2011

INFLUENCE OF TISSUE ABSORPTION AND SCATTERING ON DIFFUSE CORRELATION SPECTROSCOPY BLOOD FLOW MEASUREMENTS

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INFLUENCE OF TISSUE ABSORPTION AND SCATTERING ON DIFFUSE CORRELATION SPECTROSCOPY BLOOD FLOW MEASUREMENTS

This investigation evaluates the influences of optical property assumptions on near-infrared diffuse correlation spectroscopy (DCS) flow index measurements. Independent variation is induced in optical properties, absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$), of liquid phantoms with concurrent measurements of flow indices. A hybrid instrument is incorporated consisting of a dual-wavelength (785 and 830 nm) DCS flow device to obtain flow indices and a frequency-domain tissue-oximeter for optical properties. Flow indices are calculated with measured $\mu_a$ and $\mu_s'$ or assumed constant $\mu_a$ and $\mu_s'$. Inaccurate $\mu_s'$ assumptions produced much larger flow index errors than inaccurate $\mu_a$. Underestimated/overestimated $\mu_s'$ from $-35%/+175\%$ lead to flow index errors of $+110%/-80\%$ and underestimated/overestimated $\mu_a$ from $-40%/+150\%$ lead to $-20%/+40\%$, regardless of wavelength. Analysis of a clinical study involving human head and neck tumors indicates flow index errors due to inter-patient optical property variations up to $+280\%$. Collectively, these findings suggest that studies involving significant $\mu_a$ and $\mu_s'$ changes should measure flow index and optical properties simultaneously to accurately extract blood flow information. This study provides unique insight through the use of liquid phantoms, hybrid instrumentation, incorporation of measurement errors and a generalization into DCS flow index errors due to the influences of optical properties.

KEYWORDS: Diffuse Correlation Spectroscopy, Diffusing Wave Spectroscopy, Near Infrared Spectroscopy, Tissue Optical Properties, Blood Flow

Daniel Irwin

7/27/2011
INFLUENCE OF TISSUE ABSORPTION AND SCATTERING ON DIFFUSE CORRELATION SPECTROSCOPY BLOOD FLOW MEASUREMENTS

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7/27/2011
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Daniel Irwin

The Graduate School
University of Kentucky
2011
THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biomedical Engineering in the Graduate School at the University of Kentucky

By

Daniel Irwin

Lexington, Kentucky

Director: Dr. Guoqiang Yu, Assistant Professor of Biomedical Engineering

Lexington, Kentucky

2011

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SYMBOLS

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<td>$\alpha$</td>
<td>Ratio of Moving Scatterers to Total Scatterers</td>
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<td>$\eta$</td>
<td>Viscosity</td>
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<td>$\lambda$</td>
<td>Wavelength</td>
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<td>$\rho$</td>
<td>Source-Detector Separation</td>
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<td>$H_b$</td>
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<td>$k_0^2$</td>
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<td>$\langle \Delta r^2(\tau) \rangle$</td>
<td>Mean-Square Displacement of Scatterers during $\tau$</td>
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<td>$\mathbf{r}$</td>
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<td>$S(\mathbf{r})$</td>
<td>Source Light Distribution</td>
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<td>$S_0$</td>
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<td>APD</td>
<td>Avalanche Photodiode</td>
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<td>BFI</td>
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<td>CW</td>
<td>Continuous Wave</td>
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<td>DOCT</td>
<td>Doppler Optical Coherence Tomography</td>
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<td>IRB</td>
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<td>LDF</td>
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<td>NIR</td>
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CHAPTER 1: INTRODUCTION

1.1 Blood Flow Measurements

Blood flow is widely used as a physiological parameter in the assessment of multiple facets of disease including diagnosis [1] and treatment efficacies [2]. Analyses of blood flow extend into many applications. For example, the identification of blood flow abnormalities may be investigated for diseases such as peripheral arterial disease (PAD) [3]. The increase or decrease of blood flow to ischemic muscle tissue after surgical techniques can provide useful information as to the success of the procedures [3]. Monitoring blood flow may reveal benefits of particular treatment methods which are very important in studies involving tumors [1, 2, 4]. The tumor blood flow response to applications of radiation treatment to subjects with tumors can reveal if the treatment is working [2, 4]. The tumor site may also potentially be identified through exhibition of blood flow different from that of surrounding tissues [1]. Blood flow responses may also be followed after induced stimuli to examine activity of the brain [5].

In practice, blood flow measurements can be achieved through a variety of noninvasive technologies. Each has their own advantages and disadvantages and thus many factors must be considered including costs, portability, subject anatomy, accuracy, data acquisition time, and sensitivity. Sensitivity concerns the differentiation between vessels of varying sizes from large, such as arteries, to small such as capillaries. For superficial blood flow measurements (several μm to mm), technologies such as laser Doppler flowmetry (LDF) [6-8], optical microangiography (OMAG) [9], Doppler optical coherence tomography (DOCT) [10], and photoacoustic tomography (PAT) [11] may be
utilized. Penetration into deep tissues (e.g., several centimeters) is a limitation of employing these methods. Several modalities exist capable of penetrating into deep tissues such as Xenon-enhanced computed tomography (Xe-CT) and positron emission tomography (PET). While these are predominantly sensitive to microvasculature, they require the need for undesirable exposure to effects such as radiation. The applicability of using these deep penetration devices bedside is limited due to high costs and low portability. A new technology, near-infrared (NIR) diffuse correlation spectroscopy (DCS), has sought to fill some of the gaps in deep tissue microvasculature blood flow measurements by means of low cost, noninvasive, fast and portable instrumentation.

1.2 Near Infrared Diffuse Optical Techniques

Our investigations involve the use of NIR light to probe deep tissues. NIR light is employed in biomedical applications due to the discovery of a spectral (600 – 900 nm) window (see Fig. 1) in biological tissues [12]. This window allows deep penetration due to the low tissue absorption. Diffuse optical systems can be utilized for the extraction of information including tissue optical properties, oxygenated hemoglobin concentration (HbO₂), deoxygenated hemoglobin concentration (Hb), and blood flow [12, 13]. Note that tissue optical properties are represented by the absorption coefficient, $\mu_a$ (cm$^{-1}$ units), and reduced scattering coefficient, $\mu_s'$ (cm$^{-1}$ units). We will refer to diffuse optical equipment measuring $\mu_a$, $\mu_s'$, HbO₂, and Hb as NIR spectroscopy (NIRS) and blood flow as DCS.

Typically, NIRS is separated into three paradigms: time-domain (TD) (additionally known as time-resolved spectroscopy, TRS), frequency-domain (FD), and continuous-
wave (CW). TD systems use light pulses and contain high information content but are expensive and complex [12, 13]. Modulated light from FD systems provide less information content than TD systems but are capable of separating $\mu_a$ and $\mu_s'$ and obtaining Hb and HbO$_2$ [12, 13]. The final paradigm employs a CW light source and is generally inexpensive and simple in comparison to the TD and FD paradigms. However, CW light provides limited information content making it difficult to decouple tissue optical properties [12, 13]. In this study we are only concerned with absolute $\mu_a$ and $\mu_s'$ from FD NIRS measurements.

![Figure 1. Spectral Window](image)

The wavelength-dependent absorption coefficients ($\mu_a$) shown for oxygenated hemoglobin (HbO$_2$), deoxygenated hemoglobin (Hb), and water (H$_2$O) with the NIR spectral window denoted with dashed lines for the wavelength range of 600 – 900 nm.

For NIRS measurements, a pair of source and detector optical fibers is placed on the tissue surface separated by a distance of up to a few centimeters. An NIR laser emits light
into tissue through the source fiber. Photon migrates in the tissue following a well-known diffusive process before being detected by a photodetector connected to the detector fiber [12, 14]. The tissue optical properties, $\mu_a$ and $\mu_s'$, represent the reciprocals of absorption and scattering length, respectively. The absorption length describes the distance a photon travels before encountering an absorption event and similarly for the scattering length corresponding to a scattering event. Note that the optical properties are wavelength, $\lambda$, dependent, i.e., $\mu_a (\lambda)$ and $\mu_s' (\lambda)$, but are written without the dependence notation throughout this paper. Light scattering in biological tissues is generally much greater than that of absorption resulting in diffusive behavior.

For the determination of particle dynamics (flow), speckle fluctuations in scattered light intensity are monitored [12, 13]. Previous success in flow measurements (e.g., laser Doppler) has been obtained in optically thin samples where single scattering photon correlation spectroscopy (PCS) can be assumed [13, 15, 16]. In probing the motions in thick/deep tissues, however, additional complications are encountered. Such optically thick tissue samples result in multiple scattering and randomization of light propagation. To address this multiple scattering problem DCS [14, 15, 17, 18], also known as diffusing wave spectroscopy (DWS) [5, 19], has been developed that takes advantage of the diffusive behavior of light as it propagates through the tissue.

1.3 Diffuse Correlation Spectroscopy (DCS)

DCS flow measurements are performed from the surface of the tissue region of interest, similar to NIRS. The flow measurements are obtained by monitoring the time autocorrelation function of speckle fluctuations detected at the photodetector. To
accomplish this, a long coherence length CW NIR laser is employed to provide a constant phase both spatially and temporally [12, 14, 16]. Speckle fluctuations in non-muscular biological tissues are primarily due to the movement of red blood cells (RBC’s) in vessels [1, 2, 4, 5, 12, 18, 20-38]. However, motion artifacts and tissue shearing may arise in muscular tissues which may complicate measurements [39, 40]. DCS produces a blood flow index (BFI) and subsequent relative blood flow (rBF) from those fluctuations. The rBF produced from DCS measurements has been validated against other technologies in many tissues. These include comparisons with Xe-CT [23], Doppler ultrasound [20, 21], LDF [12], power Doppler ultrasound [4, 22], arterial spin labeled magnetic resonance imaging (ASL-MRI) [25, 41], fluorescent microsphere measurements [24], and to literatures [17, 18, 26, 27, 42]. DCS systems have had their usage expanded into many deep tissue applications including brain [5, 12, 18, 20, 21, 23-27, 29, 33-37], muscle [3, 39-41, 43, 44], and tumor [1, 2, 4, 22, 28, 30-32].

DCS can be made simple, inexpensive, and portable with short acquisition times (from 6.5 ms up to several seconds) such that bedside monitoring is made feasible [1, 27, 29, 39]. DCS is sensitive predominantly to microvasculature instead of the larger blood vessels. An inherent feature to using optical modalities is the noninvasive nature of measurements without the need for imposing undesirable effects such as radiation exposure. Spatial resolution is relatively low (~cm) governed by the volume probed where the penetration depth is approximately half of the separation between source and detector fibers. Thus, DCS fills a unique niche with the ability to simply and quickly monitor blood flow in deep tissues with robust applicability.
1.4 DCS Measurement Limitations and Current Study

Within the DCS theoretical framework, the BFI calculation has a dependence on tissue properties, \( \mu_a \) and \( \mu_s' \). Thus, calculations in the DCS flow indices will be influenced by variations in these optical properties (see details in Chapter 2.1). An additional potential influence on the flow indices is the laser source wavelength chosen. As DCS requires a CW light source it is inherently incapable of monitoring absolute tissue optical properties during flow acquisition. Two general methods commonly serve as solutions to this dilemma: using separate instrumentation for monitoring the optical properties or making assumptions of their values. In some recent studies, hybrid instrumentation has been used to monitor both sets of information in order to establish accurate blood flow data [1, 2, 21, 39, 45]. This method may be costly or unavailable and in such cases requires making assumptions as to the optical properties of the particular tissue being investigated. In studies where assumptions in optical properties are made typically either the \( \mu_s' \) is assumed constant while \( \mu_a \) changes are measured [24, 36, 43] or the values for both \( \mu_a \) and \( \mu_s' \) are obtained from literatures for the respective tissue type (e.g., brain or tumor) [20, 40]. However, by making optical property assumptions there will be an increased susceptibility to longitudinal, transient, and inter-subject deviations from the assumed values or differences between literatures.

Another concern is that flow indices in DCS tissue measurements actually correspond to a calculated effective Brownian diffusion coefficient (see Chapter 2.1). This diffusion coefficient is considered to be different from the conventional Brownian diffusion coefficient as predicted by Einstein [46]. It has been determined empirically that dynamic scatterer motions (typically microvasculature RBC’s) are best modeled by Brownian
diffusion rather than random ballistic flow, but the reasons for this are currently unknown [4, 12, 18, 20, 22, 28, 29, 36]. Thus, there is a lacking in some standard of comparison for the DCS flow index.

Currently, there has been no known generalization of potential DCS BFI errors due to inaccuracies in the estimation of the optical properties. A formal study is needed to elucidate upon the lack of optical property data while making DCS blood flow measurements. We have recently built a hybrid instrument in our lab capable of measuring flow indices using DCS and absolute $\mu_a$ and $\mu_s'$ using NIRS at several wavelengths simultaneously [44]. With this hybrid instrument we now have the capacity to evaluate and quantify optical property influences on DCS flow indices at different wavelengths. The FD NIRS system is incorporated to obtain the absolute $\mu_a$ and $\mu_s'$ at multiple wavelengths. These tissue optical properties are extracted from the modulated light AC, DC, and Phase information as it passes through the sample.

We have created homogeneous liquid phantoms allowing for manipulation of the optical property variations. NIRS and DCS techniques commonly employ liquid phantoms to emulate tissue for experimental and calibration purposes [13, 14, 18, 47-53]. Each parameter, either $\mu_a$ or $\mu_s'$, are varied individually to isolate their respective influence on DCS flow indices. By using liquid phantoms a standard for comparison with DCS is now possible. The phantoms include spherical particles suspended in liquid and undergoing Brownian motion as modeled by Einstein. Contrary to tissue, DCS measurements on these liquid phantoms should produce effective Brownian diffusion coefficients (i.e., DCS flow indices) which are now equivalent to the Einstein predictions. By utilizing this situation, the Brownian diffusion coefficient found using the Einstein-
Stokes formula [46] can be considered a true flow index. This can subsequently be used in comparison to DCS flow indices calculated using assumed or measured optical properties. Errors can then be determined in DCS flow indices due to the inaccurate optical property estimations at multiple wavelengths.

This study resolves the need for combining instrumentation to monitor both blood flow using DCS technology simultaneously with acquisition of tissue optical properties. We utilize liquid phantoms as a valuable means to investigate the influences of these optical properties on DCS flow indices. In addition, through comparisons of DCS flow indices with Brownian diffusion coefficients (true flow indices) we are capable of quantifying measurement errors in our system and methods. To further investigate the implications of the phantom study, a clinical study performed with the hybrid instrument for monitoring blood flow indices and optical properties of head and neck tumors is analyzed. Determination of in-vivo applicability involves ascertaining the measurement errors in tumor blood flow indices which are discussed and compared to the phantom study findings.

This thesis is organized into the following chapters. Chapter 2 opens with discussion of the theory used in DCS BFI calculations along with a brief overview of absolute $\mu_a$ and $\mu_s$’ measurements and hybrid instrument operation. Details pertaining to Brownian motion and liquid phantoms are also provided. The experimental protocols and methods of data analysis for the phantom study and in-vivo tumor study complete the chapter. The results of the phantom experiments and tumor study analysis are detailed in Chapter 3 with respective discussion and conclusions in Chapter 4. Finally, Chapter 5 presents the
novel contributions in this study along with directions for future research and investigations.
A hybrid NIR diffuse optical instrument combining a commercial frequency-domain NIR tissue-oximeter, the Imagent (ISS, Inc., IL, USA) [47, 48], and a custom-made NIR DCS flow-oximeter [3, 37, 44] was used in this study for simultaneous measurements of tissue optical properties and flow indices (see Fig. 2). The influences of optical properties on blood flow indices were examined in liquid phantoms with varied optical properties and in head and neck tumors using hybrid optical instruments. This chapter is organized as follows. First, the theories involved in obtaining DCS flow indices (i.e., effective Brownian diffusion coefficients) (Chapter 2.1) and tissue optical properties (i.e., $\mu_a$ and $\mu_s'$) (Chapter 2.2) are introduced along with the hybrid instrument data acquisition details (Chapter 2.3). Afterwards, the manner of determining Brownian diffusion coefficients (Chapter 2.4) is described. Next, the methods used for creating the liquid phantoms with varied optical properties are given (Chapter 2.5). Following is a discussion of the experimental protocols (Chapter 2.6) and data analysis (Chapter 2.7) in liquid phantoms. Finally, particulars concerning an analysis of real tissue data in a clinical study of head and neck tumors are provided (Chapter 2.8).
Figure 2. Hybrid Instrument

The combined Imagent and DCS hybrid instrument used during the phantom study with: (a) hybrid optical probe, (b) DCS flow-oximeter with 2 multi-mode laser source fibers (200 µm diameter) bundled together (785 and 830 nm) at 1.5 cm separation from 4 single-mode detector fibers (5.6 µm diameter) bundled together, (c) Imagent with 8 laser source fibers arranged as 1 per each wavelength at 4 separations (2.0, 2.5, 3.0, and 3.5 cm) of 780 and 830 nm along with 1 detector fiber, and (d) hybrid instrument. Note that two additional DCS detector fibers are shown, at 2.4 and 2.8 cm separations, but were not connected for this study.

2.1 Diffuse Correlation Spectroscopy (DCS) for Blood Flow Measurements

The dual-wavelength DCS system [44] with two long coherence length CW NIR laser sources at 785 and 830 nm (100 mW, Crystalaser, Inc., NV, USA) is used to quantify DCS flow indices from measurements on turbid samples such as biological tissues and liquid phantoms. DCS laser light is emitted into the tissue alternately by means of two multi-mode optical fibers (200 µm diameter). These fibers are bundled together on the tissue surface at the same position (see Fig. 2). Four single-mode detector fibers (5.6 µm diameter) are bundled and placed on the tissue surface as well with 1.5 cm separation.
from the source fiber bundle, combined into a rectangular foam pad. Each detector fiber is connected to a single photon-counting avalanche photodiode (APD) (PerkinElmer, Inc., Canada). A 4-channel autocorrelator board (Correlator.com, NJ, USA) receives the outputs of the 4 APDs and produces normalized light intensity temporal autocorrelation functions ($g_2$). The $g_2$’s from four detectors are averaged to improve the signal-noise-ratio (SNR). The averaged $g_2$ can then be related to the normalized electric field temporal autocorrelation function ($g_1$) through the Siegert relation [54],

$$g_2(\vec{r}, \tau) = 1 + \beta |g_1(\vec{r}, \tau)|^2$$  \hspace{1cm} (1)

where $\tau$ is the delay time, $\vec{r}$ is the position vector, and $\beta$ depends on laser stability and coherence length and the number of speckles detected.

Although in practice DCS measures $g_2$, the analytical solution is for $g_1$ derived from the transport of the unnormalized temporal electric field correlation function ($G_1$) through the turbid sample. The moving scatterers will contribute to the exponential decay of $G_1$. The transport of $G_1$ results in the following correlation diffusion equation, derived rigorously elsewhere [13, 14], for homogeneous media with a CW source (steady state):

$$\left( D \nabla^2 - \nu \mu_a - \frac{1}{3} \nu \mu_s' k_0^2 \alpha \left\langle \Delta r^2(\tau) \right\rangle \right) G_1(\vec{r}, \tau) = -\nu S(\vec{r})$$  \hspace{1cm} (2)

where $D = \nu / (3\mu_s')$ is the photon diffusion coefficient, $\nu$ is the speed of light in the medium, $k_0^2$ is the wavenumber, $S(\vec{r})$ is the source light distribution, and $\left\langle \Delta r^2(\tau) \right\rangle$ is the mean-square displacement of scatterers in time $\tau$. The position vector, $\vec{r}$, denotes a general vector from a source to a point of detection. Notice that $g_1$ is the normalized form of $G_1$, as used in Eq. 1, i.e., $g_1(\vec{r}, \tau) = G_1(\vec{r}, \tau) / G_1(\vec{r}, 0)$. 

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The homogeneous CW solution to Eq. 2 for semi-infinite geometry is (see Fig. 3):

\[ G_t(\rho, \tau) = \frac{v S_0}{4\pi D} \left( \frac{\exp(-K(\tau) r_1)}{r_1} - \frac{\exp(-K(\tau) r_2)}{r_2} \right) \]  

(3)

where \( S_0 \) is the source intensity, \( \rho \) is the separation distance between source and detector,

\[ K^2(\tau) = 3\mu_s \mu'_s + \mu_s^2 \kappa_0^2 \alpha \langle \Delta r^2(\tau) \rangle, \quad r_1 = \sqrt{\rho^2 + (z - z_0)^2}, \quad r_2 = \sqrt{\rho^2 + (z + z_0 + 2z_b)^2}, \quad z_0 = 1/\mu_s, \]

\[ z_b = 2(1 + R_{\text{eff}})/3\mu_s(1 - R_{\text{eff}}), \quad R_{\text{eff}} = -1.440 \ n^{-2} + 0.710 \ n^{-1} + 0.668 + 0.0636 \ n, \quad \text{and} \quad n \approx 1.33 \] (for phantoms and tissues) [12, 13, 55, 56]. The \( R_{\text{eff}} \) term accounts for the mismatch between the medium and air index of refraction with \( n \) being the ratio between them.

The collimating laser source is placed at \((0, 0, 0)\) and detector at \((\rho, 0, 0)\) on the tissue surface with \(z = 0\) (see Fig. 3) for semi-infinite geometry. The solution (Eq. 3) involves an isotropic source at \(z = z_0\) and negative isotropic imaging source at \(z = -(z_0 + 2z_b)\) with an extrapolated zero boundary condition. The position vector, \( \mathbf{r} \), from Eq. 2 regards the point source at \((0, 0, z_0)\) and negative imaging source at \((0, 0, -(z_0 + 2z_b))\). The superposition of solutions to these two sources with infinite geometry provides the resulting Eq. 3 where now the semi-infinite boundary is modeled by the scalar parameter, \( \rho \). Further details are given elsewhere [13, 56].

The movement of scatterer particles is typically characterized for biological tissues by Brownian motion with \( \langle \Delta r^2(\tau) \rangle = 6D_B \tau \), where \( D_B \) is the effective Brownian diffusion coefficient. In order to differentiate static and dynamic scatterer particles, an \( \alpha (0 - 1) \) term is added. This term is defined as the ratio of dynamic to total scatterers from the sample. The BFI produced by DCS in tissues is defined as the combined dynamic scatterer ratio with the effective Brownian diffusion coefficient, i.e., \( \alpha D_B \). The BFI can then be used to determine the rBF by comparison with the baseline BFI, \( \text{BFI}_{\text{Baseline}} \), prior
to any physiological changes, i.e., \( rBF = BFI / BFI_{baseline} \). In tissue samples, scatterers may be static (e.g., mitochondria, organelle) or dynamic (RBC). However, in liquid phantom solutions (see Chapter 2.5) all scatterers are dynamic resulting in \( \alpha \approx 1 \). Thus, the DCS flow indices in this thesis related to the phantom study are reported as just \( D_B \).

The process of DCS data acquisition beginning with tissue measurement (bottom left) and ending with (bottom right) production of a DCS flow index (\( \alpha_{DB} \)). Cartesian coordinates are shown with respect to DCS source-detector and tissue orientation with the y-axis directed into the page. A typical correlation curve is shown as an example, taken from a phantom experiment (\( \mu_a (830 \text{ nm}) = 0.05 \text{ cm}^{-1} \), \( \mu_s' (830 \text{ nm}) = 10 \text{ cm}^{-1} \)) with \( g_1 \) derived from \( g_2 \) measurements (\( g_{im} \)) using Eq. 1 and \( g_1 \) calculated (\( g_{ic} \)) using Eq. 3.

To quantify a flow index, \( g_2 (\mathbf{r}, \tau) \) is first obtained for a given sampling time (~44 ms) from the correlator where the sample is illuminated by a DCS source (either 785 or
830 nm). Multiple samples are obtained and averaged during a measuring time of ~1.2 s. From Eq. 1, \( \beta \) is found at \( \tau \approx 0 \) using measured \( g_2(\rho,0) \) and \( g_1(\rho,0) \approx 1 \) (i.e., \( g_1(\rho,0) = G_1(\rho,0)/G_1(\rho,0) = 1 \)). This leads to \( \beta = g_2(\rho,0) - 1 \). It is assumed that \( \beta \) will remain constant throughout the remaining \( \tau \) since it only depends on the optical system [15].

Now, \( g_1(\rho,\tau) \) can be calculated for all \( \tau \) with Eq. 1 using measured \( g_2(\rho,\tau) \) and calculated \( \beta \). The flow index (\( \alpha_{DB} \)) is then considered an unknown parameter in Eq. 3, which is fit with the \( g_1(\rho,\tau) \) calculated previously. For each DCS data acquisition sequence, a unique flow index is obtained for 785 and 830 nm. Two flow indices are obtained sequentially to obtain a complete frame of DCS data acquisitions at two wavelengths. The overall process of DCS data acquisition is given in Fig. 3.

### 2.2 Frequency Domain Spatially Resolved Near-Infrared Spectroscopy for Measurement of Optical Properties

A four-wavelength (690, 750, 780, and 830 nm) FD multi-distance spatially resolved spectroscopy instrument, i.e., the Imagent, is incorporated to quantify absolute \( \mu_a \) and \( \mu_s' \). Only two wavelengths (780 and 830 nm) were chosen so as to match the available DCS lasers (785 and 830 nm). Imagent measurements are obtained similar to DCS with semi-infinite geometry, by emitting NIR light into the tissue through source fibers with detector fibers up to a few cm away, both at the tissue surface. The FD Imagent system emits light sinusoidally modulated at 110 MHz. Optical fiber arrangement consists of 8 source fibers (4 per wavelength) placed at four pre-determined distances (2.0, 2.5, 3.0, and 3.5 cm) from a detector fiber bundle. The detector fiber is connected to a photomultiplier tube (PMT) (see Fig. 2). Utilizing the amplitudes of light signal
modulation (ac), average (dc), and phase ($\phi$), information can be detected from the different source-detector separations ($r$). It was found that linear relationships exist between logarithmic ac, logarithmic dc or $\phi$ and the spatial distances through solutions to a photon diffusion equation [48]. The slopes ($S_{\text{ac}}, S_{\text{dc}}, S_{\phi}$) of these linear relationships can be fit from the multi-distance measurements. From these slopes $\mu_a$ and $\mu_s'$ can be determined at both wavelengths. For the phantom study, by use of homogeneous phantoms the different source and detector separations are expected to have minimal effect on the measured optical properties despite probing different depths. In the real tissue tumor study analysis this effect may be more prominent (see Chapter 3.4).

In Imagent operation, the fitting solutions mentioned above are not ideal for real tissue measurements due to the requirement of iterative calculations. Therefore, a faster approximate solution is employed defined by the following equations [57]:

$$\ln\left(\text{dc } r^2\right) = rS_{\text{dc}}\left(\mu_a, \mu_s'\right) + \ln_{\text{dc}}\left(D, K_{\text{dc}}\right)$$

(4)

$$\Phi = rS_{\phi}\left(\mu_a, \mu_s', \omega, v\right) + \ln_{\phi}\left(K_{\phi}\right)$$

(5)

$$\ln\left(\text{ac } r^2\right) = rS_{\text{ac}}\left(\mu_a, \mu_s', \omega, v\right) + \ln_{\text{ac}}\left(D, K_{\text{ac}}\right)$$

(6)

where $\omega$ is the angular frequency of the modulation, $v$ is the speed of light in the medium, $D$ is the photon diffusion constant ($D \approx 1/3\mu_s'$), $K_{\phi}$ is the relative phase of the source plus any phase shifts outside the sample, $K_{\text{ac}}$ and $K_{\text{dc}}$ are constants dependent on detector sensitivity factors, modulation depth, and source intensity, and $\ln_{\text{dc}}, \ln_{\phi},$ and $\ln_{\text{ac}}$ are the line intercepts. Only two of the three slopes are required per wavelength to obtain corresponding $\mu_a$ and $\mu_s'$, but using the combination of $S_{\text{ac}}$ and $S_{\phi}$ eliminate influences form background light [47].
2.3 Hybrid Instrument Data Acquisition

The hybrid instrument alternates data acquisition sequentially between DCS and Imagent. An individual acquisition sequence begins with DCS activating the first laser and collecting data from the autocorrelator. The first laser is then switched off and the process is repeated for the second DCS laser. Notification is then sent from DCS to the Imagent via a TTL trigger to proceed with an iteration of data acquisition. The Imagent activates an individual laser source at the first distance collecting data from the PMT. Data is collected similarly for the Imagent laser activated alternately at each distance. The Imagent laser is then shut off and each remaining Imagent laser source (at different wavelengths) is sequentially activated to acquire data in the same manner before finally returning control to the DCS. The notification to DCS signifies the completion of one data acquisition cycle, which is then repeated until the end of the measurement. Total data acquisition time per frame is ~2.7 s (~1.2 s for each of two DCS wavelengths and ~0.3 s for Imagent). After the measurement is finished Imagent places AC, DC, and Phase information into a .txt file. The .txt file is further analyzed by supplemental Imagent processing functions, such as to produce $\mu_a$ and $\mu_s'$, and saved in a .log file.
2.4 Particle Brownian Motion in Liquid Phantoms

For comparisons with DCS flow indices, Einstein Brownian diffusion coefficients are determined for liquid phantoms. Intralipid particles provide Brownian motion within the phantoms. This motion is expected to be equivalent to the *effective* Brownian diffusion coefficient (flow index) as measured using DCS [46]. Determination of the phantom diffusion coefficient follows calculations per the Einstein-Stokes formula for spherical particles suspended in liquid defined as:

\[
D_B = \frac{k_B T}{6 \pi R \eta}
\]  

(7)

where \( R \) is the Intralipid spherical particle radius, \( \eta \) is the viscosity of the phantom, \( T \) is the temperature of the phantom, and \( k_B \) is the Boltzmann constant [46]. Due to difficulties in measuring the Intralipid particle radius, an estimation of 196 nm is used (see Chapter 2.7). The phantom viscosity is measured with a commercial viscometer (Brookfield, MA, USA). The viscosity parameter is reported in units of cP (centipoise), where 1 cP = 1 mPa·s (millipascal·second) = 0.001 kg·m⁻¹s⁻¹ [58]. Measurement of the phantom temperature is obtained via a temperature sensor (Physitemp, NJ, USA) attached near the hybrid probe. For further details on acquisition of this data see Chapter 2.6.

2.5 Liquid Phantom Creation

We follow previously published methods for creating liquid phantoms, but examine implementation details thereof here for clarity and re-creation purposes. The composition of liquid phantoms consist of India ink (Black India 44201, Higgins, MA, USA), Intralipid (30%, Fresenius Kabi, Uppsala, Sweden), and distilled water. Originally deionized water was used, but was difficult to obtain and produced similar results to
distilled water for our purposes. The complete liquid phantom setup can be seen in Fig. 4. The phantom solution is contained within a 9607.5 cm$^3$ aquarium (~9.5 L). The hybrid fiber-optic probe (see Fig. 2) is positioned on the phantom surface simulating superficial tissue measurement configuration of semi-infinite geometry. To keep the probe stationary, a custom probe holder was built by a machine shop. The holder maintains the probe near the center of the phantom solution, away from aquarium boundaries.

A 30% Intralipid solution provides particle Brownian motion, as described by Eq. 7. Additionally, it is used to manipulate the phantom reduced scattering coefficient, $\mu_s'$, while contributing minimally to phantom absorption. The phantom absorption
coefficient, $\mu_{\text{a, phantom}}$, is manipulated using the India ink with minimal influence on phantom scattering. Due to the very high contribution of absorption from pure India ink, a solution of 10% ink with 90% distilled water is created. The 10% solution is used rather than pure ink to manipulate the $\mu_{\text{a, phantom}}$. Distilled water contributes little to both phantom absorption and scattering, allowing for larger phantom volumes to facilitate satisfying the semi-infinite geometry model. The subscripts “water”, “ink”, and “Intralipid” denote distilled water, 10% ink solution, and 30% Intralipid, respectively. Prior to prediction of phantom optical properties, the $\mu_{\text{a}}$ and $\mu_{\text{s'}}$ of the contributing solutions must first be determined. These values will then be used in combination with titration equations for predicting the volumes of water, 10% ink solution, and 30% Intralipid needed to obtain the phantom optical properties required.

**Intralipid, ink, and water $\mu_{\text{a}}$.** Spectrometer (Beckman Coulter, CA, USA) measurements allow derivation of the 10% ink solution absorption coefficient, $\mu_{\text{a, ink}}$. The 10% ink solution is not within the measurable range of the spectrometer. Thus, for absorbance measurements the 10% solution is further diluted to a 0.025% ink solution (of ink and water). Multiple samples of the 0.025% ink solution are distributed into individual 1 cm path-length cuvettes and scanned by the spectrometer at 780 and 830 nm (matching the Imagent source wavelengths). These absorbances are averaged and converted to absorption by: $\mu_{\text{a, ink}}(\lambda) = \ln(10) \times \text{Absorbance}(\lambda)$ [59]. Values for $\mu_{\text{a, water}}$ at 780 and 830 nm are obtained from the literature [60]. As Intralipid is primarily comprised of water, it is assumed to have an absorption coefficient equivalent to water, i.e. $\mu_{\text{a, Intralipid}} = \mu_{\text{a, water}}$. 


Intralipid, ink, and water $\mu_s'$. The $\mu_s'$ of 30% Intralipid is derived from the theoretical values for 10% Intralipid. The $\mu_s'$ of 10% Intralipid is first calculated using a Mie theory approximation and subsequently multiplied by a factor of three to obtain the $\mu_s'$ of 30% Intralipid, $\mu_s'$ Intralipid [59]. The Intralipid particle radius and refractive index along with related theory and details are described in the original derivation [61], which has been used extensively for quantification of Intralipid-based liquid phantoms [12, 14, 59, 62, 63]. As there are expected to be no contributions to the phantom scattering by the 10% ink solution or distilled water both are ignored in calculations, i.e., $\mu_s'$ ink = $\mu_s'$ water = 0 cm$^{-1}$.

Liquid phantom $\mu_a$ variation. A list of desired $\mu_a$ and a constant $\mu_s'$ must first be chosen at a specific wavelength for creating phantoms with varied $\mu_a$ (see Chapter 2.6). Optical properties at other wavelengths are found later, once the phantom composition has been predicted. The available aquarium size is considered the total phantom volume, $V_{\text{phantom}}$. Volumes of distilled water ($V_{\text{water}}$), 10% ink solution ($V_{\text{ink}}$), and 30% Intralipid ($V_{\text{Intralipid}}$) required to obtain the set of $\mu_a$ and $\mu_s'$ are then calculated for the initial phantom. For our experiments, the initial phantom optical properties at 830 nm are: $\mu_a_{\text{phantom}}$ (830 nm) = 0.05 cm$^{-1}$ and $\mu_s'_{\text{phantom}}$ (830 nm) = 10 cm$^{-1}$. Using a titration equation, the 10% ink solution volume ($V_{\text{ink}}$) can be determined as follows [59]:

$$\mu_a_{\text{water}}(830\text{nm}) \times (V_{\text{phantom}} - V_{\text{ink}}) + \mu_a_{\text{ink}}(830\text{nm}) \times V_{\text{ink}} = \mu_a_{\text{phantom}}(830\text{nm}) \times V_{\text{phantom}}$$

(8)

The volume of 30% Intralipid ($V_{\text{Intralipid}}$) is determined using a similar titration equation [59],

$$\mu_s'_{\text{Intralipid}}(830\text{nm}) \times V_{\text{Intralipid}} = \mu_s'_{\text{phantom}}(830\text{nm}) \times V_{\text{phantom}}$$

(9)
The volume of distilled water is simply the remaining volume to be filled, calculated by: \( V_{\text{water}} = V_{\text{phantom}} - V_{\text{ink}} - V_{\text{Intralipid}} \). The 10% ink solution will be added in equivalent amounts each step to increase the phantom \( \mu_a \). Generalizing the titration equation to the volume of 10% ink solution to add, \( V_{\text{ink}}^{\text{add}} \), is given by [59]:

\[
\mu_a^{i-1}_{\text{phantom}} (830\text{nm}) \times V^{i-1}_{\text{phantom}} + \mu_a^{i}_{\text{ink}} (830\text{nm}) \times V_{\text{ink}}^{\text{add}} = \mu_a^i_{\text{phantom}} (830\text{nm}) \times (V^{i-1}_{\text{phantom}} + V_{\text{ink}}^{\text{add}})
\]

where \( i \) is the step (i.e., \( i = \) current step, \( i-1 = \) previous step). Notice that the total phantom volume for the next step will be the sum of the 10% ink solution volume added with the current volume. After the volumes have been determined Eq. 8 and Eq. 9 can be used with known volumes and constituent optical properties to predict the \( \mu_a^i_{\text{phantom}} (\lambda) \) and \( \mu_{s'}^i_{\text{phantom}} (\lambda) \) at other desired wavelengths.

**Liquid phantom \( \mu_{s'} \) variation.** The process for varying the phantom \( \mu_{s'} \) is like that of \( \mu_a \) variation, starting with selection of the constant \( \mu_a \) and list of \( \mu_{s'} \) (see Chapter 2.6). The initial phantom is created from the volumes of distilled water, 10% ink solution, and 30% Intralipid as calculated using Eq. 8 and 9. The desired initial phantom optical properties are: \( \mu_a^i_{\text{phantom}} (830 \text{ nm}) = 0.125 \text{ cm}^{-1} \) and \( \mu_{s'}^i_{\text{phantom}} (830 \text{ nm}) = 4 \text{ cm}^{-1} \). Contrary to the \( \mu_a \) variation, both 30% Intralipid and 10% ink solution need to be added at each step for \( \mu_{s'} \) variation. The additional 30% Intralipid each step contributes additional volume and lowers the phantom absorption. To account for these factors requires matching the \( \mu_a \) of the added 30% Intralipid to that of the phantom. This will maintain a constant phantom \( \mu_a \). The volume of 30% Intralipid to be added (\( V_{\text{Intralipid}}^{\text{add}} \)) is determined by the following titration equation [59]:
\[
\begin{align*}
\mu_{s,\text{phantom}}^{(830\text{nm})} \times V_{\text{phantom}} + \mu_{s,\text{Intralipid}}^{(830\text{nm})} \times V_{\text{Intralipid}}^{\text{add}} \\
= \mu_{s,\text{phantom}}^{(830\text{nm})} \times \left( V_{\text{phantom}} + V_{\text{Intralipid}}^{\text{add}} \right)
\end{align*}
\]

The quantity of 10% ink solution to be added \((V_{\text{ink}}^{\text{add}})\) is determined by a slightly modified form of Eq. 10 [59],

\[
\begin{align*}
\mu_{a,\text{phantom}}^{i-1}(830\text{nm}) \times V_{\text{phantom}} + \mu_{a,\text{ink}}^{(830\text{nm})} \times V_{\text{Ink}}^{\text{add}} + \mu_{a,\text{water}}^{(830\text{nm})} \times V_{\text{Intralipid}}^{\text{add}} \\
= \mu_{a,\text{phantom}}^{i}(830\text{nm}) \times \left( V_{\text{phantom}} + V_{\text{ink}}^{\text{add}} + V_{\text{Intralipid}}^{\text{add}} \right)
\end{align*}
\]

Once the additional volumes of 10% ink solution and 30% Intralipid are added and mixed with the phantom solution, an equivalent volume to that added \((V_{\text{remove}} = V_{\text{Intralipid}}^{\text{add}} + V_{\text{ink}}^{\text{add}})\) is removed to maintain probe submersion depth. These procedures are repeated for all remaining steps. To predict the optical properties at other wavelengths, the percentage of each constituent must first be determined. It is assumed the percentages are equivalent in the amount of volume removed as to the entire phantom. This allows one to obtain their corresponding volumes at every step. Then Eq. 8 and 9 are used as is done with \(\mu_{a}\) variation to predict the \(\mu_{a,\text{phantom}}(\lambda)\) and \(\mu_{s,\text{phantom}}'(\lambda)\) at desired wavelengths.

2.6 Phantom Experimental Protocols

\(\mu_{a}\) variation. In the first experiment, the \(\mu_{s,\text{'}}\) was maintained constant throughout: \(\mu_{s,\text{'}}(830\text{nm}) = 10\ \text{cm}^{-1}\). Conversely, \(\mu_{a}\) was varied in an increasing manner consisting of thirteen steps. The absorption range covers \(\mu_{a}(830\text{nm})\) from 0.05 to 0.20 cm\(^{-1}\) with a step size of 0.0125 cm\(^{-1}\) (i.e., \(\mu_{a}(830\text{nm}) = 0.05, 0.0625, 0.075, \ldots, 0.20\ \text{cm}^{-1}\)). Calibration of the Imagent with a phantom of known optical properties is done prior to
starting measurements. During calibration, corrections are made accounting for the
efficiency of optical coupling among the lasers/detector, optical fibers, and phantom [47,
57]. A liquid phantom of equivalent composition and optical properties as the midpoint
(step 7) was used for calibration. The initial phantom was created consisting of optical
properties at the lowest step, i.e., \( \mu_a (830 \text{ nm}) = 0.05 \text{ cm}^{-1} \). The hybrid probe with
temperature sensor was placed onto this phantom and the following actions were taken
for each of the 13 steps. The volume of 10% ink solution to add was determined as
detailed in Chapter 2.5. This volume was then added to the liquid phantom via a pipette,
mixed, and left to settle for 10 minutes while the optical properties and flow stabilized.
During the 10 minute period three 500 \( \mu \text{L} \) samples were extracted from the phantom for
viscosity measurements. The samples were taken from the right, middle, and left of the
solution to minimize spatial variations. The pipette sampled from within 1-2 cm of the
surface to prevent submersion. All lights in the room were switched off or covered with
black tape. Black plastic was used to cover the phantom setup to reduce any remaining
ambient light. Hybrid optical measurements (including temperature) were obtained for an
interval of 5 minutes duration.

\( \mu_s' \) variation. The second experiment maintained a constant \( \mu_a \) throughout: \( \mu_a (830 \text{ nm}) = 0.125 \text{ cm}^{-1} \). The \( \mu_s' \) was increased over a total of thirteen steps. The scattering
range covers \( \mu_s' (830 \text{ nm}) \) from 4 to 16 \( \text{cm}^{-1} \) with a step size of 1 \( \text{cm}^{-1} \) (i.e., \( \mu_s' (830 \text{ nm}) = 4, 5, 6, \ldots, 16 \text{ cm}^{-1} \)). This experiment immediately followed the \( \mu_a \) variation. Prior to
instantiating this second phase, the \( \mu_a \) variation phantom was disposed of and replaced by
the \( \mu_s' \) variation initial phantom. Alcohol pads were used to clean the probe before
repositioning it onto the initial phantom. The DCS and Imagent were not shut down
between protocols. Two potential difficulties arise with the addition of 30% Intralipid due to the amount required: potential reduction in the phantom $\mu_a$ and probe submersion. The addition of 10% ink solution with that of the additional 30% Intralipid allows the $\mu_a$ of the phantom to be maintained. To prevent probe submersion, after mixing at each step an equivalent amount of phantom solution is removed. Hybrid optical, temperature, and viscosity measurements were obtained in the same manner as during $\mu_a$ variation.

2.7 Phantom Data Analysis

For each 5 minute interval, the following parameters were obtained through measurements and post-analysis calculations: $\mu_a$, $\mu_s'$, temperature, viscosity, and three diffusion coefficients ($D_B$’s). Note that in all cases except viscosity, data between intervals (i.e., making additions, stirring) is not included in data analysis. Interval averages were found for $\mu_a$ and $\mu_s'$ at both wavelengths (780 and 830 nm) over each 5 minute interval. Temperature is also averaged over each 5 minute interval. Viscosity measurements were obtained at 50, 60, and 100 RPM for each of three samples and averaged. The liquid phantom was assumed a Newtonian fluid. The three sample average was then calculated to produce the mean viscosity per interval. Using the averaged $\mu_a$ and $\mu_s'$ as known parameters DCS measured $g_1$’s, calculated from Eq. 1 with measured $g_2$, are fit using Eq. 3 to produce two distinct $D_B$’s. Subscripts denote which set of optical properties (assumed or measured) were used in order to differentiate the two $D_B$’s. For assumed optical properties, the averaged $\mu_a$ and $\mu_s'$ from the middle interval (step 7), i.e., $\mu_a$ (830 nm) = 0.125 cm$^{-1}$ and $\mu_s'$ (830 nm) = 10 cm$^{-1}$, are used in calculations of the first DCS $D_B$, termed $D_B$-mid. The $D_B$-mid represents the diffusion coefficient without
consideration as to any optical property variations. Errors are induced in flow index calculations by forcing the varying optical property to be held constant. During early intervals the middle interval overestimates \( \mu_a \) (first experiment) and \( \mu_s' \) (second experiment) while at later intervals it underestimates. Corresponding interval \( \mu_a \) and \( \mu_s' \) averages are used in calculation of the second DCS \( D_B \), termed \( D_{B\text{-dynamic}} \). This \( D_B \) is considered the best evaluation of DCS flow index. \( D_{B\text{-mid}} \) and \( D_{B\text{-dynamic}} \) are calculated at both DCS wavelengths used, 785 and 830 nm. The DCS \( D_B \) calculations at 785 and 830 nm utilized the averaged optical property data from the Imagent at 780 and 830 nm, respectively. The influence from wavelength mismatch between 780 and 785 nm is considered to be minor. The two sets of DCS \( D_B \)'s, at each wavelength, are calculated then averaged over the 5 minute interval. A third \( D_B \) is calculated by Eq. 7, termed \( D_{B\text{-Einstein}} \), with the three sample averaged viscosity, interval averaged temperature, and estimated particle radius. The Intralipid particle radius estimation was found to exhibit the least errors between the measured DCS flow indices (\( D_{B\text{-dynamic}} \)) and calculated \( D_{B\text{-Einstein}} \) at the calibration point (step 7). This estimation, 196 nm, is within the Intralipid particle size range as reported in the literatures [14, 61].

Figures and tables are used for results presentation to visualize measurement variations, optical property influences on DCS flow indices, and differences between predicted and measured values. Error bars illustrate standard deviations (SD) and data are depicted as mean ± SD. Percentage errors between measured and predicted values were calculated and used to characterize measurement errors. Student t-test p-values are presented to compare measurement errors, while the significance criterion is \( p < 0.05 \).
2.8 Head and Neck Tumor Study Protocol and Data Analysis

The phantom study results provide a general view of errors in flow indices due to constant optical properties assumptions. In order to more easily visualize the implications of such assumptions on real tissue measurements, *in-vivo* data from an ongoing tumor study is analyzed. A hybrid optical instrument, similar to that in the phantom study, was employed in measuring tissue hemodynamic properties of head and neck tumors in 10 patients. Inclusion was restricted to only those patients with Stage III-IVb Squamous Cell Carcinoma of the Head and Neck (SCCHN). Further selection criteria included neck lymph nodes that measured greater than 1 cm and were clinically thought to be involved by tumor. Subjects completed consent forms and institutional review board (IRB) approval was given by the University of Kentucky prior to participation in the study.

Not all DCS and Imagent laser sources used in the phantom study were available for the tumor study and, as a result, some differences are encountered. DCS employed lasers at 785 and 854 nm whereas the Imagent had 690 and 830 nm. The best match between source wavelengths of the two instruments, for data analysis, was determined to be 854 nm for DCS and 830 nm for Imagent. Due to the large gap between the other two sources, 785 versus 690 nm, they are excluded from this analysis. The Imagent source and detector fiber separations are identical as that in the phantom study (i.e., 2.0, 2.5, 3.0, and 3.5 cm). Three source and detector separations were achieved with DCS, utilizing 3 detector fibers at 1.5, 2.4, and 2.8 cm from the bundled source fibers. For the phantom study, homogeneity was assumed for all source and detector separations. However, in the tumor study our analysis focuses on the 2.8 cm separation from DCS as it is expected to be most comparable to the tissue region/depth probed by the Imagent. The handheld
probe was placed on the tissue surface at the center of the area identified as tumor node, as applicable to the semi-infinite geometry model, and secured in position. DCS flow indices and optical property data were acquired for a duration of ~2 minutes.

Optical properties were obtained by Imagent, as done in the phantom study, and averaged over the 2 minute measurement interval for the 10 patients. Calculations with four different sets of measured optical properties produced four unique DCS flow indices. The true DCS flow index is considered that which is calculated with the true corresponding patient \( \mu_a \) and \( \mu_s' \) (i.e., averaged Imagent data over 2 minutes), and is termed \( \alpha_{DB-dynamic} \). The remaining three DCS flow indices are calculated using the overall patient minimum, mean, and maximum optical properties, termed \( \alpha_{DB-min} \), \( \alpha_{DB-mean} \), and \( \alpha_{DB-max} \), respectively. These \( D_B \) estimates are compared with the true flow index, \( \alpha_{DB-dynamic} \), for determining errors. Error bars in figures correspond to SD, and data are presented by interval mean ± SD.
CHAPTER 3: RESULTS

Presentation of results is ordered as follows. First, data related to the calculations of Brownian diffusion coefficients ($D_{\text{B-Einstein}}$) and DCS flow indices are given for $\mu_a$ variation in liquid phantoms (Chapter 3.1). In a similar fashion, these findings from $\mu_s'$ variation are then reported (Chapter 3.2). The next section (Chapter 3.3) summarizes the influence of $\mu_a$ and $\mu_s'$ variations on flow indices and is divided into three subsections: the influence of $\mu_a$ and $\mu_s'$ variations on $D_{\text{B-Einstein}}$, the measurement errors found for $\mu_a$, $\mu_s'$ and DCS flow indices compared to predicted values, and the influence of inaccurate estimations of $\mu_a$ and $\mu_s'$ on DCS flow indices. Finally, the influence of tissue optical properties on DCS blood flow indices of head and neck tumors is presented (Chapter 3.4).

3.1 $\mu_a$ Variation

The first experiment performed consists of varying the $\mu_a$ of a liquid phantom while maintaining a constant $\mu_s'$ to evaluate the influences on DCS flow indices. Thirteen steps, with a $\mu_a$ step size of 0.0125 cm$^{-1}$, were carried out over $\mu_a$ (830 nm) from 0.05 to 0.20 cm$^{-1}$ with $\mu_s'$ (830 nm) = 10 cm$^{-1}$. At 780 nm, the $\mu_a$ will also increase and have constant $\mu_s'$, but manipulations were purposely invoked at 830 nm only. Measurements were obtained for thirteen separate 5 minute intervals. These were analyzed post measurement producing data sets of mean and SD’s per interval for viscosity, temperature and three $D_{\text{B}}$’s. The viscosity, temperature and true flow index, calculated $D_{\text{B-Einstein}}$, are shown in Fig. 5 – 7, respectively, with error bars designating the SD’s. Each $D_{\text{B-Einstein}}$ (Fig. 7) is
calculated with the measured interval temperature (Fig. 6), corresponding viscosity (Fig. 5), and estimated particle radius (196 nm).

Raw data and interval means with SD’s (as error bars) of measured and true $\mu_a$, $\mu_s’$, $D_{B\text{-Einstein}}$, $D_{B\text{-mid}}$, and $D_{B\text{-dynamic}}$ during $\mu_a$ variation at the employed wavelengths are displayed in Fig. 8 – 14. For raw data figures (Fig. 8, 10, 12, and 13), the black vertical lines indicate separation between 5 minute data acquisition intervals and frame numbers serve as a generic counter as the data between intervals has been excluded for clarity. To quantify measurement errors for the optical properties, predictions using Mie theory estimations and spectrometer results are used as true values for comparisons to $\mu_s’$ and $\mu_a$, respectively. Calculated $D_{B\text{-Einstein}}$ is not wavelength dependent and thus is the same for comparisons with DCS flow indices, $D_{B\text{-dynamic}}$ and $D_{B\text{-mid}}$, at both wavelengths. Averaged optical properties at the middle interval [$\mu_a (830 \text{ nm}) = 0.125 \text{ cm}^{-1}$ and $\mu_s’ (830 \text{ nm}) = 10 \text{ cm}^{-1}$] are used for calculations of $D_{B\text{-mid}}$ and from corresponding intervals for $D_{B\text{-dynamic}}$.

![Figure 5. Viscosity During $\mu_a$ Variation](image)

Viscosity as averaged from three samples corresponding to each step, depicted as means ± SD’s (as error bars), during $\mu_a$ variation. Small variations can be seen within steps attributable to variations in sample measurements. Viscosity during step 2 has larger variations than other steps for reasons unknown, but is possibly due to operational error. The variation in viscosity across all steps is relatively low indicating stability of this parameter.
Figure 6. Temperature During $\mu_a$ Variation

Temperature as averaged over 5-minute intervals, depicted as means ± SD’s (as error bars), during $\mu_a$ variation. Temperature variations within each step are small. An increasing trend is apparent and most likely the result of increasing ambient temperature within the confined room during measurements. The overall variation is small and the parameter is considered stable.

Figure 7. Brownian Motion During $\mu_a$ Variation

Stability of particle Brownian motion, with $D_{\text{Einstein}}$ for corresponding 5-minute intervals, during $\mu_a$ variation. The stability of this variable is exhibited with only minor variations. These fluctuations are due to the small viscosity and temperature variations governed by Eq. 7.
Figure 8. Raw Data of Imagent Measured $\mu_a$ at 780 and 830 nm During $\mu_a$ Variation

Imagent measured $\mu_a$ at 780 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_a$ variation. The noise is relatively low at early intervals, but increases significantly near higher $\mu_a$ with decreases in Imagent detected intensity.

Figure 9. $\mu_a$ at 780 and 830 nm During $\mu_a$ Variation

Imagent measured $\mu_a$, shown as interval means ± SD’s, and $\mu_a$ predicted using spectrometer measurements at 780 nm (left) and 830 nm (right) for $\mu_a$ variation. Increases in Imagent measured $\mu_a$ are in agreement with spectrometer predictions. The agreement is better at 830 nm which may be due to the detection accuracy of Imagent at separate wavelengths. Measurement variations within intervals can be seen to increase with the increasing noise shown in Fig. 8.
Figure 10. Raw Data of Imagent Measured $\mu_s'$ at 780 and 830 nm During $\mu_a$ Variation

Imagent measured $\mu_s'$ at 780 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_a$ variation. The noise is low at early intervals and continually increases with the addition of ink at each step as Imagent detected intensity decreases. The $\mu_s'$ at both wavelengths are relatively stable throughout the measurement.

Figure 11. $\mu_s'$ at 780 and 830 nm During $\mu_a$ Variation

Imagent measured $\mu_s'$, shown as interval means ± SD’s, and $\mu_s'$ predicted from Mie theory approximations at 780 nm (left) and 830 nm (right) for $\mu_a$ variation. Small deviations between Imagent measured $\mu_s'$ and Mie theory predictions can be seen, but are within expectations (see Chapters 3.3 and 4.2). Measurement variations within intervals can be seen to increase with the increasing noise shown in Fig. 10.
Figure 12. Raw Data of DCS Measured $D_{B-dynamic}$ at 785 and 830 nm During $\mu_a$ Variation

DCS measured $D_{B-dynamic}$ at 785 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_a$ variation. Noise levels after averaging from four detectors is low for all intervals. A slightly increasing trend may be due to the decreases in diffusion applicability from increasing $\mu_a$ compared to $\mu_s^\prime$.

Figure 13. Raw Data of DCS Measured $D_{B-mid}$ at 785 and 830 nm During $\mu_a$ Variation

DCS measured $D_{B-mid}$ at 785 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_a$ variation. Noise levels after averaging from four detectors is low for all intervals, the same as for $D_{B-dynamic}$ (see Fig. 12). The largely decreasing trend is expected to be due to the lack of accounting for changes in the optical properties.
DCS $D_{B\text{-dynamic}}$ and $D_{B\text{-mid}}$, shown as interval means ± SD’s, and Brownian diffusion coefficients, $D_{B\text{-Einstein}}$, at 785 nm (left) and 830 nm (right) for $\mu_a$ variation. This figure overlays the $D_{B\text{-Einstein}}$ from Fig. 7 with the interval means of DCS measured $D_{B\text{-mid}}$ and $D_{B\text{-dynamic}}$ from Fig. 12 and 13. Deviations between $D_{B\text{-mid}}$ appear to be much greater than $D_{B\text{-dynamic}}$ in comparison to $D_{B\text{-Einstein}}$ (true flow index) indicating $D_{B\text{-dynamic}}$ as the more accurate flow index.

3.2 $\mu_s'$ Variation

Results of the $\mu_s'$ variation experiment are given in the same format as that for $\mu_a$ variation. Thirteen steps of increasing $\mu_s'$ were carried out with a step size of 1 cm$^{-1}$ covering $\mu_s'$ (830 nm) from 4 to 16 cm$^{-1}$ with constant $\mu_a$ (830 nm) = 0.125 cm$^{-1}$. The viscosity, temperature, and calculated $D_{B\text{-Einstein}}$ means with SD’s as error bars are displayed in Fig. 15 – 17, respectively. Note that the same $D_{B\text{-Einstein}}$ set is used as the true flow indices for comparison with both DCS source wavelengths. Raw data and interval means of measured $\mu_{as}$, $\mu_s'$, $D_{B\text{-Einstein}}$, $D_{B\text{-dynamic}}$, and $D_{B\text{-mid}}$ with SD error bars are shown in Fig. 18 – 24 along with predicted $\mu_a$ and $\mu_s'$ (as done in Chapter 3.1). Raw data figures (Fig. 18, 20, 22, and 23) are presented in a similar fashion to Chapter 3.1.
Figure 15. Viscosity During $\mu_s'$ Variation

Viscosity as averaged from three samples corresponding to each step, depicted as means ± SD’s (as error bars), during $\mu_s'$ variation. These measurements exhibit small variations within steps due to sample variations, but are stable overall. Step 2 has larger variations for reasons possibly due to operational error.

Figure 16. Temperature During $\mu_s'$ Variation

Temperature is averaged over 5-minute intervals, depicted as means ± SD’s (as error bars), during $\mu_s'$ variation. Temperature variations within each step are small, but overall stable with a generally increasing trend caused from increases in ambient temperature within the confined room throughout measurements.
Figure 17. Brownian Motion During $\mu'_s$ Variation

Stability of particle Brownian motion, with $D_{B-Einstein}$ for corresponding 5-minute intervals, during $\mu'_s$ variation. This variable shows as stable with only minor variations caused by small viscosity and temperature variations governed by Eq. 7.

Figure 18. Raw Data of Imagent Measured $\mu_a$ at 780 and 830 nm During $\mu'_s$ Variation

Imagent measured $\mu_a$ at 780 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu'_s$ variation. The noise is slightly higher than during $\mu_a$ variation at early intervals, likely due to the high Intralipid concentration, increasingly worsening throughout the measurement. The $\mu_a$ is relatively stable overall with a small jump occurring from the first and second intervals at 780 nm. This jump may be caused by near saturation of the Imagent detectors at the earliest interval.
Imagent measured $\mu_s$, shown as interval means ± SD’s, and $\mu_s$ predicted using spectrometer measurements at 780 nm (left) and 830 nm (right) for $\mu_s$’ variation. The deviations between Imagent measured $\mu_s$’ and Mie theory predictions are minor, but within expectations (see Chapters 3.3 and 4.2). Measurement variations within intervals can be seen to increase with the increasing noise shown in Fig. 18.

Imagent measured $\mu_s$’ at 780 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_s$’ variation. Noise is low early and increases with each step and the addition of Intralipid which decreases the Imagent detected intensity.
Figure 21. $\mu_s'$ at 780 and 830 nm During $\mu_s'$ Variation

Imagent measured $\mu_s'$, shown as interval means ± SD’s, and $\mu_s'$ predicted from Mie theory approximations at 780 nm (left) and 830 nm (right) for $\mu_s'$ variation. Increases in Imagent measured $\mu_s'$ are in agreement with Mie theory predictions. The agreement at 780 nm appears better which may be due to the detection accuracy of Imagent at separate wavelengths, similar to $\mu_s$ during $\mu_s$ variation (see Fig. 9). Measurement variations within intervals can be seen to increase with the increasing noise shown in Fig. 20.

Figure 22. Raw Data of DCS Measured $D_B$-dynamic at 785 and 830 nm During $\mu_s'$ Variation

DCS measured $D_B$-dynamic at 785 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during $\mu_s'$ variation. Noise levels are low for all intervals with detector averaging. The trend is decreasing at early intervals before stabilizing around the fourth and fifth intervals. This is possibly due to poor diffusion from a low $\mu_s'$ compared to $\mu_a$. 

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Figure 23. Raw Data of DCS Measured D_B-mid at 785 and 830 nm During \( \mu_s' \) Variation

DCS measured \( D_{B-mid} \) at 785 nm (left) and 830 nm (right) acquired from each data acquisition cycle (frame) during \( \mu_s' \) variation. Noise levels are low as was the case for \( D_{B-dynamic} \) (see Fig. 22). There is a largely increasing trend due to the lack of accounting for changes in the optical properties.

Figure 24. \( D_B \) at 785 and 830 nm During \( \mu_s' \) Variation

DCS \( D_{B-dynamic} \) and \( D_{B-mid} \), shown as intervals means \( \pm \) SD’s, and Brownian diffusion coefficients, \( D_{B-Einstein} \), at 785 nm (left) and 830 nm (right) for \( \mu_s' \) variation. As was done for \( \mu_a \) variation, this figure overlays the \( D_{B-Einstein} \) from Fig. 17 with the interval means of DCS measured \( D_{B-mid} \) and \( D_{B-dynamic} \) from Fig. 22 and 23. Deviations between \( D_{B-mid} \) are significantly greater than \( D_{B-dynamic} \) in comparison to \( D_{B-Einstein} \) (true flow index) indicating \( D_{B-dynamic} \) as the more accurate flow index.
3.3 Quantification of $\mu_a$ and $\mu_s'$ Influences on Flow Indices

**Influence of $\mu_a$ and $\mu_s'$ variations on $D_{B\text{-Einstein}}$.** Variations in phantom optical properties are examined to determine any potential influences on $D_{B\text{-Einstein}}$. Using the data displayed in Fig. 5 – 7 and 15 - 17, the means, SD’s, and coefficients of variation (CV) were calculated over the entire $\mu_a$ and $\mu_s'$ variation experiments for viscosity, temperature, and $D_{B\text{-Einstein}}$. The results are shown in Table 1, with experiments separated into different columns. No major influences are noticeable on $D_{B\text{-Einstein}}$ or related parameters with CV’s less than 2.2% for all three in both optical property variations. With this knowledge, the $D_{B\text{-Einstein}}$ is considered viable as the true flow index for determining measurement errors in DCS flow indices.

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\mu_a$ variation</th>
<th>$\mu_s'$ variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CV</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>0.98 ± 0.02</td>
<td>1.54%</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18.61 ± 0.20</td>
<td>1.05%</td>
</tr>
<tr>
<td>$D_{B\text{-Einstein}}$ (cm$^2$/s)</td>
<td>1.05E-08 ± 1.65E-10</td>
<td>1.57%</td>
</tr>
</tbody>
</table>

**Mean measurement errors in $\mu_a$, $\mu_s'$ and DCS flow indices.** Measurements errors are described using absolute percentage errors defined as:

\[
\text{Absolute Percentage Error} = \left| \frac{\text{Estimate} - \text{True}}{\text{True}} \right| \times 100\% \quad (13)
\]
In the case of DCS flow indices, $D_B$-Einstein is considered the true flow index whereas $D_B$-dynamic and $D_B$-mid are estimates. Estimated $\mu_a$ and $\mu_s'$ are those averaged from measurements while true values are from spectrometer and Mie theory predictions, respectively. Using the estimated and true data (shown in Fig. 9, 11, 14, 19, 21, and 24) over the complete range of $\mu_a$ and $\mu_s'$ variations, measurement errors were calculated and their means $\pm$ SD’s are shown in Table 2. P-values are also shown for 2-sample unequal variance, two-tailed Student t-tests between mean measurement errors at the wavelengths used. Significant differences are denoted with a * prefix when p-values are less than 0.05. Measurement errors for $\mu_a$ and $\mu_s'$ are less than 6% for both experiments and sets of wavelengths. DCS flow indices, however, exhibit some discrepancies amongst their measurement errors. Errors in $D_B$-mid are overall higher than those of $D_B$-dynamic, indicating optical properties influence in DCS $D_B$ calculations. Variations in $\mu_s'$ appear to contribute a greater influence on flow index errors than $\mu_a$ variations, as identified by $D_B$-mid errors up to 12.89% during $\mu_a$ variation and 49.63% during $\mu_s'$ variation. Student t-test results identified significant differences between mean measurement errors at 780 and 830 nm for $\mu_a$ during $\mu_a$ variation ($p = 0.01$) and $\mu_s'$ during $\mu_s'$ variation ($p = 0.04$). Detection accuracy of the Imagent at the different wavelengths is thought to be the greatest attributor to these differences. No other significant differences were found between the mean measurement errors at both wavelengths.
Table 2. Imagent/DCS Measurement Percentage Errors at 780/785 nm (shaded) and 830/830 nm (non-shaded)

<table>
<thead>
<tr>
<th>Variables</th>
<th>( \mu_a ) variation (Absolute % Error)</th>
<th>( \mu_s' ) variation (Absolute % Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD p-value</td>
<td>Mean ± SD p-value</td>
</tr>
<tr>
<td>( \mu_a )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>780 nm</td>
<td>3.39 ± 3.07 *0.01</td>
<td>1.86 ± 1.15</td>
</tr>
<tr>
<td>830 nm</td>
<td>0.84 ± 0.95</td>
<td>2.86 ± 2.69</td>
</tr>
<tr>
<td>( \mu_s' )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>780 nm</td>
<td>1.93 ± 1.23 0.54</td>
<td>3.14 ± 2.66 *0.04</td>
</tr>
<tr>
<td>830 nm</td>
<td>1.60 ± 1.50</td>
<td>5.29 ± 2.27</td>
</tr>
<tr>
<td>D_{B-dynamic}</td>
<td>5.52 ± 3.69 0.28</td>
<td>5.84 ± 10.73</td>
</tr>
<tr>
<td></td>
<td>4.02 ± 3.30</td>
<td>6.58 ± 6.16</td>
</tr>
<tr>
<td>D_{B-mid}</td>
<td>12.89 ± 12.00 0.64</td>
<td>49.63 ± 31.51</td>
</tr>
<tr>
<td></td>
<td>10.89 ± 8.99</td>
<td>46.76 ± 27.44</td>
</tr>
</tbody>
</table>

* p-values < 0.05

For both experiments, Student t-tests were also used for comparisons between the mean measurement errors of \( D_{B-mid} \) and \( D_{B-dynamic} \), within the same wavelengths, as shown in Table 3. Significant differences are determined and noted similarly as done in Table 2. It is readily seen that \( D_{B-mid} \) and \( D_{B-dynamic} \) are not equivalent by the significant (although for 785 nm during \( \mu_a \) variation it is borderline) differences found for both wavelengths and experiments. Additionally, \( D_{B-dynamic} \) is considered more accurate as it provides less measurement errors (see Table 2). As similarly suggested from Table 2, the \( \mu_s' \) influence on DCS flow indices is greater than \( \mu_a \) as indicated by much lower p-values during \( \mu_s' \) variation.
Table 3. P-values for Comparisons of the Mean Measurement Errors Between DCS Flow Indices Calculated with Dynamic Vs. Assumed Constant Optical Properties

<table>
<thead>
<tr>
<th></th>
<th>$\mu_a$ variation (p-value)</th>
<th>$\mu_s'$ variation (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>785 nm</td>
<td>0.0525</td>
<td>*0.0003</td>
</tr>
<tr>
<td>830 nm</td>
<td>*0.0205</td>
<td>*0.0002</td>
</tr>
</tbody>
</table>

* p-values < 0.05

**Influence of $\mu_a$ and $\mu_s'$ variations on DCS flow index.** Percentage errors (not absolute) are used to visualize the influences of the individual optical properties on flow indices, defined by the following equations:

\[
\text{% Error } \mu_a = \frac{\mu_{a\text{-mid}} - \mu_{a\text{-dynamic}}}{\mu_{a\text{-dynamic}}} \times 100\% \quad (14)
\]

\[
\text{% Error } \mu_{s'} = \frac{\mu_{s'\text{-mid}} - \mu_{s'\text{-dynamic}}}{\mu_{s'\text{-dynamic}}} \times 100\% \quad (15)
\]

\[
\text{% Error } D_B = \frac{D_{B\text{-mid}} - D_{B\text{-dynamic}}}{D_{B\text{-dynamic}}} \times 100\% \quad (16)
\]

The estimates for these errors are the assumed optical properties (middle-interval) and subsequent $D_{B\text{-mid}}$ per interval. The true values are the averaged optical properties measured by Imagent (dynamic) and subsequent $D_{B\text{-dynamic}}$ per interval. Resulting percentage errors in DCS $D_B$ due to inaccurate estimations of optical properties are overlaid for both wavelengths and experiments in Fig. 25. Note that errors in $\mu_a$ are during $\mu_a$ variation and those in $\mu_{s'}$ during $\mu_{s'}$ variation. In agreement with results thus far the $\mu_{s'}$ shows a larger influence on DCS flow indices, as visible by the wider range of $D_B$ percentage errors, than $\mu_a$. The trends in errors for $\mu_a$ and $\mu_{s'}$ with those in $D_B$ also differ. Overestimation and underestimation of $\mu_a$ result in overestimation and underestimation in
DCS $D_B$. On the contrary, $\mu_a'$ overestimation and underestimation lead to $D_B$ underestimation and overestimation. Both wavelengths display analogous behavior and are in good agreement.

**Figure 25. DCS Flow Index Errors Due to Inaccurate Optical Property Estimations**

Inaccurate estimations (percentage errors) of $\mu_a$ and $\mu_a'$ result in corresponding percentage $D_B$ errors between $D_B$-dynamic and $D_B$-mid for both wavelengths.
3.4 Influence of Tissue Optical Properties on Head/Neck Tumor Blood Flow Index

Ten patients with head and neck tumors, measured for hemodynamic information, have their DCS flow indices evaluated for optical property influences. The resulting mean optical properties over patients are: $\mu_a$ (830 nm) = 0.12 ± 0.03 cm$^{-1}$ and $\mu_s'$ (830 nm) = 7.80 ± 2.64 cm$^{-1}$. The means ± SD’s (error bars) of tumor $\mu_a$ and $\mu_s'$ are shown in Fig. 26 and 27, respectively. Red and blue dots denote the overall patient maximum and minimum optical properties, respectively. Patient blood flow indices ($\alpha_D^{B\text{-dynamic}}$, $\alpha_D^{B\text{-min}}$, $\alpha_D^{B\text{-mean}}$, and $\alpha_D^{B\text{-max}}$) along with percentage errors thereof (aside from $\alpha_D^{B\text{-dynamic}}$) are given in Fig. 28 and 29. Note that the assumption of $\alpha = 1$ from phantom experiments is no longer correct as the real tissue consists of both static and dynamic scatterers. Diffusion coefficient percentage errors are calculated using Eq. 16 by replacing the phantom study estimate, $D_B^{\text{-mid}}$, with the corresponding tumor study estimates $\alpha_D^{B\text{-min}}$, $\alpha_D^{B\text{-mean}}$, and $\alpha_D^{B\text{-max}}$. Also, the true flow index $D_B^{\text{-dynamic}}$, calculated using Imagent measured optical properties, from the phantom study is replaced in Eq. 16 by the equivalent tumor study true flow index, $\alpha_D^{B\text{-dynamic}}$. This produces percentage errors for each flow index estimate for each patient (see Fig. 29).
Figure 26. Tumor $\mu_a$ at 830 nm

Imagent measured $\mu_a$, shown as means ± SD’s, obtained at 830 nm from tumor region for 10 subjects with head and neck tumors during tumor study. Large variations can be seen to exist between the 10 subjects.

Figure 27. Tumor $\mu_s'$ at 830 nm

Imagent measured $\mu_s'$, shown as means ± SD’s, obtained at 830 nm from tumor region for 10 subjects with head and neck tumors during tumor study. Similar to $\mu_a$ in Fig. 26, large variations also exist in $\mu_s'$ between the 10 subjects.
DCS flow indices measured by DCS, shown as means ± SD’s, obtained at 854 nm from tumor region for 10 subjects with head and neck tumors and calculated using 830 nm optical properties during tumor study. Patients are listed in order of increasing $\alpha_{DB-dynamic}$ (854 nm). The underestimation of optical properties ($\alpha_{DB-min}$) overestimates the flow indices whereas overestimating optical properties ($\alpha_{DB-max}$) underestimates flow indices. These trends agree with phantom study results of a larger influence from $\mu'_s$.

Percentage errors in DCS flow indices at 854 nm from tumor region for 10 subjects with head and neck tumors. DCS flow indices $\alpha_{DB-min}$, $\alpha_{DB-mean}$, and $\alpha_{DB-max}$ are considered estimates with $\alpha_{DB-dynamic}$ as true flow indices for patients. The errors are shown to increase substantially with inaccuracies in optical properties (see Fig. 26 and 27).
To ease identification of differences in patient DCS flow index trends using the diverse optical properties sets, they are ordered by increasing $\alpha_{DB-dynamic}$ (true flow index). A black line denotes the true flow indices data. Note that the patient numbers depicted represent indices for trend illustration and are not related to those corresponding to the actual measurement sequence. After this ordering, trend changes when calculating flow indices without the true optical properties can be viewed (Fig. 28). When the $\mu_a$ and $\mu_s'$ are inaccurately estimated ($\alpha_{DB-min}$, $\alpha_{DB-mean}$, and $\alpha_{DB-max}$), the trends do not agree with the true flow index trend ($\alpha_{DB-dynamic}$). The $\alpha_{DB}$ estimates result in a wide range of percentage errors: $\alpha_{DB-min}$ from -8.07 to 278.15%, $\alpha_{DB-mean}$ from -39.48 to 149.01%, and $\alpha_{DB-max}$ from -70.26 to 22.59%. The tumor study data is consistent with expectations from phantom study results with $\mu_s'$ variation appearing to play a greater role than $\mu_a$ in DCS flow index errors. From Fig. 28, the consequential overestimation and underestimation of blood flow indices are opposite of those of $\mu_s'$ variation, as seen in Fig. 25.
CHAPTER 4: DISCUSSION AND CONCLUSIONS

This chapter begins with discussion on the influences of $\mu_a$ and $\mu_s'$ variations on $D_B$-Einstein (Chapter 4.1). Findings related to measurement errors in $\mu_a$, $\mu_s'$ and $D_B$-dynamic are then examined (Chapter 4.2). Next, the flow index errors due to the assumption of optical properties are discussed (Chapter 4.3). A comparison is then provided between the in-vivo tumor study data and phantom study results (Chapter 4.4). Finally, conclusions from this study are given (Chapter 4.5).

4.1 $\mu_a$ and $\mu_s'$ Variation Influences on $D_B$-Einstein

The Brownian motion of spherical particles, $D_B$-Einstein, from the Intralipid solution in liquid phantoms is calculated from the Einstein-Stokes formula (Eq. 7). This movement is calculated based on the liquid phantom temperature, spherical particle radius, and viscosity. The temperature has only small variations during both phantom experiments (see Table 1, Fig. 6, and Fig. 16), with CV < 1.1%. Due to the ~4.5 hour duration of each experiment, a general increase in ambient and thus phantom temperature is expected. This increase includes heat from running equipment within the small, confined room. The particle radius is assumed to be relatively constant during both experiments, with Intralipid provided from the same batch supply. Only minor variations in viscosity are evident, with CV < 2.2% for both experiments (see Table 1, Fig. 5, and Fig. 15). These variations may be the result of the viscometer sensitivity at measurements near the bottom of its range (0.2 cP) and minor sample-to-sample variations. The Newtonian fluid assumption is determined acceptable as the samples measured at three separate RPMs
showed small variation. Because of the stability of viscosity and temperature measurements as well as the assumed constant particle radius, $D_{B-Einstein}$ is also expected to be constant. This is found to be the case as is seen in Fig. 7 and 17 and with $CV < 2.2\%$ for $D_{B-Einstein}$ from Table 1.

The addition of ink and Intralipid solutions during $\mu_a$ and $\mu_s'$ variation, respectively, are not parameters expected to contribute to variations in $D_{B-Einstein}$. Ink solution should have no particles undergoing Brownian motion. On the other hand, Intralipid solution does contain particles undergoing Brownian motion, but this motion is not anticipated to change during measurement. Additions of Intralipid provide more scatterers undergoing Brownian motion, but the particle motion is equivalent for all. The ratio of moving to total scatterers ($\alpha$) should also remain unchanged ($\alpha = 1$) given that only the dynamic particles from Intralipid are scatterers. The viscosity of ink and Intralipid solution is, as mentioned, primarily water and variations thereof should not result in changing the phantom viscosity. Similarly, the ink and Intralipid solutions were at room temperature at the time of the experiments, just as the liquid phantom, having little influence on temperature variations. From Fig. 5-7, 9, 15-17, and 21 the liquid phantom optical properties exhibit independence from viscosity, temperature and $D_{B-Einstein}$. With the optical property independence and $D_{B-Einstein}$ stability, it is therefore considered reasonable to use as the true flow index for liquid phantom spherical particle motion.
4.2 Measurement Errors of $\mu_a$, $\mu_s'$, and $D_{B\text{-dynamic}}$

From Fig. 9, 11, 19, and 21 the utilization of ink and Intralipid solutions for manipulating $\mu_a$ and $\mu_s'$, respectively, is validated. The incremental addition of ink solution results in a linear increase in measured $\mu_a$ with relative stability of $\mu_s'$ at both wavelengths, as expected. Similarly, Intralipid solution linearly increases only $\mu_s'$ at both wavelengths. These measured optical properties were also found to be in good agreement with the spectrometer and Mie theory predictions. Variation patterns in Imagent optical property measurements may have been influenced by calibration at the midpoint: $\mu_a$ (830 nm) = 0.125 cm$^{-1}$, $\mu_s'$ (830 nm) = 10 cm$^{-1}$.

Measurement errors in $\mu_s'$ were less than 6% (see Table 2) for both wavelengths and experiments comparable to the literature using Mie theory estimation (see Chapter 2.7) [61]. Overall, average measurement errors for $\mu_a/\mu_s'$ are minimal with less than 4%/6% during $\mu_a/\mu_s'$ variations. Previous studies experienced measurement error levels in this range as well using frequency-domain spatially resolved NIRS [47, 48]. In comparing optical property measurement errors at 780 and 830 nm, significant differences were determined for $\mu_a$ during $\mu_a$ variation and $\mu_s'$ during $\mu_s'$ variation. The detection accuracy of the Imagent at separate wavelengths is most likely the cause of such significance. No significant differences were found between $D_{B\text{-dynamic}}$ measurement errors at 785 and 830 nm. These $D_{B\text{-dynamic}}$ errors averaged less than 7%, comparable to those of optical properties (less than 6%), for both wavelengths and experiments. This likeness implies optical property influences on DCS $D_B$. 
4.3 Resulting D_B Errors from Optical Property Assumptions

Inaccurate estimations of $\mu_a$ and $\mu_s'$ both produced higher D_B measurement errors (D_B-mid) than in comparison with the true measurements (D_B-dynamic). From Table 2, D_B-mid errors were $\sim$13\% for $\mu_a$ variation, $\sim$50\% for $\mu_s'$ variation, and only $\sim$7\% for D_B-dynamic in both cases. From Table 3, Student t-tests compliment this lack of resemblance between measured and true D_B by their significant differences. Comparisons between D_B-mid errors due to the influence of $\mu_a$ and $\mu_s'$ variations further extend this conclusion to include that $\mu_s'$ is a greater contributor than $\mu_a$. This is elicited by the higher D_B-mid measurement errors during $\mu_s'$ variation (Table 2), great differences in calculated p-values (Table 3), and larger range of D_B percentage errors due to inaccurate estimations of $\mu_s'$ (Fig. 25). The phase shifts of light due to dynamic scatterers collectively induce the light speckle fluctuations. As DCS flow indices are derived from these fluctuations it is expected that errors be more exaggerated by inaccurate scattering estimations. Coupling much larger scattering over absorption (i.e., $\mu_s' >> \mu_a$) in the liquid phantoms and biological tissues with the definition of $K^2$ (see Eq. 3) and the $\mu_s'^2$ term further support is given to $\mu_s'$ having more significance than $\mu_a$. Wavelengths were not determined to be a decisive parameter in D_B measurement errors due to optical property assumptions. During both experiments, no significant difference was found between D_B-dynamic and D_B-mid at 785 and 830 nm (Table 2) and their trends were nearly identical as seen in Fig. 25. Due to the closeness of the wavelengths employed, more information may be exposed by choosing a wider range.

Further disparity between the influences of $\mu_a$ and $\mu_s'$ variations on DCS flow index errors exists within their related trends (see Fig. 14, 24, and 25). Overestimated and
underestimated $\mu_a$ produces overestimated and underestimated $D_B$, respectively. Conversely, overestimated and underestimated $\mu_a$’ produces underestimated and overestimated $D_B$, respectively. By examination of the boundaries of our tested optical property inaccuracies (Fig. 25), the largest expected deviations in DCS flow indices can be estimated. Overestimating/underestimating $\mu_a$ by up to $\sim +150%/ -40\%$ produced $D_B$ percentage errors up to $\sim +40%/ -20\%$. Overestimating/underestimating $\mu_a$’ by up to $\sim +175%/ -35\%$ produced $D_B$ percentage errors up to $\sim -80%/ +110\%$. The phantom properties chosen for calibration may have influence over the optical properties estimation errors.

4.4 In-vivo Tumor Study Data in Comparison to Phantom Study Results

Optical property variations were large between the patients in the head and neck tumor study (see Fig. 26 and 27). This scenario proves useful for analyzing real-tissue implications of the phantom study results. The optical properties of patients covered: $\mu_a$ (830 nm) from 0.07 to 0.16 cm$^{-1}$ and $\mu_s'$ (830 nm) from 5.35 to 13.1 cm$^{-1}$. These properties are within the same range as those tested in the phantom experiments. The trends in $\alpha_{DB}$, resulting from calculations with the different optical property sets, are in agreement with conclusions from the phantom study. Overestimating/underestimating the $\mu_a$ and $\mu_s'$ (using maximum and minimum) produce underestimation/overestimation of the DCS flow index ($\alpha_{DB-max}$ and $\alpha_{DB-min}$). Thus, the variations in $\mu_s'$ appear to be the greater factor in influencing $\alpha_{DB}$ errors than that of $\mu_a$. Depending on the optical properties assumed, the errors in $\alpha_{DB}$ ranged from $\sim -70\%$ up to $\sim +280\%$. Comparisons are also made between the estimated $\alpha_{DB}$ ($\alpha_{DB-min}$, $\alpha_{DB-mean}$, and $\alpha_{DB-max}$) trends and the
true ($\alpha_{DB}$-dynamic). It is apparent that inaccurate optical property assumptions will alter the patient order based on increasing $\alpha_{DB}$ and potentially any subsequent conclusions made from this data. Invalid conclusions in similar studies may result from this lack of consideration into optical property influences on DCS flow indices.

4.5 Conclusions

DCS technology has provided deep tissue blood flow measurements in an increasing field of applications thanks to its noninvasive nature and fast data acquisition capabilities. Increasing in concert with these developments are the necessities of ensuring the proper operation and application of the technology. This includes investigating errors that are potentially encountered in measurements, notably the assumption of constant optical properties, $\mu_a$ and $\mu'_s$. DCS measurements are described by the extension of light scattering techniques into the diffusive regime of deep tissue, resulting in a correlation diffusion equation. DCS blood flow indices stem from a solution to this equation which includes the parameters of tissue $\mu_a$ and $\mu'_s$. With the advent of a hybrid optical instrument developed by our lab, we can now measure tissue optical properties and blood flow simultaneously. By also incorporating liquid phantom creation techniques to control simulated tissue optical properties and flow, investigation of optical property influences on DCS flow indices is now possible. The Brownian diffusion coefficient produced by the Einstein-Stokes formula for particles in liquid was found applicable and usable as the true flow index in comparison to DCS flow indices. The particle motions in the liquid phantoms were determined to be independent of variations in the optical properties. Although inaccurate estimations of both $\mu_a$ and $\mu'_s$ produced errors in DCS flow indices
larger than that when using true values, $\mu_s'$ was found to have a more substantial influence. This influence was not identified as being dependent on wavelengths employed. Therefore, the concurrent measurement of tissue optical properties and DCS flow indices is required within studies encountering significant $\mu_a$ and $\mu_s'$ variations to accurately determine tissue blood flow. The examination of a head and neck tumor study elucidate the possible consequences of ignoring the optical property variations in real tissue measurements.
CHAPTER 5: SUMMARY AND PERSPECTIVES

In summary of this research study, I have provided a quantified generalization of the influences of inaccurate estimations in optical properties on diffuse correlation spectroscopy blood flow measurements by means of liquid phantoms and in-vivo analysis. The recent integration by our lab of the Imagent and dual-wavelength DCS flow-oximeter into a hybrid instrument has enabled the ability to carry out this investigation. The design and completion of this study included my contributions to several facets of operation. These contributions consist of the unique phantom study experimental design (Chapter 2.6), determination of ideal hybrid instrument setup (Chapter 2.1 – 2.3), details of liquid phantom composition, measurement equipment and facilitation into our lab (Chapter 2.5), incorporation of measurement errors utilizing Brownian motion and theoretical predictions (Chapter 2.4), exemplification of generalized DCS flow errors through real tissue tumor study analysis (Chapter 2.8), and general methods of data analysis (Chapter 2.7). The results of this study have also been peer reviewed and published [64].

The experimental design of this phantom study was found capable of adequately evaluating the influences of the optical properties on DCS flow indices. The approach taken in this study sought to minimize variations other than the optical properties. The usage of our hybrid instrument performed as expected without requiring complex modifications or alterations. This was an important facet as this instrument is a means of avoidance into the errors posed by inaccurate optical property estimations. To further this study, an alternative approach would be to analyze Eq. 1 and 3 and determine the
influences of optical property variations with varying parameters, which may be the subject of future work. However, it is expected that without the knowledge of tissue optical properties such extensive quantification may not provide useful results.

The optimal hybrid instrument setup was determined from the following factors. It is expected that DCS flow indices obtained at different wavelengths provide equivalent flow information. To evaluate this aspect, 785 and 830 nm wavelengths were used. Originally, a long coherence length CW NIRS 690 nm laser was attempted instead of 785 nm, but due to technical circumstances was unavailable for this study. Utilizing different laser source wavelengths for both Imagent and DCS may extend the applicability of the results since no wavelengths at the low end of the NIR range were successfully incorporated (i.e., ~650 nm). The next factor of interest is maintaining an optimal SNR for both the Imagent and DCS equipment. The gain for the Imagent PMT was set at as high as possible (without saturation) from the initial phantoms and was not adjusted during each experiment. The source-detector separations and fiber orientation of the Imagent probe were fixed and not deemed necessary for modification. For the DCS system, the detector fibers were bundled and set at an equivalent source-detector separation of 1.5 cm. This distance was chosen due to its compliance with diffusion theory and adequate SNR. The SNR was increased through the averaging of the signal from the four detectors. The range of optical properties chosen also may affect the SNR and will be described shortly. The correlation time of DCS was found to be adequate in detecting the motion of Intralipid particles during both $\mu_a$ and $\mu'_s$ variations. The Imagent averaged 10 data acquisitions per one frame and DCS about 25 per frame (at both
wavelengths). These averaging parameters were determined to provide sufficiently stable measurements from the hybrid instrument.

There are several options currently available for creating tissue-like phantoms for NIR optical experimentation. Liquid phantoms were chosen over solid phantoms as they are typically easier to create and manipulate. In addition, there is no inherent flow in solid phantoms which would require additional design considerations. The liquid phantoms comprised of Intralipid, distilled water, and India ink proved capable of our needs thanks to their simplicity and versatility. For future studies, modifications to the liquid phantom flow can be induced using methyl cellulose or glycerin to evaluate optical property influences at different absolute DCS flow indices. However, the addition of these elements may have the undesirable effect of also altering the phantom viscosity and optical properties. The constituents were readily accessible without significant overhead and cost with Intralipid requiring the most effort to obtain. To maintain adequate SNR, the optical property ranges in this study were limited. More expansive ranges were tested but produced unreliable and unstable results. In the future, creating liquid phantoms with a broader range of $\mu_a$ and $\mu_s'$ can test the expansion of current conclusions to a greater variety of tissue types. The container size (aquarium) was chosen for adequate distance from the probe to the sides such that small variations in position did not result in interference due to boundaries. The custom probe holder was designed and put into a schematic prior to machining by the University of Kentucky machine shop. It was designed to be incorporated with the lab stand available providing stability and axial and longitudinal positioning.
Measurement errors for the DCS and Imagent were determined based on previous studies to differentiate actual optical property influences on DCS flow indices and those resulting from other factors. This includes the liquid phantom preparations and measurement variations. The methods for expected value predictions were advantageous in that they have already been discussed and used elsewhere allowing for quick implementation into our experimental design. Brownian motion for spherical particles in liquid is used for comparison with DCS. Spectrometer and Mie theory are used for comparison with the Imagent measurements. The measurements of viscosity and temperature related to Brownian motion required testing several apparatus setups. For viscosity, a spindle viscometer was tested but had inadequate range due to the low viscosity of distilled water, India ink, and Intralipid solutions. A cone-plate viscometer with better low viscosity accuracy was purchased and performed adequately. No continuous viscosity data could be acquired due to the induced motion of pumping liquid phantom solution through the viscometer. The current study employs all solutions and liquid phantoms at ambient temperature. This method provided the most stable temperature over other methods such as immersing the aquarium into a larger water filled container temperature controlled by an immersion circulator. More than one spectrometer was tested in the determination of the India ink absorption coefficient: a 96-well plate reader (Biotek), Jaz (Ocean Optics), and the Beckman Coulter as reported. Results were comparable between the three, but the Beckman Coulter device was chosen due to availability and speed of usage.

The tumor study required analyzing the hybrid equipment already in usage for that study and reasonably comparing it to the phantom study. There is inherently greater
difficulty and complexity involved in \textit{in-vivo} tissue optical property and flow measurements. Multiple DCS source-detector separations and wavelengths were used for the head and neck tumor measurements. To properly attribute the optical property influences on DCS flow indices the depth chosen was matched as closely as possible to that of the Imagent. Although the tissue is assumed homogeneous for the purposes of the theoretical models used, the best agreement between regions probed by the two systems was chosen. The optical properties of the patients were within the range used in the phantom study and it is expected that these will be similar for other tissue types, but a holistic representation is beyond the scope of this study and should be determined based on the context. More variations in absolute DCS flow indices were expected due to anatomical and other subject differences. The agreement with the phantom study results provides further insight as to the applicability of the generalized results into \textit{in-vivo} measurements.

To facilitate data analysis, a custom Matlab program was written to calculate DCS flow using various sets of optical properties and variations. Only those flows incorporating dynamic and constant property sets were included in this paper. This was done to simplify the analysis and provide a less complex, but more usable realization of the results. Data had to be extracted from the different files produced by the DCS and Imagent for processing. A robust program was written automating data analysis while providing control such as wavelengths used, source detector separations and processing methods (constant vs. dynamic optical properties). The creation of correlation curve figures each frame requires significant hard drive space which should be considered if saving is desirable. The method of analysis using averaged optical properties over the
intervals was determined acceptable as exhibited by measurement stability. However, in real tissue measurements, transient fluctuations in optical properties can occur and averaged properties may not be applicable.
REFERENCES


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