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Extracting information from optical coherence tomography images of tissues: signal attenuation and fractal analysis of speckle pattern

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ABSTRACT

Two methods for analyzing OCT images of arterial tissues are tested. These methods are applied toward two types of samples: segments of arteries collected from atherosclerosis–prone Watanabe heritable hyper-lipidemic rabbits and pieces of porcine left descending coronary arteries without atherosclerosis. The first method is based on finding the attenuation coefficients for the OCT signal that propagates through various regions of the tissue. The second method involves calculating the fractal dimensions of the OCT signal textures in the regions of interest identified within the acquired images. A box-counting algorithm is used for calculating the fractal dimensions. Both parameters, the attenuation coefficient as well as the fractal dimension correlate very well with the anatomical features of both types of samples.

Keywords: arterial tissue, optical coherence tomography, swept source, fractal analysis, clinical diagnostic, coherent speckle

1. INTRODUCTION

Atherosclerosis, which represents the build-up of plaque within arteries, is the root of many cardiovascular afflictions with stroke and heart attacks being the most prominent. The disease was once exclusively thought of as an occlusive disease where plaque accumulation resulted in the narrowing of the lumen of peripheral, coronary or cerebral arteries leading to diminished blood flow to the respective regions. Our knowledge of atherosclerosis has evolved significantly, and interest has shifted toward the biological processes occurring at the vascular wall as well as the composition of plaque rather than being focused only on luminal narrowing. Given our evolving understanding of atherosclerosis, novel techniques have been sought that enable imaging of the vessel wall beneath the luminal surface.

Intravascular optical coherence tomography (OCT) is based on the interferometric detection of low-coherence light backscattered from the tissue. OCT has an axial resolution that is determined by the coherence length of the light source, which usually is smaller than 10 μ m. This resolution offers the possibility to investigate details of the morphology of the arterial wall not resolved by other techniques currently used for intravascular probing. Despite its advantage in resolution, OCT imaging has limited soft-tissue contrast due to small differences in the optical refraction indexes of various arterial components. Reliable procedures for enhancing the soft-tissue contrast in OCT images would be beneficial. Indeed, a similar problem, limited soft-tissue contrast, is also encountered in other intravascular imaging methods so this is not a limitation that is characteristic only to OCT imaging and finding ways to overcome it could be beneficial for other intravascular imaging procedures.

It is also well known that a lot of information could be extracted from an OCT image just by analyzing its levels of grey, which are directly related to the intensities of detected light originating from specific volumes within the sample. Nevertheless there is still the need to go beyond the raw information provided by a simple OCT image. Therefore reliable quantitative parameters need to be identified for the purpose of improving the sensitivity and the specificity in detecting and distinguishing vascular pathologies.

For the mentioned purposes, the presented study will be focused on correlating the attenuation coefficients of the OCT signal with the fractal dimensions of various regions of interest within arterial tissues.

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2. SWEPT-SOURCE OPTICAL COHERENCE TOMOGRAPHY SYSTEM

The OCT data presented here was acquired by using a 3x3 Mach-Zehnder quadrature interferometer. Various parts of the system, which acquires a complex interferometric signal generated from swept-source OCT, were described in detail elsewhere ¹⁻³. The diagram of the system used in this study is shown in figure 1¹.



Figure 1. Schematic diagram of the instantaneous complex conjugate resolved swept-source OCT system using a 3x3 Mach-Zehnder interferometer (MZI) topology. The coupler ratios of the 3x3 coupler are $0.39/0.29/0.32^{-1}$.

In figure 1 the swept-source OCT system based on a 3x3 Mach-Zehnder interferometer topology with differential detection schemes is indicated. A 90/10 2x2 fiber coupler is used as a power divider of the light source: 90% power to the sample and 10% to reference arms. The possibility to guide more light toward the sample arm in order to compensate for the lower reflection of a biological sample is one advantage of the MZI configuration.

The 3x3 fiber coupler serves as a combiner of the signals coming from the sample and reference arms and, as a provider of three distinct output signals. In order to form the two separate differential detection channels needed to obtain the real and imaginary parts of the interference signal, one of the output ports of the 3x3 coupler is split using one 2x2 (50/50) fiber coupler. Each of the separate differential detection channels was constructed by combining one output of the 2x2 coupler and one of the remaining outputs of the 3x3 coupler.

The swept source (HSL2000, Santac) used in this OCT system has a central wavelength of 1320 nm and a full scan wavelength range of 110 nm. The average output power and coherence length (in air) of the swept source is 10 mW and 7 μ m, respectively. A repetition scan rate of 20 kHz is used in our system and the related duty cycle is 68%. Because the swept source operates in the 1300 nm wavelength range, commercial fiber optical couplers, circulators and polarization controllers were readily available to implement a complete fiber-based OCT system.

The light exiting the sample arm was focused onto the sample through an *in-situ* built ball-lens single mode fibers whose design and fabrication were described in previous publications^{4, 5}. A forward view probe head similar with the one used for data acquisition is shown in figure 2.



Figure 2. Ultra-small probe with ball lens used for forward view

Good control of the probe beam specifications such as the beam intensity profile, spot size, working distance, optical aberrations and losses, is also crucial for the quality of the OCT image. The optical probe used in the sample arm of the presented OCT system was fabricated at the end of a single mode optical fiber (SMF). A fiber spacer with a homogeneous refractive index and a certain length was added to the end of the SMF in order to perform the beam expansion required for establishing the focus at a specific working distance. For this purpose, a fiber spacer with the same outer diameter was fusion-spliced via arc welds to an SMF-28 fiber and then accurately cleaved to the required length. The same core-less fiber was used to fabricate the ball that subsequently auto-aligned to the fiber spacer through fused via arc welds. Finally, the whole probe head ensemble had the following specifications: working distance 1.1 mm, depth of field 0.9 mm and spot size 28.2 μ m.

The total optical power illuminating the sample was approximately 5 mW. The fiber part of the reference arm was terminated with a fiber collimator and had a variable attenuator inserted before the reference mirror to adjust the optical power in reference arm in order to limit the shot noise. A fiber circulator was used in each arm of the Mach-Zehnder interferometer for redirecting the backreflected light to the 3x3 fiber coupler. Also, two polarization controllers (PC) in both reference and sample arms were used for matching the polarization state of the two arms thus increasing the signal-to-noise ratio. The differential detection was accomplished with two photo-detectors (2117-FC, NewFocus) with adjustable band-pass filters and gains. A maximum 3 dB low-pass cutoff frequency of 10 MHz was used in our system.

The balanced detection output was recorded with a digitizer (Alazartech) at a 100 MHz sampling rate. Based on the time duration of the laser sweep, about 3200 measurement points per A-scan were recorded with a 14-bit resolution. After the records were re-sampled to equal frequency intervals, an inverse Fourier transform was performed. The end result was a depth profile (A-scan), which is the dependence on depth of the sample reflectance. The standard OCT image (B-scan) used for this study contains 900 A-scans, which amounts to a scanning width of 3 mm. The resolution of the setup is 7 µm in the axial direction. Overall, the presented Mach-Zenhder OCT system had a measured sensitivity of 107 dB.

3. IMAGE ACQUISITION

In figure 3 is presented the probe head of the OCT system during the acquisition of a B-scan. The ultra-small probe lens with forward view was covered with a plastic shield for protection. One of the samples used in this study is also shown in figure 3. The sample in the picture is a part of the left descending artery from a rabbit breed that spontaneously develops myocardial infarction. The variant specie was obtained by selective breeding of coronary atherosclerosis–prone Watanabe heritable hyper-lipidemic (WHHL-MI) rabbits ⁷. The arterial piece was cut along the direction of blood flow and it is shown in the picture with the lumen side facing the OCT probe. It can be observed from the picture that there are fatty deposits on the lumen surface. A vertical balancing motion of the OCT probe head ensures the scanning width necessary to obtain a B-scan, which is about 3 mm's wide.



Figure 3. The probe head with an ultra-small probe with ball lens used for forward view acquiring OCT data from the lumen surface of a WHHL-MI rabbit left descending arterial tissue

In optical coherence tomography the formation of an image is based on the detection of the interference of lowcoherence light that has been backscattered from the probed tissue. OCT has an axial resolution that is determined by the coherence length of the light source, which in the case presented here is about 5 µm when the optical refraction indexes of the samples investigated in this work is taken into account. The ability of OCT to image vascular pathology has been previously demonstrated⁸. An axial resolution in the micrometer range offers the possibility to investigate details such as arterial morphology, incipient plaque, fat streaks, damaged endothelial regions, lipid pools, fibrous caps and macrophage accumulations. In addition, the method is able to image features located within a two millimeter range from the lumen surface, region that has been known as the place where most of the conditions leading to arterial problems develop.

An example of an OCT image collected from an un-opened (the vessel was not cut so the lumen is not exposed directly to the probing beam) portion of the coronary left descending artery acquired from a 9-month old WHHL-MI rabbit is presented in figure 4. The sample was scanned by the beam of the swept-source OCT system while placed on a microscope slide. All the OCT images collected from the WHHL-MI rabbit samples were acquired by scanning the probing beam in the direction perpendicular on the blood flow. There are several arterial components that are very obvious in this image. First of all, there is a portion of the lumen imaged through the upper tissue of the collapsed artery. The anatomical composition of the top-side is clearly displayed through the showing of morphological details such as

serosa, adventitia, media and intima. Some vaso-vasorum elements, which is a network of vessels that feed the arterial tissue itself, can be also observed penetrating the adventitia.



Figure 4 Example of a B-scan obtained with the swept-source Mach-Zehnder OCT system. An un-opened piece of WHHL-MI rabbit artery with several anatomical details clearly shown.

A comprehensive visual inspection of the images acquired during this study demonstrated that OCT can be used successfully as a direct method to identify various anatomical features of arterial wall. Based on differences in the scattering properties of tissue constituents it has been demonstrated during in-vivo and ex-vivo trials that OCT is capable of differentiating among various types of atherosclerotic plaques⁸⁻¹². Correlations have been established between various clinical conditions and their appearance in OCT images. For example, it has been determined that both lipid and calcification plaque manifest as dark areas when compared to surrounding intimal and medial arterial walls¹². Other characteristics enter into play too; a strong specular reflection at the boundary of one of these formations indicates the presence of calcification while a diffuse interface could signal a lipid pool¹⁰.

Nevertheless, there is still a necessity to establish more quantitative parameters in order to obtain a better specificity and sensitivity in the interpretation of clinical situations captured by OCT images. Such parameters will help in achieving better diagnostics and a better understanding of the information contained within an OCT image to a level that goes beyond what can be achieved through the merely simple inspection of the distribution of signal intensity alone.

4. QUANTITATIVE PARAMETERS FOR CHARACTERIZING THE OCT SIGNAL

In this work, a quantitative analysis of OCT images is attempted by using two parameters: the attenuation of the OCT signal as a function of depth and the fractal dimensions calculated in regions of interest across the OCT images. Each OCT image used in this study is composed of 900 A-scans. An OCT A-scan is the dependence on depth of the detected signal. The OCT signal is attenuated with increasing depth due to scattering and absorption of light while it propagates within the turbid sample. In order to obtain a smooth profile that ensures a reliable estimation of an attenuation coefficient, a summation of all 900 A-scans that compose a standard B-scan image is performed thus obtaining a

compounded profile corresponding to each OCT image. Before the summation, a procedure for aligning the pixels corresponding to the air/sample interface in each A-scan was performed on every image. This procedure compensated for the non-horizontality of the sample surface. One example of the smooth compounded profiles that are the result of applying the summation procedure described above to the OCT image acquired from an un-opened piece of WHHL-MI rabbit arterial tissue is demonstrated in figure 5.



Figure 5. The compounded profile obtained from the OCT image of an un-opened rabbit artery. As the depth increases from the adventitia toward the tunica intima (from left to right in the figure), the interfaces that separate different layers can be detected as sudden reflection peaks followed by slope changes. The signal intensity local minimum located around the 600-micrometer point marks the location of the lumen channel (seen also in figure 4).

The interfaces that separate different arterial layers are not very obvious in the OCT images of the arterial samples collected from the plaque-prone rabbits. Despite that, in the compounded profiles, these interfaces are easily observed and are marked by reflectivity peaks along the compounded signal followed by changes in the slope of the profile. These features are clearly visible in the example from figure 5, where the profile obtained after compounding the OCT image of an un-opened piece of arterial tube is shown. These changes are observed as the depth increases from the exterior adventitia layer (the leftmost part of the profile) toward the tunica intima, passing through the collapsed lumen into the other side of the arterial tube.

The quantities of interest are the attenuation coefficients of the compounded profile along various regions of interest (ROI's). Their values were calculated by numerically fitting the compounded profile. For the purpose of this work, the fitting was done based on the single scattering model. In this model it was assumed that only light backscattered once within the sample contributes to the detected OCT signal. The confocal and the coherence gates of our OCT system were purposely overlapped in order to reduce the contribution from the multiple scattered part of the signal. According to this model, the mean photo-detector current that generates various grey levels at different depths within an OCT image is directly related to the OCT signal generated at the corresponding depths within the sample and it can be approximated by the following relation:

$$i(z) \propto \sqrt{h(z)} \cdot \exp(-2\mu_a z)$$
 (1)

In this relation i(z) represents the dependence on depth of the photo-current, h(z) is the axial point-spread-function of the sample arm and μ_a is the attenuation coefficient of the OCT signal. The attenuation coefficient of the compounded profile is calculated using the single scattering model with fixed focus geometry described by equation (1). Only fits with correlation factor R^2 higher than 0.85 are considered reliable and used in this report. Graphic examples of the results obtained with this numerical procedure are shown in figure 5 by the straight lines that fit various portions of the profile contained within the interfaces signaled by reflections peaks. Figure 5 has the OCT signal intensity axis plotted on a logarithmic scale so the fit of an exponential function is a straight line.

Attenuation coefficients for the OCT signal were calculated for each layer visible in the compounded profiles and the values were averaged over ten OCT images. Applying the aforementioned fitting procedure on the ensemble of acquired OCT images, an average value for the attenuation coefficient of the OCT signal propagating through the adventitia layer of the studied rabbit arterial sample was found to be $0.81\pm0.07 \text{ mm}^{-1}$. Based on the reflections peaks that were followed by slope changes along the compounded profiles, there were two sub-layers within the tissue portion recognized as the media layer. Each sub-layer was characterized by its own average attenuation coefficient: $1.77\pm0.02 \text{ mm}^{-1}$ (the sub-layer adjacent to the tunica intima) and $2.38\pm0.04 \text{ mm}^{-1}$ (the one adjacent to adventitia). The layer identified as tunica intima was too thin, only two or three coherence lengths in all OCT images, so the number of data points available for calculating a value for the attenuation coefficient within this layer was too small thus rendering the fitting procedure with large errors.

Pieces of porcine left descending coronary arteries were another class of samples used for this study. This time, all the distinct layers, i.e. intima, the media sub-layers and adventitia, structure that characterizes a normal arterial morphology, were distinguishable in most of the corresponding OCT images. There were two groups of images acquired for this sample class: one group contained images obtained by scanning the probing beam along the direction of blood flow while in the second group the B-scans were acquired through scanning that was perpendicular on the direction of blood flow.

The first layer counting from the lumen (tunica intima) in the porcine samples was also quite thin so the fitting procedure generated large errors. The errors generated by the fitting procedure for this layer are at least one order of magnitude greater than the errors corresponding to the numerical fit applied to other arterial regions. In this case, the average values calculated for the OCT signal attenuation coefficient within the intima layer were $15.88\pm2.07 \text{ mm}^{-1}$ (along the blood flow) and $14.04\pm1.72 \text{ mm}^{-1}$ (perpendicular to the blood flow).

As also observed in the rabbit arteries, there were two sub-layers contained within the media layer of the porcine samples. The existence of these two sub-layers could be observed this time by direct inspection of the OCT images and not only from the shape of the compounded profiles as it was the case for the WHHL-MI samples. Each sub-layer could be easily identified because of a different texture within the signal intensity across the OCT images. The first sub-layer of the media is composed of circular smooth muscle fibers that are crossed by the laser beam when scanning in the direction of blood flow. When scanning in the transverse direction to the blood flow, the laser beam is displaced along the muscle fiber. For the second sub-layer of the media the general direction of the muscle fibers with respect to the scanning direction is reversed when compared to the situations encountered in the scanning of the first sub-layer. Through the usage of histological staining the same change in the orientation of the smooth muscle cells versus depth in tunica media, from cells aligned predominately along the arterial circumference (the inside sub-layer) to cells oriented perpendicular to the arterial circumference (the outside sub-layer) has been observed ¹³. Changes in the orientation of the coronary muscle fibers in the media lead to a directional anisotropy of the optical properties that is visible in our OCT images and can be quantified by the attenuation coefficient of the OCT signal. Starting from the sub-layer that borders the tunica intima the values obtained for the attenuation coefficients of the OCT signal propagating through the media are the following: 4.60±0.29 mm⁻¹ and 5.63±0.05 mm⁻¹ (scanning along the blood flow), and 1.59±0.19 mm⁻¹ and 8.31 ± 0.09 mm⁻¹ (perpendicular to the flow).

Unlike the media, the adventitia layer is homogeneous and single average values for the OCT signal attenuation are obtained across its thickness for both scanning modes; along the blood flow the found value is 1.19 ± 0.02 mm⁻¹ and 1.36 ± 0.03 mm⁻¹ is the value found when scanning occurs perpendicular to the blood flow.

Another important feature that could be used to differentiate among various sample regions that appear in an OCT image is the texture. Texture refers to the physical appearance of a region. It is to be expected that the texture of an OCT image should contain, embedded within its speckle, information about the physical composition of the sample. There are three approaches for texture analysis: statistical, spectral and through structural technologies. Amongst these, the

potential of one structural technology method, also known as the fractal approach, to be used for texture analysis was tested for its potential to extract information from the OCT images acquired during this study. One method for calculating values for the fractal dimensions corresponding to textures in OCT images, i.e. the box-counting technique, was used for this study. Because a value is easily attainable through the box counting method, identifying that value with the fractal dimension is a widely used procedure.

In the work presented here, the box counting method was used to calculate fractal values for the textures of each A-scan portion that belongs to an identified region of interest (ROI). We have used the following algorithm to calculate each fractal dimension. Each individual portion of A-scan from an identified ROI was considered separately and it was "covered" with a uniform set of boxes of side length l_i . Only the non-empty boxes N_i , i.e. the boxes containing a portion of the A-scan profile, from the corresponding group of boxes of size l_i are counted. The previous steps were repeated for different box sizes while the box size has been always decreased by a factor of two. The algorithm ended when the box size became half of the coherence length of the swept source used in the OCT system. Finally the box-counting dimension is calculated as the slope of the linear fit to the graph obtained by plotting the number of non-empty boxes N_i against box size l_i on a log-log scale.

The ROI's in the OCT images acquired from the WHHL-MI rabbit arterial pieces where the ones previously identified as the media and adventitia layers based on the corresponding compounded profiles as exemplified in figure 5. The results of the box-counting algorithm were plotted in the form of a histogram graph that shows the frequency of occurrence for a fractal value versus the value of that fractal dimension. Below, the histogram plot from figure 6 shows the distributions of the calculated fractal values for the media and adventitia layers as these layers were identified from the compounded profiles corresponding to the OCT images from WHHL-MI rabbit arteries. Most of the values obtained for both layers aligned themselves along Gaussian-type curves. Gaussian numerical fits were applied to these portions and the correlation factors R^2 were 0.93 or higher for all fits. The peaks of the Gaussian profiles occurred at 1.121 in the adventitia case and at 1.360 for the Gaussian fit corresponding to the fractal values calculated for the media layer. Meanwhile the corresponding widths of the Gaussian curves are 0.065 (adventitia) and 0.077 (media), values that provide quite narrow distributions around the corresponding central peaks. Both fractal distributions presented a second peak, roughly one order of magnitude smaller than the dominant peak, secondary peaks seen also in figure 6. The secondary peaks resulted from the fact that there was not a clearly defined border between the media and the adventitia layers in the arterial samples collected from WHHL-MI rabbits.



Figure 6. Distribution of fractal values calculated in the regions identified as the media and the adventitia layers in the WHHL-MI rabbit arterial samples.

The same procedure was applied for calculating the fractal dimensions in the ROI's identified on the OCT images acquired from the porcine left descending coronary samples. For these samples, the tunica media was divided in two sub-layers that were clearly distinguishable in the OCT images and a fractal calculation was performed for each sub-layer. The fractal distributions for each ROI were also fit to Gaussian curves with correlation factors R^2 of 0.93 or higher. The values obtained for the average fractal dimensions were 1.194 (the first sub-layer of the media), 1.267 (the second sub-layer) and 1.277 (adventitia). The corresponding widths of the Gaussian distributions where as follows: 0.085 (the first sub-layer), 0.067 (the second sub-layer) and 0.054 (adventitia).



Figure 7. Distribution of fractal values calculated for the adventitia and the media sub-layers corresponding to the porcine arterial samples.

5. CONCLUSIONS

Pushing OCT image analysis to go beyond the plain assessment of the intensities recorded across acquired images can enhance its ability to distinguish tissue morphology. Two analysis methods are presented in this report, one is based on calculating the attenuation coefficient of the OCT signal as it propagates through the sample and the other involves finding the average fractal dimension of specific ROIs selected from B-scan images. In this study each of these methods was demonstrated to possess the sensitivity required to distinguish the anatomical characteristics of porcine left descending coronary arteries and of arterial samples collected from plaque-prone WHHL-MI rabbits. The attenuation coefficient of the OCT signal correlated very well with the anatomical features of the arterial wall. This parameter also presented a good degree of sensitivity to more subtle characteristics of tissue morphology. For example, based on OCT signal attenuation, smooth elastin fibers with different orientations could be identified in the sub-layers from within the tunica media. The potential of fractal analysis was also shown to provide another valuable parameter to be used for softtissue differentiation. The average fractal dimensions were different for the various layers and sub-layers of arterial wall. For all the regions of interest identified within the OCT images, the calculated fractal values narrowly grouped around the corresponding average value. Distinct average fractal values corresponding to different morphological features in combination with narrow Gaussian distributions grouped around the average values are strong indications of the specificity and sensitivity of the fractal analysis for soft-tissue segmentation. The work presented here providers a strong incentive to use fractal analysis in tandem with the OCT signal attenuation in order to further refine the classification of various regions within OCT images acquired from arterial tissues in order to achieve improved soft-tissue contrast.

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