MRI-coupled Broadband Near-infrared Tomography for Small Animal Brain Studies

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Abstract

Diffuse optical tomography (DOT) using near infrared (NIR) light has become a promising modality because it has the capability to non-invasively quantify oxygen saturation and hemoglobin content with potentially high temporal resolution and sensitivity. However, NIR imaging itself suffers poor spatial resolution due to the diffuse nature of NIR light in tissue. To improve the resolution and accuracy of the recovered physiological images, as well as being able to link the system into conventional imaging systems, one approach is to utilize more structural information from other available modalities such as MRI and another approach is to acquire more spectral information and utilize more wavelengths to achieve better quantification of chromophores.

In this thesis, both approaches were implemented in a MRI-coupled broadband near-infrared tomography system, and several new developments in utilizing the NIR data are presented and analyzed. The system can measure complete attenuation spectra over the wavelength range 700-900nm using 8 transmit fibers and 8 separate receive fibers placed around the circumference of the rat head and is the first ever to provide NIR cross-sectional images of rat head with MRI data. Second derivative spectroscopy was used to estimate optical pathlengths at the water absorption features which allows the first ever reconstruction of both absorption and reduced scattering images using broadband NIR data. A novel reconstruction algorithm for multi-wavelength DOT is presented, which has the potential to dramatically reduce image artifacts often associated with unknown measurement errors. The in vivo performance of the system and the methods is examined by undertaking simultaneous BOLD MRI and NIR data collection during graded hypoxia in the rat brain.
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Chapter 1. Introduction

Medical Imaging has been well developed in the last century. However, the recent trends in imaging reflect the shift from producing high resolution structural images to producing direct information of normal function and abnormal behaviors of biological systems (Mait et al., 2003). Currently used imaging modalities for clinical studies, such as magnetic resonance imaging (MRI), ultrasound, or X-ray CT, do not provide complete information, either because they cannot provide a sufficiently direct measurement or provide sufficient contrast of the morphology. Rarely is it possible to image at the cellular, vascular or biochemical levels, which may be better suited for diagnosis or prognosis. Optical imaging is an emerging modality in which the unique interaction, absorption and elastic scattering, between visible light or near infrared (NIR) and biological tissue, allows the study of biochemical and pathophysiological function. By probing tissue elements with NIR light, it can be used to quantify the interaction coefficients, given the correct physical model and mathematical tools (Boas et al., 2001; Gibson et al., 2005). The absorption property of tissue provides information about physiologically interesting molecules (Jobsis, 1977) and the scattering property of tissue provides information about the size, density and distribution of particles in tissue (Mourant et al., 1997; van Staveren et al., 1991). Visible light is often used for in vitro studies, or imaging superficial layers due to the limited penetration (Kohl et al., 2000; Malonek et al., 1994; Mayhew et al., 1999). Generally NIR light is better for non-invasively monitoring and quantifying tissue functions in deeper and larger structures.

There are three major reasons that NIR has been widely accepted as a promising tool to study biological tissue. The first is the non-invasive nature, because NIR photons have lower energy as compared to X-ray and visible photons, well below the threshold for ionizing of molecules, so it is safe to use intensively and repeatedly. The second is the good penetration, due
to the fact that the absorption coefficient of most tissues is very low in this NIR range. This range provides a translucent window for modern NIR technology to probe into tissue for endogenous or exogenous chromophores. The third and most important reason is the *spectral contrast* in this window. The chromophores accounting for most of the endogenous absorption are oxygenated hemoglobin (HbO₂), deoxyhemoglobin (HbR), water (H₂O) and cytochrome oxidase (Ctox), which are strongly linked to tissue oxygenation and metabolism. These chromophores can be differentiated due to their distinct absorption spectra. Near infrared spectroscopy therefore has a powerful experimental tool for assessment of hemodynamics and oxygenation *in vivo* since its modern day implementation in the 1970s (Chance et al., 1988; Jobsis, 1977; Tromberg et al., 2000; Villringer and Chance, 1997).

In the early years, NIRS was used to provide the changes in the mean concentration of chromophores in the volume sampled by a source and detector pair, or a topological map if multiple pairs were used (Gratton et al., 1995). Until the recent decade, with the development in modeling of light propagation in tissue and the current detection technology, it is possible to combine NIR spectroscopy with tomography to perform diffuse optical tomography (DOT) which can spatially resolve the distribution of chromophore concentrations and scattering properties within the volume imaged. The basic idea is to illuminate tissue from an array of sources and collect the multiply-scattered light with an array of detectors, use a model of the propagation physics to calculate spatial distributions of the optical properties, and then apply this at multiple wavelengths to fit the absorption coefficients to the chromophore concentrations (Arridge and Schweiger, 1997; Barbour, 1993; Cai et al., 1996; Pogue et al., 1995). The application of DOT has expanded into many areas: brain imaging (Benaron et al., 2000; Culver et al., 2003b; Hebden et al., 2002; Hintz et al., 1998), breast cancer (Hebden et al., 2001; Li et
al., 2005; Pogue et al., 2001), muscle tissue imaging (Hillman et al., 2001) and joint inflammation (Hielscher et al., 2004; Xu et al., 2002), to name a few.

A compelling and promising application of DOT is for the detection of tumors. Standard modalities for imaging tumors do not have a high intrinsic contrast, for example, X-ray mammography, clinical ultrasound and standard MRI have less than 10% contrast in tumor to normal tissues (Robson et al., 1995; Young, 1984). In comparison, near-infrared measurements are taken in the special “translucent” window and the abnormal tissue is expected to exhibit very high contrast due to the increased vascular bed and increased blood content. However, such high contrast may be sacrificed due to the poor resolution DOT offers.

Near-infrared imaging suffers from modest spatial resolution due to the strong scattering of the NIR light in tissue. Intensity measurements of x-ray CT are only sensitive to the mass attenuation properties on the straight line between the source and the detector, since only ballistic photons are collected with the special design in the hardware to prevent scattered light from being detected. However, for strongly scattered near-infrared light, ballistic photons or snake photons are very few for most applications. Diffuse photons are collected to meet the signal to noise ratio requirement. Therefore, the sensitivity shape of the DOT projection through the tissue is characterized as a ‘banana’ shape. Near the source and detector (surface), the sensitivity is high and narrow, but it is low and broader in the middle (deep structure). Information sensitivity is always insufficient for the deeper structures and is only weakly improved by increasing the number of source-detector pairs. The recent trend in DOT has been the inclusion of anatomical information (spatial priors) and wavelength dependent (spectral priors) into the forward and inverse problems (Brooksby et al., 2004; Li et al., ; Pogue and Paulsen, 1998; Srinivasan et al., 2005). Significant improvement in image quality is observed with this additional information.
Oxygen is critical to energy homeostasis and oxygenation is a major variable in many pathological processes, as well as in the normal functioning, especially in the brain tissue. The abnormality in tissue oxygenation, cerebral blood volume, or cerebral blood flow is an important indicator of those pathological processes (Blennow et al., 1978). Many tumors have a remarkable reduced oxygenation that is thought to be due to a rapidly growing volume with poor vascular supply of blood, thus the increased demand and decreased supply of oxygen causes areas of necrosis and hypoxia. Brain ischemia caused by stroke is a major cause of debilitation and death and its pathology is both complex and poorly understood (Siesjo, 1992), but is similarly marked by areas of damaged circulation and hypoxia.

To better identify abnormalities in the brain and understand its pathophysiology, an imaging system using NIR DOT is developed in this thesis, to spatially resolve the oxygenation and hemodynamics in the brain. It is coupled to a 7-Telsa MRI system so that the structural images provided by MRI can be used to constrain the NIR image reconstruction and maps of chromophore concentrations provided by NIR can be co-registered with functional BOLD (blood oxygen level dependent) MR images. To improve the quantification of the chromophore spectral features, the complete attenuation spectrum between 700–900nm is collected using a broadband light source and spectrograph with a charge coupled device (CCD) detector. This is quite different from most current experimental DOT systems, such as the time domain systems (TDS) (Hebden et al., 1991; Ntziachristos et al., 1998), frequency domain systems (FDS) (Franceschini et al., 1997; Pogue and Paulsen, 1998), or continuous-wave systems (CWS) (Colak et al., 1999; Schmitz et al., 2002), in which laser sources at multiple wavelengths are used. A novel theory is developed to bridge the broadband spectroscopy to the diffuse optical imaging of both absorption and reduced scattering properties (Xu et al., 2004).
In the ensuing chapters, instrumentation, theory fundamentals, experimental specifics and animal studies are presented. Special attention has been given to perform diffuse optimal tomography reconstruction with broadband spectral data. Issues pertaining to the use of the a priori structural information are discussed. A novel reconstruction algorithm providing inherent insensitivity to boundary errors is proposed. Furthermore, the theory and experiment methodology are validated by BOLD MRI through graded hypoxia. Chapter 2 introduces general methodologies for performing diffuse optical tomography in tissue and reports on the development of a MRI-coupled broadband near infrared tomography system for rat brain studies. Chapter 3 describes the details of the second derivative spectroscopy based image reconstruction theory and presents the theoretical and experiment evidence to demonstrate the robust algorithm performance and determine the accuracy of this approach for the reconstruction of absorption and reduced scattering images. Chapter 4 reviews the fundamentals of photon propagation in tissue and presents the methodology of finite element method based non-linear image reconstruction using the diffusion approximation to the radiative transport equation. Simulations and phantom experiments were performed to examine the improvement when structural priors or other constraints were incorporated. Chapter 5 presents a recent theory of direct chromophore and scattering reconstruction with multi-mode of the broadband spectral data. This derivative reconstruction mode provides inherent insensitivity to coupling and geometric errors associated with the boundary data. Chapter 6 describes preliminary in vivo results of graded acute hypoxia. A good correlation was found between the NIR images and the BOLD MR images. Chapter 7 summarizes the findings and experience of this work and points to future directions.
Chapter 2. Instrumentation

As described in the previous chapter, a novel path towards developing the system design was taken in this project. All the work that will be discussed in the ensuing chapters is with respect to the MRI-coupled broadband near-infrared tomography system described in this chapter. Here we introduce the instrumentation to illustrate the concept of MRI-coupled and broadband tomography as implemented in this work.

Section 2.1 describes the overall system design and the individual components that comprise it. Section 2.2 describes the system performance and noise characteristics. Section 2.3 describes the calibration procedure and Section 2.4 discusses the general use of the apparatus.

2.1. Apparatus

2.1.1. System overview

The intended function of the instrumentation was to measure the complete attenuation spectrum over the wavelength range 700-900nm, from 8 transmit fibers and 8 receive fibers placed alternately around the rat head in a MRI magnet. The system uses parallel detection of the receive fibers, and each transmit fiber is used for illumination in sequence. The attenuation spectra from all 8 receive fibers are collected simultaneously on a charge-coupled device (CCD, Model EEV CCD05-30-219, Wright Instruments Inc., UK) mounted on an imaging Czerny-Turner spectrograph (Triax320m, JY Horiba, Edison, NJ). Figure 2-1 depicts the schematic diagram of the MRI-coupled broadband NIR tomography system. The optical system and the MRI console are coupled at the phantom/animal interface but work independently. Light source, transmit selector wheel, balance ND filters wheel and spectrograph are placed on the bench several meters away from the 7T magnet, to avoid the high magnetic field effects. The source
selector and balance ND filters are close to the light source and spectrograph and their relative positions are always stable. Multiple single core silica fibers, having 1 mm core diameter and being 1.5 m long are used to connect these parts. From the bench to the magnet 10m long fiber bundles are used to achieve smaller bending radius and more reliable performance (Spectraflex, Schott NA Inc., NY).

![Diagram of MRI-coupled broadband NIR tomography system]

**Figure 2-1** Schematic design of the MRI-coupled broadband NIR tomography system. It has 8 source and 8 detector fibers alternately surrounding the tissue in the magnet, yielding 64 attenuation spectra from 700-900nm. The system is utilized in a 7-T MRI for cranium imaging of rodents. The 8 detection fibers are all launched into the spectrometer along the vertical slit, and wavelengths are spread horizontally onto the CCD, for parallel detection of all detectors at all wavelengths.
2.1.2. Light source

The system uses a fiber optic illuminator (model 77501, Oriel Instruments Inc.) as the light source (shown in Figure 2-3 left). It is a self-contained unit, in which a built-in regulated 12 Volt DC supply powers a 100 Watt broadband quartz-tungsten halogen lamp, emitting in the region 250-2500 nm. An iris adjusts light intensity without changing the color temperature of the lamp. A manual shutter allows zero checking when used to estimate the background signals. A built-in filter holder is located immediately in front of the lamp and accepts 25.4mm diameter filters being up to 9.5mm in thickness. Usually color, interference or neutral density filters can be loaded for variety of applications. In this project, a 610nm long pass filter (model RG610, Schott NA Inc., NY) is installed within this filter holder, and is used to remove visible wavelengths and so as to reduce the local heating of the tissue. The lamp output is collimated and refocused onto an 11mm brass ferrule (shown in Figure 2-3 right). Eight 1 mm diameter silicon fibers are
epoxyed in the ferrule and arranged in a 4x2 array to match the image of the filament. The typical integrated power coupled to each transmit fiber is about 20mW total power.

![Light source and ferrule with transmit fibers](image)

*Figure 2-3 Photographs of the light source (left) and the brass ferrule with the transmit fibers (right), shown next to 1 cent for perspective.*

### 2.1.3. Light delivery

![Source shutter wheel](image)

*Figure 2-4 Photographs of the source shutter wheel. The left is a view from outside and the right is a perspective to the rotary disk inside, allowing transmission of one source at a time, and effectively blocking all other source channels with low crosstalk.*

To switch one of the 8 source fibers on in sequence, the 8 fibers from the light source are connected to a custom made shutter wheel as the source selector (shown in Figure 2-4). Each side of the wheel 8 collimator has matching input and output source fibers, and the wheel
between is used to connect the input and output fibers one at a time, while effectively blocking the transmission at all other source fibers. Each of those fibers ends with a SMA connector which matches the collimator used (Thor Labs, NJ). A stepper motor is installed on the wheel with its shaft on the axis of the wheel. A rotary disk with an aperture is locked on the shaft. The aperture has the same diameter as the collimator. The collimator ensures a narrow beam from input to output which is parallel to the rotary axis of the wheel, and minimizes crosstalk while maximizing optical throughput. Only one fiber from the light source can be allowed to transmit and others will be blocked by the opaque disk.

Source and detector fibers are evenly distributed on a circular geometry in an alternate pattern, thus the detectors nearest to the source typically receive orders of magnitude more light than those farthest away. This raises a dynamic range issue for the detector. Imaging the rat head requires a system with a medium dynamic range because the attenuation difference between the nearest neighbors and furthest neighbors are typically 3 OD. The useful dynamic range of a CCD is typically 2 OD e.g. between spectra with a maximum signal of 100,000 and 1000 electrons. This range can be even smaller when certain SNR is required because higher SNR requires higher number electrons. The details about the SNR of the CCD will be covered in the next section. To increase the dynamic range of the CCD, the preliminary design used a custom readout mode in which the weaker channels were vertically binned over the whole height of the channel (42 pixels) and a single spectrum read out whereas the strongest channels were vertically binned over only two rows. In this plan, 21 spectra were read out and digitally binned to avoid being saturated at the A/D converter. This increased the dynamic range by approximately 1.3 OD between the lowest and highest channels. Although the imaging capabilities of the spectrograph were sufficient to completely separate the channels, it was found that imperfections on the
surface of the CCD window and retro reflections from the edges of the CCD windows produced a patterned cross talk that was not insignificant between the strongest and weakest channels. In other words, the crosstalk from the strongest channel dominated the signal in the weaker channels. In Section 2.2.2, this phenomenon will be discussed in detail. For this reason, it was decided to balance the input intensity of each channel with neutral density filters, prior to entrance into the spectrometer. Since the location of the highest detection channel changed when the source fiber moved, the location of each specific intensity received changed depending on which transmit fiber was active. Thus, the simplest implementation of ND filtering was to use a rotating wheel similar to the transmit stage but with neutral density (ND) filters instead of a single aperture. The disadvantage of this system was that the attenuation reduced the signal to noise of the measured spectrum and this was most severe on the nearest neighbor channels, which had the smallest dynamic range. On this second rotary wheel, 4 pairs of ND filters (OD=0, 0.5, 1.0, 2.0, or 2.5, Melles Griot Inc., CA) are currently installed. Sometimes different choices of ND fibers are used depending on the optical properties of the target; however when imaging rat cranium of similar size these can largely remain fixed as currently chosen. Photographs of the attenuation correction Neutral Density filter wheel are shown in Figure 2-5.
Figure 2-5 Photographs of the Neutral Density filter wheel. The left is a view from outside and the right is a view to the inside disk with light coming through the fibers.

The acquisition sequence and the synchronization of these two wheels is illustrated in Figures 2-6 and 2-7. Figure 2-6a is the diagram of the fiberoptic interface to the tissue with a circular geometry and 8 source fibers and 8 detector fibers are labeled as S1-S8 and D1-D8. Figures 2-6b and c represent the source selection wheel and the receive neutral density wheel respectively. Source fibers and detector fibers are fixed on these two wheels. A0 represents the aperture on the rotary disk and N1-4 represents the 4 pairs of ND filters. When S1 is active, positions of the two rotary disks driven by stepper motors are shown in Figure 2-6. When the next source S2 is activated, positions are changed to those shown in Figure 2-7, and so on. The computer controls and synchronizes all of these movements and ensures the data acquisition only happens when the wheels are stationary.
Figure 2-6 Diagrams are shown for when source 1 is active. Here (a) shows the fiberoptic/tissue interface, (b) shows the source shutter wheel position, and (c) shows the Neutral Density filter wheel position.

Figure 2-7 Diagrams are shown for when source 2 is active. Here (a) shows the fiberoptic/tissue interface, while (b) shows the source shutter wheel position, and (c) shows the Neutral density filter wheel position.

Both the shutter wheel and the ND wheel are driven by a stepper motors (ACS Machine Controls, MA). These are controlled through a driver broad (SMA32-A, ACS, MA), which can be programmed to provide a variety of speed options. The motor can move as fast as 57,600 steps/sec and ramp as fast as 720,000 steps/sec/sec. However due to the abrasion and the work load, the practical speed and acceleration which can provide sufficient torque for this application is about 20,000 steps/sec and 10,000 steps/sec, respectively. There are 1600 steps for one revolution and the step size for each source selection is 1/8 of revolution, i.e., 200 steps. For such a short movement, the ramp step (acceleration) determines the motion time which is about 40ms. Therefore, even ignoring the integration time required on the CCD, the minimal spin time for
projecting all 8 sources is about 320ms. This is one of the physical limitations to the temporal response of the system.

Two types of fibers are used in the system. The first is a 1mm single core silica fiber (NA = 0.3, Thor Labs Inc.). One end is terminated in a standard SMA connector and the other is epoxied in a brass ferrule. All 16 of these fibers are used for short distance delivery of light along the bench table where the main body of the system sits, i.e. between the light source and the shutter wheel or between the ND wheel and the spectrograph. The other fiber type is a flexible bundle of diameter 1.5mm sheathed in PVC and 10m long to reach the MRI magnet from the bench table (Spectraflex, Schott NA Inc., NY). The bundle is made from 50µm fibers and is more flexible and has a smaller bend radius (8mm) than a 1mm single fiber. One end of the bundle is terminated in a SMA connector and the other terminated in a ferrule (diameter: 1/8 inch, length: 5/8 inch) made of black acetal and cemented in epoxy resin. The plastic end contacts the tissue and is put inside the magnet. Ideally the end of the fiber contacting the tissue should have minimal interference to the magnetic resonance imaging; however the fiber and tissue interface introduce some susceptibility artifacts into the MRI images, as will be discussed later.

2.1.4. Fiberoptic interface and MRI coil

The MRI console made by VARIAN has an operating frequency of 15-305 MHz. The magnet was made by Magnex and is a 7-Tesla horizontal bore system with a 12cm clear bore. Everything should be fitted in this small 12cm diameter space, including animal, animal holder, RF coil, 16 optical fibers, ventilation tubes and sensors for measuring rectal temperature and arterial oxygen saturation. To collect the structural images of the animal head using MRI, a
birdcage volume RF coil is typically used as shown in Figure 2-8. The animal head to be imaged is encompassed by the coil. To minimize the image artifact due to the breathing of the animal, a holder is required in the coil to lock the animal head. To collect the NIR tomography images, optical fibers need to be placed all around the animal head and well contacted with the tissue surface. This means all the optical fibers have to be inside the coil as well. The real challenge here is the limited space available in the magnet bore. Three fiber/coil/animal interfaces are discussed in this section.

![Diagram of a low-pass birdcage coil](image)

*Figure 2-8 Diagram of a low-pass birdcage coil which produces a homogeneous field over a large region of interest, having cylindrical conductors around the periphery with capacitors to match the response in each parallel channel.*

### 2.1.4.1. Scheme A: 4cm homebuilt coil

This scheme was the preliminary interface used in the early stage of the project. A homebuilt birdcage RF coil was constructed by Mr. Sunit S. Mahajan, M.S., on a clear right-circular cylindrical plexiglass with wall thickness of 1/8 inch, having eight 6cm legs, a 4cm inner diameter, and copper traces approximately 4mm wide. The resonant coil frequency was approximately 300MHz for the Larmor frequency of protons in a magnetic field of 7 Tesla. A total of 16 holes were drilled in the wall normal to the surface in the same plane perpendicular to the bore axis and distributed equally around the coil without cutting the copper traces. Each fiber optode was manually mounted on the coil and guided through holes into gentle contact with the
phantom/tissue surface. Each hole was made to match the size of the 1/8 inch optode, so as to provide a gentle press fit inside it. This design allows each fiber to move independently, and therefore its radial position could vary by several millimeters to fit the irregular surface of the animal head. The stationary friction allows each fiber to support a certain amount of weight and thus can hold the animal head reasonably firmly, so that an additional animal holder was not required for the first phase of the study. The fibers were bent 90 degrees to exit the magnet along the bore axis in the space between the inner coil and the outer 10cm-diameter copper shield. Figure 2-9a shows the diagram of this homebuilt coil and Figure 2-9b shows a photograph of this coil when a white Teflon phantom was loaded.

![Diagram of the 4cm homebuilt birdcage coil.](a) Photograph of the coil when a white Teflon phantom is loaded](b)

*Figure 2-9 (a) Diagram of the 4cm homebuilt birdcage coil. (b) Photograph of the coil when a white Teflon phantom is loaded*

This coil was found to be impractical mainly for three reasons:

1. **Poor RF quality:** Because all the fibers penetrated the coil circuit, although they avoided breaking the copper legs, they still affected the response of the coil in the high magnetic field. As a result, the Q value (quality factor) of this coil was lower than other coils in the lab, and this parameter is crucial to the efficiency and sensitivity to collect the spin signal. Moreover the field inhomogeneity was obvious (Figure 2-10b) when this coil was used to acquire the gradient echo images which were crucial to quantify the BOLD
signal. Figure 2-10 gives examples of T2 weighted image (Spin echo) and T2* weighted image (Gradient echo) acquired using 4cm homebuilt coil.

![Figure 2-10](image)

**Figure 2-10** (a) T2 weighted image (Spin echo) (b) T2* weighted image (Gradient echo) are shown as acquired using the 4 cm custom built coil.

2. **Distorted boundaries for NIR problems**: Figure 2-10a was taken at the plane where optical fibers were positioned. The zigzag boundary resulted from the compression of the soft tissue by the solid optical fibers and also from the susceptibility artifact produced by the hard interface between fibers and tissue. This compression was necessary to maintain good light coupling. The dents that appear on the boundary can help to determine the coordinate of each fiber, which is important for modeling the light transportation but the susceptibility effect that always exists at the interface attenuates the RF signal at the interface. This phenomena is more easily observed in the T2* weighted image. Figure 2-10b was taken at the same slice as Figure 2-10a except gradient echo was used instead of spin echo. The boundary looks much smaller because of the signal loss near the fiber/tissue interface. Therefore, when the MRI images were used to generate FEM meshes for solving the light transport problem, geometry modeling error was much greater than the resolution that MRI could provide. For such a small geometry, the light propagation is heavily dependent on the boundary and even a few hundred micrometer of
error may introduce a significant mismatch in the data. The problem is not only in the estimation of the boundary but also in the coupling efficiency. For a highly distorted boundary like this, the coupling efficiency for each fiber may be very different. Such uncertainty in the coupling coefficient is unable to be exactly modeled or compensated.

3. **Fragility:** Every time the animal was loaded in the coil, each of 16 fibers needed to be manually bent and adjusted to fit the hole on the coil. It was a frequent occurrence for the fiber pressure to unintentionally break the coil or break the fiber.

In conclusion, the scheme A was found to be an unsatisfactory long term design. Modifications were made to improve the robustness and quality of the coil and avoid the highly deformed tissue boundary, and eventually Scheme B was implemented.

2.1.4.2. **Scheme B: 6cm coil with fiber holder**

Scheme B was a remedy to the problems encountered in Scheme A. A 6cm birdcage coil with superior performance was used instead of the 4cm coil. Although it was a homebuilt coil as well, it provided much better quality and robustness. When the inner diameter was increased from 4cm to 6cm, it was possible to bend the optical fibers inside of the coil without penetrating the copper loops.

The typical diameter of rat head was about 27mm. The optical fiber bundle with shields is about 3mm and has a minimal bending radius of about 8 mm. The rigid plastic tip used to encapsulate and protect the fibers was about 5mm in length. If they were assembled together as shown in Figure 2-11, the minimal radius to have everything in was about 29mm. Therefore these bended fibers would have to cross the coil when a 4cm coil was used, but with the 6cm diameter coil they could be totally enclosed inside the coil.
Another issue was how to mount these fibers. In Scheme A, fibers were anchored on the plexiglass that supports the coil circuits and locked by the stationary friction. In this scheme, instead a holder was used. The highly deformed boundary was not desirable and it was better not to use the fiber tips alone to support the tissue. To satisfy these requirements, a cylindrical holder made from black Acetal was designed, as shown in Figure 2-12. Figure 2-12a is the schematic diagram and 12b is the photograph when all the fibers were installed. Those thru-holes on the outer wall are used to guide the fiber and the smooth inner wall holds the animal and forces a circular boundary. Nylon screws are added on the end to lock the fibers.

This design brings many advantages to the imaging problem. First, because fibers and the holder are synthesized, there is no need to adjust each fiber frequently and this avoids the chance
of breakage. Second, the artificial regular tissue boundary makes the geometry modeling much easier and it is not necessary to generate an irregular forward mesh. Third, with this standard holder, the calibration phantom and the animal will have similar and consistent boundary conditions; therefore the data mismatch between the FEM simulation and the measurements is smaller.

A clear drawback of this design is that for a particular inner diameter holder, only rats of a limited range of sizes could fit in. Although it’s possible to make several holders with different size or put in a thin barrel as liner, in practice we found that the size of rat heads becomes relatively stable after a certain age, especially the skull size and the surrounding soft tissues such as skin and muscles are very good buffers for the imaging mount. Thus only one size ring was used in all the animal studies reported here. Figure 2-13 gives some examples of MRI images collected by this scheme which verify the uniformity of this coil and the smooth boundary.

![Figure 2-13 Rat cranium images are shown for (a) T2 weighted image (b) T2* weighted image using the 6cm coil with the inner circular fiber holder, forcing the exterior soft tissue of the cranium into a circle.](image)

2.1.4.3. **Scheme C: 3.8cm Varian coil with prism guided fiber holder**

This plan was a backup plan to Scheme B. A 3.8cm commercial Varian coil in the lab which provided the best performance was used in this plan. There was still not enough room for
bending fibers within this coil, however a miniature right angle prism (PS905, Thorlabs Inc.) was added onto the end of each fiber to steer the beam 90 degrees onto the tissue surface, while the fibers stayed parallel to the length of the animals body. In this design, a holder for fibers and prisms was also required. The holder was made up of two pieces, with one used to lock the fibers and the other used to hold the prisms. Their sketches are shown in Figure 2-14(a) (b). Fibers were glued in Part A and polished all together and the prisms were glued in Part B. Then parts A and B were attached as shown in Figure 2-14 (c). One of the working interfaces of the prism aligned to the fiber and the other contacted the animal.

![Figure 2-14](image)

**Figure 2-14** The fiber holder in Scheme C is shown with two pieces in (a) and (b). A schematic diagram of the pieces together is shown in (c) for the complete assembly.

Although appealing in the concept, this design is associated with many practical problems. Due to the high NA of the fiber, the prism amplified the effective aperture and reduced the spatial resolution. Because of the extra interfaces introduced in the optical path the transmission efficiency was decreased. Finally, the implementation of this design was complicated and difficult. Since Scheme B worked reasonably well, this latter scheme C was abandoned after considerable evaluation.

### 2.1.5. Spectrograph and CCD detector
The eight 1 mm fibers exiting the ND filter wheel were epoxyed into a 25mm brass ferrule in a line at a pitch of 1.5mm and height of 11.5mm. This design allowed a separation of 500µm between the edges of the neighboring fibers. The light from the fibers was collimated with a 25mm diameter, and 60mm focal length achromatic lens anti-reflection coated for 650-1000nm. The collimated light was then focused onto the slits of a spectrograph using a second achromatic lens with a diameter of 30mm and a focal length of 62mm. Achromats were used over singlet to improve the image quality at the relatively low aperture. The two lenses were mounted on a rod system allowing focusing, while the first lens was housed in a floating mount on the rod system, allowing x and y directional alignment. The combination of the two lenses gave a magnification of 0.92 so that the image of the fibers on the slits was 10.61mm high and the image of a single fiber was about 0.92mm high. The spectrograph was a Triax 320m (JY Horiba, Edison, NJ) with an aperture at F4.1, a 0.32m focal length, motorized input slits and a motorized triple grating turret allowing the gratings to be rotated and changed. For the majority of the studies, a 300g/mm grating blazed at 1000nm was used but we also had a 600g/mm grating blazed at 1000nm for use where higher throughput was needed with a reduced spectral range.

Multiple spectra from light imaged at various heights in the entrance slit of the spectrograph could be detected simultaneously with a two dimensional array. The optics permitted close vertical spacing of signals without crosstalk. Therefore the spectrograph was fitted with a CCD camera (Wright Instruments, Enfield, UK) containing an EEV CCD05-30-219 MPP CCD cooled to 205 Kelvin. The CCD has 1242 pixels in the wavelength axis (Horizontal) and 1152 pixels in the slit axis (Vertical). The pixels are 22.5x22.5 µm² and the active area is 27.9x25.9mm². The CCD was configured in frame transfer mode for which half of the chip was
used as the imaging section and the other half was used as the store section, but there was no shield over the store section. The CCD was mounted at $5^\circ$ to the focal plane in the wavelength axis of the spectrograph to optimize resolution in the wavelength and imaging planes. Furthermore, the CCD was mounted so that the center of the slit fell in the center of the image section of the CCD. Although the store section of the CCD was not shielded, the spectrograph had adequate stray light rejection and imaging capabilities to ensure negligible light fell on the store section during the readout. The image section was 12.96mm high and was adequate for the image of the fibers on the slit. The majority of the light from each fiber produced an image 42 pixels high (0.92mm).

2.2. Performance

This section discusses various issues about the performance of the instrument. All of them are closely related to the collected signals, including the noise source, crosstalk within parallel channels, system dynamic range, etc.

2.2.1. Noise

The fundamental output of the instrument is the transmitted spectrum, that is, the intensity distribution along at all wavelengths for a specific source and detector pair. This intensity is measured by the CCD device. Here the general noise model for CCD device will be discussed and the crosstalk noise for the parallel detection will be covered in the next section.

A CCD is a semiconductor device based on the MOS (Metal-Oxide-Silicon) technique. Every pixel on the chip is a potential well and capable of capturing and storing charges. It captures photons through the photoelectric effect and turns them into electrons, which are
efficiently stored in the well. Moreover the depth of the well is controlled by the applied gate voltage. With a proper sequence of the control gate voltage, electrons can be transferred entirely from one well to another, just like pouring water from a cup to another. In the end, when the charges reach the transistor, it can be amplified and converted into digital readout, after the sequential transferring process from all wells.

For a CCD device, the noise mainly comes from the following three sources:

1) *Shot noise* is due to the random nature of the event whether the photon is detected. The probability of this event is described by the quantum efficiency (QE) of the detector. The random incidence is described by Poisson statistics, where the standard deviation is the square root of the mean, i.e., \( \text{noise} = \sqrt{Q_e \cdot n_p} \), where \( n_p \) is the photon flux and \( Q_e \) is the quantum efficiency. Therefore, for a given device the shot noise is determined by the intensity of the incident light.

2) The *Dark current* generates a dark signal, that is, the signal in the absence of light. The dark signal is due to the generation of thermally excited electrons mimicking incident radiation in optoelectronic devices. It is highly temperature dependent. Around room temperature, it falls by about a factor of 2.5 for each 10 °C drop in temperature. In our system, the CCD is cooled to 200K and the dark current is extremely small, such that only \( 1.7 \times 10^{-3} \) electrons/pixel/second are generated. This dark signal introduces an offset to the true signal and it is also a source of a small amount of shot noise.

3) *Readout noise* originates in the transistors at the output amplifier of CCD. It is present in each output data of the camera. The typical value is about 3-30 electrons RMS and increases as the readout rate is increased. In our system, for the 200 kHz readout frequency, the readout noise is about 20 electrons RMS.
Assuming these three sources listed above are the dominant ones, the signal to noise ratio (SNR) of the camera is given by

\[
SNR = \frac{NQ_e n_p t}{\sqrt{NQ_e n_p t + Nn_d t + n_r^2}}
\]

(2-1)

Where \( n_d \) is the dark current (electrons/pixel/second), \( n_r \) is the readout noise (electrons RMS/pixel), \( t \) is the integration time (second) and \( N \) is number of pixels binned together. Binning is a process in which the charge from several adjacent pixels is summed before being read out. The acquisition time and the readout noise component can be reduced by binning. In our system, every fiber spans about 41 rows on the chip and they are binned together. Therefore here \( N = 41 \) for our system. Our CCD is front illuminated which has QE about 15-40\% in 700-900nm range. Because the dark current and readout noise are very small compared to shot noise and the real signal in most situations, SNR can be approximately calculated using the readout number, i.e., \( SNR \approx \sqrt{\text{readout}} \). The readout number is determined by the product of the incident light intensity and the integration time. The upper limit is the capacity of the potential well. For this camera, each well and the output register can hold 150k electrons. The maximal SNR for each readout is 387:1. Averaging the repetitive measurement is a typical method to further improve the SNR by square root of number of average. In this project, to achieve the best spectral quality, at least 10 times averaging is used for most cases. The typical averaged noise level for intensity is about 0.2\%.

2.2.2. Crosstalk

Here the crosstalk refers to the coupling of parallel channels. Ideally, every transmit channel and every receive channel should be completely isolated. Crosstalk can happen in
several places such as the source selection wheel, the receive balance ND filter wheel and the CCD. Indeed the dominant crosstalk source is the shielding glass on the CCD, which could generate noticeable stray light when the incident light is strong enough so as to overlay other channels. Although this stray light is a very small portion of the total incident light, when two channels have orders of magnitude difference, the stray light generated by the strong channel becomes a significant crosstalk signal in the weak channel. As we previously introduced, the ND filter wheel is necessary to balance the intensity between detector channels, thereby limiting this crosstalk.

Figure 2-15b shows the image on the CCD when the balance wheel was used for the acquisition geometry like Figure 2-15a. The vertical profile transect from the image is shown in Figure 2-15c. All the channels were well separated and had similar intensity. Figure 2-16 shows the situation that the other seven receive fibers were black taped. Obviously there was no noticeable crosstalk to the adjacent channels knowing that this transmitted channel was passing the 2 OD attenuation filter first. However, if the ND filter was removed, significant stray light was observed as shown in Figure 2-17b. A clear tail crossed the banned area into the adjacent channel, as shown in Figure 2-17c.
Figure 2-15 The acquisition geometry is shown in (a) and (b) shows the intensity image on the CCD with detector channel intensities balanced, with D1 to D8 from low to high. In (c) the vertical intensity profile transecting the middle of CCD is shown.
Figure 2-16 (a) The acquisition geometry and CCD signal when all other detectors are blocked is shown. In (b) the intensity profile on the CCD is shown. In (c) the vertical profile at the middle of CCD together in contrast with the one in Figure 2-16c, with all channels (red) and with only one channel (blue).
Figure 2-17 (a) The acquisition geometry is shown with the other detectors blocked and the ND filter removed. In (b) the intensity image on the CCD is shown, illustrating the stray light pattern. In (c) the vertical profile transecting the middle of CCD together with the previous two profiles.

2.2.3. Dynamic Range

With the balancing ND filter, the system dynamic range is flexible by varying the integration time as long as enough signals are collected to match the SNR requirement. For each spectrum, its dynamic range is determined by the capacity of the potential well and the minimal number of counts to satisfy the SNR requirement and typically was about 20D.
2.2.4. Repositioning error

NIR data acquired at the periphery of an object is sensitive to the coupling between the fiber and the object surface. Every time the object is repositioned, the coupling may not be exactly the same. Such repositioning error can lead to artifacts in the reconstructed image. In order to assess the magnitude of this error, the calibration cylindrical Teflon phantom was measured 6 times. The phantom was removed and repositioned between each measurement but the fiber array was fixed. The averaged measurement RMS error for a single wavelength was 3.7% (16mOD) in intensity and the relative error compared to the dynamic range of the attenuation was approximately 0.8%. The noise model used in all the simulations was the relative error which was approximately 1.0%. Compared to the noise term discussed in 2.2.1 which can be diminished by increasing the integration and number of average, this error is hard to quantify or correct and can be substantial on the soft phantoms and the animals. That’s one of the challenges for absolute imaging of small animals.

2.3. Calibration

The calibration procedure is essential to reduce the deterministic bias of the measured signal. Two kinds of calibration must be applied: (1) System calibration (2) Model calibration.

System calibration is done to eliminate the influence of the variation among all the source-detector combinations. Since our dataset is a broadband spectrum, it requires two-dimensional calibration, i.e., wavelength axis calibration and intensity calibration for each source-detector pair. Some components like the light source may change daily in its output spectrum, so the system needs to be calibrated before every use.
For the wavelength axis calibration, a neon lamp (model no. 6032, Oriel) is used to align the motorized grating stage to the right wavelength position. One channel is activated to measure the spectrum of a neon lamp that has many distinct spectral lines at particular wavelengths. Using the positions of these features, the stepper motor that drives the grating stage can be calibrated. Because all the channels are parallel to the wavelength axis on the CCD, calibrating one channel works for all.

For the intensity calibration, all the 64 source-detector pairs are calibrated simultaneously with a white Teflon cylinder phantom. The procedure is to take a measurement on this phantom as intensity reference. The measurement for the target object will be the difference to the reference dataset plus the known attenuation of the Teflon phantom, as shown in Equation 2-2.

\[ A_{obj} = -\ln\left(\frac{I_{obj}}{I_{ref}}\right) + A_{ref} \]  

(2-2)

Where \( A_{obj} \) is the desired system-independent attenuation spectrum of the target and, \( A_{ref} \) is the pre-measured attenuation spectrum on the stable reference phantom, and \( I_{obj} \) and \( I_{ref} \) are the intensity measurement of the target and the reference respectively.

Model calibration is a necessary step for image reconstruction to minimize the imperfection in the mathematical model, such as boundary conditions, discretized error, dimensional difference, etc (McBride et al., 2001a; McBride et al., 2001c). In reality, the measured data is slightly different to the data simulated by the model and could become a systematic error in the image reconstruction. The differences between data measured from a homogeneous phantom and data calculated from the model are stored and subtracted from measurements of the target object, while homogeneous bulk fitting of data provides an initial guess for image reconstruction.
2.4. Discussion

In this chapter, a novel MRI-coupled broadband near-infrared tomography system for small animal brain studies is outlined. The apparatus can collect full NIR spectra from 700 to 900nm at 64 source-detector pairs all around the target. The fiberoptic interface and MRI coil are coupled, and images can be acquired simultaneously with both modalities. The shot noise in each channel is as small as 0.2% for a typical acquisition. However the random coupling error can be as much as 3.7% for such a small geometry imaging problem. The system is limited by the broadband source power and the light delivery efficiency, thus the typical integration time for each detector is about 1-4 seconds depending on the optical properties of the object. Other operations such as transferring the data from the CCD to the computer, and switching the source selection wheel and the receiver balancing wheel costs an additional 400ms per source in total. Therefore the total acquisition for 10 averages is about 112-587 seconds. This system is not suitable for functional activation studies which require high temporal resolution but are primarily intended for relatively steady-state studies such as hypoxia, ischemia and tumor models.
Chapter 3. Second Derivative Spectroscopy Based DOT

A unique feature of this project is the capability of resolving both absorption and scattering images of tissue with the steady-state broadband measurement, without the technological complexity of time or frequency domain signal detection. The fundamental underlying approach is to utilize second derivative spectroscopy analysis (SDSA), while using the water absorption features of tissue to estimate the optical pathlength. This chapter describes the details of this theory, and presents theoretical and experimental evidence to demonstrate the robustness and accuracy of the approach for the reconstruction of NIR images. Finally, the challenging case of inhomogeneous water distributions within tissue is addressed, and the limitations of this approach are analyzed.

3.1. Introduction

The major limitation in NIR imaging has always been related to the fact that light experiences many scattering events when passing through tissue and the collected optical signal is a nonlinear function of both absorption and scattering properties of the medium. To realize the potential of near-infrared imaging, the most challenging problem is to separate absorption and scattering properties, thereby allowing interpretation of the chromophore concentration using linear absorbance spectroscopy.

Optical transport model-based image reconstruction allows accurate recovery of optical property images. In the near infrared range, the diffusion approximation of the transport equation (Section 4.2.1) is widely accepted as a practical model in which the distribution of the optical properties within the domain can be determined from measurements of the light distribution through the tissue surface. The uniqueness of this determination has been investigated (Arridge
and Lionheart, 1998) and the conclusion has been widely accepted that the solution is not unique when single wavelength steady-state diffusion-based measurements are used, resulting in an inability to separate local absorption from local scattering values. The conclusion implies that the optical pathlength has to be measured as well as the transmitted light intensity for those systems to measure both absorption and scattering properties. In general, most of these approaches are either time domain systems (TDS), in which the time-resolved tissue response to a picoseconds pulsed laser is recorded, or frequency domain systems (FDS), in which the frequency-dependent signal from a modulated laser source is detected after passing through the tissue. The equivalent measure of the optical pathlength, mean flight time or phase shift is provided together with the intensity measurement to achieve the unique solution.

Recent studies have demonstrated that the use of multiple wavelengths in image reconstruction along with a priori estimates of the spectral features can potentially resolve the problems of having a non-unique solution (Corlu et al., 2003; Li et al., 2004), through a constraint based implementation in the image reconstruction process. This needs to be taken into context with TDS or FDS systems that are thought to provide a secondary independent measurement that is more uniquely associated with the scattering coefficient, thereby providing a reliable method to separate these coefficients. Although the actual image reconstruction is an inevitable ill-posed problem due to limitations in the experimental setup and the numerical approach, least-squares iterative optimization algorithms have shown successful recovery of both absorption and scattering properties.

In this thesis, we describe a novel method to reconstruct both absorption and reduced scattering properties in the turbid medium from steady-state boundary data by applying the principles of second derivative spectroscopy to an imaging problem. Herein our transmission
data is measured by the broadband steady-state spectral CCD system introduced in the Chapter 2 and the term ‘spectral domain system’ is used to distinguish it from the others time domain systems (TDS) or frequency domain systems (FDS). The proposed method relies on broadband spectroscopy, but also provides a direct estimate of the optical pathlength, thereby providing a robust data set for quantification of absorption and scattering properties.

This approach can be explained by the fact that the differential pathlength (DP) can be consequently derived from the broadband attenuation spectrum using our second-differential spectra analysis method (SDSA) and provides another constraint to solve the inverse problem uniquely, given additional input information about the water concentration of the tissue. Since physically the DP is an equivalent measure of mean optical pathlength as that of mean flight time by TDS or phase shift by FDS (Arridge et al., 1992), our approach is essentially consistent with the other two methods.

Here, a simple derivation from the diffusion equation is provided to illustrate the equivalence of phase shift in the frequency domain and differential pathlength in the CW domain. In an infinite diffusive medium, the Green’s function solution to diffusion the equation at the distance \( r \) to a DC source or a modulated source can be derived as Equation 3-1 and Equation 3-2.

\[
\phi_0(r) = \frac{S_0}{4\pi Dr} e^{-kr}, \quad \text{where} \quad k = \sqrt{\frac{\mu_a}{D}}
\]

\[
\phi_\omega(r) = \frac{S_0}{4\pi Dr} e^{-kr}, \quad \text{where} \quad k = \sqrt{\frac{\mu_a}{D}} \sqrt{1 + i \frac{\omega}{\mu_a c}}
\]
Here, $\phi$ is the fluence rate, $D$ is the diffusion coefficient, $S_0$ is the source intensity, $\mu_a$ is the absorption coefficient, $\omega$ is the modulation frequency, and $c$ is the speed of light in the medium. In terms of the definition of the differential pathlength, which is ratio of the differential attenuation to the absorption, it can be derived from Equation 3-1:

$$DP = \frac{\partial A}{\partial \mu_a} = \frac{\partial (-\ln(\phi_0(r))))}{\partial \mu_a} = \frac{r}{2\sqrt{\mu_a}D}$$  \hspace{1cm} (3-3)$$

Here $A$ is the attenuation. The phase shift $\theta$ also can be derived from the Equation 3-2:

$$\theta = \frac{\omega}{c} \left( \frac{r}{2\sqrt{\mu_a}D} \right)_i (\omega \ll \mu_a c)$$  \hspace{1cm} (3-4)$$

It is clear that $\theta = DP \cdot \frac{\omega}{c}$ where $\frac{\omega}{c}$ is the wave number.

At Dartmouth, frequency domain tomography systems have been investigated for many years (McBride et al., 2001b; Pogue and Paulsen, 1998). It has shown promising success in breast cancer imaging, with studies ongoing currently. A finite element method based image reconstruction program has been successfully implemented in the frequency domain of the diffusion equation to recover absorption and scattering properties. Arridge et al. have shown that when the modulation frequency is lower than few hundred MHz, the attenuation of DC and AC signals are similar (Arridge et al., 1992). Therefore, if the differential pathlength and intensity measured by the steady-state broadband system are mapped to the amplitude and phase in frequency domain, the frequency domain reconstruction program can be conveniently used to recover the absorption and scattering images of tissue.

The differential pathlength is a function of wavelength, and since the estimation of the differential pathlength relies on the water absorption feature in the second derivative attenuation
3.2. Differential pathlength estimation from water features

3.2.1. Second derivative spectroscopy analysis

Second-differential spectral analysis (SDSA) is a novel approach to diffuse optical tomography (DOT) using broadband spectroscopy data (Xu et al., 2004; Xu, 2003). It not only provides a strategy to linearly separate chromophore concentrations and scattering coefficients from the attenuation spectrum but also provides a means to estimate the optical pathlengths near the water features in the second-differential spectrum (around 740nm and 840nm). This calculated pathlength is a secondary independent measurement to the intensity for solving the diffusion approximation equation for both absorption and scattering properties using amplitude and phase data types. Although several approximations need to be made during the analysis, they are reasonable assumptions based on the physics of light propagation in tissue and the makeup of the chromophores present in tissue.

This method was first introduced by Matcher et al (Matcher et al., 1994). In non-scattering media, the relationship between the concentration of absorbing chromophores and
attenuation can be modeled by a standard Beer Lambert law that states that the attenuation ($A$) is proportional to the product of the specific extinction coefficient, the chromophore concentration ($c$) and the source-detector separation ($l$). In a strongly scattering medium such as tissue, this simple expression does not hold because the light takes multiple paths between transmit and receive fibers so that the actual optical pathlength is much larger than the source-detector separation. A modified Beer-Lambert law (MBLL) is then introduced to address the scattering effect on the attenuation (Kohl et al., 1998; Matcher et al., 1994).

$$A = \gamma \cdot l \cdot \mu_a + G$$  \hspace{1cm} (3-5)

Here $A$ is the attenuation, $l$ is the optode spacing and $G$ is a term to describe the scattering losses. The term $\gamma$ describes the effect of the mean pathlength of photons being increased by multiple scattering, which is conventionally termed the differential pathlength factor (DPF). The product of $\gamma$ and $l$ is the differential pathlength. Within the framework of the modified Beer-Lambert law, the attenuation is linear with respect to $\mu_a$ though, in reality, both $\gamma$ and $G$ are function of $\mu_a$ and $\mu_s$. Matcher et al. have described how to use second-differential analysis based on the modified Beer Lambert law together with the water concentration knowledge to provide an estimate of the differential pathlength.

Although this method has been published in a few papers, we use a mathematically more rigorous derivation here, where the assumptions are more transparent and easier to examine. The light attenuation is defined as $A(\mu_a, \mu_s) = -\ln(I_d / I_s)$, where $I_d$ and $I_s$ are the detected and source light intensity respectively. It is a function of the optical properties of absorption and reduced scattering, $A(\mu_a(\lambda), \mu_s(\lambda))$, where both coefficients are wavelength dependent, so the 1st and 2nd derivatives of attenuation with respect to wavelength ($\lambda$) can be expanded as:
\[ A' = \frac{dA}{d\lambda} = \frac{\partial A}{\partial \mu_a} \frac{d\mu_a}{d\lambda} + \frac{\partial A}{\partial \mu_s} \frac{d\mu_s}{d\lambda} \]  

(3-6)

\[ A'' = \frac{d^2 A}{d\lambda^2} = \frac{\partial A}{\partial \mu_a} \frac{d^2 \mu_a}{d\lambda^2} + \frac{\partial A}{\partial \mu_s} \frac{d^2 \mu_s}{d\lambda^2} + \frac{d(\partial A/\partial \mu_a)}{d\lambda} \frac{d\mu_a}{d\lambda} + \frac{d(\partial A/\partial \mu_s)}{d\lambda} \frac{d\mu_s}{d\lambda} \]  

(3-7)

Note that the wavelength independent variables within \( A \) are now left out for clarity of presentation. Although there are five terms left in Equation 3-7 for \( A'' \), it has been found that, with the chromophores present in tissue and with the weak wavelength dependence of scattering coefficient of tissue (to be demonstrated later in this section), the last four terms are small compared to the first term. If the contribution of these four terms is defined as \( o_1 \) and dropped out, Equation 3-7 is simplified to:

\[ \frac{\partial^2 A}{\partial \lambda^2} \approx \frac{\partial A}{\partial \mu_a} \frac{\partial^2 \mu_a}{\partial \lambda^2} = DP \cdot \frac{\partial^2 \mu_a}{\partial \lambda^2} \]  

(3-8)

This is the essence of the second derivative spectroscopy analysis that the 2\textsuperscript{nd} derivative spectrum of the attenuation is the 2\textsuperscript{nd} derivative spectrum of the absorption coefficient scaled by the differential pathlength. Given that the absorption coefficient is a linear summation of the concentration of the chromophores \((C_i)\) multiplied by their known specific extinction coefficients \((\varepsilon_i)\), \( \mu_a = \sum_{i=1}^{N_c} (C_i \varepsilon_i) \), Equation 3-8 can be further expanded as:

\[ \frac{\partial^2 A}{\partial \lambda^2} = \sum_{i=1}^{N_c} \left( DP \cdot C_i \frac{\partial^2 \varepsilon_i}{\partial \lambda^2} \right) + o_1 \]  

(3-9)

Here \( N_c \) is the number of chromophores.
Because DP varies gradually with wavelength, it is possible to be approximated by the value at a particular wavelength $\lambda_o$ in a reduced wavelength range where a sharp chromophore feature exists. Therefore in this reduced wavelength range, Equation 3-9 can be transformed to:

$$\frac{\partial^2 A}{\partial \lambda^2} = DP(\lambda_o) \cdot \sum_{i=1}^{N_c} (C_i \frac{\partial^2 \varepsilon_i}{\partial \lambda^2}) + o_1 + o_2$$  \hspace{1cm} (3-10)

Here $o_2$ represents the error term due to the approximation using $DP(\lambda_o)$ to $DP(\lambda)$. Knowing that Equation 3-10 can be discretized in such reduced wavelength range $\lambda_i (i = 1, \cdots, N_\lambda)$ and discarding the small terms $o_1$ and $o_2$, its matrix form can be derived:

$$A'' = \varepsilon' \times C \times DP(\lambda_o)$$ \hspace{1cm} (3-11)

Where $A''$ is a $N_\lambda$ by 1 vector which is the 2nd derivative spectrum calculated from the measured attenuation spectrum, $\varepsilon'$ is a $N_\lambda$ by $N_c$ matrix which is the 2nd derivative specific extinction spectra of chromophores, and the scalar $DP(\lambda_o)$ and the $N_c$ by 1 vector $C$ are the unknown DP and chromophore concentrations. Since typically $N_\lambda \gg N_c$, Equation 3-11 is an over-determined multi linear problem and can be solved with multi linear regression method. Therefore, $DP(\lambda_o)$ scaled concentrations, $DP(\lambda_o) \cdot C_i (i = 1, \cdots, N_c)$ can be obtained. The differential pathlength and the chromophore concentration are coupled; to qualify either one of them, the other must be provided. Since one of the important chromophores, water, can be measured by other technologies (the nuclear magnetic resonance system) accurately, $DP(\lambda_o)$ could be extracted out from the product, $DP(\lambda_o) \cdot C_w$. Consequently other chromophore concentrations could also be derived. To distinguish this estimated differential pathlength from the true mean optical pathlength, the name $DPW$ is given.
Figure 3-1, (a) Specific extinction spectra of HbR, HbO2 and Water (b) 2nd derivative spectra of specific extinction of HbR, HbO2 and Water

In tissue, deoxyhemoglobin, oxyhemoglobin and water are the dominant absorbing chromophores in the NIR range. The absorption coefficient is linear combination of the absorption due to individual chromophores. The specific extinction coefficient spectra for these three chromophores are shown in Figure 3-1(a). At every wavelength the contribution comes from each chromophore. However, contributions of individual chromophores become sharper and more distinguishable on the wavelength axis in the second derivative spectrum as shown in Figure 3-2(b).

Savitzky-Golay filtering (Orfanidis, 1996) was used to calculate the second derivative from the noisy spectral data. This method can be thought of as a generalized moving average by performing un-weighted linear least squares fit using a polynomial of a given degree and second-derivative coefficient can be easily derived from the fitted polynomial coefficients. In our practice, a quartic polynomial with 40nm span was used to remove high frequency noise without sacrificing the low frequency spectral features.

Water has two prominent second derivative absorption features near 740nm and 840nm shown in Figure 3-1(b). To apply the relation in the Equation 3-11 and calculate the optical
pathlength near these wavelengths, two reduced wavelength ranges (700-800nm and 800-880nm) are empirically chosen to fit the measured attenuation curve. The spectrum is sampled about every 1/3 nm. Three chromophores, oxyhemoglobin, deoxy-hemoglobin and water, are typically included in the fitting. Both the measured attenuation spectrum \( A \) and the absorptivity spectrum \( \varepsilon \) are processed by a 40nm-width quartic polynomial filter to minimize the high frequency noise and the second derivatives are calculated at the same time.

### 3.2.2. Validation of the assumptions

Several assumptions and approximations were made in the SDSA approach. First, in Equation 3-7, the last 4 terms was assumed to be a small contribution, \( o_1 \). Second, in Equation 3-10, another term \( o_2 \) which represents the error term due to the approximation using \( DP(\lambda_0) \) to \( DP(\lambda) \) was assumed to be negligible on the fitted product of \( DP(\lambda_0) \) and

\[
\sum_{i=1}^{N_c} \left( C_i \frac{\partial^2 \varepsilon}{\partial \lambda^2} \right)
\]

To examine this first assumption, simulations were performed using the diffusion equation for a homogeneous infinite medium. For simplicity, assume \( D = \frac{1}{3\mu_s} \), then

\[
\frac{\partial A}{\partial \mu_s} = -3D^2 \frac{\partial A}{\partial D}
\]

The wavelength dependence of reduce scattering property is modeled by an empirical approximation to Mie theory, \( \mu_s = a\lambda^{-b} \). The fluence rate \( \Phi_{inf}(r) = \frac{S_0}{4\pi Dr} e^{-r\sqrt{\mu_s/D}} \), so the attenuation is:

\[
A = \ln(S_0 / \Phi_{inf}) = \ln(4\pi r) + \ln D + r\sqrt{\mu_s / D}
\]
Group A: 1st and 2nd derivative of optical properties to wavelength can be defined by following equations:

$$
\frac{d\mu_a}{d\lambda} = \sum d\varepsilon_i c_i \\
\frac{d\mu_i}{d\lambda} = -ab\lambda^{-(b+1)}
$$

$$
\frac{d^2 \mu_a}{d\lambda^2} = \sum d^2 \varepsilon_i c_i \\
\frac{d^2 \mu_i}{d\lambda^2} = ab(b+1)\lambda^{-(b+2)}
$$

Group B: Differentials of A to optical properties can be derived as:

$$
\frac{\partial A}{\partial \mu_a} = \frac{1}{2\sqrt{\mu_a} D} r \\
\frac{\partial A}{\partial D} = \frac{1}{D} \sqrt{\mu_a D} 2D^3 r \\
\frac{\partial A}{\partial \mu_i} = -3D + \frac{3\sqrt{\mu_a D}}{2} r
$$

$$
\frac{\partial^2 A}{\partial \mu_a^2} = -\frac{1}{4\mu_a \sqrt{\mu_a} D} r \\
\frac{\partial^2 A}{\partial \mu_a \partial \mu_i} = \frac{3}{4} \sqrt{\mu_a} r \\
\frac{\partial^2 A}{\partial \mu_i^2} = 9D^2 - \frac{9D\sqrt{\mu_a D}}{4} r
$$

Given $a, b$ and the chromophore makeup $c_i$, each term in Equation 3-6 and Equation 3-7 can be calculated. Here, these two equations are presented again.

$$
A' = \frac{dA}{d\lambda} = \frac{\partial A}{\partial \mu_a} \frac{d\mu_a}{d\lambda} + \frac{\partial A}{\partial \mu_i} \frac{d\mu_i}{d\lambda} (3-6)
$$

$$
A'' = \frac{\partial A}{\partial \mu_a} \frac{d^2 \mu_a}{d\lambda^2} + \frac{\partial A}{\partial \mu_i} \frac{d^2 \mu_i}{d\lambda^2} + \frac{\partial^2 A}{\partial \mu_a^2} \left(\frac{d\mu_a}{d\lambda}\right)^2 + \frac{\partial^2 A}{\partial \mu_i^2} \left(\frac{d\mu_i}{d\lambda}\right)^2 + 2\frac{\partial^2 A}{\partial \mu_a \partial \mu_i} \frac{d\mu_a}{d\lambda} \frac{d\mu_i}{d\lambda} (3-7)
$$

To better visualize the result, an example is given assuming HbR = 50µM, HbO2 = 50µM, Water = 100%, $a = 1$, $b = 1.1$, $r = 30$mm, $\lambda = 0.650\sim0.9$µm. Individual multiplication factors in the 1st and 2nd derivative of attenuation spectrum (Equation 3-6 and 3-7) are calculated using the equations in Group A and B and plotted in Figure 3-2 and Figure 3-4 respectively. Their inner product terms, i.e., the additive terms in the expression are plotted in the 3rd column in those
figures. In Figure 3-3 and Figure 3-4, the actual 1\textsuperscript{st} and 2\textsuperscript{nd} derivative of the attenuation spectrum are plotted with the additive terms in Equation 3-6 and Equation 3-7.

Figure 3-2. Individual multiplication factors in the 1\textsuperscript{st} derivative of attenuation spectrum (Equation 3-6) and their inner product terms (the 3\textsuperscript{rd} column), i.e., the additive terms in the expression. X axis is the wavelength in µm.

Figure 3-3. The actual 1\textsuperscript{st} derivative of attenuation spectrum and the two terms on the right side of Equation 3-6.
In the 1\textsuperscript{st} derivative spectrum, $A'$, the first term due to the differential to the absorption, dominates and the second term related to the scattering is more like a featureless offset. In the 2\textsuperscript{nd} derivative spectrum $A''$, the first term also dominates and the other four terms are insignificant at most wavelengths. The 3\textsuperscript{rd} term has a non-trivial contribution below 700nm but it is not within the wavelength of interest.

Clearly in the derivatives of the spectrum, the signals are more related to the absorption property than the scattering property. Although the diffusion equation for infinite medium was used in the analysis above, the conclusion doesn’t rely on it, because the fundamental hypothesis that validates this theory is almost independent of the details of the light transport model. Such hypothesis relies on two properties (1) the scattering parameters have weak wavelength dependence and therefore won’t produce strong signals in the derivative channels, (2) absorptivity of those chromophores such as HbR, HbO2 and water have sharp spectral features to provide intense signals in the derivative channels. In general, the SDSA could be applied in many situations, where these properties are satisfied such as a heterogeneous medium, complex boundaries, or a non-scattering medium.
Figure 3-4. Individual multiplication factors in the 2\textsuperscript{nd} derivative of attenuation spectrum (Equation 3-7) and their inner product terms (the 3\textsuperscript{rd} column), i.e., the additive terms in the expression. X axis is the wavelength in µm.
Figure 3-5. The actual 2\textsuperscript{nd} derivative of attenuation spectrum and the five terms on the right side of Equation 3-7.

Figure 3-6. (a) A measured attenuation spectrum from a homogeneous liquid phantom with 60\mu moles/L HbR and 1.0% Intralipid are shown. In (b) the raw data of its second-derivative spectrum are shown, along with the fitted spectrum using SDSA, and the residuals.

Examples of a measured attenuation spectrum, its raw second-derivative data and the fitted 2\textsuperscript{nd} derivative data from Equation 3-11, along with the residuals of fits in both ranges are shown in Figure 3-6, resulting from a liquid phantom which has 60\mu M HbR and 1.0% Intralipid. The residual results from the experimental noise and the ignored terms ($\alpha_1, \alpha_2$) and is more
consistent to random and featureless behavior. The total energy of this residual is about 5% of
the original signal, which implies our approach does capture the principle component in the
second-differential analysis and the exclusion of those small terms is a reasonable and effective
approximation.

3.2.3. **Optimal representative wavelengths**

As we discussed previously, a single value of the differential pathlength $DP(\lambda_o)$ was
used and fitted in SDSA to represent the entire differential pathlength $DP(\lambda)$ in that reduced
wavelength range. This wavelength $\lambda_o$ is named the representative wavelength. For pure
spectroscopy studies, the actual position of this $\lambda_o$ is not important. However for the purpose of
both absorption and reduced scattering properties image reconstruction, the estimated
pathlengths need to be modeled at the correct wavelengths. Otherwise, a substantial bias may
occur in the pathlength data and lead to a biased optical properties. This section investigates the
wavelength the SDSA differential pathlength represents.

As it showed in Equation 3-9, the right-hand side is equal to the product of the
differential pathlength and the 2nd derivatives of chromophores on the left-hand side. In this
reduced wavelength range, the 2nd derivative feature of water resembles a Gaussian function.
Only the pathlengths within this Gaussian function can be transferred to the right-hand side.
Intuitively, when the width of this Gaussian function approaches to zero, it becomes a delta
function and only picks up the pathlength at the center. In reality, this feature always has certain
width and a range of differential pathlengths are picked. If the differential pathlength is in the
reduced wavelength range, the representative wavelength $\lambda_o$ can be determined by the square of
2nd derivative water feature weighted average of wavelengths. Mathematically it can be described by:

\[
\lambda_0 = \frac{\int_{\lambda_1}^{\lambda_2} \lambda f(\lambda)^2 d\lambda}{\int_{\lambda_1}^{\lambda_2} f(\lambda)^2 d\lambda}
\]  

(3-12)

Here, \( f(\lambda) \) is the 2nd derivative spectrum of water extinction coefficient, \( \lambda_1 \) and \( \lambda_2 \) are the bounds of the reduced wavelength range. Using Equation 3-12, the representative wavelength \( \lambda_0 \) for both fitting ranges 700-800nm and 800-880nm can be determined. They are 735.8nm for the water feature within 700-800nm and 831.5nm for the water feature within 800-880nm.

The fact that the differential pathlength spectrum is not flat has not been considered when deducing Equation 3-12. In general, the differential pathlength decreases to longer wavelength since the scattering is a function of wavelength. Shorter wavelengths should have more contribution to the fitted differential pathlength. Hence, the representative wavelength \( \lambda_0 \) is expected to be slightly shorter than the wavelength given by Equation 3-12.

---

**Figure 3-7.** A diagram of the numerical experiment setup, where the chromophores are inputs to the diffusion model, and the calculated forward data is used to generate simulated attenuation and DP data, to test reconstruction approaches.
To investigate such a shift, an alternate method is proposed to determine the representative wavelength $\lambda_o$. In this method, the representative wavelength was determined by a statistical analysis of a numerical simulation. The numerical experiment setup can be depicted through Figure 3-7. Concentrations of total hemoglobin (HbT), Water, Intralipid solution, oxygen saturation and source-detector separation ($r$) were randomly chosen from 50~100µM, 50%-100%, 1%~5%, 0~100%, 10~40mm, respectively. These ranges mimicked the environment that might be seen in an animal. The semi-infinite medium diffusion model was used to generate the attenuation spectrum $A(\lambda)$ and the differential pathlength spectrum $DP(\lambda)$. The differential pathlength near the water feature ($DPW$) was estimated using SDSA. For a given $\lambda_o$, the relative error $e(\lambda_o)$ between $DP(\lambda_o)$ and $DPW$ was calculated. This numerical experiment was performed 1000 times. The mean and standard deviation of the relative error $e(\lambda_o)$ for a range of $\lambda_o$ is plotted in Figure 3-8. Figure 3-8(a) is for the 740nm water feature within 700-800nm range and Figure 3-8(b) is for the 840nm water feature within 800-880nm range. This was repeated with an infinite medium diffusion model, and these results are plotted in Figure 3-9(a),(b).
Figure 3-8. When using a semi-infinite diffusion modal, (a) the mean and standard deviation of the absolute relative error $e(\lambda_o)$ as a function $\lambda_o$ for 700-800nm range, (b) the mean and standard deviation of the absolute relative error $e(\lambda_o)$ as a function $\lambda_o$ for 800-880nm range.

Figure 3-9. When using an infinite diffusion modal (a) the mean and standard deviation of the absolute relative error $e(\lambda_o)$ as a function $\lambda_o$ for 700-800nm range, (b) the mean and standard deviation of the absolute relative error $e(\lambda_o)$ as a function $\lambda_o$ for 800-880nm range.

From Figure 3-8 and Figure 3-9, although different forward models were used, the error plots are similar which confirms that our SDSA is insensitive to the forward model. It is clear that the minimal error occurs when $\lambda_o = 728$nm and 830nm for 700-800nm and 800-880nm range.
respectively. The selectivity in the 800-880nm wavelength band is stronger. As expected, these optimal representative wavelengths are slightly shorter than the representative wavelengths calculated using Equation 3-12.

In general, the error for 700-800nm range (~1.0%) is greater and more variable than that of 800-880nm (~0.2%). This is partly due to the crosstalk from HbR signal and the distorted differential pathlength spectrum in this range. From 3-1(b), in 700-800nm range, there is a strong HbR feature as well as the water feature, whereas in 800-880nm range, only the water feature dominates. The 2nd derivative spectrum of HbO2 is very weak for both ranges, resulting in our SDSA being insensitive to HbO2 and so it is difficult to quantify the HbO2 concentration.

In conclusion, 728nm and 830nm are the optimal representative wavelengths for the differential pathlengths estimated from the 740nm and 840nm water features for the typical in vivo environment. These two wavelengths are modeled when the steady-state measurements are converted to the frequency domain data.

3.2.4. Robust recovery

As shown in the previous section, in a wide range of chromophore concentration, scattering amplitude and source-detector separation, the expectance of the relative error of the estimated differential pathlength is about 1.0% for 728nm and 0.2% for 830nm. The large error for 728nm is mostly the result from the crosstalk of HbR in the linear regression and the non-linearity of the differential pathlength spectrum. Some portion of the water feature may be wrongly treated as deoxyhemoglobin signal and vice versa or 2nd order term since their features overlap. To explore the correlation between the error and each input variable (concentrations and
separation), scatter plots for the previous numerical experiment are provided in Figure 3-10 and (a-e) are for HbR, HbO2, Water, Intra and \( r \) respectively.

![Figure 3-10](image)

**Figure 3-10.** Scatter plots of relative error versus each input variable for 728nm and 830nm when the semi-infinite diffusion model was used. (a) vs. HbR (b) vs. HbO2 (c) vs. Water (d) vs. Intralipid (e) vs. \( r \).

In Figure 3-10 it is clear that there is a strong correlation of relative error and chromophore concentration especially for HbR and HbO2, while changes in Intralipid
concentration and source-detector separation have little effect. Errors at 830nm are very small which means the estimate at 830nm is fairly accurate and robust. However errors at 728nm are non trivial and a large variation was observed when HbR and HbO2 concentration varied over a wide range. Also the error plot for HbR or HbO2 has a clear pattern. Because HbR and HbO2 are the two major absorbing chromophores in 700-800nm range and HbR also has a strong signal in the 2nd derivative spectrum which can influence the SDSA, shifting changes in their concentrations have a large effect on the differential pathlength spectrum therefore to shift the representative wavelength left or right. When it falls on 728nm, the error will be small. When this wavelength shifts to a shorter wavelength as HbR concentration increases, the error will be significant.

An example is given to show the robust nature of the estimate to the variation of the water, Intralipid and source-detector separation, the influence of HbR and HbO2 to the estimate, and the impact of the choice of the representative wavelength. The baseline phantom was assumed to have 37.5µM HbR, 37.5µM HbO2, 85% water, 1% Intralipid and 30mm source-detector separation. This is a set mimicking the typical in vivo environment in the rat brain. The semi-infinite diffusion model was used for this. From the baseline, each of these 5 variables was varied individually and the relative errors were calculated. Figure 3-11(a) shows the trend of the relative error when HbR concentration was varied from 12.5 to 62.5µM. The relative errors at 726nm and 730nm were calculated as well as 728nm and 830nm. Figure 3-11(b) shows the trend when only varying HbO2 concentration from 12.5 to 62.5µM. Figure 3-11(c) shows the trend when only varying water content from 50% to 100%. Figure 3-11(d) shows the trend when only varying Intralipid concentration from 1% to 5%. Figure 3-11(e) shows the trend when only varying the source-detector separation from 10mm to 40mm.
Figure 3-11. Trends of the relative error when one of the five variables was changed from baseline. (a) When HbR was varied from 12.5 to 62.5 µM. (b) When HbO2 was varied from 12.5 to 62.5 µM. (c) When water was varied from 50% to 100%. (d) When Intralipid concentration was varied from 1% to 5%. (e) When source-detector separation was varied from 10mm to 40mm. The relative errors were calculated at 726nm, 728nm, 730nm and 830nm.
Again this example clearly shows that the other two variables, Intralipid and optode separation have little effect on the error in our estimates whereas HbR, HbO2 and water do in 700-800nm range. The representative wavelength in 700-800nm is dependent on the phantom makeup. In order to minimize the error, a proper representative wavelength instead of the optimal 728nm could be applied. It is also possible to develop a lookup table for this representative wavelength in terms of HbR and HbO2 concentration since both concentrations can be estimated as the by-products in SDSA. In practice, a lookup table is not implemented. Because in the absorption and scattering properties to be recovered are smooth functions in the whole spectrum as shown in Figure 3-1a. A few nm modeling error for the representative wavelength will not have significant impact to the reconstructed images and chromophore concentration maps. Therefore, in our studies, 728nm and 830nm are fixed for the representative wavelengths for 700-800nm and 800-880nm. However, when there is very little HbR in the medium, 734nm is found to be the optimal representative wavelength for the 700-800nm range.

3.2.5. Noise influence

Another issue that needs to be examined is the noise influence on the algorithm. Because the SDSA is based on the 2nd derivative spectrum which is more sensitive to the noise than the zero-order spectrum, the noise in the spectrum may create random errors in the estimation. To investigate this issue, a noise model was added to the previous experiment setup, the relationship between relative error and SNR could be examined. In Chapter 2, we have discussed the noise model that the dominant noise source for a CCD system is the shot noise which can be modeled using the Poisson distribution. Therefore, for a given SNR, the mean light intensity in terms of the counts by CCD was determined. The intensity spectrum calculated from the forward model was scaled by this mean number of photons. The noise added intensity spectrum was generated
by a Poisson noise generator in terms of the noise free intensity spectrum. The SDSA was applied and the relative error was recorded. Here, the setup for the medium was 37.5µM HbR, 37.5µM HbO2, 85% water, 1% Intralipid and 30mm source-detector separation. For each SNR, the simulation was run for 100 times. The mean of these 100 runs was calculated to demonstrate the relation between the relative error and SNR in Figure 3-12. It is clear that the accuracy of the SDSA is highly SNR dependent. The relative error in logarithm scale decreases linearly with the increase of the SNR in logarithm scale. That is, 10 folds improvement in the SNR will improve the estimate accuracy by 10 folds as well. Therefore to have a desirable accuracy of the estimated differential pathlengths, sufficient SNR must be achieved.

![Graph showing relative error versus SNR for different wavelengths.](image)

**Figure 3-12.** The estimate error versus the SNR in the spectrum for the two wavelength values used. This was calculated for a particular case where the measurement was assumed to take on a homogeneous semi-infinite medium with 30mm source-detector separation

3.2.6. **Test using FEM-based model**

To this point, the second derivative spectroscopy analysis has been described in detail and the basic performance and characteristics have been examined through homogenous infinite
and semi-infinite diffusion models. However, our experimental data is acquired in a circular finite geometry and the finite element method (FEM) based diffusion model is used, so it is important to test SDSA using FEM-based model in the circular geometry. With this model, the heterogeneity of chromophores’ distribution and complex boundary conditions can be easily tested. In this section, some numerical experiments based our FEM forward solver are described. The accuracy of the SDSA and the robust level of the recovery will be tested in both homogeneous and heterogeneous 2D models. However, an important assumption is maintained that the water distributes uniformly in the medium.

3.2.6.1. Homogeneous medium

![FEM mesh for forward calculation](image)

*Figure 3-13. A 425-node FEM mesh for forward calculation. One source (red star) and 8 detectors (blue circles) are shown on the region boundary.*

In the first series of experiments, homogenous phantoms were considered. Figure 3-13 shows the FEM mesh of a circular geometry and one source and 8 detectors are plotted as a star and 8 circles respectively on the mesh. The diameter of this circular phantom is 27mm and meshed with 425 nodes. The makeup of the phantom was deoxyhemoglobin (HbR), oxyhemoglobin (HbO2), water and Intralipid. The concentrations of these components were chosen in the range similar to the values typical of rat brain (Hale and Querry, 1973; van
Staveren et al., 1991). The absorption coefficient spectrum $\mu_a(\lambda)$ was calculated using the extinction coefficient spectra of Hb, HbO2 and water and the reduced scattering coefficient spectrum $\mu'_s(\lambda)$ was calculated assuming all the scattering was due to Intralipid. Based on these optical properties ($\mu_a(\lambda)$, $\mu'_s(\lambda)$), the intensity spectrum $I(\lambda)$ and phase shift spectrum $\theta(\lambda)$ for each source-detector pair were calculated by FEM forward solver at each wavelength from 650nm to 930nm with interval of 1/3nm. Then use the intensity spectrum to yield the simulated attenuation spectrum $A(\lambda)$. The SDSA was applied to $A(\lambda)$ to estimate two DPWs at 728nm and 830nm. The phase shift spectrum $\theta(\lambda)$ was converted to the true differential pathlength spectrum $DP(\lambda)$ for comparison. The chromophore concentrations were randomly chosen assuming the total hemoglobin (HbT) was within 50-100$\mu$M, oxygen saturation was within 0-100%, water content was within 50-100% and Intralipid concentration is within 1-5%.

Figure 3-14. Examples of (a) attenuation spectra (b) 2nd derivative attenuation spectra (c) differential pathlength spectra at 8 detectors. In this case, $HbR = 37.5\mu$M, $HbO2 = 37.5\mu$M, Water=85%, Intralipid =1%.
Figure 3-15. Absolute relative errors at (a) 728nm and (b) 830nm for all the 8 detector positions in a homogeneous medium. Mean values (blue) are plotted with the standard deviations (red) for 1000 runs.

The FEM forward model was set in the frequency domain with the modulation frequency of 100MHz. Figure 3-14(a) shows an example of calculated attenuation spectra at the 8 detectors and the corresponding 2nd derivative spectra are shown in Figure 3-14(b). The differential pathlength spectra calculated from the phase spectra using Equation 3-3, 3-4 are shown in Figure 3-14(c). Figure 3-15 shows the statistics of the relative errors of the estimated DPWs at 728nm and 830nm for 8 detector positions. For 728nm, the absolute relative error has a 0.85% mean and a 0.75% standard deviation. For 730nm, the absolute relative error has a 0.22% mean and a 0.1% standard deviation. Relative errors have the similar distribution at different detector positions, i.e., the relative errors are not sensitive to the source-detector configurations.

3.2.6.2. Heterogeneous medium

The previous discussion was limited to a homogeneous medium. In a more practical situation, one could divide the medium into small voxels (Hiraoka et al., 1993; Matcher et al., 1994). And each voxel is treaded as a homogeneous medium and has its local differential
pathlength or partial differential pathlength \((PDP_j = \partial A / \partial \mu_{a,j})\) and local makeup of chromophores \((C_{i,j})\). Here \(i = 1, \cdots, N_c\); \(j = 1, \cdots, N_n\) \((N_n\): number of voxels). The total differential pathlength is sum of the partial differential pathlength in each voxel:

\[
DP = \sum_{j=1}^{N_n} PDP_j
\]  

Equation 3-8 now can be written as the following for the heterogeneous medium:

\[
\frac{\partial^2 A}{\partial \lambda^2} \approx \sum_{j=1}^{N_n} \left( \frac{\partial A}{\partial \mu_{a,j}} \cdot \frac{\partial^2 \mu_{a,j}}{\partial \lambda^2} \right) = \sum_{j=1}^{N_n} \left( PDP_j \cdot \frac{\partial^2 \mu_{a,j}}{\partial \lambda^2} \right)
\]  

And Equation 3-9 becomes:

\[
\frac{\partial^2 A}{\partial \lambda^2} = \sum_{j=1}^{N_n} \left[ PDP_j \cdot \sum_{i=1}^{N_c} \left( C_{i,j} \cdot \frac{\partial^2 \varepsilon_{i,j}}{\partial \lambda^2} \right) \right]
\]  

The matrix form therefore can be derived as:

\[
A' = \varepsilon' \times (C \times PDP)
\]  

Here, \(A'\) and \(\varepsilon'\) are the same as those in Equation 3-11. One is a \(N_A \times 1\) vector and the other is a \(N_A \times N_c\) matrix. \(C\) is a \(N_c \times N_n\) matrix which is the chromophore concentration map in the medium. \(PDP\) is a \(N_n \times 1\) vector which is the partial differential pathlength map. Using multilinear regression method, Equation 3-16 can be solved for \(C \times PDP\). For the component related to water, the output from the regression is the sum of the inner product of the water concentration map and the differential pathlength map, \(<C_{water} \cdot PDP>\), i.e., \(\sum_{j=1}^{N_n} (PDP_j (\lambda_0) \cdot C_{water,j})\). If the
water is uniformly distributed, the output becomes $DP(\lambda_o) \cdot C_{\text{water}}$. Once the mean water concentration is provided, the differential pathlength at the representative wavelength can be estimated.

![Figure 3-16 A mesh for the heterogeneous experiment. The red ellipse region mimics the brain in the rat head.](image)

To test the SDSA performance in the heterogeneous medium, a heterogeneous mesh was used. It has two regions as shown in Figure 3-16. The ellipses region was to mimic the rat head. The chromophore concentrations and Intralipid concentration for each region were randomly chosen as in the previous experiment for the homogeneous medium where total hemoglobin (HbT) was within 50-100µM, oxygen saturation was within 0-100%, water content was within 50-100% and Intralipid concentration is within 1-5%. The numerical experiment again was performed 1000 times. Figure 3-17 shows the statistics of the absolute relative errors of the estimated $DPWs$ at 728nm and 830nm for 8 detector positions in the heterogeneous medium. For the 728nm one, the absolute relative error has a 0.75% mean and a 0.75% standard deviation. For the 730nm one, the absolute relative error has a 0.22% mean and a 0.1% stand deviation.

It is clear that the heterogeneity doesn’t introduce additional errors in the estimation of the DP compared to the homogenous model shown in Figure 3-15. The mean values for 728nm
are even smaller possibly due to the cancellation of errors between two regions. The relative errors at different detector positions are still similar although each of them samples different portion of the included region. In conclusion, the SDSA is valid for heterogeneous medium if water is uniformly distributed.

![Graphs showing relative errors at different detector positions.](a) 728nm and (b) 830nm for all the 8 detector positions in a heterogeneous medium. The mean values (blue) are plotted with the standard deviations (red) for 1000 runs.

**Figure 3-17. Absolute relative errors at (a) 728nm and (b) 830nm for all the 8 detector positions in a heterogeneous medium. The mean values (blue) are plotted with the standard deviations (red) for 1000 runs.**

### 3.2.7. Experimental validation

Although numerical simulation provides a very friendly environment to explore the theory and predict its characteristics, a physical model presents more solid evidence to validate the theory. In this section, well-controlled phantom studies were completed to examine the question of whether $\mu_s$ and $\mu'_{s'}$ at the representative wavelengths could be successfully recovered with our measurements, i.e., the intensity measurement and the estimated differential pathlengths from the water features.

In reality, it is difficult to produce a phantom to have the exact optical properties as wished because different systems and different batches of the materials may have different
scattering properties and the extinction coefficients. Direct comparison of the estimated DPWs from the measurement and the model calculated differential pathlengths using the designed optical properties are inappropriate. Therefore the reconstructed optical properties were examined to see whether the absorption and reduced scattering values can be successfully separated and whether the values are reasonably accurate.

![Diagram](image)

**Figure 3-18. Diagrams of the liquid phantom experiment.** One fiber was fixed position and the other fiber was moved on the motorized translate stage. (a) Infinite medium. A stir bar was placed in the bottom of the tank to keep the medium homogeneous. (b) Semi-infinite medium. The stir bar was removed while collecting data.

Starting with the simplest and most predictable model, we examined the performance of our approach in the homogeneous infinite medium. A diffusing phantom with Intralipid and blood in saline/PBS was used and contained in a cylindrical tank large enough to act as an infinite medium. The absorption was considered as the contribution of water and HbO₂ since enough oxygen was provided. Two 1mm fibers were used as the source and the detector respectively. Each of them was sealed in a 20cm long needle and merged at least 10cm underneath the liquid surface. The source fiber was fixed while the detector fiber was installed on a motorized translating state with digital readout (accuracy ~ 0.1mm). The source-detector
distance \( r \) was varied. The multiple distance method was used to fit the \( \mu_a \) and \( \mu_s' \) values at 734nm and 830nm from the measured \( A(r) \) and \( \text{DP}(r) \) curves. The diagram of the experiment setup is shown in Figure 3-18a. 728nm was not used in this case because 734nm was the optimal representative wavelength when no HbR component presents in the medium. The experiment was designed to start with an Intralipid solution with concentration 0.5% and gradually increased to 1.0%, then in the second stage the Intralipid was kept constant and the HbO\(_2\) concentration was increased gradually from 0 to 20\( \mu \text{M} \). The calculated values and the expected values are plotted and compared in Figure 3-19. The recovered \( \mu_a \) had agreement within 5% difference (and more consistent to a bias offset) with the predicted values from the literature (Hollis, 2002; Horecker, 1943) and the recovered \( \mu_s' \) were within 5% of the expected values (van Staveren et al., 1991) if a 18% scaling factor was incorporated to account for the Intralipid batch difference. It is important to note that the absolute HbO\(_2\) and HbR content thus can be estimated based on the absorption at 734nm and 830nm.

\[ \begin{align*}
0 & \quad 0.002 \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01 \\
0.002 & \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01
\end{align*} \]

\[ \begin{align*}
0 & \quad 0.002 \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01 \\
0.002 & \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01
\end{align*} \]

\[ \begin{align*}
0 & \quad 0.002 \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01 \\
0.002 & \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01
\end{align*} \]

\[ \begin{align*}
0 & \quad 0.002 \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01 \\
0.002 & \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01
\end{align*} \]

\[ \begin{align*}
0 & \quad 0.002 \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01 \\
0.002 & \quad 0.004 \quad 0.006 \quad 0.008 \quad 0.01
\end{align*} \]

Figure 3-19. The predicted (dashed line) vs. reconstructed (solid line) values for \( \mu_a \) and \( \mu_s' \) when varying both Intralipid and HbO\(_2\) concentrations. (a) \( \mu_a \) vs HbO\(_2\) concentration (b) \( \mu_s' \) vs Intralipid concentration.
To include the boundary condition effect, similar experiments were performed by collecting data in a semi-infinite medium. The fibers were placed right on the liquid surface and no air space existed between liquid and the fiber tip. Because the stirring could create a curved surface and destroy the flat boundary condition, the stir bar was stopped while collecting the data. Without continuous mixing, the concentration of the solution was expected to be lower near the surface especially for blood experiment. Therefore, in this case, the blood wasn’t added and only Intralipid concentration was varied. The measured attenuation \( A(r) \) and differential pathlength \( DP(r) \) as a function of distance were fitted to a semi-infinite medium diffusion model with extrapolated boundary condition (Haskell et al., 1994). The fitted absorption values were similar to the values from the infinite medium experiment (5% difference) and the reduced scattering properties were lower at both wavelengths probably due to the stratification of Intralipid.

In both infinite medium and semi-infinite medium, a good match of absorption and reduced scattering coefficients between the predicted and the fitted values was observed. This confirmed that the differential pathlength estimated from the water feature did provide an extra independent measurement which is effective to separate absorption and reduced scattering properties.

3.3. **DOT image reconstruction in the frequency domain**

Having tested the SDSA in numerical experiments and homogeneous medium experiments with well defined geometries, we were interested in knowing whether this approach is still effective in the imaging problem where the medium is typical heterogeneous and the
geometry is complex. In this section, phantom studies are described to show that both absorption and reduced scattering inclusions can be successfully reconstructed.

3.3.1. Gelatin phantoms

The ability to characterize an embedded scattering or/and absorbing object was tested in rat head simulating phantoms. Because our approach relies on the water signals to estimate the differential pathlength, these phantoms have to be water based and the best choice is the gelatin phantom. The material to build these phantoms is a mixture of India ink (Staples), titanium dioxide powder (TiO2, Sigma Inc.) and Gelatin (G2625, Sigma Inc.), with 85% water. A recipe has been developed by colleagues (Brooksby et al.). Gelatin powder was dissolved in the deaerated water to remove any the air bubbles. TiO2 powder was gradually added while stirring the solution to avoid the aggregation of the TiO2 particles. The aggregation could substantially decrease the produced scattering property and make it difficult to control. India ink was added afterwards. The mixture was solidified by cooling to room temperature. After producing many phantoms, the procedure was optimized by mixing the TiO2 power with water before mixing the Gelatin, because the aggregation was found to be due mainly to the viscosity of the Gelatin powder. With this modification, the amount of TiO2 needed to make the same scattering property was much less than the previous procedure and the resulting properties were more reliable.
By controlling the amount of ink or TiO2, the absorption or the scattering property could be varied, respectively. These phantoms were cylinders of 27mm diameter with a 14mm inclusion near the edge, which has different optical properties to the background, similar to the geometry of a brain within the cranium of a rat. Photographs of a Gelatin phantom and its cross-section are shown in Figure 3-20. Three types of inclusion are used to build phantoms within the same background: A) an inclusion with higher scattering; B) an inclusion with higher absorption and lower scattering; and C) an inclusion with both higher properties. In Table 3-1, the “Target” column gives the optical contrast of the inclusion relative to the background. This contrast was calculated from bulk optical properties of each type of material (either inclusion or background) that were measured with a homogeneous fitting algorithm by creating a separate homogeneous cylindrical phantom with the material.
### Table 3-1

The summarized results of phantom studies are shown here. The original optical properties are converted to contrast to the background for convenience. The target values from 3 types of phantoms are listed in the “Target” column. Reconstructed peak values (“peak”) and mean values (“mean”) for the inclusion are listed and their differences (“Diff.”) to the target values are shown.

<table>
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<th>Target</th>
<th>Reconstructed</th>
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<th></th>
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<td>Diff.</td>
<td>Mean</td>
<td>Diff.</td>
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<td></td>
</tr>
<tr>
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<td>1.62</td>
<td>+4.5%</td>
<td>1.38</td>
</tr>
</tbody>
</table>

3.3.2. **Diffuse optical tomography**

Measured attenuation and differential pathlength profiles at 64 source-detector pairs were converted to the intensity and phase data type in the frequency domain using the equation 3-17 and 3-18.

\[
I = \exp(-A) \tag{3-17}
\]

\[
\theta = k\rho \tag{3-18}
\]

where \(I\) and \(\theta\) are the intensity and phased data in the frequency domain respectively, \(A\) and \(\rho\) are the measured attenuation and estimated differential pathlength by the broadband system, \(k\) is the wavelength number, namely, \(k = \frac{2n\pi f}{c}\) (where \(f\) is the modulation frequency in frequency
domain, \( n \) is the refractive index in the medium, \( c \) is the speed of light). Absorption and reduced scattering coefficients images were reconstructed using a 2D reconstruction program without using any structural \textit{a priori}. This details of the image reconstruction program (NIRFAST) and algorithm will be discussed in the next chapter.

Reconstructed cross-sectional images were generated for these phantoms and these are shown in Figures 3-21(a-c). Horizontal profile transects from cross-section plots of the images are shown in Figure 3-21(d) for better visualization of the quantitative values. Reconstructed peak and mean values of the inclusion and their difference to the target are listed in the Table 3-1 for comparison. The 3D meshing and image reconstruction are also possible and similar results to the 2D reconstruction were achieved when this was tried, so here the presentation is restricted to the 2D results for simplicity. Optical properties are converted to dimensionless contrasts to the background properties in the table and all the figures for convenience.
Figure 3-21. Absorption and reduced scattering images of phantom A, B and C are shown. (a) Phantom A, which has a higher scattering inclusion off the center. (b) Phantom B, which has a higher absorption but lower scattering inclusion off the center. (c) Phantom C, which has a higher absorption and scattering inclusion off the center. In each sub-image, the first column shows the target images for absorption (first row) and reduced scattering (second row) and the second column shows the reconstructed images. The original optical properties are converted to contrast to the background for convenience. (d) Horizontal profile transects from the images of (a) (b) (c) are shown. The contrast distribution for target images (dashed lines) was reconstructed (solid lines).
In Table 3-1, reconstructed peak values (“peak”) and mean values (“mean”) for the inclusion are listed and their differences (“Diff.”) to the target the mean and peak values. All the peak values to the inclusion are within 6% difference to the target in average and the mean values are within 12% difference of the target. This quality is similar to that of images reconstructed by other systems (Jiang et al., 1995; McBride et al., 1999).

3.4. Imaging tissue with non-uniformly distributed water concentration

3.4.1. Statement of the problem

To this point, all the discussions were limited to an assumption that water is uniformly distributed in the medium. This assumption of uniform water distribution seems reasonable in our specific application of rat brain studies (Reinoso et al., 1997), because tissues which are in the majority in the rat head, such as muscle (Water content: 0.756±0.003) and brain (Water content: 0.788±0.002) have very stable and similar water content. Although bone has relatively low water content 0.446±0.017, it only has very small volume therefore will not have a major contribution in the total optical pathlength.

However, it is important to know how the different water content in different tissue can affect the accuracy of current SDSA algorithm based on the assumption of uniform water distribution. A simulation was performed using the numerical experiment setup discussed in Section 3.2.6.2 where a heterogeneous model was used (Figure 3-16). Instead of keeping the water content the same for both regions, we allow each of them can vary within 50-100% independently like other variables. The estimated differential pathlength was calculated using the mean water content in the whole medium. After 200 runs of the random experiment, the mean and standard deviation of the absolute relative error can be summarized in Figure 3-22(a) for
both representative wavelengths and for all the detector positions. Compared to Figure 3-17, the mean errors are almost 10 times higher after introducing the water heterogeneity. Generally those detectors closer to the inclusion (red region) have higher errors. This is due to the mean water content used to calculate the differential pathlength was closer to the water content of the background (green region) since it has bigger area, whereas the optical pathlengths for these detectors were mostly within the inclusion. In Figure 3-22(b), the relation between the relative error and the water content difference of two regions (Background – Inclusion) is illustrated using a scatter plot and fitted to a linear equation. For every 1% water content difference between two regions, 0.37% error is expected in the differential pathlength estimation. If the inclusion and the background are considered to be brain tissue muscle tissue respectively, about 1% error is predicted in our SDSA approach for the 3% water content difference. This is an acceptable error level.

![Graphs showing relative error and water content difference](image)

Figure 3-22. (a) Mean and standard deviation values of absolute relative errors at 728nm and 830nm for all the 8 detector positions in a heterogeneous medium. (b) The scatter plot of the relative error versus the mean for all the detectors. A linear regression is fitted to all the data points.

3.4.2. A generalized solution with a priori of the water distribution
In reality, we always face to heterogeneous situations (Matcher et al., 1994). Although the previous analysis showed the significant error when ignoring the fact that water content is different in a variety of tissues, it is feasible to deal with such issue by incorporating the water distribution map into the reconstruction algorithm. Such water distribution map either can be estimated from the literature (Reinoso et al., 1997) or measured by MRI. In this section, a proposed algorithm is described and regarded as the generalized solution for the 2nd derivative spectroscopy based diffuse optical tomography.

Recall the physical meaning of differential pathlength which is the change of attenuation resulted from a small change of $\mu_a$ ($\partial A / \partial \mu_{a,j}$). In frequency domain image reconstruction, there is a similar term in the sensitivity function (also called the Jacobin matrix):

\[
J = \begin{bmatrix}
\delta \ln I_1 & \delta \ln I_1 & \cdots & \delta \ln I_1 & \delta \ln I_1 & \cdots & \delta \ln I_1 \\
\delta \kappa_1 & \delta \kappa_2 & \cdots & \delta \kappa_{Nn} & \delta \mu_{a1} & \delta \mu_{a2} & \cdots & \delta \mu_{aM}
\end{bmatrix}
\]

which relates the change in the boundary data (either intensity $I$ or phase $\theta$) with respect to a small change in either $\mu_a$ or $\kappa$ (diffusion coefficient). The sensitivity between log of intensity and $\mu_a$ is part of the sensitivity function. Here, $M$ is the number of measurement and $Nn$ is the number of nodes in the FEM mesh. Since log of intensity is equivalent to attenuation, it is clear
that $\frac{\delta \ln I}{\delta \mu_{a,j}}$ is equal to $-\frac{\partial A}{\partial \mu_{a,j}}$. Therefore the partial differential pathlength ($PDP_j = \frac{\partial A}{\partial \mu_{a,j}}$) can be modeled by the nodal sensitivity function ($\frac{\delta \ln I}{\delta \mu_{a,j}}$). The sum of the sensitivity at all nodes is equal to the differential pathlength which is an equivalent measure of the phase shift.

$$DP = \sum_{j=1}^{N_n} PDP_j = -\sum_{j=1}^{N_n} \frac{\delta \ln I}{\delta \mu_{a,j}}$$

(3-20)

Instead of using the phase measurement as an output of the forward model, the a priori water content map can be incorporated in the forward model to predict the water content weighted total differential pathlength ($WDP$). This $WDP$ is equal to the estimated differential pathlength ($\sum_{j=1}^{N_n} (PDP_j(\lambda_0) \cdot C_{water,j})$) in the regression model discussed in Equation 3-16. With such modification, the forward model is truly adapted to the SDSA. Once the water content map is provided, the measured attenuation and the differential pathlength estimated from the water feature can be directly used to reconstruct optical properties images without the extra step to map them into the frequency domain.

3.5. Discussion

The second derivative spectroscopy based diffuse optical tomography approach is described in this chapter. The NIR data are broadband and the steady-state data are used along with second derivative based estimation of the differential pathlength from water absorption feature. The differential pathlength estimated from water is then used along with the attenuation spectrum to recover absorption and reduced scattering coefficient images at multiple
wavelengths. Mathematical deduction and approximation based on the physics of the light-tissue interaction is detailed to provide a solid theoretical foundation. A statistical method was used along with numerical experiments to characterize the performance of the SDSA in various situations. Phantoms studies in both homogeneous medium and tissue-simulating heterogeneous phantoms were reported to show the robust nature of the approach along with analysis of the efficacy. In the end, the solution to the general case where the water content is non-uniformly distributed is proposed which can extend the SDSA to much broader applications, in future work.
Chapter 4. Diffuse optical tomography image reconstruction

In this chapter, the detail of our DOT image reconstruction algorithm is described. For the forward problem, the diffusion approximation to the radiative transport equation is used and numerically solved using finite element method (FEM). A modified Levenberg-Marquardt algorithm is used for solving the inverse problem of estimating the non-linear optical parameters. *A priori* structural information and Tikhonov regularization are incorporated into the algorithm, and both simulated and experimental data are shown to demonstrate the performance. In the end, a hybrid 2D/3D modeling is discussed, which improves the modeling accuracy of animal studies.

4.1. Introduction

Progress has been made persistently in the recent decade to develop more efficient imaging techniques along with more realistic modeling of light propagation in turbid media. In order to reconstruct a tomographic image, two kinds of problems need to be solved. One is the forward problem and the other is the inverse problem.

In the forward problem, a model must be established to describe the physics of the interaction of light with turbid media and it should be capable to predict the light distribution in the media under examination and the measurement at the detectors. With this forward model, the sensitivity map can be calculated which allows the interpretation of boundary measurement to the internal optical property distribution. Maxwell electromagnetic wave theory and transport theory are two distinct approaches in the modeling. The former treats the light as a wave phenomenon and all the characters associated with wave propagation, such as diffraction, interference, reflection, and refraction. However, in practice this is difficult to implement due to the computational complexity. Light is composed of photons which have a wave-particle dual
nature. In the second approach, the light is considered as particles with energy or packages of photons. As each package propagates in the medium, it interacts with the particles in the medium by either losing the energy (absorbed) or being redirected (scattered). As the number of scattering event increases in a large turbid medium, the incident photons lose the wave coherence and only the averaged effect can be detected, therefore the omission of the wave characteristics is a realistic strategy. The radiative transport equation especially a diffusion approximation of it, is considered as the most convenient and efficient model for highly scattering media such as biological tissue, although this approximation might be invalid in the region near the source and in the medium where the scattering is highly anisotropic.

To solve the forward problem, i.e. calculating the light fluence, three modeling methods are used; analytic methods, statistical methods and numerical methods. Analytic methods (Arridge et al., 1992; Kim, 2004) provide a solution of the Green’s function for the diffusion equation but such solutions only can be derived in a few regular and homogeneous geometries. Statistical methods such as Monte Carlo method (Boas, 2002) and random walk theory (Weiss, 1998; Weiss et al., 1998) incorporate the random nature of collected signal and provide a reliable result in the case where the diffusion approximation is invalid for the small geometry and for the weak scattering medium. However, their applications are limited due to the enormous computational cost. Numerical methods such as finite element method (FEM) and finite difference method (FDM) are most commonly used when more complex heterogeneous geometries are involved (Arridge, 1999; Culver et al., 2003a; Jiang et al., 1996; Schweiger et al., 1995).

The inverse problem can be generalized as an optimization problem. The aim is to minimize the difference of measured data and calculated data by updating the optical property
distribution in the forward model. Ideally the optimal solution is achieved when the reconstructed images match the exact optical property distributions. The calculation of this update through regularization, optimization and addition of the penalty terms in sensitivity is the key to recover the information in the image domain from the information in the measurement domain. The diffusive nature results in the poor spatial specificity of the sensitivity matrix and makes the inverse problem ill-posed; that is, the solution is not unique or does not exist for arbitrary data or depend continuously on the data which contains random noise. Although the problem itself is a non-linear problem, both linear (Boas et al., 1994; Gaudette et al., 2000) and non-linear algorithms are widely used. The advantage of the non-linear algorithms is that it gives you absolute quantitative parameters, whereas the linear algorithm can only provide qualitatively relative parameters. A variety of non-linear reconstruction methods are available, such as, conjugate-gradient methods (Arridge, 1999; Hielscher et al., 1999), quasi-Newton methods (Klose and Hielscher, 1999), Gaussian-Newton methods (Arridge, 1999; Jiang et al., 1995; Paulsen, 1995) etc. The gradients methods are stable in the updates but converge slowly. The Newton methods converge much faster but require more complex computation.

The recent trend in DOT has been the inclusion of anatomical information (spatial priors) and wavelength dependent (spectral priors) into the forward and inverse problems (Brooksby et al., 2005; Li et al., 2005; Pogue and Paulsen, 1998; Srinivasan et al., 2005). Significant improvement in image quality is observed since the introduction of additional and constraints alleviate the ill-posedness of the inverse problem.

In this thesis, the model is based on the diffusion approximation to radiative transport equation. Finite element method is implemented to numerically solve the forward problem. A synthesized Levenberg-Marquart algorithm and Tikhonov regularization is used in the inverse
problem. The use of a priori anatomical information is also investigated to allow constraints for the inverse problem.

4.2. Forward problem

4.2.1. Diffusion approximation

Light transport in random media is well described by radiative transport equation (RTE) from Boltzmann transport theory (Dunderstadt and artin, 1979). Boltzmann transport theory is a widely used tool for analyzing transport phenomena within systems that involve density and temperature gradients, such as the transport of carriers in the semiconductors. The time dependent RTE is given by

\[
\frac{1}{c} \frac{\partial L(r,s,t)}{\partial t} + \nabla \cdot L(r,s,t)s = -(\mu_s + \mu_a)L(r,s,t) + \mu_s \int \int \int (r,s,t)f(s \cdot s')d\Omega' + Q(r,s,t) (4-1)
\]

where \( L \) is the radiance as a function time \( t \), position \( r \), direction \( s \) for a given source \( Q \), speed of light in the medium \( c \), and distributions of scattering coefficient \( \mu_s \), absorption coefficients \( \mu_a \) and phase function \( f \) of the scattering event. The phase function describes the probability of a given photon density in direction \( s' \) being scattered into the direction \( s \). Many approximations are used, but the most commonly used is the Henyey–Greenstein phase function.

By integration over all solid angles and use of definitions of the fluence rate \( \Phi \) which is integral of the radiance at all the solid angles and the flux \( j \) which is the integral of the radiance at all the solid angles but at a particular direction \( s \), the continuity equation can be derived from the Equation 4-1:
\[
\frac{1}{c} \frac{\partial \Phi(r,t)}{\partial t} + \nabla \cdot j(r,t) = -\mu_a \Phi(r,t) + S(r,t) \quad (4-2)
\]

where
\[
S(r,t) = \int_{4\pi} Q(r,s,t) d\Omega \\
\Phi(r,t) = \int_{4\pi} L(r,s,t) d\Omega \\
j(r,t) = \int_{4\pi} sL(r,s,t) d\Omega
\]

Although the transport equation accurately describes the propagation of photons in the media from the statistical point of view, it is very difficult and computationally intensive to be used in practice. Equation 4-1 is a complex differential-integral equation and the analytic solution is very difficult. However, approximations can be made in some specific situations, for example, in the regions far away from the source, each photon has experienced many scattering events and has lost its original direction in the turbid medium, and their flight directions are almost uniformly distributed at all angles (isotropic), therefore the radiance can be approximated by an isotropic diffuse term and a small directional term:

\[
L(r,s,t) = \frac{1}{4\pi} \Phi(r,t) + \frac{3}{4\pi} j(r,t) \cdot s \quad (4-3)
\]

This is P1 approximation and implies that the scattering must be much greater than absorption so that the photon travels randomly in any direction before it gets absorbed. Substituting the P1 approximation (Equation 4-3) into the original RTE (Equation 4-1) and integrating over all the solid angles yields

\[
\frac{1}{c} \frac{\partial j(r,t)}{\partial t} = -\frac{1}{3} \nabla \Phi(r,t) - \frac{1}{3D} j(r,t) \quad (4-4)
\]

where
\[
D \equiv \frac{1}{3[(1-g)\mu_s + \mu_t]}
\]
and $D$ is the diffusion coefficient, $g$ is the anisotropy factor defined as the averaged cosine of the scattering angle. In steady-state or for low frequency source ($<1\text{GHz}$), the time-dependent term is dropped out in Equation 4-4 and yields an equation analogous to Fick’s law:

$$j(r,t) = -D \nabla \Phi(r,t) \quad (4-5)$$

With this relation, the flux term in Equation 4-3 can be replaced with the fluence rate:

$$\frac{1}{c} \frac{\partial \Phi(r,t)}{\partial t} - \nabla \cdot D \nabla \Phi(r,t) + \mu_a \Phi(r,t) = S(r,t) \quad (4-6)$$

This is the time-domain diffusion equation. The frequency domain diffusion equation can be obtained using the Fourier transform:

$$\nabla \cdot D \nabla \Phi(r,\omega) - (\mu_a + \frac{i\omega}{c})\Phi(r,\omega) = -S(r,\omega) \quad (4-7)$$

The boundary condition is derived using the partial-current treatment (Keijzer et al., 1988) in which the reflection due to the refractive index mismatch of the internal turbid medium and the external non-scattering medium is considered and modeled by the Fresnel’s law. Assuming there is no photon flux back into the tissue from the external medium and the outward flux normal to the boundary is proportional to the fluence rate at the boundary:

$$\Phi(r,\omega) = 2An \cdot D \nabla \Phi(r,\omega) \quad (4-8)$$

where $r$ is on the boundary, $n$ is a unit normal vector directed into the turbid medium, and $A = [2/(1 - R_0) - 1 + |\cos \theta_c|^3]/(1 - |\cos \theta_c|^2)$. $\theta_c$ is the critical angle defined by $\theta_c \equiv \arcsin(1/n_{rel})$ and $R_0 = (n_{rel} - 1)^2/(n_{rel} + 1)^2$, where $n_{rel}$ is the relative refractive index of the internal medium to the external medium.
Several assumptions are made from the RTE to the diffusion equation; however the essence is to regard the scattering as isotropic so as to reduce the scattering coefficient ($\mu_s$) and the phase function ($f$) into one parameter; the reduced scattering coefficient ($\mu'_s = (1-g)\mu_s$). The condition for the validity of the diffusion approximation has been derived (Furutsu, 1980), namely, $\frac{\mu_a}{(\mu_a + \mu_s)} < 1 - g^2$. For most biological tissue, $g$ is approximately 0.8 and $\mu_s >> \mu_a$, therefore the diffusion approximation is mostly appropriate. Deep inside the medium such approximation is more accurate than near sources, boundaries, or where dramatic changes in optical properties occur. For positions near the source, photons haven’t experienced enough scattering events to be isotropic, therefore measurements are near the source about 3~5 reduced scattering lengths ($1/\mu'_s$) are ignored to satisfy the diffusion approximation.

The argument may always exist on whether the diffusion approximation (DA) is valid for imaging problem in a small geometry such as the rat head. Since diffusion equation essentially itself is an approximation, instead this work will rather focus on alternative argument specifically whether the diffusion approximation is valid enough for this application as compared to rather than the radiative transport equation. Some issues regarding this argument are: (1) Geometric limit: The mean diameter of the rat head in the coronal section is about 27mm and the nearest source-detector distance is about 5mm for 16 equally distributed optodes in the same plane. The bulk $\mu'_s$ of the tissue in a rat head is about 1 mm$^{-1}$. To deal with such problem, one can either interlace sources and detectors to increase the minimum separation or limit the influence of these measurements by ignoring the nearest source/detector measurement or giving them less weight. (2) Heterogeneity. The rat head has a complicated structure and the DA may not be valid in some tissues or at some hard boundaries. Although the clear cerebral spinal fluid layer (CSF) is a typical obstacle for the DA in the brain studies of large mammals, it is not notable in the rat
brain. Most tissues within the animal head, such as brain, muscles and skins provide an appropriate environment for the DA to be valid. The heterogeneity implies that the image reconstruction will be a highly ill-posed problem. Even if the radiative transport equation increases the accuracy in the forward problem, whether it can provide the same amount of improvement in the inverse problem is a questionable issue itself since it increases the degree of freedom as well.

Another reason for the preference of the DA regards to the actual measurement. Given a set of data, there are two kinds of errors, namely, stochastic errors and systematic errors. The model misfit due to the DA is one component of systematic errors. Other errors such as random coupling errors, boundary condition model misfit, geometric modeling errors, are more crucial and still exist even the RTE is used.

The diffusion approximation is used in this project and it will be shown that any model inaccuracy is essentially minimized through the calibration process and the imaging techniques.

4.2.2. Finite element method and NIRFAST

To solve the diffusion equation in complex heterogeneous geometries with arbitrary boundaries, the finite element method is used. The basic idea is to break up a solution region into many small interconnected sub-regions called “finite elements”. In each element, the true solution is approximated by a finite set of mathematical basis functions whose properties are well-known. In any practical problem, the basis function has to be finite and easily computed.

For the particular problem in Equation 4-7, the FEM formulation can be derived using the Galerkin method of weighted residuals (Lynch, 2002; McBride et al., 2001b). Linear triangular elements are used in 2D applications while linear tetrahedral elements are used in 3D applications.
The medical imaging group at Dartmouth has developed a Matlab toolbox in which the FEM is implemented for solving the diffusion equation in the frequency domain, NIRFAST (Near InfraRed Frequency-domain Absorption and Scatter Tomography). In this thesis, all the forward calculations for image problems have been using NIRFAST. For a 2D mesh with 1785 nodes and 3418 elements, a forward solution for all 8 sources requires approximately 0.7 seconds on a Pentium4-1.7G windows PC. For a 3D mesh with 6166 nodes and 29059 elements, it takes about 8 seconds on the same machine.

Given $\mu'_s$ and $\mu_a$ distributions at each node within the model and source positions, NIRFAST calculates the complex field distribution due to the modulated source and converts this into the amplitude and phase measurement at detector positions. A sensitivity matrix (the Jacobian) also can be calculated which relates to the change in the boundary data (either log of amplitude or phase) with respect to a small change in either $\mu_a$ or $D$ (The reduced scattering coefficient is implicitly solved in the diffusion coefficient). The Jacobian maps the optical properties of tissue onto the measurements. This function essentially contains numerical values associated with each node of the model that are proportional to the log of amplitude and phase of light propagation between each source and detector combinations. In NIRFAST, the Jacobian matrix, $J$ is calculated using the Adjoint method (Arridge, 1995; Arridge and Schweiger, 1995) and it has the form
\[
\begin{bmatrix}
\frac{\delta \ln I_1}{\delta D_1} & \frac{\delta \ln I_1}{\delta \theta_1} & \cdots & \frac{\delta \ln I_1}{\delta D_N} & \frac{\delta \ln I_1}{\delta \theta_1} & \cdots & \frac{\delta \ln I_1}{\delta D_{a1}} & \frac{\delta \ln I_1}{\delta \theta_{a1}} & \cdots & \frac{\delta \ln I_1}{\delta D_{aN}} & \frac{\delta \ln I_1}{\delta \theta_{aN}} \\
\frac{\delta \ln I_2}{\delta D_1} & \frac{\delta \ln I_2}{\delta \theta_1} & \cdots & \frac{\delta \ln I_2}{\delta D_N} & \frac{\delta \ln I_2}{\delta \theta_1} & \cdots & \frac{\delta \ln I_2}{\delta D_{a1}} & \frac{\delta \ln I_2}{\delta \theta_{a1}} & \cdots & \frac{\delta \ln I_2}{\delta D_{aN}} & \frac{\delta \ln I_2}{\delta \theta_{aN}} \\
\vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots \\
\frac{\delta \ln I_M}{\delta D_1} & \frac{\delta \ln I_M}{\delta \theta_1} & \cdots & \frac{\delta \ln I_M}{\delta D_N} & \frac{\delta \ln I_M}{\delta \theta_1} & \cdots & \frac{\delta \ln I_M}{\delta D_{a1}} & \frac{\delta \ln I_M}{\delta \theta_{a1}} & \cdots & \frac{\delta \ln I_M}{\delta D_{aN}} & \frac{\delta \ln I_M}{\delta \theta_{aN}} \\
\end{bmatrix}
\]

(4-9)

Where \(\frac{\delta \ln I_i}{\delta D_j}\) are the sub-matrices that define the relation between the log of the amplitude \(i^{th}\) of the measurement with respect to \(D\) and \(\mu\) at the \(j^{th}\) reconstructed nodes respectively and \(\frac{\delta \theta_j}{\delta D_j}\), \(\frac{\delta \theta_j}{\delta \mu}\) are the sub-matrices that define the relation between the phase of the \(i^{th}\) measurement with respect to \(D\) and \(\mu\) at the \(j^{th}\) reconstructed nodes respectively and \(M\) is the total number of measurements (\(M = 64\) for our system), \(N\) is the total number of nodes.

### 4.3. Inverse problem

#### 4.3.1. Levenberg-Marquardt algorithm

#### 4.3.1.1. Taylor series method vs the gradient method

Levenberg-Marquardt method (LM) is an algorithm for least-squares estimation of nonlinear parameters (Levenberg, 1944; Marquardt, 1963). It performs an optimum interpolation between the Taylor series method (or Gauss-Newton method) and the gradient method so that both the divergence of the successive iterations in Taylor series method and the slow
convergence after the first few iterations in the gradient method are avoided to provide a robust solution with only few iterations.

The objective of the inverse problem is to minimize the difference between measured (observed) fluence rate $\Phi^o$ at the tissue surface and calculated data $\Phi^c$ from a given model, with a fixed geometry and a fixed source-detector configuration. The least-squares form is a minimization of $\Psi$:

$$\min\{\Psi\}, \Psi = \| \Phi^c(x) - \Phi^o \|^2 = (\Phi^c(x) - \Phi^o)^T (\Phi^c(x) - \Phi^o)$$  \hspace{1cm} (4-10)

Where column vectors $\Phi^c$ and $\Phi^o$ represent calculated and observed fluence rate at all the source-detector pairs at wavelength $\lambda$. Vector $x$ is the unknown assumed parameter and is the spatially distributed optical properties, $x = [\mu_s(\lambda), D(\lambda)]$. This requires the derivative of $\Psi$ with respect to $x$ equals zero:

$$F(x) \equiv \frac{d\psi(x)}{dx} = 0$$ \hspace{1cm} (4-11)

It can be expanded as:

$$F(x) \equiv \frac{d\psi(x)}{dx} = \frac{d(\Phi^c(x) - \Phi^o)^T (\Phi^c(x) - \Phi^o)}{dx}$$

$$= 2(\frac{\partial\Phi^c(x)}{\partial x})^T (\Phi^c(x) - \Phi^o)$$

$$= -2J^Tb$$ \hspace{1cm} (4-12)

where $J = \frac{\partial\Phi^c(x)}{\partial x}$ is the sensitivity matrix discussed in Equation 4-9 and $b = \Phi^o - \Phi^c(x)$ is defined as the data difference between observed and calculated. Equation 4-11 is a root finding
problem. Both the gradient method and the Taylor series method can be used. For the gradient method such as the steepest descent method (Arfken, 1985), the iterative update formula can be written as:

$$x_{k+1} = x_k - kF'(x_k) = x_k + 2kJ^Tb$$  \hspace{1cm} (4-13)

where $k$ is a optimal step size to provide the minimal error at current direction. Since only matrix multiplications are involved in Equation 4-13, hence it is a stable yet slowly converging process.

Newton method can be used to solve a root finding problem (Arfken, 1985). The iterative formula is:

$$x_{k+1} = x_k - \frac{F(x_k)}{F'(x_k)} = x_k - [F'(x_k)]^{-1}F(x_k)$$  \hspace{1cm} (4-14)

The first derivative of $F(x)$ can be calculated as well:

$$F'(x) \equiv \frac{dF'(x)}{dx} = \frac{d}{dx} \left( \frac{\partial \Phi^c(x)}{\partial x} \right)^T (\Phi^c(x) - \Phi^o)$$

$$= 2\left( \frac{\partial \Phi^c(x)}{\partial x} \right)^T \left( \frac{\partial \Phi^c(x)}{\partial x} \right) + 2\left( \frac{\partial^2 \Phi^c(x)}{\partial x^2} \right)^T (\Phi^c(x) - \Phi^o)$$

$$\approx 2J^TJ$$  \hspace{1cm} (4-15)

Here the approximation of ignoring the high order small term is called Gauss method. Therefore, a new update formula is achieved:

$$x_{k+1} = x_k - \frac{F(x_k)}{F'(x_k)} = x_k + (J^TJ)^{-1}J^Tb$$  \hspace{1cm} (4-16)

Typically $J^TJ$ (referred as Hessian matrix) is a singular matrix and it is not invertible. The direct use of Gauss-Newton method for update is unstable, therefore, Marquardt introduced a steering factor $\lambda$ to compromise between Gauss-Newton and the gradient method.
\[ x_{k+1} - x_k = (J^T J + \lambda I)^{-1} J^T b = \frac{J^T b}{(J^T J + \lambda I)} \]  \hspace{1cm} (4-17)

When \( \lambda \to 0 \), the updating tends towards a Gauss-Newton, and as \( \lambda \to \infty \), the updating tends towards a gradient method. \( \lambda \) is a function of the change of \( \|b\|_2 \). \( \lambda \) decreases as the least-squares of the difference decreases. This term has the steering effect, which is, allowing the algorithm to act like the gradient method when far from the global minimum but act like the Gauss-Newton method when close to the solution. The robustness and the fast convergence are therefore both achieved.

4.3.1.2. Scaling of measurements and parameters

Jacobian matrix consists of 4 sub-matrices as the result of coupled measurements (b-space) (amplitude and phase) and coupled non-linear parameters (x-space) (\( \mu_a \) and \( D \)). The magnitude of the sensitivity depends on the definition of the units of these physical quantities. More generally this is an issue which is related to the row scaling of the b-space and the column scaling of the x-space. The relevant values of the solution \( x \) in a linear system are invariant under linear transformation of the b-space. However, for a non-linear inverse problem using a gradient method, the solution \( x \) is variable if the b-space and x-space are scaled in the construction of the Jacobian matrix. \( \lambda \) is added as uniform scalar to the diagonal of the Hessian matrix shown in Equation 4-17 and its influence to each parameter in the solution \( x \) also is variable if appropriate scaling is applied.

At each iteration, the LM algorithm can be regarded as solving a linear under-determined problem:
\[ \mathbf{J} \Delta \mathbf{x} = \mathbf{b} \]

\[
\begin{bmatrix}
\mathbf{J}^{\log(1)}_{\mu_a} & \mathbf{J}^{\log(1)}_{\mu_d} \\
\mathbf{J}^0_{\mu_a} & \mathbf{J}^0_{\mu_d}
\end{bmatrix}
\begin{bmatrix}
\Delta \mu_a \\
\Delta \mu_d
\end{bmatrix}
= \begin{bmatrix}
\Delta \log(I) \\
\Delta D / W_c
\end{bmatrix}
\]

(4-18)

Here, \( \mathbf{J}^{\log(1)}_{\mu_a}, \mathbf{J}^{\log(1)}_{\mu_d}, \mathbf{J}^0_{\mu_a}, \mathbf{J}^0_{\mu_d} \) are the sub sensitivity matrices of log of amplitude and phase with respect to absorption and diffusion coefficients. In the LM scheme, it suggests to scale the x-space in units of the standard deviations of each column in the Jacobian matrix. Similarly the b-space can be scaled using the standard deviations of each row. The scaled Jacobian and Equation 4-18 can be written as:

\[
\overline{\mathbf{J}} \Delta \mathbf{x} = \overline{\mathbf{b}}
\]

\[
\begin{bmatrix}
\mathbf{J}^{\log(1)}_{\mu_a} & W_c \mathbf{J}^{\log(1)}_{\mu_d} \\
W_c \mathbf{J}^0_{\mu_a} & W_c \mathbf{J}^0_{\mu_d}
\end{bmatrix}
\begin{bmatrix}
\Delta \mu_a \\
\Delta \mu_d
\end{bmatrix}
= \begin{bmatrix}
\Delta \log(I) \\
\Delta D / W_c
\end{bmatrix}
\]

(4-19)

where,

\[ W_c = \left\| \frac{\mathbf{J}^{\log(1)}_{\mu_a} - \mathbf{J}^0_{\mu_a}}{\mathbf{J}^{\log(1)}_{\mu_d} - \mathbf{J}^0_{\mu_d}} \right\|_2 \]

\[ W_r = \left\| \frac{\mathbf{J}^{\log(1)}_{\mu_a} W_c \mathbf{J}^{\log(1)}_{\mu_d}}{\mathbf{J}^0_{\mu_a} W_c \mathbf{J}^0_{\mu_d}} \right\|_2 \]

The column scaling factor \( W_c \) normalizes the x-space where absorption and diffusion coefficients have different dimensions. The row scaling factor \( W_r \) balances the contribution of the amplitude data and the phase data. More strictly speaking, from the statistical point of view, these two scaling factors should ideally consider the covariance matrices of x-space and b-space. For the b-space, it relates to the statistical performance of the data acquisition system. For the x-space, it relates to the anatomical or physiological priors. However, in current stage, we will use the general treatment shown in Equation 4-19. In practice, the choice of \( W_c \) is different to the classic LM algorithm. \( \Delta \mathbf{x} \) is normalized by \( \mathbf{x} \) and the normalized x-space \( \overline{\Delta \mathbf{x}} \) becomes dimensionless. \( W_r = 6 \) is a typical choice of the row scaling (McBride et al., 2001b).
4.3.2. **Tikhonov regularization with L matrix**

Tikhonov regularization is a method of “regularization”, which converts an ill-posed problem into a well-posed problem. It can be applied in either linear or non-linear methods. Although the $\lambda$ introduced in the LM algorithm regularizes the problem and makes the update stable, the extra Tikhonov regularization still can be applied in parallel. Especially when $\lambda$ approaches to zero, it strengthens the algorithm’s robustness to the noise within data. Such noise can be the noise in the measured data or the numerical error in the calculated data.

The key idea in Tikhonov method is to incorporate *a priori* assumptions about the size and smoothness of the desired solution, in the form of the smoothing function in the continuous case, or the norm in the discrete case. For discrete ill-posed problems, Tikhonov regularization in general form leads to the minimization problem of:

$$\min_{x} \left\{ \|Ax - b\|_2^2 + \lambda \|Lx\|_2^2 \right\}$$

**matrix form:** \( (A^T A + \lambda L^T L)x = A^T b \)

If the L is an identity matrix, the solution of Equation 4-20 can be derived as:

$$x = \sum_{i=1}^{n} w_i \frac{\langle u_i^T, b \rangle}{s_i} v_i \quad \text{where,} \quad w_i = \frac{s_i^2}{s_i^2 + \lambda} \quad (4-21)$$

Where $u_i, s_i, v_i$ are the $i$-th left singular vector, singular value, right singular vector of matrix A. $w_i$ is a damping filter which smoothes the high frequency component when $s_i$ becomes small as $i$ increase. Without this damping filter, the solution $x$ will be dominated by the amplified high frequency components. The principle of this regularization is to stabilize the solution by adding
more constraints in the objective function (Equation 4-20). For this case, the L-2 norm of the solution is constrained.

In the LM algorithm for the iteration, an alternate objective function can be inferred from the updating formulation (Equation 4-17).

\[ \min \{ \| J\Delta x - b \|^2_2 + \lambda \| x \|^2_2 \} \]  
\[ \text{(4-22)} \]

where \( \Delta x = x_{k+1} - x_k \) and \( \lambda \) is the current steering factor in the LM algorithm. The Tikhonov regularization can be easily incorporated as:

\[ \min \{ \| J\Delta x - b \|^2_2 + \lambda \| x \|^2_2 + \lambda \| L\Delta x \|^2_2 \} \]  
\[ \text{(4-23)} \]

Obviously if \( L \) is an identity matrix, the Tikhonov regularization will be trivial. Thus the matrix \( L \) is chosen to provide a distinct constraint. Although \( \Delta x \) is a vector, it denotes an update of the optical image and consequently the neighboring nodes in the image should be correlated in the vector. The stability and performance of the inverse problem are finally judged in terms of the quality of the reconstructed image. The high frequency noise in the image is normally regarded as the artifacts due to the noise in the data amplified by the ill-posed inverse problem. Therefore the high-pass filter in the image domain can be transformed to an \( L \) matrix to suppress the noise in the inverse problem.

Two-dimensional Laplacian operator is a common operator to identify the hard boundaries (high frequency spatial variation) in images. In NIR imaging, the diffusive nature always results a low resolution image. In other words, hard boundaries should be invisible in the conventional NIR images. Any high frequency spatial variation is most likely to be the artifact. Thus if the \( L \) matrix is implemented as a Laplacian operator, the reconstructed images will be less sensitive to the noise in the data and produce less artifacts.
For a discrete 2D image $u_{i,j}$, the Laplacian operator is defined as:

$$l = \frac{\nabla^2 u}{4} = \frac{1}{4} \left( \frac{d^2 u}{dx^2} + \frac{d^2 u}{dy^2} \right),$$

where,

$$l_{i,j} = \frac{1}{4} (u_{i,j} + u_{i,j+1} + u_{i+1,j} + u_{i+1,j+1}) - u_{i,j} \quad (4-24)$$

It is the difference of the current node and the average of the neighboring nodes. It can be conveniently applied to the FEM mesh to build the matrix $L$:

$$L(i, j) = \begin{cases} 
1/\text{number of neighbouring nodes} & i = j \\
-1 & i \neq j \text{ and } i, j \text{ are neighbours} \\
0 & i \neq j \text{ and } i, j \text{ are not neighbours} 
\end{cases} \quad (4-25)$$

And the new updating formulation can be derived as:

$$x_{k+1} - x_k = (J^T J + \lambda I + \lambda_L J^T L)^{-1} J^T b \quad (4-26)$$

Since the Laplacian regularization is looking from the image perspective, it can be thought as a global constraint for the smoothness of the image. Thus the regularization parameter $\lambda_L$ can be fixed during the iteration. $\lambda_L$ can be determined by the mesh resolution and the overall smoothness. However, in practice, it is empirically chosen and a wide range of $\lambda_L$ provides similar results.

This is a very powerful and robust method to eliminate the image artifacts due to the random measurement noise and numerical error. Although in the original algorithm a low-pass filter can be applied to the image after each update, the performance is incomparable to the performance of the $L$ matrix, because the latter is directly implemented in the objective function of inverse problem and can inherently inhibit the noise during the matrix inversion.

4.3.3. **A priori information**
NIR images are often associated with low resolution and accuracy is not only due to the banana-shape photon path, but also result from the reconstruction algorithm itself. The major obstacle for NIRS imaging is that the image reconstruction problem is non-linear, resulting an ill-conditioned boundary value problem. Furthermore the presence of noise in the measurement and the fact that more unknown properties need to be recovered than the available data make the problem worse. Therefore, the image reconstruction process has to be regularized which has the effect very similar to a low-pass filter that not only attenuates the influence of noise but also suppresses the high frequency components of the original image. As a result, the actual boundary of two types of tissue will be blurred and the recovered values of a single type of tissue will have an underestimated mean and overestimated variance, although physically its properties should be more homogeneous. Moreover, the image reconstruction highly depends on the initial guess of the optical properties, even though the non-linear algorithm is used. If the initial guess is significantly different to the actual optical properties, the reconstructed optical properties will be incorrectly computed from the measured perturbation of light distribution because of the improper Jacobian matrix. This makes it even more complicated to accurately interpret the chromophore concentrations. The key problem of NIR image reconstruction is the insufficient information to accurately localize and quantify the absorber and scatter distributions in complex biological structures and arbitrary geometries. Thus there are several possible ways to improve the performance by adding more information either \textit{a priori} or \textit{a posterior}.

\textbf{4.3.3.1. Define the geometry and optode positions}

\textit{A priori} information can be used to optimally define the imaging domain in the forward problem. This step is relatively straightforward since the FEM method cannot be applied without
knowing the tissue volume and the optode positions. The outline of tissue surface can be used to create a finite element mesh either in 2D or 3D and the tissue type indicated by the MRI segmentation can be assigned as regional information to the mesh. Guided by the impressions or the susceptibility effects caused by fiber-tissue contact, the image plane where the optode ring is placed and the locations of sources and detectors can be determined.

4.3.3.2. Reduction of unknown variables

In the inverse problem, the number of unknown parameters typically is more than the number of independent measurements, since we have to define the unknown variables continuously in whole image domain in the absence of knowledge about tissue types. However, the optical properties should be approximately piecewise distributed in the tissue since the same type of tissue should have similar optical properties. Based on the structure information provided by a stack of MRI slices, if the rat head is segmented into regions with different tissue types, such as, skins, bone, muscle and brain, and each region is treated as a bulk uniform material, the inverse problem is simplified with less unknown parameters. In both simulations and some clinic studies this approach has shown the promise of improving the resolution and accuracy (Brooksby et al., 2003; Chen et al., 2001; Schweiger and Arridge, 1999). However, applying such hard constraints can easily lead to some unexpected results when the heterogeneity of the tissue is not modeled completely or errors are substantially high in the data. This can be explained from three aspects.

First, other modalities, namely, X-CT, ultrasound or MRI, are sensitive to different inherent properties of the underlying tissue and the high resolution images that they offer are not exactly the contrast seen by NIR.
Second, unlike most other modalities, NIR imaging is a non-linear reconstruction. Such non-linearity leads to the crosstalk between absorption coefficient and reduced scattering coefficient. That is, if an absorbing heterogeneity within a region is missed, instead of only affecting the mean value in the reconstructed absorption image, it can generate a significant error in both absorption and reduced scattering images. This uncertainty also reflects the poor determinacy of using diffusion equation to separation absorption and reduced scattering coefficients. Absolute reflectance (Farrell, 1992), time-resolved data (Madsen et al., 1992), multiple distances (Pogue et al., 2000), multiple modulation frequencies (Fishkin et al., 1997) are typical techniques to strength the determinacy. In the frequency domain DOT, multiple distance measurement is the key to estimate the optical properties for the bulk properties. Relationships can be derived from the analytic solution that the slopes of $\log(rl)$ and phase to distance ($r$) are functions of the absorption and reduced scattering coefficients shown in Figure 4-1. For a measured dataset, these slopes can be fitted and two contour lines can be determined from those maps (Figure 4-1 (a) (b)). The intersection of these two contour lines determines the corresponding bulk absorption and reduced scattering coefficients. However, in some regions, such as, the left-upper corner (high scattering and low absorption) or the bottom part (high absorption and low scattering), those contour lines are almost parallel to each other and almost vertical to the coordinates showing strong crosstalk between parameters. This means a slice error in the data can dramatically change the estimated coefficients.
Figure 4-1, Maps of $d\log(I)/dr$ (a) and $d\theta/dr$ (b) as functions of absorption and reduced scattering coefficients. It was calculated from a 27mm diameter 2D FEM mesh.

Third, another characteristic of NIR imaging is the broad and depth-dependent sensitivity. Measurements are more sensitive to the region close to the optode than the deep regions. In other words, a stronger or bigger absorber must occur in the deep region to produce the same effect in the boundary data as an absorber in the superficial region. If the heterogeneity within the peripheral region (muscles) is missed, besides the error in the peripheral region, an amplified error might occur for the internal region (brain).

Ideally, when the target is divided into several regions, the reconstructed values should be the mean values of all pixels. This might be true for the linear reconstructions; however, for the non-linear reconstruction, this expectation is hardly achieved since reconstructed regions and properties are not independent. Therefore, the reduction of unknown variables is proved to be impractical especially in the case where the rat head has extremely complex structures.
4.3.3.3. **Improving the initial guess**

In the absence of the structural information, the reconstruction mostly starts with a homogeneous initial guess. The difference between the measured data and the calculated data from the initial guess is then inverted to the perturbation of the optical properties from the initial guess through the sensitivity matrix calculated from the initial guess. Given a slight lack of structure, i.e., mostly homogeneous background plus a small heterogeneity, the homogeneous initial guess works well to recover the small heterogeneity. However for a medium with multiple layers and if the interested small object is deep within the internal layer, reconstruction starting with the homogeneous initial guess will probably only recover the internal layer without the detail of the small object, because the disturbance in the data is mostly due to the big internal layer not the small object. If a two-layer initial guess is used instead, the difference between the calculated data and the measured data will mostly be due to the small object and this small object can be detected. This is especially important for brain studies where the brain is surrounded by a complex structure. Basically, it needs to ensure that the initial guess is good that that small signal due to a small structure is not lost during the update of a non-linear reconstruction. Previous work has shown that the reconstruction of small heterogeneities can be greatly improved if an initial guess is close to the true value (Pogue and Paulsen, 1998; Schweiger and Arridge, 1999; Xu et al., 2003).

4.3.3.4. **Spatially variant regularization**

A second level of increasing the information is to enhance the contrast and resolution in regions of interest during the inverse process. Conventional image reconstruction treats the whole image volume with the same priority and a scalar regularization factor is used in the regularization scheme. The regularization stabilizes the matrix-inversion at the expense of
contrast and resolution. With *a priori* information of the tissue’s internal structure, the covariance matrix in the imaging domain can be estimated and therefore to direct the update of optical properties preferentially in specific regions of interest. This is accomplished by introducing spatially variant regularization, where regions with known higher average optical properties can be given lower regularization to give them more freedom to update in the inverse process (Li et al., 2003; Pogue, 1999). By defining the regularization parameter spatially, the optical contrast of region of interest can be thereby improved, yet the high-frequency interior boundaries can be preserved. The key assumption is that the probability to find the heterogeneities is higher in the regions of interest. More importantly, such probability can be estimated not only from *a priori* information, but also from *a posteriori* information. This has been shown in a three-step algorithm (Srinivasan et al., 2004) that the spatially variant regularization is determined by the first round reconstruction and applied to the second round to improve the sharpness. From the perspective of signal processing, it is similar to the idea of wiener filtering. Compared to reduction of unknown variables, the spatially variant regularization is a relatively fail-safe constraint because the spatial variation within each region still is allowed and the only assumption is that the variation is expected to be higher in a specific region.

Incorporated with the spatially variant regularization, the LM regularization term is a spatially variable and the objective function in Equation 4-23 can be written as:

\[
\min \left\{ \| J\tilde{x} - b \|_2^2 + \lambda_1 \| L\tilde{x} \|_2^2 + \lambda \| \tilde{x}(r_1) \|_2^2 + a\lambda \| \tilde{x}(r_2) \|_2^2 \right\}
\]

(4-27)

where \( r_1 \) is the background region (such as, muscles etc), \( r_2 \) is the region of interest (such as brain), and \( a \) is the amplification factor for the background region. If \( a = 1 \), Equation 4-27 simplifies to Equation 4-23. The choice of \( a \) depends on the areas of regions and their contrast.
For a linear reconstructions, Li et al (Li et al., 2003) have suggested that the optimal regularization can be determined using the L-curve technique and have chosen the amplification factor $a$ in compromise of CBNR and CONR, where CBNR stands for the contrast-to-background noise ratio defined as the ratio of the mean value of the image in the region of interest to the standard deviation in the background region and CONR stands for the contrast-to-object noise ratio defined as the ratio of the mean value of the image in the region of interest to its standard deviation. However, it was introduced in the context of the linear reconstruction scheme. For the non-linear LM scheme, the choice of the regularization $\lambda$ and the amplification factor $a$ seems not critical since it is compensated by the number of iterations. Therefore, in this thesis, the initial regularization number and the amplification factor are empirically determined and fixed.

4.4. Simulations and experiments

To examine the performance of the forward solver and the benefits of a variety of modification in the inverse algorithm, both simulations and phantom studies have been performed.

4.4.1. Optimization of fiber positions

This work investigates fiber placement issues associated with a hybrid imaging technique for small animal brain studies. Location of the optical fibers on the cranium is examined, with an emphasis on maximizing the recovered resolution and contrast in the region of interest. In a series of simulation studies, singular value decomposition of the Jacobian is used in order to determine the measurement sites which provide the most information about the region of interest. The modeling results indicate that the data collected using fibers arranged on one side of the head near the brain contain as much information about optical changes within the brain as those
positioned equally-spaced around the entire periphery of the head. It has been shown that given accurate \textit{a priori} information about structure and optical properties within the head, it is possible to computationally predict and image changes of absorption within the brain with good localization. Here the use of \textit{a priori} information means using the correct initial guess in the reconstruction which has taken care of the major heterogeneity of the tissue. The detail of this study can be found in the publication (Xu et al., 2003).

\textbf{Figure 4-2} (a) MRI image of the rat brain used for segmentation of structures within the model. To more precisely locate the external surface of the rat, the animal was enveloped in wet paper towels (which appear as a thick bright band around the animal). (b) The FEM mesh generated from the segmented data with equally spaced optical fibers on the periphery, and (c) optical fibers arranged near the brain and one on the opposite side. The circular dots represent each of the source optical fibers mounted on the surface and the diamonds represents each of the optical fibers detectors.

This study focuses on evaluating two optical fiber configurations as possible arrangements for maximizing and improving the amount of information that can be extracted from the brain parenchyma through non-invasive NIR measurements on the rat cranium. An MRI image of the rat brain has been obtained and a 2D finite element model of the head has been created shown in Figure 4-2(a). From this geometric model the sensitivity functions for the two different sets of optical fiber configurations have been calculated, one being an equally spaced arrangement around the head (Figure 4-2(b)), and the other being preferentially arranged to the side nearest to the brain (Figure 4-2(c)).
Singular value decomposition (SVD) of these sensitivity maps within the brain was used to quantify and analyze the amount of information available from each optical fiber configuration. The singular values of the sensitivity maps calculated from a heterogeneous model (Figure 4-3(b)) are shown in Figure 4-3(a). Taking noise level into consideration, Figure 4-3(a) show that more singular values exist above the practical noise threshold for each of measurement type and optical property, if the preferential fiber arrangement (configuration 2) is used. The differences become more significant for higher signal to noise ratios. The increase at the noise level indicated in Figure 4-3(a) is about 2 to 8 singular values, which suggest that the data collected using this preferential fiber array configuration contains more information about the changes within the brain region. This in-turn should correspond to more accurate image reconstruction, resulting in better resolution and quantitative localization. It was found that the preferential configuration of having the fibers grouped at the top of the head provided as much or more information from the brain region alone, relative to the evenly spaced fiber grouping. This was true whether the head model was assumed to be homogenous or whether it was updated to contain optical property heterogeneity associated with the structure in the brain. In the case where the head is imaged using MRI and DOT simultaneously, physical space limitations make it much easier to place fibers on one side of the cranium; hence, it is important from a practical stand point to conclude that this fiber array configuration can be used without losing information about the region of interest.
Figure 4-3. (a) The singular values of different sets of optical fiber configuration for the heterogeneous model shown in (b). The upper left plot is the sensitivity of log of amplitude to diffusion coefficient. The upper right is the sensitivity of log of amplitude to absorption. The lower left plot is the sensitivity of phase to diffusion coefficient. The lower right is the sensitivity of phase to absorption. In each plot the log of the normalized singular values are displayed. The solid line represents configuration 1, the dotted line is configuration 2, and the dashed line indicates the noise level in each plot. (b) The absorption images for four types of tissue (skins, muscles, skins and brain)

Reconstructed images of the rat cranium from simulated data with noise where the presence of a localized change in absorption has been included, confirm the general findings
from the singular value analysis. Here, only images of absorption were reconstructed, assuming correct *a priori* scatter distribution, but the results (images) show that the preferential fiber arrangement set provides comparable image quality to a circumferential array with equally-spaced fibers. Also notable is an artificial effect which has occurred in the heterogeneous reconstructed image, Figure 4-4(c), however it would not cause much confusion since it is not near the region of the interest, the brain.

**Figure 4-4.** (a) The target $\mu_a$ image. Reconstructed images of absorption only, from noisy data calculated in the presence of an absorbing anomaly within the top region of the brain: (b) where the data is from a single absorbing anomaly using the fiber configuration 1; (c) where the data is from a single absorbing anomaly using fiber configuration 2. (d) Horizontal transects across the images.

**4.4.2. Regularization with Laplacian L-matrix**

Spatially variant regularization and Laplacian L-matrix are two other regularization mechanisms that have been incorporated in the LM algorithm. In this work, simulations were
performed to compare the results of three methods; namely, Method 1: the original LM algorithm without any spatial priors and L-matrix, Method 2: a modified LM algorithm with spatially variant regularization, Method 3: a modified LM algorithm with spatially variant regularization as well as the Laplacian L-matrix.

Figure 4-5. Target absorption and reduced scattering images. The diameter of phantom is 27mm and the diameter of the inclusions is 6mm. Color map is the contrast to the background value. An absorbing inclusion has 3:1 contrast is located on the right side of the phantom. A scattering inclusion has 3:1 contrast is located on the left size of the phantom.

A 27mm target phantom was used as shown in Figure 4-5 having two inclusions inside. A 6mm absorbing circular blob with 3:1 contrast in absorption was located in the mid-right of the phantom. A 6mm scattering circular blob with 3:1 contrast in reduced scattering was located in the mid-left of the phantom. Dual mesh reconstruction scheme was used in which a 1785-node fine mesh was used for the forward calculation but the inverse problem was solved on a 20x20 pixel based coarse mesh. Data was simulated on the fine mesh given the true optical property distributions and 1% noise was added to both amplitude and phase data. Reconstructions were started with a homogeneous initial guess equal to the background optical properties. Referring to Equation 4-27, the regularization number $\lambda$ for the LM algorithm was always initiated at 10. If spatial priors were applied, the two regions where the absorbing and scattering inclusions located were given a smaller regularization, one tenth of the background region, i.e., $a = 0.1$ in Equation
For both absorption and scattering components, the spatial priors were identical. If the Laplacian matrix was used, $\lambda_L$ was fixed at 0.01 during the iteration. The criterion to stop the iteration was either when the projection error began to increase or the change of the project error was less than 1%. Typically the reconstruction stopped after 15 iterations.

Figure 4-6. Reconstructed images at the 8th iteration (a) and the 15th iteration (b) when Method 1 (original method without spatial priors and L-matrix) is used. Horizontal profile transects of reconstructed images and target images are plotted together below the reconstructed images. 1% noise is added.

Figure 4-7. Reconstructed images at the 8th iteration (a) and the 15th iteration (b) when Method 2 (with spatial priors) is used. Horizontal profile transects of reconstructed images and target images are plotted together below the reconstructed images. 1% noise is added.
Figure 4-8. Reconstructed images at the 8th iteration (a) and 15th iteration (b) when Method 3 (with both spatial priors and L-matrix) is used. Horizontal profile transects of reconstructed images and target images are plotted together below the reconstructed images. 1% noise is added.

Figure 4-6,7,8 show the reconstructed images of absorption and reduction scattering at the 8th iteration and 15th iteration for three methods respectively. For Method 1, the quality of the images improved as the iteration number increased. At the last iteration, the original contrast for both inclusions was almost recovered however due to the noise the shape and the center of the inclusion were distorted a little bit and some artifacts were noticeable in the background. For method 2, at the 8th iteration it provided a result with better quantitative accuracy and less distortion and artifact. However, at the 15th iteration, although the projection error of the data still was decreasing, the artifacts became severe especially where the spatial priors were applied and the contrast in the inclusions overshot. The rapid convergence of this method was due to the low regularization in the regions containing high contrast inclusions. As a negative effect of the spatially variant regularization, the original mechanism to maintain the stability in the LM algorithm was lost. Thus the artifacts could be amplified in the inclusion. For Method 3, the results at 8th iteration and 15th iteration were almost identical and so as for those in-between iterations. The overshooting in the inclusion was avoided and the artifacts in the background
were suppressed because the high frequency spatial noise was filtered out in the objective function.

![Graphs showing relative RMS error within Background, Inclusion, and Total versus iteration number.](image)

Figure 4-9. Relative RMS error within Background, Inclusion and Total versus the iteration number. (a) Method 1: without spatial priors and L-matrix. (b) Method 2: with spatial priors only (c) Method 3: with both spatial priors and L-matrix. (d) Comparison of the total errors of three methods. The red circles denote the iterations showing the better images in Figure 4-6, 7, 8.

To closely examine the quality of reconstructed images versus the iteration number, an object evaluation standard should be used. For the best result, more contrast in the inclusion is needed while the artifacts in the background are suppressed. Thus the relative RMS error in the inclusion and the relative RMS error in the background should be evaluated. In general, the relative RMS error in the background ($RMS_b$) increases as the iteration increases but the relative
RMS error in the inclusion \((RMS_i)\) decreases, since the reconstruction normally starts with an initial guess close to the background and gradually recovers the contrast in the inclusion. The sum of these two errors \((RMS)\) was adopted as a good standard to evaluate the ultimate quality. It should be noted that these two components were equally weighted instead of being weighted by their areas, since the information conceived in the image was not pixel-based but categorized in two classes. Figure 4-9 plots the relative RMS errors in background \((RMS_b)\), inclusion \((RMS_i)\) and their sum \((RMS)\) as the iteration number increases for Method 1 in (a), Method 2 in (b) and Method 3 in (c). For Method 1, the monotonous decrease of the \(RMS_i\) and increase of the \(RMS_b\) were observed as expected. The summed error \(RMS\) was observed near the last iteration. This was confirmed by the reconstructed images that the best result was obtained at the last iteration. For Method 2, the monotonous increase of the \(RMS_b\) was preserved but the \(RMS_i\) increased dramatically after several iterations. This corresponded to the overshooting in the late stage as shown in Figure 4-7(b). The \(RMS_i\) indicated that the best solution was around the 8th iteration shown in Figure 4-7(a). For Method 3, the \(RMS_i\) decreased in the first a few iterations and almost kept that level afterwards and the increase of the \(RMS_b\) was slower compared to other two methods. Thus the \(RMS\) didn’t change much from the 8th iteration to the 15th iteration. In Figure 4-9(d), the \(RMS\) of three methods are plotted together and clearly showing Method 2, 3 outperform Method 1 and Method 3 is the most stable one.

In the absence of the knowledge of the true distributions, it is difficult to determine the optimal iteration. The significance of the L-matrix is that it helps stabilize the solution and makes the final solution less dependent on the iteration number. It also greatly improves the robustness of the reconstruction to the random noise and filters the unwanted and misleading high frequency artifacts in the reconstructed image. This can be further demonstrated in the following example.
Instead of using a piecewise distributed property in the target image, 10% random variation of both optical properties was modeled to mimic the tissue environment and 5% random noise was added to the boundary data. Reconstructed images using Method 1 and Method 3 are shown in Figure 4-10 (a) and (b) respectively. Method 3 still could successfully recover the inclusion whereas the original method could not separated anomalies from the artifacts.

![Reconstructed images](image)

*Figure 4-10. Reconstructed images when 5% noise is added to the data and 10% variance is added to target optical properties. (a) for Method 1 (b) for Method 3.*

### 4.4.3. Spatial priors in Gelatin phantoms

In the previous chapter, the Gelatin phantom experiments were used to examine the performance of the 2nd derivative spectroscopy based DOT reconstruction. In this section, the spatial priors are incorporated within the reconstruction to see whether the results are improved.

The phantoms were cylinders of 27mm diameter with a 14mm inclusion near the edge, which has different optical properties to the background. Three types of inclusion are used to build phantoms within the same background: A) an inclusion with higher scattering; B) an inclusion with higher absorption and lower scattering; and C) an inclusion with higher properties in both absorption and scattering. Reconstructed images were generated using a 2D dual mesh reconstruction program for these phantoms and these are shown in Figures 4-11(a), (b), (c).
each figure, target images are shown along with reconstructed images with the conventional method having no a priori information, and reconstructed images with a priori information (spatially variant regularization) are respectively shown in the subfigure (a), (b) and (c). Profile cross-section plots of the images are shown in Figure 4-11(d) for better visualization of the quantitative values. Reconstructed peak and mean values of the inclusion by the two methods and their difference to the target are listed in the Table 1 for comparison.

![Image of reconstructed images and profile plots](image_url)

**Figure 4-11.** Absorption and reduced scattering images of phantom A, B and C are shown. (a) Phantom A, which has a higher scattering inclusion off the center. (b) Phantom B, which has a higher absorption but lower scattering inclusion off the center. (c) Phantom C, which has a higher absorption and scattering inclusion off the center. In each sub-image, the 1st column shows the target images for absorption (first row) and reduced scattering (second row), the 2nd column shows the reconstructed images without spatial priors, and the 3rd column shows the reconstruction with spatial priors. The original optical properties are converted to contrast to the background for convenience. (d) Horizontal profile transects from the images of (a) (b) (c)
are shown. The contrast distribution for target images (dashed lines) was reconstructed without spatial priors (solid lines) and with spatial priors (dotted lines).

In Figure 4-11(a-c), images reconstructed with \textit{a priori} information clearly provide higher contrast for the inclusion than those reconstructed without it. Higher contrast also allows the sharper boundary, showing better interpretation of the actual size of the inclusion. This can easily be observed in Figure 4-11(d) where profile plots of the reconstruction with \textit{a priori} information do better approximate the target profiles in the region of the inclusion. Some overshooting is observed in the images reconstructed with \textit{a priori} information but the mean value is improved. In Table 4-1, after reconstruction without \textit{a priori} information, the peak value of the inclusion is within 6\% of the target in average and the mean value is within 12\% difference of the target. With \textit{a priori} information those differences are improved to 4\% and 6\% which means better quantitative accuracy is achieved. By spatially varying the regularization using the structural information, reconstructed images have been improved both qualitatively and quantitatively, though it is not so significant since the inclusion has a relatively large size and low contrast.
Table 4-1. The summarized results of phantom studies are shown here. The original optical properties are converted to contrast to the background for convenience. The target values from 3 types of phantoms are listed in the “Target” column. Reconstructed peak values (“peak”) and mean values (“mean”) for the inclusion are listed for both reconstructions with or without a priori and their differences (“Diff.”) to the target.

4.5. Hybrid 2D/3D modeling

Image reconstruction is a truly three-dimension (3D) problem since the light diffuses in three dimensions. For a modulated laser source, the propagation of light in the tissue can be thought as the spread of a diffuse photon density wave (DPDW) from the source (Boas et al., 1994). Whether modeled in 2D or 3D the phase shift distribution of this sphere wave as the distance to the source would be similar since it is proximately a linear function of distance, whereas the intensity calculated by the 3D model will be significantly smaller than that by the 2D model since the former decays as the square of the distance but the latter decays as the distance. This has been shown by Schweiger et al (Schweiger and Arridge, 1998) that the difference in the measurement data between 2D and 3D model depends greatly on the measurement type used, giving a much better agreement for mean time-of-flight data (equivalent
to the phase measurement) than for the intensity data. Therefore if both intensity and phase measurement are used in 2D computations, the data-model match would be achieved at the expense of optical property mismatch. Such mismatch can be minimized through a calibrated 2D reconstruction which uses a calibration measurement on a homogeneous object to parameterize the model or uses difference data from two experiments with and without the inclusion present (Jiang et al., 1998; Pogue et al., 1995), yet a 3D treatment of light propagation in tissue provides a more accurate prediction of the fluence distribution in the medium (Dehghani et al., 2003). Ideally, the inverse problem should be solved in 3D as well as the forward problem, i.e., to recover the arbitrary parameter distributions all over the imaging domain. However, in the case that boundary data are only collected in the single plane, the 3D reconstruction may make the problem more ill-posed, since there is not enough information (measurement) to sample the region far from the plane.

A more practical strategy has been suggested by many researchers that a hybrid 2D/3D algorithm, or semi-3D algorithm, can be used to solve the forward and inverse problems. (Gao et al., 2002; Pogue et al., 2001; Pogue et al., 1995; Schweiger and Arridge, 1998). This approach assumed 2D heterogeneity distribution of the optical properties of the subject under investigation, i.e., the optical properties was invariable along the axial direction (z coordinate). Thus the inverse problem could be reduced to 2D and the computational cost for both memory requirement and execution time could be saved, while a 3D model was used in the forward calculation to retain the 3D nature of the photon migration. This algorithm is most realistic when the assumption about the axial uniformity of the subject is true and has been demonstrated outperforming the widely used calibrated 2D reconstruction (Gao et al., 2002). In the Gelatin experiments, the inclusion was a rod placed along the axis perpendicular to the acquisition plane,
thus the requirement for the hybrid algorithm is satisfied. In the rat head imaging, this assumption seems fairly reasonable and practicable since the brain extends on the z axis and can be regarded as a brain rod buried in the cylindrical muscle tissue particularly when the cylindrical animal holder (Discussed in Chapter 2) is used.

A similar 2D/3D hybrid reconstruction algorithm is developed in this project and it is illustrated by the diagram in Figure 4-12. Given an initial guess, the initial 2D mesh optical property is mapped to a 3D mesh assuming that the optical properties at any position \((x,y,z)\) in the 3D mesh equal to those at the position \((x,y)\) in the 2D mesh. Data and 3D Jacobian matrix are calculated from this 3D mesh through the forward solver. The 3D Jacobian matrix must be projected to a 2D Jacobian matrix by integrating along the z axis. The 2D Jacobian, the simulated data and the measured data are given to the inverse solver to compute an updated solution on the 2D basis. This is looped until the stopping criteria is met.

![Diagram of the hybrid 2D/3D reconstruction algorithm.](image)

Compared to the original reconstruction algorithm which only has two computation units (the forward solver and the inverse solver), obviously this algorithm is more complicated for additional units: the mapping from 2D to 3D and the projection from the 3D to 2D. Mathematically the mapping is an interpolation operation and the projection is an integration.
operation. The implementation of the algorithm should concern the complexity and computational cost at these two units. Another concern is the generation of the 3D mesh which should be tractable. All these concerns can be relieved with a convenient and flexible generation of the 3D mesh.

![Figure 4-13](image)

**Figure 4-13.** (a) A 3D mesh generated by 5 layers of a 2D mesh. (b) An illustration of splitting a prism to 4 tetrahedral elements. $N$ is the number of nodes in the 2D mesh.

As shown in Figure 4-13(a), 5 layers of a 2D mesh with 425 nodes and 776 triangle elements are stacked to generate a 3D mesh. The benefit of such 3D mesh generation is the convenient registration between the nodes of the 2D and 3D meshes. Any node in the 2D mesh is linked to the nodes with same x y coordinates in the 3D mesh. The interpolation operation in the mapping process is simplified to an assignment. The integration operation in the projection process is simplified to a summation. It is worth to mention that the separation of these layers is flexible thus fine elements can be created in the plane close to the sources and detectors but coarse elements can be created for those regions far from this plane.
Figure 4-14. Reconstructed images of absorption and reduced scattering coefficients for a Gelatin phantom A which has a higher scattering inclusion off the center using hybrid 2D/3D reconstruction algorithm. The profile transects (blue lines) of the images are provided below and compared with those of target images.

An example of reconstruction using this hybrid 2D/3D algorithm is shown in Figure 4-14. The Gelatin phantom used was the same as Figure 4-11(a). The forward 3D mesh was generated from 15 layers of 425-node 2D mesh. The quality of the reconstructed images was similar to those reconstructed by 2D model. However, the quantitative accuracy of this hybrid reconstruction outperforms the 2D reconstruction in terms of the absolute values of absorption and reduced scattering. This can be explained through Table 4-2.

The absorption and reduced scattering properties of the Gelatin materials are estimated using three separate methods. A) Semi-infinite approach: multiple distance data were measured on a bulk material and fitted to a homogeneous semi-infinite model to estimate two coefficients. B) 3D FEM approach: tomography data were measured on a cylindrical material and fitted to a homogeneous 3D FEM model. C) 2D FEM approach: tomography data were measured on a cylindrical material and fitted to a homogeneous 2D FEM model. Essentially all these three approaches are based on the multiple distance data. The semi-infinite approach is thought to be most accurate since it uses the analytic solution. Clearly the 3D FEM approach provides similar
results to the semi-infinite whereas the 2D FEM approach overestimates both properties. This overestimation results from the data mismatch between the truly 3D light propagation and the 2D diffusion approximation. Therefore, for the best quantification, the 3D model is necessary. However, interestingly no matter which method is used, the contrast of the inclusion to the background is similar. That may explain why in most cases, the calibrated 2D reconstruction is a good solution to a full 3D reconstruction.

<table>
<thead>
<tr>
<th></th>
<th>Background</th>
<th>Inclusion</th>
<th>Inclusion/Background</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\mu_a) (1/mm)</td>
<td>(\mu_s') (1/mm)</td>
<td>(\mu_a) (1/mm)</td>
</tr>
<tr>
<td>Semi-inf</td>
<td>0.0081</td>
<td>0.83</td>
<td>0.0085</td>
</tr>
<tr>
<td>3D FEM</td>
<td>0.0082</td>
<td>0.90</td>
<td>0.0091</td>
</tr>
<tr>
<td>2D FEM</td>
<td>0.0125</td>
<td>1.145</td>
<td>0.0129</td>
</tr>
</tbody>
</table>

Table 4-2. Fitted absorption and reduced scattering properties for the background material and the inclusion material in Phantom A and their contrast when different methods are used. Semi-inf: multiple distance data were measured on a bulk material and fitted to a homogeneous semi-infinite model. 3D FEM: tomography data were measured on a cylindrical material and fitted to a homogeneous 3D FEM model. 2D FEM: tomography data were measured on a cylindrical material and fitted to a homogeneous 2D FEM model.

4.6. Discussion

Both the simulation and phantom studies indicate that an accurate and robust reconstruction of arbitrary optical property distributions in a small turbid medium with the diffusion approximation is practicable. Solving the forward problem using finite element based approach not only makes it easily adapted to any arbitrary geometries but also makes it convenient to incorporate the spatial information from other modalities and apply the constraints to achieve better resolution and more accurate DOT images. The non-linear reconstruction algorithm allows both absorption and reduced scattering properties can be successfully
recovered. Constraints from the statistical (Spatially variant regularization) and the signal processing (Laplacian L-matrix) perspectives are elegantly implemented simultaneously into the inverse problem by modifying the regularization term within the conventional Levenberg-Marquart algorithm for least-squares estimation of nonlinear parameters. A hybrid 2D/3D reconstruction algorithm combines the accuracy of the 3D forward model and the simplicity of the 2D inverse problem with respect to the specific acquisition system and experiments.
Chapter 5. Direct chromophore and scattering reconstruction and spectral derivative based reconstruction

In this chapter, the theory of direct reconstruction of chromophore and scattering distributions with broadband intensity data is described. Consequently, a novel spectral derivative based algorithm which provides inherent insensitivity to coupling and geometric errors is proposed and demonstrated through simulations and phantom studies.

5.1. Introduction

Diffuse optical tomography (DOT) has been studied intensively for many years. Based upon propagation models of near infrared (NIR) light in tissue and the known spectral contrast of chromophores in tissue, DOT can resolve spatial distributions and temporal dynamics of hemoglobin, water, lipids, and scattering properties. The fundamental limitation of DOT has always been related to the diffuse nature of light passing through tissue making the measured light a nonlinear function of both absorption and scattering properties of the medium, as well as the exterior boundary of the tissue. This non-linear transport leads to a hypersensitivity to the boundary, and so small errors in the measurement can significantly degrade the performance by introducing artifacts within the edge of the reconstructed images. Errors such as coupling coefficient, boundary reflection mismatch, and inaccurate geometric modeling (i.e. of optical fiber positions) always exist and are unpredictable even in a reasonably well calibrated system. Although corrections have been suggested (Heino et al., 2005; Stott et al., 2003), the fundamental problem that these errors are easily coupled with the signals of interest is still a major limitation of most imaging systems. Image reconstruction algorithms that rely upon absolute transmission data are sensitive to these errors because their objectives are to match the model-calculated data with the calibrated measurement data, which contains these coupling/boundary errors.
Recently spectral reconstruction algorithms which allow direct chromophore and scattering reconstruction (DCSR) have been introduced in either continue-wave (CW) systems (Corlu et al., 2003; Li et al., 2004) or in the frequency domain system (Srinivasan et al., 2005). Corlu et al showed that the non-uniqueness of the image reconstruction with CW data (Arridge and Lionheart, 1998) is overcome by the spectral constraints of chromophores and scattering features at multiple optimal wavelengths. In their simulations, oxyhemoglobin, deoxyhemoglobin, water, scattering amplitude heterogeneity could be successfully recovered using measurement at 4 optimal wavelengths when assuming the scattering power was homogeneous. Li et al showed that more accurate quantification of hemoglobin concentration was obtained with two-wavelength CW system when spectral priors were incorporated into the inverse problem. Srinivasan et al extented Corlu’s approach in the 6-wavelength frequency system and provided both theoretical and experimental evidence to show the new method outperforms the original reconstruction (indirect method) at separate wavelengths in simultaneously recovering images of total hemoglobin, oxygen saturation, water and scattering amplitude and power. Compared to the original indirect reconstruction method which first reconstructs maps of absorption coefficient ($\mu_a$) and reduced scattering coefficient ($\mu_s'$) at individual wavelengths, this method has shown improved accuracy and is more robust in the presence of noise, since it uses the coupled spectral information to constraint the reconstruction.

Our system collects the attenuation from 670nm to 910nm continuously however such broad spectral information has not been fully utilized. This is limited by two reasons. First only two pathlengths near the water features can be estimated from the broadband spectrum. Second, the diffusion equation cannot be uniquely solved with intensity data. Cleary with the new DCSR method, by applying a priori knowledge of the spectral dependence of chromophores and
scattering effect, diffusion equations at separate wavelengths can be coupled through the chromophore concentrations and scattering parameters, and a unique solution is possible even only with the intensity measurement. With this method, the advantage of the broadband can be fully revealed. More spectral information always brings better quantification, more flexibility and stronger determinacy. In other words, we can choose optimal wavelengths for any chromophores not just limited to the ones with most interest, oxyhemoglobin, deoxyhemoglobin, water, etc.

However, spectral methods currently described rely on the absolute spectrum measurement, and therefore errors that are not common to all the optical fibers may introduce artifacts within the reconstructed parameters. In this chapter, a novel reconstruction method is proposed which is inherently insensitive to fiber coupling errors, boundary reflection mismatch and geometric modeling errors, allowing better spatial resolution and quantitative accuracy. The proposed algorithm is incorporated into a NIR reconstruction algorithm, and it may be adapted to other imaging modalities where spectral information is available.

For the proposed algorithm, the spectral derivative image reconstruction (SDIR) uses a difference of wavelengths in the DCSR approach where the reconstruction algorithm minimizes the difference between the calculated and measured first derivative of the spectrum. Currently it is limited to the first order approach to the finite differencing for simplicity but may be easily adapted to higher order approaches. The errors due to fiber and tissue coupling or external boundaries have a broadband offset effect, which is largely wavelength independent. Therefore by taking the difference at two adequate wavelengths, these common errors are canceled and the remaining signal still contains the required information regarding the internal properties being imaged.
5.2. Direct chromophore and scattering reconstruction (DCSR)

5.2.1. Forward problem

The original forward problem mentioned in Chapter 4 is based on the diffusion equation. For a given distribution of reduced scattering coefficient ($\mu'_s$), absorption coefficients ($\mu_a$), the forward solver predicts the measurement at all the detectors for all the sources. Knowing that both optical properties are wavelength dependent, this can be described as:

$$\Phi^f(x) = F(\mu_a(\lambda), \mu'_s(\lambda))$$

(5-1)

where $F$ represents the forward solver, $\mu_a$ and $\mu'_s$ are vectors that represent the optical property distributions, $\Phi^f$ is a vector and the calculated measurement at all the detectors, and the target parameter $x = [\mu_a(\lambda) \quad \mu'_s(\lambda)]$ is wavelength dependent.

The wavelength dependence of the optical properties can be modeled by two principles (Corlu et al., 2003). First, the wavelength dependent absorption is a linear combination of each component, $\mu_a(\lambda) = \sum_i \varepsilon_i(\lambda)c_i$, where $\varepsilon_i$ is the specific extinction coefficient and $c_i$ is the concentration distribution of $i^{th}$ chromophore. In matrix form, it is $\mu_a(\lambda) = \varepsilon c$. For simplicity, only three chromophores HbR ($c_1$), HbO2 ($c_2$) and H2O ($c_3$) are considered and this is reasonable for most tissues. Second, the wavelength dependence of reduced scattering is modeled by an empirical approximation to Mie theory, $\mu'_s(\lambda) = a(\lambda/\lambda_0)^{-b}$, where $\lambda_0$ is a pivot wavelength. $\lambda_0 = 1\mu m$ is used in this work and omitted in the following discussion. Substituting these two relations into Equation 5-1 yields:

$$\Phi^f(x) = F(\sum_i \varepsilon_i(\lambda)c_i, a(\lambda/\lambda_0)^{-b})$$

$$= \tilde{F}(a, b, c_1, c_2, c_3, \lambda)$$

(5-2)
where \( \tilde{F} \) is the new forward solver which includes the knowledge about the extinction coefficient of each chromophore. The new unknown parameter \( x = [a \ b \ c_1 \ c_2 \ c_3] \) is independent of wavelength \( \lambda \).

For a given distribution of chromophores and scattering amplitude and power and any combination of wavelengths, the forward model predicts the discrete spectrum at these wavelengths at all the detectors.

The Jacobian for the DCSR can be easily derived from the Jacobian in the indirect method described in Chapter 4. The detail can be found elsewhere (Srinivasan et al., 2005).

\[
\mathbf{J}_{c_1,\lambda} = \frac{\partial \Phi_{\lambda}}{\partial c_i} = \frac{\partial \Phi_{\lambda}}{\partial \mu_a} \frac{\partial \mu_a}{\partial c_i} = \mathbf{J}_{\mu_a,\lambda} F_{i,\lambda} \quad (5-3)
\]

\[
\mathbf{J}_{\lambda} = \frac{\partial \Phi_{\lambda}}{\partial a} = \frac{\partial \Phi_{\lambda}}{\partial D} \frac{\partial D}{\partial \mu_s} \frac{\partial \mu_s}{\partial a} = \mathbf{J}_{D,a} (-3D^2) \lambda^{-b} \quad (5-4)
\]

\[
\mathbf{J}_{b,\lambda} = \frac{\partial \Phi_{\lambda}}{\partial b} = \frac{\partial \Phi_{\lambda}}{\partial D} \frac{\partial D}{\partial \mu_s} \frac{\partial \mu_s}{\partial b} = \mathbf{J}_{D,b} (-3D^2) \mu_s (-\ln \lambda) \quad (5-5)
\]

where \( D \) is the diffusion coefficient, and \( \mathbf{J}_{\mu_a,\lambda}, \mathbf{J}_{D,\lambda} \) are the Jacobian matrices in the indirect method and can be conveniently calculated.

5.2.2. Inverse problem

The general DOT reconstruction algorithm is based on a standard least squares error optimization. The goal is the recovery of \( \mu_a \) and \( \mu_s \) distribution based on the measurements of light fluence at the tissue surface. The inverse solution is achieved by minimizing the difference between measured (observed) fluence \( \Phi^o \) at the tissue surface and calculated data \( \Phi^c \) from a given model. This is a minimization of \( \Psi \) :
In the DCSR approach, the unknown parameters are $x = [a \ b \ c_1 \ c_2 \ c_3]$. Clearly with measurement only at a single wavelength, it is unable to recover five individual parameters simultaneously. In general, at least five wavelengths are required and the measurement at multiple wavelengths can be coupled to yield a new objective function:

$$\Psi = \| \Phi^o_{\lambda_1}(x) - \Phi^o_{\lambda_1} \|_2^2$$  \hspace{1cm} (5-6)

where $\Psi$ is the number of wavelengths. The iterative formula for converging to a solution can be derived as $\Delta x = (\mathcal{J}^T \mathcal{J})^{-1} \mathcal{J}^T \Delta \Phi^{e-o}$, where $\mathcal{J}$ is the Jacobian matrix comprising the sensitivity for each parameter and the measurement at each wavelength:

$$\mathcal{J} = \begin{bmatrix} \mathcal{J}_a & \mathcal{J}_b & \mathcal{J}_{c_1} & \mathcal{J}_{c_2} & \mathcal{J}_{c_3} \end{bmatrix} = \begin{bmatrix} \mathcal{J}_{a,\lambda_1} & \mathcal{J}_{b,\lambda_1} & \mathcal{J}_{c_1,\lambda_1} & \mathcal{J}_{c_2,\lambda_1} & \mathcal{J}_{c_3,\lambda_1} \\ \mathcal{J}_{a,\lambda_2} & \mathcal{J}_{b,\lambda_2} & \mathcal{J}_{c_1,\lambda_2} & \mathcal{J}_{c_2,\lambda_2} & \mathcal{J}_{c_3,\lambda_2} \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ \mathcal{J}_{a,\lambda_m} & \mathcal{J}_{b,\lambda_m} & \mathcal{J}_{c_1,\lambda_m} & \mathcal{J}_{c_2,\lambda_m} & \mathcal{J}_{c_3,\lambda_m} \end{bmatrix}$$  \hspace{1cm} (5-8)

Using the Levenberg-Marquardt algorithm, the update formulation can be written as:

$$x_{k+1} = x_k + (\mathcal{J}^T \mathcal{J} + \rho I)^{-1} \mathcal{J}^T \Delta \Phi^{e-o}$$  \hspace{1cm} (5-9)

where $\rho$ is the regularization number not the wavelength.

**5.2.3. Uniqueness condition**

To examine the uniqueness condition, we’d like to follow the criteria introduced by Corlu et al. In their approach, for simplicity of formulation it was assumed that the scattering power ($b$)
was spatially constant and known. If multiple solutions exit for the measurement at single wavelength, another set of solution \( \tilde{a} = a + \Delta a \) and \( \tilde{c}_i = c_i + \Delta c_i \) must exist for the same measurement data and a given \( b \). Suppose there are \( n \) chromophores and \( m \) wavelengths, then the inverse problem is not unique only if solution exists to the matrix equation:

\[
Ax = 1
\]

where

\[
x = \frac{\Delta a}{\tilde{a} h(\tilde{a})} \begin{bmatrix} c_1 \\ \vdots \\ c_n \end{bmatrix} + \frac{1}{h(\tilde{a})} \begin{bmatrix} \Delta c_1 \\ \vdots \\ \Delta c_n \end{bmatrix}
\]

\[
A = \begin{bmatrix}
\varepsilon_1(\lambda_1) & \cdots & \varepsilon_n(\lambda_1) \\
\varepsilon_1(\lambda_2) & \cdots & \varepsilon_n(\lambda_2) \\
\vdots & \ddots & \vdots \\
\varepsilon_1(\lambda_m) & \cdots & \varepsilon_n(\lambda_m)
\end{bmatrix}
\]

\[
h(\tilde{a}) = (1/3\tilde{a}) \times \left[ \nabla^2 (1/3a)^{1/2} / (1/3a)^{1/2} - \nabla^2 (1/3\tilde{a})^{1/2} / (1/3\tilde{a})^{1/2} \right]
\]

They claimed that inverse problem has non-unique solutions if \( Ax \) is equal or very close to 1. When the number of wavelengths is more than the number of chromophores \((m>n)\), Equation 5-10 has a solution in the least-squares sense, \( x_0 = (A^T A)^{-1} A^T \). The residual norm \( R = \| I - Ax_0 \| \) can be regarded as the parameter distinguishability. The closer \( R \) is to zero, the closer \( x_0 \) is to fulfill conditions for non-uniqueness. Therefore, their first criterion for selecting optimal wavelengths is to maximize the \( R \).

Their second criterion concerns the distinguishability of chromophore concentrations among themselves. The sensitivity of the measurement to a single chromophore concentration is proportional to the smoothness of the singular value distribution of the specific extinction coefficient matrix:
The condition number $k$ of this matrix indicates its smoothness. If $k$ is larger, it means the measurements are more sensitive to some chromophores than to others. The second criterion is to minimize $k$ for having equalized sensitivities to all the chromophores.

Their approach was particularly proposed for a 4-wavelength CW laser system, with these two criteria, thus they determined an optimal set of wavelengths in the 650-930nm range. Our system is a broadband white source system, thus we are not limited by the available wavelengths of the laser source and can choose any combinations in the 670-910nm range.

For convenience, even distributed wavelengths were chosen in our approach. The $R$ and $k$ numbers referring to those two criteria were calculated when different wavelength separation ($\Delta \lambda$) was used. The number of wavelengths ($m$) is equal to the entire wavelength range ($w=910-670=240$nm) divided by the separation then plus one, that is, $m = (w/\Delta \lambda) + 1$. The samples were composed of three chromophores, HbR ($c_1$), HbO$_2$ ($c_2$) and H$_2$O ($c_3$). $b = 1.3$ was assumed. The results of $R$ and $k$ when $\Delta \lambda = 5, 10, 20, 40, 60, 80$nm, i.e., $m=49, 25, 13, 7, 5, 4$ are plotted in Figure 5-1. The results for the four optimal wavelengths [650nm, 718nm, 886nm, 930nm] determined by Corlu et al are plotted as well for comparison. Figure 5-1(a) plots the residual norm $R$ with respect to the number of wavelengths. It is clear that the residue $R$ increases as the number of wavelengths increases. This implies that more spectral information always strengthens the uniqueness. Figure 5-1(b) plots the condition number $k$ with respect to the number of wavelengths. Compared to the optimal set for 4 wavelengths in the 650-930nm range, the condition number $k$ calculated at equally spaced wavelengths in the 670-910nm range are slightly higher. This is because the HbR absorption feature at short wavelengths and the water
absorption feature at long wavelengths improve the distinguishability. If the entire range is expanded to 650-930nm, higher $R$ and lower $k$ are obtained at the same time. Even in the same range, if those wavelengths are not equally spaced, higher $R$ and lower $k$ can be obtained by choosing the optimal wavelength set. However, in general, when more than 10 wavelengths are used, both residual norm and condition number are stable and acceptable, unlike the situation shown by Corlu et al that when the four wavelength set was not optimal very small $R$ or very large $k$ might occur. In practice, it has been found that 20nm separation, i.e., 13 wavelengths, is a good option which is a tradeoff between the computation cost and the spectral information. In the simulations, we also find the phase data are not required when having enough intensity measurements. With measurement data at 13 wavelengths, the reconstructions with and without phase data are similar.

![Figure 5-1. (a) The residual norm $R$ and (b) The condition number $k$ versus the number of wavelengths for the cases of equally distributed wavelengths in range of 670-910nm, 650-930nm and the 4 wavelength set suggested by Corlu et al.](image)

5.3. **Spectral derivative image reconstruction (SDIR)**

5.3.1. **Theory**
Spectral derivative image reconstruction provides inherent insensitivity to coupling and geometric errors. The rationale is that the errors due to fiber and tissue coupling or external boundaries have a broadband offset effect, which is largely wavelength independent. Therefore by taking the difference at two adequate wavelengths, these common errors are canceled and the remaining signal still contains the required information regarding the internal properties being imaged.

Based on the concept of DCSR, the direct and explicit reconstruction parameters become the scattering amplitude \(a\), scattering power \(b\), and the chromophore concentrations (here hemoglobin \([HbR]\), oxyhemoglobin \([HbO_2]\) and water \([H_2O]\)) and the indirect and implicit parameters \(\mu_s\) and \(\mu'_s\), are not directly calculated. Using this approach, a natural extension is to utilize spectral data types which minimize the impact of edge or coupling losses, while preserving the spectral measurement of the interior, such as the first derivative of the spectrum.

In the SDIR approach, the objective function is modified from Equation 5-7:

\[
\Psi_2 = \left\| \Delta \Phi^{c-o} \right\|^2_2 = \begin{pmatrix}
\Phi^c_{\lambda_1}(x) - \Phi^c_{\lambda_2}(x) \\
\Phi^c_{\lambda_2}(x) - \Phi^c_{\lambda_3}(x) \\
\vdots \\
\Phi^c_{\lambda_{m-1}}(x) - \Phi^c_{\lambda_m}(x)
\end{pmatrix} - \begin{pmatrix}
\Phi^o_{\lambda_1} - \Phi^o_{\lambda_2} \\
\Phi^o_{\lambda_2} - \Phi^o_{\lambda_3} \\
\vdots \\
\Phi^o_{\lambda_{m-1}} - \Phi^o_{\lambda_m}
\end{pmatrix}
\]

\[= \left\| \begin{pmatrix}
\Phi^c_{\lambda_1}(x) - \Phi^c_{\lambda_2}(x) \\
\Phi^c_{\lambda_2}(x) - \Phi^c_{\lambda_3}(x) \\
\vdots \\
\Phi^c_{\lambda_{m-1}}(x) - \Phi^c_{\lambda_m}(x)
\end{pmatrix} - \begin{pmatrix}
\Phi^o_{\lambda_1} - \Phi^o_{\lambda_2} \\
\Phi^o_{\lambda_2} - \Phi^o_{\lambda_3} \\
\vdots \\
\Phi^o_{\lambda_{m-1}} - \Phi^o_{\lambda_m}
\end{pmatrix} \right\|^2_2 \quad (5-12)
\]

Here the prime denotes the finite difference operator to remind the similarity between derivative and finite difference. The Jacobian matrix for SDIR can be easily derived from the Jacobian calculated using the conventional method and is the subtraction of the first \(m-1\) row and the last \(m-1\) row of \(\mathcal{J}\) in Equation 5-8:
The sequence of pairs of wavelengths used in Equation 5-13 can be arbitrary, for example one can use \( \{\lambda_1, \lambda_2, \lambda_1, \lambda_3, \ldots\} \) or \( \{\lambda_1, \lambda_2, \lambda_2, \lambda_1, \ldots\} \).

Consider a case where coupling coefficient error exists in the measurement so that measured intensity \( I^o \) is the product of the coupling efficiency \( k \) and the real intensity \( I' \), \( I^o = kI' \). Since typically in the model \( \Phi = \log(I^o) = \log(I') + \log(k) \), the coupling error becomes an additive term. Using this measurement with coupling error with Equations 5-6 (the indirect method), Equation 5-7 (the DCSR method) or Equation 5-12 (the SDIR method), the SDIR algorithm will not be affected but the other two will treat the coupling error as part of the real signal and will lead to image artifacts.

5.3.2. **Sensitivity, spatial resolution and distinguishability**

Questions remain on whether SDIR can still provide sensitivity and specificity to resolve the chromophore and scattering properties accurately and on the choice of appropriate wavelength pairs for the difference measurement. These two facts are inherently related since the Jacobian matrix using within the SDIR algorithm is a function of the wavelength pairs chosen. Intuitively if wavelength pairs are chosen where higher absorbance contrast can be achieved for chromophores, more sensitive and independent sub-Jacobian matrices \( \mathcal{J}_a, \mathcal{J}_b, \mathcal{J}_c, \mathcal{J}_d, \mathcal{J}_e \) can be constructed to provide better separation and localization of chromophore concentrations. To precisely explore this issue, the characteristics of the Jacobian matrix, such as the magnitude, rank, condition and the correlation among sub Jacobian matrices is analyzed in depth in comparison between the SDIR method and the DCSR method.
Without losing generality, assume a homogeneous medium in a circular geometry (diameter: 27mm) and 8 sources and 8 detectors are equally distributed on the boundary. The medium is composed of HbR \((c_1=0.01\text{mM})\), HbO\(_2\) \((c_2=0.01\text{mM})\) and 100% H\(_2\)O \((c_3=1)\) and the scattering properties are \(a=1.0\) and \(b=1.4\). Jacobian matrices for the DCSR method (Equation 5-8) and the SDIR method (Equation 5-13) are calculated using the FEM solver on a 2D mesh with 425 nodes. These matrices are defined as \(\mathbf{J}_{SDIR}^a\), \(\mathbf{J}_{SDIR}^b\), \(\mathbf{J}_{SDIR}^{c_1}\), \(\mathbf{J}_{SDIR}^{c_2}\), \(\mathbf{J}_{SDIR}^{c_3}\) for the SDIR method and \(\mathbf{J}_{DCSR}^a\), \(\mathbf{J}_{DCSR}^b\), \(\mathbf{J}_{DCSR}^{c_1}\), \(\mathbf{J}_{DCSR}^{c_2}\), \(\mathbf{J}_{DCSR}^{c_3}\) for the DCSR method. 13 wavelengths are selected from 650nm to 910nm every 20nm.

The norm of each sub-Jacobian is the general sensitivity of such kind of measurement to a unit change of the chromophore or the scattering property. For both methods, the norms of the five sub-Jacobian matrices are summarized in Table 5-1.

<table>
<thead>
<tr>
<th></th>
<th>(|\mathbf{J}_{SDIR}^a|) (a=1.0)</th>
<th>(|\mathbf{J}_{SDIR}^b|) (b=1.4)</th>
<th>(|\mathbf{J}_{SDIR}^{c_1}|) (c_1=0.01\text{mM})</th>
<th>(|\mathbf{J}_{SDIR}^{c_2}|) (c_2=0.01\text{mM})</th>
<th>(|\mathbf{J}_{SDIR}^{c_3}|) (c_3=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDIR</td>
<td>0.182</td>
<td>0.112</td>
<td>0.221</td>
<td>0.0486</td>
<td>0.00333</td>
</tr>
<tr>
<td>DCSR</td>
<td>3.02</td>
<td>0.846</td>
<td>1.12</td>
<td>0.523</td>
<td>0.0103</td>
</tr>
<tr>
<td>RATIO (%)</td>
<td>6%</td>
<td>13%</td>
<td>20%</td>
<td>9%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Table 5-1, Norms of the sub-Jacobian matrices for SDIR and DCSR when \(a=1.0\), \(b=1.4\), \(c_1=0.01\text{mM}\), \(c_2=0.01\text{mM}\), \(c_3=1\). The ratio of SDIR’s to DCSR’s is calculated.

In general, the total sensitivity of the SDIR method is much lower than the DCSR method because the subtraction canceling the common signals. In the imaging mode, these common signals are not helpful in detecting the heterogeneities. The high frequency components in the Jacobian matrix are what indeed determine the spatial resolution and the cross-correlation among the sub-Jacobian matrices are the key factor related to the distinguishability of each parameter.
The spatial resolution can be investigated through relative rank of the Jacobian matrix. The relative rank is the number of singular values above a threshold which is proportional to the largest singular value. This method has been used in Chapter 4 when evaluating the optimal fiber optodes configuration. For a given threshold 0.1%, the relative rank for each sub-Jacobian matrix for both methods is summarized in Table 5-2.

<table>
<thead>
<tr>
<th></th>
<th>Rank( $\mathcal{J}_a$)</th>
<th>Rank( $\mathcal{J}_b$)</th>
<th>Rank( $\mathcal{J}_{c_1}$)</th>
<th>Rank( $\mathcal{J}_{c_2}$)</th>
<th>Rank( $\mathcal{J}_{c_3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDIR</td>
<td>133</td>
<td>135</td>
<td>77</td>
<td>86</td>
<td>70</td>
</tr>
<tr>
<td>DCSR</td>
<td>116</td>
<td>115</td>
<td>69</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>RATIO (%)</td>
<td>115%</td>
<td>117%</td>
<td>116%</td>
<td>125%</td>
<td>105%</td>
</tr>
</tbody>
</table>

Table 5-2, Relative ranks of the sub-Jacobian matrices for SDIR and DCSR when $a=1.0$, $b=1.4$, $c_1=0.010\text{mM}$, $c_2=0.010\text{mM}$, $c_3=1$. The threshold for the relative rank is 0.1% of the largest singular value. The ratio of SDIR’s to DCSR’s is calculated.

Clearly the SDIR method provides the Jacobian with higher relative rank which is equivalent to a lower condition number. Therefore better spatial resolution can be achieved by the SDIR method. The averaged improvement is about 25%. The greatest improvement is for the oxyhemoglobin ($c_2$) and this will be shown in the later in the simulations.

If any two sub-Jacobian matrices are correlated, i.e., $\mathcal{J}_i = k\mathcal{J}_j$, then it is impossible to distinguish parameter $i$ and parameter $j$. Therefore by examining the cross-correlation coefficients among these sub-matrices, the ability to separate those parameters can be predicted. Those correlation coefficients from both methods are listed in Table 5-3. Ideally the correlation coefficient matrix ($C$) should be an identity matrix, the residue norm of $C-I$ is an indicator of the distinguishability of parameters. For the SDIR method, $\|C-I\|=1.5$, whereas for the DCSR method, $\|C-I\|=2.3$. Again the SDIR method outperforms the DCSR method in terms of the distinguishability of chromophores and scattering properties. If looking into each channel, the
correlations between oxyhemoglobin ($c_2$) to other parameters are much smaller in the SDIR method than in the DCSR method and especially to water ($c_1$). In the DCSR method, usually it is hard to distinguish water and oxyhemoglobin since they have similar extinction spectrum. In the SDIR method, the derivative operation suppresses the common signal but amplifies the distinct absorption features therefore allows better separation of each chromophore.

Table 5-3. Correlation coefficients of sub-Jacobian matrices. (a) for SDIR (b) for DCSR when $a=1.0$, $b=1.4$, $c_1=0.01\text{mM}$, $c_2=0.01\text{mM}$, $c_3=1$. Closer the value to 0, more independent the two matrices are.

In conclusion, the SDIR method though has less total sensitivity than the DCSR method; it offers better spatial resolution and distinguishability for chromophores and scatter parameters. Thus the DCSR method may be better at estimating the global values but the SDIR method will perform better in the imaging problem.

5.3.3. Simulations

To demonstrate the benefits and robustness of the proposed SDIR algorithm, an example of the accuracy of the method is presented. Figure 5-2(a) shows the geometry used to measure a homogeneous diffusive blood phantom using the broadband NIR tomography system. An advantage of a broadband system is that all wavelengths are calibrated simultaneously. Figure 5-2(b) shows the measured attenuation spectra (670-910nm) at two symmetric detectors (D1, D2).
Ideally, the spectra at the symmetric detectors for a symmetric and homogenous phantom should overlap precisely, but in reality small differences are seen due to the different coupling coefficient as a function of the contact fibers and the phantom surface. Figure 5-3(c) shows the first order difference spectra with a 20nm wavelength separation. As seen, such differences are less wavelength-dependent which confirms the proposed idea of SDIR.

![Figure 5-2](image)

*Figure 5-2. (a) The experiment geometry and source-detector fiber configuration are shown. (b) The measured attenuation spectra at D1, D2 and their difference: approximately 10%. and (d) the first order finite difference spectra of D1 and D2 with a 20nm separation.*

Simulations are provided to verify the merits of the new method by comparing the results with those of DCSR. Considered a 27mm complex phantom with 5 distinct inclusions as shown in Figure 5-3(a) where each inclusion has contrast for each given parameter. The background and the inclusion properties are listed in Table 5-4. Only intensity measurement is considered. Measured data $\Phi^\omega_A$ at 13 wavelengths from 670nm to 910nm at every 20nm were simulated by solving the diffusion equation using finite element method on a linear triangular mesh with 425 nodes, having an equally distributed set of 8 sources and 8 detectors around the boundary. 0.5% Gaussian random noise is added to all synthesized data.
Figure 5-3. (a) The target phantom with 5 distinct inclusions (Diameter = 27mm). Each column corresponds to the particular parameter. (b) The target phantom for simulation where an irregular boundary was considered. The background properties are $a=1.0$, $b=1.5$, $c_1=0.01$mM, $c_2=0.01$mM, $c_3=0.5$.

<table>
<thead>
<tr>
<th>$a(10^{-3}b\text{mm}^{b-1})$</th>
<th>$b$</th>
<th>$c_1$(mM)</th>
<th>$c_2$(mM)</th>
<th>$c_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>background</td>
<td>1.0</td>
<td>1.4</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Inclusions</td>
<td>1.5</td>
<td>1.0</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 5-4. Properties for background medium and 5 inclusions.

Figure 5-4(a) shows the reconstruction of $\Phi_A^{\circ}$ using the SDIR method on the same mesh as the forward therefore simulating a perfect model and data match. Figure 5-4(b) is the set of reconstructed images where 5% Gaussian coupling coefficient error was added to $\Phi_A^{\circ}$. Figure 5-4(c) shows the reconstruction where the boundary reflection coefficient (relative reflective index $n_{rel} = 1.2$ (Keijzer et al., 1988)) was different to the one ($n_{rel} = 1.4$) used to generate $\Phi_A^{\circ}$. Figure 5-4(d) shows the reconstruction where the geometric errors in modeling are considered, by simulating the forward data on the irregular geometry shown in Figure 5-3(b) (i.e. where fibers create a displacement (1mm) on the tissue surface) but the reconstruction was still performed on the circular mesh. The reconstructions of those datasets using the DCSR method are shown in
Figure 5-5(a-d). Normalized RMS error of each reconstructed image is shown in Table 5-5. This normalized RMS error is the averaged error of inclusion and background equally weighted.

Figure 5-4. Reconstructed images of Simulations (A-D) for the SDIR method, with (A) images with 0.5% Gaussian random noise but having no data errors, (B) with 5% randomly distributed coupling errors, (C) with boundary reflection coefficient modeling error, and (D) with reconstructing data taken from a distorted boundary shape.
Figure 5-5. Reconstructed images of Simulations (A-D) for the DCSR method, with (A) images with 0.5% Gaussian random noise but having no data errors, (B) with 5% randomly distributed coupling errors, (C) with boundary reflection coefficient modeling error, and (D) with reconstructing data taken from a distorted boundary shape.

<table>
<thead>
<tr>
<th>(%)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c1</td>
<td>c2</td>
<td>c3</td>
<td>a</td>
<td>b</td>
<td>c1</td>
</tr>
<tr>
<td>SimA</td>
<td>11.2</td>
<td>11.1</td>
<td>15.2</td>
<td>17.3</td>
<td>18.4</td>
<td>8.2</td>
<td>10.3</td>
<td>13.0</td>
</tr>
<tr>
<td>SimB</td>
<td>11.2</td>
<td>11.1</td>
<td>15.2</td>
<td>17.3</td>
<td>18.4</td>
<td>31.5</td>
<td>16.0</td>
<td>16.1</td>
</tr>
<tr>
<td>SimC</td>
<td>17.2</td>
<td>7.1</td>
<td>15.1</td>
<td>14.3</td>
<td>16.5</td>
<td>23.5</td>
<td>18.3</td>
<td>21.5</td>
</tr>
<tr>
<td>SimD</td>
<td>12.6</td>
<td>7.4</td>
<td>14.8</td>
<td>17.0</td>
<td>16.6</td>
<td>23.5</td>
<td>15.6</td>
<td>90.9</td>
</tr>
</tbody>
</table>

Table 5-5. Comparison of normalized RMS error between each target image and reconstructed image for SDIR and DCSR.

As seen in this figure the original DCSR algorithm is sensitive to those errors which can introduce an error term in the absolute intensity measurement, and reconstructed artifacts are significant. Interestingly the sources of error seem to have distinct artifact pattern within each parameter channel. As expected, the SDIR algorithm shows a high tolerance to these errors and
provides consistently more accurate reconstructions. In general, SDIR provides image qualities similar to the DCSR algorithm where the error-free situation was used, shown in Figure 3(A). In some channels, such as oxyhemoglobin, SDIR appears superior in terms of the reduction of parameter cross talk and quantitative accuracy. This is a direct result of the increased spatial resolution and distinguishability for oxyhemoglobin within the differential spectrum.

5.3.4. Phantom experiments

Figure 5-6, A schematic diagram of the liquid phantom experiment setup.

Both DCSR and SDIR methods are examined in a liquid phantom experiment. The setup of this experiment can be illustrated by the diagram in Figure 5-6. 16 source fibers and detector fibers were equally placed around a cylinder container made from Acetal. The inside volume was 27mm in diameter and 54mm in height and filled with a mixed blood and Intralipid solution. A stir bar was placed in the bottom to maintain the solution homogeneous. A 6mm clear and thin straw penetrated through the container parallel to the axis of cylinder and it was placed
approximately at the half radius position. One end of the straw was connected to a reservoir and
the other end was connected to the output of a pump. The input of this pump was connected with
the bottom of the reservoir. The pump circulated the solution in the loop composed of the
reservoir and the straw. The solution within this loop was varied to provide contrast to the
background solution in the container. The container and the reservoir both were sealed and
oxygen could be perfused if needed. To fully oxygenate the blood, the oxygen supply was turned
on. To deoxygenate the blood in the external circulation, a small amount of yeasts and glucose
were added to the container while keeping the oxygen supply off.

<table>
<thead>
<tr>
<th></th>
<th>Background</th>
<th>Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExpA</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>Void</td>
</tr>
<tr>
<td>ExpB</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>30μM HbO2, 1% Intralipid</td>
</tr>
<tr>
<td>ExpC</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>30μM HbR, 1% Intralipid</td>
</tr>
<tr>
<td>ExpD</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>60μM HbR, 1% Intralipid</td>
</tr>
<tr>
<td>ExpE</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>90μM HbR, 1% Intralipid</td>
</tr>
<tr>
<td>ExpF</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>90μM HbO2, 1% Intralipid</td>
</tr>
<tr>
<td>ExpG</td>
<td>30μM HbO2, 1% Intralipid</td>
<td>90μM HbR, 2% Intralipid</td>
</tr>
</tbody>
</table>

Table 5-6. The makeup of the solution in the background and inclusion when dataset (ExpA to
ExpF) was acquired.

The background solution was 30μM HbO2 and 1% Intralipid and maintained during the
entire experiment. A reference dataset ExpR was acquired in the absence of the straw in the
container. Then the straw was added and the solution in the straw was varied. 7 imaging datasets
were acquired at different time points for different makeup of the solution in the external
circulation (referred as the inclusion). (1) In the beginning, the external circulation was empty
thus the inclusion was a void. Imaging dataset ExpA was acquired at this time point. (2) Then it
was filled with the solution same as the background which had 30µM HbO₂ and 1% Intralipid. Dataset ExpB was acquired. (3) Yeasts and glucose were added to deoxygenate the blood. Dataset ExpC wasn’t acquired until the blood was believed to be fully deoxygenated. (4) The total hemoglobin concentration in the reservoir was increased to 60µM but still fully deoxygenated. Dataset ExpD was acquired. (5) The total hemoglobin concentration in the reservoir was increased to 90µM but still fully deoxygenated. Dataset ExpE was acquired. (6) The reservoir after having been fully cleaned was filled with 90µM HbO₂ and 1% Intralipid. Dataset ExpF was acquired. (7) Yeasts and glucose were added to deoxygenate the blood. Then the Intralipid concentration in the reservoir was increased to 2%. Dataset ExpG was acquired. The makeup of the solution in the background and inclusion for dataset ExpA to ExpG was summarized in Table 5-6.

For image reconstruction, the hybrid 2D/3D method was used in which the forward model was solved on a 3D mesh composed of 15 layers of 2D mesh with 425 nodes and 776 elements and the inverse problem was solved on the corresponding 2D mesh. 13 wavelengths from 670nm to 910nm at every 20nm were used. To minimize the model misfit error and the Intralipid batch difference, the acquired dataset (ExpA to ExpG) were calibrated by the reference dataset (ExpR) and the simulated dataset (CalR) for the reference which was the output of the forward model for a given makeup of 30µM HbO₂ and 1% Intralipid. The calibrated dataset for ExpA-ExpG was calculated using CalX = ExpX – ExpR + CalR. All the reconstructions started with the same initial guess of 30µM HbO₂, 1% Intralipid and 100% water, i.e., a(SA)=0.775, b(SP)=1.113, c₁(HbR)=0.0mM, c₂(HbO₂)=0.03mM, c₃(H₂O)=1. The a and b values were calculated from the reference paper (van Staveren et al, 1991). Reconstructed images for the SDIR method and the DCSR method are shown in Figure 5-7 and Figure 5-8 respectively.
Figure 5-7. Reconstructed images (A-G) of ExpA-G for the SDIR method. Background solution was SA=0.775, SP=1.113, HbR=0.0mM, HbO₂=0.03mM, H₂O =1. (A)ExpA: The inclusion was void. (B)ExpB: The inclusion was same as the background. (C) ExpC: The inclusion was deoxygenated to HbR=0.03mM. (D) ExpD: The inclusion was increased to HbR=0.06mM. (E)ExpE: The inclusion was increased to HbR=0.09mM. (F) ExpF: The inclusion was refilled to HbO₂=0.09mM. (G) ExpG: The inclusion was deoxygenated to HbR=0.09mM and the Intralipid was increased to 2%, i.e., SA=1.55.
Figure 5-8. Reconstructed images (A-G) of ExpA-G for the DCSR method. Background solution was $SA = 0.775$, $SP = 1.113$, $HbR = 0.0\text{mM}$, $HbO_2 = 0.03\text{mM}$, $H_2O = 1$. (A) ExpA: The inclusion was void. (B) ExpB: The inclusion was same as the background. (C) ExpC: The inclusion was deoxygenated to $HbR = 0.03\text{mM}$. (D) ExpD: The inclusion was increased to $HbR = 0.06\text{mM}$. (E) ExpE: The inclusion was increased to $HbR = 0.09\text{mM}$. (F) ExpF: The inclusion was refilled to $HbO_2 = 0.09\text{mM}$. (G) ExpG: The inclusion was deoxygenated to $HbR = 0.09\text{mM}$ and the Intralipid was increased to 2%, i.e., $SA = 1.55$. 
Figure 5-7(A-G) show the reconstruction for datasets of ExpA-G using the SDIR method. Most reconstructions took 12 iterations and each iteration took approximately 10 minutes. (A) ExpA: Clearly if the void space exists, the diffuse equation is not valid for this region. However, the reconstructed images meaningfully show the lower scatter amplitude (SA) and HbO$_2$ in the inclusion. This is because the spectral method is focused on the spectral feature rather than the intensity at a single wavelength, it is less sensitive the modeling error in the forward problem. (B) ExpB: Ideally the reconstructed images should be homogeneous since the background and inclusion were the same. However, due to the reflection and the light channeling of the straw, or the inaccuracy in the external circulation, the observed SA and HbO$_2$ are slightly lower than the background. (C-E) ExpC-E: As expected, HbO$_2$ was dropped to nearly zero and stayed at the similar level for all three experiments. The residue might be real or due to the impact of the straw. In the HbR image, the reconstructed peak value was lower than the expected concentration but the peak value increased as the hemoglobin concentration increased. The underestimation is probably due to the straw, or the smoothing effect of the reconstruction, or the improper initial guess. There is no obvious crosstalk in the SA or SP image, but a small decrease is observed in the water image. (F) ExpF: As expected, only in the HbO$_2$ image the contrast to the background was observed and it was underestimated. (G) ExpG: As expected, the HbO$_2$ concentration was almost completely converted to the HbR concentration and the SA was increased. No significant artifacts especially artifacts near the boundary were observed in the reconstructed images.

Figure 5-8(A-G) show the reconstructions for datasets of ExpA-G using the DCSR method. Most reconstructions took 14 iterations except the last two with 9 iterations and each iteration took approximately 10 minutes. In general, the reconstructed images were similar to those of the SDIR method except the contrast for the HbR was lower and a crosstalk from HbR
to SA image was observed. However, some significant artifacts near 6 and 12 clock were observed throughout the experiment especially in the late stage (ExpF and ExpG). This was caused by the drift or the displacement of the fibers which was inevitable during a long experiment. These errors may have great impact to the DCSR method but have little affection to the SDIR method.

Although the straw did bring some difficulty for the interpretation, most results still are reasonable and well correlated to the expected hypothesis. Consistent with the simulation results, the SDIR method outperforms the DCSR method with much less boundary artifacts.

5.4. Discussion

In conclusion, a novel approach to solve the DOT problem is described. More importantly the SDIR method can be either a stand-alone algorithm or combined with the DCSR method. It provides another dimension in terms of how to use the spectral information. This method may help to overcome several major inherent measurement errors such as coupling coefficient variation, boundary reflection mismatch and geometric mismodeling. The rationale is to use the difference or derivative spectrum to cancel the common error term seen at each wavelength but also to maintain the scattering and chromophores spectral and spatial contrast. This method can be very useful to small animal and brain studies where fiber/tissue contact is more problematic but high spectral contrast is still available.

However, currently either the SDIR method or the DCSR method hasn’t been successfully applied to in vivo studies because of an unresolved critical issue. The spectral reconstruction method requires an initial guess close to the true value. In the frequency domain, global absorption and reduced scattering coefficients estimated at separate wavelengths, can be
used together to provide a reasonable estimate of each parameter, i.e., SA, SP, HbR, HbO₂, H₂O. However for the broadband system only with CW data, such technique is not applicable. Neither the SDIR method nor the DCSR method has shown great success in estimating 5 parameters simultaneously when model misfit error and the noise present. Also the current calibration is designed for the indirect reconstruction method and might be not suitable for the direct methods. All these issues need to be explored in the future studies.
Chapter 6. Animal studies: Graded hypoxia

The *in vivo* performance of this system is examined through a series of animal experiments during graded hypoxia. The hemoglobin dynamics, varied in response to changes of inhaled oxygen fraction, were imaged simultaneously by MRI and NIR. The functional information presented by NIR is validated by the BOLD signal measured by MRI.

6.1. Introduction

Functional MRI (fMRI) has been widely used for mapping the neuronal activity of the brain associated with various physiological stimuli and for measuring the hemodynamics and angiogenesis related to the pathophysiology. The principle of this technique is that paramagnetic deoxyhemoglobin in blood produces a magnetic susceptibility difference between the blood vessels and surrounding diamagnetic tissue. Such susceptibility affects the transverse relaxation time, thus the observed MR signal intensity in $T_2^*$ or $T_2$ weighted images is altered by the deoxyhemoglobin content. This phenomenon, called the blood oxygenation level dependent (BOLD) effect, actually involves complex mechanisms, since the deoxyhemoglobin content depends first on the mutual relationship between oxygen demand (metabolism) and supply (oxygen delivery) and second on the various hemodynamic response such as cerebral blood volume (CBV) and cerebral blood flow (CBF) changes. Furthermore, the BOLD effect depends on the strength of the main magnetic field and the size of vessels (Ogawa et al., 1993) although generally higher contrast to noise ratio can be achieved at higher magnetic field (Gati et al., 1997) and $R_2^*$ is a more sensitive measurement compared to $R_2$ (Hoppel and Minkler, 1993). Although BOLD MRI provides a high spatial and temporal sensitivity to the deoxyhemoglobin content, the interpretation of the BOLD signal to the absolute change of deoxyhemoglobin or the oxygenation state in tissue still is difficult. In the last decade, near infrared spectroscopy (NIRS)
has been successfully used to validate and explain the BOLD effect since it can provide more
direct information about the absolute content or the absolute change of oxyhemoglobin (HbO₂),
deoxyhemoglobin (HbR) and total hemoglobin (HbT). Under specific conditions, there is a
strong correlation between deoxyhemoglobin content measured by NIRS and changes in
relaxation rates measured by MRI (Chen et al., 2003; Jezzard et al., 1994; Kida et al., 1996;
Punwani et al., 1998). In these studies, such correlation was achieved during acute hypoxia
because the blood volume and the microvasculature were not expected to change significantly,
and the correlation became worse during severe hypoxia because of the decrease of the blood
volume and the vessel radius. The temporal comparison of NIRS measurement and BOLD effect
also has been investigated indicating strong correlations between fMRI changes and optical
measures during functional brain activation (Gulsen et al., 2002; Siegel et al., 2003; Strangman
et al., 2002; Toronov et al., 2001). Most of these studies have not incorporated the advance of
NIR imaging technique since the NIRS measurement was typically acquired between a single-
detector pair, thus the averaged hemodynamics within the NIR sampled volume was compared to
mean value of the MR measurement in a slice or an adjacent volume.

In order to validate the NIR image reconstructed by our system, the BOLD signal from
the MR system is used as a gold standard. The reconstructed changes of deoxyhemoglobin
content are compared to changes of the apparent transverse relaxation rates (R₂*) during graded
acute hypoxia in rat models. It is important to reproduce the well-known linear correlation under
specific conditions. Furthermore, with our dual-modality tomography imaging system, it is
possible to investigate the correlation of BOLD images and NIR images spatially. The
reconstructed image is not a simple topology of the NIRS measurement but an elegant model-
based tomography enhanced by the structural information provided by MRI.
6.2. Method

6.2.1. Experiment procedure

Male Sprague-Dawley rats (285-320 grams) were intubated, connected to a mechanical ventilator and fixed in the NIR-MRI coil with a cylindrical holder. The anaesthesia was then maintained by ventilation with a mixture of isoflurane (1-1.5%), N₂ and O₂. The inspired oxygen fraction (FiO₂) was varied using a digital gas blender which was controlled by the computer. Ventilator settings were altered to maintain arterial CO₂ within the normal range as determined by a capnogram (Capnomac Ultima, Datex-Ohmeda, Finland). The animal was placed supine in the magnet and the rectal temperature was maintained at 37~38°C during the course of the experiment using a heated fan. Continuous monitoring of heart rate and arterial oxygen saturation (SaO₂) was performed using a pulse oximeter placed on the foot. The animal was allowed a stabilization within the magnet. The baseline was collected at FiO₂ = 0.30, then FiO₂ was varied in a sequence of 0.30, 0.20, 0.15, 0.12, 0.10, 0.30, 0.0. The animal was sacrificed in the end. The imaging data acquisition was started 5 minutes after changing the inspired gas to create stable respiratory conditions. Three gradient echo images and one near infrared image were obtained for each FiO₂ condition in parallel. The optical fiber array was positioned between the bregma and the interaural line so that the light mostly traveled through the brain. Both ends of the magnet bore were covered with black plastics and the room light was turned off to minimize the interference from external light.

6.2.2. MRI acquisition
The 6cm Birdcage coil (Chapter 2) was used for both RF transmission and signal detection. Prior to the commencement of imaging, the magnet was shimmed to obtain a half-maximum linewidth of the water resonance approximately 100Hz.

In the beginning, a stack of T2-weighted images were obtained (TR = 2000ms, TE = 40ms, FOV = 3.5cm x 3.5cm, matrix size = 256 x 128, thickness = 1mm, voxel dimensions = 137µm x 137µm x 1000µm and 2 averages) in a 7T horizontal magnet (Magnex, UK) using a Unity INOVA console (Varian, CA) to guide the optical fiber placement and provide a priori structural information for diffuse optical tomography image reconstruction. The middle slice was selected at the optical fiber array. 10 slices were collected on each side of this slice so that the total number of slices was 21. Multi-slice spin echo sequence was used to acquire 21 slices in approximately 9 minutes.

To measure the BOLD effect at each oxygen fraction, three T2*-weighted gradient echo images were acquired sequentially (TR = 700ms, TE = 15ms, Flip angle = 30°, FOV = 3.5cm x 3.5cm, matrix size = 256 x 64, slice thickness = 1mm and 4 averages) and each took approximately 3mins. Only a single slice was collected and it was offset from the fiber array plane by 2~3mm to avoid the susceptibility effect of the fiber optodes. The signal intensities were averaged from three images in each condition. The differential apparent transverse relaxation rate between two conditions (ΔR₂*), can be described as:

\[
\Delta R_2^* = \frac{\ln(S_0 / S)}{TE}
\]

(6-1)

Where \( S_0 \) and \( S \) are signal intensities obtained at the baseline and the current gas condition, \( TE \) is the echo time. Equation 6-1 is based on the assumption that the differential in-flow effects are suppressed. The signal intensity images were de-noised by an adaptive filter with 7 x 7 hood and
only pixels with SNR >= 10 were used to obtain $\Delta R_2^*$ map with good quality. The average value of the ROI pixels was used to obtain a global $\Delta R_2^*$ to compare with the global $\Delta HbR$ measured by NIR.

An alternate method to obtain $\Delta R_2^*$ is to measure the $R_2^*$ directly using the multi-echo gradient echo sequence. The signal decay curve with respect to the echo time can be fitted to an exponential equation, $S = I_0 \exp(-TE \cdot R_2^*)$, to calculate the $R_2^*$. Then the $\Delta R_2^*$ can be calculated from $R_2^*$ at each condition. Both methods provided similar results when the intensity image appeared homogeneous and the acquisition time was not too long (<3 hours). In the early trials, it was very difficult to get a homogeneous gradient echo image when the imaging plane was chosen exactly at the plane containing the fiber optodes, whereas good images could be obtained if the MR imaging plane was shifted few millimeters. Although the NIR image plane was not aligned exactly to the BOLD MR image, the volume sampled by NIR still could cover the MR slice because of the diffuse nature of the NIR light.

6.2.3. Segmentation and Registration of MRI images

A package of Matlab routines has been developed to transfer MRI structural information into the NIR FEM-based image reconstruction domain. The interface of this package is shown in Figure 6-1(a). It loads the original FDF-format files from the SUN workstation on the MRI console to the image matrices in the Matlab as well as the acquisition parameters. For a T2-weighted anatomical image with arbitrary boundary, boundaries of skin, brain and bone can be sketched semi-automatically with minimal human interference so that three regions (muscle, skull and brain) can be segmented. 2D and 3D FEM meshes can be generated with these boundaries using ANSYS software (ANSYS, Inc. Solutions) or SPMESH (NML group at
An example of segmented tissue is shown in Figure 6-2(a). A 3D FEM mesh is shown in Figure 6-2(b).

**Figure 6-1.** (a) The interface of the software to segment the issue boundaries from the MRI images. (b) The grayscale histogram of the MR image in the (a).

**Figure 6-2.** (a) Iso-surface plots of the segmented tissue showing brain, skull and skin boundaries from left to right. (b) A 3D FEM mesh with dented tissue surface due to the impression of the optical fibers.

The principle of the automatic segmentation is based on the intensity contrast of different tissue at the grayscale. The histogram of the MR image in Figure 6-1(a) is shown in Figure 6-1(b). Three peaks are obvious and correspond to the background noise, muscles and brain respectively from left to right. Otsu thresholding technique (N. Otsu, 1979) was used to first separate the tissue from the background noise and then extract the brain from the bulk tissue. The
bone also can be thresholded from the remaining bulk tissue. With auto-segmentation, dozens of images can be processed in minutes and the manual modification also is available for improved accuracy.

**Figure 6-3.** (a) Nine MRI slices from $z = -4\text{mm}$ to $4\text{mm}$. (b) A 2D mesh with regional information and corrected source and detector positions.

With the latest design of the fiber optics and animal interface (Section 2.1.4.2), the tissue boundaries were confined to be a cylindrical shape with fixed size. The FEM mesh was simplified to a circular mesh in 2D case and a cylindrical mesh in 3D case. The burdensome meshing procedure was circumvented. Nine MRI slices of one subject from $z = -4\text{mm}$ to $4\text{mm}$ were shown in Figure 6-3(a). Two liquid phantoms containing copper sulfate solution ($\text{CuSO}_4$) (bright circles in the upper corner) were placed between source No. 2 and detector No. 2 (above the animal) and source No. 5 and detector No. 5 (below the bottom). In Figure 6-3(a), because the bottom one was out of field of view, it wrapped to the top of the image. The asymmetry was intended and the included angle between two phantoms was 135 degree. One phantom extended to the $Z+$ direction, the other extended to the $Z-$ direction, and they overlapped at the fiber plane. The optical fibers should be in the middle of the slices where both phantoms could be seen. With
this special design of phantoms, the animal could be adjusted to the desired location guided by the MRI images. Figure 6-3(a) confirms that the tissue boundary is very close to a cylindrical surface and the brain is similar to a cylindrical rod in the cylindrical background tissue within slices extending 10mm around the fiber plane. The geometrical center of the tissue in the middle slice was calculated and the angular coordinates of the two phantoms with respect to this center were calculated. With this center and at least one angular coordinate, the FEM mesh could be co-registered with the MRI slices and the arrangement of the source and detector positions could be determined to provide a FEM mesh shown in Figure 6-3(b).

6.2.4. NIR data acquisition and image reconstruction

Due to the high blood content in the rat head and the heterogeneity, the optical signal collected on the rat peripheral was very weak especially in the hypoxia condition. To compensate the low light fluence and achieve sufficient SNR, we had to integrate the CCD at least 4 seconds for each source and average 10~20 measurements to get a reasonable SNR especially for the differential pathlength measurement, which was sensitive to the random noise in the spectrum. The total acquisition time was approximately 5-10mins. Therefore, this system is not suitable for monitoring functional activation which requires high temporal resolution but rather for measuring a relative stable condition. The long acquisition time introduced physiological noise in the measured data. The animal movement due to the breathing and the physiological variation in tissue made measured data noisier than the measurement on the phantom which, in contrast, was stable and immobile. Although the animal was shaved to minimize the problem in light coupling between fiber and tissue, a uniform coupling efficiency for each fiber was not possible.
Fibers were fixed in the holder, if the animal size was not sufficiently large to fill all the space in the holder, poor coupling could occur at some fibers.

In current animal studies, the high level of noise in the measured data has still not been successfully modeled and suppressed, thus it was difficult to achieve a similar imaging performance to that which could be achieved in the simulations or phantom studies in terms of the absolute reconstruction of the optical property distributions. To improve the interpretation of physiological changes and limit the artifacts, a difference imaging technique was used in this graded hypoxia study. A baseline image of the initial value of latter reconstructions was created with two regions (brain and background), and the initial optical properties of each region were estimated from the mean value of the same region in the first reconstructed absolute NIR image. Then the data difference with respect to the measurement at the baseline was calculated and added to the data simulated from the baseline image and then used to recover the functional changes. This calibration procedure can be described by:

$$\Phi^C(x_i) = \Phi^C(x_0) + (\Phi^M(x_i) - \Phi^M(x_0))$$  \hspace{1cm} (6-2)

Where \(x_0\), \(x_i\) are the true images of optical property distributions at the baseline and the \(i\)th state, \(\Phi^M(x_0)\), \(\Phi^M(x_i)\) are the measured data, \(x_0^\phi\) is the estimated baseline image from the reconstruction of \(\Phi^M(x_0)\), \(\Phi^C(x_0^\phi)\) is the calibrated data for this estimated baseline \(x_0^\phi\), \(\Phi^C(x_i^\phi)\) is the calibrated data to reconstruct the image \(x_i^\phi\) at the \(i\)th state. For a linear system, if the model is accurate, the change from the baseline should be authentically retained no matter how differently the estimated baseline from the true baseline, that is, \(\Delta x = x_i^\phi - x_0^\phi = x_i - x_0 = \Delta x\). However, for the nonlinear problem, this further requires the estimated baseline \(x_0^\phi\) to be sufficiently close to the true \(x_0\), otherwise the Jacobian matrix
provided by the wrong baseline would not match the real perturbation in the data. Since the estimate of the baseline was combined with the MRI structural information and the statistics of the truly reconstructed image matched the measured data at the baseline, it was believed to be reasonably close to the true image. Therefore, the mismatch between $\Delta x'$ and $\Delta x$ should be small. This procedure provided a data set that matched the model prediction well, and yet retained all heterogeneous aspects of the rat imaging. This difference imaging technique is useful in *in-vivo* studies where many factors may degrade the accuracy of modeling.

To more accurately model the light transport in tissue, a hybrid 2D/3D model (referred in Chapter 4) was used. A circular 2D mesh with 1785 nodes was stacked in 7 layers to generate a 7-layer cylindrical 3D mesh with the height of 15mm. The forward calculation was performed on the 3D mesh but the inverse problem was solved on the 2D mesh assuming the uniform optical property distribution along the axial direction. The Laplacian L-matrix (Section 4.3.2) was used in the reconstruction but the spatially variant regularization (Section 4.3.3.4) was not applied because the physiological change might occur in the background as well as in the brain.

The reconstructed absorption images at two wavelengths were converted to the concentration maps of hemoglobin (HbO$_2$) and deoxyhemoglobin (HbR) assuming the water content was 80% and uniformly distributed. The map of the total hemoglobin concentration (HbT) and the tissue saturation (StO$_2$) were calculated from the HbO$_2$ and HbR maps. However, because the absolute concentration was sensitive to the error in the baseline image, maps of the concentration changes were believed to be more accurate. In particular, the map of the HbR changes was compared with the $\Delta R_2'$ map from the BOLD MRI.

**6.2.5. Results**
Gradient echo images at different fraction of inspired oxygen (FiO₂) for one animal are shown in Figure 6-4. At each FiO₂ condition, the image was averaged from three sequentially acquired images. \( \Delta R^*_2 \) maps shown in Figure 6-5 were calculated from these images using Equation 6-1 and only the pixels with sufficient signals (SNR>=10) were used.

![Gradient echo images at different fraction of inspired oxygen](image1)

*Figure 6-4. Gradient echo images of a coronal brain slice at different fraction of inspired oxygen (FiO₂) showing the decreasing of intensity as the FiO₂ decreases.*

![\( \Delta R^*_2 \) maps calculated from the intensity images in Figure 6-4. The values in the grayscale are in units of \( s^{-1} \). It is clear that generally the \( \Delta R^*_2 \) increases as the FiO₂ decreases. Units: \( s^{-1} \).](image2)

*Figure 6-5. \( \Delta R^*_2 \) maps calculated from the intensity images in Figure 6-4. The values in the grayscale are in units of \( s^{-1} \). It is clear that generally the \( \Delta R^*_2 \) increases as the FiO₂ decreases. Units: \( s^{-1} \).*

The reconstructed absorption and reduced scattering images at 728nm and 830nm for the baseline condition with the animal breathing normal 30% oxygen from the original measured dataset are shown in Figure 6-6 (a) and (b). The estimated baseline images shown in Figure 6-7 were calculated from these images with the structural information provided by MR images and they were used to generate a simulated dataset to calibrate the measured dataset at each condition using the difference technique.
Figure 6-6. Reconstructed images of absorption and reduced scattering coefficients at the baseline state, with the animal breathing normal 30% oxygen. (a) $\mu_a$ (left) and $\mu'_{s}$ (right) images at 728nm (b) $\mu_a$ (left) and $\mu'_{s}$ (right) images at 830nm. Units: mm$^{-1}$.

Figure 6-7. Estimated baseline images from the results in Figure 6-6 and they were used to generate the simulated dataset. (a) $\mu_a$ (left) and $\mu'_{s}$ (right) images at 728nm (b) $\mu_a$ (left) and $\mu'_{s}$ (right) images at 830nm. Units: mm$^{-1}$.

Absorption and reduced scattering images for each FiO$_2$ condition shown in Figure 6-8 were reconstructed with the calibrated difference data. With the absorption images at two wavelengths and assuming the absorption was mostly due to hemoglobin and water, the maps of oxyhemoglobin (HbO$_2$), deoxyhemoglobin (HbR), total hemoglobin (HbT) concentrations and tissue oxygen saturation ($S_{tO2}$) shown in Figure 6-9 were calculated. Therefore the changes of
HbO₂, HbR and HbT concentrations for each gas condition shown in Figure 6-10 were calculated with respect to the baseline condition.

Figure 6-8. Reconstructed \( \mu_a \) and \( \mu'_s \) images from the baseline with the difference data for each FiO₂ condition. From the left to the right, each column corresponds to FiO₂ of 30%, 20%, 15%, 12%, 30% and 0% respectively. The 1st and 2nd rows are the \( \mu_a \) images at 728nm and 830nm respectively. The 3rd and 4th rows are the \( \mu'_s \) images at 728nm and 830nm respectively. Units: \( mm^{-1} \).
Figure 6-9. Calculated maps of HbO$_2$, HbR, HbT concentrations in units of mM (millimoles per liter) and tissue oxygen saturation (StO$_2$) for each FiO$_2$ condition.

Figure 6-10. Calculated maps of the changes of HbO$_2$ (in the 1$^{st}$ row), HbR (in the 2$^{nd}$ row), HbT (in the 3$^{rd}$ row) concentrations from the baseline condition in units of mM.

The mean value of the $\Delta HbR$ and the mean value of the $\Delta R_2^*$ in the whole brain were calculated from the maps of $\Delta HbR$ (Figure 6-10) and $\Delta R_2^*$ and compared in Figure 6-12 for 6 animals. The
standard deviation in terms of the spatial variation was calculated and plotted as well for both NIR DOT and BOLD MRI. In Figure 6-12, only data at conditions of FiO₂ =0.20, 0.15, 0.12, 0.10 were used to avoid the severe hypoxia in which the decrease of the total blood volume could make $\Delta R_2^*$ not well correlated to $\Delta HbR$. A linear fit was performed to estimate the coefficients in Equation $\Delta HbR = a\Delta R_2^* + b$ and calculated the R-square error. The data for 6 animals were synthesized in Figure 6-13. As $\Delta HbR$ increased, a concurrent increase in $\Delta R_2^*$ was observed. Linear regression gave a strong correlation ($r^2 = 0.95$) between $\Delta HbR$ and $\Delta R_2^*$. Linear regressions for the six individual animals indicated stronger correlation ($0.962 < r^2 < 0.997$). The correlation between $\Delta R_2^*$ and $\Delta HbR$ generalized for all the animals was found to be $\Delta HbR = a\Delta R_2^* + b$, where $a=3.02$ and $b = 1.04$. With this relation, the $\Delta R_2^*$ map in the Figure 6-5 was converted to the $\Delta HbR$ and compared with the $\Delta HbR$ map provided by NIR. They are shown in Figure 6-11 indicating a similar pattern and trend.

![Figure 6-11](image)

**Figure 6-11.** (a) Estimated maps of $\Delta HbR$ from $\Delta R_2^*$ maps at each FiO₂ condition using the equation $\Delta HbR = a\Delta R_2^* + b$, where the coefficients, $a$ and $b$, were determined for the linear fit shown in Figure 6-13. (b) Calculated maps of $\Delta HbR$ from NIR DOT at each FiO₂ condition. Units: mM.
Figure 6-12. Graphs showing the correlation between the global changes of deoxyhemoglobin concentration $\Delta HbR$, as measured by NIR imaging, and the global changes of the apparent transverse relaxation rate, $\Delta R_2^*$, as measured by BOLD MRI in the rat brain. 6 animals were studied and the relationship for each animal is shown in (a)-(f) respectively. The error bar indicates the spatial variation within the brain. A linear fit is calculated for each graph and the fitted equation with the R-square error is plotted in the graph.
Figure 6-13. A graph showing the correlation between the global changes of deoxyhemoglobin concentration $\Delta HbR$, as measured by NIR imaging, and the global changes of the apparent transverse relaxation rate, $\Delta R_2^*$, as measured by BOLD MRI in the rat brain for 6 animals. A linear fit is calculated for each graph and the fitted equation with the R-square error is plotted in the graph.

6.3. Discussion

In the rat image reconstructed from data taken while it was in the normal resting state, a region with higher absorption (Figure 6-6)) and higher reduced scattering is observed at the position of brain, agreeing with what was expected. The shape is a little bit different to the true shape of the brain indicated by the MRI, which may be caused by the distortion from artifacts in the image but also could be real since the NIR provides different contrast to the MRI. These artifacts could result from the measurement noise, the heterogeneity of rat head, the positioning error or the calibration issue on the irregular mesh. These factors are limited by calibrating the latter reconstruction with the baseline image (Figure 6-7) created from this first image. Improved image quality is obtained in those images shown in Figure 6-8. Reconstructed optical images clearly showed changes in saturation indicating that functional changes in the rat can be
monitored. The trends of HbR and HbO₂ (Figure 6-9, 10) followed predictable trends during hypoxia and HbT (related to CBV) was observed to have a small increase during the mild hypoxia but have a small decrease in the more severe hypoxia and a significant decrease after death (>30%). A strong correlation \( r^2 = 0.95 \) (Figure 6-13) between deoxyhemoglobin and \( R_2^* \) was observed but the ratio \( \Delta HbR / \Delta R_2^* \) \( a = 3.02 \) was not same as the ratio we had found, \( a = 2.08 \) (Xu et al., 2005). This is probably because the previous case was using the 2D reconstruction and a different coil. This ratio was higher than what has been observed by others, \( a = 2.12 \), on the piglet (Punwani et al., 1998) using NIRS without image reconstruction. With imaging, the expected value should be higher, since partial volume errors would be reduced. This study is really the first step indicating that both NIR image reconstruction and MRI \( R_2^* \) could be quantifiable, and directly compared to one another in a manner which would allow spatially-resolved comparisons.

Further study is ongoing on how to use the MRI internal structures for constraints and thereby enhance the spatial resolution and potential accuracy of the NIR-derived hemoglobin and oxygen saturation. Since the MRI T2-weighted structures that can be segmented apart are the same ones that would be expected to have different optical properties, it is logical to assume that these could be used as interior a priori information. Further analysis of how to optimize the inclusion of a priori information are definitely needed, and will be completed in a systematic manner with a larger group of animals, however the working principle presented here represents the culmination of several studies in this approach (Brooksby et al., 2004; Brooksby et al., 2003; Pogue and Paulsen, 1998).
In Figure 6-12 and 6-13, it is clear that the hemodynamics measured by our system using the NIR DOT technique well reproduced the correlation between changes of deoxyhemoglobin and apparent transverse relaxation rate during graded mild hypoxia.

From Figure 6-11, in general, the $\Delta HbR$ map provided by NIR DOT almost can be co-registered with the $\Delta HbR$ map predicted by BOLD MRI. The changes of the deoxyhemoglobin concentration were observed to be higher in the brain and in the deep structure whereas relatively lower in the superficial tissue. In Figure 6-12, similar standard deviations of the spatial variation are observed for NIR and MRI, but such variation was not purely due to the physiological causes but also by different sources. The $\Delta HbR$ map predicted by BOLD MRI in Figure 6-11 (the 1st row) contains high frequency components which could be the truly high-resolution functional changes but also could be the artifacts due to the noise. In the $\Delta HbR$ map provided by NIR DOT in Figure 6-11 (the 2nd row) such spatial variation includes the smoothing effect caused by the regularization in the reconstruction algorithm by which the original hard boundary within the tissue is smoothed. Compared to global change of the oxygenation, a local change in the brain is required to investigate the real resolution of the NIR DOT in in vivo studies. Ischemia models and tumor models could be used, but it is difficult to be done with current dynamic difference imaging technique, since it requires the local changes to be introduced without moving the animal away from the fiber array. We tried a middle cerebral artery occlusion model in several animals, but neither MRI nor NIR clearly showed a local change within the brain. This topic needs to be investigated in the future.

The gradient echo images in Figure 6-4 and the $\Delta R_2^*$ maps in Figure 6-5 clearly indicate the signal to noise ratio (SNR) was not great. This is probably due to the limitation of this coil. The sensitivity of a birdeage coil is proportional to $1/r$, where $r$ is the diameter of the coil, and
decreases by a factor \((1 - r^2 / R^2)\) if it is shielded, where \(R\) is the diameter of the conduction shield (De Zanche and Allen, 2002). For the 6cm birdcage coil with 12cm shield, the sensitive is approximately 56% of a 4cm birdcage coil, thus it is difficult to achieve very good gradient echo images in the presence of susceptibility effects due to the fibers.

In the reconstructed reduced scattering images in Figure 6-8, noticeable changes are observed at 728nm, but in theory the scattering property should not have such large variation during the mild hypoxia. This might be due to the crosstalk in the reconstruction algorithm and implies that the absorption coefficient must contain some errors. In Figure 6-9, ideally after the death of the animal, the tissue saturation and oxyhemoglobin should be all zero everywhere, but we still observed some oxyhemoglobin residuals in the superficial layer and negative values in the deep region. This indicates either the initial guess or the reconstruction for a large perturbation in the data was not accurate. The quantitative accuracy needs to be examined using other techniques in the future.

6.4. Conclusion

Preliminary \textit{in vivo} results on the animal are encouraging, although the heterogeneity of rat head and the experimental complexity on a live animal bring more difficulties. Using a baseline difference calibration technique, the functional changes during graded acute hypoxia were well traced by NIR DOT images and show a good correlation to the BOLD signal. With this versatile dual-modality system, in the future, more effort should be made not only into understanding the usage of high resolution MRI structural information in diffuse image reconstruction, but also to apply it to the biological study of brain hypoxia and ischemia as well as pathologies such as stroke, tumors and asphyxia, which all induce changes in oxygenation and hemodynamics.
Chapter 7. Conclusion

This chapter summarizes the work carried out in this thesis at each stage of its development, and suggestions for subsequent work are discussed. Section 7.1 reiterates the main achievements of the work in the following five aspects: instrumentation of a MRI coupled broadband tomography system; second derivative spectroscopy based diffuse optical image reconstruction; DOT image reconstruction with \textit{a priori} information and parameter constraints; direct chromophore and scattering reconstruction with different order of broadband data; graded hypoxia with both NIR DOT and BOLD MRI. In Section 7.2, the direction for the future effort is suggested within the context of problems and difficulties encountered during these developments.

7.1. Thesis summary

With the recent trends in the diffuse optical imaging field, two pathways for technological implementation seem very attractive and scientifically sound when the goal is to improve the quantification of the DOT images. One approach is to utilize more structural information from other available modalities, to improve the resolution and accuracy of the recovered physiological images, as well as being able to link the system into conventional imaging systems. The other approach is to acquire more spectral information in terms of utilizing more wavelengths to achieve better quantification of chromophores. In this thesis, both approaches were implemented in a MRI-coupled broadband near-infrared tomography system, and several new developments in utilizing the NIR data were analyzed. The system can measure complete attenuation spectra over the wavelength range 700-900nm using 8 transmit fibers and 8 separate receive fibers placed around the circumference of the rat head. The parallel detection for 8 receive fibers was implemented on a single CCD chip, and provided sufficient signal to noise,
albeit with somewhat longer integration times that would be ideally desired. The special design of the fiber/coil/tissue interface allows simultaneous image acquisition for MRI and NIR.

A unique feature of this project is the capability of resolving both absorption and scattering images of tissue with broadband measurement, without the technological complexity of time or frequency domain systems. The fundamental underlying approach is to utilize second derivative spectroscopy analysis (SDSA) and the available water absorption features to estimate the optical pathlength in tissue. Although the SDSA is not an original idea, the implementation of this technique to the optical imaging problem is novel and the complete theory had not been addressed and clarified prior to the work in this thesis. This theory may be used to improve current approaches in broadband near infrared spectroscopy. Theoretical and experimental evidence is presented to demonstrate the robustness and accuracy of the approach for accurate reconstruction of NIR images in terms of both optical and reduced scattering coefficients.

Strategies in terms of how to utilize the \textit{a priori} structural information within the image reconstruction algorithm are described and evaluated in Chapter 4. A signal processing technique is elegantly implemented in the objective function of the inverse problem. All of these techniques are realized by modifying the regularization term within the conventional Levenberg-Marquart algorithm.

A novel reconstruction algorithm for multi-wavelength diffuse optical tomography is presented, where instead of using individual data at each wavelength separately or even simultaneously, the difference in data for multiple wavelength pairs is used. The results indicates a dramatic improvement in image reconstruction and the elimination of image artifacts, which are often associated with unknown measurement errors such as coupling coefficient and the irregular external boundary variations. This algorithm has the potential to dramatically reduce
image artifacts in multi-spectral diffuse tomography, because coupling and boundary errors are often less dependent on wavelength, and are effectively removed in the data set of the first derivative of intensity with respect to wavelength.

The \textit{in vivo} performance of this system is examined through a series of animal experiments during graded hypoxia. Preliminary \textit{in vivo} results on the animal are encouraging, in that the functional changes during graded acute hypoxia were well traced by NIR DOT images and show a good correlation to the simultaneously measured BOLD signal. These studies present a highly calibrated and accurate measurement of NIR hemoglobin and BOLD MRI taken in the same group of animals, and thus provide one of the most accurate data sets for interpretation of the BOLD signal in the rat brain.

Although this thesis has accomplished many achievements, the performance of the ultimate imaging system still is some distance away from the original expectation. Due to the error and noise from many aspects (coupling error, model mismatch, physiological error etc.), it has not been successful to accurately reconstruct small heterogeneity within the brain in the absolute optical property images, which is curial to study stroke, ischemia or tumor models. Dynamic imaging from the baseline can reliably recover the physiological changes for a relatively long period; however such changes typically are global and difficult to differentiate the normal tissue and the abnormal tissue, therefore it is difficult and not straightforward to study the pathophysiology. For the future work, first of all it is necessary to improve the performance and reliability of the acquisition system and secondly it is desirable to avoid the conversional diffuse reconstruction algorithm which is hypersensitive to the measurement errors.

7.2. \textbf{Future work}

7.2.1. \textbf{Source power and temporal response}
With the halogen white light source, the integrated power delivered to the animal in each source fiber was approximately 20mW. The average power in the 700-900nm range was only 0.1mW/nm, which is low compared to what can be achieved with laser systems (5~20mW/nm). To obtain sufficient SNR especially for the derivative analysis, we have to integrate the CCD for a long period. This poor temporal response not only limits the application for in vivo studies but also degrades the imaging performance, since the physiological noise becomes a significant component in the measured data and the non-linear reconstruction algorithm is sensitive to this noise in the data. One possible solution is to use tunable CW Ti:sapphire laser (3900S CW, Newport), which has a tunable range 700-1000nm and up to 2.2 W power, or alternatively using a white light continuum generation from a chirped femtosecond laser in a structured fiber. These laser sources could be more efficiently coupled to the fiber, albeit with more cost and infrastructure associated with them. Another solution is to use smaller animal models such as mice in which light will be less attenuated, and that also the ratio of brain to muscle is much greater than rats.

7.2.2. Model-based derivative spectral analysis

In Chapter 3, it has been shown that the current derivative spectral analysis theory does not require any explicit physical model of radiation transport. The initial assumptions in the derivation are based on the physics of light propagation in tissue and the makeup of the chromophores present in tissue: the scattering coefficient has a weak wavelength-dependence and the chromophores of interest have sharp derivative features. This leads to the first approximation in which the 2\textsuperscript{nd} differential of the attenuation within the spectrum is simply equal to the product of differential pathlength and 2\textsuperscript{nd} differential of the absorption coefficient.
(Equation 3-7 to Equation 3-9). Another assumption is that the differential pathlength is constant in the reduced wavelength range and this leads to the second approximation from Equation 3-9 to Equation 3-10.

However, as shown in Figure 3-4, the 3rd term in Equation 3-7 is not small enough to be ignored; therefore the first approximation contains some error. The second approximation is less accurate in 700-800nm range, since the differential pathlength in this range has a large curvature which may distort the chromophore extinction coefficient spectrum. This is the major reason for the crosstalk of parameters in the fitting algorithm.

If a forward model is incorporated in the analysis, the 3rd term in Equation 3-7 and the curvature of the differential pathlength could also be involved in the data fitting. Hence, smaller residuals and more accurate results could be achieved. If the chromophore concentrations can be more accurately and robustly determined, NIRS would be a much more powerful technique, and combined with the simplicity of CW detection, could have an expanded role in current clinical practice.

Another possible improvement concerns the estimate of oxyhemoglobin concentration. Figure 3-2(b) indicates the current SDSA is not sensitive to oxyhemoglobin, since its second derivative features are smaller compared to deoxyhemoglobin and water. This is calculated using approximately 1nm wavelength gap within the complete data set. However, if a wider gap is used, higher sensitivity for oxyhemoglobin is possible (Myers et al., In press). Thus, analysis of the wavelength spacing in the derivative approach may lead to improvements in the sensitivity for different chromophores, which may be found with careful analysis.

7.2.3. **Calibration and global fitting for spectral image reconstruction**
In Chapter 5, the novel idea of spectral derivative based image reconstruction is introduced which is a natural extension of the direct chromophore and scattering reconstruction method. It has shown great promise in the simulation and phantom studies; however it has not yet been implemented to \textit{in vivo} studies. The major obstacles in this are the calibration procedure and the global fitting for the initial guess. Because the modeling error between the data and the numerical diffusion model data is not easily predicted theoretically and unlikely to be wavelength independent, the effect of this model-data mismatch error in the spectral derivative image reconstruction has not been fully investigated. If the global fitting of both absorption and reduced scattering coefficients is minimum within a noisy error space, then the global fitting of 5 parameters, scattering amplitude and power, oxyhemoglobin, deoxyhemoglobin and water is considerably more challenging in an error space which is likely filled with many shallow local minima. There may be too many local minima and it is difficult to converge to the global minimum with the current non-linear least-square fitting program, without more in depth analysis of how to regularize or parameterize the problem. Clearly different orders of differentiation of the attenuation spectrum provide different sensitivities to each of the parameters. Combining them and using a multi-step fitting algorithm might improve the accuracy of the reconstructed images; however this remains to be fully tested. Another thought is to use the maximum likelihood approach, which includes a clearer statistical analysis of the convergence. Instead of searching for an absolute minimum, search for an extremum which has the highest probability to be the solution.

\textbf{7.2.4. \hspace{1em} Back projection of chromophore concentration}
In Chapter 3, it has been mentioned that the second derivative spectroscopy also could estimate the integrated concentration of chromophores other than water, which is \( \mathbf{C} \times \mathbf{PDP} \) (Equation 3-16), where \( \mathbf{C} \) is a \( N_c \times N_n \) matrix which is the chromophore concentration map in the medium. \( \mathbf{PDP} \) is a \( N_n \times 1 \) vector which is the partial differential pathlength map. If the PDP is provided, the \( \mathbf{C} \) map can be obtained through a simple linear inversion.

This is a new algorithm for imaging the chromophore concentrations. Unlike the normal NIR image reconstruction methods that reconstruct the optical properties (absorption and scattering) at many wavelengths first and then decompose the chromophore concentration, this method calculates the integrated chromophore concentration within the volume by each source detector pair using the derivative spectroscopy analysis then back projects the spatial distribution using the estimated partial differential pathlength map.
## APPENDIX A

### A. MRI toolbox

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Relevant functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>fdfbrowser</td>
<td>A GUI for browsing the FDF files on Windows. Several functions are implemented. Displaying: pick up the folder of the FDF files and display. Segmentation: Autossegmentation or Manual mode. Segmented regions are stored in the PNG picture file.</td>
<td>mfindskin, mfindbrain, mfindskull</td>
</tr>
</tbody>
</table>
| mfid2pc         | read the data from the fid file and generate the image maps.  
  [imap] = mfid2pc(fn);  
  fn is the name of the fid file  
  imap: signal intensity images; | |
| mPNG2poly       | read the data from the FDF file and generate the image maps.  
  image = mfdf2pc(fn);  
  fn : filename of the fdf file.  
  For example: image = mfdf2pc('image0001.fdf');  
  If no 'fn' input, a dialog box will let you choose the file. | |
| mfdf2pc         | convert the fdf file into 16bit tiff file  
  image = mfdf2tif(fn);  
  fn : filename of the reference file.  
  For example: image = mfdf2tif('image0001.fdf');  
  If no 'fn' input, a dialog box will let you choose the file. | |
| mfindskin       | skin = mfindskin(img)  
  automatically segment skin  
  img: intensity image  
  skin: bw mask | |
| mfindbrain      | brain = mfindbrain(img,skin)  
  automatically segment brain from the skin layer  
  img: intensity image  
  skull, skin, brain: bw mask | |
| mfindskull      | skull = mfindskull(img,skin,brain,roi,tp)  
  automatically segment skull for brain and skin tissue  
  img: intensity image  
  skull, skin, brain: bw mask  
  roi is the region been selected. | |
| mfdf_rdhd       | par = mrd_fdfhd('image0001.fdf')  
  Return the parameters in the header of the fdf file such as, pe, ro, TE, TR, ..., etc | |
| mfdf_rd         | [image,par] = mrd_fddf('image0001.fdf');  
  Read fdf MRI file into a matrix with size pe x ro  
  par: acquisition parameters | |
B. NIRFAST toolbox

| NIRFAST_SE | Setup the global variables |
| add_blob_ellipse | mesh = add_blob_ellipse (mesh,x,y,a,b,type,value) add ellipse shape inclusion to the target |
| calib_hybrid | calibrate the data using the hybrid mesh calib_hybrid(anom_data,fwd2d,fwd3d,mesh_op, data_op) |
| calibrate_3d | Calibrate the data using the 3D mesh |
| femdatahybrid | generate the forward data using the hybrid mesh data = femdatahybrid(f2d,f3d,mesh); f2d: 2d mesh , f3d: 3d mesh, mesh: input 3d mesh |
| fit_data_new | fits data to a given model to give initial guesses for reconstruction as well as data offsets Modified by Heng Xu, 2005 What's New: 1. Enable measurement selection through a link file. 2. Compatible with old data file. |
| m2Dto3Dmap | fwd_3d = m2Dto3Dmap(fwd_2d,fwd_3d); Mapping the 2D mesh to the 3D mesh |
| m2Dto3Dmesh | extrapolate the 2D mesh on the z axis to create a 3D mesh c3d = m2Dto3Dmesh(c2d,[-13:1:13],c3d_new); |
| m3Dto2Dpro | Project 3D Jacobian to 2D J3 = m3Dto2Dpro(J,fwd_3d,fwd_2d) |
| mPickOptodes | A GUI to pick up the optode locations |
| mSolver0 | inverse core using Troy’s method |
| mSolverA | inverse core using L matrix and spatially variant regularization |
| reconstruct_xu | reconstruct NIR images using different basis and controls. [fwd_mesh,pj_error,error] = reconstruct(fwd_fn,recon_basis,method,data_fn,iteration,n,lambda,output_fn,filter_n) fwd_fn: fwd_mesh recon_basis: coarse mesh or pixels method: 0, 5 data_fn: calibrated data file |
| reconstruct_xu | 2D/3D hybrid reconstruction |

m3Dto2Dpro
<table>
<thead>
<tr>
<th>Hybrid region</th>
<th>reconstruct_xu_hybrid</th>
<th>m2Dto3Dmap</th>
</tr>
</thead>
<tbody>
<tr>
<td>_hybrid</td>
<td>hybrid</td>
<td>m2Dto3Dma</td>
</tr>
<tr>
<td>[fwd_mesh,pj_error]</td>
<td>reconstruct_xu_hybrid(fwd_fn,fwd_3d,recon_basis,method,data_fn,iteration,lambda,output_fn,filter_n)</td>
<td>m2Dto3Dmap</td>
</tr>
<tr>
<td>fwd_fn: 2D forward mesh</td>
<td></td>
<td>m2Dto3Dmap</td>
</tr>
<tr>
<td>fwd_3d: 3D forward mesh</td>
<td></td>
<td>m2Dto3Dmap</td>
</tr>
<tr>
<td>recon_basis: pixel or coarse mesh</td>
<td></td>
<td>m2Dto3Dmap</td>
</tr>
<tr>
<td>regional reconstruction using the hybrid 2D/3D method</td>
<td></td>
<td>m2Dto3Dmap</td>
</tr>
</tbody>
</table>

C. Spectroscopy toolbox

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAbsp2Chrom</td>
<td>Fit oxy and deoxy hemoglobin concentration from the absorption at 728nm and 830nm using the least square method [CHbO,CHb] = mAbsp2chrom(mua1,mua2)</td>
</tr>
<tr>
<td>mAlicateRead</td>
<td>[D,R,Info,SRC,param] = mAlicanteRead(fn); Used to read the alicantedata (*.adf) D: Attenuation spectrum (OD) R: Reference spectrum (OD) Info: 16 Channels information, i.e. Attenuation,Pathlength,HbO,Hb,HbT,SaO2 at 740, 840. Src: source balance power. Obseleted param: parameters:NP,NT,NR,Noiselevel,etc.</td>
</tr>
<tr>
<td>mExtRef2</td>
<td>Calculate Extinction spectrum at specified wavelength range Ext = mExtRef(wl,M); wl: wavelength range w: half-width of polyfitting windows. order: order number of the polyfit, default 3.</td>
</tr>
<tr>
<td>mGenFilterMatrix</td>
<td>Generate the convolution matrix for the polynomial fit M = mGenFilterMatrix(w,n,dx) w: the half width of the smooth window n: the order of the polynomial, by default it's 4.</td>
</tr>
<tr>
<td>mLoadMuaMus</td>
<td>Calculate mua and mus at given wavelength based on the makeups. [mua,mus] = mLoadMuaMus(wl,Ct,Sa,CH2O,CIntrolipid) wl: wavelength Ct: total concentration of hemoglobin, unit: uM Sa: saturation of oxygenation CH2O: percentage of water CIntrolipid: concentration of Introlipid (%) type: smoothed spectrum or not</td>
</tr>
<tr>
<td>Function</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>mOptodes</td>
<td>A GUI for optimizing the optode locations</td>
</tr>
</tbody>
</table>
| mPolySmth | Smooth the spectrum and calculate the first and second differential 
\[ [y, sd, fd] = mPolySmth(y, M) \]  
x : normally wavelengths vector (not required any more)  
y : the sampled data wants to smooth  
M : the filter matrix  
dx : the separation of the wavelength, now fixed to 1 (!!!) |
| sgolay | \[ c = sgolay(x, y, f, k) \]  
savitziki-golay smooth  
\((x, y)\) are given data. \(f\) is the frame length to be taken, should be an odd number. \(k\) is the degree of polynomial filter. It should be less than \(f\).  

### D. Direct chromophore reconstruction toolbox

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
</table>
| DCForward | \[ ref1 = DCForward(fwd\_mesh, recon\_mesh, record, data\_fn) \]  
Forward calculation for the dual mesh modality  
fwd\_mesh is the fine mesh  
recon\_mesh is the coarse mesh for recording the results  
record is the saved concentration map  
data\_fn is the data file will be saved. |
| DCForwardSingle | \[ ref1 = DCForwardSingle(fwd\_mesh, record, data\_fn); \]  
Calculate the data file for given concentration map on a single mesh  
fwd\_mesh: fwd\_mesh  
record: concentration map (nnodes x 1 vector) or a file name  
data\_fn: name of the saved data file  
Modified by Heng, Feb2005 |
| DCForwardSingleH | \[ ref1 = DCForwardSingleH(fwd\_mesh, fwd\_3d, record, data\_fn); \]  
Calculate the data file for given concentration map on a single mesh using 2D/3D hybrid method  
fwd\_mesh: fwd\_mesh |

femdata mBuildOpticMaps

femdata mBuildOpticMaps

femdata mBuildOpticMaps

femdata mBuildOpticMaps

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<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>record</strong>: concentration map (nnodes x 1 vector) or a file name <strong>data_fn</strong>: name of the saved data file</td>
<td></td>
</tr>
<tr>
<td><strong>DCHomoPredit</strong></td>
<td></td>
</tr>
<tr>
<td>[xdata,ydata,zdata]= DCHomoPredit(fn)</td>
<td>This function compares the real spectrum with the predicted spectrum fn: the file name for the *.adf alicante file If a *.predit file is associated with the *.adf file, those numbers will be use. Otherwise they will be asked for.</td>
</tr>
<tr>
<td><strong>DCForward</strong></td>
<td></td>
</tr>
<tr>
<td><strong>DCHomoPredit</strong></td>
<td></td>
</tr>
<tr>
<td>DCHomoPredit(fn)</td>
<td></td>
</tr>
<tr>
<td>This function fits the given spectrum for SA using &quot;lsqcurvefit&quot; optimization and calling a user-defined function &quot;myFunSpec&quot; fn: the file name for the *.adf alicante file If a *.predit file is associated with the *.adf file, those numbers will be used. Otherwise they will be asked for.</td>
<td></td>
</tr>
<tr>
<td><strong>DCForwardSingle</strong></td>
<td></td>
</tr>
<tr>
<td><strong>DCReconstruct</strong></td>
<td>Direct Chromophore Reconstruction Program A using dual method A: Jacobian matrix is normalized by its diagonal fwd_mesh: fine mesh for forward measurement simulation recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td><strong>DCReconstructSM</strong></td>
<td>Direct Chromophore Reconstruction Program SM version (Single mesh) recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td><strong>DCReconstructSMG</strong></td>
<td>Direct Chromophore Reconstruction Program SM version (Single mesh) for global values recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td><strong>DCReconstructSML</strong></td>
<td></td>
</tr>
<tr>
<td>DCRMMDkey</td>
<td></td>
</tr>
<tr>
<td><strong>femdata mBuildOpticMaps</strong></td>
<td></td>
</tr>
<tr>
<td><strong>femdata mBuildOpticMaps</strong></td>
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<tr>
<td><strong>femdata mBuildOpticMaps</strong></td>
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</tr>
<tr>
<td><strong>femdata mBuildOpticMaps</strong></td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>Description</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>L (on, lambda)</td>
<td>Direct Chromophore Reconstruction Program SM version (Single mesh) with L matrix Jacobian matrix is normalized by G_WXW (a global control vector) recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td>DCReconstructSMH</td>
<td>DCReconstructSMH(recon_mesh, fwd_3d, data_fn, record, iteration, lambda) Direct Chromophore Reconstruction Program SM version and hybrid 2D/3D method Jacobian matrix is normalized by G_WXW (a global control vector) L matrix is implemented. recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom fwd_3d: 3d mesh associated with 2D recon_mesh data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td>DCZFHomofit</td>
<td>DCZFHomofit(fn, cmap) Fit the chromphore concentration and the scattering property using the zero order spectra and 1st order difference spectra. fn: the spectra data file cmap: the initial guess maps for 5 unknown parameters Results are stored in fn.safit and fn.residual</td>
</tr>
<tr>
<td>DCadd_noise</td>
<td>DCadd_noise(mesh, fn1, fn2, n) add n% noise to the data using gaussian distribution for log(I) = log(I) + n(1)/100*noise for phase = phase + n(2)<em>noise</em>pi/180 dtyle specifies the wavelength and measurement type</td>
</tr>
<tr>
<td>DCadd_noise_cpl</td>
<td>[data2, s, d] = DCadd_noise_cpl(mesh, fn1, fn2, n, single) add random coupling error to the spectral data mesh: mesh.dtype and mesh.link provide information about the wavelengths and source detector link map</td>
</tr>
<tr>
<td>DCinit</td>
<td>Setup the environment for the DC package (global variables)</td>
</tr>
<tr>
<td>DCmGenDataSet</td>
<td>data = DCmGenDataSet(recon, fn, fn2) Generate the data from the Alicante using the format specified by recon recon: reconstruction mesh and settings optional:</td>
</tr>
<tr>
<td>Function</td>
<td>Description</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
</tr>
<tr>
<td>fn:</td>
<td>the adf file name</td>
</tr>
<tr>
<td>fn2:</td>
<td>the name for the saved data file</td>
</tr>
<tr>
<td>DCplotimage</td>
<td>DCplotimage(recon, cmaps) plot concentration maps</td>
</tr>
<tr>
<td>DCplotspectra</td>
<td>DCplotspectra(mesh, fn) mesh is file name for the mesh like 'a4h' fn is the file name for the data file like 'sm1.saa' or fn is the data array, saa</td>
</tr>
<tr>
<td>DCread_solution</td>
<td>cmap = DCread_solution(recon, record, it) read solution from the reconstruction record file recon: 'a4h' or recon, file name or mesh structure record: '*.sol' it: iteration number</td>
</tr>
<tr>
<td>DCreadpjerror</td>
<td>[pj] = DCreadpjerror(fn) read projection error from the solution file</td>
</tr>
<tr>
<td>FDRenconstructSM</td>
<td>FDRenconstructSM(recon_mesh, data_fn, record, iteration, lambda) First derivative reconstruction based on the direct Chromophore Reconstruction Program SM version (Single mesh) recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td>FDRenconstructSM</td>
<td>FDRenconstructSML(recon_mesh, data_fn, record, iteration, lambda) First derivative reconstruction based on the direct Chromophore Reconstruction Program SM version (Single mesh) L matrix is implemented recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td>FDRenconstructSM</td>
<td>FDRenconstructSMH(recon_mesh, fwd_3d, data_fn, record, iteration, lambda) First derivative reconstruction based on the direct Chromophore Reconstruction Program SM version (Single mesh) With L matrix and Hybrid meshes recon_mesh: coarse mesh for reconstruction, also provide .dtype and .chrom data_fn: boundary data file record: the file name for storing the results.</td>
</tr>
<tr>
<td>ZFDRenconstructSM</td>
<td>ZFDRenconstructSML(recon_mesh, data_fn, record, iteration, lambda)</td>
</tr>
</tbody>
</table>
Both Zero order and First derivative reconstruction based on the direct Chromophore Reconstruction Program SM version (Single mesh)

- `recon_mesh`: coarse mesh for reconstruction, also provide .dtype and .chrom
- `data_fn`: boundary data file record: the file name for storing the results.

### mBuildOpticMaps

```python
mesh = mBuildOpticsMaps(mesh, ext, camp, wv, i)
```

Build Optical properites maps for the ith wavelength in the vector

- `ext` is the extintion coefficient matrix
- `cmap` is the concentration distribution matrix
- `wv` is the wavelength vector
- `i` is the index for the wavelength vector

### mCmapCheck

```python
cmap = mCmapCheck(cmap)
```

Check if the cmap is within the realistic range

If not, disp a warning message

### mCorluR

```python
R = mCorluR(wv, b)
```

R criterion in Corlu's OL paper 2003

- `b`: the scattering power

### mCorluk

```python
k = mCorluk(wv)
```

The criterion about the condition number in Corlu's paper

### mGetExtMatrix

```python
Ext = mGetExtMatrix(wv);
```

Load Extinction coefficient Matrix

HBR.ext, HBO.ext, and H2O.ext are pre-stored extinction spectra and have been discretized every nm and respectively have the units: 1/mm/uM,

1/mm/uM, 1/mm/100%.

However, the output EXT have the units [1/mm/mM, 1/mm/mM, 1/mm/100%]

EXT is a nw by nc matrix where nw is the number of the wavelength and c is the number of chromophores

### Mesh generation toolbox

#### m3DMapRegion

```python
mesh = m3DMapRegion(msk, mesh, reso);
```

Label regions for the 3D mesh using the provided msk

- `msk`: mask matrix (pixel based)
- `mesh`: FEM mesh file in units of
- `reso`: units conversion from FEM mesh to msk

#### mAnsys2NIR

```python
mesh = mAnsys2NIR(afn, nfn, rfn)
```

E. Mesh generation toolbox
### Convert the ansys nodes and elems file into NIRFAST format

**ANODE FILE:**
- X,Y,Z,THXY,THYZ,THZX.

**AELEM FILE:**
- I,J,K,L,M,N,O,P,MAT,TYPE,REAL,SECNUM,ESYS,IEL,
- where MAT, TYPE, REAL, and ESYS are attribute numbers, SECNUM is the beam section number, and IEL is the element number.

### mMapRegion

```plaintext
region = mMapRegion(fnlist, fnmesh, xo, yo, resolution)
```

- **mMapRegion** is used to label the mesh using the regional information provided by a series of PNG image.
- fnlist is the file name list of the PNG image.
- fnmesh is the mesh file.
- xo yo is the center in the real space (units: mm).
- resolution is the scale from the real space to the image space.
- Note: no need to provide the region 0 map.

### mNml2Ng

```plaintext
mNml2Ng(fn1, fn2)
```

- convert the NML mesh file to Netgen file.

### mSP2NIR

```plaintext
mSP2NIR(fn, fn2)
```

- Convert the SPmesh into the format for NIRFAST.

### mSetLineSize

```plaintext
nn = mSetLineSize(node, sz)
```

- reduce the number of the boundary node by giving minimal distance.

### mTecplot

```plaintext
mTecplot(mesh, data, fn)
```

- call Tecplot to plot mesh on the linux.
- mesh: the FEM mesh.
- data: [mesh.mua mesh.mus ...]
- fn: output file for tecplot.

### mresource

```plaintext
np = mresource(fnmesh, fns, fnd, d)
```

- fnmesh: the name of mesh file.
- fns: the source file required to recalculate.
- fnd: the destinate file will be written the new positions in.
- d: the distance will move in.

### mzoommesh

```plaintext
mzoommesh(org, tar, factor)
```

- org: source mesh.
- tar: target mesh.
- factor: zoom factor: from 30 to 90 then factor = 90/30.

### nmlmerge

```plaintext
nmlmerge(fn, fn1, fn2, fn3)
```

- merge several nml surface mesh files.
- each one may represent a tissue type.

### nmlread

```plaintext
[hd, nodes, elem] = nmlread(fn)
```

- read nml file to matlab.

### nmlwrite

```plaintext
nmlwrite(hd, nodes, elem, fn)
```

- write a new nml file.
## F. Other toolbox

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mFEMACModel</td>
<td>[a,p] = mFEMACModel(ua,us) calculate the attenuation and phase shift for given ua and us using the frequency domain (AC) FEM forward mesh is specified by the global FWD MESH</td>
</tr>
<tr>
<td>mFEMACModelH</td>
<td>[a,p] = mFEMACModel(ua,us) calculate the attenuation and phase shift for given ua and us using the frequency domain (AC) FEM forward mesh is specified by the global FWD MESH Heterogeneous model</td>
</tr>
<tr>
<td>mInfDCFit</td>
<td>[ua,us] = mInfDCFit(r,a,dp) Fit ua and us using the homogeneous infinite model for given attenuation (a) and differential pathlength (dp) at multiple distance (r) using DC diffusion equation</td>
</tr>
<tr>
<td>mInfDCModel</td>
<td>[a,dp] = mInfDCModel(ua,us,r) Calculating the attenuation and differential pathlength for infinite medium input, ua, us are the optical properties r is the physical distance. in mm</td>
</tr>
<tr>
<td>mSDSAFEM</td>
<td>[e1,e2,re1,re2,B1,B2] = mSDSAFEM(Cmap,CI,w1,w2) A mutant of mSDspectroscopy: second derivative spectroscopy Here FEM model is used. Cmap is concentration map CI is the intralipid concentration w1,w2 are the representitive wavelengths for 700-800nm and 800-880nm</td>
</tr>
<tr>
<td>mSDSAFEMH</td>
<td>[e1,e2,re1,re2,B1,B2] = mSDSAFEMH(Cmap,CI,w1,w2) A mutant of mSDspectroscopy Here FEM model is used. Cmap is concentration map CI is the intralipid concentration w1,w2 are the representitive wavelengths for 700-800nm and 800-880nm For heterogeneous model</td>
</tr>
<tr>
<td>mSDspectroscopy</td>
<td>[e1,e2,re1,re2,B1,B2] = mSDspectroscopy(r,Cmap,CI,w1,w2,model)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>mInfDCModel</td>
<td></td>
</tr>
</tbody>
</table>
Perform the second derivative spectroscopy analysis on the data simulated by the forward model (Inf or semi-inf) for given source detector separation \(r\) and concentration map \(Cmap, CI\), and representative wavelength \((w_1,w_2)\).

| mSDSAN \([e_1,e_2,re_1,re_2,B_1,B_2] = mSDSAN(Cmap,CI,w_1,w_2)\) | A mutant of mSD spectroscopy, but add noise to the data. Cmap is concentration map, CI is the intralipid concentration, \(w_1,w_2\) are the representative wavelengths for 700-800nm and 800-880nm. SNR is the expected SNR. |
| mSemiDCFIt \([ua,us] = mSemiDCFIt(r,a,dp)\) | Fit \(ua\) and \(us\) using the homogeneous semi-infinite model for given attenuation \(a\) and differential pathlength \(dp\) at multiple distance \(r\) using DC diffusion equation. |
| mSemiDCModel \([a,dp] = mSemiDCModel(ua,us,r)\) | Calculating the attenuation and differential pathlength for semi-infinite medium using the extrapolated boundary condition. Input, \(ua, us\) are the optical properties. \(r\) is the physical distance. in mm. |
| mSemiDCModelZero \([a,dp] = mSemiDCModelZero(ua,us,r)\) | Calculating the attenuation and differential pathlength for semi-infinite medium using the zero boundary condition. Input, \(ua, us\) are the optical properties. \(r\) is the physical distance. in mm. Because the fluence is zero on the boundary, the detected signal is flux. |
| mSDFitting \([B,BINT,R] = mSDFitting(wv,a,range)\) | Second derivative fitting using the sgolay and regress. |
| optimalwv \([e_1,e_2,re_1,re_2] = optimalwv(w_1,w_2,model)\) | Determine the optimal representative wavelength for 700-800 and 800-880 ranges. |
| optimalwvFEM \([e_1,e_2,re_1,re_2] = optimalwvFEM(w_1,w_2,seed)\) | Determine the optimal wavelength for 700-800 and 800-880 ranges using FEM. |
BIBLIOGRAPHY


