LIGHT SCATTERING SPECTROSCOPY
CLINICAL IMAGING DEVICE IMPLEMENTATION

BY

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Abstract

Recent research in Light Scattering Spectroscopy (LSS) has demonstrated it to be a powerful tool for non-invasive study of cellular structure. Cancer is associated with specific structural changes in the cell, and early detection of these features often allows for a successful treatment. Therefore LSS holds much promise for the early detection of cancer. This thesis presents and evaluates the design and implementation of a clinical imaging device based on polarized LSS for cancer detection in vivo. The device is customized for the diagnosis of cervical cancer, in particular, aiming to provide wide-area images containing size information about epithelial cell nuclei. The correct functionality of the device is evaluated by comparing experimental data obtained from tissue phantoms with theoretical predictions. It has been demonstrated that the method can accurately extract the size distribution of the particles from the scattering distribution.

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CHAPTER 1
Introduction and Background

1.1 Cancer
Cancer, one of the leading causes of death, claims one in four American lives annually. According to the American Cancer Society, about 500,000 of the 1.3 million Americans diagnosed with cancer each year are expected to die of the disease. At present, there are approximately 9 million Americans with a history of cancer [CDC].

More than 85% of all cancers originate in the cohesive and richly cellular epithelial layer lining the cavities of the body [Cotran et al., 1994]. Cancer is caused by genetic mutations leading to uncontrolled growth and proliferation of these epithelial cells. Most cancers have a well-defined precancerous stage, generally referred to as dysplasia, which is characterized by specific changes in cellular morphology. These cancer markers include increased nuclear size, increased variability in nuclear size and shape (pleomorphism), larger fraction of cell volume taken up by nuclei (“crowding”), and increased content of chromatin within nuclei (hyperchromasia) [Riddell et al., 1983].

Most cancer lesions are easily treated if diagnosed early on [Cotran et al., 1994]. Therefore, early detection of the aforementioned markers is of paramount importance in combating the disease. The difficulty is that early cancerous tissue is, in many cases,
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virtually indistinguishable from the surrounding healthy tissue. The conventional method for observing these early cancer changes relies on microscopic examination of tissue samples obtained via biopsy. The tissue samples taken from a biopsy are fixed in formalin, imbedded in paraffin, sliced into thin sections, stained with dyes, and inspected under a microscope. The dyes, such as hematoxylin and eosin, enable color-coded differentiation among morphologic structures containing substances such as nucleic acids or basic proteins. Pathologists look for enlarged and heavily stained nuclei as important diagnostic criteria. However, random biopsies are highly invasive, only sample a small fraction of tissue at risk, are prone to diagnostic error during staining evaluation, and require an extended amount of time to analyze [Cotran et al., 1994; Riddell et al., 1983].

1.2 Noninvasive Tissue Imaging and LSS

There are a number of non-invasive optical techniques promising to replace the existing invasive diagnostic procedures. By quantitatively observing light-tissue interaction, relevant structural and biochemical information about the underlying cells can be determined. Some of these techniques include absorption, fluorescence, Raman scattering, and elastic light scattering—the last being the focus of this thesis.

A number of optical modalities exist for diagnostic imaging near the surface of tissues, including fluorescence imaging, confocal microscopy imaging, optical coherence tomography, and polarization-based imaging. Some of these techniques aim to provide biochemical information associated with progression of cancer. For instance, fluorescence imaging has shown positive results in oral cancer diagnosis by detecting the presence of porphyrin compounds [Onizawa et al., 2003]. However, since the
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morphology of cell nuclei is arguably the most important diagnostic marker, the methods of more interest to work done in this thesis are those that can give insight into the size distributions of cell nuclei. Imaging methods employing elastic light scattering allow a closer look at cellular structure, including cell nuclei. One of these methods, confocal microscopy, has been used to image human tissues [Petroll et al., 1996], especially the skin and oral mucosa, both in vivo and ex vivo [Rajadhyaksha et al., 1999]. Several research groups have demonstrated the feasibility of optical coherence tomography (OCT) by imaging intra-arterial walls [Fujimoto et al., 1997] and gynecologic neoplasms [Boppart et al., 1997]. Finally, polarization-based imaging has also been used to image superficial layers of human skin [Jacques et al., 2000].

However, all of these elastic scattering methods produce physical images by detecting changes in the refractive index at boundaries and interfaces within the medium. As a result, they are diffraction-limited in resolution by the wavelength of light and the lenses used for imaging, and therefore cannot resolve fine changes in cell nuclei. Unlike these techniques, the goal of Light Scattering Spectroscopy (LSS) imaging is not to produce a physical image of a tissue and all cellular components. By using the properties of light scattering, LSS imaging aims to indirectly, yet very accurately, measure size distribution of cell nuclei within a sample of tissue. By providing this crucial insight into nuclear morphology, the LSS method can become a powerful tool in early cancer diagnosis.

LSS imaging is based upon properties of elastic light scattering. Light incident upon a medium of discrete particles scatters in a predictable manner governed by
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physical laws and boundary conditions. The spectral and angular properties of scattered light are affected by scattering parameters such as the size, shape, and refractive index of scattering particles, and the wavelength and polarization of incident light. Mie theory gives closed form solutions to the scattering distribution by spherical particles provided that the scattering parameters are known. This direct solution is useful when solving the more relevant problem to cancer diagnosis: estimating the size of the underlying scattering particles by analyzing the spectral and angular patterns of backscattered light. Moreover, LSS imaging takes the analysis one step further. By obtaining spatial images of the sample at different wavelengths, Mie theory fitting can also be used to extract the particle size in different regions of the imaged sample.

1.3 **Background Removal: Polarized LSS imaging**

In this manner, LSS imaging can be used to extract wide-area size information about nuclei within an illuminated region of epithelial cells. Moreover, the analysis is highly accurate, non-invasive, and can be done in real-time. In epithelial cells, cell organelles with higher index of refraction than the surroundings such as the nucleus, mitochondria, endosomes, and cytoplasmic vesicles account for scattering. Nuclei, the largest components with the highest index of refraction, are overwhelmingly responsible for light scattered in the nearly backward direction [Backman et al., 2001]. Therefore, the contribution of the nuclei can be highlighted by analyzing the backscattered component of light, minimizing the interference due to signals from other organelles that appear relatively inconsequential to early cancer diagnosis.
In imaging human tissue, a distinction needs to be made between single scattering and multiple scattering. The body surface of organs is comprised of a thin layer of epithelial tissue with thickness ranging from less than 10 \( \mu m \) for single layer (squamous) epithelia to several hundred microns for multiple-layer (stratified) epithelia. Beneath the epithelial layer are several layers of supporting components such as connective tissue, inflammatory cells, and neurovascular structures. A small fraction of the light incident on human tissue undergoes single scattering by the uppermost epithelial layer of cells. Most of the light is scattered multiple times by tissue beneath the epithelium or is absorbed by hemoglobin in the blood before returning to the surface in the form of diffusive background. In performing the Mie analysis, it is the backscattering signal from single scattering that allows characterization of the nuclei in the topmost layer. The multiply scattered light is, for all practical purposes, noise that interferes with the desired single scattering signal and therefore requires removal.

Previous research in LSS has demonstrated a method to remove the multiply scattered background using polarization properties of light [Backman et al., 1999]. Light that undergoes single scattering preserves its polarization whereas the polarization of multiply scattered light becomes randomized [Yoo et al., 1989]. Approximately half of the multiply scattered light is polarized parallel and the other half is polarized perpendicular to the direction of polarization of incident light. \( I_\parallel \) and \( I_\perp \) are denoted to be the overall intensity of backscattered light that is parallel and perpendicular, respectively, to the polarization of the incident light. Then \( I_\parallel \approx I_s + I_m\parallel \) and \( I_\perp \approx I_m \perp \), where \( I_s \) is the component due to single scattering and \( I_m \) is due to multiple scattering. Since it was
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proposed that $I_{m\parallel} \approx I_{m\perp}$, the single scattering component can be obtained by subtraction of the perpendicular from the parallel light: $I_s = I_{\parallel} - I_{\perp}$. Therefore, using polarized light and subtracting the orthogonal polarizations of the backscattered light, the LSS imaging instrument can effectively remove the diffusive background signal.

1.4 LSS Clinical Imaging Device and Outline of Thesis

The LSS imaging method can be applied to study a variety of cancers. One cancer of particular importance is cervical cancer—a disease that claims almost 4800 lives each year in the United States. The standard screening method for cervical cancer is through a Papanicolaou (Pap) smear that involves collecting a small sample of cervical cells. If a pathologist determines the sample to be abnormal, the woman usually undergoes additional Pap smears or a colposcopy examination. In the latter, a procedure lasting 10-15 minutes, a microscope called the colposcope is used to obtain a magnified view of the cervix. Acetic acid is used to induce water to fill the cervical cells and thus block the passage of light, thereby enhancing visualization [WH]. The physician visually scans for abnormal appearing cells, and if these cells are detected, a biopsy is performed.

As discussed earlier, these methods suffer from sampling error, rely on erroneous visual inspection, and may require substantial time.

A screening method using an LSS imaging instrument can overcome the deficiencies of these existing procedures. Such a procedure provides an in vivo, non-invasive technique for imaging the entire cervix and, in a matter of seconds, identifying the spatial size distribution of cell nuclei. The feasibility of the LSS imaging method has previously been demonstrated with T84 colon tumor cells [Gurjar et al., 2001].
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instrument presented in this thesis has been customized for the diagnosis of cervical cancer, incorporating design considerations in order to be compatible with the colposcope, and further improved for practical considerations. It is hoped that the LSS clinical imaging device will replace or at least complement existing diagnosis techniques and afford patients a more accurate, convenient, real-time diagnosis.

The remainder of this thesis documents and evaluates the LSS clinical imaging device during various stages of development and is organized as follows. Chapter 2 presents the overall design of the device and elaborates on the optical and engineering parameters. The overview of light scattering and Mie theory as well as the computer code used for fitting the experimental data is the subject of Chapter 3. Experiments with tissue phantoms and the analysis of data are presented in Chapter 4. Finally, Chapter 5 discusses future considerations and outlines the remaining work yet to be performed before the instrument can find its place in a hospital.
References


Women’s Health (WH) -

CHAPTER 2
LSS Imaging Instrumentation

2.1 Specification Overview
The goal of the LSS clinical imaging device is to provide clinically practical, accurate, real-time diagnosis of cervical cancer. The instrument allows physicians to image a circular region of cervical tissue, approximately 20 millimeters in diameter. The imaging procedure lasts 2-3 seconds, while the analysis requires a time on the order of a minute. The resultant image spatially describes the size distribution of cell nuclei, which is an important criterion in early cancer diagnosis, as described in Chapter 1. The resolution of the image can be adapted to minimize computation time for different stages of analysis. For example, the full-size image can be presented in relatively low resolution, but allows physicians to zoom in on regions of particular concern and view those with higher resolution.

2.1.1 Present Design
A schematic diagram of the LSS clinical imaging device is presented in Figure 2.1. Two high level components comprise the overall system: a desk-positioned part and a handheld part. The desk-positioned part consists of a laser light source and a computer controller for the system and image analysis. The hand-held part contains all optical
components and a charge-coupled device (CCD) camera. The two parts are connected via an optical fiber that delivers light from the source to the tissue to be diagnosed. The output of the CCD interfaces with computer software for analysis. In this way, the portability of the handheld part of the system makes the device more practical and safer for use.

**Figure 2.1.** The LSS clinical imaging device apparatus. FL: focusing lens to fiber (f=25 mm); FO: optical fiber (d=1 mm, NA=0.12); CL: collimating lens from fiber (f=14 mm); P: polarizer; L1: spatial filter focusing lens (f=73 mm); L3: collimating and imaging lens (f=300 mm); A1, A2: iris diaphragms; BS: 50/50 beam splitters; L2: viewing lens (f=148 mm); L4: inverting lens (f=65 mm); CC: calcite crystal; CCD: 1048x1392 pixel 12-bit CCD camera.

1 Adapted from a figure by Irene Georgakoudi at the G.R. Harrison Spectroscopy Laboratory
2.1.2 Improved Design

In the interest of size and weight optimization, a slight modification to the above design is proposed. The CCD camera should become part of the desk-positioned system, thus alleviating the handheld part of extra weight and bulk. Instead, a coherent optical fiber bundle shall collect the backscattered light from the imaging plane of the handheld part and guide it back to the CCD, preserving the spatial integrity of the image. There may be additional issues with noise and resolution affected by this modification. The rest of this chapter and thesis focuses on the original design described in Section 2.1.1.

2.2 Component Overview

The light source in the system consists of two modules. An Nd:YAG laser (Quantel, Les Ulis Cedex, France) with fundamental excitation at 1064 nm creates 5 ns pulses at a rate of 20 Hz. After passing the laser beam through a frequency doubler and a frequency tripler, the resultant third harmonic of the laser at 355 nm pumps an Optical Parametric Oscillator (OPO) (Opotek, Carlsbad, CA). By adjusting the phase matching conditions of its crystals, the OPO allows a tunable output of wavelengths across the visible spectrum: from 410 to 680 nm. If the OPO continuously scans across its wavelength range, it can cover the entire visible spectrum in about 3 seconds. In that manner, running the device for 3 seconds provides 60 different excitation wavelengths (at 20 Hz) for imaging and subsequent Mie analysis.

An optical fiber (Fiberguide, Stirling, NJ) carries light from the OPO to the handheld part of the instrument. The polarized light (P in Figure 2.1) passes through a
spatial filter before reaching the sample. The spatial filter (L1, A1, and L3 in Figure 2.1) serves to remove the divergent components of the beam coming out of the fiber. The aperture (A1) controls the amount of tolerable divergence. As a result, the light that hits the sample after passing through the final collimating and imaging lens (L3) will have good collimation; a well-collimated incident beam is important, as shall be seen in Chapter 3, for maintaining consistency in the scattering plane. The beam splitters (BS) allow channeling of the incident signal and the scattered signal through the system. The viewing lens (L2) allows physicians to look at tissue, just like they do during colposcopy. The focal length of the imaging lens (L3) is 300mm, identical to that of a colposcope normally used for cervical examination.

The collimated incident light hits the sample, illuminating a circular area of ~20 mm in diameter. Light that scatters in the near-backward direction passes through another spatial filter (L3, A2, and L4) before reaching the CCD camera (Roper Scientific, Tucson, AZ). The aperture (A2) allows directly backscattered light, plus a small solid angle, to pass through. Light scattered in the near-backward direction is mainly due to cell nuclei, as described in Chapter 1, and is thus vital to the imaging analysis. Before being imaged onto the CCD, the scattered light passes through a calcite crystal, a birefringent material that spatially separates the two polarizations of light. In this manner, the CCD simultaneously receives an image of both the scattered light parallel and perpendicular to the polarization of incident light. The two polarizations are necessary for background removal, as discussed in Chapter 1.
Chapter 2: LSS Imaging Instrumentation

The remainder of this chapter provides implementation details of major parts of the optical system described above. Optical parameters of components are given along with rough mathematical calculations that support the needed specifications of the system. A simulation through a ray-tracing program (ZEMAX) further corroborates the calculations that are then verified by running the system.

2.3 Instrumentation Optical Details

In analyzing the optical system for correct fulfillment of specifications, the progression of both incident and scattered light through the system must be studied closely. By using mathematical and geometrical simplifications in optics, the light can be approximately traced through the different components.

2.3.1 Incident Light Analysis

The analysis of the path of incident light in the handheld part of the device is presented in Figure 2.2. The schematic omits beam splitters and the polarizer, and it ignores the actual geometry of the device as shown in Figure 2.1. All components are assumed to be ideal and optical aberrations are ignored. Furthermore, the light is assumed to be coming out of the fiber with uniform intensity along the cross section. The pattern of light at the position of the iris is a real image of the light as it emerges from the fiber and is scaled by the magnifying factor of the ratio of the two lenses. By closing the iris aperture and cutting off some light, the light’s angle of divergence (\( \theta_2 \)) at the sample and the effective imaging diameter (\( D_{\text{eff}} \)) of the beam can be controlled.
Chapter 2: LSS Imaging Instrumentation

Using small angle approximations and geometry, the following relations are obtained:

\[ f_1 \sin(\theta_1) = f_1 NA_{\text{fiber}} \approx f_2 \sin(\theta_2) \]
\[ f_3 \sin(\theta_2) \approx \frac{D_{\text{point}}}{2} \]
\[ D_{\text{eff}} \approx D_{\text{point}} + d_1 \]

Therefore, it follows that the effective imaging diameter \(D_{\text{eff}}\) and the angle of divergence \(\theta_3\) can be expressed as:
Chapter 2: LSS Imaging Instrumentation

\[
D_{\text{eff}} = \frac{2f_1 f_3}{f_2} \text{NA}_{\text{fiber}} + d_1
\]  
(2.3.1)

\[
\theta_3 = \sin^{-1}\left(\frac{d_1}{2 f_3}\right)
\]  
(2.3.2)

With specified values of parameters \((f_1 = 14 \, \text{mm}, \ f_2 = 73 \, \text{mm}, \ f_3 = 300 \, \text{mm}, \ \text{NA}_{\text{fiber}} = 0.12,\ \text{and} \ d_1 = 5.2 \, \text{mm})\), the values obtained are \(D_{\text{eff}} = 20 \, \text{mm}\) and \(\theta_3 = 0.5^\circ\). The imaging diameter meets the specification of about 20 mm, and the angle of divergence is acceptable based on previous LSS experiments. The divergence angle can be decreased even further, improving collimation, by decreasing the iris aperture, but this is at the cost of losing power of the imaging beam.

The ray tracing simulation of incident light was performed using the ZEMAX software program. The schematic of the simulation is presented in Figure 2.3 and it confirms the mathematical calculations performed earlier.

**Figure 2.3.** Zemax simulation of the incident light beam. The left panel (a) displays the layout of all the optical components. The right panel (b) displays the profile of the beam at the position of the sample. Within one standard deviation of the peak intensity (center), the beam diameter is about 20 mm.
2.3.2 Scattered Light Analysis

The light incident on a sample will scatter in a manner described by Mie theory (cf. Chapter 3). At this moment, it is necessary to simply assume that light scatters from the sample at a variety of angles. The angles of importance are those in the near-backward direction.

A schematic of optical components relevant to the analysis of scattered light is presented in Figure 2.4. Once again, the beam splitters, actual geometry, and calcite crystal are ignored in the schematic since the primary focus is on ray tracing.

![Figure 2.4](image)

*Figure 2.4. The analysis of scattered light as it propagates from the sample to the CCD camera. L3: collimating and imaging lens (f₃=300 mm); A2: iris diaphragm (aperture diameter d₂=5 mm); L4: inverting lens (f₄=65 mm); CCD: 12-bit CCD camera 1392x1040 pixels (w=8.8 mm, h=6.6 mm).*

Ignoring azimuthal symmetry, all rays of light that scatter at some θ are mapped into the same spatial position in the plane one focal length behind the (L3) lens, which shall be referred to as the *Fourier plane* (cf. Chapter 3). By placing an iris diaphragm (A2) in the Fourier plane, the range of angles of scattering rays that pass through can be controlled.
by adjusting the aperture diameter \( (d_2) \); the range of scattering angles is important for enhancing the scattered signal from cell nuclei. Finally, the inverting lens (L4) performs an inverse Fourier transform of angular patterns at the aperture and maps an inverted image of the sample onto the CCD. The magnification factor of the two lenses (L3 and L4) scales down the real image displayed on the camera. The following equations express the diameters of the aperture and image on the CCD in terms of the system parameters:

\[
\begin{align*}
    d_2 & \approx 2f_3 \sin(\theta_b) \\
    D_{\text{image}} & \approx \frac{f_3}{f_4} D_{\text{eff}}
\end{align*}
\]

In order to emphasize the backscattering signal due to large particles (cell nuclei), the range of scattering angles should be limited to approximately \( \theta_b = 0.5^\circ \) [Gurjar et al., 2001]. Using the specified parameters of \( f_3 = 300 \text{ mm} \), \( f_4 = 65 \text{ mm} \), and \( D_{\text{eff}} = 20 \text{ mm} \), the obtained values are \( d_2 \approx 5.2 \text{ mm} \) and \( D_{\text{image}} \approx 4.3 \text{ mm} \).

Before being imaged on the CCD camera, the light will pass through the calcite crystal that will separate the parallel and perpendicular polarizations of scattered light. As a result, the two polarization components will get imaged side by side onto the CCD camera. The calculated value of \( D_{\text{image}} \) is small enough to allow both polarizations to fit on the CCD chip, as illustrated in Figure 2.5.
2.3.3 Resolution

From diffraction theory, the resolution of two objects is limited by the wavelength, distance between the objects and the observation point, and diameter of the aperture through which the objects are viewed. Below this resolution limit, the two objects appear indistinguishable [Hecht, 2002]. Using the Rayleigh criterion\(^2\), the smallest angular spacing \(\theta_{\text{min}}\) and distance \(l_{\text{min}}\) between two objects that can be resolved are:

\[
\theta_{\text{min}} \approx 1.22 \frac{\lambda}{d} \tag{2.3.5}
\]

\[
l_{\text{min}} \approx 1.22 \frac{f \lambda}{d} \tag{2.3.6}
\]

In (2.3.5), \(\lambda\) is the wavelength of light, and \(d\) is the diameter of the viewing aperture. When the objects are placed one focal length (\(f\)) away, (2.3.6) follows directly from (2.3.5). In the system, the objects (particles) are resolved in the sample plane through the

---

\(^2\)The Rayleigh criterion says that two objects are resolved when the center of Airy function diffraction pattern of one object falls over the first minimum of the Airy function of the other object.
Chapter 2: LSS Imaging Instrumentation

iris diaphragm (A2). The smallest angular spacing between the objects (as seen from L3) corresponds to the range of angles spanned by the $d_2$ diameter of the iris diaphragm (A2). Using (2.3.3) and the distance to the sample as $f_3$, (2.3.5) can be rewritten in terms of the system parameters:

\[
\frac{l_{\text{min}}}{d_2} = 1.22 \frac{f_3 \lambda}{d_2} \quad (2.3.7)
\]

\[
l_{\text{min}} = 1.22 \frac{\lambda}{2 \sin(\theta_b)} \quad (2.3.8)
\]

Keeping with the previous specification for $\theta_b = 0.5^\circ$, a resolution ranging from $l_{\text{min}} = 28$ μm at $\lambda = 410$ nm to $l_{\text{min}} = 48$ μm at $\lambda = 680$ nm can be achieved. Note that in dealing with particles of sizes comparable to cell nuclei (diameter < 10 microns) the system would be unable to correctly resolve cell nuclei on the CCD pixels due to this physical limit. However, using spectroscopy and relying on results from Mie theory, the size distribution of even particles this small can be accurately measured. Moreover, the resolution limit of tens of microns is still orders better than the 1-2 mm lower limit for detecting a tumor using present technologies [Benaron, 2002].

2.4 Status of Instrumentation

At this moment, the device has not been entirely implemented as described in this chapter. Chapter 4 evaluates the device in different stages of implementation as a function of the type of laser source used and the manner in which light is guided to the handheld optical system. Experiments and signal-to-noise analyses at each stage are used to evaluate the system performance and confirm functionality.
References


CHAPTER 3
Mie Theory and Data Analysis Tools

3.1 Introduction to Light Scattering
The topic of light scattering is well-covered in many textbooks. Much of the material presented in this Chapter follows concepts and notation from the widely used text by Bohren and Huffman (1998).

3.1.1 Physical Basis
All matter is composed of discrete electric charges: protons and electrons. If a particle, which could be a single electron, a whole atom, or a liquid molecule, interacts with an electromagnetic wave, the electric field from the incident wave sets the electric charges of the particle into oscillatory motion. These electric charges reradiate electromagnetic energy in all directions and account for what is known as scattering. Apart from scattering, some of the incident electromagnetic energy is converted to other types of energy, such as thermal energy, through a process called absorption. Although both scattering and absorption are closely linked and dependent processes, the focus in this thesis is almost exclusively on scattering.
3.1.2 Types of Scattering

All media (except for the ideal vacuum) are heterogeneous and hence scatter light. The scattering can be a result of fluctuations in density, concentration, or orientation within a medium. However, the topic of this thesis is scattering by particles, generally described as aggregates of many atoms or molecules. The problem is to describe the interaction of light at some particular wavelength with a single particle, suspended in a medium whose heterogeneity is small compared to the wavelength of the incident light. The attention is also restricted to elastic scattering, in which the wavelengths of incident and scattered light are equal. In the case of cancer detection, the particles of interest are ultimately cell nuclei and the medium is the cell cytoplasm.

3.1.3 Scattering by Single Particles vs. Collections of Particles

The fundamental problem to address is the scattering by a single particle. If the particle is divided into small regions, then the incident electromagnetic field induces a dipole in each region, which in turn scatters light in different directions. Thus the total scattered light in one particular direction is the superposition of these individual scattered wavelets in that direction. The phase of the individual dipole wavelets needs to be accounted, and it is generally a function of scattering direction. When dealing with particles that are small compared to the wavelength, all the wavelets are approximately in phase, so there is not much variation in scattering as a function of direction. However, when the scattering particles are larger, the relative phase of the wavelets deviates with direction, there are more possibilities for constructive and destructive interference. Hence as the size of the particle increases, there will be more peaks and troughs in the
scattering pattern. Since the particles of interest are cell nuclei (diameter of several microns) which are large compared to wavelength (visible light of about half a micron), these variations in scattering pattern are paramount to the size estimation.

Inasmuch as the scattering by a single particle is fundamental, of more relevant interest is scattering by a collection of particles, namely the cell nuclei within a sample tissue area. Since a rigorous theoretical treatment of scattering by many particles is extremely difficult, simplifications are often introduced. Single scattering is defined as having a sufficiently small number of particles spaced sufficiently far apart that the total field scattered by all the particles is small compared to the external field. With this assumption, each particle acts independently and in isolation from all the other particles, so that the total scattered field from the collection of particles is simply the sum of individual contributions. However, when dealing with tissue samples, there is multiple scattering, resulting from adjacent nuclei and the underlying tissue. In Section 1.3, a method was described to eliminate multiple scattering by relying on the polarization properties of incident and scattered light. For the purpose of LSS imaging, single scattering is defined experimentally and is a function of optical thickness\(^1\) \(\tau\) of the sample.

### 3.1.4 Direct vs. Inverse Problems

There are two general types of problems in dealing with interaction of electromagnetic waves and a small particle: the direct problem and the inverse problem.

\(^1\) \(\tau = C_{sca} n l\), where \(C_{sca}\) is the total scattering cross section of the (non-absorbing) particle, \(n\) is the density of the beads/mm\(^3\), and \(l\) is the thickness of the sample.
The direct problem asks to determine the field everywhere given a particle of a specified size, shape, and composition which is illuminated by a light beam of specified irradiance, polarization, and wavelength. This problem is considered “easy” as it should follow directly from physical laws and boundary conditions. The inverse problem asks to describe the particle or particles responsible for scattering given the scattered field that is produced. The inverse problem is the “hard” problem and it is the focus of attention in using light scattering to remotely estimate the nature of particles (nuclei). The general approach taken is to guess a solution for the nature underlying particles, solve the direct problem, and then compare the theoretical scattered field to the experimentally obtained scattered field. In doing so, it is useful to take advantage of a priori knowledge about certain parameters, such as the shape and the refractive index, of the underlying particles. Thus the problem is transformed into one of finding the correct particle size distribution that accounts for the observed patterns.

3.2 Scattering Notation

The scattering problem is formulated in this section, defining only the most relevant quantities. A full treatment is presented in Bohren and Huffman (1998) or Van de Hulst (1957). Given an arbitrary particle suspended in a non-absorbing medium and illuminated by an incident electromagnetic wave, the problem is to describe the scattering. The geometry of the scattering is presented in Figure 3.1. The incident wave is taken to be propagating in the \( \mathbf{e}_z \) direction and polarized in the \( \mathbf{e}_x \) direction: \( \mathbf{E}_i = \mathbf{e}_x E_0 e^{ikz} \).
The geometry defined by the scattering: the incident wave is polarized in the $e_x$ direction, and the scattered wave is polarized in the $e_r$ direction. The scattering plane is defined by the incident and the scattered wave vectors.

The direction $e_r$ of the scattered wave is defined in the spherical coordinate system by the scattering angle $\theta$ and the azimuthal angle $\phi$. The wave vectors of the incident and scattered waves define the scattering plane. In the far field, the scattering electric field is given as follows:

$$E_s \propto \frac{e^{ikr}}{-ikr} X E_0$$

The scattered wave in (3.2.1) is approximately transverse so that $e_r \cdot X = 0$, where $X$ is called the vector scattering amplitude.

\footnote{Based on Figure 3.1 on page 62 in Bohren and Huffman (1998).}
If an imaginary sphere with surface area $A$ surrounds the particle, then the total scattered electromagnetic power that is collected by this sphere is given by:

$$ W_s = \int_A \langle S_z \rangle \cdot e_z \, dA $$

(3.2.2)

In (3.2.1), $\langle S_z \rangle$ is the time averaged Poynting power of the scattered wave. The \textit{scattering cross section} $C_{sca}$ is defined as the ratio between the total scattered power $W_s$ and the incident intensity $I_i$.

$$ C_{sca} = \frac{1}{4\pi} \frac{|X|^2}{k^2} \sin(\theta) d\theta d\phi = \int_{4\pi} \frac{|X|^2}{k^2} \, d\Omega $$

(3.2.3)

The quantity $|X|^2 / k^2$ is known as the \textit{differential scattering cross section}, and will be denoted as $dC_{sca}/d\Omega$. The differential scattering cross section, which is a function of the vector scattering amplitude $X$, is of particular importance because it specifies the angular distribution of scattered light into a unit solid angle about a given direction. Therefore, given an incident intensity $I_i$, the scattered power that will reach a detector spanning some solid angle $\Delta\Omega$ can be expressed as:

$$ P_{det} = I_i \cdot \int_{\Delta\Omega} \frac{dC_{sca}}{d\Omega} \, d\Omega $$

(3.2.4)

A related term called the \textit{scattering efficiency} is defined as $Q_{sca} = C_{sca} / G$, where $G$ is the area of the cross section of the particle projected onto the plane perpendicular to the incident light.
3.3 Scattering by a Sphere: Mie Theory

The formal solution to the scattering of light by a sphere of arbitrary radius and index of refraction has been known for almost a century. In 1908, Gustav Mie developed this theory while studying the scattering by colloidal gold particles suspended in water. The mathematical theory of light scattering by spherical particles is referred to as Mie theory\(^3\). The solution to scattering by spherical particles is very useful in that many scattering objects of interest can be modeled as spherical or nearly spherical. This approximation will be used in modeling the cell nuclei.

The mathematics of Mie theory is beyond the scope of this thesis, but a good reference is Bohren and Huffman (1998). As mentioned in 3.2, the angular dependence of scattering is described physically by the differential scattering cross section. Without delving into the math, \(dC_{\text{sc}}/d\Omega\) is calculated from the vector scattering amplitude \(X\), as defined in (3.2.2) and (3.2.3). The solution is composed of spherical Bessel functions, associated Legendre polynomials, and sinusoids. The \(dC_{\text{sc}}/d\Omega\) is a function of two quantities: the relative refractive index \(m\) and the size parameter \(x\), defined as:

\[
m = \frac{n_{\text{part}}}{n_{\text{med}}}
\]

\[
x = ka = \frac{2\pi}{\lambda} a
\]

\(^3\) It is debatable that credit should have gone to Lorenz or Debye, but the term Mie theory is nonetheless used for practical consistency.
where $n_{\text{part}}$ is the index of refraction of the spherical particle, $n_{\text{med}}$ is the index of refraction of the suspending medium, $\lambda$ is the wavelength of light in the medium and $a$ is the diameter of the spheres.

### 3.4 Numerical Data Analysis Tools

Numerical simulations can be used to calculate the Mie $dC_{\text{scr}}/d\Omega$. A good reference on standard code and implementations is Barber and Hill (1990). For the purposes of analyzing data obtained from the LSS clinical imaging device, two codes were used. The first code calculates $dC_{\text{scr}}/d\Omega$ for a single wavelength at a variety of angles $\theta$ and $\phi$ near direct backscattering that correspond to the area of the detector (CCD). This *angular map* is useful for experimental data fitting if the CCD is in the Fourier plane and the excitation is with a single wavelength. The second code calculates the $dC_{\text{scr}}/d\Omega$ as a function of wavelength, integrating $\phi$ over $[0,2\pi]$ and for some specified range of $\theta$. This multi-wavelength angular map is useful when fitting data obtained with incident light over a range of wavelengths and scattering $\theta$ angles.

#### 3.4.1 Angular Maps with Single Wavelength

The computation of the angular map of backscattered light is important to our data analysis. Much of the verification of the LSS clinical imaging device functionality, described in Chapter 4, was performed by analyzing angular maps obtained with the CCD in the Fourier plane of the sample. Figure 3.2 illustrates the geometry of scattering and detection in the Fourier plane. Power scattered in the same direction specified by $\theta$ and $\phi$ from different parts of the sample gets focused onto the same point in the Fourier
Chapter 3: Mie Theory and Data Analysis Tools

plane. Note that according to the geometry in Figure 3.1, direct backscattering corresponds to $\theta = 180^\circ$. However, for notational and geometrical simplicity in the Fourier plane, it is useful to define a scattering angle $\theta_s$ relative to direct backscattering: $\theta_s = 180^\circ - \theta$. Figure 3.2 specifies how the scattered $\theta_s$ and $\phi_s$ angles as defined in Figure 3.1 correspond to radial position $r_f$ and angle $\phi_f$, respectively, in the Fourier plane.

$$r_f = f_3 \tan(\theta_s) \quad (3.4.1)$$

$$\phi_f = \phi_s \quad (3.4.2)$$

Invoking the reasoning in Section 3.1 and assuming single scattering, the power scattered by a collection of spheres (of the same size) in some direction is directly proportional to the power scattered by a single sphere, and hence to $dC_{scat}/d\Omega$ in that direction.

![Figure 3.2](image_url)

**Figure 3.2.** The geometry of scattering and the Fourier plane. Scattering and azimuthal angles $\theta_s$ and $\phi_s$ map to radial $r_f$ and angular $\phi_f$ positions on the Fourier plane as formulated by (3.4.1) and (3.4.2).
The code used for performing the angular map calculations for a single wavelength is shown in Appendix A. It is based on a standard program for the calculation of $dC_{\text{scat}}/d\Omega$ for spheres of a specified relative refractive index, size parameter, and wavelength. The code was then modified to map scattering angles to correct spatial locations in the Fourier plane$^4$. The program was further customized to partition the area of the focal plane into 131 x 175 square regions. This partition, in the interest of computational efficiency, allows for the fitting of experimental data obtained with the 1048 x 1392 pixel CCD, which after the averaged binning of 8 x 8 pixel regions results in 131 x 174 resolution.

### 3.4.2 Angular Maps with Multiple Wavelengths

It is useful to look at spectral variation of the angular maps. For example, for smaller particles, the experimental angular map with only a single wavelength $\lambda$ may not provide enough variation to form a good unique fit to theory. However, looking at the variation in scattered power over some range of angles as a function of wavelength can be more distinctly fit to theory. The spectral code integrates $dC_{\text{scat}}/d\Omega$, and hence power, over some user-specified range $[\theta_{\min}, \theta_{\max}]$ and for $\phi$ over $[0, 2\pi]$ as a function of incident light wavelength$^5$. The program also allows the specification of the wavelength range and the incremental step between wavelengths. For the purposes of the relevant LSS experiments, the range of $\theta_i$ was over $[0, 0.5^\circ]$ and the range

---

$^4$ The single wavelength angular map code was initially written by Venkatesh Gopal at the G.R. Harrison Spectroscopy Laboratory.

$^5$ The multiple-wavelength angular integration code was originally written by Vadim Backman at the G.R. Harrison Spectroscopy Laboratory.
of $\lambda$ over 410 to 680 nm. At the present time, the spectral experiments have not been performed, and hence the spectral code is not provided as part of this thesis.
References


4.1 Verification Overview

The LSS clinical imaging device was evaluated at different developmental stages. In this chapter, different experimental setups are used to analyze the scattering of polystyrene beads, which serve as the simplest phantom models for cell nuclei. In all setups, the CCD was positioned in the Fourier plane, the objective being the analysis of angular scattering patterns. Imaging, which is one additional step further than angular scattering, was ignored for the sake of simplicity. Mie theory fits were used to corroborate the experimental data with the theoretical predictions, verifying the size distribution. To complement the results, a confirmation of signal integrity and a signal to noise analysis were also performed.

4.2 Free Space System with He:Ne

4.2.1 Experimental Setup

The free space system with He:Ne was setup as shown in Figure 4.1. A He:Ne laser with 632 nm excitation is input directly into the handheld system. Note that the handheld layout is different from that illustrated in Figure 2.1, with the CCD now placed...
in the Fourier plane, one focal length behind the collimating and imaging lens (L3). The He:Ne laser provided an illumination spot with a diameter of about 6.5 mm.

![Figure 4.1. The experimental setup of the free space He:Ne system. He:Ne: laser source (632 nm, 12 mW CW); P: polarizer; L1: spatial filter focusing lens (f=73 mm); L3: collimating and imaging lens (f=300 mm); A1: iris diaphragm; BS: 50/50 beam splitters; A: analyzer polarizer; CCD: 1048x1392 pixel 12-bit CCD camera.](image)

### 4.2.2 Methods

A solution containing 44 μm polystyrene beads (n=1.54) suspended in water was prepared. A single drop of the beads solution was placed between two microscope glass cover slips. The cover slips were positioned at the sample plane so that the laser beam illuminated the sample at about 20° from normal incidence; this tilt prevented the specular reflection off the cover slips from interfering with the backscattering signal. A
neutral density filter was placed beneath the sample cover slips at a different angle to deflect the light that transmitted through the beads away from the backscattering path. The experiment was performed in a dark lab room to minimize the noise from external light sources. The CCD camera was exposed for 300 ms and 50 repetitions, for a total of 15 seconds. The parallel angular distribution was obtained with both the polarizer (P) and the analyzer (A) pointing in the upward direction (vertical). For the perpendicular angular distribution, the polarizer pointed upward (vertical) while the analyzer pointed sideways (horizontal). For each polarization state, an acquisition was performed with the beads between the slides and also a background acquisition with dry cover slips. The background was subtracted pixel by pixel from the beads data.

4.2.3 Results

Figure 4.2 shows the experimental parallel and perpendicular polarizations of backscattered light obtained after background subtraction.

Figure 4.2. The parallel (a) and perpendicular (b) polarization angular maps of backscattered light using free space He:Ne. Obtained for 44 μm beads in water, 150 seconds total exposure time. Note the granularities due to speckle.
4.2.4 Data Fitting

The patterns for both polarizations clearly have the outlining “lobes” that one would predict based on theory. To demonstrate that we can extract the size of the scattering particles from the angular maps, we needed to show that the experimental patterns for 44 μm beads closely match the theoretical predictions based on Mie theory. We used the computer program described in Section 3.4.1 to generate the corresponding angular map for 44 μm polystyrene beads (n=1.54), suspended in water (n=1.33), illuminated by 632 nm light, and where direct backscattering hits the center of a 6.6 x 8.77 mm CCD detector.

The theoretical results were compared to experimental maps from Figure 4.2, whose CCD counts were binned and averaged in 8x8 pixel regions. In the interest of clarity, we chose to compare horizontal pixel region profiles of theoretical and experimental scattering. We took the horizontal profiles in regions of the angular maps where the most lobe variation occurred. For the parallel polarization, the chosen horizontal profile was the middle row of pixels, with its midpoint at the origin (θ, = 0°). The perpendicular polarization profile was in the upper quartile of pixels, with its midpoint specified by θ, = 0.34° and ϕ, = 90°. As described in Section 1.3, diffusive background can be removed to isolate single scattering by subtracting the perpendicular from the parallel backscattering. Even though the experiments were run on beads with minimal, if any, background, we chose to fit the parallel minus perpendicular angular maps to theory as a benchmark for the future. The horizontal profile has its midpoint
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specified by $\theta_s = 0.43^\circ$ and $\phi_s = 90^\circ$. All of the aforementioned profile fits, after normalization, are shown in Figure 4.3.

![Profile with midpoint at Theta = 0](image1)

![Profile with midpoint at Theta = 0.34 and Phi = 90 degrees](image2)

![Profile with midpoint at Theta = 0.43 and Phi = 90 degrees](image3)

Figure 4.3. The fitting of experimental He:Ne profiles and theoretical profiles in the angular maps. The parallel polarization (a), perpendicular polarization (b), and the single scattering [parallel – perpendicular] (c) profiles, all normalized to scale.

4.2.5 Speckle

The intensity levels can vary greatly in even adjacent CCD pixels, as indicated by the granularities in the images and the large noise on the signal. The explanation for these granularities is owed to an effect known as laser speckle. When spatially coherent
light, as is the case with He:Ne, is scattered from a diffuse surface, it forms a stationary interference pattern in the surrounding region. The field observed at some point in space is a superposition of the scattered wavelets from different parts of the surface. All the wavelets must have a constant relative phase so that the interference is stationary. The regions of higher or lower intensity are a result of constructive or destructive interference between these wavelets [Hecht, 2002].

The speckle effect clearly affects our ability to achieve good fits between the noisy experimental data and the theoretical predictions. However, the speckle is not as pronounced with low coherence light. Therefore, it was anticipated that using the less coherent OPO and the multimode fiber, the effect of speckle would be minimized.

In assessing the correctness of the device and the experimental methods, it is necessary to verify (1) that the backscattering signal (counts) agrees with theory and (2) that the system noise is minimized, resulting in a satisfactory signal to noise ratio. Both points can be verified even in the presence of speckle and are the topics of the next two sections, respectively.

4.2.6 Signal Validation

In the interest of validating the number of counts read on the CCD, a throughput analysis of the backscattering signal was performed. The purpose was to verify that the number of counts per pixel observed at the CCD detector agreed with the physical expectations. It is argued that the speckle effect could be ignored because for a highly coherent source such as He:Ne, the speckle can be approximately modeled with a zero-mean Gaussian distribution [Dainty, 1984]. Indeed, the histogram of the images confirms
the zero-mean Gaussian assumption. So the average counts per pixel in speckled images should correspond closely to the average counts per pixel even in the absence of speckle.

The total scattered power collected by the CCD detector ($P_{\text{CCD}}$), extending from (3.2.4), can be ideally expressed as:

$$P_{\text{CCD}} = I \cdot N \cdot \int_{\Delta \Omega_{\text{CCD}}} \left( \frac{dC_{\text{sca}}}{d\Omega} \right) d\Omega$$

(4.2.1)

where $I$ is the intensity of the laser beam at the sample, $N$ is the total number of beads in the sample accounting for the scattering, $dC_{\text{sca}}/d\Omega$ is the differential scattering cross section computer from Mie theory, and $\Delta \Omega_{\text{CCD}}$ is the solid angle subtended by the detector. The intensity $I$ at the sample can be measured experimentally with a power meter. The number of beads $N$ can be determined either visually or through the optical thickness $\tau$. However, due to unknown experimental measurement accuracy of $\tau$, we decided to estimate $N$ by looking under a microscope and observing the area density of the beads between the glass cover slips.

The power collected by the detector has to take into account the optical losses incurred on the backscattering path. These losses are polarization-dependent and were measured with a power meter using a mirror as the sample. Therefore, for the parallel and perpendicular polarizations, (4.2.1) can be extended as:

$\tau = C_{\text{sca}} n l$, where $C_{\text{sca}}$ is the total scattering cross section of the (non-absorbing) particle, $n$ is the density of the beads/mm$^3$, and $l$ is the thickness of the sample. $\tau$ can be obtained from the transmitted power in the forward direction $I_t = I_0 e^{-\tau}$, where $I_0$ is the incident power.
Chapter 4: Functionality Verification

\[ P_{\text{CCD,par}} = IN \eta_{\text{par}} \cdot \int_{\text{CCD}} \left( \frac{dC_{\text{sca,par}}}{d\Omega} \right) d\Omega \approx IN \eta_{\text{par}} \cdot \sum_{i=\text{pixels}} \left[ \left( \frac{dC_{\text{sca,par}}}{d\Omega} \right)_{i} \right] d\Omega_{i} \]  \hspace{1cm} (4.2.2)

\[ P_{\text{CCD,per}} = IN \eta_{\text{per}} \cdot \int_{\text{CCD}} \left( \frac{dC_{\text{sca,per}}}{d\Omega} \right) d\Omega \approx IN \eta_{\text{per}} \cdot \sum_{i=\text{pixels}} \left[ \left( \frac{dC_{\text{sca,per}}}{d\Omega} \right)_{i} \right] d\Omega_{i} \]  \hspace{1cm} (4.2.3)

The summation approximations shown in (4.2.2) and (4.2.3) illustrate the manner in which the computer program integrates \( \frac{dC_{\text{sca}}}{d\Omega} \), projected either to the axis parallel \( \left( \frac{dC_{\text{sca,par}}}{d\Omega} \right) \) or perpendicular \( \left( \frac{dC_{\text{sca,per}}}{d\Omega} \right) \) to incident polarization. The solid angle subtended by each pixel was approximated with the expression \( d\Omega = \frac{A_{\text{pixel}}}{f_{S}^{2}} \). The optical losses for parallel and perpendicular polarizations were lumped into the parameters \( \eta_{\text{par}} \) and \( \eta_{\text{per}} \), respectively.

In converting the power received for each polarization to CCD counts, we need to specify the exposure time \( \Delta T \), the quantum efficiency of the CCD \( Q_{e} \) (photoelectrons/photon), and the conversion factor from photoelectrons to counts \( F \) (counts/photon). We therefore obtain the expressions for the average counts per pixel for the two polarizations.

\[ \frac{\text{Counts}_{\text{par}}}{\text{pixel}} = \frac{P_{\text{CCD,par}} \frac{\Delta T}{h\nu} Q_{e} F}{\# \text{ pixels}} \]  \hspace{1cm} (4.2.4)

\[ \frac{\text{Counts}_{\text{perp}}}{\text{pixel}} = \frac{P_{\text{CCD,per}} \frac{\Delta T}{h\nu} Q_{e} F}{\# \text{ pixels}} \]  \hspace{1cm} (4.2.5)

The values of all the parameters used in equations (4.2.1) – (4.2.5) are given in Table 4.1.
Table 4.1. The values and physical constants used in calculating the predicted beads signal in counts per pixel.

The values for the counts per pixel obtained by performing the described calculation were in good agreement with the experimental values. Table 4.2 summarizes and contrasts the experimental and the predicted backscattering signal from the beads. We concluded that our signal readings were justified as well as the assumption that the overall effect by speckle had zero mean.

Table 4.2. The comparison between experimental and predicted values for the average number of CCD counts per pixel.

4.2.7 Signal-to-Noise Analysis

When reading signals in the presence of noise, we need to have confidence that our signal level exceeds the noise level by an amount that eliminates ambiguities. There are several sources of additive noise that interfere with the signal. The signal is defined as the number of photoelectrons scattered into some particular pixel or regions of pixels,

\[^2\text{Using equations (4.2.4) and (4.2.5)}\]
depending on context. We shall assume that the detected light resulting from reflections from optical components and surfaces can be eliminated with background removal as described in Section 4.2.2. The remaining sources of noise are associated with the CCD. The shot noise or photon noise is associated with the random arrival of photons at the CCD and is governed by Poisson statistics. Therefore, \( \sigma_{\text{shot}} = \sqrt{S} \), where \( \sigma_{\text{shot}} \) is the standard deviation of the shot noise and \( S \) is the signal, both expressed in photoelectrons. The read noise is produced in the output amplifier and is a function of the electronics in the system. For our CCD camera, the manufacturer-specified read noise was specified as \( \sigma_{\text{read}} = 8 \) photoelectrons. The last source of noise is lumped as thermal noise resulting from dark current at the transistor junctions, which at \(-40^\circ C\) was specified as \( \sigma_{\text{thermal}} = 0.05 \) photoelectrons/pixel/second.

A standard indicator of the signal to noise strength is the signal-to-noise ratio (SNR). For the CCD, the SNR is defined as:

\[
\text{SNR} = \frac{S}{\sigma_{\text{noise}}} = \frac{S}{\sqrt{\sigma_{\text{shot}}^2 + \sigma_{\text{read}}^2 + \sigma_{\text{thermal}}^2}}
\]

In (4.2.6), the denominator represents the effective standard deviation of the total noise. For a high enough signal \( S \), the contribution to total noise by \( \sigma_{\text{read}} \) and \( \sigma_{\text{thermal}} \) is negligible compared to \( \sigma_{\text{shot}} \), and so the system is said to be shot noise limited. Under this optimal condition we have

\[
\text{SNR} = \sqrt{S}
\]

which is the best attainable SNR.
To prove that beads exposures are shot noise limited, we need to demonstrate the relation in (4.2.7). However, since the speckle interference obscured the real underlying signal in each pixel, we turn to demonstrating time dependent properties of shot noise limited measurements. Let $S_i$ be the number of photoelectrons the CCD collects in a unit time interval $\Delta t$, say 1 ms. Since shot noise follows a Poisson distribution, it is independent and identically distributed over each $\Delta t$. Let $\sigma_i$ be the standard deviation of the shot noise over $\Delta t$. If the total exposure time $\Delta T$ is some multiple $m$ of unit time intervals $\Delta t$, we have $\Delta T = m\Delta t$. The signal collected over $\Delta T$ is

$$ S = \sum_{i=1}^{m} S_i = mS_i $$  \hspace{1cm} (4.2.8)

It is assumed in (4.2.8) that the laser intensity at the sample and hence the scattering signal are constant over time. The standard deviation of shot noise over $\Delta T$ is

$$ \sigma = \sqrt{\sum_{i=1}^{m} \sigma_i^2} = \sqrt{m} \cdot \sigma_i $$  \hspace{1cm} (4.2.9)

Combining (4.2.8) and (4.2.9) we calculate the SNR over $\Delta T$:

$$ SNR = \frac{mS}{\sqrt{m\sigma_i}} = \sqrt{m} \cdot SNR_i $$  \hspace{1cm} (4.2.10)

So the signal to noise ratio increases with the square root of the exposure time $m$.

Therefore (4.2.10) is a condition equivalent to (4.2.7) for showing shot noise limited behavior. Since the speckle is stationary, any variation in the number of photoelectrons collected at a given pixel is presumably caused by the CCD noise, which should behave according to the relation in (4.2.9). Beads were not used for the noise
measurements since the microscopic movements of the beads may cause the speckle to become non-stationary, and the sample drying out over time may introduce changes in scattering. Instead, a more uniform white standard reflective surface was used. Twenty five successive exposures were taken at each one of six different exposure times: 10 ms, 50 ms, 100 ms, 200 ms, 500 ms, and 1 second. The standard deviation of the intensity was calculated across the 25 measurements for each pixel out of a sample of about 5000 pixels. A logarithmic plot of the standard deviation versus exposure time is presented in Figure 4.4. Note that taking the logarithm of both sides of equation (4.2.9) leads to the expression \( \log(\sigma) = 0.5\log(m) + \log(\sigma_f) \) for the ideal shot noise behavior. Looking at the plot, the experimental and ideal values are in good agreement.

![Figure 4.4. The standard deviation of the intensity per pixel resulting from white standard reflection. Statistics taken over 25 consecutive measurements at 6 different exposure times and ~5000 pixels.](image)

This procedure verifies that the variations in intensity follow a Poisson distribution, and therefore the system is shot noise limited, especially for exposures longer than 50 ms.
4.2 Fiber Optic System with OPO

4.3.1 Experimental Setup

The OPO was integrated into the system and coupled through the fiber optic as illustrated in Figure 4.5. The average power output of the OPO was controlled to approximately 400 mW, coupling about 150 mW of that light through the fiber. The imaging beam diameter in this system was about 20 mm, consistent with the specification and calculations presented in Section 2.3.1.

Figure 4.5. The experimental setup of the fiber optic OPO system. OPO: laser source (tunable, 20 Hz pulsed, ~30 mJ/pulse); FL: focusing lens to fiber (f=25mm); FO: fiber optic (d=1mm, NA=0.12); CL: collimating lens from fiber (f=15mm); P: polarizer; L1: spatial filter focusing lens (f=73mm); L3: collimating and imaging lens (f=300mm); A1: iris diaphragm; BS: 50/50 beam splitters; A: analyzer polarizer; CCD: 1048x1392 pixel 12-bit CCD camera.
4.3.2 Methods
An experiment analogous to one described in Section 4.2.1 was performed with this system. Once again, 44 μm beads were suspended in water and arranged at the sample to avoid backward specular reflection. The exposure time was set to 500 ms, accumulated 20 times to yield a total exposure of 10 seconds. This exposure corresponded to 200 pulses of the laser. The excitation wavelength was tuned to 632 nm, in order to simulate the conditions for comparison to the He:Ne measurements.

4.3.3 Results
The parallel and perpendicular polarizations of backscattered light using the OPO and fiber system are shown in Figure 4.6. The images appear much smoother than the ones from Figure 4.2, due to decreased effect of laser speckle.

![Figure 4.6](image)

**Figure 4.6.** The parallel (a) and perpendicular (b) polarization angular maps of backscattered light using fiber-coupled OPO. Obtained for 44 μm beads in water, 10 seconds total exposure time. Note the relative smoothness of the images.
4.3.4 Data Fitting

The angular maps were fit to Mie theory in the same manner described in Section 4.2.4. The normalized horizontal profiles of the parallel, perpendicular, and the parallel minus perpendicular angular maps are shown in Figure 4.7.

![Profiles with midpoints at different angles](image1)

**Figure 4.7.** The fitting of experimental fiber-OPO profiles and theoretical profiles in the angular maps. The parallel polarization (a), perpendicular polarization (b) and the single scattering [parallel-perpendicular] (c) profiles, all normalized to scale.

Here, the measurements seem to have very low noise, especially compared to those presented in Figure 4.3. The output of the OPO is less coherent than the He:Ne and the
mode-mixing in the multimode fiber further decreases the coherence length, resulting in the smooth images and smooth pixel profiles.

### 4.3.5 Size Distribution

Having removed the effect of laser speckle, we can still see a discrepancy between the experimental and theoretical profiles in Figure 4.7. This disagreement was almost expected since the polystyrene beads are not all perfectly uniform; the manufacturer specified a standard deviation of 7 microns from the mean of 44 microns.

We proceed to model the size distribution of the polystyrene beads with a Gaussian distribution of mean $\mu_{\text{beads}}$ and standard deviation $\sigma_{\text{beads}}$. The computer code from Appendix A was used to calculate the normalized angular maps for polystyrene beads of sizes in the range of 34 to 54 microns, with one micron increments. By taking linear combinations of these normalized angular maps, we can create a Gaussian distribution and can come closer to modeling our experimental data.

The fitting was performed by creating a Gaussian probability distribution for each $\mu_{\text{beads}}$ in the range $\mu_{\text{beads}} \in [39, 49]$ and $\sigma_{\text{beads}}$ in the range $\sigma_{\text{beads}} \in [1, 10]$ microns, both in unit increments. For each distribution, the probability of each bead size is used as a scale factor for superposition of the normalized angular maps. The overall superposed prediction is then compared to the experimental data. The code used to perform the fitting is given in Appendix B. It was determined that the values that minimized the mean squared error between the experimental profile and predicted profile were
\( \mu_{\text{beads}} = 43 \) and \( \sigma_{\text{beads}} = 7 \) microns. The experimental single scattering profile and the prediction for a Gaussian with \( \mu_{\text{beads}} = 43 \) and \( \sigma_{\text{beads}} = 7 \) are shown in Figure 4.8.

![Profile with midpoint at Theta = 0.43 and Phi = 90 degrees](image)

**Figure 4.8.** The single scattering [parallel – perpendicular] experimental data fitting to a Gaussian distribution of bead sizes with \( \mu_{\text{beads}} = 43 \) and \( \sigma_{\text{beads}} = 7 \) minimized the squared error.

In addition to being a good visual fit, the fit in Figure 4.8 is numerically very close to the experimental data; the squared error is 0.0045. The best fit represented with \( \mu_{\text{beads}} = 43 \) and \( \sigma_{\text{beads}} = 7 \) is within 3% of the manufacturer specification of \( \mu_{\text{beads}} = 44 \) and \( \sigma_{\text{beads}} = 7 \). Therefore, we can accurately extract the bead size distribution based on this experimental (par-perp) profile. By taking smaller increments in the sizes of \( \mu_{\text{beads}} \) and \( \sigma_{\text{beads}} \), we can arrive at an even more accurate assessment of the size distribution.

The Gaussian fitting of the experimental data is only a first step in the ultimate goal. When the system is modified to image the sample using 60 different wavelengths
in the visible spectrum, the objective will be to fit the spectral data with an analogous Gaussian distribution. Iterating this procedure across all parts of the sample image will produce the size distributions as a function of position in the sample. When applied ultimately to human cervical tissue, this procedure would help identify regions in which cell nuclei are larger and more variable in size, meaning regions that show signs of dysplasia.
Chapter 4: Functionality Verification

References


CHAPTER 5
Conclusion and Future Work

5.1 Conclusions

The morphology of cell nuclei, particularly size enlargement, is an important indicator of early cancer. The LSS clinical imaging device aims to provide quantitative size information about cell nuclei from a wide locus of cervical tissue. Such information helps detect dysplastic lesions and enables early, and hence more successful, treatment of cervical cancer. The major part of the device, specified and outlined in Chapter 2, has been assembled and its operation verified as described in Chapter 4. System design and experimental methodology minimize the effect of both optics-related and CCD-based noise sources thus enabling operation of the system under shot-noise limited conditions. Under this optimal condition, the Mie theory model provides a very good fit, in the least squares sense, to the experimental data for polystyrene beads. The fitting algorithm can predict the size distribution in real-time and this estimate closely agrees with the known size distribution of polystyrene beads, proving the correct functionality of the method.

The remainder of the device implementation is outlined in the next section. It is hoped that the necessary improvements will be implemented and tested immediately. The LSS clinical imaging device can then find its place in the hospital.
5.2 Overview of Future Work

The current system built and tested thus far, as described in Chapter 4, is not yet ready for clinical imaging. The following is a brief outline of the steps required to prepare the device for clinical trials.

(1) Imaging. The CCD will need to be positioned in the imaging plane as specified in Figure 2.1. This step should be a simple extension of the existing system, adding an inversion lens (L4) and a calcite crystal. Care should be taken to place the calcite correctly so that the two images of the parallel and perpendicular polarizations are clearly resolved on the CCD. The viewing lens (L2), although not necessary for imaging, can be added at that point for completeness.

(2) Spatial size distribution extraction. The single scattering image of the sample will be obtained by subtracting the image of the perpendicular from the parallel polarization component of backscattered light. Groups of pixels can then be binned according to some desired and specified resolution. Each region of pixels will contain power scattered through a solid angle of 0.5° around direct backscattering by particles in the corresponding unit area of the sample. This scattered power is a function of wavelength and is sensitive to changes in the size of the particles.

The size distribution will be extracted in the following manner. The code described in Section 3.4.2 will be used to integrate the $dC_{sc}/d\Omega$ for $\theta_i$ in the range [0, 0.5°] and for $\phi$ over [0, 2π]. For the relevant range of $\lambda$ (410 to 680 nm), with increments specified to match the experimental wavelength steps (~5 nm), the code will calculate the scattered power. Then, for a range of Gaussian distributions corresponding
to physically plausible scenarios, fits will be created for the backscattered light as a function of wavelength. For human cell nuclei, a valid range for the mean diameter may be 5 to 9 microns with a standard deviation of 0 to 3 microns, depending on the stage of dysplasia. A Gaussian distribution whose fit minimizes the squared error from the experimental data will become the estimate. The same procedure will be used to obtain estimated distributions of particles in all regions of the image. The resultant size distribution map will help identify potentially dysplastic regions of tissue.

(3) **Automation of system.** The system needs to be automated, so that the entire imaging and data analysis procedure can be done from a single software application. The automation and control will be done through the LabVIEW (National Instruments, Austin, TX) software, which is optimized for interfacing with various devices. A flowchart of the proposed sequence of commands that will need to be executed is presented in Figure 5.1. The focus of the flowchart is on the operation and synchronization of the laser source and the CCD camera detector over the tunable range of wavelengths. The pulses of the Nd:YAG flash lamp, occurring at 20 Hz, are used to trigger camera acquisition and Q-switch firing of the laser. The camera needs to be exposed for the least amount of time (1 ms) necessary to record scattering from a single pulse of light (5 ns) at a particular wavelength.
Chapter 5: Conclusion and Future Work

Figure 5.1. A flowchart of the sequence of commands necessary for the operation of the LSS clinical imaging device.

(4) **Ex vivo tissue studies.** Different types of tissue phantoms are commonly used to test the accuracy and calibrate the measurements. A simple model for tissue consists of polystyrene beads imbedded in gelatin and placed on top of a diffusive substrate. The next step is to experiment with single layers of cells and *ex vivo* tissue, such as the rat esophagus. After the feasibility of LSS imaging is confirmed and laser safety benchmarks are satisfied, the instrument can enter the stage of *in vivo* clinical trials for cervical cancer diagnosis.
Appendix A: Single Wavelength Angular Map Code

PROGRAM PLSS

Last revised on: 04/15/03 by Obrad Scepanovic

REVIZIONS:
-----------

C
C
C
C

C... subroutine "ANGLE"
COMMON /ANG/ SP, CMR, THETA, PHI, PROB, POL
COMMON /ANG2/ ETH, EPH

REAL NPART, NMED, WAVLEN, NAIR, N_MED_TO_AIR
REAL DIA
COMPLEX ET, EP
COMPLEX ETH, EPH
REAL IX, IY
REAL PIX_X(176,132), PIX_Y(176,132), PIX_T(176,132)
COMPLEX ETH, EPH, ET, EP

C... read input data
OPEN(40, FILE = 'C:\plss.in')
REWIND 40

C... read optical parameters
READ(40,*) NPART, NMED, WAVLEN
N_AIR = 1.00028
N_MED_TO_AIR = NMED/N_AIR
NPART = NPART/NMED

C... read particle parameters
READ(40,*) DIA, STDEV

C... read lens focal length
READ(40,*) FOC

C... read CCD data
READ(40,*) CCD_X, CCD_Y

C... close file
CLOSE(40)

C... calculate size parameter
C use the relative index of refraction from medium to air to adjust
C for the inputed wavelen in air

SP = 2*PI*N_MED_TO_AIR*(DIA*0.5)/WAVLEN
X = SP

C
C...backscattering
   WRITE(*,*) 'acceptance angle :', ATAND(CCD_X/FOC)
   GRIDRESX = 175
   GRIDRESY = 131
   DO J = 1,175
      DO K = 1,131
         XG = (-88 + J)*CCD_X/GRIDRESX
         YG = (-66 + K)*CCD_Y/GRIDRESY
      C...distance of source to centre of pixel of interest
         R = SQRT(XG**2 + YG**2 + FOC**2)
      C...calculate scattering angle
      C...theta
         THETA = 180 - ACOSD(FOC/R)
      C...save yourself some agony if theta = 180.
         IF (J.EQ.88) THEN
            IF (K.EQ.66) THEN
               THETA = 180
            ENDIF
         ENDIF
      C...phi
         IF (XG.GT.0.AND.YG.GT.0) THEN
            PHI = ATAND(YG/XG)
         ENDIF
         IF (XG.LT.0.AND.YG.LT.0) THEN
            PHI = ATAND(YG/XG) + 180.
         ENDIF
         IF (XG.LT.0.AND.YG.GT.0) THEN
            PHI = ATAND(ABS(YG/XG)) + 90.
         ENDIF
         IF (XG.GT.0.AND.YG.LT.0) THEN
            PHI = ATAND(ABS(YG/XG)) + 270.
         ENDIF
         IF (XG.EQ.0.AND.YG.GT.0) THEN
            PHI = 90.
         ENDIF
         IF (XG.EQ.0.AND.YG.LT.0) THEN
            PHI = 270.
         ENDIF
         IF (YG.EQ.0.AND.XG.LT.0) THEN
            PHI = 180.
         ENDIF
         IF (YG.EQ.0.AND.XG.GT.0) THEN
            PHI = 360.
         ENDIF
      C...calculate scattering cross section
      C...(*note* S1 and S2 are NOT the Bohren and Huffman variables. Just a dumb choice
      C... of dummy variable names!!)
      S1 = THETA
      S2 = PHI
      WRITE(*,*) 'j,k', J, K
      CALL ANGLE
      WRITE(*,*) 'after angle', J, K
      C...reset to degrees from radians because the subroutine, and FORTRAN, calculate in radians
      THETA = S1
      PHI = S2
      C...calculate intensities
      IX = ABS((COSD(THETA)*COSD(PHI)*ETH) * - (SIND(PHI)*EPH)**2
      IY = ABS((SIND(PHI)*COSD(THETA)*ETH) * + (EPH*COSD(PHI)))**2
      PIX_X(J,K) = IX
      PIX_Y(J,K) = IY
Appendix A: Single Wavelength Angular Map Code

\[ \text{PIX}_T(J,K) = IX - IY \]

C...save to the respective arrays
ENDDO
ENDDO

C-------------------------------------------------------------------
C
OPEN(40,FILE = 'C:\MATLABR11\work\Mie\BScat\bscat_60um.dat')
REWIND 40
DO I = 1,175
   DO J = 1,131
      WRITE(40,*)PIX_X(I,J),PIX_Y(I,J),PIX_T(I,J)
   ENDDO
ENDDO
END

SUBROUTINE ANGLE

ANGLE calculates the Mie scattering cross sections given
the scattering angles.

. ADAPTED FROM:
. Light Scattering by Particles: Computational Methods
. By P.W. Barber and S.C. Hill (program s2)
. (1990) by World Scientific Publishing Co Pte Ltd
. (Available at RRI library)
. the program has been modified to return the values of the
. scattering cross section and field amplitudes when given
. the scattering angles.
. equation numbers in columns 73-80 are references to the text.
. calculate the differential scattering cross section in all
. directions
. surface onto a rectangular coordinate system.
. theta: the theta scattering angle
. phi: the phi scattering angle, is the azimuthal angle in
. the spherical and rectangular coordinate systems
. inputs: ip = polarization parallel or perpendicular
. x = size parameter (ka)
. cm = complex index of refraction, (real,imag)
. (imag is positive for absorption)
. dimension of arrays f(*), g(*), amat(*) and cnrm(*):
. nc = int(x+4.05*x**.3333+2.0), e.g., for x = 200,
. nc = 225
. dimension of arrays bj(*), by(*), hkl(*), and
. pnmllg(*): nc+1, e.g., for x = 200, nc+1 = 226
. arrays are set for a maximum size parameter of 200
parameter (index_nc = 500)
parameter (index_nc1 = 501)
complex cm,ci,cim,f(index_nc),g(index_nc),fth,fph,eth,eph
common /cfcom/ t,g,com
common /ang/ sp,cmr,theta,phi,prob,pol
common /ang2/eth,eph
COMMON /OPTICAL/ NPART,NMED,WAVLEN
REAL NPART,NMED,WAVLEN,theta_n

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dimension pnmllg(index nc1),cnrm(index nc),ac(1000)
pi = 3.14159265358979
ci = (0.0,1.0)

C...SET MIE SCATTERING PARAMETERS FROM MAIN PROGRAM
C...set size parameter
x = SP
C
C...set real part of refractive index
cmr = npart
C
C...set absorption to zero
cmi = 0.0
C
C...set polarization to parallel (i.e. : in the x-z plane)
ip = 1
C
C...set the effective theta for scattering, taking water refraction into account
theta_n = theta / l
C
C...........................
C... set the complex index of refraction
C... for an exp(-iwt) time variation
C...........................
c = cmplx(cmr,cmi)
snorm = 1.0/(pi*x**2)
call sphere(x,cn,ip,nc)
nci = nc+1
C
phi = (pi*phi)/180
theta_n = ((pi*theta_n)/180)
C...........................
C... calculate the logarithm of the differential scattering cross section
C... at (xg,yg) and store in array ac(*)
C...........................
sinph = sin(phi)
cosph = cos(phi)
costh = cos(theta_n)
call genlgp(theta_n,pnmllg,nci)
fth = 0.0
fph = 0.0
do 20 n = 1,nc
    nl = n+1
    cim = ci**(nl)
    rn = real(n)
    pl = rn*cosph*pnmllg(nl)-(rn+1.0)*pnmllg(n)
    p2 = pnmllg(nl)
    fth = fth+cim*cosph*(p2*f(n)+ci*pl*g(n))*cnrm(n) eq 4.10a
    fph = fph-cim*sinph*(pl*f(n)+ci*p2*g(n))*cnrm(n) eq 4.10b
else
    fth = fth+sinph*(p2*f(n)+ci*pl*g(n))*cnrm(n) eq 4.11a
    fph = fph+cosph*(pl*f(n)+ci*p2*g(n))*cnrm(n) eq 4.11b
end if
20 continue
C
C...........................
C... calculate the differential scattering cross section in the (theta_n,phi) direction
C...........................
ac(jg) = (snorm*(abs(fth)**2+abs(fph)**2))
prob = ac(jg)
eth = sqrt(snorm)*fth
eph = sqrt(snorm)*fph

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Appendix A: Single Wavelength Angular Map Code

C
C..NOTE : abs(eth)**2 + abs(eph)**2 = prob
return
end
subroutine sphere(x,cm,ip,nc)

C ..................................
C..calculate the scattered field f(n) and g(n) coefficients ...
C..the f(n) and g(n) for theta_n incident polarization are, ...
C..within an n-dependent factor, the same as the b(n) and ...
C..a(n) coefficients, respectively, defined in C.F.
C..Bohren and D.R. Huffman, Absorption and Scattering of ...
C..Light by Small Particles (Wiley- Interscience,New ...
C..York,1983), p.100
parameter (indexnc = 500)
parameter (indexnc1 = 501)
complex b,cm,ci,hkl(indexnc1),an,amat(indexnc)
common /cfcom/ f,g,cnrm
dimension cnrm(indexnc)
ci = (0.0,1.0)
C..set the number of terms required for convergence ...
C ...................................................
eq 4.16
xc = x+4.05*x**.3333+2.0
nc = int(xc)
nci = nc+1
z = cm*x
C..logarithmic derivative calculation - set the ...
C..starting order for downward recursion ...
C ..................................................eq 4.20
nmx = int(max(xc,abs(z)))+15
an = 0.0
do 10 n = nmx,nci,-1
  rn = real(n)
an = rn/z-1.0/(an+rn/z)
10 continue
amat(nc) = an
do 20 n = nc,2,-1
  rn = real(n)
amat(n-1) = rn/z-1.0/(amat(n)+rn/z) eq 4.19
20 continue
C..calculate the Bessel functions - the order is ...
C..incremented by one in the hkl(*) array ...
C ................................................
call besh(x,hkl,nci)
bj = real(hkl(1))
C..calculate the coefficients ...
c ........................................
do 30 n = 1,nc
  rn = real(n)
  rf = 2.0*rn*(rn+1.0)
bjx = bj
  bj = real(hkl(n+1))
30 continue
C..scattering coefficients for theta_n ...
C..(parallel) incident polarization ...
c..f(n) = -ci**n*rf*(Bohren and Huffman's b(n))
c..g(n) = ci**(n+1)*rf*(Bohren and Huffman's a(n)) ...
C ........................................
b = cm*amat(n)+rn/x
  f(n) = -ci**n*rf*(b*bj-bjx)/(b*hkl(n+1)-hkl(n)) eq 4.18a
  g(n) = ci**(n+1)*rf*(b*bj-bjx)/(b*hkl(n+1)-hkl(n)) eq 4.18b
if(ip.eq.2) then
C..scattering coefficients for phi ...

Appendix A: Single Wavelength Angular Map Code

(-perpendicular) incident polarization

\[ f(n) = -f(n) \]  
\[ g(n) = g(n) \]  
end if

calculate the normalization factor  
(used in main program)

cnrm(n) = (2.0*rn+1.0)/(rf*rn*(rn+1.0))

continued

subroutine besh(x,hankel,nc)

calculate Hankel functions
bj = Bessel function of the first kind
by = Bessel function of the second kind
x = real argument
nc = number of orders (0 to nc-1)
the order of the functions is incremented by
one in the bj(*),by(*) and hankel(*) arrays
arrays are set for nc = 226 maximum

complex hankel(nc)
parameter (index_nc = 500)
parameter (index_ncl = 501)
dimension bj(index_ncl),by(index_ncl),t(3)

a = sin(x)/x  
by(1) = -cos(x)/x  
by(2) = by(1)/x-a

obtain the higher order functions by upward recursion

do 10 n = 3,nc
rn = real(n-2)
by(n) = (2.0*rn+1.0)*by(n-1)/x-by(n-2)
10 continue

obtain the zeroeth and first order functions

a = sin(x)/x  
by(1) = -cos(x)/x  
by(2) = by(1)/x-a

obtain the higher order functions by upward recursion

do 10 n = 3,nc
rn = real(n-2)
by(n) = (2.0*rn+1.0)*by(n-1)/x-by(n-2)
10 continue

the t(*) array is used to recur down to the two highest order functions that are needed
set starting values for the two highest orders
nst and nst-l

nst = nc+int((101.0*x)**.5)  
t(3) = 0.0
 t(2) = 1.0e-35

c = recur downward to obtain orders nc-l and nc-2

do 20 i = nst-1,nc-l,-1
ri = real(1)
t(1) = (2.0*ri+1.0)*t(2)/x-t(3)
t(3) = t(2)
t(2) = t(1)
20 continue

c = continue downward recursion to order zero

bj(nc) = t(3)
bj(nc-1) = t(2)
Appendix A: Single Wavelength Angular Map Code

do 30 i = nc-2,1,-1
   ri = real(i)
   write(*,*)'after xi',nc,i
   bj(i) = (2.0*ri+1.0)*bj(i+1)/x-bj(i+2)
   write(*,*)'bj'
30 continue

.. .................................................................
.. calculate the scale factor and the functions ...
.. .................................................................
alpha = a/bj(1)
do 40 k = 1,nc
   hankel(k) = cmplx(bj(k)*alpha,by(k))
40 continue
return
end

subroutine genlgp(theta_n,pnmllg,nc)
.. .................................................................
.. calculate associated Legendre functions (argument ...
.. cos(theta_n)) divided by sin(theta_n) for m = 1 ...
.. generate first two orders by formula and remaining ...
.. orders by recursion ...
.. .................................................................
.. pnmllg = associated Legendre function/sin(theta_n) ...
.. nc = number of orders (0 to nc-1) ...
.. the order of the associated Legendre functions is ...
.. incremented by one in the pnmllg(*) array ...
.. .................................................................
.. dimension pnmllg(nc)
costh = cos(theta_n)
.. .................................................................
.. calculate orders 0 and 1 ...
.. eq 4.70b
   pnmllg(1) = 0.0
   pnmllg(2) = 1.0
.. .................................................................
.. recur upward to obtain all remaining orders ...
.. eq 4.71
do 10 n = 3,nc
   rn = real(n-1)
   pnmllg(n) = ((2.0*rn-1.0)*costh*pnmllg(n-1)
   1 -rn*pnmllg(n-2))/(rn-1.0)
10 continue
return
end
APPENDIX B: Size Distribution Fitting Code
Appendix B: Size Distribution Fitting Code

% best_fit.m

% This program calculates and plots the best fit to the experimental
% par-perp profile by obtaining the closest fit, in the least squares sense,
% to a theoretical scattering profile of a Gaussian size distribution of
% scattering particles.

figure;

% this is the normalized experimental par-perp profile
S = single_scatter([1:173],110)/norm(single_scatter(:,110));

min_error = 1;
mse_mu = 0;
mse_sigma = 0;
for mu=39:49 % a physically plausible range of mean diameters
    for sigma=1:10 % a physically plausible range of standard deviations
        S_hat = build_gaussian(mu, sigma,'ss');
        adjust = max(S) - max(S_hat); % correct shift factor
        S_hat = S_hat + adjust;
        residual = S - S_hat;
        if norm(residual)^2 < min_error % find fit that minimizes the squared error
            min_error = norm(residual)^2;
            mse_mu = mu;
            mse_sigma = sigma;
        end
    end
end

min_error
mse_mu
mse_sigma
S_hat = build_gaussian(mse_mu, mse_sigma,'ss');
adjust = max(S) - max(S_hat);
S_hat = S_hat + adjust;
residual = S - S_hat;
plot(S, 'r.');
hold on;
plot(S_hat, 'b');
plot(residual, 'g');
legend('Experimental Par-Perp', 'Mie Ideal Par-Perp', 'Residual');
title('Profile with midpoint at Theta = 0.43 and Phi = 90 degrees','fontsize',12);
xlabel('Pixel (region) number','fontsize',12);
ylabel('Intensity (a.u.)','fontsize',12);
axis([0 180 -0.2 0.3]);
Appendix B: Size Distribution Fitting Code

% build_gaussian.m
% This function creates an angular map profile theoretically
% predicted by a Gaussian size distribution of scattering particles
% with specified mean (mu), standard deviation (sigma), and the
% polarization state (param).

function S_hat = build_gaussian(mu, sigma, param)

G = zeros(1, 100);
if sigma == 0
    G(1, round(mu)) = 1;
else
    % Gaussian distribution equation
    G(1, [1:100]) = 1/(2*pi*sigma^2) * exp(-([1:100]-mu).^2/(2*sigma^2));
    G=G./(sum(G)); % normalized as discrete pdf
end

load model.mat;
% build the Gaussian distribution by taking a linear combination of the model
if strcmp(param, 'par')
    S_hat = M_par'*G';
elseif strcmp(param, 'perp')
    S_hat = M_perp'*G';
elseif strcmp(param, 'ss')
    S_hat = M_ss'*G';
end
% build_model.m

% This program creates a model matrix M, whose rows contain the
% angular scattering profiles for various diameters of polystyrene beads,
% by extracting the necessary profile of pixels from the previously
% calculated angular map bscat_##um.dat file.

M_ss = zeros(100,173);
M_par = zeros(100,173);
M_perp = zeros(100,173);

for beads_size=34:54 % most relevant size range to polystyrene beads
    file_root = sprintf('./BScat/bscat_%dum.dat', beads_size);
    [fid_bscat2, message] = fopen(file_root, 'rt');

    % read the bscat file from Venkatesh Mie algorithm
    for x=1:175
        for y=1:131
            row = fscanf(fid_bscat2, '%e', 3);
            bscat_matrix_par2(x,y) = row(1);
            bscat_matrix_perp2(x,y) = row(2);
            bscat_matrix_pmp2(x,y) = row(3);
            end
        end
    % store the necessary horizontal pixel profile for the different polarization states
    M_ss(beads_size,:)=bscat_matrix_pmp2([3:175],110)' / norm(bscat_matrix_pmp2(:,110));
    M_par(beads_size,:)=bscat_matrix_par2([3:175],65)' / norm(bscat_matrix_par2(:,65));
    M_perp(beads_size,:)=bscat_matrix_perp2([3:175],100)' / norm(bscat_matrix_perp2(:,100));

end

save 'model.mat' M_ss M_par M_perp;