FREQUENCY RESOLVED CELL SIZES USING OPTICAL
COHERENCE TOMOGRAPHY

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ABSTRACT

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High resolution Optical Coherence Tomography (OCT) imaging is demonstrated using a superluminescent diode at 1290nm for detecting sizes of tissue cells. This system is fiber-based, built around a Michelson Interferometer. A high speed optical delay line utilizing a resonant scanning mirror at 4kHz in the reference arm allows for fast data acquisition. The choice of a superluminescent diode allows for a cost effective system capable of achieving 16μm axial resolution in air and 12μm axial resolution in tissue with a probing depth of approximately 3mm.
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Chapter 1

INTRODUCTION

Presented in this thesis is a measurement technique which can be used to characterize internal tissue structures through refractive index changes. The motivation arises from the fact that cells undergo morphological changes after becoming malignant, often characterized by the nucleus becoming enlarged [1]. Optical Coherence Tomography (OCT) is another tool that can be used to help in early diagnostics of stomach cancer in hopes of treating patients before cancer reaches its advanced stages.

This chapter provides the motivation and goals of the research presented in this thesis. It provides an introduction into some of the current technologies used in medical imaging followed by a brief introduction into OCT.

1.1 Cancer in America

In the U.S., approximately 23,000 people were diagnosed with stomach cancer in the year 2001 [2]. Cancer and its many forms, is one of the leading causes of death among people ages 45-64, along with heart disease. Part of the problem lies in the early detection of the cancer itself. Many times stomach cancer is already in advanced stages by the time it has been detected. For this reason, one in five people, overall, live for more than five years after diagnosis [3]. If stomach cancer is detected at an early stage, the survival rate increases to approximately 60%. Current detection methods include X-raying the upper gastrointestinal or GI tract, computerized axial tomography (CAT), and endoscopy.
1.2 Cancer detection methods

Some of the current methods to detect stomach cancer are: fluorescence, reflectance, and light scattering spectroscopy, autofluorescence imaging, ultrasound, and radiography. Ultrasound uses mechanical sound waves with a frequency around 10MHz. These waves travel into the tissue to be imaged and reflect off internal structures. Therefore, an echo resolved image is derived from the delay of returning sound waves. Resolution is directly related to the frequency used and for frequencies around 10MHz, a spatial resolution of up to 150μm (see Figure 1) is achievable with an imaging depth in the tens of centimeters.

Diffraction is a problem of ultrasound and distribution of energy within a sample can vary. Therefore, ultrasound systems need to make a distinction between near-field and far-field measurements.

![Comparison of OCT imaging capability versus High-frequency (30MHz) Ultrasound](image)

Figure 1: Comparison of OCT imaging capability versus High-frequency (30MHz) Ultrasound [4].

Magnetic Resonance Imaging (MRI), formally known as Magnetic Resonance Tomography, is another common diagnostic tool used in the medical fields. The way MRI works is by taking advantage of the magnetic fields found within water molecules. Since single protons from each hydrogen atom are constantly spinning, a randomly oriented magnetic field is present within the body. The MRI machine itself, essentially a large
magnet, orients these hydrogen protons in some uniform direction. Then, pulses of radiowaves are used to knock these protons from their orbits, flipping their orientation. When the pulse is off, these protons return to their original orientation and in doing so, release radio signals of a discrete frequency which can be used to generate a cross-sectional image. Resolutions for MRI techniques are in the range of 0.5 - 1mm. MRI works best in softer tissues, due to the presence of water, and can detect up to a 5% difference in the return signals.

Optical Coherence Tomography originates from Optical Time Domain Reflectometry (OTDR), which originally was used in optical component characterization and finding faults in fiber-optic cabling. OTDR provides the time-of-flight or group delay of backscattered light from index boundaries within a medium. OTDR coupled with Michelson Interferometry allows for higher-resolution measurements and became known as Optical Coherence-Domain Reflectometry (OCDR). OTDR is a one-dimensional technique whereas OCT is a two-dimensional technique as scanning is done in conjunction with a lateral scanning mechanism which provides en-face or surface scans at various depths within a medium. High speed scanning is necessary to acquire useful data that is within the time frame of biological movement. OCT is an excellent imaging technique with high spatial resolution and is an excellent choice for use in turbid media, although depth limited, such as human tissue. The advantage of OCT is its higher resolution through the use of short wavelength radiation. Common tomographic imaging techniques like MRI and ultrasound are unable to achieve as high spatial resolutions as OCT. Since more than 90% of all cancers originate in the epithelial region, OCT makes a good match since it is a high resolution, surface imaging technique.
The first application of OCDR to biological samples led to OCT as it is known today and was first demonstrated in 1991 by D. Huang, M. Hee, and J. Fujimoto for two-dimensional imaging of the retina and coronary artery [4, 5]. This original system was based on a Michelson interferometer design implemented in fiber and was the first system to be used in imaging biological samples. This system used a superluminescent diode at 830 nm, had an axial resolution of 17 μm with the reference arm containing a mirror on a translating stage. OCT needs to be performed with a low coherence light source such as diodes, lamps, or ultra-fast femtosecond pulsed lasers which have large bandwidths due to their short coherence times. Coherent lasers sources like the common HeNe collimated laser, are not used since the coherence length of these sources are much too long (order of meters and greater) to resolve anything useful.

1.3 OCT Forms

Optical Coherence Tomography can be performed either in the time or frequency domain. Systems that exist today are commonly hybrids of the two. In this thesis, the overall system is a time domain system, but the reference arm scanning mechanism used works in the frequency domain.

A time-domain system can be thought of in terms of a “time of flight delay” but OCT does not use traditional Doppler measurements as in techniques like ultrasound; rather OCT creates a gating effect with the interfering light. A widely used system design for an OCT setup is based around a Michelson interferometer. In the most basic of Michelson configurations, light from the source is divided into two paths, where one travels down what is referred to as a “sample arm” and the other a “reference arm” as seen in Figure 2.
an interferometric technique and serves as a cross-correlator between the sample and reference paths. Sources such as He-Ne lasers, have very long coherence times and the interference as seen by the detector will look rather continuous and is not of use in interferometric imaging as long coherence times lead to large or low resolutions.

![Michelson Interferometer Diagram](image)

Figure 2: Michelson Interferometer with a movable reference arm mirror and interference patterns for short and long coherent light.

One of the more popular methods used today is Swept-Source or SS-OCT, a Fourier domain method. The major difference in this type of system is the wavelength of the source is varied over some range to obtain a response of the sample over a range of wavelengths while the mirror in the reference arm is kept stationary. This can be achieved by the popular Ti:Sapphire laser combined with some method of external tuning.

One commercial product that recently became available on the market is a swept-source OCT system created by Thor Labs©. This system is powered by a light source capable of 1260-1390nm wavelengths with an output averaging 10mW and above. One
difference to note is in the operation of this system. Since the source is swept over a range of frequencies, a movable mirror is no longer required in the reference arm of the interferometer. The sweeping frequency effectively takes the place of a moving mirror so the system acquisition rate becomes a function of how quickly the source is swept.

Optical Coherence Tomography is a relatively new technology growing in popularity by its high quality imaging capabilities, low cost and relatively straightforward setup. Tomographic techniques like OCT, recreate images by combining images slices of two or three-dimensional objects. Analogous to Ultrasound, OCT is capable of far greater resolutions due to the use of light waves instead of sound waves. Another distinguishing feature of OCT is the longitudinal and lateral resolutions are independent from each other, unlike in traditional microscopy, giving OCT more flexibility in its design and operation.

OCT was initially used in ophthalmology as the transparent tissue of the eye and ocular structures is ideal for OCT's initial design based around an 830nm super-luminescent diode [5] since water absorption is low near that wavelength. Through advancements in broadband light sources, OCT can be applied to other applications such as material characterization and in-vivo imaging within living animals. Techniques such as light scattering spectroscopy are highly effective but are unable to resolve depth dependent information.

1.4 Project Goals

The goal of this project was to design an OCT system based on a Fourier-domain optical delay line capable of measuring cell sizes in the 20μm range with the equipment available. An attempt to recover the interference by examination of the frequency components will be
made. By analyzing different media, we attempt to see whether OCT is a viable supplementary tool to other cancer detecting imaging techniques currently used in the field today.
Presented in this chapter are the fundamental theories behind an OCT system. Since OCT is an interferometric technique, interference theory is covered along with the Michelson configuration of the interferometer. The light source and reference arm implementations are key areas of an OCT that determine system performance and each are discussed in this chapter.

2.1 Light Source

There are two components of an OCT system which have the largest impact on system performance, the choice of a light source and how the reference arm is implemented. The ideal source would have a large spectral bandwidth, high power and be very stable. Therefore, pulsed laser systems can be used to produce resolutions on the order of a micron as these types of systems are able to achieve bandwidths >100nm. However, these systems are typically large, very expensive and difficult to maintain, so their uses in clinical settings are limited. As an alternative to using these types of lasers, superluminescent diodes (SLDs) provide a much cheaper and still effective solution. Advancements in semiconductor processing since the early 90's when OCT was first demonstrated, allows for SLDs to be manufactured with bandwidths up to 60nm with powers ranging from 1-10mW. A summary can be seen in Table 1 of existing technologies.
Table 1: Comparison of various low-coherent light sources used in OCT systems [6].

<table>
<thead>
<tr>
<th>Light source</th>
<th>( \lambda ) (nm)</th>
<th>( \Delta \lambda ) (nm)</th>
<th>( I_C ) (( \mu )W)</th>
<th>Coherent power</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLD</td>
<td>675</td>
<td>10</td>
<td>20</td>
<td>40mW</td>
<td></td>
</tr>
<tr>
<td></td>
<td>820</td>
<td>20</td>
<td>15</td>
<td>50mW</td>
<td></td>
</tr>
<tr>
<td></td>
<td>820</td>
<td>50</td>
<td>6</td>
<td>6mW</td>
<td>Superlum Diodes Ltd.</td>
</tr>
<tr>
<td></td>
<td>930</td>
<td>70</td>
<td>6</td>
<td>30mW</td>
<td>(2002)</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td>35</td>
<td>21</td>
<td>10mW</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1550</td>
<td>70</td>
<td>15</td>
<td>5mW</td>
<td></td>
</tr>
<tr>
<td>Kerr lens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ti: sapphire laser</td>
<td>0.81 ( \mu )m</td>
<td>260</td>
<td>1.5</td>
<td>400mW</td>
<td>Drexler et al (1999)</td>
</tr>
<tr>
<td>Cr: forsterite</td>
<td>1280</td>
<td>120</td>
<td>6</td>
<td>100mW</td>
<td>Bouma et al (1996)</td>
</tr>
<tr>
<td>LED</td>
<td>1240</td>
<td>40</td>
<td>17</td>
<td>6.1mW</td>
<td>Schmitt et al (1997)</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASE fibre sources</td>
<td>1300</td>
<td>40</td>
<td>19</td>
<td>60mW</td>
<td>NTT El. Corp. (2002)</td>
</tr>
<tr>
<td></td>
<td>1550</td>
<td>80</td>
<td>13</td>
<td>40mW</td>
<td></td>
</tr>
<tr>
<td>Superfluorescence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yb-doped fibre</td>
<td>1064</td>
<td>30</td>
<td>17</td>
<td>40mW</td>
<td>Bushkunsky et al (1998)</td>
</tr>
<tr>
<td>Er-doped fibre</td>
<td>1550</td>
<td>80-100</td>
<td>16</td>
<td>100mW</td>
<td>Bouma et al (1996)</td>
</tr>
<tr>
<td>Tm-doped</td>
<td>1800</td>
<td>80</td>
<td>18</td>
<td>7mW</td>
<td>Bouma et al (1998)</td>
</tr>
<tr>
<td>Photonic crystal fibre</td>
<td>1.3 ( \mu )m</td>
<td>370</td>
<td>2.5</td>
<td>6mW</td>
<td>Hartl et al (2001)</td>
</tr>
<tr>
<td></td>
<td>725</td>
<td>370</td>
<td>0.75</td>
<td></td>
<td>Povazay et al (2002)</td>
</tr>
<tr>
<td>Thermal tungsten halogen</td>
<td>880</td>
<td>320</td>
<td>1.1</td>
<td>0.2 ( \mu )W</td>
<td>Fercher et al (2000)</td>
</tr>
</tbody>
</table>

SLD are basically diodes driven by high drive currents such that superluminescence occurs. The SLD has a broad spectrum due to the angled facets of the diode. These angled facets keep the SLD from lasing and from becoming a laser diode by providing enough feedback to keep any one mode from becoming dominant. Cavity modes are still present but no mode selection occurs.

In a real physical system, no light source can be purely monochromatic where the power spectral density (PSD) would consist of a single line at the emission wavelength. Instead all light is polychromatic and thus has a PSD of some finite width which means more than one wavelength is being emitted. As a note, no light can have an infinite PSD either as that would mean it emits light at all wavelengths. A SLD source has a Guassian
shaped output so its transform will be a Guassian as well and is the reason why sources with Gaussian spectrums are preferred.

The wavelength chosen for this system operates at 1290nm. The region around 1300nm is a good window to probe tissues as the absorption is kept to a minimum, as can be seen for melanin and hemoglobin in Figure 3. In addition, 1300nm is a wavelength with minimal dispersion in silica fiber and optical components in this range are readily available. For biological imaging in soft tissues, wavelengths ranging from 600-1300nm are commonly used [7].

![Figure 3: Wavelength dependent absorption for various light sources [7].](image)

2.2 Axial Resolution

Axial resolution, sometimes referred to as depth resolution, of an OCT system is one of its defining figures of merit. One of the main goals in imaging is to delineate the smallest
structures or achieve the highest resolution possible. The temporal coherence or depth resolution, of an OCT system is primarily determined by the properties of the light source. Spatial coherence plays a role in both depth and lateral resolution. As seen in equation 1, the axial resolution is a function of the center wavelength and the source bandwidth multiplied by a constant depending on the beam shape. For this experiment, the first term is a normalized Gaussian distribution, which is a common accepted estimation, although real physical beams are not perfect Gaussians. As (1) shows, the coherence length is inversely proportional to the spectral bandwidth and hence the second term is a function of the center wavelength squared divided by the spectral bandwidth. Once a coherence length is calculated, the axial resolution is just the coherence length divided by the group index of the material. In the case of soft tissue, common ranges for group index are roughly between 1.35-1.42 [8, 9]. Therefore, the coherence length determines the width of the interferometric signal and also defines a boundary on the axial resolution.

The axial resolution for a Gaussian source centered at $\lambda_0$ with a FWHM $\Delta\lambda$ is typically given by:

$$\Delta L = \frac{2 \cdot c \cdot \ln 2 \cdot \frac{1}{\Delta \nu}}{\pi}$$

(1)

where $\Delta \nu$ can be re-written as:

$$\Delta \nu = c \cdot \frac{\Delta \lambda}{\lambda_0^2}$$

(2)

substituting (2) into (1), yields a commonly used expression for coherence length [10]:

$$l_c = \frac{2 \cdot \ln 2}{\pi} \left( \frac{\lambda_0^2}{\Delta \lambda} \right)$$

(3)
The power spectrum density of the source, which contains the center wavelength, spectral bandwidth and shape, is the important factor in determining the resolution of the OCT.

\[ \Delta I_{FWHP} = \frac{2 \cdot \ln 2}{\pi} \left( \frac{\lambda_0^2}{\Delta \lambda_{FWHP}} \right) \]  

(4)

2.3 Transverse Resolution

Fiber based OCT systems fall under a special case of confocal microscopes. In traditional confocal microscope setups, an aperture is used to filter the light returning from a source. Light that focuses at points beyond one Rayleigh range from the focal plane is rejected by this specifically placed aperture located in front of a detector. Also, high numerical aperture (NA) lenses are typically used in the sample arm which provides a large lateral area in exchange for depth and is known as Optical Coherence Microscopy (OCM). The single mode fiber acts as this selective aperture in the OCT case and also causes light to have a Gaussian-shaped transverse shape in the far field unlike the airy disc pattern seen in free space by a circular aperture. A distinct advantage that OCT systems posses is the decoupling of axial and transverse resolutions. The axial resolution is mostly controlled by the light source while the transverse is controlled by the choice of optics for the sample arm. Since OCT does not have large penetration depths, low NA optics are normally used to give a larger axial depth range in exchange for a smaller lateral area.
The normalized Gaussian beam intensity profile focused on a sample can be expressed as:

$$I(r, z) = \frac{2}{\pi \cdot \omega^2} \exp \left( -2 \cdot \frac{r^2}{\omega^2} \right)$$  \hspace{1cm} (5)$$

The free space spot size or sometimes called the beam width of the Gaussian beam on a given sample is determined by:

$$\omega(z) = w_0 \sqrt{1 + \left( \frac{z}{z_0} \right)^2}$$  \hspace{1cm} (6)$$

where,

$$z_0 = \frac{\pi \cdot \omega_0^2}{\lambda}$$  \hspace{1cm} (7)$$

$\omega_0$ is the beam waist or the place where the beam width is minimum along the z-axis, $z_0$ is twice the Rayleigh range ($Z_R$) or sometimes called the confocal parameter. Also the Rayleigh range is commonly referred to as the depth of focus. The beam divergence is approximately the numerical aperture or related to the f-number ($f\#$) by:
The focused beam spot size on a sample is inversely proportional to the numerical aperture of the focusing lens and can be approximated by:

$$\Delta x = \frac{4 \cdot \lambda \left( \frac{f}{d} \right)}{\pi}$$

where $d$ is the spot size of the beam on the focusing lens and $f$ is the focal length of the lens. Note that this is still under the assumption of a Gaussian beam. The depth of focus is related to this spot size and the numerical aperture by:

$$b = 2 \cdot Z_R = \frac{\pi \cdot \Delta x^2}{2 \cdot \lambda}$$

As it can be seen, a large numerical aperture decreases the depth of focus and is why low numerical aperture lenses are preferable in OCT.
2.4 Michelson Interferometer

Interference is the superposition of two or more light beams in a given region of space. It presents the phase variation between the waves that created it in both time and space. OCT systems which are based on the Michelson configuration can either be done in free space or fiber. The disadvantage to using fiber is the silicate glass, a common fiber material, causes dispersion and polarization effects due to oxyhydrogen (OH) ion absorption. An advantage to using single mode fiber is its ability to cancel out higher order modes and thus reduce noise from multiple scattering in turbid tissues. Another advantage to using fiber is the sample arm can be made into structures such as probes for use in a clinical setting. While 1300nm wavelength range is good for tissue penetration due to its low absorption, it is also a great choice for fiber implementation as the dispersion within a fiber is minimized in this region. Since an OCT system is able to separate group velocity dispersion and phase delay, it can account for some of the dispersion caused by fiber by movement of the optics in the delay line [11-13]. In the end, dispersion will lead to slight blurring of the image and is not detrimental to the overall system performance for the length of fiber used in this setup.

The interference between two or more waves is a cross-correlation between all the waves involved. A special case of a cross-correlation is when a light source is made to interfere with itself, e.g. a mirror in the sample and reference arms, the cross-correlation becomes the autocorrelation function of the source. The coherence function and power spectral density (PSD) of the light source form a Fourier Transform pair which is known as the Wiener-Khinchine theorem:
\[ J(\tau) \leftrightarrow S(\omega) \quad \text{OR} \quad J(\tau) = \int_{-\infty}^{\infty} S(\nu) \cdot \exp(i2\pi \nu \cdot \tau) d\nu \]  

(11)

where \( S(\omega) \) is the PSD in the frequency domain and \( J(\tau) \) is the coherence function. This is generally why a light source with a broad bandwidth will have a short coherence length. The shape of the PSD affects the shape of the interference pattern. Since the interference pattern is partially created from the Fourier transform of the PSD, any spectral dips or bumps will lead to side lobes in the temporal coherence function and the interference pattern [14].

Since a Michelson Interferometer splits the source light, say equally, down two paths and recombines them to obtain an interference pattern, the intensity of the light at the detector can be expressed by:

\[ I_d(\Delta l) = \left( \alpha_r^2 + \alpha_s^2 \right) A^2 + 2 \cdot \alpha_r \cdot \alpha_s \cdot A^2 \Re \{ \exp(-i \cdot k \cdot 2 \cdot \Delta l) \} \]  

(12)

where \( \Delta l \) is the difference in path length between the reference and sample arm, \( \alpha_r \) and \( \alpha_s \) is the loss in amplitude of the reference and sample arm respectively, and \( A \) represents the amplitude of the two E-fields \( E_r \) and \( E_s \), which we are assuming to be equal for a 50/50 splitter. This interference contains both DC and AC components. While the DC component, the first term, is related to the amount of light present, we are interested in the AC component or the interference itself as the DC part does not carry the interference information. The interference is a sinusoid which fluctuates as a function of the phase difference between \( E_r \) and \( E_s \) as each travels down the reference or sample paths. So if we neglect the DC terms and just look at the AC part, we get:
where $S(\lambda)$ is the power spectral density of the source. A Taylor-series expansion around the center wavelength, to account for light being polychromatic, the expression then becomes:

$$ I_{AC}(\Delta l) = 2 \cdot \alpha_r \cdot \alpha_s \cdot \text{Re}\left\{ \int_{-\infty}^{\infty} S(\lambda) \exp\left\{-i \frac{2 \cdot \pi}{\lambda_0} 2 \cdot \Delta l \right\} d\lambda \right\} $$

(13)

where the higher order terms can be neglected since they are small compared to the lower ones leaving:

$$ I_{AC}(\Delta l) = 2 \cdot \alpha_r \cdot \alpha_s \cdot \text{Re}\left\{ \int_{-\infty}^{\infty} S(\lambda) \exp\left\{-i \left( \frac{2 \cdot \pi}{\lambda_0} - \frac{2 \cdot \pi}{\lambda_0^2} (\lambda - \lambda_0) \right) \frac{2 \pi}{\lambda_0^3} (\lambda - \lambda_0)^2 + ... \right\} d\lambda \right\} $$

(14)

$$ I_{AC}(\Delta l) = 2 \cdot \alpha_r \cdot \alpha_s \cdot \text{Re}\left\{ \int_{-\infty}^{\infty} S(\lambda) \exp\left\{-i \left( \frac{2 \cdot \pi}{\lambda_0} - \frac{2 \cdot \pi}{\lambda_0^2} (\lambda - \lambda_0) \right) 2 \cdot \Delta l \right\} d\lambda \right\} $$

(15)

If the reference arm contains a mirror with some velocity $\nu_m$, $\Delta l$ can be rewritten in terms of $(\nu_m \cdot t)$ and expanding the equation leaves us with:

$$ I_{AC}(\Delta l) = 2 \cdot \alpha_r \cdot \alpha_s \cdot \text{Re}\left\{ \exp\left(-i \frac{2 \cdot \pi}{\lambda_0} 2 \cdot \nu_m \cdot t \right) \cdot \int_{-\infty}^{\infty} S(\lambda) \exp\left(i \left( \frac{2 \cdot \pi}{\lambda_0^2} (\lambda - \lambda_0) \right) 2 \cdot \nu_m \cdot t \right) d\lambda \right\} $$

(16)

The first term is a phase term that is related to the velocity of the reference mirror and the center wavelength. In other words, placing a moving mirror in the reference arm generates a phase term through Doppler shifting. This Doppler shift frequency is given by:
This phase modulation caused by the Doppler shift leads to optical heterodyning of the signal. Conveniently enough, this places a modulation on our reference signal that provides two useful things: demodulation at a known frequency after detection and movement of the signal beyond baseband noise sources such as 1/f noise.

2.5 Interference

A wave’s ability to interfere is measured by its degree of coherence with other waves present. The time duration of an interfering wave can only be observed over the coherence time of the wave. The coherence time \( \Delta t \cdot e = \Delta \chi \) or the coherence length. The coherence time is inversely proportional to the frequency bandwidth \( \Delta v \). Since we are using light with large bandwidths, the waves are considered partially coherent sources. When observing the superposition of interfering waves, the amount of pathlength difference will change the intensity of the interference fringes. When the pathlengths are exactly equal, both waves will overlap exactly and maximums and minimums will coincide. When there is a path length difference, the bright fringes of one wave will shift and mix with a darker fringe of another wave. As a result, the interference will lose contrast; so therefore, the light traversing the two arms of the Michelson interferometer must ideally be pathlength matched as best as possible. Figure 6 shows the case of two interfering waves and the resultant interference wave.
Now, say we have two waves with different frequencies, \( W_1 \) and \( W_2 \), with frequencies equal to 1kHz and 1.1kHz respectively and equal amplitudes. As the two waves interfere we obtain:

\[
W_1 \cdot \cos(2 \cdot \pi \cdot f_1 \cdot t) + W_2 \cdot \cos(2 \cdot \pi \cdot f_2 \cdot t) \\
= 2 \cdot W_1 \cdot \cos\left(2 \cdot \pi \left( \frac{f_1 - f_2}{2} \right) t \right) \cos\left(2 \cdot \pi \left( \frac{f_1 + f_2}{2} \right) t \right)
\]

(18)

as a result, there will be two frequencies produced, one that is a sum and the other difference. The sum or 525Hz for this example is a fast oscillation frequency that is modulated by the slower, 50Hz one. Figure 7 shows what the interference of these two waves will look like.
Interference of two waves with different frequencies where a faster oscillation (black) that is modulated by a slower one (red).

2.6 Optical Delay Lines

In the simplest of cases, a delay line is constructed out of a reflecting mirror mounted on a translating stage. This would allow for light in the reference arm to be modulated and phase delayed as the mirror is moved with some approximate linear velocity. While this method works fine, the problem is this configuration is slow in terms of data acquisition speed and cannot be used to image any “fast” moving object. There are a number of different ways to construct an optical delay line.

Some of the earlier designs included rotating glass cubes which were able to achieve high scan velocities but suffered from non-uniform scanning rates and low duty cycles, ultimately leading to delay-dependent dispersion. Piezoelectric fiber stretching is commonly used today and is able to achieve high repetition rates (few kHz) but require large amounts of power and suffer from hysteresis and birefringence effects [15]. Methods involving moving masses such as rotating prisms and loudspeaker cones have not been able to achieve the scan rates necessary for real time OCT imaging, which is defined as 30 frames per
second. In addition, most of these mechanical methods have low duty cycles and non-linear velocities.

The rapid scanning optical delay line (RSODL) used in this experiment is derived from a technique used in femtosecond pulse shaping. The basic idea in this delay line can be seen by looking at the Fourier shifting property:

\[ x(t - t_0) \leftrightarrow X(\omega) e^{-j\omega t_0} \]  

where basically a phase ramp in the frequency domain translates to a group delay or time delay.

The configuration of this RSODL takes the form of a 4-f system from Fourier optics [16, 17]. As can be seen in Figure 8, there is an object plane, Fourier plane and an image plane. The lens between the object plane and the Fourier plane performs a Fourier transform on the light passing through it. For simplicity, if there is nothing present in the Fourier plane, then the object located in the object plane is perfectly reproduced in the image plane (inverted coordinates) as the second lens performs an inverse Fourier transform, reconstructing the original object.

![Figure 8: 4-f imaging system used in pulse shaping techniques.](image)
The RSODL used here is a folded 4-\(f\) system with a grating in the object plane and a resonant scanning mirror in the Fourier plane. By placing a scanning mirror in the Fourier plane, the angle it places on the incoming light can be mapped into a linear time delay. The resonant scanning mirror is a centrally-mounted mirror that pivots back and forth with 9° deflection under full swing.

As shown in Figure 9, light will transverse through the system once, reflect off the end mirror and return along its original path back into the fiber. This folded system provides two unique benefits: the effective delay is doubled than that from a single pass and any lateral off-set of the beam from the motion of the scanning mirror will be canceled out by the end mirror after sending the light back along its return path.

The grating is used to first spatially separate light into multiple frequencies (or wavelengths). The achromatic doublet lens performs the Fourier transform of the light
while providing dispersion compensation. The scanning mirror is the important part of the delay line as it controls several things: first, the amount of angular deflection that the mirror imposes will determine the amount of group delay that the light experiences as it offsets the light physically by some distance. This small "extra" length is what gives rise to the time delay. Second, the velocity of the mirror places a Doppler-shifted modulation on the light. Where the light strikes the mirror (Δx) determines the phase velocity of the light. The phase velocity shows up as the frequency of the interference itself. This allows one to adjust the modulation frequency applied to the interference to accommodate for detection electronics.

The interference is a cross-correlation between the two light paths consisting of the complex envelope of the cross-correlation function R_2 and a complex exponential carrier:

\[
\text{\overline{R}}_2(\Delta g, \Delta \phi) = R_2(\Delta g) \cdot e^{-jk_0 \Delta \phi} \tag{20}
\]

\(\Delta g\) and \(\Delta \phi\) are the group and phase delays respectively which are expressed in terms of a pathlength difference and \(k_0\) is the wavenumber of the source. As can be seen, the envelope is a function of the group delay, while the exponential carrier is affected by the phase delay of the system. For a system with mirrors in both sample and reference arms, (20) can also represent the autocorrelation of the source.

When the pathlength difference is scanned by the moving reference mirror, the time domain signal from the detector is related to this autocorrelation by the scan velocity of the delay. This means the carrier of the detector and autocorrelation are related through the phase delay scan speed of the reference mirror.
The scan speed is given by $V_\phi$ which is the derivative of the phase delay:

$$f_0 = \frac{V_\phi}{\lambda_0}$$  \hspace{1cm} (21)$$

$$V_\phi = \frac{d\Delta I_\phi(t)}{dt}$$  \hspace{1cm} (22)$$

The carrier frequency generated by the scanning mirror leads to a Doppler shift of the center wavelength $\lambda_0$ and affects the beat frequency of the interference signal. Basically, by changing the velocity of the scanning mirror in the reference arm, the frequency of the interference fringes can be changed. For scanning retroreflecting mirrors, $V_\phi$ is equal to $V_g$ and both are constants when the scan is linear. Unlike retroreflectors, the scanning mirror has a non-linear scanning velocity so $V_\phi$ and $V_g$ are time-varying functions. These non-linear regions of velocity occur near the maximum swing angle where the mirror slows down and reverses direction. A plot of the velocity would have a sinusoidal like pattern; so to make data processing easier, data are taken in the areas where the velocity is most linear.

To a rough approximation, this would be over the middle two-thirds of the scan range, resulting in a duty cycle of $\approx 33\%$ per direction. This is a downside to using scanning mirrors as their duty cycle is much less than retroreflectors (which have a 100% duty cycle). Another reason to take data over the middle two-thirds of the scan is due to cross-axis wobble of the mirror which should be minimized during this time, since the mirror's velocity is more linear than it is towards the edges where the mirror experiences acceleration changes.
2.6.1 Design of the delay line

The heart of an OCT system which determines its scanning range $\Delta z$ lies in pairing the grating and the lens, given a particular tilt angle and center wavelength. Using the grating equation and the small-angle approximation $\sin \theta \approx \theta$, the phase shift as a function of angular frequency is given by [9, 18]:

$$\phi(\omega) = \frac{4 \cdot \theta \cdot x \cdot \omega}{c} - \frac{8 \cdot \pi \cdot \theta \cdot l_f (\omega - \omega_0)}{p \cdot \omega}$$

where $\omega_0$ is the central angular optical frequency, $l_f$ is the focal length of the lens, $p$ is the grating pitch, $x$ is the linear offset from mirror center, and $\theta$ is the scan angle. The phase delay, given in terms of a pathlength difference, is:

$$\Delta l_\phi = 4 \cdot \theta \cdot x$$

and the group delay, given in terms of a pathlength difference, is:

$$\Delta l_g = 4 \cdot \theta \cdot x - \frac{4 \cdot \theta \cdot l_f \cdot \lambda_0}{p}$$

As (25) shows, the group pathlength difference is a function of the phase pathlength difference plus the second term which is dependent on the lens and grating. Both phase and group delay are dependent on the tilt scan angle $\theta$. The key thing here is that the second term is the dominant term and so the group pathlength difference is much larger than the phase pathlength difference for a given scan angle. Thus, the mirror only needs to be tilted slightly to generate a large group pathlength difference. Also, adjusting the offset $x$ will not significantly affect the group delay or pathlength difference.
Solving for a ratio between the focal length and grating pitch yields the expression:

\[
\frac{f}{p} = \frac{\Delta l}{4 \cdot \theta \cdot \lambda_0} = 14805
\]

(26)

where a group pathlength scanning range of 3mm with a scan angle of 3° is assumed. This results in a grating of 300 groves/mm and a lens with a focal length of 49.35mm or \(~2\) in. The diffraction off the grating can be found through the grating equation:

\[
p \cdot \sin \theta_m = m \cdot \lambda
\]

(27)

or resolving for \(\theta_m\):

\[
\theta_m = \arcsin \left( \frac{\lambda}{p} \right)
\]

(28)

This results in a diffraction angle of 22.77° for the first order mode. So if the input fiber is aligned at this angle, the grating, lens and scanning mirror can be aligned with their surfaces perpendicular to each other as can be seen in Figure 9. Differentiating the grating equation with respect to wavelength gives the expression:

\[
\Delta \theta_m = \frac{\Delta \lambda}{p \cdot \cos \theta_m}
\]

(29)

Equation (29) gives the relation between changes in diffraction angle \(\Delta \theta_m\) to changes in wavelength \(\Delta \lambda\). Solving for \(\Delta \theta_m\) yields a diffraction angle of 0.94° which converted to linear spread by:

\[
\Delta x = \Delta \theta_m \cdot f
\]

(30)
gives a linear spread of \( \sim 0.81\text{mm} \). Finally, another consideration for the lens used is its diameter. Since we are assuming a scan angle range of 3°, a maximum of 6°, the required size of the lens must be greater than:

\[
d = 2 \cdot f \cdot \tan 2\theta
\]  

(31)

\( d \) represents the diameter of the beam as it passes through the lens so solving for \( d \) yields 10.22mm minimum lens diameter size. To save space in the design, a 12.5mm (0.5in) lens was used.

Recall that for some given offset \( \Delta x \) (or \( x \)), the Doppler shift introduced by the mirror will impose a phase delay on the interferometric signal. This phase delay determines the carrier frequency of the fringes and is approximated by:

\[
f_f = \frac{2 \cdot V_m}{\lambda_0}
\]  

(32)

For a resonant scanner at 4kHz, with a scan angle of 3°, gives a scanning rate of \( \sim 420\text{mm/s} \) and a fringe frequency \( f_f \) of \( \sim 650\text{kHz} \) for a 2mm \( \Delta x \) offset. The frequency bandwidth of this signal around \( f_f \) can be approximated by:

\[
\Delta f = \frac{\Delta \lambda \cdot f_f}{\lambda_0}
\]  

(33)

For a system with a Doppler shifted frequency of 650kHz, the frequency bandwidth is calculated to be 22.9kHz. By increasing \( \Delta x \), the Doppler shifted frequency increases.
2.7 Data Detection and Processing

OCT images are formed by processing the interference between the reference arm light and the backscattered light from a sample. Since there is a modulation on the signal, demodulation of the signal is necessary to recover the information. One way to process the interferometric data obtained from the A-scan is to apply a Hilbert Transform. The Hilbert transform is a mathematical method used in signal processing that describes the complex envelope of a modulated signal and helps to show the relationship between real and imaginary parts of that complex signal. The Hilbert transform (time domain) is obtained by convolving a signal with $\sqrt{\pi \cdot t}$, approximated by the signum function. So in the time-domain, the Hilbert transform takes the form of:

$$H\left(\frac{1}{\pi \cdot t}\right) = -j \cdot \text{sgn}(f)$$

where $H(\cdot)$ represents the Hilbert transform. It is easier to understand the Hilbert transform in the frequency domain by following its effects on a cosine wave. Basically, all negative frequencies of a signal get a $+90^\circ$ phase shift and all negative frequencies get a $-90^\circ$ phase shift.

$$\cos \theta \rightarrow \sin \theta \rightarrow -\cos \theta \rightarrow -\sin \theta \rightarrow \cos \theta$$

The resulting signal after applying a Hilbert transform is the original signal plus its Hilbert-transformed part. In the case of a cosine, the resulting signal would be: $\cos \theta + j \sin \theta = e^{j\theta}$.

Many demodulation systems use lock-in amplifiers for detection since they are able to measure AC signals around a tailored frequency band. However, since the modulation of the OCT system presented is beyond the 102kHz maximum frequency range of the reference
channel for lock-in amplifier available (SR830), using a lock-in amplifier will not work. For demodulation, a demodulating logarithmic amplifier (Analog Devices, AD606) is used which amplifies and produces an envelope of the interference signal. The envelope is then displayed on the oscilloscope and Labview is used to capture and store this data set. The envelope stored represents a profile of the material the light has reflected off on one depth scan. The sample is then translated laterally so that another depth profile can be obtained. Combining these envelopes side by side will give a two-dimensional reconstruction of the internal structure of the sample being probed.
Chapter 3

EXPERIMENTAL SETUP

Presented in this chapter is the experimental setup of the OCT system. The performance of the system is characterized by the use of a mirror and glass slide, each providing different aspects of the system performance.

3.1 Characterization Setup

The purpose of the characterization step is to verify the operation of the OCT system with calculated values. Figure 10 below shows the general setup of the system.

![Optical Coherence Tomography System Diagram]

Figure 10: OCT Characterization system setup with a mirror as the sample. Dashed lines represent electrical paths.
The light source used is a Superluminescent diode (Superlum, SLD-57-HP) with a center wavelength of 1290nm, a FWHM spectral width $\Delta \lambda$ of 45.5nm and output power up to 10mW. This light source is fiber pig-tailed and is connected to a fiber isolator (Thor Labs, 4013SAFC) to protect the SLD from optical feedback. This light enters a 50/50 coupler (Newport, F-CPL-F12135) where it is split equally down the reference and sample arms.

In the reference arm, the fiber is connected to a collimator (Thor Labs, F220FC-C) which sends the light into the optical delay line. Once inside, the light reflects off a 300g/mm plane ruled grating (Newport, 10RG300-1000-2) which has an efficiency of $>80\%$ at 1300nm. The light then passes through an achromatic doublet lens (Newport, PAC028) and on to the resonant scanning mirror (Electro-Optical Products Corp., SC-30) which operates at 4kHz with a maximum swing angle of 18°. A 0.1-0.5ND filter was used in the reference arm to balance the intensity of light compared to the sample present in the sample arm.

In the sample arm, there is a custom made 5in fiber extension which was used to account for the free space distance (~21in) in the reference arm. This was done so light in the sample arm does not have to travel far in free space and thus conserves optical power. The light from this fiber is collimated again (Thor Labs, F220FC-C) and enters a microscope objective (Newport, L-10B) for focusing onto the sample. The microscope objective has a numerical aperture of 0.25 and a focal length of 12mm, which reduces the axial depth resolution to $<900\mu m$. The sample sits on two manual translation stages that provide motion in both $x$ and $y$ directions allowing movement of the sample into and out of range and also laterally across the surface. Synchronous or heterodyne detection is done via a detector. The first detector used (Newport/Picometrix, D30-IR) accepts a fiber input and eliminates the need for additional lenses. The second detector (Analog Modules, Inc. 712A-9) was used in
the later part of the experiment and had a free-space photodiode input, requiring focusing of
the light through a pair of collimators. A 0.1ND (neutral density) filter was used in front of
the photodiode to reduce source intensity and outside noises. After the detector, the
interference pattern is viewed directly on an oscilloscope (Agilent 54622A) which is connected
to a PC running Labview. Labview is able to collect 2000 sample points from the oscilloscope
via a GPIB module attached to the oscilloscope. The data that is captured by Labview is
determined by the current settings of the oscilloscope so adjusting the time/voltage scale on
the oscilloscope can yield different sets of data for the same sample.

When the scanning mirror is turned on, the group delay generated from the reference
arm can be seen on the oscilloscope in the form of a flat-topped Gaussian like shape (Figure
11). Since the shape of the delay is highly dependent on the angle of the scanning mirror,
slight movement of the scanning mirror will drastically change the shape of the delay as can be
seen in Figure 12. A flat-top Gaussian shape was chosen since the interference pattern is
simpler to visualize and analyze when the amplitude is constant. There is no difference in the
interference pattern if the interference was on a flat portion or sloped portion of the group
delay envelope. The interference will begin to decrease as it moves towards the outer edge of
the envelope or out of the depth of focus. Adjustments to the shape of the delay were
performed by slightly adjusting the reflecting end mirror in the delay line.
3.2 Mirror Results

When a mirror is placed in the sample arm, the resulting interference pattern is the autocorrelation function since the source is essentially looking at itself and reveals the point spread function (PSF) of the imaging system. Recall that the interference pattern obtained from a mirror will reveal the Gaussian shape of the source that can be seen below in Figure 13 and in the FFT in Figure 14. The period of the interference is approximately 1 μs which corresponds to a Doppler frequency of ~1MHz given a 3 mm Δx offset. The three sharp
peaks in the interference with a width of approximately 2μs are seen on the FFT as the 500kHz FWHM of the Gaussian shape.

Figure 13: Gaussian shaped interference fringes obtained from a mirror sample.

Figure 14: FFT of mirror interference pattern.
3.3 Microscope Slide

A microscope slide was used to check two performance characteristics of the OCT system: one to see how weak of a return could be detected and its ability to resolve multiple surfaces. Glass has an index of refraction of approximately 1.45 with a reflectivity of approximately 4% for NIR light [19]. Interference off the surface of the glass yields an interference pattern similar to a mirror but slightly different as the slide is not a perfect reflector like a mirror. Figures 15 and 16 show the interference patterns generated by the microscope slides placed in the sample arm. The back surface of the glass slide is more difficult to measure as the interference is much weaker. While the time-domain signal is difficult to resolve, the frequency-domain components of the interference pattern are easily seen and coincide with those from the front surface.

Figure 15: Interference pattern from the front surface of a microscope slide and the corresponding FFT signal.
Figures 15 and 16 show an interference pattern that is difficult to resolve based upon the resolution of the oscilloscope. However when the data is captured and viewed in Labview, the interference pattern can be analyzed and visualized much easier. Figure 17 shows an interference pattern with peaks located ~1mm apart confirming the two surfaces of the glass slide. However, since the working distance of the sample arm with the microscope objective is <1mm, the translation stage was manually moved forward until the interference appeared for the back surface. Moving the micrometer stage forward by 72.1 μm yields a physical slide thickness of 1.045mm. Calipers were used to measure the slide thickness and found to be 0.991mm, a difference of 5.2%. One cause for the error lies in the user's accuracy in interpreting where the interference begins to occur and the accuracy of the micrometer used on the translation stage.
Figure 17: Interference of a 1mm microscope slide where the peaks correspond to the front and back surfaces.
Chapter 4

TEST SAMPLE RESULTS

4.1 Onion

An onion makes a good sample as it is multi-layered and can demonstrate the resolution capability for this system. Typical cell thickness for onions range from 50-70μm, averaging 140x400μm in size [20]. Recall that since there is a microscope objective to focus the light onto the sample, the axial depth scanning range is reduced to a distance of about 900μm. While the actual depth range is larger, it becomes difficult to distinguish signals close to the 900μm range as the SNR decreases and the signal becomes buried into the background.

The majority of an onion is comprised of water with an index of \( n \approx 1.33 \), a 25% mismatch from air and can be approximated to have a 2% reflection. Since the glass slide had a reflection of about 4% the interference for an onion was smaller than the resolution of the oscilloscope's voltage scale (1mV) but could be seen in the FFT. Therefore, data was captured when the interference was present and analyzed via Labview. Figure 18 shows the interference pattern. From the data, the top of the onion (skin layer) can be seen along with a few of the inner layers. From the three distinct inner peaks, the approximate cell sizes calculate to be around 80μm and 70μm. There appears to be another smaller interference signal at 1150μm and 1200μm which would indicate a cell size of 50μm, still within the range. Being that the onion sample has a curved shape and not comprised of perfectly flat cells, some cells may be out of alignment and would not have been detected. Since the cell walls are \( \sim 5\mu m \) in width and the transverse resolution of the system is \( \sim 10\mu m \), distinguishing between individual cells
on a lateral plane would be extremely difficult and not possible on this system without a higher SNR. This result has been confirmed by Xu [21] as a reported onion of 50-70μm was measured and can be seen in Figure 19.

Figure 18: Interference pattern from an onion sample showing the top layer and several inner layers.
4.2 Chicken Skin

A piece of chicken skin approximately 1mm thick was placed as a sample to determine if the system could resolve the fatty tissue layer and any following tissue layers beneath the skin. Chicken skin was chosen as a sample due to its close resemblance to human tissue and for its non-rigid structure. Chicken skin is heavily comprised of fat (n≈1.45) while the tissue beneath is made up of a more dense, fibrous-like muscle tissue (n≈1.37) [22]. In this experiment, the voltage intensity of the interference pattern was difficult to observe on the oscilloscope and data was captured when a signal appeared in the FFT. Using the D-30IR, the interference and FFT signals can be seen in Figures 20 and 21.
Figure 20: Interference signal from a piece of chicken skin.

Figure 21: Corresponding FFT signal from Figure 20.
The center frequency of the FFT as captured by the 712A-9 occurred at ~810kHz so the frequency content at that point is analyzed. The electrical bandwidth of the interference signal is calculated to be ~70kHz. When observing a reflective sample, the width of the interference pattern falls between 12 and 16µs. This corresponds to a frequency width between 62-83kHz. Looking at Figure 22, there are three distinct peaks near 810kHz. The distance to the second peak is 60kHz and 80kHz to the third. Using Labview to inverse FT the signal and after applying a Hilbert transform and low pass filtering, the envelope of the three peaks is obtained. The period of these three peaks equate to 17µs and 13µs. Assuming the total width of the group delay is 30µs, the time-domain width of the interference would be a ratio of the interference width over the 30µs width, to a rough approximation. This gives a ratio of 0.566 and 0.432 respectively. Then if the total depth of focus is 900µm, then the two peaks have a calculated distance of 510µm and 390µm.

Figure 22: FFT of a chicken skin sample showing three distinct peaks at 820, 880, and 960kHz.
The envelope peaks shown in Figure 23 give an approximate distance of 460µm to the first reflection point and 380µm to the second. However, I believe this to be more of an anomaly rather than a true interference signal for several reasons. For one, the thin fatty layer found under the skin is much thinner than the 380µm distance depicted. Secondly, the second or third interference pattern does not represent a fat-to-tissue interface due to the difference in index of refraction. Given the difference between the two indexes, the reflection coefficient would be approximately 0.8% and I don’t believe the system would be able to distinguish a boundary on that resolution with the two further distinct peaks depicted in Figure 23. Finally, the magnitude of the reflection from a point almost 1mm into the sample would not yield a reflectance similar to one from half that distance. I believe that the system was only able to measure the reflectance off the surface of the skin where the reflection coefficient is closer to that of an air-glass interface at 3.4%.

Unlike the previous samples, the chicken skin was not able to be mounted in a position that was perpendicular to the incoming light. Having a perpendicular angle to the
incoming light is the optimum angle for a sample as that allows for the most direct reflection of the light but being that chicken skin is soft and has no rigid shape, the diffuse and direct reflection most likely was reflected in random angles, thus contributing to even a greater loss of the signal. The light returning from the sample would then be much weaker and while an interference signal was detected, it was not strong enough to produce a distinct detectable interference signal.
Chapter 5

SUMMARY

Presented above is an imaging system capable of high resolutions. While the data above shows marginal performance of the system, the capability of this system could be greatly improved with a few modifications. Unfortunately, this project required more resources than available on hand to be more successful. Part of the goal of this project was to see if a system could be built with the existing equipment and measure performance. While it has been demonstrated that a system can be built, this system will not quite perform as expected and several improvements to the system need to be made in order to achieve performance closer to the design specifications.

The use of a balanced detector with amplification would be ideal. The first unit used in this experiment had sufficient sensitivity and a fiber input, but offered no amplification. External methods to amplify the signal using IC amplifiers and passive components proved to be futile. In order to do so, a narrow bandpass filter exactly at the Doppler frequency with a passband of twice the electrical bandwidth would be required. I found this a bit difficult as the interference can move in frequency depending on the point at which the interference is detected. The second detector used had amplification of $10^6$ but had a free-space photodiode input. The electronics in this detector appeared to be much noisier than the first so detecting the small interference signals from the tissue samples was very difficult. Some bit of averaging on the oscilloscope would help with the visual display of data for collection but often times led to the signal being averaged out. Therefore, use of the FFT to
find interference signals became important for finding interference patterns and was explored in the methodology.

A data acquisition card needs to be used to more accurately capture the data from the scanning mirror. This system was originally designed for real-time imaging in hopes that the imaging could be displayed on a TV monitor. However in order to do so, the data must be captured quick enough to avoid a misleading representation of the data. Unfortunately, there was not a data acquisition card available and data collection using the oscilloscope did not seem quick at times as the data sometimes appeared block-like rather than composed of smooth lines. A two-dimensional image could not be created because I believe the data acquisition portion of the system led to inconsistent scans of the same area. Also, subsequent lateral movement of the biological samples did not always yield a detectable interference pattern.

Lastly and quite importantly, motorized translation stages would greatly increase the performance capability of the system. This way the stages have a fixed amount of movement and a program could be made to automate and coordinate scans with lateral scanning movement and data collection. Since stages were not available, I moved the sample manually with the micrometer at 10\(\mu\)m increments. While this rough technique is still valid, creating a cohesive picture will be difficult since lateral movement is only estimated by eye.

I have confidence in the ability to analyze the frequency information to recover the interference pattern for analysis as a viable technique for OCT imaging. While the strength of this technique lies in the accuracy of interferometric measurement, it proved to be a challenge to image a biological sample. The system was shown to distinguish sizes of greater than 50\(\mu\)m and should be able to image smaller cells. Malignant cells can become larger than
this size so with some system improvements, this OCT system should not have a problem imaging them and would be a great supplemental tool in the diagnostics of early cancer detection and high resolution biological imaging.
APPENDIX

Shown below in Figure 24 is the Labview VI, “waveformplaybackv1.1.vi”, used to analyze the collected interference pattern. This VI is mainly used to compute the fast Fourier transform, Hilbert transform, and filtering of the interference signals. Another VI, HP54622Av1.8.3.vi, was used to capture data from the oscilloscope into Labview and was built by D. Ling for another project.

Figure 24: Labview VI used to process interference signals after collection.
A-scan. Axial scan or in the longitudinal/z-direction (depth).

Achromatic Doublet Lens. A special lens created from two individual lenses which compensates for dispersion normally experienced in using a single lens.

B-scan. Lateral scan or in the x/y-direction (surface).

Coherence Gating. Distance over which light remains coherent (coherence time) where measurements are taken. An OCT system has a built-in coherence gating property due to the coherence length of the light source.

En-face scan. A lateral (area) scan at a specific depth.

Optical Coherence Tomography. Interferometric imaging technique based upon the interference of low coherence light. Similar to ultrasound B-scan.

(Rapid Scanning) Optical Delay Line. A system utilizing Fourier properties to create phase and group delays in the reference arm of the Michelson Interferometer.

Point Spread Function. Propagation of light from a point source. The PSF is determined by the imaging system and helps to describe the interaction of light with the various “layers” of the sample being imaged from the OCT system.

Power Spectral Density. The power carried by a wave per unit frequency.

Superluminescent Diode. A low coherence light source commonly used in OCT for its broad bandwidth, robustness, portability and cost versus complex pulsed laser systems.
REFERENCES


