Coherence Gated Confocal Microscopy

by

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Abstract

Optical coherence tomography (OCT) is a new, non-invasive technique for cross-sectional imaging of biological microstructures. OCT is an implementation of low-coherence interferometry, using a broad bandwidth superluminescent diode light source. By the use of high numerical aperture optics in the sample arm of the interferometer, OCT is equivalent to coherent confocal microscopy, with the added benefit of range gating of optical distance in the sample. Previous investigators have developed a single-backscatter theory which predicts the reflectance-vs.-depth profile of a sample with constant scattering density when the source is focused at the front of the sample. This single-backscatter model was modified to allow for focusing within the sample. Experimental reflectance-vs.-depth profiles were obtained in polymer microsphere suspensions of various concentrations and anisotropies of the scattering medium and compared with computer calculations from the single-backscatter model. The results help to delineate the limits imposed on coherence-gated confocal microscopy by multiple scattering.

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1 Introduction

Optical coherence tomography (OCT) is a new, non-invasive technique for cross-sectional imaging of biological microstructures [1]. OCT is essentially a single Michelson interferometer with a broad bandwidth superluminescent diode light source. When a Michelson interferometer is used with a source of narrow bandwidth, differences in the optical lengths of the arms will result in sinusoidal interference fringes superimposed on a DC offset at the detector. When the bandwidth of the source is broad, the interference fringe pattern will be multiplied by a Gaussian factor $\exp[-(l_S-l_R)^2/C]$, where $l_S$, $l_R$, and $l_C$ are the sample arm length, reference arm length, and coherence length of the source respectively. The exponential factor limits the range of differences in the path lengths of the interferometer which will contribute to the signal. If we replace one of the mirrors in the interferometer with a biological sample, the detected signal will be proportional to the reflectivity of the sample localized to within a coherence length of the reference arm length, a phenomenon we shall call coherence or range-gating. By incrementally moving the reference arm's mirror and sampling the detected signal, a reflectance profile of a sample may be obtained.

Optical coherence tomography is an extension of optical coherence domain reflectometry (OCDR), which has been used in making micron-resolution measurements in fiber-optic, integrated-optic, and biological structures [2-9]. Because the light is delivered to the sample and collected by the same single mode optical fiber, OCT has the focusing characteristics of a confocal microscope [10-11]. By combining confocal microscopy and range-gating, we can see further into tissues than we could with confocal microscopy alone because range-gating can gate out signal in front of the focusing plane instead of integrating it with the desired signal.

OCT has thus far been used to study ocular structure, but we would like to extend its use into highly scattering tissues. These tissues present difficulties in imaging since
multiply scattered light may contribute to the signal if the total pathlength travelled is the same as that travelled by singly scattered light. In the limit of purely single scattering, coherence gating could be perfectly used since the total signal received would be due to single scattering. Multiple scattering may contribute to our gated signal in a highly scattering tissue and may place a limit on how deep we can see into tissue.

We are aiming to establish the limits on coherence-gated confocal microscopy from multiple scattering and to identify any factors which may improve image quality. Our method of detecting multiple scattering contributions to the received signal is to compare experimental reflectance-vs.-depth profiles from polymer microsphere suspensions with the results from a single-backscatter model. Deviations from the single-backscatter model reflectance signal will be attributed to multiple scattering. The role of the scattering concentration and particle size on the correspondence with the single-backscatter model will be investigated.

This thesis is organized in the following format. In section 2.1 the scattering matrix formalism is described and is used in section 2.2 to explain the scanning Michelson interferometer with both narrowband and broadband sources. Section 2.3 presents the modified single backscattering model, which predicts the reflectivity versus depth of a scattering sample as a function of the scattering characteristics of the sample and the focusing characteristics of the sample arm optics. The materials and methods of generating experimental and theoretical reflectance-vs.-depth profiles are described in section 3. The experimental and theoretical reflectance-vs.-depth profiles are compared in section 4 and discussed in section 5.
2 Theory

OCT is an implementation of low-coherence interferometry, using a broad bandwidth superluminescent diode light source. The basic system and principles of OCT, and a model which predicts the single-backscattering contribution to the received signal will be explained in the following sections.

2.1 Two-port Scattering Matrix

Scattering matrices are a convenient method to relate the input and output waves of a linear system. The scattering matrix of a system with two ports of access will be briefly described since the Michelson interferometer falls into this category of systems. A schematic view of the system is shown in Figure 2.1. There is one input $a_j$ and one output $b_j$ at each port of the system. The outputs of the linear system can be described as linear combinations of the inputs as in the following equations:

$$b_1 = S_{11}a_1 + S_{12}a_2$$
$$b_2 = S_{21}a_1 + S_{22}a_2$$

where $S_{ij}$ is the weighting factor of the input $a_j$ on the output $b_i$.  

Figure 2-1: Schematic of a Two-port System
These equations can be put into matrix form:

\[ \mathbf{b} = \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} = \begin{bmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{bmatrix} \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} = \mathbf{S} \mathbf{a} \]

\( \mathbf{S} \) is called the scattering matrix of the system.

### 2.2 Scanning Michelson Interferometer

The Michelson interferometer is shown in Figure 2.2 with its two ports of access labelled [12]. An incident wave from a narrowband light source is split by a 50/50 beam splitter into two components. One of the components is reflected into arm \( R \), and the other component is transmitted into arm \( S \). Both components travel to their respective

![Figure 2.2: Michelson Interferometer](image)
respective flat mirrors and are perfectly reflected. They return to the beam splitter with an additional phase factor \(-\exp(-jk2l)\), where \(l\) is the distance from the beam splitter to the mirror, and \(k = \omega/c\). The two arms do not necessarily have the same path lengths, thus the phases of the returning waves may be different. The beam splitter recombines the two components to produce the output \(b_2\). The complete scattering matrix for the Michelson interferometer is:

\[
S = \begin{bmatrix}
-1/\sqrt{2} & j/\sqrt{2} \\
 j/\sqrt{2} & -1/\sqrt{2}
\end{bmatrix}
\begin{bmatrix}
\exp(-j2kl_S) & 0 \\
0 & \exp(-j2kl_R)
\end{bmatrix}
\begin{bmatrix}
-1/\sqrt{2} & j/\sqrt{2} \\
 j/\sqrt{2} & -1/\sqrt{2}
\end{bmatrix}
\]

The scattering matrix simplifies to:

\[
S = j\exp[-jk(l_S + l_R)]
\begin{bmatrix}
-\sin k(l_S - l_R) & \cos k(l_S - l_R) \\
\cos k(l_S - l_R) & \sin k(l_S - l_R)
\end{bmatrix}
\]

The transmitted power is:

\[
|b_2|^2 = |S_{21}|^2|a_1|^2 = (|a_1|^2/2)(1 + \cos 2k(l_S - l_R))
\]

since \(|a_1|^2\) and \(|b_1|^2\) are normalized to give the power in the waves.

For a narrowband source, periodic interference fringes will be seen at the output regardless of the magnitude of the path length difference. The fringes will have a period of a half-wavelength difference in the arm path lengths, as shown in Figure 2-3. A maximum in the transmitted power will occur when \(|l_S - l_R|\) is a multiple of a half wavelength, and a minimum occurs when \(|l_S - l_R|\) is an odd multiple of a quarter wavelength.

The previous analysis of the scattering matrix of the Michelson interferometer must be modified if the source has nonzero bandwidth. The source always consists of a band of frequencies since light is generated by electron transitions of finite duration. Further broadening of the frequency bandwidth may result from collisions of the radiating atom which change the phase of the emitted wavetrain. The temporal extent \(\Delta t_e\) over which a wave’s phase can be predicted is inversely proportional to
the frequency bandwidth, thus a broadband source will have a shorter coherence time than a narrowband source. The coherence length \( L_c \), the spatial extent over which a wave's phase can be predicted reliably, is related to the coherence time by \( L_c = c\Delta t_c \).

Let us assume that the broadband light source has a Gaussian power spectrum \( \frac{1}{\Delta k\sqrt{\pi}} \exp\left[-\left(\frac{k'}{\Delta k}\right)^2\right] \), where \( k_o \) is the center wave number, \( k' = k - k_o \), \( \Delta k \) is the wavenumber bandwidth of the light source, and \( l_C = \frac{1}{\Delta k} \) is the coherence length of the source. The time-averaged output intensity minus the DC offset is:

\[
I_{out} = \frac{1}{\Delta k\sqrt{\pi}} \int_{-\infty}^{+\infty} \exp\left[-\left(\frac{k'}{\Delta k}\right)^2\right] \cos\left[2(k_o + k')(l_S - l_R)\right]dk'
\]

\[
= \exp\left[-\left(\frac{l_S - l_R}{l_C}\right)^2\right] \cos\left[2k_o(l_S - l_R)\right]
\]
The envelope of the signal is plotted in Figure 2-4 for a source with a coherence length of 20\(\mu m\)[8], as was used in our apparatus. The output intensity is peaked when the sample arm and reference arm lengths are matched. The signal drops to \(e^{-1}\cos(2k_\omega l_c)\) of its maximum when the magnitude of the optical arm lengths differ by a coherence length. The output signal comes from a region where the arm lengths differ by a coherence length, which is 20\(\mu m\) in this case. We shall use the term coherence gating to describe the ability to choose a range defined by the coherence length over which to receive signal.

![Envelope of AC power with a coherence length of 20 microns](image)

**Figure 2-4: Interference Pattern of the Michelson Interferometer with a Broadband Source**

Now we shall place a sample in front of mirror \(M_s\), and analyze the scattering matrix once again.
With a sample in front of mirror $M_s$, the scattering matrix becomes:

$$ S = \begin{bmatrix} -1/\sqrt{2} & j/\sqrt{2} \\ j/\sqrt{2} & -1/\sqrt{2} \end{bmatrix} \begin{bmatrix} -\sqrt{R_S} \exp(-j2kI_S) & 0 \\ 0 & -\exp(-j2kI_R) \end{bmatrix} \begin{bmatrix} -1/\sqrt{2} & j/\sqrt{2} \\ j/\sqrt{2} & -1/\sqrt{2} \end{bmatrix} $$

where $R_S$ is the power reflectivity of the sample. The phase-dependent output intensity term will now be:

$$ \sqrt{R_S} \exp\left[-\left(\frac{I_S-I_R}{I_C}\right)^2\right] \cos\left[2k_o(I_S-I_R)\right] $$

The envelope of the signal is a scaled version of the plot in Figure 2-4. We can measure the output and deduce the reflectivity of the sample around the plane where the arm path lengths are matched. The path length difference can be incrementally adjusted so that the arms will be matched at a different point in the sample, and the detector output can be sampled to construct a reflectance profile.

### 2.3 Single-backscatter Model

In this section a single-backscatter model which predicts the reflectance at given plane in a sample is derived using the Fresnel integral. Schmitt, et.al. at NIH [13] have developed a single-backscatter model for optical coherence reflectometry from previous studies of atmospheric lidar [14-16]. The following analysis closely follows the atmospheric scattering work of Sonnenschein and Horrigan [16]. The main assumptions of the single-backscatter model are: (1) the arrangement of the particles is random, but the particles are separated by much more than a wavelength, and (2) only the incident light which is singly backscattered and reaches the detector remains coherent. The following analysis extends the single backscatter model of these previous investigators to include the ability to focus the source light within a sample.

A beam with a Gaussian amplitude profile is transmitted through a lens, and travels in free space to a sample where it is singly backscattered, as shown in Figure
2-5. The vector in the transmitting plane from the center of the beam to a point of interest is denoted by $r''$. Similar vectors in the scattering plane and the receiving plane are denoted by $r$ and $r'$ respectively.

![Figure 2-5: Coordinates of the Single-backscatter Model](image)

The initial distribution $u_o(r'')$ at the transmitting plane is:

$$u_o(r'') = \exp \left[ - \left( \frac{r''}{R} \right)^2 \right] \exp \left[ - \frac{i \pi (r'')^2}{\lambda f_{eff}} \right]$$

The radius $R$ of the beam is the distance from the beam center to the point where
the intensity has fallen to $e^{-2}$ of the maximum. The factor $\exp\left(-\frac{i\pi (r^m)^2}{\lambda f_{eff}}\right)$ is the phase delay caused by passing through the lens. The focal length is modified due to the change in mediums of propagation.

The focal length $f_{eff}$ can be derived by using Snell's law in the small angle limit, as shown in Figures 2.6 and 2.7.

Figure 2.6: Calculation of the Angle of Incidence

The angle of incidence can be approximated in the small angle limit as:

$$\theta_1 = \frac{D}{2f} = \frac{y}{a}$$

where $D$ is the beam diameter and $f$ is the free space focal length of the lens.

Using Snell's Law:

$$\sin \theta_2 = \frac{n_1}{n_2} \sin \theta_1$$
and considering the case when \( \theta_1 \) and \( \theta_2 \) are small, we find:

\[
\theta_2 = \frac{n_1 D}{n_2 2f}
\]

Figure 2.7: Coordinates for Calculation of \( f_{eff} \)

The total free space distance \( (a + x') \) to the point where the rays converge can be found by use of the relations \( y' + y = \frac{D}{2} \) and \( \theta_2 = y'/x' \). The total optical distance to the point of focus is:

\[
f_{eff} = a + n_2 x' = a + \frac{n_2^2 (f - a)}{n_1}
\]

In our model:

\[
f_{eff} = a + (f_{lens} - a)n_s^2
\]

where \( a \) is the freespace distance to the front of the cuvette, and \( n_s \) is the refractive index of the sample.
A wavefunction at the plane \((x, y, L_{opt})\) from the transmitting plane can be derived by using the Fresnel integral and normalizing the wavefunction to equal \(n_T\), the number of photons transmitted per second. The wavefunction \(\Psi(x, y, L_{opt})\) at the plane \((x, y, L_{opt})\) is:

\[
\frac{2n_T}{\pi \lambda RL_{opt}} \int_{-\infty}^{+\infty} dx'' \int_{-\infty}^{+\infty} dy'' u_o(r'') \exp[-\mu_s z_{opt}] \exp[i\pi(r-r'')^2/\lambda L_{opt}]
\]

where \(L_{opt} = a + z_{opt}\), and \(\mu_s\) is the attenuation coefficient. The factor \(\exp[-\mu_s z_{opt}]\) is added due to the attenuation from the transmission through the sample.

The same procedure is used to calculate the wavefunction received at the detector now using the wavefunction \(\Psi(x, y, L_{opt})\) after it has been scattered as the initial distribution. The result is:

\[
\Psi_R = \frac{S(\pi)}{L_{opt}} \exp(-\mu_s z_{opt}) \exp(-i\frac{\pi(r')^2}{\lambda f_{eff}} - \frac{\pi(r-r')^2}{\lambda L_{opt}} - kL_{opt} - \Delta \omega t))
\]

where \(\Delta \omega\) is the doppler shift caused by the moving scatterers, and \(S(\pi)\) is the scattering amplitude.

After being superimposed upon a Gaussian plane wave local oscillator \(\Psi_{REF}\), the signal is measured by a square-law detector. The heterodyne current due to one scatterer equals:

\[
i_* = 2P_o K |\gamma(\tau)| Re\int \Psi_R \Psi_{REF}^* dr'
\]

where \(P_o\) is the transmitter power, \(K\) is the optical to electronic conversion factor \(\eta e/\hbar \nu\), \(\eta\) is the quantum efficiency, \(e\) is the electronic charge, and \(\hbar \nu\) is the mean photon energy. \(|\gamma(\tau)|\) is the self-coherence function with \(\tau\) equal to the optical path time difference between the two arms. Integration of the square of the signal current over \(\tau\) and \(L_{opt}\) gives the response produced by a collection of scatterers. After integration
over $r$ we have:

$$i_s^2 = \frac{K^2 P_o^2}{4} \int_0^d \frac{\mu_b \pi D^2 \exp(-2\mu_s z_{opt}) |\gamma(\tau)|^2 dz}{2(z_{opt} + a)^2(1 + (\frac{\pi D^2}{4\lambda(a+z_{opt})})^2(1 - \frac{(a+z_{opt})}{a+(f-a)n_s^2})^2)}$$

where the backscattering coefficient of the sample $\mu_b$ [17] is the product of the uniform density of particles and the scattering amplitude, and $D$ is the $e^{-2}$ diameter of the beam. We can eliminate the integral since the response is only large when the optical path difference between the two arms is less than or equal to the source coherence length. The mean heterodyne-signal current can be approximated by,

$$i_s \approx \frac{KP_o}{2} \sqrt{R_s(z_{opt})}$$

where

$$R_s(z_{opt}) = \frac{\mu_b \pi D^2 L_c \exp(-2\mu_s z_{opt})}{2(z_{opt} + a)^2(1 + (\frac{\pi D^2}{4\lambda(a+z_{opt})})^2(1 - \frac{(a+z_{opt})}{a+(f-a)n_s^2})^2)}$$

is the reflectance of the sample at the plane $z_{opt}$. A theoretical reflectance versus optical distance into the sample plot can be made by using the equation for $R_s(z_{opt})$.

Multiple scattering degrades the quality of an image because it adds information from other planes to the image of the point of interest. We can estimate the contribution of multiple scattering to an image by finding the deviation of the signal from model which predicts the contribution of single scattering. In the following sections, experimental data obtained by using OCDR in scattering media is compared to the theoretical reflectance-vs.-optical distance profile. The experimental reflectance profile is made by incrementally adjusting the optical path length difference of the interferometer and taking measurements of the output. The comparison will help
show the contribution of the received signal from single scattering.
3 Materials and Methods

Figure 3-7 shows the confocal enhanced optical coherence domain reflectometer (OCDR) apparatus which is based on the Michelson interferometer. The light from a superluminescent diode with a center wavelength of 830nm and a coherence length of 20μm was coupled into a fiber optic.

![Diagram of OCDR apparatus]

**Figure 3-8: Confocal Enhanced OCDR Apparatus**

The fiber optic beam splitter equally divided the amplitude of the source light into the sample and reference arms. The collimated light in the sample arm was focused into a homogeneous scattering sample, and the plane of focus in the sample was adjusted by moving the sample arm’s translation stage. The path length of the reference arm was varied by moving its translation stage. Reflected light from the two arms was
recombined by the beam splitter, sensed by the detector, and digitally sampled on a Macintosh.

A 5mm glass cuvette containing a known concentration of polymer microspheres suspended in distilled water was used as the sample. The microsphere concentrations were calculated using Mie theory [17] to give the desired numbers of scattering mean free paths in the sample cuvette. Reflectance profiles of the sample were taken as a function of the location of the focusing plane. The light in the sample arm was first focused onto the surface of the tilted cuvette of microspheres, and the received signal was maximized. The reference arm's mirror was incrementally moved, adjusting the reference arm path length and the point in the sample arm where the two optical path lengths were matched, and the output at the detector was sampled.

The sample was then moved closer to the objective lens by 100μm, making the source light be focused into the sample. The path length of the reference arm was incrementally changed, the detector output was sampled, and another reflectance profile was built up. The cycle of moving the sample towards the objective lens by increments of 100μm and taking a reflectance profile was repeated until the focus position was at the noise floor.

Reflectance profiles were taken using a 5.4mm focal length 20x, 0.4 NA objective with 300nm and 0.996μm diameter polystyrene microspheres suspended in water as the sample. The focal length of the lens was experimentally determined by moving a mirror through the focus in free space to find the confocal paramter b, which is equal to $2\pi \lambda (\frac{D}{f})^2$, where D is the $e^{-2}$ diameter of the beam. Concentrations of both 100 and 200 μm mean free paths (MFPs) were used. The proportions of water to microspheres needed to achieve a given mean free path concentration were calculated using Mie theory [17].

Experimental and theoretical reflectance profiles were plotted on the same graphs for comparison. The equation for $R_s(z_{opt})$ given in Section 2.3 was used to generate the
theoretical reflectance profiles, with $D = 2.44\text{mm}$, $\lambda = 830\text{nm}$, $f=5.4\text{mm}$, and $\mu_b$ and $\mu_s$ calculated from Mie theory [17]. The diameter D of the beam was experimentally determined as the distance between the points where the output intensity had fallen to $e^{-2}$ of the maximum output voltage. The values of $\mu_b$ and $\mu_s$ for a given particle size and number of mean free paths are given in Appendix A. There was no fitting done of the theoretical curves to the experimental data.
4 Results

In this section experimental and theoretical reflectance versus optical depth into the sample profiles are shown as the position of the cuvette relative to the objective lens in the sample arm is changed. The cuvette position is equal to \( a \), the distance from the objective lens to the front of the cuvette, minus \( f_{\text{lens}} \). As the cuvette is moved closer to the lens, the focusing plane occurs deeper in the sample. All profiles were conducted with an objective lens of focal length 5.4mm.

Each experimental data set is the result of 100 averaged scans of 0.125 seconds each. The experimental curves were processed by subtracting the mean value of the noise to correct for daily variations in the voltage offset from the detection electronics and computer analog-to-digital conversion hardware. The noise floor was -120dB in the experimental plots. The logarithm of the square of the heterodyne signal is plotted, which corresponds to the power reflectivity of the sample. The experimental data was scaled into true reflectance values by dividing the reflectance of the scattering media by the reflectance from the perfectly reflecting mirror.

4.1 300nm microspheres, \( sMFP = 200\mu m \)

Figure 4-8 shows the reflectance profile for 300nm particles with a scattering mean free path of 200\( \mu m \). Towards the front of the cuvette the theoretical curves slightly overestimate the experimental data. As the distance into the sample becomes larger, the theoretical data matches the experimental data very well. A peak occurs at the focusing plane, which is much greater and sharper in the theoretical curve than in the measured value. The experimental peak appears rounded off and approximately two orders of magnitude lower than the theoretical peak. The peak at the focusing plane is still clearly visible when the cuvette has been moved forward 0.6mm (not shown).
4.2 0.996μm microspheres, sMFP = 200μm

Reflectance profiles for 0.996μm particles with a scattering mean free path of 200μm are presented in Figure 4-9. The experimental curves fit the experimental data well after the focusing plane occurs, however there is still a discrepancy between the theoretical and experimental data before the focusing plane. The theoretical peaks at the focusing plane again are much greater and sharper than the experimental data. The theoretical peaks are slightly offset from the experimental peaks. The experimental peak at the focusing plane can be recognized until the cuvette position is -0.5mm (not shown). The amount of backscattered power measured for 0.996μm particles was about an order of magnitude less than the backscattered power from the 300nm particles when the focusing plane was far into the sample.

4.3 300nm particles, sMFP = 100μm

In Figure 4-10 we see the reflectance profile for 300nm particles with a scattering mean free path of 100μm. The theoretical data underestimates the experimental data as the distance into the cuvette increases. The theoretical peaks are still sharper and greater than the experimental data. The peaks of the theoretical curves slightly precede the peaks of the experimental data. The experimental peak at the focusing plane is last seen with the cuvette position at -0.4mm. When the cuvette position is -0.5mm, the theoretical peak barely reaches above the experimental data. The peak may be hidden in the excess signal which is observed.

4.4 0.996μm particles, sMFP = 100μm

The same phenomenon of underestimation of the experimental data by the theoretical plots is shown in Figure 4-11 for 0.996μm particles with a scattering mean free path of 100μm. The underestimation of the data is less severe in the 0.996μm case. When
the cuvette position is -0.1mm, the excess signal in the 300nm case starts at 0.15mm and ends at about 1mm. In the 0.996μm case, the excess signal starts at 0.25mm and ends at about 0.7mm. The theoretical peaks are sharper and large than the experimental ones. The experimental peak at the plane of focus is last discerned easily with the cuvette at -0.2mm, which is sooner than in the 300nm case.
Figure 4-8: Reflectance Profile of 300nm particles with sMFP=200μm
Figure 4-9: Reflectance Profiles for 0.996μm particles with sMFP=200μm
Figure 4-10: Reflectance Profiles for 300nm particles with sMFP=100μm
Figure 4-11: Reflectance Profiles for 0.996μm particles with sMFP=100μm
5 Discussion

The single-backscatter model corresponded well with the experimental reflectance profiles for 300nm and 0.996μm microspheres with a scattering mean free path of 200μm. The shapes of the curves were similar, although the theoretical curves slightly overestimated the experimental data before the focusing plane. The theoretical peaks were consistently greater and sharper than the experimental peaks. More signal was received from the 300nm particles, as expected from the single-backscatter model because the backscattering coefficient μb is larger for 300nm microspheres than for 0.996μm ones. Peaks at the focusing plane were seen in the experimental data for cuvette positions up to -0.6mm for the 300nm particles and -0.5mm for the 0.996μm particles. The slight offset of the experimental and theoretical peaks may be from not fully maximizing the signal at the reference position of the cuvette.

The single-backscatter model appears insufficient in describing the reflectance profiles for 300nm and 0.996μm microspheres with a scattering mean free path of 100μm. The theoretical data underestimated the experimental data especially as the distance into the cuvette grew. Our attribution of the excess signal to multiple scattering confirms two points of the multiple scattering theory of Schmitt, et.al. [13]. The underestimation of the experimental data was more severe for the 300nm particles than for the 0.996μm particles, thus the following features of the multiple scattering theory can most clearly be seen in Figure 4.10. First, the multiple scattering contribution increases with distance into the cuvette. Second, the magnitude of the slope of the experimental reflectance-vs.-depth curves is less than that of the single-backscatter model prediction especially at large depths into the sample. The correspondence of the experimentally found increase in multiple scattering and the decrease in the magnitudes of the experimental slopes with increasing depth into the cuvette leads us to believe that the excess signal is due to multiple scattering.
In the case of a relatively low scattering mean free path, coherence gating will enhance confocal microscopy. The peaked signal at the plane of focus will be due to single scattering when the scattering mean free path is low. To make a reliable image, we want light which has only sampled the reflectivity of our point of interest to contribute to the signal. In Figures 4.8 and 4.9 we see that the size of the peak at the focusing plane is comparable to the signal closer to the front of the cuvette. Coherence-gated confocal microscopy would be advantageous to confocal microscopy in these cases since coherence-gated confocal microscopy would select the signal from the focusing plane, instead of integrating the signal before the focusing plane with the signal at the focusing plane as confocal microscopy does. Although coherence gating will improve confocal microscopy in the case of a relatively low scattering mean free path, the exponential attenuation factor in the theoretical expression for the reflectance at the plane \( z_{opt} \) may limit the depth at which signal can be received. An image cannot be made once the signal is comparable to the noise floor.

Coherence-gated confocal microscopy does not seem to provide a large improvement over confocal microscopy when the sample has a high scattering mean free path. The presence of multiple scattering at a high mean free path will degrade the image quality sinc\( z \) signal from points other than the one of interest will be included in the output signal. Selecting out the point of the focusing plane will not help when the mean free path is too high because undesired information will be a part of the signal. The presence of multiple scattering may limit us to making reliable images in lower scattering mean free path samples.
6 Conclusions

In this thesis a single-backscatter model has been developed which allows the source light to be focused within a sample. Experimental reflectance-vs.-depth profiles were obtained in polymer microsphere suspensions of various concentrations and anisotropies of the scattering medium, and were compared with the predictions of the single-backscatter model. The attribution of the deviations of the experimental data from the model to multiple scattering confirmed two points of the multiple-backscatter theory of Schmitt, et.al. [13]. Coherence-gated confocal microscopy was shown to be an improvement over confocal microscopy for a sample of a relatively low scattering mean free path, however the contribution of multiple scattering seems to decrease the benefits of coherence-gated confocal microscopy for highly scattering samples. Future studies on the role of focusing parameters on the received signal may lead to improvements in the design of coherence-gated confocal microscopy systems.
7 References


A Appendix

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<th>Scatterer Diameter ($\mu m$)</th>
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Table 1: Mie Theory Calculations