MODEL-BASED ANALYSIS OF FIBER-OPTIC EXTENDED-WAVELENGTH DIFFUSE REFLECTANCE SPECTROSCOPY FOR NERVE DETECTION

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ABSTRACT

Optical spectroscopy is a real-time technique that holds promise as a potential surgical guidance tool. Fiber-optic diffuse reflectance spectroscopy (DRS) is a technique capable of intraoperative tissue differentiation. The common DRS focuses on estimating chromophore concentrations in the visible/near-infrared (VIS/NIR) wavelength range (400-1000 nm), where spectroscopic features of the blood, pigments, and tissue densities are present between 400 and 700 nm. Recently, extended-wavelength DRS (EWDRS), which extends the spectral window from the VIS through the short wave-infrared region (SWIR) up to 1800 nm, has emerged as a promising approach for identifying nerves and nerve bundles due to the SWIR including robust tissue absorption features associated with nerve-tissue related chromophores, including lipids, water and collagen proteins. One potential application of EWDRS is guiding minimally invasive surgical techniques, such as laparoscopy, where inadvertent injury to pelvic autonomic nerves (PANs) is a primary complication that can result in over 70% of patients suffering long-term side effects, including urinary incontinence and sexual dysfunction. There is a need for objective laparoscopic surgical guidance to precisely identify PANs from other tissues, and an improved basis for EWDRS development could assist clinical translation. Prior development of Fiber-optic DRS for tissue classification in the VIS/NIR greatly benefited from the application of modeling techniques for simulation of optical measurements, analysis, and fiber-probe design. Model-based analysis can inform fundamental understanding of measured signals in different measurement scenarios, such as the varying tissue morphologies possible in laparoscopic procedures, and guide application-specific fiber-probe design through comparison of unique illumination/collection geometries;
However, the demonstration of these approaches in EWDRS is not widely reported. This dissertation focuses on the advancement of platforms for model-driven analysis of EWDRS for nerve identification. In order to advance the current state of EWDRS, a model-based characterization platform for analysis of a custom-developed fiber-optic EWDRS system was developed in Aim 1, which demonstrated agreement between data collected from optical phantoms, *ex vivo* microsurgical model, and Monte Carlo (MC) computational simulations of EWDRS measurements. In Aim 2, detailed analysis of two similar EWDRS fiber-optic probes using the model-based platform indicated subtle differences in the depth-dependent measurement performance. Finally, in Aim 3, the custom EWDRS was prepared for adapting laparoscopic use, including evaluation of placement variance and customized EWDRS package for short-distance transportation. The successful completion of this dissertation will enable improved analyses of EWDRS devices for a variety of future intraoperative applications.
DEDICATION

To My Parents,
Guoyi Zhao and Jingshun Sun
&
To My Cat,
Pineapple Bun
ACKNOWLEDGMENTS

This dissertation was completed in the Optical Diagnostics Lab, Department of Bioengineering, Temple University, during the years 2017-2022.

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CHAPTER

1. INTRODUCTION AND LITERATURE REVIEW

Minimally invasive surgical techniques, including laparoscopy, have improved surgical outcomes; however, narrow visual fields and difficulties with positive identification of tissues result in the primary complication being nerve injury and resultant loss of function. Fiber optic diffuse reflectance spectroscopy (DRS) offers potential for intraoperative tissue identification, and the emerging technique of extended-wavelength diffuse reflectance spectroscopy (EWDRS) has shown improved tissue characterization due to the inclusion of additional molecular signatures as the spectral range expands from 400-1000 nm to 400-1800 nm. Future development of EWDRS beyond current tissue classification studies in simple clinical and surgical scenarios and towards more complex situations, such as used for minimally invasive surgical guidance, raises important fundamental questions regarding detection sensitivity when target tissues may be covered with thin superficial layers of non-target tissues or fluids, and differential evaluation of fiber probe illumination/collection geometries. Model-based analysis can be beneficial for addressing these issues; however, more critical steps in the development and validation of EWDRS model systems remain un-reported and must be performed prior to more detailed analysis. Since the long-term scope of this research is to develop EWDRS for minimally invasive nerve identification, this review will include a relevant description of the minimally invasive technique of laparoscopy, as well as a detailed background of optical spectroscopy, recent work in EWDRS, and limitations in EWDRS model systems that stand in the path of analyses that could inform future laparoscopic applications.
1.1 Nerve Injury In Laparoscopy

Figure 1. Example of minimally invasive surgery and laparoscopy. Laparoscopy is a minimally invasive surgery, which is done through one or more small incisions with the aid of a camera and special laparoscopic surgical instruments [1], [2].

Laparoscopy (shown in Figure 1) is a minimally invasive surgical procedure designed to target the abdominal and pelvic organs with several small incisions using special laparoscopic instruments and devices [3]–[5]. Laparoscopy has high survival rates and provides more benefits than traditional open surgery, such as less blood loss and faster recovery [6], [7]. Laparoscopic devices are inserted via a central hollow optical trocar - a disposable and replaceable visual entry tool [8], [9]. The size of standard trocars utilized in laparoscopy is between 5 to 12 mm, depending on different operative settings [10]–[12] — the size of trocars limits that of the laparoscopic device. The development of laparoscopic devices for insertion into the laparoscopes auxiliary port, such as the Visiport (Redmond, WA) optical trocar [8], [13], allow complete visualization as the obturator passes through the abdominal wall [9], [14]. Typical applications of laparoscopy include laparoscopic prostatectomy, laparoscopic hysterectomy, and laparoscopic rectal excision [15]–[19]. Laparoscopic prostatectomy is the most common surgical approach for prostate
cancer [20], [21], which is one of the top three prevalent age-related cancer among males [20], [22]. Similarly, laparoscopic hysterectomy is a standard procedure among females; approximately 1 in 9 women over 50 will have a hysterectomy in their lifetime [23], [24], and it is also the most common treatment for patients with early-stage cervical cancer [6], [25], [26]. Laparoscopy has advantages such as less pain, shorter hospital stays, and fewer complications [6], [7].

![Figure 2. Surgeon’s view during laparoscopy. a). Laparoscopic prostatectomy: Isolated neurovascular bundles, just on top of the organ, have a similar appearance as the prostate, making it hard to recognize with the blood [27]. b) Laparoscopic hysterectomy, where again recognition of nerve from other tissues is difficult [28].]

Due to the narrow field of view and the lack of proprioception with the laparoscopic approach, nerve injury during incisions and electrocautery are common, which lead to long-term side effects including urinary incontinence and sexual dysfunction [10], [14], [29]. In some long-term, large-scale surveys, approximately greater than 80% of patients reported erectile dysfunction after prostatectomy, which resulted in the loss of reproductive function, elevated levels of anxiety, and reduced general quality of life [14], [22], [30]–
[33]. Also, the post-operative reports of hysterectomies have shown that 70% – 85% of cases occurred with bladder dysfunction [34].

The critical cause for these complications is the problematic identification of Pelvic Autonomic Nerves (PANs). PANs consist of a network of nerves, including the superior and inferior hypogastric plexuses [35], [36], where the superior hypogastric plexus (SHP) corresponds to the distal extension of the aortic and inferior mesenteric plexuses. The inferior hypogastric plexus (IHP) is a mixed plexus located in the deep pelvic viscera, which extends to all pelvic organs and perineum [37], [38]. The integrity of the pelvic autonomic nervous system is essential for proper urological and sexual function [35], [36].

The current standard to identify PANs during nerve-sparing laparoscopy is subjective and relies on surgeons’ experience and understanding of anatomy. Additionally, neurovascular bundles (NVBs) contain individual variances making it difficult to predict exact locations. Current studies have shown that along the surface of organs, the descriptions of PANs’ distribution may have a more extensive branching than a traditional assumption, which also indicates the current knowledge of the location of PANs could be limited [29], [39]. PANs contain nerves and vessels covered by fat and connective tissues lying amidst adjacent tissues and forming a multilayer structure. The current visual identification of NVB in surgical practice can be challenging due to the range of sizes, individual differences, and a wide variety of tissue arrangements [28], [33], [35], [36]. Therefore, the development of quantitative and objective techniques to assist in nerve identification could help reduce complication rates and enhance long-term potency rates resulting in inpatient benefit.
1.2 Overview Of Optical Techniques For Nerve Identification

The versatility of the laparoscopic trocar facilitates the possibility of nerve identification for surgical guidance using emerging techniques in fiber optic imaging and fiber optic spectroscopy. Numerous imaging modalities have been developed and are under examination to provide better identification of PANs [40]–[44], including dye-based fluorescence imaging [45]–[51], optical coherent tomography (OCT) [40], [52]–[62], transrectal ultrasound (TRUS) [63]–[66], multiphoton microscopy (MPM) [67], [68] and the basic white light imaging. These studies suggested the feasibility of using imaging methods to visualize PANs; however, several limitations have been reported. For instance, fluorescence imaging methods rely on exogenous agents for nerve labeling, which contains bleaching issues. Ultrasound imaging methods have poor resolutions for visualizing nerves which are subjective with low depth sensitivity.

On the other hand, optical spectroscopy is a tool to characterize biological tissues, detecting different light interactions with high spatial resolution and selectivity in real-time [69], [70]. Optical spectroscopy can provide more objective results than images with similar specificity [70], [71]; therefore, it represents a potential solution for the main challenges of laparoscopy.

1.2.1 Optical Spectroscopy

Optical spectroscopy has been utilized to characterize biological tissues [72], [73]. The system is simple: generally, a light source shines light through tissue, and a detector collects the interactions between the light and the tissues on the opposite side. Optical
spectroscopy is a mature technology with applications in several industries, including recent demonstrations in biomedical analyses. Based on different light interactions, three common types of optical spectroscopy are listed [70], Raman spectroscopy (RS), fluorescence spectroscopy (FS), and diffuse reflectance spectroscopy (DRS).

RS is a powerful optical analysis method for the biochemically specific characterization of tissues without contrast agents or exogenous dyes [74]–[76]. RS is based on molecular vibrational modes, which are many and thus allow highly specific compositional characterization, resulting in clinical applications of RS reporting the highest sensitivity and specificity to diagnose and differentiate disease among the three types of spectroscopies [77], [78]. However, the slow measurement speed increases the difficulty of use during the surgery. Moreover, the weak nature of Raman scattering results in hardware requirements that require costly instruments [78]. FS is optical spectroscopy that can only target specific fluorophores, of which there are few endogenous in biological tissues [79]–[81]. Thus, the ability of FS to identify a particular type of tissues is limited without the use of exogenous dyes or labels and also requires high-cost hardware [82], [83].

DRS is a simple, low-cost spectroscopic technique that is sensitive and primarily observes absorption features as minima in the measured spectrum. [84]–[87]. Knowledge of the wavelength-dependent spectral line shape associated with absorption from specific chromophores is used to identify tissue composition [88]. Measurement of diffuse reflectance critically relies on highly scattering samples to return sufficient light back through the surface in order to evaluate the tissue optical properties [89], and thus, unlike transmission absorbance spectroscopy, measured signals contain information that is a
combination of the tissues’ absorption and scattering properties[90]. Scattering in tissues depends on a combination of tissue morphology and the refractive index [88], [91]. Moreover, since the diffusely reflected photons travel into the tissue depth and can undergo multiple scattering events before re-emerging for detection, the diffuse reflectance signal contains information about scattering and absorbing components throughout the average photon transit volume deeper within the tissue [90]–[92]. Similar to FS, there are only a few chromophores with spectral absorbance bands in the VIS range typically used in DRS hardware configurations, and thus DRS has the lowest specificity and is unable to precisely characterize tissue as well as RS and FS [93].

As a surgical guidance approach in laparoscopy, the top goal is to remove pathological tissues altogether, maintaining normal functions without damaging PANs. The approach requires the ability to precisely identify PANs with a laparoscopic fiber probe. Also, surgery is a time-consuming procedure requiring the surgical guidance tool be as fast as possible, so it has minimal effect on the original surgery timeline. Moreover, simple operation and relatively low costs are considerations for clinical applications. The design of an aided method should adequately address surgical conditions with less complicated utilization restrictions at a minimal additional cost to the entire system. Therefore, the ideal surgical guidance in laparoscopy has high molecular specificity to detect PANs from surrounding tissues with fast measurement speed and low cost and operational complexity.

According to the critical of ideal surgical guidance, a comparison among three categories of optical spectroscopies is described in Figure 3. Traditional VIS DRS measured from 400 to 1000 nm obtains the highest collection speed and the lowest cost
with a simple setup; unfortunately, the limited ability of identification limits \textit{in vivo} applications to those which benefit primarily from knowledge of scattering and visible chromophores, such as blood and pigments. Finding a way to improve DRS's specificity to the unique compositional makeup of nerves and nerve bundles while maintaining high measurement speed and low cost would make DRS the ideal surgical guidance approach for guidance of laparoscopy.

Figure 3. Comparison among different types of optical spectroscopy being an ideal surgical guidance.

\textbf{1.3 Extended-Wavelength Diffuse Reflectance Spectroscopy}

Recent reports describe the use of EWDRS systems to expand the detected wavelength range into the short-wave infrared (SWIR) up to 1600 nm. The SWIR spectral region is unique in that molecular absorbance spectral features can arise from a combination of primary electronic absorption transitions as well as higher order overtones of vibrational absorption transitions. The combination of these optical markers provides sensitivity to additional biologically important chromophores, including lipids, water, and collagen [94]–[97]. EWDRS enables the detection of wavelength from VIS/NIR range to SWIR range and has the ability to accurately differentiate tissues [95]. Figure 4 shows absorption spectra of important chromophores in biological tissue. Most DRS studies focus
on wavelengths in the visible region of the spectrum, especially between 400 nm to 650 nm. The target of most traditional DRS studies is to characterize the functional status by measuring the concentration of blood-related chromophores [70], [92], [97], [98]. β-carotene has an absorption peak of around 500 nm. The major absorption peak of deoxygenated hemoglobin is near 556 nm; however, oxygenated hemoglobin includes two absorption peaks generating a unique W-shape near 543 and 577 nm [98]. A few studies suggested that the concentration of water and lipid at the edge of VIS could be related to breast cancer diagnosis and burn wounds [99], [100]. However, lipid and water have small absorption features near 970 nm with low differentiation ability [101].

The optical measurement range extends into NIR and may offer in the characterization of water and lipid as well as collagen [94], [95], [101], [102], which has been well studied in near-infrared spectroscopy (NIRS). NIRS is based on the fundamental vibrations of molecular groups, including overtone (< 1800 nm) and combinations (> 1800 nm) in SWIR region (from 780 nm to 2500 nm), which already been applied in nerve identification [103]. Lipid has a significant absorption feature of around 1210 nm, which is the second overtone of CH bonds [104], and a few peaks after 1300 nm which are overwhelmed by water and collagen in bulk tissues. Different types of lipids have similar absorption features described in NIR [94], [95], [105]. A minor feature of collagen overlaps with lipid around 1200 nm, and two crucial features appear around 1500 nm, which is the first overtone of water [94], [95], [104], [106]. Water contains a central absorption peak at 1430 nm [94], [95]. EWDRS thus extends the measurement wavelength providing more information on many essential components of biological tissues with the improvement of quantifying collagen and lipids.
Figure 4. The absorption spectrum of important chromophores in biological tissues across VIS and NIR range. Blood and pigment related features shown in VIS range, such as oxygenated hemoglobin and deoxygenated hemoglobin. Features of water, lipid and collagen displayed in NIR range [105].

EWDRS maintains a high measurement speed with an improved molecular specificity. Since the combination involves another spectrometer, the overall cost of the system doubles but is still in an acceptable region. Therefore, EWDRS offers many benefits in surgical guidance to identify tissues. Applications of EWDRS include skin, colorectal, and breast cancer diagnosis and the ability to differentiate tissues in skin and lung tissues [105], [107]–[117]. More importantly, EWDRS is a particularly promising technique for detecting nerves and NVBs [118]–[122].

EWDRS has been employed to characterize biological tissues in order to identify different tissues or diagnose diseases. Early EWDRS focused on optical properties measurement utilizing the integrating sphere in ex vivo studies [123]–[126]. With the widespread utilization of fiber optic probes, several groups currently reported that EWDRS had developed a fiber optic probe in the application of tissue type identifications[94], [95],
It provides an opportunity for EWDRS to be portable and flexible *in vivo* measurements.

Table 1. The list of EWDRS applications in differentiation tissue types

<table>
<thead>
<tr>
<th>Species</th>
<th>Spectral range(nm)</th>
<th>Differentiate tissue type</th>
<th>Location</th>
<th>Classification</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pig</strong></td>
<td>500-1600</td>
<td>Fat</td>
<td>NA</td>
<td>NA</td>
<td>Nachabé <em>et al.</em> 2010[94]</td>
</tr>
<tr>
<td><em>Ex vivo</em></td>
<td></td>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dura mater</td>
<td>Epidural injection</td>
<td>NA</td>
<td>Desjardins <em>et al.</em> 2011[128]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spinal cord surface</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pig</strong></td>
<td>400-1600</td>
<td>Fat</td>
<td>Breast</td>
<td>NA</td>
<td>Adank <em>et al.</em> 2018[117]</td>
</tr>
<tr>
<td><em>Ex vivo</em></td>
<td></td>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Canine</strong></td>
<td>400-1600</td>
<td>Benign canine skin</td>
<td>Skin</td>
<td>90% sensitivity 73.5% specificity</td>
<td>Cugmas <em>et al.</em> 2015[127]</td>
</tr>
<tr>
<td><em>Ex vivo</em></td>
<td></td>
<td>Malignant canine skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subcutaneous tumors</td>
<td></td>
<td>88.4% sensitivity 54.6% specificity</td>
<td></td>
</tr>
<tr>
<td><strong>Canine</strong></td>
<td>450–1550</td>
<td>Different degrees of pigment skin</td>
<td>Skin</td>
<td>96.4% sensitivity 100% specificity</td>
<td>Dahlstrand <em>et al.</em> 2019[97]</td>
</tr>
<tr>
<td><em>In vivo</em></td>
<td></td>
<td>Snout</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tongue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pig</strong></td>
<td>500–1600</td>
<td>Five different breast tissue types</td>
<td>Breast</td>
<td>89% sensitivity 79% specificity</td>
<td>Nachabé <em>et al.</em> 2011[105]</td>
</tr>
<tr>
<td><em>Ex vivo</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[97], [112], [119]–[121], [127]–[129].
<table>
<thead>
<tr>
<th>Species</th>
<th>Status</th>
<th>Tissue</th>
<th>Organ</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Normal lung tissue</td>
<td>Lung</td>
<td>89%</td>
<td>79%</td>
<td>Evers <em>et al.</em> 2012[107]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Lung parenchyma</td>
<td>Lung</td>
<td>98%</td>
<td>86%</td>
<td>Spliethof <em>et al.</em> 2013[115]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Nerve</td>
<td>Regional anesthesia</td>
<td>90%</td>
<td>90%</td>
<td>Hendriks <em>et al.</em> 2015[118]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Normal mucosa</td>
<td>Colorectum</td>
<td>93.5%</td>
<td>94%</td>
<td>Nogueira <em>et al.</em> 2021[129]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Fat</td>
<td>Colorectum</td>
<td>AUC:</td>
<td></td>
<td>Baltussen <em>et al.</em> 2019[112]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>In vivo</strong></td>
<td>Healthy colorectal wall</td>
<td>Colorectum</td>
<td>95%</td>
<td>88%</td>
<td>Langhout <em>et al.</em> 2015[87]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>In vivo</strong></td>
<td>Tumor</td>
<td>Colorectum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td><strong>In vivo</strong></td>
<td>Sciatic nerve</td>
<td>Groin</td>
<td>63%</td>
<td>82%</td>
<td>Langhout <em>et al.</em> 2018[120]</td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Slide fat</td>
<td>Groin</td>
<td>39%</td>
<td>98%</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td><strong>Ex vivo</strong></td>
<td>Muscle</td>
<td>Groin</td>
<td>61%</td>
<td>84%</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td><strong>In vivo</strong></td>
<td>Subcutaneous fat</td>
<td>Groin</td>
<td>81%</td>
<td>80%</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The table lists various tissues and organs tested for sensitivity and specificity in different studies. The studies include both ex vivo and in vivo models, as well as species ranging from human to swine.
<table>
<thead>
<tr>
<th>Human</th>
<th>In vivo</th>
<th>Peripheral nerves</th>
<th>Head and</th>
<th>Langhout et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>heck</td>
<td>2018[119]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400-1600</td>
<td></td>
<td></td>
<td>100% sensitivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>83% specificity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Femoral or sciatic nerve</td>
<td>Groin</td>
<td>85% sensitivity</td>
<td>Langhout et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>79% specificity</td>
<td>2018[121]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400-1600</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nerve</td>
<td>Adipose tissue</td>
<td>Thyroid</td>
<td>Schols et al.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td></td>
<td>2014[130]</td>
</tr>
<tr>
<td>350-1830</td>
<td></td>
<td></td>
<td>85% sensitivity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>79% specificity</td>
<td></td>
</tr>
</tbody>
</table>

Dahlstrand et al. [97] evaluated the ability of EWDRS to identify and classify different skin and tissue types in an in vivo pig model. Measurements of the skin were collected with different degrees of pigmentation, pig snout, and tongue. The overall accuracy reached 98.2% with high sensitivity and specificity for all skin and tissue types.

### 1.3.1 EWDRS For Nerve Identification

EWDRS has been applied for nerve identification in ex vivo and in vivo animal and human subjects, demonstrating promise to differentiate nerves from surrounding tissues [118]–[121], [128], [130]. Several approaches have been used to analyze spectra collected from nerves and surrounding tissues among these studies. DRS spectra are influenced by absorption and scattering coefficients within biological tissues, where absorption coefficients are related to the concentration of relevant chromophores, such as hemoglobin in the VIS range, as well as water and lipids in the NIR range [92], [94], [95]. The reduced scattering coefficient of biological tissues presents as an exponential decay function vs.
wavelength, in which the scattering decreases dramatically in the VIS/NIR region, but then tapers in the SWIR wavelength range.

Figure 5. Example of spectrum from biological tissues. a) Spectra of subcutaneous fat. The arrows indicated chromophore features: oxygenated hemoglobin (unique ‘w’ shape between 500 and 600nm), deoxygenated hemoglobin (absorption peaks between 550 and 800 nm), water (absorption peaks at 965 and 1,440 nm) and adipose tissue (absorption peaks at 1,210 nm). a) Spectra of subcutaneous fat shown features including Oxyhemoglobin: 542 and 576 nm; deoxygenated hemoglobin: 557 and 757 nm; lipids: 930 and 1210 nm; water: 976, 1197, and 1455 nm. The y axis was the intensity of diffuse reflectance [128]. b) $B_n$ was blood related points; $W_n$, was water related points; $F_n$, was fat related points [130].

Fundamental analysis of EWDRS spectra compares the intensity as a function of wavelength, in which absorption peaks are displayed as lower signal intensities for a specific wavelength. In biological spectra, major absorption features of hemoglobin from blood were observed between 500 to 600 nm, and a single dip around 550 nm was associated with the presence of deoxygenated hemoglobin commonly observed in muscle tissues [87], [119], [121], [128]. On the other hand, the oxygenated hemoglobin appeared
to have a unique ‘w’ shape between 540 and 580 nm in fat, nerve, and other tissues [119], [128]. In addition, there were reports of the deoxygenated form of hemoglobin near 757 nm. Furthermore, the existence of β-carotene around 500 nm has been reported, leading to the yellow appearance of tissue appearing in spectra of muscle [118]. There are no significant absorption features between 700 and 900 nm, named ‘optical window’. After this region, water and lipid absorption features dominated the NIR spectral line shape. A significant absorption peak at 1210 nm and 1720 nm was consistent with the presence of lipid, as well as a minor feature near 930 with much lower intensity, which was prominent in spectra collected from fat tissues [128], [130]. Water features presented a similar wavelength as lipid: a minor feature displayed at 980 nm, and a broad significant peak around 1450 nm. Prior studies and data on nerve/NVB and adipose tissues indicated that nerve/ NVB tissues contained approximately 80% of protein with the rest of lipid, but average adipose tissues contained 75% lipid, 8% protein and 17% water [131]–[133].

Figure 6. Parameters used to analyze EWDRS spectra for nerve identification. a) Box plots of relevant tissue parameters, including hemoglobin/myoglobin concentration, the oxygen saturation, fat fraction (fat/ (fat + water)), beta-carotene levels and scattering features around 800 nm. [121]. b) 36 features were calculated from predefined points related to characteristic absorption features for blood, water, and fat [130].
Different methods have been reported for quantitative analysis of EWDRS spectra in nerve identification. The most common approach that has been utilized is based on the model developed by Farrell et al. [134] which estimated the absorption and reduced scattering coefficient, including volume fractions and concentrations of various chromophores, as well as scattering parameters. This model has been implemented in Matlab (Mathworks, Co.) and modified to analyze the EWDRS spectra by Nachabé et al. [94], including water, fat, β-carotene, and hemoglobin. Desjardins et al. [128] estimated two parameters based on this model: the blood fraction and the lipid fraction, with blood fraction defined as the percentage of the concentration of hemoglobin in average normal human blood (150 g/liter), and the lipid fraction defined as the percentage of lipid from the estimated summation of lipid and water. Hendriks et al. [118] defined another parameter: blood oxygen saturation (StO2), expressed as the fraction of oxygenated hemoglobin over the total amount of hemoglobin. However, the original model was developed from a large source-to-detector (SD) distance (>1.5 mm) fiber probe, which could result in an overestimation of parameters when using a probe with a smaller SD distance. Thus, spectra highly contaminated by blood (over 20%/25%) were excluded from the analysis in these studies. Schols et al. [130] utilized another method to analyze EWDRS spectra based on characteristic absorption chromophores that proved critical in nerve differentiation, such as blood, water, and lipid. First, a total of 17 points have been defined, including 6 points related to blood between 400 and 700 nm, 2 points related to water in the NIR range, and 9 points associated with lipid from 1100 to 1800 nm. Two parameters are defined: 1) gradients were calculated as the ratio of intensity and wavelength between two points; 2) amplitude differences were calculated as the intensity difference between two points.
However, this method highly relied on individually selected spectral points and limited multivariate measures to determine chromophore composition.

Several classification algorithms have been applied in EWDRS to differentiate nerves from other tissues, including the k-Nearest Neighbor (KNN) principle, partial least squares discriminant analysis (PLS-DA), support vector machine (SVM), and classification and regression trees (CART) analysis, which is commonly combined with principal component analysis (PCA) to reduce the spectral features or defined parameters associated with specific chromophores. The overall classification results for nerve identification against surrounding tissues resulted in over 60% sensitivity and 80% specificity (accuracy > 70%) [118]–[121], [128], [130].

![Figure 7. Probe designs used for EWDRS. a) An illumination fiber connected a broadband white light and a fiber splitter connected to the single collecting fiber (SD distance = 0.8 mm) [119]. b) A ring light of seven illumination fibers were around one central collection fiber (SD distance =1 mm) [130].](image)

Previous EWDRS studies proved that features in the extended wavelength combined with those in VIS DRS were valuable in nerve detection and suggested the
potential application of EWDRS in intraoperative surgical guidance. However, there are some limitations of prior EWDRS nerve identification studies, which were more focused on simple surgical scenarios, such as head and neck surgeries, where nerve/NVB are well exposed, and the inherent anatomical structures are well understood, present in a regular fashion during surgical dissection with the size of nerve and NVB having consistent dimensions with minor variance, and also do not manifest as complex nerve networks from the multiplicity of nerve plexus as is present in the pelvic cavity. Thus, the current prominent clinical reports investigated simple tissue geometry; however, the spectral line shape changes associated with more complicated tissue geometries, such as superficial thin nerve/NVB layer and nerve/NVB beneath a thin tissue layer have not been reported. The spectral line shape is also influenced by probe configurations, the most commonly used configuration being single illumination/collection geometry with 0.8 mm SD distance [87], [118], [119], [128]. Schols et al. [130] reported another probe configuration with multiple illumination fibers around one collection fiber with the same SD distance—unfortunately, a more detailed analysis of how the probe configuration effects spectral line shape was not reported. Moreover, all the algorithmic techniques using either least squares or machine learning [130] are data driven approaches, and it is unclear the extent to which these techniques would generalize when different probe configurations are used. In general, both spectral analysis and classification techniques used in prior studies were performed in straightforward scenarios, including simple tissues geometries and highly specialized probe configurations. However, an improved theoretical understanding of how differences in these factors will influence spectral analysis and classification are critical for guiding future developments in more challenging applications such as surgical guidance.
1.4 Model-Based Techniques For Analysis Of Fiber Optic Spectroscopy

Previous EWDRS studies have described the fundamental spectral analysis approaches and performed comparison of different classification methods for identification of nerves in open surgeries where tissue geometries were elementary; however, using spectroscopy for intraoperative surgical guidance can be challenging due to the complex tissue geometry in the pelvic cavity. Therefore, the wide variety of tissue geometries could lead to complicated measurement scenarios where objective theoretical evaluation of using a model-based system has the potential to aid in understanding better how these factors influence spectral line shape.

1.4.1 Computational Modeling – Monte Carlo Model

Monte Carlo (MC) modeling [135], [136] is a widely used technique to simulate photon migration in highly scattering biological tissues having multilayered or complex structures [137], [138]. MC simulation is a computational technique to mimic the stochastic nature of photon migration in biological tissues, which relies on the optical properties of tissues along with optical illumination/collection setup. MC modeling has been utilized to investigate various spectroscopic techniques such as DRS, fluorescence, and Raman spectroscopy [137], [139], [140]. Moreover, MC modeling is a valuable approach to investigate the depth-dependency of a probe [138], [141]–[143].

Several MC studies in DRS have been used as guidance on the design of optical setups for empirical measurements, including analysis of source-to-detector (SD) distances, tilt angles of fibers, and illumination/collection geometry configurations [141]–
[149], which were primarily focused on how deep the probe could detect through the biological tissues. However, depth-dependency is imprecisely described using a single metric, such as “penetration depth”, because reflectance signal decays exponentially with depth and varies based on multiple factors, including wavelength, tissue composition, probe design, and analytical technique. Spatial localization of the origin of diffuse reflectance measurements in the EWDRS spectrum can be challenging, due to the fact that they are fundamentally related to tissue optical properties and morphology. The depth-dependency of the probe depends on several factors. Individual fiber specifications, such as diameter, numerical aperture (NA), source-to-detector (SD) distance and illumination/collection geometry configuration, are both associated with the sampling volume of a probe. In addition, depth-dependency is also a function of wavelength-dependent optical properties of biological tissues. Therefore, MC simulation could provide advanced theoretical insights to investigate, analysis and compare probe designs.

![Figure 8. Example of previous MC for EWDRS to investigate different SD distance. a) Schematic of the SD geometry simulated in MC model. b) Experimental DR spectra of fingers with different SD distances and simulated DR spectra with wide-angle numerical aperture (NA) = 1 and a numerical aperture NA = 0.2, as well as different SD distances [142].](image)
Zonios et al. [143] developed an analytical solution for a wide range of SD distances corresponding to various detector sizes on a simple homogeneous model, which investigated the properties of DR signal and guided the other modeling in more complicated tissue structures. Gomes et al. [145] investigated the depth-dependency of different probe configurations, including SD distance and illumination/collection geometries, and developed an algorithm for the automatic selection of probes based on specific applications. Overall, the previous MC simulation focused on how SD distance affected the depth-dependency of the probe and demonstrated that the SD distance was in direct proportion to the sampling volume of a probe. Kurakina et al. [142] investigated the depth-dependency of probes with various SD distances for multilayer skin model in the VIS range, emphasized the previous conclusion, and displayed the spectral maps for photon depth analysis well as photon trajectory maps. Thus, MC simulations could guide the development of fiber probes for specific applications and provide unique insights into photon maps.

1.4.2 Optical Phantoms

Optical phantoms mimic optical properties of biological tissues and aid in the development of diagnostic systems [150], [151]. A wide variety of optical phantoms have been reported consisting of various compositions, geometry, and optical properties. Optical phantoms have different forms, such as solid, hydrogel, and liquid states, in which optical properties can be controlled via fabrication processes to mimic particular features. In recent years, applications of optical systems have increased significantly, resulting in numbers of
optical phantoms that have been developed for various systems, such as optical coherence
tomography, fluorescence molecular imaging, and optical spectroscopies [150], [151].

Different types of optical phantoms have been reported in DRS, such as engineered
tissue phantoms, which can be created and cultured from actual cells and tissues containing
expected optical properties and structures [150], [152], [153]. For instance, epithelial
tissues have been cultured to generate fundamental tissue components and structure, which
was challenging to mimic via other phantoms, however these phantoms are labor-intensive
to produce, cannot produce tightly controlled optical properties due to natural biological
variability, and have short shelf-life for repeat use. Non-biological phantoms are the most
commonly used, created from tissue-mimicking compounds, similar to real tissues,
including liquid and solid formats. Plenty of non-biological phantoms have been reported
in DRS to mimic skin, which successfully mimicked the blood and pigment with different
blood concentrations [154]. Recently, benefits from 3D printing and a more complex
structure of optical phantom have been reported. Liu et al. [155] designed and fabricated a
multilayer skin-mimicking phantom with blood vessels among the epidermis, dermis, and
hypodermis. In general, optical phantoms utilized in VIS DRS mainly focused on the VIS
region, which was good at mimicking blood and pigment.
Figure 9. Example of non-biological optical phantoms reported in DRS to mimic different blood concentration, a) The two-layered skin phantoms were designed to mimic skin with various blood and pigment concentration. b) Optical properties of skin phantom, including the average reduced scattering coefficient spectrum layers and the absorption coefficient spectra of the epidermal layer and dermal layers [154].

Optical phantoms were also applied in EWDRS to evaluate the system and characterize the performance of tissue differentiation; however, the phantom only mimicked various lipid concentrations [94]. EWDRS phantoms containing chromophores features of blood, pigment, and lipid require further development for the ultimate advancement of the technique.

1.4.3 Model-Based System For EWDRS

EWDRS has demonstrated advantages over conventional DRS in the ability to perform tissue identification with improved classification performance in several open surgeries. However, a number of advanced approaches commonly used in characterization of conventional DRS have yet to be incorporated in EWRDS. Further development and characterization of EWDRS probes for the wide variety of unique intraoperative uses would benefit from characterization and analysis with model systems, including well-
characterized theoretical simulations supported by EWDRS measurements in simple optical phantoms and \textit{ex vivo} microsurgical models; however, there are few reports of specific to EWDRS range (400 – 1800 nm) spectroscopy. Finally, the application of EWDRS in laparoscopy has not been reported in previous EWDRS studies.

Due to the improved information accessible from the extended wavelength range, EWDRS has been applied to differentiate tissues in \textit{ex vivo} models as well as \textit{in vivo} models, such as pig, canine, and human [119], [127], [128]; however, there is no study using the \textit{ex vivo} microsurgical model for NVB identification. Dissection of the chicken thigh is a nonliving model system that is inexpensive, readily available, and has been widely used as a microsurgical animal model for nerve/NVB dissection due to the similar size and structure of structures similar to human arteries, veins, nerves, and connective tissues [156], [157]. Therefore, when more complex tissue models are not accessible, the chicken thigh model can be an attractive for basic validation of EWDRS performance and to guide the development of fiber optic EWDRS for nerve identification.

![Figure 10. Example of previous MC report for EWDRS, which compared two probes with different SD distances. a) Photon hitting density (PHD) map generated from Monte Carlo simulations of light propagation in mucosa tissues at 1150 nm for small SD](image-url)
distance and large SD distance. b) The depth-dependency of probe influenced by SD distance in EWDRS range, which was same results as DRS studies.[129].

A number of optical phantoms have been developed in VIS DRS to mimic different concentrations of blood, pigment, and lipid. Phantoms have also been developed in EWDRS, however, there is not a common phantom containing blood, pigment, and lipid. Reports of theoretical simulations of EWDRS and EWDRS optical phantoms are limited to one study investigating the relationship between the sampling volume in ex vivo human colorectal mucosa and the source-to-detector distance of two different probes intended for colorectal cancer detection [129]. Further, the development and validation of EWDRS MC models, including tissue-mimicking optical phantoms and simple ex vivo tissue models, would provide a valuable platform for future development of EWDRS systems, probes, and applications, similar to prior reports in conventional DRS. However, to our knowledge, multilayer optical phantoms covering the full EWDRS spectral range have yet to be reported. Overall, there remains a need for systematic model-based characterization of fiber-optic EWDRS platforms, including ex vivo microsurgical models, optical phantoms, and computational modeling. This work will be developed in Aim 1.

In clinical EWDRS studies, several probes have been used for nerve identification in open surgeries, which had different configurations, including single illumination/collection and multiple illumination/collection fiber with similar SD distance. One of the common VIS DRS probe arrangements is single illumination fiber around multiple illumination fibers, since in VIS range, the scattering features of biological tissues are at a prominent level. However, the probe for NIR spectroscopy is the inverse, due to the low scattering level of tissues, the probe requires more incident light to ensure sufficient
signals collected via the probe. EWDRS covers both VIS and NIR range, which leads to uncertainty over potential differences between these two illumination/collection geometry configurations given a similar SD distance. However, there is no EWDRS computational analysis reported for nerve/NVB identification and the only MC studies of fiber probes investigated differences between two SD distances, which lead to the limitation of theoretical studies of comparing different illumination/collection geometry. This knowledge would be critical for the interpretation of spectra collected for the purpose of surgical guidance, as well as the development of fiber-optic probes to obtain more signals from NVB during various surgical scenarios. Therefore, more research into the characterization platform of comparison among different EWDRS probes is necessary for clinical translation, and it is the subject of Aim 2.

Fiber-optic DRS is particularly well suited for incorporation with minimally invasive procedures. Fiber optic probes for needle biopsy, endoscopes, and colonoscopes have been developed with excellent success, however, published reports of EWDRS have only used general purpose fiber optic probes in open surgeries. Further development of the technique will require purpose-driven design and development of fiber optic probes and measurement protocols amenable to laparoscopic surgery. A few things need to be tested during laparoscopic surgery, such as probe placement and system short-distance transportation, while there is no report mentioned in detail. Holding the probe either by hand or laparoscopic instruments, a wide variety of probe-to-tissue geometries may change the DR spectral line shape and intensity, generating an artificial error. Unfortunately, no EWDRS studies investigated how probe-to-tissue geometry influences spectra. Pinto et al. [158] reported an application of Raman spectroscopy integrated with a robotic-assisted
surgical system. They developed a unique hemisphere phantom to investigate the integration of the entire system that allowed repeatable measurements over a sufficiently broad range of fiber optics cable curvatures. The EWDRS has not yet been reported in laparoscopic system. Therefore, important preliminary EWDRS studies in laparoscopic configurations are necessary prior to future human subjects' investigation, and this is the subject of Aim 3.
CHAPTER

2. RESEARCH OBJECTIVES AND APPROACHES

2.1 Overall Goal

Laparoscopy is a minimally invasive surgical technique commonly used during pelvic surgeries. Currently, the most common complications during laparoscopy are nerve-related injuries associated with difficulty in identification of PANs, which relies on the surgeon’s experience and best judgment. Hence, there is a need for objective laparoscopic surgical guidance to precisely identify PANs from other tissues, thus preserving overall function. Optical spectroscopy is a possible approach for surgical guidance with fast detection speed and high specificity. EWDRS is an emerging spectroscopic technique well suited for identification of neurovascular bundles because it retains the benefits of traditional DRS and appends the capability to appropriately assess important components of neurovascular bundles, including lipids, collagen, and water. EWDRS has demonstrated the capability to identify nerve-related tissues in different open surgeries, however, the challenge of laparoscopic surgical guidance presents more important fundamental questions such as detection sensitivity with depth and differential evaluation of fiber probe designs. Rigorous theoretical evaluation of these issues is impractical in surgical settings, and model-based approaches that have provided valuable insight for techniques such as diffuse optical tomography are not reported for EWDRS. Therefore, the overall project goal is to develop and validate model-based systems for analysis of fiber-optic EWDRS. The aims of this dissertation will be to 1) develop and validate a model-based characterization platform for EWDRS, then 2) investigate the performance difference
between different fiber-optic probe arrangements, and 3) adapt the custom-developed EWDRS system for use in laparoscopy.

2.2 Specific Aims

Aim 1: Develop a Model-Based Characterization Platform for Fiber Optic EWDRS

The first aim is to develop a model-based characterization platform for fiber-optic EWDRS using *ex vivo* animal model, optical phantom, and computational modeling. An EWDRS system will be developed and characterized using an *ex vivo* microsurgical animal model. A unique 2-layer EWDRS tissue mimicking optical phantom will be developed and compared with *ex vivo* measurements. Next, Monte Carlo computational modeling will be used to characterize EWDRS and evaluate the relationship between theoretical simulations and empirical measurements of both phantoms and tissues. The outcome of aim 1 is to demonstrate the agreement between a set of model-based systems that can be used to guide future investigations of EWDRS for translational applications.

Aim 2: Develop a Comparison Platform for Detection Performance Between Probes with Various Configurations

The second aim is to utilize these model-based systems for analysis of differences between two fiber-optic probe designs and evaluate potential differences for NVB identification. In this aim, two unique EWDRS reflectance probes will be developed and characterized with computational modeling and empirical measurements of NVB mimicking optical phantoms. The outcome of aim 2 is the demonstration of model-based systems for comparison of EWDRS probe spectral depth dependency and comparison of probe performance of measurement of NVB mimicking tissue layers in optical phantoms.
Aim 3: Prepare EWDRS System for Laparoscopic Use

The third aim is to develop a home built EWDRS for use in laparoscopic instruments. In this aim, the EWDRS system will be packaged for use in surgical settings and EWDRS probe measurement variability as a function of probe orientation will be performed. The outcome of the last aim is to investigate the placement variance of EWDRS probes when detecting various geometry of tissue surface and prepare EWDRS for use outside of benchtop settings and package the system in preparation for future surgical applications, as well as evaluate parameters during the measurements, such as background, noise level, integration time and probe feasibility.
CHAPTER

3. AIM 1. DEVELOP A MODEL – BASED CHARACTERIZATION PLATFORM FOR FIBER OPTIC EWDRS

Fiber-optic extended-wavelength diffuse reflectance spectroscopy (EWDRS) is an emerging technique that expands the spectral range of visible/near-infrared DRS beyond 1000 nm up to 1500-1800nm, which enables improved detection of spectral absorbances arising from lipids, water, and collagen. Recent EWDRS studies have demonstrated the promise of this approach in a variety of applications, including surgical guidance; however, reports of characterization with Monte Carlo (MC) computational simulations and other model systems remain limited. The aim of this work is to characterize agreement between EWDRS measurements and simulations and strengthen the basis for model-informed development of EWDRS systems and applications. A model-based platform consisting of an ex vivo microsurgical nerve dissection model, unique 2-layer optical phantoms, and MC models simulations of fiber-optic EWDRS spectroscopic measurements were used to characterize EWDRS and compare agreement across models.

EWDRS in the common chicken thigh femoral nerve microsurgical dissection model demonstrates understood spectral features tissue classification capabilities to identify neurovascular bundles (NVBs). A comparison of measurements from unique EWDRS issue mimicking optical phantoms and MC simulations indicates high agreement between the two in both homogenous and 2-layer optical phantoms, as well as in dissected tissues. Finally, MC simulations of an EWDRS measurement scenario are performed to provide a representative example of future analyses that can be performed.
Characterization of agreement between fiber-optic EWDRS measurements and MC simulations demonstrates strong agreement across a variety of tissues and optical phantoms, offering promise for further utilization to guide the continued development of EWDRS for translational applications.

3.1 Introduction

Diffuse reflectance spectroscopy (DRS) has been utilized as a valuable tool to characterize biological tissues [84], [85]. Visible/NIR DRS from 400 to 1000 nm has demonstrated the ability to rapidly classify tissues based on spectral differences associated with scattering and a combination of visible chromophores, such as blood and pigments [86], [159]–[161]. Recently, extended-wavelength DRS (EWDRS) systems have been reported expanding the detected wavelength range into the short-wave infrared (SWIR) up to 1600 nm and improving the ability to detect additional biologically important chromophores, including lipids, water, and collagen [94]–[96], [108]. Applications of EWDRS include skin, colorectal, and breast cancer diagnosis and the ability to differentiate tissues in skin and liver tissues [95], [105], [109], [110], [112], [162]. More importantly, EWDRS is a particularly promising technique for detecting NVB [93], [120], [121], [163], [164]. NVB consists of nerves and vessels within fat and connective tissues, lying amidst adjacent tissues and forming a multilayer structure. Visual identification of NVB in surgical practice can be challenging due to the range of sizes and scenarios they present during surgery [35], [36], [121], [122], [165]. EWDRS has demonstrated the ability to identify peripheral nerve bundles from surrounding tissues in open surgeries using a handheld probe [120], while another study reported that a combined EWDRS and fluorescence spectroscopy could identify nerves during head and neck using a needle-
shaped probe [121]. Further development and characterization of EWDRS probes for a wide variety of unique intraoperative uses would benefit from characterization and analysis with model systems, including well-characterized theoretical simulations supported by EWDRS measurements in simple optical phantoms and *ex vivo* microsurgical models; however, there are few reports of specific to EWDRS range (400 – 1800 nm) spectroscopy.

Monte Carlo (MC) modeling [135], [136] is a widely used technique to simulate photon migration in highly scattering biological tissues having multilayered or complex structures [137]. MC modeling has been utilized to investigate various spectroscopic techniques such as DRS, fluorescence, and Raman spectroscopy [137], [139], [140]. Reports of theoretical simulations of EWDRS and EWDRS optical phantoms are limited to one study investigating the relationship between the sampling volume in *ex vivo* human colorectal mucosa and the source-to-detector distance of two different probes intended for colorectal cancer detection [139]. Further, the development and validation of EWDRS MC models, including tissue-mimicking optical phantoms and simple *ex vivo* tissue models, would provide a valuable platform for future development of EWDRS systems, probes, and applications, as has been demonstrated using conventional DRS [141]. However, to our knowledge, multilayer optical phantoms covering the full EWDRS spectral range have yet to be reported.

In this Aim, we reported the characterization of a model-based characterization platform for EWDRS using an *ex vivo* microsurgical nerve dissection model, Monte Carlo modeling, and the development of 2-layer tissue simulating EWDRS phantoms. Characterization of the agreement between MC models of EWDRS and empirical measurements establishes the basis for more complex or nuanced future studies to guide
the development of EWDRS, and a representative example of studies evaluating depth dependence of an EWDRS measurement scenario is included in this work.

3.2 General Approach

In the first aim, we utilized three models to characterize the EWDRS system in order to develop a model-based platform. First, an *ex vivo* microsurgical model has been used to characterize the fiber optic EWDRS to verify the ability for NVB identification. A non-biological multilayer phantom has been developed to mimic significant features across VIS-NIR regions. At last, the computational modeling-MC model has been applied to simulate DR spectra based on the optical offset of the EWDRS system, as well as optical properties of *ex vivo* sample and phantom. Finally, the relationship between simulations and measurements has been investigated.

3.2.1 EWDRS System And Fiber Optic Probe

The EWDRS system (Figure 11) was created using a dual-spectrometer approach. The 500-1000 nm range (VIS/NIR) spectrometer utilized a silica-CCD array detector (StellarNet Inc, FL, USA), while the 1000-1500nm (SWIR) range spectrometer utilized an InGaAs array (Andor, Oxon, UK). A broadband Tungsten-Halogen illumination source (Thorlabs Inc, NJ, USA) was used for both spectral ranges. The design of the probe had a Y shape fiber bundle branch to route illumination and collection fibers to the appropriate hardware, with a total of 2-m long plastic outer jacket covering fibers. The distal end of the probe was fixed by a 20-cm long metal catheter with a metal ferrule to avoid damage from biological tissues. A custom fiber-optic probe with six collection fibers around a central
illumination fiber was developed, of which all seven fibers were 0.39 numerical aperture (NA) with a core diameter of 600 µm. Spectra in both VIS/NIR were referenced against a single 99% diffuse reflectance standard (Labsphere, USA) to ensure consistent relative intensities between measurements.

Figure 11. The EWDRS system. The fiber-optic probe connects a light source, sample, and two spectrometers.

### 3.2.2 Data Preprocessing And Analysis

All data pre-processing was implemented in MATLAB (Mathworks Inc., USA) and includes spectral calibration, InGaAs array fixed pattern noise reduction, and VIS/NIR spectral matching factor calculation.

Calibration was performed before each set of measurements using a 99% white reflectance standard (Spectralon, Inc) via the following equation:

\[
\text{Cal}(\lambda) = \frac{S(\lambda) - Bg(\lambda)}{Ref(\lambda) - Bg(\lambda)}
\] (1)
Here, $Cal(\lambda)$ and $S(\lambda)$ were the calibrated spectra and raw measured spectra. After measurement of each sample type, a reference and background spectrum were collected. The background spectrum $Bg(\lambda)$ was measured by shuttering the light input.

There was a fundamentally different detector architecture in the InGaAs detectors used in the EWDRS system compared to the CCD detectors. There was a clear difference in background signal and the different relative amounts of fixed pattern noise. Accurately, due to the readouts of 2-dimensional chip array, InGaAs signals were characterized by a unique fixed-pattern noise and typical ambient backgrounds present in spectral signals. Thus, the much noisier spectra in SWIR required an extra step to reduce noise level. The noticeable fixed pattern noise was a sequence of sinusoidal waves mixed with reflectance signal. A Savitzky–Golay filter was a filter to increase the data's precision without distorting the signal tendency. A Savitzky-Golay filter with an order of 2 and a frame length of 41 was used to reduce the noise for all SWIR spectra.

VIS and NIR spectra were acquired from two detectors, therefore, concatenating the output data requires calculation of a scalar matching to link VIS/NIR and SWIR spectra without discontinuity. A matching point of 1000 nm was chosen, and the matching factor was given by the equation:

$$M_{(1000nm)} = \frac{l_{VIS/NIR}}{l_{SWIR}}$$

Here, $l_{VIS/NIR}$ and $l_{SWIR}$ was the intensity at 1000nm from two spectrometers.

3.2.3 Ex Vivo Microsurgical Nerve Dissection Model

Dissection of the femoral neurovascular bundle in fresh chicken thighs was a common microsurgical model due to its similar size and structure to the human NVB [156],
The chicken thigh model was attractive because it avoided ethical and cost concerns associated with living animals or human subjects research, was widely available, and reasonably mimicked simple microsurgical challenges for training purposes. Future development of fiber-optic spectroscopy for nerve identification could benefit from utilization of the chicken thigh sample when more complex tissue models were not accessible. Fresh chicken thighs were obtained from a local grocer and maintained at 5°C until dissected to expose the femoral NVB against adjacent tissues, including skin and muscle (Figure 12(a)). The intact femoral NVB was then further micro-dissected into a nerve, vessels, and NVB connective tissue (Figure 12(b)). Spectra were collected from the intact NVB and the individual nerve and vascular bundles after microdissection. Five repeated measurements were made directly on each tissue type listed above at different locations. A total of 3 chicken thighs were analyzed for 85 spectra.

Figure 12. Example of fresh chicken thigh dissection. The NVB is lying under the muscle layer, which is covered by connected tissues and sliding fat. (a) Dissection exposes whole NVB. (b) The NVB is dissected into nerve, vein, artery and NVB connective tissue.
3.2.4 Statistical Analysis

The processed spectra were classified with a Bayesian machine learning algorithm, sparse multinomial logistic regression (SMLR) [165], [166]. SMLR reduced spectral dimensionality while assigning specific spectral bands responsible for classification. Moreover, SMLR produced a posterior probability of class membership and prioritizes sparsity in model construction and was thus well suited for feasibility studies where model over-fitting may be a concern. The SMLR model was trained and validated using leave-one-out cross-validation.

3.2.5 Tissue Simulating Phantoms

Two (2)-layer tissue simulating optical phantoms, shown in Figure 13 (a), were created to aid the characterization of the MC model. A gelatin-based nerve-mimicking (NM) layer was created with a mixture of water, 3.5% fat homogenized cow's milk, canola oil, and lecithin (2 wt% of canola oil), with a water to oil ratio of 1:1, which was designed to mimic lipid, water and collagen component. A gelatin-based muscle-mimicking (MM) layer was created using a mixture of water, 3.5% homogenized cow's milk, instant coffee (5 ml), red and green food dye (0.5 ml each), with a water to milk ratio of 3:1, which was designed to mimic blood, water and collagen component. NM layers were created with varying thicknesses (1-3 mm), while the MM layer was a fixed 4 cm thickness. The NM phantom was designed to mimic the spectral line shape arising from nerves and include SWIR features associated with lipid and connective tissue related spectral features, but without any blood-simulating pigments. On the other hand, the MM phantom was designed to mimic hemoglobin rich vascularized tissues, with the absorption peak near 630 nm...
intended to mimic the main spectral feature in VIS/NIR spectral window. Therefore, the NM phantom mimicked the whole NVB tissue including adipose tissues, while the MM phantom mimicked muscle tissue.

Figure 13. 2-layer phantom with different optical properties. (a) The NM layer varies in thickness from 1 mm to 3 mm, a lipid-rich and high scattering layer. The MM layer was 4 cm with high absorption features. (b) & (c) Optical properties of optical phantom: absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$). (b) The NM layer mimicked a lipid-rich tissue without blood, which scattering feature was higher than that of the MM layer, and absorption features were blank in the VIS range but a sharp lipid absorption peak around 1210 nm, as well as a water peak at 1400 nm. (c) The MM layer was designed to mimic a blood-rich tissue with minor lipid, which contained few blood-related features that appeared between 500 and 700 nm, and the 1210 nm lipid-related feature was flat.
Optical measurements were performed on 1- and 2-layer phantoms with different thicknesses of the NM layer. At each measurement location, five repeated measurements were collected. The acquisition time of the probe was 50 ms in the VIS/NIR range and 800 ms in the SWIR range. During the measurement, both probes were held by hand perpendicular to the surface of each 2-layer phantom with light pressure. A total of 5 phantoms (two 1-layer phantom and three 2-layer phantoms) were analyzed for 25 measured spectra.

3.2.6 Determination Of Optical Properties

The optical properties of all dissected tissue types (Figure 13 (b) & (c)), as well as each tissue mimicking optical phantom layer were determined using measurements of reflectance and transmittance collected via an integrating sphere (Thorlabs Inc, NJ, USA). Reflectance and transmittance measurements were then calculated using the inverse adding doubling (IAD) technique [167] to obtain optical properties: absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$). Anisotropy was kept constant at 0.9, and the refractive index was set to 1.34 for all samples. Before subsequent use in Monte Carlo Models, the reduced scattering coefficients $\mu_s'$ were fit to a first-order exponential decay function, while the absorption coefficients $\mu_a$ of chicken tissue determined by IAD was smoothed using second order Savitsky-Golay filter.

3.2.7 Monte Carlo Model

Monte Carlo models used here were created using the open-source Monte Carlo eXtreme (MCX) software package [168]. MCX allowed for GPU-parallelization to quickly
execute computer simulations of light-tissue interactions and provided a flexible framework for defining specific illumination/detection configurations that could model fiber optic probe bundles, as well as 3-dimensional tissue models, which could mimic optical phantoms.

Simulations were performed from 500 nm to 1500 nm. The light source and detectors were set up based on the fiber optic probe (Figure 11), with light illuminated in the center around six detectors. Sample volumes consisted of either homogenous layer with optical properties corresponding to those determined through IAD for each tissue phantom layer as well as each dissected tissue type, or a 2-layer optical phantom model with layer thicknesses and optical properties identical to the physical 2-layer optical phantoms. Based on the configuration parameters, the simulated EWDRS spectra were generated and compared with empirically measured spectra.

3.3 Results And Discussion

This Aim sought to complement prior reports of the ability of EWDRS measurements to differentiate nerves and NVB from adjacent tissues in human subjects [111], [112] in a microsurgical nerve dissection model, and investigate the agreement between Monte Carlo models of fiber-optic EWDRS in both optical phantoms and dissected tissues [169].

3.3.1 Ex Vivo Microsurgical Nerve Dissection Model

In all the spectra, the 500–650 nm range exhibited a spectral line shape characteristic of optical absorption by blood [96], and prior reports have characterized
tissues blood-related composition using several metrics, including the spectral ratio of 575/610 nm, where a lower ratio indicated more significant contributions from blood and heme related pigments [163]. The SWIR spectral window from 1000-1500 nm exhibited a spectral line shape characteristic of optical absorption from a mixture of water, collagen, and fat [87], [94], [95], [97], [120], [121], [128]. Water was characterized by the local reduction in optical absorption around 1000 nm. Spectral features associated with lipid absorption appear as a sharp intensity dip around 1210 nm and were particularly prominent in the spectra measured from the skin, NVB connective tissue, and NVB. Finally, the minima near 1450 nm and its rate after 1450 nm were related to water and collagen.

Figure 14. The average spectra after dissected NVB. Muscle spectra had single hemoglobin feature between 500 and 600 nm, while other spectra had ‘w’ shape hemoglobin feature. Water absorption features at 1000 and 1400 nm appeared in all spectra. Lipid feature around 1210 nm was sharp among skin, nerve and NVB connected tissue. Spectra of skin, nerve, and connective tissues were really close to each other, especially in vis range, the differences among them were significant in NIR range.
Classification of nerve and NVB vs. all other tissues (including skin, muscle, vessels, and connective tissues) was performed using SMLR. The average spectra collected from each tissue type during the microsurgical dissection of the NVB are shown in Figure 14. Spectra of skin, nerve, and NVB connective tissues had similar spectral features, especially in the VIS/NIR window. Their differences were significant in the SWIR window, as well as the absorption features associated with lipid collagen and water (All significant spectral features used for classification in Appendix A). Calculations of the mean spectral ratios for blood-related (575/610 nm) and lipid-related (1210/1270 nm) composition were shown in Table 2 and were representative of prior reports in human tissues [87], [119]–[122], [163].

Table 2. Peak ratio of measured and simulated spectra

<table>
<thead>
<tr>
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<th>VIS/NIR Peak ratio</th>
<th>SWIR Peak ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measurement</td>
<td>Simulation</td>
</tr>
<tr>
<td>MM phantom</td>
<td>0.81±0.02</td>
<td>0.653±0.003</td>
</tr>
<tr>
<td>1 mm</td>
<td>0.95±0.03</td>
<td>0.87±0.02</td>
</tr>
<tr>
<td>2-layer phantom:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2mm</td>
<td>0.98±0.01</td>
<td>0.951±0.009</td>
</tr>
<tr>
<td>3mm</td>
<td>0.988±0.002</td>
<td>0.969±0.008</td>
</tr>
<tr>
<td>NM phantom</td>
<td>1.011±0.004</td>
<td>0.95±0.01</td>
</tr>
<tr>
<td>Chicken Muscle</td>
<td>0.7±0.1</td>
<td>0.647±0.004</td>
</tr>
<tr>
<td>Chicken NVB</td>
<td>0.7±0.1</td>
<td>0.692±0.002</td>
</tr>
<tr>
<td>connective tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken NVB</td>
<td>0.7±0.2</td>
<td>0.655±0.005</td>
</tr>
</tbody>
</table>
The overall accuracy was 89.4%, with nine spectra misclassified, including six spectra collected from nerve & NVB and three spectra from other tissues, which was comparable to the prior EWDRS studies [118]–[121], [128], [130]. The classification was based on the inclusion of spectral features in both the VIS/NIR and SWIR spectral ranges, including features of blood (500-600 nm), water (900 to 1000 nm & after 1400 nm), and lipid (around 1200 nm), which were similar to those previously identified in published reports of human subjects [118]–[121]. The result of classification confirmed the similarities in EWDRS based identification of nerve and NVB from surrounding tissues, as well as the utility of the chicken thigh microsurgical model of nerve dissection in replicating both spectroscopic features of interest and microsurgical measurement scenarios. Furthermore, future work in the preliminary development of emerging EWDRS systems or fiber optic probe configurations could utilize the chicken thigh microsurgical model for the evaluation of probe performance.

<table>
<thead>
<tr>
<th></th>
<th>Nerve &amp; NVB</th>
<th>Other tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve &amp; NVB</td>
<td>19/25</td>
<td>6/25</td>
</tr>
<tr>
<td>Other tissues</td>
<td>3/60</td>
<td>57/60</td>
</tr>
</tbody>
</table>

### 3.3.2 EWDRS Of Optical Phantoms And Comparison With Ex Vivo Tissues

In order to perform further characterization of model-based systems for evaluation of EWDRS, tissue-mimicking optical phantoms were developed that exhibited diffuse
reflectance spectral line shapes similar to those observed from the tissues of interest in the nerve dissection model. The primary point of emphasis was producing phantoms that mimicked spectra obtained from tissues in the SWIR range. Both the MM and NM layers from the optical phantom were compared with spectra collected during dissection (Figure 15) and indicated the ability to approximate the spectral line shape in the SWIR range. The NM spectra only had one main feature in the VIS/NIR window, and the relatively higher concentration of lipids produced a more substantial dip around 1210 nm and was characterized by a lipid-related spectral ratio (1210/1270 nm) more similar to nerve than to muscle (All spectral ratio data in Table 2). The SWIR Peak ratio of NM phantom was 0.80 ± 0.03, compared with chicken nerve 0.88 ± 0.07. The MM spectra exhibit a minor feature around 630 nm arising from colored dyes within the phantom, which could be used to identify VIS/NIR spectral features of the underlying layer uniquely. The VIS/NIR Peak ratio of MM phantom was 0.81 ± 0.02, compared with chicken muscle 0.7 ± 0.1. Due to the minimal amount of lipid, there was only a tiny local minimum near 1210 nm wavelength in spectra measured from the MM phantom. The SWIR Peak ratio of MM phantom was 1.042 ± 0.004, compared with chicken muscle 0.93 ± 0.03. The simulated blood features in MM layer phantom were utilized red food dye, which absorption feature at 630 nm was right shifted than biological blood features around 570 nm. However, the purpose of the optical phantom was to create a mimic of the optical spectra of tissue that has a few critical features of EWDRS that had been identified as important for tissue classification. Also, the reduced spectral features provided a less complicated characterization of optical properties during computational simulation. In addition, the blood-related spectral ratio (575/610 nm) was more similar to muscle than nerve. There
remain clear differences between the spectra in the VIS/NIR range, and additional refinement of phantom fabrication mixtures would remain beneficial for future phantoms and MC models applications for more thoroughly investigating phenomena such as probe depth dependency across the spectral range.

Figure 15. Comparison of measurements from tissue and optical phantom measurements. a) The average spectra of NM phantom and ex vivo dissected nerve. Because the NM phantom did not mimic blood features in VIS range, the comparison focused on NIR region (1000 - 1500 nm), which spectral line shape between tissue and phantom were close. b) The average spectra of MM phantom and chicken muscle. The spectrum of NM phantom represents features between 500-700nm related to blood. However, compared with tissue spectra, there was a right shift from phantom spectral features, which because the absorption peak of material (red food dye) to mimic the blood feature was not perfectly match around 570 nm. Water and lipid features in NIR region displayed strong agreement between phantom and tissue.

Overall, the results indicate the ability of the phantom to mimic the EWDRS line shape from tissues arising from major chromophores in both VIS/NIR and SWIR spectral
windows and can therefore be used for evaluation of agreement between MC models and empirical measurements.

3.3.3 Comparison Of MC Simulations And Empirical EWDRS Measurements

The development of an EWDRS optical phantom provided a set of samples with controlled compositional properties, and the comparison of measurements from ex vivo dissected tissues and optical phantoms identified general similarities between the two sets of samples. Characterization of tissue spectroscopy through only empirical measurements limited the evaluation of light transit within tissues, and more rigorous analysis could be performed through computational modeling where users could specify sample architecture and optical properties, along with illumination/collection geometry. An essential yet critical step in moving forward with MC model-based characterization of EWDRS was evaluating the relative agreement between computational models of simplified samples, such as optical phantoms, and empirical measurements. The utility of EWDRS MC models could be further strengthened through the comparison of MC simulations and empirical measurements of ex vivo dissected biological tissues. Here, a comparison of empirical EWDRS measurements collected from optical phantoms and MC simulations of EWDRS identically configured samples was reported in single-layer homogenous phantoms, 2-layer phantoms, and finally dissected ex vivo tissues.
3.3.3.1 MC Simulated EWDRS vs. Measurements In Homogeneous Optical Phantoms

A comparison of homogenous, 1-layer, NM phantom and MM phantom measurements and MC simulated measurements from tissue models was shown in Figure 16.

Figure 16. Measured and simulated spectra from homogenous single layer phantoms. (a). The average spectra collected from the homogenous NM phantom and simulated spectrum. (b) The average spectra collected on the pure MM phantom and simulated spectrum. (c) The correlation between simulation and measurement diffuse reflectance from all homogenous single layer phantoms with $R^2 = 0.98$.

The MC simulation of the EWDRS spectra was based on both the geometric configuration of the fiber-optic probe as well as the optical properties of the phantom. Comparison between the measured and simulated diffuse reflectance across the entire wavelength range was evaluated using a correlation (Figure 16), which showed a robust overall agreement ($R^2 = 0.98$), which confirmed the overall validity of the configuration of the MC simulations in these simplified samples, while also identifying that there remained a degree of underestimation in the simulated diffuse reflectance above 1200 nm.
3.3.3.2 MC Simulated EWDRS vs. 2-layer Optical Phantoms

Further comparison of the measured spectra with simulated spectra was performed using an incrementally more complex 2-layer phantom, which simulated the presence of nerve tissue with various thicknesses over background muscle tissue. Similar to the comparison of homogenous phantoms, simulated vs. measured EWDRS spectra from 2-layer phantoms also were in agreement, with similar spectral line shapes and trends in calculated peak ratios in phantoms with 1 mm to 3 mm superficial NM layer thicknesses. Representative simulated and measured spectra from 1mm and 3mm thick superficial layer were shown in Figure 17 (a), (b). The correlation between simulated and measured spectra further demonstrated agreement between modeled and measured spectra and was representative of the modeled vs. measured spectra at other thicknesses of the superficial layer (Figure 17 (c)). When the superficial NM layer was 1mm, both the measured and simulated spectra showed a slight dip around 630 nm wavelength, clearly indicative of pigments unique to the underlying tissue layer with spectral features in the VIS spectral range contributing to the measured signal.

![Figure 17](image_url)

Figure 17. Measured and simulated spectra from 2-layer phantoms. (a) Spectrum collected from a 2-layer phantom with a 1mm top layer and model simulated spectrum using optical properties of phantom. (b) Spectra measured from the 3mm thick top layer
phantom and model simulated spectra. (c) Correlation of spectra between measurement and simulation from all 2-layer phantom with $R^2 = 0.99$.

However, when the thickness of the NM layer increased to 2mm and 3mm, neither measured nor simulated spectra had the unique visible peak associated with blood (comparing VIS/NIR peak ratio between 1mm vs. 2mm & 3mm for both measurement and simulation, the t-test results showed significant difference ($p$ value = 0.0259 & 0.0001)). Alternatively, the comparison of spectra in the SWIR range, both measured and modeled spectra, indicated a more prominent local minima in the lipid feature around 1210 nm, which indicates a reduced contribution from the underlying tissue layer. Due to the incrementally higher complexity of the two-layer vs. the homogenous model, a correlation between the VIS/NIR and SWIR peak ratios used for EWDRS tissue characterization was also performed to determine the extent to which trends in spectral ratio metrics are maintained with changes in sample structural composition (Figure 18). Again, a strong agreement ($R^2 = 0.99$) was observed, indicating that subtle trends in ratio metric measures are also preserved in MC models across VIS/NIR and SWIR ranges.
Figure 18. Correlation of peak ratio between measured and simulated spectra with $R^2=0.97$.

Overall, comparison between measured and simulated spectra across the entire wavelength range, indicates an excellent MC model agreeing with both homogenous and 2-layer phantom measurements, which confirms the ability of the MC models of fiber-optic probe-based EWDRS to agree with measured diffuse reflectance spectra given appropriate construction of MC tissue model and fiber-probe configuration. These results provide the necessary support for future quantitative MC based studies which use simulations to investigate more complex scenarios such as a scale of changes in spectral features corresponding to depth-dependent changes in observed EWDRS spectra, changes in EWDRS spectra with varying tissue microstructure and composition, as well as more detailed analysis of fiber-probe design/configuration.
3.3.3.3 Comparison Of MC Simulated EWDRS vs. Microsurgical Dissection Tissues

In addition to performing a comparison of simulations and measurements in optical phantoms, a comparison of empirical measurements obtained from tissues observed in the microsurgical dissection model against MC simulations of homogenous tissues with identical optical properties was performed, with the purpose of confirming the agreement between simulation and measurement in phantoms and performing simulations of more complex measurement situations. Again, simulated spectra agreed with measured data. Comparison of muscle and NVB simulated vs. measured spectra are shown in Figure 19 (a) & (b), the entire spectral correlation ($R^2 = 0.96$) between measured vs. simulated spectra is shown in Figure 19 (c), and the correlation between VIS/NIR (blood-related) and SWIR (lipid-related) spectral ratios were shown in Figure 19 (d) again what is number appearing strongly agree with the measurement result. The correlation of entire spectra and two peak ratios showed that the $R^2$ value was 0.9.
Figure 19. Measured and simulated spectra from dissection model tissues. (a)-(b) Tissue measurements vs. simulated spectra. (c) Correlation of simulation and measurement from all dissected chicken tissues with $R^2 = 0.96$. (d) Correlation of peak ratio between measured and simulated spectra with $R^2 = 0.90$.

The simulated spectra of *ex vivo* dissected tissues display similar variation from empirical measurements, with slightly lower spectral correlation and spectral ratio correlation arising from decreased quality of optical property measurement from small, delicate dissected tissues. These differences highlighted the importance of informing MC models with high-quality optical property data and motivated future work in a more comprehensive documentation of human and animal tissue optical properties across the EWDRS spectral range to better guide future MC simulations.
3.3.4 MC Simulations Of EWDRS Neurovascular Plexus Measurements

Finally, preliminary MC simulations of measurements of a nerve plexus were performed to provide a representative example of MC simulations for further applications to EWDRS. Empirical measurements from both *ex vivo* and *in vivo* animal models provide critical feasibility data to support the development of optical technologies; however, uncertainty over the precise morphological and compositional makeup of the sample can limit fundamental insight. Alternatively, optical phantom models are valuable in establishing objective fundamental characterization but can be difficult to fabricate with morphological and compositional variations that mimic the full complexity of biological tissues. As a result, MC simulations offer a critically unique path towards further analyses in some cases.

Figure 20. MC simulation of measurements from a nerve plexus. (a) Nerve plexus MC tissue model. Nerve tissues (2 mm thick) with similar geometry to human pelvic nerve plexus are represented in pink, while muscle is represented in gray. (b) The simulated spectra with nerve plexus at two different depths.
One example of MC simulations offers insight into translational scenarios is an evaluation of the depth dependency of changes in spectral signatures associated with a neurovascular plexus beneath adjacent tissues. A pelvic nerve plexus structure with dimensions and geometry informed from the microstructural anatomy typically encountered in laparoscopic surgeries of the prostate or uterus [17], [34], [36]–[40] is shown in Figure 20 (a), where one practical use of EWDRS could be intraoperative identification of nerve plexus to avoid surgical complications. In this scenario, nerve plexus with optical properties defined by NVB measurements from section 3.2.1 were placed beneath 0.2, 0.5, 1, and 2mm thick layers of muscle tissue (also defined by optical property measurements in 3.2.1). A comparison of MC simulated spectra from 0.2 mm and 2 mm thick superficial layers (Figure 20 (b)) indicated the spectral line shape with features more similar to the nerve in both the VIS/NIR and SWIR windows, while at 2 mm thick superficial layer, the spectrum was more similar to muscle in VIS/NIR windows, which blood-related peak ratio was significantly deeper than 0.2 mm superficial layer, but the spectral line shape in SWIR window was between muscle and NVB. Interestingly, the model output indicates larger magnitude spectral changes in the VIS/NIR range, which did not persist over the 2mm depth, whereas spectral differences in the SWIR window were of reduced magnitude but seem to persist over the increased 2 mm depth. These findings are simply a representative example of possible uses of MC simulations to guide the development of EWDRS systems and probes for specific applications.

3.4 Discussion

In this Aim, we developed a 2-layer EWDRS phantoms with various thickness of superficial layer, which successfully mimicked a lipid-rich NM layer on top of a blood-
rich MM layer with optical properties close to that of biological tissues across the entire EWDRS range. This unique 2-layer agar-base phantom filled the field of EWDRS phantoms for nerve identification, however, several improvements were identified. First, the 2-layer model was simple, and the superficial NM layer was hard to control under 1 mm due to the mode used. Thus, further development of NVB phantom could 3D print a complex mode to generate the structure of NVBs with various thicknesses. Moreover, the optical properties of existing phantoms were not perfectly matched with biological tissues, especially in the VIS/NIR range mimicking the blood components. Since the red food dye was utilized to mimic the absorption features of hemoglobin, there was a small right shift of spectral features from the developed MM layer phantom around 630 nm, which was different from the hemoglobin features at 570 nm. To overcome this small shift, we could change the red food dye to another red dyes or blood to mimic blood features. To better differentiate between the NM and MM layers of phantoms, there were no blood features added into the NM layer resulting in a significant difference of the spectra between NM phantom and chicken nerves in the VIS/NIR region. Thus, in future developments of optical phantom, blood features could be added into the NM layer in order to closely simulate optical properties of nerve and NVB. Moreover, the optical phantom could extend to other biological tissues beyond NVB.

Using MC modeling, we simulated DR spectra based on the offset of the probe and optical properties of samples (optical phantoms and chicken tissues), and then validated the agreement between simulations and empirical measurements of tissues and phantoms across the full EWDRS range. Overall, evaluation of the agreement between EWDRS MC simulations and measurements across model systems is a critical step towards their
expanded utilization for application development. It is important to note that the realization of close agreement between simulations and measurements requires careful specification of optical illumination/collection geometry and consistent implementation of spectral normalization from standard reference measurements. Accurate normalization is particularly important in EWDRS, where the VIS/NIR and SWIR spectral ranges are typically obtained using two spectrometers and merged in post-processing near 1000 nm. Approaches for spectral merger are not widely discussed in the EWDRS literature; however, the reduced detector performance in each spectrometer near the merging point can introduce artifacts in the final EWDRS spectra that are particularly important when considering an agreement with simulations.

3.5 Conclusions

In this Aim, an agreement between the model-based characterization of fiber optic EWDRS was evaluated for computational simulations and empirical measures. EWDRS measurements and optical property characterization of tissues from an *ex vivo* chicken femoral nerve microsurgical dissection model and unique 2-layer non-biological phantom phantoms were used to demonstrate a strong correlation ($R^2 > 0.9$) between MC simulations and empirical measures across both the entire spectral range and in important spectral VIS/NIR and SWIR ratios previously used for tissue characterization. Optical phantoms included major biological chromophore features across VIS/NIR-SWIR spectral windows, including pigments VIS/NIR blood mimicking pigments, scattering, and SWIR absorbances from lipids, collagen, and water. Empirical measurements from the chicken thigh femoral nerve microsurgical dissection model produced spectra representative that re-affirmed prior reports of tissue classification and could serve as a simple preclinical
model for future studies. Overall, these results provide a basis for further utilization of model systems for the development of EWDRS applications, such as the preliminary investigation of the depth-dependence of signal changes in neurovascular plexus identification shown here, along with the theoretical comparison of different EWDRS fiber-probe designs and more.
CHAPTER 4

4. AIM 2: DEVELOP A COMPARISON PLATFORM FOR DETECTION PERFORMANCE BETWEEN PROBES WITH VARIOUS CONFIGURATIONS

Extended-wavelength diffuse reflectance spectroscopy (EWDRS) has shown promise for identification of nerves and neurovascular bundles. However, model-based theoretical approaches for analysis and characterization of EWDRS measurements in scenarios with varying probe and sample configurations are limited. Here, a model-based characterization platform for fiber-optic EWDRS is described and used to confirm agreement ($R^2 >0.93$) between Monte Carlo simulations and empirical measurements collected using two different probe configurations from single layer and 2-layer models of nerve mimicking optical phantoms across the range from 500-1500nm, reported as both DR and absorbance spectra. The EWDRS probes had similar average source-detector separations, but subtly different illumination/collection geometries, however model-based analyses using a combination of empirical measurements from tissue phantoms and MC simulations confirmed differences in the depth-dependent contributions from tissue types. A framework from evaluating the relative contributions to measured spectra from different tissue features in the sample was described and used to evaluate the source of differences in empirical measurements between the fiber probes. Overall, these results support further use of model-based analyses in development of fiber-optic EWDRS for acceleration of translational applications, such as intra-operative surgical guidance of nerve and neurovascular bundle detection.
4.1 Introduction

Diffuse reflectance spectroscopy (DRS) is an established approach for differentiating tissue types and has been proposed for a number of applications, including disease diagnosis and surgical guidance [84], [85]. VIS/NIR DRS is typically performed using silicon-based detector arrays sensitive from 400 to 1000 nm, and thus the ability to classify tissues results from spectral features arising from visible chromophores, such as blood and pigments, as well as differences in scattering observed in the visible spectral range [86], [96], [108], [146], [159], [161]. In order to improve the molecular sensitivity of DRS, extended-wavelength DRS (EWDRS) systems have been developed, which typically use a second InGaAs detector to expand the wavelength range into the short-wave infrared (SWIR) from 1000 nm to 1800 nm. The expanded spectral range enables observation of distinct spectral features from biologically important chromophores not present in the visible range, especially for lipids, water, and collagen [95], [107], [109], [110], resulting in reports of promising potential applications of EWDRS for diagnoses of skin, colorectal, oral cavity, liver, and breast disease, as well as the ability to differentiate tissues in skin and liver [96], [107], [110], [112], [161], [170]. In addition, EWDRS is particularly well-suited for detecting nerves and neurovascular bundles (NVB) due to their unique compositional makeup including lipid and collagen features producing distinguishing spectral differences over a combination of VIS/NIR and SWIR spectral features [118]–[121], [130]. While these results suggest great promise for EWDRS as a surgical guidance tool, nerves and NVB have a wide range of sizes spanning a range from large bundles such as the femoral nerve at 8mm in diameter down to pelvic nerves at 0.180 mm [171], [172], can have a complex anatomical network, and can intraoperatively be
located underneath or between adjacent tissues in a variety of scenarios [28], [35], [36], [165]. An important consideration for future development of EWDRS for use as a surgical guidance tool for nerve identification is expanding understanding of the influence the aforementioned factors can have on measured spectra as well as their relation to fiber-optic probe optical design. Computational simulations offer a flexible and objective approach for these analyses, as well as the ability to provide indicators of factors contributing to spectral differences that cannot be empirically observed, such as the contributions to the signal depth-dependency in complex tissue microarchitecture, since simulations could provide contributions of signal from specific tissue.

Monte Carlo (MC) modeling [135], [136] is a powerful technique to simulate photon migration through highly scattering biological tissues having multilayered or complex structures [137], [138], [168]. MC modeling has been reported in various spectroscopic techniques such as DRS, fluorescence, and Raman spectroscopy [137], [139], [140]. While DR from simple scattering media decays exponentially vs. depth, MC simulations enable a more detailed inspection of the precise nature of this profile in more complex samples such as biological tissues [173]–[176]. MC simulations of VIS/NIR DRS fiber probes have demonstrated the ability of models to perform theoretical analysis of fiber-probe configurations, evaluation of signal depth dependency versus source-to-detector (SD) distances, tilt angles of fibers, and illumination/collection geometry configurations in both tissues and optical phantoms [138], [141]–[143]. Additional theoretical studies of EWDRS fiber probes using model-based in silico techniques would benefit future translational development for applications such as detection of nerves and NVB. In this manuscript, we report a comparison of theoretical MC simulations and
empirical measurements of two similar EWDRS fiber probes with subtly different illumination/collection configurations to demonstrate the capability of model-based characterization of EWDRS fiber probes. The two probes were arranged to have either central illumination or collection fibers, with either central illumination or central collection fibers, configured to uniquely accommodate both VIS and SWIR fiber channels for EWDRS.

Reports of theoretical simulations of EWDRS and EWDRS probes are limited to one study investigating the relationship between the depth-dependency of DR signals from two probes in *ex vivo* human colorectal mucosa and the different SD distance of two probes intended for colorectal cancer detection [129], and recent work from our own group demonstrating agreement between MC modeled spectra and measured spectra collected from unique 2-layer optical phantoms that successfully mimic major spectral features of biological tissues across the entire EWDRS range, as well as spectra collected from *ex vivo* microsurgical nerve detection model in the chicken thigh [169]. Fundamental development and evaluation of model-based in silico analysis of EWDRS and EWDRS probes would benefit future translational development of fiber probes for detection of nerves and NVB.

In this manuscript, we report a comparison of theoretical MC simulations and empirical measurements of EWDRS fiber probes with subtly different illumination/collection geometries to demonstrate the capability of model-based characterization of EWDRS fiber probes. While VIS DRS and NIR spectroscopy have used both designs, the central illumination fiber design is commonly observed in general-use VIS DRS fiber probes, while NIR spectroscopic probes more commonly have a central collection fiber. The agreement between MC models and empirical measurements provided
further investigation of the basis for more complex or nuanced future studies to guide the development of EWDRS. Here, an analysis of both MC simulations and empirical measurements collected from tissue simulating phantoms was performed to compare an EWDRS probe using a central illumination fiber surrounded by VIS/SWIR detection fibers against a similar probe with a pair of central VIS/SWIR detection fibers surrounded by multiple illumination fibers. This model-based characterization approach represents the demonstration of an important theoretical basis for future investigation of fiber probes with different illumination/collection geometries across the EWDRS spectral range using MC modeling. Analyses were performed to investigate differences in spectral line shape not only between probes, but also as a function of modeled nerve thickness in order to observe spatial phenomena difficult to empirically evaluate objectively in vivo. The agreement between MC models and empirical measurements supported the relative accuracy of the models and provided support for more detailed analyses of depth-dependent spectral contributions only possible using MC simulations. Overall, a model-based characterization approach for EWDRS fiber probes represents an important theoretical basis for nuanced investigation of potential applications in biomedicine using MC modeling.

4.2 General Approach

In this aim, we designed and fabricated two probes with different illumination/collection configurations but the same SD distance. We then used the MC simulations and 2-layer optical phantom model to investigate the differences in detection performance of the two probes.
4.2.1 EWDRS System And Probes

The EWDRS system (Figure 11), includes a broadband Tungsten-Halogen lamp (Thorlabs Inc, NJ, USA) and two spectrometers: VIS/NIR spectrometer (StellarNet Inc, FL, USA) covering the wavelength ranges from 500 nm to 1050 nm, and SWIR spectrometer (Wasatch Photonics, Morrisville, NC, USA) focused on 950 nm to 1500 nm, that is described in detail elsewhere in Aim 1(3.1.2) [169].

Figure 21. Probe configurations. (a) The configuration of probe 1, which is a six-around one arrangement with a single central illumination fiber. (b) The configuration of probe 2, which is a six around two arrangements, with the two central collection fibers routed to either the VIS/NIR or SWIR spectrometers.

The two probes developed for this study combine the fibers routed to the light source and respective VIS and SWIR detectors into a single 2-m long fiber jacket using a custom fabricated Y-shaped fiber bundle bifurcation adaptor. A 20-cm long steel catheter with a custom-cut steel ferrule at the tip with precisely specified fiber arrangements is used at the distal end of both probes. A custom central illumination probe (Probe 1) shown in Figure 21 (a) utilized seven 0.39 NA 600 um fibers, arranged with six collection fibers around a central illumination fiber. Three of the collection fibers were routed to the VIS/NIR spectrometer, while the remaining three were routed to the SWIR spectrometer,
with an alternating arrangement of collection fibers at the sample-facing tip of the fiber assembly. The SD distance of Probe 1 was 1 mm. The second custom probe was fabricated with a pair of central collection fibers (Probe 2) as shown in Figure 21 (b), the probe had six 0.39 NA 600 um illumination fibers around a pair of 0.22 NA 200 um collection fibers. The SD distance for this probe ranged from 0.8 to 1.2 mm, with an average value of 1 mm. Therefore, the two probes had the same average SD distance, but with different illumination/collection geometries.

All data pre-processing was implemented in MATLAB (Mathworks Inc., USA), including spectral calibration, InGaAs array fixed pattern noise reduction, and VIS/NIR spectral matching factor calculation, and is described in detail elsewhere[169].

4.2.2 Optical Phantom

1-layer homogenous optical phantom and 2-layer tissue simulating optical phantoms, shown in Figure 22 (a) & (b), were used to obtain EWDRS measurements and complement simulated measurements obtained from the MC model. An agar-based nerve-mimicking (NM) layer with thickness varying from 1 mm to 3 mm, which mimicked the spectral line shape from NVB tissues through significant spectral absorbances from lipid and water related spectral features in SWIR at 1210 nm and 1400 nm, respectively, but without any blood-simulating pigments in VIS/NIR in order to be identified from the underlying muscle-mimicking (MM) layer. On the other hand, the total agar-based model was 15 mm thick cube, including NM layer and MM layer, designed to mimic the blood-rich tissues, with a significant absorption peak near 630 nm. The simplified 2-layer model was intended to mimic the structure of various thicknesses of nerve lying on top of the background tissues and enable both empirical and MC-based evaluation of depth-
dependent contributions to the spectra observed in an intraoperative scenario where there is uncertainty as to the relative spectral changes observed in nerves of different sizes.

Optical measurements were performed on 1- and 2-layer phantoms with superficial layers thickness of 1, 2 and 3 mm. Both probes were held by hand perpendicular to the surface of each phantom with light pressure. At each measurement location, five repeated measurements were collected. A total of 5 phantoms (two 1-layer phantom and three 2-layer phantoms) were analyzed for a total of 25 measured spectra/probe. The acquisition time of Probe 1 was 100 ms in the VIS/NIR and 500 ms in the SWIR, and that of Probe 2 was 500 ms in the VIS/NIR and 300 ms in the SWIR.

4.2.3 Optical Properties

Reflectance and transmittance measurements of each optical phantom layer were measured using an integrating sphere (Thorlabs Inc, NJ, USA), then optical properties (absorption coefficient $\mu_a$, reduced scattering coefficient $\mu_s'\) were calculated using the inverse adding doubling (IAD) technique [167], and are shown in Figure 22 (c) & (d). Anisotropy was kept constant at 0.9, and the refractive index was set to 1.34 for all samples.

4.2.4 Monte Carlo Modeling

The open-source Monte Carlo eXtreme (MCX) software platform [177], was used to perform simulations. MCX provides a flexible framework for fast GPU-parallelized simulations of light-tissue interactions and includes the ability to define specific illumination/detection configurations necessary to simulate multi-fiber bundles.

Simulations were performed from 500 nm to 1500 nm in 5 nm wavelength increments. The illumination/detection geometry was based on the designs of Probe 1 and
Probe 2, described in session 4.2.1 (Figure 21(a) & (b)). Each source fiber was configured as a disk illumination source located at the surface of the tissue model with diameter corresponding to the probe core. The detection channels were configured to be circular areas in MC model with the size based on the probe’s detection fibers. MCX accumulates all photons incident on in these areas to determine the detected flux, however detected photon packets with incidence angles that exceeded the NA of collection fibers were excluded. The final detected signal was then determined as the summation of the angularly filtered detected photons across all detection fibers.

Figure 22. Tissue phantom models and optical properties used in MC simulations.
(a) The example of 1-layer homogeneous model, which used both optical properties of NM and MM layer to mimic a bulk of nerve and muscle tissue. The superficial layer varies in thickness from 1 mm to 3 mm, a lipid-rich and high scattering layer. The total tissue model is 15 mm with high absorption features. (b) The example of the 2-layer model. The superficial layer varies in thickness from 0.2 mm to 3 mm, a lipid-rich and high scattering
layer. The underlying layer is a blood-rich layer with high absorption features. (c) Optical properties of NM layer: $\mu_a$ is absorption coefficient and $\mu'_s$ is the reduced scattering coefficient. (d) Optical properties of MM layer.

Two simple tissue models were simulated, including a homogeneous model (Figure 22 (a)) using optical properties of each phantom layer shown in Figure 22 (c) & (d) and a 2-layer model identical to the physical 2-layer optical phantoms (Figure 22 (b)). First, MC simulations used models with matching properties to physical phantoms described in 4.2.2, for direct comparison with empirical measurements to verify the agreement of the MC simulation. Then, other 2-layer models with thin superficial NM layers (0.2mm and 0.5mm) which are not easily nor reliably fabricated with agar-based phantoms were simulated in the MC model only to investigate performance of probes on much thinner nerves.

The simulated EWDRS spectra were generated five times for each model, the same number of observations as performed with physical measurements from optical phantoms. The output from the MC model included flux information of incident photons, as well as "biographical" information of the detected photons [178], which included the partial pathlength (ppl) of each detected photon traveled in each unique tissue label assigned within the model space, as well as the direction vector of each detected photon. The direction of the detected photons was compared with the acceptance angles of collection fibers in order to filter out photons that could not be detected by the probe. The ppl was used to calculate the diffuse reflectance through the MCX function (mcxdref), which determines the total DR signals from the tissue surface $DR_{total}$ via the following equation [179]:

\[ \text{DR}_{\text{total}} \]
\[ DR_{total} = \frac{\sum_{k=1}^{N_{det}} w_k}{A_d N_{photon}}, k \in \{1, 2, ..., N_{det}\} \]  

(1)

where \(A_d\) was the area of detector and \(N_{inc}\) was the total number of incident simulated photons. \(w_k\) was the final weight of the \(k^{th}\) detected photon, which was described by the following equation [180]:

\[ w_k = w_0 \prod_{m=1}^{M} e^{(-\mu_{a,m} \cdot ppl_{m} \cdot \text{unit})}, \hspace{1em} m \in \{1, 2, ..., M\} \]  

(2)

The final weight of the detected photon \((w_k)\), was the product of the total attenuation across all tissue labels \((M)\), where the initial photon weight \(w_0\) for every photon was set to 1. \(\mu_{a,m}\) was the absorption coefficient of the \(m^{th}\) label, \(ppl_{(k,m)}\) was the partial pathlength each photon traveled in the \(m^{th}\) tissue label, and unit was the MC model voxel unit, which was set as 0.1 mm for all simulations.

4.2.4.1 MC Determination of Tissue-Label Specific Absorbance

As an alternative to reporting the normalized DR, measurements in infrared spectroscopy, including modern implementations in various reflectance configurations [181] continued to relate observations as the Total Absorbance \((A_{total})\):

\[ A_{total} = \log_{10} \left( \frac{l_0}{I_{det}} \right) = \log_{10} \left( \frac{1}{DR_{total}} \right) \]  

(3)

where \(l_0\) was the incident intensity, and \(I_{det}\) was the detected intensity.

Thus, the solution for the total absorbance, when substituting equations (1,2) became:

\[ A_{total} = \log_{10} \left( \frac{1}{DR_{total}} \right) = \log_{10}(1) - \log_{10}(DR_{total}) \]
\[
= 0 - \log_{10}\left(\frac{\sum_{k=1}^{N_{det}} w_k}{A_d \cdot N_{photon}}\right)
\]
\[
= \log_{10}(A_d \cdot N_{photon}) - \log_{10}\left(\sum_{k=1}^{N_{det}} w_k\right)
\]
\[
= \log_{10}(A_d \cdot N_{photon}) - \sum_{k=1}^{N_{det}} \log_{10}(w_k)
\]
\[
= \log_{10}(A_d \cdot N_{photon}) - \sum_{k=1}^{N_{det}} \log_{10}\left(e^{(-\mu_{a,m} \cdot ppl_m \cdot \text{unit})}\right)
\]
\[
= \log_{10}(A_d \cdot N_{photon}) - \sum_{k=1}^{N_{det}} \sum_{m=1}^{M} \log_{10}\left(e^{(-\mu_{a,m} \cdot ppl_m \cdot \text{unit})}\right)
\]
\[
= \log_{10}(A_d \cdot N_{photon}) - \sum_{k=1}^{N_{det}} \sum_{m=1}^{M} \left(-\mu_{a,m} \cdot ppl_m \cdot \text{unit}\right) \cdot \log_{10} e \quad (4)
\]

Thus, the total Absorbance \(A_{total}\) from the model could be calculated from total DR \(DR_{total}\), which changed the effect of different absorbing tissue types from one of exponential attenuation to one of linear summation. Moreover, the aggregate contribution from elements in the tissue model was direct in units of absorbance, whereas contributions are inversely related to the DR intensity. In the MC framework, it was important to note that the \(\log_{10}(A_d \cdot N_{photon})\) term served as a constant baseline.

A valuable feature of MC models is that they allow insight into photon migration through tissue, which is not possible empirically. Information on tissue-label-specific absorption, \(\mu_{(a,m)}\), and partial-pathlength specific tissue labels, \(ppl_{(k,m)}\), has the inherent capability to inform the relative extent to which measured absorbance from individual tissue's \(A^m\) could contribute to the observed optical measurement, \(A_{total}\).

\[
A^m = A_{total}^m - A_{total}^{m-1}, \ m \in \{1,2, \ldots, M\} \quad (5)
\]
where, $A^m_{\text{total}}$ and $A^{m-1}_{\text{total}}$ were the total absorbance from all $m$ and $m-1$ tissue labels.

$$A^m_{\text{total}} = \log_{10}(A_d \times N_{\text{photon}}) - \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{L} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e,$$

$L = m$  \hspace{1cm} (6)

$$A^{m-1}_{\text{total}} = \log_{10}(A_d \times N_{\text{photon}}) - \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{K} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e,$$

$K = m - 1$  \hspace{1cm} (7)

Thus, $A^m$, the absorbance from any individual tissue $m$, in terms of the MCX detected photon output parameters was given by,

$$A^m = A^m_{\text{total}} - A^{m-1}_{\text{total}}$$

$$= \log_{10}(A_d \times N_{\text{photon}}) - \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{L} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e$$

$$- \log_{10}(A_d \times N_{\text{photon}}) + \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{K} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e$$

$$= \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{K} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e - \sum_{k=1}^{N_{\text{det}}} \sum_{m=1}^{L} (-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e$$

$$= \sum_{k=1}^{N_{\text{det}}} [(-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e]$$

$$= \sum_{k=1}^{N_{\text{det}}}(-\mu_{a,m} \times \text{ppl}_m \times \text{unit}) \times \log_{10} e \hspace{1cm} (8)$$

After obtaining the individual tissue label absorbance, $A^{m\%}$, the percent total absorbance of each tissue label $R^m$ could be determined as:
\[ A^{m\%} = \frac{A^m}{A_{\text{total}}} \times 100\% \quad (9) \]

Evaluation of \( A^{m\%} \) represented a unique approach to evaluating depth-dependent contributions to measured signals based on the relative contributions of different chromophores distributed throughout the sample, instead of the relative signal intensity at a given depth.

4.2.4.2 Parameters For Fiber Probes Comparison

To further compare the subtle difference between probes, the lipid-related ratio [130] was calculated:

\[ R_{\text{lipid-related}} = \frac{A_{1210 \text{ nm}}}{A_{1270 \text{ nm}}} \quad (10) \]

where the absorbance at 1210 (\( A_{1210 \text{ nm}} \)) and 1270 nm (\( A_{1270 \text{ nm}} \)) for both probes measured from the total absorbance spectra. A paired \( t \)-test was utilized to investigate the difference between two probes based on the ratio of two lipid-related points \( R_{\text{lipid-related}} \).

Then, the difference of percent total absorbance between two probes was calculated by the following equation:

\[ \Delta_{\%A} = A_{\text{Probes} \ 1}^{N\%M} - A_{\text{Probes} \ 2}^{N\%M} \quad (11) \]

where \( A_{\text{Probes} \ 1,2}^{N\%M} \) were the percent absorbance from the nerve mimicking layers as determined by Probe 1 or Probe 2, respectively. \( \Delta_{\%A} \) was calculated with the intention to evaluate which probe has the ability to perform measurements with more or less contributions from the NM layer. Values greater than zero indicate Probe 1 is more sensitive to the NM layer, while values less than zero indicate Probe 2 is more sensitive.
4.3 Results

This Aim developed the characterization platform of different probes configurations using MC modeling and investigated the performance of two common probe arrangements for superficial NM layer.

4.3.1 Comparison Of MC Simulation And Measurement From 1-layer Homogeneous Model

A comparison between MC simulation and phantom measurements for 1-layer homogeneous NM and MM phantoms was shown in Figure 23. DR spectra were normalized from 0 to 1 to directly compare two probes. Qualitative similarity was observed between MC simulation and empirical measurement for both probes, in both NM and MM phantoms. A direct correlation between simulations and measurements at matching wavelengths across the entire EWDRS range for both probes (Figure 23 (c) & (f)), also demonstrated the strength of the agreement ($R^2 = 0.97$). The result verified the overall agreement between MC simulations and measurements and indicates some regions with small disagreement.
Figure 23. Measurement and simulation from 1-layer homogeneous phantom/model for Probe 1 (a-c) and for Probe 2 (d-f). The average measured and simulated normalized spectra from 1-layer NM and MM phantom for Probe 1 (a-b). Correlation between simulation and measurement across all wavelengths for Probe 1 (c). Average measured and simulated normalized spectra from 1-layer NM and MM phantom for Probe 2 (d-e). Correlation between simulation and measurement across all wavelengths for Probe 1 (f).

4.3.2 Comparison Of MC Simulation And Measurement From 2-layer Model

A comparison of measured vs. simulated spectra for the two-layer model for with NM layer thickness from 1mm to 3 mm was performed. The measured vs simulated diffuse reflectance and absorbance spectra are shown for Probe 1 (Figure 24) and Probe 2 (Figure 25). In both figures, spectral comparisons are shown for the 1mm (a,d) and the 3 mm (b,e) phantoms, as all phantoms exhibited similar qualitative trends. MC DR and Absorbance
simulations were in close agreement across the VIS/NIR. In SWIR range, simulations slightly underestimated measurements for 1mm NM layer, but overestimated measurements in the 2 and 3 mm NM layer with similar trends. Finally, for all 2-layer models, spectral regions above 1450 nm simulations were overestimated. When measured spectra were plotted as total absorbance, the spectral estimation difference artifacts were the inverse and slightly amplified due to logarithmic transformation.

Figure 24. Two-layer model comparison of measured vs simulated diffuse reflectance and absorbance spectra using Probe 1. Diffuse reflectance spectra from simulation and measurement for 2-layer phantom model with the superficial layer was 1mm and 3 mm (a,b). (c) Correlation of diffuse reflectance spectra between measurement and simulation over all wavelengths from all (1-3mm) 2-layer phantom models, with an $R^2$ value is 0.96. Absorbance spectra from simulation and measurement for 2-layer phantom model with the superficial layer was 1 mm and 3 mm (d,e) . Correlation of absorbance
spectra between measurement and simulation over all wavelengths from all (1-3 mm) 2-layer phantom models, with an \( R^2 \) value is 0.93. The differences at the extreme of the spectra caused by the reduced performance of the detector.

Regardless of differences between simulation and measurement, correlation of simulation vs. measurement across all wavelengths over the total DR spectra indicated good agreement. Both DR \( (R^2 > 0.96) \) and absorbance \( (R^2 > 0.93) \) had good agreement between simulated and measured spectra. Points with poor correlation were primarily in the spectral region above 1450 nm. Over/underestimated regions in the spectra still showed similar trends with increasing intensity as observed in the correlation.

![Graphs showing comparison of measured vs simulated diffuse reflectance and absorbance spectra](image)

Figure 25. Two-layer model comparison of measured vs simulated diffuse reflectance and absorbance spectra using Probe 2. Diffuse reflectance spectra from simulation and measurement for 2-layer phantom model with the superficial layer was 1 mm and 3 mm (a,b). (c) Correlation of diffuse reflectance spectra between measurement
and simulation over all wavelengths from all (1-3 mm) 2-layer phantom models, with an R² value is 0.97. Absorbance spectra from simulation and measurement for 2-layer phantom model with the superficial layer was 1mm and 3 mm(d,e). Correlation of absorbance spectra between measurement and simulation over all wavelengths from all (1-3 mm) 2-layer phantom models, with an R² value is 0.95.

### 4.3.3 Absorbance Comparison Of Probes In 2-layer Model

Comparison of Probe 1 vs. Probe 2 was performed using measurements and simulations of total absorbance, as well as simulations of layer-specific contributions to absorbance. First, comparison of the total measured and simulated absorbance was performed across 1, 2 and 3 mm thickness NM layers. Representative results of measurements (Figure 26 (c, d)) and simulations (Figure 26, (c, d)) from 1 mm (a, c) and 3 mm (b, d) NM layers are shown in Figure 26. Measurements are fairly similar with a slight relative increase in absorbance for 3 mm layer. 2mm layer thickness results are similar to 3 mm in both measured and simulated comparisons, and thus not shown. Simulations show slightly higher signal in Probe 1 in the 1 mm NM layer phantom, but fairly similar signals in the 2 mm and 3 mm layer spectra. Overall, variance in MC simulation measurements was much lower in MC simulations.
Figure 26. Comparison of total absorbance between two probes. Total absorbance of two probes measured from 1 mm (a) and 3 mm (b) NM layer phantom. Total absorbance of two probes simulated by MC model from 1 mm (c) and 3 mm (d) NM layer phantom. Shaded areas corresponding to measurement standard deviations are shown in all four plots, however variance is small in MC simulations.

Table 4. Lipid-related ratio of measurements for probe 1 and probe 2

<table>
<thead>
<tr>
<th></th>
<th>Probe 1: lipid peak ratio (1210/1270 nm)</th>
<th>Probe 2: lipid peak ratio (1210/1270 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mm NM layer</td>
<td>1.067±0.005</td>
<td>1.06 ± 0.02</td>
</tr>
<tr>
<td>2mm NM layer</td>
<td>1.13±0.02</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>3mm NM layer</td>
<td>1.19±0.05</td>
<td>1.142±0.02</td>
</tr>
</tbody>
</table>
To further evaluate differences between probes and NM layer thickness, the calculated lipid-related ratio is shown in Table 1 and 2. With the increasing thickness of NM layer for both measurements and simulations, peak ratio of both probes exhibited a corresponding increase. These results indicate that measurements and simulations show similar trends in the increasing relative contribution from lipid related features. Pairwise comparison of Probe 1 vs. Probe 2 indicated a significant statistical difference (P value < 0.05) in simulations only at 1 mm thickness NM layer, but not in 2 and 3 mm thickness NM layer in measurements. Overall, according to comparison of total absorbance, there was a significant difference between two probes when the NM layer was 1 mm for both simulations and measurements, which indicates that a crucial difference in the probe's sensitivity to thin NM tissue layers. However, the ability to directly evaluate contributions from the superficial NM layer using empirical measurements is possible here only due to prior knowledge of phantom composition. Direct analysis of layer-dependent signal contributions is not directly possible in most empirical measurements, and more objectively performed using simulations.

<table>
<thead>
<tr>
<th>NM Layer Thickness</th>
<th>Probe 1: Lipid Peak Ratio (1210/1270 nm)</th>
<th>Probe 2: Lipid Peak Ratio (1210/1270 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mm</td>
<td>1.140 ± 0.005</td>
<td>1.132 ± 0.005</td>
</tr>
<tr>
<td>2 mm</td>
<td>1.15 ± 0.02</td>
<td>1.144 ± 0.006</td>
</tr>
<tr>
<td>3 mm</td>
<td>1.17 ± 0.01</td>
<td>1.16 ± 0.01</td>
</tr>
</tbody>
</table>
Figure 27. Total and layer-dependent absorbance from 2-layer model for both probes. Absorbance of 1mm NM layer for probe 1 (a) and probe 2 (b). Absorbance of 3mm NM layer for Probe 1 (a) and Probe 2 (b).

Closer inspection of differences between probes can be performed using layer-specific absorbance simulations for both NM and MM layers, $A^{NM,MM}$. Comparison between two probes on signal collected from the NM layer shown in Figure 27, including total and layered absorbance. For both probes, absorbance of NM layer dominated from 800 to 1500 nm, when the NM layer is 1 mm, which indicates that the two probes could detect signal from under MM layer, but greater contribution to total absorbance comes from NM layer. When the NM layer is 3 mm, upper NM layer is the major source of absorbance. The difference between the two probes is small, thus, to better investigate them, the percentage of total absorbance, $A^{NM\%}$ of each layer is displayed in Figure 28.
Figure 28. Difference in percent absorbance from the NM layer for Probes 1 and 2. Percent total absorbance of NM layer $A_{NM}^\%$ for both probes, when NM layer is 1 mm (a) and 3 mm (b). The differences between two probes $\Delta A^\%$ of 2-layer model for 1 mm and 3 mm layers. Standard Deviation of measurements is shown in all plots, but it is too subtle to display in (a) and (b), only perceivable in (c).

When the NM layer thickness was 1 mm, the percentage of total absorbance in the NM layer from Probe 1 is larger than Probe 2 (Figure 27(a)), while, when the NM layer increased to 3 mm, the percentage of NM layer between probes were difficult to distinguish (Figure 27(b)). A comparison of the difference in percent absorbance, $\Delta A^\%$, confirms that Probe 1 did inherently have a slightly greater contribution from the superficial NM layer from thin layers than Probe 2 (Figure 27 (c)). Probe 1 was able to obtain over 4% more absorbance from NM layer than Probe 2 across the full EWDRS range when NM layer was 1 mm. When the NM layer was up to 3 mm, the ability of probe 1 at collecting signals from NM layer stayed around 1% more than probe 2 in the entire EWDRS range.

Since NVB identification in surgical guidance is a promising potential application of EWDRS, the difference between probes of absorbance from NM layer for thinner nerve layers was also investigated in simulations. In the pelvic cavity, the thickness of thin NVB networks vulnerable to inadvertent injury is on the order of 20-300 micrometers [35], [36], 81
Investigation of thin NM layer using MC models was performed for 0.2 and 0.5 mm NM layer models is shown in Figure 29 and 30, following identical methods as Figure 27 and 28 for thicker nerves. When the superficial NM layer setup was thin, both probes obtained more absorbance across all wavelengths from the underlying MM layer (Figure 29). Comparison of Probe 1 with Probe 2 (Figure 30) was similar to the results from the 1.0mm NM layer (Figure 28); when the NM layer was smaller than 1 mm, Probe 1 could obtain approximately 4% more signals from NM layer across the full EWDRS range in comparison with probe 2. Overall, based on the comparison of both total and layered absorbance, Probe 1 measurements had slightly more contributions from thin NM layers (< 1 mm) than Probe 2.

Figure 29. Total and layer-dependent simulated absorbance from thin NM layers. Absorbance of 0.2 mm NM layer for probe 1 (a) and probe 2 (b). Absorbance of 0.5 mm NM layer for Probe 1 (a) and Probe 2 (b).
Figure 30. Difference in percent simulated absorbance from thin NM layers for Probes 1 and 2. Percent total absorbance of NM layer $A_{NM}^{\%}$ for both probes, when NM layer is 0.2 mm (a) and 0.5 mm (b). The differences between two probes $\Delta_{\%A}$ of 2-layer model for 0.2 mm and 0.5 mm layers. Standard Deviation of measurements is shown in all plots, but only perceivable in (c).

4.4 Discussion

Prior reports indicate the promise of EWDRS for detection of nerves based on the development of spectral analysis algorithms developed from empirical measurements collected from different sets of measurement scenarios, using different parameters [112], [105], [119], [120]. However, it is unclear how differences in probe designs or different tissue geometries may influence measured signals and thus influence the generalization of the findings. Computational modeling in a variety of other optical spectroscopies has proven valuable in investigation of probe configuration and sample geometry/composition influence on simulated measurements [135], [138], [139]. In this manuscript, a model-based system including optical phantoms and MC simulations of EWDRS was utilized to demonstrate the similar utility in EWDRS measurements of NM tissues.
Prior studies of model-based analysis of EWDRS performed by our group demonstrated agreement between modeled and measured spectra and development of tissue mimicking EWDRS phantoms for in silico analyses [169]. Here, we demonstrate the relative agreement in measurements (in normalized diffuse reflectance absorbance) and MC simulations on homogeneous phantom/model, and 2-layer tissue mimicking phantoms. Agreement between simple measurement and MC simulations is valuable in confirming that computational simulations are configured correctly, fiber probe fabrication and function generally meets expected design parameters, and optical phantoms composition and structure are in line with expectations. Residuals in the spectral correlation between simulation and measurement occurred to some extent and can arise from a variety of factors. Differences can be due to probes fabrication mismatch with physical design, empirical variability in phantom composition, artifacts in optical property measurements, artifacts from the stitching spectra from the VIS/NIR and SWIR spectrometers near 1000 nm, and low signal quality at the upper spectral limit of the spectrometer above 1450 nm. All of these contribute to mismatch between simulations and measurements; however, the average DR and absorbance spectra for both probes between simulations and measurements were found to be in excellent agreement ($R^2 > 0.93$).

General agreement between measured and simulated spectra enables more detailed analyses, including investigation of the influences tissue makeup and fiber-probe configuration can have on EWDRS measurements. In previous studies across a range of spectroscopies, the depth dependency of a probe was the primary focus, and several factors were investigated, such as fiber tip angle, illumination/collection geometry and SD distance, which could influence the detection performance of probes, especially the
sampling volume [139], [141], [149]. The majority of MC reports suggested that SD distance could affect the depth-dependency of the interrogation [142], [143], however, it is unclear how other factors affect the detection performance of probes. In this study, two fiber probes were developed with the same mean SD distance, but different configurations. Probe 1 contained a central illumination fiber surrounded by collection fibers, while Probe 2 contained central collection fibers with smaller diameters and NA than Probe 1 collection fiber, surrounded by illumination fibers. These differences were small, however both empirical and simulated measurements confirmed differences in the total absorbance. In general, the depth-dependency differences between the two probes are subtle but observable in both empirical and simulated measurements. Empirical measurements can provide limited insight into the specific tissue label, however computational modeling provides the potential approach to investigate these scenarios which is unavailable in empirical studies. A critically important finding from this work was that Probe 1 produced measurements with roughly 4% greater contribution from superficial layers than Probe 2, which is useful in considering probe selection in future studies.

NVB identification can be challenging in minimally invasive surgeries, and even if nerve resembling structures are superficial, they can be difficult to identify with specificity. Common scenarios that appeared during surgeries are (1) NVB on top of tissues and (2) NVB underlying a thin tissue layer, and the variety of these scenarios can be difficult to investigate empirically. Here, both optical phantom measurements and MC simulations investigate differences in signal depth dependent contribution between the probes under the first common surgical scenario. Here focused on evaluating the nerve/NVB layer via single lipid-related peak ratio $R_{\text{lipid-related}}$. While many other peak ratios have been
reported and utilized in evaluating nerve/NVB [95], [118]–[120], the methods developed in this study provide a framework for more detailed analyses of model-informed contributions of different tissue types to spectral parameters used for tissue analysis in an objective, quantitative fashion.

The model-based analysis provided unique insight into individual information from specific tissue types, unavailable in empirical measurements. Whereas prior studies have focused on the penetration-depth of an optical measurements, as defined as the 1/e intensity value of the normalized attenuation profile vs. depth [173], a particularly important development reported in this manuscript is the demonstration of how MCX model outputs can be used to gain insight into the relative contribution from different tissue types to simulated measurements. Analytical development of label-specific absorbance, $A^{\text{m}}$ is a generalized concept that can be translated to different simulations of empirical measurements of DR and provided insight for measurement comparison. Here, the percent total absorbance of NM layer $A^{\text{NM}}\%$ and the difference of percent total absorbance between two probes $\Delta\%$ was also calculated, straightforwardly presenting the subtle difference in detected performance. These parameters provided a valuable framework to evaluate probe sensitivity for tissue features, which could be applied to future MC studies.

Overall, the reported model-based characterization platform of EWDRS probes allowed further comparison among different probe configurations, such as different tip angles or more complex illumination/collection geometries. In this study, we focused on investigation of NVB identification using the developed platform, which was designed to evaluate probes in different tissue types. Benefits of MC modeling provided theoretical
insights including layered information, as well as theoretical travel map of detected photons, which was unavailable from empirical measurements.

4.5 Conclusion

A model-based characterization platform for fiber-optic EWDRS has been evaluated through comparison with empirical measurements from single layer and 2-layer models. Two EWDRS probes with different illumination/collection geometry configurations were investigated and showed good agreement ($R^2 > 0.93$) of DR and absorbance spectra between simulations and measurements for both probes. The layer-specific information obtained from MC model provided an opportunity to analyze the percentage contribution from the specific sample layers in each probe. Moreover, comparisons between the two probes suggested that Probe 1 benefited more in collecting signals from superficial NM layer than Probe 2 when the top layer was less than 1 mm. Overall, these results support further model-based analyses of EWDRS fiber-optic measurement scenarios that may be challenging to accomplish via empirical measurements.
CHAPTER

5. AIM 3: PREPARE EWDRS SYSTEM FOR LAPAROSCOPIC USE

Laparoscopy is designed for applications on abdominal and pelvic cavities, where the NVB system is more compact. The previous EWDRS applications were only reported on open surgeries, and applications in laparoscopy have yet to be investigated. The last aim prepared an EWDRS system for laparoscopic procedure. The variance of two developed probes was characterized and EWDRS devices were packaged into a carry-on case for short distance transportation.

5.1 Introduction

Laparoscopy is a minimally invasive surgical procedure done with several small incisions using special laparoscopic instruments and devices, which provides an opportunity for the application of fiber optic probes to improve the surgery. Laparoscopy is designed to target the abdominal and pelvic organs, such as the uterus and prostate. Prostate cancer and uterine cancer are common diseases for people over 50 [6], [183]–[185]. Since the development of laparoscopic procedures, applications in pelvic organs have been commonly used. However, nerve injury is a common complication that requires additional surgical guidance in laparoscopy to identify nerves and NVBs. Previous EWDRS has been reported in several applications, including cancer diagnosis and surgical guidance, to differentiate nerves with improved classification performance in open surgeries. However, EWDRS has not been applied in laparoscopy.

To obtain high-quality EWDRS measurements in laparoscopy, there are several uncertain parameters and steps that need to be resolved before EWDRS can be integrated into laparoscopic procedures, such as how to package the EWDRS system and the variance.
of measurements during surgeries. In a laboratory environment, the EWDRS system was fixed on the optical table, acting as a vibration control platform to reduce vibrational noise and remain stable over time [186]. The weight of the optical table results in an inconvenient system in terms of transportation. Therefore, the entire EWDRS system must be repackaged for surgical use; unfortunately, there are no previous clinical EWDRS applications that have been reported. In addition, as of yet there is no reported literature investigating the placement variance of EWDRS probes. When the probe measures the surface of tissues, there is a wide range of probe-to-tissue contact geometries. The idea of probe-to-tissue geometry is that the probe is perpendicular to the tissue with light pressure. However, the actual probe-to-tissue geometry is hard to control, leading to the variability of measurements, which was not investigated in prior EWDRS reports.

Hence, in the last aim, we prepared the EWDRS system for laparoscopic application, including characterizing the placement variance of two probes developed in Aim 2 and developed an EWDRS packaging system for short-distance transportation.

5.2 General Approach

In this aim, we developed a hemisphere phantom to investigate the placement variance of both probes for different probe-to-tissue geometries and package the EWDRS system for future laparoscopic use.

5.2.1 Hemisphere Phantom

The single-layer phantom is utilized to validate the variance of measurement from various contact geometries, which has been reported by Pinto et al. [158]. The phantom
was a hemisphere with a flat surface on the top, and was created using a mixture of water, 2% homogenized cow's milk, and water to milk ratio of 3:1.

![Figure 31. Hemispheroid single layer phantom.](image)

This phantom having an arc surface with different angles was designed to mimic the various geometry of tissues that are encountered during surgery. Two phantoms were developed and placed on a flat plane at room temperature. At flat measurement location, five repeated measurements were collected. On the arced surface, two repeated measurements were collected at each location with various probe-to-tissue angles. The acquisition time of the probe was 50 ms in the VIS range and 800 ms in the NIR range. During the measurement, both probes were held by hand perpendicular to the surface of each location with light pressure. A total of 2 phantoms were analyzed for 15 measured spectra.

### 5.3 Results

The last aim was to investigate the fitness of the EWDRS system into a laparoscopic procedure. The performance variance of two developed probes were characterized by a
hemisphere phantom and fused as a baseline to evaluate the data collected during laparoscopy. All EWDRS devices were packaged into a carry-on case for short distance transportation between lab and surgical room. The fiber optic EWDRS system will combine with the laparoscopic instruments to evaluate the feasibility of our system in surgeries, including the background & reference measurement, noise level, integration time and probe feasibility into a laparoscopic device, as well as the probe variability during the surgery.

5.3.1 Characterization Of Placement Variance Of EWDRS Probes

The normalized spectra of the two probes and the standard deviation of each probe for the entire EWDRS wavelength on the arced and flat surface of the hemisphere phantom are shown in Figure 32. The average normalized spectra of the flat and arced surface of both probes had closed spectral line shapes with subtle differences in intensity, and the variance between the arced and flat surfaces of both probes was slight. In general, the difference of two probes on the arced surface was larger than that on a flat surface. However, the standard deviation of the entire EWDRS range was less than 2%.
Figure 32. The placement variance of two probes. a) The average normalized spectra of probe 1 collected from flat and arced surface. b) The average standard deviation of probe 1 across full EWDRS wavelength range measured on different probe-to-tissue geometry. c) The average normalized spectra of probe 2 collected from flat and arced surface. d) The average standard deviation of probe 2 across full EWDRS wavelength range measured on different probe-to-tissue geometry.

5.3.2 Package Of EWDRS System For Transportation

In the lab, the EWDRS instruments such as spectrometers and light source are fixed on the optical table to avoid unexpected vibrations from the building. The entire optical table is not available for transportation; thus, we removed the system from the optical table to a small metal broad and fixed devices on them. Then, we packaged the EWDRS system into a carryon size case and fitted all chargers, as well as connected cable into the case. The entire system was protected by customized foams, shown in Figure 33.
Figure 33. Example of package of EWDRS system, including light source and two spectrometers. a) the travel case was separated into two layers and the bottom layer contained light source and spectrometers. b) the top layer included chargers of instruments.

5.4 Discussion

In the last aim, we first developed a hemisphere phantom to characterize the placement variance of two probes, which indicated that the variance collected on the arced surface was more significant than that on the flat surface. However, the overall variance of the spectra obtained by both probes were less than 2%. The developed half-ball phantom mimicked simple situations with different curvatures; however, the probe-to-tissue geometries could be more complicated during the clinical scenarios. Thus, further investigation of the placement variance of probes could extend to a sharper radian of the arced surface. The carry-on package of the EWDRS system developed with this aim contained a light source and two spectrometers. However, due to spatial limitations, two EWDRS probes and the laptop were not included in the package, which requires an extra package to protect these devices. This aim is still ongoing, and the next step will investigate the EWDRS system for laparoscopic applications, including the development of customized EWDRS software and an investigation into the practicality of laparoscopic
EWDRS. The first laparoscopic feasibility study is scheduled for May 2022 at Temple University Lewis Katz School of Medicine.

5.5 Conclusion

In this research, the EWDRS system was prepared for laparoscopic utilization. A hemisphere phantom was developed to mimic both flat and arced surfaces to evaluate the variance of two EWDRS probes. It verified that the variance of the two probes was subtle (< 2%). We demonstrated a platform for the investigation of the various probe-to-tissue geometries. The EWDRS system has been packaged into a carry-on size case for short-distance travel, ready for laparoscopic application. Overall, these results supported that the EWDRS system is available for further laparoscopic application.
CHAPTER

6. CONCLUSION AND FUTURE DIRECTIONS

In general, this dissertation aided in the characterization of EWDRS systems using various models, bridging gaps between previous EWDRS applications and the future development of EWDRS in complex scenarios. In addition, this study utilized computational modeling and optical phantoms to characterize EWDRS fiber optic probes with different configurations. Finally, the EWDRS system was packaged into a carry-on case for practical laparoscopic use. Overall, this work addressed several critical characterization platforms of the EWDRS system and fiber optic probes in nerve identification and prepared for a transition to actual laparoscopic surgeries.

In chapter 3, a simple ex vivo microsurgical chicken thigh model was dissected to expose NVB and nerve, as well as surrounding tissues, which was used to verify the ability of the EWDRS system for nerve identification. A non-biological 2-layer optical phantom was developed to mimic nerve and muscle tissues, which successfully simulated significant chromophore features across VIS-NIR regions. The MC modeling simulated DR spectra based on the optical properties of chicken tissues and optical phantoms. A comparison between the modeling and measurements indicated substantial agreement between the computational modeling and the empirical measurements. This study further developed a characterization platform for EWDRS to investigate the agreement between Monte Carlo models of fiber optic EWDRS in both optical phantoms and dissected tissues. These results provided a basis for further utilization of model systems for developing EWDRS applications, such as the preliminary investigation of the depth-dependence of signal
changes in NVB identification shown here, along with the theoretical comparison of different EWDRS fiber-probe designs and more.

In chapter 4, two fiber optic probes were designed and fabricated to investigate the effects of different illumination/collection configurations with the same SD distance on depth-dependency of signal contribution from different tissue features and detection performance. Along with optical phantoms and MC modeling developed from the previous chapter, simulated DR spectra was generated and compared with measurements from optical phantoms to verify the functionality of the newly developed probe. The layered information provided from computational modeling benefits from theoretical insight, which is unavailable from empirical measurements. DR spectra undergoes exponential attenuation, resulting in comparison difficulty, thus DR spectra transferred to absorbance with linear summation. The total simulated absorbance was first compared with measurements to confirm that the absorbance was in agreement between simulation and measurement. After that, the layered absorbance was used to investigate the differences between probe 1 and probe 2, where we found that the central illumination probe (probe 1) was more sensitive to the thin NM layer (<1 mm) than the central collection probe (probe 2). In general, a computational modeling-based characterization platform for fiber-optic EWDRS was evaluated by empirical measurements from single layer and 2-layer models. Analysis of absorbance spectra supported the investigation of the depth-dependency performance of signal changes in NVB identification among different EWDRS fiber-optic probe designs, which could be limited via empirical measurements.

Chapter 5 developed a hemispheroidal phantom to characterize the effects on the measure spectra of variances in the placement variance of the probes on various tissue
geometries. The EWDRS system was packaged into a carry-on case for short-distance transportation. The last aim verified the variance of two probes on different surface curvatures and demonstrated a platform for characterizing differences in probe-to-tissue geometries. The EWDRS system is packaged and ready for laparoscopic applications in the summer.

Overall, this dissertation focused on using models to guide the characterization of EWDRS, using empirical measurements, such as ex vivo modeling and optical phantoms, as well as computational modeling- MC simulation. These results demonstrated that simulations were a powerful approach to investigate the further clinical characterization of EWDRS systems and fiber optic probes in different scenarios, especially in complicated clinical scenarios, which were hard to mimic by animal models and optical phantoms. Therefore, reported model-based characterization platforms could be used for theoretical analyses of the EWDRS system and various optic probes. In addition, the application of EWDRS allows for expanding the utilization in minimally invasive procedures, not only for laparoscopy to identify nerves/NVBs, but also for endoscopy to analyze multiclass tissues as well as in arthroscopy to differentiate bone and cartilage tissues.
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APPENDICES

A. SUPPLIMENTARY MATERIAL FOR AIM 1

Optical properties of biological tissues from different species can be relatively similar, due to the presence of identical chromophores and similarities in molecular composition. Figure 34 displays optical properties of chicken muscle and human gallbladder tissue, which include similar chromophores. For example, hemoglobin absorption features between 500 and 700 nm appears in both chicken and human tissues, as well as water and lipid features in NIR region. The reduced scattering coefficient of biological tissues decreases along the wavelength with a power law.

Figure 34. Example of optical properties of chicken and human tissue. a) Absorption coefficient of chicken muscle and human gallbladder across EWDRS region. There are differences in the intensity of absorption coefficient between chicken and human tissues. However, absorption chromophore features are similar: both tissues have a significant hemoglobin peak between 500 and 700 nm, as well as lipid peak around 1200 nm and water peak around 1400 nm. b) Reduced scattering coefficient of chicken muscle and human gallbladder, which has the same tendency.
SMLR can reduce spectral dimensionality while still retaining the ability to identify the wavelengths at which differences exist. Out of 2012 EWDRS data points for each chicken measurement, SMLR identified 180 spectral features that were used by the classifier. Out of these 180 features, 41 had feature importance higher than 0.4, shown in Figure 35. Spectral features of the highest importance include those related to blood (500 - 700 nm), water (around 980 nm & 1400 - 1500 nm), and lipid (1100 - 1300 nm).

![Figure 35](image)

Figure 35. Average spectra of chicken tissues with significant classification features. Vertical lines show spectral features with feature importance higher than 0.4, including major signatures of blood, water and lipid.
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B. ONGING WORK FOR AIM 3

Aim 3 is still ongoing, and several things are unknown in laparoscopic EWDRS, such as calibration steps, the integration time, and data analysis steps. To better investigate the practicality of laparoscopic EWDRS, we will first evaluate the EWDRS system, particularly fiber optic probes in laparoscopic devices. Then we will collect reference and background data in different situations in order to investigate the effect of ambient light used during surgery. The integration time will change for different samples and environments, which need to investigate the value range for laparoscopic conditions. The last part will analyze the background and noise level during the surgery.

1.1 Evaluation Of EWDRS System In Laparoscopic Devices

Optical measurements will be performed on the pelvic organs and NVB/nerve. Spectra will be measured through a laparoscopic probe inserted into a Visiport during laparoscopy. Two developed probes will be inserted into a 5-mm-ID Visiport before the surgery, analyzing the fitness of probes into the trocar and the operation inside the trocar. The monitoring camera will be utilized with an LED light to guarantee the correct measurement location and close contact with the target tissue. At each measurement location (organs and NVB/nerve), 5-8 repeated measurements will be collected from each tissue type from samples. The survey about user experience will be collected from surgeons after the operation to improve the design of probes.
1.2 Reference & Background Measurement

In *ex vivo* study, reference and background measurements were collected at the end with minimal ambient light, and the integration time was optimized during the measurements through examination of the effect on measured signals noise levels. In the case of laparoscopic use, we will collect the reference and background measurements in the cadaver specimens to optimize conditions for the measurement of tissue. Since laparoscopy has an LED light to monitor the surgery, we are going to collect reference and background spectra twice: 1) with LED light: in a high ambient light environment; 2) without LED light: in the dark environment as the lab environment. We will investigate how the LED light affects the spectra shape. These measurements will be used to specify conditions for future *in vivo* studies.

1.3 Integration Time & Averaging

The integration time and the number of averages needed for high signal-to-noise ratio (SNR) measurements may vary from *ex vivo* study and will be tested during the cadaver operations. Due to the lack of information, the optimal integration time during the surgery is unknown. Therefore, we are going to investigate the 1st sample: 5 different integration times for each spectrometer will be tried on two tissue types and 3 different times will be chosen then to apply on other samples. All integration times will be made in multiples of 3x to investigate the benefit of spectral ‘averaging’. Differences between spectra will be based on measured noise in resulting spectra.
1.4 Identification Of Additional Factors/noise Levels

The environment during the laparoscopy will be different from the lab, such as the ambient light and measurement times being obvious differences to consider. However, during the surgery, other sources of noise and complicated experimental conditions may appear, therefore, we will evaluate any other unpredictable aspects that may influence the full EWDRS spectra. If the noise level increases significantly, an extra noise reduction step will be applied on every spectrum.

Fiber-optic DRS is particularly well suited for incorporation with minimally invasive procedures. Fiber optic probes have previously been developed for needle biopsy, endoscopes, colonoscopes, however, published reports of EWDRS have only used general purpose fiber optic probes in open surgeries. Further development of the technique will require purpose-driven design and development of fiber optic probes and measurement protocols amenable to laparoscopic surgery. Generally, there are 4 parts that need to be evaluated: packaging of EWDRS system, calibration of the clinic data, experimental setup, and data analysis for surgical measurements. The ambient light is well controlled during the lab experiments, however, the environment in the laparoscopic room will have a stark difference: the ambient light will hard to control, which may influent the noise level; collection method of background and reference spectra may vary. During the laparoscopic surgery, a few things need to be tested, such as probe placement and integration time of each spectroscopy. Due to the changes of environment, data analysis process may vary from previous. Therefore, important preliminary EWDRS studies in laparoscopic configurations will be needed prior to future human subjects’ investigation.