Combining near infrared tomography and magnetic resonance imaging to improve breast tissue chromophore and scattering assessment

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Abstract

This work describes a multi-spectral frequency domain Near Infrared (NIR) tomography system which was constructed specifically for operation inside the high magnetic fields presented by Magnetic Resonance Imaging (MRI). The combined platform is used to image breast tissue morphology and function simultaneously. Image reconstruction algorithms are outlined which incorporate \textit{a priori} knowledge of breast tissue structure from MRI that could potentially improve the resolution and overall efficacy of NIR tomography. NIR spectroscopic imaging systems are used to estimate spatially resolved distributions of hemoglobin concentration and oxygenation, water, and light scattering in tissue, thereby localizing functional changes which may occur at centimeter depths. Due to the diffusive nature of NIR light propagation in tissue, computational model-based image reconstruction methods are used to provide the spatial resolution and quantification for the chromophore concentrations and scattering parameters sought. Optimizing spatial resolution is an important concern in research and clinical applications, and the combination with MRI is an important part of this.

This NIR-MRI imaging system and its complimentary modeling tools have been tested and optimized using data collected from tissue-simulating phantoms, and used to study the properties of component normal breast tissues in vivo for a pilot population of women. Total hemoglobin concentration, hemoglobin oxygen saturation, water fraction, scattering amplitude, and scattering power were estimated for adipose tissue [17 µM, 71%, 47%, 1.3, and 0.6, respectively] and fibroglandular tissue [22 µM, 70%, 60%, 0.9, and 0.8, respectively]. Images with millimeter resolution and high contrast can be reconstructed which may provide indicators of functional activity and disease processes.
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Chapter 1 : Overview

1. A. Introduction

This thesis describes the development and implementation of a hybrid imaging system which combines multi-wavelength near infrared (NIR) tomography and magnetic resonance imaging (MRI) to study breast tissue. Specifically, a NIR tomography system was constructed to be compatible with the high magnetic fields inside MRI scanners. Parallel NIR-MRI data acquisition is described, along with image reconstruction techniques which take advantage of the combined data set to produce high resolution, quantitatively accurate images of tissue optical properties and chromophore concentrations. The imaging platform, which includes data acquisition and processing, was optimized for routine clinical use and was tested extensively using computer simulations, tissue-simulating ‘phantoms’, and patient volunteers. This thesis is organized into ten chapters:

Chapter 1: Overview

Chapter 2: Instrumentation

Chapter 3: FEM reconstruction algorithm

Chapter 4: Simulations

Chapter 5: Phantom design

Chapter 6: Refractive index in NIR tomography

Chapter 7: NIR-MRI phantom imaging

Chapter 8: Functional imaging

Chapter 9: Patient imaging
Chapter 10: Concluding remarks

The work presented here builds on important research in NIR breast imaging conducted previously and concurrently at Dartmouth College and at other institutions. This overview chapter attempts to provide an abridged history of NIR imaging in order to foster an understanding of the factors which have motivated the research described herein. A summary of the key elements of this thesis is also given, including instrumentation, theory (i.e. modeling and image reconstruction), and experimental/clinical studies.

1.B. Background and significance

1.B.1. Background of optical imaging

Over the past decade the field of biomedical optics has seen steady growth. An increasing number of international research conferences and scientific journals are dedicated to the medical applications of light. One of the principle goals of the field is the development of diagnostic imaging modalities based upon visible (wavelengths 400-700 nm) and near infrared (700-1000 nm) radiation, which could potentially offer several advantages over existing radiological techniques. First, this radiation is non-ionizing, and therefore reasonable doses can be repeatedly employed without harm to the patient. Second, optical methods offer the potential to differentiate between soft tissues due to their different absorption or scatter at these wavelengths. Third, specific absorption by intrinsic chromophores allows functional information to be obtained which is indistinguishable using current clinical modalities. NIR absorption in tissue is dominated by oxy-hemoglobin, deoxy-hemoglobin, and water. Scattering is generated by
microscopic fluctuations in refractive index, and is related to the structure of the tissue resulting from membrane bound sub-cellular organelles\textsuperscript{1-6}. Mie-like scattering causes photons to follow a diffuse path in tissue, and this increased path length amplifies changes in the absorption coefficient of the final signal. Thus, even small absorption values and changes can be measured with high precision. NIR imaging and spectroscopy research has focused on a variety of potential clinical applications, including investigation of the physiological properties of breast tissue\textsuperscript{2, 7-10}, peripheral muscles and joints\textsuperscript{11, 12}, and the brain in neonates and adults\textsuperscript{13, 14}.

As early as the 1920’s, transillumination (or diaphanography) with red light was used to investigate breast tumors\textsuperscript{15}. Early experiments revealed that the multiple scattering which occurs when light propagates through tissue causes features below the surface to appear extremely blurred. Breast cancer screening demands a spatial resolution of a few millimeters or better in order to distinguish small tumors from surrounding healthy tissue. Despite attempts to improve resolution of breast transillumination by employing near infrared sources and detectors\textsuperscript{16}, clinical trials in the 1980’s demonstrated that its utility as a screening tool is severely limited\textsuperscript{17}. Around the same time, the use of near infrared for obtaining spectral information became very successful. Jobsis discovered the ability to measure oxygen saturation changes of the blood through thick tissue volumes such as the cat cranium\textsuperscript{18}. This was rapidly exploited to develop methods for oximetry of the neonate brain, and for measuring arterial oxygen saturation from the pulsatile flow in the finger, toe, or earlobe\textsuperscript{19}. Pulse oximeters measure signals from the finger, and have developed into sophisticated measurement
systems which incorporate intelligent methods to suppress movement artifacts. They are used in almost all applications of critical care monitoring.

The attraction of near infrared measurement is the ability to provide functional information about tissue blood content and oxygenation, as well as water and lipid content, using a non-invasive, compact, and inexpensive method. Similarly, the scattering spectrum and phase function of light in tissue has been shown to potentially provide information about tissue structure. Despite evidence of high contrast, optical imaging suffers from poor spatial resolution due to the diffuse nature of light propagation in tissue and the complicated path that photons travel from source to detector. To exploit these potential functional signals, researchers have attempted to improve upon the poor spatial resolution of transillumination. Clinical investigations with NIR require spatially resolved spectroscopic imaging systems, in order to localize functional changes deep within tissues. Recent advances which have led to improved NIR imaging capabilities have occurred in two main areas: (1) instrumentation, and (2) modeling and image reconstruction.

1.B.1.1. Advances in instrumentation

Laser technology in particular has greatly improved over the past 20 years. Inexpensive and stable semiconductor lasers are available at many wavelengths throughout the NIR range. These can be activated by simple wall power outlets through power regulators, and may be used in CW and frequency domain setups. Mode-locked lasers are also available for use in time domain systems. Commercial use of optical fibers has taken off during the past 20 years in concert with the widespread availability of these lasers. Solid-state photon detectors have also advanced, allowing better data
collection. Charge-coupled devices (CCD) and photodiodes (PD) are stable for low frequency or small diameter applications and have a large dynamic range. Photomultiplier tube (PMT) detectors are highly sensitive even at high frequency, and provide the attraction of having a large detection area, allowing detection from large fiber bundles. NIR light transmission can typically be measured through up to 12 cm of breast tissue, depending on the composition of the breast and the wavelength of the NIR source.

1.B.1.2. Advances in photon migration modeling

The understanding of light propagation in tissue, specifically the development of photon migration theory and the diffusion approximation to light travel in a highly scattering medium has generated an entirely new field of study. Light transport in tissue can be modeled with many different approaches, including Maxwell’s equations, Mie scattering, Monte Carlo, or radiation transport theory. The imaging task involves solving an inverse problem using an appropriate forward model of photon transport. It is well established for near infrared wavelengths that scattering dominates over absorption in tissue, and that the scattering interaction is characterized by elastic processes resulting from refractive index fluctuations at a microscopic level of cellular organelles. Under these complex circumstances, the radiative transfer equation (RTE) (a specific form of the Boltzmann transport equation) has been the most useful approach for modeling over large tissue distances. This equation can be simplified to the diffusion approximation, making realistic assumptions about the elasticity of the scattering. As instrumentation developed and time-based information became measurable, modeling techniques have focused on separating tissue absorption and scatter. The problem is highly nonlinear and analytic solutions exist only for a limited number of simple geometries. In general
iterative methods employ numerical (i.e. finite element and finite difference) model solutions\textsuperscript{25}. This process can be computationally expensive and time consuming, especially in three dimensional property estimation, but this fact may not be prohibitive considering the exponential (i.e. Moore’s Law) growth of computing power\textsuperscript{26}.

1.B.2. Optical imaging of the breast

One clinical application to which the NIR imaging community has paid special attention is the need for more efficient tools to predict, diagnose, and monitor breast diseases. The clinical standards for breast imaging—ultrasound, contrast-enhanced MRI, and x-ray mammography—provide high spatial resolution, but comparatively little information about molecular-level changes in tissue\textsuperscript{27,28}. X-ray mammography, the most common form of cancer screening, has high sensitivity in women with fatty breast composition\textsuperscript{29}, but low sensitivity in radiographically dense breasts, and low positive predictive value. Despite proven efficacy, x-ray mammography is compromised by high recall rates\textsuperscript{30,31}, its use of ionizing radiation, and uncomfortable compression. In order to improve our understanding of in vivo tissues in both research and clinical settings, it is imperative to supplement conventional imaging with functional information where possible. Adjunctive non-invasive imaging modalities could be very useful in characterizing suspicious mammographic abnormalities, especially in women with radiographically dense tissue.

NIR technologies offer several distinct advantages in terms of sensitivity to functional changes, safety, cost, and use at the bedside. They could be used to monitor variations specific to breast cancer risk, type, and treatment progress. Since the use of mammography has become the primary clinical screening tool for breast disease in older
women, large epidemiological studies have demonstrated that radiographic density is correlated to risk of developing cancer\textsuperscript{32,33}. It is a challenge to elucidate the causal link between density and risk of cancer since the tools to measure breast composition are limited. In this context, NIR measurements provide an independent measure of tissue density, based upon scattering measures, hemoglobin, and water content\textsuperscript{34}. Women with dense breasts could benefit from earlier and more frequent screening, and imaging systems which are not harmful with repeated usage (i.e. do not use ionizing radiation) such as NIR systems, could be very desirable. Characterizing tissue composition and changes in composition consistent with long-term hormone-dependent transformations that occur in breast could improve the understanding of healthy tissue properties, inter-subject variation, and variability with the menstrual cycle. NIR spectral tomography measurements offer the possibility to monitor these physiological changes, which otherwise are never quantified in vivo, and are only descriptively understood at present.

NIR techniques are of particular clinical interest for differentiation of benign from malignant disease on the bases of tissue hemoglobin concentration and oxygenation. Biopsy is generally required to determine malignancy in most women with an abnormal mammogram. In the NIR spectral window, the absorption ratio between tumor and normal breast tissue is perhaps one of the highest intrinsic biological contrasts available in medical imaging, being up to 200\%, which is a number equivalent to the contrast available in x-ray mammography imaging of microcalcifications in the breast\textsuperscript{1,10,16,35}. Hemoglobin concentration is an indicator of tissue vascularity, which is increased in breast malignancy owing to angiogenesis\textsuperscript{36-38}. Localized increases in blood vessel growth and therefore blood volume are observed in cancerous tumors. This increase has been
estimated to correspond to a two to four fold increase in hemoglobin concentration within breast cancers relative to healthy tissue\textsuperscript{16,39,40}. Lower levels of oxygen saturation have also been found in malignancies, a possible consequence of cellular proliferation\textsuperscript{35,41,42}. Also, it has been reported that a localized 1.4 to 4.4 decrease in oxygen pressure is present within breast cancers\textsuperscript{43}. This decreased oxygen pressure may correspond to a measurable decrease in hemoglobin oxygen saturation.

Recent studies have shown that scattering contrast between malignant and benign processes appears to be significant\textsuperscript{44}. The morphologic changes from normal to diseased breast tissue are seen by light microscopy in the cellular epithelial component and the surrounding support stroma. Generally, the hallmark of an epithelial malignancy is an increase in the overall epithelial cell density with increased nuclear and nucleolar size. Microscopic sub-cellular alterations exist that may not be apparent in standard pathological analysis. Hence, variations in the scattering spectral features, which can be measured tomographically, may encode morphologic and pathophysiologic changes in tissue at the microscopic level.

\textbf{1.C. NIR tomographic imaging at Dartmouth College}

1.C.1. History of the project

Since 1999 a group of engineers from Thayer School of Engineering, Dartmouth College and physicians from Dartmouth Hitchcock Medical Center has been conducting a research program dedicated to the development of alternative breast cancer imaging technologies. Four alternative modalities—magnetic resonance elastography (MRE), electrical impedance spectroscopy (EIS), microwave imaging spectroscopy (MIS), and
near infrared spectroscopic imaging (NIS)—have the potential to contribute, either alone or in combination, to breast imaging for risk assessment, early detection, differential diagnosis, treatment prognosis, and therapy monitoring. These are each ‘model-based’ imaging modalities, which rely on iterative, convergent numerical modeling to produce images from measured data. The clinical feasibility of these technologies for breast imaging has been demonstrated through evaluation of a common cohort of women with normal and abnormal breasts as defined by screening mammography and subsequently verified by biopsy.

1.C.2. Stand-alone NIR imaging system

This thesis focuses on near infrared spectroscopic imaging, and builds upon work which has gone into development and testing of a ‘stand-alone’ clinical prototype NIR tomography system currently being used in a clinical trial to determine its efficacy in characterizing breast lesions. This methodology can be summarized by looking at three different portions of the imaging process: (1) data acquisition, (2) optical property image reconstruction, and (3) functional image formation.

1.C.2.1. Data acquisition

NIR measurement systems used in research detect diffuse reflectance, transmittance, or a combination of the two when detection points surround the tissue under investigation. This latter arrangement represents a tomography system, which is the most capable of resolving subtle heterogeneity contrasts deep within tissue. Systems may also furnish measurements of the full NIR wavelength spectrum, or elect to use several discrete wavelengths, which are either pulsed or modulated, in order to separate effects of absorption from scattering.
The current data acquisition system at Dartmouth, shown in Figure 1.1, was designed for cross-sectional imaging of breast tissue. It consists of 48 source and detector optical fibers, positioned in a circular array, with three planes of 16 fibers each. The fibers are attached to a radial positioning system which allow for variable diameter fiber movement. A collection of laser diode light sources (660-850 nm) are amplitude modulated at 100 MHz. For each wavelength, measurements are taken sequentially in each plane. The light is delivered to the breast through one of the 16 in-plane fibers. The diffusely transmitted light is detected by the remaining 15 in-plane fibers that are aligned.
to output to each of 15 photomultiplier tube (PMT) detectors that sample the signal in parallel. Typical of frequency domain systems, modulated sources are used in order to extract amplitude and phase of the light remitted from the sample or breast. These two data types allow for the separation of absorption and scattering properties in the reconstruction process. This instrument has been used in multiple clinical studies involving nearly 200 patients in total, and is mentioned again in Chapter 2.

1.C.2.2. Optical property image reconstruction

The measurements acquired at a single wavelength are used in a reconstruction program which relies on a finite element (FEM) based calculation of the diffusion equation. As described in Chapter 3, images are obtained through a least squares minimization of the difference between the measured light flux amplitude and phase shift at each detector and the calculated fluence rate amplitude and phase shift determined from an estimated set of tissue optical properties. Newton’s method is employed to iteratively solve the non-linear minimization problem. Figure 1.2 is flow chart outlining image reconstruction.

Specifically, the algorithm exploits the frequency domain diffusion equation approximation to light behavior in a highly scattering medium,

$$
- \nabla \cdot D(\mathbf{r}) \nabla \Phi(\mathbf{r}, \omega) + \left( \mu_a(\mathbf{r}) + \frac{i\omega}{c} \right) \Phi(\mathbf{r}, \omega) = S(\mathbf{r}, \omega),
$$

(1.1)

where $S(\mathbf{r}, \omega)$ is an isotropic light source at position $\mathbf{r}$, $\Phi(\mathbf{r}, \omega)$ is the light fluence rate (W/mm$^2$) at $\mathbf{r}$, $c$ is the speed of light in tissue, $\omega$ is the frequency of light modulation, $\mu_a$ is the absorption coefficient (mm$^{-1}$), $D = \frac{1}{3(\mu_a + \mu'_s)}$ is the diffusion coefficient (mm), and $\mu'_s$ is the reduced (transport) scattering coefficient (mm$^{-1}$). For a given $\mu_a$ and $\mu'_s$, 

distribution, the diffusion equation is used to predict the optical flux at the detector sites for each source excitation.

In the inverse problem (image reconstruction), the goal is the recovery of optical properties at each FEM node, based on measurements of optical flux at the detector sites on the tissue surface. This is achieved numerically by minimizing the difference between the calculated data, $\Phi^C$, and measured data, $\Phi^M$, for all source/detector combinations ($NM$). Typically, 

$$\chi^2 = \sum_{i=1}^{NM} \left( \Phi^C_i - \Phi^M_i \right)^2$$

(1.2)

is minimized in a least squares sense, setting its derivative equal to zero, and using a Newton-Raphson approach. A Levenberg Marquardt algorithm is used to repeatedly solve the equation

$$\Delta \mu = \left( J^T J + \lambda I \right)^{-1} J^T \left( \Phi^C - \Phi^M \right),$$

(1.3)

defining the optical property updates, $\Delta \mu = [\delta D_j, \delta \mu_{ag}]$, at each reconstructed node $j$.

Here, $\lambda$ is a regularization factor to stabilize matrix inversion and $J$ is the Jacobian matrix for our model.

The Levenberg Marquardt inverse solution requires that the initial estimates of optical properties are sufficiently close to the true distribution. For data acquired from a real system, the initial estimate is determined based on a homogeneous fitting algorithm applied to the measurements, improving the likelihood that the convergent solution is in fact a global minimum\textsuperscript{55}. A change in $\chi^2$ (equation (1.2)) of less than 2% is used as a stopping criteria, indicating convergence.
Measure phase and amplitude data

Obtain first guess by least squares fit to data assuming homogeneous tissue

Solve forward diffusion equation using latest estimate of absorption and scattering at each finite element node to determine calculated phase and amplitude

Compare calculated and measured phase and amplitude data

Return new estimate

Does difference exceed tolerance?

No: Finished

Yes: Inverse solution of diffusion equation generates new absorption and scattering at each mesh node

Figure 1.2. Flowchart of the iterative image reconstruction process.

1.C.2.3. Functional image formation

Images of absorption and reduced scattering coefficients are typically reconstructed at six wavelengths from 660 to 850 nm. Absorption at any wavelength ($\lambda$) is assumed to be a linear combination of the absorption due to all relevant chromophores in a sample:

$$\mu_a(\lambda) = \sum_{i=1}^{N} \varepsilon(i, \lambda) C_i,$$

where $\varepsilon$ is the molar absorption coefficient for wavelength $\lambda$, and $C$ is the concentration of each chromophore$^3$. For breast tissue, we usually assume a combination due to oxygenated hemoglobin (Hb-O$_2$), deoxygenated hemoglobin (Hb-R), and water. Figure 1.3 (a) shows a plot of absorption spectra for Hb-O$_2$, Hb-R, water, and lipids for
anatomically relevant concentrations in breast tissue over the entire NIR wavelength range. Hence, given \( \mu_a \) at the \( k^{\text{th}} \) pixel for multiple wavelengths, a linear inversion of equation (1.4) determines the array of \( C \) values representing the concentrations of the three chromophores:

\[
C_k = E^{-1} \mu_{a,k} . 
\]  

(1.5)

The matrix \( E \) contains the molar absorption coefficients, having elements \( \epsilon(i, \lambda) \) for the \( i^{\text{th}} \) chromophore at the different wavelengths used.

The scattering spectrum of tissue (i.e. reduced scattering coefficients reconstructed at different wavelengths) provides information about the nature of the scattering particles and hence the composition of tissue. Since the scattering occurs predominantly from Mie-sized particles, it has been observed that scatter decreases subtly with increasing wavelength, and that this curve can be well characterized by a power law\(^1, 56, 57\). Parameters associated with this fit, termed the scattering amplitude (A) and the scattering power (b), may provide a useful summary of the overall scattering features of tissue, and relate to physiological traits such as average particle size and number density\(^20\). Figure 1.3 (b) shows a power law function fit to two representative scattering spectra for breasts measured in vivo at six wavelengths. This spectral deconvolution of chromophores and scattering parameters is discussed further in Chapter 8.
Figure 1.3. (a) Plot of NIR absorption spectra for oxy-hemoglobin (Hb-O2), deoxy-hemoglobin (Hb-R), water, and lipids. Values for absorption coefficients are displayed for anatomically relevant concentrations in breast tissue. Absorption coefficients for Hb-O2 and Hb-R were calculated using molar extinction coefficients, $\varepsilon$, in $[\text{cm}^{-1}/(\text{moles/liter})]$ compiled by Scott Prahl [http://omlc.ogi.edu/spectra/]. Lipid coefficients were measured by van Veen et al. $^{58}$, and water coefficients were measured by Hale and Querry $^{59}$. (b) Reduced scattering coefficients at six wavelengths measured from two patients are shown, along with the model function fit to the data.
1.D. Hybrid NIR-MRI breast imaging

1.D.1. Motivation

NIR methods have shown promising results in recent years, but the fundamental roadblock to clinical application is their inherent low spatial resolution. The resolution limit of NIR imaging is difficult to express with a single value. The type of reconstruction methodology described in Section 1.C tends to produce blurred images of the tissue optical properties. For example, Jiang et al. achieved 4 mm resolution for a 2:1 contrast in an 86 mm diameter phantom object. Dehghani et al. studied the resolution, contrast, and localization of small objects with a 3D reconstruction algorithm and found that the properties may vary depending on the location of the anomaly within the imaging domain. The spatial resolution is dependent on many factors, including the imaging geometry and number of projections used, tissue thickness, optical contrast, and noise in the measurements.

Advances in modeling and reconstruction techniques cannot overcome the fact that diffuse optical imaging is a non-unique, ill-posed, and often underdetermined problem. Inherent resolution limits may not be overcome by increasing the number and the signal to noise ratio of the measured data. Additional information (i.e. additional data types) may be necessary to improve imaging performance further. In medical imaging, it is beneficial to compare the same tissue volume as seen by a variety of modalities, and perhaps more importantly, there is the hypothesis that one imaging system which has high spatial resolution can be used to enhance the reconstruction of another system which has good contrast resolution. This thesis addresses this hypothesis via the development of a combined NIR-MRI imaging system. The synergistic benefits of the combined NIR-
MRI data set are explored here, specifically the ways in which MRI (i.e. high spatial resolution) could be used to enhance NIR (i.e. high contrast resolution) image reconstruction.

NIR image reconstruction may be greatly improved through the application of algorithmic constraints derived from \textit{a priori} knowledge of tissue given by other imaging modalities. Consequently, in research studies NIR techniques have been combined with several high spatial resolution, structure bearing imaging modalities including x-ray tomosynthesis\textsuperscript{63}, ultrasound\textsuperscript{64}, and MRI\textsuperscript{65-67}, to study human tissues and small animals. Priors and constraints can take a wide variety of forms, and currently there are few broadly adopted conventions. It is commonly accepted that such constraints are of great potential value. It is less well known that misguided or erroneous constraints can lead to gross solution errors. This work aims to improve upon these initial results and conventions by developing an optimized data acquisition system and reconstruction algorithm which can rigorously test this issue. The potential benefits of this type of hybrid method can only be evaluated once prototype systems are developed and optimized in a clinical setting. This type of combined system could be a valuable tool for studying poorly understood physiological processes in the breast, and may prove to be an important bridge that carries NIR imaging methods from the lab into accepted clinical application. The composite images produced could greatly benefit radiologists as they interpret mammograms, and ultimately lead to the reduction of unnecessary biopsies.
1.D.2. Hybrid imaging procedure

The integration of MRI into the three key elements of the NIR image formation process are introduced here: (1) MR-compatible NIR instrumentation, (2) MR-guided image reconstruction, and (3) Functional imaging.

1.D.2.1. Data acquisition

A multispectral, frequency domain NIR tomography system was constructed (Figure 1.4) which operates in a clinical MRI magnet, for utilization of MR-guided image reconstruction of tissue optical properties. Using long silica optical fiber bundles, and a nonferrous breast-fiber interface, measurements of light transmission can be acquired simultaneously with MRI scans. Sixteen custom designed bifurcated fiber bundles are held in a planar ring around the full circumference of the pendant breast, allowing for full cross-sectional imaging similar to the stand-alone tomography system described in Section 1.C.2.1. Six intensity modulated diode lasers are used with wavelengths from 660 to 850 nm. The source light is delivered to the tissue through the central fibers in each bundle, and the remaining fibers branch off to sixteen PMTs. There are no moving parts in the detection channels, and PMT gain levels are electronically controlled on a time scale of 200 ms, thereby allowing rapid switching of the source to locations around the tissue. The complete measurement (i.e. 240 phase and amplitude measurements at six wavelengths each) takes approximately six minutes. The optical fiber holder is housed inside an open architecture breast array coil that offers high resolution MR imaging capability. This data is acquired in parallel with NIR data acquisition. Typically, a stack of coronal T1-weighted images are acquired which provide structural information about
the full volume of breast tissue probed with NIR light. Chapter 2 gives a full description of this instrumentation.

Figure 1.4. (a) Photograph of our portable NIR-MRI tomography system computer and light generation and detection electronics. (b) Patient lies prone on MRI platform with breast pendant through hole. (c) An open architecture breast array coil houses the NIR fiber array positioning system, which can be adjusted to fit the pendant breast.

1.D.2.2. MR-guided NIR image reconstruction

In NIR image reconstruction, knowledge of tissue structure can be used to constrain/guide the iterative process, and improve the spatial resolution and quantitative accuracy of recovered physiological parameters. Past studies have implemented a variety of imaging systems, imaging geometries, and numerical reconstruction techniques, and do not offer a consensus on the optimal way of applying \textit{a priori} derived constraints. Chapter 3 explores a variety of ways which priors, derived from the MRI, can be used to guide/improve tomographic NIR image reconstruction.
A flow chart of the image formation process is shown in Figure 1.5. The steps which follow data acquisition have been automated as much as possible, and may take as little as ten minutes to complete if 2D images are desired. 3D FEM meshing and image reconstruction adds approximately 1-3 hours. The first step is to use the MRI to generate a FEM mesh for use in light propagation modeling and optical property image reconstruction. The MRI images are segmented into multiple regions based upon their grayscale intensity using image segmentation software (described in Chapter 7) written specifically for this purpose. Generally boundaries can be defined between the different tissue types visible in breast MR images. FEM meshes are generated which contain this
‘regional’ information. Also, the locations of the optical fiber source/detectors, which are individually marked with fiducials, can be defined with millimeter accuracy.

The next step is data calibration, (also described in Chapter 7) which employs the use of reference measurements taken from a homogeneous phantom in order to correct for systematic variations between detection channels, and model-data mismatch. Calibrated data then passes to an image reconstruction algorithm which uses the region information defined from segmentation to guide the iterative parameter updates. Recent work at Dartmouth has focused on developing reconstruction algorithms which utilize this region information\textsuperscript{69-72}. An algorithm which allows good flexibility upon use with \textit{a priori} data incorporates the MR spatially segmented regions into a regularization matrix which links locations with similar MR properties, and applies a Laplacian-type filter to minimize variation within each region and preserve known contrast edges. In order to introduce a spatial constraint, the least squares functional which is minimized in reconstruction includes a penalty term for \textit{a priori} information on tissue structure. The resulting objective function is given by\textsuperscript{71, 73}

\[
\chi^2 = \sum_{i=1}^{NM} \left( \Phi_i^C - \Phi_i^M \right)^2 + \beta \sum_{j=1}^{NN} L(\mu_j - \mu_{n,j})^2 , \tag{1.6}
\]

where \(\beta\) is the regularizing factor for the spatial prior and \(L\) is a matrix generated from MRI-derived spatial data, acting on the solution \(\mu\). For the \(i^{th}\) node of \(N\) in region \(R\), \(L_{ii}=1\). When nodes \(i\) and \(j\) are in the same region, \(L_{ij}=-1/N\), otherwise \(L_{ij}=0\). \(NN\) is the total number on nodes with parameters being estimated. As with conventional image reconstruction, minimization of equation (1.6) is accomplished with a Newton-Raphson iterative method\textsuperscript{54}. The optical property updating is governed by the matrix equation
\[
\Delta \mu = \left( J^T J + \beta L^T L \right)^{-1} J^T (\Phi^c - \Phi^M).
\] (1.7)

The value of \( \beta \) is automatically scaled depending on the magnitude of \( J^T J \). It was determined empirically through simulation and phantom experiments with this system that the same scaling should be used regardless of the geometry being imaged. \( L \) links all of the locations (nodes) in a particular tissue type (glandular or adipose), and \( L^T L \) applies a second differential operator within each region. Following estimation of absorption and reduced scattering coefficients at multiple wavelengths, tissue chromophore and scattering maps are derived as described in Section 1.C.2.3.

The benefits of structural information are explored in simulation studies, phantom experiments, and patient examinations. Simulations are used primarily for algorithm development. Algorithms are tested using experimental data measured from tissue-simulating phantoms. Methods which hold up at this stage are applied to the study of patients in vivo.

1.D.2.3. Functional imaging

The two step procedure which involves optical property image reconstruction, followed by spectral fitting can be referred to as ‘indirect imaging.’ Given higher resolution optical property images, higher resolution functional parameter images can be derived. While previous studies which incorporate anatomical information as a spatial prior have shown improvement in algorithm stability and convergence, and image resolution, our results indicate that functional parameter quantification by this approach may be suboptimal. The incorporation of \textit{a priori} spectral information significantly improves the parameter estimation accuracy observed in the recovered images. ‘Direct imaging,’ sometimes called ‘spectral imaging,’ incorporates the known spectral behavior
of tissue chromophores and Mie theory approximation for scattering as constraints. This type of reconstruction uses multi-wavelength measurements simultaneously to compute images of constituent parameters, without intermediate recovery of optical properties\textsuperscript{74-76}. Chapter 8 describes a reconstruction algorithm which incorporates both spatial and spectral priors simultaneously. Using this technique, high resolution, quantitatively accurate images of functional tissue parameters can be created. This procedure is used in the patient studies in Chapter 9.

1.E. Experimental studies

1.E.1. Computer simulation

The first step in testing any image reconstruction algorithm involves the use of computer simulations. ‘Measured’ data is generated by solving the forward FEM problem for a known (simulated) model and distribution of optical properties. Noise can be added to the data to better replicate experimental conditions, and the reconstruction program is applied in an attempt to recover the initial images. The parameter estimation problem is ill-posed and under-determined, so simulations can be used to test both the feasibility of the inverse solution and to evaluate methods for improving the results of the algorithm. Relevant simulation studies are performed in Chapter 4 which explore the effects of noise, regularization, imaging geometry, model heterogeneity, and anomaly contrast on spatial resolution and quantitative accuracy.
1.E.2. Phantom experiments

Following computer simulation, phantom experimentation is the second step in evaluating image reconstruction. Objects which mimic the absorption and scattering properties of breast tissue have been constructed with liquids, rigid plastics, and pliable gelatins, as described in Chapter 5. Recipes have been developed over several years at Dartmouth College, and can be tailored to give a wide range of optical properties. Gelatin phantoms are particularly well suited to the validation of this NIR-MRI imaging system. These are pliable with realistic breast-like stiffness and can be molded into arbitrary shapes. India ink or blood and titanium oxide (TiO$_2$) can be added to adjust absorption and scatter, respectively. Several different mixtures can be combined, building multi-layered structures with different properties. Because gelatin is water-based, complex designs which include internal boundaries can be visualized with MRI, allowing for the evaluation of algorithms which utilize spatial priors. Figure 1.6 shows an example of a phantom study$^{68}$. Several others are presented in Chapter 7.
Figure 1.6. (a) A cylindrical breast tissue phantom made from gelatin. (b) A T1-weighted MRI showing the layered cross-sectional structure of the phantom. (c) An FEM mesh of this geometry which contains the same boundary information visible in the MRI. (d) Reconstructed optical property images. (e) Images reconstructed using the edge information in the FEM mesh to guide optical property updates. (f) The true optical properties of each of the three material which comprise the phantom. These values were estimated from measurements of each material in its homogeneous state.

1.E.3. Patient imaging

Following an initial set of successful phantom studies, a series of female volunteers have been imaged with the combined NIR-MRI system. We demonstrate the ability to quantify tissue hemoglobin, water and subcellular organelle scattering on a spatial scale delineated by MRI, but utilizing the spectral information of NIR. In vivo

<table>
<thead>
<tr>
<th></th>
<th>Outer Layer</th>
<th>Inner Layer</th>
<th>Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_a$ (mm$^{-1}$)</td>
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<td>0.0062</td>
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<td>$\mu'_s$ (mm$^{-1}$)</td>
<td>0.51</td>
<td>0.68</td>
<td>0.9</td>
</tr>
</tbody>
</table>
results shown in Chapter 9 suggest that this combined system can quantify the properties of adipose and fibroglandular tissues present in the breast, even when they have complex spatial organization. A representative patient case is shown in Figure 1.7. Most NIR imaging systems, due to their limited spatial resolution and lack of accurate co-registration with other modalities, are only capable of imaging the properties of bulk tissues. In our study of healthy women, we present the first quantitative estimates of component tissue properties. Observed contrasts reflect known physiological differences between adipose and glandular tissue. Assessment of the normal range of temporal and spatial intra-subject variations in adipose and glandular tissue optical properties, as well as inter-subject variations could improve the understanding of breast physiology, density, and progression to disease.

Figure 1.7. (a) Anatomically axial and coronal T1-weighted MRI slices through a healthy patient with scattered radiographic density, and the 2D (coronal) FEM mesh used for image reconstruction which defines the coordinates of adipose and glandular tissue locations (nodes). (b) Reconstructed images of NIR chromophores parameters total hemoglobin concentration, hemoglobin oxygen saturation, water, scattering amplitude, and scattering power.
1.F. Future directions

Conclusions are drawn in Chapter 10 relevant to specific findings of this work, and to the clinical role of optical devices as adjuncts to other imaging systems. Improved image quality is achievable using coregistered anatomical information, increasing the potential for relevant physiological investigation—several studies are suggested for the near future. (1) Further study of the effects of prior information in 3D modeling and image reconstruction are warranted. The combined data set furnished by this system could be very useful in the developing a more complete understanding of model-data mismatch when 2D approximations are made. (2) It will be very exciting to use this combined imaging system to study breast diseases. (3) Several NIR-MRI cross-validation studies are possible. Vascular function can be assessed when contrast is used in MRI, but MR generally provides only structural information. Researchers are actively pursuing pulse sequences which do offer functional information without exogenous contrast. One such sequence is used in Chapter 9 to quantify water and lipid fractions in tissue. Additional comparison of these two fractions, as measured with NIR and MRI would be useful. MR can also be used to estimate tissue oxygenation (through $\Delta R2^*$ and $\Delta R2$ with the administration of carbogen), which can be correlated with NIR’s hemoglobin oxygen saturation.
Chapter 2 : Instrumentation

2.A. Introduction

The design and development of the NIR-MRI data acquisition system and patient interface are described in this chapter. The previous generations of stand-alone NIR systems used at Dartmouth, including the current instrumentation which is referred to here as the ‘clinical system’ because it is being tested in a clinical trial, are summarized and used as an introduction to the NIR-MRI system. Both devices, designed for tomographic (cross-sectional) breast imaging, deploy six discrete wavelengths ranging from 660 to 850 nm, and operate in the frequency domain. The NIR-MRI electronics are housed in a portable cart which is easily rolled to and from the laboratory and the clinical MRI suite in an adjacent wing of Dartmouth Hitchcock Medical Center (DHMC). Attached to this cart are 16 optical fiber bundles which extend 13 meters into the MRI scanner. The MR-compatible fiber-patient interface is also discussed—three different versions have been designed and used. Following the description of the hardware design, the detector calibration process is explained, and system performance is characterized in terms of measurement repeatability. Finally, the NIR-MRI data acquisition procedure is laid out, specifying the MR data types that are most useful, as well as the total time required for a complete in vivo examination.
2.B. Dartmouth’s stand-alone NIR tomography systems

2.B.1. Early versions

The first experimental NIR tomography system was engineered almost ten years ago at McMaster University\textsuperscript{46, 77, 78}. This system used a single amplitude modulated diode laser (751 nm) and a photomultiplier tube (PMT) detector to image liquid phantoms, and required manual repositioning of a source and a detector fiber in order to acquire multiple measurements. Data from this first system was used to show that Dartmouth’s finite element model reconstruction based on the diffusion approximation could be used to simultaneously recover images of absorption and scattering from experimental measurements. After initial success of this setup, efforts were made to create an automated data acquisition system to increase productivity of phantom studies at Dartmouth College. A system was built which used linear translation stages to multiplex the source and detector\textsuperscript{79}. Fiber bundles (16 source and 16 detector fibers arranged alternately) were held in a circle by a machined plastic ring with thumb screws over each fiber (Figure 2.1 (a)). Next, to gain spectroscopic information, the single diode laser was replaced first with a Titanium Sapphire (Ti-S) laser tunable between 700 and 850 nm, and then with a collection of diode lasers: 661, 761, 785, 805, 826, and 849 nm. Success of spectroscopic imaging studies motivated the design of an automatic fiber positioning system which could be used to image the female breast. A device which used an 8-jaw precision lathe chuck with 2 cm of travel was constructed which provided circularly symmetric radial fiber movement by rotating the base of the chuck\textsuperscript{80}. This assembly is shown in Figure 2.1 (b), and was quite successful.
Figure 2.1. (a) Early tomography setup with automatic source detector multiplexing, but manual individual fiber positioning. (b) The lathe-chuck fiber positioning system.

The clinical system, described next, allowed for fast and automatic measurement using an improved patient interface and parallel detection. Troy McBride’s Ph.D. thesis provides a complete explanation of this measurement system’s evolution. His thesis also gives details on the specifications of these systems, including heterodyning techniques and methods for preventing detector saturation given the large dynamic range of detected light levels from a tomographic geometry.

2.B.2. The ‘clinical system’

This system (Figure 2.2) has imaged nearly 200 female volunteers to date, many with breast disease. It is a frequency domain system which uses six amplitude modulated diode laser sources (660-849 nm), nominally operated at 100 MHz. The laser diodes are
driven by a single DC current source and frequency generator. The DC and AC signals are combined with a bias tee and input into a 1x6 RF switch, computer controlled through six digital lines. The laser diodes are mounted in fiber launch modules with the outputs directed into the inputs of a 6x1 fiberoptic combiner. The combined output leads to the parallel detection array where it is multiplexed using a rotation stage to the source fibers (1 cm in diameter) connected to the breast surface through the patient interface. In 2003, two additional planes, each with 16 optical fibers were added to the measurement head for 3D data collection and imaging.

The detection array consists of 15 PMTs, 15 RF mixers, and two 1x16 splitters mounted on the same rotation stage that is used for source multiplexing. A circular detection geometry is used, thus the detectors nearest to the source receive orders of magnitude more incident light intensity than those farthest away. The PMT gains are varied to account for this large variation. The rotation stage maintains each PMT’s position relative to the source, so the PMT gains are adjusted only once at the start of the imaging session. One source is initially activated, and PMT gains are each adjusted until their DC voltage signal falls within the limited dynamic range of the PMT. The signal from a second frequency generator (e.g. 100.0005 MHz) is divided 15 ways with a RF splitter, and used for electrical heterodyning the signal from the PMTs through the RF mixers. Thus, the 100 MHz PMT signal is converted down to a lower frequency (e.g. 500 Hz) which is easily read by the computer. This is similar to the mixing of AM/FM radio signals with a local oscillator signal and measuring the beat frequency.
Figure 2.2. (a) Drawing of the source and detector multiplexing system connected at a distance to the optical fiber patient interface. Sixteen linear translation stages hold three optical fibers each, giving three imaging planes which can be adjusted radially and vertically. (b) A photograph of the same optical system. (c) The patient examination table, with a hole placed over the optical measurement head. (d) A female subject positioned on the table with her breast pendant in the imaging array.

The ‘patient friendly’ optical fiber positioning system and the rotation stage which facilitates the light delivery and detection sit underneath a custom built patient bed. Sixteen motorized linear translation stages are arranged in a circle for radial positioning of the array of fiber optics. Both the radial diameter and the vertical position are varied.
by a push-button controller. All equipment is controlled through a single PC computer running LabVIEW\textsuperscript{TM} software.

2.C. NIR-MRI system design

A NIR tomography system has been designed and constructed with the same capabilities of the clinical system, and has been made portable, and compatible with MRI. This section describes the four elements that comprise the NIR-MRI system: (1) light delivery, (2) detector array, (3) fiberoptic patient interface, and (4) computer control. The system was described in detail by Brooksby et al.\textsuperscript{68}, and is shown schematically in Figure 2.3.

![Figure 2.3. Schematic design of the NIR-MRI system. NIR tomography is performed inside the MRI unit. Six diode lasers are amplitude modulated, and sixteen projections yield 240 measurements of amplitude and phase of transmitted light. The MRI simultaneously provides a full volume rendering of the tissue structure probed with light.](image)
2.C.1. Light delivery

The system deploys six diode lasers (Table 2.1). Each wavelength is amplitude modulated at 100 MHz by mixing a DC current source (LDX-3220, ILX Lightwave, Bozeman, MT) and an AC current from a frequency generator (IFR-2023A, IFR Systems, Wilmington, MA), through a bias T (#5545, Picosecond Pulse Labs, Boulder, CO).

<table>
<thead>
<tr>
<th>wavelength (nm)</th>
<th>power (mW)</th>
<th>Operating Current</th>
</tr>
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<td>100</td>
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<tr>
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<td>70</td>
</tr>
<tr>
<td>849</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 2.1. Diode laser wavelengths, powers, and nominal operating currents.

Each diode is held in a laser tube (Thorlabs, Newton, NJ), and mounted on a linear translation stage (MA2515P5-S2.5 Velmex, Bloomfield, NY). This stage directs a specified wavelength into one of sixteen bifurcated optical fiber bundles which were custom designed for this application (Ceramoptec, East Longmeadow, MA). The 248-piece bundles (0.37 N.A., 0.68 packing fraction) have pure silica core (210 µm), silicone clad (230 µm) fibers suitable for transmitting wavelengths from 400 nm to 2400 nm. The source light is delivered through the central seven fibers in each bundle, and the remaining fibers surrounding these are delivered to the detectors. The common end, which makes contact with the tissue, has a diameter of 4 mm. Each fiber bundle is 13 meters in length and extends from the instrument cart, located outside of the MR suite, into the bore of the scanner (1.5T whole body imager, GE Medical Systems, Milwaukee, WI) to the patient interface. The efficiency of the optical switching is approximately 50%, yielding an average source power of 15 mW at the tissue surface. High optical
efficiency is important to this design because the fibers used in this system, although
having more efficient transmission, are smaller than those used in the clinical system (i.e.
4 mm compared to 6 mm).

2.C.2. Light detection

The light delivery and detection systems are mounted in the portable cart in
Figure 2.4. For each source excitation, light transmission is recorded from 15 surface
locations. This signal is measured by 15 of 16 photomultiplier tubes (PMT R6357,
Hamamatsu, Japan) housed in the base of the cart, operating in parallel. The gain of the
PMTs is varied to account for the large variation in light level between detectors
depending on their distance from the source. The gains are set on PMT modules (HC120,
Hamamatsu) by applying computer generated voltages between 0.4 and 1.2 V to their
control lines, which places the anode to cathode voltage between approximately 350 V to
1000 V, respectively. Using the higher gain settings, a PMT can reliably measure optical
signals in the pW range. The optimal gain levels are determined prior to each imaging
series. Each PMT is fixed to a specific fiber, making it necessary to switch gains
electronically during the course of data collection. A 100 MΩ resistor is used in the
dynode chain discharge circuit of each PMT to achieve fast settling times after gain
adjustment (200 ms for large gain changes). Electrical heterodyning through RF mixers
(Minicircuits, Brooklyn, NY) is used to down convert the 100 MHz PMT signal to a
lower frequency (500 Hz). This offset frequency is achieved with a second frequency
generator which is synchronized to the one driving the laser current, and is set to
100.0005 MHz (3 dBm). The resulting offset frequency is filtered and amplified by a 16
channel circuit designed for this application (Audon Electronics, Nottingham, UK), then
read by the computer. Lock-in detection is executed in software to extract amplitude and phase data for each of the detectors in parallel. Unlike the clinical system, there are no moving parts in the detection channels, improving optical efficiency and detection sensitivity.

Figure 2.4. Photograph of the rack mounted, portable system showing (a) the frequency generators, (b) the linear translation optical switching stage, (c) the PMT detection plate, and (d) 13 meter bifurcated optical fiber bundles which are coiled on a hanger on the side of the cart. (e) The cart and computer stand outside of the RF-shielded MRI, and the fibers pass through a cracked door into the bore of the magnet, (f).

2.C.3. Fiberoptic patient interface

The MR exam is performed using a breast array coil (MRI Devices, Waukesha, WI) that offers high-resolution MR imaging. The coil also provides an open architecture, which allows for the integration of the NIR breast interface. Three generations of this interface have been used. The first fiber holder, shown in Figure 2.5 (c), was a circular
ring machined from black acetal (delrin) with 16 holes at equal angles (22.5 degrees) for each of the 16 fibers. Each fiber was held in place with a thumb screw, and the measurement plane was adjusted vertically and tilted if necessary. Vertical posts which support the coil’s chest platform were used to anchor the fiber ring in the desired orientation. This version of the fiber holder was useful in preliminary phantom studies.

Figure 2.5. (a-c) Photographs of the original optical fiber ring. Thumb screws held each fiber in place. The ring was manually positioned vertically, clamping to the support posts in the MRI breast array coil. This ring was used to perform initial phantom studies, but was not practical for patient imaging. (d) Grooves were cut into shaft collars and filled with MRI contrast material which could be used to identify the location of each fiber. In the center is a commercial fiducial, which proved a better solution.

At this stage different methods for locating the precise position of each of the optical fibers in the MR images were investigated. Large artifacts result in image
reconstruction if source and detector locations are not specified correctly. Because each fiber was free to move independently (before thumb screw tightening) it was important that each fiber be labeled with a fiducial (or any MRI contrast agent). However, the first attempt at constructing permanent fiducials was unsuccessful. Flexible spaghetti tubing was filled with a variety of liquids visible in MRI (i.e. water, gadolinium, and copper sulfate ($\text{CuSO}_4$) solution), and was sealed into small donuts which were inset into shaft collars (Figure 2.5 (d)) clamped close to the fiber tip. It was difficult to place a sufficient amount of liquid around the full perimeter of the fiber to make it visible in MRI.

Commercially available fiducials (#MM3005, IZI Medical Inc. Baltimore MD) proved easier to use and very easy to locate in the MRI. They are donut-shaped (i.e. annular) packages of $\text{CuSO}_4$ paste, and can be slid over the end of each fiber prior to an exam. The paste dries out after approximately two weeks, so these markers must be replaced periodically.

The second fiber holder, shown in Figure 2.6, allowed for semi-automatic fiber-positioning. The circular ring was machined from polyvinyl chloride (PVC). Phosphor bronze compression springs ($k=0.09\text{lbs/in}$, Ace Wire Spring & Form, McKees Rocks, PA) guided each fiber through holes in the ring, into light contact with the tissue surface. The ring separated into equal halves so that it could easily be moved from one breast to the other. Like the first design, this ring could be positioned vertically such that the plane of measurements intersected the region of interest in tissue. This construction was only semi-automatic because it required that a nurse or technician manually retract each fiber so that the patient could position herself on the coil platform. With the breast pendant through the opening, the fibers were then released and fiber tissue contact was confirmed.
visually. Adjustments were made if needed. This system was used to image eight women, providing useful preliminary in vivo data. However, the freedom of each fiber to move independently typically resulted in highly irregular imaging geometries.

Figure 2.6. Schematic design and photographs of the spring-loaded fiber positioning system. This semi-automatic ring was used to image several patients.

Generally, NIR data quality and image reconstruction is more effective on circular geometries than on some of the distended shapes commonly observed with the first design. This observation was anticipated by Pogue et al.\textsuperscript{82} where it was observed that geometries which maximize symmetry in the projection angles often yield the best images. This fact motivated the construction of the semi-automatic fiber positioning
system currently in use, which enforces equal fiber radii (Figure 2.7). The ring is made in three parts machined from delrin. One ring has square channels which guide the optical fibers, encased in square sheaths, toward the center of the ring. The fiber sheaths have several ‘teeth’ which fit into a spiral groove cut into a second ring. When the second ring is turned underneath the first, each fiber sheath is actuated simultaneously, and the fiber diameter is adjusted. Like the first two designs, the plane of fibers can be adjusted vertically and angled inside the MR coil. Subject feedback indicates that the examination is comfortable.

Figure 2.7. Designs and photographs of the fiber positioning system currently in use. Drawings of the three pieces involved are shown (top/center), including the fiber guide, the fiber sheath, and the sheath actuating spiral. The spiral is attached to the fiber guide, and when turned, moves all 16 sheaths simultaneously. A circular measurement geometry is rigidly maintained, and can be adjusted to accommodate phantom/tissue diameters from 6 to 12.5 cm.
2.C.4. Computer system

A PC running LabVIEW™ (National Instruments™, Austin, TX) is used to control all light delivery and detection equipment. The laser current source and frequency generator parameters are set by a general purpose interface bus (GPIB, NI). The linear translation stage is addressed through the serial port. An analog output board (NI) is used for PMT gain control. A multipurpose data acquisition (DAQ) board acquires the 16 analog input channels and the single reference channel. This board also provides six digital output lines to the high power radio-frequency switch for the laser sources. For each source position, 15 signals from the detector system are amplified by a gain of 100 and low pass filtered to prevent aliasing prior to the DAQ board using a 16 channel amplifier and filter network mounted in a BNC coupled box (Audon Electronics, Nottingham UK). Data are acquired for 500 ms, and phase and amplitude of each signal are calculated and saved. The MR exam is controlled separately, and is performed in parallel. Details related to the data acquisition protocol are given in Section 2.E.

2.D. System calibration

2.D.1. Detector calibration

Each detection channel has a different amplitude and phase response to the same optical signal due to PMT variation, mixer performance, and fixed offsets in the RF splitter. Also, PMT response varies significantly for the same optical signal detected at different gain settings. At a single gain setting, however, for the AC voltage range used, phase is approximately constant, and the detected voltage is linear with respect to the power of the optical signal.
Figure 2.8. Graphs showing measurements used to calibrate a representative PMT. (a) The log amplitude response is linear with the log of input optical power. Linear regression is performed on each line and the y-intercept and slope are used as calibration factors. (b) Over the usable range of input power, the phase response does not change significantly with input power changes. The phase offsets between different gain settings are stored as calibration factors.

A one-time calibration over the entire useful range of light levels and gains is applied to characterize detector response and remove systematic noise in the data acquisition hardware. Each detector is exposed to the same optical signal, and the differences in log amplitude and phase are used as correction factors. A single source is placed in the center of a homogeneous diffusing object and measurements are
obtained at each detector site. The light intensity alone is changed using a neutral density filter wheel (New Focus). Figure 2.8 (a) shows the log amplitude response of a representative PMT, plotted against the log of the input power for each gain setting. A log-log regression is performed and the coefficients are used to calibrate detected PMT amplitude in terms of input optical power. The phase does not fluctuate significantly with changing light level for a single gain setting (i.e. minimal phase-amplitude cross-talk), but is altered dramatically with changing gain, as shown in Figure 2.8 (b). Relative phase differences between detectors are also stored for calibration. This characterization needs to be performed only once as long as the system hardware is not modified. It is important that these calibration values be accurate considering that in a single data acquisition, measurements are taken with each PMT set to many different gains.

2.D.2. System performance

Measurement repeatability in terms of log amplitude and phase error was assessed by serially imaging a phantom with optical properties similar to those of average breast tissue (\( \mu_a =0.004, \mu_s'=1.35 \), at 785 nm). The average root-mean-square (RMS) error at each detector site was determined to be 0.26% in AC intensity and 1.04 degrees in phase. These values compare with those obtained from the clinical system described by McBride et al. \(^47\) (0.32% in AC intensity and 0.48 degrees in phase). RMS error for each of the 240 source-detector combinations for both systems is plotted versus PMT signal in Figure 2.9. As shown in Figure 2.8, the lowest detectable signal occurs in the picoWatt range. For both amplitude and phase, error sharply increases when incident light falls below approximately 0.5 pW. These points are excluded in the calculation of average RMS error.
Figure 2.9. Repeatability assessment for (a) log amplitude and (b) phase of the NIR component of the combined NIR-MRI system and the clinical system. The performance of the two systems is comparable. In routine operation, PMT voltage signals are above 1x10-5 Volts.

As an additional comparison, both devices were used to measure a collection of homogeneous phantoms (N=15) of varying diameters (73-91mm) and optical properties ($\mu_a = 0.0023-0.0102 \text{ mm}^{-1}$, $\mu_s' = 0.33-1.91 \text{ mm}^{-1}$). A homogeneous fitting algorithm described in Section 7.C, was used to determine a global $\mu_a$ and $\mu_s'$ value for each material (Figure 2.10). Table 2.2 shows the optical coefficients obtained with the NIR-MRI system, along with a measure of their discrepancy with those obtained with the
clinical system. Good agreement between the two systems is observed, consistent throughout the range of phantom properties.

Figure 2.10. Optical properties at 785 nm for a collection of homogeneous phantoms (N=15), measured by the clinical system and the NIR-MRI system. The agreement of the two systems is very good (i.e. within 10%).

<table>
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</tr>
<tr>
<td>NIR-MRI system</td>
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<td>2.5</td>
</tr>
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</table>

Table 2.2. A summary of the (homogeneous phantom) system comparison in Figure 2.10.
2.E. Examination protocol

Approval was granted from the Institutional Review Board (IRB) at the Dartmouth Hitchcock Medical Center for the clinical examination protocol, and informed consent is obtained from all volunteering women. MRI contrast agents are also approved for use with this study. The NIR and MRI data acquisition are controlled separately. All of the LabVIEW™ programs relevant to the NIR system are explained in the Appendix. Including the time required to determine optimal gain values, measurement with 6 wavelengths takes approximately five minutes. For circular fiber geometries, one source position is used to determine 15 gain settings, which are then cycled with source multiplexing in the measurement. For some of the distended breast shapes observed with the spring-loaded fiber patient interface, it was necessary to cycle through all source positions in order to determine all 240 gain settings, adding several minutes to the exam time.

The MRI console is operated by a technician. DHMC has three 1.5 T (GE) whole body scanners. The breast receiver coil is compatible with all three, but typically the Excite model is used because its pulse sequence repertoire includes ‘Spiral Hi-Res’ for quantifying water and fat. A protocol entitled ‘NIR MRI BREAST’ contains the sequences typically used and all of the related parameters are summarized in Table 2.3. The relevant receiver coil is selected (i.e. R_BREAST or L_BREAST) and the examination begins with a three plane localizer (scout) to quickly visualize the image geometry. It provides a few slices each in the axial, sagital, and coronal orientations. An axial or sagital slice which shows two fiducials at opposite ends of the imaging volume is used to prescribe the orientation of the T1-weighted 3D SPGR volume. This is done so
that one slice in the volume will be oriented in the exact plane of the optical fibers. Each slice in the volume is viewed as anatomically coronal/oblique (i.e. circles). Acquisition of the scout images and the full volume breast MRI takes approximately five minutes.

The other two sequences listed are only used in selected exams.

**NIR MRI BREAST protocol**

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</tr>
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<tr>
<td>TE</td>
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</tr>
<tr>
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</table>

Table 2.3. MR Protocol sequence parameters. The Fast SPGR Loc. and 3D SPGR sequences are always used, the other two are optional, depending on the study.

Early scans showed high levels of tissue deformation and susceptibility artifacts were suspected around the area of the plastic fiber tips. T2-weighted scans which showed more exquisite boundary detail indicated that this deformation was in fact real. T2 scans are not usually acquired—one patient described a warm sensation during this sequence, indicating possible power deposition in the acetal fiber holder.

Section 9.E discusses the measurement of water and fat content with MRI, and subsequent comparison with NIR measurement of these parameters. The Spiral High-Res sequence is used in these studies. This sequence adds approximately one extra minute to
the total exam time. The same sequence is used for both measurements, first selecting the water proton resonance peak (to get the water image) and then selecting the lipid proton resonance peak (to image fat). Manual shimming in the x, y, and z scanner directions can greatly improve the quality of these images and is recommended.

Contrast enhanced (CE) MRI is desirable for subjects with breast disease. The imaging procedure is as follows: (1) Acquire T1 structural map of tissue volume with ‘3D SPGR Volume’. (2) Repeat the same sequence with the application of fat suppression. (3) Inject 20 cc bolus of gadolinium contrast agent. (4) Repeat step (2) three times. Subtracting every slice of the pre-contrast volume from the post-contrast volumes yields images of contrast agent perfusion. (This procedure was followed for subject #1903)

In preliminary work the NIR instrumentation and computer carts stood outside of the RF shielded MR chamber and the door was kept ajar to pass the bundle of cables. On several occasions, this induced artifacts on adjacent MR scanners. A shield (of aluminum foil for example) draped over the opening in the door prevented this interference. Shielding complications can be avoided by moving the carts inside the MR chamber and closing the door. It has been confirmed that the magnetic field immediately inside the door (< 5 gauss) does not affect the computer operation or the NIR electronics.
Chapter 3 : FEM reconstruction algorithm

3.A. Introduction

This chapter explains the finite element method (FEM) image reconstruction algorithm. Time harmonic light propagation is described by the well-known diffusion equation, which can be solved numerically as a standard boundary value problem. This is the ‘forward’ problem—determining the fluence rate throughout a domain, assuming a known distribution of optical properties, appropriate boundary conditions, and a source of photons somewhere on the boundary.

The ‘inverse’ problem is the imaging process, where the optical properties are unknown, and only limited ‘boundary data’ is known. The image formation task is to make estimates (which are updated and improved iteratively) of the optical property distribution that is required to sustain the measured boundary data under the diffusion approximation. A Newton-type iteration scheme in conjunction with a finite element forward solution is used. Using FEM, image formation is a nonlinear optimization process, where the optimization parameters are coefficients in a basis function expansion for the spatial distribution of tissue optical properties. With the two data types available from a frequency domain measurement system, separation of absorption and scattering is possible.
3.B. The forward problem

3.B.1. Diffusion approximation

Understanding how radiation propagates through tissue is crucial in quantitative analysis of diagnostic measurements, and in designing certain treatments. In breast tissue, scattering events are far more likely than absorption events, and the relative probability of these two processes depends largely on the wavelength of the light. The statistical behavior of the electric field can be described using Maxwell’s equations, but this strategy is complicated and therefore widely avoided. The usual approach is radiative transport theory (the particle interpretation of light). This theory assumes that scattering is elastic, and that the transport of photons can be described by two independent coefficients—absorption ($\mu_a$) and scattering ($\mu_s$) (not to be confused with the reduced scattering coefficient $\mu'_s$) describing the likelihood of a photon being absorbed or scattered over a given distance (units of mm$^{-1}$). The phase function ($\psi$) defines the probability that a photon traveling in direction $\hat{s}$ will scatter into direction $\hat{s}'$. The most widely evoked equation in optical imaging is the radiative transfer equation (RTE)$^{84,85}$:

$$\frac{1}{c} \frac{\partial L(r,\hat{s},t)}{\partial t} + \hat{s} \cdot \nabla L(r,\hat{s},t) + (\mu_a + \mu_s)L(r,\hat{s},t) = \mu_s \int L(r,\hat{s}',t)\psi(\hat{s},\hat{s}')d^2\hat{s}'+S(r,\hat{s},t).$$ (3.1)

It describes the radiance ($L$) as a function of position ($r$), direction ($\hat{s}$), and time ($t$), for a given source $S$, speed of light in the medium ($c$), and set of coefficients ($\mu_a, \mu_s, \psi$). The radiance is in units of [Watts/m$^2$ srad].

Practical transport process modeling schemes derived from the RTE proceed either stochastically or deterministically, as described by Arridge$^{24}$. Monte Carlo
simulation is a stochastic method which models individual photon interactions explicitly. Each photon is treated probabilistically and the sum of the effects of a large number of simulated photon histories is a representation of the radiance in the medium. Monte Carlo is accurate but time-consuming, and impractical for many inverse solutions. The RTE is a deterministic equation, and simpler deterministic models can be derived from it. The standard approach is therefore to simplify the RTE to the diffusion equation through a truncated spherical harmonics expansion (P1 approximation) of the radiance, $L^{22,86-88}$. This reduces the integro-differential RTE to a differential equation which can be solved by standard techniques (i.e. analytical methods, finite-difference schemes, and finite element methods). Numerical implementations of the RTE are possible, but complex and time consuming due to the need for angular discretization.

Through simplification, the time domain diffusion equation can be written as

$$\frac{1}{c} \frac{\partial \Phi(r,t)}{\partial t} - \nabla \cdot D \nabla \Phi(r,t) + \mu_a \Phi(r,t) = S_0(r,t), \quad (3.2)$$

where $\Phi$ is the fluence rate, defined as the number of photons passing through the surface of a unit sphere per unit time, $S_0$ is an isotropic source of photons, and

$$D = \frac{1}{3(\mu_a + \mu_s')},$$

is the diffusion coefficient in units of millimeters. Here $\mu_s' = (1-g)\mu_s$ is termed the reduced scattering coefficient, where $g$ is the average cosine of the scattering angle. By performing the Fourier transform in time ($\frac{\partial}{\partial t} \rightarrow i\omega$) of equation (3.2), the frequency domain diffusion equation is obtained as,

$$-\nabla \cdot D \nabla \Phi(r,\omega) + \left(\mu_a + \frac{i\omega}{c}\right)\Phi(r,\omega) = S_0(r,\omega) \quad (3.3)$$

where $\omega$ is the angular modulation frequency in radians.
In order to solve the diffusion equation for the fluence rate ($\Phi$) throughout the imaging domain, the optical properties ($\mu_a, D$), the source term, and boundary conditions need to be defined. The best description of the air-tissue boundary is derived with an index of refraction-mismatched mixed (Type III) condition, in which the fluence normal to the edge of the tissue exits and does not return. Thus the flux crossing the boundary is equal to the fluence rate at the boundary, times a factor that accounts for the internal reflection of light back into the tissue. This relationship is described in the following equation\textsuperscript{22, 86, 88-92}:

$$\Phi(\xi, \omega) = 2A\n\cdot D\nabla \Phi(\xi, \omega),$$

(3.4)

where $\xi$ is a point on the boundary, and $A$ depends upon the relative refractive index mismatch between tissue and air. The coefficient $A$ can be derived from Fresnel’s law \textsuperscript{93}:

$$A = \frac{2/(1 - R_0) - 1 + |\cos \theta_e|^3}{1 - |\cos \theta_e|^2},$$

(3.5)

where $\theta_e = \arcsin(n_2/n_1)$ is the angle at which total internal reflection occurs for photons moving from a region with $n_1$ to a region with $n_2$, and $R_0 = \frac{(n_1/n_2 - 1)^2}{(n_1/n + 1)^2}$. At external boundaries, $n_2=1$ (the index of refraction of free space).

3.B.2. Finite element solution

The use of FEM to model the diffusion equation is attractive due to its geometric flexibility and the ability to preserve the non-linear relationship between the measurements and the properties in the partial differential equation (3.3). In finite element formalism, $\Phi(r, \omega)$ is discretized into a finite set of basis functions multiplied by weighting coefficients,
\[ \Phi(r, \omega) \approx \sum_{j=1}^{N} \Phi_j \varphi_j(r), \quad (3.6) \]

where \( N \) defines the order of the discretization. The properties of the basis functions \( \varphi_j(r) \) are well known, and the coefficients \( \Phi_j \) are the primary unknowns of the problem.

In the Galerkin method of weighted residuals, equation (3.3) is multiplied by an identical set of weighting functions, \( \varphi_i \), and it is integrated over the entire problem domain to give

\[ \left\langle -\nabla \cdot D \nabla \varphi_i \right\rangle + \left\langle \left( \mu_a + \frac{i \omega}{c} \right) \Phi \varphi_i \right\rangle = \left\langle S_{\alpha} \varphi_i \right\rangle. \quad (3.7) \]

The \( \left\langle \cdot \right\rangle \) notation represents the integration over the imaging field. In general we use linear basis and weighting functions. To avoid second-order differentiation of these basis functions, equation (3.7) is manipulated further through Green’s theorem (i.e. integration by parts) which states that

\[ \left\langle \nabla \cdot \mu \right\rangle = \oint (v \cdot \hat{n}) ds - \langle v \cdot \nabla u \rangle. \quad (3.8) \]

Using this theorem (where \( v = D \nabla \Phi \) and \( u = \varphi \)), equation (3.7) can be rewritten in the Galerkin weak form:\(^{94}\)

\[ \left\langle D \nabla \Phi \cdot \nabla \varphi_i \right\rangle + \left\langle \left( \mu_a + \frac{i \omega}{c} \right) \Phi \varphi_i \right\rangle = \left\langle S_{\alpha} \varphi_i \right\rangle + \oint \hat{n} \cdot D \nabla \Phi \varphi_i ds. \quad (3.9) \]

With this representation, boundary conditions can easily be applied through the surface integral, which defines the normal component of the flux through the boundary surface. The field is discretized into a set of points or nodes (Figure 3.1 (a)). These points are connected into elements, which can be viewed as self-contained interpolating units.

Substituting equation (3.6) into equation (3.9), the FEM discretization of the frequency domain diffusion equation for a homogeneous medium is given by:
\[
\sum_{j=1}^{N} \Phi_{j} \left[ D \nabla \varphi_{j} \cdot \nabla \varphi_{j} \right] + \left[ \mu_{a} + \frac{i \omega}{c} \right] \varphi_{j} \varphi_{j} = \left\langle \mathcal{S}_{0} \varphi_{j} \right\rangle + \sum_{j=1}^{M} D \nabla \Phi_{j} \cdot \hat{n} \int \varphi_{j} \varphi_{j} ds,
\] (3.10)

where we are solving for \( \Phi_{j} \), the fluence rate at each node \( j \). Since we are using linear elements, \( \langle \cdot \rangle \) in this case represents integration over neighboring nodes. In the application of type III boundary conditions, we approximate the outward flux at all \( M \) boundary nodes as

\[ D \nabla \Phi_{j} \cdot \hat{n} = \alpha \Phi_{j}, \] (3.11)

From equation (3.4), assuming a refractive index of 1.33 for tissue, \( \alpha = \frac{1}{2A} \approx 0.177 \) for an air-tissue interface.

For an inhomogeneous region, the absorption and diffusion coefficient are also discretized, using the same linear basis functions throughout such that,

\[ \mu_{a} = \sum_{j=1}^{N} \mu_{aj} \varphi_{j}, \] (3.12)

\[ D = \sum_{j=1}^{N} D_{j} \varphi_{j}. \] (3.13)

Substituting equations (3.11), (3.12), and (3.13) into equation (3.10), the FEM discretization becomes

\[
\sum_{j=1}^{N} \Phi_{j} \left[ \sum_{k=1}^{N} D_{k} \varphi_{k} \nabla \varphi_{j} \cdot \nabla \varphi_{j} \right] + \left[ \sum_{k=1}^{N} \mu_{ak} \varphi_{k} + \frac{i \omega}{c} \right] \varphi_{j} \varphi_{j} = \left\langle \mathcal{S}_{0} \varphi_{j} \right\rangle + \alpha \sum_{j=1}^{M} \Phi_{j} \int \varphi_{j} \varphi_{j} ds ,
\] (3.14)

The boundary term contains the unknown fluence rate and is therefore brought to the left hand side of the equation:
In practice, this becomes the matrix solution

\[
\begin{bmatrix}
A_{bb} & A_{bl}^T \\
A_{lb} & A_{ll}
\end{bmatrix}
\begin{bmatrix}
\Phi^b \\
\Phi^l
\end{bmatrix}
= \begin{bmatrix}
S^b \\
S^l
\end{bmatrix},
\]

(3.16)

where the subscript \( b \) denotes portions of the matrix associated with boundary nodes and subscript \( I \) denotes interior nodes. The matrix terms are:

\[
a_{bb}^{ij} = \left( \sum_{k=1}^{N} D_k \varphi_k \nabla \varphi_j \cdot \nabla \varphi_i \right) + \left( \sum_{k=1}^{N} \mu_{ak} \varphi_k + \frac{i\omega}{c} \varphi_j \varphi_i \right) + \alpha \oint \varphi_j \varphi_i \, ds
\]

\[
a_{lb}^{ij} = a_{lb}^{ji} = \sum_{k=1}^{N} D_k \varphi_k \nabla \varphi_j \cdot \nabla \varphi_i + \left( \sum_{k=1}^{N} \mu_{ak} \varphi_k + \frac{i\omega}{c} \varphi_j \varphi_i \right).
\]

(3.17)

\[
S_i^b = \langle S \varphi_i \rangle
\]

\[
S_i^l = 0
\]

It should be noted that equation (3.16) represents the traditional FEM solution formulation used at Dartmouth\textsuperscript{81}. Chapter 4 describes the software package used in this work, which uses a slightly different framework for this system algebraic equations\textsuperscript{95}.

The source term is defined as a distributed, Gaussian source, matching the intensity profile at the tip of the optical fiber. The source may therefore be defined over more than one node or element. Because the source is assumed spherically isotropic, modeling is more accurate when the source is centered one scattering distance within the outer boundary\textsuperscript{54,92}.

The solution of equation (3.16) in terms of the unknown fluence \( \Phi \) therefore becomes a function of matrix division, that is \( \Phi = A^{-1}S \). An example of a forward
solution is shown in Figure 3.1 (b). The complex valued fluence $\Phi$ is calculated at every mesh location (node). Using these values in equation (3.6) results in a solution over the full imaging space. The log of the absolute value of $\Phi$ is plotted. Different methods of solution are used depending on the size of the model. For computational efficiency, a direct solver is used when meshes have less than 4000 nodes. Node numbering is important, and these meshes should be optimized such that the FEM matrix has a minimum bandwidth. For more than 4000 nodes, an iterative BiConjugate Gradients Stabilized solver is used, and meshes are optimized for very sparse FEM matrices.

Important mesh details are discussed further in Section 7.B.

![Figure 3.1](image)

**Figure 3.1.** (a) A FEM mesh with 400 nodes and 700 elements. 16 sources and 16 detectors are evenly spaced around the perimeter. (b) A plot of the logarithm of the fluence generated by a single source excitation.

### 3.C. The inverse problem

In the inverse problem, the goal is the recovery of optical properties $\mu = (\mu_a, D)$ at each FEM node using measurements of light fluence at the tissue surface. The numerical way of achieving inversion is to minimize the difference between measured
fluence, $\Phi^M$, at the tissue surface and calculated data, $\Phi^C$, from the forward solver. We minimize this ‘objective’ function:

$$
\chi^2 = \sum_{i=1}^{NM} (\Phi^C_i - \Phi^M_i)^2 ,
$$

(3.18)

where $NM$ is the total number of measurements given by our imaging device. In general, $\chi^2$ will not equal zero, but instead we are interested in finding the values of $\mu$ for which $\frac{\partial \chi^2}{\partial \mu}$ is close to zero. Following the Taylor series expansion method for deriving Newton’s method, we expand $\frac{\partial \chi^2}{\partial \mu}$ based on $\mu$ for some nearby point $\mu_0$:

$$
\frac{\partial \chi^2}{\partial \mu} = \frac{\partial \chi^2}{\partial \mu} (\mu_0) + (\mu - \mu_0) \frac{d}{d\mu} \left( \frac{\partial \chi^2}{\partial \mu} (\mu_0) \right) + ... \tag{3.19}
$$

and ignore higher order terms. Assuming $\frac{\partial \chi^2}{\partial \mu} \approx 0$ we change (3.19) to an iterative scheme, where $\mu - \mu_0 = \mu_{i+1} - \mu_i$. Solving for $\mu_{i+1}$ we arrive at the standard Newton-Raphson iterative form:

$$
\mu_{i+1} = \mu_i + \left[ \frac{d}{d\mu} \left( \frac{\partial \chi^2}{\partial \mu} (\mu_i) \right) \right]^{-1} \frac{\partial \chi^2}{\partial \mu} (\mu_i) . \tag{3.20}
$$

Using equation (3.18) we solve for the derivative terms:

$$
\frac{\partial \chi^2}{\partial \mu} (\mu_i) = 2 \left( \frac{\partial \Phi^C}{\partial \mu} \right)^T (\Phi^C - \Phi^M) \tag{3.21}
$$

$$
\frac{\partial}{\partial \mu} \left( \frac{\partial \chi^2}{\partial \mu} (\mu_i) \right) = 2 \left( \frac{\partial \Phi^C}{\partial \mu} \right)^T \frac{\partial \Phi^C}{\partial \mu} + 2 \left( \frac{\partial^2 \Phi^C}{\partial \mu^2} \right)^T (\Phi^C - \Phi^M) , \tag{3.22}
$$

57
since $\Phi^M$ and $\mu_a$ are known constants based on the measurement data. In practice, $\mu_b$ is calculated using a homogeneous fitting algorithm\(^{55}\) (section 7.C). The contribution of the second derivative term $2 \left( \frac{\partial^2 \Phi^C}{\partial \mu^2} \right)^T (\Phi^C - \Phi^M)$ is thought to be small and is often discarded\(^{97}\). Inserting equation (3.21) and equation (3.22) into the Newton-Raphson equation (3.20):

$$\mu_{i+1} = \mu_i + \left( \frac{\partial \Phi^C}{\partial \mu} \right)^T \left( \frac{\partial \Phi^C}{\partial \mu} \right)^{-1} \left( \frac{\partial \Phi^C}{\partial \mu} \right)^T (\Phi^C - \Phi^M). \tag{3.23}$$

The derivative matrix $\left( \frac{\partial \Phi^C}{\partial \mu} \right)$ is the Jacobian matrix $J$. $J^TJ$ is ill-conditioned\(^{54}\), and the typical way of dealing with this problem is through regularization, which amounts to adding a quantity to the diagonal of $J^TJ$ to make it more diagonally dominant. In the standard notation, the equation for the optical property update, $\Delta \mu = \mu_{i+1} - \mu_i$, is

$$\Delta \mu = \left[ J^T J + \lambda I \right]^{-1} J^T (\Phi^C - \Phi^M). \tag{3.24}$$

In standard practice $I$ is an identity matrix, and $\lambda$ is implemented in a Levenberg-Marquardt algorithm\(^{98}\), where it starts at a high value (typically ten times the maximum value of the diagonal of $J^TJ$) and is systematically reduced at each iteration. The addition to $J^TJ$ changes the relationships in equation (3.23), and a great deal of work in the field of inverse problems has been dedicated to optimizing regularization. If $\lambda$ is too large, the update vector will be of little value, but if too small, numerical stability problems will arise, especially if $\Phi^M$ is corrupted by noise. Regularization will be discussed further in Section 3.D.3.
The Levenberg-Marquardt algorithm, equation (3.24), can be written in an alternative form known as the (regularized) underdetermined version of the Moore-Penrose generalized inverse:

\[
\Delta \mu = J^T [JJ^T + \lambda I]^{-1} (\Phi^c - \Phi^m)
\]  

(3.25)

Equation (3.25) is known to be highly suitable to problems where the number of unknowns to be recovered is much larger than the amount of information (# of measurements) available, which is the case in 3D image reconstruction. The next section describes the incorporation of spatial constraints into the iterative optical property solution. While both equation (3.24) and equation (3.25) provide a framework for applying these constraints, equation (3.24) is typically used.

The Jacobian, sometimes referred to as the sensitivity or weight matrix, defines the relationship between changes in boundary data \( \Phi^c \), and small changes in optical properties \( \mu = (\mu_a, D) \). In this case, both amplitude and phase data types are used, and the affects of absorption and diffusion must be considered, therefore the structure of the Jacobian is as follows:

\[
J = \begin{bmatrix}
\frac{\delta \ln I_1}{\delta D_1} & \frac{\delta \ln I_1}{\delta D_2} & \ldots & \frac{\delta \ln I_1}{\delta D_N} \\
\frac{\delta \ln I_1}{\delta \theta_1} & \frac{\delta \ln I_1}{\delta \theta_2} & \ldots & \frac{\delta \ln I_1}{\delta \theta_N} \\
\vdots & \vdots & \ddots & \vdots \\
\frac{\delta \ln I_{NM}}{\delta D_1} & \frac{\delta \ln I_{NM}}{\delta D_2} & \ldots & \frac{\delta \ln I_{NM}}{\delta D_N} \\
\frac{\delta \ln I_{NM}}{\delta \theta_1} & \frac{\delta \ln I_{NM}}{\delta \theta_2} & \ldots & \frac{\delta \ln I_{NM}}{\delta \theta_N}
\end{bmatrix}
\]  

(3.26)
where \( \frac{\delta \ln I_i}{\delta D_j} \) and \( \frac{\delta \ln I_i}{\delta \mu_{aj}} \) are the sub-matrices that define the relation between the log of the amplitude of the \( i^{th} \) measurement with respect to \( D \) and \( \mu_a \) at the \( j^{th} \) reconstructed nodes respectively; \( \frac{\delta \theta_i}{\delta D_j} \) and \( \frac{\delta \theta_i}{\delta \mu_{aj}} \) are the sub-matrices that define the relation between the phase of the \( i^{th} \) measurement with respect to \( D \) and \( \mu_a \) at the \( j^{th} \) node respectively.

The Jacobian is calculated using the Adjoint method\(^7\),\(^{100}\), which takes advantage of reciprocity principles, uses forward model fluence calculations, and is highly efficient. Figure 3.2 shows the four parts of the Jacobian for a simple homogeneous model. The source is on the right hand side and the detector is at the bottom. This essentially shows all of the regions within the model which affect the data measured with this source/detector pair. Measurements are clearly more sensitive to changes in optical properties which occur close to the source and close to the detector than they are to changes deep within the model/tissue.

For purposes of computational efficiency, we use two sets of basis functions in image reconstruction. All forward problems are evaluated using a fine mesh, and the update is calculated using a coarse mesh (with the same local shape and continuity characteristics). \( \mu_a(r) \) and \( D(r) \) are therefore expressed in a basis with fewer degrees of freedom. Several different strategies for defining reconstruction bases are possible, including a second mesh basis\(^54,62\), or uniform grids, which are used in this work.
3.D. Spatial priors in the inverse problem

3.D.1. Overview

It is well known that the reconstruction methodology described in the previous section tends to produce blurred, low resolution optical property maps. The inverse solutions must amplify small differences in measurement data, which has a consequence that measurement noise and model error will be amplified as well. Due to the physics of light propagation, the inverse problem is ill-posed, meaning that either it has multiple solutions, or that the relationship between the solution and the data is unstable. Small changes in the data tend to result in relatively large changes in the parameters of interest. The inverse problem is also under-determined because the number of locations in space
at which one wishes to estimate the absorption and scattering coefficients may greatly exceed the number of measurements used to do so. Objective functions are often multidimensional and multiextremal. Due to intrinsic nonlinearity, local minima exist where a solution algorithm can be trapped, thus giving a false solution. In the field of coefficient inverse problems, the presence of local minima is a major challenge. Any local method of optimization, such as the gradient or Newton-like methods, may fail if the starting vector is improperly chosen. The challenge increases as the complexity of the target image increases. The difficulty of finding global minimum of an arbitrary multiextremal multidimensional objective function motivates the development of new approaches to global optimization. Incorporating a priori information about the model/tissue structure into the reconstruction procedure could reduce the problems inherent to this ill-posedness, potentially improving resolution and quantitative accuracy.

Techniques for incorporating a priori information are relatively new, and are the subject of active research in a variety of disciplines, including medical imaging, industrial process imaging, and geophysical surveying. Spatial resolution and quantitative image accuracy can be improved when the appropriate constraints, derived from a priori information, are applied. Proper constraints reduce the effect of ill-posedness by improving the accuracy of the model, and make the problem better determined, allowing a small number of measurements to be used in a more effective way. These priors and constraints can take a wide variety of forms depending on the measurement type, geometry, and numerical reconstruction technique, and currently there are few broadly adopted conventions even though it is commonly accepted that such constraints offer significant potential value. It is less well appreciated that
mislabeled or erroneous constraints can lead to gross solution errors that are detrimental to the image outcome.

Image reconstruction techniques which utilize such prior knowledge have been largely developed for Nuclear imaging (PET, SPECT) over the last decade\textsuperscript{105, 113-116}. Simultaneous PET/CT imaging systems accurately coregister a low resolution functional image (PET) with a high resolution structural image (CT). Anatomical information is generally used to adjust PET image smoothness and reduce noise levels during reconstruction. Most of the approaches to this problem use Bayesian estimation techniques which seek smooth solutions with discontinuities which match a specified prior.

Improving NIR reconstructions by incorporating prior knowledge of tissue structure, such as MRI data, has been explored in previous work at Dartmouth\textsuperscript{62, 65, 69, 70, 117}, and by other authors\textsuperscript{13, 63, 64, 66, 67, 109, 118, 119}. In summary, priors can be applied on two levels. The first level is the initial setup of the inverse problem. Schweiger and Arridge\textsuperscript{13} demonstrated that incorporation of a correct first estimate of optical properties, and an accurate definition of the imaging volume (with a 2D/3D FEM mesh), both derived from prior structural knowledge, can significantly enhance quantitative reconstruction of localized perturbations in the absorption and scattering coefficients for a complex, multilayered neonatal brain model. The second level of structural prior incorporation occurs during the iterative process. There is a greater potential for innovation on this level. \textit{A priori} information is used to guide/constrain parameter updates. These constraints can be derived through the definition of new objective functions\textsuperscript{63, 120}. Constraints may penalize large deviations from initial estimates, penalize certain spatial
frequencies, encourage steep variations across known internal boundaries, or exclusively update regions of interest. Iterative guidance is primarily accomplished through either parameter reduction\(^66,69\), which we term as ‘hard priors’, or via regularization techniques\(^63,121\), which we refer to as ‘soft priors’. Applying hard priors, Pogue and Paulsen\(^69\) describe the use of high resolution MRI to improve simulated optical property reconstruction of a rat cranium. By accurately defining a region where heterogeneity is expected, they limit image property evolution to only those node locations. Applying soft priors, Li et al.\(^63\) use structural knowledge of the breast to define two discrete regions (anomaly and background) which they regularize differently in order to optimize NIR image contrasts. This work involves the implementation of some of these methods in an attempt to establish the optimal techniques for extracting relevant physiological information from our combined NIR-MRI data set.


Many of the first articles demonstrating improved NIR imaging capabilities using \textit{a priori} constraints implemented some form of parameter reduction\(^66,69,122,123\). This requires segmentation of the image space into regions which are assumed to be optically homogeneous. Assuming prior structural information exists to do the segmentation, this is easily implemented into the iterative optical property update equation (3.24). In general, given structural segmentation into \(n\) regions, single values of \(\mu_a\) and \(\mu'_s\) are reconstructed within each region. To do this, a new Jacobian can be defined:

\[
\tilde{J} = JK , \quad \text{(3.27)}
\]

where the dimensions of \(\tilde{J}\) are the number of measurements by two times the number of regions \((NM \times 2*NR)\). \(K\) is the \textit{a priori} matrix, given as:
In effect, a new sensitivity matrix is constructed, where all the columns corresponding to like regions are summed. A region may be very large (i.e. half the total number of nodes in the mesh), or could be comprised of a single node. As long as the number of unknowns sought does not exceed the total number of measurements, regularization is not required, and therefore

$$\Delta \mu = \left[ \tilde{J}^T \tilde{J} \right]^{-1} \tilde{J}^T \left( \Phi^c - \Phi^w \right).$$ (3.29)

In simulations using both simple models and heterogeneous optical property distributions. When applied to simulated data, this method showed some improvement relative to conventional image reconstruction. However, incorrect regionization can result in gross solution errors. Sensitivity to this kind of error increases when reconstructing small, high contrast objects. Therefore, this method is not robust when used with experimental data, even when NIR data and structural MRI data are acquired simultaneously, and co-registration is as accurate as possible.

In this work, soft priors, generally applied through involved regularization schemes have proved more flexible and effective than hard priors. This will be demonstrated in later chapters. Here, an update equation that utilizes soft priors is derived. Beginning with generic knowledge of the tissue’s makeup, the flexibility of the reconstruction procedure to accommodate a variety of constraints must be emphasized.

Beginning with a new objective function naturally alters the matrix equation that is solved\(^{70}\). The so-called Tikhonov approach\(^{124, 125}\) sets up a minimization of \(\chi\) in which a penalty term is added to the \(\chi^2\) term (equation 3.18),

\[
\chi = \sum_{i=1}^{NM} (\Phi_i^C - \Phi_i^M)^2 + \lambda \sum_{j=1}^{NN} (\mu_j)^2,
\]

(3.30)

where \(NN\) is the number of nodes, and \(\lambda\) (the regularization parameter) can be appropriately chosen, and varied in the iterative process to improve the convergence and ultimately to smooth the final solution\(^{126}\). The use of such a penalty term, which may contain \textit{a priori} information about the system, is one attempt to overcome ill-posedness in optical tomography. A spatially variant \(\lambda\) has been shown to improve image reconstruction. Pogue et al.\(^{121}\) focused on a radially variant regularization, such that this parameter had a simple exponential dependence with radial position in a circular imaging field (i.e. \(\lambda(r) = \lambda_0 + \lambda_1 \exp\{r/10\}\), which provided some correction for the radial dependence of the imaging field resolution and contrast. A number of more complex distributions can be implemented, such as those by Eppstein et al.\(^{127}\) who calculate this distribution based upon the covariance matrix at each pixel location. Projection measurement error can be a useful predictor of the regularization parameter (i.e.\)
\[ \lambda(r) = \lambda_0 + \lambda_1 \left[ (\partial \Phi / \partial \mu)^T \sigma^2 \left( \partial \Phi / \partial \mu \right) \right], \]

where \( \sigma^2 \) is the variance of each projection measurement), thus allowing adaptive regularization based upon the relative uncertainty at each node position due to the accuracy in each of the detector measurements.

If prior knowledge of tissue optical properties is available, one may choose to minimize a modified objective function with some pre-existing distribution \( \mu_0 \) input, which uses the difference between the current estimate of the optical properties \( \mu \) at each node \( j \) subtracted from the initial estimate \( \mu_0 \). This term can be thought of as a damping factor, which tends to keep the current optical property estimate from straying too far from the initial estimate. Combining this with a spatially variant regularization factor, the normal solution is sought:

\[
\overline{\mathcal{E}} = \sum_{i=1}^{NM} \left( \Phi_i^C - \Phi_i^M \right)^2 + \sum_{j=1}^{NN} \lambda_j \left( \mu_j - \mu_0 \right)^2 . \tag{3.31}
\]

The ‘initial guess’ of optical properties, \( \mu_0 \), is generally not available from co-registered structural images, and will likely be a homogeneous estimate of the bulk tissue properties. The feasibility of a heterogeneous \( \mu_0 \) is discussed in Section 4.C.1, in the context of hard priors.

These regularization factors manifest themselves primarily by adjusting image smoothness (by damping the effects of data noise), and to a lesser extent, by adjusting contrasts. Since knowledge of tissue structure is available from MRI, it would be desirable to apply ‘directional smoothing’ so that \textit{a priori} known image boundaries (assuming NIR contrasts approximately shadow MR contrasts) are preserved in the reconstruction. Directional smoothing may be applied by adding a ‘filter matrix’ to the objective function.
A ‘generalized Tikhonov’ penalty term, can be defined as

\[ \bar{\mathcal{X}} = \sum_{i=1}^{NM} (\Phi_{i}^{C} - \Phi_{i}^{M})^2 + \beta \sum_{j=1}^{NM} [L(\mu_{j} - \mu_{0})]^2. \]  

(3.32)

The constant, \( \beta \), balances the effect of the prior with the model-data mismatch.

Selection of this parameter is addressed in Section 4.C.2. The ‘filter’ matrix, \( L \), is generated using MRI-derived priors, and its construction is flexible. In this application, each node in the FEM mesh is labeled according to the region or tissue type with which it is associated (in the MR image). For the \( i^{th} \) node of \( N \) in region \( R \), \( L_{i,i}=1 \). When nodes \( i \) and \( j \) are in the same region, \( L_{i,j}=-1/N \), otherwise \( L_{i,j}=0 \):

\[
L = \begin{bmatrix}
L_{1,1} & \cdots & L_{1,NN} \\
\vdots & \ddots & \vdots \\
L_{NN,1} & \cdots & L_{NN,NN}
\end{bmatrix}
\begin{bmatrix}
0 \\
\vdots \\
0
\end{bmatrix},
\text{where } L_{i,j} = \begin{cases}
1 & i = j \\
-1/N & R_{i} = R_{j} \\
0 & R_{i} \neq R_{j}
\end{cases}.
\]

(3.33)

This effectively relaxes the smoothness constraints at the interface between different tissues, in directions normal to their common boundary. The effect on image quality is similar to that achieved through total variation minimization schemes. This procedure, however, is more robust and can easily encode internal boundary information from MR images.

As defined in Section 3.C, the Newton-Raphson iterative form is

\[
\mu_{i+1} = \mu_{i} + \left[ \frac{d}{d\mu} \left( \frac{\partial \bar{\mathcal{X}}}{\partial \mu} (\mu_{i}) \right) \right]^{-1} \frac{\partial \bar{\mathcal{X}}}{\partial \mu} (\mu_{i}).
\]

(3.34)
Using equation (3.32) we solve for the first derivative term
\[
\frac{\partial \chi}{\partial \mu}(\mu_i) = 2 \left[ \frac{\partial \Phi^C}{\partial \mu} \right]^T (\Phi^C - \Phi^M) + 2 \beta L^T L (\mu_j - \mu_0) \tag{3.35}
\]
and the second derivative term
\[
\frac{\partial}{\partial \mu} \left( \frac{\partial \chi}{\partial \mu}(\mu_i) \right) = 2 \left( \frac{\partial \Phi^C}{\partial \mu} \right)^T \frac{\partial \Phi^C}{\partial \mu} + 2 \beta L^T L . \tag{3.36}
\]
Inserting equation (3.35) and equation (3.36) into equation (3.34), recalling that
\[
\frac{\partial \Phi^C}{\partial \mu} = J \quad \text{and} \quad \Delta \mu = \mu_{i+1} - \mu_i ;
\]
\[
\Delta \mu = \left[ J^T J + \beta L^T L \right]^{-1} \left[ J^T (\Phi^C - \Phi^M) + \beta L^T L (\mu_i - \mu_0) \right]. \tag{3.37}
\]
$L^T L$ approximates a second-order Laplacian smoothing operator within each region separately. The final term in equation (3.37), $\beta L^T L (\mu_i - \mu_0)$, is not routinely used in the reconstruction. Generally our initial guess is homogeneous, and including this term would reduce the sharpness of known edges.

Two important points which warrant further discussion in later chapters include:
(1) the sensitivity of a reconstruction method (hard or soft priors) to errors in the prior, and (2) the sensitivity of a method to noise in the data. The construction of $L$ has proved flexible and effective, as demonstrated throughout this thesis.
Chapter 4 : Simulations

4.A. Introduction

A software package for performing 2D and 3D FEM image reconstruction, entitled Near Infrared Frequency domain Absorption and Scatter Tomography (NIRFAST), has been developed at Dartmouth College. The software is written in MATLAB® and C, and includes a forward solver, an inverse solver, and all of the associated subroutines. NIRFAST documentation is included in the Appendix. Simulations are generally the first step taken in algorithm development. Using NIRFAST, this chapter demonstrates their usefulness for testing image reconstruction methods.

4.B. Simulation procedure

The procedure for creating simulated forward data and for reconstructing images is to: (1) generate a test object with desired optical properties and known source/detector locations, (2) calculate data with the forward solver, (3) reconstruct images from data and compare to test objects. This section describes each step, including important details relative to NIRFAST.

4.B.1. Generation of test objects

FEM meshes are described by a node list which gives the coordinate locations of all discrete points in the mesh, and an element connectivity list which gives the node numbers associated with each element. Having generated node and element lists to describe the test geometry, absorption and scattering coefficient lists can be defined
arbitrarily, defining the optical properties associated with each node. Figure 4.1 shows three examples of optical property distributions. In cases where multiple ‘regions’ are \textit{a priori} known to exist, a region label (associated with each node) is also specified.

![Image](image.png)

Figure 4.1. (a) A NIRFAST utility used to create test objects. The coordinates of a ‘blob’ can be selected using a mouse, and its size and optical properties are entered at a user prompt. (b) Several examples of test objects with varying degrees of optical property heterogeneity.

4.B.2. Data generation

Solving the forward problem for these property distributions and a given set of source/detector locations yields simulated measurements of amplitude and phase. Figure 4.2 shows data calculated for a circular test object, with sources and detectors uniformly spaced around the outer perimeter. To further match experimental conditions, noise levels characteristic of detector behavior are also added to the data. We commonly use 1-2\% in log(amplitude) and 1-2 degrees in phase shift, added to the measurements with the
NIRFAST routine add_noise.m by multiplying by a vector of normally distributed random numbers with zero mean value.

Figure 4.2. Simulated amplitude and phase data for a homogeneous and heterogeneous circular test object. The source/detector distribution matches our NIR tomography system. Sixteen source/detector points are located around the outside, and for each active source, fifteen measurements of both data types are acquired.

4.B.3. Reconstruction program

Calculated data and a homogeneous initial guess for $\mu_a$ and $\mu'_s$ are used to initiate image reconstruction. The inverse solution described in Chapter 3 is implemented in five steps. (1) Calculate $\Phi$ and $J$ on a fine mesh to ensure numerical accuracy. (2) Interpolate $J$ onto a coarse mesh (the reconstruction basis has fewer unknowns so that parameter estimation is better determined). (3) Calculate optical
property update. (4) Interpolate back to fine mesh for smoothing. (5) Repeat until projection error is minimized.

As with the test object and the data generation, the reconstruction program is executed from the Matlab command line. Other inputs into the program include the total number of iterations, the initial regularization, the size of the reconstruction basis, and the number of filtering operations desired. If the change in the projection error between successive iterations falls below 2%, convergence is declared and the program will terminate regardless of the iteration specified. The projection error minimum typically falls between iteration seven and fourteen. As a rule of thumb, an initial regularization of ten should be used when reconstructing approximately 2000 unknowns (i.e. two parameters on a 30x30 pixel basis). This should be increased if the number of unknowns or the noise in the data increases. A uniform grid of points, connected to form elements is usually used as the reconstruction basis. This basis is easily adaptable to forward meshes with irregular shapes. Users must only specify the grid spacing. After each iteration, one filtering operation is usually performed to reduce high frequency noise in the optical property maps. Mean/median filters are typically used, and they set the optical property of each node equal to the mean/median of the properties of itself and its neighboring nodes. In cases where prior information is available, and sharp internal boundaries are expected along pre-defined lines, filtering is applied everywhere except across the boundary. Other filters can be imagined (but are rarely used in practice), including finite impulse response (FIR) filters which operate on the spatial frequency distribution of the optical coefficient images (Figure 4.3).
Figure 4.3. A FIR (Finite Impulse Response) image filter. (a) The user specifies the smallest object expected in the image, which defines the filter frequency response (smaller objects require more frequencies). (b) The filter coefficient matrix is the FFT of the frequency response. (c) Two target images (top left: diameter=10; top right: diameter=40) filtered using the coefficients in (b). The properties shown represent the difference between the original image and the filtered image.

4.C. Simulation examples

In simulation work we test algorithms which incorporate *a priori* information, using a variety of test objects, from simple to complex. Simple test objects are regular shapes (i.e. circle/cylinder) with homogeneous optical properties, except for discrete absorption/scattering inclusions placed in the interior. More complex tests involve irregular shapes, with layered and heterogeneous optical property distributions.

4.C.1. Hard priors

Parameter reduction in FEM reconstruction was described in Section 3.D.2. This method was applied to a simple test object in Figure 4.4. The target optical property
distribution contains two ‘regions’ and four unique properties which are sought in reconstruction—$\mu_{a,\text{background}}$, $\mu_{\mu,\text{background}}$, $\mu_{a,\text{inclusion}}$, and $\mu_{\mu,\text{inclusion}}$. Due to the small number of unknowns, this solution is highly over-determined and therefore computationally fast and robust to noise in the data. However, as Figure 4.4 demonstrates, the properties recovered in the region of a small, high contrast inclusion can vary dramatically given small variations in the size or location specified \textit{a priori} for the inclusion. This drawback prohibits the routine use of this algorithm for reconstructing small objects embedded in phantoms, or tumors deep in tissue. Minimal errors in the designation of spatial priors are unavoidable, considering that the domain must be discretized.

Figure 4.4. (a) Images of a simple target used in test reconstructions. (b) Conventional reconstruction, and (c) hard priors. Random noise of 2\% (log(amplitude)) and 2 degrees (phase shift) was added to the data prior to reconstruction. Hard priors are robust to noise in the data, but highly sensitive to the regionization given \textit{a priori}. (d) Large errors result when the inclusion is assumed to be 15 mm in diameter instead of the correct 20 mm.
Layered and heterogeneous test objects are used to better simulate the process of reconstructing clinically relevant images. The layered structure of breast tissue, and small-scale heterogeneity pose serious challenges in image reconstruction. There is an issue regarding how small scale heterogeneity affects the recovered location and quantitative values of an anomaly which may represent a tumor. The ability to reconstruct layered property distributions and small scale perturbations was a focus of the early work related to this thesis\textsuperscript{70, 128}.

Figure 4.5 shows various attempts at reconstructing a test object with realistic heterogeneity. Absorption coefficient variations near ±40\%, and reduced scattering coefficient variations near ±20\% were assum\textsuperscript{a priori} knowledge regarding the target optical property distribution is known except the shape of the outer boundary and locations of source and detector optical fibers. Next a two region layered structure is assumed, loosely defined from \textit{a priori} knowledge, and hard priors (equation (3.29)) were used to compute a two region initial guess to initiate conventional parameter estimation (equation (3.24)). The hard prior technique assumes that a heterogeneous media is made up of multiple homogeneous regions. The reconstruction mesh is segmented as shown in Figure 4.5 (b) by assigning all nodes within each region with the appropriate label in the region list. Next, the same two layer initial guess is used, but heterogeneity is only allowed in the interior region which contains the modeled tumor. Next, a multi-stage algorithm is utilized which performs a localized reconstruction at early iterations, followed by a conventional reconstruction at late iterations. Figure 4.5(e) shows the optical properties reconstructed from this multi-stage algorithm when the initial region
segmentation was more accurate, and expanded to include three regions. Conventional reconstruction recovers 69% of the true value of the absorption coefficient anomaly.

With the multi-stage method, which relies on hard priors to calculate an accurate initial guess, the anomaly is recovered with 99% accuracy.

Figure 4.5. (a) Modeled optical properties with a spatial pattern of heterogeneity similar to that present in tissue. An absorbing heterogeneity was added which represents a tumor. (b) and (c) show two different regionized FEM meshes which were used to guide the image reconstruction process. (d) and (e) show that recovered images are improved when prior information is used correctly in the reconstruction process.

Well chosen starting values for the assumed initial optical properties speed convergence and improve quantitative accuracy of image reconstruction. In a realistic case, ‘good’ starting values are not known except for phantoms. At Dartmouth, initial
values specified in image reconstruction are computed from measured data using a homogeneous fitting algorithm described in Section 7.C. This procedure is robust, and \( \mu_a \) and \( \mu_s \) begin as bulk averages of the media under investigation. Given a priori knowledge of tissue or phantom structure (from MRI), parameter reduction can be used to compute heterogeneous initial guesses which are more accurate. Hard priors could potentially be used in multi-step algorithms similar to those implemented in Figure 4.5. Srinivasan et al.\textsuperscript{76} used a multi-stage algorithm to improve quantification when imaging small objects. This method was fully automated, and knowledge of tissue structure was not required. One stage utilized region of interest segmentation in images obtained via conventional reconstruction.

4.C.2. Soft priors

The name ‘hard priors’ is given to the parameter reduction method because this technique applies the rigid constraint of optical property homogeneity within specified regions. When properties are not in fact homogeneous, errors will always result. Soft priors in FEM reconstruction were described in Section 3.D.3. The rigidity of the homogeneity constraint is relaxed in an attempt to reduce error biases caused by hard priors. As mentioned earlier, the soft prior in this work is implemented through a full regularization matrix which adjusts image smoothness and preserves image contrast along edges specified in the prior,

\[
\Delta \mu = \left[ J^T J + \beta L^T L \right]^{-1} J^T (\Phi^C - \Phi^M). \tag{4.1}
\]

\( L^T L \) acts as a Laplacian filter on the optical properties of each region separately. A benefit of this implementation is that complex, multi-step processes are not required to
reconstruct accurate images. Also, the definition of regularization is automatic and the same procedure is followed regardless of the model being investigated.

Simulation studies were performed in order to characterize the effect of $L$ and $\beta$ on the quality and quantitative accuracy of reconstructed images, and to establish a value of $\beta$ which can be used routinely. Data was generated from numerical phantoms with a variety of heterogeneity patterns—ranging from a simple circular anomaly in a homogeneous background to irregular distributions of regions with two or three different properties. Noise (1-3% log amplitude and 1-3 degrees) was added to simulated data in order to better replicate experimental conditions. Error was also added to the $a$ priori region designation, to account for the small loss of resolution when spatial information is transferred from MR images to FEM meshes. Images were reconstructed from this data using a range of $\beta$ from 1 to 100. A high $\beta$ value increases the impact of the spatial prior, leading to images with sharper internal boundaries, but could negatively bias solutions if this prior is not correct. By accounting for the different sources of error which are present when data is acquired with the system presented here, simulation results indicate that setting $\beta$ to ten times the maximum value of the diagonal of $J^TJ$ optimizes image quality and accuracy regardless of the level of geometric complexity present in the area under investigation. $\beta$ does not decrease during the iterative solution process. Due to the diffuse nature of photon propagation and inherent loss of spatial resolution and sensitivity at greater depths, spatially varying regularization has been used to improve recovery of deep objects\textsuperscript{121}. However, in this context, changing $\beta$ from a scalar to a vector (with smaller values associated with regions deeper in the model) does not improve reconstructed images.
Figure 4.6. Using soft priors on a three-layer model improves images quantitatively and qualitatively.

Figure 4.6 shows reconstructions of a three layered model (i.e. adipose tissue, glandular tissue, tumor tissue). Along with the target optical property distribution, the conventional reconstruction, and three reconstructions which utilize soft priors are shown. In the first soft prior, only the boundary between the outer and inner layer was specified, and the presence of the simulated tumor was ignored. In the second, only the tumor boundary was given. Finally, a ‘full prior’ was specified, which included the adipose-glandular boundary and the glandular-tumor boundary. The tables in Figure 4.6 show the mean coefficient values recovered for each region by each of these reconstructions. Overall, the best images are obtained via the ‘full prior.’ With this method, the recovered coefficients compare well with the true values, RMS image error...
is low, and contrast-to-noise-ratio (CNR) is high. CNR analysis is discussed further in the next section. It is also clear from this simulation that assuming an incomplete prior is not catastrophic.

4.C.3. Measuring performance with contrast-detail analysis

A comparison of reconstruction methods is problematic because tools for the objective assessment of image quality have yet to be clearly defined for this type of nonlinear reconstruction problem. In simulation studies, the true optical property distribution is known and can be compared to the reconstructed images. Typically RMS image error, or the mean maximum value within a region of interest is reported, but these are not comprehensive image quality measures. Contrast-detail analysis has become an accepted assessment tool to quantify x-ray mammography image quality. Pogue et al.129 and Song et al.130 have applied it to NIR tomography. This analysis provides an objective method for assessing image field response and detection and characterization limits, and can be applied to compare a collection of different reconstruction methods.

In many imaging systems, there is a modest inverse dependence between contrast and resolution. For x-ray mammography this relationship is experimentally determined through the contrast-detail plot131, where the minimum detected contrast is plotted for varying sized inclusions located within a tissue simulating breast phantom. In diffuse optical imaging there is a similar but much stronger link between object size and minimum contrast for detection, which limits the ability to resolve small objects accurately. Pogue et al.129 examined this size-contrast effect with the goal of establishing bounds on the minimum object size-contrast required for detection, as well as the minimum size-contrast required for an object to be accurately characterized. They
determined that 8 mm diameter objects can be accurately reconstructed for most absorption contrasts observed in tissue, and that objects with high contrast can be detected down to 2 mm in diameter, but cannot be accurately reconstructed. Normally contrast-detail analysis uses the subjective analysis of multiple observers to determine if an object is ‘detectable.’ Song et al.\textsuperscript{130} used contrast-to-noise ratio (CNR) to determine the detectability of objects within reconstructed images. CNR is defined as the difference between the ROI and the background region values of the optical properties divided by the average variation in the background. Given a chosen threshold value of CNR, it is fairly straightforward to find those images in which objects are detectable. Thus, contrast-detail curves can be created that show the minimum contrast required to detect objects at each size. Through experimental and simulation studies Song et al.\textsuperscript{130} established a spatial resolution limit near 4 mm and a contrast resolution limit near 1.4 for the stand-alone clinical NIR tomography system at Dartmouth College.

Contrast detail plots were constructed to characterize the ability of the reconstruction algorithms described here to detect small objects in a homogeneous background. Images were reconstructed using the conventional method, and using soft priors with $\beta=1$ and $\beta=10$. Three examples are shown in Figure 4.7: (a) small object (diameter=9 mm), high contrast (90%), (b) large object (diameter=19.5 mm), low contrast (30%), and (c) large object, high contrast. Figure 4.8 shows the surface plots of CNR for these three reconstruction methods. Assuming an image with a CNR=3 contains a detectable object, contrast detail plots defining the detection characteristics of these three algorithms are shown in Figure 4.8 (d). Curves generated from data with two noise levels indicate consistent trends. In general, $\beta=10$ is optimal, and the algorithms
which takes advantage of soft priors can detect objects with 20% less contrast. At 100% contrast, the smallest object ‘detectable’ with conventional methods is 8 mm in diameter. Using soft priors, this can be reduced to 3 mm.

Figure 4.7. Simulations used to evaluate ability of reconstruction algorithm to recover objects of different sizes and contrasts. (a) A small object (diameter=9 mm) with high contrast (90%). (b) A large object (diameter=19.5 mm) with low contrast (30%). (c) A large object (diameter=19.5 mm) with high contrast (90%). Random noise corresponding to 2% of the log amplitude data and 2 degrees of phase shift was added prior to image reconstruction. i. represents the target optical property distribution. ii. is the result obtained without spatial priors. iii. utilized spatial priors, with a weighting factor, $\beta=10$. iv. utilized spatial priors with a weighting factor, $\beta=1$.

In addition to detection characteristics, we are interested in characterizing the quantitative accuracy of these methods. Figure 4.8 (e) shows contrast-detail curves defining 60% accuracy of the mean values recovered in the ROI. Using this simple measure on a simple model, the conventional method surprisingly outperforms soft priors. However, this trend was not observed in simulations of a more complex geometry (Figure 4.6), or in phantom experiments (section 7.D), where addition of soft priors improved quantitative accuracy dramatically. When the value of $\beta$ decreases (i.e. from
ten to one), the degree of filtering applied to the images decreases, and the mean value (but not the peak value) of the ROI increases, approaching that recovered with conventional reconstruction as $\beta$ approaches zero.

Figure 4.8. Surface plots of CNR generated using simulated data (with 2% noise added) for (a) conventional reconstruction, (b) spatial priors, $\beta=1$, (c) spatial priors, $\beta=10$. (d) Contrast detail curves defining CNR=3. The solid and dashed lines correspond to data with 1% and 2% noise, respectively. (e) Curves defining the position of 60% accuracy of the reconstructed mean value for the ROI for each size object.

In completing this study, it is important to consider the potential application of the imaging system in order to tailor experiments to relevant detection features. Using spatial priors afforded through composite NIR-MRI imaging, smaller objects with lower contrasts should be identifiable. For quantitative imaging, it may be necessary to adjust the spatial prior. Chapter 8 shows that spectral priors improve quantitative accuracy more than spatial priors in the context of chromophores imaging.
Chapter 5 : Phantom design

5.A. Introduction

Following numerical simulation, the second step in evaluating an image reconstruction method, is phantom testing. In this case physical phantoms mimic the optical properties of breast tissue. These can be constructed from a homogeneous material, or through the combination of multiple materials, resulting in a heterogeneous property distribution which is well characterized and controllable. Phantom experiments test both the data acquisition component and the processing component of the imaging platform, and can be designed to assess both its abilities and limitations. Phantom studies are essential in order to gain confidence in the images acquired during clinical exams, where the true distributions are unknown.

A number of different materials have been used successfully to produce breast-mimicking phantoms for optical tomography. Troy McBride\textsuperscript{81} describes five classes of phantoms which have been used at Dartmouth: (1) liquid, (2) solid plastic, (3) gelatin, (4) semi-solid silicon, and (5) excised tissue. By adding materials which offer absorption contrast (blood, ink) or scattering contrast (Intralipid, titanium oxide), the optical properties of these phantoms can be adjusted to match typical values of bulk breast tissue ($\mu_s=0.002-0.02 \text{ mm}^{-1}$; $\mu_s'=0.5-2.0 \text{ mm}^{-1}$). Lesions have been found to have as high as 4:1 contrast in absorption and near 1.5:1 in scattering. In general, heterogeneous phantoms are created with the assumption that cancer is represented by an optically distinct inclusion suspended in a homogeneous turbid media. The benefits and drawbacks associated with the three most commonly used phantoms (i.e. liquid, solid,
and gelatin) are briefly discussed here. Gelatin phantoms have been used extensively in this work, and details regarding phantom construction are included.

Figure 5.1. (a) A liquid phantom in a bottle. (b) A hard plastic phantom with a hole drilled for addition of liquid inclusions. (c) A soft gelatin phantom.

5.B. Liquid phantoms

Liquid based phantoms are easy to make, and recipes for producing precise optical properties are well defined\(^1\). These phantoms are suspensions of lipid particles for scattering and blood or ink for absorption. Intralipid is a widely used lipid suspension which is commercially available. The absorption of lipid particles is negligible and the scattering has been characterized in the NIR range\(^56\). For 1% Intralipid in solution, \(\mu_s\) is approximately 1.0 mm\(^{-1}\) at 800 nm. The scattering of blood and ink is generally negligible and the absorption per unit concentration is easy to establish\(^81\). In general, a solution with a 2 ml/liter concentration of 0.2% India ink will yield a \(\mu_a\) of approximately 0.01 mm\(^{-1}\) at 800 nm. The absorption spectra for hemoglobin has been studied by several groups and is available in literature (see Figure 1.3)\(^132\). In order to use whole blood for absorbing contrast, the molecular weight (64500 g/mole) and the concentration of hemoglobin in the blood (mass of hemoglobin per liter) must be known.
Blood hemoglobin concentration in healthy adults is approximately 150 ± 20 g/L. Hemoglobin has a different absorption spectrum depending on its oxygen saturation, but in air hemoglobin mixed with water can be assumed to be fully oxygenated.

A concern of liquid phantoms is that it is necessary to hold the liquid in a container, which inevitably effects the measurements. In practice, Nalgene (e.g. polypropylene, Lab Safety Supply, Janesville, WI) plastic bottles are a good container for liquid phantoms. These bottles are thin walled (1 mm) and cloudy in appearance, allowing minimal light ‘channeling’. Also, the addition of an inclusion to a liquid phantom requires a separate container (or a solid material), which has an unknown effect on the diffuse light propagation.

5.C. Solid phantoms

Unlike liquid phantoms, hard plastic phantoms may last many years and are excellent for repeatability studies. Epoxy resin is combined with a hardener, and ink and titanium oxide (Sigma Chemical Co., St. Louis, MO: Titanium(IV) Oxide, TiO₂, T-8141) are added to tailor absorption and scattering. Recipe details and mixing procedures can be found elsewhere⁸¹. The hardened plastic can be shaped and drilled using standard machining equipment. The drilled holes are filled with liquid mixtures for variable contrast studies. A drawback is the mismatch in refractive index between the resin and the liquid inclusions. Refractive index effects and the modeling thereof is discussed in Chapter 6. Also, these phantoms are rigid and the flat face of the fiber optics can not make flush contact with the curved phantom surface.
5.D. Gelatin phantoms

Gelatin was first used at Dartmouth to make phantoms which could be used by all four of the model-based imaging systems which are being used to study breast cancer (magnetic resonance elastography, electrical impedance spectroscopy, microwave imaging spectroscopy, and NIR spectroscopic imaging—described in Section 1.C.1. and elsewhere\textsuperscript{45}). Li et al.\textsuperscript{133} discussed combining information collected from the three spectroscopic methods in order to provide a more complete diagnostic tool that covers the full range of the patient and pathology spectrum. It proved difficult to construct a phantom which reproduces the appropriate dielectric and optical properties over the complete spectrum spanned by these imaging modalities. Using agar based gelatin phantoms, they showed through common experiments that it was possible to correlate different types of image information. In a separate study Doyley et al.\textsuperscript{134} used gelatin derived from porcine skin to manufacture phantoms with different elasticity properties in order to characterize the ability of magnetic resonance elastography to detect small objects with different stiffness than the background.

The flexibility of gelatin for multi-modality imaging has been demonstrated, and these phantoms have been the most useful in simultaneous NIR-MRI imaging studies. The primary concern when choosing a phantom is whether or not a given material offers both NIR and MRI contrast. Similar to liquid-based phantoms, the gelatin absorption coefficient can be adjusted by adding India ink or blood. As with resin-based solid phantoms, the scattering coefficient can be adjusted by adding titanium oxide. These phantoms contain 60 to 90\% water, which provides the necessary MR contrast. Solid
plastic phantoms are largely invisible in MRI. Gelatin is also inexpensive, and a more realistic representation of tissue than liquids or solids.

Figure 5.2. Molds used for forming gelatin phantoms. Spherical inclusions from 5 to 25 mm are made to represent tumors. Bulk material may be formed into cylinders in standard beakers, or into realistic breast-like shapes using the mold shown here. Photographs of several finished phantoms are also presented.

Gelatin from porcine skin (Type A, ~175 Bloom, Sigma Chemical Co., St. Louis, MO) is predominantly used, which is mixed with water and other ingredients. As the processed gelatin (dried collagen flakes) takes up the water the phantom hardens at room temperature to tissue-like pliability. Each optical fiber therefore makes flush contact with the phantom surface, and causes realistic surface impressions. Tumors can be stiffer than glandular tissue, which is generally stiffer than adipose tissue, and mixtures with different gelatin concentrations can give these realistic mechanical properties. Before mixtures harden, they can be poured into molds to form realistic breast-like shapes. Small molds
(Figure 5.2) have been used to produce spherical and cylindrical inclusions, which are then embedded in other mixtures prior to hardening. Multi-layered phantoms can also be made to simulate the component tissues in the breast. After hardening an adipose tissue-like material in a breast or cylindrical mold, the center may be removed and filled with glandular tissue-like gelatin, which is then allowed to harden. These heterogeneous phantoms have realistic spatial variation in refractive index as well, unlike heterogeneous liquid and solid phantoms.

Ingredients which are unique to gelatin-based phantoms are Edta (Ethylendiamine-Tetraacetic acid, Sigma Chemical Co., St. Louis, MO) and Gadolinium (Omniscan™ gadodiamide). Etda is a preservative which prevents bacterial growth and Gadolinium is a routinely used MR contrast agent which is generally added to only one layer of a multi-layer phantom to make it stand out in the MR when differences in water

Figure 5.3. Axial and coronal MR slices for two multi-layer phantoms.
content of the layers are small. Gadolinium is a clear liquid and does not affect absorption or scatter. Previously, copper sulfate (CuSO$_4$) was used to improve MR contrast, but this significantly affected absorption, and was therefore undesirable. Figure 5.3 shows selected slices from the full volume MRI acquired for two multi-layered phantoms. One phantom is cylindrical in shape, and the other is breast shaped. Both are comprised of three different materials, to simulate adipose, glandular, and tumor tissue. Slight discrepancies in the amount of gadolinium in the different layers led to excellent MR contrast.

Table 5.1 shows the recipe and mixing procedure most commonly used to make gelatins representing the different tissues in the breast. The alternative recipe in Table 5.1 was developed at Dartmouth for tissue elastography studies, and uses an emulsion of water, vegetable oil, and gelatin. Triton x-100 (Sigma, St. Louis, MO) is an emulsifying agent. The mixture must be blended (on high with a household blender) for approximately 1 minute. This recipe has not been routinely used in this work because the amount of oil used affects scattering properties. Even low concentrations of oil, comparable to that listed in the glandular tissue recipe, can give reduced scattering coefficients greater than 2.0 mm$^{-1}$. It may be that this recipe is not useful for optical measurements.

Figure 5.4 shows the dependence of the absorption and reduced scattering coefficient on the amount of ink and TiO$_2$ added, respectively. For a given recipe, there can be significant variation in the optical properties produced. This variation can be reduced somewhat if the exact same mixing procedures are consistently used. The variation in scattering with respect to the amount of TiO$_2$ added is the greatest due to the
fact that TiO$_2$ does not mix well with the gelatin. If the phantom is removed from the stir plate before it becomes sufficiently viscous to hold the TiO$_2$ in suspension, a significant portion may settle to the bottom during refrigeration. This can be prevented if a working knowledge of the mixing procedure is acquired by practicing the mixing procedure.

**Gelatin phantom recipe**
Mixtures make approximately 500 ml (fills an 82 mm beaker to a height of 80 mm)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Adipose tissue</th>
<th>Glandular tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin (g)</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>450</td>
<td>425</td>
<td>400</td>
</tr>
<tr>
<td>TiO2 (g)</td>
<td>0.6</td>
<td>1.1</td>
<td>2</td>
</tr>
<tr>
<td>India ink (ml)</td>
<td>0.2</td>
<td>0.7</td>
<td>1.25</td>
</tr>
<tr>
<td>Edta (g)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gadolinium (ml)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

| Absorption coefficient (1/mm) | 0.006 | 0.01 | 0.015 |
| Reduced scattering coefficient (1/mm) | 0.6 | 1 | 1.3 |

**Alternative recipe (developed for MRE stiffness properties)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Adipose tissue</th>
<th>Glandular tissue</th>
<th>Tumor tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelatin (g)</td>
<td>50</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Vegetable oil (ml)</td>
<td>225</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>225</td>
<td>360</td>
<td>400</td>
</tr>
<tr>
<td>Triton x-100</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Mixing Procedure**
(1) Stir gelatin into water in large beaker with magnetic stir bar
(2) Microwave to approximately 40 degrees C
(3) Let stand for 5 min, then spoon off bubbles from top
(4) Mix on stir plate until slight thickening is observed
(5) Poor off 50 ml into small beaker containing TiO$_2$, mix vigorously, and replace (repeat as necessary until all TiO$_2$ is transferred)
(6) Add ink, Edta, and Gadolinium
(7) Apply thin coat of Vaseline to mold (so phantom can be easily removed)
(8) When mixture reaches 30 degrees C, poor into mold and refrigerate until semi-solid

Table 5.1. Recipe and mixing procedure for gelatin phantoms.
Figure 5.4. The optical properties of 16 gelatin phantoms (made over the course of two years) as a function of the amount of ink and TiO2 used in each recipe.

The main disadvantage of gelatin is its short shelf life. This can be prolonged for homogeneous phantoms by refrigeration in air-tight containers, or by submersion in vegetable oil. For heterogeneous phantoms however, where one gelatin object is embedded in a background with a different composition, diffusion will decrease object-to-background contrast on the time scale of days. Adding oil to individual layers could limit this diffusion. Gelatin is also somewhat fragile. Gelatin derived from porcine skin is harder to damage than that derived from agar. The surface may be deformed with optical fibers without puncturing, but only to a point. In studies where compression is required, the phantom should be wrapped in cellophane. Homogeneous gelatin mixtures take approximately 90 minutes to make. Heterogeneous phantoms, which may require multiple gel batches mixed in series can take up to 5 hours to construct.
6.A. Introduction

The refractive index \( n \) of a material medium is the ratio of the phase velocity of light in free space to that in the medium. For optical frequencies, biological tissue is reasonably considered to be a dielectric, and accordingly \( n = \varepsilon^{1/2} \) (where \( \varepsilon \) is the relative permittivity). It is known that scattering characteristics of tissues are caused by microscopic fluctuations in \( n \). While \( n \) may be called a micro-index, the concept of a macro-index must be introduced to solve various boundary problems. Refractive index is not listed in most of the literature as an optical property\(^8, 135\) because it is a difficult parameter to measure in turbid media. There have been some attempts to experimentally measure it for different tissues\(^{136-138}\) but in most numerical models, index values from 1.3 to 1.4 are assumed because the major constituent of tissue is water. In NIR imaging, the macroscopic index of refraction of most tissues is usually considered to be homogeneous. Although this extra coefficient is rarely used for in vivo imaging, it may be justified for study of phantoms comprised of materials with significantly different refractive indices (i.e. plastic cups filled with Intralipid solutions).

Numerical solutions of the frequency domain diffusion equation can be made to match experimental measurements, assuming appropriate optical coefficients and boundary conditions are specified. The best description of the air-tissue boundary is a type III condition in which the outward-normal component of the fluence at the edge of the tissue exits and does not return\(^{86, 92, 139}\). The amount of flux leaving the boundary depends on the mismatch between the index of refraction of tissue and of air. Here, we
describe FEM modeling of diffuse light transport across internal boundaries between two such media with refractive indices $n_1$ and $n_2$. Model data mismatch for phantom measurements can be reduced when internal boundary reflection and refraction is appropriately represented. This section also describes model validation with Monte Carlo and a phantom experiment.

### 6.B. Modeling internal boundary reflections with FEM

As discussed in Section 3.B.1, the diffusion equation is typically solved with the index-mismatched type III (outer) boundary condition

$$\Phi(\xi, \omega) = 2A\hat{n} \cdot D\nabla \Phi(\xi, \omega), \quad (6.1)$$

where $\xi$ is a point on the boundary, and $A$, derived from Fresnel’s law, depends upon the relative refractive index mismatch between tissue and air. Using finite element formalism, this condition is applied through Galerkin’s weak form (section 3.B.2., equation (3.9)):

$$\left\langle D\nabla \Phi \cdot \nabla \varphi_i \right\rangle + \left( \mu_a + \frac{i\omega}{c} \right) \left\langle \Phi \varphi_i \right\rangle = \left\langle S_0 \varphi_i \right\rangle + \oint_{\partial} \hat{n} \cdot D\nabla \Phi \varphi_i \, ds. \quad (6.2)$$

The integrand of the surface integral is the normal component of the flux through the boundary surface. This term does not contribute to matrix assembly at node points internal to the finite element mesh boundary because of flux continuity. At interior nodes which lie on an interface between two media with different indices of refraction, we apply the conditions used by Schmitt et al and Takatani and Graham.
\[ \hat{n} \cdot D_1 \nabla \Phi_1 (\xi, \omega) = \hat{n} \cdot D_2 \nabla \Phi_2 (\xi, \omega), \quad (6.3a) \]

\[ \frac{\Phi_1 (\xi, \omega)}{\Phi_2 (\xi, \omega)} = \left( \frac{n_1}{n_2} \right)^2. \quad (6.3b) \]

Equation (6.3a) ensures that the flux across a boundary remains continuous (so the flux integral in equation (6.2) still does not contribute at internal nodes), while equation (6.3b) establishes a discontinuity in the fluence based upon the two refractive indices defining the interface.

Figure 6.1. Meshing the interface between two regions of different refractive indices. Nodes on the boundary are duplicated to allow the enforcement of internal boundary conditions.

Implementation of (6.3b) in (6.2) occurs by separating the contributions from the domain integrations in the Galerkin weak form during matrix assembly according to which side of the interface a node belongs. A simple vehicle for accomplishing this task is to duplicate nodes on the interface (Figure 6.1) such that they possess coincident coordinate positions but unique node numbers (and therefore unique columns in the
global system of algebraic equations when serving as basis functions that define the numerical photon density solutions). This allows $\Phi_1$ and $\Phi_2$ to simultaneously exist in the list of unknowns at the index-mismatched interface. On matrix assembly, one of the node numbers is preselected to act as the weighting function, and therefore, provide the row location for the discretized version of (6.2) on the interface. The row number associated with the partnered node is initially empty and to close the algebraic system, it is ready to accept explicit enforcement of (6.3b) rewritten as the equation

$$\Phi_1 - \left(\frac{n_1}{n_2}\right)^2 \Phi_2 = 0.$$  

(6.4)

**6.C. FEM and Monte Carlo simulations**

To test the validity of the FEM model, Dehghani et al.\textsuperscript{95} compared it with Monte Carlo simulations of diffuse reflectance from the slab geometry shown in Figure 6.2. The model domain consisted of two layers with the same optical properties ($\mu_a^s$ =0.01 mm\(^{-1}\), $\mu_s^/'$=1.0 mm\(^{-1}\)) but different refractive indices. Comparable results indicated that for frequency domain measurements, both amplitude and phase are altered by internal variations in refractive index. Phase measurements, however, are more affected than amplitude measurements.
Figure 6.2. (a) Geometry of the two layered slab model used both for FEM and Monte Carlo simulations. The 5 mm thick top layer and the 45 mm thick bottom layer have the same optical absorption and scatter, but are allowed different refractive indices. The source is placed at the center of the top face, and reflectance measurements are calculated at 1 mm intervals 10–29 mm away from the source. (b) The FEM mesh which represents this geometry.

For the FEM calculation, the 3D meshes (Figure 6.2 (b)) with and without internal boundaries consisted of approximately 20,000 nodes corresponding to 70,000 linear tetrahedral elements. Figure 6.3 shows the cross-section of the internal intensity and phase plots directly under the source with and without an internal refractive index discontinuity.

The Monte Carlo model used simulated photon propagation in a stratified medium as described previously. The medium was modeled as having layers of finite thickness with distinct transport coefficients and refractive indices. Photons were incident normally at the top face of the turbid medium. For every scattering event where the calculated step-size ($s$) (along a particular direction) caused a photon to cross an index-mismatched boundary, the photon was first propagated to the point where its trajectory intersected the boundary via a shortened step-size ($s_1$). The angle of incidence was computed and used to determine if the photon suffered total internal reflection (from
Snell’s law). If the photon was internally reflected, then the $z$-component of the photon’s travel direction was reversed and the photon completed the remainder of the step ($s-s_1$) in the same layer, otherwise the reflection coefficient from Fresnel’s equations was computed and compared against a uniformly generated random number. For every sampling of the random number that was less than the reflection coefficient the photon underwent internal reflection, otherwise it was transmitted to the next layer (or escaped from the domain). On transmission into a different layer the final spatial location of the photon was calculated by propagating the photon by a distance of $s-s_1$ that was adjusted in length (to account for the difference in transport coefficients between the two layers) and its direction corrected to consider refraction. All photons emanating from the top-layer of the turbid medium were spatially and temporally binned to calculate the reflectance from a turbid medium. The resulting temporal data was Fourier transformed to give frequency-domain estimates of the amplitude and phase shift as a function of distance, in order to match the type of data given by NIRFAST.

For the Monte Carlo results, the number of simulated photons was $50 \times 10^6$, resulting in an execution time of 48 hours. Figure 6.4 shows good agreement between the Monte Carlo and FEM data. It can also be seen from the amplitude response that there is little change due to an internal refractive index mismatch. However, as the refractive index of the bottom layer ($n_2$) is increased from 1.33 to 1.58 the measured phase increases at distances greater than 10 mm from the source. Furthermore, it is evident that assuming just a change in the speed of light in tissue and ignoring internal boundary reflections does not predict the measured data accurately. The internal field and phase plots (Figure 6.3 (b)) indicate reflections at the lower face of the internal
boundary, delaying exit times at detector positions (causing an increase in phase compared to the homogeneous case).

Figure 6.3. The internal fluence amplitude and phase distribution when (a) $n_1 = n_2 = 1.33$ and (b) $n_1 = 1.33$ and $n_2 = 1.58$. The plot represents the distribution in the cross-section shown by the dashed lines in Figure 6.2 (a).

Figure 6.4. Amplitude and phase at detector positions for both the FEM and Monte Carlo models where $n_1 = n_2 = 1.33$, and $n_1 = 1.33$ and $n_2 = 1.58$. Calculated data from the FEM model with no internal boundary condition (NIB) are also shown.
6.D. Measuring phantom material refractive index

The resin used to make the solid plastic phantoms is transparent (amber in appearance). Snell’s law \( n_1 \sin \theta_1 = n_2 \sin \theta_2 \) was used to calculate its refractive index by observing the angle by which it deflects a collimated beam. Repeated measurements yielded \( n_2 = 1.58 \pm 0.01 \). Also from Snell’s law, when a wave in medium 1 is incident on a less dense medium 2 (i.e. \( n_1 > n_2 \)), \( \theta_2 > \theta_1 \). The angle \( \theta_1 \) for which \( \theta_2 = \pi/2 \) is referred to as the critical angle, \( \theta_c = \sin^{-1}\left(\frac{n_2}{n_1}\right) \). A simple apparatus was used to setup an interface between resin \( (n_1=1.58) \) and a number of materials with unknown refractive indices. A schematic is shown in Figure 6.5. A sample was fixed to the back of a half cylinder of resin which was polished on all sides. Two rotating arms swinging from the location of the sample held a HeNe laser (approximately 633 nm) and a photodetector. The arms were adjusted at equal angles from the normal direction to the resin-sample interface, and the intensity of the reflected light was measured. The critical angle was determined by measuring the angular separation of the arms corresponding the highest intensity reflection measurement. Using this apparatus, the refractive index of water \( (n_2=1.34 \pm 0.01) \), deli turkey \( (n_2=1.41 \pm 0.01) \), and mayonnaise \( (n_2=1.49 \pm 0.01) \) were measured.
6.E. Comparing FEM and phantom measurements

Two experiments were performed, which consisted of collecting data at 785 nm from cylindrical breast phantoms using the Dartmouth stand-alone NIR tomography system. In the first experiment, transmission measurements through a solid plastic cylinder with $\mu_a=0.0055$ mm$^{-1}$ and $\mu'_s=0.871$ mm$^{-1}$ with a diameter of 85 mm were obtained. The phantom’s center was then hollowed out (inner diameter=66 mm), filled with a mixture of water and Intralipid with the same $\mu_a$ and $\mu'_s$ as the plastic, and a new set of measurements was acquired. The imaging system was used to confirm differences in $\mu_a$ and $\mu'_s$ for the two materials of less than 1%. Assuming $n=1.58$ for the plastic and $n=1.33$ for the water-Intralipid emulsion, and assuming no change in absorption or scattering in the two-layer medium, the changes in the measurements were attributed to the change in $n$ within the phantom. FEM model calculations of the fluence for the two cases are shown in Figure 6.6. It is apparent that when $n_1>n_2$, light is ‘trapped’ in the $n_1$ material. As with the slab simulation, larger changes are seen in phase data than in

Figure 6.5. Schematic of apparatus used to measure refractive index of samples of water, deli turkey, and mayonnaise.
amplitude data. The agreement between the measured and calculated data is fair, and the model predicts the proper trend (i.e. phase decreases with the decrease in $n_2$).

In the second experiment a liquid phantom ($\mu_a=0.0164 \text{ mm}^{-1}$, $\mu_s'=0.33 \text{ mm}^{-1}$, $n=1.33$, $d=92 \text{ mm}$) was used for NIR measurements, and then a mismatch in refractive
index was created using a solid inclusion \((n=1.58, \, d=73 \, \text{mm})\) with the same optical properties. The results from this experiment are shown in Figure 6.7, and match expectation. Photons appear to collect in the inclusion, and both measured and calculated phase increase when \(n_2\) increases.

Figure 6.7. (a) A schematic drawing of the experiment. (b) The measured amplitude and phase data for one source position, and the FEM calculations using 2D and 3D models. (c) FEM model calculation of fluence, and the difference representing the effect of increasing \(n_2\).
6.F. Refractive index in image reconstruction

Using simulated data from this modified diffusion approximation, Dehghani et al.\textsuperscript{144} determined the effects of internal refractive index mismatch on reconstructed images of absorption and scattering coefficients. Optical property images were reconstructed assuming correct \textit{a priori} knowledge of a layered refractive index distribution to show that the modified diffusion approximation can accurately recover a simulated anomaly. Without the correct knowledge regarding the refractive index distribution, the recovered anomaly shows degraded quality, depending on the degree of refractive index mismatch. It was concluded that provided the refractive index of breast tissue is approximately 1.3-1.4, its exclusion from the model will have minimal effect in imaging. Of course this is not the case in solid-liquid phantoms described earlier in this chapter.
Chapter 7: NIR-MRI phantom imaging

7.A. Introduction

As described earlier, the motivation for developing a simultaneous NIR-MRI imaging platform is the hypothesis that high resolution MRI data can be used to improve the resolution and quantitative accuracy of NIR images. This chapter explores the validity of this hypothesis through phantom experiments. FEM meshing, data calibration, and MR-guided image reconstruction algorithms are discussed (steps two, three, and four in the image formation flowchart (Figure 1.5)). Images reconstructed from experimental data are presented as a final validation prior to patient imaging.

7.B. MRI segmentation and FEM meshing

When dealing with phantoms or tissues having arbitrary shape, the effectiveness of the (homogeneous phantom) data calibration (section 7.C) and overall image quality from reconstruction hinges on the accurate definition of the imaged area/volume with 2D/3D FEM meshes and accurate specification of source and detector fiber locations. Consider the simulation example shown in Figure 7.1. Data was generated for a circular geometry with a diameter of 86 millimeters and a simple absorption and scattering coefficient distribution. This data was used to reconstruct images based first on the correct source and detector locations and then on incorrect locations. Figure 7.1 shows that introducing 1-2 millimeter errors in the specified optode locations causes image artifacts.
In order to capitalize on the accurate coregistration of the NIR-MRI data acquisition, tools were developed to transfer MR data into the FEM modeling geometry. Software utilities have been created which perform MRI segmentation, FEM meshing, and fiber location specification. The probability of modeling errors is minimized using this tool, because each fiber is marked with an MR fiducial and the MRI clearly depicts the outer boundary surface of the phantom or tissue.
7.B.1. 2D segmentation and meshing procedure

A graphical user interface (GUI), shown in Figure 7.2, was designed in MATLAB® to accomplish MRI segmentation and FEM mesh generation: [mri2mesh.m, mri2mesh.fig]. An MRI image file (DICOM format, 256x256 pixels, 16-bit grayscale) is selected, displayed, and then segmented using functions from Matlab’s image processing toolbox to create a mask (256x256 pixels, unsigned 8-bit integer). The MRI is first converted to a binary image using a threshold value which minimizes the intraclass variance of the thresholded black and white pixels (see Matlab functions graythresh.m and im2bw.m). Through semi-automatic repeated thresholding, multiple regions can be defined, each with similar MRI grayscale values. Pixels associated with empty space in the MRI are then assigned a zero in the mask, and each material or tissue type is assigned a distinct integer representing its region number. The size and connectivity of segmented regions is also recorded so that the fiducial markers can be excluded from the mask.

Following segmentation, a mesh generator (img2mesh) is called from within the GUI to automatically create a 2D mesh given a segmented image mask and the number of nodes desired in the mesh. The mesh generator has been compiled for use under WINDOWS (img2mesh.exe), LINUX (img2mesh*), or UNIX (img2mesh_sgi*). The 2D meshes typically used contain approximately 2000 nodes (and 4000 elements), which represents sufficient discretization for identifying interior regions as small as 1 mm and ensures numerical accuracy. The output of this mesh generator is then converted to NIRFAST format (*.node, *.elem, *.region, *.param) (see Appendix B). The node locations are adjusted to match the millimeter scale of the original MRI. This mesh is optimized such that the FEM matrix has a minimum bandwidth, which is important for
computational efficiency of the direct matrix solver (the \command in Matlab) used in
the 2D forward problem. Finally, the locations of the optical fibers are specified by the
GUI user, and are saved to file (*.meas, *.source). Including MRI segmentation, the
mesh generation process takes less than five minutes.

Other mesh generators can also be used. The PDE toolbox in Matlab readily
creates meshes of specified arbitrary geometries. Depending on the algorithm employed,
bandwidth minimization may be necessary in post-processing. This can be accomplished

Figure 7.2. Graphical user interface to accomplish MRI segmentation and FEM meshing. A phantom and a patient example are shown.
using the NIRFAST routine minbnd_opt.m, which renumbers the nodes such that each 
node has a minimum number of neighboring nodes.

7.B.2. 3D segmentation and meshing procedure

A GUI was also designed to perform 3D segmentation and FEM meshing: [mri2mesh_3D.m, mri2mesh_3D.fig]. The segmentation procedure is identical to that used in 2D, and is performed serially on each 2D image slice in the MR stack. If the GUI is run from WINDOWS, mesh generation is then performed remotely using LINUX or UNIX. The volume mask is first input into a 3D surface mesh generator (surface2*) which creates a surface mesh along each interface in the mask. The surface meshes are then combined into a single file, and input into a volume mesh generator (spmesh*). Finally, boundary nodes are identified in the volume mesh with additional routines (3dtry* and bnodgen*). 3D meshes with approximately 20,000 nodes are typically used. For models of this size, NIRFAST uses an iterative solver (BiConjugate Gradients Stabilized method) for FEM matrix inversion within the forward 3D solver. The mesh is therefore optimized such that the FEM matrix is very sparse. The NIRFAST routine mindegree_opt.m accomplishes this by renumbering the nodes such that each node has a maximum number of neighboring nodes. The GUI is used to convert the mesh generator output files (volmesh.nod, volmesh.elm, volmesh.bnd) to NIRFAST format, and to locate optical fiber positions. Including MRI segmentation, the 3D mesh generation process takes approximately forty five minutes.
CNI Workbench, a software package for medical image processing, is commonly used for display purposes and solution viewing. Figure 7.3 shows typical views of 3D FEM meshes and MRI slices overlaid on one another for two phantom examples.

![Figure 7.3. 3D visualization of two phantoms using CNI Workbench.](image)

The MRI stacks used here typically have slice separations of 2 mm and pixel spacing <1 mm, so out-of-slice (z-axis) resolution is less than in-slice (x-y-axis) resolution. The ability of this serial 2D segmentation approach to consistently capture details which extend through multiple slices is limited because thresholding is applied to each slice independently, without consideration of adjacent slices. It is not uncommon to observe rough boundaries in the z-direction. In collaboration with Siemens Corporate Research, other ‘full’ 3D segmentation algorithms are being investigated, which could be more accurate than the serial 2D segmentation used here. Two different approaches have been implemented and compared to each other, using eight patient datasets. The
algorithms used were not optimized for the breast datasets, and early results could be considerably improved upon if the appropriate efforts are made to adapt these algorithms.

7.C. Data calibration

Practical data calibration issues arise when using a model-based image reconstruction to produce images from data measured with a real instrument. Once acquired, amplitude and phase data pass through a homogeneous phantom calibration process before image reconstruction.\textsuperscript{55, 148} The procedure used at Dartmouth modifies the raw data to account for systematic instrumentation-based offsets related to inter-fiber variations, source strength (i.e. multiplexing imprecision), and fiber-tissue coupling issues. These offsets need to be removed from the experimental data prior to image reconstruction in order to prevent the appearance of artifacts in the resultant tissue property profiles.

The method involves measuring a homogeneous phantom before or after measuring the heterogeneous medium, and subtracting the differences between measured and calculated homogeneous data from the heterogeneous data:

\[
\Phi_{M,\text{hetero(calibrated)}}^i = \Phi_{M,\text{hetero}}^i - \left( \Phi_{C,\text{homo}}^i - \Phi_{M,\text{homo}}^i \right). \tag{7.1}
\]

A homogeneous fitting algorithm\textsuperscript{55, 81} is used to determine the bulk optical properties (\(\mu_a\) and \(\mu'_s\)) for which calculated data, \(\Phi_{C,\text{homo}}^i\), best matches the measured data from the homogeneous calibration phantom, \(\Phi_{M,\text{homo}}^i\). Thus any differences between the best possible data fit are used as the set of calibration factors in equation (7.1) which represent the set of potentially systematic errors in each of the 240 measurements. Figure 7.4 shows data measured from a homogeneous phantom and from a heterogeneous phantom.
both with dramatically reduced ac amplitude for the 15 measurements associated with source channel #8. Calibration adjusts the heterogeneous phantom data, eliminating this systematic offset.

Figure 7.4. (a) Data measured from a homogeneous and heterogeneous phantom. For both measurements, source coupling through fiber #8 was poor, resulting in a consistent reduction in ac amplitude for 15 measurements. (b) Calibrated data exhibits relatively uniform ac amplitude for all 16 source positions (i.e. systematic offset is eliminated).

In the homogeneous fitting algorithm, the bulk $\mu_a$ and $\mu_s$ are determined using a Newton-Raphson minimization of two parameters: slope of the phase with respect to source-detector separation distance, and slope of the log of intensity times distance, $\ln(I*\text{distance})$, with respect to distance$^{50}$. These two parameters are convenient because they can be obtained from the data through linear regression, are insensitive to noise, and are constant for the analytic infinite medium diffusion equation solution. Figure 7.5 show these regressions on the data from Figure 7.4. The FEM meshes which represent the measurement geometries are also shown. The fitting algorithm involves two steps: (1) an initial solution for bulk $\mu_a$ and $\mu_s$ from a minimization based on calculated data
from an analytic solution for an infinite medium\textsuperscript{149}, and (2) the solution for bulk $\mu_a$ and $\mu_s$ in the measurement geometry from a minimization based on calculated data from the FEM forward solver, starting from the values for the infinite medium.

The image reconstruction algorithm starts from an estimated set of bulk optical properties and iteratively updates the spatially variant values based on the diffusion equation relationship and the difference between the calculated and measured data. The initial bulk estimate is obtained using the homogeneous fitting algorithm with $\Phi_{\text{hetero}}^{\text{h}}$.

Troy McBride\textsuperscript{81} discusses the impact of system drift (with respect to source
strength and initial phase) between the time of the reference measurement and the experiment. In practice, this offset can be removed by adding a factor, $\Phi_{\text{offset}}$, to the right hand side of equation (7.1). The factor can be determined in three different ways (each produce similar $\Phi_{\text{offset}}$). (1) From McBride et al.\(^5\),

$$\Phi_{\text{offset, homo}} = \frac{\sum_{i=1}^{N} (\Phi^{C}_{i, \text{homo}} - \Phi^{M}_{i, \text{homo}})}{N},$$

$$\Phi_{\text{offset, hetero}} = \frac{\sum_{i=1}^{N} (\Phi^{C}_{i, \text{hetero}} - \Phi^{M}_{i, \text{hetero}})}{N},$$

$$\Phi_{\text{offset}} = \Phi_{\text{offset, hetero}} - \Phi_{\text{offset, homo}}.$$  \hspace{1cm} (7.2)

Here, the homogeneous fitting algorithm is used to determine offsets for both measurement sets, and the factor results in the difference between these offsets. (2) Another method could simply use the mean difference between the two measurements,

$$\Phi_{\text{offset}} = \frac{\sum_{i=1}^{N} (\Phi^{M}_{i, \text{hetero}} - \Phi^{M}_{i, \text{homo}})}{N}. \hspace{1cm} (7.3)$$

(3) Or the differences between the y-intercepts of the homogeneous and heterogeneous data regressions could be used. In early work with simple phantom imaging experiments, this overall offset was small and had little effect on the calibrated data, but the correction factor was included nonetheless\(^7\). Reference measurements are generally taken immediately before or after the imaging exam, and it is reasonable to assume that system drift is small. Larger offsets were calculated in experiments on more complicated phantoms, or when the properties of the reference phantom were drastically different than the bulk properties of the experiment phantom. When included, these larger offsets created significant image artifacts, which is validated in the simulation described next.
Noise was added to simulated data from a homogeneous circular model, and to simulated data from a heterogeneous model. The calibration procedure was performed with and without the extra factor in equation (7.1). Figure 7.6 shows the target optical properties, and the images reconstructed from the two calibrated data sets. Including the extra term introduces visible edge artifacts in this simulation, and similar artifacts in reconstructions of experimental data (not shown). Therefore, $\Phi_{\text{offset}}$ is ignored here; source strength is assumed to be constant, and initial phase shift is fixed to 30 degrees in data acquisition.

Figure 7.6. Attempts to correct for system drift between the time of the reference measurement and the time of the experiment have resulted in image artifacts. The offset term is therefore ignored.
7.D. NIR-MRI gelatin phantom results

Three gelatin phantom experiments performed over the last two years have been selected for presentation in this section. Each experiment provided useful information, motivating subsequent improvements to the data acquisition system and to several image reconstruction algorithms. Generally, 2D images or absorption and scatter are shown, and ‘conventional’ Levenberg-Marquardt reconstruction (i.e. no a priori information, equation (3.24)) is compared to MR-guided reconstruction. Samples of 3D results are also shown.

All of the phantoms shown are heterogeneous, meaning that an inclusion is embedded in a homogeneous background, or in a layered background. The normal procedure is to make homogeneous phantoms from the same materials that go into the heterogeneous phantom. The homogeneous material is imaged, and the homogeneous fitting algorithm provides the ‘true’ optical properties. One of these homogeneous phantoms is generally used as the reference for data calibration. The wavelength used in all experiments shown here was 785 nm.

7.D.1. Single inclusion

The first phantom imaged in the MRI with this system is shown in Figure 7.7. A gelatin cylinder (diameter=82 mm, height=90 mm) contained a pie-wedge shaped inclusion (approximately 15 mm across, height=6 mm) with an absorption coefficient ($\mu_{a,\text{inclusion}}=0.04 \text{ mm}^{-1}$, $\mu_{s,\text{inclusion}}=0.90 \text{ mm}^{-1}$) approximately four times that of the background. The optical fibers were located in the plane of the inclusion when NIR measurements were taken. In this experiment, the fibers were not marked with fiducials,
but their position was confirmed by identifying the surface depressions in the coronal and sagittal MRI slices.

![Image of MRI experiment](image)

**Figure 7.7.** An early NIR-MRI experiment. A cylindrical gelatin phantom (diameter=82 mm) had a single wedge-shaped absorbing inclusion (4:1 contrast with background). Images obtained with conventional reconstruction, and using hard priors are shown. With hard priors, the anomaly’s true absorption coefficient is recovered with 92.5% accuracy.

<table>
<thead>
<tr>
<th>Target optical properties</th>
<th>Background</th>
<th>Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_a$ (mm$^{-1}$)</td>
<td>0.0095</td>
<td>0.04</td>
</tr>
<tr>
<td>$\mu_s$ (mm$^{-1}$)</td>
<td>0.80</td>
<td>0.90</td>
</tr>
</tbody>
</table>

The conventional reconstruction reproduces a peak absorption and scatter in the vicinity of the inclusion. The recovered peak values for the inclusion region, $\mu_a = 0.025$
mm$^{-1}$ and $\mu_s'=2.57$ mm$^{-1}$, are too low and too high, respectively. Using hard priors (equation (3.29)) to calculate $\mu_{a,\text{background}}$, $\mu_{s,\text{background}}$, $\mu_{a,\text{inclusion}}$, and $\mu_{s,\text{inclusion}}$, applying a global mean filter at each iteration, the inclusion is recovered with greater accuracy ($\mu_{a,\text{inclusion}}=0.037$ mm$^{-1}$ and $\mu_{s,\text{inclusion}}=0.75$ mm$^{-1}$). Soft priors (equation (3.37)) produce a similar result, recovering $\mu_{a,\text{inclusion}}$ and $\mu_{s,\text{inclusion}}$ with 85% accuracy.

When reconstructing these images, the same fiber locations were assumed for both the reference and the experimental measurements. Figure 7.8 plots the conventional image from Figure 7.7 on a tighter colorbar, revealing edge artifacts caused by movement of the optical fibers between the reference measurement and the experiment. These artifacts were a hazard of the thumb-screw fiber holder. Figure 7.8 also shows a conventional reconstruction on a uniform circular mesh having the same artifacts, indicating that accurate definition of source/detector locations may be more important than small boundary details in the FEM mesh. When boundary impressions are larger, as they are with breast exams, the recovered optical properties in the image periphery change if they are not included in the model (as shown in Section 9.D). Figure 7.8 also shows the phantom’s absorption coefficient reconstructed (with the conventional method) in 3D from the same data. The peak absorption coefficient ($\mu_a=0.022$ mm$^{-1}$) is slightly reduced relative to the 2D case. Image detail is limited in the z-direction, but fewer artifacts appear in the plane of interest.
Figure 7.8. (a) Edge artifacts (‘hot spots’) are caused primarily by errors in the source and detector locations. Reconstructions in this case are only minimally affected by meshing fine surface indentation details. (b) Conventional 3D reconstruction ($\mu_a$) using a single measurement plane.

7.D.2. Inclusion and inner layer

A three layer phantom was built to better replicate the optical signature of tumor tissue, embedded in fibroglandular tissue, surrounded by adipose tissue. Layered property distributions pose a key challenge for optical imaging systems, and this experiment shows they can be resolved with the help of coregistered MRI. Two phantoms were made with the same background (diameter=82 mm, $\mu_a=0.0055$ mm$^{-1}$, $\mu_s'=0.76$ mm$^{-1}$) and the same inclusion ($\mu_a=0.02$ mm$^{-1}$, $\mu_s'=0.76$ mm$^{-1}$), and one was given an inner layer with different properties ($\mu_a=0.01$ mm$^{-1}$, $\mu_s'=1.2$ mm$^{-1}$). The outer layer (and inner layer) extended the full height of the phantom (10 cm) while the inclusion (diameter=15 mm, height=25 mm,) was embedded half-way from top to bottom. Equal fiber radii were maintained for all measurements which resulted in
minimal phantom surface deformation. Circular and cylindrical models are assumed. Reconstructed 2D images of the phantom without the layer are shown in Figure 7.9.

![Photograph of a gelatin phantom](image1)

![Cross-sectional MRI](image2)

![Phantom images reconstructed using soft priors](image3)

**Figure 7.9.** (a) Photograph of a gelatin phantom. A cylinder (diameter=15 mm, height=25 mm) is embedded under the pin. (b) A cross-sectional MRI through the plane of the inclusion. (c) Phantom images reconstructed using soft priors are superior. The true object shape is recovered with greater quantitative accuracy.

<table>
<thead>
<tr>
<th>Target optical properties</th>
<th>Background</th>
<th>Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_a$ ($\text{mm}^{-1}$)</td>
<td>0.0055</td>
<td>0.02</td>
</tr>
<tr>
<td>$\mu_s$ ($\text{mm}^{-1}$)</td>
<td>0.76</td>
<td>0.76</td>
</tr>
</tbody>
</table>

The conventional reconstruction underestimates $\mu_{a,\text{inclusion}}$ and overestimates $\mu_{s,\text{inclusion}}$. In this case there should be no scattering contrast, and so the increase in $\mu_{s,\text{inclusion}}$ relative to $\mu_{s,\text{background}}$ likely represents $\mu_a$ cross-talk. The conventional reconstruction also distorts the circular shape of the inclusion. It should be noted that
hard priors failed in this case, converging to the erroneous $\mu_{a,\text{inclusion}} = 0.2\ \text{mm}^{-1}$ and $\mu_{s,\text{inclusion}} ' = -0.5\ \text{mm}^{-1}$. Soft priors yield both qualitative and quantitative improvements relative to the conventional method. Cross-talk is reduced, and a circular anomaly appears in the absorption coefficient image with a peak value matching 99\% of the ‘true’ value expected.

Figure 7.10. (a) Conventional 3D reconstructions using three planes of data. (b) Soft prior reconstruction.

Three planes of NIR data approximately 2.0 cm apart were collected along the 10 cm height of this phantom. The middle measurement plane cut through the center of the
2.5 cm tall inclusion. This data was used to reconstruct the 3D images in Figure 7.10. As noted in the previous section, the peak absorption recovered for the ROI is again slightly reduced relative to the 2D reconstruction. The conventional solution places the object closer to the boundary than expected. Soft priors remove a noticeable artifact close to the edge opposite the inclusion.

![Image](image_url)

**Figure 7.11.** (a) MRI slice through the three-layer gelatin phantom. (b) 2D FEM mesh with layer information from MR intensity. (c) Phantom images reconstructed using soft priors are superior. The true layered structure is recovered with greater quantitative accuracy.
For the layered phantom, 2D images reconstructed from measurements in the plane of the inclusion are shown in Figure 7.11. Parameter reduction failed again in this case and is not shown. The third pair of images illustrates the performance of the algorithm when soft priors are incomplete—the presence of the inclusion was not specified. The bottom pair of images is produced using the full MRI data to form the regularization matrix, including the presence of the inclusion.

Conventional image reconstruction is greatly improved when the layered structure of this phantom is used in the regularization matrix. When the MRI is ignored, the calculated images only slightly resemble the true spatial structure of the phantom. Scattering of the inclusion incorrectly appears to increase relative to the inner layer. The RMS error of the recovered distributions of the absorption and reduced scattering coefficients are estimated to be 0.0023 and 0.230, respectively. When a subset of the prior knowledge of the phantom’s structure provided by MRI is used in image reconstruction, the images which result indicate the presence of three material types, but quantitative accuracy is not optimal. When the full MRI data set is utilized to derive soft priors, distinct boundaries separating each of the phantom layers are recovered. In this case, the RMS error of the absorption and reduced scattering coefficient images decreases 43% to 0.0014, and 55% to 0.104, respectively. The mean value of the absorption coefficient estimated in the region of the inclusion is accurate within 10% (0.018 mm\(^{-1}\) compared to the expected 0.02 mm\(^{-1}\)). Estimation of the reduced scattering coefficient improved to within 20% (0.9 mm\(^{-1}\) compared to the expected 0.76 mm\(^{-1}\)). These values compare very well to those derived from the phantom without the added layer \((\mu_{a,\text{inclusion}}=0.017 \text{ mm}^{-1} \text{ and } \mu_{s,\text{inclusion}}=0.85 \text{ mm}^{-1})\). This indicates that quantification
through reconstruction is not degraded as the complexity of the geometry increases, as long as prior knowledge of that complexity is available.

Measurements were also taken at two planes along the height of the layered phantom. The first was used for 2D reconstruction, and the second was 5 mm below the bottom edge of the inclusion. This data was used to reconstruct the 3D images in Figure 7.12. These images are comparable to the 3D reconstructions of the phantom without the added layer.

![Figure 7.12](image)

**Figure 7.12.** (a) Conventional 3D reconstructions using 2 planes of data. (b) Soft prior reconstruction.
7.D.3. Variable inclusion contrast

To further characterize the performance of the system and the quality of the defined algorithms, a phantom with inclusions of different contrast was imaged\textsuperscript{71}. A gelatin solution ($\mu_s=0.005 \text{ mm}^{-1}$, $\mu_s'=0.85 \text{ mm}^{-1}$) was hardened inside an 82 mm cup, with a 22 mm diameter cylindrical rod included in the interior. After the gelatin hardened, the rod was removed and the empty column was filled with Intralipid solutions having absorption coefficients ranging from 0.005-0.015 mm$^{-1}$. A photograph of the phantom is shown in Figure 7.13 (a). Another photograph (not shown) taken of the phantom in the imaging array was used as a surrogate MRI to define \textit{a priori} information on the phantom’s structure, and provided the necessary detail to carry out region of interest analysis to assess reconstruction accuracy.

For the images shown in Figure 7.13 (b), when soft priors are used in the reconstruction, the RMS error of the absorption and reduced scattering coefficient images decreases from 0.0019 to 0.0014 (26\%) and from 0.1444 to 0.0613 (58\%), respectively, relative to conventional reconstruction. The data plotted in Figure 7.13 (c) and (d) indicates that linearity exists between the estimated and the true contrast for the two reconstruction methods used. MRI-guided reconstructions recovered more accurate property values over the entire range of contrast. The error bars represent the NIR image pixel standard deviation indicating that the variation in the background and inclusion were reduced by using MRI priors. This experiment was repeated using several layered phantoms with cavities for the addition of Intralipid inclusions. One is shown in Figure 7.14.
Figure 7.13. (a) Photograph of a homogeneous gelatin phantom with a 22 mm cylindrical cavity slightly off-center. (b) Reconstructed optical property images for this phantom when an Intralipid solution with 3:1 absorption contrast fills the cavity. Image artifacts appear in the form of artificial background heterogeneity when priors are not utilized. A more accurate estimate of the true optical properties, and shape of the inclusion, is obtained with the MR-guided iterative algorithm. (c-d) Mean absorption and reduced scattering coefficients for both the background (bg) and inclusion (inc) recovered using the two algorithms for eight Intralipid solutions.
Figure 7.14. (a) Photographs of two-layered gelatin phantoms with cylindrical cavities. (b) Target optical property maps for one of these, with an Intralipid inclusion, and reconstructed images. A more accurate estimate of the true optical properties is obtained with the MR-guided iterative algorithm. (c) From top to bottom, absorption coefficients for the background, inner region, and inclusion for eight Intralipid solutions. In this case, hard priors work as well as soft priors.
7. E. Summary of algorithm performance in phantom experiments

These phantom experiments dealt with locating and quantifying inclusions with higher absorption coefficients than the background media. With conventional reconstruction of measured data it is possible to locate the approximate position and shape of the anomaly. Peak values in the reconstructed 2D images are generally in good agreement with the true values expected for the inclusion. However, some absorption coefficient cross-talk is usually observed in the reduced scattering coefficient images. Also, noise in the data manifests itself in the form of artificial heterogeneity, especially around the periphery of the image, where model sensitivity is high.

When incorporating a priori information, derived from phantom MRI slices, soft priors (i.e. regularization schemes) outperform hard priors (i.e. parameter reduction). Applying hard priors in the iterative coefficient estimation sometimes produce accurate results, but may also yield convergent solutions with large quantitative errors. These are rigid constraints, and spatial resolution is not allowed to deviate from the pattern specified a priori. Discrepancies between the boundaries in the true optical property distribution and those modeled are difficult to avoid in physical measurements, and are most likely the cause of biased solutions. The performance of hard priors is difficult to predict—the method has succeeded and failed for both simple and layered phantoms. The one scenario where it appears robust is when two regions of similar size can be specified. Hard priors are universally avoided in the clinical applications presented in Chapter 9.

These experiments indicate that resolution and accuracy of conventional image reconstruction can be improved through soft priors—integrating the spatial pattern
observed in the MRI into a full regularization matrix. Spatial NIR image patterns correctly resemble those of the phantom MR images. Soft priors are robust to noise, and their region-specific filtering reduces artificial spatial variation in both the background and the inclusion. Unlike the rigid constraints of hard priors, soft priors allow some disagreement between the input prior and the algorithm output, and are much more robust. Contrasts should be dictated primarily by the measured data, not by the prior introduced from MRI. The contrast mechanisms of optical imaging and MRI are different, and so an MRI edge does not guarantee absorption or scattering edges. Soft priors preserve sharp contrast boundaries, if they happen to coincide with the prior. It should be noted, however, that when a localized region is specified to exist, but no absorption or scattering contrast actually exists, the algorithm does not introduce artificial contrast within the image space matching the prior. In terms of inclusion quantification, the average value throughout the inclusion ROI is closer to the true value when soft priors are included. However, using the same stopping criteria (projection error change less than 2%) it is likely that conventional reconstruction will yield equal or greater peak values.
Chapter 8 : Functional imaging

8.A. Introduction

Thus far, this thesis has addressed the reconstruction of spatially resolved images of absorption and reduced scattering coefficients. The clinical value of NIR tomography lies in its spectroscopic quantification of intrinsic tissue chromophore concentrations and scattering properties. These parameters are important indicators of metabolic activity, functional processes, or presence and staging of disease. Their physiological significance is discussed in the next chapter on patient imaging. This chapter focuses on the methods of their derivation from measurements at multiple NIR wavelengths.

Conventionally, reconstruction of optical absorption and scatter at each discrete wavelength is an intermediate step in functional parameter estimation. Absorption and scattering coefficient images at different wavelengths are combined through spectral analysis to determine the concentration of oxy-hemoglobin, deoxy-hemoglobin, water fraction, scattering amplitude, and scattering power. This procedure, termed ‘indirect’ chromophore estimation, is described in Section 8.B. Given prior knowledge of how optical properties vary as a function of source wavelength, chromophore absorption spectra and scattering spectra can be exploited as a prior in the image reconstruction, leading to a new algorithm for reconstructing functional images directly from the optical measurements at multiple wavelengths. Section 8.C describes this ‘direct’ chromophore estimation algorithm. Section 8.D describes a framework for simultaneously utilizing spectral and spatial priors, which is then tested with a phantom experiment in Section 8.E.
8.B. Indirect chromophore estimation

The absorption coefficient at any wavelength is assumed to be a linear combination of the absorption due to all relevant chromophores (at that wavelength) in a sample:

\[ \mu_a(\lambda) = \sum_{i=1}^{N} \varepsilon(i, \lambda)c_i, \tag{8.1} \]

where \( \varepsilon \) is the molar absorption spectra, and \( c \) is the concentration of each chromophore\(^3\). For the near infrared wavelength range of the imaging system described here (661-849 nm) most absorption by breast tissue can be attributed to oxy-hemoglobin (Hb-O\(_2\)), deoxy-hemoglobin (Hb-R), and water (H\(_2\)O). Figure 8.1 (a) shows a plot of NIR absorption spectra in our wavelength range for Hb-O\(_2\), Hb-R, water, and lipids for anatomically relevant concentrations in breast tissue. Lipid is generally not considered in equation (8.1). However, measurement systems which use more wavelengths, including those longer than 850 nm, do account for lipid absorption\(^{35,150}\). The feasibility of lipid estimation in this application is discussed further in Section 9.E.
Figure 8.1. (a) Plot of NIR absorption spectra for oxy-hemoglobin (Hb-O2), deoxy-hemoglobin (Hb-R), water, and lipids. Values for absorption coefficients are displayed for anatomically relevant concentrations for breast tissue. Absorption coefficients for Hb-O2 and Hb-R were calculated using molar extinction coefficients, \( \varepsilon \), in \([\text{cm}^{-1}/(\text{moles/liter})]\) compiled by Scott Prahl (http://omlc.ogi.edu/spectra/). Lipid coefficients were measured by van Veen et al, and water coefficients were measured by Hale and Querry. (b) Reduced scattering coefficients at six wavelengths measured from two patients are shown, along with the model function fit to the data.
As outlined by Srinivasan et al., molar absorption spectra of tissue chromophores have been published in several studies, and may vary by as much as 10%. Most imaging systems introduce systematic errors in obtaining absorption coefficients, which could be due to the measuring system or the reconstruction procedure. To compensate for these errors in converting light attenuation into chromophore concentrations, molar absorption spectra should be measured specific to an imaging system. In phantom experiments, Srinivasan et al. measured the molar absorption spectra specific to the NIR tomography procedure described here.

Given \( \varepsilon \), and absorption coefficients at multiple wavelengths, the chromophore concentrations can be obtained by a linear least squares constrained fit to the matrix equation

\[
[a] = [E][C]. \quad (8.2)
\]

The size of \([a]\) is \(nw \times nn\), where \(nw\) is the number of wavelengths and \(nn\) is the number of image points, or nodes, assigned a unique coefficient. \(E\) (size of \(nw \times 3\)) contains the molar absorption coefficients for the three chromophores at each wavelength, having elements \(\varepsilon(i, \lambda)\). \(C\) is the concentrations of the three chromophores, which is to be determined. Hence, given \(\mu_a\) at the \(k^{th}\) node for multiple wavelengths, a linear inversion of equation (8.2) determines the array of \(C\) values representing the concentrations of the three chromophores:

\[
C_k = E^{-1}\mu_{a,k}. \quad (8.3)
\]

Having calculated \(C\), total hemoglobin concentration

\[
[Hb_T] = [Hb-O_2]+[Hb-R], \quad (8.4)
\]

in units of \(\mu M\), and percent hemoglobin oxygen saturation
Equation (8.3) is solved using Matlab’s function (lsqlin.m) for constrained linear least squares, while water and \( S_\text{rO}_2 \) are constrained to fall between 0 and 100%.

The spectral character of the reduced scattering coefficient also provides information about the composition and structure of tissue. Origins of the transport scattering coefficient, which can be measured tomographically, are likely the result of differences in the index of refraction between the extracellular or cytoplasmic fractions of tissue and the lipid composition of the membranes bounding each cell and cellular organelle. Mie scattering theory is a model of light scattering which is applicable when the scatterer particle size is near the same dimension as the wavelength of radiation being scattered. Mie theory\textsuperscript{5, 56, 152} predicts the scattering reasonably well but strictly applies only for spheres in a homogenous background, and the use of this theory in random media such as tissue can only be an approximation. Furthermore, when using this theory assumptions must be made regarding the histogram of particle number density per unit particle size (i.e. exponential, gaussian, or step\textsuperscript{20}). The alternate approach for this type of fitting is to approximate the spectrum as a power law, rather than an explicit fit to Mie scattering theory\textsuperscript{56}:

\[
\mu'_i(\lambda) = A \lambda^{-b},
\]

where \( A \) is the scattering amplitude and \( b \) is the scattering power. A number of groups have adopted this approach to characterize the spectrum of the reduced scattering coefficient observed in tissues\textsuperscript{1, 40, 52, 152}. A method for extracting the mean particle size and number density from this spectrum is proposed by Wang et al.\textsuperscript{20, 153}. Section 9.B

\[
S_\text{rO}_2 = \frac{[\text{Hb-O}_2]}{[\text{Hb_T}]} \times 100,
\]
discusses further how scattering features may provide fundamental insight into these pathophysiologic changes.

Equation (8.6) describes a smooth function with no oscillations in the spectrum, and conveniently restricts the fitting process to only two parameters. A nonlinear least squares curve fit in Matlab (lsqcurvefit.m) is used to solve for \( A \) and \( b \), with wavelengths in \( \mu \text{m} \). The coefficient \( \mu_s' \) has units \( \text{mm}^{-1} \), \( b \) is dimensionless, and \( A \) has units given by \( 10^{-3b}(\text{mm})^{b-1} \). Scattering power is governed primarily by the shape or slope of the \( \mu_s' \) spectrum which is thought to be predominantly affected by the size distribution of membrane bound scatterers within the tissue. Scattering amplitude relates to the number density of these scatterers\(^{20}\). Scattering and absorption spectral analysis have been combined in a single program entitled calibrate_spectral_bb.m. Multi-wavelength absorption and scattering coefficient solution files (from image reconstruction) are its input arguments, and \([\text{HbT, S}_2\text{O}_2, \text{water, A, b]}\) at every image location are its outputs.

### 8.C. Direct chromophore estimation

The quantification of chromophores and scattering parameters relies upon the spectral decomposition of the images acquired at a sparse number of discrete wavelengths instead of a complete spectrum. This sparse spectral sampling, coupled with an ill-posed image reconstruction process, tends to amplify errors in quantifying spatially resolved parameters in tissue. Corlu et al.\(^{74}\) and Li et al.\(^{75}\) showed that measurements at multiple wavelengths can be used simultaneously to compute images of constituent parameters, without the intermediate recovery of optical properties. Applying the technique to continuous-wave data, with suitable assumptions regarding scatter, they showed images
with improved parameter independence in simulations. Srinivasan et al.\textsuperscript{154,155} extended the approach to the frequency domain, and showed experimental evidence of improved quantification. This spectrally-constrained approach proved very robust in the presence of high levels of measurement noise (up to 5\%). It suppresses artifacts, especially those significant in water and scatter power images, and reduces cross-talk between chromophore and scatter parameters. Cross-talk can be reduced further with the addition of measurements at more wavelengths. The development and characteristics of direct chromophore reconstruction is the primary emphasis of Subhadra Srinivasan’s Ph.D. thesis\textsuperscript{156}.

As discussed in Chapter 3, optical property image reconstruction minimizes the least squares functional (equation (3.18))

\[
\chi^2 = \sum_{i=1}^{NM} \left( \Phi_i^C - \Phi_i^M \right)^2 ,
\]

(8.7)

for \(NM\) measurements, with a Newton-Raphson iteration scheme, along with Levenberg Marquardt regularization. The optical property updating is governed by the matrix equation (equation (3.24))

\[
\Delta \mu = \left[ J^T J + \lambda I \right]^{-1} J^T \left( \partial \Phi \right) ,
\]

(8.8)

where \(J = \left[ J_{\mu}, J_D \right]\) is the Jacobian containing the derivatives of \(\Phi^C\) with respect to the optical properties \(\mu = (\mu, D)\), and \(\partial \Phi = \Phi_k^C - \Phi_k^M\) at the \(k^\text{th}\) iteration\textsuperscript{54}. To incorporate spectral relationships into the reconstruction directly, the least squares functional is modified to be,

\[
\chi^2 = \sum_{i=1}^{NM} \left( \Phi_i^C - \Phi_i^M \right)^2
\]

(8.9)
so that \( i \) includes all wavelength measurements \((NMn=240\times6)\), where \( n=6 \) is the number of wavelengths available. Newton’s method then gives a different relationship, which for each wavelength is represented by

\[
\partial \Phi_A = \mathcal{J}_{c,\lambda} \partial \sigma + \mathcal{J}_{A,\lambda} \partial A + \mathcal{J}_{b,\lambda} \partial b
\]  

(8.10)

where \( \mathcal{J}_{c,\lambda} \), \( \mathcal{J}_{A,\lambda} \) and \( \mathcal{J}_{b,\lambda} \) represent the Jacobians for each of the chromophore and scattering parameters. The relationships between these Jacobians and \( J_{\mu_a} = \frac{\partial \Phi}{\partial \mu_a} \) and \( J_D = \frac{\partial \Phi}{\partial D} \) calculated before have been derived by Srinivasan et al.\(^{155}\). Equation (8.8) is suitably modified so that the update in chromophores and scatter parameters \( \Delta c \) occurs directly:

\[
\Delta c = \left[ \mathcal{J}^T \mathcal{J} + \lambda I \right]^{-1} \mathcal{J}^T \partial \Phi ,
\]  

(8.11)

where \( \partial \Phi = \left\{ \Phi_k^{c,\lambda} - \Phi_k^{M,\lambda} \right\}_{\lambda=1}^{\lambda_n} \) and \( \mathcal{J} = \left[ \mathcal{J}_{c,\lambda}, \mathcal{J}_{A,\lambda}, \mathcal{J}_{b,\lambda} \right]_{\lambda=1}^{\lambda_n} \).

### 8.D. Combining spectral and spatial priors

In direct chromophore image reconstruction, chromophore and scattering spectra are applied directly in the reconstruction algorithm, thereby reducing the parameter space of the inversion process. These spectral relationships are a form of prior information which inherently enforces a natural spectral consistency in the reconstructed images. Since both spectral and spatial priors individually improve image quality, the next natural step involves combining them to further optimize the reconstruction.

Li et al.\(^{157}\) described a framework for incorporating multiple (i.e. spectral and spatial) priors in diffuse optical tomography in the context of breast imaging, and
demonstrated significant improvement in image quality in simulations. In their work on spatial priors, Li et al. incorporated three-dimensional x-ray mammography (i.e. tomosynthesis) in the NIR reconstruction of a breast lesion. Their spatial prior was incorporated into a modified objective function containing a structural prior term. They used two different regularization parameters for the background and for the lesion. A smaller regularization in the area of the lesion reduces the penalty for the reconstruction of the optical contrast, and thus increases the probability of finding contrast in the designated region. Their spectral prior enforces spectral consistency in the reconstructed images. Assuming that absorption is dominated by hemoglobin, a modified matrix equation is solved such that these parameters are reconstructed directly. Intes et al. also showed in simulation studies that functional parameter estimation can be enhanced with multiple priors.

This section describes the combination of spectral priors (from Section 8.C) and spatial priors (from Section 3.D.3), developed separately for our application. The least squares functional which includes the penalty term for spatial priors (equation (3.32)) is modified to include measurements taken at \( n \) wavelengths:

\[
\mathcal{X} = \sum_{i=1}^{NM} (\Phi_i^C - \Phi_i^M)^2 + \beta \sum_{j=1}^{NN} [L(\mu_j - \mu_0)]^2. \tag{8.12}
\]

\( \beta \) is the regularizing factor for the spatial prior, and \( L \) is a matrix generated from MRI-derived spatial data acting on the solution \( \mu \), which in this case is represented by the parameter list: Hb-O2, Hb-R, water, A, and b. Setting the derivatives of \( \mathcal{X} \) with respect to each of these parameters equal to zero, the final matrix equation which is solved iteratively becomes
\[
\Delta c = \left[ \mathbf{Z}^T \mathbf{Z} + \beta \mathbf{L}^T \mathbf{L} \right]^{-1} \mathbf{Z}^T \delta \mathbf{\Phi}.
\]  

(8.13)

**8.E. Simulation and phantom results**

The algorithm which utilizes both spectral and spatial priors was tested using simulated and measured data. A simulated model was defined having a separate localized contrast in each parameter (Figure 8.2 (target)). Using the defined chromophore concentrations, scattering amplitude, and scattering power, maps of absorption and reduced scattering coefficients at six wavelengths were calculated by equations (8.2) and (8.6), respectively. From each of these maps, amplitude and phase data was generated using the FEM forward solver, and randomly distributed Gaussian noise (1% in AC amplitude and 1 degree in phase shift) was added. Using this data, several algorithms were then used to reconstruct the initial model (Figure 8.2). Conventional methods yield (noisy) images with high spatial frequency variations. Spatial priors alone produce smoother images with mild cross talk between several of the parameters, especially between deoxy-hemoglobin and scatter power. Spectral priors moderately improve quantification (especially of deoxy-hemoglobin and water) and reduce cross talk. Combining spectral and spatial priors gives the best image resolution and quantitative accuracy.
having localized contrast in oxy-hemoglobin ([HbO], µM), deoxy-hemoglobin ([Hb], µM), water (H2O, %), scattering amplitude (A), and scattering power (b). Combining spectral and spatial priors improves reconstruction of wavelength-dependent measurements.

The benefits of spectral and spatial priors, applied independently and together, were also compared using measurements of a gelatin phantom containing hemoglobin. Figure 8.3 shows a photograph of the phantom placed inside Dartmouth’s clinical NIR tomography system. The properties of the gelatin, estimated from measurements in its homogeneous state are displayed in the table next to the photograph. Pig blood (1% by volume) was added to the gelatin to generate a wavelength dependent absorption coefficient. Near the edge is a 25 mm cylindrical cavity, which was filled with Intralipid
solutions with varying [HbT]. The hematocrit level of the solution was measured with a clinical co-oximeter. NIR data was acquired—240 measurements of amplitude and phase of transmitted light at six wavelengths (661 nm, 761 nm, 785 nm, 808 nm, 829 nm, and 849 nm).

**Gelatin properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hemoglobin concentration</td>
<td>[HbT]=23 μM</td>
</tr>
<tr>
<td>Hemoglobin oxygen saturation</td>
<td>S\textsubscript{a}O\textsubscript{2}=72%</td>
</tr>
<tr>
<td>Water fraction</td>
<td>H\textsubscript{2}O=57%</td>
</tr>
<tr>
<td>Scattering amplitude</td>
<td>A=0.65</td>
</tr>
<tr>
<td>Scattering power</td>
<td>b=1.35</td>
</tr>
</tbody>
</table>

Figure 8.3. Photograph (from top) of a cylindrical breast tissue phantom containing blood with a 25 mm cavity placed inside the stand-alone NIR imaging system. Sixteen optical fibers are in contact with the phantom surface.

Figure 8.4 shows the true properties of the phantom with an inclusion containing [HbT] nearly twice that of the background (top row), along with images reconstructed with four algorithms. The first uses no priors—absorption and reduced scattering coefficients are reconstructed at each wavelength, then spectrally decomposed to produce images of [HbT], S\textsubscript{a}O\textsubscript{2}, water fraction, A, and b (2\textsuperscript{nd} row). The second, third, and fourth algorithms incorporate spatial, spectral, and both spectral and spatial priors, respectively, and their corresponding images appear in the third, fourth, and fifth rows of Figure 8.4. Spatial priors were derived from the photograph in Figure 8.3. The conventional method (no priors) yields images with considerable artifacts. Spatial priors remove these, so that the inclusion is clearly visible and matches the expected size and shape. However, [HbT]
contrast is significantly underestimated. The recovered mean in the region of the anomaly reaches only 57% of the true value (maximum reaches 63% of truth). Spectral priors show substantial improvement in the quantification with mean [HbT] at 78% of the true value (maximum at 91%). Finally, the application of both constraints results in images with further reduction in artifacts close to the boundary, and mean [HbT] reaches 88% of the expected value (maximum at 98%).

![Figure 8.4](image)

Figure 8.4. Reconstructed images of total hemoglobin concentration (µM), percent hemoglobin oxygen saturation, water fraction, scattering amplitude, and scattering power, for the gelatin phantom in Figure 8.3 using algorithms which incorporate different degrees of a priori information.
Figure 8.5. Mean reconstructed values of [HbT] (µM), StO2 (%), Water (%), A, and b, in the region of the inclusion using four algorithms. As the total hemoglobin concentration in the phantom cavity increases, so does the reconstructed value. When the blood concentration is high, the best results are achieved when both spectral and spatial priors are used in the reconstruction. As expected, StO2, Water, A, and b within the cavity remain constant for each solution. Generally, when no priors are used, the standard deviation of the property values within the region is the highest.

The experiment was carried further, using four different blood concentrations in Intralipid solution as inclusions (22-43 µM [HbT], S_tO_2=100%, Water=100%, A=0.65, b=1.35). Figure 8.5 shows the mean property values recovered in the region of interest for each change in [HbT], by applying the four methods of reconstruction. As suggested in Figure 8.4, while the standard deviation in the region of interest is reduced using
spatial constraints (leading to much smoother images), the quantitative values are underestimated, and may even be degraded relative to the reconstruction without any priors. The spectrally constrained technique produces more accurate results for [HbT], and including both priors gives the best results: accurate quantification along with reduced standard deviation.

While previous studies which incorporate anatomical information as a spatial prior have shown improvement in algorithm stability and convergence, and image resolution\textsuperscript{13, 69, 70, 109, 118}, results shown here indicate that functional parameter estimation by this approach can be suboptimal\textsuperscript{72}. The incorporation of \textit{a priori} spectral information significantly improves the parameter estimation accuracy observed in recovered images. Results show that (1) spatial priors improve image resolution, but can underestimate the [HbT] of a heterogeneity, (2) spectral priors generate superior quantification of all estimated NIR parameters, and (3) the use of both simultaneously produces images that are quantitatively accurate and spatially superior. Intes et al.\textsuperscript{110} and Li\textsuperscript{111} showed in simulation studies that the combination of spatial and spectral priors improves the accuracy and quality of NIR images. This experimental work\textsuperscript{72} provides the first comparative analysis of their individual benefits. With this implementation, anatomical information improves image quality by reducing artifacts, but does not significantly improve functional parameter quantification. The spectral prior obtained by including the intrinsic behavior of tissue chromophores and scattering plays a more important role in preserving quantitative functional parameter estimates. A synergy between these two priors could yield the most accurate characterization of breast tissue properties currently available.
Chapter 9 : Patient Imaging

9.A. Introduction

The clinical prototype hybrid imaging system discussed in this thesis has been developed to investigate breast tissue physiology—both the hardware and software designs have been optimized for breast imaging. Methods which incorporate a priori information have been tested and validated in well controlled phantom studies. MRI is used to determine tissue structure with excellent spatial resolution, and NIR is used to quantify the absorption of oxy-hemoglobin, deoxy-hemoglobin, and water, as well as the scattering from cellular organelles.

In this chapter, in vivo results from clinical exams show that this combined system can produce images with resolution sufficient to quantify the properties of adipose and fibroglandular tissues present in the breast, even when they have complex spatial organization. The internal adipose/fibroglandular structure varied considerably between the different women examined. Assessment of the normal range of intra-subject variations in adipose and glandular tissue properties, as well as inter-subject variations could help improve the understanding of breast physiology, density, and progression to disease. This tissue specific property analysis is performed using an algorithm which incorporates spectral and spatial priors, and would not be possible with stand-alone NIR imaging systems.
9.B. Normal patient study

9.B.1. Data acquisition

Volunteers were recruited from a pool of women having received a routine screening mammogram, and all human subject studies were carried out under informed consent. The data acquisition protocol described in Section 2.E was followed when acquiring NIR and MRI data in a clinical exam. At the time of the first NIR-MRI patient exam, four wavelengths were operational (785 nm, 808 nm, 829 nm, and 849 nm). A fifth (661 nm) was added prior to the third exam, and a sixth (761 nm) was added prior to the eighth exam. Typically, a full volume T1-weighted gradient echo MRI was acquired (approximately 50 coronal slices, 2 mm slice thickness), which provides a map of the structure of the full breast volume. In-slice resolution is sub-millimeter (approximately 0.8 mm pixel spacing). Figure 9.1 shows NIR data at 785 nm, and several slices from the MRI volume for a representative subject (ID #506). In the BI-RADS (American College of Radiology Breast Imaging Reporting and Data System) classification system, this participant had breasts with scattered radiographic density (i.e. fatty tissue containing scattered fibroglandular densities). The MR slices revealed an area of blood vessels and vascularized glandular tissue near the center of the breast (dark in the MR image), surrounded by a subsurface layer of adipose tissue (light gray in the MRI). The coronal slice which best matches the NIR measurement plane is chosen to create a 2D FEM mesh by the procedure outlined in Section 7.B.1. The MRI clearly differentiates adipose from fibroglandular tissue, and for asymptomatic women these two tissue types are assigned different ‘region labels’ in the mesh. The mesh accurately describes the convoluted outer breast boundary, the size and shape of the glandular region, and the location of the 16...
NIR measurement sites. The bright spots around the tissue perimeter represent fiducial markers attached to each optode.

Figure 9.1. (a) Amplitude and phase measurements at 785 nm, taken from a homogeneous reference phantom, and from a subject (ID #506) with scattered radiographic density. (b) Anatomically axial (cranial-caudal; slice 6 to slice 1) T1-weighted MR images of the breast (slice thickness is 5 mm and the space between slices is 10 mm). (c) Oblique coronal T1 weighted MR images. Slice 1 is toward the chest wall, and slice 6 is toward the nipple (slice thickness is 2 mm and the space between slices is 10 mm). Coronal slice 3 is the plane of the optical fibers, and was used to create a ‘regionized’ FEM mesh. A region of glandular tissue (dark gray) appears surrounded by a layer of adipose tissue (light gray). Fiber locations are indicated by fiducial markers in the MRI.
9.B.2. Image reconstruction

Having obtained NIR measurements at the periphery of the breast, image reconstruction is carried out by repeated solution of the diffusion equation to estimate either optical properties or functional parameters. For the subject in Figure 9.1, five wavelengths were used. Figure 9.2 displays results from the indirect chromophore and scattering estimation procedure described in Section 8.B. Absorption and reduced scattering coefficients are reconstructed at each wavelength, and then processed through spectral analysis. The three sets of functional parameter images in Figure 9.2 (b) are derived using different $\mu_a$ and $\mu'_s$ reconstructions. The first set of images (top row) results when conventional Newton minimization (equation (3.24)) is stopped prior to convergence at iteration 5, which is the approach commonly used in Dartmouth’s NIR breast studies. The second set of images (in the middle row) results when the algorithm continues to the projection error minimum (iteration 9-11). The third set of images (bottom row) is obtained from the convergent solution MRI-guided Newton minimization (equation (3.37)). In this case the full knowledge of tissue structure, provided by MRI, is brought to bear in the NIR image reconstruction. When the algorithm is unconstrained by MR, inter-tissue contrast appears to develop at later iterations, but spatial noise also increases (especially in scatter). The estimates which rely on constraints from MRI data suppress artifacts and produce images which exhibit high contrast and resolution.\(^{71}\)
Figure 9.2. A clinical example of indirect chromophore and scattering estimation. (a) Optical property images at five different wavelengths derived through (left) conventional (standard Newton-minimization) image reconstruction and (right) spatial priors. Coefficients are in units of mm$^{-1}$. (b) Images which result from spectral decomposition of coefficient images. Two sets were derived from coefficients reconstructed with standard Newton-minimization. The top set used the fifth iteration while the second used projection error change less than 2% as a stopping criteria (iteration 9-11, also shown in (a)-left). The third set (bottom) was derived from the coefficients estimated with MR-guided reconstruction (which also used 2% projection error change as a stopping criteria).
Figure 9.3 shows results for another clinical example\textsuperscript{72}. Six NIR wavelengths were measured in this case, from a woman with heterogeneously dense breasts (ID #501c). Figure 9.3 shows the results obtained from four different parameter estimation procedures: (a) conventional indirect (equation (3.24)$\rightarrow$equation (8.3) & equation (8.6)), (b) MRI-guided indirect (equation (3.37)$\rightarrow$equation (8.3) & equation (8.6)), (c) direct (i.e. spectral priors, equation (8.11)), and (d) MRI-guided direct (i.e. spectral and spatial priors, equation (8.13)). The images in (a) are noisy and exhibit boundary artifacts. The spatial priors (b) act on these images, making them spatially smoother, but preserve the trends in chromophore and scattering quantification. For example, the scatter power shows a decrease in the glandular tissue similar in value to that obtained without priors. Previous studies suggest that glandular tissue has a higher number density of scatterers, and may therefore have a greater scatter power than fat\textsuperscript{20}. Hence, the results from the spatially constrained reconstruction, while appearing smoother, may be misleading. The scatter power image obtained by the spectrally constrained method (c) is more quantitatively acceptable. Including the spatial priors within this spectral method (d) produces the most intuitively appealing image for this parameter by also showing the layered structure of the breast. Elevated [Hb\textsubscript{T}] (25:13 µM), water (91:49%), and scattering power (1.0:0.5) is observed in glandular relative to adipose tissue using the combined priors, which matches the higher degree of vascularization expected in fibroglandular tissue than in fibrous and adipose stroma. High contrast is observed with MR-like resolution, and a tissue interface can be identified. Given its superior
performance, the algorithm which incorporates spectral and spatial priors is routinely used to reconstruct clinical images.

Figure 9.3. (top) Axial and coronal MR images, along with the 2D (coronal) FEM mesh which includes the coordinate locations of pixels associated with adipose and glandular tissue. (bottom) Breast tissue property images estimated using four different reconstruction methods. (a) Only the outer boundary of the imaging domain, and the location of the optical fiber measurement sites are specified. (b) A spatially constrained (indirect) algorithm was used. The spatial constraints are applied automatically by the algorithm, using the regionized mesh, and relate to the internal distribution of adipose and glandular tissues. (c) Spectral constraints were applied and chromophore concentrations and scattering parameters were reconstructed directly. (d) Both spatial and spectral constraints were combined.
9.B.3. Tissue property summary

Twelve exams have been performed (on ten women) in total. Upon each visit, the participant is assigned a unique exam number. Demographic information on each subject, and the number of NIR wavelengths used in the exam are listed in Table 9.1. The average age of the volunteers was 53 years, ranging from 43 to 69 years. Three of the women were premenopausal and seven were postmenopausal, none of whom were taking hormone replacement therapy. Details on the subject’s menstrual cycle phase at the time of the exam were not recorded. Six of the women had scattered radiographic density, three were heterogeneously dense (HD), and one was extremely dense (ED).

Subject #501 was imaged three times over 10 months. Exam 501 and 501b were performed on the right breast, and 501c was on the left breast. Subjects 1903 and 1907 had clinically diagnosed abnormalities, but these were spatially removed from the NIR measurement area.

<table>
<thead>
<tr>
<th>ID #</th>
<th>Radiographic Density</th>
<th>Years of Age</th>
<th>% Adipose tissue by area</th>
<th>Menopausal status</th>
<th>Exam date</th>
<th>NIR wavelengths used</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>Scattered</td>
<td>61</td>
<td>75.93</td>
<td>post</td>
<td>1/29/2004</td>
<td>4</td>
</tr>
<tr>
<td>501</td>
<td>HD</td>
<td>43</td>
<td>53.74</td>
<td>pre</td>
<td>1/29/2004</td>
<td>4</td>
</tr>
<tr>
<td>501b</td>
<td>HD</td>
<td>43</td>
<td>52.33</td>
<td>pre</td>
<td>6/8/2004</td>
<td>6</td>
</tr>
<tr>
<td>501c</td>
<td>HD</td>
<td>43</td>
<td>57.47</td>
<td>pre</td>
<td>10/28/2004</td>
<td>6</td>
</tr>
<tr>
<td>502</td>
<td>ED</td>
<td>52</td>
<td>53.50</td>
<td>pre</td>
<td>2/12/2004</td>
<td>5</td>
</tr>
<tr>
<td>503</td>
<td>HD</td>
<td>44</td>
<td>49.29</td>
<td>post</td>
<td>2/12/2004</td>
<td>5</td>
</tr>
<tr>
<td>505</td>
<td>Scattered</td>
<td>69</td>
<td>82.25</td>
<td>post</td>
<td>3/4/2004</td>
<td>5</td>
</tr>
<tr>
<td>506</td>
<td>Scattered</td>
<td>69</td>
<td>81.08</td>
<td>post</td>
<td>4/1/2004</td>
<td>5</td>
</tr>
<tr>
<td>507</td>
<td>Scattered</td>
<td>56</td>
<td>86.10</td>
<td>post</td>
<td>7/8/2004</td>
<td>6</td>
</tr>
<tr>
<td>1903</td>
<td>Scattered</td>
<td>65</td>
<td>82.51</td>
<td>post</td>
<td>9/16/2004</td>
<td>6</td>
</tr>
<tr>
<td>1907</td>
<td>HD</td>
<td>43</td>
<td>68.56</td>
<td>pre</td>
<td>1/6/2005</td>
<td>6</td>
</tr>
<tr>
<td>Average</td>
<td>n/a</td>
<td>53</td>
<td>70</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table 9.1. Patient demographic information and exam details.
Axial and oblique coronal MR slices for each of these women are shown in Figure 9.4 and Figure 9.5. These images can be used to assess tissue composition in terms of the relative percentages of the two tissue types case by case. Specifically, coronal MR slices were used to define percent adipose tissue by area in the NIR imaging plane (see Table 9.1), which is used to guide data analysis. While age and radiographic density have some bearing on the overall appearance of the MRI, these breasts share few structural similarities. Scattered breasts are of predominantly adipose composition, intermixed with small amounts of vasculature and glandular tissue. Heterogeneously dense breasts contain more glandular tissue, and sometimes have a well differentiated layer of surrounding fat. The Extremely dense subject (ID #502) contains predominantly glandular tissue, intermixed with fat.
Figure 9.4. Anatomically axial (left) and oblique coronal (center) T1-weighted gradient echo MR images from six exams. The 2D FEM meshes generated from the coronal MRI are also shown (right). Each mesh contains approximately 2000 nodes, connected by 4000 linear triangular elements, and defines two regions based upon the spatial organization of adipose and glandular tissue. Each participant is also issued a study number (ID #, shown far left), which is referenced later when tissue properties are analyzed and correlated with demographic factors.
Figure 9.5. Anatomically axial (left) and oblique coronal (center) MR images from six different exams. The 2D FEM meshes generated from the coronal MRI are also shown (right).
Figure 9.6. Coronal MRI slices defining the imaging geometry (same as Figure 9.4), and NIR parameter images: total hemoglobin concentration ([HbT], µM), hemoglobin oxygen saturation (SO2, %), water fraction (H2O, %), scattering amplitude (A), and scattering power (b).
Figure 9.7. Coronal MRI slices defining the imaging geometry (same as Figure 9.5), and reconstructed NIR parameter images.
Figure 9.8. NIR parameters extracted from the images in Figure 9.6 and Figure 9.7. For each subject (N=12), average NIR properties are reported for both adipose and glandular tissue. Error bars represent property standard deviations within a tissue region. These standard deviations represent intra-tissue variation for each exam, which can be as high as 31% (scattering amplitude), indicating the presence of heterogeneity within each tissue type. The average properties of each tissue type from all exams is also shown (bottom). Error bars in these graph represent inter-subject variations.

Reconstructed NIR property images are shown in Figure 9.6 and Figure 9.7. From these images, average property values and standard deviations were extracted for both adipose and fibroglandular tissue. This data is graphed in Figure 9.8. Average values for the subject population and inter-subject variation are also shown in Figure 9.8,
and listed in Table 9.2. Subject #501b was excluded from the mean and standard deviation calculations, because the exam resulted from the same breast as #501—#501c was contralateral and was included. It should be noted that the variation between the three exams of patient 501 is comparable to the variation across the total population.

<table>
<thead>
<tr>
<th>Property</th>
<th>Adipose tissue (N=11)</th>
<th>Glandular tissue (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Total range</td>
</tr>
<tr>
<td>[HbT], µM</td>
<td>17.1 ± 3.2</td>
<td>11.7-22.9</td>
</tr>
<tr>
<td>StO2, %</td>
<td>70.7 ± 8.6</td>
<td>51.8-77.4</td>
</tr>
<tr>
<td>water, %</td>
<td>46.8 ± 18.5</td>
<td>23.0-78.5</td>
</tr>
<tr>
<td>A</td>
<td>1.34 ± 0.54</td>
<td>0.86-2.77</td>
</tr>
<tr>
<td>b</td>
<td>0.56 ± 0.32</td>
<td>0.00-0.94</td>
</tr>
</tbody>
</table>

Table 9.2. The average values, standard deviations, and total ranges observed for total hemoglobin concentration, hemoglobin oxygen saturation, water fraction, scattering amplitude, and scattering power of adipose and glandular tissue.

<table>
<thead>
<tr>
<th>Property</th>
<th>Quaresima et al. (Parallel plate, 680-1100 nm, 5 subjects)</th>
<th>Cerussi et al. (Probe, 7 wvl, 28 subjects)</th>
<th>Shah et al. (Probe, 4 wvl, 14 subjects)</th>
<th>Durduran et al. (Parallel plate, 3 wvl, 52 subjects)</th>
<th>Srinivasan et al. (Tomog., 6 wvl, 24 subjects)</th>
<th>Spinelli et al. (Parallel plate, 4-7 wvl (637-985), 113 subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HbT]</td>
<td>(2.9-20.4)</td>
<td>(5-60)</td>
<td>19.2</td>
<td>7</td>
<td>34±9 (9-41)</td>
<td>15.7±5.1 (7.9-36.2)</td>
</tr>
<tr>
<td>S&lt;sub&gt;O&lt;/sub&gt;2</td>
<td>(0-90)</td>
<td></td>
<td>68±8</td>
<td>58±9 (32-75)</td>
<td></td>
<td>66.4±9.2 (44.7-84.4)</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>(11-74)</td>
<td>(10-70)</td>
<td>26±6</td>
<td>48±12 (21-82)</td>
<td></td>
<td>14.5±10.7 (1.2-57.2)</td>
</tr>
<tr>
<td>Lipid</td>
<td>(26-90)</td>
<td>(15-80)</td>
<td>11±2</td>
<td></td>
<td></td>
<td>58.0±12.1 (20.0-79.7)</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>(0.3-1.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.1-1.9)</td>
</tr>
</tbody>
</table>

Table 9.3. A partial summary of literature values measured for healthy female breast tissue.
All NIR parameters listed in Table 9.2 lie within, or overlap the ranges of bulk average breast properties measured in previous studies (see Table 9.3)\textsuperscript{1-3, 8, 40, 52, 159, 160}, which uniformly report large inter-subject variations in estimated NIR parameters. This reported variation reflects the complex anatomy and physiology of normal breast tissue, which has been shown to vary considerably depending on demographic factors. The system used here has a unique ability to image heterogeneity and localize sources of variation which may be tissue specific.

Adipose tissue appeared to be composed of approximately 17 µM hemoglobin (approximately 0.75% blood volume, assuming an average 15.6 dL/L hematocrit), 71% oxygen saturation of the blood, 47% water fraction, 1.3 scattering amplitude, and 0.6 scattering power. Glandular tissue appeared to be composed of approximately 22 µM hemoglobin (0.93% blood volume), 70% oxygen saturation of the blood, 60% water fraction, 0.9 scattering amplitude, and 0.8 scattering power. Based on Table 9.2, scattering power shows the highest relative variability (inter-subject variation is 61% in glandular tissue), and hemoglobin oxygen saturation shows the lowest (8% in adipose tissue). Based upon its greater blood supply, glandular tissue NIR parameters are thought to vary with the menstrual cycle more than those for adipose tissue. A trend towards greater chromophore concentration inter-subject variation was present in glandular as compared to adipose tissue, but the difference was not statistically significant.

The differences between the optically relevant parameters of the two tissue types in the images were analyzed. Table 9.4 shows that hemoglobin oxygen saturation, S\textsubscript{O2}, is the only parameter not significantly different in adipose versus glandular tissue for these 11 exams (again, 501b is excluded). Glandular tissue shows significantly elevated...
[HbT], water, and b, and reduced A, relative to adipose tissue. These trends match several expectations based upon physiology. Glandular tissue is known to contain more blood vessels than adipose tissue, and to have a greater blood supply and water content\textsuperscript{36}. The connection between physiology and NIR scattering parameters A and b is less clear\textsuperscript{20,52}. Peters et al.\textsuperscript{161} measured the scattering spectrum of excised glandular and adipose tissue in the near infrared wavelength range and observed that glandular tissue scatters light with a steeper spectral dependence. Thus, higher scatter powers are expected in glandular tissue.

<table>
<thead>
<tr>
<th></th>
<th>Adipose vs. Glandular (N=11)</th>
<th>Scattered (N=6) vs. HD/ED (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Diff. (p-value)</td>
<td>Mean Diff. (p-value)</td>
</tr>
<tr>
<td>[HbT] (µM)</td>
<td>5.4 (0.022*)</td>
<td>0.3 (0.902)</td>
</tr>
<tr>
<td>StO2 (%)</td>
<td>0.9 (0.798)</td>
<td>8.3 (0.166)</td>
</tr>
<tr>
<td>Water (%)</td>
<td>13.5 (0.040*)</td>
<td>2.6 (0.769)</td>
</tr>
<tr>
<td>A</td>
<td>0.4 (0.005*)</td>
<td>0.2 (0.673)</td>
</tr>
<tr>
<td>b</td>
<td>0.2 (0.045*)</td>
<td>0.2 (0.324)</td>
</tr>
</tbody>
</table>

Table 9.4. Paired t-test p-values indicating differences between NIR derived properties associated with (left) different tissue types, (center) adipose tissue in women with different radiographic densities, and (right) glandular tissue in women with different densities.

Previous studies have reported correlations between NIR parameters and subject demographic factors. Substantial quantitative differences have been observed in both absorption and scattering spectra of breast as a function of subject age. In particular, decreases in absorption and reduced scattering coefficients\textsuperscript{162}, [HbT]\textsuperscript{1,2}, water\textsuperscript{1,2,160}, and scatter power\textsuperscript{1,52}, were seen with increased age. Decreases in absorption and reduced scattering coefficients\textsuperscript{8,162}, [HbT]\textsuperscript{8,52}, and water\textsuperscript{160}, were observed with increased body
mass index. Increases in scattering amplitude and power\textsuperscript{3, 52} were observed to correlate with increased radiographic density and decreased breast diameter. Increases in [Hb\textsubscript{T}]\textsuperscript{3} and water\textsuperscript{160} have also been reported to correlate with increased radiographic density. Studies report measuring elevated [Hb\textsubscript{T}], water, and scattering power\textsuperscript{1, 2} in premenopausal relative to postmenopausal subjects, and systematic changes in [Hb\textsubscript{T}] correlating to the menstrual cycle of premenopausal women\textsuperscript{2, 3}. Given the nature of measurement systems used, all reported correlations have used bulk averages of tissue properties. Because the imaging system used here provides high resolution MRI images which show the distributions of adipose and glandular tissues in the breast, it is unique in its ability to investigate the same correlations related to each tissue separately, in order to go beyond bulk correlation in an attempt to understand the basic physiological reasons for the correlations.

The segmented MRI in the NIR imaging plane provided estimates of the percent adipose tissue by area, which could possibly be related to body mass index. For these 11 subjects, the percent adipose tissue by area ranged from 49 to 86, with an average of 70. A correlation between age and percent adipose tissue was observed (r=0.69, P=0.02), and a paired t-test confirmed that subjects with scattered radiographic density had a higher percent adipose content than those with heterogeneously dense, or extremely dense breast tissue (r=0.30, P=0.001). This is reasonable considering that adipose tissue is more transparent to low keV x-rays than glandular tissue. The adipose tissue fraction can also be used as an indicator of body mass index. Stand-alone NIR imaging systems have seen correlations with age, radiographic density, and body mass index\textsuperscript{1, 2, 52, 160, 162}, and could, therefore, be measuring changes in the volume fraction of these two tissues, rather than
changes in their individual compositions. A multivariate test was performed to estimate the correlation coefficient and the p-value for relationships between age, percent adipose tissue by area, and the NIR parameters derived for adipose and glandular tissue. No correlation was observed between subject age, and any of these tissue characteristics, supporting the assumption regarding previous studies. Percent adipose tissue correlates only with adipose tissue oxygen saturation (r=0.66, P=0.027). It is not immediately obvious why these two parameters should vary together, and due to the limited sample size these results are not unequivocal. In addition to age, the relative abundance of adipose and glandular tissue is known to depend upon the subject’s weight, heritage, and general habitus, but these factors were not considered here.

Relationships between the different NIR parameters derived from the two tissue types were also studied. The only significant connection appears to be between water and oxygen saturation for glandular tissue (r=0.70, P=0.015). The relationship between radiographic density and NIR properties for adipose and glandular tissue was also examined. Table 9.4 shows that none of the adipose tissue NIR parameters were significantly different between women with different breast density. For glandular tissue, however, differences in [HbT] did correlate with density. It is unknown whether the composition of glandular tissue is truly different for women in these different density categories, or if it changes as a woman ages. One explanation for the difference observed here can be found by referring to the MRIs in Figure 9.4 and Figure 9.5. It is clear that parenchyma patterns for women with different densities can vary dramatically. It is well known that during menopause, with the cessation of ovarian hormonal function, breast lobules in glandular tissue atrophy, and relative adipose and fibrous stroma volumes may
increase. All of the women with scattered density imaged here were postmenopausal. In several scattered cases, small pockets of glandular tissue can be found throughout an adipose background. It is possible that the resolution of the FEM mesh is too coarse to accurately transcribe this distribution for image reconstruction. The size of the glandular regions may be overestimated, leading to an underestimation of the localized absorption contrast due to hemoglobin. Additional studies are required to confirm that the vasculature in the segmented region of glandular tissue is not fundamentally different for women with different ages and hormonal status, despite similar MR intensity.

9.C. Comments on tissue deformation

Figure 9.4 and Figure 9.5 show that the external pressures applied by optical fibers deform the tissue in the measurement plane. Dehghani et al.\textsuperscript{163} described this deformation process with a model which accounts for external pressures and tissue mechanical properties, and examined the extent to which external boundary changes, when ignored in modeling, degrade image quality. In most image reconstruction algorithms, the general assumption is made that the region under investigation is a uniform circular (2D) or conical or cylindrical (3D) domain. In this work, a model of tissue deformation is not required because the MRI gives accurate boundaries. For one patient (501c), images were reconstructed using both the correct tissue map, which includes tissue bulging between fiber contact points, and a circular approximation. Total hemoglobin images are shown in Figure 9.9. In this case the periphery of the image is adipose tissue and the center is glandular tissue. When tissue bulging is ignored, average [Hb\textsubscript{T}] in adipose tissue changes significantly from 9.0 µM to 5.0 µM. The change in the
glandular tissue region is much smaller (29.1 µM to 29.6 µM). Changes to the other NIR properties in the adipose region occur on a similar scale.

![Figure 9.9.](image)

Figure 9.9. (left) A circular mesh overlaid on a mesh generated from the MRI—both contain the same internal region and source/detector locations. (right) Total hemoglobin concentration images (µM) reconstructed on the two meshes. The circular approximation affects the recovered subsurface values more than those in the interior of the mesh.

Soft tissue elastic properties can be directly measured with elastography imaging in vivo, thereby providing the key information needed for predicting the deformation of tissue under force or displacement conditions. In practice, for these imaging exams efforts are made to minimize breast deformation through soft fiber compressions. However, there are strategic benefits to compressing in that the signal transmitted can be higher, and there can be pressure-induced changes which might
provide meaningful contrast about tissue composition. With this combined NIR-MRI system, component tissue displacements can be correlated with changes in NIR signals which result in the application of pressure. In two clinical exams, MR scans were performed without and with fiber-tissue contact. Although registration is only approximate, changes are recognized in the volume fraction of the component tissues in the imaging plane (Figure 9.10).

Figure 9.10. Segmented MRI of (a) subject #504 and (b) subject #505 with optical fibers retracted (left) and with fiber compression (right). (a) Percent adipose tissue decreases from 91.9 to 81.3. (b) Percent adipose tissue decreases from 88.8 to 82.3. The subjects were repositioned on the bed between scans, so these planes (with and without compression) are only approximately matched.
9.D. Validating NIR images with MRI: water and lipid quantification

In addition to direct imaging methods, other spectral priors can be mined from a combined NIR-MRI data set. MRI measurements could be very useful in both validating and improving the ability of NIR methods to estimate water and lipid content in tissue. Structurally, contrast in MRI originates from intrinsic tissue factors related to micromagnetic structural inhomogeneities. Relaxation times vary substantially for different tissues and are strongly dependent on their physical characteristics. Water and fat are the principle endogenous contrast elements in MR images. At high-strength, high homogeneity magnetic fields (i.e. >1.0 T, <1.0 ppm inhomogeneity), the difference in resonant frequency between water and fat protons is sufficiently large that a variety of proton MR spectroscopic imaging techniques have shown the ability to quantify their concentrations separately. Chemical shift selective (CHESS) pulse sequences image the spatial distribution of water (or fat) by preceding a standard spin-echo sequence by a saturation pulse which targets fat (or water)\(^{166}\). The three-point Dixon technique can provide decomposition of water and fat proton images even in the presence of off-resonance conditions which result from susceptibility differences, demagnetization, or shim errors\(^{167}\). Dixon methods, however, generally require multiple acquisitions at different phase shifts relative to the two resonances and can be slow.

A MRI sequence has been implemented here which is similar to the ‘spatial-spectral’ pulse sequence described by Duerk\(^{166}\), and by Meyer et al.\(^{168}\). This technique yields images of either fat or water in the slice of interest by locally targeting the appropriate resonance. K-space is filled in a spiral pattern to allow for rapid 3D imaging of large volumes. The ability to quantify water and fat using MRI was tested by imaging
a collection of water-vegetable oil (soybean oil) emulsion phantoms constructed using the
technique described by Merritt et al. Absolute concentrations of water and oil (which
has the same magnetic properties as fat) ranged from 40-90% and 60-10% respectively.
Figure 9.11 shows water-only and fat-only MR images. Samples of pure water (top
left) and pure oil (bottom left) were included in the imaging field and used to normalize
the signal intensity of the other samples. Reference samples are desirable, and may be
used to compensate for field inhomogeneity-induced image lighting. The imaged
centration concentrations extracted from image intensity ROI analysis are in good agreement
(within 10%) with the actual concentrations of the individual emulsions.

![Image](image.png)

Figure 9.11. Validation of a MR sequence used to quantify water and fat. (a) Water
images of vegetable oil-water emulsions of different concentrations. (b) Fat images of
the same emulsions. (c) Plot of fat versus water content from ROI analysis of (a) and (b).
The assumed concentrations are known from the recipe.
This MR sequence was used to quantify water and fat content in four of the subjects presented in Section 9.C.3. Figure 9.12 shows fat and water images, along with the reconstructed water fraction from NIR data. Recall that lipid absorption is considered negligible in this NIR imaging procedure. The fat and water MR images contain a higher noise level than the corresponding T2 slices. MR slices with relatively high signal to noise ratio were selected for processing, and do not match the exact NIR
measurement plane in three of the four cases. The susceptibility discontinuities in the plane of our plastic fiber holder caused these images to be blurry. Image quality can be greatly improved through manual shimming along the three scanner axes. Automatic shimming was used in these acquisitions.

The water fraction recovered by MRI and by NIR are compared in Figure 9.13. The mean values are presented separately for adipose and glandular tissue. The tissue areas were defined by the thresholding performed in the creation of the FEM meshes. As expected, higher fat content was observed in adipose relative to glandular tissue via MRI, and higher water content was seen in glandular relative to adipose tissue with both MRI and NIR. In the MRI, more inter-subject variation of glandular tissue properties than of adipose tissue properties was observed. Also, NIR consistently overestimates water content, relative to MRI.

![Figure 9.13. Water and fat content in adipose and fibroglandular tissue estimated with MRI and water estimated with NIR for four subjects.](image)

Because the tomography system utilizes six NIR wavelengths, six measurements are effectively used to solve for five unknowns. Given that lipid absorption is low and that its spectrum is fairly featureless between 661 and 850 nm, adding lipids as a sixth unknown is difficult. In a case study, McBride et al. attempted to quantify the
hemoglobin of an invasive cancer using different methods to compensate for water and lipid absorption. Different methods led to hemoglobin values which varied by 15%. Pifferi et al.\textsuperscript{48} characterized breast tissue of a small group of healthy women using broadband spectroscopy and concluded that absorption by water and lipid is significant in the wavelength range 700 to 850 nm, and that lipids should not be ignored, especially in fatty tissue. In their work on combining spectral and spatial priors, Li et al.\textsuperscript{157} proposed obtaining the spatial distributions of water and lipid from MRI, and then to use these as a spatial prior in the spectrally-constrained reconstruction of hemoglobin images. The data set described here allows this to be accomplished, and the example of subject #501c is shown in Figure 9.14. It has been shown that direct chromophore estimation is more accurate than indirect estimation, but the indirect method (equation (8.3)) was used here in a simple test in order to assess chromophore cross talk when the water and lipid concentrations in adipose and glandular tissue are constrained to the values measured by MRI (in Figure 9.13). The molar extinction coefficients used for lipids were taken from Quaresima et al.\textsuperscript{159}. When lipid is added to the list of unknown chromophores, its derived concentration does not agree well with MRI or intuition (i.e. near 100% levels are estimated for glandular tissue). When both water and lipid are constrained to match the distributions recovered with MRI (within 1% error), the estimated deoxy-hemoglobin concentration is minimally effected, but nodal changes in oxy-hemoglobin reach 35%. Given a spatial prior for some of the chromophores, an improvement in the reconstruction of all of the chromophores is expected.
Figure 9.14. Indirect chromophore estimation using six absorption coefficient images reconstructed for subject #501c with MR-derived spatial priors. (a) Three chromophores are assumed to be present: oxy-hemoglobin ([Hb-O], μM), deoxy-hemoglobin ([Hb-R], μM), and water (H2O, %). (b) Lipids were added as a fourth unknown and constrained to lie between 0 and 100%. (c) Water and lipid content maps measured from MRI are assumed in an attempt to derive more accurate [Hb-O] and [Hb-R]. Nodal values of [Hb-O] are affected by as much 35%.
Chapter 10: Concluding remarks

10.A. Conclusion

The ‘normal’ female breast is an organ with complex anatomy and physiology which varies significantly between different subjects. Thousands of optical breast examinations have now been carried out world wide as part of many clinical trials testing a variety of instruments and techniques. Still, the in vivo optical properties of healthy breast tissues and common lesions are not well understood. Advances in NIR tomography have made it possible to map volumetric functional changes in tissue, and the use of anatomical prior information indicates that improved image quality is achievable, and may increase the potential for relevant physiological investigation. It seems increasingly probable that optical techniques could play a major clinical role when used as adjuncts to other imaging systems. Integrating optical imaging with MRI may provide enhanced information about hemodynamics and metabolism at minimal additional cost and complexity compared to the MRI system, itself.

This thesis describes the design, construction, and testing of a NIR tomography system for measuring breast tissue properties in conjunction with MRI. NIR data acquisition requires approximately 45 seconds for a single tomographic slice at one (of six) optical wavelength with measurement standard deviation on average of 0.26% in AC intensity and 1.04 degrees in phase. The methodology of how to optimally combine the image data of a high resolution system into the image of a diffuse field reconstruction is discussed. Simultaneously-acquired MRI is used to improve NIR image resolution through the application of MRI-guided iterative reconstruction algorithms. In phantom
studies, optical property reconstruction accuracy is shown to improve, and resolution approaches that of the structural images. In addition, spectral priors were combined with spatial priors, and shown through phantom studies to yield superior spatial resolution and quantitative accuracy of reconstructed functional parameters ([HbT], S\textsubscript{O2}, water, A, b). The system has been used to study tissue composition for a small pilot population of asymptomatic female volunteers. Images with millimeter resolution and high contrast reflect known physiological differences between adipose and glandular tissue—glandular tissue shows significantly elevated [HbT], water, and b, and reduced A, relative to adipose tissue. Hemoglobin oxygen saturation, S\textsubscript{O2}, is the only parameter not significantly different in adipose versus glandular tissue. It is reasonable to conclude that the tissue characterization offered is preferred over stand-alone systems, and research with this novel imaging system may be useful for improving disease prediction and diagnosis, and for understanding its progression.

10.B. Future studies

10.B.1. 3D imaging

NIR tomography is inherently a three dimensional process, and the same software used for 2D studies can be used for 3D studies. This NIR-MRI data set is well suited for 3D modeling/reconstruction because asymmetries normal to the measurement plane are accurately captured by MRI. 3D views of a patient with a 2 cm tumor out of the NIR measurement plane are shown in Figure 10.1 (a). The best hope for gaining information about this tumor is through 3D reconstruction. Figure 10.1 (b) shows a simulation which loosely represents this case—an absorbing and scattering object was placed above the
plane of optical fibers, and 3D images were reconstructed without and with prior knowledge of the object size and location. Using priors, the object can be localized, but its true coefficient values are underestimated. Future work should focus on improving quantitative accuracy of 3D reconstructions, and phantom studies similar to the one shown in Figure 10.2 could be useful in the process. Gelatin can be molded into breast-like shapes, and out-of-plane asymmetries and fiber-induced deformation can be represented.

Figure 10.1. (a) MRI slices and overlaid (3 region) FEM mesh of subject #1907 (2 cm tumor, centered 3 cm above NIR measurement plane. (b) 3D simulations testing the ability to quantify anomalies out of the measurement plane. (top) A 2 cm sphere with 2:1 absorption and reduced scattering coefficient contrast was placed just above the fiber ring (at z=0). (middle) Conventional reconstruction shows anomalies in the measurement plane with less than expected contrast. (bottom) With spatial priors, the correct location is recovered, but contrast is still underestimated.
Figure 10.2. (a) Photograph of a realistic breast-shaped, three layer gelatin phantom. (b) A cross-sectional MRI through the plane of the inclusion. (c) The same slice as in (b), but with increased fiber compression. In this case the tips punctured the phantom’s surface. (d) Reconstructed phantom images. This experiment was not a complete success because reduced scattering coefficient of the component gelatins was very low.

10.B.2. Cancer studies

Breast disease was not studied in detail through the course of this work, but should be a significant focus of future research. Two subjects with abnormalities (#1903 and #1907) were imaged, but the fiber interface in use at the time was not capable of maintaining a position near enough to the chest wall to localize the tumor in the imaging plane. Modifications were made to reduce the distance from the chest wall to the fiber plane (now approximately 2 cm). Future work should emphasize the characterization of different diseases, and correlations between NIR findings and pathology. Efforts are currently being made to recruit women with breast abnormalities.
10.B.3. NIR-MRI cross-validation

Clinical MRI is known for providing structural information. However, this is a vast research field, and MR methods for gaining insight into functional processes are being developed. It would be desirable to avoid contrast agents, but they are approved for use with this protocol, and could provide useful information, especially in disease studies\textsuperscript{171}. In Section 9.E, a MRI sequence for quantifying tissue water and fat fractions was tested in four participant exams. These measures can be used first to validate the ability to quantify water and fat with NIR measurements at a sparse number of wavelengths, and second to improve NIR spectral imaging (of hemoglobin) through spectral-spatial constraints of these two quantities. In future clinical exams, it would be useful to acquire these extra scans in order to expand on this preliminary analysis. Once the MRI technician is familiar with the sequence, it should not add extra time to the exam.

Other MR sequences capable of measuring blood volume\textsuperscript{172} and tissue/blood oxygen\textsuperscript{173, 174} should be investigated in order to validate the NIR component of this system. Regions of hypoxia in tumors are resistant to radiotherapy and chemotherapy\textsuperscript{175}, and noninvasive techniques for assessing hypoxia could be very useful in treatment planning. Blood oxygenation level dependent (BOLD) \textsuperscript{1}H MRI has been applied to monitor the effects of changes in blood oxygenation of tumors using deoxy-hemoglobin as an endogenous contrast agent. Paramagnetic deoxy-hemoglobin increases the transverse MR relaxation rate $R_2^*$ (=1/$T_2^*$). Breathing high-oxygen content gasses such as carbogen (95% O\textsubscript{2}, 5% CO\textsubscript{2}) increases blood and tissue oxygen. Several studies have quantified carbogen-induced decreases in $R_2^*$ in tumors. The oxygenation state of
hemoglobin is related to the arterial blood $p_aO_2$, which is in equilibrium with tissue $pO_2$
(although this equilibrium is not fully understood), hence tumor $R_2^*$ may provide an
index related to tissue oxygenation. Carbogen is known to increase blood oxygenation,
and the magnitude of the change in tumor $R_2^*$ ($\Delta R_2^*$) will be dependent on blood
volume, which in turn will be a factor in determining hypoxic fractions. A flexible
protocol for MR imaging with administration of gasses with different $O_2$ concentrations
is in place at Dartmouth Hitchcock Medical Center, and this NIR-MRI system could be
very useful in this context. It would be useful to coregister changes in $[HbT]$ and $S_O2$
with $\Delta R_2$ and $\Delta R_2^*$. 


Appendix A : Data acquisition software

This appendix lists the main programs used in NIR data acquisition. All equipment is controlled through a single PC computer running LabVIEW™ software. Several lower-level subroutines developed for use with the clinical system, including the lock-in detection routine, are used. Many others were adapted to this instrumentation. The file naming convention is also consistent with that system.

A.1. Major data acquisition programs

The main program used for patient imaging acquires measurements serially at the NIR wavelengths specified by the operator. This program is entitled ‘Automatic Patient Imaging MRI.vi.’ A library of containing all of the supporting VI’s (virtual instruments) was compiled with the same name. The program and library are located in the directory ‘C:\LabVIEW\MRIsystem\’ on the data acquisition computer.

1. Automatic Patient Imaging MRI.vi

   On the VI front panel, the desired wavelengths are selected, the patient identification number/name is entered, the left/right breast is specified, and the repetition number is entered. These parameters are concatenated to form the data file names. This program calls each of the subroutines described next (2-5).

2. MRINIR System slider main program.vi

   This is the main program for system operation. Each call to this program (or when run independently) results in measurements at a single NIR wavelength. A wavelength is chosen and a file name for output data is entered. This program
can be used to acquire a reference measurement, and to directly call FORTRAN programs for calibration and image reconstruction, described by Troy McBride 81.

3. Find Optimal PMT gain slider MRI.vi

If elected for use, this routine will determine the optimal gain settings for a particular measurement geometry. 240 gain settings can be found, or 15 can be found for one source location, which are then cycled together with source switching. A file is then written which contains the gain setting associated with each source-detector pair. This file is named according to the wavelength used (i.e. C:\LabVIEW\Autoimag\Gain_set_785.dat), and should be replaced anew when the measured sample changes.

4. Find Gain MRI.vi

This routine is called by ‘Find Optimal PMT gain slider MRI.vi’ for each source position. For a given source, the gain is adjusted (using repeated calls of ‘DAQ&LockInDet16 MRI.vi’ in a loop structure) until the AC voltage from all fifteen PMTs lies between 0.1 and 1.0 V.

5. Parallel Acquisition slider MRI.vi

This is the routine which actually acquires and saves the data. The root file name is specified by the user, in ‘MRINIR System slider main program.vi’, or generated by ‘Automatic Patient Imaging MRI.vi.’ See the next section for more details on data file structure. It reads in the file containing the gain settings, extracts the associated PMT calibration factors (C:\LabVIEW\Autoimag\PMT_761_calib_program_mod.asc), and applies them to the raw PMT measurements.
There are several routines used repeatedly in the above mentioned VIs which are particularly useful for system testing and laser power monitoring.

i. All_on_MRI.vi

In the main programs, the user specifies whether the system should be turned on at the beginning or turned off following acquisition. With this VI, only the wavelength to be activated must be chosen, and both AC and DC currents are used to activate this diode laser.

ii. All_off_MRI.vi

This VI turns off any activated light sources, and moves the optical translation stage to its ‘home’ position if prompted by the user.

iii. move chosen laser to chosen fiber.vi

This is the primary VI used to control light multiplexing (i.e. the translation stage). The light source number and the output optical fiber must be chosen.

iv. auto home slider MRI.vi

Each time the system is turned off from the main programs, the translation stage is sent to ‘home.’ If power to the system cart is turned off when the stage is not at home, it must be recalibrated when used the next time. This program achieves this calibration automatically by making fine adjustments to the stage position until coupling power is maximized.
A.2. Relevant data files and naming conventions

In patient imaging, a typical root file name is patID_left/right_wavelength_rep. Parallel Acquisition slider MRI.vi appends this root with *.paa, and writes (hardware) calibrated amplitude and phase data to this file. The format of ‘rootname.paa’ is as follows:

ac amplitude, phase (Source1, Detector2)
ac amplitude, phase (Source1, Detector3)
.
ac amplitude, phase (Source 1, Detector 16)
ac amplitude, phase (Source 2, Detector 3)
ac amplitude, phase (Source 2, Detector 4)
.
.
ac amplitude, phase (Source 2, Detector 16)
ac amplitude, phase (Source 2, Detector 1)
ac amplitude, phase (Source 3, Detector 4)
.
.
ac amplitude, phase (Source 3, Detector 16)
ac amplitude, phase (Source 3, Detector 1)
ac amplitude, phase (Source 3, Detector 2)
ac amplitude, phase (Source 4, Detector 5)
.
.
.
The root is also appended with *.asc, and real and imaginary data is written to this file. Finally, *.dat acts as a log file, containing system settings, gain settings, and all measured data.
Appendix B : NIRFAST (v1.08.2004)

This appendix provides the key documentation related to the software package used in this thesis for modeling and image reconstruction: Near Infrared Frequency Domain Absorption and Scatter Tomography (NIRFAST). This package solves the frequency domain Diffusion Approximation in 2D or 3D, and solves the image reconstruction to provide simultaneous solution of absorption and reduced scatter properties using log amplitude and phase data. Running MATLAB® and NIRFAST under LINUX is recommended; WINDOWS is permitted. This appendix was largely written by Hamid Dehghani.

B.1. Installation

Unzip NIRFAST.zip onto local directory and make sure that the path in Matlab is set for the NIRFAST directory and all its sub-directories. This can be done from the command line:

```
path(path,'/home/dehghani/NIRFAST/FEM');
path(path,'/home/dehghani/NIRFAST/FEM/mex');
path(path,'/home/dehghani/NIRFAST/FEM/c');
path(path,'/home/dehghani/NIRFAST/meshes');
```

Or, do this from the Matlab Main GUI,
File → Set Path → Add with Sub-folder, and then select the NIRFAST directory.

B.2. Directory Structure

NIRFAST\FEM contains call m files.
NIRFAST\FEM\mex contains all mex files.
NIRFAST\FEM\c is empty. This is the original directory for c files used to create mex files.
NIRFAST\Example contains some 2D and 3D examples for running problems set in:
- test2d.m (2D circular model with 3 anomalies)
- test3d.m (3D cylindrical model with 3 anomalies and a single plane of data)
- test3d_3planes.m (3D cylindrical model with 3 anomalies and a 3 planes of data)
NIRFAST\meshes contains some sample meshes, in 2D and 3D
• circle2000_86 is a circle of approximately 2000 nodes, diameter of 86 mm with 16 sources and 16 detectors
• circle400_86 is a circle of approximately 400 nodes, diameter of 86 mm with 16 sources and 16 detectors
• circle220_86 is a circle of approximately 220 nodes, diameter of 86 mm with 16 sources and 16 detectors
• cylinder_43_40 is a cylinder of radius 43 mm, height of 40 mm (±20 mm) with a single plane of 16 sources and 16 detectors at z = 0 mm
• cylinder_43_40_3planes is a cylinder of radius 43 mm, height of 40 mm (±20 mm) with 3 planes of 16 sources and 16 detectors each at z = +10, 0 and −10 mm
• some sample NETGEN input files

NIRFAST\doc contains very useful documentations.
NIRFAST\bin contains binary of NETGEN to NIRFAST converter.

B.3. File Formats

Each mesh, in order to load correctly, needs a total of 6 associated files:

1. *.node: Contains nodal information.
   a. In 2D this can either be a NN × 3 matrix (or NN × 4 where the 4th Column is all 0, i.e. z coordinate = 0), where NN is total number of nodes.
   b. In 3D it is an NN × 4, where NN is total number of nodes.

   For example:

   
   0  \( N_x \) \( N_y \) \( N_z \)
   1  \( N_x \) \( N_y \) \( N_z \)
   \vdots \vdots \vdots
   1  \( NN_x \) \( NN_y \) \( NN_z \)

   The first 1\(^{st}\) column is a flag of either 0 (internal node) or 1 (external boundary node). This is essential to ensure correct Type III boundary condition formulation. The 2\(^{nd}\) column is the x co-ordinates, 3\(^{rd}\) is the y co-ordinates and 4\(^{th}\) is the z co-ordinates.

2. *.elem: Contains the element connectivity list.
   a. In 2D this is a MM × 3 matrix (i.e. 3 nodes make one triangle), where MM is the total number of elements.
   b. In 3D this is a MM × 4 matrix (i.e. 4 nodes make one tetrahedral), where MM is the total number of elements.
3. *.param: Contains the nodal optical parameter values. This is a $NN \times 3$ matrix, where $NN$ is the total number of nodes.

For example:

\[
\begin{array}{ccc}
\mu_{a1} & D_1 & RI_1 \\
\mu_{a2} & D_2 & RI_2 \\
\vdots & \vdots & \vdots \\
\mu_{aNN} & D_{NN} & RI_{NN}
\end{array}
\]

The 1$^{st}$ column is the absorption coefficient, 2$^{nd}$ column is the diffusion coefficient and 3$^{rd}$ is the Refractive Index (RI).

4. *.source: Contains the x, y and/or z co-ordinates of each optical source. The sources need to be assigned as near to the external boundary as possible. During mesh load-up, the software automatically finds nearest boundary, finds the associated reduced scatter value at that region and places the source at 1 scattering distance inside the boundary.

5. *.meas: Contains the x, y and/or z co-ordinates of each optical detector. The detectors need to be assigned as near to the external boundary as possible. During mesh load-up, the software automatically finds nearest boundary, and placed the detectors on the boundary.

6. *.link: Contains the linking protocol for each source detectors pair. This is a matrix of integers, with a size of $NS \times (ND-1)$, where $NS$ is total number of sources, and $ND$ is total number of detectors. The integers in a given row refer to the detector numbers which are active for the corresponding source position. Any element in *.link can be set to zero in order to discard a corrupted (i.e. low SNR) measurement.

For example:
B.4. Using the program

1. Loading meshes: To load a mesh, use `load_mesh`:

   ```matlab
   Mesh = load_mesh('circle2000_86');
   ```

   This loads the mesh into a structured array `mesh`:

   ```matlab
   Mesh =
   nodes: [1785x3 double]
   bndvtx: [1785x1 double]
   elements: [3418x3 double]
   dimension: 2
   mua: [1785x1 double]
   kappa: [1785x1 double]
   ri: [1785x1 double]
   mus: [1785x1 double]
   region: [1785x1 double]
   source: [16x2 double]
   meas: [16x2 double]
   link: [16x16 double]
   c: [1785x1 double]
   ksi: [1785x1 double]
   element_area: [3418x1 double]
   support: [1785x1 double]
   meas_int_func: [16x4 double]
   ```

2. Running forward solver: To run the forward solver use `femdata`
[data, mesh] = femdata('circle2000_86', 100);

data =
phi: [1785x16 double]
complex: [240x1 double]
amplitude: [240x1 double]
phase: [240x1 double]
paa: [240x2 double]

- 1st input is the FEM mesh, 2D or 3D
- 2nd input is the modulation frequency (MHz)
- data.phi is the internal complex field
- data.complex is complex boundary data
- data.amplitude is amplitude of boundary data
- data.phase is phase of the boundary data
- data.paa is amplitude and phase together

To view internal solution, everywhere, use plotimage

plotimage(mesh, log(abs(data.phi(:, 1))));

This plots log of amplitude for all nodes for source 1.

To view boundary data:

semilogy(data.amplitude);
plot(data.phase);

To save data:
mysave('anomaly.paa', data.paa);

Saves the amplitude and phase to filename ‘anomaly.paa’.

3. Adding anomalies: To add anomalies to a mesh:

a. In 2D use add_blob_2d or add_on

    mesh_anomaly = add_blob_2d(mesh)
    mesh_anomaly = add_on(mesh)

b. In 3D use add_blob_3d

    mesh_anomaly = add_blob_3d(mesh)
In both cases above, circular (2D) or spherical (3D) blobs can be added by following the prompts.

4. Adding noise to saved data: To add noise to saved *.paa data use:

```matlab
add_noise('anomaly.paa','anomaly_noise.paa',1,1);
```

- 1st argument is your saved *.paa file
- 2nd is the *.paa file you like saved after adding noise
- 3rd is % amplitude noise
- 4th is phase noise (degrees).

Calculating Jacobian: To calculate the Jacobian for a given mesh use:

```matlab
[J,data,mesh] = jacobian('circle2000_86',100);
```

```
J =
complex: [240x3570 double]
complete: [480x3570 double]
```

- J.complex is the complex Jacobian
- J.complete is Jacobian for log amplitude and phase for absorption and diffusion coefficient.

To view Jacobian solution, for 8th measurement, log amplitude and phase for absorption and diffusion everywhere, use `plotimage`

```matlab
figure;
subplot(2,2,1);
plotimage(mesh,J.complete(15,1:end/2));
title('log I / \kappa')
subplot(2,2,2);
plotimage(mesh,J.complete(16,1:end/2));
title('\theta / \kappa')
subplot(2,2,3);
plotimage(mesh,J.complete(15,end/2+1:end));
title('log I / \mu_a')
subplot(2,2,4);
plotimage(mesh,J.complete(16,end/2+1:end));
title('\theta / \mu_a')
```

5. Data calibration: To calibrate ‘experimental’ or ‘anomaly’ data, use `calibrate`:

```matlab
calibrate(homog_data,...
anom_data,...
```
mesh_fn_homog,...
mesh_fn_anom,...
mesh_op,...
data_op,...
frequency,...
iteration)

- 1\textsuperscript{st} input is homogeneous data (*.paa)
- 2\textsuperscript{nd} input is heterogeneous data (*.paa)
- 3\textsuperscript{rd} input is name of homogeneous model or mesh of homogeneous phantom
- 4\textsuperscript{th} input is heterogeneous model
- 5\textsuperscript{th} input is name of model to be saved for use in reconstruction, containing region information from mesh_fn_anom, and initial guess of optical properties
- 6\textsuperscript{th} input is name of calibrated data to be saved (*.paa)
- 7\textsuperscript{th} input is modulation frequency (i.e. 100 MHz)
- 8\textsuperscript{th} input is iterations for homogeneous fitting algorithm, typically set to 5

6. Conventional Image Reconstruction: To reconstruct images use: reconstruct

```matlab
[mesh,pj] = reconstruct('circle2000_8',...
[30 30],...
100,...
'test2d_anom_noise.paa',...
30,...
10,...
'test2d_recon_noise',...
0);
```

- 1\textsuperscript{st} input is the mesh to use,
- 2\textsuperscript{nd} is the reconstruction basis: (either a second mesh, or pixel basis). If a second mesh, the second mesh needs be of same geometry and dimensions. If pixel basis, in 2D use a 2D pixel, like 20×20 or 30×30 etc. In 3D, use a 3D pixel, like 20×20×10.)
- 3\textsuperscript{rd} is the modulation frequency
- 4\textsuperscript{th} is the boundary data
- 5\textsuperscript{th} is max iteration
- 6\textsuperscript{th} is the initial regularization value
- 7\textsuperscript{th} is the root output filename. It will save rootname_mua.sol, rootname_mus.sol and rootname.log
- 8\textsuperscript{th} is number of mean filters per iteration
- outputs: mesh is the solution at final iteration and pj is the projection error
7. MRI-guided Image Reconstruction: To reconstruct images use:

```matlab
[mesh,pj] = reconstruct_L('circle2000_86',... [30 30],... 100,... 'test2d_anom_noise.paa',... 30,... 10,... 'test2d_recon_noise',... 0,... 10);
```

- Inputs 1-8, and outputs, are the same as those used in `reconstruct
- A 9th input to this routine is $\beta$ (see equation (3.37)), the weighting factor of the spatial prior, which is commonly set to 10.
- This program requires that the input mesh contain the ‘region’ information of the spatial prior. `create_L`, within `reconstruct_L` generates the filter matrix, L.

8. Viewing solutions: To view solutions save in `rootname_mua.sol`, `rootname_mus.sol` for 2D use `read_solution.m`

```matlab
read_solution(mesh,'test2d_recon_noise',15)
```

- 1st input argument ‘mesh’ is the mesh variable in your workspace
- 2nd is the root filename for mua and mus
- 3rd is the iteration number.

For 3D, either use NETGEN, or you can use `rasterized_3d`

```matlab
rasterize_3d(mesh,'test3d_anom1_recon_noise',10,4);
```

- 1st, 2nd and 3rd inputs are same as before, and last input is the number of slices you like.

9. Spectral analysis (i.e. indirect chromophore estimation)

```matlab
calibrate_spectral_bb(mua_dat,... mus_dat,... 'case_sol.prop');
```

- 1st input is mua solution (NNxNwavelengths)
- 2nd input is mus solution
• 3rd input is solution file to be saved (*.prop) (NNx5). The five columns in this file are (1) [HbT], (2) S_02, (3) water, (4) A, (5) b

To include the lipid absorption (i.e. fit for an extra chromophore), use `calibrate_spectral_lipid_bb`.

### 10. Direct chromophore reconstruction (i.e. spectral reconstruction)

First, data at each wavelength is calibrated with `calibrate`. Then the mua and mus values at each wavelength are used to calculate initial guesses for the five functional parameters sought with:

```matlab
[ mesh_cal,...
  fexcoeff ];
```

- 1st input is a vector of wavelengths used
- 2nd input is the list of mesh filenames for the individual wavelength calibrations
- 3rd is the file containing the extinction coefficients at the appropriate wavelengths
- This routine saves a series of files containing initial guesses for [HbT], S_02, water, A, and b

```matlab
[mesh,pj] = reconstruct_spectral('mesh_fn',...[26,26],...
100,...
'pat_id',...
'excoff.dat',...
wv_array,...
20,...
10,...
'pat_id_output_spectral',...
1);;
```

- 1st input is the mesh to use,
- 2nd is the reconstruction basis: (either a second mesh, or pixel basis)
- 3rd is the modulation frequency
- 4th is the file name of the initial guesses and data to be loaded
- 5th is file name of the extinction coefficient matrix
- 6th is the vector of wavelengths used
- 7th is the max number of iterations
- 8th is the regularization
9\textsuperscript{th} is the root output filename. It will save rootname_hbl.sol (oxy-), rootname_hb2.sol (deoxygenated), rootname_wat.sol, rootname_sa.sol, rootname_sp.sol, and rootname.log

10\textsuperscript{th} is number of mean filters per iteration

outputs: mesh is the solution at final iteration and pj is the projection error

11. Spectral-spatial reconstruction

```
[mesh,pj] = reconstruct_spec_spat('mesh_fn',...
[26,26],...
100,...
'pat_id',...
'excoeff.dat',...
wv_array,...
20,...
10,...
'pat_id_output_spectral',...
1);
```

All inputs and outputs are the same as those for `reconstruct_spectral`

12. Reading functional data and plotting images

```
read_spectral_hbt(mesh,...
'pat_id_output',...
iter,...
'pat_id.prop');
```

1\textsuperscript{st} input is mesh used in reconstruction
2\textsuperscript{nd} input is root output filename from reconstruction
3\textsuperscript{rd} is the desired iteration
4\textsuperscript{th} is the solution file to be saved (NNxN parameters)

To plot these images, use

```
plot_chrom(mesh,'pat_id.prop')
```
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