POLARIMETRIC EXPLORATORY DATA ANALYSIS (pEDA) USING DUAL ROTATING RETARDER POLARIMETRY FOR IN VITRO DETECTION OF EARLY STAGE LUNG CANCER

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POLARIMETRIC EXPLORATORY DATA ANALYSIS (pEDA) USING DUAL 
ROTATING RETARDER POLARIMETRY FOR IN VITRO DETECTION OF EARLY 
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Thesis

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ii
ABSTRACT

According to the National Cancer Institute, in 2010 there were approximately 222,520 new cases of lung cancer and 157,300 deaths. Of the two main types of lung cancer, small cell and non-small cell, 87% are diagnosed as non-small cell lung cancer (NSCLC) [1]. There are currently no generally accepted screening tests for lung cancer. Therefore, a more sensitive and specific imaging technique is required that can play an important role in the diagnosis and treatment of cancer. Efficient imaging procedures allow diagnosis and therapy to be addressed selectively to the tumor and can be used to better facilitate localized surgical interventions that allow limited disease areas to be treated more drastically.

A NIR backscattered optical polarimetric imaging technique utilizing the Dual Rotating Retarder Method was used in this study to find discriminate signatures between early stages of NSCLC, namely carcinoma in-situ (CIS), and stage I carcinoma (S1C), from normal, healthy human lung tissue. The experiments were performed on formalin fixed tissue samples in vitro. A new optical polarimetric metric definition, polarimetric Exploratory Data Analysis (pEDA), which combines polarimetry with histogram data analysis, was implemented. The pEDA aims to quantify the signal characteristics of photons transmitted or backscattered through/from optically active media and tissue in terms of enhanced contrast to potentially discriminate optical signatures.
Statistically significant differences (p < 0.05) in the optical properties, namely depolarization, diattenuation, and retardance, between normal and early stage cancer were revealed. Stage I carcinoma (S1C) and carcinoma \textit{in-situ} (CIS) were found to depolarize light less than normal healthy lung tissue. This result, along with others obtained for diattenuation and retardance are consistent with previous research [12-13, 24]. The FWHM and Dynamic Range (DR) calculated from histograms of backscattered signals proved to be another important metrics for the characterization of cancerous tissue. From the results obtained in this study, using pEDA as a diagnostic tool to provide discriminate optical information regarding normal, precancerous, and cancerous lung tissue can eventually aid in the early detection of Non-Small Cell Lung Cancer.
DEDICATION

I would like to dedicate this work to my parents for supporting me in my quest for higher education and to my fiancé, for his constant love and support throughout my academic career.
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## TABLE OF CONTENTS

| LIST OF TABLES | xi |
| LIST OF FIGURES | xiii |

### CHAPTER

#### I. INTRODUCTION

1.1 Motivation ................................................................. 1
1.2 Problem Definition ...................................................... 5
1.3 Objectives of the Study .................................................... 6
1.4 Hypotheses ........................................................................ 6
   1.4.1 Null Hypotheses .......................................................... 6
   1.4.2 Alternate Hypotheses .................................................... 7

#### II. LUNG CANCER FORMATION AND CANCER DETECTION TECHNIQUES

2.1 Types of Lung Cancer ....................................................... 8
   2.1.1 Adenocarcinoma ........................................................... 8
   2.1.2 Squamous Cell Carcinoma (SCC) ...................................... 9
   2.1.3 Large Cell Carcinoma (LCC) ............................................ 9
2.2 Polarized Light Interaction with Tissue .................................. 10
2.3 Polarimetric Phenomenology ............................................... 12
2.4 Stokes Parameters and Mueller Matrix Calculus ........................ 14
2.5 The Mueller Matrix .......................................................... 16
2.6 Dual Rotating Retarder Polarimetry ......................................................... 17
  2.6.1 Error Compensation ................................................................. 19
  2.6.2 Dual Rotating Retarder Algorithms ............................................. 21
2.7 Calculating Depolarization, Diattenuation, and Retardance from the Mueller Matrix ................................................................. 21
  2.7.1 Depolarization ........................................................................ 22
  2.7.2 Diattenuation ....................................................................... 23
  2.7.3 Retardance ......................................................................... 23
2.8 Polarimetric Exploratory Data Analysis (pEDA) ......................... 24

III. EXPERIMENTAL ARRANGEMENT AND TECHNIQUES
3.1 Beam Profile and Receiver Sensitivity Measurements ................. 28
  3.1.1 Graphing Noise of the System .................................................. 30
3.2 Calibration of System Components ............................................... 31
  3.2.1 Calibration of Polarizers ......................................................... 31
  3.2.2 Calibration of Quarter-Wave Retarders .................................... 33
  3.2.3 Calibration of the Dual-Rotating Retarder Method .................... 34
  3.2.4 Obtaining Mueller Matrix Elements of an LVP and LHP .......... 35
3.3 Backscattered Polarimetric Measurements From Tissue .............. 36
  3.3.1 Tissue Slide Preparation and Mounting .................................... 37
  3.3.2 Linear Polarization Tissue Data ............................................... 40
  3.3.3 Histogram Capture of Each Sample ......................................... 41
  3.3.4 Obtaining Mueller Matrix Elements of a Sample ..................... 42
  3.3.5 Calculating Depolarization, Diattenuation, and Retardance
      of Each Sample ..................................................................... 42
IV. RESULTS

4.1 Beam Profile and Receiver Sensitivity Measurements

4.2 Calibration of Polarimetric System using Dual-Rotating Retarder Method

4.3 Testing Two Known Samples in the Polarimeter

4.3.1 Testing a Linear Horizontal Polarizer (LHP)

4.3.2 Testing a Linear Vertical Polarizer (LVP)

4.4 Linear Polarimetric Tissue Data

4.4.1 Calculating Depolarization, Degree of Linear Polarization, and Residual Intensity

4.5 Tissue Mueller Matrices using the Dual Rotating Retarder Method

4.5.1 Statistical Analysis

4.6 Histogram Analysis

V. DISCUSSION

5.1 Calibration Results for the Dual-Rotating Retarder Polarimeter

5.2 Linear Polarization Tissue Data

5.3 Tissue Mueller Matrix using the Dual Rotating Retarder Method

5.3.1 Diattenuation

5.3.2 Depolarization

5.3.3 Retardance

5.4 Histogram Analysis
VI. CONCLUSION AND FUTURE WORK

6.1 Conclusion.................................................................................................................89

6.2 Future Work...............................................................................................................90

REFERENCES..................................................................................................................92

APPENDIX A....................................................................................................................95
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Pathologic description of the lung tissue.................................38 samples.</td>
<td></td>
</tr>
<tr>
<td>4.1 Theoretical versus calculated Mueller Matrices ..........................50 from experimental data for Air, Linear Horizontal Polarizer (LHP), and Linear Vertical Polarizer (LVP)</td>
<td></td>
</tr>
<tr>
<td>4.2 Orientation errors calculated for the generator........................51 retarder (ε3), analyzer polarizer (ε4), and analyzer retarder (ε5).</td>
<td></td>
</tr>
<tr>
<td>4.3 Depolarization, Degree of Linear Polarization.............................60 (DOLP), and Residual Intensity calculated for Normal lung tissue and Stage I lung Carcinoma with the standard error of the mean.</td>
<td></td>
</tr>
<tr>
<td>4.4 The 16 element Mueller matrices for each................................64 sample are calculated from the modulated intensities obtained through experimentation.</td>
<td></td>
</tr>
<tr>
<td>4.5 Raw Mueller matrices decomposed into the..................................65-67 submatrices Depolarization, Diattenuation, and Retardance for (a) Normal lung tissue (b) Carcinoma In-Situ and (c) Stage I lung Carcinoma.</td>
<td></td>
</tr>
<tr>
<td>4.6 Average diattenuation, depolarization, total..............................67 retardance and the standard error of the mean calculated from the Mueller matrices of each sample of lung tissue (Normal, CIS, S1C).</td>
<td></td>
</tr>
</tbody>
</table>
4.7 A Single Factor ANOVA with a 95% confidence level ($\alpha = 0.05$) is performed on the data to reveal if there is a significant difference between samples for Diattenuation (a), Depolarization (b), and Retardance (c).

4.8 Confidence intervals calculated at a 95% confidence level revealing where significant differences in optical parameters exist.

4.9 Histogram statistical analysis for the three lung samples: Normal, CIS, and S1C.

4.10 A Single Factor ANOVA with a 95% confidence level is performed on the data for FWHM revealing a significant difference between samples.
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 An electromagnetic light wave propagating in the z-direction.</td>
<td>12</td>
</tr>
<tr>
<td>2.2 An elliptically polarized light wave with amplitudes $E_{ox}, E_{oy}$, and angle of rotation, $\psi$.</td>
<td>13</td>
</tr>
<tr>
<td>2.3 Dual-rotating retarder polarimeter calibration setup.</td>
<td>19</td>
</tr>
<tr>
<td>2.4 Errors associated with a dual-rotating retarder polarimeter.</td>
<td>20</td>
</tr>
<tr>
<td>3.1 Overhead view of the experimental setup to obtain laser radiation pattern and detector pattern by varying the angle of rotation, $\varphi$, of the laser or detector.</td>
<td>29</td>
</tr>
<tr>
<td>3.2 Overhead view of the experimental setup to obtain the detector sensitivity pattern by varying the angle of rotation of the detector, $\varphi$, by 30º.</td>
<td>30</td>
</tr>
<tr>
<td>3.3 Calibration setup for the high contrast linear polarizer 1 (P1).</td>
<td>32</td>
</tr>
<tr>
<td>3.4 Calibration setup for linear polarizer 2 (P2).</td>
<td>32</td>
</tr>
<tr>
<td>3.5 Calibration setup for quarter-wave retarders (R).</td>
<td>34</td>
</tr>
<tr>
<td>3.6 Calibration setup for Dual-Rotating Retarder Method.</td>
<td>35</td>
</tr>
</tbody>
</table>
3.7 Optical backscattered experimental setup .................................................. 37

3.8 Microphotograph of (A) normal, (B) lung Carcinoma .......................... 39
    In Situ (CIS), and (C) Stage I non-small cell lung carcinoma (NSCLC) tissue.

3.9 Graphical interpretation of the Full Width at .............................................. 44
    Half Maximum (FWHM) of a sample from a Gaussian curve fit to the observed data (Obs. Data).

3.10 Flow chart of the steps involved in the DRR .............................................. 46

4.1 Normalized 785nm Laser Rotation ......................................................... 47
    Femtowatt Detector

4.2 Normalized Femtowatt Detector ............................................................. 48
    Rotation 785nm Laser

4.3 Calibration of Polarimetric System ......................................................... 49
    No Sample (Air)

4.4 Theoretical vs Experimental Mueller ....................................................... 51
    Matrix Elements: No Sample (AIR)

4.5 Theoretical vs Experimental Modulated ................................................ 53
    Intensities: Linear Horizontal Polarizer

4.6 Theoretical vs Experimental Mueller ....................................................... 54
    Matrix Elements: Linear Horizontal Polarizer (LHP)

4.7 Theoretical vs Experimental Modulated ................................................ 55
    Intensities: Linear Vertical Polarizer

4.8 Theoretical vs Experimental Mueller ....................................................... 56
    Matrix Elements: Linear Vertical Polarizer (LVP)

4.9 Normal versus Stage I Carcinoma Lung ................................................... 57
    Tissue: Copolarized Polarizers

4.10 Normal versus Stage I Carcinoma Lung .................................................. 58
    Tissue: Crosspolarized Polarizers
4.26 Normal Lung Tissue: Probability Plot of..................................................74
Data fit with Normal Distribution

4.27 Carcinoma In-Situ Lung Tissue: Histogram.............................................75
of Data fit with Normal Distribution

4.28 Carcinoma In-Situ Lung Tissue: Probability.............................................75
Plot of Data fit with Normal Distribution

4.29 Stage I Carcinoma Lung Tissue: Histogram..............................................76
of Data fit with Normal Distribution

4.30 Stage I Carcinoma Lung Tissue: Probability.............................................76
Plot of Data fit with Normal Distribution

4.31 Gaussian Fits to Detected Amplitudes from..........................................77
Normal Lung, Carcinoma In-Situ, and
Stage I Carcinoma

4.32 Correlation Coefficient of Data fit with..................................................78
Gaussian Distribution

4.33 Full Width Half Maximum from Histograms..........................................78
of Lung Tissue Samples

4.34 Dynamic Range from Histograms of Lung..............................................79
Tissue Samples
CHAPTER I
INTRODUCTION

1.1 Motivation

According to the National Cancer Institute, in 2010 there were approximately 222,520 new cases of lung cancer and 157,300 deaths. Of the two main types of lung cancer, small cell and non-small cell, 87% are diagnosed as non-small cell lung cancer (NSCLC) [1]. The majority of patients are diagnosed with advanced stages of this cancer that are inoperable since early stages present no symptoms. The five year survival rate of those diagnosed at an advanced stage is between 7-19% [1], [2]. There are currently no generally accepted screening tests for lung cancer.

The most common detection mechanisms used for lung cancer are classical imaging techniques such as chest radiography (film or digital) and computed tomography (CT). When compared to classical radiography, digital radiography provides better contrast resolution with equal or better spatial resolution [1], [3]; however, these techniques still do not provide meaningful imaging information that can be utilized towards the early detection of tumors. On the other hand, low-dose, spiral / helical CT can detect earlier stages of cancer 6-10 times more frequently than radiographic imaging and can provide improved image resolution, detecting tumors less than 5mm in diameter [3]. However, spiral CT is limited to small peripheral lesions and, further, has a high
false positive rate due to increased sensitivity which comes from high scatter rejection capabilities. As a result, spiral CT may lead to unnecessary interventional procedures. Although these caveats, spiral CT remains the golden standard for early detection of lung cancer.

By referring to early cancer detection and treatment, it is well established that the use of low contrast-low-spatial resolution, or low-specificity imaging procedures contribute to inaccurate staging and treatment assessment of cancer. Efficient imaging procedures allow diagnosis and therapy to be addressed selectively to the tumor and can be used to better facilitate localized surgical interventions that allow limited disease areas to be treated more drastically. Therefore, more sensitive and specific imaging is imperative to the diagnosis and treatment of cancer.

Optical imaging is an advancing diagnostic technique that can provide metabolic information which, combined with anatomical information offered by other imaging modalities, would enhance the detection process of cancer and overcome individual imaging limitations. Currently diagnostic optical imaging techniques that have been developed to combat these limitations and detect pre-invasive cancer include bronchoscopic technologies that utilize white light (WLB) or auto-fluorescence imaging (AFI), narrow-band imaging (NBI), high magnification bronchovideoscope, endobronchial ultrasound (EBUS) and optical coherence tomography (OCT). Most peripheral tumors are adenocarcinomas or large cell carcinomas. Because of their peripheral location, adenocarcinoma may not be caught early until they have developed extrathoracic metastases.
Optical imaging involves probing tissue with non-ionizing radiation in the visible and near-infrared region (400nm-1500nm). Studying the optical properties of tissue reveals information that can potentially characterize diseases, be used to study drug treatment effects, and analyze molecular pathways leading to disease [4]-[6]. Optical imaging combined with polarimetry has the ability to detect weakly backscattered linearly polarized light in the presence of highly backscattered depolarized light. It has the capability of producing high contrast, high specificity images under low light conditions [4], [7]-[8]. Information on the surface smoothness, orientation, drug-cell interactions, and biochemical and metabolic composition of the tissue can be obtained [4].

Upon interaction with tissue, an optically turbid media, photons propagate through the tissue and are scattered and absorbed. The intrinsic properties of tissue determine the scattering behavior [9]. Scattering and absorption are penetration depth and wavelength dependent. A main disadvantage of optical imaging is its minimal penetration depth and poor image contrast of deep structures due to high absorption coefficients [4]. Near infrared (NIR) interrogation of tissue appears attractive for clinical studies since the main absorbers of light, namely hemoglobin, water, and lipids, have their lowest absorption coefficients in this region (700nm-900nm), offering deeper penetration in tissue. By using NIR light in an optical system, the above mentioned parameters can be monitored and used to detect angiogenesis (the growth of new blood vessels), metabolic activity, and blood flow/concentration that can lead to early cancer detection [10].
In addition to using a NIR polarimetric technique to determine differences in optical properties of tissue, distribution fitting of histograms of the acquired backscattered signal is also a powerful tool for studying tissue properties. Histograms provide a visual discriminator for detecting outliers or unusual behavior from a sample. By associating a distribution model with a distinct tissue sample, the remainder of the samples may be characterized by a similar model with different parameters [11].

Previous work on the interrogation of living epithelial cells based on light scattering spectroscopy with polarized light has been reported by Sokolov et al. [12]. In that specific methodology, single backscattering from the uppermost epithelial cells and multiply scattered light were obtained. The index of refraction and size distribution of the epithelial cells were assessed through single backscattering.

In another study conducted by Backman et al [13], a method for selective detection of size-dependent scattering characteristics of epithelial cells in vivo based on polarized illumination and polarization sensitive detection of scattered light was presented. The finding of that study revealed that reflectance spectroscopy with polarized light can provide quantitative morphological information which could potentially be used for non-invasive detection of neoplastic changes.

Further, the combination of a wavelength-dependent and polarization-dependent method to study changes in light scattering was measured by Mourant et al. [14]. This study revealed that the difference in scattering is attributed to the average dimension of the “scatterers”, being a few tens of nanometers smaller in the healthy cells compared with the cancerous cells. This work highlights the significance of developing noninvasive, optical tissue diagnostic methods based on the sensitivity of wavelength-
dependent and polarization-dependent light scattering measurements to cell morphology.

Finally, classification of cultured human lung cancer cells has been performed by Gakuin [15] using Raman spectroscopy, principal component analysis (PCA), and linear discrimination analysis (LDA). Raman spectra of single, normal lung cells, along with four cancer cells with different pathological types, were successfully obtained with an excitation laser at 532nm.

1.2 Problem Definition

The purpose of this research is to use a NIR backscattered optical polarimetric imaging technique to obtain the intrinsic optical properties of cancerous tissue. These properties will then be used to find discriminate signatures between early stages of lung cancer, namely carcinoma in-situ (CIS), and stage 1 carcinoma (S1C), from normal, healthy human lung tissue. Recently, a new optical polarimetric metrics has been introduced by Giakos [16], namely the polarimetric Exploratory Data Analysis (pEDA), which combines polarimetry with histogram data analysis.

The pEDA aims to quantify the signal characteristics of photons transmitted or backscattered through/from optically active media and tissue in terms of enhanced contrast to potentially discriminate optical signatures. Specifically, combining polarimetric analysis with exploratory data analysis (EDA) would offer the opportunity to relate a physical process to categorize signatures by grouping and separating different parts of the histograms, eliminating outliers, and applying thereafter fittings of different statistical curves. In this study, pEDA will be explored using the Dual Rotating Retarder
Method [17] and the results compared to those obtained using a simplified linear polarimetric method.

1.3 Objectives of the Study

1. To evaluate the capabilities of the pEDA based NIR optical polarimetric system to reveal changes in the optical properties (depolarization, diattenuation, and retardance) of early stage lung cancer by measuring the backscattered intensity signal from each sample in vitro using the Dual-Rotating Retarder Method.

2. To analyze the histograms of obtained backscattered signals to determine if departures from normality and/or changes in the spread of the histogram between different stages of lung cancer (Full Width at Half Maximum and Dynamic Range) can be observed in vitro.

3. Overall, to assess whether pEDA can be shown an effective metric to characterize early cancer detection rather than using only polarimetric or histogramming techniques.

1.4 Hypotheses

1.4.1 Null Hypotheses

1. There is no significant difference in optical properties: depolarization (DP), diattenuation (D), and retardance (R) between the three samples of lung tissue: normal, carcinoma in-situ (CIS), and stage 1 carcinoma (S1C).
2. There is no significant difference in the Full Width at Half Maximum (FWHM) and Dynamic Range (DR) calculated from the histograms of the backscattered signals between the three samples of lung tissue: normal, carcinoma \textit{in-situ} (CIS), and stage 1 carcinoma (S1C).

1.4.2 Alternate Hypotheses

1. There is a significant difference in optical properties: depolarization (DP), diattenuation (D), and retardance (R), between the three samples of lung tissue: normal, carcinoma \textit{in-situ} (CIS), and stage 1 carcinoma (S1C).

2. There is a significant difference in the Full Width Half Maximum (FWHM) and Dynamic Range (DR) calculated from the histograms of the backscattered signals between the three samples of lung tissue: normal, carcinoma \textit{in-situ} (CIS), and stage 1 carcinoma (S1C).
CHAPTER II
LUNG CANCER FORMATION AND CLINICAL DETECTION TECHNIQUES

2.1 Types of Lung Cancer

According to most recent reports from the National Cancer Institute [1], lung cancer can be divided up into two main types, namely Small Cell Lung Cancer and Non-Small Cell Lung Cancer (NSCLC). Of these two cases, more than 80% are diagnosed as NSCLC [1]. There are three main types of NSCLC that have similar growth patterns: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma.

2.1.1 Adenocarcinoma

Adenocarcinoma is the most common type of NSCLC contributing to almost 50% of all cases. It is most common in female non-smokers under the age of 50. It can be further broken down into other subtypes of adenocarcinoma: acinar, papillary, and bronchioloalveolar [18]. Some adenocarcinoma cells are glandular in appearance and the epithelial tissues produce mucin, a thick fluid-like substance. In most cases of adenocarcinoma, the tumor arises in the peripheral areas of the lungs.
2.1.2 Squamous Cell Carcinoma (SCC)

SCC is the second most common type of NSLC and accounts for approximately 30% of all cases. It is most commonly seen in men and people over the age of 65 who are cigarette smokers [1]. Squamous cells are flat in structure and form in the trachea and bronchi of the chest area. The cells have a lower tissue hemoglobin oxygen saturation compared to non-cancerous cells in tissue and most express high levels of keratin.

SCC is thought of as a multistep process starting from squamous metaplasia progressing to dysplasia, to carcinoma in-situ (CIS), and finally to invasive cancer. At the stage of CIS, the cell-structure of the epithelial layer is altered and the nuclei become enlarged and crowded. The average size of non-dysplastic cell nuclei is between 5-10µm as compared to dysplastic cell nuclei which can be as large as 20µm. This size is much larger than the incident wavelength of light (Visible to NIR) resulting in Mie Scattering [13]. Further, the optical index of refraction increases compared to the surrounding cytoplasm due to the increased metabolic activity of the cells. These changes affect the amount and type of scattering that occurs upon light interacting with tissue and is further explained in the following sections [2, 15].

2.1.3 Large Cell Carcinoma (LCC)

LCC is undifferentiated carcinoma that occurs anywhere in the lung and makes up only 10-15% of all NSCLC cases [1]. It is most common in men and smokers of age 60 or older. Unlike SCC or adenocarcinoma, LCC does not possess any glandular or squamous features. Samples within this study are adenocarcinoma and SCC and, therefore, LCC is not discussed further in this thesis.
2.2 Polarized Light Interaction with Tissue

Polarized light and polarimetric systems have been used for years to study biological samples and their optical properties for medical diagnostic purposes. The majority of linearly polarized light incident upon tissue loses its polarization, however, a small portion is backscattered by epithelial cells such that polarization is retained. The remainder diffuses further into the tissue and is depolarized due to multiple scattering from the cells and organelles in tissue that act as scattering structures [4, 7, 10, 18].

There are several factors that affect the polarization of scattered light such as the incident polarization state, size of scatterer, concentration of scatterer, shape of scatterer, and the index of refraction of the scatterer and surrounding media [7, 19-21]. The size of these scattering structures and the incident wavelength of light determine the type of scattering that will occur. This scattering dependence on wavelength and scatterer size was studied by Sokolov et al. [12] using a polarization sensitive light scattering spectroscopic system. For cell nuclei of the epithelial layer, the diameter (approximately 5-10 µm) is much larger than the optical wavelength of light used to interrogate the tissue (around 0.5-0.9 µm) resulting in Mie scattering [13].

The degree of scattering is determined by the optical parameters of the tissue. Changes to the cells and organelles, such as enlarged nuclei and increased mitochondria content, take place in cancerous tissue. Previous work by Backman et al. [13] utilized light scattering spectroscopy (LSS) to interrogate epithelial cells in vivo through single backscattering to determine the index of refraction and size distribution of the uppermost epithelial cells. This technique revealed cellular changes affect the degree of scattering
which results in different polarization information (absorption, birefringence, dichroism, optical activity) obtained between normal and pre-cancerous samples [4-8].

In tissue, collagen, for example, is linear birefringent due to the orientation of the fibers. Birefringence refers to the retardance associated with light propagation through an anisotropic medium that possesses two different refractive indices [10]. The slow axis (the axis with the higher index of refraction and, therefore, slower phase velocity) is considered to be in the direction of fiber extension, while the fast axis (the axis with the lower refractive index and, therefore, the faster phase velocity) is the cross-sectional direction [16]. The development of cancer may result in the denaturization of collagen fibers, which can therefore be detected with a loss in birefringence calculated from the Mueller matrix measurements. This has the potential of being utilized to distinguish between malignant or benign tumorous structures imaged. The same analysis can be applied to glucose, which possesses circular birefringence due to its asymmetric chiral structure. Glucose causes rotation of the plane of linearly polarized light about the axis of propagation, referred to as optical activity. An increase in glucose intake in cancerous tissue will reveal an increase in the optical activity of the media [23].

Recently, many investigations have used optical polarimetric sensing systems to potentially aid in the diagnosis of early stage cancer [20, 23-24]. In the particular case of lung cancer, the majority of cases arise in the epithelium and have a well-defined precancerous stage characterized by nuclear dysplasia [13]. Bard et al. performed a study using white light reflectance spectroscopy to measure the microvascular oxygenation of normal and neoplastic endobronchial mucosa [25]. Further, Backman et al. [13] obtained single and multiple scattering from uppermost epithelial cells in the lung to determine the
index of refraction and size distribution of the cells for normal and precancerous tissue and concluded that nuclei determine the scattering structure of the epithelial layer.

2.3 Polarimetric Phenomenology

Light is an electromagnetic wave characterized by its polarization, wavelength, and intensity. The electric and magnetic field components of the wave are depicted in Figure 2.1. From the figure it can be seen that the light wave is propagating in the \( z \)-direction along the \( \mathbf{k} \) vector and contains transverse components, propagation in the \( x \) and \( y \) plane. The electric field \( \mathbf{E} \) is oscillating in the \( x \)-direction with an amplitude of \( E_{ox} \). The magnetic field \( \mathbf{B} \) is oscillating in the \( y \)-direction with an amplitude of \( E_{oy} \).

\[
E_x(z,t) = E_{ox} \cos(\tau + \delta_x) \quad (2.1)
\]

Figure 2.1 An electromagnetic light wave propagating in the \( z \)-direction. The electric field component \( (\mathbf{E}) \) is oscillating in the \( x \)-direction and the magnetic field component \( (\mathbf{B}) \) is oscillating in the \( y \)-direction [26].

The wave can best be described in terms of amplitude and phase shift

\[
E_x(z,t) = E_{ox} \cos(\tau + \delta_x) \quad (2.1)
\]
where \( \tau = \omega t - k z \), \( E_{ox} \) and \( E_{oy} \) are the maximum amplitudes in the x and y plane respectively, and \( \delta = \delta_y - \delta_x \) is the phase shift between electric and magnetic fields.

By expanding equations 2.1 and 2.2 using trigonometric identities, squaring them, and adding them together, you obtain the equation of an ellipse

\[
\frac{E_x^2}{E_{ox}^2} + \frac{E_y^2}{E_{oy}^2} - 2\frac{E_x}{E_{ox}} \frac{E_y}{E_{oy}} \cos \delta = \sin^2 \delta
\]  

The polarization of light refers to the direction of oscillation of its electric field component. A perfectly polarized light source is elliptically polarized as shown in Figure 2.2 [27]. The polarization is actually a rotated ellipse, rotated by an angle \( \psi \) from the major axis X, Y. The wave is propagating out of the page in the z-direction.

If there is oscillation only in the x-direction, \( E_{oy} = 0 \), the state of polarization is said to be linear horizontal. If there is oscillation only in the y-direction, \( E_{ox} = 0 \), the state of
polarization is said to be linear vertical. When there is no phase shift introduced, \( \delta = 0 \), or a phase shift of \( 180^\circ \), \( \delta = \pi \), and \( E_{ox} = E_{oy} \), then equation 2.3 reduces to

\[
E_y = \pm E_x
\]  

(2.4)

which corresponds to \( \pm 45^\circ \) linear polarization, respectively. Introducing a phase shift of \( \delta = \pi/2 \) or \( 3\pi/2 \) and \( E_{ox} = E_{oy} = E_0 \) results in equation 2.3 reducing to the equation of a circle, thereby producing circularly polarized light. Finally, if \( \delta = \pi/2 \) or \( 3\pi/2 \), elliptically polarized light is produced [27].

2.4 Stokes Parameters and Mueller Matrix Calculus

Mueller matrix calculus was developed to analyze polarization of light and obtain complete polarization information about an interrogated target. The Stokes parameters allow for any state of polarized light to be described by four measurements [11]. This mathematical representation to describe the polarization state of a light beam can be displayed in vector form as:

\[
S = \begin{pmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{pmatrix} = \begin{pmatrix} E_{ox}^2 + E_{oy}^2 \\ E_{ox}^2 - E_{oy}^2 \\ 2E_{ox}E_{oy}\cos\delta \\ 2E_{ox}E_{oy}\sin\delta \end{pmatrix} = \begin{pmatrix} I_0 \\ I_H - I_V \\ I_P - I_M \\ I_R - I_L \end{pmatrix}
\]  

(2.5)

\( S_0 \) represents the total intensity (\( I_0 \)) of the light beam. \( S_1 \) represents the difference between linear horizontal and vertical polarized states (\( I_H \) and \( I_V \)). If \( S_1 \) is greater than 0, the light has a tendency towards being in a linear horizontal polarized state while \( S_1 \) less than 0 suggests that the light is in a linear vertical polarized state. The same is true for \( S_2 \).
and $S_3$ representing $\pm 45^\circ$ linear polarization ($I_P$ and $I_M$) and Right and Left circular polarization ($I_R$ and $I_L$), respectively.

The Stokes parameters can be obtained in several ways. One method in particular involves the Rotating Quarter-Wave Retarder Method [17] and analyzing eight intensity measurements using Fourier analysis. The light in question passes through a quarter-wave retarder followed by a linear polarizer onto a detector. The quarter-wave retarder is rotated through eight steps such that $\omega t = n\theta_j$ where $\theta_j$ is the step size of $22.5^\circ$. The total intensity of light emerging from the quarter-wave retarder can be expressed as:

$$I(\theta) = \frac{1}{2}\left( A + B \sin(2n\theta_j) + C \cos(4n\theta_j) + D \sin(4n\theta_j) \right)$$

(2.6)

where

$$A = \frac{1}{4} \sum_{n=1}^{8} I\left( n \frac{\pi}{8} \right)$$

(2.7)

$$B = \frac{1}{2} \sum_{n=1}^{8} I\left( n \frac{\pi}{8} \right) \sin\left( n \frac{\pi}{4} \right)$$

(2.8)

$$C = \frac{1}{2} \sum_{n=1}^{8} I\left( n \frac{\pi}{8} \right) \cos\left( n \frac{\pi}{2} \right)$$

(2.9)

$$D = \frac{1}{2} \sum_{n=1}^{8} I\left( n \frac{\pi}{8} \right) \sin\left( n \frac{\pi}{2} \right)$$

(2.10)

The elements of the Stokes parameters can be calculated using equations 2.11-2.14:

$$S_0 = A - C$$

(2.11)

$$S_1 = 2C$$

(2.12)

$$S_2 = 2D$$

(2.13)

$$S_3 = B$$

(2.14)
From the Stokes parameters, the Degree of Polarization (DOP), Degree of Linear Polarization (DOLP), and Degree of Circular Polarization (DOCP) can be calculated using equations 2.15-2.17. For unpolarized light, DOP=0, 0 < DOP < 1 for a partially polarized beam, and DOP=1 for a completely polarized beam [27-28]. The same convention is true when observing DOLP and DOCP.

\[
DOP = \frac{\sqrt{S_1^2 + S_2^2 + S_3^2}}{S_0} \tag{2.15}
\]

\[
DOLP = \frac{\sqrt{S_1^2 + S_2^2}}{S_0} \tag{2.16}
\]

\[
DOCP = \frac{S_3}{S_0} \tag{2.17}
\]

2.5 The Mueller Matrix

The Mueller matrix, developed by Hans Mueller in 1943, can provide valuable information on the optical characteristics of the sample, in particular how the sample transforms the incident light beam and therefore causes a change in polarization [27-29]. Four changes can occur to the incoming optical beam upon interaction with matter: a change in amplitude, phase, polarization, and direction of the orthogonal field components.

The Mueller matrix is thought of as the transfer function of the sample \( M_{\text{sample}} \) acting on the input Stokes vector \( S_{in} \) and producing the output Stokes vector \( S_{out} \)

\[
S_{out} = M_{\text{sample}} S_{in} \tag{2.18}
\]
If the input and output Stokes vectors are known, the Mueller matrix of the sample can be calculated. By obtaining the Mueller matrix of the sample, information can be revealed such as depolarization, retardance, and diattenuation that would otherwise be unattainable with standard imaging methods.

The Mueller matrix is a 4x4 matrix as shown in equation 2.19 whose elements can be obtained through experimental and mathematical techniques.

$$M_{sample} = \begin{pmatrix} m_{11} & m_{12} & m_{13} & m_{14} \\ m_{21} & m_{22} & m_{23} & m_{24} \\ m_{31} & m_{32} & m_{33} & m_{34} \\ m_{41} & m_{42} & m_{43} & m_{44} \end{pmatrix} \tag{2.19}$$

Different methods to obtain the Mueller matrix of samples have been widely used by researchers to study the change in polarization of light upon interaction with normal and cancerous tissue [20, 23-24]. The method being utilized in this study is the Dual-Rotating Retarder Method [17]. It is chosen for its simplicity and straightforward experimental techniques and is further explained in the next section.

2.6 Dual Rotating Retarder Polarimetry

The Dual Rotating Retarder (DRR) Method was originally proposed by Azzam [17] in 1978. He suggested that rotating two quarter-wave retarders at different angular speeds $\omega$ and $5\omega$ would generate a periodic signal. A discrete Fourier transform (DFT) could be performed on the signal to obtain Fourier coefficients ($a_0, a_n, b_n$) which could then be used to obtain the 16 elements of the sample Mueller matrix. Goldstein [30] expanded the work of Azzam and developed a polarimetric system and algorithms that could be applied to any laser source in the 3 to 12\(\mu\)m spectral range. In addition he
developed data reduction equations that included systematic errors due to orientation
errors of the polarizing elements within 0.3°, as well as, retardance errors within 2°.

Chenault et al. [31] and Smith [32] later expanded upon his work and developed
data reduction algorithms that included larger orientation errors of the components and
errors in retardance values. Their data reduction algorithms are applicable for orientation
errors within 22.5° and retardance values within λ/8 (11.25°) of the actual λ/4 retardance
value for a quarter-wave retarder. The algorithms developed by Chenault et al. [31] are
applied in this study.

The DRR method consists of an optical light source followed by a generator
linear polarizer that determines the polarization state of the light. A rotating linear
quarter-wave retarder follows the polarizer. Light leaving the retarder interacts with the
sample of interest and is then analyzed by another linear rotating quarter-wave retarder
and a linear polarizer and then incident upon a detector. The linear polarizers are fixed
with their transmission axes parallel to one another. Only the generator and analyzer
retarders are moved as shown in Figure 2.3.

The generator and analyzer retarders are rotated in 5:1 angular increments. The
5:1 ratio allows for the 16 elements of the Mueller matrix to be encoded onto twelve
harmonics of the signal detected (n=12) [31]. By performing a discrete Fourier transform
(DFT) of the detected signal, the Fourier coefficients of the signal are found. The
elements of the Mueller matrix of the sample are functions of the Fourier coefficients.
While the 5:1 ratio is not the only ratio that can be used, it is the lowest ratio that can be
implemented in which the expressions for the Fourier coefficients can be inverted to
obtain the Mueller matrix elements and reduce elemental errors [27]. Also, Smith [32]
revealed the rms error for Mueller matrix elements increases when a ratio smaller than 5:1 is chosen. Further, choosing a ratio greater than 5:1, though reducing error in elemental measurements, also increases the data acquisition time.

The advantage in using the Dual-Rotating Retarder Method [17] is that it is straightforward with few unknowns. The generator linear polarizer removes any polarization errors that may arise from a partially polarized light source. Also, the fixed linear polarizer on the analyzer side of the system removes potential error due to the polarization sensitivity of the detector. Further, by performing a discrete Fourier transform on the detected signal, a least squares solution is found for the over determined data obtained [27-31].

Figure 2.3: Dual-rotating retarder polarimeter calibration setup. \( \Theta_2 \), the angular speed of the analyzer retarder, is 5\( \Theta_1 \).

2.6.1 Error Compensation

Five systematic errors were considered by Chenault et al. [31] and can be seen in Figure 2.4. Orientation errors must be considered for the generator and analyzer retarders, R1 and R2 respectively, as well as the analyzer polarizer, P2 (\( \varepsilon_3, \varepsilon_4, \varepsilon_5 \)). The generator polarizer, P1, sets the transmission axis to which the other components must be oriented to minimize error. The orientation errors of R1 and R2 (e.g. ±1°) are then due to their optical axes being misaligned slightly with the transmission axis set by P1. The
same orientation error can be present for the analyzer polarizer P2 when its transmission axis is misaligned.

In addition to orientation errors of the last three components, R1, R2, and P2, the retarders have additional error related to the true value of their retardance. Both generator and analyzer retarders are quarter-wave retarders. This means that an ideal quarter-wave retarder produces a phase shift of 90° between the orthogonal components of the incoming light wave. True retardance values may not be exactly 90° and therefore deviations (\(\varepsilon_1, \varepsilon_2\)) from this value must be considered in the calculations of the Mueller matrix. Adding these deviations to the expected 90° phase shift of an ideal retarder results in the true retardance value, i.e. phase shift, of the retarder (\(\delta_1, \delta_2\)).

![Diagram showing errors associated with a dual-rotating retarder polarimeter. P1, P2 are fixed linear polarizers. R1, R2 are quarter-wave retarders. S is the sample of interest. Retardance errors (\(\varepsilon_1, \varepsilon_2\)) determine true retardance values (\(\delta_1, \delta_2\)). Orientation errors of R1, R2, and P2 (\(\varepsilon_3, \varepsilon_4, \varepsilon_5\)) [31].](image)

Figure 2.4: Errors associated with a dual-rotating retarder polarimeter. P1, P2 are fixed linear polarizers. R1, R2 are quarter-wave retarders. S is the sample of interest. Retardance errors (\(\varepsilon_1, \varepsilon_2\)) determine true retardance values (\(\delta_1, \delta_2\)). Orientation errors of R1, R2, and P2 (\(\varepsilon_3, \varepsilon_4, \varepsilon_5\)) [31].
2.6.2 Dual Rotating Retarder Algorithms

The Mueller matrix of the sample is a 4x4 matrix whose elements can be determined from the Fourier coefficients obtained from measured intensities from the system.

\[
M_{\text{sample}} = \begin{pmatrix}
m_{11} & m_{12} & m_{13} & m_{14} \\
m_{21} & m_{22} & m_{23} & m_{24} \\
m_{31} & m_{32} & m_{33} & m_{34} \\
m_{41} & m_{42} & m_{43} & m_{44}
\end{pmatrix}
\]

(2.20)

The output intensity measured by the detector can be rewritten to produce a Fourier series expansion,

\[
I_q = \frac{a_0}{2} + \sum_{n=1}^{12} (a_n \cos(2n\gamma q) + b_n \sin(2n\gamma q))
\]

(2.21)

where \( n \) is the harmonic \( (n=1, 2, \ldots 12) \). \( Q \) is the total number of measurements taken where the \( q^{th} \) measurements is from 1 to \( Q \). The angular speed step size, \( \gamma \), is equal to \( T/Q \), where \( T \) is the total rotation of the generator retarder, \( R1 \), being \( 180^\circ \). A minimum of 24 measurements \( (Q = 24) \) must be taken to apply the data reduction equations. By taking the discrete Fourier transform of the modulated intensity signals, the Fourier coefficients \( a_0, a_n, \) and \( b_n \) can be found. The Mueller matrix elements can be written in terms of the Fourier coefficients. A full list of equations developed and implemented in the current setup can be found in the paper by Chenault et al. [31].

2.7 Calculating Depolarization, Diattenuation, and Retardance from the Mueller Matrix

A problem arises when analyzing the parameters obtained in the 4x4 Mueller matrix since the optical polarization effects occur simultaneously in the sample. The
Mueller matrix elements more accurately represent lumped parameters and require decomposition of the matrix. Decomposition into three smaller matrices (Equation 2.22), namely retardance ($M_R$), diattenuation ($M_D$), and depolarization ($M_\Delta$), was first proposed by Lu and Chipman [29] and has been carried out to obtain information about biological samples by other authors [20, 23].

$$M = M_\Delta M_R M_D$$  \hspace{1cm} (2.22)

Chung et al. (2007) [24] decomposed the Mueller matrix to diagnose oral precancer. Depolarization and retardance images of hamster oral mucosa were obtained. They observed changes in the elements of the Mueller matrix and its decomposed matrices and correlated that with changes in tissue structure. Their findings suggested that cancerous tissue depolarizes light less than the healthy tissue surrounding the tumor. They found no significant change in depolarization between noncancerous tissue and the surrounding tissue. The same was true for the retardance images.

2.7.1 Depolarization

Depolarization is a process that turns polarized light into unpolarized light in the beam exiting the sample. Due to inhomogeneities and asymmetries in the makeup of a sample, all matter depolarizes light to an extent. From the Mueller matrix elements, equation 2.23 is used to calculate the depolarization of the sample.

$$DP = 1 - \frac{\sqrt{\sum_{ij} m_{ij}^2} - m_{11}^2}{m_{11} \sqrt{3}}$$  \hspace{1cm} (2.23)
where \(i\) and \(j\) are index integers for elements of the matrix. \(DP\) can have a value between 0 for an ideal polarizer, to 1 for an ideal depolarizer [27] – [28].

2.7.2 Diattenuation

Diattenuation occurs when the intensity transmittance of the beam exiting the system depends on the polarization state of the incident beam. Diattenuation also varies from 1 for an ideal polarizer, thereby completely blocking one polarization component of incident light, to 0 for an ideal retarder or depolarizer, thereby all incident states have the same intensity transmittance [27- 28]. The diattenuation can be found from the maximum and minimum intensity transmittances (\(T_{\text{max}}\) and \(T_{\text{min}}\)):

\[
D = \frac{T_{\text{max}} - T_{\text{min}}}{T_{\text{max}} + T_{\text{min}}} \tag{2.24}
\]

From the elements of the sample Mueller matrix:

\[
T_{\text{max}} = m_{11} + \sqrt{m_{12}^2 + m_{13}^2 + m_{14}^2} \tag{2.25}
\]

\[
T_{\text{min}} = m_{11} - \sqrt{m_{12}^2 + m_{13}^2 + m_{14}^2} \tag{2.26}
\]

By substituting equations 2.25 and 2.26 into equation 2.24 the diattenuation can be rewritten as:

\[
D = \frac{\sqrt{m_{12}^2 + m_{13}^2 + m_{14}^2}}{m_{11}} \tag{2.27}
\]

2.7.3 Retardance

The elements of the Mueller matrix of the sample can also be used to calculate the retardance that is introduced from the anisotropic tissue [27]. A retarder introduces a
phase shift between the electric and magnetic fields of the incoming light while maintaining constant intensity transmittance. From the elements of the sample Mueller matrix:

\[
R = \cos^{-1}\left(\frac{1}{2a} \left[ m_{22} + m_{33} + m_{44} - b(m_{21}m_{12} + m_{31}m_{13} + m_{41}m_{14}) - a \right] \right)
\]  

(2.28)

\[
a = \sqrt{1 - \left( \frac{m_{12}^2 + m_{13}^2 + m_{14}^2}{m_{12}^2 + m_{13}^2 + m_{14}^2} \right)}
\]  

(2.29)

\[
b = \frac{1}{m_{12}^2 + m_{13}^2 + m_{14}^2} \left( m_{12}^2 + m_{13}^2 + m_{14}^2 \right)
\]  

(2.30)

2.8 Polarimetric Exploratory Data Analysis (pEDA)

Polarimetric Exploratory Data Analysis (pEDA) aims to extract additional signatures from tissues by combining polarimetric analysis explained in the previous sections of this chapter with histogram analysis [16]; offering the opportunity to relate a physical process to discriminate signatures by grouping and separating different parts of a histogram and thereafter fit different statistical curves. Histograms provide a visual discriminator for detecting outliers or unusual behavior from a sample.

Spatial information of the tissue can be obtained from the histograms of backscattered signals. Depending on the physical phenomenology of the sample, Gaussian or other suitable interpolation curves can be used to fit histogram data. A Gaussian distribution is used in the current single-pixel detection experiments to fit the amplitude measurements of the backscattered signals. The Gaussian (normal) probability density function (pdf) in 1-d is
\[ f(x, \mu, \sigma^2) = \frac{1}{\sqrt{2\pi}\sigma^2} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right) \] (2.31)

A form of validation is carried out to determine the goodness of fit between the fitted Gaussian curve and the original data. This is done by creating a probability plot of the original data with the fitted Gaussian curve. Linearity between the two sets of data suggests a good fit. The correlation coefficient between the observed and fitted data is found to further validate the goodness of fit.

The Full Width at Half Maximum (FWHM, Equation 2.32) can be calculated from the Gaussian model used to fit the data. The FWHM is used to determine the Dynamic Range (DR, Equation 2.33) of the system for a particular sample

\[ FWHM = 2.354 \times \sigma \] (2.32)

\[ DR(dB) = 20 \times \log \left( \frac{V_{\text{max}}}{V_{\text{min}}} \right) \] (2.33)

where \( \sigma \) is the standard deviation of the data and \( V_{\text{max}} \) and \( V_{\text{min}} \) are the maximum and minimum voltages, respectively. They correspond to the x-values (amplitudes) of the points on the Gaussian curve that are at 50% of the maximum on either side of the peak.

The x-axis is normalized with respect to the sample mean, \( \mu \).

\[ V_{\text{max}} = +\sigma \sqrt{2 \ln 2} + \mu \] (2.34)

\[ V_{\text{min}} = -\sigma \sqrt{2 \ln 2} + \mu \] (2.35)

The angular dynamic range of a sample is similar to equation 2.33 but is based on interrogation of the heterogeneous sample at different aspect angles

\[ DR(\theta) = 20 \times \log \left( \frac{V_{\text{max}}}{V_{\text{min}}} \right) \] (2.36)
Angular dynamic range can be further broken down into copolarized and crosspolarized angular dynamic ranges defined below

\[
DR(\theta)_{\parallel} = 20 \times \log \left( \frac{V_{\text{max} \parallel}}{V_{\text{min} \parallel}} \right) \tag{2.37}
\]

\[
DR(\theta)_{\perp} = 20 \times \log \left( \frac{V_{\text{max} \perp}}{V_{\text{min} \perp}} \right) \tag{2.38}
\]

In single-pixel detection, a single pixel displays the intensity of a one-dimensional imaging signal. The dynamic range for single-pixel detection expresses the gray-level range associated with this one-dimensional imaging signal. An increase in the backscattered signal will result in an increase in the dynamic range [16].

Dynamic range and angular dynamic range can aid in characterizing each of the 16 elements of the Mueller matrix under different aspect angles. As mentioned in Section 2.4, the Mueller matrix \( M \) transforms an input Stokes vectors, \( S_{\text{in}} \), into an output Stokes vector, \( S_{\text{out}} \) according to equation 2.39:

\[
\begin{pmatrix}
S_0' \\
S_1' \\
S_2' \\
S_3'
\end{pmatrix} =
\begin{pmatrix}
m_{11} & m_{12} & m_{13} & m_{14} \\
m_{21} & m_{22} & m_{23} & m_{24} \\
m_{31} & m_{32} & m_{33} & m_{34} \\
m_{41} & m_{42} & m_{43} & m_{44}
\end{pmatrix}
\begin{pmatrix}
S_0 \\
S_1 \\
S_2 \\
S_3
\end{pmatrix}
\tag{2.39}
\]

Rotating the sample in reflection configuration, the rotated Mueller matrix of the sample, \( M_R \), is obtained by

\[
M_R(\theta) = R(\theta) \cdot M \cdot R(\theta)
\tag{2.40}
\]

where \( \theta \) is the rotation of the sample, and \( R(\theta) \) is the change in the basis matrix for Stokes vectors and Mueller matrices for the optical components of the system [28].
Applying the polar Mueller matrix decomposition explained in Section 2.6 allows for the calculation of depolarization, retardance, and diattenuation of the Mueller matrices of the sample. Typically, the Mueller matrix elements have been used to provide a polarimetric intensity value that mainly contributes to the image contrast; however, the polarimetric Exploratory Data Analysis (pEDA) would provide both polarimetric contrast and enhanced discrimination potential.
Prior to any backscattering experimental measurements, the following preliminary experiments, aimed to measure the beam and receiver’s quality parameters as well as characterize the optical system, took place:

a. Beam profile and receiver sensitivity measurements
b. Histogramming noise system measurements
c. Optical polarimetric calibration

3.1 Beam Profile and Receiver Sensitivity Measurements

The beam profile of three NIR lasers were experimentally assessed as well as the sensitivity pattern of the receiver at each wavelength. Both laser and detector were mounted on translational and rotational mounts that allowed for adjustment in the xy plane. The experimental setup is shown in Figure 3.1. Detailed alignment took place so that the laser-detector arrangement was in line-of-sight. Keeping the detector in a stationary position, the laser was rotated about its center and mean DC voltage measurements were made every tenth of a degree. The radiation pattern, which practically represents the beam profile of the laser, was recorded with a high contrast
linear polarizer paired to the laser and without it. When the high contrast linear polarizer was introduced into the system, its transmission axis was aligned with the laser to allow maximum light transmission.

The laser and detector were then placed in the line-of-sight again and the laser was kept stationary. The detector was then rotated at small incremental angles and the sensitivity pattern of the detector was recorded, as shown in Figure 3.2. The detector pattern was recorded both with and without a high contrast linear polarizer to determine the effects of polarimetry.

![Figure 3.1 Overhead view of the experimental setup to obtain laser radiation pattern and detector pattern by varying the angle of rotation, $\varphi$, of the laser or detector.](image)
3.1.1 Graphing Noise of the System

While the radiation pattern of the laser was being taken, measurements of noise were also recorded and displayed in a histogram on the oscilloscope. All noise measurements were taken when the laser and detector were aligned so that $\varphi = 0^\circ$. A histogram of the mean DC voltage was detected and displayed on the oscilloscope over the period of two milliseconds at a sampling rate of 5 Msamples/second therefore resulting in an averaging of 100K samples per acquisition or sweep. The histogram displayed the last 1,000 sweeps acquired from the parameter chosen to be graphed, in this case the mean DC voltage of the signal detected. The bin width in volts was chosen to be consistent for all histograms acquired. The bin values and counts were stored in a Microsoft Excel® document and used in post-processing algorithms described later in this chapter.

The background noise was recorded with the laser turned off and detector still on, thereby picking up spurious signals from other system components and the surrounding
area in the lab. The electronic noise displayed overall system noise consisting of the oscilloscope and associated connecting cables. This noise measurement was taken with both laser and detector turned off. Finally, signal noise was obtained which technically refers to the signal plus background and electronic noise combined.

3.2 Calibration of System Components

Before experimentation, the system components, namely polarizers and retarders, were calibrated. An ideal linear polarizer allows transmittance of light oscillating parallel to its transmission axis while rejecting those components that are in the perpendicular plane [28]. The calibration of polarizers refers to finding the points at which the polarizer’s transmission axis was aligned parallel or perpendicular with the incident light. A quarter-wave retarder is also known as a phase shifter since it changes the phase of an incident optical beam. It can transform a linearly polarized beam of light whose axis is at ±45° into right or left circularly polarized light, respectively [4]. The calibration of the retarders refers to that point at which the output signal is extinguished, or at a minimum. This point is termed the “zero” of the retarder. It is at this point that the retarder introduces no phase shift.

3.2.1 Calibration of Polarizers

Two high contrast linear polarizers were used in the experimental setup, P1 and P2. To calibrate the polarizers, the 785nm laser, polarizer (P1), and detector were arranged in the same plane as depicted in Figure 3.3. The optical chopper (C) was used to generate a pulsed laser beam with a frequency of 500Hz. The pulsed laser beam was
sent through P1 and incident upon the femtowatt single pixel detector. This signal was then sent to the oscilloscope for observation.

![Diagram of calibration setup for high contrast linear polarizer 1 (P1).](image)

**Figure 3.3** Calibration setup for the high contrast linear polarizer 1 (P1). C is an optical chopper.

To calibrate P1, the polarizer was rotated 360° until a signal with maximum amplitude was obtained on the oscilloscope. This angle on the polarizer was recorded as its *maximum* and was located where the fast axis (the axis with a smaller index of refraction allowing light to travel faster) of the polarizer was aligned parallel to the incident light. Rotating the polarizer 90° from this angle results in a minimum signal and was therefore recorded as the polarizer’s *minimum*. Once these data were obtained, a second linear polarizer, P2, was introduced into the system for calibration, as shown in Figure 3.4.

![Diagram of calibration setup for linear polarizer 2 (P2).](image)

**Figure 3.4** Calibration setup for linear polarizer 2 (P2). C is an optical chopper and P1 is the generator linear polarizer. ‘Tr’ is the transmission axis of the two polarizers.
P1 was set to its maximum position as recorded in the previous step. The second polarizer, P2, was then rotated in a similar fashion as P1, 360°, until a maximum signal was obtained on the oscilloscope. This angle was recorded as P2’s maximum. Rotating P2 90° from this point results in a minimum signal and therefore P2’s minimum. When both P1 and P2 were oriented at their maximums (P1 and P2 were parallel to each other), maximum transmission of light was allowed and it was referred to as co-polarized. When P1 and P2 were set at angles 90° apart from one another (i.e. P1 is set to its maximum and P2 to its minimum or vice versa) they were perpendicular to one another and were considered to be cross-polarized and minimum light was transmitted.

3.2.2 Calibration of Quarter-Wave Retarders

To calibrate the quarter-wave retarders, R1 and R2, the polarizers P1 and P2 were cross-polarized to obtain minimum light intensity. Retarder 1, R1, which was used on the generator side of the experimental setup, was introduced into the system between both polarizers as shown in Figure 3.5. R1 was then rotated around its axis until minimum light intensity was obtained. This position was recorded as 0° of the retarder. From 0°, R1 was rotated 45° clockwise looking in the direction of light transmission. This value was read from the retarder and recorded as the position at which linear polarized light was converted to circular polarized light. Retarder 1 was then taken out and replaced with retarder 2, R2, and the same procedure was followed until 0° of R2 was obtained.
3.2.3 Calibration of the Dual-Rotating Retarder Method

The Dual-Rotating Retarder Method [17, 30-32] is an inexpensive and easy method to obtain the Mueller matrix elements of a sample. Before measurements using the sample of interest, a calibration of the system to account for imperfections in the generator and analyzer retarders (R1 and R2) and alignment errors was done. A procedure for calibration of the system is well explained in [31] but is briefly described below.

To determine systematic errors, a sample with a known Mueller matrix was tested. Air has a well known Mueller matrix being the identity matrix, and was therefore tested for calibration. With the experimental setup as shown in Figure 3.6, both generator and analyzer retarders were rotated in a 5:1 ratio. A total of 30 intensity measurements were taken every 6° rotated on the generator retarder (R1) and every 30° on the analyzer retarder (R2) until R1 was rotated a full 180°. For each rotation, an intensity measurement corresponding to the amplitude of the waveform detected was recorded. Once R1 was rotated through 180° and a total 30 measurements were taken, the data was stored in Microsoft Excel® files on the oscilloscope.
The files were transferred to a computer where they were analyzed using a program developed in Matlab® R2007a to obtain the Fourier coefficients \((a_0, a_n, b_n)\) for \(n=1\) to 12. The coefficients were then used to calculate the retarder values \(\delta_1, \delta_2\) and orientation errors \(\varepsilon_3, \varepsilon_4, \varepsilon_5\). Efforts to minimize orientation errors were done by rotating the polarizing elements. Once this was completed, another calibration experiment was run following the same steps described above. The new intensity measurements were analyzed using Fourier analysis and new retarder values and orientation errors were calculated. These error values were substituted back into equations derived by Chenault et al. [31] to find the Mueller matrix elements of each sample, providing a more accurate representation of the optical properties of the sample. For an ideal system, retarder values are \(90^\circ\) \((\delta=90^\circ)\) and orientation errors are 0 \((\varepsilon=0)\).

### 3.2.4 Obtaining Mueller Matrix Elements of an LVP and LHP

The Mueller matrix of known samples, in particular a linear vertical polarizer (LVP) and linear horizontal polarizer (LHP), were found with the setup in transmission mode, as shown in Figure 3.6. The same DRR method described in section 3.2.3 was applied here to obtain the sample 30 intensity measurements used to calculate the Mueller
matrices. The Mueller matrices of an ideal linear vertical and linear horizontal polarizer are shown in equations 3.4 and 3.5, respectively.

\[
MM_{\text{LVP}} = \begin{pmatrix}
1 & -1 & 0 & 0 \\
-1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0
\end{pmatrix}
\]

(3.4)

\[
MM_{\text{LHP}} = \begin{pmatrix}
1 & 1 & 0 & 0 \\
1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0
\end{pmatrix}
\]

(3.5)

3.3 Backscattered Polarimetric Measurements From Tissue.

The backscattered experimental layout that was implemented using the DRR method is shown in Fig. 3.7. For a complete list of experimental components used and a description of each including serial number and company, refer to Appendix A. A 785nm continuous wave solid state laser (IS785-100, Intellite, Inc.) was used for excitation of the tissue samples. An optical chopper (3501, New Focus, Inc.) set to 500Hz was used to modulate the light source. The generator side of the optical system consisted of a high contrast linear polarizer, P1, and a quarter-wave retarder, R1. The analyzer side consisted of another quarter-wave retarder, R2, and another high contrast linear polarizer, P2, followed by a single-pixel femtowatt photoreceiver (2151, New Focus, Inc.). The output of the single pixel detector was observed on a 3GHz bandwidth oscilloscope via a BNC cable (Wavepro 7300A, LeCroy).

The target was mounted to a motor driven rotational and translational stage with a rotation accuracy of 0.01°. The stage allowed for adjustment of the samples for proper
alignment in the xy plane as well as rotation of the sample to allow for different incident angles of light. A flowchart of the experimental procedure is shown in Figure 3.10 at the end of this chapter and is explained in detail in the upcoming sections.

Figure 3.7 Optical backscattered experimental setup. Optical components boxed in blue are the generator and red are the analyzer of the system. C is an optical chopper. P1 and P2 are high contrast linear polarizers. R1 and R2 are quarter-wave retarders.

3.3.1 Tissue Slide Preparation and Mounting

Normal (healthy) lung tissue, Carcinoma In-Situ (CIS), and Stage I lung Carcinoma (S1C) tissue samples were studied. The tissue samples were obtained from and prepared by Folio Biosciences (Columbus, OH). They were formalin fixed, paraffin embedded (FFPE) human lung tissue samples, unstained, and five microns thick.
Paraffin was removed for each tissue sample and no slip covers were used to eliminate specular reflection. Further explanation into the origin of the samples is provided in Table 3.1.

A microphotograph was taken of each sample, shown in Figure 3.8. The normal tissue (Figure 3.8A) revealed thin-walled alveoli (small air spaces) composed of a single layer of squamous epithelium cells (indicated by the red arrows). The sample of Carcinoma In-Situ (Figure 3.8B) showed malignant squamous cells with hyperchromatic and pleomorphic characteristics (indicated by the red arrows). Stage I Carcinoma (Figure 3.8C) consisted of enlarged and vesicular nuclei and prominent nucleoli (indicated by the red arrows).

Table 3.1 Pathologic description of the lung tissue samples.

<table>
<thead>
<tr>
<th>Pathology Description</th>
<th>Normal Lung</th>
<th>Carcinoma In-Situ</th>
<th>Stage I Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>28</td>
<td>62</td>
<td>60</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>Unknown</td>
<td>Caucasian</td>
<td>Caucasian</td>
</tr>
<tr>
<td><strong>Pathology Description</strong></td>
<td>Normal Lung</td>
<td>Poorly differentiated NSC lung carcinoma, with glandular and squamous features.</td>
<td>NSC lung carcinoma – adenocarcinoma with papillary / bronchioloalveolar pattern. T1, N0, G2</td>
</tr>
</tbody>
</table>
Figure 3.8 Microphotograph of (A) normal, (B) lung Carcinoma In Situ (CIS), and (C) Stage I non-small cell lung carcinoma (NSCLC) tissue. Solid bars equal 100 μm in each panel.
The tissue slides were mounted on a rotating stage that has an accuracy of 0.01°. Each slide was interrogated with linear horizontal polarized light at 0° (face to face). The Dual-Rotating Retarder method is implemented for each sample to obtain the sample Mueller matrix and histogram of the data.

3.3.2 Linear Polarization Tissue Data

Linear data using only high contrast linear polarizers was obtained from Normal lung tissue (healthy tissue) and Stage I Carcinoma (S1C) using a backscattered geometry similar to Figure 3.7 but with the removal of the quarter wave retarders in the generator and analyzer sides. With the transmission axes of both generator and analyzer polarizers parallel to one another (co-polarized), and parallel to the polarization of the light source, amplitude measurements were made for each tissue. The analyzer polarizer was then rotated 90° to its minimum such that the polarizers were cross-polarized and amplitude measurements were taken again. As explained in section 3.1.1, each amplitude measurement was an average of 10,000 samples with a coefficient of variation of approximately 0.033. Five tests were taken for each sample at each orientation (co and cross-polarized) and the average amplitude was recorded. An average of the five tests for co-polarized amplitudes and cross-polarized amplitudes was calculated. The standard error of the mean was calculated from the five tests.

From these amplitude measurements for co and cross-polarized configurations, the degree of linear polarization (DOLP), residual intensity (R.I.), and depolarization ratio were calculated according to equations 3.1 through 3.3.
\[ DOLP = \frac{\begin{bmatrix} I_{II, \text{tissue}} & I_{\perp, \text{tissue}} \\ I_{II, \text{glass}} & I_{\perp, \text{glass}} \end{bmatrix}}{\begin{bmatrix} I_{II, \text{glass}} + I_{\perp, \text{glass}} \\ I_{II, \text{glass}} \end{bmatrix}} \]  

(3.1)

\[ R.I. = \frac{\begin{bmatrix} I_{II, \text{tissue}} & I_{\perp, \text{tissue}} \\ I_{II, \text{glass}} & I_{\perp, \text{glass}} \end{bmatrix}}{\begin{bmatrix} I_{II, \text{tissue}} & I_{\perp, \text{tissue}} \\ I_{II, \text{glass}} & I_{\perp, \text{glass}} \end{bmatrix}} \]  

(3.2)

\[ \text{Depolarization Ratio} = \frac{I_{\perp, \text{glass}}}{I_{II, \text{glass}}} \]  

(3.3)

3.3.3 Histogram Capture of Each Sample

The experimental setup shown in Figure 3.6 was used with the target at a rotation angle of \( \varphi = 0^\circ \). Data to calculate the full width at half maximum (FWHM) and also dynamic range of the system for each sample was obtained by recording the histogram of the backscattered signal detected by the single-pixel photodetector. The same procedure to obtain the histogram mentioned in section 3.1.1 Graphing Noise of the System was used here. With the target at a rotation angle of 0\(^\circ\), polarizers parallel with one another, and both generator and analyzer retarders (R1 and R2) set to their zero position, a histogram of the amplitude of the signal detected was created on the oscilloscope.

The data point displayed in the histogram was taken over a period of two milliseconds, \( 1/f_c \), where \( f_c \) is 500Hz, the frequency of the optical chopper. Each amplitude measurement that was displayed on the histogram, 1000 total, was actually an average of 10Ksamples since the system was sampling at a rate of 5M samples per second for two milliseconds. The histogram as a whole was an average of 100Ksamples. The
data from the histogram was saved in a Microsoft Excel® file and used for post-processing later.

3.3.4 Obtaining Mueller Matrix Elements of a Sample

With the experimental setup as shown in Figure 3.6 and the target aligned so that $\varphi = 0^\circ$ both generator and analyzer retarders were rotated in a 5:1 ratio. The same procedure described in section 3.2.3 was applied here. Once the coefficients were obtained using Fourier analysis, they were plugged into equations derived in [31] and were used to calculate the 16 elements of the sample Mueller matrix taking the error found in section 3.2.3 into consideration.

Once a total of 30 intensity measurements $\times$ 3 tests for each sample = 90 measurements were taken for the first sample containing normal tissue, the slide was removed and replaced with one containing Carcinoma In-Situ and then Stage I Carcinoma. The same experiments described above were performed again. This was done until all tissue samples (n=3) have 90 intensity measurements (3 tests) recorded.

3.3.5 Calculating Depolarization, Diattenuation, and Retardance of Each Sample

Once the error compensated Mueller matrix of each sample was found, the matrix was decomposed into three submatrices representing diattenuation, depolarization, and retardance. The equations used to decompose the calculated sample Mueller matrix were derived in [11] and [29]. It was from these three submatrices that the optical properties of the sample were calculated. Using equations 2.23-2.30 explained in Chapter 2, diattenuation, depolarization, and retardance were calculated for each sample.
3.3.6 Histogram Analysis

The histograms of the backscattered signals obtained from each sample were obtained on the LeCroy oscilloscope and imported into a Microsoft Excel® file. A short program developed in Matlab® was used to read the histogram data from the Microsoft Excel® file. To re-create the histogram in Matlab®, the raw data was uploaded into the Distribution Fitting Tool from the Statistics Toolbox. Using this GUI, the bin width and number of bins were selected. In this study, the Freedman-Diaconis rule was applied to the data (an option in the Distribution Fitting Tool) where the bin size was determined by equation 3.6:

\[ \text{Binsize} = 2IQR(x)n^{-\frac{1}{3}} \]  

(3.6)

where \( IQR \) was the interquartile range and \( n \) was the number of observations in the sample \( x \).

In this particular experiment, \( x \) was the detected amplitude vector from a tissue sample and \( n \) was the frequency of detection. Once the histogram was created, a distribution was fit to the data. In this experiment a Gaussian distribution was fit to all data. The probability plot was created from the same GUI which showed the goodness-of-fit between observed raw data and the fit distribution. Further validation to show the goodness of fit was done by finding the correlation coefficient. This was calculated by downsampling the fit Gaussian curve such that the length of the fit curve was equal to the length of the observed data in the histogram. From here, the Matlab® command ‘corrcoef’ was performed on these two signals to obtain the correlation coefficient.
The Full Width Half Maximum (FWHM) was calculated from the fit curve for the data that was then used to calculate the Dynamic Range (DR), in decibels, of the system for a particular sample using equations 2.32 thru 2.35 from Chapter 2. Figure 3.9 displays a graphical interpretation of finding the FWHM, $V_{\text{max}}$, and $V_{\text{min}}$ on the Gaussian curve fitting the data.

![Graphical interpretation of the FWHM](image)

Figure 3.9 Graphical interpretation of the Full Width at Half Maximum (FWHM) of a sample from a Gaussian curve fit to the observed data (Obs. Data).

3.3.7 Statistical Analysis

Once the Mueller matrix of each sample was obtained and the optical parameters (depolarization (DP), diattenuation (D), retardance (R), and FWHM) were calculated, a single-factor ANOVA at a 95% confidence level, 5% significance level ($\alpha=0.05$), was performed to test the null hypotheses proposed in Chapter 1. The single factor ANOVA followed the general model.
\[ Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \]  

(3.7)

where \( Y_{ij} \) is the response of the \( j^{th} \) subject in the \( i^{th} \) group, \( \mu \) is the fixed effect, \( \alpha_i \) is the effect of the \( i^{th} \) group (treatment effect), and \( \varepsilon_{ij} \) is random error associated with the \( j^{th} \) subject in the \( i^{th} \) group. A post priori test of the means was performed on the data using Tukey’s honest significance test to reveal which sample means were significantly different from one another.
Figure 3.10 Flow chart of the steps involved in the DRR experiment. MM is the Mueller matrix, D is diattenuation, DP is depolarization, R is retardance, FWHM is Full Width at Half Maximum, and DR is Dynamic Range.

**Error ranges are specified by Chenault et al. [31]**
CHAPTER IV
RESULTS

4.1 Beam Profile and Receiver Sensitivity Measurements

The beam profile of the 785nm solid state CW laser was experimentally assessed with and without a high contrast linear polarizer (Figure 4.1). The sensitivity pattern of the femtowatt photoreceiver was also found with and without a linear horizontal polarizer (Figure 4.2).

![Normalized 785nm Laser Rotation](image)

**Figure 4.1** Beam profile of the 785nm solid state laser with and without a high contrast linear polarizer.
4.2 Calibration of Polarimetric System using Dual-Rotating Retarder Method

The following results were taken using transmission geometry with no sample in the polarimeter to determine the orientation errors, in degrees, of the generator retarder (R1) and the analyzer polarizer and retarder (P2 and R2, respectively). An ideal polarizer and retarder, properly aligned, have an orientation error of 0°. The retardance values, in degrees, were also calculated for both generator and analyzer retarders. An ideal quarter-wave retarder has a retardance value of 90°; it introduces a 90° phase shift between orthogonal components of the light wave.

The experimental modulated intensity recorded with no sample in the polarimeter (air) is plotted in Figure 4.3 versus theoretical values. The theoretical and experimental Mueller matrices derived from these modulated signals are reported in Table 4.1. Matrix

Figure 4.2 Femtowatt photoreceiver sensitivity measurements with and without a high contrast linear polarizer.
elemental error between theoretical and experimental values are seen in Figure 4.4. The calculated orientation and retardance values for the components from the Mueller matrices are reported in Table 4.2.

Figure 4.3 Normalized experimentally detected modulated intensities with no sample (air) in the polarimeter (red). The theoretical values (blue) are plotted overtop to show the accuracy of the system.
Table 4.1 Theoretical versus calculated Mueller Matrices from experimental data for Air, Linear Horizontal Polarizer (LHP), and Linear Vertical Polarizer (LVP).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Theoretical Mueller Matrix</th>
<th>Experimental Mueller Matrix</th>
</tr>
</thead>
</table>
| AIR    | \[
\begin{pmatrix}
1 & 0 & 0 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 \\
\end{pmatrix}
\] | \[
\begin{pmatrix}
1 & -0.0032 & -0.0846 & -0.0246 \\
-0.0244 & 1.0542 & -0.0141 & -0.001 \\
0.0174 & -0.0419 & 1.0348 & -0.0019 \\
-0.0298 & 0.016 & 0.0436 & 1.009 \\
\end{pmatrix}
\] |
| LHP    | \[
\begin{pmatrix}
1 & 1 & 0 & 0 \\
1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
\end{pmatrix}
\] | \[
\begin{pmatrix}
1 & 1.1265 & 0.2479 & -0.0281 \\
0.9669 & 0.9753 & 0.2487 & -0.0275 \\
0.3966 & 0.4292 & 0.1119 & -0.0337 \\
-0.0115 & -0.0072 & 0.0031 & -0.0224 \\
\end{pmatrix}
\] |
| LVP    | \[
\begin{pmatrix}
1 & -1 & 0 & 0 \\
-1 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 \\
\end{pmatrix}
\] | \[
\begin{pmatrix}
1 & -1.0043 & 0.0031 & -0.0395 \\
-0.9762 & 0.9989 & -0.0014 & -0.0389 \\
-0.1155 & 0.1183 & -0.0426 & -0.0008 \\
0.0057 & -0.0092 & 0.0167 & -0.0164 \\
\end{pmatrix}
\] |
Figure 4.4 Experimental Mueller matrix elements calculated for no sample (air) in the polarimeter plotted (blue) along with the theoretical Mueller matrix elements of air (red), the identity matrix.

Table 4.2 Orientation errors calculated for the generator retarder (ε3), analyzer polarizer (ε4), and analyzer retarder (ε5). The true retardance values in degrees were calculated for the generator (δ1) and analyzer (δ2) retarders.

<table>
<thead>
<tr>
<th>Orientation Errors (°)</th>
<th>Retardance Values (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε3</td>
<td>ε4</td>
</tr>
<tr>
<td>-0.089</td>
<td>-3.443</td>
</tr>
</tbody>
</table>
4.3 Testing Two Known Samples in the Polarimeter

To test the accuracy of the system once calibration was performed and orientation errors and retardance values were found, samples with known Mueller matrices were tested. In particular, a linear horizontal polarizer (LHP) and a linear vertical polarizer (LVP) were tested.

4.3.1 Testing a Linear Horizontal Polarizer (LHP)

Setting the transmission axis of a high contrast linear polarizer parallel to the polarization of the 785nm laser light source created a linear horizontal polarizer (LHP) as the sample of interest. The detected modulated intensities compared with theoretical intensities are plotted in Figure 4.5. The experimental Mueller matrix derived from this modulated signal after error compensation is shown in Table 4.1 along with the theoretical Mueller matrix of a LHP. Matrix elemental error between theoretical and experimental values can be seen in Figure 4.6.
Figure 4.5 Normalized experimentally detected modulated intensities with a linear horizontal polarizer (LHP) in the polarimeter (red). The theoretical values (blue) are plotted overttop to show the accuracy of the system.
4.3.2 Testing a Linear Vertical Polarizer (LVP)

Setting the transmission axis of a high contrast linear polarizer perpendicular to the polarization of the 785nm laser light source created a linear vertical polarizer (LVP) as the sample of interest. The detected modulated intensities compared with theoretical intensities are plotted in Figure 4.7. The experimental Mueller matrix derived from this modulated signal is expressed in Table 4.1 along with the theoretical Mueller matrix for a LVP. Matrix elemental error between theoretical and experimental values is plotted in Figure 4.8.
Figure 4.7 Normalized experimentally detected modulated intensities with a linear vertical polarizer (LVP) in the polarimeter (red). The theoretical values (blue) are plotted overttop to show the accuracy of the system.
4.4 Linear Polarimetric Tissue Data

The first set of experiments with the sample lung tissues were performed under the geometry described in Chapter 3, Section 3.3 in the previous chapter. Only two samples were considered for this experiment involving only high contrast linear polarizers, namely Normal lung tissue and S1C, for simplicity and time constraints. Amplitudes of backscattered signals were obtained under co-polarized and cross-polarized configurations, as well as with polarizers removed. The results of using a co-polarized configuration and taking five measurements for each sample are shown in Figure 4.9. The amplitudes are reported as normalized amplitudes, normalized with respect to the background slide (glass). By taking the average of the five measurements
for each sample, there is a 34.27% increase in signal amplitude observed for Stage I Carcinoma from Normal (healthy) lung tissue. When the polarizers were rotated to a cross-polarized configuration, the difference decreases to 12.68% as can be seen in Figure 4.10. The standard error of the mean is much higher in this cross-polarized configuration as compared to the co-polarized configuration because of the low signal-to-noise ratio. Similar results were seen with no polarizers present, shown in Figure 4.11, with a percent decrease from Normal to SIC of 13.22%. In all plots, the error bars represent the standard error of the mean. Plotting the mean detected amplitude from all five measurements taken for each sample in each configuration (Figure 4.12) shows the effects of polarimetry on the detection process.

Figure 4.9 Normalized detected amplitudes of Normal lung tissue, S1C, and glass with polarizers in a co-polarized configuration. Data is normalized with respect to the mean amplitude detected of the background slide (glass).
Figure 4.10 Normalized detected amplitudes of Normal lung tissue, S1C, and glass with polarizers in a cross-polarized configuration. Data was normalized with respect to the mean amplitude detected of the background slide (glass).

Figure 4.11 Normalized detected amplitudes of Normal lung tissue, S1C, and glass with no polarizers in the polarimeter. Data was normalized with respect to the mean amplitude detected of the background slide (glass).
4.4.1 Calculating Depolarization, Degree of Linear Polarization, and Residual Intensity

With the detected mean normalized amplitudes found in Section 4.3, the residual intensity (RI), degree of linear polarization (DOLP), and linear depolarization (Dep) were calculated according to Equations 3.1 through 3.3 in Chapter 3. The results are reported in Table 4.3 and plotted in Figures 4.13 through 4.15. There was an 11.15% decrease in depolarization from Normal Lung to S1C (Figure 4.14). There was a 20.19% increase in the residual intensity and a 16.53% increase in DOLP from Normal Lung to S1C as shown in Figures 4.13 and 4.15, respectively.
Table 4.3 Depolarization, Degree of Linear Polarization (DOLP), and Residual Intensity calculated for Normal lung tissue and Stage I lung Carcinoma with the standard error of the mean.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>St. Error of Mean</th>
<th>Stage I</th>
<th>St. Error of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depolarization</td>
<td>0.6569</td>
<td>0.0067</td>
<td>0.4172</td>
<td>0.0195</td>
</tr>
<tr>
<td>DOLP</td>
<td>0.2071</td>
<td>0.0049</td>
<td>0.4118</td>
<td>0.0194</td>
</tr>
<tr>
<td>Residual Intensity</td>
<td>0.6930</td>
<td>0.0174</td>
<td>1.6318</td>
<td>0.0583</td>
</tr>
</tbody>
</table>

Figure 4.63 Residual Intensity calculated from the detected backscattered amplitudes normalized with respect to the background glass. The error bars represent the standard error of the mean.
Figure 4.74 Linear Depolarization calculated from the detected backscattered amplitudes normalized with respect to the background glass. The error bars represent the standard error of the mean.

Figure 4.85 Degree of Linear Polarization (DOLP) calculated from the detected backscattered amplitudes normalized with respect to the background glass. The error bars represent the standard error of the mean.
4.5 Tissue Mueller Matrices using the Dual Rotating Retarder Method

Using the experimental setup shown in Chapter 3, Section 3.3, Figure 3.6, tissue samples (Normal Lung, Carcinoma In-Situ, and Stage I Carcinoma) were interrogated with NIR light and the backscattered signal is analyzed. Using the DRR method described in Chapter 3, 30 intensity measurements were recorded during one test for each sample, forming a modulated intensity signal. The modulated intensity signals obtained from each sample from one test are shown in Figure 4.16. The data is normalized with respect to the maximum detected amplitude from that sample during that test since data was taken on separate days for each sample. Not all plots of the modulated intensities obtained for all tests for each sample are shown.

![Normalized Detected Modulated Intensities from Lung Tissue Samples](image)

Figure 4.16 Normalized detected modulated intensities from lung tissue samples (Normal, CIS, S1C) and the background (glass). The plots shown are of only one test for each sample. A total of three tests were taken.
From the normalized modulated intensities obtained from the experiment, the Mueller matrix of each sample was calculated. Three tests were performed on each sample resulting in three experimental Mueller matrices. The 16 elements of the matrix for each test were averaged and the result plus the standard error of the mean is reported in Table 4.4.

Once the Mueller matrix has been found for each sample, the matrices were decomposed into Depolarization, Diattenuation, and Retardance matrices according to Equations 2.21 through 2.29 in Chapter 2. The normalized intensity versus matrix element for each sample for the decomposed submatrices is plotted in Figure 4.17 through 4.19. From the decomposed matrices, the optical parameters were calculated. This resulted in three sets of values for each parameter for each sample. For easier viewing, these values were averaged and reported in Table 4.5 and plotted in Figures 4.20 through 4.22. The error bars represent the standard error of the mean.
Table 4.4 The 16 element Mueller matrices for each sample were calculated from the modulated intensities obtained through experimentation. The table displays the average matrix elements for each tissue along with the standard error of the mean calculated from all three tests performed.

<table>
<thead>
<tr>
<th>Mueller Matrix Elements</th>
<th>Normal Lung</th>
<th></th>
<th>Carcinoma In-Situ</th>
<th></th>
<th>Stage I Carcinoma</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>St. Error of Mean</td>
<td>Average</td>
<td>St. Error of Mean</td>
<td>Average</td>
<td>St. Error of Mean</td>
</tr>
<tr>
<td>m11</td>
<td>1.0000</td>
<td>0.0000</td>
<td>1.0000</td>
<td>0.0000</td>
<td>1.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>m12</td>
<td>0.0443</td>
<td>0.0406</td>
<td>0.2757</td>
<td>0.0080</td>
<td>-0.0218</td>
<td>0.0149</td>
</tr>
<tr>
<td>m13</td>
<td>-0.1892</td>
<td>0.0424</td>
<td>-0.2267</td>
<td>0.0022</td>
<td>-0.1127</td>
<td>0.0081</td>
</tr>
<tr>
<td>m14</td>
<td>0.0056</td>
<td>0.0373</td>
<td>-0.0748</td>
<td>0.0038</td>
<td>-0.0732</td>
<td>0.0041</td>
</tr>
<tr>
<td>m21</td>
<td>0.0343</td>
<td>0.0505</td>
<td>0.2145</td>
<td>0.0031</td>
<td>0.1296</td>
<td>0.0056</td>
</tr>
<tr>
<td>m22</td>
<td>0.4820</td>
<td>0.0134</td>
<td>0.2686</td>
<td>0.0154</td>
<td>0.4946</td>
<td>0.0120</td>
</tr>
<tr>
<td>m23</td>
<td>0.3685</td>
<td>0.0048</td>
<td>0.3023</td>
<td>0.0026</td>
<td>0.3797</td>
<td>0.0096</td>
</tr>
<tr>
<td>m24</td>
<td>0.0032</td>
<td>0.0056</td>
<td>-0.0218</td>
<td>0.0039</td>
<td>-0.0046</td>
<td>0.0035</td>
</tr>
<tr>
<td>m31</td>
<td>-0.0024</td>
<td>0.0165</td>
<td>-0.0345</td>
<td>0.0112</td>
<td>-0.0268</td>
<td>0.0085</td>
</tr>
<tr>
<td>m32</td>
<td>0.4711</td>
<td>0.0518</td>
<td>0.4394</td>
<td>0.0290</td>
<td>0.5551</td>
<td>0.0195</td>
</tr>
<tr>
<td>m33</td>
<td>-0.4798</td>
<td>0.0101</td>
<td>-0.5521</td>
<td>0.0163</td>
<td>-0.4864</td>
<td>0.0025</td>
</tr>
<tr>
<td>m34</td>
<td>0.0468</td>
<td>0.0088</td>
<td>-0.0466</td>
<td>0.0917</td>
<td>0.0298</td>
<td>0.0001</td>
</tr>
<tr>
<td>m41</td>
<td>0.0050</td>
<td>0.0417</td>
<td>0.0346</td>
<td>0.0049</td>
<td>0.0856</td>
<td>0.0028</td>
</tr>
<tr>
<td>m42</td>
<td>0.0765</td>
<td>0.0886</td>
<td>-0.0315</td>
<td>0.0098</td>
<td>-0.0755</td>
<td>0.0253</td>
</tr>
<tr>
<td>m43</td>
<td>-0.0381</td>
<td>0.0056</td>
<td>-0.0590</td>
<td>0.0040</td>
<td>-0.0331</td>
<td>0.0085</td>
</tr>
<tr>
<td>m44</td>
<td>-0.5236</td>
<td>0.0136</td>
<td>-0.6501</td>
<td>0.0054</td>
<td>-0.5507</td>
<td>0.0002</td>
</tr>
</tbody>
</table>
Table 4.5 Raw Mueller matrices decomposed into the submatrices Depolarization, Diattenuation, and Retardance for (a) Normal lung tissue (b) Carcinoma In-Situ and (c) Stage I lung Carcinoma.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Normal Lung Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>1 0.0443 −0.1892 0.0056</td>
</tr>
<tr>
<td></td>
<td>0.0343 0.4820 0.3685 0.0032</td>
</tr>
<tr>
<td></td>
<td>−0.0024 0.4711 −0.4798 0.0468</td>
</tr>
<tr>
<td></td>
<td>0.0050 0.0765 −0.0381 −0.5236</td>
</tr>
<tr>
<td>Depolarization</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td></td>
<td>0.0329 0.6068 0.0021 0.0502</td>
</tr>
<tr>
<td></td>
<td>−0.1469 0.0021 0.6759 0.0580</td>
</tr>
<tr>
<td></td>
<td>−0.0401 0.0021 0.6759 0.0580</td>
</tr>
<tr>
<td>Diattenuation</td>
<td>1 0.0904 −0.2325 0.0443</td>
</tr>
<tr>
<td></td>
<td>0.0904 0.9715 −0.0107 0.0020</td>
</tr>
<tr>
<td></td>
<td>−0.2325 −0.0107 0.9948 −0.0052</td>
</tr>
<tr>
<td></td>
<td>0.0443 0.0020 −0.0052 0.9683</td>
</tr>
<tr>
<td>Retardance</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td></td>
<td>0 0.7547 0.6492 0.0929</td>
</tr>
<tr>
<td></td>
<td>0 0.6297 −0.7569 0.1741</td>
</tr>
<tr>
<td></td>
<td>0 0.1834 −0.0729 −0.9803</td>
</tr>
<tr>
<td>Matrix</td>
<td>Carcinoma In-Situ</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Raw</strong></td>
<td></td>
</tr>
</tbody>
</table>
|             | \[
|             | \begin{bmatrix} 1 & 0.2757 & -0.2267 & -0.0748 \\
|             | 0.2145 & 0.2686 & 0.3023 & -0.0218 \\
|             | -0.0345 & 0.4394 & -0.5521 & -0.0466 \\
|             | 0.0346 & -0.0315 & -0.0590 & -0.6501 \\
|             | \end{bmatrix} \] |
| **Depolarization** | |
|             | \[
|             | \begin{bmatrix} 1 & 0 & 0 & 0 \\
|             | 0.2376 & 0.4398 & -0.0975 & -0.0208 \\
|             | -0.3156 & -0.0975 & 0.7973 & 0.0841 \\
|             | -0.0152 & -0.0208 & 0.0841 & 0.7042 \\
|             | \end{bmatrix} \] |
| **Diattenuation** | |
|             | \[
|             | \begin{bmatrix} 1 & 0.2757 & -0.2267 & -0.0748 \\
|             | 0.2757 & 0.9705 & -0.0324 & -0.0107 \\
|             | -0.2267 & -0.0324 & 0.9577 & 0.0088 \\
|             | -0.0748 & -0.0107 & 0.0088 & 0.9340 \\
|             | \end{bmatrix} \] |
| **Retardance** | |
|             | \[
|             | \begin{bmatrix} 1 & 0 & 0 & 0 \\
|             | 0 & 0.6779 & 0.7310 & -0.0755 \\
|             | 0 & 0.7240 & -0.6818 & -0.1015 \\
|             | 0 & -0.1254 & 0.0144 & -0.9918 \\
|             | \end{bmatrix} \] |

(b)
Table 4.6 Average diattenuation, depolarization, total retardance and the standard error of the mean calculated from the Mueller matrices of each sample of lung tissue (Normal, CIS, S1C).

<table>
<thead>
<tr>
<th>Optical Parameters</th>
<th>Normal Lung</th>
<th>Carcinoma In-Situ</th>
<th>Stage I Carcinoma</th>
<th>Glass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>St. Error of Mean</td>
<td>Average</td>
<td>St. Error of Mean</td>
</tr>
<tr>
<td>Diattenuation</td>
<td>0.2535</td>
<td>0.0038</td>
<td>0.3648</td>
<td>0.0061</td>
</tr>
<tr>
<td>Depolarization</td>
<td>0.3886</td>
<td>0.0064</td>
<td>0.3447</td>
<td>0.0046</td>
</tr>
<tr>
<td>Total Retardance (rad)</td>
<td>3.0093</td>
<td>0.0056</td>
<td>3.0783</td>
<td>0.0112</td>
</tr>
</tbody>
</table>
Figure 4.97 Normalized intensity calculated for the Mueller matrix elements of the decomposed Depolarization matrix for each lung tissue sample (Normal, CIS, and S1C).

Figure 4.18 Normalized intensity calculated for the Mueller matrix elements of the decomposed Diattenuation matrix for each lung tissue sample (Normal, CIS, and S1C).
Figure 4.19 Normalized intensity calculated for the Mueller matrix elements of the decomposed Retardance matrix for each lung tissue sample (Normal, CIS, and S1C).

Figure 4.20 Average diattenuation introduced by each tissue sample (Normal, Carcinoma In-Situ, and Stage I Carcinoma) and the background (glass).
Figure 4.21 Average depolarization introduced by each tissue sample (Normal, Carcinoma In-Situ, and Stage I Carcinoma) and the background (glass).

Figure 4.22 Average total retardance introduced by each tissue sample (Normal, Carcinoma In-Situ, and Stage I Carcinoma) and the background (glass).
4.5.1 Statistical Analysis

A single factor ANOVA was performed on the data with a post priori test of the means using Tukey’s honest significance test. It was found that there exists a significant difference (p < 0.05) in Diattenuation between all three samples (Table 4.7(a) and Table 4.8). The same is true for the Total Retardance (Table 4.7(c)). There exists a significant difference in Depolarization (Table 4.7(b)), however from Table 4.8 it can been seen that it is only between Normal and CIS and Normal and S1C. No significant difference was found between CIS and S1C.

Table 4.7 A Single Factor ANOVA with a 95% confidence level (α = 0.05) was performed on the data to reveal if there is a significant difference between samples for Diattenuation (a), Depolarization (b), and Retardance (c).

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>0.06303</td>
<td>2</td>
<td>0.03152</td>
<td>395.15</td>
<td>3.13E-06</td>
</tr>
<tr>
<td>Error</td>
<td>0.0004</td>
<td>5</td>
<td>0.00008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.06343</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a)

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>0.00316</td>
<td>2</td>
<td>0.00158</td>
<td>18.67</td>
<td>0.0048</td>
</tr>
<tr>
<td>Error</td>
<td>0.00042</td>
<td>5</td>
<td>0.00008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.00358</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>0.01657</td>
<td>2</td>
<td>0.00829</td>
<td>43.86</td>
<td>0.0007</td>
</tr>
<tr>
<td>Error</td>
<td>0.00094</td>
<td>5</td>
<td>0.00019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.01752</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(c)
Table 4.8 Post priori test of the means Confidence intervals calculated at a 95% confidence level revealing where significant differences in optical parameters exist. Cells highlighted in yellow indicate where a significant difference was not found.

<table>
<thead>
<tr>
<th>Optical Parameter</th>
<th>Normal vs CIS*†</th>
<th>Normal vs. Stage I</th>
<th>CIS vs Stage I*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retardance</td>
<td>[-0.1055, -0.0689, -0.0324]</td>
<td>[-0.1546, -0.1138, -0.0730]</td>
<td>[-0.0857, -0.0449, -0.0041]</td>
</tr>
<tr>
<td>Diattenuation</td>
<td>[-0.1350, -0.1113, -0.0875]</td>
<td>[0.0900, 0.1165, 0.1431]</td>
<td>[0.2013, 0.2278, 0.2543]</td>
</tr>
<tr>
<td>Depolarization</td>
<td>[0.0195, 0.0440, 0.0684]</td>
<td>[0.0078, 0.0351, 0.0624]</td>
<td>[-0.0362, -0.0089, 0.0184]</td>
</tr>
<tr>
<td>FWHM</td>
<td>[-7.3977, -3.3918, 0.6141]</td>
<td>[-9.3940, -4.9153, -0.4366]</td>
<td>[-6.0022, -1.5235, 2.9552]</td>
</tr>
</tbody>
</table>

* Depolarization and FWHM are not significantly different between samples
† FWHM is not significantly different between samples

4.6 Histogram Analysis

Histograms were recorded for each sample for all three tests performed and fit with a Gaussian curve as shown in Figures 4.23, 4.25, 4.27, and 4.29. A probability plot displaying the goodness-of-fit of the Gaussian curve to the experimental data was also created and shown in Figures 4.24, 4.26, 4.28, and 4.30. An overlay of histograms obtained from each sample displaying the difference in intensity distributions is shown in Figure 4.31.

The correlation coefficient, another measure of the goodness-of-fit between the Gaussian curve and the experimental data, is shown in Figure 4.32. The FWHM and Dynamic Range (DR) were also calculated for each test in each sample from the fitted Gaussian curve and the averaged results were displayed in Figures 4.33 and 4.34, respectively. Table 4.9 reports the average values of all statistical parameters calculated from the histograms.
Figure 4.23 Histogram of observed amplitude data from Glass (background) fit with a Gaussian (Normal) Distribution.

Figure 4.24 Probability plot of observed amplitude data from Glass (background) fit with a Gaussian (Normal) Distribution.
Figure 4.105 Histogram of observed amplitude data from Normal lung tissue fit with a Gaussian (Normal) Distribution.

Figure 4.26 Probability plot of observed amplitude data from Normal lung tissue fit with a Gaussian (Normal) Distribution.
Figure 4.27 Histogram of observed amplitude data from Carcinoma In-Situ fit with a Gaussian (Normal) Distribution.

Figure 4.28 Probability plot of observed amplitude data from Carcinoma In-Situ fit with a Gaussian (Normal) Distribution.
Figure 4.29 Histogram of observed amplitude data from Stage I Carcinoma fit with a Gaussian (Normal) Distribution.

Figure 4.30 Probability plot of observed amplitude data from Stage I Carcinoma fit with a Gaussian (Normal) Distribution.
Gaussian Fits to Detected Amplitudes from Normal Lung, Carcinoma In-Situ, and Stage I Carcinoma

Figure 4.31 Gaussian fits to detected amplitudes from Normal Lung Tissue, Carcinoma In-Situ (CIS), and Stage I Carcinoma (S1C) to display the differences in intensity spreads.

Table 4.9 Histogram statistical analysis for the three lung samples: Normal, CIS, and S1C.

<table>
<thead>
<tr>
<th></th>
<th>Normal Lung Tissue</th>
<th>Carcinoma In-Situ</th>
<th>Stage I Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>St. Error of Mean</td>
<td>Average</td>
</tr>
<tr>
<td>μ (mV)</td>
<td>500.36</td>
<td>2.4700</td>
<td>390.33</td>
</tr>
<tr>
<td>St. Error of μ</td>
<td>0.2461</td>
<td>0.0041</td>
<td>0.2600</td>
</tr>
<tr>
<td>σ (mV)</td>
<td>7.7842</td>
<td>0.1299</td>
<td>8.2231</td>
</tr>
<tr>
<td>St. Error of σ</td>
<td>0.1742</td>
<td>0.0029</td>
<td>0.1840</td>
</tr>
<tr>
<td>FWHM (mV)</td>
<td>17.5165</td>
<td>0.2535</td>
<td>19.1820</td>
</tr>
<tr>
<td>D.R. (dB)</td>
<td>0.1521</td>
<td>0.0027</td>
<td>0.1666</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.8563</td>
<td>0.0010</td>
<td>0.8997</td>
</tr>
</tbody>
</table>
Figure 4.32 Correlation Coefficient calculated between the observed histogram data and the fitted Gaussian curve for each sample: Normal, CIS, S1C and the background (Glass). The error bars represent the standard error of the mean.

Figure 4.33 Full width at half maximum (FWHM) calculated from the histograms of each sample: Normal, CIS, and S1C and the background (Glass). The error bars represent the standard error of the mean.
Figure 4.34 Dynamic Range (D.R.) in decibels calculated from the histograms of each sample: Normal, CIS, S1C, and the background (Glass). The error bars represent the standard error of the mean.

A single factor ANOVA was also performed on the FWHM at a 95% confidence level ($\alpha = 0.05$) shown in Table 4.10. A significant difference ($p < 0.05$) exists between Normal and S1C.

Table 4.10 A Single Factor ANOVA with a 95% confidence level was performed on the data for FWHM revealing a significant difference between samples.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>32.8036</td>
<td>2</td>
<td>16.4018</td>
<td>7.21</td>
<td>0.0336</td>
</tr>
<tr>
<td>Error</td>
<td>11.367</td>
<td>5</td>
<td>2.2734</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44.1707</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

Optical parameters of normal and pre-cancerous tissue using polarimetric Exploratory Data Analysis (pEDA) were obtained in this study. *In vitro* measurements of both normal and precancerous lung cancer tissues were performed with a NIR optical polarimeter imaging system. The experimental results indicate the following:

- Backscattered intensities from stage I lung adenocarcinoma are higher than those of normal tissue as shown in Figures 4.9-4.12 and 4.13;
- Stage I lung adenocarcinoma depolarizes incident photons less than normal tissue as shown in Figure 4.21;
- Similarly, backscattered photons maintain their original linear polarization state at a higher degree with respect to the normal tissue, as shown in Figure 4.15;
- Both cancerous and normal tissues partially depolarize linearly-polarized incident photons.

5.1 Calibration Results for the Dual-Rotating Retarder Polarimeter

Beam profile and receiver sensitivity measurements were obtained (Figures 4.1 – 4.2) revealing the wider sensitivity pattern of the femtowatt photoreceiver. The narrow
beam profile of the laser indicates the light source is sufficient for illumination of a small area of tissue for examination.

With this 785nm laser and femtowatt photoreceiver, modulated signals of air and known samples (LHP and LVP) were obtained using the DRR Method for system level calibrations (Figures 4.3 – 4.8). The accuracy of the system is shown in Table 4.1, listing the experimental versus theoretical Mueller matrices for the known samples. In particular, the calibration done with no sample in the polarimeter, air, was used to calculate the orientation errors of the optical components (Table 4.2). The errors were within the limits specified by Chenault et al. [31] in order to use the Mueller matrix algorithms they derived. These small orientation errors highlight the high degree of system calibration of the optical system. Specifically, the small error in retardance values suggests good wavelength matching between the source laser (785nm) and peak transmission wavelength of the quarter-wave retarders.

When testing known samples, the larger error present in the LHP (Figure 4.3) as compared to LVP (Figure 4.5) could be due in large part to the intrinsic orientation of the sample. A linear polarizer whose transmission axis was aligned parallel to the input light source (being horizontally polarized) was used for the LHP sample of interest. It was possible that the orientation of the polarizer did not meet the technical specifications as it should have; therefore it was not an ideal LHP.
5.2 Linear Polarization Tissue Data

The results from the linear experimental data exhibit a difference in Normal (healthy) lung tissue and Stage I lung Carcinoma (S1C) as shown in Figures 4.9 – 4.11. Small fluctuations between all five measurements show good repeatability and reveal the reliability of the polarimetric system. With the polarimeter in a cross-polarized configuration (Figure 4.10), the signal from S1C is slightly smaller than from Normal (healthy) lung tissue, suggesting that light interacting with S1C lung tissue maintains its polarization more so than when light interacts with Normal lung tissue.

The results of the system without polarizers (Figure 4.11) were similar to those obtained with the polarizers perpendicular to one another. The closeness in backscattered intensities between Normal lung tissue and S1C without polarizers compared to the large differences seen with polarizers (Figure 4.9) suggests that the introduction of polarizing elements (i.e. high contrast linear polarizers) offer the capability to distinguish between normal and cancerous tissues. The average of all five trials taken for each sample for each configuration (co-polarization, cross-polarization, and no polarizers), highlighting their difference in the detection of backscattered intensities, is shown in Figure 4.12.

The results of Figures 4.11 and 4.12 indicate that part of the backscattered light originates from singly scattered light from epithelial nuclei. In fact, considering a scattering coefficient of $\mu_0=170\times10^{-2}$ m$^{-1}$ (a typical value for a cancerous tissue), for a 5 $\mu$m epithelial length leads to an optical thickness $\tau=8.5\times10^{-6}<<1$, the linearly polarized photons incident on the cancerous tissue maintain their degree of linear polarization (DOLP) by approximately 40%. Similar results were also observed in previous studies.
by Chung et al. [24]. They found that cancerous tissue depolarized light less than the surrounding healthy tissue. This can be due to the changes in the surface of cancerous tissue, such as increased cellular components of the epithelial tissue.

In all graphs mentioned above, the normalized intensity is reported. This normalized intensity is the raw intensity recorded by the detector and normalized with respect to the average intensity obtained from the background slide (glass) the tissue samples were mounted on. The background slide or glass measurement on the graph is actually the glass plus black background. The glass depolarizes light more than S1C lung tissue since a black surface absorbs light and is highly depolarizing. This is similar to what occurs in thicker tissue samples that undergo multiple scattering and therefore loss of polarization. This is observable mostly under cross-polarized configuration which typically reveals backscattered light from deeper tissue layers.

On the other hand, light backscattered by the epithelial layer maintains polarization of incident light. By subtracting the cross and co-polarized intensities from each other, the scattered signal from the epithelial layer only is revealed and is referred to as the Residual Intensity [13]. The principal of residual intensity, used to distinguish epithelial cells from the deeper tissue layers, was redefined in this study to distinguish tissue from the background glass and was calculated using Equation 3.2. Eliminating the background results in the Residual Intensity plot shown in Figure 4.13.
5.3 Tissue Mueller Matrix using the Dual Rotating Retarder Method

Decomposition into three submatrices, namely Depolarization matrix, Diattenuation matrix, and Retardance matrix is performed. Table 4.5 (a) – (c) shows the normalized raw sample Mueller matrices decomposed into their corresponding submatrices. The normalized intensity of the Mueller matrix elements versus sample is plotted in Figures 4.17 through 4.19. Each decomposition emphasized different tissue optical properties. There is a difference in Depolarization (Figure 4.21) between samples in the elements $m_{23}$ through $m_{32}$. Diattenuation (Figure 4.20) reveals differences between samples in the elements $m_{12}$ through $m_{21}$. Retardance (Figure 4.22) reveals differences between samples in the matrix elements $m_{34}$ through $m_{43}$.

Referring to the epithelial structure of precancerous and early cancer stages, formation of clustered large-size cells take place leading to the thickening of the epithelium [14]-[16]. These effects prevent light from penetrating the tissue as deeply as it would in normal tissue giving rise to high photon backscattering, as shown in 4.9-4.13. In addition, because of the reduced amount in collagen, the early-stage cancerous epithelial structure tends to depolarize the light less than normal tissues [14], as shown in Figures 4.11 and 4.21.

Similar experimental observations were made in polar decomposition measurements for early oral cancer detection, although higher retardance differences among different stages of disease were observed [15]. By referring to Figure 21, it can be seen that the depolarization Mueller matrix elements for different lung cancer tissue
pathologies indicate that enhanced discrimination among different lung cancer is obtained through circularly type polarized waves rather than linearly polarized waves.

Referring to the bronchioloalveolar structure of the carcinoma, the presence of alveoli (cavities) would result in a lower percentage of light absorbing components than a non-cancerous tissue, due to the fact that cancerous tissue exhibits a lower percentage of oxygen saturation than a non-cancerous tissue. As result, this could be one of the reasons that cancerous tissue exhibits higher reflectance characteristics than healthy tissue; although this claim cannot be supported at this time at given operating optical wavelength.

5.3.1 Diattenuation

The chart of Diattenuation versus sample, shown in Figure 4.20, reveals a decrease in diattenuation introduced by the sample between Normal lung tissue and S1C. Diattenuation is when the intensity transmittance depends on the polarization state of the incident beam. For example, a polarizer has a diattenuation of 1, therefore one can deduce that a sample with high diattenuation will ideally have a lower depolarization value. The same follows for retardance. CIS resulted in a higher diattenuation than both Normal lung tissue and S1C. This could be due to the morphology changes of the tissue between CIS and Normal and S1C. Diattenuation was found to be significantly different between all three samples \( p < 0.05 \) (Tables 4.7(a) and 4.8) and therefore the null hypothesis proposed in Chapter 1 regarding Diattenuation is rejected.
5.3.2 Depolarization

Depolarization versus sample, shown in Figure 4.21, supports the data obtained earlier from the Linear polarization datasets described in Section 5.2 of this chapter. There exists a significant difference ($p < 0.05$) (Table 4.7(b)) between Normal lung tissue and both cancerous stages (CIS and S1C), therefore the null hypothesis regarding Depolarization is rejected. Normal (healthy) lung tissue depolarized light to a greater extent than cancerous tissue. The background (glass) showed little depolarization, which was expected, and follows with the understanding from the higher diattenuation value. Similar results were seen in previous studies by Chung et al. [24]. The increase in size of the cellular structures, in particular the nuclei, causes a denser epithelial layer (or the nuclei to cytoplasm ratio increases). This creates more of a hard barrier making it difficult for light to penetrate deeper, eliminating the occurrence of multiple scattering which is what depolarizes light [12, 13]. No significant difference was found in depolarization between CIS and S1C.

5.3.3 Retardance

Finally, Total Retardance is shown in Figure 4.22 as an increasing trend from Normal lung tissue through S1C. Retardance was found to be significantly different between all three samples. Our findings suggest that the cancerous structures do introduce a slight phase shift between the incoming and backscattered light. Further investigation on this matter is required as these results do not follow what other researchers have observed. It would be expected that normal tissue would have a higher
retardance value than cancerous tissue due to the denaturization of collagen fibers in cancerous tissue.

5.4 Histogram Analysis

Using the pEDA and incorporating histogram analysis in with polarimetric analysis just described above, further information about each tissue was revealed. From Figures 4.23 through 4.30 it can be seen that a Gaussian distribution is a good fit to the backscattered amplitudes obtained from all three samples (Normal lung, CIS, and S1C) as well as the background (glass). The probability plot, one measure of the goodness of fit, revealed a relatively linear relationship for all samples. The correlation coefficient is plotted in Figure 4.32 and the averages are shown in Table 4.9. For all three samples, the correlation coefficient is greater than 0.85, suggesting a relatively good fit between the chosen Gaussian distribution and the experimental backscattered signal.

The Full Width at Half Maximum (FWHM) was calculated from the Gaussian distribution. (Table 4.9). The average FWHM versus sample is plotted in Figure 4.33. The error bars represent the standard error of the mean due to the small sample size. There is an increase in FWHM between normal lung tissue and precancerous tissue, as well as an increase from pre-cancer (CIS) to S1C. The Dynamic Range (DR) is linearly related to the FWHM and therefore also increases as the stage of cancer progresses, as shown in Figure 4.34. However, the above statement applies only for precancerous or early cancer stages, since at more advanced stages pronounced vasculature of tissue would lead to reversed patterns.
The FWHM and DR are metrics that are directly proportional to the degree of polarization observed for each sample. A wider spread DR indicates a higher intensity signal (observed by S1C) while a narrow DR indicates a lower intensity signal (observed by healthy tissue). Comparing all three visually on the same graph, as shown in Figure 4.30, helps to see the increase in FWHM and therefore DR between normal tissue, CIS, and S1C. Although there is an increase in FWHM for all samples, a statistically significant difference is only found between Normal and S1C. This may be due, in part, to the noise of the system or a narrow data acquisition gate. Further elimination of the background noise or use of a larger gate must be implemented for further study.

The experimental findings of this study support the observation trends of other studies on different types of cancer, like oral and colon cancers [14]-[15]. However, this statement should be applied with caution as these findings are applicable only for certain types of cancers, because of the diverse histological, morphological and molecular information exhibited by different cancer types.

The outcome of this study supports that backscattered depolarization and retardance signatures could be significant not only to discriminate among different tissue pathologies but also for margin identification, although this study is still in its early stages.
CHAPTER VI

CONCLUSION AND FUTURE WORK

6.1 Conclusion

Light interaction with healthy and cancerous lung tissue \textit{in vitro} using polarimetric Exploratory Data Analysis (pEDA) was presented in this study. Changes in the optical properties, namely depolarization, diattenuation, and retardance, between normal and early stage cancer were revealed. The FWHM and DR calculated from the histograms of the backscattered signals also proved to be another important metrics for the characterization of cancerous tissue.

Statistically significant differences were seen in optical parameters between tissues (p < 0.05) and, therefore, the null hypotheses presented in Chapter 1 were rejected. The difference in diattenuation, depolarization, retardance, and FWHM revealed results that support the trends observed in previous research on different cancer types [12-13, 24]. However, this statement should be applied with caution as these findings are applicable only for certain types of cancers, because of the diverse histological, morphological and molecular information exhibited by different cancer types. The outcome of this study supports that backscattered depolarization and retardance signatures could be significant not only to discriminate among different tissue
pathologies but also for margin identification, although this study is still in its early stages.

6.2 Future Work

There are several factors that were not considered in this study due to time constraints. The rotation of the retarders in the DRR method was done manually; resulting in slower than desired rotation speeds and data acquisition times. Because of this, only one tissue sample was studied for each category of cancer (Normal, CIS, and S1C). Increasing the number of samples tested could increase the power of the tests, potentially revealing more information in areas where no significant differences were found since more data was needed (e.g. depolarization between CIS and S1C). Testing more samples will then allow for a blind study to be performed eliminating the possibility of false positives.

Varying the angle of incidence of the light interacting with the tissue can also be studied as it will result in different optical parameters. The sample was mounted on a rotational stage with an accuracy of 0.01°. Rotating the sample and performing the same experiments as described in this thesis will reveal how optical parameters change with a change in the angle of incidence.

Further, the samples in this experiment were fixed samples, not fresh. As far as we were aware prior to experimenting, no study had been performed using the polarimetric system and the Dual Rotating Retarder Method for either fixed or fresh samples. However, fresh lung tissue samples have been studied using other methods by
Bard et al. [25] and Gakuin [15]. As future work, in addition to increasing the sample size, using fresh samples may provide different optical information.

Finally, the experiments in this study were performed in vitro since this is a phenomenology study. Any implementation of this setup to a real clinical setting is impractical and requires a change in experimental design. However, using the information obtained from this experiment about healthy and cancerous lung tissue, one can go on to develop a clinical, endoscopic / bronchoscopic procedure that will aid in the diagnosis of NSCLC.

Currently, narrow band imaging and autofluorescence bronchoscopy utilize this endoscopic procedure and have already been introduced into the clinical setting to aid in early cancer detection and staging [1]. From the work in this thesis, the developed NIR optical polarimetric system utilizing pEDA also has the potential to contribute additional discriminate information to aid in advancements in cancer research.
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## APPENDIX A

### OPTICAL COMPONENTS USED IN EXPERIMENTATION

<table>
<thead>
<tr>
<th>Component</th>
<th>Manufacturer</th>
<th>Model</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Contrast Linear Polarizer (P1,P2)</td>
<td>Meadowlark Optics</td>
<td>UPM-100-VIS</td>
<td>An ideal linear polarizer allows transmittance of light oscillating parallel to its transmission axis while rejecting those components that are in the perpendicular plane [10].</td>
<td>2</td>
</tr>
<tr>
<td>Quarter-Wave Retarder (R1,R2)</td>
<td>Meadowlark Optics</td>
<td>AQM-100-0840</td>
<td>A quarter-wave retarder is also known as a phase shifter since it changes the phase of an incident optical beam. It transforms a linearly polarized beam of light whose axis is at ±45° into right or left circularly polarized light, respectively [3]. An ideal quarter-wave retarder has a phase shift of 90°.</td>
<td>2</td>
</tr>
<tr>
<td>785nm pulse-modulated, solid state laser</td>
<td>Intellite Inc.</td>
<td>IS785-100</td>
<td>The laser has a maximum power output of 100mW. The beam divergence is 2mrad and the size is adjustable.</td>
<td>1</td>
</tr>
<tr>
<td>Optical Chopper (C)</td>
<td>New Focus</td>
<td>3501</td>
<td>Modulates the continuous wave laser at a frequency of 500Hz.</td>
<td>1</td>
</tr>
<tr>
<td>Femtowatt Photoreceiver</td>
<td>New Focus</td>
<td>2151</td>
<td>1mm diameter aperture (NEP) ≤ 15 fW/Hz^{1/2} Maximum conversion gain of 1x10^{11}</td>
<td>1</td>
</tr>
<tr>
<td>3GHz Bandwidth Oscilloscope</td>
<td>LeCroy</td>
<td>Wavepro 7300A</td>
<td>Used to display transmitted signals detected by the single-pixel detector. The oscilloscope can also create a histogram of amplitudes of the incoming signal and save them to a Microsoft Excel® file.</td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
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<td>---------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Optics Table</td>
<td>Melles Griot</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Optic Mounts</td>
<td>Thorlabs</td>
<td></td>
<td>Rotational mounts to hold the high contrast linear polarizers and quarter wave retarders. The mounts have an accuracy of ±1°.</td>
<td></td>
</tr>
<tr>
<td>Translational Stage with motor controller</td>
<td>Vexta; Velmex, Inc</td>
<td>Stepping Motor – PK245-01AA; Controller VP900</td>
<td>Used to mount the rectangular optics mount holding the tissue slide. The controller has an accuracy of 0.01°.</td>
<td></td>
</tr>
<tr>
<td>Rectangular Slide Mount</td>
<td>Edmund Optics</td>
<td>NT54-997</td>
<td>Used to hold the tissue slides.</td>
<td></td>
</tr>
<tr>
<td>Optics Table Posts</td>
<td>New Focus</td>
<td>9912</td>
<td>Rigid posts that attach optical components to the optics table.</td>
<td></td>
</tr>
<tr>
<td>Filter Wheel</td>
<td>Thorlabs</td>
<td>FW1</td>
<td>Used to hold the filters.</td>
<td></td>
</tr>
<tr>
<td>Filters</td>
<td>Coherent</td>
<td></td>
<td>Used to attenuate the output laser power.</td>
<td></td>
</tr>
</tbody>
</table>