References and links
1. Introduction

Optical coherence tomography (OCT) is an emerging optical imaging technique which is able to generate micron-level resolution images of biological tissues [1]. OCT uses interferometric techniques, in either broadband low coherence light source configuration (time-domain OCT, TD-OCT and spectral-domain OCT, SD-OCT) or swept-source laser configuration (swept-source OCT, SS-OCT), to provide depth gating and a localized measurement of tissue reflectivity. Systems typically reach an imaging depth of 2-3 mm. This high resolution imaging and versatile platform technology has allowed OCT to be applied in numerous applications such as retinal imaging, gastrointestinal tract imaging and skin imaging or [2–4] functionally in Doppler flow imaging and microcirculation imaging [5,6]. In all these applications, OCT is able to distinguish between anatomical layers and identify abnormalities to give accurate diagnosis on the onset or progress of various diseases [7,8]. With the recent introduction of SS-OCT, imaging speed has been greatly increased when compared with previous generation OCT technologies. The most prominent endovascular OCT (EV-OCT) application has thus far been focused to the coronary artery.
Numerous studies have demonstrated the ability of OCT to distinguish the intima, media and adventitia layers of normal coronary arteries [9]. EV-OCT is also able to image intra coronary atherosclerotic plaques and can identify multiple tissue structures such as fibrous cap, intra-plaque lipid deposition, and intra-plaque calcification nodules [9]. In addition, various research studies have reported the imaging of intra-plaque neovascularization [10–12]. Imaging of these features are important in determining the stage and progress of atherosclerosis and their determination techniques can readily be extended to the carotid artery, in which the rupture of vulnerable plaques can lead to thrombosis and consequently stroke occurrence. Moreover, OCT has been shown to image coronary stenting and evaluate the occurrence of improper stent apposition, thrombosis and restenosis [13,14].

However, all the studies have only been devoted to imaging the inner layers of the arteries close to the lumen. Different layers have been distinguished in normal arteries including the intima, the media, the external elastic lamina and the adventitia [9,15]. To our knowledge, no attempts have yet been made to characterize finer features that are present in the arterial wall as detected by EV-OCT. In particular, there are various morphological features present in the adventitia, albeit with a significantly lower intensity due to the intrinsic optical absorption and scattering properties of tissue. Furthermore, the thicker vessel wall and larger diameter of the carotid artery add to the challenge of EV-OCT due to limited penetration depth and reduced lateral resolution.

One particular anatomical structure of considerable interest in the adventitia is the vasa vasorum, the blood vessels of blood vessels. A number of physiological studies have elucidated the importance of the vasa vasorum and their roles in the supply of nutrients and the removal of waste from endothelial cells and tissues, in their blockage or damage leading to atherosclerotic plaque formation and in their reduction or increase in number in correlation to the vulnerability of vessel walls [16]. Additional studies have also imaged the vasa vasorum of the aorta, coronary artery and carotid artery of humans and pigs using micro-computed tomography (Micro-CT) [16]. However, most of these studies to date have used either ex vivo cadaveric vessel samples or in situ euthanized animals to obtain the Micro-CT vascular maps, where the technique encounters significant difficulty in obtaining in vivo patient data. Intravascular ultrasound (IVUS) has also been shown to map out areas perfused by vasa vasorum but it relies on the injection of micro-bubble contrast agent [17]. Moreover, IVUS does not possess sufficient resolution to map out individual vasa vasorum clearly. Another novel imaging modality, namely photoacoustic tomography (PAT), has been used to map out microvasculature [18,19]. Different configurations of PAT differ drastically in both time and spatial resolution. For endoscopic configuration, PAT has a spatial resolution in the range of 47-65μm and a temporal resolution of ~2.6Hz [18]. Thus, in this and similar configurations, with the present resolution, PAT may still be insufficient for vasa vasorum detection. When the future development of PAT allows a resolution and temporal measurements similar to that of EV-OCT, we believe the same algorithm could then be applied to PAT images. Therefore, there exists an unique opportunity for EV-OCT to offer the combination of in vivo imaging with a resolution suitable to clearly identify this important vascular structure. Initial results have been presented where several morphological features of the carotid artery were visible in the adventitial layer via EV-OCT [20]. However, few studies have investigated vascular components of the adventitial layer as it can be difficult to clearly identify the presence of the vasa vasorum due to the high motion artifacts associated with carotid imaging. Therefore, in this pilot study, EV-OCT images of in vivo porcine carotid arteries were imaged and analyzed to detect the presence of dynamic vasa vasorum and a method was developed to distinguish these important features from their surrounding structures with histological comparison.

2. Materials and methods

During endovascular imaging, multiple factors degrade structural EV-OCT with respect to motion artifacts, due to saline or contrast agent flushing, movement of the imaging and guide
catheters, a beating heart, aortic and arterial pulsations, and breathing motion. Therefore, significant bulk motion induced speckle modulation was detected during the imaging procedures and resulted in poor results when attempting to derive vascular maps through standard techniques such as color-Doppler or speckle variance detection of blood flow [21–23]. Thus a new approach was required to detect the vasa vasorum within the adventitial layer.

Variance is the second moment of a random variance about the mean, (i.e. second order statistic). Speckle variance measures the spread of intensity values between frames such that blood vessels can be detected via the induced time varying speckle field pattern. Therefore, when this technique failed to distinguish the microvasculature from surrounding tissues, a higher order statistical moment was applied to detect these high intensity modulations due to blood flow. Kurtosis, $K$ [24], is defined as

$$K = \frac{\mu_4}{\sigma^4} - 3$$

$$\mu_4(m, x, y) = \frac{1}{N} \sum_{n=m}^{m+N-1} [I(n, x, y) - \mu_0(m, N, x, y)]^4$$

$$\sigma^4(m, x, y) = \left( \frac{1}{N} \sum_{n=m}^{m+N-1} [I(n, x, y) - \mu_0(m, N, x, y)]^2 \right)^2$$

$$\mu_0(m, x, y) = \frac{1}{N} \sum_{n=m}^{m+N-1} I(n, x, y)$$

where $\mu_4$ is the fourth order moment about the mean intensity recorded at pixel $(x,y)$, $\sigma$ is the standard deviation at pixel $(x,y)$, $m$ is the m-th frame of kurtosis signal, $N$ is the number of frames used to compute the kurtosis, $I$ is the intensity of each frame at pixel $(x,y)$ and $\mu_0$ is the mean between $N$ frames at pixel $(x,y)$. Kurtosis is a fourth order statistical measure normalized by standard deviation to identify the deviation of a statistical distribution from Gaussian distribution. It has been shown that additional biomedical imaging modalities have taken advantage of this metric to quantify non-Gaussian diffusion [25]. In another study, kurtosis was used for motion detection in video analysis [26]. In EV-OCT, the kurtosis between sequential frames of a stationary target evaluates the distribution of intensity along the time axis. Any area of the tissue with significant changes over time will manifest as a high kurtosis signal value. Moreover, since from Eq. (1) the fourth moment about the mean is normalized by the fourth power of standard deviation of the structural OCT intensity, kurtosis results from areas with weak intensity signal are not masked by strong reflections from the pulsating vessel wall or the guidewire.

For our work, the kurtosis algorithm was first verified using a flow phantom. A pulsatile flow was mimicked manually using titanium oxide solution in a 30 gauge plastic tube, so that the intensity inside the tube imaged by OCT (Thorlabs Inc.) underwent sudden and huge changes. The image data obtained were then processed with the kurtosis algorithm.

Yorkshire pigs weighing ~50kg were housed in animal vivarium for one week prior to the experiment. Upon sedation with intramuscular injection of ketamine, the pig was anesthetized with continuous inhalation of isoflurane and monitored by a physiologic monitor for any potential complications. A surgical incision exposing the groin of the pig to gain access to the femoral artery and 8-French (8F) sheath were established with subsequent heparinization at 100U per kg. A 5-Fr diagnostic catheter was then inserted into the femoral artery with guidewire to select the common carotid artery. The 5-Fr catheter was then exchanged with a 6F Shuttle guide catheter, allowing placement of an embolic protection device (AngioGuard, Cordis) distally in the carotid artery. The AngioGuard contained a proximal monorail guidewire upon which the EV-OCT imaging catheter (Dragonfly, LightLab Imaging) was
installed. Variable mixtures of saline and contrast agent (Omnipag) using an automated pump were injected during EV-OCT imaging using a SS-OCT system (LightLab C7-XR, LightLab Imaging), with customized high-speed data acquisition. All animal procedures were approved by St. Michael’s Hospital (Toronto, ON) Animal Care Committee.

For the detection of pulsating vasa vasorum using intensity kurtosis, the resolution of the OCT system must be sufficient to detect the actual vessels from the surrounding structures. In addition, the frame rate of the system must be of a greater value than the inherent pulsation rate such that the kurtosis algorithm can capture sudden intensity changes. The C7-XR SS-OCT system provided an axial resolution of 15μm where images were acquired at 100Hz. The spot size was ~25μm. For the vasa vasorum, which typically are ~40μm in size [27], a 15μm axial resolution was adequate to detect their presence. The frame rate of the system was also much higher than the heart rate and therefore the pulsation rate of the vasa vasorum.

Both the speckle variance and the kurtosis algorithms were applied to EV-OCT images acquired at 100 frames per second. A particular scenario occurs when the contrast/saline mixture injection partially flushes the pulsatile blood flow in a time varying manner, during which boluses of blood enters the imaging field of view, causing large intensity variations throughout the image, especially from the vessel wall. This is chosen as an extreme scenario to test kurtosis algorithm, specifically the ability to suppress such artifacts. From a clinical perspective, this is the threshold condition for determining the minimal contrast/saline flush injection rate for a particular blood vessel and its associated flow rate, to achieve optical clearing condition for imaging while maintaining flow.

With full displacement of blood during imaging, the imaging catheter is maneuvered close to the vessel wall to improve lateral resolution and OCT intensity. Identification of vasa vasorum is performed by searching for areas of reduced image intensity in the adventitia and verified with kurtosis signal processing, for temporal variation in synchrony with heart beat yet distinct from vessel wall pulsations. The results are presented as follows.

3. Results

Figure 1 demonstrates the result from the flow phantom experiment using SS-OCT in microscopic configuration. In Fig. 1a, there is no flow in the flow phantom tube and as a consequence there is no kurtosis signal (Fig. 1b). However, when titanium oxide solution is injected into the phantom (Fig. 1c), a strong kurtosis signal shows up due to the sudden surge in intensity inside the flow phantom (Fig. 1d).

![Fig. 1. 30 gauge flow phantom results: (a) Flow phantom with air inside; (b) Kurtosis signal corresponding to (a); (c) Flow phantom with sudden injection of titanium oxide solution; (d) Kurtosis signal corresponding to (c). Scale bar represents 0.2mm. The tube appeared larger when titanium oxide solution was imaged due to refractive index changes.](image)

During in vivo porcine experiments when investigating the threshold condition of appropriate flush injection rate, EV-OCT signal intensity varied significantly depending on completeness of blood displacement by flushing fluid pump injections (contrast/saline mixture). Figure 2 shows one frame of the EV-OCT image sequence obtained with the blood-saline flush experiment (Media 1). Figure 3 shows a speckle variance OCT (SV-OCT) map computed with the blood-saline flushing EV-OCT image sequence (Media 2). Figure 4
demonstrates a normalized kurtosis map computed with 32 frames of the blood-saline flushing EV-OCT image sequence (Media 3). In (Media 3), the kurtosis algorithm clearly gives strong kurtosis signal whenever the blood flushed in. It also demonstrates that the algorithm is extremely sensitive to outliers. Even one of the 32 frames takes on a dramatic difference with the other 31 frames, this difference would manifest as a strong kurtosis signal in the vicinity.

Figure 5 shows one frame of a porcine carotid EV-OCT image sequence (Media 4). The imaging catheter was placed close to the artery wall, where the carotid artery abluminal surface was not supported, as evident from the loose structure shown in the image. As the image demonstrates, a number of low intensity regions can be found throughout the image.
Fig. 4. One example kurtosis map of the blood-saline flush experiment when the flow took on a sudden change.

Typically, these low-signal areas depict the presence of a blood vessel [12], where the question remains: Are these in fact vasa vasorum vessels or are the dark areas associated with fat deposits (or equivalent low signal tissue structures) as typically imaged by OCT.

Speckle variance (SV) imaging was also investigated in this part of the experiment, with a typical SV image displayed in Fig. 6. This is computed with the same data sequence as previously presented in Fig. 5. As seen in Fig. 6, large SV-OCT signals around the vasa vasorum were observed, yet may not be adequately distinguished from large bulk tissue

Fig. 5. Stationary EV-OCT image of porcine carotid artery when the imaging catheter is placed close to the vessel wall (Media 4). The supposed vasa vasorum is indicated by the white arrow. Scale bar = 1mm. Inset is the zoomed in version of the red box.

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movements associated with carotid vessel wall pulsations, especially with imaging catheter in close proximity of the vessel wall and high backscatters. The brightest region is due to high reflectivity from the guidewire.

Figure 7 demonstrates a normalized kurtosis map from a sequence of maps each computed with 32 frames of the porcine carotid EV-OCT image sequence (Media 5). Neglecting the entire signal caused by motion artifact on the lumen wall and the catheter, a high kurtosis
Fig. 8. Average intensity of a region of interest around the vasa vasorum and an arbitrary section of the vessel wall versus time. Although periodic variation in intensity is also observed in the vasa vasorum, these intensity fluctuations are of the same approximate value, making it extremely difficult to identify vessel wall structures such as the vasa vasorum micro-vessels (Media 4).

The signal region appears right above the arterial wall, and approximately at the luminal side of the adventitia. The location of this high kurtosis signal region coincides with one of the low-signal regions appearing in the EV-OCT structural image (Fig. 5). However, as can be seen in Fig. 7, not all low-intensity regions in the EV-OCT image correspond to high signal intensity in the kurtosis map. (Media 4) and (Media 5) are examined carefully, and a region of interest (ROI) is drawn around the area outlined above. The average intensity of EV-OCT images, the average speckle variance and the average kurtosis signal in the ROI are plotted versus time in Fig. 8, Fig. 9 and Fig. 10, respectively. The average speckle variance in the ROI (Fig. 9) did not follow any pattern. Speckle variance was much higher in the ROI misleadingly because of its proximity to the imaging catheter. The average kurtosis signal in the ROI (Fig. 10) would follow a periodic pattern with respect to time, where the frequency of this modulation correlated to the pig heart rate of ~85 beats per minute. Therefore, the continuous EV-OCT data set was processed via the kurtosis algorithm to identify this periodic blood flow, which we believe to correspond to the vasa vasorum.

Figure 11 shows an example Hematoxylin & Eosin (H&E) staining of a representative section of porcine carotid artery. As can be discerned in the image, in the adventitia area, there are a number of blood vessels that correspond to the location of the detected micro-vessel in the adventitia of the kurtosis maps and EV-OCT images. However, there are also a considerable number of void regions (unstained regions or regions filled with blood but without proper tissue surrounding them) that do not correspond to micro-vessels. Moreover, nerves are also present in the adventitia area, as seen in Fig. 11. Thus, the intensity kurtosis detection method provides a simple and fairly robust way to identify the dynamic vasa vasorum and analyze their blood flow dynamics.

An additional benefit of implementing the intensity kurtosis algorithm is that it could be used to identify the presence of blood clots and thrombus flowing along the lumen of the artery during imaging. The flow of blood clot debris creates a large and sudden jump in
Fig. 9. Average speckle variance of suspected vasa vasorum region and arbitrary vessel wall versus time. At the vasa vasorum, misleadingly, the speckle variance is much larger due to its proximity to the imaging catheter. Speckle variance was computed within a moving time window of 8 frames, updated at 100fps.

Fig. 10. Average kurtosis of suspected vasa vasorum region and arbitrary vessel wall versus time. At the vasa vasorum, the kurtosis is much larger due to pulsation of blood filling and emptying the micro-vessel. Note the increase in pixel intensity of the vasa vasorum region, which acts as a contrast mechanism such that these vessels become visible and stand out when compared to the surrounding tissue (Media 5). Kurtosis was computed within a moving time window of 32 frames, updated at 100fps.
intensity. As can be viewed in Fig. 7, the clot debris generates very high kurtosis signal in the lumen area. If this algorithm can be displayed in real-time, the technique not only offers additional vessel wall information, but could also be used to identify threats such as the dislodging of thrombi, where the surgeon may have an opportunity to take appropriate measures to protect patients in these situations.

4. Discussion

From (Media 2), the SV-OCT signal of the guidewire and of the vessel wall in the vicinity of the imaging catheter masks out all the blood-saline flush signal in the lumen. In (Media 3), however, the blood-saline flush gives out a strong kurtosis signal. Moreover, the kurtosis measurement strongly rejects both the guidewire and quasi-static carotid vessel wall. This shows that the kurtosis algorithm clearly has overwhelming advantage when measuring pulsed flow. It can strongly reject stationary objects even in high clutter scenario, yet giving out strong signal in areas corresponding to strong changes in intensity. This method might prove to be useful in imaging lymphatic vessels in which one can have time varying concentrations.

From (Media 4), it can be clearly seen that the speckle pattern fluctuates randomly, throughout the image. This is due to the fact that speckle depends on the interference of randomly scattered photons throughout the volume of interrogated tissue [21]. Therefore, any induced tissue motion such as the smooth muscle tone, flushing that vibrates the catheters, and the vibration of the catheter itself can alter the coherence of the photons. When these artifacts add up together, a bulk overall time varying speckle pattern results, rendering speckle variance detection of microvasculature, via our current hardware setup, impossible.

The kurtosis measurement, on the other hand, does not depend on speckle. The metric measures the deviation from a Gaussian distribution. It is sensitive to outliers, and therefore, only sensitive to sudden, drastic changes of intensity between imaging frames. As shown in Fig. 10, the cycle of the kurtosis signal consist of two peaks with a dip in the middle. The first peak corresponds to the blood filling the vasa vasorum and therefore induces a sudden increase in intensity in the EV-OCT image. The second peak corresponds to the blood emptying the vasa vasorum and thereby induces a sudden decrease in intensity in EV-OCT image.
Various studies have been devoted to the understanding of the vasa vasorum. It is believed to be an important role in atherosclerosis [16]. One very important aspect is that vasa vasorum are dynamic. The flow in these structures can be transiently obstructed by the blood pressure of the main vessel wall, can undergo vasodilation or vasoconstriction and most importantly they can undergo angiogenesis in response to external factors. In the presented results, the filling of blood due to the arrival of the systolic pressure pulse into the vasa vasorum was clearly observed. Via our kurtosis detection method, this study has demonstrated to our knowledge, the first OCT technique, which is capable of identifying vasa vasorum in vivo. This method may prove to be useful in evaluating the health of the artery as it can clearly delineate a periodic pattern directly correlating to the heart beat rhythm. If there are any abnormalities with the artery, this periodic pattern may be altered to become irregular. For example, in hypertension patients, one could expect that the pressure on the vasa vasorum is higher and therefore, the duration of blood filling in the kurtosis signal pattern may be dramatically reduced. Thus the intensity kurtosis could be a useful way to assess the health of the dynamic vasa vasorum, which in turn can be indicative on the risk, the onset or even the progression of atherosclerosis.

5. Conclusion

Stationary sequences of EV-OCT images of in vivo porcine carotid artery were obtained and analyzed using intensity kurtosis measurement. The average intensity kurtosis in the vicinity of the dynamic vasa vasorum took on a periodic pattern that correlated to the animal heart rate. The kurtosis method has shown to be able to distinguish the dynamic vasa vasorum from surrounding morphological structures, especially in the adventitia.

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