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| Citation          | van Soest, Gijs et al. “Atherosclerotic tissue characterization in vivo by optical coherence tomography attenuation imaging.” Journal of Biomedical Optics 15.1 (2010): 011105-9. ©2010 Society of Photo-Optical Instrumentation Engineers. |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| As Published      | http://dx.doi.org/10.1117/1.3280271                                                                                                                                                             |
| Publisher         | SPIE                                                                                                                                                                                             |
| Version           | Final published version                                                                                                                                                                         |
| Citable link      | http://hdl.handle.net/1721.1/58483                                                                                                                                                             |
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Atherosclerotic tissue characterization in vivo by optical coherence tomography attenuation imaging

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Abstract. Optical coherence tomography (OCT) is rapidly becoming the method of choice for assessing arterial wall pathology in vivo. Atherosclerotic plaques can be diagnosed with high accuracy, including measurement of the thickness of fibrous caps, enabling an assessment of the risk of rupture. While the OCT image presents morphological information in highly resolved detail, it relies on interpretation of the images by trained readers for the identification of vessel wall components and tissue type. We present a framework to enable systematic and automatic classification of atherosclerotic plaque constituents, based on the optical attenuation coefficient $\mu_t$ of the tissue. OCT images of 65 coronary artery segments in vitro, obtained from 14 vessels harvested at autopsy, are analyzed and correlated with histology. Vessel wall components can be distinguished based on their optical properties: necrotic core and macrophage infiltration exhibit strong attenuation, $\mu_t \approx 10 \text{ mm}^{-1}$, while calcific and fibrous tissue have a lower $\mu_t \approx 2 - 5 \text{ mm}^{-1}$. The algorithm is successfully applied to OCT patient data, demonstrating that the analysis can be used in a clinical setting and assist diagnostics of vessel wall pathology. © 2010 Society of Photo-Optical Instrumentation Engineers.

Keywords: biomedical optics; attenuation; endoscopy; tissues; medicine; medical imaging.

Paper 09170SSR received Apr. 29, 2009; revised manuscript received Aug. 10, 2009; accepted for publication Aug. 18, 2009; published online Jan. 15, 2010.

1 Introduction
1.1 Background

It is generally accepted that the majority of acute coronary events is precipitated by the rupture of a vulnerable atherosclerotic plaque in the coronary system, and subsequent thrombogenesis. The thin-cap fibroatheroma is currently hypothesized to be the most likely class of arterial wall pathology to constitute a vulnerable plaque. The key to plaque

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Journal of Biomedical Optics 011105-1
January/February 2010 • Vol. 15(1)
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vulnerability is still elusive,\textsuperscript{6} even though recent technological advances in intravascular imaging technology have enabled the collection of a wealth of data on unstable atherosclerosis in all its stages of development,\textsuperscript{7} both in clinical and in ex vivo settings. It appears very likely that combined information on physiological, anatomical, chemical, and mechanical parameters\textsuperscript{8–10} is required for a reliable assessment of the proneness of a specific lesion to rupture. Some of these parameters may be accessible through intravascular imaging methods.\textsuperscript{11–19} In addition, plaque type and morphology prior to intervention influence the long-term procedural outcome significantly.\textsuperscript{20} Among the parameters that influence plaque vulnerability are the thickness of the fibrous cap overlying the necrotic core, inflammation, intraplaque hemorrhage, and composition.\textsuperscript{9,21} Data on plaque composition and stability can complement the image, and can aid decisions regarding whether a particular section of coronary artery should be treated and how.

The advent of optical coherence tomography (OCT) for cardiovascular applications has boosted the resolving power of intravascular imaging to a level where imaging the thin cap of a vulnerable plaque is possible.\textsuperscript{22} Insight into the physiology of a plaque is complementary to the structural information offered by the OCT grayscale image.

In intravascular ultrasound (IVUS) imaging, parametric imaging techniques have been developed that quantify properties of the underlying tissue in the image. Examples of this approach are IVUS elastography, characterizing local elasticity,\textsuperscript{11,12,23,24} and spectral analysis methods for tissue identification.\textsuperscript{25–27} In intravascular OCT, published research into parametric imaging methods so far has been very limited.

Recent publications have highlighted the possibility of tissue identification in OCT images. Yabushita et al.\textsuperscript{28} published a qualitative image classification scheme, which has become the de facto standard, distinguishing fibrous, calcified, and lipid-rich tissues. A comparison with later work by Kume et al.\textsuperscript{29} reveals, however, that the interpretation of OCT images based on qualitative criteria can be ambiguous in some cases. In this study, we attempt to resolve some of the ambiguity associated with qualitative OCT tissue classification by developing a parametric imaging technique that images the optical extinction coefficient (sum of scattering and absorption: $\mu_t = \mu_s + \mu_a$) in atherosclerotic plaques in situ.

A few papers have been published recently\textsuperscript{30–32} that targeted the optical attenuation coefficient for arterial tissue identification with OCT. These studies were performed on longitudinally sectioned arteries, imaged with a scanning-stage type OCT setup. Xu et al.\textsuperscript{33} published a study in which both the backscatter coefficient $\mu_s$ and the extinction coefficient $\mu_a$ were measured on transverse sections of paraffin-embedded, intact human coronaries. The latter study can be interpreted as a quantitative basis for the qualitative scheme of Yabushita et al.\textsuperscript{28} For this work, we used an intracoronary, catheter-based OCT system, enabling us to image the optical attenuation in situ and even in vivo.

1.2 OCT Signal Model

The expectation value of the detected OCT signal $\langle I_d(r) \rangle$ can be modeled using a single scattering model that incorporates the axial point spread function due to the coupling of the emitted and backscattered intensity to the Gaussian mode profile of a single-mode fiber\textsuperscript{34} (SMF).

$$\langle I_d(r) \rangle = I_0 T(r) \hat{S}(r) \exp(-\mu_t r), \quad (1)$$

$$T(r) = \left[ \frac{r - z_0}{z_R} \right]^2 + 1 \right]^{-1/2}, \quad (2)$$

$$\hat{S}(r) = \begin{cases} 1 & \text{time domain OCT} \\ \exp \left( - \frac{r - z_C}{z_{sw}} \right) & \text{swept-source OCT.} \end{cases} \quad (3)$$

In this equation, the OCT signal is modeled by a Lambert-Beer exponential decay curve, multiplied by the coupling factor. The expectation value operator $\langle \cdot \rangle$ denotes the ensemble average over many realizations of the speckle generated by the beam in the tissue. Here $T(r)$ is the longitudinal point spread function (PSF) for an SMF-based OCT system, governed by the position of the beam waist $z_0$ and the Rayleigh length $z_R$. For analysis of Fourier-domain OCT (FD-OCT), Eq. (1) models the amplitude reflectivity.

In FD-OCT, one must also account for the spectral coherence of the source.\textsuperscript{35} In this study, we use a swept-source system for in vivo imaging. A Gaussian coherence function $\hat{S}(r)$ can be assumed in the frequency domain during the linear scan. The finite width of $\hat{S}(r)$ introduces an extra factor $\hat{S}(r)$ (the Fourier transform of $\hat{S}$), describing the signal roll-off with depth. In Eq. (3), $z_C$ is the center of the scan, which has been moved to positive $r$ by frequency shifting,\textsuperscript{36} and $z_{sw}$ is the half width of the roll-off function.

Our objective is the attenuation coefficient $\mu_t$, appearing in the exponent of Eq. (1). The scale factor $I_0$ is the locally available intensity multiplied by the backscattering coefficient $\mu_s I_0 = \mu_s(r) I'(r)$. The backscatter efficiency is a tissue property that can be measured independently in homogeneous media.\textsuperscript{33} The local intensity is equal to the source intensity incident on the vessel wall $I_{in}$ diminished by the attenuation along the path from the lumen border to the imaged depth, $I'(r) = I_{in} \int_0^r \exp(-\mu_s r') dr'$. As the OCT image traces provide only one intensity measurement for every depth, it is not possible to extract two depth-dependent parameters $\mu_s(r)$ and $\mu_a(r)$ from the analysis.

Equation (1) describes the OCT signal in a homogeneous medium. The vessel wall, and biological tissue in general, are heterogeneous structures. An OCT A-line $I_d(r)$ usually samples more than one tissue type. Hence, the signal intensity must be fitted in windows of a length that is unknown a priori.\textsuperscript{31} Here, variations in $\mu_a(r)$ may confound the analysis of $\mu_s(r)$. In addition, the presence of speckle means that the signal-to-noise ratio (SNR) in the image is inherently low, and several frames must be averaged to reduce speckle. Averaging is complicated by artifacts due to cardiac motion, and by nonuniform catheter rotation, which can usually be corrected.\textsuperscript{39,40}

In this paper, we present a method for intracoronary imaging of the optical extinction coefficient $\mu_t$. The principle is demonstrated in human coronary arteries ex vivo. The optical attenuation maps are compared with histopathology to iden-
tify the correspondence between tissue type and extinction coefficient. After validation, we recorded suitable intracoronary OCT sequence in patients and applied the algorithm to the data. Analysis was successful and the resulting optical attenuation maps both corroborated and complemented the qualitative interpretations of plaque type.

2 Materials and Methods

2.1 Coronary Artery Specimens

We examined 14 human coronary arteries, acquired during autopsy at the Department of Pathology of the Erasmus Medical Center (MC), from 14 human hearts (57% men, 12 left anterior descending coronary arteries, 2 right coronary arteries, mean age 64). Inclusion criteria were age >40 yr and noncardiac death. Permission to use autopsy material for scientific study was obtained from the relatives; this study was approved by the Medical Ethics Committee of the Erasmus MC.

The artery segments were excised from the heart and varied in length between 5 and 9 cm. In these vessels, 65 sites were identified (up to 5 per segment) for imaging and further histologic processing. All vessels except one were observed to be atherosclerotic based on gross pathology. During excision of the arteries, side branches were closed with sutures. OCT imaging was performed within 24 h postmortem.

2.2 Imaging Protocol In Vitro

OCT imaging was performed with an M2CV time-domain OCT system, and ImageWire 200 catheters (Lightlab Imaging Inc., Westford, Massachusetts). A vessel cross section was imaged by an IR light beam, central wavelength 1310 nm, that was swept along the vessel wall by the rotating fiber inside the catheter sheath. The OCT system had an axial resolution of 14 μm and a tangential resolution of 25 μm in the focus. The imaging depth was 3.3 mm. Each frame consisted of 312 lines × 752 pixels, corresponding to 4.5 μm per pixel. Imaging was performed at a rate of 10 frames/ps (fps).

The artery under investigation was placed in a water bath filled with saline solution at 37 °C. Both ends were mounted on plastic sheaths, and the vessel was pressurized to 100 mmHg using a water column system filled with saline. The vessel was inspected longitudinally by moving the imaging catheter along the lumen. Sites of interest were marked with a needle, visible in the OCT images. At these sites, a stationary sequence of 40 to 50 frames was recorded and stored in polar coordinates in stacked TIFF (tagged image file format). After removal from the water tank, the needles were replaced by a color-coded suture.

2.3 Histopathology

After imaging, the artery sections were pressure fixed at 100 mmHg in formaldehyde for 24 h at room temperature, and subsequently stored in formaldehyde at 4 °C for further processing. Vessels were partially decalcified for 24 h in formic acid. After fixation and decalcification, sutures marking the imaged cross sections were replaced by ink dots. The tissue was embedded in paraffin and serially sectioned for histological staining. Each imaged cross section was stained with hematoxylin-eosin (H&E), picrosirius red, elastic van Gieson (EvG), and immunohistochemical stain CD68 for macrophage identification.

Histological cross sections were characterized by two pathologists blinded for the OCT results. Characterization was done by making a map of all the cross sections, called cartoon histology, with color coding for different types of tissue. We distinguished fibrous tissue, including intimal smooth muscle cells (SMCs), necrotic core in early or advanced stage, hemorrhage, and dense calcium. In case of disagreement, the two pathologists reevaluated the slides and reached a consensus diagnosis. A summary of the color coding used in the cartoon histology is given in Table 1.

2.4 Imaging Protocol In Vivo

Quantitative analysis of intracoronary OCT imagery is complicated by motion and other artifacts, resulting from catheter eccentricity or blood remnants in the lumen. The influence of cardiac motion can be mitigated by increasing the imaging speed. The diastolic filling phase, where coronary motion is minimal, lasts about 0.2 s, measured from the end of the T wave in the electrocardiogram (ECG) to the beginning of atrial systole, marked by the start of the P wave. At a frame rate of 100 fps, each heart cycle will have 15 to 20 frames in which the effects of cardiac motion are limited, and enable averaging of those frames.

In vivo imaging was performed in the catheterization laboratory of the Thorax Center of the Erasmus MC (Rotterdam, The Netherlands) using an optical frequency domain imaging (OFDI) system built at the Wellman Center for Photomedicine (Boston, Massachusetts). This swept-source system had a center wavelength of 1310 nm and an axial resolution of 10 μm. The frame rate was 105 fps, and the ranging depth in tissue is about 4.7 mm. The resulting images were sampled at 512 lines × 1024 pixels per frame.

Patients undergoing percutaneous coronary intervention (PCI) were enrolled in this study, having given informed consent. In the OFDI pullback and x-ray angiography data, lesions of interest were identified for optical attenuation imaging. The catheter imaging tip was then positioned at such a site, and a stationary (i.e., no pullback) sequence was recorded. The blood was cleared from the artery by flushing with 100% ioxitalam 370 (Visipaque™, GE Healthcare, Cork, Ireland) at 37 °C, either manually or using an injection pump (Mark-V Pro Vis, Medrad Inc., Indianapolis, Pennsylvania) at 3 to 4 ml/s. Recording was started as soon as clearing was achieved, and stopped after about five heart cycles; flushing was also stopped at this time. An ECG was recorded syn-

| Table 1 Color coding used for tissue type in the cartoon histology. |
|-----------------|-----------------|
| Green           | Fibrous and smooth muscle cells |
| Yellow          | Early necrotic core |
| Red             | Advanced necrotic core |
| Gray            | Calcification    |
| Black           | Hemorrhage       |
chronically with the OCT acquisition at a sampling rate equal to the A-line frequency (54 kHz). In the acquired data, we selected the frames corresponding to the cardiac phase between the T and P waves for further analysis. The imaged plaques were classified by two experienced OCT readers for comparison with the assessment based on optical attenuation.

### 2.5 Catheter Characterization

Individual catheters of the type used in this study may have nonidentical focal length $z_0$ and Rayleigh length $z_R$. It is essential to characterize the catheters before attempting to extract quantitative data from the OCT image. Catheter beam parameters were determined by recording the OCT signal in a series of Intralipid (Fresenius Kabi, ’s-Hertogenbosch, The Netherlands) dilutions (base solution 20% w/w; diluted 1:4 to 1:128) in water. Every recording consisted of about 15000 A-lines. These were averaged to reduce speckle. An $r$-dependent (catheter-independent) intensity offset $I_{\text{offset}}$ was measured in water, with negligible attenuation and backscattering. This offset was subtracted from the averaged OCT traces.

To reduce the dynamic range of the fitted signal and reduce sensitivity to noise, we linearized Eq. (1) by logarithmic compression:

$$\log(I_d(r)) - I_{\text{offset}}(r) - \log[T(r; z_0, z_R)] - \log[\hat{S}(r)] = \log(I_0) - \mu_r r.$$  \hspace{1cm} (4)

The second term in Eq. (4), representing the catheter-specific PSF, is calculated with Eq. (2) for a matrix of $(z_0, z_R)$ combinations, with $0.7 \leq z_0 \leq 3$ mm and $0.2 \leq z_R \leq 1.2$ mm, both with 0.01-mm increments, and added to the offset-corrected measured intensity profile. We minimized the root mean square (rms) difference between the fitted line and measurement, compensated for roll-off if applicable, see Eq. (3). All catheters yielded a unique, paraboloid minimum. The values of $z_0$ and $z_R$ at the minimum of the cost function represent the best estimate of the beam parameters.

### 2.6 Data Analysis

All data analysis was performed in MATLAB R2007b (The Mathworks, Natick, Massachusetts). Frames from the OCT movies were aligned using our nonlinear rotation correction algorithm, and then averaged for speckle reduction. This mean OCT image was used as the basis for analysis of the optical attenuation coefficients. If there was evidence of remaining motion artifacts (e.g., blurring in the averaged frame) in the corrected sequence, the data were discarded.

The intensity traces were linearized in the manner of Eq. (4), using the beam parameters specific for the catheter that was used for imaging the vessel.

$$i_d = \log(I_d(r)) - I_{\text{offset}}(r) - \log[T(r)] - \log[\hat{S}(r)].$$  \hspace{1cm} (5)

Starting at the lumen boundary, the log-compressed data $i_d$ was least-squares fitted with a linear model,

$$\tilde{i}(r) = \log(I_0) - \mu_r r,$$  \hspace{1cm} (6)

in a window of variable length $L$. With a linear optimization, there is a unique optimum for each set of data, and results are independent of the initial guess required for an iterative nonlinear model. Speed is another advantage.

We extracted $\mu_r$ from the data, using a fitting procedure optimized to search for homogeneous tissue regions. The fitting window was extended until an inhomogeneity was encountered that degraded the result. Searching for the longest window maximizes the accuracy of the fitted attenuation coefficient, and reduces sensitivity to intensity fluctuations that result from $\mu_0$ inhomogeneities.

A schematic representing the data flow in the fitting procedure is shown in Fig. 1. Using an adaptive threshold criterion, the lumen boundary is determined in the mean image. A linear least-squares (lsq) fit is performed in a window of minimum length $L_{\text{min}} = 200 \mu$m, corresponding to $w = 44$ pixels (px). Results with $\mu_r < 0$ are discarded. The cost function (fxn) $\delta$ is defined as the root of mean square (rms) difference between the measured OCT trace $i_d$ [Eq. (5)] and the model $\tilde{i}$. The window is iteratively extended until a decrease in fit quality is detected. The optimum values (for smallest $\delta$) are stored, and the window is moved forward. This procedure is repeated until the window encounters the end of the A-line. All parameters in the fitting procedure were optimized in a simulation study.

This algorithm results in an estimate of $\mu_r(r)$ for every A-line. The attenuation coefficients are color coded on a linear scale and plotted in the regular gray-scale OCT image as an overlay or side by side. The in vitro attenuation image is then compared with the cartoon histology to identify a correspondence between tissue type and $\mu_r$. As histological tissue slices are much thinner (5 $\mu$m) than the accuracy of the needle marker (0.5 mm) there is an unavoidable sampling error. Imaged cross sections that were obviously mismatched with histology, based on anatomical features, were removed from the data set before analysis.
3 Results

3.1 In Vitro Imaging

We analyzed OCT data of 39 lesions. Other sites had either too much catheter motion, preventing the averaging of the data, or the match between the OCT data and histology was inaccurate. Results for different lesion types are shown in Figs. 2–4. Figure 2 displays data for a lesion containing a large calcification and an advanced necrotic core. Low attenuation is found in areas with fibrous tissue and calcification. The necrotic core exhibits a high attenuation coefficient, and stands out much more clearly in the attenuation image than in the gray-scale OCT. The adventitia also has a high attenuation, but can be distinguished from pathological features by its morphology and location: the high attenuation region occurs outside the media and is circumferential. A concentric fibrous lesion shows up as a homogenous, circumferential, low-attenuation region in Fig. 3. Again, the attenuation coefficient rises in the adventitial region.

A more complex plaque type is analyzed in Fig. 4. The OCT image is very heterogeneous, and this is reflected by the morphology in the histology. The cartoon histology shows intraplaque hemorrhage and necrotic core, embedded in a mostly fibrous matrix. The green and orange boxes highlight areas with high attenuation coefficient. The green box contains some necrotic core, which is in accordance with the example in Fig. 2. The orange box, however, is only fibrous tissue and SMC. The CD68 stain reveals strong topical macrophage infiltration in both these areas. Macrophages, being strong optical scatterers, attenuate the OCT beam. An example of intimal xanthoma is shown in Fig. 5. Macrophages in a lesion that is mostly fibrous otherwise cause strong attenuation.

The limited size and composition of our data set does not permit formulation of a statistically sound, quantitative classification scheme for automatic identification of necrotic core and macrophages, or plaque type in general. We can, however, formulate tentative criteria based on the appearance of certain pathologies in our in vitro optical attenuation data. The characteristics we observed are summarized in Table 2.

3.2 In Vivo Imaging

Stationary OCT sequences with ECG registration were obtained from nine sites in four patients. Of these, eight sites had OFDI data with small enough catheter motion that the residual shifts could be corrected and the frames could be averaged in at least one heart cycle. Of the imaged plaques, five were classified as fibroatheroma, and three were fibrous lesions. Figure 6 presents optical attenuation imaging of atherosclerotic lesions in vivo in two plaques of different types.

The measured optical attenuation reproduces accurately if a single site is imaged repeatedly. In Fig. 7, we show attenuation data from a necrotic-core coronary lesion in vivo, imaged over three consecutive heart cycles. The features observed in the plaque, as well as the absolute values of the...
attenuation coefficient, appear very similar in the different images. A quantitative estimate of the reproducibility is not straightforward as the data from different heart cycles are not exactly aligned angularly and radially.

The characteristics noted in Table 2 were also observed in vivo, as evidenced by Fig. 6. Plaque identification by application of these criteria agreed with the interpretation by two OCT readers for seven out of eight cases. Interpretation of the last one was complicated by inhomogeneity in both the gray-scale and attenuation images.

4 Discussion

4.1 Study Results

We compared the optical attenuation coefficient, measured by OCT, with the composition of atherosclerotic lesions, based on histology. High optical attenuation—\( \mu_t \geq 10 \text{ mm}^{-1} \)—is associated with two important markers of atherosclerotic plaque vulnerability: necrotic core and macrophage infiltration. Other common plaque tissue types, fibrous and calcific, were found to have a low attenuation coefficient—\( \mu_t \approx 2 \text{ to } 4 \text{ mm}^{-1} \). The in vitro measurements were done on intact excised human coronary arteries, with a commercially available, clinically approved OCT system and catheters.

We demonstrated that the analysis can be performed, with similar results, on patient data. The application in interventional procedures is easy, quick, and does not cause any patient discomfort. It requires the recording of a stationary se-

![Fig. 4 Complex lesion containing necrotic core, hemorrhage, and macrophages: (A) OCT image, scale bar corresponds to 1 mm; (B) attenuation coefficient, scale from 0 to 15 mm\(^{-1}\); (C) histology, H&E stain; (D) cartoon indicating an early stage necrotic lesion (yellow) with a thin fibrous cap and intraplaque hemorrhage (black); and (E) CD68 stain, showing macrophage infiltration. The green and orange boxes are magnified (2.25× with respect to the overview) above, and correspond to the indicated areas in (B). Both areas show high attenuation coefficients (10 to 12 mm\(^{-1}\)), coinciding with the presence of necrotic core (green box) and macrophages (green and orange boxes).](image)

Table 2 Appearance of vessel wall condition in the attenuation image.

| Condition                  | Appearance                                                                 | Typical \( \mu_t \) |
|----------------------------|---------------------------------------------------------------------------|---------------------|
| Healthy vessel wall        | Thin (< 300 \( \mu \)m) circumferential layer of low attenuation, adjacent to the lumen; enveloped by a circumferential high-attenuation layer | 2 to 5 mm\(^{-1}\)  |
| Intimal thickening         | Circumferential layer of low attenuation adjacent to the lumen; thickness \( \geq 500 \mu \)m | 2 to 5 mm\(^{-1}\)  |
| Necrotic core              | Asymmetric area of high attenuation                                        | \( \geq 10 \text{ mm}^{-1} \) |
| Macrophage infiltration    | Layer of high attenuation, possibly missing data behind it                  | \( > 12 \text{ mm}^{-1} \) |

![Fig. 5 Intimal xanthoma case. High attenuation (color scale 0 to 15 mm\(^{-1}\) as before) in a fibrous lesion occurs due to macrophage infiltration, as evidenced by the CD68 stain in the inset.](image)
Plaque, with values up to $15 \text{ mm}^{-1}$ in the macrophage area. Detection did not produce any errors. Data processing is computationally light: analysis of a cross section takes $1$ to $2$ s (depending on frame size) in MATLAB on an office PC, without any custom optimization.

This study is the first to report in situ tissue typing of atherosclerotic lesions in intact human coronary arteries by means of the optical attenuation coefficient. It is also the first to demonstrate the in vivo feasibility of such an approach, and to assess the diagnostic potential of this parameter for plaque type in a clinical setting. One of the main difficulties in OCT-based plaque characterization is the distinction between necrotic core and calcified lesions, as the difference is only in border definition. In optical attenuation, there is a marked contrast between the two plaque components.

The analyzed attenuation coefficients for the different tissue types are similar in vivo compared to in vitro. Given that the two data sets were acquired with fundamentally different OCT systems, and different catheters, this inspires confidence in the data. It also suggests that the tissue optical properties did not change significantly after excision of the arteries at autopsy and storage in saline during the experiment.

**4.2 Comparison with Literature**

Our results are in general agreement with those presented by Xu et al.\textsuperscript{33} Using a benchtop OCT system, they measured low $\mu$, for fibrous and calcific tissue and high $\mu$, for necrotic core. Macrophage infiltration was not identified in that study. The scale is also comparable, taking uncertainty margins into account and reading the "lipid" category of Ref.\textsuperscript{33} as our category "necrotic." Necrotic core has $\mu > 10 \text{ mm}^{-1}$, Xu et al. report a value of the fibrous and calcific, $\mu \approx 6 \text{ mm}^{-1}$, that is slightly larger than the one we found ($\mu \approx 2$ to $5 \text{ mm}^{-1}$). This may be a result of the fixation procedure applied in Ref.\textsuperscript{33}.

Other literature reports both higher\textsuperscript{30} and lower\textsuperscript{31} values for necrotic core attenuation. Our data do confirm the observation\textsuperscript{33} that lipid-rich tissue attenuates more strongly than other atherosclerotic tissue types, or healthy vascular tissue.

**4.3 Image Artifacts and Study Limitations**

We were unable to determine the backscatter coefficient $\mu_b$ from our data because of tissue heterogeneity and the lack of an intensity calibration ($I_0$ in Sec. 1.2) that is constant for all lines and frames. We put in a significant effort to reduce the sensitivity of the fitting algorithm to sudden steps in intensity that result from a drop in $\mu_b$ at a sharp interface between different tissues. This approach has been successful, by and large, but evidence of artifacts, resulting from changes in $\mu_b$ being interpreted as large $\mu_s$, remains in the data. An example can be seen in Fig. 2, where fibrous and calcific tissue are adjacent. Both fibrous and calcified tissue have a low $\mu_s$, but the transition from high $\mu_b$ (fibrous) to low $\mu_b$ (calcified) produces a spot of high fitted $\mu_s$ near $3 \text{o’clock}$. This illustrates that the attenuation display should always be interpreted in conjunction with the gray-scale OCT image.

The model we used for analysis is a single-scattering model, assuming straight light paths to and from the scattering site where backreflection occurs, and not incorporating interfaces between different tissues. In reality, specular interface reflections at the boundary between different tissues—with differing refractive indices\textsuperscript{34}—may produce a strong signal.
locally, which cannot be accounted for by Eq. (1). Likewise, there is evidence of multiple scattering, causing a higher signal than predicted by the model, depending on beam parameters and tissue optical properties. More sophisticated optical modeling could reduce these artifacts, but will substantially complicate the analysis and increase dependence on parameter guesses.

Two aspects of the comparison with histology merit discussion. The first is in-plane (as opposed to longitudinal, pointed out in Sec. 2.6) image registration: the histologic processing inevitably deforms the tissue. This complicates automated correlation and one-to-one mapping between tissue type and optical attenuation.

Second, density variations are apparent in the histology slides, particularly in fibrous tissue. Certain areas seem to be more tightly packed than others. While in some cases this may be an artifact due to histologic processing, these regions can often be identified in the OCT images as well, thus representing real tissue inhomogeneities. They will undoubtedly reflect in the attenuation coefficient. Our standard—tissue type, color coded in the cartoon histology—does not represent these density variations. This is a source of variation that our analysis cannot account for. Likewise, for the in vivo data, the plaque classification is more coarse-grained than our image analysis. It qualifies an entire cross section as a certain plaque type and cannot account for possible variability within a plaque that will be apparent in our data.

Routine clinical use of the tissue characterization method is limited in its present implementation by the frame averaging used for speckle reduction. Multiple frame processing is incompatible with imaging of a coronary artery in a pullback, where each frame samples a different vascular cross section. The required amount of speckle reduction depends on the minimum acceptable SNR for the fit results to be meaningful. An unpolarized OCT image theoretically has $SNR^2 = \langle I \rangle / \sigma^2 = 1.4$; we observe SNR = 1.8 to 2.0 in our in vitro data and SNR = 1.7 to 1.9 in vivo. Averaging $N$ independent speckles increases the SNR as $\sqrt{N}$. We found heuristically that, with the present algorithm, about 30 frames are required in the in vitro data, while 15 to 20 frames suffice in vivo. The difference may be attributed to residual cardiac motion, assisting speckle decorrelation between frames. In the future, we will investigate more sophisticated speckle reduction and data processing techniques, such as frequency compounding in FD-OCT data or digital filtering, to enable comprehensive imaging of the optical attenuation in a coronary artery.

5 Conclusions

We developed a framework for imaging the optical attenuation coefficient $\mu_t$ in human coronary arteries using intravascular OCT. In vitro studies demonstrate that $\mu_t$ is a suitable parameter to distinguish between different tissue types in atherosclerotic lesions. Two markers of plaque vulnerability, the presence of necrotic core and macrophage infiltration, can be identified as areas of high attenuation ($\mu_t = 10 \text{ mm}^{-1}$). Fibrous and calcified tissue have $\mu_t = 2-5 \text{ mm}^{-1}$. The analysis was successfully applied to OCT images acquired in patients. Optical-attenuation-based plaque classification, using the criteria developed with the in vitro analysis, corroborated the plaque classification based on the gray-scale OCT.

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