OPTICAL BIOPSY OF IN VIVO TISSUE USING OPTICAL COHERENCE TOMOGRAPHY

by

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ABSTRACT

The ability to obtain optical biopsies, or micron scale, cross-sectional, optical images of tissue microstructure in situ, would aid the diagnosis and clinical management of many diseases. Optical coherence tomography (OCT) is an optical imaging technique that uses low coherence interferometry to perform high resolution, cross-sectional imaging in biological systems. The goal of this thesis is to investigate the use of OCT for obtaining optical biopsies of in vivo tissue. The first part of this thesis describes an in vitro tissue survey for establishing clinically pertinent areas for performing OCT based diagnostics. The clinical relevance of OCT images of tissue microstructure in the gastrointestinal tract, the urinary tract, and the cardiovascular system is discussed. The results of the tissue survey indicate that OCT is a viable technique for obtaining optical biopsies in a variety of in vivo tissues. Until recently, however, state of the art OCT systems have not been capable of imaging in vivo tissue for the following three reasons; (1) lack of a catheter or endoscope for enabling OCT imaging access to internal organ systems, (2) lack of an adequate light source for high speed scanning and (3) insufficient image acquisition rates. In the second part of this thesis, recently developed technological advances which address these issues are presented. In addition, these key technologies which include an OCT compatible catheter-endoscope, a high power low coherence source, and a rapidly scanning optical path length delay line have been integrated into an OCT system. This system has been used to perform the first in vivo endoscopic OCT imaging study. By obtaining micron scale images of the respiratory and gastrointestinal tracts of a living New Zealand White Rabbit, the potential of OCT to become a powerful tool for obtaining in vivo optical biopsies has been realized.

Thesis Supervisor: James G. Fujimoto
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like to express my appreciation to Mark for investing so much time to help me mature as a clinician and a scientist.

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Chapter 1

INTRODUCTION

1.1 Overview

Medical imaging technology has advanced over the last twenty years to provide physicians with indispensable information on the macroscopic anatomy of patients. Imaging techniques such as radiography, magnetic resonance imaging, computed tomography, and ultrasound non-invasively allow investigation of large-scale structures in the human body with resolutions ranging from 100 μm to 1 mm. However, for many disease processes, including the early stages of cancer and atherosclerotic disease, higher resolution is necessary for proper diagnosis. In addition, clinical procedures such as screening for carcinoma in situ in Barrett’s esophagus and the surgical detection of tumor margins require higher resolution diagnostic imaging methods. To address these and other clinical problems in situ, a non-invasive imaging technology with a resolution that approaches the diagnostic “gold standard,” excisional biopsy, must be used. A new imaging technology that is capable of high resolution diagnosis must be able to provide information about the microscopic anatomy of tissue, including architectural morphologic structure and cellular features. This thesis presents optical coherence tomography (OCT), an optical imaging technique for non-invasively obtaining high resolution images of scattering tissue microstructure.

1.2 Excisional Biopsy

Excisional biopsy is the “gold standard” for diagnosis of disease in medicine[1]. Typically, an excisional biopsy is acquired by first removing the tissue from the patient. The specimen is fixed by precipitating or cross-linking the proteins in formalin. Following fixation, the specimen is dehydrated in alcohol and the alcohol is subsequently replaced with xylene. These steps
are necessary to preserve the tissue structure during the remainder of the histologic processing, but they may introduce artifacts due to alteration of tissue composition. The tissue is then placed in an embedding medium and sectioned using a microtome. The embedding medium, usually paraffin, is used to stabilize the specimen during the sectioning process. Thin, 5-10 μm, sections are then placed on a microscope slide. The slide is stained with several dyes that are preferentially localized to different intracellular or extracellular components. Commonly used dyes are Hematoxylin and Eosin (H&E). Eosin stains components of the cytoplasm red while Hematoxylin stains the nuclear material of the cell blue. A histologic slide may be prepared in as little as a few hours by an experienced technician. Once the biopsy is sectioned and stained, the pathologist views the slide under a microscope to form a diagnosis[1].

1.3 Optical Biopsy

The term “optical biopsy” refers to cross-sectional images of the microscopic structure of human tissue obtained using the detection of optical radiation. As opposed to excisional biopsy, optical biopsies are obtained in situ, without removal of the tissue from the patient. The capability to perform optical biopsy would allow biopsies to be obtained without the scarring, pain, time, and expense associated with excisional biopsy. The patients that are likely to benefit from optical biopsy have diseases that occur in locations where it is hazardous to remove tissue (e.g. the vascular system and nervous system) and cases where many biopsies have to be acquired (e.g. the detection of adenocarcinoma for patients with Barrett’s esophagus). In addition, optical biopsies could be useful for non-invasive guidance during surgery. Finally, optical biopsies would allow the evolution of diseases to be followed over time. In this thesis, the potential to perform optical biopsies in different tissues using a technique known as OCT will be investigated.

1.4 Optical Coherence Tomography

OCT is a new optical imaging technique that uses low coherence interferometry to obtain high resolution, cross-sectional images in biological systems[2, 3]. OCT employs a low coherence light source to interferometrically perform micron scale resolution ranging and imaging. For an interferometric signal to be detected, the optical path lengths of the object and reference beam must be matched to within the coherence length of the source. Tissue reflectance versus axial depth is obtained by mechanically varying the reference arm and digitizing the magnitude of the interference. A cross-sectional image of backscattered reflectance from architectural and cellular
structures within tissue is produced by recording axial reflectance profiles while the beam on the tissue specimen is scanned. Typical OCT images have approximately 10 μm axial and lateral resolution, up to 10 times higher than any other clinically available non-invasive diagnostic imaging modality.

1.5 Background

1.5.1 Optical Time Domain Reflectometry (OTDR)

Before low coherence interferometry techniques were fully developed, optical ranging in tissue had been attempted in the time domain. This temporal analog of low coherence interferometry, known as optical time domain reflectometry (OTDR), performed optical ranging by detecting the reflected time-of-flight of ultrashort pulses within biological media[4, 5]. While this technique enabled optical ranging of the skin and human cornea with high (15 μm) resolution and a detection sensitivity of 70 dB, it was expensive and complex.

1.5.2 Optical Coherence Domain Reflectometry (OCDR)

At the same time, the one dimensional precursor to OCT was being developed, optical coherence domain reflectometry (OCDR). Like OCT, OCDR uses a low coherence source to perform optical ranging by detecting interference reflected from the reference and sample arms. This technique was the first to use low coherence interferometric ranging to obtain high resolution axial scans. Experimental work demonstrated the use of OCDR for evaluating optical components and measuring faults in waveguide structures[6, 7]. Resolutions of up to 10 μm and a dynamic range of 120 dB (detection of $10^{-12}$ of the incident power) were demonstrated using this technique[8].

1.5.3 OCDR and OCT in Ophthalmology

Because the eye is a transparent structure, the primary biological applications of OCDR were in the field of ophthalmology. OCDR was first used in the eye to obtain structural measurements, including the axial eye length and corneal thickness measurements[9-12]. Later, OCDR was extended to obtain two dimensional images of the eye. This technique was coined optical coherence tomography[3] and subsequently developed into a clinically viable instrument. OCT has been extensively applied in ophthalmology to provide tomographic images of the transparent structures in the eye.
Clinical studies have shown that OCT provides high resolution, cross-sectional images of the retina and can be used to diagnose a wide range of retinal macular and anterior eye diseases\cite{13, 14}. Greater than 3000 patients with a variety of macular diseases and diseases of the optic nerve head have been examined at the New England Eye Center, Tufts University School of Medicine. In patients with macular pathology, OCT images have been correlated with the conventional clinical techniques of slit-lamp biomicroscopy and fluorescein angiography. The cross-sectional view of OCT has been found to be effective in the diagnosis and monitoring of macular holes, retinal detachment, macular edema, epiretinal membrane formation, and age-related macular degeneration\cite{13, 15}. In patients with glaucoma, the ability of OCT to directly measure retinal nerve fiber layer thickness with micron scale resolution may lead to the first truly objective diagnostic for the presence or progression of this degenerative disease\cite{16}.

1.5.4 OCT in Multiply Scattering Tissues

Optical imaging in non-transparent tissues is complicated by multiple scattering. However, in the single scattering limit, OCT can reject multiply scattered photons. For a signal to be detected, the optical path lengths of the object and reference beam must be matched to within the coherence length of the source. Since most multiply scattered photons from the object have traveled different optical path lengths than the reference arm photons, multiple scattering effects are minimized in the OCT image.

Due to the capability of OCT to reject multiply scattered light, several investigators have pursued the use of OCT to image non-transparent \textit{in vitro} tissue samples. Since the skin is the most easily accessed of all human organ systems, most researchers in this area have published images of the skin. These studies uniformly report the capability to image the architectural layers of the skin, including the epidermis, and dermis\cite{17-19}. In addition, OCT imaging of other architectural morphologic features such as sweat glands and hair follicles have been demonstrated\cite{19}. To date, the only \textit{in vivo} study of human tissue has been performed in the skin. In this work, OCT imaging of \textit{in vivo} normal human skin with 10 \textmu m resolution and 1 mm penetration depth enabled visualization of the epidermis, dermis, and dermal-epidermal junction\cite{20}.

Recent \textit{in vitro} studies have shown that OCT can image architectural morphology in other highly optically scattering biological tissues such as the vascular system, gastrointestinal tract, urinary tract, and developing embryos. Imaging of atherosclerotic plaque morphology has been
demonstrated on in vitro samples with a resolution that is approximately 10 times greater than the current high resolution cross-sectional imaging technology, intravascular ultrasound[21, 22]. OCT images of gastrointestinal and urinary tract tissues have been recently presented, and demonstrate the capability of OCT to image the architectural microstructure of these layered organs[19, 23, 24]. Finally, OCT imaging of developing embryos has recently been shown to be a valuable tool for analyzing embryonic development[25].

Since the resolution of OCT is an order of magnitude greater than other currently available cross-sectional imaging technologies, it provides information on the microstructure of biological specimens which could only previously be obtained with conventional excisional biopsy. However, most of the studies to date have been performed on in vitro samples taken post mortem. In vivo studies in multiply scattering tissues have not been attempted for two reasons. First, in order to access internal organ systems in vivo, an OCT compatible catheter-endoscope must be constructed. Another reason for the lack of in vivo studies is that the acquisition times of state-of-the-art OCT systems are too slow to prevent artifacts caused by the motion of in vivo specimens.

1.6 Statement of Work

The goal of this thesis is to investigate the use of OCT for obtaining optical biopsies of in vivo tissue. The first part of this thesis will consist of an in vitro tissue survey for establishing clinically pertinent areas for performing OCT based diagnostics. A tissue survey that includes in vitro tissues from the gastrointestinal tract, the urinary tract, and the cardiovascular system, has been conducted. The clinical relevance of OCT images of tissue microstructure in each of these systems will be discussed.

The potential capability of OCT to perform optical biopsy, or high resolution non-invasive optical diagnostic imaging of a variety of in vivo tissues, has motivated advances in OCT technology that will enable in vivo diagnostic imaging. In the second part of this thesis, these advances which include the development of an OCT compatible catheter-endoscope and high speed OCT technology are described. The OCT compatible catheter-endoscope allows OCT imaging to be performed in previously inaccessible internal organ systems. High speed image acquisition is essential for allowing motion artifact free imaging of living subjects to be performed. Finally, the catheter-endoscope and high speed OCT imaging are integrated into a system that has the potential to be used in a clinical environment for obtaining optical biopsies.
These developments represent essential steps towards enabling OCT to become a powerful tool for performing \textit{in vivo} non-excisional optical biopsy of internal human organ systems.
Chapter 2

OPTICAL COHERENCE TOMOGRAPHY

2.1 Introduction

A high resolution cross-sectional optical imaging device for obtaining non-invasive optical biopsies of human tissue could revolutionize the field of medical imaging. Optical coherence tomography, an optical ranging technique based on low coherence interferometry, may have the potential to image tissue with sufficient resolution to perform optical biopsy[3]. This chapter presents an overview of the OCT system used for the tissue survey, the theory behind low coherence interferometry, and an analysis of the OCT signal in multiply scattering tissue.

2.2 OCT System Overview

OCT is similar to B mode ultrasonic imaging except that it measures interference from reflected infrared light rather than the reflection of acoustical waves. To obtain a spatial ranging resolution on the order of 10 μm by measuring the echo delay time would require a temporal resolution of 30 fs (10^-15 seconds). OCT avoids this problematic constraint by working in the coherence domain using low coherence interferometry.

The OCT system consists of a single mode fiber optic Michelson interferometer which may accommodate different, short coherence length sources (Figure 2-1). The source is coupled into a fiber optic 50/50 beam splitter. Half of the light is directed towards the reference arm mirror while the remaining light is incident on the sample. Light reflected from both the reference arm mirror and the sample recombine at the beam splitter. Precise measurements of distance are possible since interference is only observed at the detector when the path lengths of the sample and the reference arms match to within the coherence length of the light. OCT measures the mag-
Figure 2-1. Schematic of the OCT system.

magnitude of the interference, which is proportional to the backscattered light from the tissue. Since interference is only detected when the sample and reference arms are matched to within the coherence length of the light, the coherence length defines the axial resolution of the OCT system. In addition, to being path length matched, the polarization states in each arm must be matched in order to maximize interference contrast. Polarization matching is accomplished by use of a polarization controller (PC) in the reference arm (Figure 2-1).

Reflectivity as a function of depth within the sample is obtained by varying the reference arm length and digitizing the magnitude of the demodulated interference. Tomographic images are produced in a manner similar to radar, by scanning the position of the optical beam across the sample and generating two dimensional data sets. Thus, an OCT image represents a cross sectional picture of the optical reflectivity or backscatter from within the tissue.

2.3 Low Coherence Interferometry Theory

This section presents an in-depth theoretical treatment of low coherence interferometry. The groundwork for this description is laid by first considering the function of an interferometer that uses narrow band light for the source. The theory is then extended to include broadband Gaussian optical sources and sources with spectra that deviate from Gaussian.
2.3.1 Coherent Source

OCT is based on a common optical two port network known as the Michelson interferometer (Figure 2-2.).

![Schematic of the Michelson interferometer.](image)

**Figure 2-2.** Schematic of the Michelson interferometer.

We first will define the source electromagnetic field to be,

\[ a_1 = A_1 \exp(-j kz). \]  \hspace{1cm} (2-1)

If the illuminating radiation contains only one wavelength, then the normalized field amplitude at the detector can be written as:

\[ \frac{b_2}{a_1} = \frac{j}{2} \left[ r_s \exp(-2j k l_s) + r_r \exp(-2j k l_r) \right] \]  \hspace{1cm} (2-2)
where $r_s$ and $r_r$ are the reflectivities at the sample and reference arm positions, respectively. The intensity at the detector is the magnitude squared of the fields combined at the detector,

$$\left| \frac{b_2}{a_1} \right|^2 = \frac{1}{4} \left[ |r_s|^2 + |r_r|^2 + 2 r_s r_r \cos(2k\Delta l) \right], \tag{2-3}$$

where

$$\Delta l = l_r - l_s \tag{2-4}$$

is the optical path length mismatch between the two arms in free space. The detected intensity of Eq. (2-3) shows that the interferometric signal consists of a DC term that is proportional to the sum of the reflectances from each arm. In addition, the interferometric signal contains an AC term which represents a sinusoidal modulation as a function of $\Delta l$ and an amplitude that is proportional to the sample arm reflectivity.

### 2.3.2 Low Coherence Source

If the source consists of more than one wavelength, then the intensity at the detector is simply the sum of Eq. (2-3) for all of the wave vectors, $k$:

$$\left| \frac{b_2}{a_1} \right|^2 = \frac{1}{4} \int S(k) \left[ |r_s|^2 + |r_r|^2 + 2 r_s r_r \cos(2k\Delta l) \right] \, dk, \tag{2-5}$$

where $S(k)$ is the power spectrum of the source. The autocorrelation function, or the AC coupled photon current representing the interference, is then proportional to,

$$I(\Delta l) \propto \text{Re} \left[ \int S(\omega) r_s r_r \exp(-2 jk(\omega)\Delta l) \, d\omega \right], \tag{2-6}$$

and $\omega = kc$. 

22
In a non-dispersive media, the propagation constants $k$ in each arm can be considered to be equal and can be rewritten using a first order Taylor series expansion around $\omega_0$,

$$ k(\omega) = k(\omega_0) + k'(\omega_0)(\omega - \omega_0). \quad (2-7) $$

Thus, Eq. (2-6) can be reformulated to read

$$ I(\Delta l) \propto \text{Re} \left[ r_s r_r \exp(-2j k(\omega_0) \Delta l) \int S(\omega - \omega_0) \exp(-2j k' (\omega - \omega_0) \Delta l) \partial(\omega - \omega_0) \right], \quad (2-8) $$

or

$$ I(\Delta l) \propto \text{Re} \left[ r_s r_r \exp(-j \omega_0 \tau_p) \int S(\omega - \omega_0) \exp(-j(\omega - \omega_0) \tau_g) \partial(\omega - \omega_0) \right], \quad (2-9) $$

where the phase delay is,

$$ \tau_p = \frac{2\Delta l}{v_p}, \quad (2-10) $$

and the group delay is

$$ \tau_g = \frac{2\Delta l}{v_g}, \quad (2-11) $$

and $v_p = c$ and $v_g = k^{-1}\big|_{\omega_0}$ are the phase and group velocities of the light ($v_g = c$ in free space). Eq. (2-9) can now be written as

$$ I(\Delta l) \propto r_s r_r \text{Re} \{ F\{S(\omega)\}\} \cos(\omega_0 \tau_p), \quad (2-12) $$

and $F\{S(\omega)\}$ represents the Fourier transform of the power spectrum. Eq. (2-12) describes the operation of the low coherence interferometer. If the lengths of the interferometer sample and the reference arms are perfectly matched, a maximum interference signal forms at the detector. As
the reference arm is moved, the detected autocorrelation function consists of the Fourier transform of the power spectrum, modulated by a sinusoid that varies with the path length difference between the two arms, $\Delta l$. Since the width of the spectrum and the width of the autocorrelation function, the coherence length, are related to one another by the Fourier transform, the width of the spectrum is inversely proportional to the width of the autocorrelation function. This relationship implies that as the spectral width of the source increases, the coherence length decreases and the spatial resolution of the low coherence interferometer increases.

2.3.3 Gaussian Light Source

If the source in the interferometer has a Gaussian profile, its spectral shape may be defined as the unity normalized distribution,

$$S(\omega) = \frac{1}{2\pi\sigma_\omega^2} \exp\left(\frac{(\omega - \omega_0)^2}{2\sigma_\omega^2}\right),$$  \hspace{1cm} (2-13)

where $\omega_0$ is the center frequency of the source and $2\sigma_\omega$ is the standard deviation width of the Gaussian distribution. If the Gaussian definition for the power spectrum is input into the formula for the autocorrelation function, Eq. (2-12), the autocorrelation is also a Gaussian:

$$I(\Delta l) \propto \exp\left(-\frac{\Delta \tau^2}{2\sigma_\tau^2}\right)\cos(\omega_0 \Delta \tau_p),$$  \hspace{1cm} (2-14)

where the standard deviation width of the Gaussian envelope, $\sigma_\tau = \sigma_\omega^{-1}$, is a measure of the coherence length or axial resolution of the OCT system for a Gaussian light source.

2.3.4 Non-Gaussian Light Source

For a non-Gaussian spectral distribution, the spectral bandwidth-duration product is greater than one, implying a greater spatial extent of the autocorrelation function for a given bandwidth, relative to the Gaussian. A result of this greater uncertainty in the measurement of the autocorrelation function is the appearance of sidelobes in the wings of the autocorrelation function. These sidelobes are best demonstrated by investigating the autocorrelation function.
that would be produced by using a rectangular spectral distribution with an equivalent width, \( \sigma_r = 2\sigma_\omega \):

\[
S(\omega) = \begin{cases} 
\frac{1}{\sqrt{2\pi}\sigma_r^2}, & -\sigma_r \leq \omega \leq \sigma_r \\
0, & -\sigma_r \geq \omega \geq \sigma_r
\end{cases}
\]  

(2-15)

The autocorrelation function then can be calculated to be

\[
I(\Delta l) = \frac{1}{\sqrt{2\pi}} \text{sinc} \left( \frac{\tau_g \sigma_r}{2} \right) \cos (\omega_0 \Delta \tau_g).
\]  

(2-16)

Plots of the autocorrelation function for the Gaussian power spectrum and the rectangular power spectrum are shown in Figure 2-3. The autocorrelation function for the rectangular power spectrum is narrower at the half maximum level than the Gaussian, but has higher sidelobes. On
the log scale, the Gaussian spectrum autocorrelation function decays quadratically, while the maximum sidelobe values present in the rectangular spectrum autocorrelation function decay linearly. In general, as the power spectrum of the input light source to the interferometer becomes more rectangular, the sidelobes of the autocorrelation function increase and can obscure adjacent scattering points in the image. This shadowing is also known as “blindness” and presents limitations for the imaging capability of the interferometer.

2.3.5 Gaussian Light Source with Modulation

Commonly, light sources are modulated with noise. This noise may cause symmetric peaks to appear in the autocorrelation function. If we assume that the unmodulated source is a Gaussian, and the noise is expressed as a percentage modulation of the Gaussian distribution, then the source spectrum may be expressed as

\[ S'(\omega) = S(\omega)(1 + Mn(\omega)), \]  

(2-17)

where \( M \) is the percent modulation and \( n(\omega) \) is the modulation noise on the power spectrum. In a non-dispersive medium, the envelope of the autocorrelation function is then described by:

\[ I(\Delta l) = \exp\left( -\frac{\Delta\tau_g^2}{2\sigma^2} \right) + M \exp\left( -\frac{\Delta\tau_g^2}{2\sigma_t^2} \right) \otimes n(\Delta\tau_g), \]  

(2-18)

which consists of an ideal Gaussian autocorrelation envelope convolved with the Fourier transform of the spectral modulation noise. As an approximation, if we consider the noise to be sinusoidal with a frequency of \( \omega_n \), then sidelobes with relative intensity, \( M \), appear at \( \Delta\tau_g \pm \omega_n \) in the autocorrelation function. These sidelobes may either cause symmetric artifacts in the OCT image, if \( \omega_n \) is large, or blur the envelope of the autocorrelation function if \( \omega_n \) is small.

2.4 Detection Electronics

The detection scheme for the OCT system involves detection of the sum of the returning electrical fields from the sample and reference arms of the interferometer with a photodetector. The photodetector current is converted to voltage by a transimpedance amplifier. The amplified signal is then band pass filtered to remove both the DC component of the signal and the noise.
outside of the bandwidth of the signal. Finally, the band passed signal is demodulated using a square law demodulator (RMS converter) followed by a low pass filter (Figure 2-4).

![Schematic of OCT detection electronics.](image)

**Figure 2-4.** Schematic of OCT detection electronics.

As the reference mirror of the interferometer is moved, the optical path length mismatch between the reference and sample arms changes. Translation of the reference arm mirror at a constant velocity induces a linear phase modulation on the reference arm electric field. The OCT signal in free space can be described as a DC component corresponding to the reflectance of the sample and reference arm mirrors and an AC component which consists of an envelope modulated by a sinusoid:

\[
I(t) = \frac{|r_r|^2}{4} + \frac{|r_s|^2}{4} + \frac{1}{2}(r_r r_s \text{Re}[F(S(\omega))] \cos(2\pi f_d t)).
\]  

(2-19)

The Doppler frequency induced by the moving reference arm is,

\[
f_d = \frac{2v}{\lambda},
\]  

(2-20)

and \(v\) is the velocity of the reference arm. The intensity is converted to current, \(i\), by detecting the incident power on a photodetector:

\[
i(t) = \frac{ne}{h\nu} I(t),
\]  

(2-21)
where the term, $\eta$, is the detector quantum efficiency, $e$ is the electronic charge, and $h\nu$ is the photon energy.

### 2.4.1 Transimpedance Amplifier

The current from the detector is then converted to voltage using a transimpedance amplifier with a cutoff frequency, $\omega_c = (RC)^{-1}$ (Figure 2-5). The capacitor is used to reduce amplifier noise and increase the amplifier stability. Below the cutoff frequency of the transimpedance amplifier, the voltage, $V = IR$.

![Transimpedance Amplifier](image)

**Figure 2-5.** Transimpedance amplifier used to convert voltage to current in the OCT system.

### 2.4.2 Band Pass Filter

Once the light is converted to voltage by the photodetector-transimpedance amplifier pair, band pass filtering is performed to reduce excess signal noise and remove the DC component from the signal. The voltage is filtered using a passive second order Butterworth band pass filter(Figure 2-6). The shape of the response function of the Butterworth filter is plotted in Figure 2-7. The center frequency of the band pass filter is the doppler frequency, $f_d$. The full-width-half-maximum or noise equivalent bandwidth ($NEB$) of the filter is chosen to match the source bandwidth using the following simple relationship:

$$\Delta f = NEB = \frac{\Delta\lambda f}{\lambda}.$$  \hfill (2-22)


Figure 2-6. Second order Butterworth band pass filter used in the OCT system

Figure 2-7. Frequency response function for Butterworth band pass filter. The center frequency, \( f_0 \), is 4.1 MHz, and the bandwidth is 410 KHz.

2.4.3 Demodulation

After the signal is band pass filtered, the envelope of the autocorrelation function is removed from the carrier, \( f_d \), by using an RMS converter and a low pass filter. The RMS converter squares the band passed signal, causing the frequency spectrum of the signal to be convolved with itself. The RMS converted signal is then low pass filtered to remove the sidebands at \( \pm 2f_d \).
2.4.4 Analog to Digital Conversion and Display

Following the RMS converter and low pass filter, the signal is digitized using an A/D converter. The A/D converter is typically triggered by the start of scan pulse that indicates the beginning of a reference arm optical path length sweep. For every reference arm sweep, an axial reflectivity profile is digitized. A cross-sectional image is produced by recording sequential axial reflectivity profiles while the beam position is scanned across the sample. For in vitro studies, the beam position is scanned across the sample by moving the sample using a computer controlled scanning stage. If the image is acquired in vivo, the beam is scanned in one dimension across the sample using a galvanometer or another type of beam scanning mechanism.

2.4.5 Image Processing

After the two-dimensional data is collected, the image may be displayed using a gray scale or false color lookup table. Typically, only contrast and brightness adjustments are used to enhance the contrast in the OCT images. However, since the detected reflectivity in tissue under-
goes an exponential decay as a function of penetration depth, a base ten logarithm is often applied to the image data values to improve the displayed dynamic range.

2.5 System Parameters

2.5.1 Axial Resolution

Resolution is determined by the minimal distance between two point scatterers that can be distinguished. The axial resolution of the OCT system in free space is defined as the full-width-half-maximum (FWHM) width of the autocorrelation envelope. If the source has a Gaussian spectrum with a FWHM bandwidth, $\Delta \lambda$, and a center wavelength, $\lambda_0$, then the coherence length, or the axial resolution, $\Delta L$ may be calculated to be[26]:

$$\Delta L = \frac{2\ln(2)}{\pi} \left| \frac{\lambda_0^2}{\Delta \lambda} \right|. \quad (2-23)$$

If the refractive index of the sample medium, $n$, is greater than one, then the effective axial resolution is $\Delta L/n$.

2.5.2 Lateral Resolution

The lateral point spread function of the OCT system is determined by the focusing lens in the sample arm (Figure 2-1). The spot size of the lowest order mode Gaussian beam focused by the imaging lens can be determined using the Fresnel diffraction formula in the paraxial limit[27]. The FWHM of the gaussian spatial distribution, or spot diameter, $d$, can be calculated from the wavelength,

$$d = 2w_0 = \sqrt{\frac{2b\lambda}{\pi}}, \quad (2-24)$$

where the confocal parameter, $b$, is twice the Rayleigh parameter. The confocal parameter is a good approximation of the imaging depth of focus that can be achieved for a given spot size.

Typically, the lateral resolution of the OCT system is chosen to match the axial resolution to form a 1:1 resolution aspect ratio. However, an important trade-off in OCT imaging occurs
when the source spectral bandwidth is large enough to allow extremely high axial resolutions (e.g. 4 μm). In this case, if the lateral resolution is chosen to match the axial resolution, the depth of focus becomes too small to be useful for many clinical applications (Figure 2-9). Thus, a compromise must be made so that the lateral resolution is large enough to allow a reasonable depth of focus.

**Figure 2-9.** Beam diameter vs. confocal parameter at λ = 1300 nm.

### 2.5.3 Noise

OCT detection has been optimized to perform in the shot noise limit by choosing a doppler frequency above 10 KHz to avoid 1/f noise and a proper transimpedance amplifier resistance and reference arm voltage to overcome thermal noise in the transimpedance amplifier resistance[28]. Shot noise arises from current fluctuations due to the conversion of light into charge. This form of noise is a wide sense stationary (WSS) stochastic process[28]. A WSS process has a constant mean. The shot noise associated with any given mean photocurrent, \( \langle i \rangle \), has a constant power spectral density, commonly referred to as white noise.

In general, the shot noise power spectral density is proportional to the mean photocurrent, \( \langle i \rangle \):

\[
N_i(\omega) = e \langle i \rangle,
\]  

(2-25)
where $e$ is the electron charge and $N_i(\omega)$ is the shot noise power spectral density. In OCT, the reference power returned to the detector is typically much larger than the sample power because the reflectivity in the reference arm is much greater than the reflectivity of the sample ($r_r \gg r_s$). Since the reference power remains relatively constant during the scan, the total photocurrent in the system varies only slightly about $\langle \dot{i} \rangle$. Thus, the shot noise of the OCT system can be treated as additive white noise[28].

2.5.4 Dynamic Range

The dynamic range is a measure of the ratio of the maximum signal to the minimum detectable change in signal magnitude. For OCT, the overall system dynamic range is determined by the signal to noise ratio (SNR) of the detection and band pass filtering electronics, the dynamic reserve of the RMS converter, and the analog to digital (A/D) converter dynamic range.

Detection Signal to Noise Ratio

For shot noise limited detection, the theoretical maximum SNR that can be achieved with OCT under the assumption of infinite linearity of the electronics and infinite dynamic range of the digitization electronics can be expressed:

$$\text{SNR} = 10\log\left(\frac{\eta P_s}{2h\nu NEB}\right)$$

(2-26)

where $\eta P_s/2h\nu$ describes the number of electrons per unit time generated by the photodetector due to light returning from the sample and $1/NEB$ is the time interval corresponding to the band pass filter bandwidth[26]. The result of Eq. (2-26) indicates that, depending on the quantum efficiency of the detector, $\eta$, only a few photons are required for detection. The SNR is empirically measured by comparing the signal from a 100% reflector (mirror) to the variance of the signal with the sample arm blocked. A typical value of the SNR for the SLD-based OCT system is 111 dB ($\eta = 0.8$, $P_s = 150\mu W$, $NEB = 3\text{kHz}$).
Analog to Digital Dynamic Range

While Eq. (2-26) accurately predicts the minimum detectable signal, the finite linearity of the electronics and the finite dynamic range of the digitization typically limit the SNR of the acquired OCT image. The dynamic range of the analog to digital converter is given by

\[ \text{SNR}_{AD} = 10 \log(2^n), \]  
(2-27)

where \( n \) is the number of bits in the A/D converter. For the 12 bit A/D converter used in the SLD system, this yields a dynamic range of 36 dB. If a base ten logarithm demodulator is used, the dynamic range is

\[ \text{SNR}_{AD} = 10(2^n - \log(90)), \]  
(2-28)

and the dynamic range of the entire system is limited by the dynamic reserve of the demodulator (60-80 dB).

2.5.5 OCT System Trade-off Between Resolution and Scanning Speed

An increase in scanning speed, \( v \), increases the bandwidth of the OCT signal. Thus, the bandwidth of the band pass filter (NEB) in the detection electronics must also be increased to preserve the resolution of the demodulated signal.

\[ \text{NEB} = \frac{2 \Delta \lambda \nu}{\lambda^2} \]  
(2-29)

If the resolution of the OCT system is increased by using a spectral source with a broader spectral bandwidth (\( \Delta \lambda \)), the NEB also increases. In both cases, an increase of the NEB of the band pass filter decreases the SNR because the band pass filter accepts more shot noise (Eq. (2-26)). Thus, for either an increase in scanning speed or resolution, the power incident on the sample must be raised commensurately to maintain a constant SNR.
2.6 OCT in Multiply Scattering Tissue

The previous section has treated OCT theory by analyzing a simple Michelson interferometer in free space. Analysis of the OCT signal reflected from within tissue is considerably more complicated. The complications arise from the heterogeneous scattering and absorbing properties of tissue. These complications which include speckle, refractive index scaling, focusing, attenuation, and multiple scattering all serve to provide artifacts within the OCT image of a turbid media. An understanding of these artifacts is necessary for proper interpretation of the OCT image data.

2.6.1 Speckle

Speckle is a form of multiplicative noise which arises when two or more scattering loci are separated by less than one coherence length away from one another. Multiple electrical fields can interfere at the output port and the OCT signal detected at a given point is sensitive to the phase difference between the scattering points. Speckle affects the image on a size scale near that of the coherence length, so speckle in the OCT image is a major difficulty when trying to resolve structures that are near the resolution of the OCT system.

The OCT signal in a turbid medium can be obtained from the Michelson analysis by replacing the sample arm reflectivity, \( r_s \), with a reflectivity function, \( r_s(z) \). Assuming the reference arm reflectivity is 1.0, and \( z = \Delta L \), the intensity at the detector for a single optical frequency may be written as:

\[
I_{\parallel, k} = \frac{1}{4} \left[ 1 + \left| r_s(z) \right|^2 dz + 2 \int r_s(z) \cos(2kz) dz + \int \int r_s(z) r_s(z') \cos[2k(z - z')] dz' dz \right].
\]  

(2-30)

If the DC term is eliminated and the single frequency intensity is integrated over the entire spectrum, the intensity at an axial position, \( z_0 \),

\[
I(z_0) \propto 2 \int S(k) r_s(z) \cos[2k(z - z_0)] dk dz + \int \int S(k) r_s(z) r_s(z') \cos[2k(z - z' - z_0)] dz' dk dz
\]

(2-31)
can be expressed as two interference terms. The first interference term causes speckle when the
sample reflectivities are separated by less than a coherence length. The second interference term,
is similar to the autocorrelation function of the reflectivity function within the coherent envelope
and adds a constant value to the reflectivity at $z_0$.

Speckle may be removed from the image by averaging the OCT signal if the movement of
the structures inside the sample is sufficient to induce a phase change of $\pi$. If the image is aver-
aged the SNR is increased by $\sqrt{N}$, where $N$ is the number of averages. However, frame averag-
ing has the disadvantage that it takes $N$ times longer to capture the data.

2.6.2 Group Refractive Index

Since the group refractive index, $n_g$, is greater than one, and OCT measures the group
delay imposed by the sample(Eq. (2-14)), the axial dimension in the OCT image is scaled by $n_g$.
Thus, to obtain the true axial distances between scattering points in the OCT image, the $z$ axis
must be scaled by $1/n_g$.

Moreover, if the thickness of the sample is known, the group refractive index can be cal-
culated from the group delay. The refractive index of a tissue sample can be measured by placing
the tissue on top of a planar reflecting surface and acquiring an OCT image. The thickness of the
sample, $z$, may be determined by subtracting the axial position of the reflector from the axial
position of the tissue surface in the OCT image. The additional optical path length delay, $z'$, may
be measured by subtracting the axial position of the reflector outside the tissue from the axial
position of the reflector imaged through the tissue (Figure 2-10). The refractive index of the sam-
ple, $n_g$, may then computed to be:

$$n_g = \frac{z' + z}{z}.$$  \hspace{1cm} (2-32)

Figure 2-10 demonstrates the use of OCT to measure the group refractive index of an in vitro
human full-thickness skin specimen.
Figure 2-10. Group refractive index calculation using an OCT image of an in vitro piece of full thickness human skin. The group refractive index of the skin was calculated to be 1.39 at 1300 nm. The area to the left of the skin is a water meniscus. The group refractive index for the water was calculated to be 1.33 which is the expected value.

2.6.3 Optical Properties of Tissue

Optical Property Definitions

The backreflected light scattered from within a turbid medium, such as tissue, is affected by the optical properties of the medium. The optical properties that determine the propagation of light in tissue are the absorption coefficient, $\mu_a$, the scattering coefficient $\mu_s$, and the total attenuation coefficient, $\mu_t$, where

$$\mu_t = \mu_a + \mu_s.$$

(2-33)

The absorption coefficient is linearly related to the concentration of the absorber, such that

$$\mu_a = \varepsilon [Ab]$$

(2-34)

where $\varepsilon$ is the molar extinction coefficient for the absorber and $[Ab]$ is the molar concentration of the absorber.
The optical properties of tissue, $\mu_a$, $\mu_s$, and $\mu_t$, can also be expressed in terms of transport mean free paths such that

$$l_a = 1/\mu_a,$$  \hspace{1cm} (2-35)

$$l_s = 1/\mu_s,$$  \hspace{1cm} (2-36)

and

$$l_t = 1/\mu_t$$  \hspace{1cm} (2-37)

where $l_a$ is the absorption mean free path, $l_s$ is the transport scattering mean free path, and $l_t$ is the total attenuation mean free path.

Often, the mean cosine of the scattering phase function, $g$, is combined with $\mu_s$ to form the transport scattering coefficient:

$$\mu_s' = \mu_s(1 - g).$$  \hspace{1cm} (2-38)

Propagation of light described using the transport scattering coefficient can be considered isotropic since the scattering coefficient has been normalized by the anisotropy coefficient, $g$.

**Optical Property Values**

The primary constituents that contribute to absorption in tissue from the ultraviolet (UV) to the infrared (IR) are given in Figure 2-11. In tissue, melanin and hemoglobin absorption dominate scattering ($\mu_a \geq 10\mu_s'$) in the UV (below 300 nm) and water absorption dominates scattering in the far IR (above 2800 nm). Scattering dominates absorption ($\mu_s' \geq 10\mu_a$) in the so called “optical window” (600-1300 nm). Research performed to measure the scattering coefficient of different tissues have found that scattering varies inversely with wavelength[30]. Thus, one would expect that in the optical window, OCT image penetration depth is greater at longer wavelengths. Figure 2-12 shows a comparison between an OCT image of an in vitro human epiglottis taken with a source wavelength centered at 800 nm and 1300 nm. The penetration at the
FIGURE 2-11. Absorption spectra of water, melanin, and oxygenated hemoglobin[29].

FIGURE 2-12. OCT image of an *in vitro* human epiglottis taken at 800 nm (left) and 1300 nm (right). The penetration depth in the 1300 nm image enables clearer visualization of the cartilage (c) below the epithelium. Glandular structures are seen in both images. Bars represent 500 μm.

longer wavelength is more than two times greater than at 800 nm. For this reason, the tissue surveys were conducted using a superluminescent diode with a center wavelength at 1300 nm. Furthermore, the decreased attenuation due to scattering at longer wavelengths may indicate other wavelengths for imaging with optimal penetration depth, such as the 1600 nm - 1800 nm range.
2.6.4 Single Backscattering OCT Theory

Single backscattering theory is an analytical solution for the detector current given a heterodyne detection scheme and the focusing geometry shown in Figure 2-13. The amplitude of the incident beam of the sample arm at the scattering media is computed using the Fresnel Integral through the imaging lens. A spherical particle in the medium then backscatters the incoming wavefunction and this scattered wavefunction is projected back through the imaging system. The sample backscattered reflectance is combined with the local oscillator signal from the reference arm to produce the detector current. The two assumptions of single backscattering theory are: 1) multiply scattered light does not reach the detector; 2) the particle separation is much greater than the wavelength[31].

![Figure 2-13. Geometry for single backscattering theory.](image)

Scattered wavefunction

Using the Fresnel diffraction integral, the wavefunction through the imaging objective at the sample can be written as:

\[
\Psi(x, y, L) = \frac{2n_T}{\pi LR\lambda} \int \exp \left[ j \frac{\pi}{L} (\tilde{r} - \tilde{r}'')^2 - j \frac{\pi \tilde{r}''^2}{\lambda_f} - \frac{(r'')^2}{R} \right] dx'' dy'',
\]  

(2-39)
where \( n_T \) is the number of photons transferred per second. This equation can be integrated by completing the square to give:

\[
\Psi(x, y, L) = \frac{R_A \sqrt{2n_T \pi}}{\lambda L \left[ 1 - \frac{j \pi R^2}{\lambda L} \left( 1 - \frac{L}{f} \right) \right]} \exp \left( -\frac{\left( \frac{\pi R \hat{r}}{\lambda L} \right)^2}{\left[ 1 + \left( \frac{j \pi R^2}{\lambda L} \right)^2 \left( 1 - \frac{L}{f} \right)^2 \right]} - j \phi \right), \tag{2-40}
\]

where \( f \) is the focal length of the lens and \( \phi \) is the phase offset[31].

Eq. (2-40) gives the wavefunction at the point \( x, y, L \). The scattering matrix for a spherical particle determines the electric field such that

\[
\begin{bmatrix}
E_{s||} \\
E_{s\perp}
\end{bmatrix} = \exp(jk[\hat{r} - z]) \begin{bmatrix}
S_1 & S_2 \\
S_3 & S_4
\end{bmatrix}
\begin{bmatrix}
E_{i||} \\
E_{i\perp}
\end{bmatrix}, \tag{2-41}
\]

where \( S \) is the scattering matrix of the particle and \( S \) is dependent on the scattering angle, \( \theta \) [32]. Since this theory is formulated for spherical particles,

\[
S_3(\theta) = S_4(\theta) = 0 \tag{2-42}
\]

and

\[
S_1(\pi) = S_2(\pi) \tag{2-43}
\]

Finally, the return wavefunction propagated back through the imaging lens becomes[31]:

\[
\Psi_s = \frac{S(\pi)\Psi}{L} \exp \left( -j \left[ \frac{\pi}{\lambda f} \hat{r}^2 - \frac{\pi}{\lambda L} (\hat{r} - \hat{r}')^2 - \phi \right] \right). \tag{2-44}
\]
Heterodyne Signal

The AC coupled current at the detector with quantum efficiency, \( \eta \), and a gain, \( G \), is determined by the superposition of the sample wave function and the local oscillator wavefunction[31]:

\[
i_s = 2\eta GeRe \left[ \int_A \Psi_S \Psi^*_{REF} d\vec{r}' \right], \tag{2-45}
\]

where \( A \) is the collection aperture and \( \Psi_{REF} \) is the reference wavefunction:

\[
\Psi_{REF} = \sqrt{\frac{n_{REF}}{\pi R}} \exp \left( -j \phi + \left( \frac{\vec{r}'}{R} \right)^2 \right). \tag{2-46}
\]

When \( \Psi_{REF} \) and \( \Psi_S \) are substituted into Eq. (2-45), the mean squared current from the heterodyne signal becomes:

\[
\langle i_s^2 \rangle = \pi n_T n_{REF} R^2 \mu_b \eta^2 G^2 e^2 \int_0^\infty \frac{dL}{L^2 \left[ 1 + \left( \frac{j\pi R^2}{\lambda L} \right)^2 \left( 1 - \frac{L}{f} \right)^2 \right]}, \tag{2-47}
\]

where the sample beam has been integrated over the spot radius, \( \vec{r}' \), for a uniform density of particles and \( \mu_b \) is the radar backscattering coefficient[31]. The reflectance from the sample derived from the mean squared heterodyne current may be written as:

\[
R_s(z) = \frac{\langle i_s(z) \rangle^2}{i_0^2} = \mu_b \int_0^\infty \frac{\pi R^2 dL}{L^2 \left[ 1 + \left( \frac{j\pi R^2}{\lambda L} \right)^2 \left( 1 - \frac{L}{f} \right)^2 \right]}. \tag{2-48}
\]

In this form, the single backscattering model does not account for the exponential attenuation in a scattering medium, the effects of the sample refractive index, or the effects of the finite
coherence length of the source. After these parameters have been included, the single backscattered reflectance becomes:

\[
R_s(z) = \mu_b \int_0^\infty \frac{\pi R^2 \exp(-2\mu_z z) |G(\tau)|^2 dL}{(f + n_z z)^2 \left[ 1 + \left( \frac{j\pi R^2}{\lambda(f + n_z z)} \right)^2 \left( 1 - \frac{(f + n_z z)^2}{f} \right)^2 \right]},
\]

(2-49)

where the \( \exp(-2\mu_z z) \) term corresponds to the exponential decay to and from the scattering position \( z \)[33]. The term \( (f + n_z z) \) accounts for the refractive index change in \( L \) due to the sample. Finally, \( |G(\tau)|^2 \) incorporates the effect of the finite coherence length in the single backscatter model.

Since the integral in Eq. (2-49) yields large values only when the reference and sample arm lengths are matched, the reflectance becomes:

\[
R_s(z) = \frac{\mu_b \pi R^2 L_c \exp(-2\mu_z z)}{(f + n_z z)^2 \left[ 1 + \left( \frac{j\pi R^2}{\lambda(f + n_z z)} \right)^2 \left( 1 - \frac{(f + n_z z)^2}{f} \right)^2 \right]},
\]

(2-50)

with \( L_c \), the coherence length defined as:

\[
L_c = \int_{-\infty}^{\infty} |G(\tau)|^2 d\tau.
\]

(2-51)

Single backscatter theory can be further extended to include focusing of the imaging objective at an arbitrary position in the sample. Eq. (2-50) is valid for an imaging lens with a small numerical aperture if we allow[34]:

\[
n_z z \Rightarrow a + n_z z
\]

(2-52)
and

\[ f \Rightarrow a + n_s^2(f - a). \]  \hspace{1cm} (2-53)

Plots of the reflectance as a function of axial position, \( z \), obtained using single backscatter theory, are shown in Figure 2-14.

![Graph showing reflectance as a function of axial position for different total attenuation coefficients for 0.3 \( \mu \)m diameter spheres.](image)

**Figure 2-14.** Reflectance calculated from single backscatter heterodyne theory for different total attenuation coefficients for 0.3 \( \mu \)m diameter spheres. Sample arm is focused on the surface of the medium. \( R = 0.391 \) mm, \( f = 14.5 \) mm, \( \lambda = 1300 \) nm, \( L_c = 0.02 \) mm.

### 2.6.5 Single Scattering Penetration Depth Limitations

The ability of OCT to detect singly scattered light from a multiply scattering media has not yet been fully investigated. However, several important upper limits on the ability of OCT to image through multiply scattering media have been defined, both for coherence gated transillumination[35] and OCT enhanced confocal microscopy[34].
Using a femtosecond mode locked laser transillumination OCT system, the upper limit for the detection depth for ballistic or unscattered light has been determined to be:

\[ L = \frac{1}{\mu_i} \ln\left( \frac{E}{2h\nu} \right), \]  

(2-54)

where \( E/\hbar \nu \) is the number of incident photons[28]. This is the maximum sample thickness through which an image using unscattered light may be obtained[28]. According to this limit, ballistic imaging can be performed through 20 MFP's in a scattering medium[35]. However, this limit does not hold for low coherence imaging because it does not include the effects of multiply scattered coherent light reaching the detector.

Past research using the heterodyne single scattered backscattering theory presented in Section 2.6.4, determined that OCT imaging has a SNR = 1 for a depth of

\[ L = \frac{1}{2\mu_i} \left( \frac{\pi^2}{4n_s^2 M^2} \right), \]  

(2-55)

where \( M \) is the magnification of the imaging objective and \( n_s \) is the refractive index of the medium[34]. Using this limit, the total number of MFP's through which single scattering OCT can image was found to be 15-20 MFP's[34]. Since Eq. (2-55) is obtained using single scattering theory, it is the best case limit for OCT in multiply scattering media. However, it does not consider how multiple scattering affects the resolution as a function of depth. This issue will be addressed in the next section.

2.6.6 Multiple Scattering

Multiple scattering adversely affects the resolution in OCT images. As the ratio of single scattering to multiple scattering decreases, the PSF of the OCT system broadens. This section describes a framework for understanding multiple scattering using the spatial mutual coherence function.
The Spatial Mutual Coherence Function

The spatial mutual coherence function determines the degree of spatial coherence between two optical fields separated by a distance in a plane perpendicular to the direction of propagation[36].

![Diagram of U(r,z) and U(r+\rho,z)](image)

**Figure 2-15.** Geometry for calculating the plane wave mutual coherence function.

The spatial mutual coherence function is defined as the cross-correlation between these two optical fields[36]:

\[
M(\rho, z) = \langle U(r, z), U^*(r + \rho, z) \rangle.
\]  
(2-56)

Several important limits of the mutual coherence function must be defined in order to interpret the mutual coherence function in the general case. The mutual coherence function for reflected radiation for two coincident points, \( \rho = 0 \), is only dependent on the absorption coefficient[36]:

\[
M(0, z) = \langle |U(r, z)|^2 \rangle = U_0^2 \exp(-2\mu_0 z).
\]  
(2-57)

When \( \rho \to \infty \), radiation cannot have reached both \( r \) and \( r + \rho \) without having been scattered, so

\[
M(\infty, z) = \left| \langle U(r, z) \rangle \right|^2 = U_0^2 \exp(-2\mu z)
\]  
(2-58)
and the mutual coherence function is dependent on the total attenuation coefficient[36]. For this case, the mutual coherence function is independent of $\rho$ and is equal to the unscattered attenuation of the reflected field.

The mutual coherence function for values of $\rho$ between 0 and $\infty$ has been computed to be[36]:

$$M(\rho, z) = U_0^2 \exp(-2[\mu_d z + \mu_r z(1 - f(\rho))]), \quad (2-59)$$

where for small angle scattering,

$$f(\rho) = 1 - \frac{1}{3}(1 - g^2)\pi^2 \frac{p^2}{\lambda^2}. \quad (2-60)$$

The heterodyne power detected by the low coherence interferometry system is proportional to the effective receiver area[37]. For a circular aperture,

$$A_{RE}(z) = 2\pi \int_0^D M(\rho, z)R_0(\rho)\rho d\rho, \quad (2-61)$$

where $R_0(\rho)$ is the transfer function of the circular aperture and $D$ is the beam diameter at the sample[37].

Using these formulae for the spatial mutual coherence functions for forward scattering media, the ratio of multiple to single scattered components of the interference signal can be defined as[38]:

$$S_\tau(z) = \frac{A_{RE, MS}}{A_{RE, SS}} = \frac{\int_0^D M(\rho, z)R_0(\rho)\rho d\rho}{\int_0^D M(\infty, z)R_0(\rho)\rho d\rho}. \quad (2-62)$$
Thus, the ratio of multiple to single scattering due to mutual coherence between two scatterers in the medium is dependent on the spot size, $D$, the anisotropy coefficient, $g$, and the imaging depth, $z$. A plot of $S_r(z)$ for different values of $g$, computed from Eq. (2-62), is shown in Figure 2-16.

![Graph showing $S_r(z)$ for different values of $g$.](image)

**Figure 2-16.** Multiple to single scattering ratio, $S_r(z)$ for different values of $g$. The spot diameter, $D$, at the sample is 30 $\mu$m. The total attenuation coefficient, $\mu_r$, is 5.1 mm$^{-1}$.

Using this definition of the percentage of multiple scattering as a function of $z$ and the reflectance calculated from the single backscattering theory (Section 2.6.4), single backscattering theory can be corrected to include the effects of multiple scattering due to spatial mutual coherence:

$$R_m(z) = R_s(z)[1 + S_r(z)].$$

(2-63)

Figure 2-17 depicts the single backscatter reflectance corrected for multiple scattering. The multiple scattering contribution to the OCT signal increases with imaging depth, $z$, and the anisotropy coefficient, $g$. The increase in the multiple scattering contribution with increasing forward scattering can be explained by the fact that forward scattered photons have a greater probability of not being rejected by the coherence gate of the OCT system.

The implication of multiple scattering detection by the OCT system is an increase in the axial point spread function or a decrease in axial resolution as a function of increasing depth in
Figure 2-17. Reflectance computed using single backscatter theory corrected for multiple scattering. Values of $g$ below 0.76 show no appreciable multiple scattering effects. The spot diameter at the sample is 30 $\mu$m and the total attenuation coefficient, $\mu$, is 5.1 mm$^{-1}$.

tissue for high $g$ media (Figure 2-18). The plot in Figure 2-18 was created by measuring the OCT

Figure 2-18. Full width half maxima of the axial point spread functions for in vitro human tissue specimens.

axial point spread function as a function of depth in various tissue types[39].
Chapter 3

OCT IN GASTROINTESTINAL TISSUES

3.1 Introduction

In order to assess the capability of OCT imaging to obtain clinically useful diagnostic information for optical biopsy, *in vitro* tissue studies have been performed. The following three chapters will present tissue surveys from several major organ systems. The OCT images of *in vitro* tissues will be compared with their corresponding histology to verify the structures imaged by OCT and establish a foundation for understanding sources of tissue contrast. The first survey presented is a comprehensive study of OCT in the gastrointestinal system.

A need exists in gastroenterology for imaging at or near the cellular level to address limitations in diagnostics of both neoplastic (cancer) and non-neoplastic disorders. Recently, high frequency ultrasound transducers have been attached to the distal portions of endoscopes with the objective of improving the resolution available for clinical diagnostics. However, the relatively low resolution achieved with high frequency ultrasound, well above the cellular level at 100 microns, has resulted in only limited clinical utility[40-43]. An imaging technology which can yield resolutions in the micron range can provide information on tissue microstructure that could only previously be obtained with conventional biopsy. In this chapter, the capability of optical coherence tomography for obtaining high resolution, cross-sectional images or optical biopsies of the gastrointestinal tissues is investigated.
3.1.1 Gastrointestinal System Anatomy and Histology

The gastrointestinal tract is the organ system principally responsible for the processing of ingested food and water and consists of both luminal and solid organs. The luminal structures, which each have discrete function and are susceptible to different disorders, include the esophagus, stomach, small bowel (duodenum, jejunum, and ileum), large bowel (or colon), sigmoid colon, rectum, and the ductal systems of the solid organs. The solid organ systems of the gastrointestinal tract, whose function largely surrounds the production of biomolecules capable of digesting food, include the liver, gallbladder, and pancreas.

At the level of resolution that OCT yields, most luminal tissues of the digestive system contain four distinct functional layers. The ability to assess and analyze these different regions have important clinical implications. From the surface of the tissue (lumen) outward, these layers include the mucosa, the submucosa, the muscularis, and the adventitia[1]. The cellular and architectural morphology of the mucosa varies from the oropharynx to the colon due to different functional requirements[1]. The muscular layers, in general, serve to aid in rhythmic contractions (referred to as peristalsis) that aid in moving nutrients along the digestive tract. The vascular adventitia consists of adipose (fat) and supportive tissue[1].

As stated, the solid organs consists of the gallbladder, pancreas, and the liver. These organs produce biomolecules which are critical in the digestion of food. The biomolecules are transported through ducts which connect the organs to the small bowel lumen. The gallbladder stores bile, which emulsifies fat. The gallbladder has three distinct layers, the mucosa, consisting of folds known as villi, the submucosa, and the serosa[1]. The serosa is similar to the adventitia of the gastrointestinal tract. Bile ducts, which connect both the liver and gall bladder to the duodenum, have a mucosa, a submucosa, and an adventitia[1].

In addition to the production of digestive enzymes, the pancreas is the source of life sustaining hormones such as insulin. These hormones are secreted into the bloodstream, where they exert their effects throughout the body. These hormones are produced by a group of cells in the pancreas termed islet cells, images of which will be generated with the OCT system.
3.1.2 Gastrointestinal Tract Imaging Modalities

Disorders of the gastrointestinal tract include both neoplastic and non-neoplastic lesions. With respect to neoplastic disorders, high resolution imaging could have a substantial impact on the morbidity and mortality. Since most cancers are diagnosed late in their course (after they have metastasized or migrated) the ability to identify changes at a microstructural level could represent a powerful tool to the clinician. Currently, CCD based endoscopic imaging is the most commonly used method for identifying early changes. However, it is plagued by a relatively low resolution and inability to image below the tissue surface. Even when combined with high frequency ultrasound, the current clinical technology with the highest resolution (100 μm), magnification is inadequate for early diagnostics[40, 42-46]. In addition, ultrasound imaging is complicated by the need for a transducing medium since propagation of the signal is relatively poor through air. This is typically accomplished by filling the lumen with saline or using a liquid filled balloon to couple the ultrasonic probe to the tissue surface, a procedure which substantially complicates in vivo imaging. Furthermore, other difficulties associated with ultrasound imaging, including the expense of the system and the relatively large size of the equipment necessary in the operative field, often make it impractical for routine integration with endoscopy.

3.1.3 Optical Coherence Tomography

Though the penetration is limited to a few millimeters, the resolution of OCT is 5 to 25 times higher than high frequency ultrasound. In addition to the high resolution, several features of OCT make it well suited for intraluminal diagnostics. Because OCT is based on mature technology used in optical communication, it can be constructed with common optical fiber components. Therefore, OCT imaging can be performed at sites within the gastrointestinal tract through optical fibers without the need for a distal transducer. Unlike magnetic resonance imaging (MRI) or computed tomography (CT), OCT is compact and portable. Finally, OCT does not require contact during imaging and can be performed directly through air without the need for a transducing media.

The purpose of this study is to investigate the capability of OCT to image the microstructure of the non-transparent tissue of the gastrointestinal tract. OCT imaging of gastrointestinal tissues was performed in vitro and correlated with histology. The results of this study suggest the feasibility of using OCT for high resolution intraluminal imaging or optical biopsy of the gastrointestinal tract and serve as a foundation for future in vivo investigations. In particular, the
high resolution and fiber optic based design of OCT make it attractive for diagnostic imaging during endoscopic procedures.

3.2 Materials and Methods

3.2.1 OCT System

The tissue survey of the gastrointestinal tract was performed using two low coherence sources. The first, a superluminescent diode (SLD), had a center wavelength of 1300 nm and a spectral bandwidth of 50 nm which provided an axial resolution of 16 μm. The axial resolution was measured by inserting a mirror in the sample arm and digitizing the OCT signal. For high resolution, a Kerr lens modelocked (KLM) Chromium doped Forsterite laser was used. This solid state source had a center wavelength of 1260 nm, a bandwidth of 150 nm, and enabled OCT imaging with 4 μm axial resolution. The OCT system used in this tissue survey had a free space lateral resolution of 30 μm, a confocal parameter of 1.1 mm, a sample arm power of 150 μW, and a signal to noise ratio of 110 dB. The dimensions of the OCT images in this study were 3 (axial) X 6 (transverse) mm which corresponded to 250 (axial) X 500 (transverse) pixels. The image acquisition time was approximately 50 seconds. After acquisition, the logarithm of the two-dimensional image data is displayed using an inverse gray scale lookup table.

3.2.2 Sample Preparation

Normal and diseased gastrointestinal tissue were obtained within 5 hours of the initiation of autopsy. These tissue types have been examined since current in vivo imaging with high frequency ultrasound has met with limited success[44]. More than 35 different samples from 5 patients were examined. The tissue samples were placed in isotonic saline with 0.05% Sodium Azide and stored at 0° C. The tissues were dissected to dimensions of approximately 10 mm x 5 mm and imaged with the luminal surfaces exposed. During imaging, the tissues were partially immersed in isotonic saline to prevent dehydration. The saline did not cover the surface of the tissue.

Imaging was performed through air at room temperature. The position of the beam on the sample was monitored using a visible light guiding beam (633 nm Helium Neon laser) that was coincident with the 1300 nm infrared OCT beam on the sample. The imaging planes were marked using small injections of dye. The samples then underwent routine histologic process-
ing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin (H&E) or Trichrome Blue. The stained histologic sections enabled verification of tissue identity, and in most instances, allowed identification of sources of tissue contrast in the OCT images.

3.2.3 Measurement Statistics

Distance measurements were compared on one OCT image and the corresponding histology. From the artery in Figure 3-1, fourteen sites were identified on the OCT image and histology image that represented the intima-internal elastic membrane width. Fourteen additional sites were selected for measuring the width from the internal to external elastic membrane. The OCT image was scaled in the axial direction by the estimated refractive index \( n = 1.4 \). Distance measurements were then performed on the marked regions by two blinded investigators, one measuring histologic distances while the other assessing OCT distances. Mean distances were determined ± the standard error. Statistical differences were determined via a simple correlation coefficient test or the Pearson’s product-moment correlation coefficient[47].

3.3 Results

Intrahepatic Artery

An OCT image of an intrahepatic artery with its corresponding histology is depicted in Figure 3-1. The intima and media of the artery can be clearly differentiated. Other architectural morphologic features present in the OCT image include the presence of veins and nerves. The stroma of liver is unremarkable at this resolution. The vessel width from the internal surface of the intima to the external surface of the internal elastic membrane (int-iel) and the width from the internal surface of the internal elastic membrane to the external surface of the external elastic membrane (iel-eel) were both assessed at 14 locations on the OCT image and histology by independent investigators. The average int-iel distance on the OCT image was 51± 4 \( \mu \)m and 50± 5 \( \mu \)m (r<0.008) on the histology. The average iel-eel distance was 119±4 \( \mu \)m for the OCT image and 122±5 \( \mu \)m (r<0.007) for the histology.

Pancreatic Duct

In the pancreatic duct, the dense connective tissue layer containing elastic fibers is well differentiated from the underlying pancreas (Figure 3-2). Adipose tissue is clearly distinguish-
Figure 3-1. OCT image of intrahepatic artery (top) with its corresponding histology (bottom). The artery lumen (a), nerve bundles (n), and vein (v) are present in the OCT image. Bar represents 500 µm.

able from both the stroma of the pancreas and the connective tissue beneath the epithelium. The outlines of entire subepithelial adipose cells can be visualized in the OCT image of the pancreatic duct.

Pancreas

The high resolution of OCT enables the imaging of Islet cells borders and the interiors of Islet cells. In Figure 3-3, a portion of the pancreas has been excised and imaged. Normal glandular tissue appears homogenous at this resolution. Visualization of the larger Islets of Langerhans can be seen. However, individual acini cannot be resolved with this source. Islets in the OCT image appear as highly backscattering rings with a much lower backscattering interior. A high resolution image of the pancreas is also provided (Figure 3-4). This high resolution OCT image demonstrates the capability of OCT to image through the pancreatic duct to the pancreatic stroma. The Islet cells are more clearly differentiated in the high resolution image.

Esophagus

OCT imaging of the esophagus allows visualization of the morphology of the mucosa and submucosa (Figure 3-5). The upper portion of the mucosa appears homogenous in the OCT image. The muscularis mucosa is more highly reflective than the mucosa. A gap can be seen
between the muscularis mucosa and the submucosa. The submucosa is more irregular than the mucosa. Microstructure including veins can be seen in the submucosa as highly scattering foci. Figure 3-6 contains two high resolution OCT images of esophageal varices. Abnormally enlarged veins containing clotted blood in the submucosa can be visualized in the high resolution OCT images of these dilated veins in the submucosa.

**Gallbladder**

It is possible to differentiate several anatomic layers of the gallbladder in the OCT image, including the mucosa-submucosa, muscularis, and serosa (Figure 3-7). Villi on the surface of the OCT image can be clearly visualized. These outpocketings of tissue represent the columnar epithelium and lamina propria of the mucosa of the gallbladder. Diverticula or infoldings of the mucosa can also be seen at the surface of the OCT image. Vascular structures in the submucosa and muscularis can be identified in the image and demonstrate the capability to image through the serosa of the gallbladder. Areas of relatively low backscatter in the serosa represent the presence of adipose tissue, which has been previously demonstrated to have a low backreflection intensity[21].
**Common Bile Duct**

OCT images of the layered common bile duct also demonstrate the capability of OCT to resolve the submucosa-muscularis and muscularis-adventitia boundaries (Figure 3-8). Differentiation of the submucosa, muscularis, and adventitial layers is made possible by visualization of the different backscattering characteristics within each layer. The adventitial layer seems to have a lower and more irregular backscattering intensity than the submucosa or muscularis. This irregular backscattering pattern is most likely due to the presence of adipose tissue in the adventitial layer. The high resolution of OCT enables tissue microstructure such as secretions within individual glands to be visualized (Figure 3-8). In addition, invagination of gland ducts from the mucosal surface can be identified in the submucosa (Figure 3-8). Histology is provided only to confirm tissue identification.
**FIGURE 3-4.** High resolution OCT image of pancreatic duct and underlying pancreatic stroma. Arrows point to Islets of Langerhans. Bar represents 150 μm.

**FIGURE 3-5.** OCT imaging of the esophagus allows visualization of the morphology of the mucosa and submucosa (left image). Surface folds in the corresponding histology (right image) represent artifacts during histologic processing. Bar represents 500 μm.
FIGURE 3-6. A. High resolution OCT image of esophageal varices containing clotted blood. B. High resolution OCT image of varices containing no blood. Bar represents 500 μm.

FIGURE 3-7. OCT image of gallbladder (top) with corresponding histology (bottom). The submucosa, muscularis, and serosa can be visualized in the OCT image. Arrows point to a small muscular artery in the muscularis. Bar represents 500 μm.
**FIGURE 3-8.** OCT images of the common bile duct (top) with histology provided to confirm tissue identification (bottom). A. Arrows show glands containing secretions in the submucosa. B. Arrow points to a duct invaginating from the luminal surface. Bars represent 500 μm.
Colonic Inflammation

Colonic pathologic morphology can be visualized in OCT images of colonic inflammation (Figure 3-9). Normal glands can be seen to the right of the OCT image. These glands appear circular because the OCT system imaged these structures in cross-section. Inflammation is depicted by a high backscattering area in the OCT image (arrow). Areas of hemorrhage are noted in the OCT image as disruption of the normal mucosal morphology and resultant loss of crypt structure.

![Image of colonic inflammation](image)

**Figure 3-9.** Inflammation of Colonic Mucosa. Normal glands are noted on the right of both the OCT image (top) and histology (bottom). The arrow identifies an area of inflammation in both the OCT image and the histology, with resultant loss of crypt structure. Areas of hemorrhage, left, are noted in the OCT image as disruption of the normal mucosal morphology, and resultant loss of crypt structure. Bar represents 500 μm.

Colonic mucosa with pseudomembrane

This OCT image of the colon also demonstrates the capability of OCT to delineate mucosal pathologic microstructure (Figure 3-10). The mucosa and muscularis mucosa can be differentiated due to the different backscattering characteristics within each layer. Architectural morphology, such as crypts or glands, within the mucosa can be seen. A pseudomembrane can be visualized as the low reflectance layer overlying the mucosa proper. Deeper inflammatory
lesions also distort the normal colonic glands. The initial clinical diagnosis of this lesion on gross pathology was an adenoma. The misdiagnosis illustrates the limitations of diagnostic assessments based on visual inspection.

**FIGURE 3-10.** OCT image of the colon (left) demonstrate the capability of OCT to delineate pathologic microstructure cushion as a colonic pseudomembrane. Top arrows point to the pseudomembrane, while the bottom arrows mark a locus of inflammation. Bar represents 500 microns.

**FIGURE 3-11.** OCT image of an ampullary carcinoma (left) adjacent to normal duodenum (right). Bar represents 500 μm.

**Ampullary Carcinoma**

Figure 3-11 is an OCT image of ampullary carcinoma adjacent to normal duodenal mucosa. The normal duodenum consists of villous projections of mucosa. The adenocarcinoma shows a marked disruption of the duodenal architectural morphology, including loss of the vil-
lous structure and a thinning of the mucosa. The transition zone between the carcinoma and the normal mucosa shows a transformation of the mucosa to carcinoma.

3.4 Discussion

OCT has the potential to allow the endoscopist to delineate tissue microstructure at an axial resolution (4 to 16 µm) not available with current imaging technologies. In this work, the ability of OCT to resolve tissue morphology was demonstrated with a 1300 nm SLD source (16 µm) and a 1260 nm solid state KLM Chromium doped Forsterite laser (4 µm). The feasibility of using OCT for gastrointestinal imaging was suggested in this chapter by work performed on both normal and abnormal clinically relevant tissue.

In addition to its high resolution, OCT is attractive as an adjunct to endoscopic imaging due to its compact design and ability to image through air. Unlike MRI or CT, the fiber based design of OCT allows for compact and portable construction which can be engineered into a unit size smaller than a personal computer or standard defibrillator. The size of the instrumentation is particularly important in view of the physical constraints within the endoscopy suite. Furthermore, since OCT is based on light rather than sound, imaging is possible through air and does not require a transducing medium or direct contact with the tissue surface. Therefore, the use of an imaging balloon or saline injections, which can lead to impractical time demands on the gastroenterologist, is not necessary as it is with ultrasound.

Clinical applications of OCT imaging in gastrointestinal tissues can now be suggested. An important future application for OCT may be the screening for adenocarcinoma in patients with Barrett’s esophagus. Barrett’s esophagus is a disease where the mucosa of the esophagus transforms to gastric mucosa. Patients with Barrett’s esophagus have a 50 times higher incidence of developing esophageal adenocarcinoma than normal patients[48]. Excisional biopsy screening is very time consuming and prone to inaccuracy due to few sampling sites[44]. OCT based optical biopsy would enable screening of a much larger area without the recurrent costs associated with regular excisional biopsies and histology processing.

In conclusion, high resolution, cross-sectional OCT images of in vitro normal and pathologic gastrointestinal tissues have been obtained and correlated with conventional histopathology. The images acquired in this study provide information on tissue microstructure that could
only previously be obtained with conventional biopsy. Improvements in acquisition time and the development of a fiber optic endoscope are technically feasible and are the source of future investigations. These results suggest that OCT may become a powerful adjunct diagnostic imaging technology to conventional endoscopy, enabling non-excisional high resolution diagnostics to be performed in gastrointestinal tissue.
Chapter 4

OCT IN URINARY TRACT TISSUES

4.1 Introduction

The introduction of CCD based intraluminal endoscopic imaging of the urinary tract surface has led to substantial reductions in the morbidity and mortality associated with disorders in this organ system[49-52]. However, endoscopic imaging is frequently limited by the relatively low resolution and the inability to access the structure of tissue below the luminal surface[44]. A technology able to obtain optical biopsies, or high resolution, cross-sectional images of tissue, could substantially improve the diagnosis and treatment of urinary tract disorders. An important example would be a reduction in morbidity in the removal of hypertrophic prostatic tissue, referred to as transurethral prostatectomy (TURP), one of the most common of all surgical procedures[53]. As men age, the prostate grows (hypertrophies) and often obstructs urination. When a TURP is performed, this tissue is removed with an endoscope based surgical device. In a high percentage of these procedures, impotence (sexual dysfunction) or incontinence (inability to control urination) result from damage to small adjacent nerves [53]. An imaging technology which can yield resolutions in the micron range can potentially provide information on tissue microstructure that would enable localization of the capsule and aid in preventing damage to the neurovascular bundles. In this chapter, we will investigate the use of OCT for obtaining non-excisional, high resolution, cross-sectional images of the urinary tract, in particular, the small nerves in the immediate vicinity of the prostate.
4.1.1 Urinary Tract Anatomy and Histology

Urinary System

The urinary system is critical for controlling water and salt balance in the body. Electrolytes and water filtered from the kidneys flow from the kidney through the ureters, and are collected by the bladder[1]. As with the gastrointestinal system, the ability to image microstructure within organs of the urinary system is important for the diagnosis of many disease states. The wall of the ureter contains specialized transitional epithelium, muscular layers, and a surrounding adventitia[1]. The architectural morphology of the bladder wall consists of transitional epithelium, loose connective tissue below the epithelium, and several muscular layers[1]. The bladder is connected to the external environment by the urethra.

Urethra and Prostate

In the male, the bladder empties into the prostatic urethra, the final conducting portion of the urinary tract. The urethra is lubricated by mucous secretions from the paraurethral glands below the epithelium[1]. The prostatic urethra is surrounded by the prostate gland, which produces fluid essential for sexual function. The prostate gland consists of glandular lobules which open into ducts that empty into the prostatic urethra. Surrounding the prostate is the prostatic capsule, a dense fibroelastic connective tissue[1]. The supporting stroma of the prostate contains neurovascular bundles that primarily contain the sympathetic innervation for erectile function and control of urine excretion[1]. As mentioned earlier, the significance of prostate microstructure is related to the serious problem of nerve damage associated with prostatic surgery.

4.1.2 Urinary Tract Imaging Modalities

High resolution cross-sectional endoluminal imaging technologies, such as endoscopic ultrasound (EUS), have recently been developed to address clinical scenarios such as the diagnosis of early malignancies and the prevention of nerve damage in prostate surgery[50]. The current clinical technology with the highest resolution is transluminal ultrasound[50]. This technology uses small high frequency ultrasound transducers (10-20 MHz) to generate axial resolutions in the range of 100μm. A cross-sectional ultrasonic image is typically produced by rotating the transducer 360 degrees. Imaging of the urologic tissues is performed by filling the lumen with sterile water to couple the ultrasonic probe to the tissue surface. High frequency endoluminal ultrasound has been reported to be useful for evaluating urethral diverticula, the urethral
sphincter, staging ureteral and bladder carcinoma, and quantifying the dimensions and locations of ureteral calculi[49-52, 54]. However, since axial resolutions below 100 μm cannot be achieved, microstructure within tissue layers and cellular structure are poorly differentiated, particularly small nerves and the presence of early changes associated with cancers.

4.1.3 Optical Coherence Tomography

OCT has the potential to allow the urologist to delineate tissue microstructure at an axial resolution up to 25 times higher (4 to 16 μm) then high frequency ultrasound. In addition to high resolution, several features of OCT make it well suited for intraluminal diagnostics. As mentioned in the previous chapter, since OCT is based on technology used in optical communication, OCT can be constructed with common optical fiber components and integrated with conventional endoscopes. In addition, unlike MRI or CT, OCT is compact and portable. Finally, OCT does not require contact during imaging and can be performed directly through air without the need for a transducing media.

The purpose of this study is to investigate the capability of OCT to image in vitro microstructure of the urinary tract, with the focus on a critical region consisting of the prostatic capsule, surrounding adipose tissue, and neurovascular bundles. The results of this study will suggest the feasibility of OCT for high resolution imaging of the urinary tract and serve as a foundation for future in vivo investigations.

4.2 Materials and Methods

The OCT system used in this experiment is implemented by coupling a superluminescent diode (SLD) with a center wavelength of 1300 nm and a spectral bandwidth of 50 nm into a single mode fiber optic Michelson interferometer. The OCT system had a free space axial resolution of 16 μm. The transverse resolution, determined by the spot size of the focused beam incident on the sample, was measured to be 30 μm. The spot size was chosen so that the transverse resolution was comparable to the axial OCT resolution, while maintaining an appropriate depth of focus (a confocal parameter of approximately 1.1 mm). The power incident on the sample was 150 μW which provided a signal to noise ratio (SNR) of 110 dB. The dimensions of the OCT images acquired in this study were 3 (axial) x 6 (transverse) mm which corresponded to 250 (axial) x 500 (transverse) pixels. The image acquisition time was 50 seconds. After acquisition, the
logarithm of the two-dimensional image data was displayed using an inverse gray scale lookup table.

Normal urologic tissue, including the prostatic urethra, prostate, bladder, and ureters, were obtained within 5 hours of the initiation of autopsy. More than 20 different samples from 5 patients were examined. The tissue samples were placed in isotonic saline with 0.05% Sodium Azide and stored at 0°C. The tissues were dissected to dimensions of approximately 10 mm x 5 mm and imaged with the luminal surfaces exposed. During imaging, the tissues were partially immersed in isotonic saline to prevent dehydration. The saline did not cover the surface of the sample.

Imaging was performed through air at room temperature. The position of the beam on the sample was monitored using a visible light guiding beam (633 nm Helium Neon laser) that was coincident with the 1300 nm infrared OCT beam on the sample. The imaging planes were marked using small injections of dye. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were then processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with hematoxylin and eosin (H&E). The stained histologic sections enabled verification of the microstructure of the different samples and allowed identification of sources of tissue contrast in the OCT images.

4.3 Results

Prostatic Urethra

OCT enables visualization of the architectural microstructure of the prostatic urethra and the periurethral prostate (Figure 4-1). Differentiation between the prostatic urethra and the prostate is possible due to the different backreflection characteristics of the two tissue types. Paraurethral gland ducts can be visualized within the urethra (Figure 4-1). Prostatic glands can be identified in both images and demonstrate the capability to image completely through the ure-
thra to the prostate. Areas of relatively low backscatter within the prostatic glands represent the presence of prostatic secretions.

**Figure 4-1.** OCT image of *in vitro* human prostatic urethra and periurethral prostate. The top arrow points to a periurethral gland duct in the urethra. Lower arrows indicate prostatic glands containing secretions. Bar represents 500 μm.

**Neurovascular Bundle**

The high resolution of OCT allows imaging of neurovascular bundles near the capsule at the prostate-adipose tissue border. In Figure 4-2, a portion of the prostate has been excised and imaged. The prostate tissue appears relatively homogenous at this resolution. The outlines of entire adipose cells can be visualized in this OCT image. A neurovascular bundle can be seen within the adipose tissue adjacent to the prostate (Figure 4-2). Neurovascular bundles appear to have a high backreflection intensity relative to that of the surrounding adipose tissue. Thus, OCT provides high contrast between neurovascular bundles and adipose tissue at the prostate-adipose border.

**Prostatic Capsule**

OCT images of the exterior surface of the prostate demonstrate the capability of OCT to resolve and locate the prostatic capsule (Figure 4-3). Differentiation of the collagenous layers of the capsule is made possible by the differences in backreflection between the capsule and the
FIGURE 4-2. OCT image of *in vitro* human prostate-adipose border (left) and histology (right). Entire outlines of adipose cells may be seen in the OCT image. Top arrows demarcate the prostate-adipose border. The lower arrows point to a neurovascular bundle. Bar represents 500 μm.

FIGURE 4-3. *In vitro* OCT image of the prostatic capsule (top) and corresponding histology (bottom). The top arrow points to fibrous prostatic capsule. Microstructure such as the border between the intima and media of an artery can be seen below the capsule (lower arrow). Bar represents 500 μm.
prostate. The axial thickness of the capsule in this image can be measured from the OCT image and is approximately 50 µm. Figure 4-3 also shows an artery below the fibrous capsule. Microstructure visible within the artery include the intima and media of the vessel.

**Figure 4-4.** OCT image of an *in vitro* human bladder wall (top) and histology (bottom). The borders between the mucosa (m), submucosa (sm), and the muscularis propria (mp) can be clearly resolved in the OCT image. Bar represents 500 µm.

**Bladder and Ureter**

Different anatomic layers in the bladder can be identified in the OCT image, including the mucosa, submucosa, and muscularis propria (Figure 4-4). OCT images of the ureter demonstrate the capability of OCT to resolve the mucosa, muscular layers, and adventitia (Figure 4-5). Differentiation of the mucosa and the muscular and adventitial layers is made possible by visualization of the different backreflection characteristics within each layer. The muscular layers seem
to have a higher and more regular backreflection intensity than either the mucosal or adventitial layers.

4.4 Discussion

OCT has the potential to allow the urologist to delineate tissue microstructure at an axial resolution up to 25 times higher (4 to 16 μm) then high frequency ultrasound. In this work, the ability of OCT to resolve tissue morphology was demonstrated with a 1300 nm diode source which provided an axial resolution of 16 μm. The feasibility of using OCT for high resolution urologic imaging was suggested by studies performed on representative tissues of the urinary tract. Microstructural details such as periurethral exocrine ducts, prostatic glandular secretions, and ureteral muscular layers could be easily identified.

A role for OCT in guiding the resection of hyperplastic prostatic parenchyma has also been suggested. OCT images, with an axial resolution of 16 μm, sharply delineate the prostate capsule border. Furthermore, neurovascular bundles were identified in close approximation to the prostatic capsule. Since postoperative impotence and incontinence have been linked to traumatic transection of these neurovascular bundles, OCT guidance may substantially reduce the morbidity associated with mechanical interventions.

In conclusion, we have demonstrated high resolution, cross-sectional OCT imaging of several different urologic tissues in vitro and have correlated these images with conventional histopathology. The images acquired in this study provide information on tissue microstructure
that could only previously be obtained with conventional biopsy. These results demonstrate that OCT may become a powerful diagnostic technology during urologic procedures, particularly the guidance of prostatic resection.
Chapter 5

OCT IN THE CARDIOVASCULAR SYSTEM

5.1 Introduction

Atherosclerosis (abnormal fat or fibrous tissue formation in the blood vessel wall) is the most prevalent cause of death and serious morbidity in the Western world[55]. Myocardial infarction, which results from atherosclerosis of the arteries of the heart and is commonly referred to as a heart attack, accounts for 20 - 25% of all deaths in the United States. It most commonly occurs when the coronary vessels (heart blood vessels) are occluded by the rupture of small atherosclerotic plaques. After rupture, fat is released into the blood stream which can cause clot formation or thrombosis[55]. These small plaques which rupture can not be detected reliably by currently available imaging technologies. Because of the widespread prevalence of this disease and its severity, the development of imaging methods to diagnose plaques likely to rupture is one of the most active areas of present day medical imaging research. In this chapter, we will investigate the use of OCT for obtaining high resolution, diagnostic information on atherosclerotic plaque morphology[21, 22].

5.1.1 Cardiovascular Histology and Histopathology

The fundamental histologic layers of the heart arteries (coronary arteries) include the intima, media, and adventitia. The surface of the intima is covered by a single cell layer, the endothelium, which is supported by an underlying thin layer of collagenous connective tissue[1]. The subendothelial layer, the layer of the intima below the endothelium, can accumulate lipid and leads to the process of atherosclerosis or plaque formation. Thickening of the intima represents one of the earliest changes of atherosclerosis[55]. The media is a broad layer that con-
sists of elastin, collagen, and smooth muscle cells[1]. Below the media, the adventitia contains adipose tissue and small blood vessels[1].

5.1.2 Acute Coronary Syndromes

The mechanisms which result in myocardial infarction explain the need for high resolution imaging. Recently, research performed by several groups have shown that most myocardial infarctions result from the rupture of a lipid-laden plaque in a coronary artery followed by a cascading sequence of biochemical reactions resulting in thrombosis and vessel occlusion[56-58]. The plaques at highest risk for rupture are those which contain a large core of lipid covered by a structurally weak fibrous cap[56]. The likelihood of rupture increases when the junction between the lipid and the fibrous cap is thin. Several studies have shown that these plaques generally do not significantly alter blood flow prior to rupture[59-61], and are thus difficult to diagnose by conventional radiological techniques. In fact, the large, near occlusive plaques which are seen on angiography serve predominately as markers of severe disease, rather than initiators of acute myocardial infarctions[59-61]. Predicting future events is particularly important in view of the high percentage of occlusions resulting in sudden death[62]. However, to date, no modality other than postmortem histology has provided the level of resolution and contrast necessary to diagnose the small thin walled lesions likely to undergo rupture[63].

5.1.3 Coronary Imaging

The most commonly used procedure for diagnosing coronary atherosclerosis is angiography. Angiography is the direct injection of opaque contrast medium into the artery while monitoring the procedure with fluoroscopic imaging. This procedure has a limited ability to predict future acute events since it primarily only identifies large near occlusive lesions[59-61]. Moreover, its level of resolution remains greater than 500 microns and is not likely to improve significantly. Coronary wall thickness, luminal area, lipid content, and the extent of atherosclerotic disease would be better assessed by viewing the artery in cross section, an advantage of intravascular ultrasound and potentially OCT. For these reasons, other methods of imaging have recently been aggressively pursued to overcome these limitations, including magnetic resonance imaging(MRI), angioscopy, and intravascular ultrasound(IVUS).

MRI (and closely related magnetic resonance angiography) is a promising technology to screen for the presence of coronary artery disease[64]. It has the advantage of being non-inva-
sive, can give information on flow in addition to anatomical obstruction, and has a limited ability to provide spectroscopic information on tissue composition. Its limitations are low resolution (greater than 100 μm even in animal studies), very poor visualization of the circumflex, expensive instrumentation, and the inability to allow simultaneous deployment of mechanical interventions[65]. Furthermore, though it may eventually be able to screen for areas suspicious for unstable plaques, its low resolution makes definitive identification of these areas unlikely.

Angioscopy is the direct visualization of the surface of the blood vessel with a fiber optic bundle. It has the advantage over other imaging methods in diagnosing surface fissuring and the presence of thrombus[66, 67]. Its disadvantages are its dependence on a clear field of vision, it cannot accurately measure luminal dimensions for guiding interventions, and it cannot image though the vessel wall[68]).

High frequency ultrasonic transducers (20-30 MHz) located at the tips of catheters have recently been introduced for intravascular imaging[69-71]). The ability of this technique to image the vessel in cross section and delineate structure within the wall have made it the current gold standard for assessing the severity of atherosclerotic disease. It is superior to angiography in its ability to diagnose dissections, assess the adequacy of coronary interventions, guide stent deployment, and determine the extent of arterial stenosis, most notably at ostial sites[72, 73]. Catheters are currently under investigation which allow imaging to be performed simultaneously with interventions. However, the maximum attainable resolution of 100 μm, which decreases linearly with distance, limits its ability to delineate structure[73]. Furthermore, it is limited in its ability to assess lipid content (lower limit 250 μm) and has a poor interobserver agreement as to whether lipid is present in vivo (in some instances not significantly different from chance)[73-75]. Also, IVUS imaging through calcified tissue is problematic due to the attenuation of ultrasound by calcifications.

5.1.4 Optical Coherence Tomography

Although the penetration of OCT imaging in nontransparent tissue is limited to a few millimeters, the typical axial resolution of OCT can range from 16 μm, almost 10 times greater than ultrasound or MRI. In addition to the high resolution, several features of OCT make it well-suited for intraluminal diagnostics. Because OCT is based on technology used in optical communication, it can be constructed with common optical fiber components. Therefore, OCT imaging
can be performed at sites within the cardiovascular system through optical fibers without the need for a distal transducer. Finally, unlike MRI, OCT is compact and portable.

5.2 Materials and Methods

The OCT system used in this survey of the cardiovascular system is implemented by coupling a superluminescent diode (SLD) with a center wavelength of 1300 nm and a spectral bandwidth of 50 nm into a single mode fiber optic Michelson interferometer. As in the previous studies, the OCT system had a free space axial resolution of 16 μm. The transverse resolution, determined by the spot size of the focused beam incident on the sample, was measured to be 30 μm. The spot size was chosen so that the transverse resolution was comparable to the axial OCT resolution, while maintaining an appropriate depth of focus (a confocal parameter of approximately 1.1 mm). The power incident on the sample was 150 μW which provided a signal to noise ratio (SNR) of 110 dB. The dimensions of the OCT images acquired in this study were 3 (axial) x 6 (transverse) mm which corresponded to 250 (axial) x 500 (transverse) pixels. The image acquisition time was 50 seconds. After acquisition, the logarithm of the two-dimensional image data is displayed using an inverse gray scale lookup table.

Normal and pathologic tissues including the left ventricular muscle, adipose, cortical bone, coronary arteries, and aortas were obtained within 5 hours of the initiation of autopsy. More than 50 different samples from 10 patients were examined. The tissue samples were placed in isotonic saline with 0.05% Sodium Azide and stored at 0° C. The tissues were dissected to dimensions of approximately 10 mm x 5 mm and imaged with the luminal surfaces exposed. During imaging, the tissues were partially immersed in isotonic saline to prevent dehydration.

Imaging was performed through air at room temperature. The position of the beam on the sample was monitored using a visible light guiding beam (633 nm Helium Neon laser) that was coincident with the 1300 nm infrared OCT beam on the sample. The imaging planes were marked using small injections of dye. The samples then underwent routine histologic processing. Samples were immersed in 10% buffered formalin for 48 hours. The tissues were subsequently processed for standard paraffin embedding. Five micron thick sections were cut at the marked imaging sites and stained with H&E. The stained histologic sections enabled verification of the microstructure of the different samples and allowed identification of sources of tissue contrast in the OCT images.
5.3 Results

Lipid Laden Tissue

OCT can be clinically useful for identifying high risk atherosclerotic plaques because it is able to differentiate lipid and water-based tissue, a limitation of conventional cardiovascular imaging technologies[69]. Figure 5-1 has been added to demonstrate this feature. Figure 5-1 shows an in vitro OCT image of cardiac muscle abutting adipose tissue. Because the backscattered optical reflectance of fat cells is significantly lower than muscle, the contrast between these two tissues is high. The large reflection peaks observed in the measurement of adipose tissue may be attributed to supportive tissue structures, while the intracellular lipid has a very low reflectivity compared with muscle. Thus, in contrast to ultrasound imaging, lipid and water based tissues have distinct optical reflectance properties which enables OCT imaging to sharply delineate microstructure within lipid laden atherosclerotic arteries.

![Image of OCT Image of left ventricular muscle (left) adjacent to adipose tissue (right). Bar represents 500 μm.](image)

Heavily Calcified Tissue

A major limitation of ultrasound for intravascular imaging is that it cannot penetrate heavily calcified tissue[69]. Figure 5-2 demonstrates the ability of OCT to image in highly calcified tissue. An OCT image of an in vitro human clavicle is shown, demonstrating imaging of
Haversian systems at depths of over 2.0 mm into the cortical bone. Unlike sound waves, infrared light is less strongly reflected from calcified tissues and thus OCT imaging is possible even within these structures. Similar results were achieved in calcified aortic lesions.

**Aortic Atherosclerotic Plaques**

Figure 5-4 through Figure 5-7 demonstrate imaging of atherosclerotic lesions in human abdominal aorta specimens. They have been added to emphasize specific features of imaging atherosclerotic plaques. An intramural collection of lipid can be seen in Figure 5-3 where the arrows identify corresponding areas in the histology and OCT image. In Figure 5-4, a small intimal layer is covering a large atherosclerotic plaque which is heavily calcified and has a relatively low lipid content. The ability to identify fine structural detail such as the width of intimal caps in
**Figure 5-3.** OCT image of an aortic atherosclerotic plaque (top) and histology (bottom). Arrows point to corresponding locations in both images. Bar represents 500 μm.

*In vivo* will likely lead to significant improvements in patient risk stratification[76]. Figure 5-5 illustrates a fissure within the vessel wall extending into heavily calcified plaque. In Figure 5-6, the arrow marks a particularly thin area at the base of the plaque. The thin wall relative to lipid content increases the likelihood of rupture[76]. Both Figure 5-5 and Figure 5-6 demonstrate the ability of OCT to image structural details within plaques such as fissuring. Fissures, which extend from the surface into the media, often result in intramural thrombus formation and a rapid increase in plaque size, occasionally resulting in complete occlusion[76]. A small deeper plaque is present in Figure 5-7. The existence of a thick intimal cap renders this plaque less likely to rupture than the atheroma in Figure 5-6.
**FIGURE 5-4.** OCT image of a thin intramural aortic plaque (top) and corresponding histology (bottom). Arrows point to the thinned intima. Bar represents 500 μm.

**FIGURE 5-5.** OCT image of an aortic plaque with fissuring. Black arrows indicate a fissure in the media of the aorta. Bar represents 500 μm.
FIGURE 5-6. OCT image of a thin walled aortic atherosclerotic plaque. Arrow points to location of intimal thinning. Bar represents 500 μm.

FIGURE 5-7. OCT image of a thick walled aortic atherosclerotic plaque. Bar represents 500 μm.
Coronary Artery

Figure 5-8 is an image of a right coronary artery with a small plaque. Penetration through the artery to the underlying coverslip is seen. This image demonstrates the capability of OCT to image through the entire width of the normal coronary artery.

![Image of coronary artery](image)

**Figure 5-8.** OCT image of *in vitro* human coronary artery. All of the structural features of the coronary artery can be visualized, including the intima (i), the media (m), and the adventitia (a). The area marked by (p) is an atherosclerotic plaque. Imaging through the entire coronary artery to the underlying coverslip (c) is demonstrated. Bar represents 500 µm.

5.4 Discussion

Rupture of lipid filled atherosclerotic plaques in coronary arteries is now believed to be the most common mechanism initiating acute myocardial infarction[58-60]. However, current imaging techniques only have a limited ability to identify these lesions. For this reason, other methods of imaging have recently been aggressively pursued to overcome these limitations, the most significant being intravascular ultrasound (IVUS).

High frequency ultrasound (20-30 MHz) is superior to angiography in its ability to diagnose dissections, assess the adequacy of coronary interventions, guide stent deployment, and determine the extent of arterial stenosis, most notably at ostial sites[77]. However, the maximum attainable resolution of 100 microns, which decreases linearly with distance, limits its ability to...
delineate structure[73-75]. In addition, IVUS has limited capability to image through calcified lesions.

The intravascular use of OCT has the potential of overcoming these limitations. This study demonstrates the ability of OCT to delineate in vitro plaque morphology. Structural details such as the thickness of intimal caps, extent of lipid collections, and presence of fissures were assessed at a level of resolution (16 microns) not achievable by other imaging modalities. In addition, OCT has the capability to image through heavily calcified tissue. Finally, because OCT is based on fiber optic technology, it can potentially be incorporated into a fiber optic catheter for intraluminal imaging. To conclude, the ability of OCT to enable high resolution imaging makes it a promising new technology for the intravascular evaluation of high risk atherosclerotic lesions.
Chapter 6

IN VIVO OPTICAL COHERENCE TOMOGRAPHY TECHNOLOGY

6.1 Introduction

The potential capability of OCT to obtain optical biopsies, or non-invasive optical diagnostic images of in vivo tissue architectural morphology, has been demonstrated by in vitro tissue surveys. The images acquired in these studies provide high resolution structural information that could only previously be obtained with conventional biopsy. In addition, OCT images of the architectural morphology are useful because the differences in optical scattering in tissue provides sufficient tissue contrast to identify clinically relevant microstructural features. Finally, since many important pathologies can be visualized in the first two or three millimeters from the epithelial surface, OCT imaging penetration depth is sufficient to enable the acquisition of powerful diagnostic information without resorting to surgical exploration and excisional biopsy.

However, new technologies must be developed to enable in vivo OCT imaging of external and internal organs systems. Until recently, the state of the art OCT systems have not been capable of imaging in vivo tissue for the following three reasons; (1) lack of a catheter or endoscope for enabling OCT imaging access to internal organ systems, (2) lack of an adequate light source for high speed scanning, and (3) insufficient image acquisition rates. This chapter serves to introduce each of these issues and their potential solutions. The next five chapters of this thesis will provide a detailed description of advances in OCT technology that address these problems and enable in vivo OCT-based optical biopsy of external and internal organs systems.
6.2 OCT Compatible Catheter-Endoscope

A key technology which is necessary in order to apply OCT for imaging of internal organ systems is a catheter-endoscope which is capable of delivering, focusing, scanning, and collecting a single spatial mode optical beam. In addition, the catheter must be flexible and have a small diameter to facilitate its entry into internal channels such as coronary arteries that have inner diameters of approximately 1 mm. In Chapter 7, the design and performance of a prototype single mode fiber optic scanning OCT catheter is described. This device is an enabling technology for developing a wide range of catheters and endoscopes and will permit the OCT imaging of many tissue and organ systems which were not previously accessible.

6.3 Low Coherence Light Sources

6.3.1 Source Development

An additional technology necessary for producing an OCT system capable of imaging in vivo tissues is a high power, single mode, low coherence source. In order to maintain a useful imaging penetration depth in multiply scattering tissues (2-3 mm), the SNR of the OCT system must be greater than 100 dB[21]. For shot noise limited detection, the SNR is proportional to the power incident on the sample and inversely proportional to the signal bandwidth. Since the bandwidth increases with scanning velocity, the power incident on the sample must be increased commensurately with the increase in scanning speed to preserve the SNR. Chapter 8 summarizes work performed to identify and build light sources for high speed OCT.

6.3.2 Power Limitations Due to Tissue Damage Thresholds

The ultimate limitation that affects the maximum scanning rate that can be achieved is the maximum incident power that results in tissue damage. This restriction places an upper limit on OCT scanning speed for a given SNR. The tissue damage threshold is dependent on the wavelength, the spot size, the scanning rate, and the scan length. Using the ANSI standard, the maximum permissible exposure (MPE) for human skin is approximately 40 mW, for a spot size of 30 μm, a center wavelength of 1300 nm, a scanning rate of 30 fps, and a scan length of 10 mm[78]. Since this standard only applies to skin, the MPE for other tissues need to be determined in future experiments. Nevertheless, using a reasonable approximation of half the MPE of skin, a sample arm power limit of 20 mW will enable OCT imaging at real time rates without substantial tissue damage and an acceptable SNR (~100 dB).
6.4 High Speed Optical Delay Scanning

The development of techniques for high speed image acquisition in OCT systems is essential for suppressing motion artifacts when imaging living systems (Figure 6-1). The OCT system used in the tissue survey utilized a mechanically translated reference arm mirror to perform axial scanning. Because no cost effective mechanical translator capable of achieving high scanning rates is currently available, alternative optical delay scanning technologies must be developed. Chapter 9 and Chapter 10 describe two new methodologies for achieving high speed optical delay scanning.

6.5 In Vivo OCT (IVOCT) System Integration

The OCT compatible catheter-endoscope, low coherence source, and high speed optical delay scanning, have been implemented and integrated into an in vivo OCT system (IVOCT). Chapter 11 discusses the technology involved in constructing the IVOCT system which include video circuitry, high speed data acquisition, high speed image formatting, and display. IVOCT is the first OCT system to acquire in vivo images of internal organs. The capability of IVOCT to acquire optical biopsies is demonstrated by capturing motion artifact free images of the respiratory and gastrointestinal tracts of a living rabbit (Chapter 12).
Chapter 7

SCANNING SINGLE MODE OPTICAL FIBER CATHETER-ENDOSCOPE

7.1 Introduction

The design and construction of a catheter-endoscope which is capable of delivering, focusing, scanning, and collecting a single spatial mode optical beam is necessary in order to use OCT to image internal organ systems. The catheter-endoscope must also have a small diameter and be flexible to facilitate its entry into lumen such as coronary arteries that have inner diameters of approximately 1 mm. In this chapter the design and performance of an OCT compatible single mode fiber optic scanning catheter-endoscope is presented[79].

Ultrasound catheters and endoscopes are currently being used to obtain cross-sectional images of internal channels in the body. Ultrasound images are acquired by sending an acoustic pulse into the tissue and measuring the temporal delay of the backscattered acoustic reflections from within the surrounding media. For ultrasound imaging devices, the transducer is the source of the ultrasonic radiation as well as the detector of the reflected acoustic energy. In most modern ultrasound catheters, the transducer is directed towards a mirror[41, 42]. The entire transducer/mirror apparatus is attached to an inner sheath which is free to rotate within an external sheath[41, 42]. The state of the art ultrasonic imaging device, intravascular ultrasound, uses a 30 MHz transducer which provides an axial resolution of approximately 100 μm[41, 42].
7.2 Overview

The ability of OCT to provide cross-sectional imaging of tissue microstructure with approximately 10 times greater resolution than ultrasound has motivated the design and construction of an OCT compatible catheter-endoscope. The OCT catheter consists of an optical coupling element at its proximal end, a single mode fiber running the length of the catheter, and optical focusing and beam directing elements at the distal end (Figure 7-1). The catheter is designed to scan the beam in a circumferential pattern in order to cross sectionally image through the vessel (or other biological structure) into which it is inserted (Figure 7-2).

Beginning at the proximal end of the device, incident light from a fixed single mode optical fiber is coupled across a small air gap into a second single mode fiber which can rotate (Figure 7-3). The drive assembly of the catheter, located at the proximal end, uses an optical fiber connector (modified AT&T connector). A gear is attached to the connector and a shaft assembly consisting of the connector, the fiber in the catheter, and the distal focusing elements. A DC motor is used to drive the shaft assembly through a gear mechanism (Figure 7-3).

The catheter scans the field of view by rotating the optical fiber and the distal optics. Precise alignment of the optical coupling between the fixed and rotating fiber is ensured by mounting the fibers in a precision mechanical ferrule assembly. This is accomplished using two male fiber connectors (AT&T type connectors) and a male-to-male optical fiber coupler typically used for rigidly connecting two fibers. The input fiber connector is fixed to the coupler while the catheter fiber connector is free to rotate. The precise tolerances of the coupler ensures alignment and
Figure 7-2. General use of the OCT catheter-endoscope. The catheter-endoscope is inserted in the center of the lumen of a vessel and scans in a circumferential pattern perpendicular to the axis of the catheter[79].

Figure 7-3. Proximal end of the OCT compatible catheter-endoscope[79].

coupling of light from the fixed to rotating fiber as the drive shaft rotates. This approach is simpler and cheaper than optically coupling across the rotating junction using a pair of lenses and free space.

The body of the catheter is comprised of a flexible, rotating inner sleeve that fits loosely inside a stationary outer Teflon sheath (Figure 7-4). The body is flexible to allow for bending dur-
Figure 7-4. Body and distal end of OCT compatible catheter-endoscope[79].

Passage through the contours of internal channels, such as blood vessels. The outer sheath allows the inner sleeve to rotate freely and provides a smooth exterior for passage through internal organ systems. The inner sleeve utilizes a hollow flexible cable similar to one found in a speedometer. The optical fiber is fixed within the center of the inner sleeve which is rotated as a unit with the optical connector at the proximal end of the catheter-endoscope. Rotational torque exerted at the proximal end by the drive mechanism rotates the shaft assembly consisting of the fiber and cable within the outer Teflon sleeve.

The distal end of the catheter is composed of miniature beam focusing and directing optics. The single mode fiber is attached to a gradient index (GRIN) lens (Figure 7-4). Analysis of the Gaussian beam using the ABCD matrix formalism is a convenient method for determining the fiber-GRIN separation that yields an appropriate spot size and working distance for the catheter-endoscope (Appendix A). The fiber-GRIN separation is usually chosen so that the spot size (or transverse resolution) is comparable to the axial OCT resolution while maintaining an appropriate working distance (approximately 2 mm). Plots of the working distance, beam diameter, and confocal parameter ($2z_R$) versus the fiber-GRIN separation for a commercially available 700 µm diameter GRIN lens with a length of 1.9 mm are given in Figure 7-5, Figure 7-6, and Figure 7-8. For a typical beam diameter of 30 µm, for example, the fiber-GRIN separation is approximately 250 µm, the resultant working distance is approximately 2 mm, and the confocal parameter, $2z_R$, is approximately 1.1 mm.
FIGURE 7-5. Working distance of the distal end of the catheter-endoscope as a function of fiber-GRIN separation.

FIGURE 7-6. Focused beam diameter of the distal end of the catheter-endoscope as a function of fiber-GRIN separation.

After the single mode optical fiber has been fixed to the GRIN lens, a micro prism is mounted at the distal surface of the GRIN lens to direct the beam perpendicular to the axis of the catheter (Figure 7-4). The distal end of the catheter is enclosed by a transparent sleeve which is continuous with the Teflon sleeve housing the body of the catheter (Figure 7-4). The micro prism, GRIN lens, inner sleeve, optical fiber, and proximal rotating optical connector are all attached together with ultraviolet curing optical cement to form a single unit, the rotating shaft portion of the catheter.
During image acquisition, the catheter is inserted into the tissue structure being imaged (the artery or other internal tissue channel) and, as the drive motor turns, the shaft of the catheter and distal optics circumferentially scan the focused beam perpendicular to the axis of the catheter (Figure 7-2). An OCT image is acquired as the beam angle of rotation is varied over some range (usually 360 degrees). The speed of imaging depends on the speed of the rotation and the OCT unit acquisition speed. Thus, this catheter design should be scalable to real time imaging speeds of greater than 30 frames per second (fps).

7.3 Catheter-Endoscope Materials and Methods

7.3.1 OCT System

To demonstrate the operation of the catheter-endoscope, a 1300 nm SLD based OCT system was employed. Without the catheter-endoscope, the OCT system had a free space axial resolution of 16 µm, a sample arm power of 150 µW, and a signal to noise ratio (SNR) of 110 dB. The OCT system was modified to include the catheter endoscope. An optical fiber with a length near that of the catheter fiber was placed in the reference arm to match the optical path lengths and dispersion in both arms (Figure 7-8).
7.3.2 Internal Reflection

**Power Loss**

Since the refractive indices of the commercially available optical elements in the different components of the distal end were not equal,

\[ n_{fiber} = 1.4, \quad n_s = 1.5, \quad n_G = 1.6, \quad n_p = 1.8, \quad n_{air} = 1.0, \quad n_{window} = 1.4, \quad n_{air} = 1.0, \quad (7-1) \]

internal reflections from each interface result in a loss of sample arm power. The percentage loss of sample arm power returned to the detector is simply twice the summation of the Fresnel reflection at each of the interfaces,

\[ P_{loss} = 2 \sum_{i=n_i}^{n_{air}} \frac{(\frac{n_i}{n_i-1} - 1)^2}{(\frac{n_i}{n_i-1} + 1)^2}. \quad (7-2) \]

For the catheter elements used in the distal end, the power returned from the sample arm is approximately 30% lower due to reflections at the optical interfaces. This loss can be minimized by custom designing optical elements that have the same refractive indices.
Returned Internal Reflection

Sample arm power loss is less serious than returned internal reflections because the loss can be compensated by increasing the source power. However, internal reflections that reach the detector increase the shot noise level of the system and can saturate the detector if the sample arm power is high enough. The magnitude of the internal reflection signal not only depends on the refractive index differences at the interfaces, but it is also a function of the beam diameter and radius of curvature at any given interface. The focussing properties of the beam at the interfaces determines the amount of light coupled back into the single mode fiber. The sources of the back-reflected light can be determined by analyzing an OCT plot of the internal reflections from the distal end of the catheter. The backreflected light from within the distal optics of the first prototype catheter is shown in Figure 7-9. The primary sources of returned internal reflection seem to arise from the optical fiber-optical adhesive interface and the optical adhesive-GRIN interface. The reflection from the optical fiber-optical adhesive interface is sufficiently large in magnitude that it saturates the A/D converter. Symmetric reflections around this interface represent echoes from the facets of the SLD. This reflection must be removed for high power applications because

![OCT Signal](image)

**Figure 7-9.** OCT signal from internal reflections within the distal end of the catheter-endoscope.
an increase in source power will saturate the detector. The other echoes within the catheter are less significant and can be windowed out of the OCT signal by increasing the coherence gate. The multiple internal reflections are of some concern, however. While they are small, they will appear in the image and obscure tissue structure.

7.3.3 Catheter-Endoscope

A prototype OCT catheter has been designed, constructed, and analyzed. At the proximal end, the catheter uses a 1 rpm DC motor mechanically coupled to a modified AT&T connector through a custom 1:1 ratio gear box. A single mode optical fiber (Corning SMF-28) with a core diameter of 9 \( \mu \)m guides the light through the body of the catheter. The distal end is comprised of a 0.7 mm diameter, 0.2725 pitch GRIN lens, and a 0.5 mm right angle micro prism. The catheter outer diameter is 1.1 mm at the transparent window of the catheter. The confocal parameter and the focused beam diameter were experimentally measured by measuring the full-width-half-maximum reflectance from a mirror as a function of distance perpendicular to the catheter axis. The confocal parameter was 2.42 mm and the spot size was 45 \( \mu \)m at a wavelength of 1300 nm. The working distance from the microprism face was approximately 3.0 mm. A photograph of the distal optics of the catheter is shown in Figure 7-10.

![Figure 7-10](image)

**Figure 7-10.** Photograph of the distal optics of the OCT compatible catheter-endoscope.
Variations in optical coupling at the proximal end produced loss as the fiber was rotated. Angular losses were determined by measuring the change in reflectance from the fiber-GRIN interface as a function of angle. The loss as a function of angle was found to be ±1.5 dB. These angular variations can be reduced by using higher mechanical tolerances or can be renormalized out of the imaging by simple processing techniques. Power loss caused by index mismatches within the catheter was 5 dB. Thus, the overall SNR of the OCT system coupled into the catheter was approximately 105 dB.

7.4 Results

In order to demonstrate imaging with the catheter, OCT was performed in several intact luminal tissues. These samples included an in vitro human ureter, fallopian tube, and coronary artery. The intact specimens were taken postmortem and imaged with the catheter in the center of the lumen of the tissues. The rectangular raw data image arrays were displayed in polar coordinates using a linear interpolation algorithm. These images represent micron scale tomographic images taken transluminally through the tissue and are the first OCT images of this type.

Ureter and Fallopian Tube

An OCT image of an intact in vitro human ureter was acquired using the catheter-endoscope (Figure 7-11). The OCT image allows visualization of the architectural structure of the ureter including the epithelium, which appears as a highly backscattering band near the lumen. The muscularis can be visualized as circular striations below the transitional epithelium. Imaging penetration of more than 2 mm is demonstrated by the ability of OCT to image the outer wall of the ureter. A portion of the distal fallopian tube was also imaged with the OCT catheter-endoscope (Figure 7-12). Fimbriae, or villous mucosal projections, can be seen below the epithelium of the fallopian tube.

Coronary Artery

Figure 7-12 demonstrates OCT imaging of an intact human coronary artery using the prototype catheter-endoscope. The adventitia and media of the in vitro coronary artery are well differentiated in addition to thickening of the intima, known as moderate intimal hyperplasia. A 3.2 French, 30MHz IVUS transducer (Cardiovascular Instrument Sys., Sunnyvale, CA) was used to image the same arterial segment. Data was processed and displayed with an Insight III ultra-
**Figure 7-11.** OCT image of an intact in vitro human ureter (left) and fallopian tube (right). The epithelial (e) layers, muscularis (ms), and the outer wall of the ureter can be clearly visualized. Fimbriae from the mucosal wall of the fallopian tube can be seen. Bars represent 1 mm.

**Figure 7-12.** OCT image of human coronary artery taken with the catheter-endoscope (A) compared with an intravascular ultrasound image of the same coronary artery (B). Bar represents 1 mm.

sound system (Cardiovascular Instrument Sys., Sunnyvale, CA). The OCT image shows a significant enhancement in resolution and capability to differentiate tissue morphology.
An important topic in cardiology research is the imaging of the three-dimensional structure of the coronary lumen. The three-dimensional structure of the coronary artery is significant because it supplies the cardiologist with additional information about plaque morphology and hemodynamics. For example, locations on plaques where the blood flow is highly turbulent are more likely to rupture. The design of an OCT catheter-endoscope provides the ability to obtain three-dimensional reconstructions of coronary artery lumen with higher resolution than any other method. Serial sections of the lumen were obtained by sequentially acquiring OCT images with the catheter-endoscope while pulling the catheter back at a constant rate with a computer controlled mechanical stage. The serial sections were then segmented to identify the lumen of the artery. Finally, to create a three-dimensional representation of the coronary lumen, the segmented serial sections were surface rendered using the marching cubes algorithm[80]. A three-dimensional reconstruction of a coronary artery acquired with the OCT compatible catheter-endoscope is shown in Figure 7-13.

7.5 Discussion

A prototype single mode optical fiber scanning catheter-endoscope for OCT imaging of tissue architectural morphology within human internal organ systems has been designed and constructed. The catheter is compact, inexpensive, and can be readily engineered into a clinically viable form. This device is an enabling technology which will permit the development of OCT based optical biopsy techniques for a wide range of diagnostic imaging applications in tissues such as the vascular system, the gastrointestinal tract, the urinary tract, and the respiratory tract.

7.5.1 Second Generation Catheter-Endoscope

A second generation OCT compatible catheter-endoscope has been designed and constructed for use in in vivo studies which require higher sample arm power. The second generation catheter removes the dominant source of returned internal reflections from the distal optics. In addition, this catheter-endoscope contains a saline irrigation port that will enable it to be used as a stand-alone imaging device for in vivo applications. A schematic of the second generation catheter-endoscope is shown in Figure 7-14.

To eliminate the internal reflections from the optical fiber-adhesive interface, the optical fiber was angle cleaved with an angle of 10 degrees. The angle cleaving prevents most of the internal reflections from the optical fiber-optical adhesive interface from being coupled back into
**Figure 7-13.** Three-dimensional reconstruction of a coronary artery lumen using the OCT compatible catheter-endoscope.

**Figure 7-14.** Schematic of the distal end of the second generation catheter.
the fiber. To eliminate the unwanted internal reflections from the adhesive-GRIN interface, the GRIN was angle polished at approximately 10 degrees, again preventing internal reflections from being coupled back into the optical fiber. An OCT axial profile of the internal reflections from within the catheter is shown in Figure 7-15. Comparison of this figure with Figure 7-9 shows that angle cleaving the optical fiber and angle polishing of the GRIN lens have reduced the backreflections significantly. In fact, the fiber-optical adhesive and the optical adhesive-GRIN interfaces are no longer the principal sources of backreflection from the distal optics. For the second generation catheter-endoscope, reflection from the prism-air interface dominates.

The second generation catheter-endoscope is designed to be filled with saline. Saline injection into the catheter will minimize unwanted reflections from the inside of the transparent sleeve-air interface. An irrigation port is provided at the tip of the transparent sleeve for simultaneous saline injections into the surrounding media. Saline injection into the surrounding media removes blood or other debris from the field of view. Moreover, the presence of saline in the surrounding media eliminates unwanted reflections from the transparent sleeve-air interface.
Finally, a guidewire can be passed through the tip of the catheter to aid in guiding the catheter to the area of interest without disruption of the lumen wall.

These modifications to the first prototype brings OCT compatible catheter-endoscope technology to a mature level that enables the \textit{in vivo} imaging of internal organ systems. As a result, the second generation catheter-endoscope was used as the imaging transducer for the first \textit{in vivo} endoscopic OCT imaging study (Chapter 12).
Chapter 8

LOW COHERENCE LIGHT SOURCES

8.1 Introduction

In order to perform in vivo OCT imaging, the scanning rate must be increased to avoid artifacts due to patient motion. One important technology necessary for producing a high speed OCT system is a high power, single mode, low coherence source. High source power is essential because the bandwidth of the OCT signal increases with scanning velocity[26]. Thus, the power incident on the sample must be increased commensurately with the increase in scanning speed to preserve the SNR.

Commercially available SLD’s coupled to single mode fibers are typically limited to output powers of approximately 1 mW. Mode locked solid state lasers, however, are capable of delivering hundreds of milliwatts of short coherence length light with a single transverse spatial mode, ideal for coupling to optical fiber. A Kerr lens mode locked titanium doped sapphire laser operating at 820 nm has already been demonstrated as a high resolution, high power source for OCT[81]. Although this laser source would be appropriate for fast scanning OCT, previous investigations have shown that imaging in human tissues with a source wavelength of 1300 nm enables significantly deeper image penetration than at 820 nm[21, 38]. To access this wavelength range, a solid-state Kerr lens mode locked Chromium doped Forsterite laser has been constructed[82].

While solid-state lasers, such as the titanium sapphire and the Chromium Forsterite lasers, produce high power, broad bandwidth, single mode light, they are difficult and expensive to construct. Moreover, these femtosecond lasers are too large to be integrated into a clinical
environment. For these reasons, research has been conducted to identify high power, low coherence light sources that are less expensive and have a smaller footprint. The most likely candidates are diode-pumped rare-earth-doped silica optical fibers (REDF). If the fiber geometry prevents backreflection and resultant lasing, these fibers can produce superluminescent light through amplified spontaneous emission (ASE). This chapter describes results obtained using both the solid state sources and several different rare-earth-doped fibers including Neodymium (Nd), Ytterbium (Yb), and Thulium (Tm). Analysis of OCT images using REDF's composed of different rare-earth ions has the additional advantage that it enables first-order determination of the performance of OCT imaging in tissue at different wavelengths.

8.2 Spectral Shaping

While the superluminescent diode does not have high enough power to enable in vivo OCT imaging, it does have a Gaussian spectral shape. Many of the high power optical sources described in this chapter require some form of spectral shaping, either self-phase modulation or spectral filtering to broaden the source bandwidth.

8.2.1 Self-phase Modulation

Self-phase modulation is based on a third order nonlinear effect known as the optical Kerr effect. The nonlinear polarization component at frequency, \( \omega \), produces a change in the susceptibility of the medium, \( \Delta \chi \), where,

\[
\Delta \chi = \frac{P_{NL}(\omega)}{\varepsilon_0 E(\omega)} = \frac{3\chi^{(3)}|E(\omega)|^2}{\varepsilon_0} = 6\chi^{(3)} \eta I, \tag{8-1}
\]

and

\[
I = \frac{|E(\omega)|^2}{2\eta}, \tag{8-2}
\]

is the intensity of the incident optical wave[27]. Since the refractive index

\[
n^2 = 1 + \chi, \tag{8-3}
\]
the incremental nonlinear refractive index,

$$\Delta n = \left( \frac{\partial n}{\partial \chi} \right) \Delta \chi = \frac{\Delta \chi}{2n},$$  \hspace{1cm} (8-4)

or

$$\Delta n = \frac{3n}{\varepsilon_0 n^3} \chi^{(3)} I = n_2 I,$$  \hspace{1cm} (8-5)

and can be defined as the product of a constant of proportionality, $n_2$, and the incident optical intensity[27]. Because of the optical Kerr effect, a pulse of light confined in an optical fiber is modulated in phase such that

$$\Delta \phi(t) = kn_2 l I(t),$$  \hspace{1cm} (8-6)

where $l$ is the length of the optical fiber. The frequency is then modulated by,

$$\Delta \omega(t) = \frac{\partial}{\partial t} \Delta \phi(t) = kn_2 l \frac{\partial}{\partial t} I(t).$$  \hspace{1cm} (8-7)

If the peak pulse intensity is large, a broadened spectrum results from the frequency modulation[83].

### 8.2.2 Spectral Filtering

In several of the superfluorescent sources described in this chapter, the spectrum was broadened using spectral filtering. As opposed to self-phase modulation, filtering shapes the spectrum by rejecting specified source wavelengths, resulting in a reduction of source power. Spectral filtering is necessary for some REDF sources because the spectrum narrows when the pump power increases. Typically, in-line filters, or filters that reside within the optical fiber itself, were used as opposed to a free-space interference or absorption filters. The implementation of in-line filters avoids coupling losses and spurious reflections within the REDF. Two types of in-line
filters have been employed in these REDF studies, long period Bragg gratings[84], and cascaded customized fiber optic wavelength division multiplexers (WDM).

### 8.3 Solid State Sources

#### 8.3.1 Titanium Sapphire

Recently, modelocked solid state lasers have been used as high power and high resolution sources for OCT. Kerr lens modelocking (KLM) in Ti:Al₂O₃ oscillators has been shown to produce high average power near-infrared pulses with durations <10 fs[85, 86]. A Ti:Al₂O₃ oscillator has been constructed and used to perform OCT imaging. This laser has a center wavelength of 800 nm, an output power of 400 mW, and a spectral width of 145 nm corresponding to a coherence length of 1.9 μm[81]. The resolution of this KLM source is an order of magnitude greater than commercially available superluminescent diodes.

![Figure 8-1. Schematic of KLM Ti:Al₂O₃ oscillator. The crystal pump source is the 514 nm line of an Argon laser.](image)

To maintain the high resolution of the Ti:Al₂O₃ source, dispersion imbalance between the interferometer arms must be precisely canceled[87]. To perform dispersion balancing, a fused silica prism pair with faces contacted and index matched to form a variable thickness window is inserted in the reference arm (Figure 8-2)[81]. The width of the autocorrelation function is minimized by translating the prisms along their contacted faces. This simple adjustment compensates for differences in fiber length, collimating lens and microscope objectives between the interfer-
ometer arms[81]. The Ti:Al₂O₃ laser has high amplitude noise relative to that of SLD sources. Thus, a dual balanced detection scheme is used to attain a shot noise limited signal to noise ratio (SNR) (Figure 8-2)[81].

![Diagram of OCT system](image)

**Figure 8-2.** Schematic of Ti:Al₂O₃ oscillator coupled into the OCT system.

OCT images of an onion performed with the standard resolution 1300 nm SLD source system used in the tissue surveys (Figure 2-1) and with the KLM Ti:Al₂O₃ system (Figure 8-2) demonstrate a marked improvement in resolution provided by the Ti:Al₂O₃ laser[81]. In both images, the transverse resolution is approximately matched to the axial resolution. In the image acquired using the Ti:Al₂O₃ laser (Figure 8-3) the confocal parameter is 40 μm, corresponding to a spot size of 5 μm. Resolution degradation due to beam divergence becomes apparent at greater depths. The confocal parameter for the 1300 nm SLD image in Figure 8-3 is 350 μm, corresponding to a spot size of approximately 17 μm. Both of these images have dimensions of 120 vertical and 360 horizontal pixels. The image acquisition time was 2.5 seconds[81].

The KLM Ti:Al₂O₃ laser coupled to an optimized OCT system enables high resolution, high power, imaging of biological structures. Additionally, the application of KLM to other solid state laser materials will provide high power, short coherence length sources at wavelengths with greater penetration in tissue such as 1.3 μm from Cr⁴⁺:Forsterite and 1.5 μm from Cr⁴⁺:YAG.
8.3.2 Chromium Doped Forsterite

For the spectral optical window in tissue, OCT image penetration depth is greater at longer wavelengths due to decreased tissue scattering. Thus, the construction of a solid state low coherence source with a center wavelength longer than that of Ti:Al$_2$O$_3$ would be more appropriate for OCT applications in tissue. Like Ti:Al$_2$O$_3$, Cr$^{4+}$:Forsterite is a phonon broadband tunable solid state laser material which can be used for the generation of femtosecond optical pulses. Application of Kerr-lens modelocking to a Nd:YAG pumped Cr$^{4+}$:Forsterite oscillator has been achieved with pulse durations as short as 25 fs[88, 89].

A Cr$^{4+}$:Forsterite laser has been constructed for use as a high power, high resolution OCT source by pumping a Cr$^{4+}$:Forsterite with 6.0 W of 1.06 mm light from a diode excited Nd:YAG laser (Figure 8-4)[82]. The KLM Cr$^{4+}$:Forsterite laser produces 300 mW of modelocked output power at 1280 nm[82]. In addition, while the Ti:Al$_2$O$_3$ laser has a high amplitude noise relative to
that of SLD sources, the Cr\textsuperscript{4+}:Forsterite amplitude fluctuations are much lower and comparable to that of the SLD. Therefore, a dual balanced detection scheme is not necessary to attain shot noise limited detection (Figure 8-4). The single transverse mode output from this oscillator is well-suited for coupling to standard single mode optical fiber with sufficient power to enable rapid acquisition of OCT images while preserving high signal to noise ratios. The spectrum emitted from this laser has a FWHM bandwidth of 50 nm which corresponds to a coherence length of 15 \textmu m[82].

![Diagram](image)

**Figure 8-4.** Schematic of the Cr\textsuperscript{4+}:Forsterite laser coupled into the OCT system.

For high resolution imaging applications, the high peak power of the pulses from this laser have been used to nonlinearly broaden the spectrum through self-phase modulation[82]. The modelocked output of the Cr\textsuperscript{4+}:Forsterite laser at 100 mW of average power coupled into a single mode Corning SMF/DS\textsuperscript{TM} CPC3 dispersion shifted fiber (zero group velocity dispersion at 1.55 \textmu m) is shown in Figure 8-5[82]. Figure 8-5 also displays the demodulated autocorrelation function corresponding to the spectrum[82]. The FWHM coherence length of light produced from this source measures 5.7 \textmu m[82]. Sidelobes in the autocorrelation function due to the rectangular shape of the spectrum are almost 20 dB lower than the maximum peak intensity.
**Figure 8-5.** Spectrum and autocorrelation function for the self-phase modulated Cr\textsuperscript{4+}:Forsterite laser[82].

A cross-sectional tomographic image of *in vitro* human adipose tissue generated with the Cr\textsuperscript{4+}:Forsterite self-phase modulated source is shown in Figure 8-6[82]. Again, the transverse resolution, determined by the spot size on the sample, was chosen to approximately match the axial resolution of the self-phase modulated laser source, 6 \( \mu \)m. The power incident on the sample was 2 mW, corresponding to a measured signal to noise ratio is 115 dB[82]. The image dimensions were 1.8 mm transverse (600 pixels) by 5 mm axial (200 pixels). The entire image was acquired in 30 seconds[82]. The high resolution of this system permits the visualization of tissue microstructure including cell membranes and intercellular spaces.

**Figure 8-6.** OCT image of *in vitro* human adipose tissue acquired using the self-phase modulated Cr\textsuperscript{4+}:Forsterite source[82]. Bar represents 100 \( \mu \)m.
The high peak power of the solid state KLM Cr\textsuperscript{4+}:Forsterite laser source allows self-phase modulation in a single mode optical fiber. This self-phase modulated source is capable of enabling high resolution, high speed optical coherence tomographic imaging. In addition, the 1.3 \(\mu\)m wavelength of this source is better suited for OCT imaging because it enables deeper imaging penetration due to the decreased scattering and absorption in tissue at this wavelength.

### 8.3.3 Chromium Doped YAG

Nd:YAG pumped Cr\textsuperscript{4+}:YAG KLM oscillators have recently been demonstrated\cite{90, 91}. These lasers can be modelocked over the range of 1.34-1.58 \(\mu\)m and have been shown to produce high intensity 70 fs pulses (37 nm bandwidth)\cite{90, 91}. Like the Cr\textsuperscript{4+}:Forsterite, high peak intensity pulses from the Cr\textsuperscript{4+}:YAG are capable of being spectrally broadened through self-phase modulation in optical fibers. Some degree of uncertainty exists in the utility of Cr\textsuperscript{4+}:YAG for OCT in tissue. While tissue scattering in the wavelength range, 1.34-1.58 \(\mu\)m, is low, this range coincides with the water absorption peak at 1.4 \(\mu\)m (Figure 2-11). The increased tissue absorption at these wavelengths may decrease the imaging penetration of OCT systems using the KLM Cr\textsuperscript{4+}:YAG oscillator.

### 8.4 Doped Fiber Sources

Solid state lasers are excellent sources of low coherence light due to their high average power and potential for high resolution. However, these sources are difficult and expensive to construct and are too large to be incorporated into a clinical environment. The lack of a high power, clinically viable low coherence source has motivated the investigation of diode-pumped rare-earth-doped single mode fibers (REDF). These single mode, broad bandwidth superfluorescent light sources are compact, relatively simple, and inexpensive. Besides evaluating the capability of the REDF's as a high resolution, high power source for OCT, research using REDF's at different wavelengths allows the determination of appropriate wavelength for OCT imaging in tissue.

#### 8.4.1 Amplified Spontaneous Emission (ASE)

If the excitation of the REDF is strong, the fluorescent light emitted from the fibers is amplified by spontaneous emission. The pump energy is primarily stored as a population inversion which in turn amplifies the guided fluorescence\cite{92}. Because the fluorescence is amplified by the population inversion, the fluorescence spectrum narrows. In addition, if any backreflec-
tions are present in the fiber, either from a backscattering site within the device or from Rayleigh scattering from the fiber, lasing will commence and further reduce the spectral width[92]. For these reasons, many REDF's require spectral filtering because significant spectral narrowing can occur before the ASE power reaches a useful level.

8.4.2 Superfluorescent Source Design

SFS Configurations

Figure 8-6 depicts several different fiber configurations used for creating REDF superfluorescent sources[93]. In order to suppress lasing, feedback from either one or both fiber ends can be eliminated by angle cleaving or polishing. The forward superfluorescent signal (SFS) configuration is the simplest to implement, but reduces the useful superfluorescent power by 50% (Figure 8-6a). In the single-pass forward SFS case, both fiber ends are cleaved and the superfluorescence is detected from the fiber face opposite the pump. Many of the REDF sources presented in this chapter use the single-pass forward SFS configuration due to its simplicity and complete elimination of feedback from both fiber ends. Figures 8-6 b,c depict double pass configurations. Double pass SFS geometries enable use of both forward and backward propagating superfluorescence.

Cladding Geometries

One problem with REDF superfluorescent sources is the need for a high power (~500 mW) single mode pump for optimal coupling into the doped single mode core. While some compact semiconductor diode sources produce a single mode output with these powers, they are typically very expensive. Because of the need for an inexpensive and compact pump, a cladding pumped geometry has been adopted to enable coupling into the fibers with less expensive multimode diode arrays (Figure 8-8). The cladding pumped REDF consists of an asymmetric cladding surrounding the doped core[93]. The shape of the inner cladding can be tailored to match the geometry of the pump diode array. For a given dopant density, the length of the doped fiber must be much longer than the single mode doped fiber, because the absorption of the pump light per unit length is much lower than for standard single mode REDF's. For four level systems, such as Nd:silica and Yb:silica, which exhibit little signal absorption, the increased fiber length does not pose a problem. For three level systems or quasi-four level systems, however, such as Er:silica and Tm:silica, the increased length of the fiber causes increased absorption of the super-
fluorescence[93]. For this reason, rare earth ions with three level or quasi-four level transitions, cannot be used for the cladding pumped geometry.

8.4.3 Neodymium (Nd)

Nd:silica, excited at 800 nm, has a strong four level transition at 1060 nm, and is therefore well-suited as a dopant for the cladding pumped fiber geometry. Initial experiments were performed using a double clad fiber to evaluate the suitability of this source for OCT. Because optical scattering in tissue at 1060 nm is significantly higher than the scattering at 1300 nm, one
would expect the penetration to be less. However, the absorption at 1060 is lower than the absorption at 1300 nm (Figure 2-11). Possibly, the change in attenuation due to increased scattering and decreased absorption cancel and OCT imaging at 1060 nm may have an acceptable penetration depth.

**Single Mode Nd:silica**

Figure 8-8 depicts the fluorescence spectrum of a 10 m single mode Nd:silica REDF pumped at 800 nm with 100 mW Ti:Al₂O₃ power. The Nd:silica REDF was obtained from J. Minelli, University of Southampton, UK. While the shape of the fluorescence spectrum seems reasonable for OCT imaging without significant sidelobes in the autocorrelation function, the total integrated power emitted was only 16 μW (Figure 8-9). At higher pump powers, ASE occurs. The Nd:silica REDF pumped with 600 mW produces 3.5 mW of SFS, but the spectrum contains significant gain narrowing at the dominant emission line, 1060 nm (Figure 8-10). The width of the dominant peak is now only 8 nm, corresponding to a coherence length of 62 μm. If this peak narrowing can be suppressed, the wings of the SFS can be amplified and the REDF spectrum can be broadened. The next section describes the use of an in-line long period Bragg grating to broaden the Nd:silica REDF SFS spectrum.
Figure 8-9. Spectrum of single mode Nd:silica REDF pumped with 100 mW. The integrated emitted power was 16 µW.

Figure 8-10. Gain narrowed superfluorescent spectrum for a double clad Nd:silica fiber pumped with 600 mW of 800 nm power. The total integrated power is 3.5 mW.
Bragg Grating Filtered, Cladding Pumped Nd:silica REDF

Because of gain narrowing in Nd:silica at high pump power, a Bragg grating filtered, cladding pumped Nd:silica REDF has been developed[94]. The Bragg grating filtered Nd:silica REDF was obtained from E.A. Swanson, Lincoln Laboratory, MA. A schematic of the system is shown in Figure 8-11. This system uses 3.7 m of cladding pumped Nd:silica fiber in a double

passed backward SFS configuration (Figure 8-11). To eliminate the spectral gain narrowing at 1060 nm, an in-line optical notch filter is spliced between the REDF and the mirror. The notch filter is a long period Bragg grating that is etched in a single mode optical fiber (Figure 8-12). The Bragg grating flattens the spectrum by diminishing the peak narrowing at 1060 nm while allow-

\[ \text{Figure 8-11. Schematic of the Nd:silica REDF superfluorescent source with the long period Bragg fiber grating[94].} \]

\[ \text{Figure 8-12. Long period Bragg grating notch filter transmission spectrum[94].} \]
ing ASE to pass unfiltered outside of the bandwidth of the notch filter (Figure 8-14). The output

**Figure 8-13.** Double clad SFS spectrum of Nd:silica REDF without notch filter (left) and with notch filter (right) [94].

power from the filtered REDF is 7 mW, with a multimode diode array pump power of 200 mW at 810 nm. The bandwidth of the filtered spectra is 39 nm, corresponding to a measured FWHM coherence length of 16 μm.

The notch filtered Nd:silica REDF effectively broadens the SFS spectrum to provide a FWHM coherence length that is comparable to the 1300 nm SLD coherence length. However, the filtered Nd:silica REDF spectral shape deviates significantly from Gaussian. As discussed in Section 2.3.4, the rectangular shape of the filtered Nd:silica REDF will cause sidelobes of non-negligible magnitude to appear in the autocorrelation function. The autocorrelation functions for both a 1300 nm SLD and the double clad, filtered, Nd:silica filtered REDF are shown in Figure 8-14. The sidelobes in the REDF autocorrelation function are only 15 dB down from the main peak.

After measurement of the spectra and autocorrelation functions, the filtered, double clad Nd:silica source was coupled into an OCT system. Images of a calcified aortic plaque were compared to images of the same plaque taken with the 1300 nm SLD source. The source powers were adjusted so that the SNR of the OCT system was 105 dB for both sources. The images are shown in Figure 8-15. In the REDF image, the sidelobes in the autocorrelation function cause a blurred air-tissue interface at the top of the image. Because of the high reflectivity at this interface, the
**Figure 8-14.** Autocorrelation functions for the 1300 nm SLD and the filtered 1060 REDF. The top curve in both images represents a magnification of the displacement axis by 100× [94].

**Figure 8-15.** OCT images of a calcified aortic atherosclerotic plaque taken with the 1060 filtered REDF and the SLD. Bar represents 500 μm.

The magnitude of the sidelobes in the autocorrelation function are comparable to the tissue signal levels. This artifact can be perceived in all OCT images that have been acquired with a non-Gaus-
sian source. In addition, the internal structure of the plaque, such as highly calcified foci, also appear blurred compared to the same features in the SLD image. Again, this artifact is due to sidelobes caused by the rectangular shape of the filtered REDF spectrum. Finally, in this sample, the penetration is approximately equal for the two wavelengths. Possibly, the increase in scattering at 1060 nm may be offset by the decrease in water absorption at this wavelength. This result may indicate that the OCT imaging penetration depth at 1060 nm may be similar to the penetration depth at 1300 nm. Based on these results, a second generation source should be constructed with a spectral filter that not only decreases line narrowing, but also shapes the spectrum to be more Gaussian.

### 8.4.4 Praesodymium (Pr)

Praesodymium doped silica fiber has a gain narrowed spectrum that is significantly broader than the gain narrowed spectrum of Nd:silica REDF. Recently, a 590 nm pumped Pr:silica REDF ASE source has been reported to have a broad Gaussian spectrum with a FWHM of 25 nm[95]. With a pump power of 250 mW, the Pr:silica REDF produces 60 mW SFS at 1049 nm[95]. The primary problem with the Pr:silica REDF is that there are no inexpensive high power single mode diodes at 590 nm. Instead, Shi et. al. used a dye laser to pump the Pr:silica REDF[95]. Until an inexpensive diode source at 590 nm is available, the Pr:silica REDF is unlikely to become a clinically viable source for OCT.

### 8.4.5 Ytterbium (Yb)

Ytterbium (Yb) doped silica has a broad four state transition ranging from 1000-1200 nm, with an absorption spectrum ranging from 850-1000 nm. Typically, Yb:silica is pumped at 980 nm because low cost high power diodes are available at this wavelength. However, the gain narrowed bandwidth for a cladding pumped Yb:silica REDF in the single-passed SFS configuration is approximately 15 nm which corresponds to a coherence length of only 35 μm(Figure 8-16). The cladding pumped Yb:silica REDF was obtained from M. Meundel, Polaroid Corporation, MA. Like the Nd:silica REDF, a higher coherence length is desirable, and spectral notch filtering must be used to decrease peak narrowing at 1100 nm while allowing ASE to pass unfiltered outside of the bandwidth of the notch filter.
WDM Filtered Yb:silica REDF Source

As opposed to the Bragg grating filtered Nd:silica REDF, a cladding pumped Yb:silica REDF ASE source has been constructed that uses a cascade of custom wavelength division multiplexers (WDM) to perform the notch filtering[97]. The WDM filtered Yb:silica REDF was obtained from S. Chernikov, Imperial College, UK. A schematic of the WDM filtered Yb:silica source is shown in Figure 8-17. The ASE source consists of two lengths of Yb:silica fiber pumped using 3 separate 980 nm diodes in a double-passed SFS configuration.

The spectrum of the WDM filtered Yb:silica REDF source is given in Figure 8-18. The spectrum is very rectangular, which should lead to severe artifacts in the autocorrelation func-
Figure 8-18. Spectrum of the WDM filtered Yb:silica REDF source.

tion. The autocorrelation function for this WDM filtered Yb:silica REDF source is shown in Figure 8-19. The 75 nm bandwidth centered at 1075 nm, produced by the Yb:silica REDF source

Figure 8-19. Autocorrelation function of the WDM filtered Yb:silica REDF ASE source. The FWHM bandwidth is 13 μm.
provides a FWHM coherence length of 13 μm with an output SFS power of 10 mW. Like the Bragg grating filtered Nd:silica REDF, the WDM filtered Yb:silica source contains sidelobes due to the rectangular shape of the spectrum. These sidelobes are similar in magnitude to those of the Bragg grating filtered Nd:silica REDF.

Images of an in vitro human breast carcinoma were acquired with the WDM filtered Yb:silica REDF ASE source (Figure 8-20). While some tissue features can be identified, such as adipose cells near the surface of the image, sidelobes present in the autocorrelation function cause blurring of the surface and the internal structure. Because of these severe artifacts, a second generation source must be designed that uses WDM filters to not only reduce gain peaking, but also shape the spectrum to be more Gaussian.

8.4.6 Erbium (Er)

A very common single mode doped fiber used in telecommunications applications is the Er:silica REDF. The erbium fiber is pumped at 980 nm and has a three state transition with a peak emission at 1550 nm with a bandwidth of approximately 50 nm[93]. The Er:silica REDF exhibits gain narrowing, so spectrally filtering would be necessary to produce a sufficient coherence length. Since the Er:silica emission is a three level transition, the cladding pumped geometry cannot be used due to absorption of the signal[93]. In addition, while scattering is lower at 1550 than at any of the previous wavelengths, the erbium emission band overlaps with the high water absorption peak at 1500 nm (Figure 2-11). For this reason, it is expected that OCT imaging with
Er:silica REDF ASE would have a lower penetration depth than imaging with other sources discussed in this chapter.

8.4.7 Thulium (Tm)

An intriguing emission wavelength range exists after the water absorption peak, at wavelengths greater than 1500 nm. Between 1600 and 1800 nm, water absorption decreases, and increases at 2.0 μm. Because of the inverse dependence of scattering on wavelength, the attenuation in tissue due to scattering in this wavelength range is low. Thus, sources between 1650 and 1800 nm could give rise to OCT imaging penetration depths equal to or greater than the penetration at 1300 nm.

Thulium doped silica is a REDF with a quasi-four level transition at 1800 nm[98]. The maximum absorption peak is very narrow and is located at 785 nm. Figure 8-21 shows a plot of a single mode Tm:silica REDF emission spectra pumped at 785 nm in the single passed SFS configuration[98]. The Tm:silica REDF was obtained from L. Nelson and E.P. Ippen, Massachusetts Institute of Technology, MA. The single mode doped fiber was 2 m long and was pumped with

![Figure 8-21. Superfluorescent spectrum of Tm:silica REDF[98].](image)

500 mW of Ti:Al₂O₃ power at 785 nm[98]. The SFS bandwidth is 80 nm which gives rise to a
FWHM coherence length of 18 μm. The gain narrowed spectrum is asymmetric, but more Gaussian than the spectrally filtered ASE sources. The Tm:silica REDF total integrated SFS output power was 4 mW. Figure 8-22 shows a comparison between an image of a calcified aortic plaque adjacent to a 1300 nm SLD image of the same plaque. Both OCT images were taken with the same SNR, 102 dB.

**Figure 8-22.** OCT images of a calcified aortic atherosclerotic plaque taken with the 1800 Tm:silica REDF source and the 1300 nm SLD. Bar represents 500 μm.

As can be seen in these images, the Tm:silica REDF shows sharp delineation of boundaries in the image, such as the calcified foci within the plaque. In addition, no blurring due to autocorrelation sidelobes is seen at the surface. Finally, the penetration depth in this particular
image of the heavily calcified aortic plaque seems to be at least equal to the penetration depth of the 1300 nm SLD image.

One major disadvantage to Tm:silica is that it cannot be used in a cladding pumped configuration because it is a quasi-four level system[98]. As a result, the ground state absorption of the SFS signal extinguishes the emission over a long length of fiber. Single mode pumps at 785 nm are commercially available, but expensive, and for this reason Tm:silica REDF ASE sources may not be as desirable as other less-expensive, high power REDF ASE sources.

8.5 Discussion

Table 8-1 has been provided in order to summarize the source development described in this chapter.

<table>
<thead>
<tr>
<th>Source</th>
<th>Pump $\lambda$ (nm)</th>
<th>Pump Power (W)</th>
<th>Emission $\lambda$ (nm)</th>
<th>Emission $\Delta\lambda$ (nm) (Coherence Length)</th>
<th>Emission Power (mW)</th>
<th>Sidelobes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti:Al$_2$O$_3$</td>
<td>514</td>
<td>6.0</td>
<td>800</td>
<td>145 (1.9 $\mu$m)</td>
<td>400</td>
<td>-</td>
</tr>
<tr>
<td>Cr$^{4+}$:Forsterite (self-phase mod.)</td>
<td>1064</td>
<td>6.0</td>
<td>1280</td>
<td>200 (5 $\mu$m)</td>
<td>300</td>
<td>+</td>
</tr>
<tr>
<td>Nd:silica (Bragg filtered)</td>
<td>810</td>
<td>0.2 (cladding pumped)</td>
<td>1060</td>
<td>39 (16 $\mu$m)</td>
<td>7</td>
<td>+++</td>
</tr>
<tr>
<td>Yb:silica (WDM filtered)</td>
<td>980</td>
<td>0.3 (cladding pumped)</td>
<td>1075</td>
<td>75 (13 $\mu$m)</td>
<td>10</td>
<td>+++</td>
</tr>
<tr>
<td>Tm:silica</td>
<td>785</td>
<td>1.0 (single mode)</td>
<td>1800</td>
<td>80 (18 $\mu$m)</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

**TABLE 8-1.** Low coherence source summary.

Solid state lasers provide high power, broad spectrum low coherence light that enable both high speed and high resolution OCT. In their current state of development, they are valuable tools for performing initial *in vivo* OCT studies. However, the cost and size of these devices make their use impractical outside of a research setting. Development performed to miniaturize
KLM solid state lasers would greatly increase the likelihood that these sources could be integrated into a clinical setting.

The state of the art REDF ASE sources also require improvement. Both the filtered Nd:silica and the Yb:silica REDF's suffer from sidelobes due to a rectangularly shaped spectrum. Distortions in the OCT images caused by autocorrelation sidelobes should be improved in second generation ASE devices that not only diminish gain peaking, but shape the spectrum to avoid sidelobes. Pr:silica and Tm:silica REDF sources have broad enough gain narrowed bandwidths to be used without filtering, and their spectra is much closer to being Gaussian. However, no 590 nm semiconductor diode is available to pump the Pr:silica ASE source. 785 nm diodes are available to pump the Tm:silica source, but because the emission at 1800 for Tm:silica is quasi-four level, cladding pumped geometries cannot be used. While the shape of the Tm:silica REDF ASE spectrum and the optical properties of tissue at 1800 nm make the Tm:silica ASE source the most desirable of all of the REDF's, the cost of a single mode pump may prohibit its widespread use. Most likely, the next generation clinically viable REDF source will either be a spectrally shaped Nd:silica or Yb:silica REDF with shaping that not only accounts for the spectral narrowing, but also makes the spectrum more Gaussian.
Chapter 9

PIEZOELECTRIC OPTICAL FIBER STRETCHING FOR HIGH SPEED OCT

9.1 Introduction

The ability to obtain non-invasive optical biopsies in living patients could significantly impact the diagnosis and treatment of disease. Since recently constructed OCT systems cannot perform imaging in vivo, due to artifacts caused by the motion of the patient, high speed OCT imaging systems must be devised. Because of the trade-off between scanning speed and source power, the development of a high power, low coherence source is one important advance that makes the construction of a high speed OCT imaging system possible. The other essential technology is a rapidly scanning optical path length delay line capable of enabling axial scan rates one or two orders of magnitude greater than provided by OCT systems used for in vitro studies.

Previous OCT systems have utilized a mechanically translated reference arm mirror to perform axial scanning[19, 21, 33, 38]. The fastest mechanical scanning systems to date use a galvanometer with a lever arm attached to the axis of rotation (Figure 9-1). A retroreflecting mirror is mounted on the distal end of the lever arm, allowing small angles of rotation to be converted to linear displacement of the retroreflector. The mirror velocity for these systems is typically in the range of 30 mm/s which corresponds to 10 axial scans per second. This velocity is limited by the force needed to rotate the lever arm-retroreflector pair and mechanical resonances that occur when the scanning frequency is raised. At this mirror velocity, the scanning optical path length delay produces a Doppler frequency shift in the reference arm of 46 kHz at 1300 nm.
Figure 9-1. Schematic of mechanical optical delay scanner using a galvanometer, a lever arm, and a retroreflector (top view).

For an image with 250 axial scans, to enable image acquisition rates of four frames per second, the reference mirror scanning velocity must be increased by approximately two orders of magnitude to 3 m/s. Moreover, this velocity must be held constant for distances greater than a few mm and repetition rates of approximately 500 Hz.

Because no cost effective mechanical translator meeting these specifications is currently available, an alternative technology based on the use of piezoelectric transducers to induce stretch in an optical fiber has been implemented. A long length of single mode fiber is wound around several piezoelectric transducers (PZT) fixed to a cylindrical housing. The composite device is termed a piezoelectric modulator (PZM). As a voltage is applied to the piezoelectric transducers, the fiber expands and creates an optical path length delay in the guided light. In this chapter, a new high speed OCT system that incorporates a PZM to induce stretch in a single mode optical fiber is presented. This OCT system achieves an acquisition rate of 1200 axial scans per second. The capabilities of the rapid scanning system are demonstrated by the motion artifact free, in vivo imaging of a Xenopus laevis (African frog) heart and in vivo human skin.

9.2 Piezoelectric Transducers

Piezoelectric transducers are crystalline materials that produce an electric field when a force is applied to the material. This phenomenon is known as the piezoelectric effect. The con-
Figure 9-2. Schematic of the fiber wound PZM used to create a rapidly scanning optical delay line.

verse situation also occurs. When electrical charge is applied the materials expand by $\Delta d$ according to,

$$\Delta d = d_{33}V,$$

(9-1)

where $d_{33}$ is the piezoelectric strain coefficient tensor along the axis of the applied voltage, $V$[99].

The physics behind the force generated by these materials is related to a missing axis of symmetry in the crystal[99]. Upon application of an external charge across the ceramic, the centers of the crystal elements can separate causing force to be generated between the crystals and expansion of the bulk material[99]. The most commonly used piezoelectric materials are polycrystalline ceramics which provide a maximal expansion for a given external applied voltage[99]. These ceramics are prepared by cooling the piezoelectric material to well below the Curie temperature and polarizing the material by applying a strong electrical filed along one
axis[99]. Once this process, known as poling, is complete, the piezoelectric effect can occur in the material[99].

Both high voltage PZT’s, also known as hard PZT’s, and low voltage piezoelectric transducers, sometimes referred to as soft PZT’s, are commercially available[99]. Low voltage piezoelectric transducers are generally more widely used because the electrical drive units (100 - 200 V) are easier to design[100]. Most low voltage piezoelectric transducers are constructed as stack assemblies. As can be seen from Eq. (9-1), the magnitude of the expansion of the material is not dependent on the geometry of the crystal. If a stack of piezoelectric transducers is constructed, the expansion for a given voltage is increased by the number of transducers in the stack. One important disadvantage to low voltage piezoelectric stacks is overheating due to friction between the individual piezoelectric elements[100]. Because of heat generation, large voltage drives at high frequencies can destroy the transducer[99].

9.2.1 Properties

Resonance Frequency

The propagation of the mechanical wave at the driven frequency can couple into the natural mechanical resonance frequencies of the device. The resonance frequency of the PZT is a function of both the elastic properties and mass of the device. For a stacked piezoelectric transducer, the resonance frequency, $f_r$, given a mass, $m$, and a translator stiffness, $c_T$ (N/m) is[99]

$$f_r = \frac{1}{2\pi} \sqrt{\frac{c_T}{m}}.$$  \hspace{1cm} (9-2)

Coupling into the resonance frequency causes sinusoidal oscillations which distort the mechanical expansion. Typically, for sinusoidal drive waveforms, the maximum drive frequency should always be 60% below $f_r$, [100]. Thus, for high frequency applications, the use of PZT’s with high resonance frequencies is necessary.
Power Requirements

The power necessary to drive piezoelectric trasnducers is primarily dependent on the capacitive impedance of the crystal. For a sinusoidal drive of frequency, \( f \), the power required to drive the piezoelectric transducer with a capacitance, \( C \), is[100]

\[
P = \frac{V^2}{Z} = 2\pi f CV^2.
\] (9-3)

For the four PZT’s used in the PZM for the scanning optical delay line, the total capacitance, \( C = 5.6 \mu F \). The power necessary to provide a 7.5 \( \mu m \) expansion with a 600 Hz sinusoidal drive voltage with an amplitude of 60 \( V_{pp} \) is about 76 \( W \). A triangle voltage waveform requires more power since the triangle wave consists of odd higher order harmonics,

\[
P_{tri} = \sum_{n, \text{odd}} 2\pi f C \left( \frac{V}{n} \right)^2.
\] (9-4)

Using the same parameters as above, the power necessary to drive the triangle waveform is greater than 400 \( W \). These power requirements are not unreasonable for a research setting, but may not be acceptable in a clinical environment.

Hysteresis

In reality, the mechanical expansion of the piezoelectric material is not proportional to the strength of the applied electric field. The crystalline structure of piezoelectric ceramics contain microscopic domains or subunits[101]. When the electric field is applied to the bulk crystal, the mechanical movement of the domains may be dampened by resistance between the domain walls[101]. The resistance provided by the domains causes hysteresis, or a condition where the polarization state reflects the previous states of polarization. A typical hysteresis loop obtained by driving the fiber wound PZM with a triangle waveform is given in Figure 9-3.
**Figure 9-3.** Measured hysteresis curve for the fiber wound PZM driven with a triangle voltage waveform.

**Thermal Properties**

The temperature of the piezoelectric transducer is proportional to the power dissipation in the crystal,

\[ P_d = \frac{V^2}{Z} = 2\pi fCV^2\tan(\delta), \]

(9-5)

where \( \delta \) is a constant known as the loss angle of the material[99]. A typical value of \( \delta \) for low voltage PZT stacks is 0.04[99]. Figure 9-4 shows a plot of the heat generated in the PZT’s used in this chapter as a function of frequency for a 150 V\textsubscript{pp} sinusoidal waveform. A triangle waveform will increase the generated heat by a factor of 5.33. PZT breakdown occurs at approximately 80° C[99]. When an oscillating voltage waveform is applied to the PZT, the PZT expands from its resting mechanical operating point due to a slow increase in temperature. This drift will stabilize at a mechanical equilibrium point after approximately 1 hour of continuous operation. An increase in temperature also decreases the piezoelectric effect. This temperature dependent decrease in mechanical expansion is approximately 0.2% per °C.
9.3 Birefringence in Wound Optical Fibers

9.3.1 Bending Induced Birefringence

Birefringence is induced in the reference arm when a long length of fiber is wrapped around the cylindrical housing of the PZM (Figure 9-2). The birefringence in the wound single mode fiber imposes a phase delay between the two polarization eigenmodes in the fiber. As discussed in Chapter 2, the polarization states of each arm can be matched by use of a polarization controller in the reference arm (Figure 2-1). The polarization controller consists of three discs that tightly hold loops of the optical fiber. If the radius of the loop is chosen properly, then the loop can become a fiber optic quarter or half wave retarder. Adjusting the angles of the loops with respect to one another changes the output polarization of the light emitted from the fiber, allowing complete control of the transmitted polarization state[103].

9.3.2 Polarization Mode Dispersion

While the polarization controllers can adjust the polarization state of the wound fiber in the reference arm, they do not compensate polarization mode dispersion. Polarization mode dispersion occurs in wound optical fiber when broad bandwidth light is used. If the input source is unpolarized, two separate wavelength dependent group refractive indices exist in the fiber, analogous to the slow and fast axes of an anisotropic medium[104]. Since the group delay for the two
eigenmodes are different, the eigenmodes that propagate through the wound fiber are delayed with respect to each other. This phenomenon is known as polarization mode dispersion. Polarization mode dispersion increases with the length of fiber and as a result is a very significant problem in the fiber wound PZM[104].

9.3.3 Effects of Birefringence Mismatch on OCT Operation

The fiber wrapped around the PZM acts as a linear retarder. The retardation of the optical fiber winding, $\psi(\omega)$, is wavelength dependent and related to the group delays of the fast and slow axes of the birefringent medium, $\tau_f$ and $\tau_s$ [104],

$$\psi(\omega) \equiv 2\omega(\tau_f - \tau_s).$$ (9-6)

When one winding is placed in the reference arm, and another matching length of fiber is placed in the sample arm, the two eigenmodes of the reference arm and the sample arm give rise to four combinations of group delay mismatch. If the source is unpolarized, four separate autocorrelation functions can be formed, located at $2\Delta \tau$, where $\Delta \tau$ is the difference in group delay between each combination of interfering eigenmodes[104]. The presence of multiple autocorrelation functions can be severe when a long length of fiber is used to wrap the PZM and must be eliminated with some form of polarization mode dispersion compensation.

9.3.4 Birefringence Mismatch Compensation

Faraday Rotators

One approach to reducing the polarization mode dispersion is the placement of Faraday rotators after the wound fiber. Faraday rotators are composed of materials that rotate the polarization of light when placed in a static magnetic field. The angle of rotation for a crystal of length, $l$, is

$$\theta = VBL,$$ (9-7)
where $V$ is the Verdet constant and $B$ is the strength of the magnetic field[27]. An analytical expression for the Verdet constant is,

$$V = \frac{\pi \gamma}{\lambda n'}$$  \hspace{0.5cm} (9-8)

where $\gamma$ is a property of the material known as the magnetogyration constant[27]. It is important to note that the Verdet constant is a function of wavelength. Therefore, a Faraday rotator is specified at one angle for one wavelength only.

A unique property of Faraday rotators is the capability to rotate the polarization of light in a non-reciprocal manner[105]. In other words, a Faraday rotator rotates the input polarization by the same angle for both forward and backward propagating light. Thus, a double passed configuration rotates the polarization by $2\theta$ (Figure 9-5).

![Figure 9-5. Use of the Faraday rotator in a double passed configuration.](image)

Operation of the Faraday rotator can be conveniently described by use of Jones matrices, $\mathbf{J}$ [105],

$$\mathbf{P}_t = \mathbf{J}\mathbf{P}_i,$$  \hspace{0.5cm} (9-9)
where $P_t$ and $P_i$ are the Jones column vectors describing the transmitted and incident polarization states, respectively. The Jones matrix for the Faraday rotator is defined as

$$
\mathcal{J}_F(\theta) = \begin{bmatrix}
\cos(\theta) & -\sin(\theta) \\
\sin(\theta) & \cos(\theta)
\end{bmatrix},
$$

(9-10)

which is equivalent to the Jones matrix for a linear retarder with a coordinate axis inversion[105]. When light is reflected from the reference mirror (Figure 9-5) it propagates in the reverse direction. The Jones matrix for backward propagating light for the Faraday rotator becomes its transpose, $\mathcal{J}_F'(\theta) = \mathcal{J}_F(\theta)^T$. In order to conjugate the birefringence induced by the wound fiber, a total double passed polarization rotation of $\pi/2$, or $\theta = \pi/4$ is chosen.

The Jones matrix for a retroreflecting mirror is simply a matrix that performs a coordinate transformation on the x axis, or[106],

$$
\mathcal{J}_R = \begin{bmatrix}
-1 & 0 \\
0 & 1
\end{bmatrix}.
$$

(9-11)

In the double passed configuration, the Jones matrix for the Faraday rotator with a polarization rotation angle of $\pi/4$ reflecting off of a mirror, and propagating through the Faraday rotator in the reverse direction is simply the product of the three Jones matrices,

$$
\mathcal{J}_F \mathcal{J}_R \mathcal{J}_F' = \begin{bmatrix}
1 & 1 \\
\sqrt{2} & -\sqrt{2}
\end{bmatrix} \begin{bmatrix}
-1 & 0 \\
0 & 1
\end{bmatrix} \begin{bmatrix}
1 & 1 \\
\sqrt{2} & \sqrt{2}
\end{bmatrix} = \begin{bmatrix}
0 & -1 \\
-1 & 0
\end{bmatrix},
$$

(9-12)

which will invert the coordinate axes of the incident polarization vector[106].
The fiber wound PZM can be expressed as a generalized elliptical retarder with a Jones matrix,

\[
\mathbf{J}_w = \frac{1}{\sqrt{(|a|^2 + |b|^2)}} \begin{bmatrix} a & -b^* \\ b & a^* \end{bmatrix}.
\]  

(9-13)

The propagation of light in the reverse direction for the fiber wound piezoelectric cylinder is,

\[
\begin{bmatrix} -1 & 0 \\ 0 & 1 \end{bmatrix} \mathbf{J}_w^{T} \begin{bmatrix} -1 & 0 \\ 0 & 1 \end{bmatrix} = \frac{1}{\sqrt{(|a|^2 + |b|^2)}} \begin{bmatrix} a & -b \\ b^* & a^* \end{bmatrix},
\]  

(9-14)

where \( a \) and \( b \) are arbitrary complex numbers determined by the bend radius and number of turns in the PZM[107].

The polarization state returned to the beam splitter thought the entire system is then defined by the cascade of Jones matrices,

\[
\frac{1}{\sqrt{(|a|^2 + |b|^2)}} \begin{bmatrix} a & -b \\ b^* & a^* \end{bmatrix} \begin{bmatrix} 0 & -1 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} a & -b^* \\ b^* & a^* \end{bmatrix} = \begin{bmatrix} 0 & -1 \\ -1 & 0 \end{bmatrix},
\]  

(9-15)

which is independent of the polarization induced by the long length of wrapped fiber[107].

Unfortunately, for broad bandwidth light, the Faraday rotator does not completely cancel the polarization due to the wavelength dependence of the Verdet constant. In this case, the Jones matrix for the double passed Faraday rotator, mirror combination is

\[
\mathbf{J}_F \mathbf{J}_R \mathbf{J}_F' = \begin{bmatrix} \sin^2(\theta(\lambda)) - \cos^2(\theta(\lambda)) & -2\cos(\theta(\lambda))\sin(\theta(\lambda)) \\ -2\cos(\theta(\lambda))\sin(\theta(\lambda)) & \cos^2(\theta(\lambda)) - \sin^2(\theta(\lambda)) \end{bmatrix},
\]  

(9-16)
where

\[ \theta(\lambda) \equiv \frac{B\pi y l}{\lambda n}. \] (9-17)

Since the angle of rotation for the Faraday rotator is only \( \pi / 4 \) for one wavelength, the polarization mode dispersion is not fully corrected.

The first experiment performed to analyze the polarization mode dispersion cancellation used a fiber wound PZM in the reference arm and an arbitrarily wound matching length of fiber in the sample arm. Faraday rotators were placed after the long lengths of fiber in each arm. In this case, the Faraday rotation was sufficient to eliminate polarization mode dispersion autocorrelation splitting, but the fringe contrast ratio was only 50% and the coherence length was broadened to twice the predicted value.

**Sample Arm Winding**

In order to obtain high interference fringe contrast and the minimum obtainable coherence length, polarization mode dispersion does not need to be eliminated. Instead, the polarization mode dispersion must be closely matched between the reference and sample arms. Thus, the equivalent elliptical retarder formed by the windings in both the reference and sample arms should be designed so that they match as closely as possible[104]. If the complex numbers, \( a \) and \( b \), that comprise the Jones matrix for the elliptical retardation induced by the windings, are matched in both arms, then the polarization mode dispersion mismatch can be corrected. If the retardation in both windings do not closely match, then the wavelength dependence of the Faraday rotator prevents complete compensation of the polarization mode dispersion.

To better match the retardation of the windings in the two arms, a wound PZM was placed in the sample arm. The sample arm winding contained the same number of turns and bend radius as the winding in the reference arm. Faraday rotators were again placed after the windings in both arms. These simple steps enabled full compensation of the polarization mode dispersion resulting in both 100% fringe contrast and minimal autocorrelation function broadening.
9.4 The Piezoelectric Optical Fiber Stretcher

9.4.1 Overview

The PZM rapid scanning optical delay line is depicted in Figure 9-2. The PZM consists of a long, 40 m length of single mode optical fiber wrapped under constant tension around a cylinder supported by four PZT stacks (Figure 9-2). Approximately 300 turns were used. Each piezoelectric transducer was capable of producing 10 μm of translation with 60 V applied across the stacks. The capacitance of each PZT was 1.8 μF and the resonance frequency of the PZT’s were 68 kHz. The fiber was stretched as the PZT’s expanded, inducing a temporal delay on the light propagating within it. Multiple wraps of the long length of fiber allowed the small expansion of the PZT’s to be magnified to a total optical path length delay of approximately 3 mm.

9.4.2 Drive Waveform

400 W of power were necessary to drive the PZT’s with a 60 V_{pp} 600 Hz triangular wave. Use of a triangular wave allows two scans to be acquired in one period of the waveform, thus enabling the generation of 1200 axial scans. The spatial inversion of every other axial scan was corrected after the data acquisition.

Hysteresis, or nonlinearity of the PZT motion as a function of drive voltage, presented a significant problem. As described in Section 2.4, the OCT detection electronics use a band pass filter centered at the Doppler frequency. The Doppler frequency is produced by the scanning optical path length delay in the reference arm. Since the center frequency and the bandwidth of the band pass filter is fixed, any deviation from linearity in the scanning optical delay line will shift the Doppler frequency away from the center frequency of the band pass filter, causing signal loss. A first order attempt was made to correct for the hysteresis nonlinearity. This was accomplished by measuring the hysteresis, h(t), as a triangle waveform, tri(t) was applied to the PZM. The corrected function,

\[ tri'(t) = 2tri(t) - h(t), \]  

was then used as the driving waveform of the PZM. A plot of the corrected waveform is shown in Figure 9-6.
Figure 9-6. PZT drive waveform for the rapid optical delay scanning line corrected to compensate for hysteresis.

One difficulty with using the corrected function (Figure 9-6) as the drive waveform for the PZT's was that the correction function contained high frequency components which increased the power requirements for the PZM. Since the maximum power output of the amplifier used in this experiment was only 300 W, the high frequency components of the corrected waveform could not be used. Thus, the corrected waveform output from the power amplifier was only a rough approximation to the calculated corrected waveform.

9.4.3 Birefringence Compensation

Interferometric detection requires that the degree and state of polarization returned from each arm of the interferometer be identical. In the fiber wound PZT optical delay line, three separate impediments to maintaining polarization mode dispersion balance in the returned polarizations existed: static polarization mode dispersion mismatch that was inherent to wrapping the fiber around the PZM, slow drift which resulted from frictional heating of the piezoelectric transducers, and fast modulation (within each scan) which resulted from applying a voltage to the reference arm PZM. Each of these effects must be either canceled within the interferometer arm in which it is induced, or identically balanced in the other interferometer arm.
Matching of the static and dynamic polarization mode dispersion in the reference arm was accomplished by wrapping a duplicate PZM with an identical length of fiber and inserting it in the sample arm[104]. The duplicate PZM was not modulated. The remaining birefringence was canceled by inserting Faraday rotators in the free space region of each interferometer arm. Thermal expansion of the PZT's caused an increase in the bend radius of the fibers wound around the reference arm PZM. The expansion of the reference arm PZM contributed to a greater polarization mode dispersion imbalance between the reference arm PZM and the sample arm PZM. The slow thermal drift was canceled by controlling the temperature of the driven PZM in the reference arm. All three of these corrective mechanisms were necessary to provide complete polarization matching over the entire laser spectrum and collectively resulted nearly 100% fringe contrast.

9.5 Fast Scanning OCT

9.5.1 Methods

OCT System

The PZT optical fiber stretcher was incorporated into the reference arm of the OCT system (Figure 9-7)[108]. An unmodulated duplicate PZM was placed in the sample arm. In order to

![Diagram](image)

**Figure 9-7.** Schematic of the fast scanning OCT system incorporating the PZT optical fiber stretcher and an identical non-modulated cylinder in the sample arm. Faraday rotators (F) were placed in the free space regions in the reference and sample arms. An OCT image was created by recording axial reflectivity profiles while the focused ample arm beam was scanned across the specimen with a galvanometer (G).
maintain a high SNR, the solid state Cr$^{4+}$:Forsterite laser was used. The Cr$^{4+}$:Forsterite oscillator was set to produce 200 mW of single mode output power with a bandwidth of 50 nm centered at 1290 nm[108]. The free space axial resolution or coherence length of the Cr$^{4+}$:Forsterite source coupled into the fast scanning OCT system was 15 μm. For actual measurements, the laser power was attenuated to 30 mW. The power incident on the sample was 2 mW, providing a measured SNR of 112 dB[108]. A cross-sectional image was produced by recording axial reflectance profiles while the beam on the sample was scanned with a galvanometer.

9.5.2 Results

The image acquisition rate of the fast scanning OCT system was sufficient to allow motion artifact free imaging of in vivo biological specimens. Figure 9-8 demonstrates this by showing the beating heart of an in vivo embryonic Xenopus laevis (African frog) taken using both a SLD-based mechanical scanning OCT system and the high speed OCT system[25]. The heart was imaged through the skin of the ventral side of the frog. Each image consisted of 300 x 250 pixels covering a sample area of 3 mm x 2.2 mm. The images were cropped and enlarged to display structures of interest. The focused transverse spot size was 33 μm, corresponding to a confocal parameter of 1.32 mm. The SLD-based mechanical scanning OCT image was acquired in 30 seconds while the high speed OCT image was collected in 250 milliseconds. The mechanical scanning OCT image of the heart is blurred due to motion artifacts. These artifacts can be seen as

![Figure 9-8](image_url)

**Figure 9-8.** A. SLD-based mechanical scanning OCT image of an in vivo Xenopus laevis heart. B. Fast scanning OCT image of in vivo Xenopus heart. Bar represents 500 μm.

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vertical striations in the OCT image (Figure 9-8A). The images acquired by the fast scanning OCT system, however, show clear delineation of the anatomy of the heart (Figure 9-8B). This anatomy can also be seen in Figure 9-9 and include the pericardial sac, atrium, ventricle, and bulbus arteriosus of the beating embryonic *Xenopus* heart.

![Figure 9-9. Piezoelectric fiber stretcher rapid scanning OCT image of an in vivo *Xenopus* heart showing clear delineation of anatomy, including the pericardium (pc), ventricle (v), atrium (a), and bulbus arteriosus (ba). Bar represents 500 μm.](image)

The PZM-based high speed OCT system also allows imaging of different stages of the cardiac cycle, such as diastole (ventricular relaxation and filling) and systole (ventricular contraction). The capability of the high speed OCT system to rapidly image functional microanatomy of *in vivo* biological specimens is shown in Figure 9-10. This figure shows a series of images of the beating heart of an *in vivo Xenopus laevis*, taken at different phases of the cardiac cycle. Sequences similar to that of Figure 9-10 have been subsequently combined to form the first known OCT movies of microanatomical function of *in vivo* specimens.

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**Figure 9-10.** Series of OCT images taken during different phases of the *Xenopus* cardiac cycle using the fiber stretching OCT system. Image 1-6 vary from the beginning of diastole to the mid systole. Bar represents 500 μm.

*In vivo* human tissue has also been imaged by the PZM-based high speed OCT system. Figure 9-11 depicts an OCT image of an *in vivo* human forefinger acquired by the high speed OCT system. The image enables visualization of the microstructure of the skin, including the stratum corneum, the epidermis, the dermal-epidermal junction, and the dermis. The backscattering characteristics of structures such as the epidermis are considerably different than those observed in previous *in vitro* OCT studies of human skin.

### 9.6 Discussion

A fast scanning OCT system capable of *in vivo* imaging of human tissue without image degradation due to motion of the specimen has been constructed. The system integrates a high power short coherence length KLM solid state laser source and a PZM driven optical fiber delay line. 3 x 2.2 mm images consisting of 300 x 250 pixels were acquired at a rate of four images per second with a SNR of 112 dB and an axial resolution of 15 μm[108]. The rapid acquisition rates of the high speed OCT system allows motion-artifact-free imaging of living biological specimens such as the embryonic *Xenopus laevis*. In addition, the high signal to noise ratio was shown to allow penetration to a depth of greater than 2 mm in *in vivo* human skin. The capabilities of this
fast scanning OCT system should also enable in vivo optical biopsy of other tissue systems, including the cardiovascular system and gastrointestinal and urinary tracts.

While the fiber wound PZM optical delay line provides the capability to perform high speed OCT imaging, it has several limitations. One of the most important problems with the PZM scanning system is the need for a high power electronics to drive the piezoelectric transducers. High power electronics may be difficult to integrate in a clinical environment because of cost and safety concerns. Moreover, in highly motion sensitive scenarios such as intravascular imaging, OCT scanning speeds may have to increase by an order of magnitude. In these cases, the power needs for the PZM driven optical delay line could approach several kiloWatts. Recently, specialized low voltage piezoelectric transducers have been developed that have a 5 times lower capacitance than the PZT’s used in this experiment. This will help in reducing the power requirements of the PZM scanning optical delay line, but probably not to a level that would be acceptable for integration in a clinical environment.
A more severe limitation to this method may be PZT stack breakdown due to overheating. As depicted in Figure 9-4, the heating of the PZT stacks increases as the square of the driving frequency. Thus, it may not be possible to drive the PZT’s above 1 kHz before transducer breakdown. The only solution to this problem is to design a heat conduction system for the PZT’s which will increase the complexity of the PZM optical delay line considerably.

Next, for higher resolution systems, implementation of polarization mode dispersion compensation will be significantly more difficult. While the three birefringence compensation mechanisms used in this experiment were sufficient for a source with a spectral bandwidth of 50 nm, the wavelength dependence of the Faraday rotators will place an upper limit on the spectral width that can be compensated. In addition, the tolerances for both the Faraday rotators and the matching of the piezoelectric windings in both arms of the interferometer will be more extreme in order to match polarization mode dispersion for a spectral bandwidth that produces 5 µm resolution.

Finally, the method described in this chapter for linearizing the nonlinear mechanical motion of the piezoelectric transducers is less than optimal. Figure 9-11 shows one of the artifacts that occurs due to suboptimal hysteresis correction. The arrow in Figure 9-11 marks an artificial boundary that is caused by uncorrected PZT hysteresis. Since the change in optical delay is not linear, the Doppler frequency is not constant across the scan. Over the length of the scan, portions of the OCT signal are clipped by the band pass filter as the heterodyne frequency changes. Proper correction of the hysteresis will involve the use of a closed loop feedback system. To date, however, no commercially available piezoelectric closed loop feedback systems are available that operate above 1 kHz. In addition, any form of hysteresis correction will require the power requirements for driving the PZT’s to be significantly higher because the correction waveform contains higher frequencies than the uncorrected triangle waveform.

Therefore, the work presented in this chapter may represent the upper limit for a clinically fieldable high speed optical delay line using an PZM-based optical fiber stretcher. The complexity of the hysteresis correction, the high power requirements, and the temperature-related breakdown of the piezoelectric transducers place an upper limit on the maximum scanning rate that this method can achieve. Because of these limitations and the overall complexity of this high
speed scanning optical delay line solution, a search for other, less complex and less power intensive methods is merited.
Chapter 10

PHASE CONTROL FOR HIGH SPEED OCT

10.1 Introduction

Piezoelectric optical fiber stretching is an effective method for rapidly scanning the group delay in the reference arm of the OCT system. However, the limitations of the PZM optical fiber stretcher, including its large power requirements, hysteresis, and PZT breakdown due to heat generation, have motivated the investigation of other scanning optical delay line methods. One promising alternative to PZM-based fiber stretching is a novel optical delay line based on wavelength dependent phase manipulation.

A phase control optical delay line produces an optical group delay by dispersing the spectrum with a grating, and applying a temporally modulated linear wavelength dependent phase. The linear wavelength dependent phase can be achieved by reflecting the spread spectrum from a tilted mirror. If the angle of the mirror is rapidly scanned, a time dependent optical group delay line is produced. The phase control optical delay line can then be inserted into the reference arm of an interferometer for performing high speed OCT. This technique is more versatile than the piezoelectric method, does not have large drive power requirements, does not suffer from piezoelectric hysteresis and drift, and has better potential to enable real time (24-30 frames per second) video rates.

In this chapter, the use of the phase control apparatus to produce an optical group delay is presented. In addition, other different group delay scanning configurations are proposed. An analysis of the phase delay is also provided to understand the local oscillator signal that is used for heterodyne detection. Finally, a rapidly scanning optical group delay line has been con-
structured and incorporated into an OCT system. This high speed system will be used for the first
*in vivo* OCT studies of animal internal organ systems, described in the final chapter of this thesis.

10.2 Beam Scanners

The phase control paradigm is powerful because it allows group delay to be produced by
scanning the angle of a beam, instead of employing mechanical linear translation. Commercially
available mechanical beam scanners such as the galvanometer, resonant scanner, rotating polygon
mirror, and scanning holographic optical elements are one to two orders of magnitude faster
than the galvanometric linear translator. In addition, rapid optical beam scanning can be per-
formed by devices such as acoustooptic modulators which contain no moving parts. This section
describes common angular optical scanning instruments. These components are used in a vari-
ety of applications such as bar code readers, laser printers, and real time video scanning sub-
systems. Since this technology is mature, the incorporation of these devices into an OCT system
is relatively simple and cost-effective.

10.2.1 Mechanical Beam Scanners

Galvanometer

The galvanometer is used in linear mechanical scanning OCT systems. However, due to
the large force required to drive a retroreflector mounted to a lever arm and to mechanical reso-
nances, the maximum frequency of galvanometer-based linear translators is only approximately
100 Hz. The galvanometer is similar in structure to a torque motor, consisting of a mirror
mounted to a moving magnet rotor positioned between stator coils[109]. The stator coils can pro-
vide a variable magnetic field which causes the rotor to turn. Without the large mass of a lever
arm, this device is capable of angular scanning with high linearity and frequencies up to 1 kHz.
Scanning frequencies are maximized by reducing the mass of the mirror. Thus, for high scan fre-
quencies, the mirror must be small in size, limiting the maximum beam size on the mirror. A lin-
ear angular scan is possible because the galvanometer is heavily damped to prevent coupling
into its natural mechanical resonances[109].

Resonant Scanner

A resonant scanner is a galvanometer without damping. The resonant scanner only oscil-
lates at or near its mechanical resonance frequency. For this reason, resonant scanners produce a
sinusoidal change in angle as a function of time. If the most linear portions of the rising and falling edges of the sinusoidal angular scan are used, a 66% duty cycle can be achieved with a 2:1 slope change[109]. Thus, for applications which require a linear angular scan, such as OCT with a fixed band pass filter, the resonant scanner can provide a 66% duty cycle with a SNR loss that is dependent on the NEB. However, the resonant scanner can oscillate at speeds up to 20 kHz, so if the loss can be tolerated, it could be used for real time (24-30 frames per second) applications.

**Polygonal Scanning Mirror**

The polygonal scanning mirror consists of a machined polygon with highly reflecting facets (Figure 10-1). A high speed motor is used to rotate the polygon. As the polygon rotates, the input beam is reflected off of one of the facets, producing an angular scan. Since air bearing motors are available that can scan at up to 40,000 rpm, a polygonal scanning mirror with 24 facets can produce 16000 angular scans per second. This technology is well-suited for obtaining linear angular scans at high speeds.

![Fig. 10-1. Schematic of the polygonal scanning mirror.](image)

**Holographic Optical Element (HOE)**

A holographic scanner is a device that changes the transmitted diffraction angle of a beam as it is rotated. One simple configuration consists of a circular element with wedge subsections (Figure 10-2a). Each wedge consists of a diffraction grating with grating spacing that varies as a function of angle(Figure 10-2b). If the holographic scanner is rotated using a high speed motor, the change in grating spacing diffracts the beam at different angle. Usually, the holographic scanner is only used with monochromatic light because the grating will disperse a broad
bandwidth source. This property is advantageous for phase control, for the HOE could, in principle, take the place of both the grating and the angular scanner.

10.2.2 Acoustooptic Modulator

The acoustooptic modulator (AOM) is capable of beam scanning without using any moving parts. It functions by forming a variable refractive index grating in a crystal through the interaction of the crystal with sound. Sound energy is transferred to the crystal by means of a small piezoelectric transducer attached to one end. When a radiofrequency (RF) signal is applied to the PZT, a sound wave is created in the crystal. This sound wave varies the refractive index of the crystal to produce a Bragg grating. The light diffracted by the grating is transmitted through the crystal at an angle determined by the grating spacing. If the RF frequency is scanned, the grating spacing changes, altering the angle of transmittance. Like the HOE, the AOM performs scanning of both the transmitted angle and the grating spacing.

10.3 Phase control

When combined with angular beam scanning, the phase control paradigm can be a versatile method for producing a scanning optical group delay[110]. Phase control is a technique that uses a lens-grating pair to alter the temporal properties of ultrafast pulses by manipulating the spectrum. This technique has typically been used for the temporal shaping of ultrafast pulses[111, 112]. A schematic of the generic pulse shaping apparatus is shown in Figure 10-3. The pulse shaping apparatus consists of a two identical reflection grating-lens pairs and an amplitude, \(A(x)\), and/or phase, \(\phi(x)\) mask placed in the center, one focal length, \(f\), away from both lenses (Figure 10-3). The grating disperses the spectrum of the incident optical beam. If
Figure 10-3. Apparatus for performing pulse shaping.

$L = f$, the Fourier transform of the dispersed optical beam occurs at the mask. The mask modifies the spectrum either by phase or amplitude modulation. The modified spectrum is then inverse Fourier transformed by the second lens, causing an alteration of the temporal profile of the pulse.

Figure 10-4. Folded phase control geometry.

The work presented in this chapter employs a folded geometry version of the standard pulse shaping apparatus (Figure 10-4). This scheme has two advantages. First, the folded geome-
try only uses one grating-lens pair. In addition, the folded geometry enables coupling back into the reference arm collimating lens without additional optical components.

10.3.1 Optical Group Delay

Phase manipulation is capable of producing an optical group delay by dispersing the spectrum with a grating, and then applying a temporally modulated linear wavelength dependent phase. The wavelength dependent angular diffraction of the incident collimated beam is given by the grating equation[113],

\[ \theta(\lambda) = \sin^{-1}\left(\frac{m\lambda}{d} - \sin(\theta_i)\right), \]  \hspace{1cm} (10-1)

where \( m \) is the diffracted order of the reflected beam, \( d \) is the ruling spacing of the grating, and \( \theta_i \) is the incident angle on the grating. If \( L = f \), each wavelength is distributed along the x axis after the lens, at the position,

\[ x(\lambda) = f \tan(\theta_0 - \theta(\lambda)), \]  \hspace{1cm} (10-2)

where \( \theta_0 \) is the diffracted angle at the center wavelength of the source, \( \lambda_0 \). The Fourier transform of the input beam now resides at the plane of the mirror. Since the Fourier transform of a linear phase ramp in the spectral domain corresponds to a delay in the time domain, a temporal group delay is obtained by placing a phase mask at the mirror,

\[ \phi(x(\lambda)) = -x(\lambda)\tau. \]  \hspace{1cm} (10-3)

The modified spectrum is then inverse Fourier transformed by propagating back through the folded phase control apparatus, creating a temporal delay of the input beam. The magnitude of the optical delay is proportional to the spectral dispersion of the grating, the focal length of the lens, and the slope of the phase ramp, \( \tau \). Note that this phase control device can be used to independently modulate phase delay and group delay (Section 10.5).

Several groups have proposed the use of an arbitrary phase mask, such as a liquid crystal array for pulse shaping[112, 114]. However, for producing an optical group delay only, a com-
plicated phase mask is not necessary. Instead, the phase-mask-mirror combination can be replaced with a single tilted mirror (Figure 10-5)[110]. If the mirror is tilted with an angle, $\gamma$, a linear wavelength dependent phase is applied to the incident beam. A 100 Hz linear scanning group delay line using a piezoelectric mirror tilter has been previously presented for construction of a high speed autocorrelator to measure pulse durations[110].

One difficulty with using a tilted mirror to produce the group delay is that the light reflected from the tilted mirror is no longer colinear with the incident beam. Beam walkoff due to deflection by the tilted mirror prevents coupling of the reflected beam back into the reference arm collimating lens. A solution to this problem is the use of a double-passed configuration (Figure 10-6). In the double passed configuration, the beam emergent from the collimating lens is incident high on the grating so the diffracted beam is offset from the lens along the y axis(Figure 10-6). The beam is refracted by the lens, which is corrected for spherical aberration, down onto the tilted mirror. The tilted mirror reflects the beam through the lower portion of the lens. The light is then diffracted off the grating, and onto the double pass mirror(Figure 10-6). The double pass mirror is aligned to allow the beam to retrace its path back to the collimator. This scheme enables the folded configuration to be used with a tilted mirror while avoiding beam walkoff and resultant coupling losses. In addition, since the phase control apparatus is double passed, the delay produced for a given set of components and mirror tilt is also doubled.
10.3.2 Dispersion Compensation

In addition to enabling high speed group delay scanning, another advantage of the phase control apparatus for OCT is the capability to compensate dispersion mismatch between the reference and sample arms. An analysis performed to determine the group velocity dispersion (GVD) for a grating compressor has described the dispersion in the double passed configuration to be[115],

\[
\frac{d^2 \phi}{d\omega^2} \bigg|_{\omega_0} = \frac{\lambda_0^3 (L - f)}{\pi c^2 d^2} [\cos(\theta_0)]^{-\frac{3}{2}}.
\]  

(10-4)

When the lens is not one focal length away from the grating, an additional wavelength dependent phase delay is added to the pulse, creating positive dispersion for \(L < f\) or negative dispersion for \(L > f\). This property of the phase control apparatus enables compensation of the dispersion imbalance between the reference and sample arms in the OCT system by simply changing the lens-grating separation.
10.4 Group Delay Scanning Configurations

One powerful aspect of the phase control paradigm is its versatility. Analysis of this method has revealed that altering any one of several optical comments in the phase control apparatus can produce a change in group delay (Figure 10-7). Specifically, a scanning group delay can be obtained by tilting the mirror, $\gamma$, changing the incident angle on the grating, $\theta_i$, tilting the grating, or changing the grating spacing, $d$. This section presents an analysis of the group delay that can be achieved by these different configurations in a folded double passed phase control apparatus.

10.4.1 Scanning the Mirror Angle, $\gamma$

A simple ray tracing analysis can be used to determine an analytical expression for the group delay produced by changing the Fourier plane mirror tilt by an angle, $\gamma$. The wavelength dependent phase shift produced by the tilted mirror can be easily determined from the geometry of Figure 10-7,

$$\phi(\lambda) = -2kz(\lambda),$$  \hspace{1cm} (10-5)

or

$$\phi(\lambda) = -2kx(\lambda)\tan(\gamma).$$  \hspace{1cm} (10-6)

The diffracted angle for the center wavelength of the source is

$$\theta_0 = \theta(\lambda_0) = \sin^{-1}\left(\frac{\lambda_0}{d} - \sin(\theta_i)\right).$$  \hspace{1cm} (10-7)

If the phase delay is reformulated as a function of frequency, the wavelength dependent phase shift induced by the folded phase control apparatus is,

$$\phi(\omega) = -2\frac{\omega}{c}f\tan(\gamma)\tan\left(\theta_0 - \sin^{-1}\left(\frac{2\pi c}{\omega d} - \sin(\theta_i)\right)\right).$$  \hspace{1cm} (10-8)
Since the group delay is defined as

\[ \tau_g(\gamma) = \frac{\partial \phi}{\partial \omega} \bigg|_{\omega_0}, \]  

(10-9)

after differentiation and substitution of the center wavelength,

\[ \lambda_0 = \frac{2\pi c}{\omega_0}, \]  

(10-10)

the group delay becomes

\[ \tau_g(\gamma) = \frac{2f\lambda_0 \tan(\gamma)}{cd \cos(\theta_0)}. \]  

(10-11)

The change in group delay is twice that of Eq. (10-11) for the double passed phase control system. Given the double passed group delay length, \( l_g = 2\tau_g c \), for small angular deviations of the mirror by \( \gamma \), the total group delay length is a linear function of the scan angle,

\[ l_g(\gamma) = \frac{4f\lambda_0 \gamma}{d \cos(\theta_0)}. \]  

(10-12)

For values of \( \theta_i \) at Littrow's angle,

\[ \theta_i = \theta_L = \sin^{-1}\left(\frac{\lambda}{2d}\right), \]  

(10-13)

d = 150 lines per mm, \( \Delta \gamma = 10^\circ \), and \( f = 10 \) cm, the total group delay length calculated using Eq. (10-12) is 14 mm.
Since rapid scanning requires a small mirror, vignetting of the spectrum is a potential problem. The beam spread on the mirror,

\[ \Delta x(\lambda_{\text{max}}, \lambda_{\text{min}}) = f(\tan[\theta(\lambda_{\text{max}})] - \tan[\theta(\lambda_{\text{min}})]), \tag{10-14} \]
determines the maximum allowable mirror size for the rapid scanning delay line. For the parameters given above with a \( \lambda_{\text{max}} - \lambda_{\text{min}} \) bandwidth of 200 nm, the beam spread is 3 mm. Thus, the mirror must be at least 3 mm, or clipping of the spectrum will result, resulting in the convolution of the autocorrelation function with a sinc function. For this reason, other configurations of the phase control apparatus that do not require a moving \( \gamma \) mirror, should be investigated for high resolution applications.

10.4.2 Scanning the Grating Incident Angle

Optical group delay may also be produced by scanning the grating incident beam angle, \( \theta_i \), using a rotating polygon mirror, a galvanometer, or a resonant scanner. The configuration dif-

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**Figure 10-7.** Optical group delay obtained by scanning the grating incident angle. Notable differences are the insertion of an additional scanning mirror and a telescope between the scanning mirror and the grating to prevent walkoff at the grating.
fers from the previous method by the inclusion of a fixed angle, $\gamma$, a device for scanning $\theta_i$, and a telescope between the scanning mirror and the grating (Figure 10-7). Since the tilted mirror is fixed, it can be large enough to accommodate any bandwidth source. The telescope is inserted between the scanning component and the grating to prevent beam walkoff at the grating. To accomplish this, the image and object planes of the telescope must match the positions of the scanning mirror and the grating.

An analytical expression for the optical group delay produced by this configuration can be formulated in a similar manner to the scanning $\gamma$ mirror configuration, except the independent variable is now $\theta_i$. If $\gamma$ is small, after differentiation of the wavelength dependent phase and evaluation at the center wavelength, the double passed group delay length,

$$I_g(\theta_i) = -4f \tan \gamma \left[ \theta_{i0} - \left( -\sin^{-1}\left( \frac{\lambda_{0}}{d} - \sin(\theta_i) \right) \right) \right] + \frac{\lambda_{0}}{d \sqrt{1 - \left( \frac{\lambda_{0}}{d} - \sin(\theta_i) \right)^2} }, \quad (10-15)$$

where $\theta_{i0}$ is the angle of diffraction from the grating for $\lambda_0$ for the central scan position. A plot of the path length delay calculated from Eq. (10-15) is shown in Figure 10-8 for $d=150$ lines per mm, $f=10$ cm, $\gamma=3^\circ$, and a variation of the grating incident angle by $10^\circ$. For these parameters, the delay obtained using this method is also a linear function of the independent variable, $\theta_i$.

### 10.4.3 Scanning the Grating

Angular scanning of the grating in the folded phase control apparatus also creates a group delay (Figure 10-9). The primary advantage to this configuration is that a telescope is not necessary because beam walkoff at the grating does not occur. However, placing a grating on a galvanometer mirror or polygon scanning mirror is difficult, so this method was not explored further in deference to more plausible methods which perform grating scanning using an HOE or an AOM.

### 10.4.4 Changing the Grating Spacing (HOE)

Another interesting modification of the phase control apparatus enables high speed group delay scanning. If the grating is a transmission HOE, like that shown in Figure 10-2, the
groove density of the grating may be scanned in a rapid fashion. This may be accomplished by using a rotating circular HOE with grating spacing that varies as a function of angle. As the HOE is rotated, the change in grating spacing alters the extent of the spectral spreading. Since the wavelength dependent phase delay is proportional to ruling of the grating, rotating the HOE also produces a scanning group delay.
10.4.5 Changing the Grating Spacing (AOM)

A possibly more elegant method for changing the grating groove density is the use of an AOM (Figure 10-11). In this configuration, the wavelength spread is augmented by directing the light transmitted through the AOM through a diffraction grating (Figure 10-11). The diffraction grating is necessary because the change in grating spacing (RF bandwidth) for commercially available AOM's are not sufficient to produce a large enough group delay scan for OCT. A telescope with a high magnification may also be used to enhance the change in diffraction provided by the AOM[116].
A plot of the path length delay produced by an AOM-diffraction grating pair as a function of the RF driving frequency is presented in Figure 10-12. To generate this data, an analytical expression of the group delay for a changing grating spacing, \( d \), was formulated. The parameters used for generating the data include the use of a slow shear wave TeO\(_2\) AOM (\( c_s = 0.6 \text{ km/s}, n = 2.35[105] \)), an RF center frequency 50 MHz, \( f = 5 \text{ cm} \), and \( \gamma = 4^\circ \). The secondary diffraction grating had a ruling of 1200 lines per mm. The group delay produced by this configuration is nonlinear. This nonlinearity can be corrected during a group delay scan by modifying the RF waveform sweep frequency. In addition, changes in frequency dependent diffraction efficiency can be compensated for by altering the RF signal amplitude. Another difference between the AOM scanning method is that a Doppler shift (2\( v_{RF} \)) is transferred to the local oscillator signal. This modulation frequency may be removed by double passing the AOM[117]. The AOM configuration is preferable over the mechanical angular scanning configurations because it allows real time (15 kHz) path length scanning with no moving parts.

### 10.5 Phase Delay

In order to use the scanning path length delay lines presented in the previous section, the phase delay must be analyzed to determine the heterodyne modulation frequency. Unlike other rapid scanning optical delay lines, such as the linear mechanical translator or the piezoelectric
optical fiber stretcher, the change in phase delay using the phase control method is not directly related to the change in group delay. Consider Figure 10-13 where the center wavelength is directed towards the tilting mirror, offset from the axis of rotation by $x_0$. If the mirror surface approximately intersects the axis of rotation, the phase delay can be written as,

$$
\phi(\lambda, t) = \frac{4\pi f v}{\lambda} \left[ \theta_0 - \sin^{-1}\left(\frac{\lambda}{d} - \sin(\theta_0)\right) + \frac{x_0}{f} \right],
$$

which is simply a modification of Eq. (10-8) that incorporates a lateral offset of the galvanometer. As discussed in Chapter 2, the heterodyne modulation frequency for a source with a Gaussian spectral distribution, is determined by the phase shift at the center wavelength,

$$
\phi(\lambda, t)|_{\lambda_0}.
$$
The phase shift for the scanning mirror configuration with a linear change in angle as a function of time, $\gamma t$, is then

$$\phi(\lambda_0, t) = \frac{4\pi\gamma t x_0}{\lambda_0},$$  \hspace{1cm} (10-18)

because for this case,

$$\theta_0 = \sin^t \left( \frac{\lambda_0}{d} - \sin(\theta_i) \right).$$  \hspace{1cm} (10-19)

Thus, the envelope of the autocorrelation function produced by the scanning linear group delay is modulated by a sinusoid,

$$\cos(2\pi f_p t),$$  \hspace{1cm} (10-20)

where the modulation frequency,

$$f_p = \frac{2\gamma x_0}{\lambda_0}.$$  \hspace{1cm} (10-21)

As can be seen by Eq. (10-18), if the center wavelength of the spectrum is incident on the mirror axis of rotation ($x_0 = 0$), no modulation frequency is applied to the local oscillator, even though a scanning linear group delay is produced. Thus, the interferometric signal consists only of the envelope of the autocorrelation function without any modulation. This feature of the tilting $\gamma$ mirror configuration may be advantageous. If an independent phase modulation is applied to the local oscillator, the system would be capable of scanning at different speeds without changing the center frequency of the band pass filter before demodulation.

Furthermore, by translating the scanning mirror so that the center wavelength is offset from the axis of rotation ($x_0 \neq 0$), an arbitrary modulation frequency can be applied to the local oscillator. This feature allows complete control over the center frequency of the local oscillator. The modulation frequency may be varied by simply translating the tilting mirror perpendicular
to the optical axis of the beam. The range of center modulation frequencies that may be achieved is only limited by spectral vignetting due to the finite size of the scanning mirror.

The group-phase delay independence of the phase control apparatus is an advantage for scanning mirrors with an axis of rotation that intersects the mirror surface. However, when the mirror surface is separated from the axis of rotation by a distance, \( r \), this property of the phase control method is disadvantageous. For real time OCT applications (> 1 kHz), a polygon mirror is the optimal scanning device for rapidly changing the angle, \( \gamma \) (Figure 10-14). In this case, to a first order approximation, \( x_0 \) changes across a single scan,

\[
x_0(t) \equiv r \tan \left( (\Omega - \Omega_0)t \right), \tag{10-22}
\]

where \( \Omega \) is the rotation angle and \( \Omega_0 \) is the angle at which the center wavelength of the source is coincident with the center of the polygon mirror facet. In the limit of small \( \Omega - \Omega_0 \), \( x_0 \) is a linear function of \( t \). The modulation frequency in this case becomes,

\[
f_p(t) = \frac{2r(\Omega - \Omega_0)t}{\lambda_0}. \tag{10-23}
\]

\textbf{Figure 10-14.} Schematic used for determining phase delay produced by a polygonal scanning mirror.
While the change in group delay produced by the polygon scanning mirror is linear, the change in phase is quadratic as a function of time. Since the modulation frequency shifts linearly over the scan, the polygon scanning mirror cannot be used in conjunction with a demodulation method that incorporates a fixed band pass filter. This is an unfortunate result because the polygon scanning mirror is the best mechanical option for obtaining high speed (> 1 kHz) linear group delays. The varying modulation frequency could be overcome, however, by using an alternative demodulation scheme, such as adaptive frequency mixing detection.

Alternative phase control configurations such as scanning the grating angle of incidence or the grating ruling density, also produce a nonlinear phase delay. By evaluating Eq. (10-8) at \( \lambda_0 \), for these scanning methods, the phase shift becomes

\[
\phi(t) = \frac{4\pi f_{\gamma}}{\lambda_0} \left[ \theta_{i0} - \sin^{-1} \left( \frac{\lambda_0}{d(t)} - \sin(\theta_{i}(t)) \right) \right]. \tag{10-24}
\]

As with the polygon scanning mirror, the phase is a nonlinear function of time and again, these methods can only be used in conjunction with an adaptive frequency mixing demodulation scheme. Based on the phase produced by the polygon scanner, scanning the grating angle of incidence, and the grating ruling density, it is clear that to exploit the full potential of the phase control paradigm, a change in the demodulation method is warranted.

### 10.6 Scanning Optical Delay Line for High Speed OCT

Scanning the angle, \( \gamma \), with a galvanometer produces a linear optical group delay scan with a constant modulation frequency. Because the demodulation electronics used in this study required a constant modulation frequency, a folded double passed scanning \( \gamma \) mirror configuration was used to perform coherence gating in the second generation high speed OCT system. The galvanometer was driven with a 1 kHz triangle waveform, enabling 2000 scans per second, twice the speed of the PZT-based high speed OCT system. This rapid scanning rate enabled image acquisition at 4 frames per second for an image size of 512 (lateral) x 256 (axial) pixels or 8 frames per second for an image size of 256 (lateral) x 256 (axial) pixels. Unlike the PZT fiber stretcher optical delay line, the phase control method produced axial scans that were not corrupted by dropout artifacts due to hysteresis. The total galvanometer scan angle of 3°, provided an optical path length delay of 3 mm.
The self-phase modulated KLM Cr\textsuperscript{4+}:Forsterite laser (Chapter 8) was used as the source of low coherence light for the high speed OCT system. The laser was set to an output power of 30 mW. After being transmitted by the fiber optic beamsplitter, the sample arm power was 12 mW. The FWHM spectrum of the source was 75 nm, corresponding to a Gaussian autocorrelation FWHM of 10 µm.

The center of the spectrum was offset on the scanning mirror to produce a modulation frequency of 750 kHz. The FWHM bandwidth of the signal was approximately 350 kHz. This modulation frequency was chosen to enable band pass filtering of the interferometric signal without accepting any contributions from low frequency noise. In order to produce linear angular scans at 1 KHz, the mirror size was minimized to have a width of 6 mm. Because of this constraint, the full bandwidth of the self-phase modulated source was not used. If the entire spectrum was employed (200 nm), one side of the spectrum would have been clipped by the edges of the scanning mirror.

10.7 Discussion

Most current mechanical scanning optical delay lines are not rapid enough to allow in vivo imaging owing to the presence of motion artifacts. Piezoelectric fiber stretchers allow rapid scanning, but they suffer from high power requirements, nonlinear fringe modulation due to hysteresis and drift, uncompensated dispersion mismatches, and poor mechanical and temperature stability. The phase control optical delay line enables rapid scanning OCT without the limitations of the piezoelectric method. It can be constructed with common optical components, has modest power requirements, is repeatable, and temperature stable.

The double passed folded phase control apparatus is versatile, since many different scanning configurations may be used to produce an optical path length delay. Optical group delay may be produced by scanning the angle, γ, varying the grating incident angle, physically tilting the grating, or changing the grating spacing. Scanning the angle, γ, using a galvanometer or a resonant scanner, is the only method that is compatible with fixed band pass filter detection electronics. The primary disadvantage of this configuration is that the mirror size places a limit on the spectral bandwidth and resultant axial resolution. Because the other scanning configurations produce a nonlinear phase delay, they must be used in conjunction with an alternative demodulation scheme, such as adaptive mixing. However, these methods do not constrain the usable
spectral bandwidth. Once the new demodulation scheme is implemented, the production of a rapidly scanning optical path length delay line using an AOM is particularly desirable, as it contains no moveable parts.

A phase control optical delay line based on the folded double passed $\gamma$ scanning configuration has been constructed. The scan rate provided by this device is twice that of the PZT fiber stretcher presented in Chapter 9 (2000 axial scans per second) and its axial resolution is 50% greater (10 $\mu$m). This system was much simpler to construct, has modest power requirements, and does not suffer from dropout artifacts due to hysteresis. As a result, the phase control optical delay line has become the foundation for the fastest scanning OCT system to date. Because of the superior performance of this method, it was incorporated into a high speed OCT system (IVOCT) and used to obtain the first in vivo OCT images of internal organs systems.
Chapter 11

IN VIVO OCT SYSTEM

11.1 Introduction

Detailed technical descriptions of the system integration for OCT imaging devices have not been emphasized in the literature due to their reliance on common components and their primary use in research settings. However, the new in vivo OCT imaging system (IVOCT), described in this chapter, differs significantly from previous OCT systems, justifying a discussion of system integration issues.

Since the information transfer rate of IVOCT is more than two orders of magnitude greater than previous OCT systems, IVOCT must be capable of both acquiring and storing images in a different manner. To accomplish this, a new digitization system based on a variable rate frame grabber is employed. In order to coordinate the frame grabber with the other system components, video synchronization circuitry has been designed and constructed. This circuit synchronizes the video capture to both the galvanometer in the optical delay line and the scanning transducer (i.e. OCT compatible catheter-endoscope) in the sample arm. In addition, images can be saved on both digital (single image acquisition) and analog media (continuous image acquisition) to store the large amount of data acquired by the high speed IVOCT system.

The IVOCT system is designed to be used in a clinical environment. Thus, issues such as cost, portability, image presentation, and ease of use have been considered in the design. Aside from the source, all of the components of the IVOCT system are relatively inexpensive and are constructed to fit into a portable, self-contained medical enclosure. The low coherence source can be incorporated into the enclosure either when an adequate second generation REDF is devel-
oped or the Cr$^{4+}$:Forsterite source is made compact. Effective image presentation is essential for real time interaction during a diagnostic imaging procedure. The software of the IVOCT system accommodates different lookup tables and display formats depending on the imaging transducer that is used. Moreover, important display controls, such as the black and white levels can be adjusted interactively in the software environment. These and other features of the IVOCT system enable its use in a medical setting for obtaining in vivo diagnostic OCT images, or optical biopsies, of tissue.

11.2 IVOCT System Overview

A block diagram of the IVOCT diagnostic imaging apparatus is shown in Figure 11-1. The fundamental components of this system include the low coherence source, fiber optic Michelson interferometer, imaging transducer, phase control optical delay line, and demodulation electronics. The rapid scanning phase control optical delay line enables a 4 frames per second (fps) image acquisition rate for 512 (lateral) x 256 (axial) pixels and 8 fps acquisition rate for 256 (lateral) x 256 (axial) pixels. At this speed, using the KLM Cr$^{4+}$:Forsterite laser as the source, the IVOCT system achieves these high speed imaging rates while maintaining a SNR of 110 dB and 10 μm axial resolution. IVOCT has been designed to be used with the OCT compatible catheter-endoscope (Chapter 7) for optical biopsy of internal organ systems and other imaging transducers such as a forward scanning hand-held probe for skin applications[118].

A computer controlled master clock is input into a custom-built circuit which synchronizes the frame grabber, the galvanometer in the optical delay line, and the imaging transducer. Once the data is acquired, it is displayed on a computer screen, using software that interfaces with the variable rate frame grabber. The data displayed on the computer screen may be digitally captured, mirrored to another video monitor and/or stored on an analog recording medium. Finally, the IVOCT software and electronics have been designed to be compatible with future developments in OCT technology, such as the capability to support higher frame rates (> 30 fps) if a faster optical delay line is implemented.

11.3 Low Coherence Source

The self-phase modulated KLM Cr$^{4+}$:Forsterite laser (Chapter 8) is used as the low coherence source for the IVOCT imaging system. The center wavelength of the KLM Cr$^{4+}$:Forsterite laser wavelength is 1280 nm, allowing for optimal imaging penetration in multiply scattering tis-
FIGURE 11-1. IVOCT system block diagram.

sue. The laser is set to produce an output power of 30 mW with a Gaussian FWHM spectral bandwidth of 75 nm, corresponding to a free space axial resolution of 10 μm.
11.4 Optical Layout

The optical layout of the IVOCT system is shown in Figure 11-1. The IVOCT system consists of a single mode fiber optic Michelson interferometer. A portion of the sample arm fiber is wound around polarization controllers to match the polarization states in each arm. The sample arm also contains a free space region that can be varied to change the coherence gate location. The sample arm is then coupled into an imaging transducer which may be either an OCT compatible catheter-endoscope (Chapter 7), or a forward scanning hand held probe[118]. Since the imaging transducer typically contains a long length of optical fiber, optical fiber must be introduced into the reference arm to keep the two arms path length matched. As mentioned earlier, the recently developed high speed phase control optical delay line is also incorporated in the reference arm.

11.5 Imaging Transducers

11.5.1 Second Generation Catheter-Endoscope

A second generation OCT compatible catheter-endoscope has been constructed to be used with the IVOCT system. This catheter-endoscope is similar in design to the catheter used to image intact in vitro tissue (Chapter 7) with the exception of two important differences. First, a constant velocity motor is employed in order to synchronize the motor with the frame rate. The use of this motor, which is controlled by closed loop feedback electronics, enables synchronization of the motor rotation with the IVOCT frame rate. In addition, the second generation catheter has been designed to remove the dominant sources of returned internal reflections from the distal optics (Chapter 7).

11.5.2 Forward Imaging Probe

A hand held forward scanning probe has also been constructed for skin and laparoscopic applications[118]. The forward probe focuses and scans the sample arm beam across the specimen at a fixed working distance[118]. Proximal end rotation is not necessary because the scanning is performed at the distal end of the device. The distal optics of the probe are identical to those of the catheter-endoscope, except the right angle prism is omitted. The scanning is performed by mounting the GRIN lens to a piezoelectric controlled cantilever arm[118]. The fiber length of the hand-held probe is equivalent to the fiber length of the OCT compatible catheter-endoscope, so either device can be conveniently interchanged and used with the IVOCT system.
11.6 Rapid Scanning Optical Delay Line

A rapid scanning optical delay line based on the phase control paradigm has been incorporated into the IVOCT system. The high speed IVOCT imaging system uses a folded double passed scanning γ mirror phase control apparatus to perform optical ranging. The galvanometer is driven with a 1 kHz triangle waveform, enabling 2000 scans per second, twice the speed of the PZT-based high speed OCT system. The total galvanometer scan angle of 3°, provides an optical path length delay of 3 mm.

11.7 Demodulation Electronics

In the folded double passed scanning γ mirror configuration, the center of the source spectrum is offset on the scanning mirror to produce a modulation frequency of 750 kHz. This modulation frequency was chosen to enable band pass filtering of the interferometric signal without accepting any contributions from low frequency noise. At an optical path length delay rate of 6 m/s, the FWHM bandwidth of the signal is approximately 350 kHz. The band pass filter of the demodulation electronics has a center frequency of 750 kHz and a bandwidth of 450 kHz. The band passed signal is demodulated using a base 10 logarithmic RMS converter.

11.8 A/D Converter

One important aspect of a diagnostic imaging system is its ability to allow the user to view the image data as it is acquired. This feature enables efficient use of the imaging apparatus, as areas of interest can be rapidly identified. In the IVOCT system, images are acquired and displayed at 4 (512x256) or 8 (256x256) frames per second using an 8 bit variable rate frame grabber. Since a base 10 logarithmic RMS converter is used to demodulate the OCT signal, an 8 bit frame grabber has sufficient dynamic range to capture all levels of the demodulated OCT signal. The frame grabber is resident on the host PCI bus of an Apple Computer Power Macintosh 8500/120. The variable rate PCI bus frame grabber enables immediate acquisition and display by transferring the data from the on-board memory of the frame grabber to the host computer memory using direct memory access (DMA). DMA over the PCI bus can accommodate acquisition rates that are greater than 30 fps if the scanning speed of the IVOCT system increases in the future.
11.9 Synchronization Electronics

11.9.1 PCLK, HSYNC, VSYNC

In order to digitize an image, the variable rate frame grabber requires an external pixel clock (PCLK), horizontal sync pulse (HSYNC), vertical sync pulse (VSYNC), and the video signal. The board uses the PCLK to determine when to digitize each pixel in the image. An HSYNC pulse initiates the start of a horizontal scan in the image. The HSYNC pulse must be synchronized with the galvanometer in the optical delay line. To accomplish this, the HSYNC pulses trigger an arbitrary waveform generator that controls the galvanometer in the reference arm. Since the HSYNC pulse triggers the acquisition of a horizontal line of data, corresponding to an OCT axial scan, an unprocessed video image acquired by the frame grabber is rotated by 90°. Remapping of the image axes to correct for the image rotation will be discussed later in this chapter. The VSYNC pulse triggers the start of a single image acquisition and also is capable of synchronizing the forward scanning hand held probe. Synchronization of the catheter-endoscope is obtained by adjustment of the angular velocity of a closed-loop constant velocity motor until the image is stable. Use of the HSYNC and VSYNC signals to trigger the frame grabber, the scanning optical delay line, and the imaging transducer, assures that the captured images will be synchronized with the rest of the IVOCT system.

The relationships between the PCLK, HSYNC, and VSYNC frequencies, the number of frames per second, and the image size are as follows:

\[ \text{VSYNC} = \text{the number of frames per second}, \]  \hspace{1cm} (11-1)

\[ \frac{\text{PCLK}}{\text{HSYNC}} = \text{the number of digitized pixels in one axial scan}, \]  \hspace{1cm} (11-2)

and \[ \frac{\text{HSYNC}}{\text{VSYNC}} = \text{the number of axial scans per image}. \]  \hspace{1cm} (11-3)

Once the image size and the number of frames per second are chosen, the PCLK, HSYNC, and VSYNC frequencies can be calculated from Eq. (11-1) through Eq. (11-3). Figure 11-2 shows an example of video synchronization pulses used to capture a 256 (horizontal) x 512 (vertical) pixel image at a rate of 4 frames per second. The PCLK is typically a square wave, while the HSYNC and VSYNC signals consist of short pulses with a negative polarity.
One important video signal formatting issue remains. The frame grabber board typically receives a blanked video signal directly after the HSYNC pulse. The blanking period lasts for 40 cycles of the PCLK. Since the blanking period occurs when the galvanometer reverses direction, it is likely that the demodulated OCT signal will only contain background noise. For this reason, the OCT signal is not altered after an HSYNC pulse. However, during the blanking period, the analog signal input to the board is not digitized. Thus, approximately 40 pixels need to be discarded from each horizontal line. To overcome this reduction of data, the PCLK is increased to allow for 288 digitized pixels in one axial scan. Exactly 40 of these pixels are thrown out to create a displayed image size of 248 (horizontal) x 512 (vertical) pixels.

11.9.2 Video Synchronization Circuitry

An efficient method for generating the HSYNC and VSYNC signals incorporates counting down from a master clock (MCLK). This scheme has the advantage that all of the synchronization signals must be in phase because they are derived from the same clock. The video synchronization circuitry constructed for the IVOCT system uses this principle. A schematic of the video control circuit is given in Figure 11-3. The circuit uses CMOS 74HC191 and 74HC4040 IC's to count down from the master clock. The ripple clock from the 74HC191 is used to provide the short duration negative polarity HSYNC and VSYNC pulses. Specific load bits of the 74HC191 counters are enabled to provide the additional 40 pixels clocks for each horizontal scan. In order to vary the frame rate, the master clock must be increased. The master clock is provided
by a voltage controlled oscillator (VCO). The voltage input to the VCO is designed to be generated by an analog output board that resides in the computer.

The video circuitry also has the capability to enable image acquisition for one of four different image sizes at a constant frame rate. The HSYNC, VSYNC, and PCLK signals are switched
<table>
<thead>
<tr>
<th>Visible Image Size (HxV)</th>
<th>PCLK</th>
<th>HSYNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>248x256</td>
<td>MCLK/4</td>
<td>M_HSYNC/2</td>
</tr>
<tr>
<td>248x512</td>
<td>MCLK/2</td>
<td>M_HSYNC</td>
</tr>
<tr>
<td>536x256</td>
<td>MCLK/2</td>
<td>M_HSYNC/2</td>
</tr>
<tr>
<td>536x512</td>
<td>MCLK</td>
<td>M_HSYNC</td>
</tr>
</tbody>
</table>

**Table 11-1.** PCLK and HSYNC frequencies for different image sizes. M_HSYNC refers to the horizontal synchronization frequency derived from the master clock (MCLK) for the largest image size.

by a dual, 4 input multiplexer, the CMOS 74HC153. A two bit word, generated by a digital output board in the computer, is input into the multiplexer, and switches the synchronization signals. A table of the different PCLK and HSYNC combinations for a particular image size is given in Table 11-1. The combination of the digital multiplexer and the VCO control of the pixel clock allows computer control of the frame rate for any one of four image sizes.

### 11.10 Data Storage

In comparison to previous OCT systems, imaging at rates of 4 or 8 frames per second dramatically increases the data storage requirements. For this reason, acquisition data is continuously saved on video tape, using a Super-VHS (SVHS) recorder. The SVHS recorder uses a high resolution video format and is capable of storing approximately 400 lines of video data per frame. The input to the SVHS recorder is a video signal generated by the computer that mirrors the information displayed on the computer screen. In addition, at any time during acquisition, the displayed information can be stored on the hard disk of the computer, enabling single high quality digital images to be recorded.

### 11.11 Portable Enclosure

Except for the solid state laser source, the entire IV OCT system is designed to be enclosed in a portable, rugged cart (Figure 11-4). This enclosure contains the computer, the phase control optical delay line controls, and the custom video synchronization circuitry. The computer moni-
Figure 11-4. Photograph of the portable enclosure containing the IVOCT system.

The IVOCT software is designed to provide the capability to perform OCT imaging within the constraints of a medical setting. Ease of use, control over the OCT acquisition process, and user interactivity are the key strengths of this program that should facilitate its use in diverse clinical environments. The software is written in C and runs on a PowerPC® 604, Power
Macintosh 8500/120 personal computer. The speed of this RISC computer enables real time image formatting and display, which would otherwise be possible only on more expensive workstations.

11.12.1 Features

Hardware Control

IVOCT is an integrated hardware/software solution. Therefore, the IVOCT software has been written to provide control over the acquisition process. Computer control of the MCLK frequency and the resultant frame rate is provided by the IVOCT program. Once the video synchronization circuitry receives the MCLK, it generates the remainder of the synchronization signals. The rapid scanning optical delay line is also controlled by the IVOCT software. The waveform used to drive the galvanometer is generated by the IVOCT program and input into an arbitrary waveform generator using GPIB (RS-422) output. The HSYNC triggers the arbitrary waveform generator to output cycles of the computer generated waveform to the galvanometer controller. Computer control of other functions with GPIB output can be easily accomplished, such as catheter-endoscope pullback for obtaining three-dimensional OCT images. The IVOCT system software also enables selection of the image size by sending a two bit word to the multiplexer in the video synchronization circuitry.

Interactive Operation

In order to efficiently perform high speed imaging in a medical setting, interaction with the imaging system is necessary to optimize the captured images. The IVOCT software provides interactive adjustment of two key parameters, the black and white levels of the displayed image. These parameters represent the analog voltage values that are digitized to black (digitized pixel value = 0) and white (digitized pixel value = 255), respectively. Manipulation of these values while continuous acquisition is in progress enables interactive control of the brightness and contrast of the displayed image. In addition, real time displays of the image histogram, the maximum digitized data value, and the image variance allow the user to dynamically adjust the brightness and contrast to optimize the dynamic range of the image.

Image Display

One of the most important aspects of the IVOCT software is its ability to allow optima-
program allows the user to choose from one of two different gray scale lookup tables, standard and inverse gray scale. In addition, the coordinate system of the displayed images are formatted according to the imaging transducer used in the IVOCT system. For example, a rectangular to polar coordinate transformation is used to display the image for OCT compatible catheter-endoscope imaging of internal organ system lumen. The polar coordinate display configuration also includes scaling markings that are separated by 500 µm.

11.12.2 The User Interface

The IVOCT program uses the Macintosh System 7.5 graphical user interface for simple and intuitive operation. All of the program functions are accessed by means of pull-down menus from the main menu bar.

Menu Options

The main menu bar controls four pull-down menus, File, Edit, Capture, and Video Parameters (Figure 11-5). The File and Edit menus provide standard operations such as saving, preferences, quitting, and clipboard functions (Figure 11-5). The Capture pull-down menu

![Menu Options](image)

**FIGURE 11-5.** IVOCT software main menu bar and File and Edit pull-down menus.

enables initiation of the acquisition process, including the ability to select from three different
display formats, and the capability to capture image data that has been stored on the SVHS recorder.

![Capture Menu](image1.png)

**Figure 11-6.** Contents of the Capture pull-down menu.

Functions used to alter the IVOCT video capture session can be accessed using the Video Parameters pull-down menu. The important adjustable parameters that can be edited and saved

![Video Parameters Menu](image2.png)

**Figure 11-7.** Contents of the Video Parameters pull-down menu.
to a configuration file include the lookup table and the start-up black/white levels. The Frame Rate menu selection is intended to allow the user to change the acquisition rate of the IVOCT system. The image dimensions can be selected using the Frame Size hierarchical menu. Below the Frame Size selection, the interactive black and white levels (brightness and contrast) controls, image statistics, and the histogram can be toggled on or off for the subsequent acquisition session. Finally, the Mirror to VCR function forces the computer display monitor to be mirrored to the SVHS recorder, enabling storage of continuous acquisition data.

**Figure 11-8.** RTheta acquisition screen. This image is intended to show the features of the user interface during the acquisition session, and thus contains no data. The white markers in the image window are separated by 500 μm.

**Acquisition Session**

An image acquisition session begins when the user selects the desired display mode from the Capture pull-down menu. The IVOCT program then prompts the user to input a patient name and identification number. Once these parameters are entered, the IVOCT software displays the acquisition screen, shown in Figure 11-8. The screen contains four different subcompo-
ments, acquisition information, real time statistics and histogram, the image display window, and the interactive level adjustment controls. During continuous acquisition, the image data, the real time statistics and the histogram will update at the frame rate of the IVOCT system. At any point during the acquisition, an 'S' or 's' key may be pressed to save the digital image data. If the space bar is pressed, the acquisition session terminates.

**Display Modes**

The Capture pull-down menu allows the selection of three different display modes or image coordinate transformations, XY, YX, and RTheta. The XY and YX display modes are

![XY and YX Images](image)

**Figure 11-9.** Screen appearance for the different display modes supported by the IVOCT program.

intended to be used with a lateral scanning probe. The surface of the sample in the XY mode is located at top of the image, while in the YX display mode it resides at the right of the displayed image. The RTheta display mode is used in conjunction with the catheter-endoscope. This rectangular to polar coordinate transformation produces a circular image with the center of the image representing the location of the catheter-endoscope.

11.12.3 Image Formatting and Display

Since various display modes are supported by the IVOCT software, DMA from the frame grabber memory directly to the display memory is not possible. In addition, reformatting of the image after acquisition must occur because every other horizontal line is spatially reversed due to the triangle waveform used to drive the galvanometer. While the use of a triangular waveform doubles the number of scans per galvanometer cycle, the direction of optical delay scanning is
reversed with respect to the previous scan. Thus, for all of the display modes, every other line must be spatially reversed before being transferred to the screen.

The image formatting is accomplished by the IVOCT software using a double buffered data transfer scheme. Before the acquisition begins, once the display mode and image size are chosen, the IVOCT software directs the variable rate frame grabber board to DMA the acquired data to an offscreen buffer. Subsequently, a memory address lookup table is allocated that has the same dimensions as the displayed image. Each value of the lookup table array corresponds to a memory location in the offscreen buffer. The memory location is calculated using the coordinate transformation (XY, YX, or RTheta) and the horizontal line reversal. Continuous acquisition is then initiated and data is transferred to the offscreen buffer from the frame grabber. The data is then copied to the screen using the memory address lookup table to determine which offscreen buffer pixel value is to be placed at a given location in the displayed image. This method is computationally efficient because time intensive calculations, such as the coordinate transformation and horizontal scan reversals, are only computed once, before continuous acquisition begins.

Discussion

An OCT system designed for in vivo imaging has been constructed. The major components of this system include a phase control optical path length delay line, a KLM Chromium doped Forsterite laser, and an imaging transducer, such as the OCT compatible catheter-endoscope. The optical components are integrated into a computer system that contains a variable rate frame grabber. Custom electronics provide the master pixel clock, the video synchronization signals, and synchronization to the optical delay line. Video mirroring allows the images to be stored on a Super VHS (SVHS) video cassette recorder. Most of the hardware components reside within a portable enclosure, enabling easy transport of the imaging system.

Image presentation and ease of use have been emphasized in the design of the IVOCT software. Many of the hardware and optical components are controlled by the software resident on the computer. In addition, the IVOCT software is flexible, for it accommodates different lookup tables, display formats, and image sizes, depending upon the application. Image display controls, such as the black and white levels used to capture the OCT images, can be adjusted interactively during an acquisition session. Real time feedback is supplied to the operator by means of a continuously updated histogram and image statistics.
The key new optical components of the IVOCT system, including the KLM Cr\textsuperscript{4+}:Forsterite laser, the rapid scanning phase control optical delay line, and the image transducers make optical biopsy possible. Integrating these essential technologies into an portable, easy to use, imaging workstation, enables these technologies to be effectively used for \textit{in vivo} diagnostic imaging. As a result, this system is the first ever to acquire \textit{in vivo} OCT images of internal organ systems. The capability of IVOCT to acquire optical biopsies is demonstrated in the next chapter. By capturing micron scale, motion artifact free OCT images from the respiratory and gastrointestinal tracts of a living New Zealand White Rabbit, this system has set a new standard for non-invasive high resolution medical imaging technology.
Chapter 12

*IN VIVO OCT IMAGING OF INTERNAL ORGAN SYSTEMS*

12.1 Introduction

The potential of OCT to greatly improve diagnostic capabilities in medicine has been established in a variety of *in vitro* studies. The research presented in Chapters 7-11 describe new technologies that have been developed to enable and implement *in vivo* OCT imaging of external and internal organ systems. These advances in the state of the art include the construction of a catheter-endoscope for enabling OCT imaging access to internal organ systems, a high power low coherence source, a rapid scanning optical delay line, and integration of the components into a portable imaging workstation (IVOCT). The work presented in this chapter represents the culmination of these efforts. The ability of the IVOCT system to perform optical biopsy is demonstrated by capturing OCT images of the respiratory and gastrointestinal tracts of the living New Zealand White Rabbit.

12.2 Methods

12.2.1 OCT System

The *in vivo* imaging study was performed using the IVOCT system described in Chapter 11. The self-phase modulated KLM Cr^4+:Forsterite laser (Chapter 8) was used as the low coherence source for the IVOCT imaging system. The laser was set to produce an output power of 30 mW with a Gaussian FWHM spectral bandwidth of 75 nm, centered at 1280 nm. These parameters corresponded to a measured free space axial resolution of 10 μm and an SNR of 110 dB. The sample arm power was 12 mW. The phase control rapid scanning optical delay line enabled the
acquisition of 2000 axial scans per second. The total optical path length delay produced was approximately 2.8 mm. In the organ systems that were investigated, the lumen radius was generally larger than the total path length delay provided by the phase control system. To compensate for this, the length of the free space region in the sample arm (Figure 11-1) was varied to change the coherence gate to match the diameter of the organ lumen under investigation. As a result, the displayed lumen diameters in the OCT images may not accurately represent the actual diameter.

The second generation OCT compatible catheter-endoscope was inserted in the sample arm. The catheter-endoscope was 2.9 French or had an outer diameter of 1.0 mm. The measured working distance of the catheter-endoscope was approximately 3.0 mm from the central axis. The confocal parameter was 1.9 mm which corresponded to a focused beam diameter of 40 μm. The closed loop feedback electronics of the motor controller unit enabled synchronization of the motor rotation with the IVOCT frame acquisition. Power loss caused by suboptimal coupling and internal reflection within the catheter was 3-4 dB. Thus, the overall SNR of the OCT system coupled into the catheter-endoscope was approximately 106 dB. The axial dimension of each OCT image was 2.3 mm which was digitized to 248 pixels. The number of acquired lateral pixels or axial scans in each image was 512. The image acquisition time was approximately 0.25 seconds. Since a base 10 logarithm RMS converter was used in the detection electronics, all of the image data represented the logarithm of the reflectivity within the tissue. The two-dimensional image data was displayed using a polar coordinate transformation and an inverse gray scale lookup table.

12.2.2 Animal Protocol

Since the tissue microscopic architectural morphology of the New Zealand White Rabbit is similar in structure to humans, this animal model was ideal for the in vivo OCT studies. Animal handling was performed in accordance with MIT Committee on Animal Care guidelines, protocol # 90-125-3. At the age of 12 weeks, a normal New Zealand White Rabbit was anesthetized with ketamine (35 mg/kg), xylazine (5 mg/kg), and glycopyrrolate (0.01 mg/kg), given intramuscularly. Maintenance of the anesthesia was accomplished with intravenous ketamine (8 mg/kg) and xylazine (1 mg/kg), administered via a marginal ear vein. The animal was then placed on a table adjacent to the catheter-endoscope imaging transducer. During the procedure, local injections of lidocaine were given when necessary.

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Imaging of the gastrointestinal tract was performed first because it could be accessed less invasively. Before imaging, the total length of a guiding catheter with an inner diameter larger than 2.9 French was marked on the catheter-endoscope. The guiding catheter was then inserted through the oropharynx. Bite blocks were placed bilaterally, centering between the second and third molar. The chin of the rabbit was placed forward and anterior with the tongue maximally extended to reduce the probability of entering the trachea. When the guiding catheter was advanced to the esophagus, the imaging catheter-endoscope was introduced through the center of the guiding catheter. The catheter-endoscope was fed down the guiding catheter until the marked location on the imaging catheter-endoscope was no longer visible, assuring that the distal optics were outside of the guiding catheter. At this time, the acquisition session commenced.

Imaging of the respiratory tract was performed next. A 1.3 mm inner diameter guiding plastic sheath was introduced into the trachea. The 1 mm diameter OCT catheter-endoscope was then advanced through the sheath to the trachea and the main bronchi. This procedure was repeated 3 or 4 times, washing the transparent window of the endoscope with each pass. For this first experiment, only the trachea and the bronchi were imaged.

After imaging, the rabbit was sacrificed with an intra-arterial injection of 5 ml sodium pentobarbital (65 mg/ml). The imaging sites were determined by introducing the marked catheter-endoscope postmortem. The imaged regions were excised and immersed in 10% formalin in preparation for routine histologic processing.
12.3 Results

12.3.1 Respiratory Tract

Trachea

OCT images of the living New Zealand White Rabbit trachea enable clear differentiation of the epithelium, mucosa, and surrounding cartilage (Figure 12-1). The most superficial layer of

![Image of the in vivo New Zealand White Rabbit trachea.](image)

**Figure 12-1.** OCT image of the *in vivo* New Zealand White Rabbit trachea.

the trachea, which consists of pseudostratified ciliated epithelium (e), can be visualized on the OCT image as a thin structure adjacent to the lumen (Figure 12-1 and Figure 12-2). Since the pseudostratified epithelium is tall, it can be resolved by the OCT system. The light reflected from within the epithelium is relatively low, a finding that is consistent with *in vitro* OCT studies of epithelial surfaces [19, 33]. Below the epithelium, the mucosal stroma (m) can be identified due to
its higher backscattering properties (Figure 12-2). Glands and blood vessels may be found within the mucosal stroma. These mucous-filled glands may be distinguished in OCT images of the trachea as regions of low backscatter in the surrounding highly backscattering stroma (Figure 12-3). The mucosa is supported by hyaline cartilage rings which can be easily seen in the OCT images as large poorly reflecting structures external to the mucosa (Figure 12-1 - Figure 12-3). The cartilage rings are structures that completely surround the circumference of the tracheal mucosa, except for the dorsal regions that contain bands of the trachealis muscle (tm). These bands of smooth muscle aid in expanding and contracting the diameter of the trachea for the regulation of air flow. These muscles can be distinguished from the adjacent cartilage in images acquired by the IVOCT system(Figure 12-4). As can be seen in all of the images, the dynamic range of the IVOCT system is sufficient to enable imaging through the entire rabbit trachea to the underlying adipose and supportive tissue.

**Mainstem Bronchus**

After imaging the rabbit trachea, the catheter-endoscope was introduced into the respiratory tract at a lower level, to view the mainstem bronchus. The diameter of the mainstem bronchus is smaller than the diameter of the trachea. In addition, the columnar epithelium of the
**Figure 12-3.** An OCT image of *in vivo* rabbit glands (g) above the cartilage (c) of the trachea. The inset shows an enlargement of a portion of the main OCT image to better enable visualization of these structures.

Bronchus is much shorter than that of the trachea and thus less likely to be resolved with 10 μm axial resolution. OCT images of the mainstem bronchus display these features, as the epithelium, the mucosal stroma, and the muscular layers below the stroma cannot be distinguished from one another (Figure 12-5). However, the bronchial cartilage which is formed in several histologically distinct plates located ventrally and laterally, can still be seen below the surface (Figure 12-5).
Figure 12-4. OCT image of the *in vivo* rabbit trachea showing the trachealis muscle (tm) and adjacent cartilage (c).
**Figure 12-5.** Image of the rabbit mainstem bronchus taken with the IVOCT system. Arrows point to cartilage (black arrows) below the epithelium and muscular layers. The strong backreflection artifacts in this figure (white arrows) should not be confused with luminal structure and are most likely due to reflection from a defect on the interior of the transparent window of the catheter-endoscope.
12.3.2 Gastrointestinal Tract

Esophagus

Images of the living rabbit esophagus acquired by the IVOCT system enable differentiation of all of the layers of the esophageal wall (Figure 12-6). A portion of Figure 12-6 has been expanded to show the layered structure of the esophagus in greater detail (Figure 12-7). The layers, including the mucosa (m), submucosa (sm), inner muscularis propria (im), outer muscularis propria (om), serosa (s), and the surrounding adipose tissue (a), can be easily differentiated. The high degree of contrast between the layers is due to the difference in backscattering properties of these structures. Visualization of the layer boundaries is further enhanced by reflectance from the refractive index interfaces between adjacent layers. The innermost layer, the mucosa, is

**Figure 12-6.** OCT image of in vivo rabbit esophagus.
Figure 12-7. A portion of Figure 12-6 enlarged to enable better visualization of the esophageal layers of the rabbit. These structures include the mucosa (m), the submucosa (sm), the inner muscular layer (im), the outer muscular layer (om), the serosa (s) and the adipose and vascular supportive tissues (a). The rippled structure of the surface of the mucosa does not represent real cellular structure. It is an artifact caused by the nonlinearity of the galvanometer of the phase control delay line.

readily distinguished owing to its low reflectivity compared with the submucosa. The highly reflecting submucosa (sm) of the rabbit esophagus is located directly below the mucosa. Separation of the submucosa is visible in portions of the OCT image. This separation may be due to the presence of glandular, lymphoid, and vascular structures within the submucosa (Figure 12-7 and Figure 12-8). Glandular structures within the submucosa can be more clearly seen in Figure 12-9. The next two layers beneath the submucosa are the inner muscularis propria layer (im) and outer muscularis propria (om)(Figure 12-7). The inner muscularis is more reflective than the outer muscularis. The thin outermost layer most likely represents the two refractive index boundaries of the serosa (s)(Figure 12-7). Supporting tissues external to the serosa, include adipose tissue and vascular structures (a)(Figure 12-7). These high resolution images demonstrate the capability of OCT to image through the entire rabbit esophagus to the supporting adipose and vascular tissues.
**Figure 12-8.** OCT image of the rabbit esophagus demonstrates the presence of glandular and vascular structures within the submucosa (arrows).
FIGURE 12-9. OCT image of the *in vivo* rabbit esophagus showing a large gland in the submucosa.
12.4 Discussion

For the first time, OCT has been successfully used to produce high resolution (10 μm) images of the architectural morphology of in vivo internal organ systems. Architectural morphologic features of the rabbit esophagus and trachea, including histologic layers, glands, and cartilage, can be clearly identified in the OCT images. Other available cross sectional imaging technologies would not have been able to resolve all of these structures because many of them were separated by less than 100 μm. Remarkably, the quality of the images of the in vivo gastrointestinal and respiratory tracts rivaled that of the best images that have been obtained from in vitro specimens. While this may be due to the smaller dimensions of the rabbits' organs, structures within in vivo images seem to contain higher contrast because in vitro tissue usually has been degraded by cell death and dehydration. Moreover, the OCT system penetration depth allowed complete imaging through the walls of the esophagus and the trachea without any visible degradation due to multiple scattering.

While the results of this first experiment were extremely encouraging, several issues must still be addressed to achieve optimal imaging performance. First, the catheter-endoscope contains internal reflections that can potentially obscure image structure. This can be easily remedied in the next generation catheter-endoscope by applying anti-reflection coating to some of the optical elements in the distal end. In addition, since the catheter had a fixed focal length (approximately 3.5 mm), imaging was only optimal when the lumen diameter was approximately 6.0 mm. In the future, several catheter-endoscopes will be constructed with different focal lengths and the imaging transducer will be selected to best match the dimensions of the organ under investigation. Next, a small artifact that appears as serrated edges can be seen in the OCT images(Figure 12-7). This artifact is caused by nonlinearity of the angular scanning galvanometer in the optical delay line and will be corrected by adjusting the galvanometer to produce a more linear scan. Finally, this experiment showed that for in vivo imaging, specimen motion was significant at 4 fps. In the esophagus and trachea, this motion did not significantly blur the images, but it often did cause misregistration of the polar coordinate transformation (Figure 12-9). The results of this study suggest that for motion sensitive applications, such as imaging the cardiovascular system, the speed of the OCT system must be increased. The most likely candidate for producing an increased scanning optical delay rate is a phase control apparatus that uses a resonant scanner or an AOM.
This OCT imaging study demonstrates an integrated device for obtaining diagnostic information from living subjects that could only previously be obtained by excisional biopsy. High contrast, cross-sectional in vivo images of internal organ systems have been acquired with an axial resolution of 10 μm, representing an important landmark in medical imaging science. The success of this initial experiment indicates that the capability to obtain in vivo optical biopsies of human tissue is not far off in the future.
Chapter 13

SUMMARY AND CONCLUSION

13.1  *In Vitro* Tissue Studies

13.1.1 Gastrointestinal Tract

In order to assess the capability of OCT imaging to obtain clinically useful diagnostic information for optical biopsy, *in vitro* tissue studies have been performed. *In vitro* OCT images of the gastrointestinal tract demonstrate the potential to allow an endoscopist to delineate tissue microstructure at an axial resolution (4 to 16 µm) not available with current imaging technologies. Chapter 3 presents the ability of OCT to resolve gastrointestinal tissue morphology with both a 1300 nm SLD source (16 µm) and a 1260 nm solid state KLM Chromium doped Forsterite laser (4 µm). The high resolution and contrast in the OCT images enable visualization of the histologic tissue layers and morphologic features such as crypts, glands, and vessels. An important future application for OCT in the gastrointestinal tract may be the screening for adenocarcinoma in patients with Barrett’s esophagus. OCT based optical biopsy would enable screening of a much larger area without the recurrent costs associated with regular excisional biopsies and histology processing. These results suggest that OCT may become a useful adjunct diagnostic imaging technology to conventional gastrointestinal endoscopy.

13.1.2 Urinary Tract

The images of the *in vitro* urinary tract show the potential of OCT to allow the urologist to delineate tissue microstructure at an axial resolution up to 25 times higher (4 to 16 µm) than the most commonly used cross-sectional diagnostic imaging device, high frequency ultrasound. In Chapter 4, the ability of OCT to resolve urinary tract tissue morphology with an axial resolution
of 16 μm is demonstrated. Microstructural details such as periurethral exocrine ducts, prostatic glandular secretions, and ureteral muscular layers can be easily identified. A role for OCT in guiding the resection of hyperplastic prostatic parenchyma has been suggested. OCT images, with an axial resolution of 16 μm, sharply delineate the prostate capsule border. Furthermore, neurovascular bundles can be identified in close approximation to the prostatic capsule. Since postoperative impotence and incontinence have been linked to traumatic transsection of these neurovascular bundles, OCT guidance may substantially reduce the morbidity associated with mechanical interventions. The results of this in vitro study demonstrate that OCT may become a powerful diagnostic technology during urologic procedures, particularly the guidance of prostatic resection.

13.1.3 Cardiovascular System

The intravascular use of OCT for imaging atherosclerotic plaque morphology has the potential of overcoming the resolution limitations of intravascular ultrasound. The in vitro study presented in Chapter 5 demonstrates the ability of OCT to delineate in vitro plaque morphology. Structural details such as the thickness of intimal caps, extent of lipid collections, and presence of fissures have been assessed at a level of resolution (16 microns) not achievable by other imaging modalities. In addition, OCT has the capability to image through heavily calcified tissue. The ability of OCT to enable high resolution imaging makes it a promising new technology for intravascular imaging and the diagnosis of high risk coronary lesions.

13.1.4 Other Tissue Systems

The OCT studies of the gastrointestinal tract, urinary tract, and cardiovascular system demonstrate that OCT holds great promise for diagnosing disease on a microstructural level. Furthermore, due to the high resolution of this technique, OCT imaging of other human tissues such as the skin, respiratory tract, and reproductive tracts is also likely to be clinically useful. In addition, OCT may be useful as a tool for guiding surgery. For example, the capability of OCT to enable differentiation normal and cancerous tissues (Chapter 3) may enable its use as a tool for the detection of tumor margins during surgical resection. Further improvements in OCT resolution may provide the capability to image cellular structures and potentially allow this imaging modality to be used for the diagnosis of early stages of cancer.
13.2 In Vivo OCT Technology

OCT has the capability to provide the physician with a tool for obtaining high resolution cross-sectional diagnostic images in situ. However, new technologies must be developed to enable in vivo OCT imaging of external and internal organs systems. The state of the art OCT systems have not been capable of imaging in vivo tissue for the following three reasons; (1) lack of a catheter or endoscope for enabling OCT imaging access to internal organ systems, (2) lack of an adequate source for high speed scanning, and (3) insufficient image acquisition rates. This thesis has addressed each of these issues.

13.2.1 OCT Compatible Catheter-Endoscope

A prototype single mode optical fiber scanning catheter-endoscope for OCT imaging of tissue architectural morphology within human internal organ systems has been designed and constructed. The first generation OCT catheter-endoscope consists of an optical coupling element at its proximal end, a single mode fiber running the length of the catheter, and optical focusing (GRIN lens) and a beam directing element (right angle prism) at the distal end. The catheter-endoscope is designed to scan the beam in a circumferential pattern in order to cross sectionally image through the vessel (or other biological structure) into which it is inserted. The prototype catheter-endoscope has a small diameter (1 mm) to facilitate its entry into lumen such as coronary arteries. Images of an intact ureter, fallopian tube, and coronary artery acquired using the catheter-endoscope are presented in Chapter 7. While these images demonstrate the capability of this imaging transducer, internal reflections from the distal optics prevent the use of the first prototype for high source power applications.

A second generation OCT compatible catheter-endoscope has been designed and constructed for use in in vivo studies which require higher sample arm power. The second generation catheter removes the dominant sources of returned internal reflections from the distal optics. The reflections were removed by angle cleaving the distal end of the single mode fiber and angle polishing the GRIN lens. These modifications to the first prototype have improved OCT compatible catheter-endoscope technology to a mature level that enables the in vivo imaging of internal organ systems.
13.2.2 Low Coherence Sources

In order to perform in vivo OCT imaging, the scanning rate must be increased to avoid artifacts due to patient motion. One important technology necessary for producing a high speed OCT system is a high power, single mode, low coherence source. High source power is essential because the power incident on the sample must be increased commensurately with the increase in scanning speed to preserve the sensitivity of the OCT imaging system. This thesis presents OCT images obtained using two types of optical input, solid state lasers and rare-earth-doped fiber sources.

Solid state lasers provide high power, broad spectrum low coherence light that enable both high speed and high resolution OCT. In their current state of development, they are valuable tools for performing initial in vivo OCT studies. However, the cost and size of these devices make their use impractical outside of a research setting. Development performed to miniaturize KLM solid state lasers would greatly increase the likelihood that these sources could be integrated into a clinical environment.

Results obtained using several different rare-earth-doped fibers including Neodymium (Nd), Ytterbium (Yb), and Thulium (Tm) are described in Chapter 8. The state of the art REDF ASE sources require improvement. Both the filtered Nd:silica and the Yb:silica REDF’s suffer from sidelobes due to a rectangular shaped spectrum. Distortions in the OCT images caused by autocorrelation sidelobes should be improved in second generation ASE devices that not only diminish gain peaking, but shape the spectrum to avoid sidelobes. The Tm:silica REDF sources has a broad enough gain narrowed bandwidth to be used without filtering. While the shape of the Tm:silica REDF ASE spectrum and the optical properties of tissue at 1800 nm make the Tm:silica ASE source the most desirable of all of the REDF’s, the cost of a single mode pump may prohibit its widespread use. Most likely, the next generation clinically viable REDF source will either be a spectrally shaped Nd:silica or Yb:silica REDF with shaping that not only accounts for the spectral narrowing, but also makes the spectrum more Gaussian.

13.2.3 Piezoelectric Optical Fiber Stretching for High Speed OCT

A fast scanning OCT system capable of in vivo imaging of human tissue without image degradation due to motion of the specimen has been constructed. The system integrates a high power short coherence length mode locked solid state laser source and a PZM driven optical
fiber delay line. Images consisting of 300 x 250 pixels with a size of 3 x 2.2 mm were acquired at a rate of four frames per second with a SNR of 112 dB and an axial resolution of 15 μm[108]. The rapid acquisition rates of the high speed OCT system allows motion-artifact-free imaging of living biological specimens such as the embryonic Xenopus laevis. In addition, the high signal to noise ratio was shown to allow penetration to a depth of greater than 2 mm in in vivo human skin.

However, the work presented in Chapter 9 may represent the upper limit for a clinically fieldable high speed optical delay line using an PZM-based optical fiber stretcher. The complexity of the hysteresis correction, the high power requirements, and the temperature-related breakdown of the of the piezoelectric transducers place significant restrictions on the maximum scanning rate that this method can achieve.

13.2.4 Phase Control for High Speed OCT

The phase control optical delay line enables rapid scanning OCT without the limitations of the piezoelectric method. It can be constructed with common optical components, has modest power requirements, is repeatable, and temperature stable. The double passed phase control apparatus is versatile, since many different scanning configurations may be used to produce an optical path length delay. Optical group delay may be produced by scanning the angle, γ, varying the grating incident angle, physically tilting the grating, or changing the grating spacing. Scanning the angle, γ, using a galvanometer or resonant scanner is the only method that is compatible with fixed band pass filter detection electronics. The primary disadvantage of this configuration is that the mirror size places a limit on the spectral bandwidth and resultant axial resolution. Because the other scanning configurations produce a nonlinear phase delay, they must be used in conjunction with an alternative demodulation scheme, such as adaptive mixing. However, these methods do not constrain the usable spectral bandwidth. Once the new demodulation scheme is implemented, the production of a rapidly scanning optical path length delay line using an AOM is particularly desirable, as it contains no moveable parts.

A phase control optical delay line based on the folded double passed γ scanning configuration has been constructed. The scan rate provided by this device is twice that of the PZT fiber stretcher presented in Chapter 9 (2000 axial scans per second) and its axial resolution is 50% greater (10 μm). This system is much simpler to construct, has modest power requirements, and
does not suffer from dropout artifacts due to hysteresis. As a result, the phase control optical delay line has become the foundation for the fastest scanning OCT system to date. Because of the superior performance of this method, it was incorporated into a high speed OCT system (IVOCT) and used to obtain the first in vivo OCT images of internal organs systems.

13.2.5 IVOCT System Integration

An OCT system designed for in vivo imaging has been constructed. The major components of this system include a phase control optical path length delay line, a KLM Chromium doped Forsterite laser, and an image transducer, such as the OCT compatible catheter-endoscope. The optical components are integrated into a computer system that contains a variable rate frame grabber. Custom electronics provide the master pixel clock, the video synchronization signals, and synchronization to the optical delay line. Video mirroring allows the images to be stored on a Super VHS (SVHS) video cassette recorder. Most of the hardware components reside within a portable enclosure, enabling easy transport of the imaging system.

Image presentation, and ease of use have been emphasized in the design of the IVOCT software. Many of the hardware and optical components are controlled by the computer. In addition, the IVOCT software is flexible, for it accommodates different lookup tables, display formats, and image sizes, depending upon the application. Image display controls, such as the black and white levels used for capturing the OCT images can be adjusted interactively during an acquisition session. Real time feedback is supplied to the operator by means of a continuously updated histogram and image statistics.

The key new optical components of the IVOCT system, including the KLM Cr$^{4+}$:Forsterite laser, the rapid scanning phase control optical delay line, and the image transducers make optical biopsy possible. Integrating these essential technologies into an portable, easy to use, imaging workstation, enables these technologies to be effectively used for in vivo diagnostic imaging. As a result, this system is the first ever to acquire in vivo OCT images of internal organ systems. The capability of IVOCT to acquire optical biopsies is demonstrated in Chapter 12. By capturing micron scale, motion artifact free OCT images from the respiratory and gastrointestinal tracts of a living New Zealand White Rabbit, this system has set a new standard for non-invasive high resolution medical imaging technology.
13.3 *In Vivo* OCT Imaging of Internal Organ Systems

For the first time, OCT has been successfully used to produce high resolution (10 \( \mu \text{m} \)) images of the architectural morphology of *in vivo* internal organ systems. Architectural morphologic features of the rabbit esophagus and trachea, including histologic layers, glands, and cartilage, can be clearly identified in the OCT images. The penetration depth of the OCT system allowed complete imaging through the walls of the esophagus and the trachea without any visible degradation due to multiple scattering.

In this experiment, *in vivo* specimen motion was non-negligible at an acquisition rate of 4 fps. While the specimen motion did not significantly blur the images of the respiratory and gastrointestinal tracts, it often did cause misregistration of the display coordinate transformations. The results of this study suggest that for motion sensitive applications, such as imaging the cardiovascular system, the speed of the OCT system must be increased. The most likely candidate for producing an increased scanning optical delay rate is a phase control apparatus that uses a resonant scanner or an AOM.

This OCT imaging study demonstrates an integrated device for obtaining diagnostic information from living subjects that could only previously be obtained by excisional biopsy. High contrast, cross-sectional *in vivo* images of internal organ systems have been acquired with an axial resolution of 10 \( \mu \text{m} \), representing an important landmark in medical imaging science.

13.4 Conclusion

This thesis has presented the use of OCT for obtaining optical biopsies of *in vivo* tissue. An *in vitro* tissue survey has been conducted for establishing several clinically pertinent areas for performing OCT based diagnostics. The results of this tissue survey have motivated the design and construction of new OCT technology that enable *in vivo* diagnostic imaging. This technology has been integrated into an OCT system that has been employed to perform the first *in vivo* endoscopic OCT imaging study. By obtaining micron scale images of the respiratory and gastrointestinal tracts of a living New Zealand White Rabbit, the potential of OCT to become a powerful tool for obtaining *in vivo* optical biopsies has finally been realized.
Appendix A

DISTAL OCT CATHETER-ENDOSCOPE DESIGN

A.1 ABCD Matrix Formalism

The two important design parameters for the distal end of the catheter-endoscope are the focused beam diameter and working distance of the GRIN lens. Analysis of the Gaussian beam using the ABCD matrix formalism is a convenient method for choosing the fiber-GRIN separation to yield an appropriate spot size and working distance for the catheter-endoscope.

A.1.1 The Gaussian Beam

The normalized zeroth order solution of the paraxial wave equation that describes the Gaussian beam profile is defined as,

\[ u(r) = \sqrt{\frac{k z_R}{\pi}} \frac{j}{\lambda(z + j z_R)} \exp \left( -\frac{j k r^2}{2(z + j z_R)} \right), \quad (A-1) \]

where \( r = x^2 + y^2 \) and \( z_R \) is the Rayleigh range[113]. If we separate Eq. (A-1) into real and imaginary components the Gaussian beam profile can be reformulated as,

\[ u(r) = \sqrt{\frac{2}{\pi}} \frac{\exp(j \phi)}{w(z)} \left( \frac{1}{z + j z_R} \right) \exp \left( -\frac{r^2}{w^2(z)} \right) \exp \left( -\frac{j k r^2}{2 R(z)} \right), \quad (A-2) \]
where the beam radius,

$$w(z)^2 = \frac{2z_R}{k} \left(1 + \frac{z^2}{z_R^2}\right), \tag{A-3}$$

the radius of curvature,

$$R(z) = \frac{z^2 + z_R^2}{z}, \tag{A-4}$$

and

$$\tan(\phi) = \frac{z}{z_R}. \tag{A-5}$$

The minimum beam radius, $w_0$, can be found by minimizing Eq. (A-3)[113],

$$w_0 = \sqrt{\frac{2z_R}{k}}. \tag{A-6}$$

**A.1.2 ABCD Matrix**

By defining the $q$ parameter,

$$q = z + jz_R, \tag{A-7}$$

we can completely describe the propagation of the Gaussian beam within the constraints of the paraxial approximation[113]. The real part of the $q$ parameter gives the radius of curvature, while the imaginary part gives the beam radius[113],

$$\frac{1}{z + jz_R} = \frac{z - jz_R}{z^2 + z_R^2} = \frac{1}{R(z)} - \frac{j\lambda}{\pi w^2(z)} = \frac{1}{q}. \tag{A-8}$$
The propagation of the Gaussian beam and thus transformation of $q$ by any optical element may be expressed as the bilinear transformation,

$$q' = \frac{Aq + B}{Cq + D}, \quad (A-9)$$

where ABCD transformation matrices $\begin{bmatrix} A & B \\ C & D \end{bmatrix}$ can be cascaded for many different elements\[113].

The ABCD transformation matrices for several common optical elements are given below. For free space propagation, over a distance, $d$,

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} 1 & d \\ 0 & 1 \end{bmatrix}, \quad (A-10)$$

If the medium has a refractive index, $n$, the ABCD matrix for propagation over a distance, $d$, is

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} 1 & \frac{d}{n} \\ 0 & 1 \end{bmatrix}, \quad (A-11)$$

The ABCD matrix for a thin lens is\[113]

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ \frac{1}{f} & 1 \end{bmatrix}, \quad (A-12)$$

### A.1.3 The GRIN Lens

A GRIN lens consists of a dielectric rod with a parabolic refractive index profile,

$$n = n_0 \left(1 - \frac{r^2}{2h^2}\right), \quad (A-13)$$
where $n_0$ is the refractive index in the center of the rod and $1/h$ is related to the parabolic refractive index profile in the rod[113]. The parabolic profile of the refractive index in the cylinder curves the phase front in a manner similar to a lens. The similarity of the GRIN to a lens can be seen by looking at the refractive index change of the wavefront as a function of a differential element of the lens, $\Delta z$,

$$
\frac{k}{2f} r^2 = \frac{1}{2} \frac{n_0 r^2}{h^2} \Delta z.
$$

(A-14)

By analogy to a lens, we can see that

$$
\frac{1}{f} \equiv \frac{n_0}{h^2} \Delta z.
$$

(A-15)

The ABCD matrix for the GRIN lens must also include the propagation through a medium with a refractive index, $n_0$[113]. This is accomplished by representing two slabs of optical thickness, $\frac{\Delta z}{2n_0}$ before and after the lens ABCD matrix, such that[113]

$$
\begin{bmatrix}
A & B \\
C & D
\end{bmatrix}_{GRIN} = \begin{bmatrix}
1 & \frac{\Delta z}{2n_0} \\
0 & 1
\end{bmatrix} \begin{bmatrix}
1 & 0 \\
\frac{-n_0 \Delta z}{h^2} & 1
\end{bmatrix} \equiv \begin{bmatrix}
1 - \frac{\Delta z^2}{2h^2} & \frac{\Delta z}{n_0} \\
\frac{-n_0 \Delta z}{h^2} & 1 - \frac{\Delta z^2}{2h^2}
\end{bmatrix}.
$$

(A-16)

If the length of the GRIN lens, $l = m \Delta z$, is inserted into (A-16), then the final formula for the ABCD matrix of the GRIN lens can be written as[113]

$$
\begin{bmatrix}
A & B \\
C & D
\end{bmatrix}_{GRIN} = \begin{bmatrix}
\cos \left( \frac{l}{h} \right) & \frac{h}{n_0} \sin \left( \frac{l}{h} \right) \\
\frac{n_0}{h} \sin \left( \frac{l}{h} \right) & \cos \left( \frac{l}{h} \right)
\end{bmatrix}.
$$

(A-17)
The lens-like nature of the GRIN lens can be visualized by identifying the ABCD matrix for a GRIN lens with length $\pi h/2$,

$$
\begin{bmatrix}
A & B \\
C & D
\end{bmatrix}
\begin{bmatrix}
l_
\\n\hphantom{l}\hphantom{\pi}
\end{bmatrix}
= 
\begin{bmatrix}
0 & h \\
-n_0 & h
\end{bmatrix}
$$

(A-18)

which is simply the ABCD matrix for a lens of focal distance, $h/n_0$ with a free space distance, $d = h/n_0$, before and after the lens[113].

**A.1.4 ABCD Matrix for Distal End of Catheter-Endoscope**

The optical elements contained in the distal end of the catheter-endoscope are shown in Figure A-1. Given the definitions in Figure A-1, the ABCD transformation matrix for the distal end of the catheter is

$$
\begin{bmatrix}
A & B \\
C & D
\end{bmatrix}
= 
\begin{bmatrix}
1 & l_d \\
0 & 1
\end{bmatrix}
\begin{bmatrix}
l_p \\
n_p
\end{bmatrix}
\begin{bmatrix}
\cos\left(\frac{l_G}{h}\right) & \frac{h}{n_G} \sin\left(\frac{l_G}{h}\right) \\
-n_G \frac{h}{n_G} \sin\left(\frac{l_G}{h}\right) & \cos\left(\frac{l_G}{h}\right)
\end{bmatrix}
\begin{bmatrix}
l_s \\
n_s
\end{bmatrix}

\begin{bmatrix}
l_s \\
0 & 1
\end{bmatrix}
\begin{bmatrix}
l_d \\
l_d
\end{bmatrix}

(A-19)

**Figure A-1.** Schematic of the distal end of catheter-endoscope.
Given the \( h \) parameter for the GRIN lens and the length of the GRIN lens, the fiber-GRIN separation that gives us the desired spot size and working distance, \( l_f \), needs to be determined. This is accomplished by allowing the \( q \) parameter at both the optical fiber tip and the focus of the GRIN to be entirely imaginary, \( q = jz_R \) and \( q' = jz_R' \). The spot diameter at the optical fiber tip is the mode field diameter,

\[
d = 2w_0 = \sqrt{\frac{2z_R \lambda}{\pi}}, \tag{A-20}
\]

and the spot size at the focus,

\[
d' = 2w_0' = \sqrt{\frac{2z_R' \lambda}{\pi}}. \tag{A-21}
\]

To obtain all of the parameters of the ABCD matrix, the working distance, \( l_f \), must be found. This is accomplished by setting the real part of Eq. (A-9) to zero,

\[
z_R = \sqrt{\frac{-BD}{AC}}, \tag{A-22}
\]

and solving for \( l_f \). The spot diameter at the focus of the GRIN lens can then be calculated from the ABCD matrix,

\[
d' = \sqrt{\frac{2}{\lambda \pi z_R} \left( \frac{1}{D^2 + C^2 z_R^2} \right)}. \tag{A-23}
\]
REFERENCES


