CHAPTER 4

Parametric imaging of attenuation by optical coherence tomography: a review of models, methods and clinical applications

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Optical coherence tomography provides cross-sectional and volumetric images of scattering from biological tissue that reveal, to a greater or lesser extent, the morphology of the sample. The strength of the scattering represents a potentially complementary source of information on the tissue’s optical properties, which can be characterized by parametric imaging of the OCT attenuation coefficient. Over the last 15 years, there have been many works published seeking to advance methods to determine the OCT attenuation coefficient and applications utilizing it. This review provides an overview of the main models and methods, their assumptions and applicability, together with a survey of clinical applications and accompanying reported values of the OCT attenuation coefficient. The use of the attenuation coefficient is shown to be applicable in various medical fields. Most studies show promising results towards the usability of the OCT attenuation coefficient to differentiate between tissues of clinical interest. As a future step, larger scale studies are desirable, which will benefit from a consensus on the model and method used for the determination of the attenuation coefficient from the OCT signal. With this review we hope to provide a basis for discussion towards establishing this consensus.
INTRODUCTION

Optical coherence tomography (OCT) discriminates the backscattered field from the sample based on transit time, being exquisitely sensitive to light which has undergone one or a few scattering events. Depth-resolved images of this backscattering in tissue can be obtained, ex vivo and in vivo, with a resolution commonly in the range 5-15 μm, although sub-1 μm has been demonstrated. Currently, OCT is primarily used to visualize the morphology of tissue, which can be used to differentiate pathology in some circumstances. There is further clinical value, in addition to visualization, to use OCT to differentiate pathology based on the altered structure and organization not readily visible with conventional clinically available techniques. The structure and organization of a tissue is reflected in its optical properties, and perhaps the most accessible such property in OCT is the attenuation coefficient, describing the extinction with depth of the detected OCT signal. To measure the OCT attenuation coefficient (μOCT) and obtain useful information from this measure, a model of the OCT signal and a model correlating μOCT to the tissue structure are developed.

Pre-clinical and clinical studies in a wide variety of medical fields, including dermatology, cardiology and urology, have shown promising results on the use of μOCT for tissue characterization. This literature presents multiple models and methods to determine μOCT, and to relate it to tissue properties. To advance the application of μOCT for tissue characterization, standardized and validated approach to obtaining reliable μOCT data, and to dealing with issues such as the heterogeneity of tissue and the length scales on which this occurs is needed. The aim of this review, then, is to present an overview of the models, methods and applications of parametric imaging of attenuation by OCT and to discuss issues in the determination of μOCT with the ultimate goal of establishing a unified basis for future clinical research on using μOCT. To this end, the review is divided into three sections. In Section I, the relationship between tissue properties and the OCT attenuation coefficient is discussed. In Section II, commonly used models for the OCT signal are summarized. In Section III, an overview of pre-clinical and clinical applications of μOCT is given, accompanied with a summary of the reported μOCT values. Finally, in the discussion section, the limitations of the discussed models and methods, together with clinical challenges, and future perspectives are discussed.

Tissue optical properties

The interaction of light with tissue includes absorption and scattering of the incident light, both being dependent on the wavelength. Tissue scattering forms the basis for contrast in OCT. The decay of the OCT signal in depth (i.e. the attenuation coefficient) is due to both scattering and absorption. Here, we will focus on tissue scattering properties as absorption by biological tissue is minimal at commonly used wavelengths for
OCT, and quantification of absorption is outside the scope of this thesis. The origin of scattering in biological tissue is the spatial variation in refractive index, which is known to exhibit fractal properties because of the wide range of particle sizes present in the tissue that interact with light.\textsuperscript{17} The range of sizes and refractive indices of tissue components such as cells, fibers and the surrounding fluid contributing to tissue scattering have been the subject of ongoing research.\textsuperscript{18} For example, novel studies have demonstrated a lower refractive index of the cell nuclei compared to the cell cytoplasm, contrary to previous assumptions.\textsuperscript{19,20} Measurements of tissue scattering are of clinical interest since changes in tissue composition, structure and organization are reflected in tissue scattering, which can potentially be probed using non-invasive imaging techniques such as OCT. As the composition of tissue and its structure and organization varies between tissue types, and is altered due to disease, probing tissue scattering properties can enable tissue characterization and detection of pathologies.\textsuperscript{21} For example, it has been shown that subtle changes between pre-invasive cancer cells compared to normal cells,\textsuperscript{4} and for apoptotic compared to necrotic cells\textsuperscript{5} can be detected by measurement of optical scattering properties.

The scattering properties of tissue are wavelength dependent and commonly expressed by the scattering coefficient ($\mu_s$), and the phase function ($P(\theta)$). The scattering coefficient quantifies the amount of scattering. The multiplicative inverse of the scattering coefficient ($1/\mu_s$) defines the average path length light can travel in a medium without scattering. In general $\mu_s$ decreases for increasing wavelength. The phase function expressed the probability of scattering angle. Usually the scattering angle is specified by the anisotropy factor ($g$), which is the average cosine of the scattering angle ($<\cos(\theta)>$). The anisotropy factor has values between -1 to 1, where $g = -1$ corresponds to fully backward scattering and $g = 1$ corresponds to fully forward scattering. In general, $g$ increases for larger particles (e.g. red blood cells) and $g$ approaches 0 for particle diameters smaller than the wavelength. Furthermore the backscattering coefficient ($\mu_B$) can be defined, which is the light scattered back under an angle of 180°, in the direction of the incident light. In the description of diffuse light transport, the reduced scattering coefficient is frequently used, given by $\mu_s' = \mu_s(1-g)$.\textsuperscript{22} For spherical particles the scattering properties can be straightforwardly calculated using Mie-theory to obtain the scattering cross section ($\sigma_s$) and the phase function ($P(\theta)$). For dilute solutions $\mu_s$ is obtained by multiplying $\sigma_s$ with the particle concentration. The by Mie-theory calculated scattering properties are a function of particle size, refractive index mismatch of the particle and the surrounding medium, and the illumination wavelength.

Scattering properties of tissue can be quantified from the OCT signal; perhaps the most accessible scattering property in OCT is the attenuation coefficient. The OCT attenuation coefficient (or extinction coefficient) is conventionally referred to as $\mu_T$, given as the summation of the scattering coefficient and the absorption coefficient: $\mu_T = \mu_s + \mu_a$. Here,
it is assumed that that only singly scattered light contributes to the OCT single, as the multiple scattered light is suppressed by the coherence and confocal gate. However, for high scattering and high forward scattering media (for example red blood cells) multiple scattered light contributes to the OCT, which causes an underestimation of $\mu_t$.

Here, the symbol $\mu_{OCT}$ will be used to explicitly denote the OCT attenuation coefficient. At commonly used OCT wavelengths (e.g., 850, 1050 and 1300 nm) $\mu_a$ for most biological tissues is assumed to be negligible compared to the scattering ($\mu_s \gg \mu_a$). Consequently, at these wavelengths and assuming single scattering $\mu_{OCT}$ give an estimate of $\mu_s$.

The resolution at which $\mu_{OCT}$ is determined depends on the resolution of the OCT system and the amount of pixels that is averaged prior to analysis. Averaging is required to suppress speckle and create a smooth depth profile suitable for analysis such as curve fitting. A crucial assumption is that $\mu_{OCT}$ is determined over a homogeneous region of interest of tissue. The tissue commonly comprises different structures that may invalidate attenuation estimation when the model fitting window covers structures with significantly different scattering properties. For example, many tissues present layered structures within the OCT imaging depth range, such as the skin, arteries and retinal tissue. Tissue heterogeneities on the scale of the imaging hamper determination of $\mu_{OCT}$ and need to be dealt with in the analysis and interpretation of obtained data. Approaches to deal with tissue heterogeneity will be discussed in more detail in the discussion section.

Models of the OCT signal

To quantify $\mu_{OCT}$ and link it to tissue properties a model for the OCT signal is required. This section summarizes the most commonly described models for the OCT signal. First, the single scattering model will be discussed, followed by means to correct the OCT data for system-dependent parameters, continuing with a description of the fitting method and depth resolved method to determine $\mu_{OCT}$ based on the single scattering model. Second, approaches to incorporate multiple scattering into the OCT signal will be discussed. Finally, full-wave modelling of the OCT signal based on Maxwell’s equations will be reviewed.

Single scattering model

Applying the first-order Born approximation, the OCT signal amplitude in a homogeneous turbid medium is commonly modelled as a single exponential decay function, assuming the detected backscattered light has only interacted with the sample in one scattering event. Further, assuming no absorption or noise, the mean OCT amplitude at depth $z$ is expressed as:

$$\langle A(z) \rangle \propto A_0 \exp(-\mu_{OCT}(z))$$

where, $A(z)$ is the OCT signal amplitude; $A_0$ is the amplitude at $z=0$; and $\mu_{OCT}$ is the attenuation coefficient. For expressions of the intensity of the OCT signal, Eq. (1) can be

$$\langle I(z) \rangle = I_0 \exp(-2\mu_{OCT}(z))$$

where $I(z)$ is the OCT signal power at depth $z$.
squared \( <I(z) > = \langle A(z) \rangle^2 \)), introducing a factor of 2 in the exponent. Eq. (1) does not take into account the optical system involved, which has a collection efficiency determined by its numerical aperture, confocal point spread function, as well as other factors influencing its sensitivity versus depth. Factoring in the system’s coupling efficiency \( \alpha \), confocal point spread function\(^{24,25} \), and the sensitivity roll-off\(^{26,27} \) to Eq. (1), the mean OCT amplitude, neglecting noise, is expressed as Eq. (2):

\[
\langle A(z) \rangle = \alpha \cdot t(z) \cdot h(z) \cdot \sqrt{\mu_{b,NA}} \exp(-\mu_{OCT}(z-z_0))
\]  

Introducing calibration factor \( \alpha \), \( A_0 \) in Eq. (1) can be written as the backscattering coefficient \( (\mu_{b,NA}) \) within the numerical aperture (NA) of the system. \( z \) is the depth from zero-delay, \( z_0 \) is the depth from the sample boundary, \( h(z) \) describes the sensitivity roll-off of the spectral-domain and swept-source system. \( t(z) \) is the confocal point spread function:

\[
t(z) = \frac{1}{\sqrt{\left(\frac{z-z_f}{2nz_{R0}}\right)^2 + 1}}
\]  

where \( z_f \) is the location of the focus; \( z_{R0} \) is the Rayleigh length; and the refractive index of the medium is denoted by \( n \). The factor of 2 in \( 2nz_{R0} \) takes into account the increase in \( z_{R0} \) for a diffuse reflector compared to specular reflector.\(^{24,25} \) This factor was found by van Leeuwen et al.\(^{24} \) when comparing the axial response from well-controlled scattering samples and validated with data from different locations of the focal plane using the single scattering model.\(^{25} \) The sensitivity roll-off of the system, \( h(z) \), depends on the sampling of the interference fringes. For spectral-domain OCT systems, this is determined by the pixel resolution and spectral resolution of the spectrometer. For swept-source OCT systems, the integration time of the detector and the instantaneous linewidth of the

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**Figure 1.** Propagation of a focused OCT beam and scattering in the sample.
source determine the roll-off. These contributions are described by a sinc function and a Gaussian function, respectively:

\[
h(z) = \text{sinc} \left( \frac{\pi}{2} \cdot \frac{z}{z_{\text{max}}} \right) \cdot \exp \left( - \frac{\pi^2 s^2}{16 \ln(2)} \cdot \left( \frac{z}{z_{\text{max}}} \right)^2 \right)
\]

(4)

where \(z_{\text{max}}\) is the maximum imaging depth of the system; and \(s\) is the ratio of spectral resolution to the sampling interval.\(^{26}\) The decrease of the OCT signal amplitude in depth due to the system’s sensitivity roll-off is usually less for swept-source systems compared to spectral-domain systems, resulting in a higher SNR at greater depths.

**Confocal PSF and sensitivity roll-off correction**

To determine \(\mu_{\text{OCT}}\), the OCT depth response needs to be corrected for confocal PSF and sensitivity roll-off given in Eqs. (3) and (4), respectively. The instrumental response function (confocal PSF and sensitivity roll-off) can be experimentally determined by the measurement of a reflector at increasing depths\(^{26}\) and a knife-edge measurement\(^{28}\), or using a very weakly scattering calibration sample, the confocal PSF and sensitivity roll-off functions can be obtained by fitting Eq. (2) to the OCT signal amplitude, with \(s\), \(z_i\) and \(z_{R0}\) as free parameters. These system-dependent parameters can be fixed when fitting the OCT data to Eq. (2). Alternatively, the OCT data can be corrected by dividing the OCT data by the instrumental response function.\(^{11,13}\) Because the measured axial resolution is influenced by the refractive index of the sample, it is desirable for the calibration sample to have a refractive index similar to the samples to be studied.

**Calibration samples and tissue-like phantoms**

For reliable calibration of OCT systems and analysis methods, samples with well-controlled optical properties are often used. By tweaking the optical properties to match those of tissue, systems and algorithms can be tested on tissue-like samples, often referred to as tissue-like phantoms, to mimic real measurements and study changes of system properties on those measurements.\(^{29}\) Tissue like phantoms are usually made from a non-scattering matrix to which scattering particles and possibly absorbing particles are added.\(^{29}\) The concentration of the scattering particles is commonly calculated using Mie theory. However, when dealing with concentrations of approximately > 2% by volume, concentration-dependent scattering effects have to be considered to accurately evaluate the scattering properties – which is not taken in to account by Mie theory alone. For homogenous and isotropic samples and phantoms for which spherical monodisperse scattering particles are used, which is commonly the case (silica and polystyrene microspheres), the concentration-dependent scattering properties can be calculated by combining the Percus-Yevick pair correlation function with Mie theory.\(^{23,30}\)
Nonlinear least squares curve fitting is the most frequently applied and most straightforward approach to obtaining $\mu_{\text{OCT}}$ using the single scattering model. After selection of the region of interest (ROI), Eq. (2) is fitted to the OCT amplitude data. The free parameters $A_0$ and $\mu_{\text{OCT}}$ are then obtained together with uncertainty and goodness-of-fit estimates (e.g., $R^2$, residue). Alternatively, a linear fit to the logarithmic OCT data can be performed after subtraction of the logarithmic confocal PSF, logarithmic sensitivity roll-off and noise from the data. The linear fit on logarithmic data is the computationally more efficient, but can be less robust than the exponential fit on linear data; Yuan et al.\textsuperscript{31} show that high attenuation coefficients (~5 and ~7 mm\textsuperscript{-1}) are underestimated by this approach for ROIs larger than 800 and 600 μm, respectively due to the larger weight of the low amplitudes at the end of the ROI. This could be readily dealt with by weighing the data point by their SNR.\textsuperscript{32} Challenges within the fitting method are the influence of speckle noise on the goodness of the fit, the ROI selection, and noise level selection.

To suppress speckle and smooth the axial profile in order to achieve better fits lateral and/or axial averaging can be applied. By averaging the resolution at $\mu_{\text{OCT}}$ which is determined is decreased.

The range in which the fit can be considered valid can be assisted by a comparison between the modeled signal decay within the ROI, compared to the noise level of the OCT system; the range typically is in the order of a few times the attenuation length. By performing multiple fits with a small depth variation of the ROI, an average and standard deviation of $\mu_{\text{OCT}}$ can be obtained, in this way the influence of the ROI selection on the obtained value of $\mu_{\text{OCT}}$ can be taken into account.\textsuperscript{23} Heterogeneity in the tissue and buildup of multiple scattering can be mitigated by adaptively choosing a fitting ROI and

Figure 2. Upper graph shows a plot of the confocal point spread function estimated by Eq. (3) for a lens with a Rayleigh length of 0.56 mm with the focus at 0.56 mm in depth. Lower graph shows the sensitivity roll off function for OCT system with $s^2=2.7$. 

Fitting method
analyzing each A-line in sections. A requirement for the fitting method is that enough data points in depth must be included in the ROI to obtain a sufficiently reliable fit and a good estimate of the attenuation coefficient. The optimal ROI length depends on the attenuation coefficient and the smoothness of the averaged A-line. Consequently, fitting of very thin tissue layers (e.g., retinal layers) can be problematic.

**Depth-resolved method**

The depth-resolved method was proposed by Vermeer et al. for pixel-based determination of $\mu_{OCT}$ inspired by the analysis of ultrasound data. It has been much more recently proposed and much less investigated, but is extremely appealing as it does not require an axial fitting range beyond the OCT axial resolution. Based on the single scattering model, $\mu_{OCT}$ is calculated based on two main assumptions: 1) all the light is attenuated within the OCT image depth range; and 2) the backscattered light is a fixed fraction of the attenuation coefficient, i.e., the ratio $\mu_{b,NA}$ over $\mu_{OCT}$ within every pixel is constant. Assuming a constant intensity over a pixel, the attenuation is expressed as:

$$\mu_{OCT}[i] = \frac{1}{2\Delta} \log \left(1 + \frac{I[i]}{\sum_{i+1}^{\infty} I[i]}\right)$$  \hspace{1cm} (5)

Where $i$ is the $i_{th}$ pixel along an A-scan, $I[i]$ is the intensity of the signal at the $i_{th}$ pixel. This equation can be simplified by applying a first-order linearization of $\log(1+x)$ around $x=0$ to:

$$\mu_{OCT}[i] \approx \frac{I[i]}{2\Delta \sum_{i+1}^{\infty} I[i]}$$  \hspace{1cm} (6)

Please note, that the summation approaches infinity, which in experiments is not possible, therefore, the values of the OCT signal at the last pixel in the image should be close to 0, which corresponds to the first assumption of fully attenuated light within OCT imaging depth, in order to obtain reliable $\mu_{OCT}$ values. System parameters (sensitivity roll-off and confocal PSF) were not corrected for, however an estimate of the confocal point spread function for their system was made and demonstrated to be relatively small compared to the scale of $\mu_{OCT}$ estimates. Smith et al. applied the depth-resolved method and introduced terms to considering both the sensitivity roll-off and confocal PSF to the method. The depth-resolved method was validated on homogeneous and layered phantoms by Vermeer et al. and showed to provide a way to estimate the attenuation coefficients with a higher axial resolution compared to the fitting method. Moreover, the depth-resolved method was implemented for analysis of intravascular OCT data. As pointed out above, the depth-resolved method rests on the assumptions of complete attenuation on an A-scan and fixed ratio between $\mu_{OCT}$ and $\mu_{b,NA}$. The former becomes more problematic for pixels towards the end of the A-scan and when prominent multiple scattering background is present, which can be eliminated by carefully choosing a cutoff constant. The latter assumption does not hold in case of absorption.
Comparison between fitting and depth-resolved method
The fitting method requires a user-selected or automatically delineated ROI, and depends on averaging or other form of smoothing to enable the analysis of speckle data. This processing is not required by the depth-resolved analysis, which thus preserves image resolution in the attenuation images. It does mean that speckle is still present in the attenuation data, which can lead to a prominent artifact or is suppressed by median filtering after analysis. As the ROIs in the fitting analysis are independent, extraction of the backscattering coefficient is difficult as it depends critically on the accuracy of the estimate of attenuation in the overlaying tissue, and on the intensity calibration of the OCT system. The fitting and depth-resolved methods are formally equivalent, but their practical implementations introduce some differences in the results. In summary, the depth-resolved method shows much more detail in axial direction, but requires assumptions on the light decay and does not work in case of absorption. Coincidentally, the depth-resolved method has been formulated in terms of the OCT intensity, while studies using the fitting approach have used the interferogram amplitude. This detail results in a factor 2 difference in the reported $\mu_{OCT}$ values.35

Multiple scattering model
In addition to single scattered light, multiple scattered light that matches the detected optical path length set by the reference delay contributes to the OCT signal.36–39 In highly forward scattering tissues, and tissues containing blood vessels multiple scattering can be expected due to highly forward scattering of red blood cells.40–42 Figure 3 shows the difference in OCT amplitude for an anisotropic and forward scattering sample in order to demonstrate the contribution of multiple scattered light. The contribution of multiply scattered light leads to a lower resolution and introduces a signal additional to singly scattered light. The contribution from multiply scattered light increases for: 1) larger depths43; 2) samples with strong forward scattering23; 3) samples with high scattering coefficients23; and 4) for low numerical apertures.43 Faber et al. have shown that, for scattering media with $\mu_s<6$ mm$^{-1}$, the single scattering model-based $\mu_{OCT}$ gives a good estimate of $\mu_s$.25,44,45 Experiments on samples with controlled optical properties show that multiple scattering starts to contribute significantly to the OCT signal for samples with $\mu_s>10$ mm$^{-1}$ or $g>0.8$.23 To date, there are two main approaches to take into account multiple scattering in OCT, probabilistically with Monte Carlo simulations46,47 and analytically with the extended- Huygens Fresnel model for OCT.38,48,49

Monte Carlo simulations
Monte-Carlo simulation is a probabilistic approach to simulate the backscatter trajectory of photons from the sample. The simulation tracks the trajectory of photons in the sample arm and outputs the photon count and corresponding path lengths. An assumption for
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the phase function of the sample is needed as an input to the simulation, and restrictions on photon count and trajectory are required to create time efficient simulations. Multiple studies have been done on Monte Carlo-based simulations of the OCT signal. Jacques et al. applied Monte Carlo simulation to derive a general equation to correct the OCT attenuation coefficient for the contribution of multiple scattering to determine tissue optical properties, including μs and g. This approach was applied in subsequent studies by Levitz et al. to study the growth of collagen gels. In general, Monte Carlo simulations do not model the interference of the reference with the sample light explicitly. Karamate et al. combine their analytical model, in which the coherence is taken into account, with Monte Carlo simulations to account for both single and multiple scattered light. A limitation of Monte Carlo simulations is that the obtained results depend on the specific chosen input parameters, such as the system specific optical geometry, and the phase function, which is generally not well known for tissue.

Extended Huygens-Fresnel model
The extended Huygens-Fresnel model (EHF) for OCT was introduced by Schmitt and Knutte, and elaborated by Thrane. The model assumes the paraxial approximation (i.e., sin(θ) ≈ θ, where θ is the angle of the wavefront relative to the optical axis) and theory is applicable to samples with g > 0.7. The mean OCT intensity, is expressed in

Figure 3. Average OCT amplitude for samples of silica beads in water demonstrating the difference in the decay of the slope due to multiple scattering. 0.5 μm beads (black) with scattering coefficient of 5 mm⁻¹ and anisotropy factor of 0.1 and 1.5 μm beads (red) with scattering coefficient of 5 mm⁻¹ and anisotropy factor of 0.9. The OCT data is collected using a swept source 1300 nm system with a 150 focal length detection lens.
three terms: the singly backscattered field, the multiply (forward) scattered field, and
the coherent cross-term between these two fields. The expression for the mean squared
OCT amplitude (which is equal to OCT intensity) is:

\[
\langle A^2(z)\rangle \propto \frac{1}{w_i^2(z)} \left[ \exp(-2\mu_z) + \frac{4\exp(-\mu_z)[1-\exp(-\mu_z)]}{1+w_s^2(z)/w_i^2(z)} + [1-\exp(-\mu_z)]^2 \right] w_i^2(z)
\]

(7)

\[w_i^2(z) = w_0^2 \left( \frac{z-z_f}{2n Z_{RO}} \right)^2 + 1\]

(8)

\[w_s^2(z) = w_i^2(z) + \frac{1}{3} (\mu_z) \theta_{RMS}^2 (\ell_\perp).
\]

(9)

where \(z\) is the depth coordinate in tissue measured from the sample boundary at \(z_0\).
Equation (8) is the expression for the local beam waist in the absence of forward scattering, \(w_i\). \(w_0\) is the beam waist at the focus in air. The factor of two in front of the
Rayleigh length of the beam is introduced to account for the doubling of the Rayleigh
length for diffuse reflection, as described above.25 Equation (9) is the expression for
the local beam waist in the presence of multiple forward scattering, \(w_s\).49 Based on the
EHF model, multiple scattered light influences the OCT signal at all depths. Assuming
highly forward scattering media, the EHF model can be fitted to the OCT data using
Eq. (7) to obtain tissue optical properties, including \(\mu_s\) and \(\theta_{RMS}\). \(\theta_{RMS}\) is the root mean
square of the average scattering angle and related to the scattering anisotropy through
\[\theta_{RMS} = \sqrt{2(1 - g)}.\] A drawback of the EHF model is that \(\theta_{RMS}\) and \(\mu_s\) are codependent
parameters, which means that a change in \(\theta_{RMS}\) can be compensated with a change in
\(\mu_s\) without any change in the outcome of the fit statistics.25 A priori knowledge of \(\theta_{RMS}\)
and/or \(\mu_s\) of the sample can be used to restrict the fit.30 Alternatively, the EHF model is
used with a priori knowledge of \(\theta_{RMS}\) and \(\mu_s\) for controlled silica bead samples to simulate
the OCT signal, in order to estimate the contribution of multiple scattering to the single
scattering model obtained \(\mu_{OCT}\). The model-based estimations were in good agreement
with the experimental data for a large range of scattering and anisotropy values in silica
bead samples.23

Modeling of the OCT signal with Maxwell’s equations

A full wave mathematical model, based on Maxwell’s equations, of the OCT image
formation is introduced by Munro et al.54-56 Using this model, the 3D OCT images can
be simulated. Compared to the above mentioned models, the Maxwell’s equation based
model does not need to assume the first-order Born approximation or consider an en-
ssemble average of the scattering particles. This full wave approach could in general allow
modeling of the OCT signal for a variety of system configurations, beam geometries
(Gaussian/Bessel) and (sub-resolution) sample parameters without violating any approxi-
mations made in the model. The refractive index distribution of the sample is used as an input parameter, which works well for controlled phantoms, however at the moment not known for biological tissue. In the latest publication on this model, Munro\textsuperscript{55} reports on current limitations of modelling high numerical apertures and the limited computational speed. Improving the model on these main disadvantages in addition to accessibility and user-friendliness of the software, this rigorous model had the potential to be a useful tool for verification of quantitative imaging and approximated models.

In summary, a variety of models for the OCT signal are proposed in literature, ranging in complexity from a single exponential fit to a full wave mathematical model based on Maxwell’s equations. The most simple models lack consideration of system parameters and the contribution of multiple scattered light. However, as the model increases in complexity, more input parameters are required. For the EHF model this result in codependent sample related parameters, which can’t be determined independently without a priori knowledge of $\mu_s$ or $g$. For Monte Carlo based estimations the same problem arises for which the scattering phase function has to be assumed. Although multiple models for the OCT signal are studied in literature, the most frequently applied is the single exponential model. The single exponential model together with accurate correction for system parameters provides a comprehensive approach without mutual dependence of the fit parameters. Alternatively to fitting the single scattering model, the pixel method can be applied to estimate $\mu_{OCT}$ per pixel in depth, avoiding assumptions of tissue homogeneity in the axial direction. However, more research to validation and determination of the limitations and challenges of this method seem needed.

APPLICATIONS OF OCT ATTENUATION

OCT attenuation has been extensively used to provide additional contrast to the OCT structural information for the characterization of tissue. These applications include the analysis of individual A-scans or B-scans to quantify the attenuation values, or of volumetric scans to map the 2-D distribution of the attenuation in the lateral plane as en face attenuation images, which in general terms is referred to as parametric imaging.\textsuperscript{8,57} This section presents a survey of such applications to various tissues, including skin, arteries and tissues with cancer, summarizing the characteristic attenuation coefficients of normal and diseased tissues.

Applications in dermatology
Cutaneous tissue in humans comprises a superficial thin cellular epidermis overlaying a thicker layer of dermis with various scatterers, mainly the abundant collagen fibers in the dermal extracellular matrix. The OCT attenuation in the dermis has been assessed
in vivo but there is generally limited data in the epidermis\textsuperscript{1}, because on many parts of the body it is too thin (~100 μm) to be readily amenable to measurement. Shortly following the early development of OCT, Schmitt et al. presented the single-scattering model incorporating a beam-divergence function in the OCT signal model to quantify the attenuation coefficient of tissue.\textsuperscript{1} This beam-divergence function is similar to, but different from the confocal PSF as a diverging beam is employed instead of a focusing beam in common OCT systems. They applied their method to normal cutaneous dermal tissue in vivo at multiple body locations on two human subjects, including the forearm (mean $\mu_t$: 4.6-4.7 mm$^{-1}$), finger (mean $\mu_t$: 3.7-5.0 mm$^{-1}$) and lip (mean $\mu_t$: 2.0 mm$^{-1}$). Later, Kholodnykh et al. studied and corrected the systematic errors in the quantified attenuation coefficient due to the confocal PSF.\textsuperscript{44} They applied their method to human forearm in vivo, reporting much higher attenuation values of dermis ($\mu_t$: 10-13 mm$^{-1}$) than Schmitt et al. at the same mean wavelength (1300 nm). They attributed this difference to the different experimental protocols, such as the pressure due to the contacting probe and the use of clearing agent (i.e., glycerol) in Schmitt et al.’s work. It is well known that compression of a sample induces more scattering. This phenomenon is largely the simple consequence of increasing the gradient of the refractive index by reducing the axial dimensions between scatterers, but its effect on the OCT attenuation coefficient has not been well studied. On the other hand, optical clearing as an approach to enhance the light penetration into tissue has been shown to give rise to decreased attenuation coefficients in rat skin.\textsuperscript{58}

The single-scattering model used in these studies assumes tissue homogeneity over the depth range used to estimate the attenuation coefficient. However, the extracellular matrix in the dermis is perfused with a network of blood vessels with highly distinct optical properties (determination of the attenuation coefficient of whole blood is challenging due to high forward scattering of red blood cells\textsuperscript{40}. By using appropriate models to calculate optical properties of whole blood a value of $\sim$0.96 is found for the scattering anisotropy and a value of 35 mm$^{-1}$ for the scattering coefficient at 1300 nm, which is close to complied data from literature of 40 mm$^{-1}$ at 1300 nm),\textsuperscript{59} signifying a distinct lack of homogeneity of the dermis. Additionally, many conditions are characterized by visible redness of the skin, indicating higher levels of blood than in surrounding tissues. This inhomogeneity lead to artifacts in the estimated attenuation coefficients when the vessel is contained in the fitting window, such as shown in Figs. 4(b) and (c).\textsuperscript{60} Such artifacts include either underestimation or overestimation of the attenuation, depending on the size of the fitting window and its depth position relative to the vessels. To mitigate this obvious source of inhomogeneity, Gong et al. presented a method to identify and mask the blood vessels from the attenuation estimation, using OCT speckle decorrelation for their detection, and mapped the 2-D spatial distribution of the attenuation coefficient of the remaining tissue.\textsuperscript{60} The resulting dermal attenuation coefficient from normal hu-
Parametric imaging of attenuation by OCT: a review

Man subjects (n = 6) is 6.3±0.5 mm\(^{-1}\). Another distinction of their work is the use of a polarization-sensitive OCT (PS-OCT) scanner to mitigate the possible errors in the quantified attenuation coefficients due to the birefringence of dermal collagen, measured to be 0.4-1.3 × 10\(^{-3}\) by Gong et al. at 1325 nm wavelength,\(^61\) and 0.5-1.1 × 10\(^{-3}\) by Pierce et al. at 1300 nm wavelength.\(^62\)

Using the attenuation coefficient of normal dermis as the baseline, OCT attenuation has been applied to the assessment of cutaneous conditions, such as psoriasis, which is characterized as patches of abnormal skin resulting from autoimmune disease. Welzel et al. demonstrated a lower attenuation coefficient (μ\(_t\): 2.9±0.9 mm\(^{-1}\); n = 28) in the upper dermis at 1300 nm than in normal human skin (μ\(_t\): 3.6±1.5 mm\(^{-1}\); n = 28).\(^64\) Their longitudinal monitoring further indicated the increase of the OCT attenuation in psoriasis after therapy (μ\(_t\): 3.8±1.7 mm\(^{-1}\)), approaching the normal skin attenuation (μ\(_t\): 4.2±1.6 mm\(^{-1}\)). They believe these characteristic attenuation coefficients are associated with inflammation in psoriasis, which can impact the density and distribution of collagen fibers and, thus, the scattering properties of dermis.

Another example of the application of OCT attenuation to cutaneous conditions is the assessment of human burn scars. Burns arise from various causes and lead to scarring, which presents as the proliferation of collagen and blood vessels in pathological scarring, including hypertrophic scars and keloids. In contrast to pathological scars, normotrophic scars present similar characteristics to the surrounding normal skin and represent the best clinical endpoint. To investigate the optical properties of burn scars,
Gong et al. quantified the OCT attenuation of dermis with masking of blood vessels and found significantly lower values (hypertrophic scar $\mu_t$: 3.8±0.4 mm$^{-1}$; normotrophic scar $\mu_t$: 4.2±0.9 mm$^{-1}$) than those of the contralateral or adjacent normal skin tissue ($\mu_t$: 6.3±0.5 mm$^{-1}$; n = 6), using a PS-OCT scanner. They attributed this difference to the reduced scattering in scar tissue arising from the higher water content$^{65,66}$ and supported this assertion with corresponding optical propagation simulations showing a similar trend. Es'haghian et al. further extended OCT attenuation to longitudinal assessment of hypertrophic scars undergoing fractional laser ablation treatment, as shown in Figs. 4(d) and (e)$^{63}$. They reported characteristic changes in the scar attenuation after treatment: an increase (31±27%) and decrease (13±5%) in the attenuation coefficient, respectively, in immature and mature scars; whilst there was minimal change in the higher OCT attenuation coefficient ($\mu_t$: 5.1±0.7 mm$^{-1}$) of the normal untreated skin (n = 7). The difference of these attenuation coefficients of the normal skin from those estimated by Gong et al. is likely due to the large variation of the skin locations and the use of PS-OCT by Gong et al. Overall, the characteristic attenuation coefficients of normal skin and skin conditions (Table 1), and their changes during treatment, indicate the great potential of OCT attenuation for clinical monitoring of skin conditions.

Table 1. OCT attenuation coefficient of human dermis in vivo. CPSF: confocal point spread function; SRF: sensitivity roll-off function. N/A: not given.

<table>
<thead>
<tr>
<th>Cutaneous tissue</th>
<th>Reference</th>
<th>Wavelength (nm)</th>
<th>Model</th>
<th>Correction CPSF</th>
<th>SRF</th>
<th>Location</th>
<th>Attenuation coefficient (mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal skin</td>
<td>Schmitt et al.$^1$</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Forearm</td>
<td>4.6/4.7 (mean)</td>
</tr>
<tr>
<td></td>
<td>Finger</td>
<td>3.7/5.0 (mean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lip</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kholodnykh et al.$^{44}$</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Forearm</td>
<td>10-13</td>
</tr>
<tr>
<td></td>
<td>Gong et al.$^{60}$</td>
<td>1325</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Forearm, thigh and lower leg</td>
<td>6.3±0.5</td>
</tr>
<tr>
<td></td>
<td>Es'haghian et al.$^{63}$</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Upper arm, abdomen, back, thigh and calf</td>
<td>5.1±0.7</td>
</tr>
<tr>
<td></td>
<td>Welzel et al.$^{64}$</td>
<td>1300</td>
<td>SSM</td>
<td>-</td>
<td>-</td>
<td>Including forearm</td>
<td>3.6-4.2 (mean)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Welzel et al.$^{64}$</td>
<td>1300</td>
<td>SSM</td>
<td>-</td>
<td>-</td>
<td>Including forearm</td>
<td>2.9±0.9 (untreated) 3.8±1.7 (treated)</td>
</tr>
<tr>
<td>Burn scar</td>
<td>Gong et al.$^{60}$</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Forearm, thigh and lower leg</td>
<td>3.8±0.4 (hypertrophic) 4.2±0.9 (normotrophic)</td>
</tr>
</tbody>
</table>

Applications in cardiology

The arterial system can be affected by atherosclerosis, a systemic inflammatory disease that gives rise to focal formations of fatty deposits in the vessel wall. This is a problem,
in particular in the carotid and coronary arteries, where disruption of those “plaques” can trigger thromboembolism, leading to ischemia in the brain (stroke) or heart muscle (heart attack). Catheter-based intravascular OCT is a powerful method for visualization of atherosclerosis, and is routinely applied in guidance of minimally-invasive coronary interventions. The normal structure of healthy coronary artery wall consists of three layers: a thin, bright intima; a darker muscular media that has a thickness of 200-300 μm, and the connective adventitia, which has a signal-rich and heterogeneous appearance on OCT.

Atherosclerotic plaques form in the intimal layer, which thickens under the influence of the deposition of cholesterol and related compounds. Accumulation of these species triggers an inflammatory response, which leads to hypoxic conditions and subsequent necrosis. Detection of these lipid-rich necrotic cores in atherosclerotic plaque potentially enables pre-emptive interventions by medication or stenting. Quantitative characterization of the different tissue types, including calcification and fibrous tissues, is a potentially important application of attenuation imaging.

Quantitative OCT analysis of atherosclerosis was first explored by Levitz et al. who demonstrated that there is quantitative OCT contrast in different arterial tissue components. Two studies by Van der Meer et al. showed that such information could be extracted locally and, thus, be used for imaging tissue types. The single-scattering model was applied to OCT data acquired from carotid arteries (n = 13) ex vivo with an 800 nm scanner to quantify the attenuation of various tissue constituents. Differences between tissue types (lipid-rich, fibrous intimal thickening, calcification, and thrombus) were attributed to the different scatterers in these tissues, such as the highly scattering red blood cells, which lead to a high attenuation in the thrombi. These results were compounded by imaging data of atherosclerosis at an OCT wavelength of 1300 nm. They demonstrate the feasibility of OCT attenuation to differentiate different tissue types. Angle dependence of scattering parameters was investigated by Xu et al., who demonstrated a strong dependence on imaging orientation for the highly oriented media.

Van Soest et al. further implemented OCT attenuation analysis of coronary arteries (n = 65) in a catheter-based OCT imaging, approximating in vivo clinical imaging of coronary arteries. They demonstrated the differentiation of necrotic core and macrophage infiltration (μ ≥ 10 mm⁻¹) from calcific and fibrous arterial tissue μt: 2-5 mm⁻¹) using the OCT attenuation. In vitro data were acquired with a time-domain OCT system. Figure 5 shows an example of a coronary atherosclerotic lesion with a necrotic core behind a calcified region, identified from histology in Fig. 5(b) and marked in Fig. 5(c) (red). The necrotic core and calcified region have similar signal in the structural OCT image in Fig. 5(a). Aided by OCT attenuation imaging in Fig. 5(d), the necrotic core is better contrasted with the calcified region than the original OCT image. In vivo data from this study, recorded with a prototype FD-OCT scanner, showed qualitatively and quantitatively similar attenuation patterns. These results illustrate the promise of OCT attenuation to
complement the qualitative arterial tissue classification that relies on interpretation of image texture and structural features for determination of tissue composition and plaque type. Table 2 summarizes the quantified attenuation coefficients of various tissue types.

Very recently, Liu et al.\textsuperscript{35} implemented the depth-resolved method on intravascular OCT scans acquired \textit{in vitro} on 135 images from coronary arteries on two cadaver hearts. Using a variety of signal descriptors (intensity, attenuation and backscatter), they were able to distinguish up to six different tissue types (mixed, calcified, fibrous, lipid-rich macrophages and necrotic). No correction for confocal function or sensitivity roll-off was applied.

The attenuation coefficients vary quite significantly between different studies. Qualitative identification of vascular tissue has generally followed the classification of Yabushita \textit{et al.}\textsuperscript{69}: fibrous tissue is homogeneous and signal-rich; calcified tissue is signal-poor with well-defined borders; and lipid-rich/necrotic tissue is signal-poor with diffuse borders. This set of criteria implicates low attenuation for both fibrous and calcified tissues, with high backscattering for fibrous tissue, and low for calcifications. Lipid-rich/necrotic tissue would have strong attenuation, based on a tissue optical interpretation of the qualitative classification. The attenuation (and backscattering, if available) values reported by Xu \textit{et al.}\textsuperscript{8}, Van Soest \textit{et al.}\textsuperscript{12}, and Liu \textit{et al.}\textsuperscript{35} follow this general pattern, while the contrast measured by Van der Meer \textit{et al.}\textsuperscript{7,68} appears to be inverted. A possible explanation of this difference may lie in the selection of fitting regions, which in the case of van der Meer \textit{et al.} seems to exclude the signal-rich proximal areas in attenuating tissues, causing them to derive data from the slowly varying multiple-scattering background.

Table 2 replicates the mean/median attenuation coefficient values reported by Liu \textit{et al.}\textsuperscript{35}, which are much smaller than those reported by other studies. Half of this difference is explained by their adoption of the intensity-based depth-resolved model, which results in attenuation values that are a factor two smaller than the ones extracted from the amplitude-based fitting formalism. But even when accounting for this discrepancy, their attenuation coefficients are significantly smaller. They also report maximum and 95th
percentile values for the parameters they compute. These quantifiers for the top of the distribution are in good agreement with the values reported by Xu et al.\textsuperscript{8} and Van Soest et al.\textsuperscript{12} The attenuation values computed by the depth-resolved model are affected by OCT speckle, which was filtered post-hoc by application of a median filter. It is unknown which part of the speckle-modulated attenuation distribution is representative of the true tissue-optical parameters, and how this measurement is modified by filtering.

When different arterial tissue types present similar attenuation properties, tissue characterization with OCT attenuation coefficient is ineffective. A combination of the OCT attenuation with additional OCT-derived optical properties by Xu et al.\textsuperscript{8} and Liu et al.\textsuperscript{35} resulted in statistically significant discrimination between tissues types. An example from Xu et al. is shown in Fig. 6, where the calcific (red) and fibrous (green) tissues are better differentiated using the combined attenuation and backscattering coefficient image in Fig. 6(d) than using only the attenuation in Fig. 6(b). Such combination of multiple parameters provides one promising approach to enhance tissue contrast and thus better tissue classification for future applications, if they can be reliably extracted form catheter-based measurements.

<table>
<thead>
<tr>
<th>Arterial tissue</th>
<th>Reference</th>
<th>Wavelength (nm)</th>
<th>Model</th>
<th>Correction for CPSF</th>
<th>SRF</th>
<th>Location</th>
<th>Attenuation coefficient (mm\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal thickening/fibrous</td>
<td>van der Meer et al.\textsuperscript{68}</td>
<td>800</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Carotid</td>
<td>5.5±1.2</td>
</tr>
<tr>
<td></td>
<td>van der Meer et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>3.2±1.2</td>
</tr>
<tr>
<td></td>
<td>Xu et al.\textsuperscript{8}</td>
<td>1320</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>6.4±1.2</td>
</tr>
<tr>
<td></td>
<td>van Soest et al.\textsuperscript{12}</td>
<td>1310</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Coronary</td>
<td>2±5</td>
</tr>
<tr>
<td></td>
<td>Liu et al.\textsuperscript{35}</td>
<td>1310</td>
<td>DRM</td>
<td>N</td>
<td>N</td>
<td>Coronary</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>Lipid-rich region</td>
<td>van der Meer et al.\textsuperscript{68}</td>
<td>800</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Carotid</td>
<td>3.2±1.1</td>
</tr>
<tr>
<td></td>
<td>van der Meer et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>2.3±0.5</td>
</tr>
<tr>
<td></td>
<td>Xu et al.\textsuperscript{8}</td>
<td>1320</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>13.7±4.5</td>
</tr>
<tr>
<td></td>
<td>van Soest et al.\textsuperscript{12}</td>
<td>1310</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Coronary</td>
<td>≥10</td>
</tr>
<tr>
<td></td>
<td>Liu et al.\textsuperscript{35}</td>
<td>1310</td>
<td>DRM</td>
<td>N</td>
<td>N</td>
<td>Coronary</td>
<td>2.6±0.1</td>
</tr>
<tr>
<td>Calcification</td>
<td>van der Meer et al.\textsuperscript{68}</td>
<td>800</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Carotid</td>
<td>11.1±4.9</td>
</tr>
<tr>
<td></td>
<td>van der Meer et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>26±3.2</td>
</tr>
<tr>
<td></td>
<td>Xu et al.</td>
<td>1320</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Coronary</td>
<td>5.7±1.4</td>
</tr>
<tr>
<td></td>
<td>Liu et al.\textsuperscript{35}</td>
<td>1310</td>
<td>DRM</td>
<td>N</td>
<td>N</td>
<td>Coronary</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Macrophage infiltration</td>
<td>van Soest et al.\textsuperscript{12}</td>
<td>1310</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Coronary</td>
<td>&gt;12</td>
</tr>
<tr>
<td></td>
<td>Liu et al.\textsuperscript{35}</td>
<td>1310</td>
<td>DRM</td>
<td>N</td>
<td>N</td>
<td>Coronary</td>
<td>3.4±0.4</td>
</tr>
<tr>
<td>Thrombus</td>
<td>van der Meer et al.\textsuperscript{68}</td>
<td>800</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Carotid</td>
<td>11.2±2.3</td>
</tr>
<tr>
<td></td>
<td>Kume et al.\textsuperscript{70}</td>
<td>1300</td>
<td>SSM</td>
<td>N</td>
<td>N/A</td>
<td>Coronary</td>
<td>3.8±1.0 (red)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.1±0.3 (white)</td>
</tr>
</tbody>
</table>

Table 2. Reported measured OCT attenuation coefficient of human arterial tissue ex vivo. CPSF: confocal point spread function; SRF: sensitivity roll-off function.
Applications in oncology

A promising application of OCT attenuation is the characterization of tissue in the presence of cancer. Expected changes in tissue due to abnormal cell and tissue growth caused by cancer include altered cell arrangement, density, size and size distribution of nuclei and organelles, and proliferation and changes in the organization of the extracellular matrix. McLaughlin et al. were the first to apply parametric OCT attenuation imaging to assess cancer ex vivo, extracting an attenuation coefficient for each A-scan and visualizing the spatially distributed attenuation values as an en face image (Fig. 7). Although they quantified only the relative values of attenuation coefficient, the demonstration of their method on malignant human axillary lymph nodes from breast cancer patients (n = 2) indicated the significant contrast between malignancy and healthy non-neoplastic tissue. Figure 7 shows one such example indicating the differentiation of residual healthy tissue (circled regions) in a malignant lymph node as the low attenuation coefficient regions in Fig. 7(b), which is difficult to identify using the original OCT image in Fig. 5(c). Their method was further developed and applied by Scolaro et al. for imaging the absolute attenuation coefficient in axillary lymph nodes (n = 4). They summarized the OCT attenuation coefficients of various tissue sub-types, as shown in Table 3, to guide the classification of different tissue types within the lymph node.

Figure 7. OCT attenuation imaging of a malignant human axillary lymph node. (a), (b) and (c) Histology, OCT attenuation and structural OCT image of the lymph node. The circles highlight the residual, noncancerous cortical tissue. Scale bar: 1 mm. Adapted from Ref. 14
OCT attenuation has also been used to characterize ovarian cancer, which is difficult to diagnose at an early stage due to the lack of symptoms. Yang et al. applied OCT attenuation to ovaries (n = 18) ex vivo, showing lower attenuation values in malignant tissue ($\mu_t: 1.55\pm0.46 \text{ mm}^{-1}$) than normal tissue ($\mu_t: 2.41\pm0.59 \text{ mm}^{-1}$). In a subsequent study, they demonstrated consistent contrast between the malignant ($\mu_t: 1.74\pm0.55 \text{ mm}^{-1}$) and normal tissue ($\mu_t: 2.38\pm0.67 \text{ mm}^{-1}$) in ovaries (normal: n = 26; malignant: n = 7), and further augmented the attenuation with measurement of the phase retardation caused by the optical birefringence of collagen tissue. The combination of these two parameters shows high sensitivity and specificity for the classification of malignant and normal ovary tissue. Interestingly, they correlated the two contrast mechanisms in these quantified optical properties with the lower collagen area fraction in the malignant than the normal tissue to explain the contribution of the collagen tissue to these mechanisms.

Analysis of urothelial carcinoma by OCT attenuation has also investigated. Cauberg et al. applied OCT attenuation to human bladder samples (n = 54) to assist grading of urothelial carcinoma tissue ex vivo. They reported the attenuation coefficients for different tissues, including benign tissue (median $\mu_t: 5.75 \text{ mm}^{-1}$), grade 1 (median $\mu_t: 5.52 \text{ mm}^{-1}$), 2 (median $\mu_t: 4.85 \text{ mm}^{-1}$), and 3 (median $\mu_t: 5.62 \text{ mm}^{-1}$) urothelial carcinoma at 850 $\mu$m. Bus et al. extended this analysis to patients (n = 7) in vivo, and reported the higher attenuation in grade 3 (median $\mu_t: 3.53 \text{ mm}^{-1}$) than 2 (median $\mu_t: 1.97 \text{ mm}^{-1}$) lesions. A following study on a large number of patients (n = 26) by Bus et al. further validated the higher attenuation of high grade lesions ($\mu_t: 2.9-3.7 \text{ mm}^{-1}$) than low grade lesions (median $\mu_t: 1.7-2.3 \text{ mm}^{-1}$), demonstrating the feasibility of OCT attenuation for grading low and high grade lesions.

Recently, Muller et al. used OCT attenuation to aid the characterization of prostate cancer, reporting attenuation coefficients of benign (mean $\mu_t: 3.8 \text{ mm}^{-1}$) and malignant (mean $\mu_t: 4.1 \text{ mm}^{-1}$) tissue. Kut et al. investigated the OCT attenuation of cancer and non-cancerous brain tissue from human patients (n = 37), and reported lower attenuation coefficients in cancer tissue (infiltrated zone mean $\mu_t: 2.7$ and 3.5 $\text{ mm}^{-1}$, cancer core mean $\mu_t: 3.9$ and 4.0 $\text{ mm}^{-1}$) than non-cancerous tissue (mean $\mu_t: 6.2 \text{ mm}^{-1}$). Wessels et al. applied OCT attenuation analysis to vulvar intraepithelial neoplasia (VIN), which can progress to vulvar squamous cell carcinoma. The results showed high epidermal attenuation coefficients in VIN ($\mu_t: 6.2\pm2.1 \text{ mm}^{-1}$) than the healthy skin ($\mu_t: 2.1\pm1.4 \text{ mm}^{-1}$). They also investigated cutaneous melanoma and reported lower attenuation in melanomas (mean $\mu_t: 4.28 \text{ mm}^{-1}$) than in benign lesions (mean $\mu_t: 5.49 \text{ mm}^{-1}$), indicating the promising application of attenuation for better differentiation of melanoma. The results in these studies indicate the promise of OCT attenuation as a biomarker to characterize cancer tissue.

Following the wide applications of OCT to ophthalmology, OCT attenuation analysis has also been explored to investigate eye diseases in vivo, such as glaucoma, which
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Paper</th>
<th>Wavelength (nm)</th>
<th>Model</th>
<th>Correction</th>
<th>Attenuation coefficient (mm⁻¹)</th>
<th>Tissue type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axillary lymph nodes in breast cancer</td>
<td>Scolaro et al.</td>
<td>1320</td>
<td>SSM</td>
<td>Y</td>
<td>N/A</td>
<td>Paracortex (All tissue types are from 4 axillary lymph nodes)</td>
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<tr>
<td></td>
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<td>Primary inactive cortical follicles</td>
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<td>Medullary sinus</td>
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<td>Fibrous capsule</td>
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<td>Dystrophic calcification</td>
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<td>Ovarian cancer</td>
<td>Yang et al.</td>
<td>1310</td>
<td>SSM</td>
<td>Y</td>
<td>N</td>
<td>Normal tissue</td>
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<td></td>
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<td></td>
<td>Malignant tissue (All tissue types are from 51 ovaries.)</td>
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<tr>
<td>Urothelial carcinoma (UC)</td>
<td>Cauberg et al.</td>
<td>850</td>
<td>SSM</td>
<td>N*</td>
<td>N/A</td>
<td>Benign tissue</td>
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<td>UC (All tissue types are from 54 bladder samples of 18 patients)</td>
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<tr>
<td></td>
<td>Bus et al.</td>
<td></td>
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<td>Garde 2 UC (23 lesions)</td>
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<td>Grade 3 UC (13 lesions)</td>
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<tr>
<td></td>
<td>Bus et al.</td>
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<td>Low grade UC (All tissue types are of 26 patients.)</td>
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<td>High grade UC</td>
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<tr>
<td>Prostate cancer</td>
<td>Muller et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Benign tissue</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>Malignant tissue</td>
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<td>Brain cancer</td>
<td>Kut et al.</td>
<td>1310</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Non-cancer white matter of seizure patients</td>
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<td></td>
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<td>Infiltrated zone</td>
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<td>Cancer core (All tissue types are from 16 patients in the training group.)</td>
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<td>Non -cancer gray matter of seizure patients</td>
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<tr>
<td>Kidney cancer</td>
<td>Barwari et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>Normal tissue (All tissue types are from 14 patients)</td>
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<td></td>
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<td>Renal cell carcinoma</td>
</tr>
</tbody>
</table>

* N/A: Not applicable

### References

- Scolaro et al. 13
- Yang et al. 9, 73
- Cauberg et al. 15
- Bus et al. 3
- Bus et al. 74
- Muller et al. 75
- Kut et al. 76
- Barwari et al. 77
Parametric imaging of attenuation by OCT: a review

can lead to the damage of the retina and vision loss. The OCT attenuation in the retinal nerve fiber layer (RNFL) of retina of healthy (n = 10) and glaucomatous subjects (n = 30) has been analyzed by van der Schoot et al., showing the significantly decreasing values in glaucoma (mild glaucoma $\mu_t$: 4.09±0.34 mm$^{-1}$; moderate glaucoma $\mu_t$: 3.14±0.22 mm$^{-1}$; advanced glaucoma $\mu_t$: 2.93±0.33 mm$^{-1}$) as compared to normal subjects ($\mu_t$: 4.78±0.46 mm$^{-1}$). Consistent contrast was later presented by Vermeer et al. in a study on healthy (n = 10) and glaucomatous (n = 8) eyes. Additionally, OCT attenuation coefficients of the multiple layers (including RNFL) in retina have been extracted by DeBuc et al. and Sun et al. as a tool to assess other diseases closely related to the changes in retina, including diabetics and pituitary adenoma. However, a large body of work is still needed to further study the feasibility of OCT attenuation for assessing these diseases.

Additionally, OCT attenuation has been applied to the characterization of various other tissue pathologies, such as dystrophic muscle tissue, to identify necrotic lesions in mouse models of muscular dystrophy (necrotic lesion $\mu_t$: 9.6±0.3 mm$^{-1}$ and necrotic myofiber $\mu_t$: 7.0±0.6 mm$^{-1}$ vs healthy tissue $\mu_t$: 3.9±0.2 mm$^{-1}$); and cartilage tissue to quantify the differences between the healthy cartilage ($\mu_t$: 9.7±3.3 mm$^{-1}$), repair tissue ($\mu_t$: 3.1±1.4 mm$^{-1}$) and bone ($\mu_t$: 4.5±0.5 mm$^{-1}$) in goats with induced osteochondral defects. All of these studies on OCT attenuation both at the level of initial demonstration and relatively large-scale clinical data sets have shown good potential for improved quantitative tissue characterization as compared to the use of only the qualitative OCT images.

<table>
<thead>
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<th>Skin cancer</th>
<th>Wessels et al.</th>
<th>1300</th>
<th>SSM</th>
<th>Y</th>
<th>Y</th>
<th>5.49±0.24</th>
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<td>Benign lesions (All tissue types are from 33 patients)</td>
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<td>Healthy skin (All tissue types are from 20 lesions of 16 patients.)</td>
<td>Wessels et al.</td>
<td>1300</td>
<td>SSM</td>
<td>Y</td>
<td>Y</td>
<td>2.1±1.4</td>
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<td>Vulvar intraepithelial neoplasia</td>
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<td>6.2±2.1</td>
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</table>

Table 3. OCT attenuation coefficient of human cancer tissue for various tissue and cancer types. (All results were acquired ex vivo except those from the skin cancer by Wessel et al. and UC by Bus et al.) The depth scan response in the time-domain OCT scanner was corrected. Dynamic focusing was used. Sensitivity fall-off was corrected together with the confocal function using the phantom signal. CPSF: confocal point spread function; SRF: sensitivity roll-off function.
DISCUSSION

Accuracy and precision

To extract $\mu_{\text{OCT}}$ from OCT data an appropriate model for the OCT signal is required. The ideal model would be an accurate and complete representation of the OCT signal, both including and separating system dependent parameters and tissue scattering dependent parameters. In literature multiple models describing the OCT signal are available, ranging in sophistication, with extra input parameters for which although the number of output physical parameters does not change. However, unnecessary complexity of the model should be prevented to avoid mutual dependence between parameters and the need for a priori knowledge of tissue scattering properties. Furthermore, precise determination of $\mu_{\text{OCT}}$ is desired, with a low sensitivity to the selected input parameters.

All studies discussed in the application section of this review use the single scattering model to extract $\mu_{\text{OCT}}$ from OCT data. This model assumes the contribution of only single scattered light and tissue homogeneity. The most frequently used approach to extract $\mu_{\text{OCT}}$ is by means of Non-Linear Least Squares (NLLS) curve-fitting of the single scattering model to the measured OCT data. As no mutual dependence exists between the fit parameters, the covariance matrix obtained from the NLLS fit procedure yields uncertainty estimates on the fitted parameters. These uncertainty estimates quantify how well the data is represented by the fitted model in order to estimate the accuracy of the model.\textsuperscript{23} Moreover, based on these uncertainty estimates, poorly fitted data can be excluded. Approaches to correct for system dependent parameters while fitting the single scattering model to OCT data are elaborately discussed in literature.\textsuperscript{23–25,31} The precision of this approach depends mostly on the selection of the input parameters (e.g. noise floor, ROI selection). The largest influence on variation in the extracted $\mu_{\text{OCT}}$ value are caused by the selection of the ROI for the fit.\textsuperscript{84} An average standard deviation of 1.0 mm\textsuperscript{-1} is found in the data discussed in the application section of this thesis. By performing multiple fits and changing the ROI selection by a couple of percent in the axial direction, an estimate for the precision of the $\mu_{\text{OCT}}$ value can be obtained.

Depth-resolved determination of $\mu_{\text{OCT}}$ could provide a solution for tissue inhomogeneity. Vermeer et al.\textsuperscript{33} propose such method. However, two main assumptions should be considered when applying this depth-resolved approach; 1) full attenuation of light in the A-scan and 2) absence of absorption. The first assumption can be corrected for by taking into account accurate estimates of the noise level. The obtained $\mu_{\text{OCT}}$ values are sensitive to the estimation of the noise level and thereby less reliable in case of contribution of multiple scattering. The correction of confocal point spread function, sensitivity roll-off and noise are not yet fully described for the depth-resolved method.\textsuperscript{34} It is also less clear how to quantify whether the obtained $\mu_{\text{OCT}}$ is an accurate representation of the tissue attenuation coefficient. Further research is needed to address reliable correction for the
noise level and system dependent parameters and the use of the depth resolved method on samples containing absorption.

**Limitations of the single scattering model**

Although for weakly scattering media the single scattering approximation gives an good estimate of sample scattering coefficient (i.e., $\mu_{\text{OCT}} = \mu_s$), in highly forward-scattering samples, the contribution of multiple scattering becomes significant; therefore, we use the notation of $\mu_{\text{OCT}}$ instead of $\mu_s$ to address the possible difference between both parameters.\textsuperscript{23,25,44,45} As no multiple scattering model for the OCT signal without the need for a priori assumptions on optical properties is available at the moment, the single scattering model is commonly used to obtain $\mu_{\text{OCT}}$ from clinical OCT data. Two approaches to include multiple scattering in the description of the OCT signal are proposed in literature: Monte Carlo simulations and the extended-Huygens Fresnel (EHF) model. Monte Carlo simulations are based on probabilistic photon counting simulations for which the outcome depends strongly on the chosen input parameters such as systems numerical aperture and the phase function of the sample. The EHF model is an analytical model on light propagation through homogeneous turbid media, valid for particles with a scattering anisotropy larger than $0.7$. The strength of the EHF model is that the variation of any parameter in the model may be changed easily to study its influence on the retrieved optical properties. However, because of competing parameters in the EHF model, a priori input of sample properties was needed for the simulations and estimation of multiple scattering.

**Clinical applicability and limitations**

**Tissue heterogeneity**

One crucial assumption in determination of $\mu_{\text{OCT}}$ is the homogeneity of the sample over the A-scan depth range used to estimate the attenuation coefficient. Many biological tissues possess a layered structure and are heterogeneous in the axial direction. The depth ROI of the tissue is preferably chosen such that the obtained attenuation coefficient is obtained from a homogenous region. The capacity to find such a region is highly dependent on the specific tissue. Usually the borders of tissue layers and structures are not well defined due to low scattering contrast, which makes the selection of ROI for fitting challenging. Pre-analysis segmentation of tissue layers and the use of pixel-based methods can provide for a solution. Segmentation of tissue structures forms an important part of an automated workflow for OCT attenuation analysis, using a priori knowledge of expected morphology to restrict the analysis to homogeneous tissue regions. Identification of regions for attenuation analysis typically proceeds with the first step being detection of the tissue surface in OCT images. Yuan et al. highlighted the significance of reliable surface segmentation, showing the resulting artificial nonphysical negative attenuation
coefficients from the inclusion of low signal above the tissue surface by incorrect surface detection. Tissue surface segmentation has been performed via different approaches, mainly using the high reflectance and corresponding high OCT signal at the surface. Hori et al. and Liew et al., respectively, adopted the detection of the local maximum intensity pixels and Canny edge detector to segment the skin surface. Zahnd et al. have implemented a gradient-based dynamic programming approach for layer segmentation in arterial images. Smoothing the OCT images with median or averaging filters prior to surface detection is typically important for suppressing the noise in the detected surface in these methods. The identified surface is then used to initiate the start depth for model fitting in attenuation analysis at different lateral tissue locations.

The capability to differentiate and segment distinct tissue layers in the OCT images depends on their contrast in the reflectance of OCT light. In skin tissue, the superficial epidermal layer presents lower signal than the underlying dermal layer compromising strong scatterers, such as the collagen fibers, which allows ready segmentation. In contrast, the papillary dermis and reticular dermis in the dermal layer is difficult to differentiate and segment due to the lack of contrast in the OCT images. Hori et al. segmented the epidermis by locating the pixel with local minimum intensity below the tissue surface, which is corresponding to the bottom margin of the epidermis. By estimating the thickness of the epidermis below the tissue surface, Gong et al. restricted the attenuation analysis to the dermis to avoid the possible artifacts by mixing these two different layers. Moreover, it is shown that the OCT amplitude distribution (i.e., speckle) relates to tissue homogeneity, and could be used as a measure to optimize ROI selection.

When OCT attenuation analysis is applied to tissue in vivo, the OCT scan commonly contains blood and lymphatic vessels with different scattering properties from the surrounding fibrous or stromal tissue. The blood vessels have very high scattering with strong forward scattering of the OCT light due to the large scatters (i.e., blood cells) relative to the OCT wavelength. This leads to the lower OCT signal of the blood vessels than that of the surrounding tissues, and the artifacts in the estimated attenuation coefficients if the fitting window covers the vessel signal as demonstrated by Gong et al. The lymphatic vessels have been observed by Blatter et al. to present even lower signals than the blood vessels, almost approaching noise floor due to the optical transparency of the lymph (i.e., lack of scatterers), which can lead to similar artifacts in attenuation analysis. Gong et al. presented a vessel masking approach to mitigate such artifacts by segmenting and masking the blood vessels using speckle decorrelation angiography and restricting the attenuation analysis to A-scans without vessels. The improvement of the accuracy of attenuation estimation by such vessel masking depends on the size and depth of the blood vessels relative to those of the fitting window. Their method can be further improved by additional masking of the lymphatic vessels with segmentation of vessels using OCT lymphangiography.
Data processing algorithms and representation

For clinical applicability, especially for guidance in real-time during procedures, automated analysis and real-time visualization of $\mu_{\text{OCT}}$ is necessary. The main challenges in achieving this are the processing speed of detection and segmentation of layers, whilst achieving adequate robustness and validity. A depth-resolved method is appealing in this context, as the requirements of layer and ROI selection are avoided. Linear fitting of the log OCT amplitude is faster compared to the exponential fitting of the linear OCT amplitude, as mentioned; however, the goodness of fit decreases rapidly as multiple scattering becomes significant. The expected $\mu_{\text{OCT}}$ values are then underestimated by the obtained values compared to exponential fitting. There has been considerable work on approaches and models designed to achieve fast and robust analysis. Turchin et al. used an algorithm based on a small-angle approximation of the radiative transfer equation and validated their approach on layered tissues. Yuan et al. presented a frequency-domain model, to which they compare linear log fitting and exponential fitting of the single-scattering model on silica phantoms. They conclude that their method is more robust compared to both fitting methods when dealing with incorrect surface detection. Their method is 22 times faster than an exponential fit, and more robust than the linear log fit when dealing with multiple scattering. The same method has been used to analyze ex vivo brain tumor OCT data. Reduction of the dimensionality of attenuation data, of which en face projection is an example, is a feasible method to reduce the complexity of the imaging data, which has significant advantages for validation purposes. For analysis of atherosclerotic plaques in OCT data, Gnanadesigan et al. proposed a so-called index of plaque attenuation, which represents the fraction of pixels in an en face image above a certain attenuation threshold. By comparing with histopathological analysis or human annotation of clinical pullback data, and applying receiver-operator characteristic analysis, they were able to derive attenuation thresholds for different clinically relevant plaque types. The reduction of complex, 3D attenuation data to a single parameter greatly facilitates validation and application of attenuation imaging as a basis for intervention guidance and clinical decision making. Depending on the application and clinical question, the $\mu_{\text{OCT}}$ values can be represented in an overlay on top of cross sectional B-scans or en face projection.

Requirements for successful clinical application

For the application of $\mu_{\text{OCT}}$ for tissue characterization in the clinic determination of the added clinical value is needed. Therefore, the obtained $\mu_{\text{OCT}}$ should be reliable and robust, and the sensitivity and specificity in comparison with the current gold standard should be determined.

First, sufficiently accurate knowledge of the system parameters and data output format of the OCT system are needed to ensure the use of the correct description of the OCT
signal (e.g., amplitude, intensity or dB). Such knowledge may require careful calibration for a commercial system. Calibration of the OCT system, probe and validation of the analysis method should be performed using calibrated samples with known attenuation coefficients (i.e., a phantom). Secondly, consensus on the applied model and method to extract quantitative parameters from OCT images is needed. Automated analysis software could be used to avoid human introduced biases. Moreover, open source analysis software, sample preparation guidelines and OCT data sets could be a helpful towards standardization of the data analysis.

Comparison of \( \mu_{\text{OCT}} \) results with the current clinical gold standard, which in many cases is histopathology, is needed and can be very challenging. Progress has been made in recent studies in which ex vivo OCT images and quantitative outcomes are matched as closely as possible with histopathological outcomes. Matching of in vivo data introduces additional challenges. Studies comparing both in vivo, ex vivo OCT data with histological results can be helpful, although resection of in vivo tissue is always restricted to diseased tissue.

**Additional OCT-derived optical parameters**

To enhance contrast between tissue types and detect pathology additional parameters can be obtained from the (conventional) OCT intensity images, such as amplitude/backscattering coefficient, speckle distribution. The backscattering coefficient (\( \mu_b, \text{NA} \)) of the sample detected within the detection NA of the OCT system can be obtained from the OCT amplitude after calibrating for system parameters. Changes in the backscattering coefficient compared to changes in the total scattering can be applied to estimate changes in the phase function of the sample. Kodach et al. combine measurements of \( \mu_S \) and \( \mu_B \) to obtain the anisotropy factor (\( g \)) of Intralipid. Levitz et al. experimentally obtain attenuation and amplitude (which Levitz et al. refer to as reflectivity \( \rho \)), of the development of collagen gels. Combining these measurements with Monte Carlo simulations, they conclude that changes in the architecture of the gels can be measured and quantified with OCT. Schneider et al. used OCT derived \( \mu_S \) and \( \mu_B \) combined with Mie theory calculations to determine the size of polystyrene nanoparticles. The pixel-to-pixel signal amplitude fluctuations, related to OCT speckle, are often suppressed to enhance image quality. However, speckle also carries information on sub-resolution structural sample properties. Therefore, analysis of static OCT speckle can be applied for tissue characterization. Several studies have studied the relation between speckle statistics within a ROI to structural sample properties in controlled phantoms. The relation between the OCT amplitude distribution and \( \mu_B \) for samples of silica microspheres is derived and validated. Seguita et al. map a speckle distribution-derived parameter onto OCT images. They demonstrate added contrast compared to the OCT intensity images in liver and brain tissue of rats. Moreover, other additional parameters
can be extracted from OCT data to gain information of sample properties through the interaction of tissue with light. Such as Doppler OCT, polarization sensitive OCT and spectroscopic OCT.

Future perspectives
Simulations of OCT images\textsuperscript{39,50,51,56,113} can be used to obtain information on the expected OCT image and OCT-derived parameters of tissues of interest. Monte Carlo based simulation\textsuperscript{39,50,51} for which the optical geometry needs to be modelled and assumptions of the phase function of the tissue are needed as an input. Munro et al.\textsuperscript{55,56} demonstrate a 3D mathematical model of OCT image formation, based on Maxwell’s equations. Such algorithms are a promising tool, however the structural and optical properties of the sample are needed as an input for the calculations, which is challenging.\textsuperscript{114–117} For example the refractive index of cell nuclei, for which different studies show discrepancies.\textsuperscript{4,20,21} A promising model for tissue optical properties is the continuous random media model.\textsuperscript{21} The optical properties are expressed as statistical properties such as variance, correlation length and fractal dimension. Challenging is the physical interpretation of the fractal dimension of a tissue. Further research and validation of this model will potentially enable modelling of tissue optical properties. Information on the structural and optical properties of biological tissue may potentially be obtained using a high resolution imaging modality, which enables imaging of structural changes in cells during disease progression. Knowledge on cellular changes at different stages of disease can be used to build up a library. Combined with understanding how these cellular changes influence optical properties the expected OCT signal and OCT-derived parameters can be simulated beforehand and the experimental protocol can be adjusted accordingly.

CONCLUSION
Microscopic changes in tissue structure and organization due to disease progression indicate altered tissue optical properties, which can possibly be probed by extracting the attenuation coefficient form OCT data in order to discriminate between normal and diseased tissue. In order to obtain the attenuation coefficient, multiple models describing the OCT signal are available in literature, ranging in sophistication, with extra input parameters for which although the number of output physical parameters does not change. The most commonly used model is the single scattering model, which is used in all studies on clinically relevant tissues reported in this review. The overview of these studies shows that most studies are on small sample sizes and in many cases on ex vivo samples. The reported values of the OCT attenuation coefficient suggest that although most pathologies show a change in $\mu_{\text{OCT}}$, the difference between normal
and diseased tissue is not always significant. While in some studies relative $\mu_{OCT}$ values provide added contrast, other studies combine $\mu_{OCT}$ with other OCT-derived parameters to obtain significant difference healthy and diseased tissue. Furthermore, a wide spread in $\mu_{OCT}$ values is obtained, which can be understood as a result of the use of different systems, methodologies and sample preparation. Effort towards standardization is crucial in further research.
REFERENCES


