POLARIZATION-MAINTAINING FIBER-BASED OPTICAL COHERENCE TOMOGRAPHY AND ITS APPLICATIONS

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DEDICATION

To my beloved parents,
Yiun-Long and Mei-Ling
ABSTRACT

Recent advances in Optical Coherence Tomography (OCT) have enabled wide ranges of attractive applications, including characterization of biological tissues, morphological tracking, intravascular imaging, developmental biology and in functional spectroscopic aspects. Neural activity has been studied using optical methods in the past few decades. The use of light to probe neural activity allows outstanding resolution, and the possibility for simultaneous measurement from a range of targeted locations without physical contact with the tissue. Optically detectable signals, such as transient changes with phase and backscattered light intensity, are captured using spectral-domain OCT system. In this dissertation, a fundamental introduction to polarization-maintaining fiber-based OCT system is overviewed with particular emphasis on our research.

We have developed polarization-maintaining fiber-based polarization-sensitive OCT systems for various applications. The system is advantageous in that it simultaneously measures the depth-resolved reflectivity, retardance, and axis orientation information. We present the minute Faraday rotations in clear liquids and tissue-mimicking phantoms, and the capability to detect and measure rapid transient structural changes in nerves during activity using spectral-domain OCT with and without exogenous contrast agents. Transient phase changes from backscattered light during action potential propagation show that the scanner can provide high spatiotemporal resolution cross-sectional images of neural activity and the two-dimensional neural functionality will open the possibility for high spatiotemporal functional imaging in a small volume.
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CHAPTER 1 INTRODUCTION

1.1 Motivation

Hundreds of millions of people are affected by neurological disorders worldwide. Neural diseases are a major concern in healthcare and research environments. The causes of neural disorders can be complicated and range from minor ailments to fatal conditions. Neural diseases and nerve cell death is irreversible, and early detection of disease is crucial. Although medication is available for the treatment of different disorders, the need for efficient and accurate diagnostics is pressing because the nervous system cannot replenish dead neuronal cells in most cases. There is not a single non-invasive device that can measure individual action potentials in clinics. As a consequence, understanding of nerve functionality and developing real-time monitoring techniques will be beneficial to fundamental neurophysiological studies.

Currently, the most extensively employed technique for measuring the neural activity is the use of a variety of electrodes. However, bringing electrodes into direct contact with a nerve to perform measurements is an invasive process which can damage the nerve permanently. Over the past few decades, the optical detection of neural activity has been widely reported. Recent advances in depth-resolved optical imaging techniques have enabled non-invasive measurement in tissue. Optical coherence tomography (OCT) is an interferometric imaging technique that provides images of biological tissues with micrometer axial resolution. The non-contact nature of OCT can minimize potential damage to tissue during diagnosis. Among various techniques, spectral-domain optical coherence tomography (SD-OCT) provides high-speed acquisition, and
with better sensitivity and stability, thereby allowing simultaneous recordings of depth-resolved intensity and phase information. In this dissertation, polarization-maintaining fiber-based SD-OCT has been utilized as a functional extension to detect nerve functionality. SD-OCT has proven to be capable of measuring the changes in backscattered light intensity and displacements associated with the nerve activity. Optical detection of the small changes is useful in a broad spectrum of biomedical imaging and sensing applications.

An in-depth understanding of the physiological basis of neural activity from the cellular to brain level requires integration of multi-scale knowledge. Both structural and functional information are valuable for combining an anatomical map with precise physiological details. In functional aspects, optical imaging techniques have been widely used in studies of vasculature and hemodynamics. Efforts to measure the intrinsic optical signal and transient structural changes using OCT have been reported in various isolated nerve preparations during action potential propagation. Despite the diverse set of approaches that can be applied to monitor the neural activity, monitoring with high spatiotemporal resolution at multiple sites simultaneously at different depths is still limited. In addition, intrinsic optical signal changes are often very small, thus, the use of added molecular probes as a contrast agent to monitor nerve activity needs further investigation. In the past few years, we have attempted to extend functional OCT applications from a single depth-profile to a cross-section. Moreover, the integration of dual-wavelength OCT systems and the use of different spectroscopic contrast-enhancing voltage-sensitive dyes were explored. All the experiments reported in Chapter 5 and Chapter 6 were conducted in the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts as part of this dissertation.
1.2 Scope of the Dissertation

The goal of this dissertation is to introduce various optical coherence tomography applications which can be potentially used as a measure of neural functionality. This dissertation is organized in eight chapters.

Chapter 2 provides a review of the fundamentals, principles, limitations and different modalities of OCT. The principles and mathematical representations of time-domain OCT (TD-OCT) and Fourier-domain (FD-OCT) systems are described. The last section of this chapter summarizes and discusses the functional extensions of OCT and their applications.

Chapter 3 describes the Polarization-maintaining fiber-based (PMF) polarization-sensitive OCT (PS-OCT) instrumentation. A PMF based PS-OCT system was built and characterized. In this OCT system, an area-scan camera was introduced for spectral acquisition of two polarization channels. The optical setup, calibration of spectrometer, signal processing and system performance and optical problems are explained.

Chapter 4 demonstrates the application of the PMF based PS-OCT system presented in Chapter 3. The optical system is slightly modified to measure the minute Faraday rotations of clear liquids and turbid media. Unlike conventional transmission-mode measurements, this approach is capable of probing multiple depths simultaneously in reflection-mode, which is attractive for biological assessments.

Chapter 5 investigates a dual-wavelength OCT system for neural activity with voltage-sensitive dyes. In this work, we designed and constructed a dual-wavelength spectral-domain OCT system using two wavelength bands at 690 and 840 nm. Eighteen different voltage-sensitive dyes were used and tested for in vitro experiments. The observations of transient changes in light scattering and displacements of the stained nerves during excitation are reported.
Chapter 6 presents a cross-sectional imaging technique for transient phase changes during neural activity. This chapter demonstrates the feasibility of extending of from a single depth-profile to a cross-section to detect neural activity. Transient phase changes from backscattered light were detected during action potential propagation using both stained and unstained squid nerves. Compared to a single depth-profile, this technique can provide cross-sectional images of neural activity with high spatiotemporal resolution.

Chapter 7 presents the use of OCT for measuring the neural activity from the pike olfactory nerves, which is densely packed with unmyelinated small axons. The optical system is slightly modified and acquisition parameters are adjusted to measure the neural activity associated with compound action potentials. Both m-mode and cross-sectional imaging of transient changes during neural activity with and without dyes as contrast agents are reported. Transient structural changes and cross-polarized transmission-mode intensity change were both detected along with action potential propagation.

Chapter 8 summarizes the overall findings of this study and highlights the significant contributions that can lead to future research studies.
CHAPTER 2 OPTICAL COHERENCE TOMOGRAPHY

Optical coherence tomography (OCT) is a rapidly developing high resolution, non-invasive, cross-sectional imaging technique with a wide range of applications in biomedical fields, including dermatology, ophthalmology, cardiology, developmental biology. OCT utilizes low coherence light to image tissues up to a depth of a few millimeter with an axial resolution on the order of micrometers [1]. Recent revolution in OCT techniques based on spectral rather than scanning interferometry (time-domain) has opened up the possibility of faster scanning for biomedical applications. Extensions of OCT systems, including polarization-sensitive OCT, functional OCT and wavelength-dependent OCT, expand the basic imaging modality to functional perspectives. The diversified extensions of OCT systems would enable a broad spectrum of research and clinical applications.
2.1 Introduction

Interferometry is a conventional optical method of using interference to measure physical parameters, such as amplitude and phase of electromagnetic waves. The fundamental principle behind interferometry is the superposition of two waves where they superimpose constructively or destructively. Optical coherence tomography is first introduced by Fujimoto’s group in 1991 [2]. It is crucial to mention that the origin of OCT is based on white-light interferometry which led to the advances of optical coherence-domain reflectometry [3], [4]. OCT is analogous to ultrasound except that it uses light (transverse wave) as opposed to sound (longitudinal wave). In terms of imaging depth and spatial resolution, OCT bridges the gap between confocal microscopy and high frequency ultrasound. The advantages of OCT over other imaging systems are high-resolution, non-invasive technique, non-ionized radiation, high speed, fiber-optics delivery and the potential for performing additional contrasts [5]. The fiber-based OCT system allows the integration with catheter and endoscope, which would be capable of clinical applications.

The aim of this chapter is to state the basic concepts of OCT system, including the signal properties, characteristics and principles. The extensions of OCT are described toward the end of this chapter.

2.2 OCT Signal origins

OCT has been demonstrated to be a powerful method for medical imaging. The image contrast originates from optical scattering by illuminating a sample with low-coherence light. Light penetration within a tissue is limited by the scattering and absorption properties. In addition, the polarization states of propagation light come into play if the sample is birefringent.

Due to the fact that the optical properties of the tissue vary with wavelength [6], the wavelength band of the light source of OCT systems should be carefully selected. In general, the
scattering decreases with the increasing wavelength in the range of 600 nm to 1400 nm [7]. Therefore, near infrared (NIR) wavelength range is potentially more beneficial for deeper imaging. Traditionally, OCT has been operated at three wavelength bands (840, 1060 and 1310 nm). 1310 nm has been extensively used for imaging highly scattering tissues for an improved imaging depth. However, the use of longer wavelengths for improving imaging depth is limited by the increased optical absorption of water [6].

2.3 OCT Characteristics

2.3.1 Resolution

The limiting factors include the axial and lateral resolution, which are independent from each other. The axial resolution $\Delta z$ (or coherence length $\lambda_c$) of an OCT system in a medium with refractive index $n$ is governed by the source parameters,

$$\Delta z = l_c \sim \frac{2 \ln 2 \lambda_0^2}{n \pi \Delta \lambda}$$

(2.1)

where $\lambda_0$ and $\Delta \lambda$ are the central wavelength and the FWHM (full width at half maximum) bandwidth of the source with Gaussian spectral distribution, respectively. Therefore, to achieve a better axial resolution, broadband sources are required.

A cross-sectional image is generated when the sample path is scanned laterally. The lateral resolution $\Delta x$ is determined by the focusing optics of the sample arm and is given by

$$\Delta x = \frac{4 \lambda_0}{\pi} \left( \frac{f}{d} \right)$$

(2.2)

where $d$ is the spot size on the focusing lens and $f$ is its focal length. Higher lateral resolution can be obtained by using higher numerical aperture. However, with a higher lateral resolution, the depth of field will be decreased.
2.3.2 Depth of Range

The trade-off between the lateral resolution and the depth of focus (DOF) or the confocal parameter $b$ is given by

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

(2.3)

where $Z_R$ is Rayleigh range. Thus, the axial image range is limited by Rayleigh range of the sample beam. It is crucial to design the sample optics to obtain the best possible lateral resolution without sacrificing the DOF. On the contrary, the depth of field can be shorter than the coherence length, i.e. $b < l_c$. This operating regime has been referred to as optical coherence microscopy (OCM). It is feasible to extend the DOF by acquiring multiple images with different focusing zones and fusing the images together.

2.3.3 Signal to Noise

The quality of an optical system is usually characterized in terms of signal-to-noise ratio. The main noise sources in OCT system are the detector noise (thermal, readout noise) $\sigma_{det}^2$, the relative intensity noise $\sigma_{RIN}^2$, and the shot noise $\sigma_{shot}^2$. The SNR can be given by the following expression [8]

$$\text{SNR} \propto \frac{P}{\sigma_{det}^2 + \sigma_{RIN}^2 + \sigma_{shot}^2}$$

(2.4)

where $P$ is the power of the signal. In the optimal configuration, i.e. shot-noise limited system, the SNR performance can be calculated by

$$SNR = \frac{\eta P}{2h\nu(NEB)}$$

(2.5)

where $\eta$ is the detector quantum efficiency, $h\nu$ is the photon energy, and NEB is the noise equivalent bandwidth of the detection. For a shot-noise limited OCT system, the contributions of other noises thus have minimum impact on the sensitivity. The desired signal can be amplified by
increasing the optical power onto the sample or gaining the integration time of the detector. However, the power on the sample is regulated by safety standards for biomedical imaging and the efficiency of the available light sources.

2.3.4 Sensitivity

OCT can achieve high sensitivity by using optical heterodyne detection. The interference term is the multiplication of the backscattered electric field from the sample and reference arm. Thus, the interferometer produces heterodyne gain for weak signals from the sample. The quality of an OCT system is usually stated in terms of sensitivity, defined as a minimum reflected signal that can be detected over the noise floor (i.e. SNR~1) [9]. Typically, the sensitivity of OCT can be easily over 90 dB [10], [11].

2.3.5 Phase Noise

The phase of the complex depth profile yields quantification of optical path length variations. The phase values are largely affected by environmental perturbations, including mechanical and thermal vibrations. The phase noise is related to the intensity SNR, and the phase sensitivity can be characterized by the variance of the phase SNR [12], [13]. The variance of the phase noise $\langle \sigma_{\phi}^2 \rangle$ (unit: radian) is inversely proportional to the SNR of the intensity as

$$\langle \sigma_{\phi}^2 \rangle \approx \frac{1}{2SNR}$$  \hspace{1cm} (2.6)

2.4 Principles of optical coherence tomography

The basics of OCT employ a low-temporal-coherence Michelson interferometer, where interference fringes are observed when the optical path length of light reflected from the sample matches that from the reference within the coherence length of the source. A depth profile of
sample is acquired by scanning the length of the reference arm. Typically, OCT systems can be categorized into time-domain and Fourier-domain OCT. A brief description of time-domain and Fourier-domain OCT is presented below.

2.4.1 Time-domain OCT (TD-OCT)

During the early stage of OCT imaging, the depth-resolved signal (A-scan) was accomplished by moving the mirror in the reference arm of the interferometer in order to coherently get backscattered light from different depths within the sample.

![A common TD-OCT system.](image)

A common TD-OCT system schematic is depicted in Figure 2.1. Light from a broadband source with center wavelength $\lambda_0$ and bandwidth $\Delta\lambda$ is split into two parts $E_{\text{ref}}$ and $E_{\text{sam}}$ by the beam splitter (BS). $E_{\text{ref}}$ is focused onto the reference reflector and reflected back; whereas Field $E_{\text{sam}}$ goes through the sample path and is focused on the sample. Light traveling back from sample
and reference arms recombines and interferes and is directed to the detection arm. The detection of light waves from the interferometer is expressed as,

\[
I_d = \langle |E_{ref} + E_{sam}|^2 \rangle = \frac{1}{2} (I_{ref} + I_{sam}) + \sqrt{I_{ref} I_{sam}} \Re \{\langle E_{ref}^* (t + \tau) E_{sam}(t) \rangle \}
\]

(2.7)

where \(I_{ref}\) and \(I_{sam}\) are the intensities of time-averaged electric fields returning from reference and sample path, and \(\Re\) represents the real part of a complex function. The cross-correlation term in equation (2.1) is the real part of the mutual coherence function of the interference with their optical time delay \(\tau\).

\[
\Re \{\langle E_{ref}^* (t + \tau) E_{sam}(t) \rangle \} = |I(F)| \cos \left[ \frac{2\pi c}{\lambda_0} \tau + \varphi(\tau) \right]
\]

(2.8)

where \(|G(\tau)|\) is the complex temporal coherence function modulated by fringes of frequency determined by the center wavelength of the source \(\lambda_0\) with the path length difference between reference and sample path \(\varphi(\tau)\). The envelope of the temporal coherence function is weighed by the square root of the time averaged intensities from reference and sample path \(\sqrt{I_{ref} I_{sam}}\).

According to Wiener-Khinchin theorem, the relation between the complex temporal coherence function \(G(\tau)\) and the power spectral density of the source \(S(\nu)\) is

\[
G(\tau) = \int_0^\infty S(\nu) e^{-i2\pi \nu \tau} d\nu
\]

(2.9)

Figure 2.2 shows a sample of two reflectors and the corresponding A-line. The two reflectors cannot be resolved if they are closer than the width of the coherence function, which is limited by the bandwidth and the wavelength of the light source.
Figure 2.2 The A-line of the sample shows a coherence function corresponding to each reflector in the sample. The DC level is ignored.

In a TD-OCT system, depth information is acquired by scanning the mirror in the reference arm, and the axial resolution is determined by the coherence length of the light source being used. Due to the limitation for mechanically scanning the mirror required for a typical A-line of OCT, TD-OCT is relatively slow in practical applications. With the technological advances in sources and detectors, SD-OCT is developed with a faster acquisition rate, which is introduced below.

2.4.2 Fourier-domain OCT (FD-OCT)

Instead of scanning a reference reflector to obtain a single depth profile, Fourier/spectral-domain OCT (FD-OCT/SD-OCT) uses a stationary in reference arm which provides faster data
acquisition and better sensitivity and stability than TD-OCT [14]. The other technique is swept-source OCT (SS-OCT), which realized by sweeping the source spectrum rapidly in the source arm and using a single detector in the detection arm in order to achieve spectral discrimination. Spectral-domain OCT utilizes the spectrometer in the detection arm to simultaneously capture the spectrum of the interferometric signals from reference and sample arms. The detected wavelength-dependent intensity $I(k)$ can be written as [15]

$$I(k) = I_{\text{ref}}(k) + I_{\text{sam}}(k) + 2 \sqrt{I_{\text{ref}}(k)I_{\text{sam}}(k)} \sum R_n(z_n) \cos(kz_n)$$  \hspace{1cm} (2.10)

where $k$ is the wavenumber, $I_{\text{ref}}(k)$ and $I_{\text{sam}}(k)$ are the intensities reflected from reference and sample arms. $R_n$ is the reflectivity from depth $z_n$. Different path lengths $z_n$ in the sample arm originate from the scattering centers at different depths within the sample. Spectral interference of the two arms exhibits a spectral modulation as a function of wavenumber. The depth profile (A-line) is calculated by an inverse Fourier transform, which is given by [15],

$$|\delta^{-1}[I(k)]|^2 = \Gamma^2(z) \otimes \left\{ \delta(0) + \sum \alpha_n^2 \delta(z - z_n) + \sum \alpha_n^2 \delta(z + z_n) + O\left[\frac{I_{\text{sam}}^2}{I_{\text{ref}}^2}\right]\right\}$$  \hspace{1cm} (2.11)

where $\Gamma(z)$ is the envelope of the coherence function, the $\delta(0)$ is the autocorrelation signal, $\sum \alpha_n^2 \delta(z - z_n)$ and $\sum \alpha_n^2 \delta(z + z_n)$ are mirror images of the interference from the depth $z_n$, and $O\left[\frac{I_{\text{sam}}^2}{I_{\text{ref}}^2}\right]$ is the autocorrelation noise of interference within the sample arm, which can be ignored.

Figure 2.3 gives an example of the concept of the SD-OCT. A single reflector placed in the sample path shows the interference of the spectrum at the custom-built spectrometer when the optical path difference between the sample and reference arm is within the imaging depth. The spectral modulation frequency increases when the optical path length difference between sample and reference arms increases. Considering more complex structures, the scatterers from different
depths will add more modulation frequencies. All spectral modulation frequencies are contributed by back-scattered photons from all the depths within the sample.

SD-OCT technique yields simultaneous intensity and phase information of a depth profile, which are useful for functional imaging.

![Figure 2.3 Interference fringes and the depth profiles after FFT in FD-OCT](image)

2.5 Polarization-sensitive OCT (PS-OCT)

Polarization-sensitive OCT (PS-OCT), a functional extension of OCT which can measure reflectivity, retardance, optic axis orientation and form-biattenuation [16], provides additional contrasts in OCT based on these parameters. PS-OCT can be used to characterize optical anisotropy properties from biological tissues, which is important for early clinical implications and basic understanding of pathological studies. The polarization of light, birefringence, form birefringence and Jones matrix formalism will be described below.

2.5.1 Polarization of Light

A light wave is a traverse electromagnetic field oscillation contained in a plane \((x, y)\) to the propagation direction \(z\). Consider the case of a monochromatic wave, in which the two orthogonal electric field oscillations of the wave can be written as
\begin{align*}
\vec{E}_x(z, t) &= |E_x| \cos(kz - \omega t) \hat{x} \\
\vec{E}_y(z, t) &= |E_y| \cos(kz - \omega t + \phi) \hat{y}
\end{align*}

where \(k\) is the wavenumber, \(|E_x|\) and \(|E_y|\) are the amplitudes, and \(\phi\) is the relative phase difference between the waves.

The resultant electric field oscillation is given by

\[ \vec{E}(z, t) = \vec{E}_x(z, t) + \vec{E}_y(z, t) \]

If \(\phi = \pm 2n\pi\) \((n=0, 1, 2, 3\ldots)\), the waves are in-phase and the polarization is linear with orientation determined by the ratio between \(|E_x|\) and \(|E_y|\). When \(\phi = \pm \pi/2 + 2m\pi\) \((m=0, 1, 2, 3\ldots)\), and \(|E_x|=|E_y|\), the polarization state is circular. When \(\phi \neq 0\), the polarization state is elliptical. The biological samples and other inhomogeneous media cause scattering that may alter the propagation direction, polarization states and phase of light.

### 2.5.2 Multi-contrast imaging

PS-OCT images retardance in depth, and optic axis orientation for fiber alignment. Figure 2.4 demonstrates the reflectivity with a dynamic range of 55 dB, phase retardance and relative axis orientation images from facet capsular ligament, respectively. High-contrast banding patterns can be found in the retardance image because of birefringence natures in the facet capsular ligament. These contrasts can be simultaneously acquired by using polarization-maintaining fiber-based OCT system.
Figure 2.4 Reflectivity, phase retardance (in degree) and relative axis orientation (in degree) images of the facet capsular ligament (FCL).

2.5.3 Birefringence

Birefringence ($\Delta n$) is the optical property of optical anisotropic materials having refractive indices that are not the same in all directions. A birefringent material experiences two refractive indices in different directions and the difference can be measured as,

$$\Delta n = n_e - n_o$$

---

1 The related images were published in the following peer-reviewed article:
where \( n_o \) is the ordinary refractive index and \( n_e \) is the extraordinary refractive index. The well-knowned birefringent crystals are calcite and quartz. A popular demonstration of birefringence is the production of a double image from calcite as shown in Fig 2.2.

![Calcite](http://www.newvisiondisplay.com/re_polarization.htm)

Intrinsic birefringence is resulting from the anisotropic properties of the molecules in the crystal itself, whereas form-birefringence originated from a medium containing well-aligned elongated structures whose period is smaller than the wavelength of incident light.

Highly organized structures in tissue, particularly parallel linear structures, can exhibit form-birefringence that is sensitive to light polarization states. Form-birefringence appears in various biological samples, including collagen fibers, muscle, cartilage, retina nerve fiber (RNFL) [17] and tendons.

Incremental phase retardations \( \delta(z) \) accumulate through birefringent samples linearly and the retardation (\( \delta \)) between the orthogonal oscillating fields after propagating a distance \( z \) is given by

\[
\delta(z) = \frac{2\pi \Delta n}{\lambda_o} z
\]

(2.12)

where unit of \( \delta \) is in radian. Extracting the birefringence information of tissue may detect the change of the fiber organization and indicate disease or abnormality.

Figure 2.5 Calcite produces a double image because of its birefringent property (http://www.newvisiondisplay.com/re_polarization.html)
2.6 Functional OCT

Although OCT provides morphological investigations, functional OCT recognizes that optical properties change in tissues during physiological events. “Functional” means medically or physiologically relevant information about the condition of the tissue and the processes occurring in the tissue. Therefore, quantitative measurement of optical properties can be used for diagnosis and treatment monitoring. Functional OCT is particularly useful for applications, such as imaging blood flow velocity in human skin [18] and action potential-related neural activity [19]–[21].

2.7 Spectroscopic OCT

In addition to providing structural, functional and physiological information, OCT techniques can be used for extracting spectroscopic information [22]–[25]. Two samples with the same amount of backscattered light intensity cannot be differentiated by using conventional OCT. To improve the contrast of the system, spectroscopic OCT targets on imaging spectral absorption and spectral scattering that can retrieve molecular information. Spectroscopic OCT can apply to extract functional information from tissue, for example, cross-sectional mapping of tissue oxygenation saturation level or differentiation of tissue pathologies by localized spectroscopic properties or functional states.
CHAPTER 3 PMF-BASED SPECTRAL DOMIAN PS-OCT

We have introduced the basic background of optical coherence tomography previously. Polarization-sensitive OCT, being a functional extension of OCT, provides depth-resolved information of the polarization states from a sample. Fiber-based implementations offer easy alignment, flexibility and compact size, compared to bulk optical system setup. A polarization-maintaining fiber-based spectral-domain interferometer that utilizes a low-coherent light source and a single camera is presented. A custom spectrometer using an area-scan camera separates the orthogonal channels and records the interference-related oscillations on both spectra. This design simplifies the camera alignment. The implementation of the fiber-based optical coherence tomography was described in this Chapter.
3.1 Introduction

Polarization-sensitive optical coherence tomography (PS-OCT) provides multiple contrasts by measuring the polarization state of back-reflected light from the sample. PS-OCT quantitatively images depth-resolved tissue birefringent properties originating from the difference of refractive indices between the polarized light propagating parallel and perpendicular to the axis of anisotropy. This enables the extraction of not only structural but also functional information of tissues. To control the polarization state of light with single-mode fiber-based optical system is difficult owing to the random changes that occur in the single-mode fiber as the light propagating through [26]. To overcome this limitation, polarization-maintaining (PM) fibers utilize the stress elements to maintain the polarization states of the propagation light.

Free-space PS-OCT systems with dual [27] or single [28] detector setups have been demonstrated earlier. However, bulk systems are not easy to be incorporated with endoscopic applications. Fiber-based systems offer ease of alignment, flexibility and compact size. In SMF, the polarization state of the propagating light can be changed by several factors, including environmental disturbance, fiber movement and fiber imperfections. Also, multiple measurements of different polarization states impinging onto the sample need to be acquired. The presence of polarization-maintaining fiber (PMF) opens up the possibility to simultaneously detect two spectrally interferometric fringes holding different polarization information. As the earlier chapter stated, Fourier-domain OCT systems have the superior speed and sensitivity [10], [11] over the time-domain OCT systems. We designed and constructed the visible PS-OCT in spectral domain. The potential applications for using the visible spectral domain PS-OCT for Faraday rotation and extracting functional information are explored in the later chapters.

In this chapter, Optical setup, Jones matrix calculation, and system performance tests are described and presented as follows.
3.2 Polarization-maintaining fibers (PMF)

Polarization-maintaining fibers (PMF) are a special type of single-mode fibers utilizing two stress rods inserted to the fiber core along the cladding to induce birefringence. The two polarization modes are forced to travel in different velocities due to the birefringence introduced within the core. Therefore, PMF preserves two orthogonal polarization states even if the fiber is bent. PMF is used in special applications where maintaining polarization is crucial. Several different geometries, such as PANDA, Bow-tie, and elliptical jacket, are used to produce stress birefringence in a fiber. Figure 3.1 shows the cross-section of Panda PM fiber.

![Figure 3.1 The cross-section of Panda PM fiber. S: slow axis, F: fast axis. The two stress rods create the stress on the core of the fiber in order to preserve two simultaneously propagating polarization states.](image)

The polarization isolation of PMF, which we used to build the visible OCT system, is roughly 25 dB from the test data provided by the manufacturer.

3.3 System setup

A 5 mW superluminescent diode (SLD) (Superlum, Russia) centered at 687 nm with a FWHM of 8.9 nm is protected and polarized by the isolator (IOB-3D-690-VLP, Thorlabs). A 2x2
PM coupler (Model 954P, Evanescent Optics Inc.) directs the input light equally to the reference and sample arm. In the reference arm, light is collimated and passes through a quarter-wave plate (QWP) oriented at 22.5° with respect to the input polarization state, and is reflected from a movable reflector. After a roundtrip, the polarization state becomes linear state oriented at 45°, which is coupled back equally to the slow and fast channels of the PMF. The sample arm comprises a collimator, a QWP oriented at 45°, a galvanometer mirror and an achromatic focusing lens (f = 30 mm). The reason for the QWP oriented at 45° is to provide circular polarized light to the sample. Backreflected light from the sample propagating back through QWP oriented at 45° carries the information of birefringence and optic axis orientation of the sample.

The reference and sample light are interfered and recombined at the coupler and directed to the detection arm. The detection arm consists of a collimator, a transmission grating (1800 lines/mm, Wasatch Photonics) with the incident angle of 37.7° to the surface normal, a Wollaston prism with splitting angle of 0.5° oriented at 45°, an achromatic focusing lens (f = 75 mm) and an area scan camera (acA2000-340km, Basler, Germany). The area scan camera has the advantage of easy alignment.

![Schematic diagram of the PMF-based PSOCT.](image)

**Figure 3.2** Schematic diagram of the PMF-based PSOCT. SLD – Superluminescent diode, PC – polarization controller, C – collimator, FB – fiber bench, PM – polarization maintaining, QWP – quarter-wave plate, L – lens, R – reflector, M – mirror, G – transmission grating, WP – Wollaston prism, ASC – area scan camera.
3.3.1 Jones Matrix Calculation

Jones matrix method, which is concerned with the polarization state of the light, is invented by American Physicist R. Clark Jones in 1941. The Jones vector representation of various polarization states is listed in Table 3.1.

<table>
<thead>
<tr>
<th>Polarization State</th>
<th>Jones Vector</th>
</tr>
</thead>
</table>
| ![Diagram 1](image1.png) | \[
\begin{bmatrix}
\cos \Phi \\
\sin \Phi
\end{bmatrix}
\] |
| ![Diagram 2](image2.png) | \[
\frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ -i \end{bmatrix}
\] |
| ![Diagram 3](image3.png) | \[
\frac{1}{\sqrt{2}} \begin{bmatrix} 1 \\ i \end{bmatrix}
\] |
| ![Diagram 4](image4.png) | \[
\begin{bmatrix} a \cos \Phi + ib \sin \Phi \\ a \sin \Phi - ib \cos \Phi \end{bmatrix}
\] |
The input light polarization after passing through the polarizer is described by the Jones vector as

\[ \overrightarrow{E_{in}} = E_o \begin{bmatrix} 1 \\ 0 \end{bmatrix} \]

where \( \overrightarrow{E_{in}} \) is the input electric field and \( E_o \) is the input scalar electric field.

The polarization state of a beam propagating through the optical components can be calculated by multiplying the Jones vector of the incident light by the 2x2 Jones matrix corresponding to the optical components. If the light passes through a series of optical components represented by the matrices \( J_1, J_2, \ldots, J_N \), then the transmitted polarization state can be calculated as

\[ \overrightarrow{E'} = J_N J_{N-1} \cdots J_2 J_1 \overrightarrow{E} \]

where \( J_N \) is the Jones matrix of the \( N^{th} \) optical component and \( \overrightarrow{E} \) is the Jones vector of the incident light. Note that the matrices do not commute.

The Jones matrix of a retarder with phase retardance \( \delta \) and fast axis orientation \( \theta \) is given by [29]

\[
J(\delta, \theta) = \begin{bmatrix}
cos^2 \theta + \sin^2 \theta e^{-i\delta} & \cos \theta \sin \theta (1 - e^{-i\delta}) \\
\cos \theta \sin \theta (1 - e^{-i\delta}) & \cos^2 \theta e^{-i\delta} + \sin^2 \theta
\end{bmatrix}
\]

For the reference arm, the polarization state after double passing the QWP with orientation at 22.5° (\( \delta = \frac{\pi}{2} \) and \( \theta = \frac{\pi}{8} \)) is
where $\vec{E}_{\text{ref}}$ is the Jones vector of the light entering the reference arm. Note that depending on the splitting ratio of the coupler, the proportionality constant is a portion of the input scalar electric field.

In the sample arm, the light passes through QWP with orientation at 45° ($\delta = \frac{\pi}{2}$ and $\theta = \frac{\pi}{4}$) and the sample (reflectivity $R$, phase retardance $\delta$, and fast axis orientation $\theta$) twice. The Jones vector of light returning from the sample arm can be written as

$$
\vec{E}_{\text{sample}} = J_{\text{QWP}}^T (45^\circ) \cdot J_{\text{sample}} (\delta, \theta) \cdot \sqrt{R} \cdot J_{\text{QWP}} (45^\circ) \vec{E}_{\text{si}}
$$

where $\vec{E}_{\text{si}}$ is the Jones vector of the light entering the sample arm.

Solving the equation above yields

$$
\vec{E}_{\text{sample}} \propto \sqrt{R} \begin{bmatrix} \sin \delta \\ \cos \delta e^{(2\theta+i\pi)} \end{bmatrix}
$$

The Jones vector in the sample arm gives the information about reflectivity, phase retardance and the optic axis orientation of the sample.

### 3.3.2 Spectrometer calibration

A custom spectrometer, which is designed to simplify camera alignment, separates the orthogonal channels and records the interference-related oscillations on both spectra. The spectrally encoded fringes from the area scan camera contain the depth-resolved amplitude and phase information. However, the wavelengths $\lambda_1, \lambda_2, \ldots, \lambda_{N-1}, \lambda_N$ incident on a given pixel 1, 2, 3, ..., N-1, N of the camera for both channels are yet to be determined. In principle, grating equation and geometric optics can be employed to calculate the relationship correspondingly.

Here, the grating equation is given as

$$
d (\sin \theta_i + \sin \theta_e) = m \lambda
$$

25
where \( d \) is distance between adjacent grooves in the grating, \( \lambda \) is the wavelength, and \( \theta_i \) and \( \theta_m \) are the incident angle and the diffracted angle with respect to the grating surface normal of order \( m \). 

However, the nonlinearity from the combination of the optical elements renders it hard to get an accurate mapping from camera pixel domain to wavelength domain. The calibration process could be done by replacing the SLD with a tunable laser and simultaneously recording the pixel number on the camera and the wavelength from the commercial spectrometer. However, we could not get the appropriate bandwidth laser source to calibrate the spectra. Therefore, we calibrated the custom-built spectrometer with a commercial one by developing a method that launches various wavelengths of the source onto these spectrometers consecutively. Figure 3.3 depicts the spectrometer calibration setup. The selection of wavelengths is achieved by placing a diffraction grating (GR50-1208, Thorlabs) in the reference arm. The spectral component, whose optical axis is orthogonal to an end mirror, is coupled back into the PMF; hence, selection of a single wavelength is realized by adjusting the mirror. Figure 3.4 shows the spectra of two channels on the area scan camera. The displacement of the wavelength between the spectra is due to the 45° orientation of the Wollaston prism. For 19 different wavelengths, corresponding pixel numbers are identified for the two channels. At the same time, the zero-order component of the transmission grating is coupled to the multi-mode fiber of the commercial spectrometer for actual reading of the wavelength. The readings are fitted to a curve, which is then interpolated to determine the wavelengths for all pixels. Because the empirical data links an individual wavelength to a corresponding pixel on the camera, thus it provides more accurate results to use the calibration method described above than using the grating equation for remapping the spectra for the spectrometer.

Figure 3.4 The spectra of two channels on the area scan camera when launching the selected single wavelength realized by adjusting the mirror

Further, we calculated $\sum |\lambda_{\text{est}} - \lambda_{\text{measured}}|^2$ to evaluate the first-order and second-order least square fitting to the measured data and we found that second-order least square fitting gives
less error listed in Table 3.2. Thus, second-order least square fitting is employed in signal processing.

**Table 3.2 The comparison of first-order and second-order fitting for the interpolation**

<table>
<thead>
<tr>
<th></th>
<th>1(^{st}) order least square fitting error</th>
<th>2(^{nd}) order least square fitting error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Channel 1</td>
<td>0.0945 nm</td>
<td>0.0176 nm</td>
</tr>
<tr>
<td>Channel 2</td>
<td>0.0919 nm</td>
<td>0.0172 nm</td>
</tr>
</tbody>
</table>

3.3.3 Dispersion compensation

By virtue of the fact that the speed of light depends on the refractive index of the materials, chromatic dispersion is induced by using a broadband light source in the interferometric configuration. Different wavenumbers of the propagating light experience different refractive indices. Dispersion imbalance is introduced when the sample and reference arms contain different lengths of dispersive media, which greatly degrade the axial resolution [9]. Dispersion mismatch can be physically compensated by implementing a rapid scanning optical delay [30], [31] or inserting variable-thickness BK7 and fused silica prisms in the reference arm [32]. Alternatively, dispersion can be compensated using a numerical method in software which is advantageous to simplify data acquisition [33].

A dispersion imbalance between sample and reference arms induces a phase shift $e^{i\theta(k)}$ in the complex cross-spectral density $I(k)$ as a function of wavenumber. A linear interpolation from wavelength-space to wavenumber-space is implemented to calculate the phase shift as a function of wavenumber. The phase term can be expanded by a Taylor series near the center wavenumber of the light source [34]:

28
\[
\theta(k) = \theta(k_o) + \frac{\partial \theta(k)}{\partial k} \bigg|_{k_o} (k_o - k) + \frac{1}{2} \frac{\partial^2 \theta(k)}{\partial^2 k} \bigg|_{k_o} (k_o - k)^2 + \cdots + \frac{1}{n!} \frac{\partial^n \theta(k)}{\partial^n k} \bigg|_{k_o} (k_o - k)^n
\]

where \(k_o = \frac{2\pi}{\lambda_o}\) (\(\lambda_o\) is the central wavelength of the light source). Dispersion compensation can be realized by multiplying the complex cross-spectral density \(I(k)\) with a phase \(e^{-i\theta(k)}\) where the phase was calculated by shifting the coherence function to the origin in z-space. After applying an inverse Fourier transform, a complex spectrum in k-space \(I'(k)\) is retrieved. In this case, phase \(\theta(k)\) is equal to arctangent of the imaginary part divided by the real part of \(I'(k)\). The phase \(\theta(k)\) was fitted to a 9th polynomial expression [34].

### 3.4 Signal Processing

Corresponding polarization states of the reference and sample arms light interfere in the 2x2 PM coupler within the imaging depth. The spectra of the two channels were simultaneously acquired from LabVIEW software using the optical setup in Figure 3.2. Spectral interferometric data for both channels are subtracted by a background to remove the fixed pattern noise. Then interpolation by second-order least square fitting from camera pixel-space to wavenumber-space (k-space) is performed [35]. Numerical dispersion correction is applied in software. Finally, Fast Fourier Transform relates the wavenumber-space to in-depth position space to get the complex valued depth profiles, which are in the form of \(A_m(z) \exp\{i\phi_m(z)\}\) where \(A\) is the amplitude and \(\phi\) is the phase at particular depth \(z\) and the subscript \(m\) refers to channel number (1 or 2). The sample reflectivity \(R(z)\), phase retardance \(\delta(z)\) and relative optic axis orientation \(\theta(z)\) can be obtained by,

\[
R(z) \propto A_1^2(z) + A_2^2(z) \quad (3.1)
\]

\[
\delta(z) = tan^{-1}\left(\frac{A_1(z)}{A_2(z)}\right) \quad (3.2)
\]
\[ \theta(z) = \pi \cdot (\phi_1(z) - \phi_2(z)) / 2 \] (3.3)

The flow chart of the signal processing steps is shown in Figure 3.5.

---

**Signal acquisition**
Frames acquired from LabVIEW software

**Subtract background**
Remove fixed pattern noise

**Resample the spectra**
From pixel to wavelength by second-order interpolation

**Dispersion compensation**
Correct dispersion mismatch between reference and sample arm

**Inverse Fast Fourier Transform**
Calculate the amplitudes and phases from two orthogonal channels

**Signal extractions**
Calculate reflectivity, phase retardance and optic axis orientation etc

---

Figure 3.5 Signal processing method applied to extract the signals from the raw data.
3.5 System performance

Setting up of the visible PS-OCT system for performing the measurements, including depth-resolved minute Faraday rotation, functional imaging experiments, is the main task of this dissertation. Thus, it is essential to evaluate the basic system performance, such as axial resolution, sensitivity, and phase sensitivity.

3.5.1 Axial resolution

The center wavelength and FHWM bandwidth are 687 nm and 9 nm, corresponding to the theoretical axial resolution of 23.2 µm in air. A single reflector is placed in the sample arm to characterize the measured axial resolution. After compensating the dispersion mismatch between sample and reference path by software correction, the measured axial resolution is about 26.5 µm in air. Considering the refractive index in tissue \((n \approx 1.4)\), the axial resolution is about 18.9 µm.

3.5.2 Sensitivity

To evaluate the dynamic range and sensitivity of the reflectivity measurement, a silver mirror was placed under the sample arm. The dynamic range (in decibel [dB]) is defined as the range between the peak reflectivity and the baseline level, which is calculated as

\[
\text{Dynamic range} = (\text{peak reflectivity} - \text{baseline})_{\text{dB}}
\]

As shown in Figure 3.6, the dynamic range of the system is 40 dB, which is measured as the range between the peak value of the first peak and the noise level. With 472 µW incident on the sample and a calibrated 55.4 dB of attenuation contribution from the sample arm, a system sensitivity of 95.4 dB was measured.
3.5.3 Phase sensitivity

The phase sensitivity determines the minimum path length change that can be detected in a phase-sensitive PS-OCT system. The phase information is generally used for phase-sensitive imaging, for instance, relative optic axis orientation, Doppler flow, and functional measurement. Therefore, the phase sensitivity plays an important role in the phase-resolved OCT systems. The phase sensitivity of this system is quantified by placing a cover slip under the sample arm with the optimized reflectivity without saturating the area scan camera. The standard deviation of the phase difference $\Delta \varphi(z)$ between the front and back surface of the cover slip was $\sim 878.8$ pm (averaged of 100 trials), demonstrating sub-nanometer sensitivity. The phase variance is related to SNR by equation 2.6. Generally, in biological samples, the SNR decreases owing to low reflectivity. Bulk motion artifacts can induce noise on the measurements. Therefore, averaging a sufficient number of trials is required to observe sub-nanometer changes. Also, environmental...
perturbations, such as mechanical vibration and temperature fluctuation, will lead to excessive noise. Thus, characterization of the phase sensitivity is required prior to the experiment.2

3.6 Discussion

Plot the axial resolution in depth and discuss the local dispersion compensation from the software. Because the source FWHM bandwidth is comparably small, thus the dispersion is acceptable. Nonetheless, for the sources with larger FWHM bandwidth, additional considerations, such as focusing lens design to reduce the chromatic aberration and residual dispersion imbalance, must be taken carefully.

3.6.1 Ghost Lines

Although PMF based PS-OCT is a promising imaging modality with additional contrasts, it is problematic in terms of ghost images. Ghost images are unwanted patterns which restrict the imaging depth range of the system. Because ghost lines can be misleading for imaging the tissue samples, eliminating the ghost lines within the imaging depth range of the system is a major challenge. Imprecise coupling of the input polarization state will lead to a fixed pattern in the image. Therefore, the isolator and the exiting angle of collimator in the detection path were optimized to minimize the fixed pattern. Figure 3.7 shows the two ghost lines outside of the imaging depth due to the cross-coupling in the 2x2 PM coupler. By using longer PMFs (~10 m) in the reference and sample arm, the ghost lines are displaced away from imaging depth range.

2 Due to shipping and reconstruction the system from our lab to Marine Biological Lab (Woods Hole, MA), calibration of the spectrometer and characterization of the phase sensitivity is needed.
3.6.2 Back-reflection from fiber tips

Back-reflection can be one of the sources of measurement errors. We tried to minimize the amount of back-reflection light from the reference and sample arm fiber tips by polishing the FC/APC fiber tips of the polish angle of 8 degrees. The fiber polishing sheets from sizes 3, 1, and 0.3 μm grits were used. The back reflection light level from fiber tips was minimized by polishing the angle of the fiber tips, which is shown in Figure 3.7.

Figure 3.7 The ghost lines outside of the imaging depth

Figure 3.8 Back-reflection of fiber tips of the two channels were minimized
3.7 Conclusion

To summarize, we constructed a polarization-maintaining fiber-based spectral-domain interferometer which utilizes a low-coherent light source and a single camera using PM fibers. A custom spectrometer, which is designed to simplify camera alignment, separates the orthogonal channels and records the interference related oscillations on both spectra. The system performance was tested and reported. The PMF based spectral domain PS-OCT system is robust, simple, and flexible to be modified for various applications, which is reported in the later chapters.
We described a polarization-maintaining optical coherence tomography with center wavelength of 687 nm and bandwidth of 8.9 nm. In this chapter, the optical setup was slightly modified to demonstrate the feasibility for differential phase measurement of Faraday rotation from multiple depth locations simultaneously in both clear liquids and turbid media. The differential phase signal dramatically reduces the phase noise from the cross-coupled channels. A single-shot acquisition of spectra is adequate to obtain depth-resolved Faraday rotations in reflection mode, making it attractive for biological assessments. System sensitivity of 0.86 minutes of arc for the Faraday rotation measurement was achieved.  

3 The content of this chapter was published in the following peer-reviewed article:
4.1 Introduction

The Faraday effect is a magneto-optical phenomenon in which the rotation of the plane of light polarization in a Faraday-active medium is affected by a longitudinal magnetic field. Faraday rotation arises from the different propagation velocities for left-handed and right-handed circularly polarized light. The Faraday rotation ($\theta$) is quantified by multiplying the Verdet constant ($V$) of the medium, magnetic field intensity ($B$) parallel to the light propagation axis, and the length of propagation ($d$) in the medium.

$$\theta = V B d$$

The Verdet constant depends on material properties, temperature [36] and wavelength [37]. Faraday-active materials are employed in broad spectrum of applications, such as optical isolators [38], glucose sensors [39], magnetic field sensors [40] and displacement sensors [41]. Faraday rotation measurements can be applied to characterize and analyze the stress distribution in materials including glass [42]. A reciprocal fiber optic interferometer [43] and a combination of Sagnac and Mach-Zehnder interferometers [44] have been utilized to measure Faraday rotation for remote sensing of electrical current.

Faraday rotation is typically measured in transmission-mode by launching monochromatic light through a polarizer, then through the sample (contained within a solenoid) and finally the light is collected after it passes through an analyzer, as shown in Figure 4.1.

Figure 4.1 Traditionally, Faraday rotation is measured in transmission-mode
An external AC or DC current is applied to the solenoid to generate a magnetic field parallel to the light propagation axis to rotate the plane of light polarization [45]. Once the Faraday rotation is measured, the Verdet constant of the sample can be calculated by dividing the measurement to the field-depth factor, which is the multiplication of magnetic field strength and sample thickness. Due to the fact that the Verdet constant is a small quantity for most materials, a high field-depth factor is needed for a measurable Faraday rotation. This is especially concerning at longer wavelengths at which the Verdet constant decays to dramatic values.

As an alternative approach, we introduced a phase-sensitive technique for measuring Faraday rotation in reflection-mode [46]. Because a time-domain implementation of low-coherence interferometry was utilized, only one surface at a particular depth was probed at a time. In this paper, we present a spectral-domain implementation of the technique, which allows simultaneous measurements of Faraday rotation from multiple surfaces. Light decorrelated by the orthogonal channels of the fiber is launched on a sample as two oppositely polarized circular states. These states reflect from sample surfaces and interfere with the corresponding states of the reference arm. Inverse Fourier transform of the spectral oscillations in k-space yields complex depth profiles, whose amplitudes and phase difference are related to reflectivity and Faraday rotation within the sample, respectively. A single-shot acquisition of spectra is adequate to obtain depth-resolved Faraday rotations, which enables successful removal of stray rotations that are induced outside the target medium. Since we alternated the direction of magnetic field by rotating magnets under the sample, we employed frequency domain analysis to extract the rotations more accurately. Verdet constants of clear and turbid liquids placed in a glass chamber with 780 µm internal thickness are measured at a wavelength of 687 nm.
4.2 Approach

Linearily polarized light can be represented as two oppositely polarized circular polarization states. The phase shift between these states is related to the rotation of the linear state. The phase shift due to the Faraday effect is caused by a change in the difference between dextrorotary and levorotary refractive indices of the medium under the influence of magnetic field that is parallel to the light propagation axis. Therefore, Faraday rotation can be represented by phase analysis of left and right circularly polarized light that are decorrelated.

Polarization-maintaining-fiber (PMF) ensures the isolation of two orthogonal polarization states. When low-coherent polarized light is coupled to fast and slow channels of PMF, two decorrelated states are created by propagation due to unequal time delays experienced in these channels. As a result, orthogonally polarized linear and decorrelated states emerge from the PMF.

Assuming the slow and fast channels to be in the horizontal and vertical axes, respectively, we can represent the polarization states in the PMF with Jones vectors as

$$E_s = \begin{bmatrix} 1 \\ 0 \end{bmatrix}$$

$$E_f = \begin{bmatrix} 0 \\ 1 \end{bmatrix}$$

In order to obtain circular states a quarter-wave plate can be introduced with axis oriented at 45º, which is represented by the Jones matrix

$$J_{QWP} = \frac{1}{\sqrt{2}} \begin{bmatrix} 1 & -i \\ -i & 1 \end{bmatrix}$$

The polarization state coupled back into the PMF after a round-trip travel through the quarter-wave plate and the sample can be written as

$$E_{PMF} = J_{QWP}^T J_{sample} J_{QWP} E_{PMF}$$

where $E_{PMF}$ is either $E_s$ or $E_f$, and $J_{sample}$ represents the Jones matrix of the sample.
For a Faraday-active medium $J_{\text{sample}}$ can be written as a rotation matrix with a double-pass rotation of $\theta$.

Solving the equation gives $e^{i\theta} \begin{bmatrix} 0 \\ 1 \end{bmatrix}$ and $e^{-i\theta} \begin{bmatrix} 1 \\ 0 \end{bmatrix}$ with respect to $E_x$ and $E_y$.

Since, the left-handed and right-handed circular states undergo phase shift in opposite directions, i.e. $\theta$ and $-\theta$, a system capable of measuring the phase difference ($\Delta \phi$) between these states will measure $2\theta$. Therefore, the Faraday rotation is calculated as

$$\theta = \frac{\Delta \phi}{2}$$

where $\Delta \phi$ is the measured phase difference.

4.3 Method

4.3.1 System setup

The experimental setup is depicted in Figure 4.2. The light source is a 5 mW superluminescent diode (Superlum, Ireland) with a full-width-half maximum (FWHM) bandwidth of 8.9 nm centered at 687 nm. Light transmitted through a free-space isolator in a fiber bench is attenuated and coupled equally into the PMF channels. The incoming light is split into the reference and sample arms equally by a 2×2 PM-coupler (Evanescent Optics Inc., Canada).

In the reference arm, collimated light passes through a QWP oriented at 45º with respect to the PMF channels. The resulting circular states reflect back from a partial reflector (surface of a glass wedge) and pass through the QWP again. As described in the previous section, the optical configuration ensures that polarized light entering in the slow channel couples back to the fast channel in its return, and vice versa. Therefore, decorrelated states experience the same double-pass optical path length in the reference arm. In the sample arm, left and right circular states are created in the same way. Then light is focused on a sample by an achromatic lens ($f = 30$ mm).
Power incident on the sample is 9.7 \( \mu \text{W} \), but it can be increased to a milliwatt level easily by coupling more light to the PMF in the input arm. Underneath the sample, four NdFeB magnets (\( B_r = 12.6 \text{ kG} \), 0.25 in. thickness, 0.75 in. diameter) are mounted on a rotating disk with alternating poles facing upward and with 90\(^\circ\) angular separations as illustrated in Figure 4.2. The controller is set to 25 rps yielding a magnetic field alternating at 50 Hz. The decorrelated states in PMF experience slightly different double-pass optical path lengths due to the Faraday effect in the sample.

Decorrelated states returned from the sample arm interfere with the corresponding states of the reference arm. Interference of each state is formed in the coupler and maintained on a PMF channel. A home-built spectrometer in the detection arm separates the two channels and their spectral components that contain interference related oscillations. The spectrometer has an achromatic lens (\( f=60 \text{ mm} \)) to collimate light, which is directed to a transmission grating (1800 lines/mm). The direction of the grating lines is aligned at 45\(^\circ\) with respect to the PMF channels so each polarization channel encounters equal grating efficiency. A Wollaston prism (Karl Lambrecht Corp., Illinois) with a splitting angle of 0.5\(^\circ\) separates the orthogonal polarization states, whose spectra are then focused by an achromatic lens (\( f=75 \text{ mm} \)) onto an area scan camera (acA2000-340km, Basler, Germany). The camera contains 2048 (horizontal) \( \times \) 1088 (vertical) pixels with 5.5 x 5.5 \( \mu \text{m}^2 \) pixel size and allows selection of two lines, one for each PMF channel as shown in Figure 4.3. We used an integration time of 90 \( \mu \text{s} \) and an acquisition rate of 5 kHz for both lines (maximum rate: 6.6 kHz). Vertical binning of 4 pixels was used to maximize photon collection. Pixel binning and region-of-interest selection capabilities of the camera drastically simplify the alignment. Moreover, selection of vertically separated lines gives flexibility in the design of single-camera based systems. For instance, selections of source spectrum, number of grating lines and split angle of Wollaston prism do not produce a spectral overlap. This may
occur, if the components are not chosen carefully, when the spectra are only split horizontally to form a line for acquisition with a line scan camera.


Figure 4.3 The area scan camera is configured to select and output two spectral lines (white) with a vertical binning of 4 pixels.
4.3.2 Signal processing

Similar to spectral domain optical coherence tomography (OCT), interference-related oscillations on optical spectra carry depth-resolved information. First, data in wavelength-space are interpolated and resampled in wave number (k)-space for both channels. Then, dispersion mismatch between the reference and sample arms was compensated in software [34]. Next, inverse Fourier transform is applied to the spectra in k-space to obtain the complex depth profiles. Amplitude of the complex signals is related to the reflectivity; therefore, sample surfaces at particular depths are localized with an axial resolution determined by the width of the coherence function. For an identified surface, phases of the two channels are subtracted, since the phase difference is related to the Faraday rotation experienced up to that point.

As we alternated the magnetic field at a given frequency, Faraday rotation at a particular point was varied accordingly. To quantify Faraday rotation, we first unwrapped the phases and calculated the phase difference

$$\Delta \varphi(t) = \varphi_1(t) - \varphi_2(t)$$

which was then zero-padded by a factor of 4 to facilitate accurate readings in the frequency domain. Fast Fourier Transform of $\Delta \varphi(t)$ had peak amplitude at the modulation frequency of the magnetic field. After reading the peak value, double-pass Faraday rotation was calculated as

$$\theta = \frac{\Delta \varphi}{2}$$

4.4 Results

4.4.1 System characterization

The system was characterized by placing a glass surface at a depth of 580 µm as a sample. Spectra from two channels were recorded using custom LabVIEW software. The sum of the dynamic range and the attenuation contribution yields a sensitivity of 95.4 dB. Analysis
showed that the axial resolution determined from FWHM of the coherence function was 26.5 $\mu$m, which is close to the theoretical value of 23.4 $\mu$m. By observing the peak of the coherence functions in M-mode, phase variations on the individual channels and noise on the differential phase signal are demonstrated in time and frequency domains. Figure 4.4 (a) and (b) show that the individual phases ($\phi_1$ and $\phi_2$) fluctuate by a large amount due to optical path changes in the reference and sample paths. The environmental perturbations, especially the vibrations affecting the bulk optics portions of these paths, induce such fluctuations that are common on both channels. The differential phase ($\Delta \phi$) signal, Figure 4.4 (c) and (d), dramatically reduces the phase noise with a common-mode rejection ratio of 28.7 dB. The dominant noise source appears to be the mechanical perturbations of the rotating disk. The probability distribution of the noise on $\Delta \phi$ follows a Gaussian distribution. The sensitivity for measuring a Faraday rotation angle ($\frac{\Delta \phi}{2}$) is found to be 0.86 minutes of arc.
Figure 4.4 Time (a) and frequency (b) domain representations of phase variations on individual channels (φ₁ and φ₂). The differential phase (Δφ) signal in time (c) and (d) frequency domains indicate removal of common noise terms.

To demonstrate feasibility of Faraday rotation measurement from multiple surfaces, a cell consisting of two parallel microscope slides separated by spacers was constructed. An inner cell thickness (d) of 780 µm was measured by the system. Figure 4.5 illustrates the cell and the depth profile showing its internal surfaces when the cell was filled with deionized (DI) water. The internal surfaces, bottom surface of the top glass and top surface of the bottom glass, were probed
simultaneously by the optical system while the magnetic field was modulated at 50 Hz. During a 200 ms recording time, one acquisition contained 10 cycles of phase variation, which is adequate for Fourier-domain analysis. Faraday rotations at the glass-water and water-glass interfaces were band-pass filtered (30-100 Hz) for visualization purposes and plotted in Figure 4.6 (a). The time trace shows the cycles, while the Fourier transforms yield the frequency components and the magnitudes of the rotations shown in Figure 4.6 (b).

Figure 4.5 The glass cell and the depth profile showing its internal surfaces. Surface 1: glass-solution interface. Surface 2: solution-glass interface.
4.4.2 Faraday Rotations in Clear Liquids

We used DI water, acetone, isopropyl alcohol (IPA) and methanol samples at room temperature, and recorded 120 measurements for each liquid. After every 10 measurement, the fluid in the cell was flushed and replaced with new fluid sample by using a syringe. Faraday rotation in a fluid sample is quantified by subtracting the Faraday rotations measured at the two surfaces since the value for surface-1 corresponds to stray rotation from the top glass. Figure 4.7 shows the mean values and standard deviations of the Faraday rotations in the liquids. Because the magnets with opposite polarities and the round-trip path of light in the sample contribute to the peak-to-peak values of Faraday rotation ($\theta_{pp}$), the Verdet constants of the liquids are calculated by

$$V = \frac{\theta_{pp}}{(2B)(2d)}$$
The magnetic field strength in the cell was approximately $B=2.05$ kG, which is measured by a gauss meter (AlphaLab Inc.) along the axis of the magnets and at the corresponding height. The calculated values of Verdet constants are listed in Table 4.1. The values are in good agreement with those reported in literature [47].

![Graph showing Faraday rotation measurements in various liquids](image)

**Figure 4.7** Faraday rotation measurements in various liquids ($B=2.05$ kG, $d=780\ \mu m$, $\lambda=687$ nm).

### Table 4.1 Verdet constants of liquids at 687 nm wavelength are calculated from measurements

<table>
<thead>
<tr>
<th>Liquid</th>
<th>Verdet constant min.kG$^{-1}.cm^{-1}$</th>
<th>deg.kG$^{-1}.cm^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water</td>
<td>9.2233</td>
<td>0.1537</td>
</tr>
<tr>
<td>Acetone</td>
<td>8.1950</td>
<td>0.1366</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>8.3901</td>
<td>0.1398</td>
</tr>
<tr>
<td>Methanol</td>
<td>6.5894</td>
<td>0.1098</td>
</tr>
</tbody>
</table>
4.4.3 Faraday Rotations in Turbid Media

Intralipid solutions are often used for tissue-mimicking experiments [48]. To investigate Faraday rotations in scattering media, we diluted 20% intralipid solution with DI water to obtain intralipid concentrations of 0.125%, 0.25% and 0.375%. Faraday rotations in these samples were measured by using the same optical setup and sample cell. Because of a different distance between the magnets and the cell, the magnetic field strength in the cell was about 1.81 kG. High concentrations of the intralipid solution reduced light collection from the second surface, which significantly decreases the signal-to-noise ratio. To minimize this effect, the optical power on the sample was increased up to 35.4 μW by adjusting light coupling to PMF. Figure 4.8 shows the mean values and standard deviations of Faraday rotation for different intralipid concentrations. An increase in scattering limits the penetration depth and elevates the noise as predicted. A sample cell with a highly reflecting bottom surface would allow measurement of Faraday rotation in highly scattering media [46]. Also, some biological tissues and materials can be birefringent. This system can be converted to polarization-sensitive OCT by controlling the input polarization state and the orientation angle of the quarter-wave plate in reference arm to assess birefringence. The presence of birefringence can affect the electromagnetic wave propagation which is considered different from pure Faraday-active media [49]. Assessment of Faraday rotation in birefringent tissues as well as influences of linear dichroism and other polarization effects require further investigation.
4.4.4 Faraday Rotations in Nanowires solution

Nickle-gold nanowires solution\(^4\) was injected to the chamber to measure the Faraday rotation. The chamber (thickness \(\sim 1.1 \ \mu m\)) used here for this experiment is thicker than the one used previously. Also, the magnetic field used here is different. Figure 4.9 shows the mean values and standard deviations of DI water and nanowires solution without removing the stray effect of the top glass. The preliminary results indicate Faraday rotation enhancement in nickle-gold nanowires solution. However, only one sample solution was tested. The qualitative observation of enhanced optical Faraday rotation from nickle-gold nanowires solution were found in this preliminary data set.

\(^4\) The sample solution (Courtesy from Prof. Beth Stadler’s lab) number: 127
4.5 Discussion

A high field-depth (Bd) factor and the use of shorter wavelengths (larger Verdet constant) increase the amount of Faraday rotation in a medium. However, there are limitations and constraints in each application. We demonstrated our depth-resolved results with a small field-depth factor. If applicable, magnetic fields such as in new MRI machines and thicker transparent or semi-transparent samples would greatly increase the signal to noise ratio. Development of new light sources, such as blue SLDs, is also promising for our method. Another way to enhance Faraday rotation would be to introduce nanoparticles such as gold-coated maghemite nanoparticles [50], which might also be used as a contrast agent for OCT imaging. As Faraday rotation for horse spleen ferritin, magnetoferritin and nanoscale magnetite and Fe$_3$O$_4$ suspensions has been reported [51], biomedical applications of the measure may be possible. In addition, water might be a target for assessing hydration levels of biological tissues. Quantitative measurements for enhanced optical Faraday rotation with different concentrations from nickle-
gold nanowires solution need to be further studied and investigated. This may also be useful for contrast-enhancing probes for OCT imaging.

4.6 Conclusion

We have developed a PMF-based spectral-domain low-coherence interferometer for differential-phase measurement of Faraday rotation from multiple depth locations simultaneously. The development includes design of a polarization-sensitive spectrometer and a method for calibration, both of which can be adapted by other systems including spectral-domain polarization-sensitive OCT. The sensitivity for Faraday rotation measurement is 0.86 minutes of arc. Verdet constants obtained for various liquids and turbid media agree with literature. Fast and simultaneous acquisition of a full depth profile makes this technique attractive, as it provides removal of stray rotation and multi-point assessment of Faraday-active media. It is also possible to incorporate lateral scanning for 3D imaging.
Harnessing light to study neural activity can be traced back to the 1940s. The development of optical imaging allows visualization of cell functioning and the study of physiological processes. An interdisciplinary research field has emerged that involves optical methodology and neuroscience, which is called neurophotonics. Over the past decades, observations of a transient change in optical properties of a nerve during excitation, including fluorescence, turbidity, birefringence, light scattering, absorption, and volume changes [19], [20], [52], have been reported. However, intrinsic optical signals are often very small. Thus, voltage-sensitive dyes were introduced to further investigate action-potential-related optical changes. In order to study spectroscopic features with different voltage-sensitive dyes during action potential propagation, we designed and constructed a dual-wavelength spectral-domain OCT system using two wavelength bands at 690 and 840 nm.\(^5\)

\(^5\) The content of this chapter was presented in 2015 conference:
5.1 Introduction

The use of light to detect functional neural activity non-invasively has been tremendously studied for decades. In early stage research, the use of microelectrodes was the only approach for achieving the spatiotemporal resolution required to probe the neural activity. Tasaki et al. [53] observed fluorescence changes during nerve excitation with dye staining. Optical imaging techniques in combination with contrast-enhanced voltage-dependent molecular transducers provide visualization of neural activation.

The use of light to detect neural activity dates back to the late 1940’s. In 1949, Hill and Keynes [54] detected an increase in scattering followed by a decrease due to stimulation of shore crab nerve. Cohen and collaborators reported light scattering and birefringence changes due to stimulation in squid giant axon as well as crab nerve [55]. In 1968, Tasaki et al. demonstrated the first optical detection of neural activity from the nerves stained with an extrinsic voltage-sensitive dye. The changes in fluorescence, light scattering and birefringence during excitation of crab and lobster nerves were observed [53]. In recent years, Akkin et al. published the depth-localized neural activity utilizing a voltage-sensitive dye, which provides significant changes in local back-scattered light intensity [21].

In addition to AP-related intensity, birefringence and fluorescence changes, there have been several observations of rapid mechanical changes during neural activity. Using an optical technique, Hill et al. studied the volume change due to the repetitive stimulation and showed a cumulative increase in radius of a cuttlefish axon of about 0.1 µm for 10,000 impulses [56]. Since then, various methods had been proposed to investigate axon swelling during action potential propagation. In 1955, Bryant and Tobias observed a transient shortening in the length of crab and lobster nerves due to stimulation [57]. Hill et al. demonstrated small contraction (~ 1.8 nm) of the axon followed by a slow swelling in the diameter of crayfish axon corresponding to the action
potential propagation using a laser interferometer and gold particles [58]. In another study, Iwasa et al. used a fiber-based sensing system with gold-particles on squid giant axons to examine small mechanical changes. They observed a rapid swelling of axon surface (~0.5 nm) followed by a slow contraction along with action potential propagation over 1 ms in duration [59]. In 2003, Yao et al. designed the optical lever recording using a knife edge to study physical displacements associated with electrophysiological activation of Nitella internodes and revealed a rapid swelling displacement (~ 10 nm) followed by a large and slow shrinkage (~ 100 nm) along with a compound action potential [60].

In addition to structural imaging, optical coherence tomography (OCT) allows the measurement of changes in depth-localized back-scattering intensity and phase, which can be related to neural functionality. The idea of employing phase-sensitive optical low-coherence reflectometry to provide non-contact and depth-resolved detection of neural activity was proposed by Akkin et al. [61] and Fang-Yen et al. [62]. A surface displacement of ~ 1 nm with ~ 1ms duration coincident with the action potential in a nerve bundle of crayfish was reported using differential phase interferometry [61]. Another non-contact measurement utilizing a dual-beam low-coherence interferometer showed swelling displacements of ~ 5 nm with ~ 10 ms duration from lobster nerves [62]. The depth-resolved measurements of transient structural changes associated with action potentials in crustacean nerves have been demonstrated using SD-OCT, showing mechanical changes in the range of 0.5-2.5 nm [13]. Also, action potential-related changes in backscattered light and phase from multiple depth locations have been reported. Absorption and scattering changes induced by voltage-sensitive dyes during action potential propagation may enhance the image contrast [21].

Early development of OCT focused on static and morphological imaging modalities. Later, the applications have included the use of functional OCT systems, such as Doppler OCT [63], PS-OCT [65], [66], and detection of neural activation [19], [62]. These functional
detection systems present additional information depending on the optical changes which indicate physiological events. Optical probes, such as scattering, modulating, absorbing, and plasma-resonant probes have been employed in a wide variety of optical detection schemes. The use of exogenous probes to enhance the intrinsic optical signals (\textit{i.e.} contrast arising from endogenous tissue properties) dramatically expands the imaging capability of OCT systems [67].

A motivation for this project is to employ functional OCT with and without the use of exogenous voltage-sensitive dyes for detecting contrast-enhanced intensity-based optical changes associated with neural activity. In addition, the dual-wavelength spectral-domain OCT system provides depth-resolved spectroscopic information, which is advantageous for testing different dyes since the dyes have distinct action spectra. Developing contrast-enhancing molecular imaging techniques could be potentially useful for replacing multi-electrode array recordings to detect nerve functionality in electrophysiology.

5.1.1 The action potential

Excitable cells, including neurons and muscle cells, are able to generate action potentials. An action potential is a short-lived series of processes between depolarization and the exchange of ions on a cellular level. Figure 5.1 illustrates the intracellular recording of a neuron action potential and the processes of ion exchange. The resting membrane potential is -70 mV.

When the cell is stimulated, the cell membrane becomes depolarized and the sodium (Na\(^+\)) and potassium (K\(^+\)) ionic permeability changes, allowing more Na\(^+\) to enter into the membrane. If the stimulus is below the threshold voltage, no action potential is triggered. All action potentials are about the same size regardless of the stimulus strength once the minimum required threshold is reached. This is called the “all-or-nothing” law; a neuron either fires completely or not at all.
Generally, there are two types of nerve fibers, myelinated and unmyelinated. The unmyelinated axons are enveloped by Schwann cells whereas myelinated fibers are wrapped in a myelin sheath (layers of Schwann cell membranes). The speed of conduction in unmyelinated axons is proportional to the square root of the axon diameter; on the other hand, myelinated axons conduct faster because only the axon membrane at the node of Ranvier needs to be depolarized. Thus the action potential appears to “jump” when propagating along the axon. The conduction velocity in myelinated axons is proportional to the axon diameter.

Traditionally, the predominant methods for measuring action potentials require electrodes inserted into the target tissues. Nevertheless, these methods are invasive and provide limited spatial resolution. Reliable detection of neural activity non-invasively has been one of the long-lasting challenges in neuroscience.

Figure 5.1 The action potential and the processes of ion exchange

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6 This picture is reprinted from http://hyperphysics.phy-astr.gsu.edu/hbase/biology/actpot.html
5.1.2 Voltage-Sensitive Dye

Optical measurements have tremendous advantages due to their minimally invasive nature, thereby avoiding unintentional damage to tissue. However, intrinsic optical signals are usually small and hard to measure. Because the neural activity is directly related to the changes in membrane potential, the use of exogenous voltage-sensitive dyes which bind to the surface of excitable membranes serve as molecular transducers that translate the changes in membrane potential into optical signals. Voltage-sensitive dyes might serve to amplify intrinsic signals. In principle, the concept is rather simple. However, imaging with voltage-sensitive dyes remains an exceptionally challenging problem owing to multiple factors that need to be considered, such as specificity, sensitivity, toxicity, photodynamic damage (i.e. photobleaching properties) and pharmacological damage. Furthermore, the precise voltage sensitivity depends on the light source as well as the staining procedure and may vary from species to species.

5.2 Approach

5.2.1 Optical system

To study neural activity, dual-wavelength polarization-sensitive spectral-domain optical coherence tomography system was constructed. Figure 5.2 depicts the system configuration.

A polarization-maintaining-fiber (PMF) based OCT systems (690 nm and 840 nm) was used for this project.

The light source is a 25 mW broadband near-infrared superluminescent diode (SLD) with a full width at half maximum (FWHM) bandwidth of 50 nm centered at 840 nm (Broadlighter S840, Superlum, Ireland), yielding an axial resolution of ~5.5 μm in tissue. After passing through a polarizer in the fiber bench, linearly polarized light is coupled to one of the orthogonal channels of PMF and then split into reference (70 %) and sample (30 %) arms by a 2x2 polarization-maintaining (PM) coupler (Canadian Instrumentation & Research Ltd., Canada). For 690 nm
system, a 5 mW superluminescent diode (SLD) (Superlum, Russia) centered at 687 nm with a FWHM of 8.9 nm is protected and polarized by the isolator (IOB-3D-690-VLP, Thorlabs). A 2x2 PM coupler (Model 954P, Evanescent Optics Inc.) directs the input light equally to the reference and sample arm.

In the reference arm of the 840 nm system, collimated light passes through a quarter-wave plate (QWP) oriented at 22.5° with respect to the PMF channel and an achromatic lens \((f = 30 \text{ mm})\), and returns from a reflector, which is the front surface of a glass wedge. The returning light becomes linearly polarized at 45°; and therefore, couples back to the fast and slow axis of PMF equally.

In the reference arm of the 690 nm system, light is collimated and passes through a quarter-wave plate (QWP) oriented at 22.5° with respect to the input polarization state, and is reflected from a movable reflector. After a roundtrip, the polarization state becomes linear state oriented at 45°, which is coupled back equally to the slow and fast channels of the PMF.

A dichroic mirror (T760lpxr, Chroma Technology Corp.) combines the sample arm of two OCT systems onto the sample. The quarter-wave plate (QWP) and half-wave plate (HWP) were placed before and after the dichroic mirror to isolate the two orthogonal polarization states. Thus, linear polarization states were impinged onto the sample with a 19 mm focusing lens for both the 690 and 840 nm OCT systems, yielding a lateral resolution of 7 µm and 8.5 µm. The optical power onto the sample of 690 nm OCT is 0.5 mW whereas that for 840 nm OCT is 1 mW. Transmission light passing through the nerve chamber was coupled into a multimode fiber (Thorlabs Inc.) by a 20X objective lens. Light exiting the multimode fiber was collimated and passed through the filters (Filter A: FF01-650/100, Semrock; Filter B: FF01-794/160, Semrock; and dichroic filter: FF801-Di02, Semrock) that help screen for source-related light intensity change recordings. Photodetector D1, D2 and D3 recorded the non-depth-resolved transmitted light intensity of 690 nm, fluorescence and 840 nm, respectively.
All signals were synchronized and recorded using custom LabVIEW software (National Instruments, Austin, Texas). Light returning from the reference and sample arms mixes in the PM coupler of 690 nm and 840 nm system separately. In the spectrometer of 840 system, light collimated by an achromatic lens (f = 75 mm) is dispersed to its spectral components by a 1200 lines/mm transmission grating (Wasatch photonics, Logan, UT). A Wollaston prism (Karl Lambrecht Corp., Chicago, IL) with a 6º splitting angle separates the two channels, which are then focused by an achromatic lens (f = 200 mm) onto a CMOS line-scan camera (Sprint, spl4096-140km Basler, Ahrensburg, Germany). The spectrometer was calibrated with a commercial spectrometer (HR2000+, Ocean Optics, Dunedin, FL) by using a diffraction grating coupling selected wavelengths of the light source to these spectrometers and fitting the readings to a curve. The spectra were acquired by a CMOS linescan camera (spl4096-140km Basler, Germany) and CMOS area scan camera (acA2000-340 km, Basler, Germany) at a rate of 20 kHz (50 µs/A-Line) and 5 kHz (200 µs/A-Line) for 840 nm and 690 nm PS-OCT, correspondingly. The spectrometer construction and design of 690 nm system can be found in section 4.3.1. During the experiments, cross-sectional images of the squid nerve in the chamber are displayed in real-time to search for the optimal positions for functional recordings. Then, by terminating the controller input (0V) of the lateral scanner, a single depth profile \( z \) of interest is recorded over time.

The phase sensitivities of 537.4 pm and 878.8 pm for 840 nm and 690 nm systems were characterized by simultaneously recording the phases reflected from the front and back surfaces of a coverslip with averaging of 100 times. Note that the reference arms of these systems were separate. Thus, the system noise and vibration would be different. The isolations of two orthogonal channels are 16 and 18 dB for 840 and 690 nm PS-OCT when using a glass surface as a sample.

5.2.2 Voltage-Sensitive Dyes used for nerve staining

Table 5.1 lists all dyes used with the respective staining conditions, such as staining concentration, staining period and additional solutions for the experiments. The structures, properties and characteristics of the dyes listed below can be found in [70]–[74].

<table>
<thead>
<tr>
<th>Dye</th>
<th>Structure</th>
<th>Staining concentration (mg/mL)</th>
<th>Staining period (minutes)</th>
<th>Additional solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>JPW6030</td>
<td>N/A</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>JPW5021</td>
<td>styryl</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>NK2761</td>
<td>Merocyanine-rhodanine</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
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<td>-------</td>
<td>---</td>
<td>----</td>
<td>---------</td>
</tr>
<tr>
<td>NK3630</td>
<td>Oxonol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGH1</td>
<td>styryl</td>
<td></td>
<td></td>
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<tr>
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<td>styryl</td>
<td></td>
<td></td>
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<tr>
<td>PGH6</td>
<td>styryl</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PGH4</td>
<td>styryl</td>
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<tr>
<td>PGH7</td>
<td>styryl</td>
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<td>PGH8</td>
<td>styryl</td>
<td></td>
<td></td>
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<td>styryl</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MJP114</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR123</td>
<td>Tricarbocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR132</td>
<td>Tricarbocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR140</td>
<td>Tricarbocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR144</td>
<td>Tricarbocyanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RH1691</td>
<td>oxonol</td>
<td></td>
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</tr>
<tr>
<td>RH237</td>
<td>styryl</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

5.3 Nerve Preparation

The giant axon and surrounding small nerve fibers (Figure 5.3) were dissected from squid 
(Loligo pealeii, Marine Biological Laboratory, Woods Hole, MA). The nerve was kept immersed in sea water during and after dissection. Both ends of the squid nerve were tied with threads. After immersing in sea water solution for rinsing, the nerve was stained for 20 minutes with a voltage-sensitive dye (1 mg/mL in seawater with 1% dimethyl sulfoxide, DMSO) and placed in the groove of the nerve chamber containing sea water. We performed measurements on at least 2 squid nerves (except for MJP114, only 1 squid nerve was measured) for each voltage-sensitive dye. We conducted 3-trial stimuli for each location and recorded 100 times to aid averaging. Multiple depth profiles were recorded from each squid nerve.

Figure 5.3 depicts the cross sectional image of squid giant axon and its surrounding small fibers. The large size of the axon can be up to 1 mm in diameter. Thus, it allows researchers to investigate some of the underlying neurophysiology.
5.3.1 The nerve chamber and electrical stimulation

Figure 5.4 illustrates a nerve chamber made of plexi glass that can deliver and record the action potentials from the electrodes embedded inside. The platinum electrodes are fixed to the chamber with epoxy to stimulate the nerve and to obtain electrical action potential recordings. A cover glass is attached on top of the chamber in the optical recording area in order to provide an optical reference signal to measure surface and subsurface displacements. The nerve, stained or unstained, is placed in the groove (~ 1 mm wide, 1 mm deep) and the chamber is oriented such that the long axis of the nerve is at 45 degrees with respect to the linear light polarization of the incoming light. This will ensure the maximum cross-polarized light intensity that can be detected [75]. The nerve chamber wall contains tubes through which cold water is circulated to vary the axon temperature if needed. The chamber is attached to a three-dimensional stage in order to perform fine and precise adjustment for optimizing the signals.

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Figure 5.3 Squid giant axon and surrounding nerve bundles

7 This picture is reprinted from http://hermes.mbl.edu/publications/pub_archive/Loligo/squid/images/axon.large.jpg
Figure 5.4 Schematic of the nerve chamber used to perform the experiments. A squid nerve is cleaned and placed in the groove of the nerve chamber. The chamber is oriented with the length of the nerve at 45° to the incident polarization state. Note: Chilled water is circulated through the water circulation tubes for cold temperature experiments.

Between the stimulating and recording electrodes, the electrical isolation pool is filled with petroleum jelly in order to isolate the two electrodes and reduce stimulation artifact in the electrical recordings. A cover glass is placed on the exposed parts of the axon to prevent it from drying out. An isolated pulse stimulator (Model 2100, A-M system, Sequim, WA) is used to apply adjustable electrical current pulses that are 50 μs in duration to the nerve in the chamber. In order to measure and amplify the action potential, an AC differential amplifier (Model 1800, A-M systems, Sequim, WA) with low- and high-pass cutoff frequencies set to 10 Hz and 10 kHz were connected to the recording electrodes. The action potential recording is recorded by the analog input from a data acquisition card (Model PCI 6110, National Instruments NI, Austin, TX). The action potential is displayed on an oscilloscope to monitor the functioning of the nerve over time. The Labview data acquisition software provides real-time display and acquisition of optical and electrical signals.

5.4 Signal Processing

After background subtraction, the spectra were resampled from pixel space to k-space by interpolation, and dispersion imbalance between the reference and sample arm was compensated
in software [34]. Then, an inverse Fourier Transform is applied to obtain the complex-valued depth profile as described in Chapter 3, section 3.4. The sample reflectivity $R(z)$, phase change $\Delta p(z)$, fractional intensity change $\Delta I(z)$, and fractional intensity change of transmission-mode photodetector signals $\Delta T(z)$ can be extracted as,

\begin{align}
R(z) &\propto A_1^2(z) + A_2^2(z) \\
\Delta p(z) &\equiv \frac{A_2}{4\pi} \left( \varphi_1(z) - \varphi_1(z_r) \right) \\
\Delta I_m(z) &\equiv \frac{A_m(z) - \bar{A}_m(z)}{A_m(z)} \\
\Delta T(z) &\equiv \frac{T(z) - \bar{T}(z)}{\bar{T}(z)}
\end{align}

Phase signals were extracted by subtracting the reference glass signals from the main channel. The fractional intensity changes of main and crossed-polarized channels were calculated by equation 5.3, where $A$ is the amplitude at a particular depth $z$ and the subscript $m$ refers to channel number (1 or 2). The fractional intensity changes of transmission-mode detectors were processed by equation 5.4, where $T$ denotes the transmitted light intensity.

The signals were averaged by 100 responses in time to improve the SNR. These signals are then passed through forward and reverse low-pass filtering with the cut-off frequency at 2 kHz and 5 kHz for 840 nm and 690 nm systems to obtain the zero phase distortion signals.

A semi-automatic detection method for prescreening the fractional intensity change was developed for faster and better analysis. The signals were averaged 100 times and the possible fractional intensity change of a particular depth was identified by the classifier. The feature extraction of this project is using the three timing-windows of the 3-trial stimuli of action potential recording to define the region of interest. Within the timing window, the duration lengths of the zero-crossings are used to determine if the trace is identified as signal or noise. The threshold was set to screen out the possible fractional intensity change. Once the possible
fractional intensity change was selected and saved only when the three timing-windows all return
the true Boolean signals. After this pre-selection stage, the fractional intensity change that are
misclassified due to artifacts or imperfection of the design of this classifier are manually ruled out
by a visual decision.

5.5 Results

Spectral domain PS-OCT provides depth-resolved nerve displacement by phase
measurements and backscattered light intensity changes in main and cross polarization channels.
Staining the nerve with voltage-sensitive dye enables measurement of voltage induced absorption
and scattering changes in transmission-mode or reflection-mode geometries. Figure 5.5 shows a
cross-sectional image of a squid nerve with a dynamic range of 45 dB using the 840 nm system,
including the giant axon and its surrounding small nerve fibers. The OCT reflectivity image also
shows the glass-saline and saline-glass interfaces above and below the nerve, which can be used
as a reference for differential phase measurements. There exists no change in optical path length
between the two fixed interfaces during neural activity. Optical path length change observed from
the nerve is due to structural changes within the nerves.

The dual-wavelength OCT system (840 nm and 690 nm) offers simultaneous cross-sectional
images as shown in Figure 5.6 (b) and (d) from 840 and 690 nm OCT systems. The darkness of
the structural image indicates the reflectivity of the squid nerve. This is a region with only small
nerve fibers of the squid whereas Figure 5.5 shows the squid giant axon and the surrounding
small fibers. After examining the cross-section, single depth-profiles shown in Figure 5.6 (a) and
(c) were optimized with sufficient SNR and then selected for M-mode recordings. Since M-mode
records the same lateral position over time, the spectra and the depth profile remain nearly
identical over time. Within the same measurement, the single-pixel intensity and phase, and their
time-varying characteristics were obtained.
Figure 5.5 A cross-sectional image of the giant axon and its surrounding small nerve fibers of a squid. The surfaces (above and below the axon) represent the glass-saline and saline-glass interfaces. The black arrow indicates the squid giant axon.

Figure 5.6 (a) depth profile of the yellow dashed line in the cross-sectional image (b) from 840 nm OCT system and (c) depth profile of the yellow dashed line in the cross-sectional image (d) from 690 nm OCT system, respectively.
5.5.1 Intrinsic Signals

The intrinsic optical signals associated with neural activity are found to be very small mostly. The $\Delta p$ response measured between the top and bottom surfaces of the nerve can be constantly observed. The detailed discussion can be found in [20]. In addition to the $\Delta p$ responses, we focused on analyzing the changes in backscattered light intensity of the main channel. Figure 5.7 shows the AP recording and $\Delta I$ from main channel at a particular depth. Unlike the $\Delta p$ response, the intrinsic signals are merely observed.

![Figure 5.7](image1)

Figure 5.7 The action potential recording and the fractional intensity change of main channel from a particular depth.

5.5.2 Voltage-Sensitive Dyes

Since intrinsic signals are often very small, extrinsic optical imaging techniques have increasingly become a widely used method to monitor neural activity in various preparations. The previous published results [21], [72] demonstrate the feasibility of this approach. In this study, the dual-wavelength OCT system allows simultaneous measurements of depth-resolved changes in back-scattered light intensity and non-depth-resolved transmitted light intensity from 840 nm and
690 nm wavelength bands. We employed the dual-wavelength OCT to investigate dye dependent light intensity changes.

Figure 5.8 (a) and (b) show the depth profiles from the two wavelength bands probed in this configuration with labels indicating points, whose \( \Delta p \) and \( \Delta I_1 \) responses were presented in this figures. Figure 5.8 (c) shows the logarithmic reflectivity image from the 840 nm system with a dynamic range of 45 dB. The dots indicate the depth locations where both \( \Delta p \) and \( \Delta I_1 \) responses were observed. The amplitude of the stimulus current pulses was 2 mA. The AP recording is given in Figure 5.8 (d). To extract the \( \Delta p \) responses, common-mode phase noise was removed by subtracting the phase of the reference bottom surface from the phase of the nerve points. Then the phase difference \( (\Delta \varphi_1) \) from the main channel was converted to single-pass optical path length change \( (\Delta p) \) using equation 5.2 and plotted as a function of time in (e) and (g). The \( \Delta p \) responses associated with action potential propagation from multiple depth points of 840 and 690 nm systems present the same polarity and similar amplitudes for the four points chosen from the same nerve bundle. such traces can be obtained without staining as well [20]. On the other hand, (f) and (h) show the backscattered light intensity change of main channels \( (\Delta I_1) \) from both systems. From these locations, the sign of the \( \Delta I_1 \) responses of 690 nm and 840 nm systems are in opposite directions and their amplitudes are different from each other. Each trace is an average of 100 trials so as to have a better SNR. The traces have 5-kHz bandwidth for the 840 nm system and 2-kHz bandwidth for the 690 nm system and the fractional transmitted light intensity change \( \Delta T(z) \).

Both \( \Delta p \) and \( \Delta I_1 \) responses are correlated with the AP propagation. Moreover, fractional intensity change of transmission-mode photodetector signals \( \Delta T(z) \) were observed for both wavelength bands (i).
Figure 5.8 Depth profiles of the stained (IR144) squid nerve probed by the dual wavelength OCT from 840 nm (a), and 690 nm (b). The blue in (a) and magenta in (b) are the amplitudes of main channel, whereas black lines are the amplitudes of cross-polarized channel. The dots indicate the depth locations where both $\Delta p$ and $\Delta I$ responses were observed. (c) Cross-sectional image from 840 nm system. The AP is given in (d). The insets show the labels of points, whose $\Delta p$ and $\Delta I$ responses are given in (e, g), and (f, h) respectively. (i) displays the fraction transmitted intensity changes from the 690 nm (red) and 840 nm (blue) wavelength bands. Each trace is an average of 100 responses. The traces have 5 kHz bandwidth for the 840 nm system shown in (g, h) and 2 kHz bandwidth for the 690 nm system in (e, f) and the fractional transmitted light intensity change in (i), respectively.

The results can be further illustrated by plotting the main channel intensity in the depth/time plane. Figure 5.9 (a) and (b) show the main channel intensity and fractional intensity change (main channel) in the depth/time plane, correspondingly. The three hot spots correspond to AP-induced backscattered light intensity change.
Using another voltage-sensitive dye NK3630, we observed the AP-evoked phase signal ($\Delta p$ response) and the backscattered light intensity change ($\Delta I_1$) from the main channel. Similarly, Figure 5.10 (a) shows the depth profile using the 840 nm OCT system with labels indicating points, whose $\Delta p$ and $\Delta I_1$ traces are shown in (c) and (d) accordingly. The AP recording is presented in (b). The amplitude of the stimulus current pulses was 4 mA. Figure 5.10 (e) shows that the non-depth-resolved fractional intensity change of transmission-mode photodetector signals $\Delta T(z)$ observed using the 840 nm wavelength band. The $\Delta I_1$ traces show positive and negative polarities in the nerve bundle whereas $\Delta p$ responses remain in the same direction.

In Figure 5.11, the depth-resolved $\Delta p$ responses of the same nerve locations are plotted in (c). The corresponding depth points are labeled in yellow in (b) and with red dots in (c), respectively. This is the same location shown in Figure 5.10. Both decreased and increased phases in $\Delta p$ were observed during AP propagation. In addition, it shows the biphasic and monophasic behaviors along the same depth profile with AP propagation. Interestingly, the transient displacements near the edge of the giant axon give larger $\Delta p$ responses. However, this may not be due to the firing of the giant axon as the time course of the signal corresponds to the small fibers.
Figure 5.10 Depth profile of the stained (NK3630) squid nerve probed by the dual wavelength OCT system from 840 nm (a). The blue trace in (a) is the amplitude of main channel, whereas black lines is the amplitude of cross-polarized channel. The AP is plotted in (b). The red dots indicate the depth locations where both $\Delta p$ and $\Delta I_{\text{main}}/I_{\text{main}}$ responses were observed in (c) and (d), respectively. (e) displays the fraction transmitted intensity changes from the 690 nm (red) and 840 nm (blue) wavelength bands. Each trace is an average of 100 responses and has 5 kHz bandwidth for the 840 nm system and 2 kHz bandwidth for the 690 nm system and the fractional transmitted light intensity change, respectively.
The fractional transmitted light intensity changes of the 690 wavelength band were found from the squid nerves frequently. The transmission light intensity change of 690 nm from NK3630 shows the decreases in transmission predominately. Figure 5.12 shows the percentage of trials from each nerve for experiments stained with NK3630. Among all the detected signals, we found that the polarities are mostly negative except for the small amount of biphasic signals shown. This is consistent with the results from the literature [72] as shown in Figure 5.13 where around 690 nm the fractional intensity change of transmitted light gives the largest fractional changes.
Figure 5.12 Comparison between the percentage of trials where we observed the fractional transmitted light intensity changes of 690 wavelength band in different nerves.

Figure 5.13 Wavelength dependence of the optical signals. The amplitudes of the largest fast optical signal were plotted as a function of different wavelengths of the incident light [72].

5.5.3 Image registration

The scattering and absorption changes of the nerves stained with voltage-sensitive dye are known to be wavelength-dependent with respect to the incident light. We performed the image registration of 840 and 690 systems (red: 840, green: 690nm). The overlap region indicates
that the dual-wavelength system is probing the same region of interest. Although the lateral resolutions are comparable, the axial resolutions are different for 690 and 840 nm systems. The strategies for accurate registration and signal processing of the OCT images between the 840 nm and 690 nm systems need to be further studied.

5.6 Discussion and Future Directions

Although OCT does not require exogenous molecules for detecting neural activity, we used a voltage-sensitive dye, which transduces the change of membrane potential to light intensity change, as an OCT contrast agent. We examined eighteen different voltage-sensitive dyes in this project. An earlier study [21] in our group reported the enhancement from a near-infrared dye, IR144. The IR144 was used again in this study and showed very promising results for the dual-wavelength system. The NK3630 demonstrated the prominent backscattered signal changes mainly from the 840 nm OCT system and showed a decrease in transmission of 690 nm light during AP arrival to the measurement site, which is consistent with the results reported in [72].
Signal changes when using a voltage-sensitive dye depend on the wavelength of the incident light and the action spectrum of the membrane potential-related optical signal varies from species to species [76]. Thus, the dual-wavelength OCT system is advantageous for imaging membrane potential ratiometrically provided that the dye and/or the wavelength regions are properly chosen. The idea is proposed by different research groups [77], [78] previously taking the merit of the opposite changes in the voltage-dependent optical signals at the lower and higher halves of the action spectrum. In addition, the shape and the polarity of these intensity and phase signals are different in different nerve bundles probed by the dual-wavelength system. Nevertheless, the origins and the mechanisms still remained elusive. While further investigations are needed to understand the mechanisms of the AP-induced phase and intensity signals of OCT, its depth-resolved backscattered signals from both wavelength bands of 690 nm and 840 nm apparently provide more information than the conventional transmission-mode recording.

Quantitative evaluation of light attenuation in stained nerves by either fitting the slope of the logarithmic reflectivity of OCT signal in depth or using the depth-resolved model-based reconstruction of attenuation coefficients [79] can also be investigated as some of the voltage-sensitive dyes are known as “absorption dyes”. This may also possibly provide the localized AP-related attenuation coefficients enabling a contrast-enhancing method for neural detection.

Although this study reports the results from a depth profile (M-mode imaging), function in cross-sectional slices may be attained by employing a fast and stable scanner together with high-speed cameras. The cross-sectional imaging of neural activity is presented in the next Chapter.

5.7 Conclusion

We have designed and constructed dual-wavelength polarization-sensitive spectral-domain optical coherence tomography for functional neural activity studies with voltage-sensitive
dyes from two wavelength bands at 690 nm and 840 nm. Of all the dyes we tested, NK3630 (N = 4) and IR144 (N = 2) demonstrated the most prominent signal changes. It is important to note that limited numbers of nerves (~N = 2) were used for testing each dye. A semi-automatic detection and signal processing algorithm were developed for faster and better analysis, respectively. This optical measurement scheme extracts functional spectroscopic features with contrast-enhancing voltage-sensitive dye.

With further developments in the analysis methodology and experimental staining procedures, our observations can be used to obtain a multi-contrast enhancing approach of non-contact neural detection as well as in-depth understanding of neural activity. Subsequently this research may lead to early detection of neural diseases as the reliability and amplification of the optical signals are required for functional neural imaging.
We have presented the action potential related transient backscattered light intensity and phase changes. In this chapter, the extension of previous work from a single depth-profile to a cross-section is presented. We built a functional optical coherence tomography (OCT) cross-sectional scanner to detect neural activity using both stained and unstained unmyelinated nerves dissected from squid. Transient phase changes from backscattered light were detected during action potential propagation. The results demonstrate that the scanner can provide high spatiotemporal resolution cross-sectional images of neural activity (15 µs/A-line; 0.25 ms/B-scan; ~8.5 x 5.5 µm² in xz). The advantage of this proposed method is a dramatic increase in the number of available sites that can be measured and demonstrates that two-dimensional monitoring of small scale functional activity would also be feasible.⁸

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⁸ The content of this chapter has been published in the following peer-reviewed article:
6.1 Introduction

In recent decades, beginning with the pioneering work of Professor Lawrence B. Cohen and his colleagues, there has been active investigation into the use of light to probe neural function. Light scattering, birefringence, fluorescence and structural changes can be optically detected during action potential propagation [52]. Transient displacements were demonstrated from crustacean nerves [58], [60]–[62], garfish olfactory nerves [80], squid giant axons [20], [59] and mammalian nerve terminals [81]. However, intrinsic optical signals are often very small, which has stimulated the development and the use of added molecular probes to monitor nerve activity [82]. Fast voltage-sensitive dyes, that report changes in membrane potential as changes in absorbance, birefringence, dichroism, fluorescence, fluorescence resonance energy transfer (FRET), second harmonic generation and two-photon fluorescence, have been used to greatly enhance the signal-to-noise ratio (SNR) [83]–[86]. In order to detect the intrinsic or extrinsic signals of neural activity, optical setups varying in measurement geometry, spectral characteristics of light source and temporal and spatial resolutions have been utilized. Optical methods can have high horizontal spatial resolution and can record from many cells simultaneously. Depth-resolved measurement of nerve activity has been achieved primarily by microelectrode and multisite electrical recordings [87]. Simultaneously monitoring neural activity with optical resolution at multiple sites at different depths is still a major task needing further investigation.

Optical coherence tomography (OCT) [2] is a non-contact depth-resolved imaging technique with high resolution and sensitivity that has been widely used in the biomedical field. Spectral-domain implementations of OCT can provide real-time video-rate visualization of tissue microstructures in optical cross-sections. While the amplitude of the interferometric signal is generally used for structural imaging, the phase-sensitive OCT measurements utilize small
variations in the optical path length that might be induced by physical or refractive index changes. The Doppler and polarization-sensitive OCT systems primarily target the blood flow and tissue birefringence, respectively, to provide additional information. Previous OCT functional studies demonstrated intrinsic changes in the intensity and phase of the back-scattered/reflected light from neural tissue during activity in various preparations.

The OCT intensity measurements have been used to detect visually evoked cortical functions in cat brain [88]–[90], electrically evoked (forepaw stimulation) somatosensory changes in rat brain [91], [92], and light activated changes in frog [93], rabbit [94], rat [95], chicken [96] and human [97] retinas. Also, the intensity signals due to electrical stimulation of the abdominal ganglion of sea slug [19], [98] and squid giant axon [21] have been reported. The initial OCT phase measurements have utilized dual-channel low-coherence reflectometers to measure action potential related optical path length changes (\(\Delta p\) response) from a single observation point. The \(\Delta p\) response was about 1 nm in amplitude and 1 ms in duration for nerve bundles dissected from crayfish claw [61], and about 5 nm in amplitude and 10 ms in duration for nerve bundles dissected from lobster leg [62]. Then, the spectral-domain OCT, which monitors a full depth profile simultaneously, has been utilized to measure 0.5-2.5 nm \(\Delta p\) responses from lobster and crayfish nerve preparations [13], and to study the \(\Delta p\) response in detail with the squid giant axon preparation [20]. A common-path phase-resolved OCT has also been used to detect 20-30 nm signal from the optic nerve of horseshoe crab [99]. In addition to these intrinsic signals, the simultaneous phase and intensity measurements of spectral domain OCT have shown action potential related signals from squid giant axon stained with a voltage sensitive dye [21].

In this chapter, we present cross-sectional imaging of activity associated with neural action potentials. Electrical pulses were administered to initiate action potential propagations through squid nerve with and without voltage-sensitive dye staining, while a polarization
maintaining fiber (PMF) based spectral-domain OCT laterally and repeatedly scanned the beam over the nerve. Transient changes in the phase signal were detected at the time of action potential propagation through the measurement site. The advantage of the scanner is to extend the previous action potential related neural activity studies at a point or along a depth profile (z) to two-dimensional (xz) functional imaging studies at high-resolution.

6.2 Approach

6.2.1 System setup

A polarization-maintaining-fiber (PMF) based OCT system (Figure 6.1) was used for cross-sectional imaging of neural activity. The light source is a 25 mW broadband near-infrared superluminescent diode (SLD) with a full width at half maximum (FWHM) bandwidth of 50 nm centered at 840 nm (Broadlighter S840, Superlum, Ireland), yielding an axial resolution of ~5.5 μm in tissue. After passing through a polarizer in the fiber bench, linearly polarized light is coupled to one of the orthogonal channels of PMF and then split into reference (70 %) and sample (30 %) arms by a 2×2 polarization-maintaining (PM) coupler (Canadian Instrumentation & Research Ltd., Canada).

In the reference arm, collimated light passes through a quarter-wave plate (QWP) oriented at 22.5º with respect to the PMF channel and an achromatic lens (f = 30 mm), and returns from a reflector, which is the front surface of a glass wedge. As in conventional polarization-sensitive OCT [66], the returning light becomes linearly polarized at 45º; and therefore, couples back to the fast and slow axis of PMF equally.

In the sample arm, light from 690 nm SLD and 840 nm system were combined by a dichroic mirror (T760lpxr, cut-off wavelength: 760 nm, Chroma Technology Corp., Bellows Falls, VT). This configuration was already setup for m-mode dual-wavelength study described in Chapter 5. For this experiment, visible light (690 nm) was only used for
positioning the sample prior to data acquisition. A galvanometer-controlled mirror (Cambridge Technologies, Cambridge, MA) directed the light onto the nerve, whose axis was aligned 45º to the light polarization. Polarization effects of imperfect optical components in the sample arm were minimized by using wave-plates before and after the dichroic mirror yielding approximately 22 dB isolation between the main and cross-polarized channels. The phase measurements from the squid nerve, which has low birefringence, do not require polarization sensitivity, as the phase information is obtained from the main channel. A 19-mm focal-length lens focused the beam on the sample yielding ~8.5 µm lateral resolution.

Light returning from the reference and sample arms mixes in the PM coupler. The interference occurs when the optical path difference between these arms is within the imaging depth. The PM fiber directs the interferometric signal in its slow and fast channels to a high-speed custom-built spectrometer. In the spectrometer, light collimated by an achromatic lens \((f = 75 \text{ mm})\) is dispersed to its spectral components by a 1200 lines/mm transmission grating (Wasatch photonics, Logan, UT). A Wollaston prism (Karl Lambrecht Corp., Chicago, IL) with a 6º splitting angle separates the two channels, which are then focused by an achromatic lens \((f = 200 \text{ mm})\) onto a CMOS line-scan camera (Sprint, spl4096-140km Basler, Ahrensburg, Germany). Since the spectra of the main and cross channels are positioned side by side, a single acquisition of the camera is sufficient to capture the interference related spectral oscillations on both channels (1024 pixels per channel with 2x2 binning). The spectrometer was calibrated with a commercial spectrometer (HR2000+, Ocean Optics, Dunedin, FL) by using a diffraction grating coupling selected wavelengths of the light source to these spectrometers and fitting the readings to a curve [68].

The spectra carrying interference related oscillations on both PMF channels were acquired at the rate of 66.67 kHz (15 µs/A-Line) simultaneously. The galvanometer-controlled mirror was scanning a triangular wave with a 0.5 millisecond period continuously during the
acquisition for 16 ms. The optical power on the sample was 1 mW. An isolated pulse stimulator (Model 2100, A-M Systems, Sequim, WA) was used to deliver electrical current pulses of varying amplitudes to the squid nerve for the duration of 50 µs. Electrical signals from the nerve were amplified and recorded simultaneously by an AC differential amplifier (Model 1800, A-M Systems, Sequim, WA) and a data acquisition card (PCI 6110, National Instruments, Austin, TX), respectively. The action potential traces were displayed on the oscilloscope to monitor the nerve functionality over time.


6.2.2 Electrical stimulation and functional imaging

Detailed nerve chamber design and electrode recording settings can be found in section 5.3.1. The linear light polarization impinges 45 degrees (or 135 degrees) to the long axis of the nerve, which results in maximum cross-polarized light intensity [75]. The nerves were cooled prior to recording to prolong the viability of the nerve and increase action potential duration. The
amplitude of the stimulus current pulses was 4 mA. The imaging system simultaneously provided both structural and functional information due to the high sampling rate of the line-scan camera and the small scan area which was repeatedly scanned rapidly (0.5 ms period).

To choose a location for probing the functional activity, we monitored the larger cross sectional scanning range of the squid nerve in the nerve chamber shown in Figure 6.2(a). The arrow indicates the axis of symmetry of the cross sectional images as we apply one cycle of the triangular wave to the galvanometer. The black dash lines of Figure 6.2(a) show the approximate location of the smaller range functional scanning. A 20-μm outer sheath was observed between the giant axon and the small nerve fibers of the stained squid nerve. The dark sloping line near the top is the glass-solution interface. The chamber was tilted to avoid saturation of specular reflection from glass surface. Once the area was selected, the smaller scanning range of the functional site (Figure 6.2(b)) was recorded.

Figure 6.2 OCT image (reflectivity contrast) of a squid nerve containing the small fibers and the giant axon (*) within a larger scanning range, scale bars: 100 μm in (a). The arrow indicates the axis of symmetry of the cross sectional image and the black dash lines indicate the approximate location of small scan, scale bars: horizontal, 10 μm; vertical, 100 μm in (b). Dynamic range: 45 dB.
6.2.3 Data processing and analysis

The spectra acquired by the line-scan camera for the orthogonal polarization channels were resampled to k-space and dispersion imbalance between the reference and sample arms was compensated in software. An inverse Fourier Transform was then applied to the k-space spectra to get the complex valued depth profiles, which are in the form of $A_m(z)\exp\{i\phi_m(z)\}$ where $A_m$ is the amplitude and $\phi_m$ is the phase at particular depth $z$ and the subscript $m$ refers to channel number (1 or 2). The reflectivity information $R$ is proportional to the square of the amplitudes as follows,

$$R(z) \propto A_1^2(z) + A_2^2(z)$$  \hspace{1cm} (6.1)

OCT images (Figure 6.2) show the microstructure with the reflectivity contrast, typically in logarithmic scale. The images are formed by stacking several depth profiles acquired during lateral scanning of light over the sample. The phase of the complex depth profile allows quantification of optical path length variations at particular depths. The phase values are affected by environmental perturbations changing the path difference between the reference and sample arms. Hence, we removed the common-mode phase noise by subtracting the phase of a reference surface (glass-solution interface above the nerve) from the phase of the nerve locations. To calculate $\Delta p(z)$, we used the phase of the main channel, $\phi_1$, as

$$\Delta p(z) = \frac{\lambda_0}{4\pi} (\phi_1(z) - \phi_1(z_r))$$  \hspace{1cm} (6.2)

where $\lambda_0$ is the center wavelength, and $\phi_1(z)$ and $\phi_1(z_r)$ denote the phases of the observation points and the reference glass surface, respectively.

Intensity contrasts can be served as AP indicators to localize the neural activity. The intensity change of main channel was calculated by

$$\Delta I_1(z) = I_1(z) - \overline{I_1(z)}$$  \hspace{1cm} (6.3)
where $A_1$ is the amplitude of the main channel at a particular depth $z$.

A functional back-and-forth scan consisted of 32 consecutive optical cross-sections (32 triangular waves) during the recording. The acquisition scheme of the cross-sectional neural activity is shown in Figure 6.3. The scan range was adjusted by the amplitude of the triangular wave applied to the galvanometer controller. Optical contrasts of the cross-section were acquired before, during and after the action potential propagation. The signals for 10 and 90 responses were averaged to improve the SNR for analysis. A mask was created from the reflectivity image with a 25 dB threshold and applied to the phase image to exclude the low SNR regions since low reflection regions will result in unreliable phase values. To better illustrate the structural changes inside the squid nerve, the mean-subtracted absolute values of the $\Delta p$ responses were taken due to the fact that the $\Delta p$ responses can exhibit positive or negative deflections.

Figure 6.3 The acquisition scheme of the cross-sectional neural activity. Scale bars: 100 µm.
6.3 Nerve Preparation

Live squids (*Loligo pealeii*) were obtained and dissected at the Marine Biological Laboratory, Woods Hole, MA. The giant axon together with surrounding small fibers was extracted under a microscope. The solution was seawater. The staining was with an oxonol voltage-sensitive dye NK3630 (RH482, from Nippon Kankoh-Shikiso Kenkyusho Co., Ltd., Japan) 0.25 mg/mL with 1% DMSO for 20 minutes in dark environment. The nerve, stained or unstained, was placed in the nerve chamber and covered by a glass window in order to provide a reference surface for phase measurements.

6.4 Results

6.4.1 Structural and functional imaging

The PMF-based OCT simultaneously provides the main channel backscattered light intensity, cross-polarized channel light intensity, the structural image (equation 6.1), and the phase signals (equation 6.2) which may vary due to neural activity. Figure 6.4(a) and (b) depict the larger scope of reflectivity and the smaller scope of reflectivity with a dynamic range of 45 dB. The darkness of the structural image indicates the reflectivity from the squid nerve. This is a different location and a narrower region than Figure 6.2. It is a region with only small nerve fibers of the stained nerve, whereas Figure 6.2 shows the stained squid giant axon and small nerve fibers. The pixelated image in Figure 6.4(b) contains fewer number of depth profiles (34 A-lines) due to the fast scanning speed. The reflectivity contrast shows the tissue light scattering properties of the squid small fibers within the smaller region of interest. Within the same measurement, the single-pixel reflectivity and phase, and their time-varying characteristics can be obtained.
Figure 6.4 (a) Larger scope (scale bars: 100 µm) and (b) smaller scope (scale bars: horizontal, 10 µm; vertical, 100 µm) of reflectivity showing the small fibers of a stained squid nerve. The smaller scope was scanned repeatedly for functional recording. The arrow indicates the turning point and the black dash lines show the approximate location of the smaller range functional scanning in (b). Dynamic range: 45 dB.

6.4.2 $\Delta p$ response

Figure 6.5 demonstrates the $|\Delta p|$ response in cross-sectional slices before (~3 ms), during (~8 ms) and after (~12 ms) the arrival of action potential to the measurement site. The electrical action potential recording is provided as well. Ninety trials ($N = 90$) from a stained squid nerve were averaged to increase the SNR, although averaging ten trials ($N = 10$) was shown to be sufficient to identify neural activity as shown in Fig. 6.6(c). The image size in Figure 6.5 is about 90 µm × 305 µm. Since the galvanometer-controlled mirror was scanning a triangular wave signal for each cross-section, the images show the mirror image at the turning point. Considering two mirrored images, the actual scan range was 45 µm in the lateral direction. The black arrow indicates the turning point found by post-image correlation. The cross-correlation of the ramp-up and ramp-down images within the acquisition time indicates that the lateral scanning was not drifting or shifting during the recording time.
The conduction velocity of squid small fiber bundles is slower than the squid giant axon due to the smaller axon diameters. The electrical current stimulation pulse was applied at \(\sim 4\) ms after the start of the recording, resulting in stimulus artifact due to the residual current propagating from the stimulus electrodes to the recording electrodes. Unlike electrical recordings, optical recordings were free of stimulus artifacts. When the action potential reached the optical recording window at \(\sim 8\) ms, the \(|\Delta p|\) response from multiple sites occurred correspondingly. The recovery after the action potential is shown at \(\sim 12\) ms. The \(|\Delta p|\) response has not completely returned to baseline at \(\sim 12\) ms, which is consistent with previous recordings with squid giant axons [20]. We interpret the \(\Delta p\) signal shown in Figure 6.5 (b) as indicating regions of the fiber bundle that were active.

![Figure 6.5 |\(\Delta p|\) response of a stained squid nerve at about (a) 3 ms, (b) 8 ms and (c) 12 ms, and the corresponding action potential recording (scale bars: 0.2 mV). Stimulus is presented at about 4 ms. The arrows indicate turning point of the galvanometer. Size of the region: 45 \(\mu m\) (from 0 to arrow) \(\times\) 305 \(\mu m\). Ninety trials were averaged.](image)
Figure 6.6 visualizes the action potential trace and the details of $\Delta p$ response from the same scan. The red dashed line in Figure 6.6 (a) indicates the timing of the cross-sectional images. Figure 6.6 (b) and (c) represent the $|\Delta p|$ response at ~8 ms by averaging 90 trials ($N = 90$) and 10 trials ($N = 10$), respectively. The image dimensions are about $90 \, \mu m \times 771 \, \mu m$. With the 10-trial average, the responses can be detected from the stained squid nerve. The functional cross-sectional images do not carry the sign information of the $\Delta p$ responses since the absolute values of $\Delta p$ signals were used to better illustrate the $\Delta p$ responses. Figure 6.6 (d) and (e) show the time course of the $\Delta p$ response for selected pixels with $N = 90$ and $N = 10$, respectively. The actual locations of the pixels are given by pixel numbers in the coordinate system defined as (lateral index, depth index). The pixels are also marked by the light blue circles in Figure 6.6 (b). The action potential traces are given in blue for the time registration with the optical signals. Pixel $(20,123)$ has the high standard deviation of the $\Delta p$ signal during the 16 ms acquisition time. Pixels like this are masked out (dark pixels) in Figure 6.6 (b) and (c) due to the low reflectivity values. Averaging multiple trials reduces the noise as shown in Figure 6.6 (d) and (e). The noise on $\Delta p$ measurement was analyzed for the pre-stimulus interval at multiple locations within the cross-section. Averaging 90 trials resulted in ~2.64-fold improvement over averaging 10 trials, which agreed with the theoretical 3-fold improvement.
Figure 6.6 Details of the $\Delta p$ response. The data was also presented in Figure 6.5. Action potential (a) and $|\Delta p|$ response at ~8 ms is given by averaging 90 trials ($N = 90$) (b) and 10 trials ($N = 10$) (c). In b and c, arrow indicates the turning point of the galvanometer, and pixel numbers are given in x-axis and y-axis. Scale bars: horizontal, 10 µm; vertical, 100 µm. Signal traces in time (d, e) are given for selected pixels (lateral index, depth index), which are marked by blue circles in b, with averaging 90 and 10 trials. Action potential traces in blue are for guidance.
Using another location of the same stained nerve, the $|\Delta p|$ images at two different times and the $\Delta p$ traces during the recording time for four pixels are demonstrated in Figure 6.7. The results are averaged over 90 trials. The time points show different $|\Delta p|$ image at ~7 ms and ~8.5 ms as the red dashed lines on the action potential indicate. The light blue circles are the actual locations plotted in Figure 6.7 (d). Monophasic and biphasic signals were observed with increase and decrease phases in $\Delta p$ response. Also, the maximum $\Delta p$ responses occurred at different timing, e.g. ~7-9 ms. Interestingly, pixel (9,62) shows the upward phase response whereas pixel (17,64) and (21,121) show predominately downward phase responses. Typically, we observed that the $\Delta p$ responses gradually change from upward to downward direction from the top to the deeper nerve areas across all the recording sites. Also, pixel (11,64) and (17,64) have the same depth pixel index but different lateral pixel index corresponding to a separation of ~16 $\mu$m. (17,64) has a predominately downward direction $\Delta p$ response whereas (11,64) has the biphasic $\Delta p$ response. This suggests different structural changes during the neural activity are happening within a very small range.
Figure 6.7 Action potential recording (a), $|\Delta p|$ images at ~7 ms (b) and ~8.5 ms (c), and $\Delta p$ traces of selected pixels (d) recorded from a stained squid nerve. Ninety responses were averaged. Arrow indicates the turning point of the galvanometer. Scale bars in b and c: horizontal, 10 µm; vertical, 100 µm. The blue circles in b are the actual locations plotted in d. Pixel coordinates are given as (lateral index, depth index). Blue trace is the action potential recording for registering time.
Without staining, we also observed the $\Delta p$ responses from squid nerves. Figure 6.8 shows the $\Delta p$ responses from an unstained nerve. The $|\Delta p|$ image in Figure 6.8 (b) represents the response at ~10.5 ms. The light blue circles on the image are the actual locations plotted in Figure 6.8 (c). In this set of recordings, the scanning range (90 $\mu$m) was doubled compared to the stained cases. This might result in higher noise level due to wider range of scan at the same scanning rate.

![Figure 6.8 Action potential recording (a), $|\Delta p|$ image at ~10.5 ms (b), and $\Delta p$ traces of selected pixels (c) from an unstained nerve with averaging of 90 trials. Arrow in b indicates the turning point of the galvanometer mirror, scale bars: horizontal, 10 $\mu$m; vertical, 100 $\mu$m. Pixel coordinates in c are in the form of (lateral index, depth index), and the locations are marked by blue circles in b. The blue trace is the action potential recording for registering time.]

We chose five cross-sections exhibiting $\Delta p$ signals from a stained nerve and an unstained nerve. During the action potential propagation, the mean and standard deviation of the $|\Delta p|$ responses for a stained and an unstained nerve were 5.54 ± 3.39 nm (N = 6834 pixels) and 4.01 ± 2.65 nm (N = 7638 pixels), respectively. Low SNR regions (< 25 dB) were excluded from the analysis. On average the $|\Delta p|$ responses were larger from the stained nerves and their distribution was broader and more skewed towards larger values. Two of the cross-sections, one presented...
here, for the stained nerve demonstrated larger $\Delta p$ signals, but the other three cross-sections were similar to the unstained values. However, a potential effect of dye on $\Delta p$ signals remain obscure as the number of squids used in the study should be increased.

6.4.3 Assessments on intensity and phase measurements

The PMF-based OCT system offers phase and intensity-based contrasts. For example, the backscattering intensity on the main and cross-polarized channels forms reflectivity (equation 6.1). These channels can also be used to analyze retardance or birefringence. In this study, we could not reliably obtain a detectable functional change on the intensity-based contrasts during neural activity. We analyzed the intensity signal on the main channel, $A_1(z)$, by randomly selecting 10 A-lines from a nerve cross-section and excluding the low SNR regions (reflectivity < 30 dB). Figure 6.9 illustrates the intensity signal on the main channel from one of the randomly selected A-line in a cross-section. The red dashed line is the noise level and the blue dashed line is the 30 dB threshold. The amplitude fluctuation due to perturbations owing to vibrations and noise were calculated by the standard deviation divided by the mean of the peak values of the coherence functions. For a single trial, the amplitude fluctuation was 16.9% for the unstained nerves and 15.6% for the stained nerves. The numbers for M-mode imaging, 8.9% and 8.0%, showed better stability of the intensity measurements. Averaging 90-trials reduced the amplitude fluctuations due to perturbations for both unstained and stained nerves (2.5% and 3.1% for lateral scanning, and 1.8% and 2.2% for M-mode imaging, respectively).
Vibrations that change the optical path length difference between the reference and sample arms shift an entire depth profile up and down by a small amount with time. As a result, a bulk motion along the axis of the light beam due to mechanical noise appears to exist between the images. A stationary surface (glass-solution interface), with its phase multiplied by $\lambda/(4\pi)$, can be used to quantify the axial bulk motion. For a single trial, the standard deviation of this noise within 16 ms measurement time can exceed 10 nm (more with longer recordings). The $\Delta p$ response did not suffer from this noise because of the differential operation. However, the intensity signal of a particular pixel could require software stabilization of the depth profile. After obtaining the axial bulk motion, we eliminated it from the images by reprocessing the spectral (k-space) data using the translation property of Fourier transform. However, fluctuations of the intensity signal due to other noise sources were much larger and action potential propagation signals could not be identified. In addition, the action potential-induced phase signals indicate local transient structural changes in the nerve, which can also influence the intensity signal. For
instance, a 20 nm shift of a 5.5 µm wide Gaussian-shaped coherence function could induce a 0.004% fractional amplitude change at the peak, and ~1% change at the FWHM. These numbers are much smaller than the amplitude fluctuation (noise) discussed above; nevertheless, the effect of Δp response can be decoupled from intensity change when such a signal is detected.

To assess the sensitivity of the Δp measurement we imaged a coverslip sample by both M-mode and cross-sectional scanning with the same experimental settings and Δp was calculated from the phases of the top and bottom surfaces from the coverslip. The phase sensitivity is calculated as the standard deviation of this Δp. Analyzing the Δp signals for each A-line over 16 ms recording time resulted in an average standard deviation of 425 pm within a cross-section. The optical path length variation for both top and bottom surfaces of the coverslip (bulk movement) was much larger than the differential signal (Δp) resulting in a common-mode rejection ratio of approximately 13 dB. The average standard deviation for M-mode imaging was 341 pm. An unpaired student’s t-test suggested that the standard deviations for m-mode and scanning configurations were not statistically significant (p = 0.21). However, owing to surface non-uniformity in biological tissues, the sensitivity values for a nerve sample could degrade compared to a simpler structure. A potential advantage of scanning might be the reduction of light exposure onto the same nerve location, which might avoid photobleaching in some studies. Scanning also supports application of image processing techniques such as clustering and spatial averaging.

6.4.4 Complex averaging and traditional averaging

We further processed the data using complex averaging [100]. For complex averaging, the complex-valued depth profiles is averaged in the complex plane (i.e. a + bi) rather than in the polar coordinate form (i.e. \( A_m(z)\exp\{i\varphi_m(z)\} \)). In further detail, the real part and the imaginary part are realized by 90-trial averaging the real parts and imaginary parts of the complex-valued depth profiles, separately. Then, the average phase is obtained as the argument of the complex-
valued depth profiles. Figure 6.10 (b) and (c) visualize the neural activity at ~ 10.5 ms using the 
traditional averaging described in section 6.4.2 and complex averaging, respectively. The blue 
circles on the image are the actual locations plotted in Figure 6.10 (d) and (e).

Figure 6.10  Action potential recording (a), $|\Delta \rho|$ image at ~10.5 ms (b), complex averaging $|\Delta \rho|$ image 
with the same time points (c), and $\Delta \rho$ traces of selected pixels (d) and (e) from an unstained nerve 
with averaging of 90 trials from (b) and (c). Arrow in (b) and (c) indicates the turning point of the 
galvanometer mirror, scale bars: horizontal, 10 µm; vertical, 100 µm. Pixel coordinates in (d, e) are 
in the form of (lateral index, depth index), and the locations are marked by blue circles in b. The 
blue trace is the action potential recording for registering time.
We also quantitatively assessed the SNR of the two averaging methods. Using the same evaluation criteria: for pixels exhibiting $|\Delta \rho|$ responses of 10 nm or more, the SNR was calculated by the ratio of the magnitude of the response (signal) and the standard deviation in the pre-stimulus interval (noise), which resulted in an average SNR of 6.7 using the traditional averaging with averaging 10 trials.

Figure 6.11 shows the scatterplots of standard deviation of pre-stimulus interval versus signal level of complex averaging and traditional averaging for 10-trial and 90-trial averaging. Complex averaging with 10 trials (Figure 6.11(a)) shows the SNR of ~7.5 for the phase signals for those pixels having reflectivity values over 25 dB; whereas, traditional averaging (Figure 6.11(c)) with 10 trials yields an SNR of ~6.7. It is important to note that the 25 dB reflectivity threshold resulted in more pixels for traditional averaging which would degrade the SNR value (6.7). Figure 6.11(b) and (d) show the results with 90-trial averaging which improve the SNR to ~20 and ~18.7 for complex and traditional averaging, respectively. However, the traditional averaging with the same pixels as in complex averaging resulted in SNR of ~21. Therefore, the two averaging methods in this study were found comparable in terms of SNR values. Different averaging methods may be investigated for functional neural imaging studies.
6.4.5 Possible Cross-Sectional Intensity Change

We further processed the data from the intensity contrast of the main channel, $A_i(z)$, which might produce a change in backscattered light intensity as an AP indicator to localize the neural activity if the contrast-enhancing voltage-sensitive dye is properly selected. Due to the fact that the intensity change can have positive and negative values, the absolute value of the intensity change was taken for illustration purposes. Figure 6.12 shows the 90-trial averaged reflectivity signal in cross-sectional slices before (~3 ms), during (~8 ms) and after (~12 ms) the arrival of
action potential to the measurement site from the same location as Figure 6.6. When the action potential arrived at \( \sim 8 \) ms, the \( |\Delta I|^2 \) response from multiple sites can be merely observed.

![Figure 6.12 |\Delta I|^2 response of a stained squid nerve at about (a) 3 ms, (b) 8 ms and (c) 12 ms, and the corresponding action potential recording (scale bars: 0.2 mV). Stimulus is presented at about 4 ms. The arrows indicate turning point of the galvanometer. Ninety trials were averaged.](image)

Unlike the \( |\Delta p| \) responses shown in the previous section 6.4.2, the cross-sectional \( |\Delta I|^2 \) responses do not carry out the promising results. Thus, we plotted the fractional intensity changes from the main channel of each single pixel, accordingly. Figure 6.13 shows the possible fractional intensity changes from the same location presented in Figure 6.12. Pixel (27,140) shows \(-12\%\) fractional intensity change. Whereas pixel (16,140), (31,140) and (3,165) have the unreliable values before \( \sim 2 \) ms, which may coming from the unreliability of the start of the scanner. Also, pixel (31,140) and (32,140) might not be resolved by the lateral resolution. However, spatial averaging can be employed for improving SNR. The pixels shown in Figure 6.13 are all near the low reflectivity level. In addition, the signal range and the SNR of the fractional changes are too small to visualize in the cross-sectional frames.
Figure 6.13 Possible fractional intensity changes from the same location presented in Figure 6.12. Pixel coordinates are in the form of (lateral index, depth index.)
6.5 Discussion

We have demonstrated cross-sectional imaging of interferometric phase changes ($\Delta p$ response) during action potential propagation using the spectral-domain OCT. The squid nerve contained small fibers, and appeared to display larger $\Delta p$ responses compared to the squid giant axon [20]. However, $4.01 \pm 2.65$ nm response of the unstained nerve (this study) should not be directly compared to $1.65 \pm 2.01$ nm response of the giant axon in cold environment [20]. The smallest detectable response was better for the giant axon study due to increased number of averaging and M-mode imaging that allowed temporal filtering. Transient structural changes during nerve activity are typically small (on the order of nanometers), and traditionally require averaging. Although the sensitivity of phase-sensitive imaging systems are less than a nanometer, several factors such as SNR decrease, perturbations and heterogeneities in nerves affect the measurement. In the case of M-mode imaging, neural activity is probed by fixing the sample beam on a region of interest. Functional nerve measurement from an entire depth profile (A-line) is available when the M-mode approach is used with spectral-domain OCT [13]. Without averaging, it was able to clearly measure a $\sim 21$ nm $\Delta p$ response from a squid giant axon that was in a cold hypertonic solution [20]. In the current study, we showed imaging of a similar magnitude $\Delta p$ response in an optical cross-section with an average of 10 trials. The advantage of this method is a dramatic increase in the number of available sites that can indicate action potential related activity in cross-sections.

Future OCT studies may target the intensity and polarization signals of neural activity by implementing several improvements. These include increasing the stability, decoupling phase and intensity signals, designing a system at another wavelength that voltage-sensitive dye responds favorably, and testing with different dyes. The stability issue could be addressed by minimizing the vibrations of the nerve chamber/holder, optimizing repeatability of the scanner, or avoiding
the use of a physical scanner. The latter would only allow for M-mode imaging or require a bulk optics system with a high-speed area-scan camera that records spectra from a line focused beam on a sample.

Contrast enhancements of voltage-sensitive dyes are recognized to be wavelength and nerve-type dependent. The action spectrum of NK3630 (RH482) absorption dye, as reported for hippocampal slice preparation [72], suggests that the maximum fractional change of transmitted light intensity is at around 700 nm (~0.12%). Although the OCT measurement of back-scattered light from particular depths can yield larger fraction at particular depth locations with dye staining [21], the measurements are still expected to vary with design wavelength, species and staining procedure. The development of low-noise OCT light sources at visible wavelengths could facilitate light intensity and polarization-based detection of neural activity. Moreover, the opposite sign changes in the dye action spectra might suggest a dual-wavelength detection scheme for differential measurements.

6.6 Conclusion and Future Directions

We demonstrated cross-sectional imaging of transient structural changes due to action potential propagation. PMF-based PS-OCT was employed to image squid nerves with and without voltage-sensitive dye staining. Phase-sensitive measurement of optical path length change (Δp response) was shown to be useful for imaging the activity. In addition, possible fractional backscattered intensity change from main channel and complex averaging methods were explored. The imaging method increases the number of recording sites to yield neural activity in optical cross-sections at high resolution, which may support neurophysiological studies in future.

This functional imaging technology provides high-resolution, depth-resolved assessment of neural activity in cross-sectional images. Dual-wavelength cross-sectional scanner could be
developed to study the ratiometric information for different voltage-sensitive dyes. In addition, the scanner could be further expanded to record function in a three-dimensional volume by employing an additional scanner if camera can support higher acquisition rate. On the software side, complex averaging needs to be further investigated and evaluated for functional applications.
The pioneering work of Dr. Cohen and Keynes [55] and Dr. Muralt [101] encouraged other scientists to further investigate the transient optical changes that occurring during action potential propagation. Observations of a variety of optical properties, including absorption, fluorescence, scattering, structural and birefringence changes, associated with the activation of nerves were reported. However, fast intrinsic signals are often very small. Thus, we introduced fast voltage-sensitive dyes to enhance the action-potential related signal changes. In this chapter, the optical system is slightly modified to measure from the pike olfactory nerve. Both m-mode and cross-sectional imaging of transient changes during neural activity with and without dyes as contrast agents were presented.
7.1 Introduction

Optical imaging of neuronal function is a promising approach that avoids direct contact with tissue since light can propagate through biological membranes. Also, optical methods offer an outstanding spatial resolution and the possibility for three dimensional measurements from a wide range of spatial locations. Neural activity alters a variety of physical properties, such as birefringence, retardance, intensity and phase changes. The action potential (AP)-related biochemical and structural changes may be caused from reorientation of membrane molecules, influx and outflux of ions, and mechanical changes from the axonal surfaces.

Fast intrinsic signals, including light scattering and birefringence changes associated with action potential propagation from crab nerve [52] and squid giant axon [55] are observed. There have been a number of studies which utilize changes in transmitted cross-polarized light intensity to obtain changes in retardance. This is done by using a polarizer at 45° to the nerve axis and an analyzer which minimized the transmitted light intensity onto a detector. Measurements in transmission and reflection setups are demonstrated previously [102]. Cohen et al. [55] proposed that the transient retardance change (\(\Delta R\)) during action potential propagation can be calculated from

\[
\Delta R = \frac{1}{2} R \left( \frac{\Delta I}{I} \right)
\]

where \(R\) is the retardance due to the resting birefringence from the sample and \(\frac{\Delta I}{I}\) is the light fractional intensity change.

Muralt et al. [101] reported an AP-dependent rapid retardance increase of 37 pm with a resting retardance of \(R=23\) nm for pike olfactory nerve containing densely packed 4.2 million unmyelinated axons (95% are smaller fibers and 5% are larger fibers). The retardance change can
be a result of conformational changes in the membrane channels, influx and outflux of ions during excitation, and mechanical changes occurring on the axon surface.

In another study, Yao et al. suggested that birefringence changes with the passage of an action potential from lobster leg by using a cross-polarized reflected light measurement. These measurements, both utilizing transmission and reflection mode, showed these changes in functioning nerve. However, depth-resolved measurements in reflection geometry have not yet been demonstrated.

Retardance is a product of the birefringence of the medium with its thickness. As a result, variations in both birefringence and thickness can contribute towards retardance changes. Retardance changes entirely due to thickness change may be too small to explain the change in transmitted light intensity results present in literature; however thickness changes could alter light scattering and contribute to the intrinsic signals of neural activity.

In this chapter, we employed the optical setup in Chapter 5 and 6 to investigate the retardance, backscattered light intensity, and structural changes with both m-mode and cross-sectional scan.

### 7.1.1 Pike Olfactory Nerve

Earlier literature [101] reported that the pike olfactory nerve is about 1 mm in diameter and 5-7 cm long. The cross-sectional image of pike olfactory nerve in Figure 7.1 shows the densely packed nerve bundles comprising 95% of small axons (diameter between 0.1 μm and 0.3μm) and 5% larger fibers. The number of nerve fibers is about 4 million for the unmyelinated smaller axons and 0.2 million for larger axons. Measurements with pike olfactory nerve resulted in a fractional cross-polarized light intensity change of $3.3 \times 10^{-3}$, showing nearly $10^3$ larger than those from squid giant axon [55].
7.2 Nerve Preparation

The olfactory nerve of a freshly sacrificed northern pike (*Esox lucius*) is dissected under the microscope. A detailed description of the preparation can be found in ref [101]. After careful dissection, the olfactory nerves were extracted and placed in a dish with Ringer solution. Both ends of the nerves were tied with threads for positioning in the groove of the nerve chamber. Ringer solutions were prepared by dissolving solutes, which comprised of 120 mM NaCl, 4 mM KCl, 11 mM CaCl$_2$, 3 mM NaHCO$_3$ and 5 mM glucose. The solution was oxygenated using an aerator with a bubbling tip. The nerve was immersed in the solution during and after dissection.

Several pike olfactory nerves are treated with different staining conditions. The staining conditions are listed in Table 7.1.
Table 7.1 The dyes for staining the nerve and the staining conditions for each nerve

<table>
<thead>
<tr>
<th>Dye for staining</th>
<th>Nerve number</th>
<th>Staining concentration (mg/mL)</th>
<th>Staining period (minutes)</th>
<th>Additional solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK 3630</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>NK 3630</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>IR 144</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>IR 144</td>
<td>2</td>
<td>1</td>
<td>20</td>
<td>1% DMSO</td>
</tr>
<tr>
<td>PGH 6</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>1% Pluronic</td>
</tr>
<tr>
<td>PGH 6</td>
<td>2</td>
<td>1</td>
<td>30-35</td>
<td>1% Pluronic</td>
</tr>
</tbody>
</table>

All the nerves were stained in a dark environment. The nerve was placed in the nerve chamber and covered by a glass window in order to provide a reference surface for phase measurements.

7.3 Hardware Setup

Two different acquisition settings and optical setups were employed to study the pike olfactory nerves in m-mode (a single depth profile) and cross-sectional scanning mode.

7.3.1 M-mode Dual-Wavelength Optical Setup

In this study a system for dual-wavelength polarization-sensitive spectral-domain OCT was constructed. Figure 7.2 shows a sketch of the instrument that was used for both m-mode and cross-sectional data acquisition. This setup is very similar to the one presented in CHAPTER 5 and 6; therefore, only differences in the optics for the transmission path and the changes in
acquisition rates will be described in detail. Briefly, the two light sources with center wavelengths of 840 and 690 nm were used. A dichroic mirror (Chroma Technology Corp.) combines the sample arm of the two OCT systems onto the sample. The purpose of half-wave plate (HWP) placed after the dichroic mirror is to isolate the two orthogonal polarization states for both systems. Linear polarization states were impinged onto the sample with a 19 mm focusing lens for both 690 and 840 nm OCT systems, yielding lateral resolutions of ~7 µm and ~8.5 µm, respectively. The optical power onto the sample of the 690 nm OCT was 505 μW whereas that in 840 nm OCT was 750 μW. The long axis of the nerve was aligned 45º to the linear polarization states of both OCT systems. Transmission light was guided through the nerve chamber and the crossed-polarizer in order to detect the crossed-polarized light intensity changes during neural activity. The crossed-polarizer was aligned beneath the nerve chamber to minimize the light intensity detected by two detectors. The light leakage from the crossed-polarizer was measured to be 0.97% and 0.007% for 690 and 840 wavelength bands, respectively. Then, the light was coupled to the multimode fiber (Thorlabs Inc.) by a 16X objective lens. Light exiting from the multimode fiber was collimated and passed through filters (Filter A: FF01-650/100, Semrock; Filter B: FF01-794/160, Semrock; DF: FF801-Di02, Semrock) that help screen for wavelength-related light intensity changes. Photodetectors D1 and D2 record non-depth-resolved transmitted light intensity of 690 nm and 840 nm at the rate of 20 kHz, accordingly. All signals were synchronized and recorded using custom LabVIEW software (National Instruments, Austin, Texas). The spectra were acquired by a CMOS linescan camera (spl4096-140km Basler, Germany) and CMOS area scan camera (acA2000-340 km, Basler, Germany) at the rate of 4 kHz (250 μs/A-Line) and 1 kHz (1000 μs/A-Line) for 840 nm and 690 nm PS-OCT systems, respectively. The data acquisition software performed real-time display of the cross-sectional images to search for the optimized positions of the nerve before functional measurements. A cross-sectional image was obtained by scanning the beam laterally across the width of the pike
olfactory nerve. After identifying a nerve region, the beam was positioned at a selected lateral position. Once the position was set, the galvanometer controller was electrically terminated by using a 50 Ω connector at the input and a single depth profile of interest was recorded for both OCT systems simultaneously. The acquisition period was 250 ms.

7.3.2 Cross-sectional Imaging Optical Setup

This setup is similar to the one reported in CHAPTER 6 with slight modification. Briefly, the light source is a 25 mW broadband near-infrared superluminescent diode (SLD) with a full width at half maximum (FWHM) bandwidth of 50 nm centered at 840 nm (Broadlighter S840, Superlum, Ireland), yielding an axial resolution of \(~5.5\, \mu m\) in tissue. After passing through a polarizer in the fiber bench, linearly polarized light is coupled to one of the orthogonal channels of PMF and then split into reference (70 %) and sample (30%) arms by a 2×2 polarization-maintaining (PM) coupler (Canadian Instrumentation & Research Ltd., Canada).

In the reference arm, collimated light passes through a quarter-wave plate (QWP) oriented at 22.5° with respect to the PMF channel followed by an achromatic lens (f = 30 mm), and subsequently returns from a reflector. The returning light becomes linearly polarized at 45° and couples back to the fast and slow axis of PMF equally (derived from the Jones calculation).

In the sample arm, light from 690 nm and 840 nm systems were combined by a dichroic mirror (T760lpxr, cut-off wavelength: 760 nm, Chroma Technology Corp., Bellows Falls, VT). This configuration was already setup for the m-mode dual-wavelength optical setup described in the previous section 7.3.1. In this experiment, only visible light was used for measurements of crossed-polarized transmission light intensity.

A galvanometer-controlled mirror (Cambridge Technologies, Cambridge, MA) directs the light passing through a 19-mm focal-length lens onto the nerve, whose axis was aligned 45° with respect to the light polarization, resulting in \(~8.5\, \mu m\) lateral resolution. Polarization effects
of imperfect optical components in the sample arm were minimized by using an achromatic half wave-plate after the dichroic mirror. The transmission detection setup is described in section 7.3.1.

The PM fiber directs the interferometric signal returning from the reference and sample arms in its slow and fast channels to a high-speed custom-built spectrometer. The interference induced spectral oscillations occurs only when the propagation distance of reference and sample arms match within the imaging depth. In the detection arm, light carrying the interferometric information is collimated by an achromatic lens (f = 75 mm) and dispersed to its spectral components by a 1200 lines/mm transmission grating (Wasatch photonics, Logan, UT). The orthogonal polarization channels are separated by a Wollaston prism (Karl Lambrecht Corp., Chicago, IL) with a 6° splitting angle. The spectra are then focused by an achromatic lens (f = 200 mm) onto a CMOS line-scan camera (Sprint, spl4096-140km Basler, Ahrensburg, Germany), which consisted of 2048 pixels for two channels (1024 pixels/channel, 2x2 pixel binning). The spectrometer was calibrated with a commercial spectrometer (HR2000+, Ocean Optics, Dunedin, FL) by using a diffraction grating. A more detailed spectrometer calibration was described in section 6.2.1.

The spectra carrying interference related oscillations on both PMF channels were simultaneously acquired at a rate of 33.33 kHz (30 µs/A-Line) by the custom-built spectrometer. The galvanometer-controlled mirror was continuous scanned during the acquisition a triangular wave with a 6 millisecond period for 180 ms. The optical power onto the sample was 750 µW. The data packing process of the scanning configuration is illustrated in Figure 7.3.
7.3.3 The nerve chamber and electrical stimulation

The nerve chamber used is made of acrylic glass. The platinum electrodes are fixed to the chamber with epoxy to stimulate the sample and to record the action potentials. A cover glass is placed on top of the chamber in the optical window to provide an optical reference signal for extracting the differential phase signals. The chamber is oriented such that the long axis of the nerve is at 45 degrees with respect to the linear polarization of the incoming light. This ensures that the maximum cross-polarized light intensity can be detected. After placing the nerve in the groove of the chamber, petroleum jelly is used to isolate the stimulating electrodes from the recording electrodes.
Figure 7.4 The nerve chamber. The dissected nerve is laid along the groove. The groove is filled with the appropriate solution. Direct conduction of the stimulating signal is prevented by filling the isolation pool with petroleum jelly. The preparation is covered with a microscope slide.

An isolated pulse stimulator (Model 2100, A-M Systems, Sequim, WA) was used to deliver the electrical current pulses of adjustable amplitude to stimulate the AP. The differential electrode recordings from the nerve were amplified by an AC differential amplifier (Model 1800, A-M Systems, Sequim, WA) and recorded by a data acquisition card (National Instruments, Austin, TX). The action potential traces were displayed on the oscilloscope to monitor nerve functionality and repeatability over time. The SD-OCT system, the non-depth-resolved photodetectors, the electrical stimulation and recording equipments are controlled by a computer to ensure simultaneous recording of the optical and electrical signals.

7.4 Signal Processing

Similar to the signal processing method described previously in Chapter 5 and 6, the raw spectra acquired by the line-scan camera and the area-scan camera are re-mapped from the wavelength domain to k-space. Dispersion imbalance is then compensated for and then an inverse
Fourier Transform is applied to obtain the complex valued depth profiles, which are expressed in the form of $A_{1,2}(z)\exp\{i\phi_{1,2}(z)\}$ where $A$ is the amplitude and $\phi$ is the phase at particular depth $z$. The subscript refers to orthogonal channels of the optical system. The reflectivity information $R$, optical path length variations at particular depths ($\Delta p$ response) fractional intensity change $\Delta I(z)$, and fractional intensity change of transmission-mode photodetector signals $\Delta T(z)$ are extracted as follows,

$$R(z) \propto A_{1}^2(z) + A_{2}^2(z) \tag{7.2}$$

$$\Delta p(z) = \frac{\lambda_0}{4\pi} (\phi_1(z) - \phi_1(z_r)) \tag{7.3}$$

$$\Delta I_1(z) = \frac{A_1(z) - \overline{A_1(z)}}{A_1(z)} \tag{7.4}$$

$$\Delta T = \frac{T - \overline{T}}{\overline{T}} \tag{7.5}$$

where $\lambda_0$ is the center wavelength, and $\phi_1(z)$ and $\phi_1(z_r)$ denote the phases of the observation points and the reference glass surface from main channel, respectively.

Post-processing for extracting the $\Delta p$ responses as equation (7.3) were performed. Phase signals were selected by applying two-stage masking process to generate the cross-sectional phase images. First, a reflectivity mask was created with a threshold of SNR > 20 dB to exclude the low SNR regions since low reflection regions will result in unreliable phase values. The second mask was created to extract the reliable phase signals by ignoring the high standard deviation ( > 10 nm) of the phase signal during the 180 ms data acquisition time.

Environmental perturbations, such as vibrations, can reduce the phase stability and thus change the path difference between the two arms. The differential phase calculation is made immune to these perturbations by removing the common-mode phase noise. This is accomplished by subtracting the phase of a reference surface from the phase of the nerve locations.
7.5 Results

7.5.1 System Characterization for M-mode Setup

To determine the minimal displacement that can be measured, a coverslip was placed in the sample arm of the system. The phase sensitivities were 293 pm and 894 pm for 840 nm and 690 nm systems. The numbers are obtained by using the phases reflected from front and back surfaces of a coverslip.

7.5.2 System Characterization for Cross-Sectional Imaging Setup

For the cross-sectional imaging setup, $\Delta p$ was calculated from the phases of the top and bottom surfaces of the coverslip. The front surface of the glass slide served as a reference surface, and the back surface of the glass slide mimicking as the location where the displacement that was measured. Theoretically, the displacement is supposed to be zero. The phase sensitivity is characterized as the standard deviation of $\Delta p$ for each A-line over 180 ms of recording time. By averaging over 50 trials, the $\Delta p$ was determined to be $2.4 \pm 1.5$ nm within a cross-section. This number is even higher than the one shown in CHAPTER 6 with smaller scan, different scanner and shorter recording. The phase sensitivity is an explicit function of SNR [12], and the phase variance is related to the SNR by equation 2.6. The fundamental limitation on the minimum detectable phase difference is related to the vibrations of the system and the signal-to-noise ratio of a measurement.

The turning point of the scanning mirror is at a-line number 103 in the cross section. A common-mode rejection ratio of 10.6 dB was quantified by calculating the standard deviation of the differential signal ($\Delta p$) divided by the standard deviation the average of optical path length change between top and bottom surfaces of the coverslip. The phases of the top and bottom
surfaces from a-line number 150 are shown in Figure 7.5. The differential phase is calculated by taking the phase difference from top and bottom surfaces.

Figure 7.5 The phases of the top (blue) and bottom (red) surfaces from a-line number 97 are converted to optical path length. The differential signal (black) shows common mode noise rejection.

Figure 7.6 (a) shows the cross-sectional image of a coverslip used for characterizing the phase sensitivity of the system. The measured thickness of the coverslip was 155 μm which is consistent with the specification provided by the manufacturer. To characterize the direction of the phase changes, the glass side was tilted. We found that the decrease of the phase value corresponds to a downward movement of the glass slide, and the increase of the phase value corresponds to an upward movement of the glass slide.
Figure 7.6 (a) The cross-sectional image of a coverslip. The black arrow indicates the turning point of the scanning mirror. The orange arrow indicates the top surface that plotted in (b). (b) Phase of the top surface of the small segment from the recording was converted to optical path length, P.

7.5.3 M-mode phase changes

An OCT cross-sectional image of pike olfactory nerve is formed from a number of a-lines acquired consecutively during a scan in the lateral direction. Figure 7.7 (b) and (d) show the structural images (equation 7.2) from 840 and 690 OCT systems with a dynamic range of 50 dB
and 45 dB, respectively. The darkness of the structural image indicates the reflectivity from the unstained pike nerve. Figure 7.7 (a) and (c) depict the amplitude of the reflectivity from a single A-line as indicated by the yellow dashed lines. The single depth profiles show the glass-solution interfaces and the nerve from both 840 and 690 nm SD-OCT systems. Figure 7.8 shows how M-mode (continuously monitor the same depth profile over the acquisition time) data are presented. From a single depth profile, multiple depth points are analyzed by using equation 7.3 and 7.4. Averaging of 50 trials has been performed to improve the SNR and extract the phase changes.

Figure 7.7 structural images from 840 nm (a, b) and 690 nm (c, d) OCT systems and the corresponding depth profiles of an unstained pike olfactory nerve

Figure 7.8 Depth-resolved activity of M-mode intensity image and the depth profile to monitor neural activity along a depth profile
After real-time SD-OCT imaging of the nerve cross-sections in a chamber, the nerve sample and the reference glass were recorded during AP propagation. Overall, the $\Delta p$ responses can be constantly detected either for unstained and stained nerves when the glass surface was reliable during the recording. Figure 7.9 illustrates the depth profile recorded in this particular location, compound AP recording, the $\Delta p$ traces, and the change in fractional backscattered light intensity of main channel of five traces chosen within the unstained nerve bundles. AP was recorded using stimulation currents of 7 mA with pulse duration of 5 ms. The red dots in the depth profile indicate the depth points plotted on the right panels. As described earlier, the glass surface was used for differential phase measurement in order to remove the phase noise in the signals. According to the characterization, an upward change in $\Delta p$ indicates an increase whereas a downward change indicates a decrease in optical path length between a surface and the reference glass. Since the compound action potential is slower, we only acquired one action potential within the recording time. The $\Delta p$ responses were monophasic with the downward directions, indicating to a decrease in optical path length change between the nerve surfaces and reference glass. The change in fractional backscattered light intensity of the main channel of the unstained nerve can be hardly detected. Also, the electrical current stimulation pulse resulted in stimulus artifacts due to the residual current propagating from the stimulus electrodes to the recording electrodes.

In another set of experiments, the nerves were stained with voltage-sensitive dye PGH6. Figure 7.10 shows the depth profile, compound AP recording, the $\Delta p$ traces, and the fractional backscattered light intensity change of main channel of multiple traces chosen within the stained nerve bundles. In general, more $\Delta I_1$ traces were observed in the stained nerves and their values were larger. Each trace in Figure 7.9 - 7.13 has a bandwidth of 500 Hz and represent an average of 50 trials.
Figure 7.9 (a) depth profile of the unstained pike olfactory nerve. Black: main channel, blue: cross-polarized channel. The red dots indicate the depth locations where both $\Delta p$ and $\Delta I_1$ responses were observed. The AP recording is plotted in (b). (c) $\Delta p$ responses and (d) $\Delta I_1$ traces.
Figure 7.10 (a) depth profile of the pike olfactory nerve stained with PGH6. Black: main channel, blue: cross-polarized channel. The red dots indicate the depth locations where both $\Delta p$ and $\Delta I_1$ responses were observed. The AP recording is plotted in (b). (c) $\Delta p$ responses and (d) $\Delta I_1$ traces.
Figure 7.11 to 7.13 further exhibit the three-dimensional plots of $\Delta p$ responses in the nerve stained with PGH6. All the observed signals are stacked together; thus, the depth index is not the actual depth index shown in Figure 7.10. Figure 7.11 displays the monophasic $\Delta p$ responses across depth. This is the same location plotted in Figure 7.10. Figure 7.12 demonstrates that transient changes are altered from positive deflections to negative deflections in a depth profile. In another location, the traces present biphasic (two directions) signals as shown in Figure 7.13. The $\Delta p$ first increased and then decreased, resulting in a bi-directional trend with a return to baseline.

Figure 7.11 Three-dimensional plots of $\Delta p$ responses in the nerve stained with PGH6. This is the location-1 (the same location in Figure 7.10)
Figure 7.12 Three-dimensional plots of $\Delta p$ responses in the nerve stained with PGH6, location-2

Figure 7.13 Three-dimensional plots of $\Delta p$ responses in the nerve stained with PGH6, location-3
We quantified and compared $\Delta p$ signals between two stained nerves (PGH6) and an unstained nerve. The mean and standard deviation of the $\Delta p_{\text{peak-to-peak}}$ values for the stained nerves and an unstained nerve were $9.95 \pm 1.21$ nm ($N = 1609$ pixels) and $5.85 \pm 2.29$ nm ($N = 676$ pixels), respectively. Figure 7.14 shows the $\Delta p_{\text{peak-to-peak}}$ magnitude for the (a) unstained nerve and (b) the two nerves stained with PGH6. On average the $|\Delta p|$ responses were larger from the stained nerves. Nevertheless, the transient changes measured from the same nerve in different depth profiles are highly correlated. The potential enhancement of dye on $\Delta p$ signals still needs further investigation.

![Figure 7.14](image)

Figure 7.14 Histograms of peak-to-peak magnitude of $\Delta p$ during action potential propagation of (a) unstained and (b) stained with PGH6 of pike nerves

The changes in cross-polarized transmitted light intensity associated with AP propagation from both unstained and stained nerves were observed. Figure 7.15 shows the compound AP (a), $\Delta T/T$ in transmission-mode of 840 nm detector (b) and 690 nm detector (c). Fifty records are averaged in each trace to further improve the signal to noise ratio. The traces have a bandwidth of 50 Hz. Clearly, the transmission intensity changes detected from 840 nm and 690 nm detectors are associated with the AP arrival to the optical measurement sites. Their values are comparable to the value reported in [101].
7.5.4 Cross-sectional phase changes with axial bulk motion correction

The transient phase changes are found in PGH6 stained nerves. Figure 7.16 visualizes the compound action potential trace and the details of $\Delta p$ response and fractional intensity change of main channel with and without axial bulk motion correction. The stimulus strength is 2 mA. In this set of recording, the lateral range is larger to feature the $\Delta p$ responses for nearly the whole nerve. Spatial averaging with a kernel size of 8x8 pixel was applied to the fractional intensity change from main channel.

As discussed earlier in section 6.5, the axial bulk motion due to mechanical vibrations can have more effect because the recording time in this configuration is longer. Hence, the software correction of bulk motion induced artifacts on the intensity signal is crucial to extract the bulk-motion free backscattered light intensity change. Briefly, the axial bulk motion was obtained by taking the phase values for each A-line and then reprocessing the data in k-space using the translation property of the Fourier transform to remove the fluctuation.
Figure 7.16 Compound action potential trace and the details of $|\Delta p|$ response from the stained nerve with and without axial bulk motion correction. Scale bars: horizontal, 50 µm; vertical, 100 µm. (N = 50 trials)

The red dashed line in Figure 7.16 indicates the timing of the cross-sectional images. The fractional main channel intensity change, corrected fractional main channel intensity change and $|\Delta p|$ response at ~36, 102 and 172 ms are plotted by averaging 50 trials. The recovery after the action potential is shown at ~172 ms. We interpret the $\Delta p$ signal as indicating regions of the fiber.
bundle that were active. The cross-sectional images of fractional intensity change with and without axial bulk motion correction presented in Figure 7.16 demonstrate that the spatial pattern/variation of the fractional intensity change is owing to the axial bulk motion during the recording.

We further illustrate the Δp responses by plotting the traces in Figure 7.17 for selected pixels. The coordinate system is defined as (lateral index, depth index). These locations present negative deflections of Δp response. This suggests the transient shrinkage of this location of the stained pike nerve. Nevertheless, the cross-sectional images of Δp response show more information than the single traces.

![Figure 7.17 AP and the Δp responses from three pixels. Coordinate is defined as (lateral index, depth index)](image-url)
7.6 Discussion and Future Directions

The Δp and ΔI1 transients measured from the stained and unstained nerves in different locations/cross sections can be highly correlated. Due to the limited number of nerves recorded in this project, it would not be fair to conclude the enhancement of the Δp transient amplitude with staining. It will be possible to assess the dye effect if the number of nerves tested can be increased. In this case, the automatic program will be needed for faster signal extraction eventually.

The stability of the cross-sectional scanner using different ranges or galvanometer controller would result in different phase sensitivities. The galvanometer controller used in this project is a different one than the one used in Chapter 6. It is essential to characterize the scanner’s performance and stability for different scanning ranges.

We have demonstrated the axial bulk motion correction. Motion effects can arise from various sources, such as mechanical vibration, sound waves, and temperature drift can alter the path length difference in the interferometer [103]. A detailed understanding of motion effects is an essential step to further develop characterization methods to compensate the resulting artifacts.

In addition, construction of the dual-wavelength band scanner would be possible for the system due to the fact that the compound AP of pike olfactory signal is relatively slower. This could be beneficial for ratiometric study using different voltage-sensitive dyes.

7.7 Conclusion

Transient changes of unmyelinated nerves are investigated using the PMF-based depth-resolved and cross-sectional scanner. An axial bulk motion correction method is presented and implemented to stabilize the Δp responses. Retardance images are different from the stained and unstained pike olfactory nerves. The experiments presented here prove that the transient changes of entire nerve in cross section would be feasible. In addition, the PMF-based PS system suggests
the cross-sectional retardance changes can be possibly detected by using such models providing larger retardance changes.
CHAPTER 8 SUMMARY AND FUTURE DIRECTIONS

We have demonstrated several applications by using PMF-based PS-OCT. PMF-based PS-OCT was constructed and characterized at the beginning of the dissertation. PMF preserves the two orthogonal polarization states propagating in the fiber, making it feasible to obtain tissue reflectivity and birefringence in a single measurement. PMF-based PS-OCT is advantageous over standard bulk optics PS-OCT and conventional single-mode fiber-based PS-OCT in that it simultaneously measures the birefringent properties of biological tissues as an additional contrast. PS-OCT provides micrometer resolution reflectivity, phase retardance, and relative optic axis orientation as characteristics of anisotropic tissues. Retardance and axis orientation measurements from PS-OCT have been utilized to differentiate the gray matters and white matters and identify fiber pathways in the brain [104]. The multi-contrast OCT uses the intrinsic optical contrasts, such as backscattered light intensity, phase retardance, light attenuation, to map the neural pathways. PS-OCT has revealed significant information about nerve anatomy and its intrinsic optical properties. Despite the structural imaging of tissue, the OCT system used in this study is to mainly investigate its capability for providing quantitative measurements of optical changes associated with physiological events of the nerves. Efforts here have also contributed to the introduction of the voltage-sensitive dyes as exogenous contrast agents.

At the early stage of this research, the spectral-domain OCT system was used for phase-sensitive measurements. We have employed the PMF-based spectral-domain low-coherence interferometry for minute Faraday rotation from multiple-depth simultaneously. The reflection-mode measurement was demonstrated in clear liquids and the results are in good agreement with
the previously published report. In addition, Faraday rotation in tissue-mimicking phantoms and the magnetic nickel-gold nanowires solution were presented. The common measurement techniques are often in transmission-mode which is not applicable to tissue imaging. There are not much information that can be found about the Verdet constant in biological tissues. Therefore, this proposed technique is potentially applicable for imaging of Faraday rotation in tissues and understanding of magneto-optical interactions in biological samples. It is essential to investigate the feasibility of using Faraday rotation to image the biological tissues.

Optical detection of neural activity is a favorable technique for applications where no physical contact between electrode and nerve is desired. In this study, PMF-based PS-OCT systems of two different wavelengths (840 nm and 690 nm) were integrated along with transmission-mode detectors to demonstrate the depth-resolved optical detection of neural activity. This system is capable of simultaneously measuring the backscattered light intensity changes of main and cross-polarization channels, and phase changes associated with transient structural changes during action potential propagation. Additionally, 18 different voltage-sensitive dyes were tested to measure the optical changes in backscattered and transmission light intensity changes. Using a molecular probe for measuring the activity has advantages and disadvantages over monitoring intrinsic signals. The concept of dual-band spectral-domain OCT system is potentially applicable for depth-resolved spectroscopic imaging providing ratiometric information.

OCT provides the transient changes associated with action potential propagation along a depth profile. However, the activity in other dimensions was elusive. Consequently, we proposed and demonstrated a method by which the neural activity can be captured in a cross section by scanning the galvanometer-controlled mirror and acquiring the spectra at a high rate. The cross-sectional OCT scanner is advantageous over depth-resolved OCT since it dramatic increases the number of available sites that can be measured. Because of the undesired fluctuations in phase measurement due to environmental perturbations and the action potential-induced phase signals
indicate local transient structural changes in the nerve, the effect of motion related phase change can be decoupled from intensity measurement when such a response is detected. The results from the stained and unstained squid nerves provide enormous information about transient phase responses due to action potential propagation. However, increasing the number of squids in this study is necessary to unbiasedly evaluate the potential effect of dye on phase signals.

Besides, different acquisition settings and slightly modification of dual-wavelength OCT and cross-sectional OCT scanner were employed to study the pike olfactory nerves. To the best of our knowledge, these results have shown that transient phase signals can be detected by both systems for the first time. Also, complex averaging were used and averaged with traditional averages for the study. Since our understanding of backscattered light intensity changes with voltage-sensitive dyes that occur during neural activity is still primitive, current and future efforts are targeted towards the quantitative measures of the effect of voltage-sensitive dyes in intensity and phase signals from various preparations to apply them for development of novel voltage imaging tools for monitoring neural activity in a minimally invasive manner.
References


