Sub-surface Diffuse Fluorescence Tomography: System Development and Feasibility Studies

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Committee: Brian W. Pogue (Chair), Keith D. Paulsen, Roger Springett, and Hamid Dehghani

ABSTRACT

Sub-surface tomography with diffuse light has been investigated with a non-contact approach to characterize the performance of optical absorption and fluorescence imaging. Using both simulations and experimental studies, the reconstructed images of local sub-surface heterogeneities was demonstrated, using diffusion properties characteristic of near-infrared light in mammalian tissue. The results indicate that the recovery of target size and fluorophore concentration is not linear when changes in depth occur, whereas the mean position of the object for experimental fluorescent and absorber targets is accurate to approximately 1mm when located within the first 10 mm below the surface. Improvements in the linearity of the response with depth appear to remain challenging and may ultimately limit this tomographic imaging approach to detection rather than characterization applications. However, increases in tissue curvature and/or the addition of prior information are expected to improve the linearity of the response. The potential for this type of imaging technique to serve as a surgical guide is highlighted.
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Chapter 1: Introduction

1.1 Overview

During the past decade, diffuse optical tomography (DOT) has emerged as a noninvasive technology for many imaging applications, but its routine use in humans is still limited by seemingly insurmountable geometric and attenuation constraints [1]. Development of remission-mode tomographic imaging techniques would improve the opportunities for use in human tissues, since they only require direct access from one side of the tissue, and could be added into standard systems that already use this geometry, such as microscopy, endoscopy and colonoscopy. This type of imaging has been explored extensively in prior studies, but with each investigation typically using different geometries and detection schemes. Examples include experiments in animals [2-4], as well as breast cancer imaging [5], and functional imaging of the brain [4, 6, 7]. Much of what is currently explored in this remission mode is ‘topography’ of the tissue, where single source-detector pairs are used to sample the tissue with limited depth, and simply recover a superficial map of the tissue optical properties. This has been extensively studied in brain function studies. In this thesis, the focus is on developing a ‘tomography’ system, which can use multiple overlapping source-detector projections to reconstruct images of the tissue volume below the surface. This remission-mode tomography is still an area of development and the performance of tomography in this geometry is still poorly understood.

Extension of this imaging geometry into fluorescence has not been fully evaluated experimentally either, nor have the strengths and weaknesses of different source-detector geometries been systematically examined. In this study a non-contact raster scanning fluorescence diffuse optical tomography system to detect subsurface lesions in tissue is presented. In particular, the approach was developed and optimized to image Protoporphyrin IX
(Pp-IX) fluorescence, so that it could be used as a guide for intrasurgical brain tumor resection which appears to be a very promising and immediate application [8]. However the system has been developed in a generalized way, such that performance characteristics can be extrapolated to imaging other chromophores and fluorophores as much as is possible.

1.2 Background

The potential for absorption-based diffuse optical tomography as a noninvasive modality for functional and metabolic imaging of tissue has been comprehensively analyzed for clinical use [9-12]. The majority of these systems, however, utilize a transmission-based approach for measuring the absorption and scattering of light in tissue, as illustrated in Figure 1 (a). Subsequently, this type of implementation limits DOT to a few specific anatomical sites, most commonly the female breast. To this point, there has been no clear evidence that the development of remission, epi-illumination or B-scan modes (Figure 1 (b)) will provide useful imaging geometries. In remission mode, an A-scan would comprise a single excitation point where the emissive field is then collected. A B-scan would consist of the light source scanned across a line of points on the surface, and the remitted field captured at each successive location. The successful implementation of standalone remission-mode systems has thus far been limited to functional imaging in the brain [4, 7] [6, 13], and as a diagnostic tool for breast cancer screening [14, 15]. While it has been shown that these systems can detect relative changes in various parameters that are correlated with functional activity, a systematic study of the ability to exploit remission mode data to quantify parameters such as target size, position and concentration has yet to be definitively demonstrated.
Imaging with fluorescence in vivo is being investigated to monitor drug uptake, enhance contrast in diseased tissue [16-18], and resolve molecular activity [19, 20]. Though encouraging, many of these studies utilize planar imaging techniques, which are inherently incapable of imaging deeper than perhaps a centimeter due to the diffusive nature of light propagation in tissue [21]. To extend and improve depth resolution, spatially-resolved diffuse fluorescence tomography has been successfully demonstrated in several important applications, most notably in small animal transmittance tomography [22-24], and in some pilot remission studies performed on breast phantoms [25, 26]. In the present study, the geometry of a B-scan or epi-illumination mode of depth tomography is examined, with a focus on defining the clinically relevant and accurate information it can provide. The results have important implications for a B-scan form of depth tomography which may be the most promising geometry for use in human studies with specific contrast agents.

Of particular interest in this study is fluorescence guided surgical resection, which has recently been shown to improve progression free survival compared to conventional white-light guided surgical resection [8]. Several studies have used 5-aminolevulinic acid, a prodrug that is

Figure 1.1: An illustration presenting the two most common geometries that have been previously investigated for fluorescence diffuse optical tomography. Examples of transmission tomography (a) and remission or sub-surface tomography (b). As one might expect transmission tomography systems collect light transmitted at through the tissue whereas remission mode systems deliver and collect light from the same side of the tissue surface.
biochemically synthesized into Protoporphyrin IX (Pp-IX) in many tissues. Since normal brain tissue has a very low production of Pp-IX, this contrast can be used to enhance fluorescence from glioma tumors relative to normal brain. Intraoperative planar remission imaging techniques allow fluorescence intensity maps of the parenchymal surface to be generated. Using these maps as a guide, surgeons can more easily identify and resect malignant tissue on the basis of its fluorescence intensity [8]. Because the technology as presently practiced simply uses a broad beam excitation and fluorescence imaging approach, the ability to detect subsurface disease is severely limited by the diffusive scatter of tissue. A tomography system, on the other hand, would provide increased depth resolution in addition to improved sensitivity to smaller targets and lower contrasts. An imaging system with tumor-specific depth resolution may further extend patient survival by improving the sensitivity to smaller, lower contrast targets at depth.

Here, a non-contact, B-scan absorption and fluorescence diffuse tomography system is evaluated both theoretically and experimentally, and its potential for providing clinically relevant information is evaluated. This optical fluorescence system utilizes a 635 nm diode laser and two orthogonal galvanometers to raster scan the position of the source along the tissue (or phantom) surface. Using Protoporphyrin IX (Pp-IX) as a fluorescent agent, the amplitude of the absorbance and remitted fluorescence signal is separated by optical filters, and detected by a cooled CCD camera. Intensity data is acquired for all source positions along the surface of the region of interest. Calibrated data sets are generated and then used to reconstruct the sub-surface volume, via a finite element model of diffusion. System performance, in terms of the ability to quantify target size, position, and concentration, is evaluated as a function of depth for both absorption and fluorescence diffuse optical imaging. Biologically relevant contrasts are
considered in simulations as well as 'best case' experimental scenarios. The results can be
generalized to most planar depth tomography geometries.

1.3 Thesis Objectives

Although the ability to image the absorption of various tissue chromophores in remission
mode has previously been demonstrated [4, 6, 7], the literature has lacked a systematic study
evaluating the ability to quantify absorbers at varying depths. In an earlier simulation study,
Pogue et al.[1] showed that remission mode tomography would not perform as linearly as
transmission diffuse optical tomography. In this study, the hardware realization of these
simulations is studied for non-contact remission-mode imaging. The ability to quantify
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measure of depth in the range 0-10 mm below the surface. This may be adequate for
applications where target detection and depth may be more important than quantification, such as
in some clinical surgical guidance applications [8, 27, 28]. In addition, the direct comparison of
detectability between broad beam imaging and depth tomography are compared, to show that
depth tomography increases the range of what is detectable down to smaller sizes and lower
contrasts than broad beam imaging could achieve.
Chapter 2: Image Formation

2.1 Overview

Images in this thesis were reconstructed using an iterative, non-linear solver, which uses the finite-element method to model light transport in tissue. Specifically, the frequency domain diffusion approximation to the radiative transport equation (RTE) was utilized as the forward model. Finite-element meshes used in simulations as well as experiments were created using the MATLAB Partial Differential Equation Toolbox and then converted to NIRFAST/FluoroFAST format using the NIRFAST ‘mesh_tool’ function. The mesh used to reconstruct all experimental images in this thesis contained 1600 elements and had dimensions of 60 mm (X-axis) by 30 mm (Y-axis), as shown in Figure 2.1. The Y-axis dimension was selected to correspond with the volume used in the liquid phantom experiments. In the X-dimension however, the boundary was selected to be 1 cm greater on either side than the actual scan area, to decrease any effect of the mesh edges upon the solution in the interior. This was done because the liquid phantom is essentially a semi-infinite media, with respect to the volume that is actually imaged. When a smaller bounded domain with the dimensions of the actual scan area was used, boundary artifacts were observed due to inaccurate forward calculations. Simulation experiments utilized a 2000 node mesh with the same Y-dimension, but an X-dimension that matched the 4 cm size of the imaging field of view (FOV). Here, boundary artifacts were not observed due to the fact that the simulation generated noisy data matched the model.
2.2 Finite-element Image Reconstruction

A number of researchers have demonstrated the feasibility of the diffusion approximation to model both NIR [29-32] and fluorescence light transport[33-37] in tissue. Here, similar techniques were extended to model fluorescence diffusion to recover the spatial distributions of absorption and fluorescence yield. Image reconstruction was achieved using FluoroFAST, a finite element method (FEM)-based software package developed and distributed at Dartmouth [38]. FluoroFAST utilizes a nonlinear Newton-minimization approach [39] to obtain inverse solutions to the coupled frequency-domain diffusion equations [40]:

\[
\nabla \cdot D_x(\vec{r})\nabla \Phi_x(\vec{r},\omega) - \left[\mu_{ax}(\vec{r}) + i\omega/c\right]\Phi_x(\vec{r},\omega) = -q_0(\vec{r},\omega) \tag{2.1}
\]

\[
\nabla \cdot D_m(\vec{r})\nabla \Phi_m(\vec{r},\omega) - \left[\mu_{am}(\vec{r}) + i\omega/c\right]\Phi_m(\vec{r},\omega) = -\Phi_x(\vec{r},\omega)\mu_{am}(\vec{r})\frac{1-i\omega\tau(\vec{r})}{1-\omega\tau(\vec{r})} \tag{2.2}
\]

where Eq. (2.1) is the excitation field and Eq. (2.2) is the fluorescence emission field. The subscripts \(x\) and \(m\) denote the excitation and emission wavelengths respectively; \(q_0(\vec{r},\omega)\) is an isotropic excitation source term at position \(\vec{r}\); \(c\) is the speed of light in the medium; \(\omega\) is the
modulation frequency at excitation; \( \Phi_{x,m}(\vec{r}, \omega) \) are the excitation and emission fields at position \( \vec{r} \); \( \mu_{ax} \) and \( \mu_{am} \) represent the absorption coefficients; \( \mu_{af} \) is the absorption due to fluorophore; \( D_{x,m} = 1/[3(\mu_{ax,m} + \mu'_{sx,m})] \) are the diffusion coefficients; \( \tau \) is the fluorophore lifetime; and \( \eta \) is the fluorophore quantum efficiency. The reduced scattering coefficient, \( \mu'_{s} \), is equivalent to \( \mu_{s}(1-g) \), where \( g \) is the anisotropy factor \( \mu_{s} \) is the scattering coefficient. Implementation of a Robin-type (type III) condition accounts for the refractive index mismatch at the boundary.

Given initial estimates of \( \mu_{a} \) and \( \eta_{af} \), the model optical properties are calculated by non-linear iterative solutions to Eqs. (2.3) and (2.4).

\[
\mathfrak{S}_x \partial \mu = \partial \Phi_x \\
\mathfrak{S}_m \partial \gamma = \partial \Phi_m
\]  

(2.3) 

(2.4)

Here, \( \partial \Phi_x \) and \( \partial \Phi_m \) are small changes in the measured boundary data with respect to an initial estimate of optical properties \( \mu \) and \( \gamma \) where \( \gamma = \eta_{af} \frac{1}{1 + (i \omega \tau)^2} \) is the fluorescence source term; optical property update solutions are given by \( \partial \mu \) and \( \partial \gamma \). In both cases, \( \mathfrak{S}_{x,m} \), the Jacobian matrix, is calculated via the adjoint-method [36, 41] and it maps changes in the logarithm of the signal intensity to small changes in the optical properties at each node within the finite-element model. Having the dimensions of \( (S \times D) \) by \( N \), \( (S \) is the number of sources; \( D \) is the number of detectors; and \( N \) is the number of nodes), the Jacobian matrix elements are defined as:

\[
\mathfrak{S}_{ij,x} = \frac{\partial \ln(\Phi_{x,ij})}{\partial \mu_a}
\]  

(2.5)
9

\[ \mathcal{I}_{ij,m} = \frac{\partial \ln(\Phi_{m,ij})}{\partial \gamma} \]  

(2.6)

where \( \Phi_{ij} \) is the intensity of the diffuse remission measurements from source \( i \), to detector \( j \), in Eqs. (2.5) and (2.6), respectively.

The inverse solutions are then obtained by a modified Tikhonov minimization, where the objective function is:

\[ \chi^2 = \sum_{i=1}^{M} (\phi_{\text{meas}}^i - \phi_{\text{calc}}^i)^2 + \lambda \sum_{i=1}^{N} (\mu_i - \mu_0)^2 \]  

(2.7)

Here, \( M \) is the number of measurements, \( N \) is the number of nodes, \( \phi_{\text{meas}}^i \) is the measured intensity data, \( \lambda \) is the regularization parameter, and \( \phi_{\text{calc}}^i \) is the model intensity data. Values of \( \mu_{a,s} \) and \( \eta \mu_{a,f} \) at each node within the finite-element-model are given by \( \mu \) and \( \mu_0 \) is the initial parameter estimate. A spatially variant Levenberg-Marquadt type regularization scheme (Eq. 2.8) has been implemented to improve image contrast and resolution at a distance from the source/detector boundary [42].

\[ \hat{\mu} = \left( \mathbf{Z}^T \mathbf{Z} + \lambda(p_j) I \right)^{-1} \mathbf{Z}^T (\phi_{\text{meas}} - \phi_{\text{calc}}) \]  

(2.8)

Again, \( \mu \) is a generic symbol for the optical property of interest, \( \lambda(p_j) \) is a spatially variant regularization parameter; and \( I \) is the identity matrix. At each iteration, the spatially variant regularization term is calculated by:

\[ \lambda(p_j) = \lambda_{hf} \exp(-p_j / \alpha) \]  

(2.9)

where \( p_j \) is the y-axis location of node \( j \), \( \lambda_{hf} \) is an empirically determined free regularization parameter multiplied by the maximum diagonal of the Hessian matrix at each iteration, and \( \alpha \) is an empirically determined decay coefficient.
2.3 Improving Depth Resolution with Spatially Variant Regularization

It has been shown that image contrast and resolution degrade with increasing distance from the sources and detectors and that these parameters can be significantly improved using a spatially variant regularization technique [5]. Here, in reconstructing absorbance and fluorescence yield we implement the spatially variant form of the Levenberg-Marquadt regularization scheme (Eq. 2.9) is employed. The regularization parameter at each node will exponentially decay with increasing distance from the source detector boundary. Spatially variant regularization is implemented here in an effort to extend the depth resolution of B-scan mode imaging and improve the quantitative accuracy in target recovery. One problem exists however. Depending on the choice of $\alpha$, the quantitative accuracy in the recovered image can be substantially worse than in the standard Levenberg-Marquadt type of regularization, which applies the same regularization value to each element within the model.

![Main Effects Plot](image)

Figure 2.2: A Main Effects Plot is shown for the parameters $\lambda_H$ and $\alpha$. In these graphs the y-axis represents the error in the recovered centroid location (mm) and the x-axis represents the initial regularizations (left) and the decay parameter values (right). From these plots, it is clear that the decay term ($\alpha$) is the most important parameter in minimizing the error in the recovered location. Furthermore, we can conclude that $\alpha=25$ is the optimal decay value.
To find an optimal $\alpha$, a statistical Design of Experiment (DOE) analysis was implemented using MINITAB. The experiment was comprised of two levels, $\lambda_H$ and $\alpha$, having 3 ($\lambda_H = 10, 25, 50$) and 5 ($\alpha = 2.5, 5, 7.5, 10, 25, 50$) factors, respectively. The error in the recovered centroid position at 0, 2.5, 5, 7.5, and 10 mm were used as replicates for each, as the error in the recovered depth is what we wish to minimize. This analysis was performed for a 5 mm target having 5:1 target to background fluorescence contrast. The MINITAB generated “Main Effects Plot” is shown in Figure 2.2. From this we can infer that the free regularization parameter, ($\lambda_H$) has little to no effect on minimizing the error, but that the decay term ($\alpha$) strongly impacts the centroid error in reconstructed images as suspected. By inspection of the plot, Error vs. Decay, it is apparent that the optimal value, in terms of recovering the centroid position, is $\alpha = 25$. In the simulations and experiments presented in this thesis values of $\alpha = 25$ and $\lambda_H = 25$ are used.

To illustrate how spatially variant regularization improves subsurface imaging, simulations were performed to compare the standard and spatially variant forms of the Levenberg-Marquadt type regularization implemented here. Both types of regularization were used to reconstruct simulated data (1% Gaussian noise) for an 8mm target over a range of depths (0 mm, 2.5 mm, 5 mm, 7.5 mm, and 10 mm) and contrasts (2:1, 3.5:1, 5:1, and 10:1). For this set of simulations, the error in recovered centroid position was determined to be 0.88 mm 0.64 mm for the normal and spatially variant regularization formulations respectively. A representative set of images, shown for 3.5:1 fluorescence contrast, are shown in Figure 2.3. From this set of images it is apparent that spatially variant regularization substantially improves the reconstruction accuracy as the object moves deeper into the medium. In general, this performance enhancement has been demonstrated to be effective in most simulation and
experimental settings, with the exception of large experimental targets. Chapter 5, Phantom Studies, provides greater insight and potential explanation regarding this large target phenomenon.

Figure 2.3: A series of images illustrating the power of spatially variant regularization to improve reconstructions and quantification at depth. The non-spatially variant reconstruction is shown in the top row, with test image shown in the bottom row. Then images recovered with spatially dependent regularization are shown in the middle row. As the object goes deeper into the medium, away from the source-detector array, the object becomes more difficult to recover.
Chapter 3: Experimental Setup

3.1 Theoretical Determination of the Optimal Imaging Geometry

3.1.1 Sensitivity Analysis

Sensitivity analysis was employed here in an effort to determine the imaging biases and the optimal number of sources and measurements for the given slab remission geometry [3] [43]. In general, the hypothesis underlying this analysis is that the optimal geometry is that which provides the most uniform sensitivity deepest within the finite-element image domain. The analysis is performed on a 2000 node mesh for an increasing number of sources and measurements as well as for a fixed number of sources with an increasing number of measurements. Specifically, the sensitivity for 8 sources & 7 detectors, 16 sources & 15 detectors, 32 sources & 31 detectors, and 16 sources & 31 detectors is examined here. Other source detector configurations were also examined, but excluded due to the fact that the results are similar and many are not practical – either in terms of the computation time necessary to reconstruct the images or due to hardware-related implementation limitations.

The Jacobian matrix maps changes in the logarithm of the intensity of fluorescence intensity to small changes in the optical properties at each node within the finite-element model. Having the dimensions of $S \times D$ by $N$, ($S$ is the number of sources; $D$ is the number of detectors; and $N$ is the number of nodes), the absorbance and fluorescence Jacobian matrices are computed by equations 2.5 and 2.6 respectively. In creating a sensitivity plot, the entire Jacobian matrix is first computed, and then the absorption/fluorescence component is separated and summed column by column. The result is a 1-D array the same size as the number of measurements. Here, each is normalized to 1 by dividing each sensitivity array by its maximum value. This
normalized sensitivity provides insight regarding the detection uniformity and imaging biases. The results for both absorbance and fluorescence are depicted in Figure 3.1 (a) and (b) respectively.

![Graphs](image)

**Figure 3.1:** Sensitivity analysis results for absorbance (a) and fluorescence (b), both indicate that sensitivity does not increase significantly for any source/detector configuration in the remission geometry. Similarly, the detection uniformity does not improve.

The results indicate that:

- sensitivity does not increase for any of the various source/detector configurations under examination.
- detection uniformity does not significantly improve for any source/detector combination in the remission geometry.
- the accuracy in quantifying the depth of anomalies beyond 10mm will be poor and anomalies beyond 15mm will not be recovered at all.
- a hypersensitivity exists at approximately 2.5 mm. This is problematic because targets are biased in the reconstruction to be recovered at this point in the model, as demonstrated in the Experimental Results section of Chapter 5. Because the uniformity degrades more rapidly for fluorescence, biasing will be more apparent in reconstructing fluorescence that absorbance.
Overall, none of the various source/detector configurations examined provide improvements in
detection uniformity or depth sensitivity.

3.1.2 Singular Value Analysis

Culver, et. al. [44] as well as others [3,43] have shown that singular-value analysis is a useful technique for determining the optimal number and placement of optodes in diffuse imaging. In general, Singular Value Decomposition (SVD) can be used to provide information regarding the number of measurements above the system’s noise floor; a greater number of singular-values, or eigenvalues, indicates an improvement in the image contrast to noise ratio (CNR). Singular-value analysis is performed by decomposing the Jacobian into:

\[ \mathbf{J} = \mathbf{USV}^T \] (3.1)

where \( \mathbf{J} \) is the Jacobian, \( \mathbf{U} \) and \( \mathbf{V} \) are orthonormal singular-vector matrices of \( \mathbf{J} \), and \( \mathbf{S} \), a diagonal matrix, contains the eigenvalues of \( \mathbf{J} \). Here, SVD was applied to the same geometries considered in the section 3.1.1 Sensitivity Analysis, but with 1% noise added.

Singular-value analysis results for the geometries under consideration are illustrated in Table 3.1 for absorbance (a) and fluorescence (b). The results indicate that increasing the number of sources and measurements as well as increasing the number of measurements for a fixed number of sources both increase the number of singular values. Here the problem comes down to an image improvement vs. computation time tradeoff. To quantify this cost benefit relationship we can examine the useable number of measurements by:

\[ \text{Useful Measurements (\%)} = \left[ \frac{\text{Useful no. Singular Values}}{\text{Total no. Singular Values}} \right] \times 100\% \quad [43] \] (3.2)
Table 3.1: Absorbance (a) and fluorescence (b) singular-value analysis results for various source detector geometries. These results indicate 32 sources & 31 detectors and 16 sources & 31 detectors will produce images with the best CNR. However, as the number of source-detectors increases, the fraction of truly useful measurements decreases proportionately.

These results are shown in the far right column of Table 3.1. We notice that though 16 sources & 31 detectors provide the greatest number of singular-values, it does not provide the greatest percentage of useful measurements. Using 16 sources & 15 detectors will result in a slight degradation in image contrast to noise (compared to 16 sources and 31 detectors), but requires less computation. Considering these results as well as the sensitivity analysis conclusions, 16 sources & 15 measurements was chosen as a reasonable compromise geometry for diagnostic imaging.

3.2 System Configuration

3.2.1 Instrumentation

The non-contact fluorescence diffuse optical tomography (DOT) imaging system is depicted in Figure 3.2 (a) and is schematically shown in Figure 3.2 (b). A 635 nm collimated diode laser (Model CPS196, Thor Labs) was coupled to an automated filter wheel (Model AB301-T, Spectral Products) and two orthogonal galvanometers (Model 6220, Cambridge Technology), which were used to perform the raster scan along the surface of the imaging plane.
Diffuse reflectance and remission intensity signals were separated using a 632 nm narrow bandpass interference filter (Model NT43-185, Edmund Optics) and a 650 nm long-pass filter (Omega Optical), directed through a lens of f 1.2, and collected using a cooled CCD camera (Sensicam QE, Cooke Corporation). Camera specifics include electrical cooling to -12°C, a 12bit dynamic range, and a CCD chip size of 1376 x 1040 pixels. Individual pixels have dimensions of 6.45 x 6.45 μm² and the manufacturer specifies a frame rate of 10 fps. Custom LabVIEW control software, implemented on a 2.4 GHz computer with 1 GB of RAM, allows for full automation of the galvanometers, filter wheel, and CCD.

For this application, the most suitable 2D imaging geometry was previously determined to consist of 16 source and 15 detector locations (240 measurements) by performing sensitivity and singular-value decomposition analysis for various source/detector combinations. Here, for each source position, 16 virtual detectors [45] of size 2.5×2.5 mm² were used to collect diffuse projections over a 4×0.25 cm² field of view (FOV), as illustrated in Figure 3.2 (c). In the imaging plane, detectors in the locations of the active sources were omitted to ensure the data obeyed the constraints imposed by the diffusion regime. To collect absorbance data at the excitation wavelength, a 2.0 optical density neutral density filter was used in the automated filter wheel to attenuate the laser source intensity; this was necessary to ensure that the reflectance and remission signals collected by the CCD fell within the same dynamic range. Acquisition of each dataset required approximately 1.5 minutes, or 6 minutes to build the calibrated datasets necessary for reconstructing the spatial distribution of absorption and fluorescence yield. The rationale and signal-to-noise considerations behind the virtual detection scheme implemented here is highlighted in the following section.
Figure 3.2: The experimental setup is comprised of an excitation light source (LD), two orthogonal galvanometers (XY) for raster scanning the source position, a filter for attenuating the excitation light (F1), filters for separating the excitation and emission light (F2/F3), and a charge coupled device (CCD) camera for detection. A photograph of the hardware set up is shown in (a) with a schematic in (b), illustrating the remittance geometry with laser source and detector camera pointing upwards at a glass plate, upon which phantoms and animals can be placed. A schematic of the virtual detector scheme (c) that was experimentally implemented graphically illustrates the intensity from a group of pixels (256) being averaged together to form a virtual detector. Intensity data is collected at all detector positions (gray) for each source position (white).

3.2.2 Optimizing Non-contact CCD-based Detection

In reconstructing DOT images it is pertinent that the experimental data matches the mathematical model. Poor signal-to-noise data or improper calibration will inevitably result in a failed reconstruction or an image dominated by artifacts. To measure the diffuse projections using a CCD, several approaches to data acquisition and CCD-based detector configuration were considered. These approaches to CCD detection include creating CCD detectors via: pixel binning; virtual detector creation in conjunction with multiple integration times (to improve the
SNR at detectors far from the source); and video-rate averaging of virtual detectors. The strengths and weaknesses of each will be discussed.

Pixel binning is a CCD clocking scheme which can be implemented to reduce readout noise, improve the SNR, allow for faster frame rates, and increase sensitivity in low light applications. Binning works by exploiting the CCD clock capabilities to combine the quantum wells of many pixels into a single well. As an example, Figure 3.3 provides insight as to how 2x2 binning is accomplished using the CCD’s parallel and serial shift registers. Images (b) and (c) illustrate how the charge is shifted by the parallel registers into the serial registers where it is summed. In steps (d) and (e) the charges are shifted from the serial register to the output node, where one packet is then processed by the CCD’s electronics and converted from analog to digital format. It is important to note that full-well capacity of the serial register is many times that of each individual pixel well and that the well depth of the output node is many times that of the serial register (they have a much larger dynamic range). Knowing this, it is easy to understand how binning can be used to improve the SNR and increases the frame rate because it reduces the amount of serial clock cycles. In this FDOT system, a binning scheme was implemented and assessed for its ability to improve readout time and improve signal-to-noise, but it was quickly dismissed because the Cooke Sensicam QE camera used in this system is not cooled to a low enough negative temperature. Subsequently, it was observed that between 25-50% of the serial shift registers well capacity was being consumed by noise.
Virtual detection refers to a group of CCD pixels being combined via averaging to yield a single measurement. Here, 256 pixels are combined in a 2.5 mm x 2.5 mm area as depicted in Figure 3.2 (c). This approach, when compared to binning, provides the advantage that only 1-2% of the signal will be comprised of noise. The ability of virtual detection combined with video rate averaging of individual detectors was examined for its ability to improve the SNR in non-contact DOT measurements. For a single source position, twenty images of the ROI under examination were acquired using a 250 ms integration time, averaged together, and then individual groups of 256 pixels were averaged to form 15 individual detectors. The idea behind this scheme was that if only a few photons were being counted at the detectors located beyond 3 cm from the active source, averaging virtual detectors would improve the SNR at these measurement points. This theory was experimentally disproved however. Figure 3.4 depicts the
fluence as measured at each detector graphically for diffusion theory as well as experimentally when virtual detection is combined with averaging and when virtual detection is employed with integration binning (to be discussed shortly).

![Graph showing fluence vs. distance for different detection techniques and their comparison with diffusion theory.](image)

Figure 3.4: The fluence as measured at each detector for virtual detection combined with averaging (VD Mean), virtual detection using integration binning, or multiple integration times (VD Int Bin), and the results from diffusion theory (Model). Clearly, the technique which averages many virtual detector signals is unable to improve the SNR at the far detectors. Dividing the integration time into distinct bins based on distance from the source does however provide an adequate SNR. Subsequently, it is this technique which was applied to DOT experiments in this thesis. Note: the slight mismatch between the measured and model fluence can be likely be attributed to a mismatch in the optical properties.

To combat the signal-to-noise issues in read-noise limited scenarios (when only a few photons are being counted), the imaging ROI was divided into three distinct bins, according to integration time. A 10x increase in integration time was used for detectors located between 1-2cm of the source and a 100x integration time was used for detectors located within the 2-4cm region. This is illustrated in Figure 3.5, which depicts the detector integration scheme for sources 1 (a), 8 (b), and 16 (c). In this system, which was optimized for imaging Pp-IX fluorescence, a \( t_{int} \) of 50ms was selected under the assumption that the target fluorophore concentration would be no greater of 10 \( \mu \)g/mL. Assuming this constraint is obeyed, the diffuse
intensity at each virtual detector will be within the linear operating range of the CCD and blooming artifacts are not likely to occur. For large fluorescent targets (> 8 mm) however, blooming was prevalent and a stable signal was unable to be obtained. This data collection scheme is likely the most promising for non-contact CCD-based imaging because it permits fast acquisition and adequate signal-to-noise, as depicted in Figure 3.4. However, steps must be taken to physically block the position of the active so that blooming artifacts can be eliminated. Section 3.2.4 System Performance Evaluation provides a more thorough explanation of blooming and some possible solutions for preventing blooming are highlighted in Chapter 6, Future Directions.

![Image](image-url)

**Figure 3.5:** Examples of the optimal data acquisition scheme which involved reading the data out in three distinct bins. Here, the white rectangle represents the position of the active laser source, and the yellow, green, and red blocks represent integration time increases of 1x, 10x, and 100x respectively. This approach was necessary to maintain a good SNR at the detectors located far from the source. A $t_{\text{int}}$ of 50ms was used in this work.

In summary, the integration binning technique was utilized in the experimental work presented in this thesis. The data collection geometry was intentionally restricted to a single 1D line to focus on the depth reconstruction below this line of data, thereby avoiding the complications and partial volume effects known to exist with 3D reconstructions. The collection of the remitted light at all points along the line using the CCD was efficient, and the point where the laser source hit the phantom/tissue was always excluded due to saturation of the camera and to ensure the data obeyed the constraints imposed by diffusion theory. As such, with 16 source locations, it was found that 15 detector positions could be measured accurately. The data provided multiple overlapping projections through the depth of the tissue but focused along the plane below this line.
3.2.3 Instrumentation Hardware Design Specifics

A block diagram illustrating the communications and electrical connections between the system’s primary components is illustrated in Figure 3.6. The heart of the system control is a PC controlled National Instruments 6014 PCI-type data acquisition (DAQ) card. This DAQ device permits bidirectional communication between remote devices and the PC, but here it is used in an open-loop type of control. The control software dynamically adjusts the analog and digital signals needed to control the galvanometers and relay for the laser according to the demands imposed by the user. Specifically, the DAQ voltage signals delivered to the galvanometer are not a set constant, but a function of the system calibration that is to be performed each day, as described in 3.3 LabVIEW Control Software. Powered by a bipolar 24V power supply, high performance closed-loop servo controllers (Cambridge Technology), supply power to the galvanometers and provide fast and accurate positioning of the mirrors. To turn the laser on/off a digital TTL signal is sent to a single pull single throw relay connected to the AC input of the laser’s power supply. The alternative to this approach for switching the laser would have been electronically bringing the laser below threshold, which is dangerous in the sense that it is somewhat difficult to reliably suppress transients and avoid damaging the laser, especially through a DAQ device. A table containing the specifics of the DAQ terminal block pin connections to each component can be found in Appendix A.
3.2.4 System Performance Evaluation

Because this system is fully non-contact, the greatest source of data error is not due to coupling of the sources and detectors at the phantom or tissue interface, but rather problems inherent to the CCD device itself – most prominently CCD noise and blooming.

The primary sources of noise in a CCD camera include shot noise \( n_{\text{shot}} \), readout noise \( n_{\text{read}} \), and dark noise \( n_{\text{dark}} \). Shot noise is due to the statistical variation in the rate at which photons arrive at and interact with the CCD’s semiconductive material. In general, CCD shot noise follows a Poisson distribution and can be equivalently expressed as:

\[
 n_{\text{shot}} \equiv \text{electrons}^{1/2}
\]  

(3.6)

For most applications, shot noise is the dominant source of noise because it is a function of the intensity of the incident light. The exception however would be low light applications; in this scenario readout noise, or noise produced during the analog-to-digital conversion is dominant. Typically, read-noise is on the order of a few electrons per ‘count’, and in the case of the Sensicam QE the manufacturer specifies a readout noise of 5-6 electrons (rms). The A/D
conversion factor for this camera is 4 e⁻/count. In the Sensicam QE an offset of 49 counts is used to ensure that a positive intensity count value is given in low-light scenarios. The final source of noise, the dark noise signal is due to thermally generated electrons within the semiconductor substrate. Because the dark noise is a random variation in the dark signal it cannot be removed. The raw datasets must however remove the dark signal which is highly dependent on temperature and increases linearly with increasing integration. This is typically done experimentally by recording the dark signal as a function of increasing integration time and performing a linear polynomial fit. Most CCD devices are cooled to very low negative temperatures in an effort to reduce the dark signal to only a few electrons. In the Sensicam however the dark signal is a somewhat serious problem for two reasons:

1. The QE is only cooled to -12°C the dark signal rapidly increases with integration time. This is evident upon inspection of Figure 3.7 which demonstrates a 67% increase in dark noise over a 9 second period.

2. The relative intensity in the number of dark counts is spatially dependent. That is, it appears that the Sensicam employs a cylindrical style cooling device which does not cool the CCD uniformly. This is demonstrated in Figure 3.8 by images depicting the spatial distribution of the dark and readout signals for a 250ms exposure (a) and a 60s exposure (b). This problem worsens when the camera is left on for long periods of time, such as when a large number of experiments are being performed.

Taking all of these various constraints into consideration, this system should truly remove the dark signal in a virtual detector specific manor. So, instead of always using the predetermined signal values for a specific region, the dark signal should be determined before each raster scan. For this work and in the experiments performed herein, an effort was made to constrain the
imaging ROI to the center of the CCD chip. By doing this, using the noise values obtained from the linear fit to mean dark signal for detectors in that region, and by performing integration binning, it was found that a stable signal could be obtained.

![Graph](image)

Figure 3.7: Sensicam QE dark noise and offset signal together as a function of integration time. In collecting tomographic data, the noise was removed from the diffuse intensity signal using the equation obtained from a polynomial fit to this data which represents the dark and read noise as a function of integration time.

![Image](image)

Figure 3.8: The spatial distribution of noise across the Sensicam QE CCD chip. From these it is apparent that the camera cooling is non-uniform.

When individual pixels in the CCD reach saturation the quantum wells which store the charge are actually at their full well capacity. As a direct result, a phenomenon known as
blooming occurs in the neighboring pixels. Blooming is due to the overflow of electrons from saturated quantum wells into the neighbors. In example of CCD blooming as observed using this system is demonstrated in Figure 3.9. In the first image (a) an integration time of 50ms is used and it appears that the full well capacity (red pixels) of a small group of pixels within that virtual detector has been reached. Here, blooming does not occur as this is the actual spot size of the laser diode source. In the second image (b), an integration time of 500ms is used and the blooming phenomenon is apparent. It now appears that the laser spot actually occupies the area of several virtual detectors. Blooming is a major problem that must be addressed for non-contact CCD-based diffuse optical imaging to be successful. Potential solutions are highlighted in Chapter 6 of this thesis.

![Figure 3.9: A pair of images illustrating what is known as CCD blooming. The approximate size of the laser beam is an ellipse of size 1mm x 1.5 (a). When the camera’s integration time is increased the charge overflows from pixel wells in the position of the laser spot and into the neighboring wells (b). In this system blooming artifacts in the data make imaging large fluorescent targets difficult.](image)

### 3.3 LabVIEW Control Software

#### 3.3.1 Software Overview

System control software was created using the National Instruments LabVIEW 7.1 software development environment. The software allows users to easily calibrate the system, interactively select a region of interest (ROI) to image, acquire 1-D or 2-D B-scan mode absorbance and fluorescence datasets, generate calibrated datasets, and reconstruct images. Each
of these functions and the hierarchy of each is summarized in the flow chart depicted in Figure 3.10; a LabVIEW produced VI hierarchy table can be found in Appendix B. The remainder of this chapter is dedicated to explaining the capabilities and functionality of the system software.

Figure 3.10: A flow chart illustrating the steps which must be followed to acquire data at all laser positions, and use the data for subsurface tomography.
3.3.2 System Calibration

The purpose of system calibration is to determine the voltages that the DAQ device must supply to each galvanometer in order to move the laser spot 1 pixel. Calibration is necessary to ensure accurate positioning of the laser during raster-scans as well as to ensure that the correct regions of interest on the CCD are identified. A screenshot of the LabVIEW system calibration GUI is depicted in Figure 3.11 (a). To calibrate, users begin by placing a piece of black felt over the surface of the entire glass imaging surface. One must then input the maximum galvanometer voltages, $X_1$ & $Y_1$ for the X and Y galvanometers respectively. This will define the ‘calibration region’ or the area within which users will be permitted to select an ROI. After this information has been entered, the program automatically adjusts the laser to the ‘home’ location by positioning the galvanometer at voltages (0 Volts, 0 Volts). The centroid pixel location of the laser spot in the camera’s entire FOV is then calculated in both dimensions. This procedure is repeated for galvanometer voltages ($X_1$ Volts, 0 Volts) and (0 Volts, $Y_1$ Volts) as illustrated in Figure 3.11 (b). Finally, these coordinates are used to determine the voltages required to move each galvanometer 1 pixel. A calibration file containing this voltage information, the coordinates at each calibration point and the voltages defining the calibration region are saved to the file ‘Calibration.txt’ in the default Labview Data directory. In the event that no changes in the magnification or the focal distance have occurred this file can be loaded and used.

3.3.3 Scan Settings

Post system calibration users are permitted to choose between 1-D and 2-D raster scans as well as select the axis about which the scan will be performed, as illustrated in Figure 3.13. The various other features here, such as binning, the number of sources and detectors, and source
detector spacing are fixed in the current version of the software, but could easily be made functional with some simple modifications to a few sub-VIs.

Figure 3.11: A screenshot of the system calibration aspect of the system software. By inputting maximum voltages for the X & Y galvanometers, the system defines a calibration region (b), automatically adjusts the laser to the calibration points, and determines the galvanometer voltages required to move the laser spot 1 pixel. In performing this calibration, the laser can now be accurately positioned anywhere within the calibration region. In the case that no changes in the magnification or focal distance have occurred the previous calibration file can be loaded and used.

Figure 3.12: Users can easily choose between 1-D or 2-D data acquisition for 2-D and 3-D image reconstructions respectively. Data can be collected in either the X or Y plane. The number of sources/detectors, binning, and source/detector spacing is fixed.
3.3.4 ROI Selection

In selecting an ROI, an image of the entire imaging FOV is acquired and displayed in a popup window with the user defined calibration region shown in red. This permits users to dynamically select an ROI to image. For the case of a 1-D line scan, the user simply selects a single point in the center of the line, or ROI, that is to be scanned. Knowing that a single 1-D line of data requires 10 camera ROIs of size 32 x 32 pixels to be read out, the software automatically adjusts the user selected point to an ROI that can be physically realized and readout by the CCD. In doing this, the image of the entire imaging FOV, with images of the calibration region and a yellow rectangle representing the region to be raster-scanned are superimposed and saved to file. This procedure is summarized in Figure 13 (a) along with an image illustrating how the user selected region will be transformed into virtual detectors (b). Finally, the initial X-Y position of the laser source for the raster scan is found, and the laser is adjusted. The procedure for selecting a 2-D region is identical, but a user selects a rectangle that is translated into a 2-D region of ROIs that can be realized by the CCD.

![Figure 3.13](image.png)

(a) Figure 3.13: ROI selection permits a user to select a single point within the calibration region (a). The program will automatically translate this point into a group of ROI’s that will be readout by the CCD and used to create virtual detectors (b). In this step, the initial position of laser is determined and the galvanometer voltages are adjusted such that the laser source is in the position of ‘Source 1’.
3.3.5 Raster Scan

In performing a raster scan a data file, containing 240 a noise-free data points scaled by the integration time will ultimately be saved to disk. First, a folder for the given date must first be created to store files. This is accomplished by selecting the ‘Create Folder’ button. In doing so, a time stamped folder with the data will be created in the default LabVIEW Data directory. When a filename is entered and the radio button of the scan-type is selected, i.e. absorbance or fluorescence, the program will automatically adjust the filter wheel to the appropriate position and adjust the integration time, \( t_{\text{int}} \) to be used in the integration binning. Before initiating a scan, the appropriate filter must manually be placed over the lens and the lights must be turned off. Because the room is essentially pitch black during data acquisition, a beep feature was implemented to alert users when a scan has completed. This can be stopped by pressing the ‘Stop Beeping’ button after the lights and monitor are turned back on.

Figure 3.14: Before initiating a raster scan, a time stamped folder must be created to store data collected. The radio button indicating the type of scan to perform must also be selected so that the appropriate ND filter can be used to attenuate the laser source.
3.3.6 Data Calibration and Image Reconstruction

The data calibration feature of the software, Figure 3.15 (a) is capable creating calibrated absorption and fluorescence data files (paaxfl) or solely fluorescence data files (paafl). A user selects homogenous and heterogeneous absorbance and fluorescence datasets, that are then displayed in the appropriate tab windows, and calibrated using the formulations described in Section 5.3, Data Calibration and Fitting. Currently, data calibration performed through the LabVIEW system software is limited to phantom experiments that utilize the optical properties, $\mu_a = 0.00205 \text{ mm}^{-1}$, $\mu_s = 0.0071 \text{ mm}^{-1}$, $\mu_c = 1 \text{ mm}^{-1}$, and an isotropic diffusion coefficient ($D_s = 0.331 \text{ mm}$). This is because in calibrating the experimental data, it is scaled by a data file that was creating using a mesh containing these optical properties. It is possible to implement a function which would permit users to adjust the mesh optical properties and then calibrate based on these values, but problems were encountered trying to save files in MATLAB through the Active-X link between it and LabVIEW. Using these calibrated data sets, the image reconstruction portion of the code will fit the fluorescence data and then reconstruct the images by calling FluoroFASTS’s `reconstruct_rect` function. Users are able to select between paafl and paaxfl type reconstructions which reconstruct for extrinsic and both intrinsic and extrinsic optical properties respectively. The end result, jpeg format subsurface images, are saved in the time stamped folder under a user specified name.
Figure 3.15: Screenshots of the data calibration (a) and image reconstruction aspects of the system software.
Chapter 4: Simulation Studies

4.1 Overview

Simulations in the reflectance/remittance slab geometry were performed using NIRFAST [47] and FluorFAST, using noisy data estimates (1% Gaussian distributed). Forward data was generated for various target sizes and contrasts corresponding to the actual experimental setup. The centroid location of the target was varied over a range of 0-15 mm, in 2.5 mm increments. A dual mesh basis [48] was deployed to recover the spatial distributions of $\mu_a$ and $\eta\mu_a$ using the reconstruction techniques described Chapter 2.

4.2 Absorbance Simulation Results

To examine the feasibility of imaging absorbance in the reflectance-slab geometry, simulations were performed for physiologically relevant target-to-background absorption contrasts. A mesh background absorption coefficient of $\mu_a = 0.01$ mm$^{-1}$ was used to simulate the bulk tissue while the concentration in various target sizes was adjusted between $\mu_a = 0.02–0.1$ mm$^{-1}$ (2:1 – 10:1 contrasts). Reconstructions were performed as the target centroid location was systematically adjusted from 0 – 15 mm. Phantom background optical property values for the absorption coefficient ($\mu_a = 0.01$ mm$^{-1}$), reduced scattering coefficient ($\mu_s' = 1.0$ mm$^{-1}$), and diffusion coefficient ($D = 0.33$ mm) were held constant for all contrasts.

A representative series of images produced by simulated data for the case of a 6mm target is illustrated in Figure 4.1. Here, the reconstructed images for 10:1 (effectively infinite), 4:1, and 2:1 contrast are shown alongside the true images.
Figure 4.1: A representative set of simulated absorbance images. Simulated data was produced for a 6mm diameter cylindrical target placed at a range of depths from 0-10mm while target to background contrast was varied between 10 to 1 (effectively infinite), 4:1, and 2:1; the 'true' images are shown at the bottom of this figure. From these results, it is evident that the ability to recover target size and $\mu_a$ concentration degrade when the target moves deeper into the tissue, however the location of the target can be accurately estimated.

From these, it is apparent that the ability to quantify the size and magnitude of $\mu_a$ degrades with increasing distance from the source detector boundary; however, the centroid position of the target can be recovered accurately. Figure 4.2 validates these empirical observations by quantifying (a) target concentration, (b) target size, and (c) target location as a function of depth for all contrasts under consideration. It is evident that the target concentration and size are poorly determined if at all. However, here the target centroid position can be recovered with less
than 1 mm of error for all contrasts above 4:1 having true locations no greater than 10 mm in depth.

Figure 4.2: Quantitative analysis of absorption images in the slab geometry. Tomographic images were produced from simulated data for a 6mm target in the range of 0-15mm having contrasts of 10:1, 4:1, and 2:1. Results, in terms of the true target $\mu_a$ concentration (a), size (b), and centroid location (c) indicate that the target location is the only parameter that can be recovered with reasonable accuracy over the depth range 0-10 mm.

The signal attenuation constraint inherent to the remission mode geometry is the greatest limiting factor in this type of imaging, since the deeper projections through the tissue are simply too low to provide sufficient sensitivity to depths deeper than 1.5 cm. Though similar results can be achieved for additional target sizes and contrasts within the range examined here, the centroid error is a function of target size, contrast, and depth. The following section rigorously demonstrates this point for the case of fluorescence B-scan mode imaging.
4.3 Fluorescence Simulation Results

FluorFAST was used to generate simulated fluorescence amplitude boundary data and to produce subsurface fluorescence images. Various targets diameters in the range 4-14 mm having 10:1, 5:1, and 3.5:1 fluorescence contrasts were considered. Again, the target centroid position was systematically adjusted over the range 0-15 mm for each. Mesh optical properties, \( \mu_a = 0.00205 \text{ mm}^{-1} \), \( \mu_a = 0.0071 \text{ mm}^{-1} \), \( \mu_s = 1 \text{ mm}^{-1} \), and an isotropic diffusion coefficient (\( D_x = 0.331 \text{ mm} \)) were selected to mimic the background optical properties used in the liquid phantom experiments. Optical properties at the emission wavelength were set equal to those of the excitation wavelength. The refractive index for tissue was assumed to be \( n = 1.33 \) and the fluorophore lifetime (\( \tau \)) was set to zero because amplitude only reconstructions were performed.

Target and background fluorophore concentrations used in the model were calculated by:

\[
\mu_a = 2.303(\varepsilon \cdot c)
\]

where \( \mu_a \) is the absorbance, \( \varepsilon \) represents the molar extinction coefficient of Pp-IX, and \( c \) is the fluorophore concentration. In this work a Pp-IX molar extinction coefficient of 500 mm\(^{-1}\) M\(^{-1}\) [49] was used along with a Pp-IX molecular weight of 562.6 g/M in calculating the target and background fluorophore absorption values for use in these simulations; they are summarized in Table 4.1.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>( \mu_{af} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ( \mu \text{g/mL} )</td>
<td>0.0205 mm(^{-1})</td>
</tr>
<tr>
<td>5 ( \mu \text{g/mL} )</td>
<td>0.0102 mm(^{-1})</td>
</tr>
<tr>
<td>3.5 ( \mu \text{g/mL} )</td>
<td>0.0072 mm(^{-1})</td>
</tr>
<tr>
<td>1 ( \mu \text{g/mL} )</td>
<td>0.0021 mm(^{-1})</td>
</tr>
</tbody>
</table>

Table 4.2: The Pp-IX fluorophore concentrations used in the liquid phantom experiments along with their corresponding fluorophore absorbance values.
Results highlighting the recovered spatial distribution of fluorescence yield for an 8mm diameter target are depicted in Figure 4.3 for 3.5:1, 5:1, and 10:1 contrasts when the anomaly was located between 0 and 10mm.

![Simulation results for the recovered spatial distribution of fluorescence yield are depicted here for an 8 mm anomaly having 10:1 ('effectively infinite'), 5:1, and 3.5:1 fluorescent contrast. In these images the location of the target is adjusted between 0 and 10mm, as indicated by the ‘true images’ shown at the bottom of this figure. This representative set of images illustrates that the ability to recover target size and concentration degrades with increasing distance from the source/detector boundary. Again, it appears that the actual target location can be accurately estimated by the centroid technique.](image)

The results for fluorescence reconstructions in the slab geometry indicate that the recovered size and concentration degrade with increasing distance from the source/detector boundary. Again, the true centroid location of the inclusion appears to be accurate for realistic contrasts. Specifically, for the case of an 8 mm target, the mean error in centroid recovery was
found to be 0.42 mm over the range 0-10 mm when all contrasts were considered. When the target was adjusted to a depth greater than 1 cm quantitative accuracy was found to degrade significantly; mean error in the centroid recovery increased to 1.67 mm in the range 0-15 mm. Figure 4.4 quantifies these results by depicting the recovered: $\eta \mu_{af}$ concentration (a), size (b), and centroid location (c) for 3.5:1, 5:1, & 10:1 contrasts as a function of target depth. Again, similar results are possible for different target sizes and contrasts.

Figure 4.4: A quantitative analysis of fluorescence imaging performance in the remission-slab geometry. Tomographic images were produced by reconstructing data which simulated the submersion of an 8mm target in a liquid phantom. The depth of the target was systematically adjusted between 0 and 15 mm while target to background contrasts of 10:1, 5:1, and 3.5:1 being considered at each depth. Results, in terms of the true target $\eta \mu_{af}$ concentration (a), size (b), and centroid location (c) were quantified. These findings indicate that the target location is the only parameter that can accurately be recovered over the depth range of 0-10 mm.
In general, a linear increase in the calculated error can be observed with increasing target depth. Figure 4.5 (a) demonstrates this by depicting the total centroid error as a function of depth. Here, centroid error is calculated by considering the comprehensive mean in calculated error for various target sizes (4mm, 6mm, 8mm, 10mm, 12mm, & 14mm) and contrasts (3.5:1, 5:1, & 10:1) as function of depth. Conversely, a non-linear response in centroid error can be observed when target contrast and size are considered. In the case of error induced by variations in target contrast (Figure 4.5 (b)), a centroid error of less than 1.1 mm can be expected for biologically feasible fluorescent contrasts. The biphasic centroid error response induced by changes in target size can be observed in Figure 4.5 (c). From this one can infer that the recovered position for target diameters in the range 4-10 mm will be accurate to less than 1mm for all contrasts above 3.5:1 and centroid positions less than 1 cm. Increases in centroid error for larger targets (>1 cm) can likely be attributed to the size of the sampling region and the limited depths being probed. So, by increasing the source/detector separation distance and thereby expanding the size of the sampling region, it is likely that the large target centroid error will improve.

The ability to provide an image reconstruction approach which is linear with depth in B-scan mode may not be possible, without the addition of further constraints (such as object location from prior information). The response of sub-surface diffuse reconstruction algorithms are depth dependent because of the ill-posed nature of diffuse imaging and the fact that the sensitivity decreases by orders of magnitude with increasing distance from the tissue surface. Here, this shortcoming was improved via the implementation of a spatially variant regularization parameter [42] which enhanced the overall image quality and allowed the recovery of deeper
lesions to be realized; yet, it was still insufficient for maintaining linear sensitivity with depth beyond 1.0 cm deep.

Figure 4.5: Plots depicting the centroid error response as a function of depth (a), contrast (b), and size (c). From these it is evident that error increases linearly with increases in target depth and non-linearly with changes in size contrast. Positional accuracy induced by changes in contrast are acceptable, but error increases rapidly for larger targets.
Chapter 5: Phantom Studies

5.1 Overview

Experiments were performed by submerging a cylindrical target in a liquid phantom, as depicted in Figure 5.1. Targets of varying diameter were filled with background fluid and absorber or fluorescent contrast agents, and were attached at varying depths, as illustrated. To simulate tissue optical properties, a mixture composed of water, 2% India ink & 5% Tween-20 (\(\mu_a = 0.0071 \text{ mm}^{-1}\)), and 1% Intralipid™ (\(\mu_s = 1 \text{ mm}^{-1}\)) was used. The tween was required to maintain a monomeric fluorescent solution of the fluorophore Pp-IX. The target centroid position was adjusted to 7 different locations in the range 0-10 mm. In the case of fluorescence imaging, 1.0 \(\mu\text{g/mL}\) of Pp-IX (\(\mu_a = 0.00205 \text{ mm}^{-1}\)) was added to the background. The target fluorophore concentration was then systematically increased to yield target-to-background contrasts of 3.5:1, 5:1, and 10:1. Following data acquisition and image reconstruction, performance was characterized by an assessment of target size, concentration, and centroid location as a function of depth. A single line of intensity data was collected along the surface of the imaging plane through the center of the target. Excitation and fluorescence intensity data were collected in the presence and absence of increased fluorescence contrast, calibrated, and finally, used as boundary data in the recovery of the subsurface images of \(\mu_a\) and \(\eta\mu_a\).
Figure 5.1: A schematic of the liquid phantom used in these experiments is shown in (a) and a photograph of the phantom on the imaging plane is shown pictorially in (b). All experiments were performed by submerging a cylindrical target in the liquid phantom shown here. Targets of varying diameter were filled with background fluid and absorber or fluorescent contrast agents, adjusted at varying depths, and then a 1-D cross section was raster scanned to collect datasets used in the reconstruction of subsurface images.

### 5.2 Data Calibration and Fitting

It is well known that proper calibration and fitting of experimental data are necessary precursors to image reconstruction [50]. Here, data calibration was inherently simplified because the experimental diffuse optical tomography system acquired non-contact measurements; subsequently, there was no need to account for individual detector coupling errors. In calibration, each raw dataset was scaled by the respective integration time and the magnitude of source attenuation before undergoing the calibration procedures described below. Ultimately, absorption and fluorescence datasets were expressed in terms of the model fluence. In the case of fluorescence reconstructions, an initial estimate of the homogeneous value was needed. This was achieved by fitting the calibrated data to a model which minimized the squared difference error between the data and model predictions, to give a homogeneous value for the quantum yield.
A calibrated absorption dataset was generated by scaling the homogenous and heterogeneous reflectance data by their respective dynamic ranges and then by the model data:

\[
\phi^i_{\text{cal}_-\text{abs}} = \left( \frac{\phi^i_{\text{meas(hetero}_-x)}}{(\max - \min) \phi^i_{\text{meas(hetero}_-x)}} \frac{\phi^i_{\text{calc}_-x}}{(\max - \min) \phi^i_{\text{meas(homo}_-x)}} \right)
\]  

(5.1)

where the subscript \( x \) denotes the excitation wavelength. The intensity amplitude for each measurement, \( i \), in both the presence (hetero) and absence (homo) of a fluorescent object is given by \( \phi^i_{\text{meas}} \). The corresponding calculated amplitude data (calc) is generated by a forward solution to a finite-element model of the homogenous phantom. The calibrated absorption data, \( \phi^i_{\text{cal}_-\text{abs}} \), was then used as the intensity data to recover the spatial distribution of absorption at the excitation wavelength (\( \mu_{a_x} \)).

Calibration of the fluorescence data is more complicated because of the endogenous background fluorescence and the excitation light that is not entirely rejected by the filter. However, we found that these issues could be corrected by first acquiring a homogenous emission dataset and then by performing a point-by-point subtraction from the heterogeneous measurements:

\[
\phi^i_{fl} = \left( \phi^i_{\text{meas(hetero}_-m)} - \phi^i_{\text{meas(homo}_-m)} \right)
\]  

(5.2)

where the subscript \( m \) denotes the emission wavelength. Example homogenous (Fl Homo) and heterogeneous (Fl Hetero) fluorescence datasets for the case of an 8mm target having 10:1 contrast are depicted in Figure 5.3 (a) along with the adjusted fluorescence dataset (Fl Data). In equation 5.2, the adjusted fluorescence dataset was given by \( \phi^i_{fl} \) and the amplitude of each
intensity measurement, $i$, was given by $\phi^i_{\text{meas}}$. The fluorescence data was then scaled to the magnitude of the model by:

$$
\phi^i_{\text{cal, fl}} = \phi^i_{\text{fl}} \times SF^i \quad \text{where} \quad SF^i = \frac{\phi^i_{\text{calc, x}}}{\phi^i_{\text{meas(homo, x)}}} \quad (5.3)
$$

Here, the scaling factor ($SF^i$) was produced via the point-by-point division of the model generated absorption data by the measured absorption data. The subsequent multiplication by $SF^i$ yielded a fluence dataset, $\phi^i_{\text{cal, fl}}$, that was used as the fluorescence intensity amplitude in the recovery of fluorescence yield ($\eta\mu_f$) images. Examples of calibrated data ($Cal$), and the scaling factor ($SF$), for the case of an 8mm target are shown along side a forward generated fluorescence data set ($Fl\ Model$) in Figure 5.3 (b). Finally, we show our experimental data and a forward generated data set on our solution mesh (c). We see that they are in very close agreement with the only discrepancies likely due to artifacts caused by the acrylic target used in the phantom experimented.
Figure 5.3: The steps of fluorescence data calibration are shown here for the case of an 8mm target having 10:1 contrast with respect to the background. Experimental homogenous fluorescence data (Fl Homo) is subtracted from heterogeneous fluorescence data (Fl Hetero) to yield an adjusted fluorescence dataset (Fl Data) that is free of filter bleed-through and endogenous background fluorescence. By scaling this data to the model by a scaling factor (SF), a calibrated dataset (Cal) is produced. The model generated dataset (Fl Model) is shown alongside the calibrated data for comparison. From this figure it is obvious that they are in close agreement and that the calibration was successful. In (c) we can observe that our experimental data very closely matches our solution after the model has converged to a solution.

5.3 Experimental Results

5.3.1 Absorbance

In an effort provide a simple validation scheme for the performance of the model and the reconstruction algorithm, experiments were performed for the infinitely-absorbing 6mm diameter target submerged in the liquid phantom previously described. Again, target position from the
phantom surface was varied to a depth of 15 mm. The measured absorption intensity data was then calibrated and used to reconstruct for the subsurface spatial distribution of absorption. Image reconstructions for targets in the range 0 – 10mm are depicted at the top of Figure 5.4 while images showing the true target are provided at the bottom of this figure. From these images it appears that our simulation and experimental studies are in close agreement, and that target location is the only parameter that can be recovered with reasonable accuracy from experimental data. Figure 5.5 presents a graphical comparison of these simulated and experimental centroid results. When adjusting the target over a range of 1cm, the mean accuracy in centroid recovery was determined to be 1.4 mm and 0.86 mm under the experimental and simulated infinite contrast scenarios respectively.

![Figure 5.4: Experimental absorbance images produced by reconstructing tomographic data collected from a liquid phantom containing a 6 mm diameter, 'effectively infinite' absorbing target are shown for a range of depths at the top of this image. The true locations of the target are shown at the bottom.](image)

![Figure 5.5: A comparison between experimental and simulated absorbance imaging centroid results for the case of 'effectively infinite' contrast. Experimental and simulated results are in close accordance within the first 1cm below the tissue surface - mean positional error was determined to be 1.4 mm and 0.86 mm, respectively.](image)
5.4.2 Fluorescence

Fluorescence liquid phantom experiments utilized the same mixture of water, intralipid, ink, Tween-20 and Pp-IX. The absorption due to fluorophore in the target was increased to 3.5:1 ($\mu_a = 0.00716 \text{ mm}^{-1}$), 5:1 ($\mu_a = 0.01023 \text{ mm}^{-1}$), and 10:1 ($\mu_a = 0.002047 \text{ mm}^{-1}$) contrasts. Using cylindrical targets of size 4mm, 8mm, and 14mm, data was acquired and calibrated fluorescence data sets were reconstructed to produce subsurface tomographic images. To avoid performing an exhaustive number of experiments, two experimental scenarios were selected such that the take away messages would still be apparent. These scenarios include:

1. The target used in the liquid phantom experiment was fixed at 8mm while the contrast and depth were adjusted. This experiment provides insight regarding the ability to image biologically feasible contrasts over a range of depths.

2. The target to background contrast was fixed at 10:1 while target size and depth were systematically adjusted. From this, we can begin to understand the significance of target size on the ability to recover the centroid position.

Results for the scenario of a fixed 8mm target being considered over a range of depths and contrasts are shown in Figure 5.6. From these results, it is apparent that the target can be localized, but that this ability degrades with decreasing contrast. In addition, it is evident that the target concentration and size can not be recovered, as indicated by the large variation in these parameters with depth. Overall, the mean centroid error in this set of images reconstructed from experimental data was determined to be 0.87 mm in the first 1 cm below the surface when target contrasts of 5:1 and greater were considered. These results are highlighted in Figure 5.7, which provides a quantitative evaluation of the centroid recovery for this experiment. The experimental
results appear to be within reason, as the simulation studies report a mean error of 0.38 mm when the same size, depth, and contrast constraints are imposed.

Figure 5.6: A representative set of fluorescence image reconstructions in the remission-slab geometry. Images were reconstructed from experimental data collected for an 8 mm target submerged in a liquid phantom, when the target had 10:1, 5:1, and 3.5:1 fluorescent contrast with respect to the background. The true target locations are shown at the bottom of this figure.

The centroid results for 3.5:1 contrast were excluded in the quantification of mean error because of the large discrepancy between the simulated and experimental results. One explanation for this could be a poor SNR in the experimental data. Two factors could be contributing to this, including light piping around the outside of the acrylic cylindrical target and an inadequate CCD integration time. Regarding the light piping issue, because a clear acrylic cylinder with a 1mm wall thickness was used as the target, the propagation of the incident diffuse light would be inhibited from scattering through the acrylic walls into the region of
increased fluorescent contrast. Specifically, the refractive index change between the intralipid mixture and the acrylic wall of the target gives rise to a reflection and a refraction of the incident light, with a significantly smaller percentage of the light being refracted. Subsequently, only a very small portion of the incident light reaches the inside of the target. By using a scattering material instead of an acrylic for the target, one can hypothesize that the experimental results could be improved significantly due to the improved SNR. In terms of SNR issues arising from a suboptimal integration time, the current integration scheme is optimized for imaging high contrast targets on the surface. So, in imaging lower contrasts at depth, only a very small fraction of the camera’s dynamic range is being utilized. Exploiting the full dynamic range of the camera is essential for maintaining a good SNR, but it is challenging because the fluorescence intensity rapidly degrades with decreasing target size, contrast, and depth. One possible solution for this would be to performing a series of ‘fast-scans’ where the same integration time would be utilized for all detectors. In doing this, a poor signal-to-noise would exist at the read-noise limited detectors, but a good SNR would exist at the near-source detectors, which are the measurements of interest here. By then performing a linear fit to the maximum fluorescence intensity measurement in each fast-scan, the optimal integration time can easily be determined. To ensure linear operation of the CCD device, the integration time should be selected such that the maximum measurement consumes approximately 80-90% of the full well capacity. In dynamically adjusting the integration time an adequate SNR could easily be achieved in an in-vivo setting where no prior knowledge of the target size, contrast or depth is known.
Figure 5.7: Experimental fluorescence centroid results when the depth of an 8mm target was adjusted between contrasts of 10:1, 5:1, and 3.5:1. When contrasts of 5:1 and above were considered, mean positional error in the recovered centroid was determined to be 0.87 mm and 0.38 mm for experimental and simulated fluorescence images.

The impact of target size on centroid accuracy was evaluated experimentally by fixing the fluorescence contrast at 10:1 while the size and position of the target were adjusted. Image reconstructions are depicted in Figure 5.8 for 14mm (top), 8mm (center), and 4mm (bottom) targets in the depth range of 0 – 10mm. Figure 5.9 (a) quantifies these results and compares them to simulation results (b) by graphically portraying the recovered centroid position for each size as a function of depth into the medium. Though the experimental results are substantially less accurate than the simulation results for the 4mm and 14mm targets, the same trends in the data are apparent – the recovered centroid location is underestimated for the 14mm target and overestimated for the 4mm target.

Figure 5.8: Images generated from experimental data for targets of size 14mm (top), 8mm (center), and 4mm (bottom). The contrast was fixed at 10:1 for each as the depth was adjusted from 0mm to 1cm.
There are several plausible explanations for the centroid underestimation in the case of a 14mm target including, the fact that the spatially variant regularization implemented here was not optimized for recovering large targets. Also, because the top of the target is actually at a depth of 24mm when the target is located 1 cm from the surface, it is likely that the lack of sensitivity in this region inhibits accurate recovery by biasing the target to a more shallow depth. From an experimental perspective, the size of the imaging FOV and the source/detector separation distance used here may be hindering the target recovery because the entire depth of the target is not being probed.

Figure 5.9: Experimental (a) and simulated (b) centroid results for a fixed target contrast of 10:1 for a range of sizes (4mm, 8mm, and 14mm) and depths (0-10mm).

In the case of the 4mm target, recovered centroid inaccuracies can likely be attributed to partial volume effects and light piping around the acrylic target. Partial volume effects are prevalent when small targets are being imaged because the acquired diffuse intensity signals at each detector constitute sampling from the background more so than from the target. Subsequently, the ability to detect and quantify subsurface lesions is inhibited. Increasing the number of detectors will reduce partial volume effects, but conversely it will result in an increased computation. Again, light piping around the acrylic target is most likely reducing the
SNR or distorting the signal by preventing light from reaching the inside of the target. Some possible solutions for improving the liquid phantom setup are discussed in Chapter 6.

5.4.3 Experimental Evaluation of Broadbeam and Tomographic Fluorescence Imaging

Fluorescence surgical resection has recently been shown to significantly improve survival time, as compared to conventional white-light guided surgical resection. Thereby demonstrating its ability to enhance progression free survival and increase the number of full malignant glioma resections [8]. The imaging approach adopted in this type of guidance uses the fluorescence provided by preoperative intravenous injection of patients with a prodrug, 5-aminolevulinic acid, that the body biochemically synthesizes into Protoporphyrin IX (Pp-IX). The technology used for guidance in these studies simply maps the surface, and does not take advantage of the sub-surface light propagation to allow tomography. Tomographic imaging of subsurface fluorescence regions provides distinct advantages over broadbeam imaging including increased sensitivity to lower contrasts and the ability to localize targets at depth. Here, we demonstrate the strengths and weaknesses of each by performing image contrast-to-noise (CNR) and contrast analysis on experimental datasets.

The primary objective of this experiment was to provide a direct comparison between the two types of fluorescence imaging modalities. Tomographic images were generated using the data acquisition and image reconstruction techniques previously described in this thesis. Specifically, the images from the ‘fixed target size’ experiment (Figure 5.6) were used in this analysis. In performing the broadbeam imaging experiments, the previously described liquid phantom was filled with the same mixture of water, intralipid, ink, Tween-20 and Pp-IX to as the tomographic experiment. To match the other constraints imposed by the tomographic technique, the imaging field-of-view was fixed at 4cm and the fluence of 633nm HeNe was set to 0.8 mW/mm². Again, excitation and emission signals were separated using a 650nm long-pass optical filter. A photograph of the experimental setup used for broadbeam imaging is depicted in Figure 5.10 and the resultant experimental broadbeam images are illustrated in Figure 5.11.
Figure 5.10: A photograph of the fluorescence broadbeam instrumentation is shown. Also operating in the remittance geometry, the broadbeam system is comprised of a 633nm HeNe laser for exciting the fluorophore, a 650 long-pass optical filter (650 LP) for separating the excitation and emission signals, and a CCD for detection.

Figure 5.11: Experimental fluorescence broadbeam imaging results when the depth and target-to-background contrast of an 8mm target was adjusted. It is clear that the ability to recover targets using this technique degrades rapidly with distance from the surface. Moreover, it provides broadbeam imaging provides no means of quantifying the actual depth location of the lesion.
Image contrast-to-noise analysis in diffuse optical tomography has been rigorously investigated and proven as a means to quantify detectability in reconstructed images [51]. Here, we examine image contrast-to-noise as a function of target position from the surface for both tomographic and broadbeam imaging techniques. In performing contrast-to-noise analysis the following equation was selected to ensure proper weighting of the noise in the target and background [51]:

\[
CNR = \frac{\mu_{af}^{ROI} - \mu_{af}^{bkg}}{\left( W_{ROI} \sigma_{ROI}^2 - W_{bkg} \sigma_{bkg}^2 \right)}
\]  

(5.1)

where \( \mu_{af}^{ROI} \) & \( \mu_{af}^{bkg} \) are the mean node values in the target and background respectively, \( W_{ROI} \) & \( W_{bkg} \) are weighting factors to account for the area of each ROI, and \( \sigma_{ROI}^2 \) & \( \sigma_{bkg}^2 \) are the calculated noise values in the target and background. Image contrast, the relative difference between the fluorescence intensity in the target and the background was then calculated by:

\[
Contrast = \frac{\mu_{af}^{ROI} - \mu_{af}^{bkg}}{\mu_{bkg}^{af}}
\]

(5.2)

To calculate the contrast and CNR in the tomographic images, the image was interpolated onto a 10,000 node fine mesh to ensure an adequate number of nodes existed in the target location. In computing the mean signal and noise in the target ROI, the area encompassing a circle of radius = 4mm from the recovered centroid location was used for each. The entire area outside of each target region was then used in the computing the background signal and noise levels. In the broadbeam analysis, the pixel locations corresponding to the target and background as determined from the ‘True’ image (Figure 5.11) were used. Examples of the region segmentation used in the ROI analysis are shown in Figure 5.12 for tomographic (a) and broadbeam (b) images.
Figure 5.12: Examples of the region segmentation used in the CNR and contrast calculations for tomographic (a) and broadbeam (b) images. In the tomographic images the target ROI was determined based on the position of the recovered centroid whereas the regions were fixed for the broadbeam analysis.

Results for the tomographic and broadbeam contrast (a) and CNR (b) analysis are highlighted in Figure 5.13 and Figure 5.14 respectively. Upon inspection of these figures it is clear that though FDOT yields images of greater contrast, the CNR is substantially better in broadbeam imaging due to the low noise levels. This is not surprising as tomographic images are inherently noisy due to the ill-posed nature of the problem and non-linear reconstruction techniques that are used to generate images. It is interesting to note that the maximum CNR in the tomographic images occurs at around 2.5mm and not at the surface where the SNR is the highest. This seems counterintuitive unless we recall the sensitivity analysis results from Chapter 3 which indicate a hypersensitivity in this region. Subsequently, targets in this location will actually yield the best reconstructed image. Also worthy of mention is the increasing CNR trend that can be observed for most contrasts around target depths of 7.5 to 10 mm in the surface imaging experiment. It is extremely likely that this is not the case as it makes no physical sense...
whatsoever. Most likely, this obscurity can be attributed to the incident angle of the laser beam orientation relative to the phantom. In both cases image contrast and CNR are governed by depth of the target and the tissue/phantom optical properties due to the diffuse nature of the light propagation.

Figure 5.13 Experimental fluorescence tomography contrast (a) and CNR (b) results when the depth of an 8mm target was adjusted between contrasts of 10:1, 5:1, and 3.5:1. To compute the image contrast and CNR the area encompassing r=4 from the recovered centroid was assumed to be the ROI. The remainder of the image served as the Background in these calculations.

Figure 5.14: Experimental contrast (a) and CNR (b) analysis for the set of fluorescence broadbeam images depicted in Figure 5.11. Here, in computing the CNR (Eq. 5.1) and contrast (Eq 5.2), the ROI is considered to be the True location of the target and the remainder of the image is treated as the Background.

The ideal surgical guidance system would utilize both surface imaging and tomography, particularly in the case of resecting residuals where the ROI is not necessarily located on the
surface. When compared to surface imaging, FDOT provides the distinct advantage of providing specific information regarding the depth and position of sub-surface lesions. Moreover, an argument can be made that deep targets imaged using the surface imaging technique could possibly be interpreted as noise or simply intrinsic fluorophore heterogeneities due to the highly diffuse fluorescence intensity that can be observed in the resultant images. For these reasons, neurosurgical tumor resection under combined surface/FDOT guidance has the potential to further improve the number of full resections, as a FDOT based system is capable of localizing residual tumors undetectable or potentially misinterpreted when broadbeam imaging alone is utilized.
Chapter 6: Concluding Remarks

6.1 Discussion and Conclusion

In summary, a non-contact, fluorescence diffuse optical tomography approach capable of B-scan or epi-illumination mode absorption and fluorescence subsurface imaging was examined for its potential to provide clinically relevant information through both simulations and experiments. The particular focus was emission from the fluorophore Pp-IX, but the observations can be generalized to appreciate how the geometry affects both absorption and emission tomography. In generating tomographic images, multiple source positions are required, so two orthogonal galvanometers were used to raster scan a 635nm laser source across 16 positions over the phantom surface. For the purpose of keeping the analysis relatively simple, the data collection and reconstruction was entirely two dimensional, only using a single mirror to raster scan the laser source back and forth across a line, and the 2D plane below this line was the reconstructed area. For each source position, a CCD camera focused on the region of interest and collected the diffuse intensity data at 15 ‘virtual’ detector locations along the line of the source being scanned. The full dataset containing 240 measurements was calibrated, and subsurface planar images of absorption and fluorescence were generated via a non-linear, finite-element approach to modeling the diffusion. Image analysis was performed to quantify the accuracy of recovering parameters, including $\mu_a$ & $\eta\mu_{af}$ values, target size, and target centroid location as a function of depth for both absorption and fluorescence imaging. Simulated and experimental results are in close accordance. They reveal that the target centroid location is the only parameter that can accurately be quantified. That is, the calculated centroid location can serve as an accurate measure of depth in the range 0-10 mm below the surface. This may be adequate for applications where target detection and depth may be more important than
quantification, such as in some clinical surgical guidance applications [8, 27, 28]. Implementing larger source-detector separation distances may extend the depth resolution [44]. It is also known that if the upper surface possesses some convex curvature, then sensitivity to absorbers at greater depths is increased [1]. It is important to clarify that imaging below 1 cm is certainly feasible, but the positional accuracy degrades. Using larger source-detector separations and elimination of nearer source-detector separations may help the sensing of deeper structures in the tissue; however, improvement in the linearity of response is likely to be more challenging.

The ability to provide an image reconstruction approach which is linear with depth in B-scan mode may not be possible, without the addition of further constraints (such as object location from prior information). The response of sub-surface diffuse reconstruction algorithms are depth dependent because of the ill-posed nature of diffuse imaging and the fact that the sensitivity decreases by orders of magnitude with increasing distance from the tissue surface. Here, this shortcoming was improved via the implementation of a spatially variant regularization parameter [42] which enhanced the overall image quality and allowed the recovery of deeper lesions to be realized; yet, it was still insufficient for maintaining linear sensitivity with depth. This system should be adequate for most surgical guidance applications where the goal might be detection over quantification of the absorption and fluorescence properties of tissue. However, it would not be useful in applications which seek to quantify features of the regions within the tissue at an unknown depth. This problem could likely be overcome by multi-modality imaging where the DOT-based reconstruction algorithm would utilize the structural information obtained from another modality, such as ultrasound [5, 52], optical coherence tomography, x-ray, CT or MRI.
The results show that the location of lesions can accurately be determined for sub-surface absorption and fluorescence B-scan imaging both in simulations and experiments. In general, the ability to recover the position of a target within a turbid media in the remission mode is a function of target size and contrast. Experimental centroid errors in position were found to be 0.87 mm and 1.4 mm for fluorescent and absorber targets within the first 10 mm of tissue below the surface when contrasts above 5:1 were considered. Thus, it appears at least based on the data and techniques presently available, that diffuse tomography when used in a sub-surface imaging geometry, is limited to applications where localization is the primary goal, or that if the location can be known by other methods then linear-quantitative imaging might become possible.

6.2 Future Directions

One possible future direction might entail making the system spectrally resolved. This may prove useful in applications such as sub-surface imaging of the brain where the endogenous background fluorescence is relatively low and high contrasts are possible. While these features will improve the contrast to background performance of the system, they may not improve the linearity of the response with depth to a significant degree.

In order for the system to serve as a surgical guide it would be necessary for the data acquisition and image reconstruction to be performed in real-time. For this to become a reality, one could envision dual low-temperature stabilizing CCD cameras for data acquisition. In this setup, if the two cameras were be equipped with the proper optical filters, absorbance and fluorescence data sets could be simultaneously acquired within milliseconds. In addition, one could exploit the availability of the two cameras by using them to stereoscopically map the surface of the FOV. Thus, meshes of the region under investigation could be created in real-time. Because we now know that the ability to quantify in B-scan mode is not a reality, and
detection would be the primary objective for this type of system, it can also be hypothesized that a linear reconstruction technique would suffice for the image formation. Thus, given the proper system hardware, and enough computational power, it is completely reasonable to propose a B-scan mode system for real-time surgical guidance.
References


## Appendix A – DAQ Device Pin Outs

<table>
<thead>
<tr>
<th>Connection Description</th>
<th>68-LP Terminal #</th>
<th>Terminal Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor A (+)</td>
<td>21</td>
<td>DAC1 Out</td>
</tr>
<tr>
<td>Motor A (-)</td>
<td>54</td>
<td>AOGND</td>
</tr>
<tr>
<td>Motor B (+)</td>
<td>22</td>
<td>DAC0 Out</td>
</tr>
<tr>
<td>Motor B (-)</td>
<td>55</td>
<td>AOGND</td>
</tr>
<tr>
<td>SPST Laser Relay (+)</td>
<td>52</td>
<td>DIO0</td>
</tr>
<tr>
<td>SPST Laser Relay (-)</td>
<td>35</td>
<td>DGND</td>
</tr>
</tbody>
</table>
Appendix B – Labview VI Hierarchy Table
VI Summary

The following provides an outline of the major Labview Virtual Instrument files.

NOTE: Subsurface_FDOT_Software_v1.vi is the main program.

Calibration

- **Move_Filter_Wheel.vi** – Adjust the ND filter position to the OD appropriate for calibration (1 OD). Note in calibrating, a piece of black felt must be present on the glass plate.
- **System_Calibration.vi** – Determine the galvanometer voltages required to move each galvanometer 1 pixel.
  - **Determine_Home_Location.vi** – Find centroid pixel position of laser for galvanometer voltages (0 Volts, 0 Volts)
  - **Determine_Y_Location.vi** – Find centroid position of laser at (X Volts, 0 Volts)
  - **Determine_X_location.vi** – Find centroid position of laser at (0 Volts, Y Volts)
  - **Calibration_Change_Laser_Position.vi** – Input X and Y galvanometer voltages to adjust the position of each.
- **Determine_CALIBRATED_ROIS.vi** – Automatically finds the bounds for the camera defined ROIs (32 pixels x 32 pixels for each ROI). These ROIs are defined by the calibration region selected and thus the maximum and minimum galvanometer voltages.
- **Beep_Indicator.vi** – Alerts the user that calibration has completed.
User ROI Selection

- **Get_User_Input_Line.vi** – Allows users to select a single point in the center of the ROI they wish to image.
  - *Get_Image.vi* – Acquires an image of the entire FOV.
  - *ROI_Draw_Rect.vi* – Draws a red rectangle on the image acquired using *Get_Image.vi*. The red rectangle illustrates the ‘calibration region.’
- **ROI_Get_ROIs_Line_Scan.vi** – Locates the camera defined ROIs within the user selected ROI region. The corresponding pixel values are also determined so that the laser can be appropriately positioned during the raster scan. The ROIs to be imaged are then highlighted with a yellow mask.

Raster Scan

- **Create_Folder_Time_Stamp.vi** – Gets the current date and creates a ‘date-stamped’ folder in the default Labview data directory; data will be saved here.
- **Move_Filter_Wheel.vi** – Adjust the ND filter position to the OD appropriate for fluorescence (0 OD) or absorbance (2 OD) imaging.
- **Determine_Init_Line_Scan_Pos.vi** – Adjusts the position of the laser so that it is in the location of ‘Source 1’.
- **Determine_Inc_Voltage.vi** – Determine what incremental voltages are required to accurately reposition the laser in the location of each source.
- **Scan_Line_Basic_Mar1** – Raster scans the laser source while acquiring intensity measurements at each detector. Raw datasets with the dark signal removed are created here.
- **Beep_Indicator.vi** – Alerts the user that data acquisition has completed.