A Diffuse Reflectance Spectroscopy
Instrument for use in the Optical Biopsy of Brain
Tumour Margins
A Diffuse Reflectance Spectroscopy Instrument for use in the Optical Biopsy of Brain Tumour Margins

by

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Abstract

Optical biopsy is a medical technique that uses light to perform non-invasive analysis of tissue *in-situ*. This technology has many applications in the medical profession, opening up exciting new possibilities for surgical guidance and diagnosis of malignancies and other conditions. Optical biopsy allows a medical professional to perform near instantaneous, real time analysis of tissue composition without the need to physically remove tissue from the body, as required in traditional biopsy.

A technique frequently used for this purpose is diffuse reflectance spectroscopy (DRS): collection and analysis of the spectrum of light reflected from a material. Another technique frequently used for optical biopsy is laser induced fluorescence spectroscopy (LIFS): analysis of the fluorescence spectrum returned by a material when illuminated at a specific wavelength.

This thesis discusses the design and construction of a spatially resolved DRS system intended for use in a dual modality DRS and time resolved LIFS optical biopsy instrument for clinical analysis of brain tissue. This instrument is specifically intended for use in the surgical removal of malignant gliomas: infiltrating tumours associated with a poor patient prognosis.

Theoretical simulation based studies were used to optimize the design of a compact, dual modality fibre optic probe for use in the system and a novel algorithm was developed to allow recovery of the optical properties of tissue from reflectance spectra obtained with this probe. This probe was manufactured and a corresponding spectrometer based system was created for the acquisition of diffuse reflectance spectra. Components
were designed to allow sterilization and thus clinical use in an operating room environment. A laboratory trial of this system demonstrated its range and ability to recover the optical properties of lipid emulsion optical phantoms.
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Chapter 1

Introduction

1.1 Optical Biopsy

Biomedical optics is a field that combines exciting new advances in optical technology with well-established knowledge of the interactions between light and tissue and applies this to the world of healthcare and medicine. This is often used for diagnostic purposes to help a healthcare professional visualize or obtain information about a volume of tissue, but can also be used for the treatment of certain conditions, using techniques such as photodynamic therapy.

Optical biopsy is a promising technique that uses light to non-invasively analyze a volume of tissue. By illuminating the tissue and analyzing the resultant reflectance or fluorescence, valuable diagnostic information can be obtained on its composition or unique characteristics. This is often used to distinguish malignant tissue from healthy tissue throughout the body. Optical biopsy offers advantages over traditional biopsy techniques, which require the surgical removal of a piece of tissue and subsequent processing and analysis by a trained pathologist. Optical biopsy results are obtained rapidly, since illumination, acquisition and processing can typically be performed within seconds; and the analysis can be performed in place, therefore eliminating the trauma associated with physical removal of tissue from the body.

Two technologies that are often used for optical biopsy are known as Diffuse Reflectance Spectroscopy (DRS) and Time Resolved Fluorescence Spectroscopy (TRFS).
While each technology can be used separately, they complement each other and combining them within one instrument opens up valuable new possibilities for analyzing data and improving the utility of the overall optical biopsy system (Muller, Georgakoudi, Zhang, Wu, & Feld, 2001).

A broad overview of diffuse reflectance and fluorescence spectroscopy techniques and brief introduction to the underlying theory is provided in this chapter. The three studies presented in each of the following chapters focus specifically on the development of the diffuse reflectance component for use in a combined DRS/TRFS instrument for optical biopsy of brain tissue.

1.2 Diffuse Reflectance Spectroscopy

The different biomedical optics techniques used for diagnostic purposes can be broadly categorized into two different groups: imaging, and spectroscopy. Imaging techniques allow the user to better visualize the structure and appearance of the tissue under investigation, either on a microscopic or macroscopic level, and generally provide qualitative information. Spectroscopic techniques, on the other hand, measure the amount of light that is collected at different wavelengths, and are able to provide the user with quantitative information that can be used to determine the biochemical or functional properties of the tissue (Boas, Pitris, & Ramanujam, 2011).

When illuminating a sample, a portion of the incident light will undergo specular reflection, the amount of which is determined by the angle of incidence and the refractive index of the material. The light that penetrates the surface is refracted and will travel some distance in the material, undergoing both scattering and absorption. Scattering
causes some of the light to return to the surface and emerge in random directions through a process known as diffuse reflection. By collecting and analyzing this diffusely reflected signal, the composition of the material can be inferred. Using a broadband light source to illuminate the material and a spectrometer to measure the reflectance allows one to obtain this data across a desired spectral range.

The diffusely reflected signal from tissue results from the interactions of light with a variety of different molecules and structures. Significant sources of scattering include the structural proteins keratin and collagen and cellular components such as mitochondria, lysosomes and membranes (McGee, Mirkovic, & Feld, 2011). Significant absorbers within the UV/visible spectrum include amino and nucleic acids, hemoglobin and various pigments (McGee et al., 2011).

A variety of diffuse reflectance measurement devices have been built for a wide variety of biomedical applications including the detection of epileptic brain tissue (Yadav et al., 2013), real-time monitoring of hypoxia in the brain (Kawauchi et al., 2013), diagnosis of skin cancers (Garcia-Uribe et al., 2012) and detection of the optical nerve during oral surgery (Stelzle et al., 2011). A feature of all of these examples, and the system discussed in this thesis is the use of optical fibres to guide light from an illuminator to the tissue, and collect the reflectance at one or more source-detector distances (SDD).

1.3 Optical Properties

The effect that a material has on light travelling through it is quantified by its optical properties: the degree to which the intensity of a beam of light is reduced due to
scattering and absorption. Two properties that are of interest when performing diffuse reflectance spectroscopy of tissue are the absorption coefficient $\mu_a$ and the scattering coefficient $\mu_s$. These describe the degree to which the intensity of a beam of light is reduced due to absorption or scattering as it passes through the tissue. If the transport coefficient, $\mu_t = \mu_s + \mu_a$ of the tissue is known, the total unscattered intensity of the beam after travelling a distance $x$ can be calculated using the Beer-Lambert law, Equation 1.1. $\mu_a$ or $\mu_s$ can be substituted for $\mu_t$ in this equation if one is only interested in absorption or scattering.

$$I(x) = I_0 e^{-\mu_t x}$$  
Equation 1.1

The above quantities are sufficient to determine the fraction of an initial collimated beam lost to scattering or absorption. To fully model diffuse reflectance in tissue, however, a more thorough description of scattering is required. This requires knowledge of the phase function: a function describing the expected angular distribution of scattering, the angle between an individual photon’s initial trajectory and its trajectory after a single scattering event. Light in the optical region of the spectrum is not isotropic in tissue – photons are instead scattered in a forward directed manner, described by models such as the Henyey Greenstein phase function or Mie theory. The Henyey-Greenstein phase function, Equation 1.2, is commonly used to describe photon transport in tissue (Binzoni, Leung, Gandjbakhche, Rufenacht, & Delpy, 2006). This function depends on a single variable: $g$, the mean cosine of the scattering angle, also known as the anisotropy coefficient (Patterson, Wilson, & Wyman, 1991). Knowledge of the three optical properties $\mu_s$, $\mu_a$ and $g$ is sufficient to develop a basic model of light transport.
through tissue and predict the diffuse reflectance distribution created by an incident light source. All of these properties are wavelength-dependent.

$$P_{HG}(\cos \theta) = \frac{1}{2} \frac{1 - g^2}{(1 + g^2 - 2g \cos \theta)^2}$$  \hspace{1cm} \text{Equation 1.2}

Another optical property commonly referred to during DRS is the reduced scattering coefficient, $\mu_s'$. This is a combination of the scattering coefficient and anisotropy factor, given by Equation 1.3. This provides an approximation of the scattering behaviour of light based on isotropic scattering (Farrell, Patterson, & Wilson, 1992). The inverse of this quantity is the transport scattering mean free path: the average depth a photon penetrates before its orientation can be considered isotropic.

$$\mu_s' = \mu_s (1 - g)$$  \hspace{1cm} \text{Equation 1.3}

A number of different mathematical models can be used to describe the relationship between the optical properties of a material and photon transport. These can be used to model the expected diffuse reflectance given a material’s optical properties, or inversely to determine its optical properties from measurements of diffuse reflectance. The models discussed below have unique advantages or disadvantages, and are used in different situations.

1.4  \hspace{1cm} \textbf{Numerical Approaches for Light Transport in Turbid Media}

1.4.1  \hspace{1cm} \textbf{Mie Theory}

Maxwell’s equations are a set of partial differential equations that describe electric and magnetic fields and how they are altered by local charges and currents. Given
appropriate boundary and initial conditions, solutions to these equations can be found that describe the propagation of electromagnetic waves (light) through space. Mie scattering (or Mie theory) is a particular solution to these equations that describes the scattering of light by a sphere (Wriedt, 2012).

When the diameter of a sphere is much smaller than the wavelength of the incident light, the electric field is approximately constant across its surface and the Rayleigh scattering approximation can be used to describe the propagation of light interacting with (scattered by) the sphere. Alternately, if the diameter of the sphere is much larger than the wavelength of incident light, interactions can be described using geometrical optics and Fresnel refraction/reflection. A method known as anomalous diffraction can be used if the refractive index is close to that of its surroundings (van de Hulst, 1981). Because of their relative simplicity, these approaches are used in place of Mie theory for very small or large spheres. Mie theory therefore typically refers only to the scattering of light from spheres of a size comparable to the wavelength.

In practical applications, the Mie solutions can be used to describe the scattering of light in a medium consisting of many identical regularly shaped particles (Avanaki, Podoleanu, Price, Corr, & Hojjatoleslami, 2013). These solutions take the form of an infinite series which can be solved using numerical methods and have been used successfully to describe the propagation of light through tissue. Saidi et al. (1995) modelled the scattering of light from collagen fibres in neonatal skin by treating the fibres as a collection of identically shaped cylindrical objects and performing Mie theory calculations. Schmitt & Kumar (1998) used Mie theory to calculate optical parameters
from a theoretical distribution of spherical particles derived from a model of microscopic variations in refractive index caused by the many different organelles in soft tissue.

More commonly, Mie theory is used indirectly in biomedical optics to model the reflectance of tissue-simulating phantoms. These are custom made materials or mixtures that have scattering and absorption properties similar to those expected in tissue. Polystyrene microspheres, mixtures of equally sized microscopic spheres are a popular scattering medium used in the creation of optical phantoms. Mie theory is especially effective for predicting the scattering properties of these phantoms. Mie theory has been successfully used either on its own to model the bulk reflectance from a scattering medium as described by Gordon (1985) or to approximate the phase function of a material for Monte Carlo modelling (Mourant, Boyer, Hielscher, & Bigio, 1996).

While Mie theory offers a method for directly calculating the results of light scattering by particles, practical models of light interaction with tissue usually take a broader approach and deal with the overall transport of light through a turbid medium through use of diffusion theory or Monte Carlo modelling.

1.4.2 Diffusion Theory

If the interactions of unpolarised photons with a turbid medium are limited to absorption or elastic scattering, as is the case for light in the visible and near visible spectrum, then the transport of these photons can be described by the Boltzmann radiative transfer equation (Keijzer, Star, & Storchi, 1988). If scattering is the predominant form of interaction ($\mu_s \gg \mu_a$), then this equation can be simplified to the diffusion equation, Equation 1.4 (Farrell et al., 1992). $D$ is the diffusion constant given by Equation 1.5, and
\( S_0 \) and \( S_1 \) are spherical harmonics of order zero and one, representing photon sources within the medium.

\[
\nabla^2 \psi(r) - \frac{\mu_a}{D} \psi(r) = -\frac{S_0(r)}{D} + 3 \nabla \cdot S_1(r)
\]

Equation 1.4

\[
D = \frac{1}{3(\mu_a + \mu_s')}
\]

Equation 1.5

Solutions to the diffusion equation can be obtained for simple geometries if appropriate source terms and boundary conditions are used. A common measurement geometry that provides a simplified, one dimensional solution is the following.

Photons enter in an infinitely narrow, forward directed beam (a pencil beam).

The medium is homogeneous and semi-infinite: bounded at the surface by a plane perpendicular to the beam but extending infinitely in all other directions.

In this situation, the total reflectance can be expressed in a closed form solution that depends only on \( \mu_a, \mu_s' \) and the relative index of refraction of the material and its surroundings, usually air (Farrell et al., 1992).

One issue encountered when applying diffusion theory is that it breaks down for source-detector separations less than approximately one mean free path (Hull & Foster, 2001). Several groups have proposed other approximations to the Boltzmann equation described earlier that allow its use at shorter SDDs and potentially lower values of \( \mu_s' \). Bevilacqua et al. (1999) suggest that considering the second moment of the phase function allows for more accurate determination of the reflectance at short SDDs. Hull & Foster (2001) take a different approach, and consider higher order Legendre polynomials when approximating the Boltzmann transport equation. This allows for more accurate
modelling of anisotropy in the light distribution, as observed in regions closer to the source.

1.4.3 Monte Carlo Modelling

The two approaches to modelling diffuse reflectance discussed thus far have been mathematical models that can be used to calculate the light distribution given specific assumptions and limitations on the geometry or material composition. An alternative to these approaches is computer modelling of photon transport by Monte Carlo simulation. This powerful tool allows great flexibility in terms of the geometry being studied, and can be applied over a wide range of optical properties. The trade-off of this method, however, is increased computation time. Monte Carlo modelling takes a probabilistic approach to photon transport by simulating large numbers of photons individually as they travel through tissue. Each simulated photon is tracked as it travels from its source until it is either absorbed or it exits the tissue. In this way, a map can be obtained showing the exact density of photons exiting each region of tissue. Because simulating each individual photon in this matter would be impractical, groups of photons (photon ‘bundles’) are used instead: this variance reduction technique is known as survival weighting (Patterson et al., 1991).

The typical approach used for Monte Carlo modelling of photon transport is described in detail by Wang, Jacques & Zheng (1995); the basics of which are summarized as follows. Each photon is injected into the medium with an initial weight of one, and an orientation determined by the initial conditions of the simulation. The bundle is allowed to travel a random distance (one ‘step’) given by Equation 1.6, derived from
the Beer-Lambert law. $\xi$ is a random number drawn from a uniform probability distribution on the interval (0,1).

$$d = \frac{-\ln(\xi)}{\mu_t}$$  \hspace{1cm} \text{Equation 1.6}

After each step, the weight of the photon is reduced by a factor given by the albedo of the medium: the ratio of $\mu_a$ to $\mu_t$. A new direction is chosen from the probability distribution described by the scatter phase function, and the photon travels a step in this new orientation. This process continues until the photon’s weight becomes so low that it is no longer worth tracking (the designer sets this threshold based on the conditions or requirements of the simulation) or it exits the medium. A roulette technique can be used to determine when to stop tracking the photon: when its weight falls below a given threshold, the photon is given a one in x chance of surviving. If it survives this process, the weight of the photon is increased by a factor of $x^{-1}$. In this manner the overall energy (total weight of all photons) is conserved on average.

Despite being more flexible and requiring fewer assumptions than methods such as diffusion theory, Monte Carlo modelling is not exact, and still requires some simplifications regarding the properties of the tissue. A common assumption is the choice of phase function. Because it represents the scattering process using a closed form equation, the Henyey-Greenstein phase function is frequently used. Although this has been shown to closely mimic the scattering properties of tissue, there are limits to its accuracy and the detection geometries with which it can be used (Mourant et al., 1996). To further reduce the computation time required, many groups have assumed an idealized
geometry including a semi-infinite medium and pencil beam source of photons (Wang et al., 1995). This topic, and the inversion of the Monte Carlo model to recover optical properties are discussed in more detail in the paper presented in Chapter 2.

Different groups have proposed methods to reduce the time required to run Monte Carlo computations. One particular method that will be discussed in detail in Chapter 2 is the use of Monte Carlo simulations to generate a look-up table (LUT) of the reflectance expected for a wide range of optical properties. Although each simulation takes a long time to run, this only has to be done once, ahead of time, to generate the table.

1.5 Measurement Techniques

Different types of devices have been developed to measure the diffuse reflectance spectrum of a sample and determine its optical properties. These can be broadly categorized into time and frequency domain techniques, or spatially resolved techniques. A general overview of these methods is provided in Chapter 2 with an emphasis on spatially resolved techniques. The basic principle behind all these methods is the use of one of the previously discussed models (diffusion theory, Mie scattering or Monte Carlo) to fit the modelled (theoretical) reflectance to data measured from a sample. The optical properties that produce the best fit of the modelled data to the experimental data are accepted as the optical properties of the sample.

1.6 Tissue-Simulating Phantoms

When studying optical properties or developing an instrument for optical analysis of tissue, a necessary step is the construction of an optical phantom: a synthetic object with optical properties similar to those of tissue. These tissue simulating phantoms can be
solid or liquid, depending on the required application. Often the phantom will contain both a scattering material and an absorbing material. This allows one to control the scattering and absorption properties independently by adjusting the concentration of each component. These phantoms are essential in optical studies of biological tissues, as they allow the researcher to control the parameters of the experiment and independently test the desired variables.

Liquid phantoms are the most widely used in optical imaging or spectroscopy studies (Pogue & Patterson, 2006). This is because they are very easy and quick to produce, and the properties can be adjusted as needed by adding more of the appropriate material. Lipid emulsions and India ink are often used as the scattering and absorbing components, respectively (Pogue & Patterson, 2006) and will be discussed in much further detail in chapter 4. Alternate scattering materials include titanium dioxide (TiO$_2$) and polystyrene microspheres. Alternate absorbers include molecular dyes such as Evans Blue (van Staveren, Moes, van Marie, Prahl, & van Gemert, 1991). These can be used to produce specific spectral features, since they exhibit strong absorption ‘peaks’ at specific wavelengths. These dyes often fluoresce, however, re-emitting absorbed light at a different wavelength. This can prove advantageous if one is interested in studying fluorescence effects, but is undesirable if one is only interested in the reflectance.

Solid phantoms with optical properties similar to tissue are also used in optical spectroscopy studies (Avanaki et al., 2013; Pekar & Patterson, 2010; Pogue & Patterson, 2006). Because they can be machined to a desired shape, these phantoms are particularly useful in applications that require geometries other than a semi-infinite medium.
Additionally, the mechanical properties of these phantoms can be made to mimic those of tissue. An example of this is given by Bays et al. (1997), who use a silicone based phantom to produce a three-dimensional model of the bronchial tree. Common scattering materials include metal oxides or polystyrene microspheres. As with liquid phantoms, ink or dyes (molecular absorbers) are often used as the absorber (Pogue & Patterson, 2006). These scattering and absorbing additives are mixed with a host material, often an epoxy resin or polymer that hardens to produce a final, solid phantom.

### 1.7 Time-Resolved Fluorescence Spectroscopy

Another technique that has shown great promise for optical biopsy applications is fluorescence spectroscopy. When a molecule interacts with an incoming photon, one of three basic phenomena can occur: the molecule can scatter the photon in a new direction, leaving the energy unchanged; the molecule can absorb the energy; or, the molecule can return to its ground state through the fluorescence process. In the case of fluorescence, the excited molecule first converts a portion of its energy to heat (internal conversion through molecular vibration), then returns to its ground state by emitting a new, fluorescent photon. Because some of its initial energy was absorbed by the molecule, the emitted photon has a longer wavelength than the initial excitation photon. This process is illustrated in the Jablonski diagram of Figure 1.1. Note that more complex processes not discussed here can also occur, including phosphorescence, Raman scattering or triplet production.
Figure 1.1: A Jablonski diagram illustrating the process of fluorescence. A photon is absorbed, raising the energy of the molecule to a higher state. A portion of this energy is converted to heat through molecular vibration. The molecule then returns to the ground state by emitting the remaining energy as a fluorescent photon.

Fluorescence imaging or analysis is performed by illuminating a sample with light at the target molecule’s excitation wavelength and collecting the emitted light. Light at the illumination wavelength is filtered out, leaving only the fluorescent signal. If intended for imaging purposes, a fluorescent dye or other molecule ‘tagged’ with a fluorophore is often injected into the body, allowing one to view the areas in which it concentrates using specialized goggles or instrumentation. This approach has been used for intraoperative imaging throughout the body, with clinical applications including treatment of malignant gliomas, sentinel lymph node mapping, liver resection and angiography (Schaafsma et al., 2011). Fluorescent dyes used for these purposes include Indocyanine Green and 5-Aminolevulinic Acid (W. Stummer et al., 2000).

Injection of an external fluorophore allows for imaging or determination of physiological or metabolic information from the quantity or rate of uptake. It does not,
however, provide direct information on the composition of tissue. Certain naturally occurring biological molecules are known to fluoresce when illuminated at near-UV wavelengths in a process known as autofluorescence. Examples include components of connective tissue: collagen and elastin; and molecules essential to metabolic processes: NADH and FADH. The presence of these components can be detected by illuminating an area of interest with the appropriate wavelength and analyzing the resultant fluorescence spectrum. This technique is known as laser-induced fluorescence spectroscopy (LIFS).

As a tumour develops, the tissue undergoes many changes to its biochemical and morphological characteristics. Tissue near the centre of a tumour often becomes deprived of oxygen, which changes the redox state of NADH and alters its fluorescent properties (Georgakoudi et al., 2002). The amount of collagen rich connective tissue also changes, with the amount of collagen increasing significantly in glioblastoma multiforme (Mammoto et al., 2013) and breast cancer (Luparello, 2013). Detection of these changes using LIFS at near-UV wavelengths (330 – 370 nm) has proven to be an effective tool for identifying malignant tissue and distinguishing tumours or tumour margins from their surroundings. This type of approach has been investigated in a clinical setting for the diagnosis of many different types of cancers, including those of the brain (Butte et al., 2010), bladder (Schäfauer et al., 2013) and oral tissue (Meier et al., 2010).

This technique can be further refined by adopting a time-resolved approach. The fluorescence signal is collected discretely or continuously over a short period of time following illumination. This allows observation of the decay of the signal and calculation of the lifetime: the average time that the molecule remains in the excited state before
emitting a fluorescent photon. Because the average fluorescent lifetime of a molecule depends on its chemical composition, this technique provides a second source of contrast for the identification of specific fluorophores in a sample. The addition of time-resolved capabilities to an LIFS system can prove especially beneficial when the emission spectra of distinct fluorophores overlap and are therefore difficult to distinguish.

1.8 Intrinsic Fluorescence Spectroscopy

When performing fluorescence spectroscopy on tissue, the signal received by the detector is not the raw (intrinsic) fluorescence signal emitted directly by the fluorophore. The photons emitted during the fluorescence process have been scattered and absorbed while passing through optically thick tissue (Wu, Feld, & Rava, 1993). This distortion can be modelled and compensated for if the optical properties of the tissue are known. Integration of DRS for optical property recovery together with LIFS can therefore allow for analysis of the intrinsic fluorescence spectrum from tissue.

Muller et al. (2001) use photon migration theory to develop a mathematical expression relating the intrinsic fluorescence to the measured fluorescence and diffuse reflectance at a given wavelength. The model’s ability to recover intrinsic fluorescence is demonstrated on liquid phantoms containing a water soluble dye mixed with polystyrene microspheres and powdered hemoglobin. An advantage to this approach is that the entire model is reduced to a single closed form mathematical expression, making it relatively simple to apply in the analysis of optical biopsy data. This requires, however that the fluorescence and reflectance are emitted and collected in the same configuration.
1.9 Clinical Problem

An area of medicine that can benefit greatly from the use of optical biopsy is the surgical treatment of malignant gliomas. Despite regular improvements in treatment options, these locally invasive tumours are associated with a poor prognosis, especially for patients presenting with high grade gliomas (Walter Stummer et al., 2006). Depending on the tumour type, standard protocol is to surgically remove the tumour and to follow with chemotherapy and/or radiation therapy (Baehring & Piepmeier, 2006). Because multiple studies have linked the extent of tumour resection to the probability of patient survival (Lacroix et al., 2001; McGirt et al., 2009; Walter Stummer et al., 2008; Yamaguchi et al., 2012), it is essential that a surgeon is able to identify malignant tissue in the surgical cavity and remove as much as possible.

Current practice frequently involves extensive pre-operative imaging to identify tumour volumes and plan a strategy for removal (Inoue, Ogasawara, Beppu, Ogawa, & Kabasawa, 2005; Roux, Berger, Wang, Mack, & Ojemann, 1992). Once the surgery has begun, however, the procedure is highly dependent on the surgeon’s ability to visually identify malignant tissue. This is especially true after the primary tumour volume has been removed and the surgeon is working in the margins of the tumour, an area in which healthy and malignant tissue coexist and often blend together. This blending of tissue makes it very difficult to identify where the tumour fully ends, thus making the decision on where to halt resection somewhat subjective. The need to visually distinguish tumour from healthy tissue within the margins makes the entire process highly dependent on the surgeon’s previous experience.
An optical biopsy instrument can be used to improve this process, by allowing the surgeon to scan the cavity after the primary tumour volume has been removed, and identify any residual malignant tissue. The use of this instrument has the potential to remove some of the subjectivity from the surgical process, allowing the surgeon to make informed decisions on how to proceed with the resection. This could prove especially advantageous during the surgical treatment of glioblastoma, which are highly diffuse and infiltrative tumours (Zong, Verhaak, & Canoll, 2012). Such tumours have extensive, difficult to identify margins, a feature which makes full resection difficult to achieve.

This thesis forms a part of a larger overall project: the design and development of an optical biopsy system to be used for guidance during surgical resection of gliomas. The system is to consist of two components: a DRS system for reflectance analysis and recovery of tissue optical properties and a TR-LIFS system for fluorescence analysis. Separately, each component is able to obtain diagnostic information: DRS systems can detect changes in hemoglobin or other chromophore levels while TR-LIFS systems can detect changes in collagen levels and/or metabolic activity. Combining these two systems into one instrument not only increases the number of sources of contrast available for identifying malignancies versus healthy tissue, but also opens up the potential for recovery of intrinsic fluorescence characteristics from the combined raw data.

1.10 Thesis Objective and Organization

The objective of this thesis was the development of a diffuse reflectance component for use in this optical biopsy instrument. Specific objectives included:
1. Design and produce a combined, compact fibre-optic probe for acquisition of both DRS and TR-LIFS measurements with a fibre layout optimized for optical property recovery in brain tissue.

2. Design and produce a standalone DRS system able to acquire reflectance spectra from brain tissue.

3. Develop an algorithm capable of recovering the optical properties expected in human brain tissue from reflectance spectra acquired by this system.

4. Test this equipment and algorithm on tissue-simulating optical phantoms, comparing results to those from a well-established reference steady state DRS system used in the lab.

Chapter 2 is the author’s published work (Cappon, Farrell, Fang, & Hayward, 2013), reformatted to the style of this thesis:


This chapter discusses the design and construction of the integrated fibre-optic probe for acquisition of DRS and TR-LIFS signals. It also provides a detailed discussion on the development of a Monte Carlo based algorithm for the recovery of optical properties from spatially resolved diffuse reflectance spectra acquired using this probe.

Chapter 3 is the author’s work submitted to the Journal of Biomedical Optics:

This chapter is indirectly related to the development of the optical biopsy system described in this thesis, as it discusses improvements made to another diffuse reflectance instrument used in the lab. This system was used as a standard reference for all diffuse reflectance measurements performed in this thesis, and the improvements discussed in this chapter were considered necessary for its use in this project.

Chapter 4 is the author’s work submitted to the Journal of Biomedical Optics:


This chapter discusses the development of the standalone diffuse reflectance instrument and liquid phantoms used in this project. The hardware used is presented and the operation of the system was confirmed through measurements of the optical properties of phantoms made from mixtures of lipid emulsions and ink.

Chapter 5 summarizes the research presented in this thesis and discusses future direction for the project.

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Chapter 2

Paper I – Fiber-optic probe design and optical biopsy algorithm for optical biopsy of brain tissue

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2.1 Introduction to Paper I

This paper presents the design and development of a novel fibre optic probe to be used for optical biopsy of brain tissue. The diameter of the probe was minimized to allow ease of use in small surgical cavities, and an additional fibre was embedded in the probe to allow future use with a time resolved laser induced fluorescence system under development by our group. A Monte Carlo based algorithm designed for the recovery of optical properties from reflectance data collected with this probe is also presented.

The optical property recovery algorithm presented in this paper is designed to eliminate many of the simplifying assumptions inherent to the scalable single Monte Carlo simulation based algorithm used previously in our lab. A hybrid algorithm combining aspects of the single simulation approach with a look up table algorithm is developed and presented for use with the fibre optic probe.

The simulations, experiments and analysis presented in this paper were performed by Derek Cappon, under the co-supervision of Dr. Hayward, Dr. Farrell and Dr. Fang. The manuscript was written by Derek Cappon, and edited by Dr. Hayward and Dr. Farrell. The manuscript has been altered slightly to match the style of this thesis.
2.2 Abstract

Optical biopsy techniques offer a minimally invasive, real time alternative to traditional biopsy and pathology during tumour resection surgery. Diffuse reflectance spectroscopy (DRS) is a commonly used technique in optical biopsy. Optical property recovery from spatially resolved DRS data allows quantification of the scattering and absorption properties of tissue. Monte Carlo simulation methods were used to evaluate a unique fibre optic probe design for a DRS instrument to be used specifically for optical biopsy of the brain. The probe diameter was kept to a minimum to allow usage in small surgical cavities at least 1 cm in diameter. Simulations showed that the close proximity of fibres to the edge of the probe resulted in boundary effects from reflection of photons from the surrounding air-tissue interface. A novel algorithm for rapid optical property recovery was developed which accounts for this reflection and therefore overcomes these effects. The parameters of the algorithm were adjusted for use over the wide range of optical properties encountered in brain tissue and the performance when subjected to random noise was evaluated. This algorithm can be adapted to work with any probe geometry to allow optical property recovery in small surgical cavities.
Content of Paper I

2.3 Introduction

Intraoperative guidance of tumour resection surgery is an emerging technology that is being adopted in many different forms in operating rooms around the world (Davis, Hsu, Bouton, Wilhelmson, & Komenaka, 2011; Eljamel, Leese, & Moseley, 2009; J.-S. Wu et al., 2009). This has widespread applications, but can be especially important during the resection of glioblastomas: grade IV brain tumours with a poor prognosis. These infiltrating glial tumours originate from normal brain cells and have an appearance that is similar to the surrounding tissue (Oh & Black, 2005), especially in the margins, meaning that optimal resection is difficult to achieve. Studies have indicated that complete resection is beneficial to patients presenting with both low and high-grade gliomas (Stummer et al., 2008; van Veelen, Avezaat, Kros, van Putten, & Vecht, 1998).

Optical spectroscopy is a promising technique that has been investigated for intraoperative use. It allows rapid, minimally invasive analysis of the composition of tissue; a process commonly referred to as optical biopsy. Various optical biopsy modalities have been developed and investigated in different diseases, including cancers of the breast (Brown et al., 2009; Volynskaya et al., 2008), oral mucosa (de Veld et al., 2005; Schwarz et al., 2009) and brain (Toms et al., 2005). Fibre optic probes are typically used to both deliver light to the target tissue and collect the resulting optical signal for analysis. These spectroscopy devices offer a low cost alternative to more expensive imaging techniques such as intraoperative MRI (Oh & Black, 2005; J.-S. Wu et al., 2009) and can be designed so that they do not require the injection of exogenous fluorophores as
is often the case in intraoperative fluorescence imaging (Stummer et al., 2006; Valdes et al., 2011). Because the area of tissue investigated by these devices is limited, they are not suited for wide field applications but are ideal for localized investigations within small, well-defined areas such as surgical cavities.

Diffuse reflectance spectroscopy (DRS) is a technique that is often used during optical biopsy (Bigio et al., 2000; Dhar et al., 2006; Keller et al., 2010). The reflectance spectra obtained with this modality can be used to extract quantitative information on the scattering and absorption of light in tissue: a process known as optical property recovery. The optical properties recovered by this process are the absorption coefficient $\mu_a$ and reduced scattering coefficient $\mu'_s = \mu_s(1-g)$, where $\mu_s$ is the scattering coefficient and $g$ is the anisotropy coefficient of the tissue.

Many different methods have been developed for the determination of tissue optical properties. Some rely on time resolved (time or frequency domain) methods while others are based on steady state measurement of DRS signals. Time resolved measurements use time dependent diffusion theory or Monte Carlo modeling to predict the 'time of flight' of photons, and fit experimental data to these predictions. Time domain methods use a very short (picosecond) light pulse and measure how long it takes photons to reach the detector, while frequency domain methods use a modulated light source and match the measured phase shift, amplitude modulation and/or steady state intensity of the reflectance with those predicted by the fourier transform of the time domain diffusion theory formula. An example of a time domain system for optical property recovery in
human tissue is described by Svensson et al. (2005) while examples of frequency domain systems are given by Fishkin et al. (1997) and Gurfinkel et al. (2004).

Some of the methods used to measure steady state tissue reflectance include: total reflectance measurements with integration spheres, spectrally constrained diffuse reflectance measurements and spatially resolved diffuse reflectance measurements. When performing spectrally constrained diffuse reflectance measurement, the reflectance spectrum is measured using a single detector fibre located some distance from a source (illumination) fibre. A diffusion theory model of the reflectance spectrum, calculated using \textit{a priori} knowledge of the scattering spectrum and the spectra of the chromophores assumed to be present in the tissue is then fit to this measured spectrum (Kim & Wilson, 2011). Recently, this method was expanded to more than one radial distance to allow use over a wider range of optical properties (Kim, Roy, Dadani, & Wilson, 2010).

Spatially resolved steady state DRS (SR-DRS), on the other hand, uses multiple detector fibres placed at different distances from a source to obtain measurements of the reflectance versus radial distance. Spatial reflectance curves predicted using diffusion theory or Monte Carlo modeling are then fit to these data. When using a broadband light source and spectrometer, fitting must be performed at each different wavelength of interest to determine the absorption and scattering spectra.

In this study, optical properties are recovered from SR-DRS data. When compared to time resolved techniques, this method uses simpler, generally less expensive equipment, requiring only a fibre optic probe and spectrometer (either an imaging spectrometer, or a separate single channel spectrometer for each source-detector
This method differs from the spectrally constrained technique in that it can be used to extract optical properties without advance knowledge or assumptions of which chromophores are present in the tissue.

Monte Carlo simulation is an effective tool to study photon transport in tissue (Flock, Patterson, Wilson, & Wyman, 1989; Wang, Jacques, & Zheng, 1995). It offers advantages over diffusion theory and other numerical methods that are accurate only for large source to detector separations and correspondingly large probes. Probability distributions are used to describe local interactions of photons with their surroundings, and the individual histories of many different photons are simulated as they travel through tissue. Optical properties can be effectively recovered from SR-DRS data using Monte Carlo simulations (Graaff et al., 1993; Kienle & Patterson, 1996).

Our group is working to develop an optical biopsy instrument that makes use of SR-DRS measurements to recover the optical properties of brain tissue. This instrument will later be combined with a time resolved fluorescence spectroscopy (TRFS) system that is being developed concurrently (Yuan et al., 2009). Because both DRS and TRFS make use of fibre optic probes, integrating two such systems is a relatively straightforward task. Potential benefits of this integration include: improved sensitivity and specificity for diagnosis of malignancies due to the combination of these modalities (Georgakoudi et al., 2001) and the possibility of intrinsic fluorescence spectroscopy (IFS). IFS is a technique explored by many other groups in this field (Gardner, Jacques, & Welch, 1996; Kanick, Robinson, Sterenborg, & Amelink, 2012; Muller, Georgakoudi, Zhang, Wu, & Feld, 2001; Palmer & Ramanujam, 2008; Volynskaya et al., 2008; J. Wu,
Feld, & Rava, 1993) that uses DRS data to correct distortions in fluorescence spectra that result from scattering and absorption of light in tissue.

Other groups have previously demonstrated that there are quantitative differences between the optical properties of malignant versus healthy brain tissue. Yaroslavsky et al. (2002) used an integrating sphere setup to recover the optical properties of a variety of brain tissue samples in vitro, including healthy samples from various areas of the brain and samples of two different tumour types: astrocytoma and meningioma. The authors demonstrate a measurable increase in both the anisotropy factor and scattering coefficients for both tumour types as compared to healthy grey matter tissue over a wavelength range of 360–1100 nm. Gebhart, Lin, & Mahadevan-Jansen (2006) used an integrating sphere setup and inverse adding doubling method to recover the optical properties of 83 in vitro brain tissue samples recovered from patients undergoing tumour resection surgery. Histologic classification of each sample identified it as white matter, grey matter or glioma. This group demonstrated that the reduced scattering coefficient of glioma samples lies between the scattering coefficient of both white matter and grey matter consistently over a wavelength range of 400–1300 nm. The results of both of these groups show a monotonic decrease in the scattering coefficient across the wavelength range studied, with Gebhart et al.’s results showing reduced scattering values decreasing from approximately 4 mm⁻¹ at 400 nm to 1 mm⁻¹ at 1300 nm.

The purpose of this paper is to present the development of a unique small diameter fibre optic probe design meant for DRS of brain tissue in small surgical cavities, and to present a modified optical property recovery algorithm developed for use with this probe.
design. This expands upon previous results (Cappon, Nie, Farrell, Fang, & Hayward, 2012) by demonstrating (through simulations) the incompatibility of previous optical property recovery algorithms with the new probe design and documents the optimization of the parameters of the Monte Carlo simulations used in the algorithm.

2.4 Models and Methods

2.4.1 Monte Carlo Models

All Monte Carlo codes used in this study simulated a single layer of tissue with homogeneous optical properties and a refractive index of 1.4. The same procedures were used for selecting photon step size, absorption/scattering and boundary interactions as those described by Wang et al. (1995). The Henyey-Greenstein phase function (Henyey & Greenstein, 1941) was used to calculate the scattering angle of photons (Prahl, Keijzer, Jacques, & Welch, 1989; Wang et al., 1995; Zhu, Palmer, Breslin, Harter, & Ramanujam, 2008) and all simulations assumed an anisotropy factor (g) of 0.9. This was considered appropriate as the anisotropy factor of brain tissue is close to 0.9 across the spectral range of interest (Yaroslavsky et al., 2002).

The use of a single anisotropy coefficient is a common approach taken when modeling reflectance data (Dhar et al., 2006; Keller et al., 2010; Muller et al., 2001). Although decreasing $|g|$ means that scattering is more isotropic and therefore shifts spatial reflectance curves towards shorter source-detector separations, it has been demonstrated that when $g > 0.8$ changes to the anisotropy factor have a minor effect on the reflectance (Graaff et al., 1993; Kienle & Patterson, 1996).
The absorption and reduced scattering coefficients ($\mu_a$ and $\mu'_s$) used in these simulations were chosen to be similar to those of human brain tissue (both grey and white matter) at various wavelengths. The wavelengths investigated in this study were from 360 to 950 nm, as these are the limits of the spectrometer to be used in the final DRS instrument. The optical properties of brain tissue at these wavelengths were taken from results published in a study by Yaroslavsky et al. (2002), and representative values are shown in Table 2.1. These values were used to define the range over which a DRS instrument used in the brain would be expected to operate, and therefore ensure that optical property algorithms are able to recover such values.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$\lambda$ (nm)</th>
<th>$\mu_a$ (mm$^{-1}$)</th>
<th>$\mu'_s$ (mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter</td>
<td>360</td>
<td>0.31</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.02</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>360</td>
<td>0.23</td>
<td>12.30</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.08</td>
<td>6.54</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.10</td>
<td>3.60</td>
</tr>
</tbody>
</table>

A number of Monte Carlo based techniques are described in this section. The first is the 'Mono Monte Carlo' method, a technique for determining optical properties from SR-DRS data using the results of a single Monte Carlo simulation. This technique was used to simulate how removing detector fibres from a DRS fibre optic probe design would affect the recovery of optical properties. As will be shown in the results section, simulations indicated that the close proximity of detector fibres to the edge of the probe
invalidated the assumption of a uniform refractive index at the tissue surface. This is a common assumption when modeling reflectance in tissue (Farrell, Patterson, & Wilson, 1992; Palmer & Ramanujam, 2006; Wang et al., 1995) and is necessary for the spatial invariance required by the 'Mono Monte Carlo' method. This motivated the development of a modified algorithm for the recovery of optical properties from DRS data, one which considers the true geometry of the probe and the different refractive indices encountered by photons leaving the tissue surface (described in section 2.4.5).

To test the validity of these Monte Carlo methods, results of the basic Monte Carlo code were compared with those from the MCML code published by Wang et al. (1995) and the results of diffusion theory calculations. The extended source diffusion theory model described by Farrell et al. (1992) was used.

2.4.2 Optical Property Recovery from the Results of a Single Monte Carlo Simulation

An inverse Monte Carlo algorithm was used to recover optical properties from spatially resolved diffuse reflectance spectra. This algorithm uses the iterative Marquardt-Levenberg curve fitting routine to fit Monte Carlo generated spatial reflectance curves to experimental data (in this work, simulated data were used in place of experimental data). Since running repeated Monte Carlo simulations is prohibitively slow, a ‘Mono Monte Carlo’ technique was used, based on the condensed simulation approach introduced by Graaf et al. (1993) and adapted by others (Kienle & Patterson, 1996; Palmer & Ramanujam, 2006).

This approach used a single reference simulation with optical properties $\mu_{a,\text{ref}}$ and $\mu'_{s,\text{ref}}$ to determine the number of interactions of each photon within the medium. In such a
simulation, changing the scattering coefficient only affects the path lengths followed by photons between interactions. For this reason, the results of a single Monte Carlo simulation can be applied for a sample with new optical properties so long as the distances in the original simulation can be scaled and the new albedo \( c = \frac{\mu_s}{\mu_a + \mu_s} \) is less than or equal to \( c_{\text{ref}} \), the albedo of the reference simulation (Graaff et al., 1993).

The reference simulation was run for \( 2 \times 10^9 \) photons with optical properties \( \mu_{a \text{ ref}} = 0 \) and \( \mu_{s \text{ ref}} \). Note that any value of \( \mu_{a \text{ ref}} \) could be used, but setting the absorption to 0 guaranteed that \( c_{\text{ref}} \) was always higher than or equal to \( c \). Upon exiting the tissue, each photon was binned in a grid location defined by \( \rho_{\text{ref}} \), the photon’s radial distance from the source; and \( n_{\text{scat}} \), the number of scattering events it experienced while travelling through the tissue. This provided a table of the reflectance \( R_{\text{ref}} \) at discrete values of \( \rho_{\text{ref}} \) and \( n_{\text{scat}} \) as illustrated in Figure 2.1.

![Photon scoring grid used to set up the ‘Mono Monte Carlo’ method. \( \rho_{\text{ref}} \) is the radial distance from the source at which a photon exited the tissue and \( n_{\text{scat}} \) is the total number of scattering events the photon experienced.](image)

This grid could then be used to determine the reflectance for a sample with optical properties \( \mu_a \) and \( \mu_s' \) by applying Equation 2.1. First, each value of \( R_{\text{ref}} \) was multiplied by the new albedo raised to the power of \( n_{\text{scat}} \). This could be done since the albedo provides a
measure of the fraction of photons that are scattered out of a collimated beam in one path length. The grid was then ‘collapsed’ down to one dimension by taking the sum along each column.

\[ R(\rho) = \sum_{n_{\text{wa}}} R_{\text{ref}} \left( \rho \cdot \left[ \frac{\mu_t}{\mu_{t\text{ref}}} \right] n_{\text{scat}} \left[ \frac{\mu_t}{\mu_a + \mu_s} \right]^{n_{\text{wa}}} \right) \quad \text{Equation 2.1} \]

The reflectance at a given source to detector distance \( R(\rho) \) could be found after first scaling the distances in the collapsed grid by the ratio of the new mean free path \((1/\mu_t)\) to the mean free path used in the reference simulation \((1/\mu_{t\text{ref}})\). Because scoring was performed radially, the boundaries of each bin were concentric circles around a central (source) point. The scaling procedure moved the boundaries of the bins inwards or outwards and therefore changed the bin positions and widths (areas). To compensate for this, each extracted reflectance value was normalized by the total area of the scaled bin in which it was scored (found by subtracting the area of the circle defined by the inner boundary from the area of the circle defined by the outer boundary).

Values of \( \rho \) were used that corresponded to the positions of optical fibres in a DRS probe and extracted spatial reflectance curves were fit to SR-DRS data using the Marquardt-Levenberg curve-fitting algorithm.

2.4.3 Reducing the Probe Size

Practically, to allow ease of use in small surgical cavities, the diameter of the fibre optic probe will be kept quite small. This meant that any data being fit could contain only a few short source-detector distances near the front of a radially resolved reflectance
A simulation-based procedure was used to observe the precision in the recovered optical properties as the outermost source-detector distance (SDD) was reduced.

To do so, a low noise reflectance curve was generated (using Monte Carlo methods) and random Gaussian noise was added with standard deviations of 5, 10 and 15% commensurate with experimental conditions observed during preliminary investigations. These data were fit using the 'Mono Monte Carlo' method to recover $\mu_a$ and $\mu'$. This was repeated 1000 times and the standard deviation in the recovered optical properties was determined. Previous investigations suggested that one thousand repetitions was sufficient to ensure that the uncertainties in the results approached their limiting values. This entire process was repeated as the maximum radial distance was reduced, for various input optical properties. In these simulations, reflectance data were generated at evenly spaced distances of 0.3 mm, corresponding to typical fibre diameters.

Reducing the maximum SDD in the probe had the additional effect of changing the depth of tissue probed by reflectance measurements. This was investigated by simulating a probe containing a single source and detector and placing the detector at varying distances from the source. For any photon that was eventually collected by the detector, its depth was recorded each time it passed below a target radial distance. These results were then used to find the average depth probed below a target radial distance versus detector position.

2.4.4 **True Probe Forward Monte Carlo Model**

Because of its ability to recover the reflectance at arbitrary source-detector distances, the ‘Mono Monte Carlo’ method was effective for investigating how the
number of fibres and their positioning affects optical property recovery in a custom probe. However, certain simplifying assumptions were required during the Monte Carlo simulation. These included:

1. A pencil beam photon source instead of a realistic fibre
2. Does not use finite size detectors with typical acceptance angles
3. A semi-infinite medium, which does not account for refractive index variations due to different materials

To keep within the small diameter constraint while maximizing the outermost SDD, detector fibres were placed very close to the outer edge of the probe. It was therefore expected that inhomogeneous boundary conditions (the difference in the refractive index of the probe and surrounding air) would have an effect on the reflectance. To investigate this effect, a forward Monte Carlo model was developed which explicitly simulates the true probe design as closely as possible.

Each fibre was given a circular profile with a refractive index of 1.46, a representative value provided by an optical fibre manufacturer (CeramOptec Industries, Inc., East Longmeadow, MA, USA). Each fibre used for diffuse reflectance measurements was given a core diameter of 200 microns. This size was chosen as a trade-off between maximizing the SDD of the outermost fibre and minimizing the required exposure time. Larger fibres would reduce the maximum achievable SDD and affect the performance of the optical property recovery algorithm, while smaller fibres would require longer measurement times to achieve an acceptable signal to noise ratio.
The source fibre was simulated by emitting photons randomly at any point within its core, at any angle within its numerical aperture. Similarly, only photons incident on a detector fibre core at a (refracted) angle within the numerical aperture were scored by the model. Photons incident on the epoxy region of the probe were removed from the simulation (absorbed by the epoxy), while those reaching the tissue surface outside of the probe diameter were reflected according to the Fresnel equations at a boundary with mismatched refractive indices of 1.4 and 1.

The results of this simulation were fit using the previously described 'Mono Monte Carlo' method to see if it was capable of recovering the input optical properties. This was done over a range of optical properties.

2.4.5 Hybrid Reflectance Recovery Algorithm

To accurately model the reflectance that will be measured with our probe design, a 'true probe' Monte Carlo simulation is required. Because it is not spatially invariant, however, such a simulation cannot be used to generate the scoring grid in the 'Mono Monte Carlo' model, and therefore a new algorithm for rapid generation of reflectance curves was required. One possible solution would be to generate a two dimensional look-up table using the forward Monte Carlo model of section 2.4.4 over a range of \( \mu_s \) and \( \mu_a \) values. Achieving sufficient resolution over the wide range of optical properties listed in Table 2.1, however, would require a very large number of simulations and the time required to implement this was considered impractical. Instead, a hybrid look up table/Mono Monte Carlo method was developed for rapid generation of reflectance
curves. This allowed the use of 'true probe' simulations but required fewer simulations than a pure look-up table approach.

The Monte Carlo simulations described in section 2.4.4 were run for selected values of $\mu'_s$, but with $\mu_a$ set to 0. Detected photons were scored according to the detector in which they were collected and $x_t$, the total three-dimensional path length traversed while in the tissue. Combining the results of these simulations produced the three-dimensional grid shown in Figure 2.2: the reflectance in each detector for each different value of $\mu'_s$ and $x_t$.

In a process similar to the one in section 2.4.2, this grid could later be used to recover the reflectance corresponding to a measurement on a sample with arbitrary optical properties. The absorption coefficient of the sample was incorporated by applying Equation 2.2, thus collapsing the grid in one dimension. This created a look-up table of
the reflectance collected by each detector versus $\mu'_s_{\text{ref}}$. Finally, reflectance values were extracted from this table by interpolating between $\mu'_s_{\text{ref}}$ values by cubic splines. This procedure is shown graphically in section 2.7.

$$R(\mu_a) = \sum_{x_t} R_{\text{ref}}(x_t) \cdot e^{-\mu_a \cdot x_t}$$  \hspace{1cm} \text{Equation 2.2}

In an experimental situation, the optical properties of a tissue sample can be recovered by generating successive spatially resolved reflectance curves with this algorithm and using Marquardt-Levenberg curve-fitting to find the best fit to measured spatial reflectance data. By changing the geometry used in the initial Monte Carlo simulations, this algorithm could be applied to any desired probe design.

To ensure accuracy in reflectance curves generated with this algorithm, a number of parameters were investigated and optimized. Because photons were scored in discrete bins based on the total path length, both the maximum path length scored and the width of the bins (path length resolution) were parameters of the model. Simulations were run in which photons were scored at very high resolutions of $1 \times 10^4$ bins/mm and maximum path lengths of 150 mm. While these values would be impractical for use in a final algorithm due to time and file size constraints, they were used in initial investigations to allow for independent evaluation of each parameter. The optical properties used in these investigations were $\mu_a = \{0.01, 0.5\}$ and $\mu'_s = \{0.5, 13\}$. The four combinations of these values covered the range of optical properties of brain tissue listed in Table 2.1.

The influence of the maximum path length ($x_m$) was investigated by finding the total reflectance recovered for a series of $x_m$ values and comparing this to the reflectance
when the path length was not limited. The path length resolution was investigated by progressively summing the contents of adjacent bins to simulate coarser binning. Since each simulation used to generate the grid was run with a different value of \( \mu' \), the resolution in \( \mu' \) was the final parameter of the model (the range was previously defined as 0.5-13 mm\(^{-1} \)). This resolution was progressively improved by running additional simulations and observing plots of the total reflectance versus \( \mu' \). This procedure was continued until intermediate data points could be accurately interpolated by cubic splines. The error in interpolation was estimated by consecutively removing each point from the data (except the endpoints) and interpolating for the value of the missing data point.

2.4.6 Optical Property Recovery Performance

Using the Marquardt-Levenberg curve-fitting routine with absolute reflectance data from the hybrid reflectance recovery algorithm will allow recovery of \( \mu_a \) and \( \mu' \) from measured SR-DRS data. A simulation based testing procedure was used to characterize the precision of the optical property algorithm. The relative uncertainty (defined here as the standard deviation divided by the mean of 1000 runs) in the recovered optical properties was observed when simulated reflectance curves corrupted by random noise were input to the algorithm.

2.5 Results and Discussion

2.5.1 Monte Carlo Model

Results of the comparison between our basic Monte Carlo model, the MCML code (Wang et al., 1995) and diffusion theory are shown in Figure 2.3 for selected optical properties. It can be observed that at short source-detector distances (Figure 2.3 inset) the
results are consistent with MCML, while at longer distances the results are consistent with both MCML and diffusion theory.

Figure 2.3 Validation of Monte Carlo codes. Solid lines are SR-DRS reflectance curves generated using the Mono Monte Carlo method. Dots indicate the results of the MCML code by Wang et al. (1995) Crosses show the results of diffusion theory calculations using the extended source model described by Farrell et al. (1992)

2.5.2 Reduced Probe Size

The results of the investigation described in section 2.4.3 (reducing the probe diameter) are shown in Figure 2.4, which plots the percent uncertainty in the recovered optical properties versus the maximum source-detector distance in the simulated probe design. These results indicated that the presence of random noise in the reflectance curves had a strong effect on the precision of the optical properties recovered by the ‘Mono Monte Carlo’ algorithm. The relative uncertainty in the recovered optical properties was found to increase approximately linearly with the standard deviation of the added noise, and to vary approximately inversely with the SDD of the outermost detector. Examination of Figure 2.4 suggests that, for the optical properties shown, an outermost distance of 1.5
mm would demonstrate acceptable precision. This was consistent with other simulations using different optical properties (data not shown). It can also be observed that at short SDDs the uncertainty in $\mu_a$ is significantly greater than $\mu'_s$. At short SDDs, collected photons have experienced short path lengths in tissue and therefore the influence of small changes in $\mu_a$ is insignificant.

The effect that reducing the maximum SDD had on the depth probed by reflectance measurements is demonstrated by the simulation results in Figure 2.5. This shows the average depth probed by photons as they passed below a radial distance of 0.89 mm when the detector was placed at SDDs ranging from 0.89 to 3 mm.

Using these data alongside other practical constraints, a design with three detectors placed up to a maximum SDD of 1.67 mm was chosen for use in brain tissue. The furthest detector was placed near to the outer edge of the probe to maximize its SDD, and the diameter of the probe was limited to less than 2 mm to allow easy manipulation in a small surgical cavity.
Multiple detector fibres were placed at each SDD and their outputs were bundled together to improve the photon collection efficiency. More fibres were placed in bundles at further SDDs to help compensate for the decreased signal at these distances. The design chosen for the probe is shown in Figure 2.6 and the relative signal strength collected by each detector bundle is shown in Table 2.2.

A 0.4 mm diameter fibre was included in the probe design for future use in a combined diffuse reflectance/time resolved fluorescence instrument. Although photons entering this fibre were not scored during Monte Carlo simulations, its presence was considered because it altered the reflectance profile of the probe due to a difference in the index of refraction of the fibre and the surrounding probe surface.
Figure 2.6 The proposed design for the fibre optic probe. DRS detector fibres are bundled together into three groups, each at the indicated distance from the DRS source fibre (measured from the centres of each fibre). The central fibre is used for both illumination and detection in TRFS measurements.

Table 2.2 Relative signal strength in each of the outer two DRS detector bundles of the probe shown in Figure 2.6, obtained through Monte Carlo simulations. This was defined as the total reflectance (photons per unit area) collected in all fibres of the indicated bundle divided by the total reflectance in the detector bundle 0.23 mm from the source.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>λ (nm)</th>
<th>$R(0.59 \text{ mm}) / R(0.23 \text{ mm})$</th>
<th>$R(1.67 \text{ mm}) / R(0.23 \text{ mm})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter</td>
<td>360</td>
<td>0.337</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.547</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.526</td>
<td>0.236</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>360</td>
<td>0.073</td>
<td>5x10^{-4}</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.179</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.304</td>
<td>0.023</td>
</tr>
</tbody>
</table>
2.5.3 True Probe Forward Monte Carlo Model

The consequences of ignoring the change in refractive index at the probe’s edge (boundary effects) are demonstrated in Figure 2.7. These results show that although the 'Mono Monte Carlo' algorithm was able to generate a good fit to the (simulated) true probe reflectance, this fit corresponds to incorrect optical properties. This analysis was performed over a range of optical properties, and results are listed in Table 2.3. The discrepancies between the target and recovered absorption coefficients in Table 2.3 show the strong influence of boundary effects on this probe design. This motivated the development of a new optical property recovery algorithm; one which considers the true geometry of the probe surface. This algorithm was described in section 2.4.5.

![Figure 2.7](image-url)

Figure 2.7 The solid line is the reflectance curve predicted by the ‘Mono Monte Carlo’ model for optical properties $\mu_a = 0.02$ mm$^{-1}$ and $\mu'_s = 0.99$ mm$^{-1}$. Points marked with an ‘x’ show the reflectance predicted by the 'true probe' forward Monte Carlo model for the same optical properties. This model takes into account reflections due to refractive index mismatches around the boundary of the probe and the surface of each fibre. The dotted line is the fit generated with the ‘Mono Monte Carlo’ algorithm, which corresponds to optical properties $\mu_a = 2.8 \times 10^{-6}$ mm$^{-1}$ and $\mu'_s = 1.0$ mm$^{-1}$.

2.5.4 Parameter Optimization: Hybrid Reflectance Recovery Algorithm

Selected results from the investigation of the maximum path length parameter are shown in Figure 2.8. It was found that for path lengths <150 mm, the reflectance in each
detector reached >99% of its maximum value: the total when the path length was unlimited. This was true for all investigated optical properties (data not shown).

Table 2.3 The effects of boundary conditions on the Mono Monte Carlo algorithm’s ability to recover optical properties. The ‘Target Optical Properties’ were used to generate reflectance curves with a model that simulates the probe boundary. ‘Recovered Optical Properties’ are the values returned when fitting this simulated data with a model that does not consider boundary conditions. SDDs of 0.23, 0.59 and 1.67 mm were used.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>λ (nm)</th>
<th>Target Optical Properties (mm(^{-1}))</th>
<th>Recovered Optical Properties (mm(^{-1}))</th>
<th>Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter</td>
<td>360</td>
<td>0.31</td>
<td>2.34</td>
<td>-6.45 2.17</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.02</td>
<td>0.99</td>
<td>-99.98 1.54</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.04</td>
<td>0.61</td>
<td>-39.40 1.54</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>360</td>
<td>0.23</td>
<td>12.30</td>
<td>-0.92 2.79</td>
</tr>
<tr>
<td></td>
<td>630</td>
<td>0.08</td>
<td>6.54</td>
<td>-31.86 8.90</td>
</tr>
<tr>
<td></td>
<td>950</td>
<td>0.10</td>
<td>3.60</td>
<td>-23.75 3.53</td>
</tr>
</tbody>
</table>

Figure 2.8 Investigation of the maximum path length parameter for simulations with optical properties a) \(\mu_a = 0.01\) mm\(^{-1}\), \(\mu'_s = 13\) mm\(^{-1}\) and b) \(\mu_a = 0.5\) mm\(^{-1}\), \(\mu'_s = 0.5\) mm\(^{-1}\). Each curve corresponds to a simulated detector bundle with the indicated source to detector distance. \(N_p(x)\) is the number of photons collected that travelled a total path length equal to \(x\). \(N_{p,ref}\) is the total number of photons collected for each SDD when the path length was not limited.
Investigation of the path length resolution parameter showed that when it was decreased to 13 bins/mm, the simulated reflectance remained within 1% of the reflectance at 1x10^4 bins/mm. This was true across the range of investigated optical properties, and therefore a resolution of 13 bins per mm was selected for $x_t$. Since the maximum path length recorded was 150 mm, 1950 path length bins were used in total.

The resolution in $\mu'$ is shown in Figure 2.9 for $\mu_a = 0.02$ and 0.5 mm$^{-1}$. The error in each point was found to be less than 2% when the resolution in $\mu'$ was set to 0.5 mm$^{-1}$.

![Figure 2.9](image)

Figure 2.9 The reflectance recovered using the method described in section 2.5 for each detector bundle in the probe layout of Figure 2.6. Results are shown over a range of desired $\mu'$ values, with $\mu_a =$ a) 0.01 mm$^{-1}$ and b) 0.5 mm$^{-1}$. Data points indicate the $\mu'_s$ values used to generate the grid and the lines are the results of interpolation by cubic splines. Reflectance values are plotted as a fraction of the total reflectance collected by all three fibre bundles. The error in each data point was less than 2%.

### 2.5.5 Optical Property Recovery Performance

Results characterizing the precision of the optical property recovery algorithm are shown in Figure 2.10. All reflectance data used in the fitting procedure corresponded to the probe design of Figure 2.6. As expected, the highest uncertainty in the results occurs when recovering the absorption coefficient from data corresponding to low $\mu_a$ values. This is a consequence of using a probe with short SDD values: the algorithm is less
sensitive to changes in $\mu_a$ than $\mu_s$. This is especially true for tissues with very low absorption, because of the shape of the corresponding reflectance curve.

![Relative Uncertainty in Recovered $\mu_a$ (mm$^{-1}$)](image)

**Figure 2.10** Performance of the optical property recovery algorithm when presented with simulated reflectance curves corrupted by random noise with a standard deviation of 5%. The values on the x and y axes are the target optical properties while the contour lines represent the relative uncertainty in the recovered optical properties, defined as the standard deviation of the results divided by the mean over 1000 runs.

### 2.6 Conclusion

Monte Carlo simulations were used to determine the minimum diameter of a fibre optic probe to be used to recover optical properties from spatially resolved diffuse reflectance spectra. This was investigated over the wide range of optical properties expected in brain tissue, and the minimum diameter was found to be 1.5 mm. It was discovered that for small probe designs in which detector fibres are placed close to the edge, the true geometry of the probe must be simulated and the different refractive indices at the tissue surface must be considered. A hybrid algorithm was developed to efficiently generate reflectance data that account for the true geometry of the probe. Fitting these data to measured SR-DRS data allows optical property recovery in brain tissue.

These methods were used to develop a novel, small diameter fibre-optic probe design for use in a diffuse reflectance spectroscopy instrument. Simulations with the
expected signal to noise ratio indicated acceptable performance across the range of optical properties of brain tissue. Although the data presented here are for a specific probe design, the algorithm could be readily adapted for any desired probe geometry. The algorithm is compatible with small diameter probes that are easy to manipulate in surgical cavities.

2.7 Appendix: Reflectance Recovery Example

A worked example showing the process of recovering the reflectance from the true probe simulating grid is shown in Figure 2.11 and Figure 2.12 for optical properties $\mu_a = 0.01$ and $\mu'_s = 0.75$.

![Image](image.png)

Figure 2.11 The true probe inverse reflectance recovery process. a) The grid is collapsed in one dimension by applying Equation 2.2 to incorporate the target $\mu_a$ value. b) The target value of $\mu'_s$ is incorporated by interpolating between $\mu'_s$ ref values, thus collapsing the grid further. c) The expected reflectance collected by each detector when performing a measurement on a sample with optical properties $\mu_a$ and $\mu'_s$. 
Figure 2.12 A worked example showing the process of reflectance recovery from the true probe simulating grid for optical properties $\mu_a = 0.01$ and $\mu'_s = 0.75$. a) The reflectance collected by the closest detector (SDD = 0.24 mm) is recovered. Selected data are shown in the table for $\mu'_s = 0.5$ to 2, and path lengths of 1.462 to 1.692 mm. b) Equation 2.2 is applied to incorporate the absorption $\mu_a = 0.01$ thus collapsing each row of the table. c) Data is interpolated by cubic splines to incorporate the scattering, $\mu'_s = 0.75$. d) This procedure is applied for all three source to detector separations in the probe.
References


brain tissues in vitro in the visible and near infrared spectral range. *Physics in Medicine and Biology, 47*(12), 2059.


Chapter 3

Paper II – Distortion correction and cross-talk compensation algorithm for use with an imaging spectrometer based spatially resolved diffuse reflectance system

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This manuscript has been submitted to Review of Scientific Instruments
3.1 Introduction to Paper II

In this paper, the development of a novel algorithm to correct for distortion and crosstalk in diffuse reflectance data collected using the Mark II, an imaging-spectrometer based DRS system, is presented. While the Mark II system is not the primary focus of this thesis, it is used throughout as the primary reference system, used to establish the optical properties of unknown materials. Reliable operation of the Mark II is therefore essential for the work described in this thesis, and for all users performing optics research within our lab.

The algorithm presented in this paper makes use of a combination of image processing and curve fitting techniques to adaptively process reflectance data obtained with the Mark II system.

The simulations, experiments and analysis presented in this paper were performed by Derek Cappon, under the co-supervision of Dr. Hayward, Dr. Farrell and Dr. Fang. The manuscript was written by Derek Cappon, and edited by Dr. Hayward and Dr. Farrell. The manuscript has been altered slightly to match the style of this thesis.
3.2 Abstract

Optical spectroscopy of human tissue has been widely applied within the field of biomedical optics to allow rapid, *in-vivo* characterization and analysis of tissue. When designing an instrument of this type, an imaging spectrometer is often employed to allow for simultaneous analysis of distinct signals. This is especially important when performing spatially resolved diffuse reflectance spectroscopy. In this paper, an algorithm is presented that allows for the automated processing of 2 dimensional images acquired from an imaging spectrometer. The algorithm automatically defines distinct spectrometer tracks and adaptively compensates for distortion introduced by optical components in the imaging chain. Crosstalk resulting from overlap of adjacent spectrometer tracks in the image is detected and subtracted from each signal. The algorithm’s performance is demonstrated in the processing of spatially resolved diffuse reflectance spectra recovered from an Intralipid and ink liquid phantom, and is shown to increase the range of wavelengths over which usable data can be recovered.
3.3 Content of Paper II

Diffuse reflectance spectroscopy (DRS) is an optical technique commonly used to analyze the composition of human tissue. It has been investigated for use in many different applications of biomedical optics, from the characterization of skin lesions (Garcia-Uribe et al., 2011) to discrimination of esophageal malignancies (Douplik et al., 2014) to the in-vivo assessment of colon tissue in colorectal cancer patients (Wang et al., 2009). A broadband light source (ultraviolet, visible or near infrared depending on the application) illuminates the tissue, and a spectrometer is used to collect the reflectance spectrum allowing for quantitative analysis of the results (Karsten, Singh, Karsten, & Braun, 2013; Nickell et al., 2000; Qian, Victor, Gu, Giller, & Liu, 2003). Optical fibres are often used both to deliver light to the tissue and collect the resulting reflectance. The features of the reflectance spectrum can be observed directly to analyze tissue composition (Palmer et al., 2003; Zhu, Palmer, Breslin, Harter, & Ramanujam, 2006), or used to recover the optical properties of the sample, providing a quantified measure of how light interacts with the tissue (Mourant, Marina, Hebert, Kaur, & Smith, 2014; Valdes et al., 2011; Wang et al., 2009). Optical properties recovered from diffuse reflectance data include the reduced scattering coefficient (\(\mu'_s\)) and the absorption coefficient (\(\mu_a\)).

Methods for recovering optical properties from diffuse reflectance data include frequency (or time) domain analysis (Gurfinkel, Pan, & Sevick-Muraca, 2004; Svensson et al., 2005), and spatial domain analysis (Valdes et al., 2011; Wang et al., 2009). In either of these approaches, measured reflectance curves are fit with the results of a
theoretical model generated from approximate solutions of the diffusion equation or Monte Carlo simulation. The optical properties that generate the best fit to the measured data are accepted as the properties of the tissue.

A well-established method for measuring spatially resolved diffuse reflectance data uses an imaging spectrometer and CCD camera to capture the output from multiple optical fibres (Hyde, Farrell, Patterson, & Wilson, 2001; Nickell et al., 2000; Pekar & Patterson, 2010). Detector fibres are placed at different distances from a source fibre so the system is able to simultaneously capture both spatially and spectrally resolved data in a single measurement. The fibres are embedded in a probe, making for an easy to use measurement device with fixed source-detector distances (SDDs). At the opposite end, the fibers are connected to the imaging spectrometer and CCD. Each detector fibre is physically separated from the others at the entrance to the spectrometer, as demonstrated in Figure 3.1a. As the spectrometer resolves each fibre’s output by wavelength, this produces an image of ‘tracks’ on the CCD, Figure 3.1b. Some image processing is required to separate each track from its neighbours and determine the spectral intensity of each detector fibre.

In this letter, an automatic image processing routine used to define spectrometer tracks in an imaging spectrometer based spatially resolved diffuse reflectance system is described. This algorithm is a flexible improvement over previous algorithms, as it compensates for distortion introduced by the optical chain and adaptively subtracts crosstalk resulting from the overlap of detector tracks. This algorithm was designed
specifically for the setup described below, it can be readily adapted for use with other imaging spectrometer based systems.

Figure 3.1 a) Individual fibres are physically separated before entering the spectrometer. b) An example of the image acquired by the CCD. Each track corresponds to a separate fibre and the source-detector distances of each fibre are indicated in mm.

The equipment used by our group has been described previously by Pekar and Patterson (2010). In summary, a broadband tungsten-halogen light source (Oriel Instruments, Stratford, CT) was used to illuminate a sample through a 100 μm optical fibre embedded in a handheld epoxy probe. Detector fibres were arranged across the surface of this probe according to the layout shown in Figure 3.2. Multiple detector fibres were used at the outermost distances to partially compensate for the decreased signal at these distances. Each detector fibre was attached to a variable attenuator (OZ optics, Ottawa, Ontario), to allow further balancing of the signals and fit the entire image within the dynamic range of the CCD. The outputs of the attenuators were sent through an imaging spectrometer (Kaiser Optical Systems, Ann Arbor, MI) and the image was acquired using an attached cooled CCD (Princeton Instruments, Trenton, NJ). The digitized signal was collected using Princeton Instrument’s WinSpec software package and
all analysis was performed using Matlab (The Mathworks, Natick, MA). A diagram of the system is shown in Figure 3.3. For every acquisition, a dark background image was also collected and subtracted from the data.

![Diagram](image)

**Figure 3.2** The arrangement of fibres across the surface of the epoxy probe. The source fibre is used to deliver light to the tissue, and the distance of each detector from the source is indicated in mm. Each fibre has a core diameter of 100μm.

![Diagram](image)

**Figure 3.3** The equipment used to acquire diffuse reflectance spectra.
The reflectance from each detector fibre broadens as it travels from the spectrometer grating to the CCD such that the signal from two separate fibres overlaps slightly. This undesired effect is referred to as crosstalk in this letter. To reduce the influence of this crosstalk on the final image, the fibres were arranged such that the fibres which have the strongest signal (at shorter SDDs) were located at the top and bottom of the image, while those with the least intensity were clustered near the middle. The positioning of each fibre on the CCD image is shown in Figure 3.1b.

Inspection of the CCD images reveals that each track follows a curved shape. This is demonstrated in Figure 3.4, a plot of the peak intensity of selected tracks across the spectrum. The curvature is more pronounced for the outermost tracks and most likely results from image distortion between the spectrometer and CCD. To compensate for this distortion and other potential irregularities in track size, an algorithm was developed to automatically define track boundaries. Although these boundaries are not expected to vary significantly for different measurements, the algorithm is applied to CCD images on an individual basis.
The curvature of the tracks on the spectrometer image. The locations of peak intensity of selected tracks are plotted in red. Examination of the tracks reveals a distinct curvature towards the top and bottom of the image, most significant for the outermost tracks.

The initial steps in the analysis were applied at each wavelength along lines perpendicular to the intensity tracks, as demonstrated in Figure 3.5. The midpoint between each pair of adjacent tracks was first identified by finding a local minimum (the zero-crossing of the first derivative). The edges of each adjacent track were then defined as the points where the data crossed a threshold of 1.05 times the pixel value at this midpoint. A threshold of 5% was chosen as it was observed to be consistently above the noise level of the data in a large training set. This process is illustrated in Figure 3.5.
Figure 3.5 Track edge identification. This step in the algorithm operates along vertical lines through the image, with each line having a width of 1 pixel, approximately 0.5nm. The plot displays the image intensity along the 650nm line. Inset: the midpoint between two adjacent tracks is identified by finding the local minimum within this region (red circle). The edges of the two adjacent tracks are defined as the points at which the intensity is 1.05 times the intensity at the minimum (the two lines at 675 and 685 nm).

Because the algorithm depended on the data reaching a local minimum, it could not be used to find the top and bottom edges of the two outermost tracks. Instead, the outermost boundaries of these tracks were estimated at selected wavelengths in a series of training images and averaged to provide an estimate of the track boundaries across the spectrum. The final track boundaries produced by this algorithm at each wavelength are shown in Figure 3.6. Each boundary was smoothed by fitting with a two term Gaussian function (Equation 3.1), which produced an $R^2$ value greater than 0.9 in all investigated examples.

$$a_1 e^{-\left(\frac{x-b_1}{c_1}\right)^2} + a_2 e^{-\left(\frac{x-b_2}{c_2}\right)^2} \quad \text{Equation 3.1}$$
Despite the attempt to minimize crosstalk through the physical arrangement of the fibre outputs across the CCD, crosstalk was still present in the data. This can be observed in the plot of Figure 3.5, in which the base signal level between each adjacent track depends on the tracks’ intensity. The amount of crosstalk in each track was estimated by finding the line connecting the data at both boundaries of each track, as illustrated in Figure 3.7. Final reflectance values were obtained by integrating across each track and subtracting the crosstalk. Applying this method at each wavelength collected by the spectrometer provided a final spectrum for each distance on the fibre optic probe head.
Figure 3.7 Crosstalk subtraction. The algorithm identified the highlighted areas as crosstalk resulting from overlap of the signal from adjacent tracks. These areas were subtracted when integrating the total intensity of each track.

A comparison between the image processing algorithm described here and a basic approach that uses straight lines for track boundaries was performed. A liquid phantom containing a lipid emulsion (Intralipid 20%, Fresenius Kabi AG, Bad Homburg, Germany) diluted to 0.65% was measured, and the images were processed and corrected for wavelength-dependent spectrometer transmission and detector response. The final spatially resolved spectra using each method are shown in Figure 3.8. The data show a clear difference in results, especially at the outer ends of the spectrum. In fact, the algorithm extends the spectral range of the system since when using the basic algorithm (straight line boundaries, no crosstalk), the data for some of the tracks is unusable below 575nm and above 925nm.
Figure 3.8 Algorithm comparison. The spatially resolved spectra after correction for spectrometer transmission and detector response. Each line corresponds to a different source-detector distance on the probe surface. a) The spectrum after processing using the algorithm described in this letter. b) The spectrum after processing with a very basic algorithm that uses straight line track boundaries and does not correct for crosstalk.

A technique for recovering spatially resolved spectra from diffuse reflectance images collected with an imaging spectrometer and CCD has been presented. This image processing routine automatically detects track edges on the raw CCD images and corrects for distortion introduced by the equipment. Crosstalk due to overlap of distinct spatially resolved signals is estimated and subtracted from the data. The spectra produced by this algorithm can be used with a steady state version of the Monte Carlo fitting routine described by Kienle and Patterson (1996) to determine the optical properties of turbid media.
Acknowledgements

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References


Chapter 4


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4.1 Introduction to Paper III

This paper presents the design of a novel instrument for optical property recovery in brain tissue. A unique calibration procedure designed to allow the use of this instrument in a clinical setting, and a modified algorithm for recovery of optical properties are also presented. Results of a laboratory trial using lipid based optical phantoms are presented and used to demonstrate the performance and range of the system.

The algorithm used for optical property recovery in this paper differs from the original algorithm presented in chapter 2. Testing of this original algorithm on liquid phantoms demonstrated incompatibility with the uncertainty in experimentally recovered data. To compensate for this, an empirical approach was adopted, in which look up tables were generated from experimentally obtained data, rather than analytical, Monte Carlo simulated data.

Results documented in this paper are presented over the range 500 – 850 nm as this corresponds to the limitations of the tungsten halogen light source used for illumination. A future clinical iteration of this system will be required to recover optical properties in the range 400 – 600 nm. The spectrometers used in the system are sensitive to wavelengths in the 350 to 1150 nm range, and thus a light source with stronger output in the near UV portion of the spectrum is all that is required to achieve the desired range of operation.

The simulations, experiments and analysis presented in this paper were performed by Derek Cappon, under the co-supervision of Dr. Hayward, Dr. Farrell and Dr. Fang.
The manuscript was written by Derek Cappon, and edited by Dr. Hayward and Dr. Farrell. The manuscript has been altered slightly to match the style of this thesis.

4.2 Abstract

Optical biopsy for the guidance of surgical tumour resection allows a surgeon to rapidly analyze and assess an area of tissue \textit{in-vivo} and distinguish healthy from malignant tissue. This allows a surgeon to make informed decisions on how to proceed with the surgical operation. Advantages over the current gold standard of biopsy sampling for analysis in the pathology lab include its non-invasive nature and ability to perform near instantaneous analysis. A diffuse reflectance system intended for use in optical biopsy of the brain is presented. This instrument allows for the recovery of optical properties of brain tissue from spatially resolved diffuse reflectance spectra, and is intended for integration with a time resolved laser induced fluorescence system for use during surgical resection of brain tumours. The system uses a compact probe designed for easy manipulation within small surgical cavities. This necessarily limits the source to detector distances available for spatially resolved analysis. The performance of the system is demonstrated through the analysis of liquid phantoms created from lipid emulsions and ink. Optical properties recovered using this compact system are shown to agree with those recovered using a reference laboratory system that uses much larger source to detector distances within ±15% across a wide spectral range.
Content of Paper III

4.3 Introduction

Optical biopsy for the analysis of human tissue is a promising technique that can be used intra-operatively during tumour resection to help distinguish malignant from healthy tissue (Lin et al., 2010; Lin, Toms, Johnson, Jansen, & Mahadevan-Jansen, 2001; Stummer et al., 1998; Valdes et al., 2011). Light in the ultra-violet, visible or near infrared spectrum is shone on an area of interest, and the resultant reflectance or fluorescence is analyzed to determine the composition or properties of the tissue under investigation (George, Chandrasekaran, Brewer, Hatch, & Utzinger, 2010; Mourant, Marina, Hebert, Kaur, & Smith, 2014; Palmer et al., 2003; Rolfe, 2000; van Veen, Amelink, Menke-Pluymers, van der Pol, & Sterenborg, 2005; Wang & Van Dam, 2004). This offers advantages over traditional biopsy techniques, which require the removal of tissue samples for analysis in the pathology lab. Optical biopsy techniques are minimally invasive, since the tissue is analyzed in situ, and rapid, since illumination, collection and analysis of the results can often be done within seconds (Foschum, Jager, & Kienle, 2011).

One well-established measurement technique used in optical biopsy is known as diffuse reflectance spectroscopy (DRS). Spectra collected using DRS are often used to recover the optical properties of tissue: typically the absorption ($\mu_a$) and reduced scattering ($\mu'_s$) coefficients (Kienle et al., 1996). Knowledge of optical properties has been used successfully both in-vivo and ex-vivo to help distinguish malignant or pre-malignant tissue from healthy tissue in many different areas of the body (Bydلون et al.,
2010; Gebhart, Lin, & Mahadevan-Jansen, 2006; Marin et al., 2005; Salomatina, Jiang, Novak, & Yaroslavsky, 2006). Different techniques and systems have been reported by various groups for the collection of diffuse reflectance, and a wide variety of algorithms have been developed to recover optical properties from these data. The algorithms can be analytical: fitting measured reflectance data to the reflectance predicted by diffusion theory (Farrell, Patterson, & Wilson, 1992; Finlay & Foster, 2004; Zonios et al., 1999) or Monte Carlo modelling (Kienle et al., 1996; Palmer & Ramanujam, 2006; Thueiler et al., 2003); or empirical: fitting the measured reflectance from a sample under investigation to the reflectance collected from other samples with known optical properties (Glennie, Hayward, & Farrell, 2015; Pfefer et al., 2003).

Many DRS systems use optical fibres to deliver light to the tissue and collect reflectance (Cletus, Kunnemeyer, Martinsen, McGlone, & Jordan, 2009; Dimofte, Finlay, & Zhu, 2005). By embedding detector fibres in a probe at different, fixed distances from a source fibre, using a broadband light source to illuminate the tissue and using a spectrometer to analyze the results a single measurement can provide both spectrally and spatially resolved data that can be used to recover absorption and scattering coefficients across the spectrum (Cappon, Farrell, Fang, & Hayward, 2013; Hyde, Farrell, Patterson, & Wilson, 2001). This is known as spatially resolved diffuse reflectance spectroscopy.

Lipid emulsions are often used to prepare tissue-simulating optical phantoms (Michels, Foschum, & Kienle, 2008). They act as an almost purely scattering medium with a combination of a high $\mu'$ and low $\mu_a$ and exhibit a smooth scattering spectrum across a wide spectral range (Pogue & Patterson, 2006). The optical properties of these
emulsions have been studied extensively by many different groups (Aernouts et al., 2013; Di Ninni, Martelli, & Zaccanti, 2011; Michels et al., 2008; van Staveren, Moes, van Marie, Prahl, & van Gemert, 1991), and have been shown to remain stable over long periods of time (Di Ninni et al., 2011). India ink is often used with lipid emulsions when preparing liquid phantoms since it acts as an almost purely absorbing medium and features a smooth absorption spectrum across a wide spectral range. The absorption coefficient of India ink is also stable over long periods of time (Di Ninni, Martelli, & Zaccanti, 2010).

There is significant variation in the values of $\mu'_s$ and $\mu_a$ for Intralipid and ink reported by groups using different methods/equipment for optical property recovery. Some groups have even demonstrated that optical properties vary with different brands of Intralipid or ink (Di Ninni et al., 2010; Michels et al., 2008). A recent study comparing the optical properties of Intralipid and ink phantoms recovered by different laboratories found a range in the reduced scattering coefficient equal to 25% of the mean, and a range in the absorption coefficient of 18% of the mean (Spinelli et al., 2012). Despite this variation in the optical properties of these materials they remain in common use today, partly due to their relatively cheap cost and widespread availability.

In this paper, we present a diffuse reflectance system designed for the recovery of optical properties of human tissue. This system is intended for clinical use in an optical biopsy instrument that will discriminate between glioma margins and healthy tissue during brain tumour resection surgery (Cappon et al., 2013). The calibration procedure and optical property recovery algorithms developed for use with this system are
described, and the performance of the system was evaluated through measurements on liquid lipid emulsion/ink phantoms.

As discussed in a previous publication (Cappon et al., 2013), this system will be used in conjunction with a time resolved fluorescence system that operates primarily within a 400 – 600nm spectral band. At this range, both the scattering and absorption coefficients of human brain tissue are quite high, especially for white matter (Gebhart et al., 2006; Yaroslavsky et al., 2002). The system and optical property algorithm have therefore been designed to be able to handle high values of $\mu_a$ and $\mu_s'$. This ability and the compact size of the probe are unique features that help set this system apart from other spatially resolved DRS systems.

4.4 Materials and Methods

The basic components of the DRS system include a broadband tungsten-halogen light source (Oriel Instruments, Stratford, CT), a handheld epoxy fibre-optic probe (Walsh Medical Devices, Oakville, Ontario, Canada), and three single-channel spectrometers (Stellarnet Inc., Tampa, FL) to measure the reflectance collected by the detector fibres, Figure 4.1. The detector fibres are organized into three separate bundles, where each fibre in a bundle is at the same source-detector distance (SDD). The choice of SDDs and the reasoning behind this particular fibre layout have been described previously (Cappon et al., 2013). The rapidly decreasing reflectance intensity at outer SDDs was compensated for by adjusting the integration times of each spectrometer, allowing use of the full dynamic range.
The diffuse reflectance spectroscopy system. Fibres in the handheld probe are grouped into 3 bundles at distinct source to detector distances. The combined output of each fibre in a bundle is collected and analyzed using a separate single channel spectrometer. Another spectrometer is used to view the output of the light source (from the 5% branch of the fibre splitter), and allows monitoring of fluctuations in its intensity.

All liquid phantoms used in these experiments were made by varying the concentrations of Intralipid (Fresenius Kabi AG, Bad Homburg, Germany) and India ink (Chartpak Inc., Leeds, MA).

4.4.1 Calibration

To monitor variations in the light source intensity over time, a 5/95 splitter (Fibersense & Signals Inc., Toronto, Canada) was connected to the output of the light source, splitting it into two branches: one (95%) coupled to the source fibre of the probe, and the other (5%) connected to a fourth spectrometer, as indicated in Figure 4.1. At the beginning of the measurement period, a single reference spectrum, $LS_{\text{ref}}$, was acquired from the light source. For each subsequent measurement, the ratio of the spectrum from the fourth spectrometer (connected to the light source branch) to $LS_{\text{ref}}$ was calculated. All spectra collected from the detector fibres were adjusted by this ratio to compensate for instability in the illumination intensity.
For storage and sterilization purposes, the fibre optic probe will be disconnected from the system between uses on different patients. This introduces the possibility that the fibre to spectrometer coupling may change. To compensate, all measured spectra are calibrated using an integrating sphere. The light source is connected directly to the entrance port of the sphere, and the probe is placed at the exit port. After starting the system, reference spectra $I_{S_{\text{ref}}}$ are acquired (one spectrum per SDD) from the integrating sphere. All subsequent measurements are then calibrated by dividing by $I_{S_{\text{ref}}}$. The calibration process is summarized by Figure 4.2.

### 4.4.2 Optical property recovery

Although the DRS system described here was originally designed for use with an analytical, Monte Carlo based algorithm for optical property recovery (Cappon et al., 2013), higher than expected uncertainty in the data made this approach infeasible with experimental results. An empirical algorithm for optical property recovery was adopted instead. This algorithm relies on a ‘grid’ of the reflectance measured from a series of reference Intralipid/ink phantoms of varying concentrations, covering a wide range of optical properties. The concentrations of Intralipid and Ink used in these phantoms are shown in Table 4.1.
Figure 4.2 The calibration process. Before any other measurements are performed, reference spectra \( I_{Sref} \) are collected from an integrating sphere. The light source spectrum collected at this time, \( L_{Sref} \) is saved for later use. When measuring a sample, detector spectra are calibrated for variations in light source intensity by adjusting by the ratio of the current light source spectrum to \( L_{Sref} \). Spectra are then calibrated to account for any variations in fibre coupling between measurement periods by dividing by the \( I_{Sref} \) spectra, giving the final calibrated reflectance \( R_{cal} \).

4.4.3 Reflectance Grid

The optical properties of the Intralipid/ink phantoms were determined using an existing steady state DRS system in our lab, referred to in this paper as the Mark II. This system and the optical property recovery algorithm used with it have been described previously (Hyde et al., 2001; Kienle et al., 1996). Because the optical properties corresponding to brain tissue were too high to measure directly with this system, samples
with lower concentrations were measured and these data were extrapolated to calculate the optical properties at high concentrations.

Table 4.1 Liquid phantoms used to generate the reflectance grid. 42 phantoms were created with every combination of the listed Intralipid and ink concentrations. The optical properties listed were determined using linear fits to $\mu_a$ and $\mu'_s$ versus concentration for phantoms measured with the reference, Mark II system, as shown in Figure 4.4.

<table>
<thead>
<tr>
<th>Intralipid concentration (%)</th>
<th>$\mu'_s$ at 550nm (mm$^{-1}$)</th>
<th>$\mu'_s$ at 750nm (mm$^{-1}$)</th>
<th>Ink concentration (%)</th>
<th>$\mu_a$ at 550nm (mm$^{-1}$)</th>
<th>$\mu_a$ at 750nm (mm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>1.21</td>
<td>0.82</td>
<td>0.015</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>1.8</td>
<td>2.84</td>
<td>1.74</td>
<td>0.040</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>2.8</td>
<td>4.47</td>
<td>2.66</td>
<td>0.065</td>
<td>0.31</td>
<td>0.23</td>
</tr>
<tr>
<td>3.8</td>
<td>6.10</td>
<td>3.57</td>
<td>0.115</td>
<td>0.56</td>
<td>0.41</td>
</tr>
<tr>
<td>4.8</td>
<td>7.73</td>
<td>4.49</td>
<td>0.165</td>
<td>0.80</td>
<td>0.59</td>
</tr>
<tr>
<td>5.8</td>
<td>9.36</td>
<td>5.41</td>
<td>0.215</td>
<td>1.04</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Each liquid phantom was measured using the compact probe DRS system described in this paper and the calibrated data were analyzed at each wavelength and SDD to produce a table of the calibrated reflectance $R_{\text{ref}}$ versus $\mu_a$ and $\mu'_s$. A separate set of tables was created for each wavelength within the range of the DRS system: 475nm to 850nm at increments of approximately 0.5nm, defined by the spectrum of the halogen light source and the resolution of the spectrometer. Each set contained three tables: one for each source-detector distance in the probe.

4.4.4 Optical Property Recovery

The set of grids corresponding to a desired wavelength were used with a least squares fitting routine to recover the optical properties of a sample material. The reflectance of the sample $R_{\text{meas}}$, was measured and calibrated using the previously
described procedure. The calibrated reflectance was then fit by extracting reflectance values from the grids for successive combinations of optical properties chosen according to the Levenberg-Marquardt fitting algorithm (Bevington & Robinson, 2003). Applying this procedure at each individual wavelength within a range of interest allowed recovery of the sample’s optical property spectrum.

Five additional Intralipid/ink phantoms with the concentrations listed in Table 4.2 were created to test the performance of the optical property recovery algorithm. These phantoms were measured and calibrated using the same procedure, but were not used when generating the reflectance grids. Measurements taken from these phantoms were input to the fitting algorithm, and the recovered optical properties were compared with the expected optical properties of the samples.

Table 4.2 Liquid phantoms used to test the performance of the optical property recovery algorithm. 5 phantoms were created with the listed concentrations of Intralipid and ink. The optical properties listed were determined using linear fits to \( \mu_a \) and \( \mu' \) versus concentration for phantoms measured with the reference, Mark II system, as shown in Figure 4.4.

<table>
<thead>
<tr>
<th>Concentrations (%)</th>
<th>Expected optical properties at 550nm (mm(^{-1}))</th>
<th>Expected optical properties at 750nm (mm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intralipid</td>
<td>Ink</td>
<td>( \mu'_s )</td>
</tr>
<tr>
<td>1.3</td>
<td>.04</td>
<td>2.03</td>
</tr>
<tr>
<td>1.3</td>
<td>.13</td>
<td>2.03</td>
</tr>
<tr>
<td>2.3</td>
<td>.08</td>
<td>3.66</td>
</tr>
<tr>
<td>4.3</td>
<td>.09</td>
<td>6.92</td>
</tr>
<tr>
<td>5.3</td>
<td>.04</td>
<td>8.54</td>
</tr>
</tbody>
</table>
4.5 Results and Discussion

4.5.1 Reference Optical Properties

The $\mu_s$ spectra recovered using the Mark II system for a series of low concentration Intralipid phantoms (Intralipid only, no ink) are plotted in Figure 4.3a. The absorption spectra of selected Intralipid/ink phantoms are plotted in Figure 4.3b.

The optical properties of these phantoms versus concentration at 650nm are plotted in Figure 4.4. This demonstrates that $\mu_a$ and $\mu_s$ increase linearly with ink and Intralipid concentration, respectively. Linear fits at each wavelength were used to predict the optical properties of the higher concentration phantoms used to generate the reflectance grid.

4.5.2 Compact Probe DRS System

The measured, calibrated reflectance from the phantoms of Table 4.1 is shown at 650nm by the plots of Figure 4.5a to c. These data make up the reflectance ‘grids’ used to later recover the optical properties of unknown samples. The data at other wavelengths are not shown, but were observed to follow a similar shape. The reflectance predicted for these same concentrations using the forward Monte Carlo model described previously by Cappon et al. (2013), are shown in Figure 4.5d to f for comparison. A qualitative comparison of these data shows that both the experimental and theoretical (Monte Carlo generated) reflectance have similar shapes across the range of optical properties.
Figure 4.3 Mark II optical property spectra. The optical property spectra measured for series of different low concentration liquid phantoms, measured directly using the reference, Mark II system. The concentrations are indicated on the figures. a) The $\mu$'s spectra of phantoms with the indicated concentrations of Intralipid, and no ink. b) The $\mu_a$ spectra of phantoms with the given concentrations of India ink, and varying concentrations of Intralipid.
Figure 4.4 Linear fits of a) $\mu'$ versus Intralipid concentration and b) $\mu_a$ versus Ink concentration at 650nm. The solid lines are linear fits to the data.
Figure 4.5 a) through c) the reflectance grids measured at 650nm. a) Reflectance from detector bundle 1, SDD = 0.34mm b) Detector bundle 2, SDD = 0.64mm c) Detector bundle 3, SDD = 1.43mm. d) through f) the reflectance predicted using the Monte Carlo model of Cappon et al. (2013).
Figure 4.6 shows the optical property spectrum recovered for one of the test phantoms using the reflectance tables and Marquardt-Levenberg fitting algorithm. Also shown is the optical property spectrum predicted from the phantom’s concentration, extrapolated from Mark II results. These are within ±10% agreement across the spectrum (inset). This comparison was repeated for all of the test phantoms listed in Table 4.2, and the maximum difference observed (the worst agreement out of all five phantoms) is plotted in Figure 4.7.

Overall, the results agree to within ±20% across the spectrum. While the difference in these results is fairly high, similar results have been observed by other groups performing diffuse reflectance spectroscopy on Intralipid phantoms (Pfefer et al., 2003).

4.6 Conclusion

A system for performing diffuse reflectance spectroscopy on tissue and a corresponding calibration and optical property recovery algorithm have been presented. The algorithm was designed to recover the optical properties of human brain tissue at 400-600nm, and is able to handle samples with very high absorption and reduced scattering coefficients. The compact size of the probe used by this system makes it easy to manipulate in space-restricted environments such as a surgical cavity, offering advantages over other spatially resolved systems that use larger source-detector separations.
Figure 4.6 The optical property spectrum recovered for the test phantom with Intralipid and ink concentrations of 4.3% and 0.09%, respectively. The lines marked ‘expected’ are the optical properties predicted using an extrapolation of the Mark II results, as in Figure 4.4. The lines marked ‘recovered’ are the optical properties recovered using the algorithm described in this paper, using the reflectance grids of Figure 4.5 and a least squares fitting routine.
Figure 4.7 The percent difference (error) between the optical properties recovered using the reflectance grid/least squares fitting algorithm and the expected optical properties as predicted by the Mark II data for the indicated ink and intralipid concentrations. The $\mu'$ value fell within ±20% for all five concentration values listed in Table 4.2, while the $\mu_a$ values fell within ±15%.
The performance of the system was evaluated through measurements on tissue-simulating phantoms made from Intralipid and India ink with optical properties similar to those expected for human brain at the wavelengths of interest. These results show agreement with those predicted by measurements using an established reference system, with differences remaining within ±20% across the spectrum. This is comparable to the differences observed in an inter-laboratory comparison of other groups working with Intralipid/ink phantoms at lower concentrations. This work represents a step towards the production of a clinical optical biopsy instrument for characterization of human brain tissue.

References


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http://doi.org/10.1364/AO.38.006628
Chapter 5

Conclusion and Future Direction

5.1 Summary and Conclusions

Optical biopsy for the rapid, *in vivo* analysis of human tissue is a technique that has been steadily developed over the past decades. As new technology and techniques for data analysis are created, the capabilities of such instruments expand, opening up exciting new possibilities for real time diagnostics and surgical guidance. The ultimate goal of an instrument that provides instant diagnosis of a wide variety of disease conditions without further trauma due to physical removal of tissue samples has prompted researchers around the world to investigate and develop optical biopsy technologies. Much of this research, and the focus of this thesis, has been on spectroscopic techniques such as diffuse reflectance spectroscopy and fluorescence spectroscopy. These two principles: one the simple scattering and absorption of photons, the other absorption and subsequent re-emission at new wavelengths, can be combined in such a way that they complement each other and provide a new analysis perspective that could not be obtained if implemented separately.

The goals of this thesis were to develop and test instrumentation for the collection of diffuse reflectance spectra from brain tissue, develop an algorithm for the recovery of optical properties from these data, and facilitate the integration of this technology with instrumentation for the recovery of time resolved fluorescence spectra. The details of this
process have been discussed in the papers presented in previous chapters, and summaries of the conclusions that can be drawn from each are presented here.

In Chapter 2, the design of a novel fibre optic probe intended for use in an optical biopsy system was discussed, and the development of an algorithm for recovery of optical properties from diffuse reflectance spectra collected with the probe. With separate fibres dedicated to reflectance and fluorescence, this probe allows for the integration of these systems and the recovery of both types of spectra from overlapping areas of tissue. The diameter of the probe was kept to a minimum to improve the ease of use in small surgical cavities. The positioning of DRS detector fibres was optimized for the recovery of optical properties from measured reflectance spectra using a Monte Carlo simulation based approach.

Two algorithms for the recovery of optical properties from diffuse reflectance data were discussed in this chapter; the Mono Monte Carlo method and the Hybrid Reflectance Recovery model. Each algorithm is based on Monte Carlo simulation of photon transport through tissue, similar to the method originally published by Wang, Jacques, & Zheng (1995). The Mono Monte Carlo method is based on the condensed simulation approach described by Graaf et al. (1993), and was used to determine the optimal layout of fibres on the probe surface. The Hybrid Reflectance Recovery model is an adaptation of this approach designed to eliminate many simplifying assumptions inherent in previous Monte Carlo methods, and simulate the experimental setup as closely as possible. This was developed to allow optical property recovery from tissue despite the presence of non-
uniform boundary conditions, a complication observed when simulating application of the 

Mono Monte Carlo method with a small diameter probe.

In Chapter 3 an image processing method used to correct for distortion in an imaging spectrometer based system for collection of DRS spectra was presented. It was discovered that the lenses used to focus the output of the spectrometer onto a CCD introduced distortion into the data, especially at the outer edges of the spectrum. This cut off some of the data at high and low wavelengths, and therefore reduced the spectral range of the system.

Although not a component of the DRS system that is the primary subject of this thesis, the imaging spectrometer described in this paper was used extensively throughout the project as a calibration standard and reference, used to recover the optical properties of various reference materials. This spectrometer operates within a higher wavelength range than the system discussed in this thesis, with a limited region of overlap near the bottom end of the spectrum. To extend this region of overlap as much as possible and therefore optimize the ability to calibrate the new system, it was important to correct for distortion in the shorter wavelength portion of the reference spectrum.

The work described in this paper was essential to the progression of the project since it improved the reliability of the reference and calibration data used in the work described in chapter 4. The image processing algorithm and the software developed for its implementation will directly benefit researchers who continue to work with the imaging spectrometer based reference system. This method could prove valuable to outside groups
working with lens based imaging spectrometer systems for DRS or other spectroscopic applications.

In Chapter 4 experimental results for the recovery of optical properties from DRS data were presented. This paper used a modified approach to recover optical properties different than that proposed in Chapter 2. An empirical method was used to generate the reflectance table used for optical property recovery, instead of a Monte Carlo approach. A series of optical phantoms were created with varying concentrations of absorbing and scattering media. The optical properties of each sample were established using the reference system and methods presented in chapter 3. The reflectance of each of the phantoms was then measured using the new, compact-probe DRS system. These data were combined to create an optical property look up table similar to the end product of the Mono Monte Carlo method of Chapter 2. This look up table can be used to determine the optical properties of samples or tissue measured with the compact probe system using a Levenberg-Marquardt curve fitting algorithm. This empirically generated look up table is less flexible than the concept presented in Chapter 2 since it is not scalable: each reflectance data set in the table corresponds to a single pair of optical properties. Because of this, measurements of many different samples covering a wide range of optical properties are required to provide adequate resolution and dynamic range.

The reflectance grid generated through this approach was qualitatively compared with the results of Monte Carlo simulations to verify its consistency with expectations. Measurements on selected Intralipid/ink optical phantoms were used to validate results obtained using this algorithm.
Overall, the work presented in this thesis represents a significant step towards the deployment of a novel clinical system for optical biopsy of human brain tissue. This device will allow for the collection of co-registered fluorescence and reflectance spectra in small surgical cavities. This opens up exciting new possibilities for surgical guidance during tumour resection procedures, aiding surgeons by allowing real time histological analysis and characterization of malignant versus healthy tissue. This has the potential to improve patient outcomes by reducing the time required for surgery, and increasing the chances of achieving full tumour resection. During pre-clinical, laboratory use, such a device will allow for the analysis of co-registered fluorescence and reflectance spectra collected directly from tissue samples, contributing to the field of biomedical optics by improving our understanding of light-tissue interactions and further characterizing the optical properties of tissue.

The equipment and algorithms for optical property recovery developed in this thesis, although intended specifically for use in brain tissue, can be adapted for use in a wide variety of different situations. Because of the large difference in scattering and absorption coefficients in white versus grey matter, the system was designed to handle a wide range of optical properties similar to those found in a variety of different tissue types. The small diameter of the probe may allow use in a various surgical applications, including laparoscopic procedures in which the site is accessed through a narrow opening. The Monte Carlo simulations and algorithms presented in this thesis, although designed specifically for use with the probe described in chapter 2, can be readily adapted to simulate a wide variety of fibre layouts and probe designs or materials. This opens up
further possibilities for use with custom probes designed to accommodate the unique constraints of an intended application.

5.2 Optical Property Recovery: Challenges and Limitations

Although studied extensively for many years, challenges persist when attempting to recover optical properties from measured reflectance data. No simulation or theoretical model perfectly represents or predicts the realities of the complex interactions between light and tissue, and assumptions or simplifications are required to make the problem manageable. Disagreement between theory and experiment is unavoidable, and the extent that can be tolerated depends on the constraints of the intended application.

The degree of accuracy required for optical properties recovered using this system is not yet known. This partly due to the fact that the end use of these optical properties is twofold: to provide an independent analysis of brain tissue under investigation; and to aid in the interpretation of TR-LIFS results, potentially allowing for intrinsic fluorescence analysis. As an independent source of contrast for tissue analysis, it is not the accuracy of the optical properties that is critical, but the precision: so long as the system is able to consistently return one set of results for malignant tissue, and another for healthy tissue, it can be applied successfully. When used in intrinsic fluorescence analysis however, the accuracy of recovered optical properties becomes more important. In this case, the optical properties are used to quantify the number of scattering and absorption events experienced by both excitation and fluorescent photons, and eliminate this influence from the resultant fluorescent signal. Inaccuracies in the optical properties can lead to an
incorrect description of the bulk tissue, and therefore affect the modelled intrinsic fluorescence.

One challenge encountered when modelling photon transport through tissue is the choice of phase function (Calabro & Bigio, 2014; Kienle, Forster, & Hibst, 2001). The Henyey-Greenstein phase function, used in this thesis, has been shown to under-represent the number of high-angle scattering events: events which have more influence on the final reflectance as the source-detector distance is decreased (Mourant, Boyer, Hielscher, & Bigio, 1996). Mie theory may demonstrate better performance at short distances, but is complex and requires additional assumptions including the need to treat all scattering centres as uniformly sized spherical or cylindrical objects. Various groups have proposed hybrid phase-functions which consider higher orders of the phase function (Bevilacqua & Depeursinge, 1999) or even a unique phase function corrected solution to the diffusion equation (Vitkin et al., 2011). Each approach has its own advantages, but continues to involve some level of approximation with associated trade-offs between accuracy and complexity and/or computation time.

The Henyey-Greenstein phase function was chosen for this work, as it provides a closed form solution that can easily be sampled to provide data points for use in Monte Carlo code. It was felt that the source to detector distances in this probe were large enough to obtain acceptable performance. Other reasons for sticking with the Henyey Greenstein model included its familiarity and establishment within biomedical optics research, and its existing integration in simulation code used by this lab. Calabro & Bigio (2014) have demonstrated that reflectance results are insensitive to the specific form of
the phase functions when the product of the reduced scattering coefficient and the source-detector separation, \( \mu' \cdot \rho \) is approximately 0.7. For the probe design chosen in this work, this is realized in one or more fibres when the reduced scattering coefficient is between approximately 0.5 and 2 mm\(^{-1}\), the range used during investigations of Intralipid phantoms.

Although the model developed in Chapter 2 was intended to simulate experimental conditions as closely as possible, certain assumptions about the physical environment were required for practical use. This included the assumption of a uniform, flat sample surface with no gaps between the studied material and the probe. During initial investigations with a large diameter probe, occasional inconsistencies were realized when the probe was placed into a liquid solution too quickly, trapping air bubbles between the probe surface and the phantom material. A similar situation was encountered when working with solid phantoms. It is suspected that roughness in the surface of the phantom, or non-parallel alignment between the probe and the phantom surface introduced non-uniform air gaps between fibres and the surface of the phantom. This changed the relative refractive index encountered by photons exiting the material, and introduced further variations between the theoretical model and physical setup.

In clinical applications, physical restrictions or inconsistent technique may sometimes mean that the probe is not placed flat against the tissue, causing similar complications. Although a more uniform boundary can be created by applying more pressure to a pliable surface such as brain tissue, the need to avoid further injury or puncture of the tissue may prevent this in a clinical setting. Applying additional pressure
to tissue can also be undesirable when interpreting results, as compression has been shown to alter the optical properties of tissue by altering cellular shape and changing the blood volume within the area (Chan et al., 1996; Lim, Nichols, Rajaram, & Tunnell, 2011; Ti & Lin, 2008).

A common assumption during Monte Carlo modelling of tissue optics is the use of homogeneous optical properties to represent the entire volume of the tissue/phantom material. This assumption is reasonable when using solid phantoms provided that the initial suspension is well mixed and that little to no settling of the scattering or absorbing additives occurred during hardening. Although this assumption seems reasonable for well mixed liquid phantoms, recent research by Bodenschatz et al. (2015) suggests that the situation is more complicated when working with Intralipid phantoms such as those of Chapter 4. Their research demonstrated that Intralipid-water emulsions begin to form a time-dependent, thin but highly scattering layer at its surface. The authors estimate that a homogeneous model for the optical properties of Intralipid introduces errors with a magnitude increasing from a minimum of 7% immediately following stirring up to 25% an hour later when using a spatial frequency domain system. For a spatially resolved DRS system similar to the type discussed in this thesis, the authors estimated errors ranging from 3% to 10% over an hour. The authors also note when performing spatially resolved measurements that small source detector separations show much stronger sensitivity to inhomogeneities and surface flow than larger SSDs.

Although none of the issues discussed thus far are insurmountable, the combination of these and other minor but noticeable discrepancies between the model and
experiment can limit the effectiveness of model-based predictions and analysis. During the course of this work, the algorithm used to interpret results progressed from a theoretical approach based on simulation alone, to an empirical model dependent on repeated measurements of optical phantoms. This progression towards an empirical approach was necessitated by inconsistencies between model-based predictions and data recovered using the DRS system. Some of these inconsistencies resulted from the challenges inherent to optical property recovery discussed in this section, while others may be a consequence of the equipment used.

One equipment related challenge discovered during the work of Chapter 4 was obtaining suitable signal strength from the DRS detector fibres across the targeted spectral range. Two priorities were identified when initially designing the probe: minimizing the total diameter of the probe (a constraint of the intended application), and achieving an optimal fibre layout for use with a Monte Carlo based optical property recovery algorithm. Diameters of the DRS detector fibres were limited to 200 μm both to minimize the size of the probe and to approximate a point source and detector geometry as closely as practical. The goal at this time was to make use of the Mono Monte Carlo method for recovery of optical properties: a model that depends on the point source and detector approximation to allow scaling of the simulation results.

A consequence of using small diameter DRS fibres however, was low signal strength at the spectrometers. This was especially noticeable for fibres at the outermost SDD. This was mitigated to some extent by increasing the number of detectors at further distances. Although this improved signal strength at these distances, the 200x1000 μm
spectrometer entrance slit set a hard limit on the number of fibres that could be used. With 5 detectors at the outermost SDD aligned vertically within the connector, the height of the combination is 1.1 mm including cladding. This meant that, even assuming perfect alignment of the fibres and the entrance slit, at least 10% of the overall signal collected by these fibres was unusable. In reality, imperfect fibre spacing and mismatches between the angle of the fibre bundle and the spectrometer entrance slit produced an estimated maximum collection efficiency of 80%. Low signal strength at outer SDDs had the effect of reducing the signal to noise ratio (SNR) at these distances, and limited the ability of the algorithm to fit the data effectively. This was of particular concern when measuring samples with a high effective attenuation coefficient: attenuation within tissue sometimes reduced signal strength at the outermost SDD below usable levels.

Despite the challenges discussed in this section, the results published in this thesis appear to be consistent with those published by other groups. Clinical investigation and evaluation are required to draw proper conclusions on the efficacy of this equipment for 

\textit{in-vivo}, surgical use.

\section{Future Work and Directions}

The end goal for the equipment developed during the course of this thesis is \textit{in-vivo} clinical use within an operating room setting. The work presented here has demonstrated the development of both the equipment and the algorithms used to extract meaningful results from the data collected by this equipment. Suggestions for improvements and refinements that could be made to overcome challenges of \textit{in-vivo}
diffuse reflectance spectroscopy and optical property recovery and achieve this end goal are presented in this section.

One of the first goals that should be accomplished in pursuit of a clinically viable instrument is the testing of existing equipment in an operating room setting. This will identify aspects of the device that may be currently unsuited for \textit{in vivo} use, and identify logistical challenges involved in assembling and using the instrument in the already crowded space surrounding the operating room table. Additional constraints that may be identified during this process will help to shape further development of the equipment.

Part of the initial groundwork has been laid for a clinical, operating room trial during the pursuit of this thesis. An application for Research Ethics Board (REB) approval has been prepared, and the process has been initiated. This will have to be completed, and approval received before an evaluation can begin. The materials used in the construction of the probe and a solid optical phantom intended for in-procedure calibration of the DRS system were selected specifically such that they can be sterilized before being brought into the operating room. The phantom was sent to an outside lab for testing and certification of its sterilizability, while the manufacturer of the probe has certified the same. This documentation will have to be presented to the infection control department at Hamilton Health Sciences, and a case made to allow its use in the operating room environment.

A challenge specific to the operating room environment that may be encountered when using this equipment in clinical studies is high ambient light. Operating theatres are generally well lit environments, with movable spotlights and headlamps used by the
surgical team to maximize their ability to see every detail during the procedure. This can interfere with DRS results, as ambient light raises the intensity of the background in each acquired spectrum. This decreases the signal to noise ratio (SNR), an all-important quantity in signal analysis that determines the ability of the system to distinguish spectral features from random noise. To date, testing has been performed in a lab with the lights dimmed or turned completely off, to maximize SNR. In a clinical trial of a DRS system published by Lin, Toms, Johnson, Jansen, & Mahadevan-Jansen (2001), researchers temporarily turned off the room lighting and instructed the surgical team to redirect spotlights away from the patient when taking measurements in the surgical cavity. If low SNR due to background light proves to be an obstacle in clinical testing and usage of the instrument discussed in this thesis, this approach should be adopted and the general room lighting turned off if possible. The surgical team’s use of fibre optic headlamps, designed to provide high intensity illumination to the area the surgeon is currently working on, will cause additional complications. Since it may prove impractical to dedicate a member of the surgical team to switch this light on or off as needed during measurements, it may be necessary to ask the surgeon to position the probe and look away as a measurements are acquired.

If testing shows that ambient light levels are still too high despite the measures proposed above, equipment changes may prove necessary. The SNR at the outermost SDD could be improved by increasing the illumination power of the light source. This would increase the ratio of diffusely reflected light (the signal) to ambient background. Simply increasing the power of the source, however, would increase the total power
collected by every detector, and could cause saturation of the spectrometer at shorter SDDs. This problem has been experienced when measuring highly scattering samples. In these cases, the spectrometer at the shortest SDD remained saturated even when its integration time was reduced to the minimum possible setting. The only option in this situation was to reduce the intensity of the light source, thus decreasing the SNR at outer SDDs. Increasing the illumination power of the system would therefore require corresponding upgrades to the dynamic range of the detection system to continue practical use with highly scattering samples.

When designing the light source described in Chapter 4, a requirement was emission in the near UV portion of the spectrum. This was intended to provide overlap in the spectral ranges analyzed by the DRS and TR-LIFS components. The solution described in Chapter 4, while effective in terms of spectral coverage, is dependent on a series of optical components that defocus, combine, and refocus the light from four different distinct-wavelength sources (three LEDs, one halogen lamp) in two separate housings. Inherent losses in the optics due to reflection at material boundaries combined with imperfect focussing due to the use of non-point sources (filaments and LED dies) resulted in significant power losses before the signal enters the source fibre.

If higher illumination power proves necessary during clinical evaluation of this instrument, alternate sources of broad spectrum illumination should be considered. Xenon arc lamps have been used effectively by other research groups (de Veld et al., 2005; Zonios et al., 1999), providing high power illumination across a wide spectral range as demonstrated in, adapted from Petrasch et al.(2006). Due to their small arc length, short-
arc xenon lamps can be more readily focussed, an advantage over long filament sources. For very high output power across a wide spectral range, a laser driven light source (LDLS) could prove advantageous. This unique light source uses a focussed, continuous wave laser to create a plasma inside a xenon bulb. Photons emitted by this plasma form the output of the light source. At the time of writing, a patent for this technology was held by Energetiq Technology, Inc., Woburn, MA (Smith, 2015). This manufacturer advertises an intensity of up to 20 mW·mm$^{-2}$·nm$^{-1}$·sr$^{-1}$, with a relatively flat output spectrum across a range of interest in this thesis (365-750 nm). Although considered during the initial stages of this project, xenon arc lamps and LDLS were rejected due to high cost. Given the experience of this thesis, and the anticipation of higher ambient levels in practical application, however, a xenon light source may prove necessary to improve signal power while maintaining a wide spectral range.

![Figure 5.1: Example output spectrum from a Xenon arc lamp. Adapted from Petrasch et. al (2006)](image-url)
While a high-power source with a wide spectral range is desirable, practical considerations such as the cost of the system and spectral limitations of other components in the optical chain may necessitate trade-offs. Target wavelength ranges should be identified, and high power but narrow band illumination options such as fibre coupled LED sources considered. This could reduce costs while allowing system performance to be optimized within target spectral bands.

Another option that could be considered to increase signal power would be an increase in the diameters of the source and/or detector fibres. While this would require a re-make of the fibre optic probe, this option could prove more cost-effective than an upgrade to the light source. Both options should be considered.

As discussed, increased illumination power will require a corresponding increase in the dynamic range of the detection system in order to balance the response across all SDDs. Two options for increased dynamic range should be considered: improvements to the inherent range of the spectrometers, and selectable attenuation of individual signals.

The first option is the purchase of spectrometers with a higher dynamic range: a measure of the difference between the saturation point of the detectors (maximum power) and the dark signal, the reading generated when the detectors are shielded (unexposed). The spectrometers used in this project to date contained a charge-coupled device (CCD) sensor. These detectors have seen wide spread use in photography, and are commonly used in spectrometers. An alternative technology that should be considered is the photodiode array (PDA). A PDA consists of many photodiodes spaced linearly throughout a silicon wafer, each one individually connected to a dedicated
biasing/readout circuit. The current generated in each diode while illuminated is measured individually. This is in contrast to a CCD, which collects charge in each pixel, then transfers the charge between pixels to a readout circuit, thus reading multiple pixels with one readout circuit. The photodiodes in a PDA are able to measure a higher signal before saturation, thus producing a detector with a high dynamic range.

A second option for increasing the dynamic range of the overall system is the use of individual attenuators, one for each SDD. This requires the installation of external attenuators at the input to each spectrometer in the system. This allows fine control over the signal collected by each spectrometer: if the intensity of the light source has been increased to improve the SNR of the outermost fibre bundle, the attenuator on the innermost bundle can be reduced to prevent saturation. Although this type of adjustment is possible in the existing system by changing the integration time of each spectrometer, control is limited by the timing resolution and minimum shutter time of the spectrometers. Better control over the signal power could be achieved through the combination of spectrometer integration time (coarse adjustment) and attenuators (fine adjustment). This possibility was explored during the course of this work, using the same attenuators as the Mark II, the reference system discussed in Chapter 3. These attenuators used a combination of a collimating lens, an adjustable iris, and a focusing lens to couple the signal between two SMA terminated optical fibres. These were found to be unsuitable for the intended application, however as the vertical layout of fibres in the detector cables was incompatible with this attenuator design. Additionally, the lenses used in this model
were optimized for 750nm and exhibited very low transmission in the 400 to 500 nm range.

In order to increase the dynamic range of the DRS system for future clinical use, alternate attenuator designs and/or a rearrangement of fibres should be explored. For practical usage in a clinical environment, motorized attenuators should be acquired, thus allowing rapid, computer controlled balancing of the signal during each measurement.

As previously discussed in Chapter 4, a challenge already encountered during this work was inconsistency in the coupling between optical fibres and spectrometers. Application constraints required that the probe assembly be detachable from the spectrometers to allow sterilization of the probe and cables before and after each surgical procedure. Each time the system was reassembled, significant variations in the coupling were observed. This inconsistency was worsened as a result of the fibre positioning in each cable, since coupling was dependent on the angle between the spectrometer entrance slit and the fibre bundle.

To further complicate matters, four different spectrometers were in use: three to collect the signal from the fibre bundles (one bundle per SDD), and a fourth monitoring the output power of the light source. Independent drift in the response of each spectrometer led to inconsistencies in the results returned for a given sample. The calibration procedure discussed in Chapter 4 was designed to compensate for variability in coupling and illumination intensity through the use of a single, reference phantom to which all measurements were normalized. Although valid in theory, in practice the sources of variability discussed here combined with inconsistent coupling at the fibre-
phantom boundary frequently led to an inability to relate measurements taken on one occasion to measurements on another.

During clinical usage of this instrument there will likely be a limited time period during which equipment can be assembled before surgery. This may further exacerbate the challenges discussed above, since there will be less time available to optimize positioning of the connectors for all three SDDs. A single spectrometer solution similar to that used by the Mark II system should be re-evaluated. This would serve to eliminate variability in spectrometer response, and would allow the use of a single connector coupling the signal from the detector fibres in to the spectrometer, thus ensuring consistent coupling for all SDDs. A single spectrometer solution would require the use of external attenuators to maximize the dynamic range of the system.

5.3.1 Summary of Recommendations

Moving forward, the following multi-step approach is recommended during clinical investigations of the DRS component of this optical biopsy instrument.

1) Using the existing equipment, assess conditions in the operating room environment. Observe ambient lighting conditions, collect diffuse reflectance spectra from human brain tissue in-vivo. Communicate with the surgical team and discuss the feasibility of background reduction measures such as shutting off room lighting and personal headlamps.

2) If increased signal power is required to overcome ambient lighting conditions, investigate the use of a higher power light source. Broadband options such as
xenon light sources should be considered alongside less expensive but spectrally limited solutions such as multiple fibre coupled LEDs.

3) Should a higher power light source prove insufficient or impractical, consider modifications to the fibre-optic probe. Increasing the diameter of the source and/or detector fibres will improve signal power.

4) With increased signal power, evaluate the dynamic range of the detection system. Consider the use of photo diode arrays to improve the intrinsic dynamic range of the detectors, or variable attenuators to allow balancing of the signal from each SDD.

5) Should inconsistent probe-spectrometer coupling and/or drift in spectrometer response prove incompatible with clinical usage, consider implementation of a single-spectrometer solution.

References


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Appendix A

In-Vitro Measurements of the Diffuse Reflectance of Bovine Tissue

This section presents an investigation on the consequences of using small volume tissue samples for \textit{in vitro} diffuse reflectance measurements. This was intended to investigate the hypothesis that DRS measurements are affected by the leakage of light from very small volume tissue samples by examining the effect of decreasing sample volume on the DRS signal from bovine brains. This work was presented as a scientific poster at the 2010 annual scientific meeting of the Canadian Organization of Medical Physicists.

A.1 Methods

Food grade bovine brain was purchased from a local butcher shop. The age of the brains was unknown, but they were kept refrigerated from the time of slaughter to the time of the experiment. Large samples in the shape of a rectangular prism were cut out of the bulk tissue. The samples were large enough to be considered semi-infinite, with little to no light exiting out from the sides or bottom. The sizes of the samples were measured and they were covered with saline to prevent drying. The top layer of each sample consisted entirely of white or grey matter.

The probe of the DRS system was placed in direct contact with the top of each sample, near to the centre. A background measurement was taken with the light source off. The light source was then turned on and multiple reflectance measurements were acquired. Each measurement was processed by subtracting the background and normalizing to a reference signal from the light source. This was repeated at five or six
different locations, in order to estimate the signal variation across the centre of the sample. The sample was then decreased in size by removing a small amount of tissue and re-measured. This process was continued until the sample was too small for easy manipulation. Data were collected for sample volumes ranging from as high as 10 000 mm\(^3\) to as low as 30 mm\(^3\).

The probe used in this study was designed for TR-LIFS data collection. The source fibre is located in the centre, surrounded by 12 detector fibres in a circular pattern. Since the signals from the detector fibres were summed together, the probe cannot provide spatially resolved reflectance information.

The range of radial distances used to generate the reflectance data was initially set at 0.3 to 3.9 mm. The maximum distance was decreased in increments of 0.3 mm to simulate the use of probes with smaller footprints, and the previous analysis was repeated.
A.2 Results and Discussion

![Graph showing DRS Spectrum](image)

A typical diffuse reflectance spectrum for bovine brain tissue is shown in Figure A.1. The green line is the reflectance and the flat line (blue) is the reference signal used for normalization. The plotted values are the mean across each measurement for a sample volume of 4725 mm$^3$, and the error bars are standard deviation. Plots of the reflectance signal versus sample volume are shown in Figure A.2 for a wavelength of 496 nm.

The results for the tissue sample presented here suggest that the DRS signal strength drops when the sample volume is decreased below 500 mm$^3$. This trend was not seen for all samples. Some showed similar results while others demonstrated an increase in reflectance at small volumes. Any final conclusions on the relationship between sample size and DRS signal need further investigation.
Figure A.2 DRS signal at a wavelength of 496 nm (a) white matter (b) grey matter