Optical Imaging of Breast Cancers

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Breast cancer is the most common cancer and the second major cause of cancer death among women. Screening tries to detect the disease in the early stage, and can save more lives. Diffuse optical tomography (DOT) in the near-infrared (NIR) spectrum is a promising noninvasive approach for functional diagnostic breast imaging. Our group has explored the use of ultrasound (US)-guided DOT to improve lesion localization and light quantification accuracy. In this dissertation, several special cases of breast cancer imaging were studied with the US guided DOT technique.

Firstly, the heterogeneous absorption distributions of advanced cancers were characterized. A series of simulations and phantom experiments were then performed to systematically evaluate the effects of target parameters, target locations, and target optical properties on imaging the periphery enhancement absorption distribution using a reflection geometry. A clinical example is given to demonstrate the complexity of tumor vasculature. Secondly, to improve the light quantification of clustered lesions, a new multi-zone reconstruction algorithm guided by co-registered US image is investigated using simulations and phantoms experiments. The performance of the algorithm is demonstrated with clinical examples. Thirdly, the DOT mapping of tumor deoxy-hemoglobin (deoxyHb) and oxy-hemoglobin (oxyHb) concentrations in blood
phantoms and in in-vivo patients is presented. Targets of different sizes located at
different depths were used to validate the accuracy of oxygen saturation estimation.
Clinical examples are given to demonstrate the mapping of heterogeneous deoxyHb and
oxyHb distributions in breast cancers. Fourthly, in vivo mouse tumor imaging using
fluorescence DOT were conducted; this demonstrated an improved imaging capability of
a new synthesized 2-nitroimidazole-indocyanine green conjugate using a piperazine
linker (piperazine-2-nitroimidazole-ICG) relative to an earlier version with an
ethanolamine linker (ethanolamine-2-nitroimidazole-ICG). All the findings have been
supported with the fluorescence images of histological sections of tumor samples and an
immunohistochemistry technique for identifying tumor hypoxia. Lastly, a two-step
imaging model was set up for the two-layer tissue structure of breast imaging. Absorption
and scattering distributions of the lesions in the layered structure could be reconstructed
simultaneously. Simulation and phantom experiments show promising accuracy and
clinical examples were applied to demonstrate the utility of this imaging method.
Optical Imaging of Breast Cancers

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1 Introduction

1.1 Breast cancer imaging

The breast consists of lobules (glands that make breast milk), duct (small tubes that carry milk from the lobules to the nipple), fatty and connective tissue, blood vessels and lymph vessels. The milk-producing ducts and glands are the two most likely areas to develop cancerous cells.\(^1\) Breast cancer is the most common cancer and the second major cause of cancer death for women.\(^2-5\) Biomedical imaging plays important roles for breast cancer on the detection, diagnosis, treatment and monitoring.\(^4\) Traditional imaging modalities such as X ray imaging\(^6, 7\), nuclear imaging\(^8-11\), ultrasound (US)\(^12-14\) and magnetic resonance imaging (MRI)\(^15,16\), are well developed and widely used in clinic. However, due to drawbacks like high cost, use of ionizing radiation, etc., new biomedical optical imaging techniques based on the rapid development of laser, is currently being researched and applied to clinical practice. One of them is optical coherence tomography (OCT), a high resolution technique for imaging thin layers of tissues in a turbid medium at micrometer levels.\(^17\) Another one is photoacoustic imaging (PAI), and provides optical contrast at ultrasonic resolution.\(^18,19\) Diffuse optical tomography (DOT) in near infrared (NIR) spectrum has shown clinical potential of probing the tumor vasculature up to few centimeters in biological tissues.\(^20-24\) Figure 1-1 shows the spectrum of typical biomedical imaging modalities. In this dissertation, several special cases of breast cancer imaging using US guided DOT technique were systematically studied.
1.2 Diffuse optical tomography (DOT)

The NIR spectrum from 680 nm to 900 nm shown in Figure 1-2 shows is called the optical window due to the high absorption of oxygenated and deoxygenated hemoglobin (Hb). Meanwhile, the absorption of water is relatively low in this window, making light penetrate deep into biological tissue. Additionally, the oxygenated and deoxygenated Hb absorption curve has an intersection point; this means that if multiple wavelengths were detected, the concentration of oxygenated and deoxygenated Hb could be calculated. From that, the total hemoglobin (tHb) can be determined. Since NIR light has been identified as a potential diagnostic radiation, and Hb contrast has been confirmed an indicator between breast cancerous and normal tissues, DOT in NIR spectrum as a non-invasive imaging technique, has demonstrated the clinical potential of probing tumor angiogenesis which can be quantitatively assessed by the tHb concentration. However, the primary limitation of DOT is related to the intense light scattering in tissue which dominates NIR light propagation. As a result, the localization of lesions is poor and the accurate quantification of lesion optical properties is difficult. To overcome these drawbacks, a priori information provided by a coregistered modality, such as MRI, X-ray
and US, has been investigated and proved to be a practical method for the clinical translation. 22, 32-38

![Absorption spectrum of water, oxygenated Hb, and deoxygenated Hb in NIR range.](image)

Figure 1-2 Absorption spectrum of water, oxygenated Hb, and deoxygenated Hb in NIR range.

In our group, we adopt the US to guide the lesion localization and use DOT to map the lesion vasculature. This approach has successfully overcome the location uncertainty of pure DOT approach. 39-41 The current system we are using for the clinical experiments is shown in Figure 1-3. In the middle, there is a bed for patients; the commercial US machine is on the left side, and the DOT imaging system is on the right side. The US transducer, source fibers, and detection fibers are combined to make a hand-held probe and lies on the bed as shown in the figure. The center slot of the probe is used to fit the US transducer and the sources and detectors are distributed on both sides. The DOT system is a frequency domain system with a modulation frequency of 140 MHz and
consists of 14 parallel detectors and 4 laser diodes of wavelength 740, 780, 808 and 830 nm, respectively. Each laser diode is sequentially switched to 9 positions on the probe.

Figure 1-3 The DOT imaging system in the operating room.

1.3 Fluorescence DOT

Fluorescence is the phenomenon whereby a molecule in the ground electronic state is excited and jumps to a higher energy state of the excited electronic state by absorbing a photon. Then the molecule drops down to the lowest vibrational state of the excited electronic state. Following this, the molecule drops again to one of the vibrational levels of the ground electronic state and emits a photon in the process. Typically, in an experiment, the absorber is excited with light at one wavelength and the emission light goes through a bandpass or longpass filter to be detected as the emission spectrum.

The frequency domain fluorescence imaging system we use for the in vivo experiments consists of 14 parallel detectors and 4 laser diodes of 690, 780, 808 830 nm. Each laser diode is sequentially switched to nine positions on the imaging probe. The
14-channel parallel detection system has two modes: fluorescence mode and absorption mode. The two modes can be easily switched by moving a mechanical handle. In order to remove the excitation and stray light, a bandpass filter is placed in the light path in the fluorescence mode and in the absorption mode, it is moved out of the light path. A sketch of the detection optics is shown in Figure 1-4.

Figure 1-4 The sketch of the fluorescence detection optics

1.4 Dissertation organization

The rest of the dissertation organized as follows: Chapter 1 gives a brief introduction of the history, development and the principles of the imaging modalities used in this dissertation for breast cancer. In chapter 2, a series simulation and phantom experiments were performed to systemically evaluate the effects of target parameters, target locations, and target optical properties on imaging periphery enhancement absorption distribution using reflection geometry. Large tumors modeled as concentric semi-ellipsoidal targets of different outer shell and inner core optical properties were analyzed as well. In chapter 3, clustered breast lesions imaging will be introduced. Clustered small breast lesions may be present in the neighboring areas which are difficult to resolve and quantify accurately in diffuse optical tomography. In addition, advanced breast cancers are often accompanied by clustered satellite lesions in the neighboring
areas, which are also difficult to resolve and quantify. To improve the light quantification of clustered lesions, a multi-zone reconstruction algorithm guided by co-registered ultrasound image was investigated using simulations and phantoms. The performance of the algorithm is demonstrated using clinical examples. In Chapter 4, an US-guided DOT for mapping tumor deoxy-hemoglobin (deoxyHb) and oxy-hemoglobin (oxyHb) concentrations in blood phantoms and in in-vivo patients will be presented. Targets of different sizes and located at different depths were used to validate the accuracy of oxygen saturation estimation. An inhomogeneous concentric blood phantom of deoxygenated center core and oxygenated outer shell was imaged and deoxyHb and oxyHb maps revealed corresponding distributions which correlated well with inhomogeneous deoxy- and oxy- distributions frequently seen in breast cancers. Clinical examples are given to demonstrate the utility of US-guided optical tomography in mapping heterogeneous deoxyHb and oxyHb distributions in breast cancers. In Chapter 5, the synthesis of a 2-nitroimidazole-indocyanine green conjugate using a piperazine linker (piperazine-2-nitroimidazole-ICG) capable of robust fluorescent imaging of tumor hypoxia will be described. In vivo mouse tumor imaging studies were completed and demonstrated an improved imaging capability of the new dye relative to an earlier version of the dye that was synthesized with an ethanolamine linker (ethanolamine-2-nitroimidazole-ICG). All the findings have been supported with fluorescence images of histological sections of tumor samples and an immunohistochemistry technique for identifying tumor hypoxia. In Chapter 6, a two-step imaging model is set up for the two-layer tissue structure of breast. First, a small probe with short source-detector pairs is used to collect the diffused light only from the first
layer tissue of the normal breast; Then, a clinical imaging probe is used to collect the perturbation data from the breast with the lesion, and the reference from the contralateral breast. With the relatively accurate information of the first layer, the optical properties of the second-layer are fitted. After that, a two-step imaging reconstruction using genetic algorithm is performed for the two-layer model with the fitted bulk tissue properties. Absorption and scattering distributions of the lesions in the layered structure can be reconstructed simultaneously. Simulation and phantom experiments showed promising results. Clinical examples have been also applied to demonstrate the utility of this imaging method. Finally in Chapter 7, the dissertation is briefly summarized.

References


2 Imaging Heterogeneous Absorption Distribution of Advanced Breast Cancer

2.1 Introduction

Optical tomography has tremendous potential to provide clinically useful functional information about tumor angiogenesis and tumor hypoxia.\textsuperscript{1-22} However, the process of tumor angiogenesis is complex, resulting in a highly distorted and heterogeneous distribution of blood vessels in advanced cancers.\textsuperscript{23} This distorted distribution is dependent on angiogenic factors and related to the incorporation of existing host blood vessels into tumor and the creation of new tumor microvessels.\textsuperscript{24, 25} The distribution of these tumor vessels is highly heterogeneous. Some areas may have high microvessel density especially at the periphery of the tumor; other areas may develop necrosis especially in the central region of the tumor.\textsuperscript{26, 27} In our pilot studies of imaging tumor vasculature using ultrasound-guided optical tomography, we have observed heterogeneous absorption distribution in advanced cancers.\textsuperscript{19, 21, 28} In this dissertation, we have systematically investigated the capability of optical tomography to accurately image heterogeneous absorption distributions using Monte Carlo simulations and phantom experiments. A clinical example is given to show the complex vasculature distributions seen from an advanced cancer. To the best of our knowledge, this study is the first one to characterize and quantify the effects of target parameters, target locations and target optical properties on imaging heterogeneous absorption distributions of large targets in reflection geometry. Understanding and characterizing the features of advanced breast cancers imaged by optical tomography is a critical step toward translating this promising technique into clinical practice. It is also important to understand the initial angiogenesis
distributions and potential angiogenesis changes of advanced cancers when patients are undergone neoadjuvant chemotherapy.

2.2 Methods

2.2.1 Monte Carlo simulation

The Time-Domain Monte Carlo (MC) method was used to generate the forward data with a target inside the turbid medium located at different depths. The details of the time-domain MC simulation developed by our group can be found in our previous works.\textsuperscript{29, 30} Briefly, the reflection geometry with multiple sources and detectors distributed on a probe of 10 cm diameter was used in the MC simulation (see Figure 2-1). At each source location, a delta pulse consisting of 30 millions of photons was launched into the medium. Initially, each photon was assigned a unity weight $W$, which is analogous to light intensity. Each photon went through many steps of absorption and scattering processes. After each step, a part of the weight $\Delta W$ was absorbed by the medium and the weight of the photon was decreased. The photon was scattered following the Henyey-Greenstein function. The Roulette technique was used to terminate the photon when the residual weight was less than a threshold value. For each photon, it was either absorbed in the medium, detected at the reflecting surface, or left from the transmitting surface. After the migration of one particular photon halted, a new photon was launched into the medium at the source location. In this simulation, the absorption boundary was used between the scattering medium and the outer surface. Each photon's energy and arrival time were recorded when the photon reached the outer surface (boundary). The distribution or time profile of the recorded photons at each detector position for each delta pulse at a source position was stored and the resulting temporal
data were Fourier transformed to provide frequency domain amplitude and phase shift at 140 MHz which was used in simulations.

Figure 2-1 Probe geometry used for simulation and phantom experiments (a) without closer-to-center center sources and (b) with two closer-to-center sources.

The MC program has been extended to include a larger semi-ellipsoidal inhomogeneous target embedded inside a turbid medium, which closely models large breast lesions when patients are imaged in a supine position using the conventional pulse-echo ultrasound and our hand-held combined probe. In addition, most of the large lesions are squashed into a semi-ellipsoidal shape against the chest-wall under the slight probe compression. Therefore, a semi-ellipsoidal target is a reasonable model for these lesions. As shown in Figure 2-2 (a), the inhomogeneous target has two concentric semi-ellipsoids of different outer shell and inner core optical properties. The boundary conditions between target layers and medium could be easily controlled by mapping corresponding refractive indexes in an input file.
Figure 2-2 (a) Geometry of the photon propagation to the boundary of a concentric semi-ellipsoidal target. (b) $\Delta W$ inside the 3-D volume was summed in x direction (left), y direction (middle) and z direction (right), respectively and projected into the y-z plane, x-z plane and x-y plane, respectively.

When a photon propagated to one of the boundaries between the target outer layer and the medium, the target outer layer and the core or the target core and the medium, the intersection point (point $P$ in Figure 2-2 (a)) was computed and the shortened step size ($s_1$) of this photon from $B$ to $P$ was calculated. At the incident angle ($\angle NPB$), Snell’s law was applied to calculate the reflection angle ($\angle NPC$) and the refraction angle ($\angle MPD$). After this the photon travels a distance $s_1$, with the traveling direction being changed depending on different boundary conditions. If the photon experienced total internal reflection, the photon was propagated in the same medium in the reflection direction. If
the photon experienced partial internal reflection, one simplification was made by assuming that the photon would be either all reflected or all refracted. By comparing the reflection coefficient from Fresnel’s equations and a random number generated from a uniform distribution, the photon would be refracted only if the random number is larger than the reflection coefficient; otherwise, it would be reflected. Thus, the remaining step size ($s_2$) of the photon was propagated in the corresponding medium determined above. As the photon propagated in the medium, part of the weight $\Delta W$ was absorbed after each step. The absorbed $\Delta W$ inside the 3-D volume was summed in x direction, y direction, and z direction, respectively, and projected into the y-z plane, x-z plane, and x-y plane, respectively, as shown in Figure 2-2 (b).

2.2.2 Imaging Reconstruction

Born method was used to approximate the received photon density wave as a linear superposition of homogeneous incident and scattered fields originated from a source located at $r_s$ and evaluated inside the medium at $r$.

$$U(r_s, r) = U_0(r_s, r) + U_{sc}(r_s, r)$$  \hspace{1cm} (1)

Under the approximation that $U_0(r_s, r) >> U_{sc}(r_s, r)$, $U_{sc}$ could be derived as

$$U_{sc}(r_s, r_d) = -1/D_0 \int G(r-r_d)\Delta\mu_a(r)U_0(r_s, r)d^3r$$  \hspace{1cm} (2)

Where $G(r-r_d)$ is the Green’s function, which relates the scattered field measured at the detector $r_d$ to the field point $r$. $\Delta\mu_a(r)$ represents the absorption variation at the voxel $r$; and $D_0$ is the diffusion coefficient of the homogenous medium.
The dual-zone mesh scheme introduced by us earlier was used for inversion.\textsuperscript{31} Briefly, the imaging volume was segmented into two regions consisting of the lesion (ROI) as identified by the co-registered ultrasound and the background region. We used a smaller fine mesh size for the lesion region and a larger coarse mesh size for the background region, so that the total voxels with unknown optical properties was significantly reduced and the inversion converged quickly in three to four iterations. The conjugate gradient method was used for the iterative optimization. Typically, in simulations and phantom experiments, the fine mesh region was chosen about 3-4 times larger than the true target area, and in clinical data, the fine mesh region was about 4.5 times larger than the lesion area estimated by ultrasound. Using this dual-zone mesh method, the scattered field can be related to the total absorption distribution as:

\[ [U_{SC}]_{M\times1} = [W_L, W_B]_{M\times N} [M_L, M_B]^T \]  

where \( W_L \) and \( W_B \) are the weight matrices for lesion region and background region, respectively; \( M_L = \left[ \int_{N_L} \Delta \mu_a (r') d^3r', \cdots \int_{N_L} \Delta \mu_a (r') d^3r' \right] \) and \( M_B = \left[ \int_{N_B} \Delta \mu_a (r') d^3r', \cdots \int_{N_B} \Delta \mu_a (r') d^3r' \right] \) are the total absorption distribution of the lesion and the background regions, respectively. Here, the total absorption distribution was reconstructed rather than \( \Delta \mu_a (r') \). At the end of the iterative optimization, the total absorption distribution is divided by the different voxel sizes of the lesion and background to obtain \( \Delta \mu_a (r') \). This method significantly reduced the background artifacts because the voxel size in the background area was much larger than that in the lesion area.
For inhomogeneous concentric target, the inner core size of the reconstructed image was measured as 2 times of the mean value of $r_x$ and $r_y$ (as shown in Figure 3), where $r_x$ was measured between the $(\text{maximum} + \text{minimum})/2$ and minimum of the absorption curve along the x axis and $r_y$ was measured similarly along the y axis.

Figure 2-3 Illustration of measuring reconstructed inner radius.

2.2.3 Experiment

Phantoms of different size with different optical properties were made using polyvinyl chloride-plastisol (PVCP) solution, which was a white opaque solution and became translucent when it was heated to a high temperature. When the solution was gradually heated, the Indian ink and titanium dioxide (TiO2) powder were added to control the optical absorption and scattering coefficients of the phantom. The heated solution was poured into molds of a semi-ellipsoidal shape and solidified after cooling for several hours. For the special case of a larger target with different inner core optical properties, we made smaller targets first, and then proceeded the second time to make the larger target by
embedding the smaller ones inside. Thus the target outer shell and inner core had different optical properties. An example of a concentric semi-ellipsoidal target phantom is given in Figure 2-4. The refractive index of the PVCP was reported in the literature\textsuperscript{33,34} and the range at closer to room temperatures was from 1.44 to 1.54. MC simulations were performed to evaluate the effect of refractive index difference between the PVCP target and Intralipid solution on reconstructed target absorption. The maximum difference in reconstructed absorption was about 0.02 cm\textsuperscript{−1} when the solid phantom of $\mu_a=0.25$ cm\textsuperscript{−1} and liquid background and inner core of $\mu_a=0.03$ cm\textsuperscript{−1} were used. No change in target absorption distribution was observed.

![Figure 2-4 Example of a concentric semi-ellipsoidal target phantom. (a) The front view of the phantom. (b) The bottom view of the phantom.](image)

Our frequency domain system consisted of 14 parallel detectors and 4 laser diodes of 740nm, 780nm, 808nm and 830nm. Each laser diode was sequentially switched to 9 positions on the probe (see Figure 2-1 (a)). The center slot on the probe was used for ultrasound transducer and the sources and detectors were distributed on both sides.
Intralipid solution was used to emulate the background tissue. Measurements were made with the target inside the intralipid (target data) and intralipid alone as a reference. The target was supported by a 6 to 7 cm long optical fiber mounted on a piece of white clay located at the bottom of a 2 liter capacity tank filled with intralipid. The perturbation between the target data and the reference was used for imaging reconstruction. To compare reconstruction results with the closer to center source illumination when imaging a large semi-ellipsoidal inhomogeneous target, we also imaged the same target using the probe geometry shown in Figure 2-1 (b). In this geometry, two sources from the left side of the probe were moved to the top and bottom sides of the ultrasound transducer location. The objective of the second set measurements was to estimate the effect of sources near the center on imaging reconstruction.

Clinical experiments were performed with the system of the same design as that used for phantom experiments. The study protocol was approved by the local Institution Review Board (IRB) committee. All patients who participated in our study signed the informed consent. The patients’ data were taken at the lesion area and the contralateral breast of the same quadrant as the lesion. Contralateral data set was used to estimate background optical properties for weight matrix computation. The perturbation was computed between lesion data and contralateral data and used for imaging reconstruction.

2.3 Results

In this section, five sets of simulation and experiments have been reported in sections 2.3.1-2.3.5 and the clinical example has been given in section 2.3.6. The first set simulation and experiments was performed using a homogeneous target. The result can serve as a baseline to compare with that obtained from an inhomogeneous target of a
semi-ellipsoidal shape of different outer shell and inner core optical properties. The second set of simulation and experiments was performed to investigate two important target parameters that affect the resolving capability of the optical tomography. The third set of simulation and experiments was designed to emulate the condition that a tumor with fixed outer-shell to inner-core contrast was located at different depths. The range of the target depths investigated was from 1.0 to 3.0 cm which was often encountered in the clinical studies when patients were scanned in a supine position. The fourth set of simulations and experiments was targeted to evaluate how the absorption of the inner core could affect the reconstructed contrast ratio of outer-shell to inner-core as well as the measured inner core size. In clinical studies, the center tumor core could have different absorption lower than the periphery due to rapid growth of the malignant cancer. The fifth set of the simulation and experiment was designed to investigate the effect of inner core diameter change on reconstructed contrast ratio of outer-shell to inner-core as well as measured inner core size. In clinical studies, we often encounter the necrotic core of dead tumor tissue of different size while the malignant tumor cells grow outward. At the end, we show a clinical example of a larger cancer imaged by our system.

2.3.1 Homogeneous targets

In this section, we show MC simulation and phantom results of a homogeneous target which can be used as a baseline to compare with that of inhomogeneous targets.

In the MC simulation, a 5-cm-diameter of 2-cm-height semi-ellipsoidal target had optical properties of absorption coefficient $\mu_a = 0.25 \text{ cm}^{-1}$ and reduced scattering coefficient $\mu_s' = 6.0 \text{ cm}^{-1}$. The background properties were set to $\mu_a = 0.03 \text{ cm}^{-1}$ and
\( \mu_s' = 6.0 \text{ cm}^{-1} \). The target bottom was located at 2.5 cm depth from the surface. Figure 2-5 (a) shows the reconstructed target absorption map. The probe shown in Figure 2-1 (b) was used for image reconstruction. Tomography images are shown in seven slices at different depths from 0.5 cm to 3.5 cm with 0.5 cm increment in depth. The ROI is shown from the second slice and distributed in three layers in depth of 1.0, 1.5 and 2.0 cm. The distribution at each layer is quite uniform. The reconstructed maximum \( \mu_a \) was 0.13 cm\(^{-1} \). A similar result was obtained from a phantom experiment as shown in Figure 2-5 (b). The target had the same size and same optical properties as the simulation and reconstructed maximum absorption coefficient is 0.14 cm\(^{-1} \). The absorption map at each layer is quite uniform.

![Reconstruction results of a concentric semi-ellipsoidal target with diameter of 5 cm and height of 2 cm. (a) simulation and (b) phantom experiment.](image)

**2.3.2 Effects of target parameters on imaging concentric inhomogeneous targets**

In this section, we evaluate two target parameters which are critical for imaging periphery enhancement absorption distribution. The thickness between the outer shell and the inner core shown in Figure 2-2 (a) is an important parameter for resolving the target
inhomogeneity. In this set of simulation, a semi-ellipsoidal target was presented with outer diameter of 5.0 cm, inner core diameter of 2.5 cm, h of 2.0 cm and t of 0.5, 0.8 and 1.0 cm, respectively. The target outer shell and inner core had same reduced scattering coefficient of $\mu_s' = 6.0 \text{ cm}^{-1}$ and different absorption coefficients of $\mu_a = 0.25 \text{ cm}^{-1}$ and $\mu_a = 0.03 \text{ cm}^{-1}$. The target bottom was located at 2.5 cm depth from the surface. The background optical properties were $\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$. Error!

Reference source not found. shows the reconstructed target absorption maps of different layer thickness of 0.5, 0.8 and 1.0 cm, respectively. The probe shown in Figure 2-1 (b) was used for image reconstruction. The reconstructed maximum $\mu_a$ s were 0.13, 0.14 and 0.13 cm$^{-1}$, respectively. Using the method introduced in section 2.2, the measured inner diameter of the target region was 1.9, 1.4 and 0.3 cm.
The corresponding phantom experiments were performed for the same size targets with layer thickness of 0.5 cm and 1.0 cm, respectively. Figure 2-7 shows the reconstruction results which had the maximum $\mu_a = 0.15 \text{ cm}^{-1}$ and $\mu_s = 0.13 \text{ cm}^{-1}$, respectively. When the thickness $t$ is less than 1.0 cm, the inner core is visible. However, when $t$ is larger than 1 cm, the inner core cannot be resolved, even the core diameter is relatively large. This is caused by the increased scattering events when photons pass the outer shell of more than 1.0 cm thick and the information loss between the two groups of photons passing outer shell only and passing the outer shell and the inner core.
Figure 2-7 Phantom experimental results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, and height 2 cm of different layer thickness (a)-(b) 0.5 cm and (c)-(d) 1.0 cm. (a) and (c) are ultrasound images and (b) and (d) are corresponding absorption maps.
Another important parameter is the target size. A series of MC simulations was performed by fixing $t = 0.5$ cm and varying the target size from 2.5 cm to 5.0 cm. The reconstruction results are shown in Figure 2-8. Figure 2-8 (a)-(d) are the absorption maps of the 2.5, 3.0, 3.5 and 5.0 cm diameter target with 1.5, 2.0, 2.5 and 4.0 cm inner core size, respectively. The probe shown in Figure 2-1 (b) was used for the imaging reconstruction. The target bottom was located at 2.5 cm. The maximum absorption coefficients were 0.14, 0.14, 0.15 and 0.14 cm$^{-1}$, respectively. The reconstructed inner diameters were 0.0, 1.2, 1.8 and 2.6 cm, respectively. As one can see, the target size has
to be larger than 3.0 cm for resolving target outer shell and inner core. The simulation results were validated by experiments. An example of 2.5 cm diameter target of 1.5 cm inner core size is shown in Figure 2-9. In this example, the target inner core cannot be resolved.

Based on the simulations and experiments, we used \( t = 0.5 \) cm layer thickness and 5.0 cm target diameter for all simulations and experiments reported in the following sections to investigate the effect of other parameters on imaging target inhomogeneity.

![Figure 2-9 Phantom result of a small concentric semi-ellipsoidal target with outer shell diameter of 2.5 cm, inner core diameter of 1.5 cm, height of 1.5 cm and layer thickness of 0.5 cm. (a) US image, (b) reconstructed absorption map](image)

2.3.3 An inhomogeneous target located in different depths

This set of experiments was designed to investigate the effect of target depth on the reconstructed contrast of a target having a fixed outer shell to inner core absorption ratio, and the measured inner core diameter. The target depths evaluated were in the range of 1.0 to 3.0 cm and correspond to those often encountered in the clinical studies.
A semi-ellipsoidal target presented in this set of simulation had outer diameter of 5.0 cm, inner core diameter of 2.5 cm, h of 2.0 cm and t of 0.5 cm. The target outer shell and inner core had same reduced scattering coefficient of $\mu'_s = 6.0 \text{ cm}^{-1}$ and different absorption coefficients of $\mu_a = 0.25 \text{ cm}^{-1}$ and $\mu_a = 0.06 \text{ cm}^{-1}$, respectively. The background optical properties were set to $\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu'_s = 6.0 \text{ cm}^{-1}$. Figure 2-10 shows the reconstructed absorption maps of the target with the bottom of the target located at 2.3, 2.5, 2.8 and 3.0 cm from the probe surface, respectively. The probe with closer to center sources shown in Figure 2-1 (b) was used to obtain the measurements. The reconstructed maximum $\mu_a$ s were 0.12, 0.13, 0.12 and 0.11 cm$^{-1}$, respectively, which were quite close at all depths studied. The measured inner diameters were 1.9, 1.9, 1.5 and 0.0 cm, respectively. The periphery of the target showed higher absorption than the inner core for the first three sets of experiments and the periphery enhancement was disappeared when the target was located deeper (Figure 2-10 (d)). The ring shape was ideally seen in the reconstructed absorption map at different target layers in Figure 2-10 (a)-(c). In our clinical studies, the center slot dimension was adapted to the commercial ultrasound transducer used and the space for center sources was limited. To evaluate how much closer-to-center sources can affect the reconstructed target inhomogeneity, we used the probe without center sources to perform the simulation again. Figure 2-11 shows the reconstruction results of the same target located at the corresponding depths as that of Figure 2-10. The reconstructed maximum $\mu_a$ s were $\mu_a = 0.14, 0.14, 0.14$ and $0.11 \text{ cm}^{-1}$, respectively. We can clearly see that the absorption at the first target layer was higher at the two sides where the sources were distributed (Figure 2-11 (a)-(c)). However, the higher absorption at both sides of the target was clearly visible. We can see that the
maximum reconstructed values were quite close at the depths studied, however, the measured inner diameter was getting smaller and smaller when the target was deeper. When the target bottom reached 3.0 cm, the outer and inner layers merged together.

![Simulation results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, height 2 cm and layer thickness 0.5 cm located at different depths. Distance between the bottom of the target and the probe surface was (a) 2.3 cm (b) 2.5 cm (c) 2.8 cm, and (d) 3.0 cm. Probe of Figure 2-1 (b) was used for image reconstruction.](image)

Figure 2-10 Simulation results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, height 2 cm and layer thickness 0.5 cm located at different depths. Distance between the bottom of the target and the probe surface was (a) 2.3 cm (b) 2.5 cm (c) 2.8 cm, and (d) 3.0 cm. Probe of Figure 2-1 (b) was used for image reconstruction.

A phantom experiment was performed under a similar condition as the simulation. Intralipid solution of calibrated $\mu_a = 0.03$ cm$^{-1}$ and $\mu_s' = 7.2$ cm$^{-1}$ at 780 nm wavelength was used as the background. The target shown in Figure 4 had outer shell diameter of 5.0 cm, inner core diameter of 2.5 cm, height $h$ of 2.0 cm and $t$ of 0.5 cm. Shell had the $\mu_a = 0.25$ cm$^{-1}$ and $\mu_s' = 7.1$ cm$^{-1}$ and the core had $\mu_a = 0.06$ cm$^{-1}$ and $\mu_s' = 5.2$ cm$^{-1}$. Figure

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2-12 shows the reconstruction results of the target located at different depths using the probe shown in Figure 2-1 (b). Figure 2-12 (a)-(d) show the reconstructed absorption maps when the target was located at the corresponding depths as Figure 2-10. The reconstructed maximum $\mu_a$ s were 0.15, 0.14, 0.13 and 0.15 cm$^{-1}$, respectively. The reconstructed inner core diameters were 2.0, 2.0, 1.4 and 0.0 cm, respectively. When the target bottom was located at 3.0 cm, the outer and inner regions were merged together and target appeared as a homogeneous one.

Figure 2-11 Simulation results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, height 2 cm and layer thickness 0.5 cm located at different depths. Distance between the bottom of the target and the probe surface was (a) 2.3 cm (b) 2.5 cm (c) 2.8 cm, and (d) 3.0 cm. Probe of Figure 2-1 (a) was used for image reconstruction.
Figure 2-12 Phantom results of a concentric semi-ellipsoidal target with outer shell diameter of 5 cm, inner core diameter of 2.5 cm, height of 2 cm and layer thickness of 0.5 cm located at different depths. Distance between the bottom of the target and the probe surface was (a) 2.3 cm (b) 2.5 cm (c) 2.8 cm, and (d) 3.0 cm. Probe of Figure 2-1 (b) was used for image reconstruction.

To verify the simulation results without center sources, we used the probe shown in Figure 2-1 (a) to obtain data of the same target at the same depth as used for Figure 2-12. The result is shown in Figure 2-13. The reconstructed maximum $\mu_s$ were 0.14, 0.14, 0.13 and 0.14 cm$^{-1}$, respectively, which were very close to that obtained from Figure 2-12. The ring pattern is clearly visible, however, the shape is not as good as the one obtained with the center sources shown in Figure 2-12.
Figure 2-13 Phantom results of a semi-ellipsoidal target with outer shell diameter of 5 cm, inner diameter of 2.5 cm, height of 2 cm and layer thickness of 0.5 cm located at different depths. Distance between the bottom of the target and the probe surface was (a) 2.3 cm, (b) 2.5 cm, (c) 2.8 cm, and (d) 3.0 cm. Probe of Figure 2-1 (a) was used for image reconstruction.

Figure 2-14 provides more quantitative comparison of both simulation and phantom experiments. As given before the true $\mu_a$'s of the outer shell and inner core for both simulation and experiment were $\mu_a=0.25$ cm$^{-1}$ and $\mu_a=0.06$ cm$^{-1}$, so the true ratio was 4.17. The reconstructed ratio was defined as the maximum value over the minimum value inside the target region. The reconstructed ratios for simulation shown in Figure 2-10 (a)-(d) were 2.12, 2.08, 1.66 and 1, respectively. The corresponding ratios for experiment shown in Figure 2-12 were 1.83, 1.53, 1.32 and 1.0, respectively. The true inner diameters for both simulation and phantom experiments were 2.5 cm. The measured inner diameters were 1.9, 1.9 and 1.5 cm, respectively, in Figure 2-10 (a)-(c); and the
corresponding measured values were 2.0, 2.0 and 1.4 cm, respectively, in Figure 2-12 (a)-(c). As expected, when the target depth increased, the reconstructed ratio as well as the measured inner diameter reduced because the received photons that passed through the outer shell only and the outer shell and inner core went through more scattering events. As a result, the location information that these two groups of photons carried was lost in the scattering process.

Figure 2-14 Plot of contrast ratio (left y axis) and reconstructed inner diameter (right y axis) vs. target depths.

2.3.4 An inhomogeneous target with different inner core absorption

This set of simulation and phantom experiment demonstrates how the absorption of the inner core affects the reconstructed contrast ratio of outer shell to inner core as well as
the measured inner core size. Both MC simulation and phantom experiments were performed under the similar conditions. In clinical studies, the center tumor core could have different absorption lower than the periphery due to rapid growth of the malignant cancer.

In this set of simulation, the semi-ellipsoidal target had the outer diameter of 5 cm, the inner core diameter of 2.5 cm, h of 2 cm and t of 0.5 cm. The absorption coefficient of the outer layer was fixed to $\mu_a = 0.25 \text{ cm}^{-1}$ and the inner core $\mu_a$ was changed to 0.03, 0.06, 0.08 and 0.15 cm$^{-1}$, respectively. The reduced scattering coefficient of both shell and core is the same as $\mu_s' = 6.0 \text{ cm}^{-1}$. The target bottom was located at 2.5 cm from the probe surface. The background properties were $\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$. Figure 2-15 shows the reconstruction results of the target with fixed outer layer absorption and different inner core absorption using the probe shown in Figure 2-1 (b). As shown in Figure 2-15, the reconstructed maximum $\mu_a$'s were 0.14, 0.13, 0.13 and 0.13 cm$^{-1}$, respectively. The reconstructed inner diameter of the center region was 2.0, 1.9, 1.5 and 1.5 cm as shown in Figure 2-15 (a)-(d). As expected, the inner core absorption does not affect the reconstructed maximum value because the outer shell absorption was fixed. However, as the inner core absorption increases, the contrast ratio of the shell and the core decreases and the measured inner diameter reduces. If we use the probe without center sources as shown in Figure 2-1 (a), the maximum $\mu_a$'s were 0.14, 0.14, 0.13 and 0.13 cm$^{-1}$, respectively. Similar as the results shown in section 2.3.3, the probe shown in Figure 2-1 (b) with the center sources reconstructs the target shape better than that of probe shown in Figure 2-1 (a); while the reconstructed maximum $\mu_a$'s were very similar by using both probes.
Figure 2-15 Simulation results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, height 2 cm and layer thickness 0.5 cm of different inner core absorption coefficients. Probe shown in Figure 2-1 (b) was used for image reconstruction. Inner core absorption coefficient was (a) 0.03 cm\(^{-1}\), (b) 0.06 cm\(^{-1}\), (c) 0.08 cm\(^{-1}\), and (d) 0.15 cm\(^{-1}\), respectively.

The phantom target was made of a fixed outer shell absorption \( \mu_a = 0.25 \) cm\(^{-1}\) and reduced scattering coefficient \( \mu_s' = 7.1 \) cm\(^{-1}\), but of different inner core \( \mu_a = 0.03, 0.06, 0.08 \) and 0.15 cm\(^{-1}\), respectively, and the reduced scattering coefficients of \( \mu_s' = 7.2, 5.2, 7.9, 9.0 \) cm\(^{-1}\), respectively. Similar to the simulation, the outer target diameter was 5 cm, the inner core 2.5 cm, \( h = 2 \) cm and \( t = 0.5 \) cm. Figure 2-16 shows the reconstruction results of the target of different inner core absorption coefficients. The intralipid solution used as the background had \( \mu_a = 0.03 \) cm\(^{-1}\) and \( \mu_s' = 7.2 \) cm\(^{-1}\) at 780 nm wavelength. The probe shown in Figure 2-1 (b) with center sources was used. In Figure 2-16 (a)-(d),
the reconstructed maximum $\mu_a$ s were 0.16, 0.17, 0.17 and 0.17 cm$^{-1}$, respectively. Similar to the simulation, the maximum values do not change much with the different inner core absorption coefficients. However, the measured inner core diameters were 2.2, 1.9, 1.6 and 1.3 cm accordingly. The measured inner core diameter reduces as the target contrast of outer shell and inner core decreases. The reconstructed absorption map using probe given in Figure 2-16 (a) has maximum $\mu_a$ s 0.15, 0.16, 0.16, and 0.17 cm$^{-1}$, respectively, which were very close to the corresponding values given in Figure 2-16. However, the ring-shape of the target was not as good as shown in Figure 2-16.

Figure 2-16 Phantom results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner diameter 2.5 cm, height 2cm and layer thickness 0.5 cm of different inner core absorption imaged. Probe shown in Figure 2-1 (b) was used for image reconstruction. Inner core absorption coefficient was (a) 0.03 cm$^{-1}$, (b) 0.06 cm$^{-1}$, (c) 0.08 cm$^{-1}$, and (d) 0.15 cm$^{-1}$, respectively.
Figure 2-17 compares the simulation and phantom experiments for targets with different outer shell and inner core contrast. The dash lines represent simulation results and the solid lines are that of phantom experiments. Three groups of data are: true contrast ratio, reconstructed ratio of outer shell over inner core absorption coefficients, and measured inner core diameters. As one can see, the experimental results agree well with simulation data.

![Figure 2-17](image)

**Figure 2-17** Plot of contrast ratio (left y axis) and reconstructed inner diameter (right y axis) vs. different inner core absorption.

For both simulation and phantom experiments, the true ratios of outer shell to inner core absorption were 8.33, 4.17, 3.13 and 1.67, respectively. The reconstructed ratio was the measured maximum absorption over the minimum inside the target region. The reconstructed ratios of simulation were 2.25, 2.08, 1.75 and 1.59 for inner core $\mu_a=0.03$, \ldots
0.06, 0.08 and 0.15 cm\(^{-1}\), respectively. These ratios were 2.46, 2.10, 1.78 and 1.77, respectively, as measured in experiments. The measured inner diameters were 2.0, 1.9, 1.5 and 1.5 cm in simulation, respectively, while the measured diameters were 2.2, 1.9, 1.6 and 1.3 cm, respectively in experiments. It is interesting to note that the true ratio of outer shell to inner core absorption reduces rapidly with the increase of inner core absorption; however, the reconstructed ratio in both simulation and experiments reduces gradually. Because of the intense scattering of photons in the turbid medium, the reconstructed ratios can only reach about a factor of two. The measured inner diameter also reduces with the increase of inner core absorption.

2.3.5 An inhomogeneous target with different inner diameter

This set of experiments was designed to investigate the effect of inner core diameter change on reconstructed contrast ratio of outer shell and inner core optical absorptions as well as measured inner core size.

The target had fixed outer shell and inner core optical properties of \( \mu_a = 0.25 \) cm\(^{-1}\), \( \mu_s' = 6.0 \) cm\(^{-1}\) and \( \mu_a = 0.03 \) cm\(^{-1}\), \( \mu_s' = 6.0 \) cm\(^{-1}\), respectively. The outer diameter and layer thickness \( t \) were fixed to 5 cm and 0.5 cm, respectively and the core diameters were changed from 1.0 to 1.5, 2 and 2.5 cm, respectively. The background properties were \( \mu_a = 0.03 \) cm\(^{-1}\) and \( \mu_s' = 6.0 \) cm\(^{-1}\). The simulations were processed with center sources as shown in Figure 2-1 (b). Figure 2-18 shows the reconstructed image. Figure 2-18 (a) is the image of target with 1.0 cm inner core diameter. The maximum value of \( \mu_a \) was 0.12 cm\(^{-1}\) and the measured inner core diameter was 1.5 cm. Figure 2-18 (b)-(d) are the reconstructed images with 1.5, 2.0, and 2.5 cm inner core diameters, respectively. The
corresponding measured maximum absorption were 0.13, 0.13 and 0.13 cm\(^{-1}\); while the measured inner core diameters were 1.52, 1.9, and 1.9 cm, respectively. As expected, the maximum reconstructed values are similar because of the fixed absorption of the outer shell, and the reconstructed inner core diameter increases with the increase of the inner core diameter size.

Figure 2-18 Simulation results of a concentric semi-ellipsoidal target of outer shell diameter 5 cm, inner core diameter 2.5 cm, height 2 cm and layer thickness 0.5 cm of different inner core diameters. Probe shown in Figure 2-1 (b) was used for image reconstruction. Inner core diameter was (a) 1.0 cm, (b) 1.5 cm, (c) 2.0 cm, and (d) 2.5 cm.

To validate the simulation results, phantom experiments with the similar condition were performed. Because of the difficulty of making different phantoms with exactly the same inner core size as the simulation, we used the same phantom by gradually enlarging
the inner core size to perform the experiments. To do so, the optical properties of the outer shell and inner core did not change and the only variable was the inner core size.

![Image](image_url)

Figure 2-19 Phantom results of a concentric semi-ellipsoidal target of outer shell diameter of 5 cm, inner core diameter of 2.5 cm, height of 2 cm and layer thickness 0.5 cm of different inner core diameters. Probe shown in Figure 2-1 (b) was used for image reconstruction. Inner diameter was (a) 1.0 cm, (b) 1.5 cm, (c) 2.0 cm, and (d) 2.5 cm.

The optical properties of the outer shell were \( \mu_a = 0.25 \text{ cm}^{-1} \), \( \mu_s' = 7.1 \text{ cm}^{-1} \), the diameter was 5 cm and the t was 0.5 cm. The inner core was filled with intralipid solution of calibrated \( \mu_a = 0.03 \text{ cm}^{-1} \) and \( \mu_s' = 7.2 \text{ cm}^{-1} \). Experiments of four different inner core diameters of 1.0, 1.5, 2 and 2.5 cm were performed with results shown in Figure 2-19, respectively. The probe of Figure 2-1 (b) was used for this set of experiments. The reconstructed maximum \( \mu_s \) s in Figure 2-19 (a)-(d) were 0.14, 0.15, 0.14 and 0.15 cm\(^{-1}\), respectively; while the measured inner core diameters were 1.3, 1.6, 1.9 and 2.2 cm, respectively. The phantom experiments closely follow the simulation, and further
demonstrate that maximum values do not change much for the fixed outer layer, however, the measured inner diameter increases with the increase of the true hollow inner core size.

Figure 2-20 Plot of contrast ratio (left y axis) and reconstructed inner diameter (right y axis) vs. the inner core diameter.

Figure 2-20 shows the simulation and phantom experimental results for targets with different inner diameters. The dash lines represent simulation results and the solid lines are that of phantom experiments. The three groups of data are: true absorption ratio, reconstructed ratio of outer shell over inner core absorptions, and measured inner core diameter for target of different inner core size. The true contrast ratio of outer shell over inner core absorption was 7.6. The reconstructed ratios from simulations were 1.52, 1.60, 1.84 and 2.08, respectively; these values were 1.28, 1.34, 1.68 and 1.84 for experiments. The measured diameters were 1.5, 1.5, 1.9 and 1.9 cm in simulation, and were 1.3, 1.6,
1.9 and 2.2 cm in experiments, respectively. Comparing the reconstructed ratios with the true ratio, we see that the reconstructed ratios can only reach a factor of two and increase with the increase of the inner core diameter. Correspondingly, the measured inner core diameter increases with the increase of the true diameter size.

Figure 2-21 Clinical example of an advanced cancer. (a) Co-registered US showed a suspicious mass of a semi-spherical shape with top and bottom located at 0.5 cm and 2.5 cm from the skin surface. Core-biopsy revealed a high grade ductal carcinoma. (b) Optical absorption map reconstructed at 780 nm; (c) Absorption map reconstructed at 830 nm, and (d) Computed total hemoglobin concentration map.
2.3.6 Clinical example

A clinical example of an advanced cancer of approximately 4cm size is given in Figure 2-21. The co-registered ultrasound showed a highly suspicious mass located at the left breast of 3 o’clock position of an 86-year old woman. The lesion top and bottom were approximately located at 0.5 cm and 2.5 cm depth from the skin surface. Ultrasound-guided biopsy revealed that the mass was a high grade (nuclear grade II, histology grade III) invasive ductal carcinoma. Further evaluation of H&E stained histology slides showed extensive tumor necrosis which occupied about 40% of the core biopsy samples. Optical absorption maps obtained at 780nm (b) and 830nm (c) showed higher periphery contrast than the inner core area. The computed total hemoglobin concentration showed similar periphery enhancement of maximum and average concentrations of 86 $\mu$Mol/Liter and 55 $\mu$Mol/Liter, respectively. This type of periphery enhancement is often seen in advanced cancers.

2.4 Discussion and summary

Angiogenesis patterns of advanced breast cancers are complex, and there is no unique feature that can uniquely characterize these tumors. From our on-going clinical studies, we have seen two typical types of absorption or vasculature distribution patterns of advanced cancers: periphery enhancements or posterior shadowing. More interestingly, the periphery enhancements are often seen in high grade tumors which have been reported in MRI literature as well. Posterior shadowing is caused by significant light absorption of a highly vascularized tumor, which causes a dramatic reduction of the reflected light received from the deeper portion of the tumor. As a result, the reconstructed absorption maps of these large tumors have shown much higher light absorption at the top
portion than that of the deeper portion.\textsuperscript{30} This shadowing effect is similar to the posterior shadowing seen in pulse-echo ultrasound when imaging larger tumors. The presence of significant posterior shadowing of a lesion in ultrasound images suggests malignance.\textsuperscript{35}

To characterize and quantify periphery enhancement features of optical tomography when imaging these larger cancers, we performed five sets of simulations and phantom experiments in the reported study. As shown in the studies, two target parameters affect the resolving capability of optical tomography: the layer thickness between the outer shell and the inner core and the target size. In order to resolve the inner core, the layer thickness has to be less than 1 cm and the target size has to be larger than 3 cm. Concentric inhomogeneous targets of thicker layers and smaller diameters may not show periphery enhancement features in images. This result may explain why we have observed the periphery enhancement in advanced cancers of high grade tumors which grow rapidly outward with extensive dead necrotic core. In addition, the periphery enhancement occurs at target depths less than 3 cm. Deep concentric inhomogeneous targets may not show this feature in images due to intense light scattering in tissue. Therefore, quantifying target parameters and target depth is critically important when using periphery enhancement feature to assist ultrasound diagnosis of advance cancers. Certainly, their parameters may vary to some extend based on individually patient’s bulk absorption and scattering coefficients. The inner core optical absorption and size also affect imaging periphery enhancement features to some extend. The reported investigation on near center source illumination also provides an important guideline for the probe design. As shown from the studies, two near center sources at the top and
bottom sides of the ultrasound probe are needed for improving visualization of the target heterogeneity. This is feasible in the probe design using ultrasound guidance.

Because of the large target size studied, multiple fine-mesh target layers in depth were used in image reconstruction. As a result, more voxels with unknown optical properties were reconstructed and the lesion quantification was about 50-60% which was lower than that of 70-85% obtained from smaller targets.\textsuperscript{29,36} The measured contrast of the higher target absorption periphery over the lower absorption core was about a factor of 2 at the best imaging condition. This is due to the intense scattering events of diffused photons which cause the information loss between the two groups of photons passing outer shell only and passing the outer shell and the inner core. Interestingly, several groups have reported the factor of 2 contrast between malignant lesions and background tissues using different instrumentation and different measurement methods.\textsuperscript{10,37}

In summary, we have shown that large inhomogeneous concentric semi-ellipsoidal targets with outer shell thickness less than 1 cm can be resolved at the typical depth range for breast imaging when reflection geometry is used. For large breast lesions of more than 3 cm in size, the periphery enhancement feature can be used to assist ultrasound diagnosis of benign versus malignance.

References


3 Clustered targets imaged by optical tomography guided by ultrasound

3.1 Introduction

DOT in the NIR spectrum provides a unique approach for functional diagnostic breast imaging and for monitoring chemotherapy response of advanced breast cancers.\textsuperscript{1-19} However, the primary limitation of DOT is related to the intense light scattering in tissue which dominates NIR light propagation. As a result, the resolution of the DOT is low and the localization of lesions is poorer. In addition, the accurate quantification of lesion optical properties is difficult with DOT. Optical tomography guided by co-registered US, MRI, and x-ray has demonstrated a great clinical potential to overcome lesion location uncertainty and to improve light quantification accuracy.\textsuperscript{1, 5, 17, 20-24} In the co-registration approach, a region of interest (ROI) containing a suspicious lesion seen by US or other imaging modalities is used to guide the DOT image reconstruction.

Our group has developed a unique approach of using co-registered US to guide the lesion localization and using DOT to map the lesion vasculature. This approach has successfully overcome the location uncertainty of pure DOT approach.\textsuperscript{18, 25, 26} For a single target, this approach can achieve 61-77\% accuracy for high contrast 1 cm targets and 79-113\% for low contrast targets of the same size. The accuracy for larger targets is lower in general in the range of 33-48\% and 81-101\% for high and low contrast targets, respectively.\textsuperscript{27} A recent paper by Ghadyani et al. has reported 85\% accuracy in simulations when a contrast details analysis is used to recover the optical properties of anomalies guided by the MR imaging.\textsuperscript{1, 5} Studies using x-ray as the prior information demonstrated 90\% accuracy for small volume problems.\textsuperscript{22} There are other groups using
spatial prior information to guide DOT imaging, for example, Tian et al has presented a best recovering rate of 64% of dual targets with a new depth compensation algorithm in simulations.28, 29

In the dual-zone mesh method we introduced earlier,30 the ROI was segmented into a finer mesh and the background region was segmented into a coarse mesh. The inversion was well conditioned by this dual-zone mesh scheme and converged quickly in three to four iterations. In the presence of multiple targets, multiple ROIs are needed to guide the DOT image reconstruction. In this paper, we extend the dual-zone mesh method to include multiple ROIs when multiple targets are present. We evaluate the performance of the multi-zone method using simulations and phantom experiments. Clinical examples are given to demonstrate the improvement of lesion characterization using this method. Although the capability of DOT alone in distinguishing multiple targets was evaluated by several research groups,16, 28, 31-33 the use of a prior knowledge of multiple lesions seen by US has not been investigated. This study will systemically evaluate the performance of the multi-zone algorithm and the improvement of this method in target quantification.

3.2 Methods

3.2.1 Reconstruction algorithms

In the single ROI dual-zone-mesh based image reconstruction, the imaging volume was segmented into two regions consisting of the lesion (L) as identified by the co-registered ultrasound and the background (B) region. A finer imaging voxel is used for lesion region and a coarse imaging voxel is used for background region. A modified Born approximation is used to relate the scattered field $U_{sd}(r_d, r_l, \omega)$ measured at the source
(s) and detector (d) pair i to absorption variations $\Delta \mu_a(r')$ in each volume element of the two regions within the sample, where $r_{si}$ and $r_{di}$ are the source and detector positions, respectively. The matrix form of the image reconstruction is given as:

$$[U_{sc}]_{M \times 1} = [W_L, W_B]_{M \times N} [M_L, M_B]^{T}$$

where $W_L$ and $W_B$ are weight matrices for the target and background regions, respectively. Instead of reconstructing $\Delta \mu_a$, the integral absorption distribution $M$ is reconstructed, which is the product of $\Delta \mu_a$ times voxel size in discrete form. The $M$ matrix is segmented into two regions, L and B, where

$$M_L = \left[ \int_{L_1} \Delta \mu_a(r')d^3r', \cdots, \int_{L_n} \Delta \mu_a(r')d^3r' \right]$$

and

$$M_B = \left[ \int_{B_1} \Delta \mu_a(r')d^3r', \cdots, \int_{B_n} \Delta \mu_a(r')d^3r' \right].$$

After reconstructing $M$, we divide it by different voxel sizes of L and B to obtain $\Delta \mu_a$ distribution. By using a finer mesh for L region and a coarser mesh for B region, we reduce the total number of voxels with unknown optical properties for improving inversion. In addition, the $M$ is reconstructed rather than absorption distribution per se, which further conditions the inversion because the $M$ in the lesion region with a higher absorption and a finer voxel grid is in the same scale as the $M$ in the background region with a lower absorption and a courser grid. The weight matrices are calculated based on the background absorption coefficient $\overline{\mu_a}$ and reduced scattering coefficient $\overline{\mu'}_s$ measured from the homogeneous intralipid solution in phantom studies and normal contralateral tissue in clinical studies.

For more than one target, the performance of the single ROI based dual-mesh scheme is degraded because of the increased number of fine-mesh grids with unknown optical properties. As a result, the lesion quantification is poor. We have extended the
dual-zone mesh method to multiple zones based on the ROIs identified by ultrasound.
If N targets present, we divide the region of interest (ROI) into N zones either in a single
layer or multiple layers in depth denoted as ROI#1, ROI#2, … ROI#N, respectively.
The equation (1) is modified as:

$$\begin{bmatrix}
U_{SC}^{(c)}
\end{bmatrix}_{M \times 1} = 
\begin{bmatrix}
W_{L1}, W_{L2}, \ldots, W_{LN}, W_B
\end{bmatrix}_{M \times N}
\begin{bmatrix}
M_{L1}, M_{L2}, \ldots, M_{LN}, M_B
\end{bmatrix}^T,$$

(2)

where the $W_{L1}$, $W_{L2}$, …… and $W_{LN}$ are the weight matrices for the targets, and $W_B$ is
the weight matrix for the background tissue. $M_{L1}$, $M_{L2}$, …. and $M_{LN}$ are the integral
absorption distributions of the corresponding targets and $M_B$ is that of the background
region. The absorption distributions can be computed by dividing the corresponding
voxel size in ROIs and the background regions.

For experiments and clinical cases, we have used 0.25 x 0.25 x 0.5 cm$^3$ for the finer
grid inside the lesion and 1.0 x 1.0 x 1.0 cm$^3$ in the background region. The total imaging
volume is 9.0 x 9.0 x 4.0 cm$^3$. The choice of the imaging grids is based on the
considerations of the system signal to noise ratio and the total number of voxels with
unknown optical properties. The total number of finer and coarser voxels depends on
target size. For typical 1-cm dual targets, the total numbers of finer and coarser voxels are
242 and 324 (total 566), respectively, when a dual ROI is used. These numbers are 441
and 321 (total 762), respectively, when a single ROI is used. For simulations, we have
used 0.15 x 0.15 x 0.5 cm$^3$ for the finer grid and the same coarse grid as the experiments.
The corresponding numbers are 402 and 324 (total 726) for a dual ROI scheme, and 1258
and 324 (total 1582) for a single ROI scheme.
3.2.2 Simulations and phantom experiments

In the simulation, a commercial finite-element (FEM) package COMSOL was employed to solve the forward diffusion equation in the frequency domain.\textsuperscript{34,35} 140 MHz modulation frequency was used in all the simulations. A cylinder of 20 cm diameter and 10 cm height was used to model the semi-infinite medium, and 9 sources and 14 detectors were distributed on the surface in reflection geometry as shown in Figure 3-1. Two spherical absorbers of same or different absorption coefficients were embedded in the scattering medium. The optical properties of the medium were $\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$, which were typical values of fatty breast tissue.\textsuperscript{23,35,36} Targets’ $\mu_a$ and $\mu_s'$ were changed based on the different types of tumor targets simulated, for example, the high contrast target of $\mu_a = 0.25 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$ was used to simulate malignant tumors and the low contrast target of $\mu_a = 0.07 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$ was used to simulate benign lesions.

![Figure 3-1 Probe geometry used for simulations and phantom experiments. The probe is of 1 cm thickness and the center slot is used for US transducer.](image-url)
In the phantom experiments, the frequency domain system\textsuperscript{36} consisted of 14 parallel detectors and 4 laser diodes of wavelength 740nm, 780nm, 808nm and 830nm, respectively. Each laser diode was sequentially switched to 9 positions on the probe. The central slot on the probe shown in Figure 3-1 was used to fit the ultrasound transducer, and the sources and detectors were distributed on both sides. Polyester resin spheres of calibrated values $\mu_a = 0.23 \text{ cm}^{-1}$ and $\mu'_s = 5.45 \text{ cm}^{-1}$, and $\mu_a = 0.07 \text{ cm}^{-1}$ and $\mu'_s = 5.50 \text{ cm}^{-1}$ were used to emulate high contrast tumors and low contrast benign lesions. Intralipid solution of $\mu_a = 0.03 \text{ cm}^{-1}$ and $\mu'_s = 7.2 \text{ cm}^{-1}$ was used to emulate the background tissue. Measurements were made with the target inside the intralipid (target data) and intralipid alone as a reference. The perturbation between the target data and the reference was used for imaging reconstruction.

Clinical experiments were performed at University of Connecticut Health Center. The study protocol was approved by the local Institution Review Board (IRB) committee. All patients who participated in our study signed the informed consent. The data were taken at the patients’ lesion area and the contralateral breast of the same quadrant of the lesion. Contralateral data set was used to estimate background optical properties for weight matrix computation. The perturbation computed between the lesion data and the contralateral data was used for imaging reconstruction.

3.3 Results

3.3.1 Two targets with different contrast

In some clinical cases, patients may have multiple lesions with malignant or benign characteristics. To evaluate the performance of our multi-zone ROIs approach, we have
performed simulations and experiments by using two targets of same size but different contrast.

A series of FEM simulations were conducted for two types of targets, one high and one low contrast absorbers. Both targets had the same diameter of 1.0 cm and located at same depth with center-to-center separations of 1.5, 2.0, 2.5, and 3.0 cm, respectively. The depth from the target center to the probe surface varied from 1.0 cm to 2.5 cm in 0.5 cm increment. Tomography images are shown in six slices at different depths from 0.5 cm to 3.0 cm with 0.5 cm increment. The targets are shown at the corresponding depth. The single ROI and the multi-zone ROIs algorithms were used to reconstruct the images as shown in Figure 3-2. In the figure, the separation of the two targets is 2.5 cm and target center depths are 1.0, 1.5, 2.0 and 2.5 cm, respectively. The images in the left column ((a), (c), (e) and (g)) are the reconstructed absorption distributions using the single ROI method and the images in the right column ((b), (d), (f) and (h)) are the reconstruction results using the multi-zone ROIs algorithm. For single ROI method, the reconstructed maximum absorption value of the high contrast target was 0.09 cm$^{-1}$ (36% accuracy), and that of the low contrast target was 0.04 cm$^{-1}$ (57%) at the depth of 1.5 cm; but the accuracy could reach 0.17 cm$^{-1}$ (68%) for the high contrast target and 0.06 cm$^{-1}$ (86%) for the low contrast target when the multi-zone method was used. With respect to target resolving capability, the two targets could not be separated using the single ROI method when they were deeper than 2.0 cm. However, the targets up to 2.5 cm depth could be separated with the guidance of the prior target locations when the multi-zone method was used. Briefly, the average reconstruction accuracy of four target separations of the high contrast targets using the single ROI method was 36%, 38%, 35% and 33% at 1.0, 1.5,
Figure 3-2 Simulated absorption maps of two targets of different contrast (\( \mu_a = 0.25 \text{ cm}^{-1} \) and \( \mu_s = 0.07 \text{ cm}^{-1} \)) separated by 2.5 cm and located at 1.0, 1.5, 2.0, 2.5 cm depth, respectively, using single ROI shown in (a), (c), (e) and (g) and multi-zone method shown in (b), (d), (f) and (h). For each figure, there were six subfigures reconstructed at different depths marked on the figure title. Each subfigure is a spatial x-y image of 9 cm by 9 cm in spatial dimensions. The rest of the figures consisting reconstructed absorption maps were displayed with the same dimensions as Figure 3-2.
2.0 and 2.5 cm center depth, respectively. The average accuracy of the corresponding separations was 63%, 67%, 58% and 47%, respectively, using the multi-zone method. A 23% average improvement was achieved compared with that obtained using the single ROI method. To further evaluate the performance of the multi-zone method with targets of different contrast, another set of simulation with $\mu_a = 0.15 \text{ cm}^{-1}$ as the low contrast target and $\mu_o = 0.25 \text{ cm}^{-1}$ as the high contrast target, and $\mu_s' = 6.0 \text{ cm}^{-1}$ for both was performed. For single ROI method, the average reconstruction accuracy of four separations was 31%, 46%, 38% and 22% at 1.0 to 2.5 cm center depth, respectively. The average accuracy was 41%, 73%, 68% and 44%, respectively, and an average 22% improvement was obtained compared with that using the single ROI method.

To validate the simulation results, a phantom experiment using two 1-cm polyester resin spheres with different contrasts was performed. The intralipid solution was used as the background. The experiment was designed using similar conditions as the simulation. Figure 3-3 shows the reconstructed absorption maps of the two targets which were separated by 2.5 cm and located from 1.0 to 2.5 cm center depths. Targets could not be resolved when they were located deeper than 2.0 cm if the single ROI method was used (Figure 3-3 (e) and (g)), while in the right column (Figure 3-3 (b), (d), (f) and (h)), they could be separated at all depths when the multi-zone method was used. The average accuracy of four target separations of the high contrast target was 39%, 44%, 41% and 34% at depth of 1.0, 1.5, 2.0 and 2.5 cm using the single ROI method, respectively, and that was 66%, 75%, 64% and 56%, respectively, using the multi-zone method. The average improvement was 26%. Both simulations and phantom experiments
demonstrated that the multi-zone algorithm could improve the capability of resolving two targets with improved quantification.

Figure 3-3 Phantom experimental results of two targets of different contrast \( \mu_a = 0.23 \text{ cm}^{-1} \) and \( \mu_a = 0.07 \text{ cm}^{-1} \) separated by 2.5 cm and located at 1.0, 1.5, 2.0, 2.5 cm depth, respectively, using single ROI shown in (a), (c), (e) and (g) and multi-zone method shown in (b), (d), (f) and (h).
Figure 3-4 shows the analysis of reconstruction accuracy quantified using accuracy versus different depths. The solid curves are for high contrast target using the multi-zone method, while the dotted lines are obtained from the single ROI method. The dashed lines are for the low contrast target using the multi-zone method. Because of the limited resolving ability of the single ROI method for the low contrast target, the accuracy could not be accurately measured and therefore no values are given. The 100% is plotted as the reference using dash-dot line. Figure 3-4 (a) and (b) show the results of the simulations, where (a) corresponds to the two targets with $\mu_a = 0.25 \text{ cm}^{-1}$ and $\mu_a = 0.07 \text{ cm}^{-1}$, and (b) $\mu_a = 0.25 \text{ cm}^{-1}$ and $\mu_a = 0.15 \text{ cm}^{-1}$. Figure 3-4 (c) shows the results of the phantom experiments. The improvement of the multi-zone method is shown by its higher reconstruction accuracy (solid lines) than that of the single ROI method (dotted lines). From the comparison between the figures, one can see that the reconstructed absorption values of the high contrast target remain similar and the most accurate reconstructions occur at the depth range beyond 1.0 cm and less than 2.5 cm. For the low contrast target (true $\mu_a = 0.07 \text{ cm}^{-1}$), the reconstruction values were around or slightly over the true value 100% (Figure 3-4 (a) and (c)). For the medium contrast target (true $\mu_a = 0.15 \text{ cm}^{-1}$) imaged with the high contrast target together, both targets were under reconstructed but the medium contrast target had higher accuracy than that of the high contrast target (Figure 3-4 (b)).
Figure 3-4 Plot of reconstruction accuracy vs. target depths of two targets using single ROI and multi-zone method as labeled in the figures. (a) and (b) were from simulations and (c) was from experiments. (a) two targets of $\mu_a = 0.25$ cm$^{-1}$ and $\mu_a = 0.07$ cm$^{-1}$; (b) $\mu_a = 0.25$ cm$^{-1}$ and $\mu_a = 0.15$ cm$^{-1}$. (c) $\mu_a = 0.23$ cm$^{-1}$ and $\mu_a = 0.07$ cm$^{-1}$.

A clinical example was given to demonstrate the improvement by using the multi-zone method. This 52 years old patient had two suspicious lesions as shown in US Figure 3-5 (a). When the single ROI was used, two targets of one slightly higher contrast (left) than the other (right) showed in the absorption map reconstructed at 780 nm (Figure 3-5 (b)) and 830 nm (not shown). The calculated maximum absorption coefficients were 0.13 and 0.10 cm$^{-1}$, respectively. When the multi-zone method was used, the calculated maximum absorption coefficients were 0.22 and 0.12 cm$^{-1}$ (Figure 3-5 (c)).
calculated maximum tHb concentrations were 80 and 63 $\mu$mol/Liter by using the single ROI method (Figure 3-5 (d)), and 97 and 62 $\mu$mol/Liter by using the multi–zone method (Figure 3-5 (e)). Biopsy results showed that the higher contrast lesion was a ductal carcinoma in situ (left in US) and the lower contrast one was a benign fibrocystic lesion.

Figure 3-5 A clinical example of two different contrast lesions. (a) Co-registered US showed two suspicious masses with centers located at 1.2 cm from the skin surface. (b)-(c) Optical absorption map reconstructed at 780 nm using (b) single ROI and (c) multi-zone method; (d)-(e) Computed total hemoglobin concentration map using (d) single ROI, and (e) multi-zone method.
3.3.2 Two targets with same contrast

In this section, the resolving ability of the multi-zone ROIs method in imaging clustered tumors with the same contrast was evaluated using simulations and phantom experiments. Two small targets of 1.0 cm diameter and same contrast located at the same or different depths were characterized.

In the first set of FEM simulations, two high contrast targets of 1.0 cm diameter were imaged. The target center-to-center separations were 1.5, 2.0, 2.5, and 3.0 cm, and center depths varied from 1.0 to 2.5 cm. Figure 3-6 shows an example when the two targets with 2.5 cm separation are located at same depths from 1.0 to 2.5 cm. A comparison of the results in the left column ((a), (c), (e) and (g)) using the single ROI method and in the right column ((b), (d), (f) and (h)) using the multi-zone method clearly demonstrates that the capability of multi-zone method in resolving two targets is much better. Quantitatively, the single ROI method had the best reconstructed absorption value of 0.11 cm$^{-1}$ (44%) at 1.5 cm depth; the resolving ability was diminished when the target was deeper than 2.0 cm. In contrast, the multi-zone method can reach 0.19 cm$^{-1}$ (76%) reconstruction accuracy and extend the resolving ability up to 2.5 cm. The average reconstruction accuracy of four different target separations was 33%, 42%, 40% and 27% from 1.0 to 2.5 cm center depth, respectively, using the single ROI method. The average accuracy was 45%, 74%, 60% and 41%, respectively, using the multi-zone method. An average 20% improvement was achieved compared with that using the single ROI method.
Figure 3-6 Simulated absorption maps of two targets with same contrast ($\mu = 0.25 \text{ cm}^{-1}$) separated by 2.5 cm and located at 1.0, 1.5, 2.0, 2.5 cm depth, respectively. (a), (c), (e) and (g) obtained using single ROI and (b), (d), (f) and (h) using multi-zone method.
Figure 3-7 Phantom results of two targets with same contrast ($\mu_a = 0.23$ cm$^{-1}$) separated by 2.5 cm and located at 1.0, 1.5, 2.0, 2.5 cm depth, respectively. (a), (c), (e) and (g) obtained using single ROI method, and (b), (d), (f) and (h) using multi-zone method.

Phantom experiments were performed to verify the simulation results. Two high contrast phantom targets of 1.0 cm diameter were embedded inside the intralipid solution
with the same experimental conditions as the simulation. Figure 3-7 shows one example of the phantom targets separated by 2.5 cm at all depths. The average reconstruction accuracy was 29%, 37%, 41% and 28% at 1.0, 1.5, 2.0 and 2.5 cm center depth, respectively, using the single ROI method and the value was 47%, 84%, 65% and 42%, respectively, using the multi-zone method. An average 26% improvement was achieved compared with that using the single ROI method. Figure 3-8 plots the accuracy ratio vs. different depths. The reconstruction accuracy of the multi-zone method (solid lines) is much higher than that of the single ROI method (dotted lines). The more accurate reconstruction occurred in depth range beyond 1.0 cm and less than 2.5 cm in both simulations (Figure 3-8 (a)) and phantom experiments (Figure 3-8 (b)).

Figure 3-8 Plot of reconstruction accuracy vs. target depths of two targets of same contrast. (a) Simulation results of two targets of $\mu_a = 0.25 \text{ cm}^{-1}$, and (b) phantom results of $\mu_a = 0.23 \text{ cm}^{-1}$.

The second set of simulation was performed with two targets located at different depths. The two targets were the same as before but one was located at 1.0 cm depth while another was at 1.5 cm. Different center-to-center separations of 1.5, 2.0, 2.5 and 3.0...
cm were simulated and shown in Figure 3-9. None of the images shown in the left column ((a), (c), (e) and (g)) obtained from the single ROI method could separate the two targets; while the images shown in the right column ((b), (d), (f) and (h)) obtained from the multi-zone method could resolve them when the center-to-center separation was larger than 2.0 cm. Same as other cases, the reconstructed absorption coefficients were more accurate when the multi-zone method was used. The absorption coefficients were 0.12 (48%), 0.10 (40%), 0.10 (40%) and 0.10 (40%) for 1.5, 2.0, 2.5 and 3.0 cm center-to-center separations, respectively, using the single ROI method; and those were 0.16 (64%), 0.18 (72%), 0.15 (60%) and 0.16 (64%) using the multi-zone method with an average 23% improvement.

To verify the simulation results, phantom experiments were performed using the similar condition as the simulation. Two high contrast phantom targets of 1.0 cm diameter were located at 1.0 cm and at 1.5 cm center depths. Figure 3-10 shows the US and the reconstructed images of the two targets separated by 1.5, 2.0, 2.5 and 3.0 cm. The reconstructed absorption coefficients were 0.10 (43%), 0.09 (39%), 0.08 (35%) and 0.09 (39%) for 1.5, 2.0, 2.5 and 3.0 cm center-to-center separations, respectively, using the single ROI method ((a), (c), (e) and (g)); and those were 0.16 (70%), 0.14 (61%), 0.17 (74%) and 0.15 (65%) using the multi-zone method ((b), (d), (f) and (h)). An average 29% improvement was achieved compared with that of the single ROI method. When the separation of the two targets was larger than 2.0 cm, the multi-zone reconstruction method could separate them, but the single ROI method could not.
Figure 3-9 Simulated absorption maps of two targets of same contrast ($\mu_a = 0.25$ cm$^{-1}$) separated by 2.5 cm with one located at 1.0 cm and another located at 1.5 cm center depths. (a), (c), (e) and (g) obtained using single ROI method, and (b), (d), (f) and (h) using multi-zone method.
Figure 3-10 Phantom experimental results of two targets of same contrast ($\mu_a = 0.23$ cm$^{-1}$) separated by 2.5 cm with one located at 1.0 cm and another located at 1.5 cm center depths. (a), (c), (e) and (g) obtained from the single ROI method, and (b), (d), (f) and (h) from multi-zone method. The corresponding co-registered US images were shown in the first column.
Figure 3-11 A clinical example of two malignant tumors. (a)-(b) Co-registered US image of 9 o’clock lesion and (b) 7-8 o’clock lesion with center location of approximately 2.0 cm; (c)-(d) Absorption maps reconstructed at 780 nm using (c) single ROI, and (d) multi-zone method; (e)-(f) Total hemoglobin concentration maps using (e) single ROI, and (f) multi-zone method.

A clinical example of 59 year-old woman with two suspicious lesions located at the 9 and 7-8 o’clock positions is given in Figure 3-11. US images of the two lesions are shown in different views in Figure 3-11 (a) and (b) with the center depth of 2.0 cm.
approximately. Because the NIR probe is much bigger (9 cm diameter) than the US probe (5 cm x 1 cm), the two targets are visible in the absorption maps reconstructed using a single ROI at 780nm (c) and 830 nm (not shown). The calculated maximum absorption coefficients were 0.18 and 0.14 cm\(^{-1}\) for 9 and 7-8 o’clock lesions, respectively. When the multi-zone method was used as shown in (d), the calculated maximum absorption coefficients were 0.23 and 0.19 cm\(^{-1}\), respectively. The calculated maximum tHb concentrations were 90 and 81 \(\mu\) mol/Liter by using the single ROI method, and 105 and 101 \(\mu\) mol/Liter by using the multi–zone method. Biopsy results showed that both lesions were invasive carcinomas.

3.3.3 Large target surrounded by a small satellite target

Figure 3-12 Simulated absorption maps of one larger target with a smaller target closer to its top half ((a) and (b)), and bottom half ((c) and (d)) using single ROI ((a) and (c)) and multi-zone method ((b) and (d)). The center to center distance between the two targets was 3.0 cm. (The arrow head points to the smaller target.)
Large cancers may have smaller satellite lesions in the neighboring areas. To quantify the reconstruction accuracy under this imaging condition, two high-contrast targets of 2 cm and 1 cm diameter next to each other were simulated using FEM method. Two sets of simulations were performed where the larger target was surrounded by the smaller one closer to its top half or bottom half in depth. In the first set of simulations, the larger target was located at 1.5 or 2.0 cm center depth and the smaller target located closer to its top half with the center-to-center separation of 2.1, 2.5, 3.0 and 3.5 cm, respectively. Figure 3-12 (a) and (b) shows the absorption maps of the two targets with 3.0 cm separation when the larger one is located at 1.5 cm center depth. The reconstructed maximum absorption of the larger target using the single ROI method (a) was 0.10 cm$^{-1}$ (40%), and the multi-zone method (b) was 0.16 cm$^{-1}$ (64%). The smaller target was not resolved using the single ROI method (a) and that was 0.09 cm$^{-1}$ (36%) using the multi-zone method (b). More results of different target separations and depths are given in Table 3-1. The average reconstruction accuracy of the larger target was 51.5% and the satellite target could not be resolved until the separation was larger than 3.5 cm if the single ROI method was used. The accuracy of the larger target could reach 78.5% using the multi-zone method and the satellite target was visible when the separation reached 3.0 cm with the average accuracy of 39%. In the second set of simulation, the larger target with the small satellite target closer to its bottom half was simulated with 2.1 to 3.5 cm separations and the center depths of 1.5 and 2.0 cm. The reconstructed absorption maps are shown in Figure 3-12 (c)-(d). Using the single ROI method, the smaller target could not be resolved and the maximum reconstructed $\mu_a$ was 0.10 cm$^{-1}$ (40%) as shown in (c). Using the multi-zone method, the reconstructed
maximum absorption of the larger target was 0.15 cm\(^{-1}\) (60%) and that of the smaller one was 0.05 cm\(^{-1}\) (20%) (d). The satellite target is visible in (d) but reconstruction accuracy (20%) is far lower than that of the larger one. As shown in Table 3-1, the average reconstruction accuracy of the larger target is 51.5% using the single ROI method and that is 76% using the multi-zone method. The smaller target could not be resolved until the target separation reached 3.5 cm using the single ROI method and it could be resolved when the separation was larger than 3.0 cm with the average accuracy of 23% using the multi-zone method.

We also performed phantom experiments using two high contrast polyester resin spheres. Two sets of experiments were done and the targets were located at the same coordinates as used in the simulation described earlier. The left column of the US images in Figure 3-13 show the locations of the two targets when they are separated by 3.0 cm, and the reconstructed absorption maps are shown in the right column. When the smaller target was closer to the top half of the larger one, the reconstructed maximum \(\mu_a\) of the larger target was 0.09 cm\(^{-1}\) (39%) using the single ROI method (a) and 0.17 cm\(^{-1}\) (74%) using the multi-zone method (b), and the maximum \(\mu_a\) was 0.11 cm\(^{-1}\) (48%) in (b) of the smaller target using the multi-zone method. The reconstructed maximum \(\mu_a\) was 0.09 cm\(^{-1}\) (39%) in (c) using the single ROI method and 0.16 cm\(^{-1}\) (70%) of the larger target, and 0.11 cm\(^{-1}\) (48%) of the smaller one in (d) using the multi-zone method when the smaller target was located closer to the bottom half of the larger one. The smaller target can not be resolved in both (a) and (c). In Table 3-2, the average reconstruction accuracy at all separations and depths is 46.7% and 80.5% (larger target) and 26% and 48% (smaller target) using the single ROI method and the multi-zone method,
respectively, when the smaller target is closer to the top half of the larger one. The average values are 49% and 78% (larger target) and 24% and 36% (smaller target) when the smaller target is closer to the bottom half of the larger one. This set of simulations and phantom experiments demonstrated the improvement and limitations when the clustered lesions of different sizes were imaged by the multi-zone algorithm.

Figure 3-13 Phantom experiments of one larger target with a smaller target located close to its top half ((a) and (b)), and bottom half ((c) and (d)) using single ROI ((a) and (c)) and multi-zone method ((b) and (d)). The corresponding co-registered US images were shown in the left column. (The arrow head points to the smaller target.)
Table 3-1 Simulation results of reconstructed absorption coefficient and accuracy of one larger target surrounded by a smaller satellite target with same contrast ($\mu_a = 0.25$ cm$^{-1}$) separated by 2.0 to 3.5 cm at 1.0 to 2.5 cm center depth, respectively, using single ROI and multi-zone method.

<table>
<thead>
<tr>
<th>Reconstructed $\mu_a$ Unit: (cm$^{-1}$)</th>
<th>Smaller target closer to the top half</th>
<th>Smaller target closer to the bottom half</th>
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</thead>
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<tr>
<td></td>
<td>Larger target depth 1.5 cm</td>
<td>Larger target depth 2.0 cm</td>
</tr>
<tr>
<td></td>
<td>Single ROI</td>
<td>Multi-zone</td>
</tr>
<tr>
<td>Target separation 2.0 cm</td>
<td>Max(L): Max(S):</td>
<td>0.11* (44%)</td>
</tr>
<tr>
<td>Target separation 2.5 cm</td>
<td>Max(L): Max(S):</td>
<td>0.11* (44%)</td>
</tr>
<tr>
<td>Target separation 3.0 cm</td>
<td>Max(L): Max(S):</td>
<td>0.10* (40%)</td>
</tr>
<tr>
<td>Target separation 3.5 cm</td>
<td>Max(L): Max(S):</td>
<td>0.10 (40%)</td>
</tr>
</tbody>
</table>

* Two targets merged together as one. Max(L) and Max(S) referred to maximum reconstructed absorption coefficient of larger target and smaller target, respectively.
Table 3-2 Phantom experimental results of absorption coefficient and accuracy of one larger target surrounded by a smaller satellite target of same contrast ($\mu_r = 0.25$ cm$^{-1}$) separated by 2.0 to 3.5 cm at 1.0 to 2.5 cm center depth, respectively, using single ROI and multi-zone method.

<table>
<thead>
<tr>
<th>Reconstructed $\mu_c$ Unit: (cm$^{-1}$)</th>
<th>Smaller target closer to the top half</th>
<th>Smaller target closer to the bottom half</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larger target depth 1.5 cm</td>
<td>Larger target depth 2.0 cm</td>
</tr>
<tr>
<td></td>
<td>Single ROI</td>
<td>Multi-zone</td>
</tr>
<tr>
<td>Target separation 2.0 cm</td>
<td>Max(L): Max(S):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.08)* (35%)</td>
<td>(0.12)* (52%)</td>
</tr>
<tr>
<td>Target separation 2.5 cm</td>
<td>Max(L): Max(S):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.10)* (43%)</td>
<td>(0.19)* (83%)</td>
</tr>
<tr>
<td>Target separation 3.0 cm</td>
<td>Max(L): Max(S):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.09)* (39%)</td>
<td>0.17 (74%) 0.11 (48%)</td>
</tr>
<tr>
<td>Target separation 3.5 cm</td>
<td>Max(L): Max(S):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.09 (39%) 0.06 (25%)</td>
<td>0.15 (65%) 0.11 (48%)</td>
</tr>
</tbody>
</table>

* Two targets merged together as one. Max(L) and Max(S) referred to maximum reconstructed absorption coefficient of larger target and smaller target, respectively.
3.4 Discussion and Summary

We have qualitatively evaluated the performance of the single ROI reconstruction method and the new multi-zone reconstruction method with respect to the resolving ability and accuracy when multiple targets were present. In general, the single ROI method cannot resolve two small targets when their separations were less than 2.5 cm, and the target depth was greater than 2.0 cm. The highest reconstruction accuracy of the single ROI method for small dual targets was about 50% for high contrast targets. The multi-zone reconstruction method improved both the resolving ability and accuracy when the priori lesion location information was given. As a result, two targets located at 2.5 cm depth with separation greater than 2.0 cm could be distinguished. With respect to accuracy at all depths and separations, the multi-zone method improved the accuracy and the highest reconstruction could reach 91% for high contrast targets.

Due to the intense light scattering in a turbid medium, the light quantification of targets located at deeper depths of more than 2.5 cm was lower and the resolving capability of the multiple targets was poorer. In addition, because the diffusion approximation was used in reconstruction and the lacking of the center source, targets located at shallower depth were not quantified as accurately as the targets located beyond 1.0 and less than 2.5 cm depth range. The multi-zone method improved the light quantification for smaller 1 cm dual targets of different separations located at all depths and the improvement was more dramatic when targets were in the depth range beyond 1.0 cm and less than 2.5 cm. Another issue is related to the reconstruction accuracy of different contrast targets. In this paper, we have three sets of targets which have high (0.25 cm\(^{-1}\)), medium (0.15 cm\(^{-1}\)) and low contrast (0.07 cm\(^{-1}\)). Over reconstruction
happened more to low contrast target and under reconstruction occurred to high and medium contrast targets, however, medium contrast targets had higher reconstruction accuracy than that of high contrast targets because of the use of linear Born approximation. In clinical studies, multiple lesions located closer to each other is not uncommon, a diagnostic modality should be able to characterize multiple lesions more accurately. In all simulations and phantom experiments, the multi-zone method achieved about 20% improvement compared to that of the single ROI method. Thus, it is more accurate in diagnosis of clustered lesions. When two targets one larger and one smaller were located closer to each other, the location of the reconstructed absorption mass was shifted toward the larger target. A smaller target located more than 3 cm away from the larger one may be resolved when the multi-zone algorithm was used, however, the reconstruction accuracy was low. Therefore, it is not reliable to use the multi-zone method to characterize the clustered lesions with a primary larger tumor that dominates the reconstruction. In other words, when the primary tumor is malignant, the smaller lesions around it cannot be diagnosed correctly using the diffused optical tomography even with a priori target information.

In summary, we have introduced a new multi-zone reconstruction method and compared its performance with the single ROI method. Simulation and phantom studies have showed more than 20% improvement in target quantification as compared to that of the single ROI method. Clinical examples were given to demonstrate the potential of the new method in accurate characterization of the malignant and benign lesions.
References


4 Imaging tumor oxyhemoglobin and deoxyhemoglobin concentrations with ultrasound-guided diffuse optical tomography

4.1 Introduction

Tumor vasculature is directly related to tumor proliferation, growth, and metastasis; while tumor hypoxia alters the pattern of gene expression leading to more aggressive behavior with increased metastatic potential and treatment resistance.\textsuperscript{1-11} Tumor oxygen partial pressure measurements have shown that hypoxia conditions often exist in malignant tumors but not in benign lesions.\textsuperscript{4, 11} When tumor cells are exposed to chronic hypoxia as they outgrow their vascular supply, they develop an adaptation that has a negative effect on their response to treatment.\textsuperscript{2, 5} If the tumor is not well perfused, chemotherapeutic drugs cannot easily reach the tumor cells. Furthermore, tumor cells rapidly adapt to hypoxia by slowing their growth rate, and conventional chemotherapy generally is toxic to cells in proportion to their proliferation Most chemotherapeutic drugs are essentially anti-proliferation agents rather than specific anti-cancer agents so that when cells enter a resting phase they are insensitive to these cytotoxic agents. Thus, the ability to image tumor hypoxia in general has important clinical utility, providing additional diagnosis information for distinguishing malignant and benign lesions, selection of a cohort of patients who may benefit from hypoxia-directed therapies and monitoring tumor response using longitudinal imaging.\textsuperscript{7}

NIR DOT or Diffuse Optical Spectroscopy (DOS), a non-invasive imaging or spectroscopic technique, has demonstrated its clinical potential of probing tumor angiogenesis which can be quantitatively assessed by tHb concentration.\textsuperscript{12-23} When
multiple wavelengths are used, DOT/ DOS can be used to map deoxyHb and oxyHb concentrations or tumor oxygen saturation. Numbers of publications have reported a lower oxygen saturation SO$_2$ in breast tumors with respect to normal tissues,$^{24-26}$ whereas others have observed no difference.$^{27, 28}$ Publications involving a larger number of subjects have shown that oxygen saturation itself may not be a statistically significant discriminator to differentiate malignant from benign breast lesions.$^{12, 13, 15, 16, 19, 20}$ Chance et al. showed that SO$_2$, when combined with tHb could provide sensitivity and specificity of 96% and 93%, respectively, for classifying malignant from benign lesions.$^{14}$ Rinneberg et al. concluded from a study involving 154 patients that the tumor SO$_2$ provided additional information in distinguishing malignant from benign breast lesions, however, the tumor SO$_2$ itself was a poor discriminator.$^{19}$ By using x-ray tomosynthesis guided DOT, Fang et al. reported lower SO$_2$ in some cancer cases, however, the statistically significance of SO$_2$ was found only between cysts and solid benign lesions.$^{16}$ Choe et al. found statistical significance of oxyHb not SO$_2$ in differentiating malignant cancers from normal tissues.$^{15}$ Cerussi et al. reported statistical significance of oxyHb and deoxyHb not SO$_2$ in malignant cancers as compared to the background normal tissues.$^{13}$ Using DOS to follow patient response to neoadjuvant chemotherapy, Cerussi et al. found that deoxyHb was a significant predictor to treatment response.$^{12}$

Our group has explored the use of US-guided optical tomography to improve the lesion localization and light quantification accuracy.$^{22, 23, 29}$ In our clinical studies, we have observed that large tumors often presented with more deoxygenated center core and oxygenated peripheral distribution.$^{30, 31}$ This type of heterogeneous distribution
sometimes can be seen in smaller 1 - 2 cm tumors as well, however, it has not been observed in benign lesions. It has been reported in the literature that as a solid tumor progresses, tumor angiogenesis produces abnormal vasculature that is characterized by hypervascularity at the tumor periphery, where the tumor center can be hypovascular. Peripheral enhancement patterns are frequently used in contrast-enhanced MRI to differentiate malignant from benign lesions.

This study was motivated by the lack of phantom studies correlating inhomogeneous oxygenation distribution with our in vivo observations. Additionally, there are no systemic phantom studies quantifying the accuracy of reconstructed deoxyHb and oxyHb distributions or oxygen saturation as a function of tumor size and tumor depth with different oxygen conditions. In this paper, we have evaluated the capability of US-guided DOT in imaging deoxyHb and oxyHb distributions of blood phantoms having different controlled oxygen conditions. Different size phantoms located at different depths were imaged and the estimated oxygen saturations were compared with the measurements obtained using a pO2 electrode. A blood hypoxia phantom with deoxygenated core and oxygenated periphery was imaged and the deoxyHb and oxyHb distributions correlate with the heterogeneous distributions frequently seen in breast cancers. Clinical examples of benign and breast cancer cases are given to demonstrate the capability of US-guided optical tomography in mapping heterogeneous deoxyHb and oxyHb distributions in breast cancers and in assisting diagnosis. To the best of our knowledge, this manuscript is the first to report the utility of US-guided DOT in mapping heterogeneous deoxyHb and oxyHb distributions in phantoms and in patients.
4.2 Materials and methods

4.2.1 US guided DOT imaging system and imaging algorithm

Our frequency domain system consists of 14 parallel detectors and 4 laser diodes of 740nm, 780nm, 808nm and 830nm. Each laser diode was sequentially switched to 9 positions on the probe (see Figure 4-1). The center slot on the probe was used for ultrasound transducer and the sources and detectors were distributed on both sides. Intralipid solution of 0.8\% was used to emulate the background tissue and the calibrated absorption coefficient ($\mu_a$) was in the range of 0.01-0.03 cm$^{-1}$ and the reduced scattering coefficient ($\mu_s'$) was in the range of 7-8 cm$^{-1}$ for the four wavelengths employed. Measurements were made with the target inside the intralipid (target data) and intralipid alone as a reference. The perturbation between the target data and the reference was used for image reconstruction.

Figure 4-1 Probe geometry used for phantom experiments. The source and detector pairs were distributed between 2 cm to 7.5 cm. Due to saturation of PMT detectors at near source-detector pairs, only those pairs with distance greater than 2.5 cm were used for imaging reconstruction.
The dual-zone mesh scheme introduced by us earlier was used for reconstruction of absorption maps.\textsuperscript{35} Briefly, the imaging volume was segmented into two regions consisting of the lesion (ROI) as identified by the co-registered ultrasound and the background region. We used a smaller fine mesh size for the lesion region and a larger coarse mesh size for the background region, so that the total voxels with unknown optical properties was significantly reduced and the inversion converged quickly in three to four iterations. The conjugate gradient method was used for the iterative optimization. Using this method, the scattered field can be related to the integral or total absorption distribution as:

\[ [U_{SC}]_{M \times 1} = [W_L, W_B]_{M \times N} [M_L, M_B]^T \]

where \( W_L \) and \( W_B \) are the weight matrices for lesion region and background region, respectively; \( M_L = [\int_{V_L} \Delta \mu_a(r')d^3r', \ldots, \int_{V_L} \Delta \mu_a(r')d^3r'] \) and \( M_B = [\int_{V_B} \Delta \mu_a(r')d^3r', \ldots, \int_{V_B} \Delta \mu_a(r')d^3r'] \) are the total absorption distribution of the lesion and the background regions, respectively. Here, the total absorption distribution was reconstructed rather than \( \Delta \mu_a(r') \). At the end of the iterative optimization, the total absorption distribution is divided by the different voxel sizes of the lesion and background to obtain \( \Delta \mu_a(r') \). \( \mu_a(r') = \Delta \mu_a(r') + \overline{\mu}_a \),

where \( \overline{\mu}_a \) is the background absorption obtained from intralipid for phantom experiments and contralateral normal breast for patients.

Both deoxyHb and oxyHb are the major endogenous chromophores in tissue that absorb light in the NIR range. The absorption between 700 nm - 800 nm is dominated by deoxyHb and that between 800 nm - 900 nm is dominated by oxyHb (Figure 4-2). The
deoxyHb and oxyHb concentrations at each imaging voxel \( r' \) are related to the reconstructed absorption coefficients, \( \mu_a^{\lambda_i}(r') \)'s, by the following equations:

\[
\begin{bmatrix}
\mu^{\lambda_1}(r') \\
\mu_a^{\lambda_2}(r') \\
\mu_a^{\lambda_3}(r') \\
\mu_a^{\lambda_4}(r') \\
\end{bmatrix} =
\begin{bmatrix}
\varepsilon_{\text{Hb}, \lambda_i}^{\lambda_1}, \varepsilon_{\text{HbO}_2, \lambda_i}^{\lambda_1} \\
\varepsilon_{\text{Hb}, \lambda_i}^{\lambda_2}, \varepsilon_{\text{HbO}_2, \lambda_i}^{\lambda_2} \\
\varepsilon_{\text{Hb}, \lambda_i}^{\lambda_3}, \varepsilon_{\text{HbO}_2, \lambda_i}^{\lambda_3} \\
\varepsilon_{\text{Hb}, \lambda_i}^{\lambda_4}, \varepsilon_{\text{HbO}_2, \lambda_i}^{\lambda_4} \\
\end{bmatrix} \times
deoxyHb(r') = \varepsilon \times oxyHb(r')
\]

(2)

where \( \varepsilon_{\text{Hb}, \lambda_i}^{\lambda_1} \) and \( \varepsilon_{\text{HbO}_2, \lambda_i}^{\lambda_1} \) are extinction coefficients at wavelength \( \lambda_i \). The deoxyHb and oxyHb concentrations were computed as:

\[
\begin{bmatrix}
deoxyHb \ (r') \\
oxyHb \ (r')
\end{bmatrix} = [\varepsilon^T \varepsilon]^{-1} \varepsilon^T \times
deoxyHb(r') = \mu_a^{\lambda_1}(r')
\]

\[
\begin{bmatrix}
\mu_a^{\lambda_2}(r') \\
\mu_a^{\lambda_3}(r') \\
\mu_a^{\lambda_4}(r')
\end{bmatrix}
\]

(3)

where wavelengths \( \lambda_1, \lambda_2, \lambda_3, \lambda_4 \) correspond to 740, 780, 808 and 830 nm, respectively. The tHb concentration is computed as:

\[
tHb(r') = deoxyHb(r') + oxyHb(r')
\]

(4)

and oxygenation saturation as:

\[
SO_2(r') = \frac{oxyHb(r')}{tHb(r')} \times 100\%
\]

(5)

Equation (5) provides oxygen saturation mapping at each voxel \( r' \), which is not robust at the locations where the tHb concentration is low. Therefore, the maps of \( deoxyHb(r') \) and \( oxyHb(r') \) computed from four wavelengths were used to visualize the deoxy- and oxy- components of the hemoglobin distribution. Furthermore, the mean values of oxyHb and tHb computed within the full width and half maximum from the maximum value of
the corresponding distribution were used to obtain the estimated $SO_2$ of phantom targets as:

$$SO_2 = \frac{\text{mean}(\text{oxyHb})}{\text{mean}(\text{totalHb})} \times 100\%$$  \hspace{1cm} (6)

The estimated or computed $SO_2$ was used to compare with measured $SO_2$ obtained from a \(pO_2\) electrode discussed in the next section.

![Absorption spectra of tissue chromophores in NIR region with highlighted four wavelengths used in reported experiments.](image)

Figure 4-2 Absorption spectra of tissue chromophores in NIR region with highlighted four wavelengths used in reported experiments.

4.2.2 Hypoxia model experiments

Hypoxia model experiments were performed to validate the accuracy of the tumor hypoxia mapping and oxygen saturation quantification using the US-guided DOT technique. In the model experiment, blood phantoms at different controlled oxygenation conditions were imaged by the combined system. Hollow glass spheres of sizes 1.0 cm to 3.0 cm (0.5 cm increment) were customized (Glass Technology Center, University of
Connecticut, Storrs, CT) to hold the blood samples for mimicking different tumor sizes. Blood was collected from 2-3 months old fresh euthanized rats by cardiac puncture and preserved in 7.0 ml EDTA tubes (BD Vacutainer, Franklin Lakes, NJ; K3 EDTA 12 mg blood collection tubes: 8018346). The blood was centrifuged at 2000 rpm for 15 minutes to collect red blood cells (RBC). The supernatants of plasma and serum were aspirated. Similar amount of sterile saline solution was added to RBC and centrifuged again until the supernatant was clearer. The RBCs were then free from the clotting proteins which were originally present in the plasma. For the optical imaging experiment, the RBCs were diluted 4 times with sterile saline solution and the diluted RBCs were filled in the customized glass spheres (2.0 mm openings). The diluted RBCs referred as diluted blood, considered as oxygenated blood, were placed in glass spheres and imaged with the combined system.

The diluted blood was deoxygenated by passing nitrogen gas (Airgas East, Cheshire, CT) at 1.5 psi for 10 minutes in a tonometer. The deoxygenated blood was then transferred to the previously evacuated glass spheres for imaging. Absorption maps were reconstructed at four wavelengths with oxygenated and deoxygenated blood phantoms located at different depths. Following the collection of one set of data at one deoxygenated condition, the blood was transferred to the original tonometer and slowly oxygenated by adding small volumes of oxygen and gently shaking it. Using this method, different intermediate deoxygenated conditions were achieved.

We have taken care to avoid the concentration change caused by water evaporation during gas bubbling. As explained by Cope et al., hemoglobin can become denaturized and precipitate (produce scattering), if it is deoxygenated vigorously or remains
deoxygenated for a long period of time. The concentration change can shift the isobestic point to different wavelengths. For deoxygenation, the blood was filled in the tonometer sealed with a rubber stopper. Two 18 gage needles were attached to the rubber for inlet and outlet of gases. There was an additional port for a pO$_2$ electrode, for measuring oxygen saturation. The nitrogen gas was passed to the tonometer at 1.5 psi and the tonometer was gently shaked for proper mixing. During gas passing precautions were taken not to form bubbles which can evaporate and changes the density of blood hence the absorbance. We have observed this effect when the gas was passed vigorously and kept the gas passing for a long period of time. In our procedure we always limited the gas passing time to 10 minutes. To bring back to oxygenated blood and for getting intermediate oxygen saturation points, air from the environment was injected to the tonometer and the tonometer was shaked gently.

Three different types of model experiments were performed to evaluate our approach; (1) same blood phantom with different oxygenation conditions located at different depths, (2) blood phantoms of different sizes with similar oxygenation conditions, and (3) a smaller target with deoxygenated blood inside a bigger target with oxygenated blood. The third set of experiment emulated the condition of a large cancer where the core of the tumor could be deoxygenated and periphery was oxygenated. All the targets were embedded in a 0.8% homogeneous intralipid medium (Fresenius Kabi, Uppsala, Sweden). The intralipid background was first calibrated before imaging the targets. The oxygen saturation was also measured by a commercial pO$_2$ electrode (DO-166MT-1, Lazar Research Laboratory, CA), and was considered as measured SO$_2$ throughout the paper.
4.2.3 Clinical example

Clinical experiments were performed at the Health Center of the University of Connecticut. The study protocol was approved by the local Institution Review Board (IRB) committee. All patients who participated in our study signed the informed consent. The patients’ data were taken at the lesion area and the contralateral breast of the same quadrant as the lesion. Contralateral data set was used to estimate background optical properties for weight matrix computation. The perturbation was computed between lesion data and contralateral data and used for image reconstruction.

4.3 Results

4.3.1 Model hypoxia experiments

One set of experiment was done with a 2.0 cm target of different oxygen saturations. The target was imaged at depths from 1.5 cm to 3.0 cm with 0.5 cm increments. From the NIR optical spectrum of chromophores, it is well known that oxyHb and deoxyHb show significant differences in absorption coefficients at 740 nm and 830 nm. The reconstructed absorption images at four wavelengths, tHb, oxy- and deoxy- hemoglobin concentrations, for an oxy- and deoxy- blood phantom are shown in Figure 4-3 (A) and (B), respectively. The colorbar represents the hemoglobin concentration in µM. The maximum absorption coefficients reconstructed at four wavelengths for both oxy- and deoxy- blood located at 2.0 cm depth is shown in Figure 4-3 (C), which is in accordance with the standard NIR spectrum. The isobestic point was estimated around 798 nm.
(A)

90
Figure 4-3 (A)-(d) Reconstructed absorption maps for target of SO2 = 92% at different wavelength of 740 nm (a), 780 nm (b), 808 nm (c), and 830 nm (d), respectively. (e) tHb, (f) oxyHb, (g) deoxyHb. Each map has 7 sub-images. Each sub-image shows a spatial x-y absorption distribution at the corresponding depth marked on the title of the sub-image. The depth increment is 0.5 cm. The dimensions of the sub-image are 9 cm by 9 cm. In the following figures, the dimensions of the absorption and hemoglobin maps are the same as Figure 4-3 (A). In addition, the color bars for absorption coefficients were from 0 to 0.22 cm$^{-1}$, and the values for hemoglobin concentrations were from 0 to 100 µM unless noted in the figure caption. (B) Reconstructed absorption maps for target of SO2 = 31% at different wavelength of 740 nm (a), 780 nm (a), 808 nm (a), and 830 nm (a), respectively. (e) tHb, (f) oxyHb, (g) deoxyHb. (C) Measured absorption coefficients at 4 wavelengths under two different oxygen conditions. The isobestic point is estimated at 798 nm.

In this set of experiments, we used equation (6) to estimate the average oxygen saturation from the reconstructed absorption maps and referred it as estimated SO2. The target oxygen saturation was also measured by the SO2 meter prior to and post-experiment and the former measurements were taken as measured SO2. The target oxygen saturation was varied from 14 % to 92 %. The estimated SO2 verses the measured SO2 of a 2.0 cm target located at different experimental depths is plotted in Figure 4-4.
The experiments, with different sets of phantoms, were repeated at a different day to evaluate the consistency of our procedures and variations related to environmental pO$_2$, and both results are plotted as ‘day: 1’ and ‘day: 2’. The estimated values matched well to the measured values (solid dark line) when the targets were at depths of 2.0 and 2.5 cm and the SO$_2$ was within 14-89%. The absolute deviations in the estimated SO$_2$ from the measured values were within 7% (mean error of 3.5% over all the saturation ranges). The deviations were higher when the targets were at shallower depths (1.5 cm) and deeper (3 cm) from the probe. At 1.5 cm depth, the measurement deviations were from 0 to 7% with one exception of 11% deviation at 89% measured SO$_2$, where as for deeper targets (3.0 cm), the deviations were from 2-8% (mean error of 5.5% over all the oxygen saturation ranges). It is noted here that, the errors were larger in reconstructed SO$_2$ if the oxygen saturation was less than 10% (not shown in the figure). This problem was earlier observed in theoretically generated data (large estimation errors for SO$_2$ < 30%) by Heffer et. al.$^{39}$ and experimentally blood phantom data by McBride et. al.$^{38}$ and Srinivasan et. al.$^{25}$ (oxygen saturation was accurate to within 15% for pO$_2$ < 20 mmHg).

We have studied the effects of target size on SO$_2$ estimation. This set of experiment mimicking different development stages of breast tumors. To model this process, glass spheres of diameters from 1.0 cm to 2.5 cm in 0.5 cm increments were imaged at different oxygenation conditions. The top portions of the targets were placed at 1.0 cm depth from the probe. The measured oxygen saturations for targets of different sizes at two different oxygenation conditions are shown in Figure 4-5. The reconstructed values were very close to that of measured values with absolute deviations of 1-5% for 14% measured SO$_2$ and 1-8% for 70% measured SO$_2$. The deviations between reconstructed
and measured SO$_2$ values were the least for targets of 1.5cm and 2.0 cm, and the deviations were slightly higher for 1.0 cm and 2.5 cm targets. From Figure 4-5, it is noted that the SO$_2$ is over estimated for deoxy-targets and underestimated for oxy-targets. For the deoxy-targets, the deoxy-hemoglobin is slightly underestimated because the power of 740nm laser diode in our system is about 60% of that at other three wavelengths. The underestimation of deoxy-hemoglobin causes overestimation of SO$_2$ (see equation 6). For the oxy-targets, the oxy-hemoglobin is underestimated because stronger absorption at 808 nm and 830 nm (dominated by oxy-absorption) causes the saturation in the reconstructed target absorption maps when Born approximation is used.

![Graphs showing measured versus estimated SO$_2$ for different target depths and days.](image)

Figure 4-4 Measured oxygen saturation versus estimated oxygen saturation obtained using equation (6). The 2.0 cm target was located at different depth.
Figure 4-5 Target size versus estimated SO2 obtained at two oxygen saturation conditions.

Figure 4-6 Sketch of the concentric target and experimental set-up.
To emulate the real clinical scenario where the core of the large tumor could be hypoxic, we have made a phantom with a 4.0 cm diameter ball (2.2 cm opening) filled with oxygenated blood. A smaller 2.0 cm diameter ball filled with deoxygenated blood was placed inside the bigger ball (see Figure 4-6). The thickness between the outer shell and the inner core was 0.5 cm. In our previous study of imaging inhomogeneous or heterogeneous absorbing targets, we found that when the thickness between the outer shell and the inner core was less than 1.0 cm and the targets located between depth range of 1-3 cm, the inner core could be visualized. However, when the thickness was larger than 1 cm, the inner core could not be resolved, even the core diameter was relatively large. This is caused by the increased scattering events when photons pass the outer shell of more than 1.0 cm thick and the information loss between the two groups of photons passing outer shell only and passing the outer shell and the inner core. The filled smaller ball was mounted on an 8.0 cm long thin transparent glass rod and introduced into the bigger ball. The 4.0 cm bigger ball was filled with oxygenated blood. A transparent plastic cap with center hole was used to seal the bigger ball. The plastic cap had a transparent ventilated extension (a hollow glass cylinder) at the center, some part to inside and remaining to outside of the bigger ball, helped guiding the smaller ball to stay at the center of the bigger ball. The smaller ball was at the center of the bigger ball when the thin transparent glass rod holding the smaller ball was well fitted inside the transparent ventilated cylindrical extension of the bigger ball. Based on the length of the glass rod coming out of the whole assembly, we were able to control the gap between the top surfaces of the bigger and smaller balls. Ultrasound images were taken to estimate the exact gap between the balls. The measured SO₂ of the outer target was 70% and that of
the inner target was 14%. The 4.0 cm oxy-target was imaged first, and a 2.0 cm deoxy-blood target was introduced after and the combination was imaged again.

The absorption images of the 4.0 cm oxy-target are shown in Figure 4-7 (A) (a-d). Since light at 830 nm is absorbed more by oxy-hemoglobin and light at 740 nm is absorbed more by deoxyHb, the 830 nm image (Figure 4-7 (A) (d)) shows highest contrast and the reconstructed images at all wavelengths show one target. The combined target image at 740 nm (Figure 4-7 (B)) shows that the core has higher absorption than the periphery and the image at 830 nm shows that the periphery is higher than the core. The tHb, oxyHb and deoxyHb maps are shown in Figure 4-7 (B) (e)-(g), where the colorbar represents the hemoglobin concentration in µM. As seen from the maps, the center is higher in deoxyHb map and the periphery is higher in oxyHb map. The quantitative SO₂ using equation 6 is calculated as 64.2%, however, it is not accurate because the distributions of hemoglobin (total, oxy-, deoxy-) are not homogeneous. The maxima of total, oxy- and deoxy- hemoglobin concentrations were 58, 46 and 25 µM. The maximum was measured at the periphery in total and oxyHb maps and at the core in the deoxyHb map. This example demonstrates that our four wavelength imager is capable of mapping out inhomogeneous oxyHb and deoxyHb distributions of the target.

4.3.2 Clinical examples

The first clinical example is shown in Figure 4-8. The patient is a 26-years-old woman having a suspicious solid mass measured 2.2 cm (lateral) x 1.5 cm (depth) by US (a). Near infrared and ultrasound scans were performed before her core biopsy and biopsy result revealed a benign fibroadenoma. The distributions of absorption maps are quite homogeneous for all wavelengths with increasing absorption from 740 to 830 nm (not
shown). The reconstructed maximum absorption coefficients were 0.11, 0.13, 0.15, and 0.16 cm\(^{-1}\) at 740, 780, 808 and 830 nm, respectively. The computed tHb, oxyHb and deoxyHb maps are shown in Figure 4-8 (b)-(d). The color bar is in µM from 0 to 100 µM. The reconstructed maximum and mean tHb were 70.6 µM and 42.2 µM. The lesion is highly oxygenated which can be seen from oxyHb and deoxy maps. Quantitatively, the reconstructed maximum and mean oxyHb were 56.5 µM and 34.8 µM, and corresponding values for deoxyHb were 14.7 µM and 9.0 µM. The estimated SO\(_2\) was 83%. It is noted that when the absorption maps are homogeneous, the sum of maximum and mean oxyHb and deoxyHb are approximately equal to maximum and mean tHb.

(A)
(B)
Figure 4-7 (A) (a)-(d) Reconstructed absorption maps of the 4.0 cm oxy-target at four wavelengths of 740 nm (a), 780 nm (b), 808 nm (c), and 830 nm (d), respectively. The color bars for absorption coefficients were from 0 to 0.12 cm\(^{-1}\). (B) (a)-(d) Reconstructed absorption maps of the concentric target at four wavelengths of 740 nm (a), 780 nm (b), 808 nm (c), and 830 nm (d), respectively. The color bars for absorption coefficients were from 0 to 0.14 cm\(^{-1}\); and the values for hemoglobin concentrations were from 0 to 70 µM.

![Image](image.png)

(a) co-registered US
(b) tHb Concentration
(c) Oxy_hemoglobin
(d) Deoxy_hemoglobin

Figure 4-8 Clinical example of a benign fibroadenoma. (a) Co-registered US showed a suspicious 2 cm solid mass on the right breast of a 26-years-old woman. (b)-(d) computed tHb, oxyHb and deoxyHb maps. The absorption maps at all wavelengths (not shown) and computed hemoglobin concentration maps were homogeneous.

The second clinical example of a stage I breast cancer is given in Figure 4-9 (a). This 61-year old woman had a suspicious 1 cm mass seen by US on her left breast. Near infrared and ultrasound scans were performed before her core biopsy and biopsy result
revealed an intermediate grade invasive carcinoma with ductal and lobular features. The reconstructed absorption maps of 740-808 nm are quite homogeneous, while the distribution of 830 nm shows higher absorption at the periphery (not shown). The computed tHb, deoxyHb and oxyHb maps are shown in Figure 4-9 (b)-(d). As seen from the deoxyHb and oxyHb maps, the tumor core is more deoxygenated and the periphery is more oxygenated. The computed maximum and mean of tHb, deoxyHb, and oxyHb were 99.9 µM and 71.3 µM, 61.8 µM and 42.7 µM, 45.3 µM and 33.5 µM, respectively. The estimated SO₂ was 60% which was much lower than the benign case. It is noted that when the absorption maps are inhomogeneous, the sum of maximum oxyHb and deoxyHb may not approximately equal to maximum tHb, because maximum values of these maps may appear at different spatial locations. The sum of mean values of oxyHb and deoxyHb may or may not equal to mean of tHb depending on the spatial distributions of deoxyHb and oxyHb masses.

The third clinical example of an advanced breast cancer is shown in Figure 4-10. This 64-years-old woman had a highly suspicious 5 x 4 cm mass on her right breast measured 5 cm (lateral) x 3 cm (depth) by ultrasound (a) and 3.9 cm (cranial caudal) x 5.5 cm (transverse) x 4.3 cm (anterior-posterior) by contrast enhanced MRI (b). MRI showed periphery enhancement. Near infrared and ultrasound scans were performed before her core biopsy and biopsy result revealed an advanced high grade infiltrating carcinoma. The reconstructed absorption maps at 740 nm shows higher absorption at the core, while 830 nm map shows higher absorption at the periphery. The 780 nm and 808 nm show absorption distributions between 740 nm and 830 nm. The 360 reconstructed maximum absorption coefficients were 0.19, 0.13, 0.13, and 0.25 cm⁻¹ at 740, 780, 808 and 830 nm,
respectively. The computed tHb, oxyHb and deoxyHb maps are shown in Figure 4-10 (c)-(e). The color bar is in μM from 0 to 70 μM. The oxyHb map showed periphery distribution, while the deoxyHb map showed higher value at the core. The maximum total hemoglobin concentration was 82.6 μM measured at periphery. The maximum oxyHb and deoxyHb were 56.4 μM and 39.8 μM measured at periphery and core respectively. The mean values of tHb, oxyHb, and deoxyHb were 52.5, 35.2 and 27.4 μM and estimated SO2 was 67%. This example demonstrates the unique tumor vasculature and oxygen consumption patterns that can be revealed by our US-guided DOT.

Figure 4-9 Clinical example of a stage I breast cancer. (a) Co-registered US showed a suspicious 1 cm mass on the left breast of a 61-years-old woman. (b)-(d) computed tHb, oxyHb and deoxyHb maps. The absorption maps of 740nm, 780nm and 808nm are quite homogeneous, however, the map at 830nm shows more periphery distribution (not shown). As a result, the computed oxyHb shows more periphery distribution.
Figure 4-10 Ultrasound image of an advanced breast cancer located at the right breast of a 64-years-old woman (a), and MRI image of the cancer (b) marked by the arrow head. (c)-(f) computed 600 tHb, oxyHb, and deoxyHb maps, respectively. The absorption map at 740nm shows higher value at the core; while the absorption at 830nm reveals higher value at periphery (not shown). As a result, the deoxyHb shows a higher value at the core and oxyHb map reveals periphery distribution. The color bars for hemoglobin concentrations are from 0 to 70 μM.
4.4 Discussion and Summary

We validated the US-guided optical tomography with hypoxia model experiments using blood phantoms of different oxygenation conditions, targets of different sizes mimicking different developmental stages of breast tumors. The estimated SO₂ was within 92-100 % of the measured SO₂ by using an invasive pO₂ electrode as a standard. An inhomogeneous concentric blood phantom of deoxygenated center core and oxygenated outer shell was imaged and deoxyHb and oxyHb maps revealed corresponding distributions which correlate with inhomogeneous deoxy- and oxy-distributions frequently seen in large breast cancers. Our results suggest that in addition to quantitative tHb concentration, tumor oxyHb and deoxyHb distributions can add more diagnostic information for distinguishing malignant from benign breast lesions. Our initial findings will need to be further validated by a larger patient population.

From the reconstructed optical absorption maps at four wavelengths, we were able to estimate SO₂ in addition to tHb accurately. Compared with using two wavelengths of 780 nm and 830nm, the target deoxyHb and oxyHb maps obtained from the selected four wavelengths were more robust. There are several factors that may affect the estimated SO₂ obtained from reconstructed absorption maps. Because Born approximation was used, the target absorption coefficient could be under reconstructed due to saturation at the wavelength where the absorption was high under certain oxygen conditions. For example, for oxygenated blood, the reconstructed target absorption was higher at 808 nm and 830nm. The under reconstruction of target absorption at these wavelengths may cause under estimation of SO₂. For the diluted blood we used, the larger error up to 8% occurred at higher SO₂ end beyond 70%. The experimental errors in measuring oxygen
saturation include the time gap between the DOT experiments and SO\textsubscript{2} measurement by electrode. There might be a minor change in oxygen saturation during this time frame. Small reflections from the transparent glass balls might have slightly changed the absorption coefficients causing some minor errors in SO\textsubscript{2} estimation. We have conducted testing with thin transparent balloons, transparent gloves, thin glass balls, and thin clear plastics, but the transparent glass balls were easier for mounting and controlling the oxygenation level while maintaining lower material effect on image reconstruction. Some numbers of RBCs might have died during all the procedures and getting the intermediate SO\textsubscript{2}s, causing different SO\textsubscript{2} in blood environment and RBC molecules. The electrode measured SO\textsubscript{2} of any whole or diluted blood was always less than 92%. By nitrogen passing, we were able to bring the oxygen saturation up to 6%, however our reconstructed optical absorption coefficients never estimated any SO\textsubscript{2} less than 14% for any measured SO\textsubscript{2} at any depths and any target size. This might be due to the relative lower power of our 740nm source than that of the other three wavelengths.

In summary, we have demonstrated the utility of US-guided diffuse optical tomography for mapping tumor deoxyHb and oxyHb concentrations in blood phantoms and in in-vivo patients. Targets of different sizes and located at different depths with different oxygen conditions were used to validate the accuracy of oxygen saturation estimation. The absolute deviations between the estimated hemoglobin oxygen saturations obtained from reconstructed absorption maps and oxygen measurements obtained using a pO\textsubscript{2} electrode were less than 8% over the measured range of oxygen saturation. An inhomogeneous concentric blood phantom of deoxygenated core and oxygenated outer shell was imaged and deoxyHb and oxyHb maps revealed
corresponding distributions which correlated well with inhomogeneous deoxy- and oxy-distributions frequently seen in breast cancers. Such distributions may reveal hypovascular core and hypervascular peripheral enhancement that correspond to tumor angiogenesis development.

References:


5 Targeting Tumor Hypoxia with 2-Nitroimidazole-ICG dye Conjugates

5.1 Introduction

Tumor hypoxia is a major indicator of cancer resistance to chemotherapeutic drugs.\(^1\) Non-invasive imaging of tumor hypoxia has important clinical utility in predicting cancer treatment outcomes and offers the possibility of tailored chemotherapeutic regimens that may enhance the probability of complete tumor remission. Nitroimidazoles are a well studied class of molecular probes that target tumor hypoxia by nucleophilic covalent binding to proteins in environments of low pO\(_2\) (\(<1.5\%\) O\(_2\)).\(^2\)-\(^4\) Among all the imidazole compounds, 2-nitroimidazoles have higher electron affinities and are the most commonly used hypoxia markers when labeled with positron emission tomography (PET) radionuclides.\(^5\),\(^6\) Problems associated with PET radionuclides include low resolution, high background counts and the use of radioactive tracers. In addition, PET systems are expensive for routine clinical use in longitudinal imaging assessments over the course of chemotherapy, thus there is a need for the development of safer, alternative techniques for imaging tumor hypoxia and improving cancer patient outcomes.

The development of fluorescence diffused optical tomography (FDOT) techniques in the near infrared (NIR) spectrum is expected to have significant impact on future personalized oncology treatments owing to the very low tissue autofluorescence and high tissue penetration depth in the NIR spectrum window.\(^7\)-\(^9\) Cancer NIR molecular imaging relies greatly on the development of stable, highly specific and sensitive molecular probes.\(^10\)-\(^15\) Organic dyes such as indocyanine green have been used as non-targeted
agents for optical imaging of cancer and have been used clinically for many years.\textsuperscript{16, 17} By combining these two molecules we have developed a novel nitroimidazole indocyanine dye conjugate (2-nitroimidazole-ICG dye conjugate) for tumor-targeted hypoxia fluorescence tomography.\textsuperscript{18} The \textit{bis}-carboxylic acid ICG derivative was synthesized and used in our studies (hereafter cited as ICG). The 2-nitroimidazole-ICG dye conjugate hypoxia probe has been evaluated in vitro using tumor cells and in vivo using a mouse tumor model to confirm the capability of the novel probe to target and identify hypoxic environments.\textsuperscript{19} In vivo tumor targeting studies in mice showed that the fluorescence signals measured at the tumor site were twice those at the normal site after 150 minutes post-injection of the hypoxia probe. The fluorescence signals measured after injection of ICG alone were the same at the tumor and normal sites indicating a lack of tumor targeting and further proving the importance of the 2-nitroimidazole. In vivo fluorescence tomography images of mice injected with the hypoxia probe showed that the probe remained for more than 5-7 hours in the tumors, however, the images of mice injected with ICG confirmed that the unbound dye washed out in less than 3 hours. These findings were supported with fluorescence images of histological sections of tumor samples using a commercial infrared scanner and immunohistochemistry to independently identify tumor hypoxia. In this paper, we report on the synthesis of a second generation 2-nitroimidazole-ICG conjugate using a more stable piperazine linker to conjugate the 2-nitroimidazole and \textit{bis}-carboxylic acid ICG (piperazine-2-nitroimidazole-ICG) moieties. We have compared the performance of the second generation hypoxia probe with the earlier version and systemically evaluated its sensitivity in an in vivo murine tumor model with tumors located at depths of 1.5 and 2
cm inside turbid medium emulating biological tissue. These studies demonstrate that the new piperazine linker significantly improved in vivo fluorescence signal strength relative to that of the first generation dye synthesized with an ethanolamine linker (ethanolamine-2-nitroimidazole-ICG).

\[ \text{Scheme 1. (a) Methylbromo acetate, TBAI, K_2CO_3, CH}_2\text{CN, 80°C, 45mts, 79% (b) Methanol, ethanolamine, rt, overnight, 60% (c) DMF, PyBOP, DIPEA, HOObt, 2, 0°C-rt, 48hr, 35%.} \]

Figure 5-1 Synthesis Scheme 1.
5.2 Materials and Methods

5.2.1 Synthesis of 2-nitro-ICG dye conjugates

The details of the synthetic procedures used to prepare the dye conjugates, as well as the photophysical and chemical properties and the optical stability of the first generation dye, ethanolamine-2-nitroimidazole-ICG (compound 4), and related compounds have been previously described. Briefly, as shown in Error! Reference source not found., the methyl 2-nitroimidazoleacetate (compound 1) was coupled to ethanolamine to give 2. Subsequent dehydrative coupling with indocyanine dicarboxylic acid 3 gave 4. This work describes the preparation of 2, the synthesis of 3, and coupling procedures to prepare 4. Both the bis-carboxylic acid ICG derivative and the 2-nitroimidazole-ICG have an absorption peak at 755 nm, and a fluorescent emission peak at 778 nm, with a quantum yield of 0.066, which is 5.5 times higher than that commercially available ICG purchased from Sigma-Aldrich. For the studies described in this manuscript, the synthesis was modified to change the ethanolamine linker to a piperazine linker. This modification of the original sequence gave compound 9 (see Figure 5-2). The goal of this modification was to produce a dye-conjugate that was more robust in vivo, with two amide linkages rather than an amide and an ester. As shown in Figure 5-2, the synthesis of compound 9 began by the preparation of the Boc protected piperazine in 35% yield using a literature procedure. Subsequent treatment with bromoacetyl bromide gave compound 6 in 81% yield, and subsequent coupling to 2-nitroimidazole gave 7 in 94% yield. Deprotection of Boc group with TFA gave trifluoroacetyl salt 8 in 86% yield, which was coupled to ICG-bis(carboxylic acid) 3 by
the same coupling procedure used to prepare 4, giving 2-nitropiperazine ICG dye conjugate (9) in 21% yield based on 3.

Figure 5-2 Synthesis Scheme 2.

The piperazine-2-nitroimidazole-ICG, ethanamine-2-nitroimidazole-ICG and bis-carboxylic acid ICG were spectrally characterized by using an UV-Vis spectrophotometer and a fluorescence spectrophotometer (Varian Analytical Instruments,
Walnut Creek, CA). The wavelength range of both spectrophotometers is 250 nm-1100 nm. The relative quantum yield of each dye was calculated as \( \Phi_x = \Phi_{ST} \) (\( \text{Grad}_x/\text{Grad}_{ST} \))\( (\eta_x^2/\eta_{ST}^2)^{23, 24} \) where Grad was the gradient or slope from the plot of the integrated fluorescence intensity vs. absorbance measured at different concentrations, x is any unknown dye and ST is the standard dye, \( \eta \) is the refractive index of the solvent. ICG from Sigma-Aldrich of quantum yield 0.012 \(^{25} \) was used as a standard. The excitation wavelength of 755 nm was used for all quantum yield measurements. Since the standard and unknown dyes were measured in the same solvent, so the refractive index effects were canceled. All the dyes were measured at very low concentration to avoid the self-quenching effects. The extinction coefficient of each dye was measured using a certain amount of dye powder weighed and diluted in known volume of sucrose solution to maintain a fixed concentration (\( \mu \text{M/l} \)). The sample path length was 10 mm. The absorbance was measured in the UV-Vis Spectrophotometer and Beer-Lambart’s law was used to calculate the extinction coefficient from the peak absorbance of the dye with a known concentration. The optical properties of all the compounds are given in Table 1.

Table 1  The optical properties of commercial Sigma-Aldrich ICG, bis-carboxylic acid ICG, ethanolamine-2-nitroimidazole-ICG and piperazine-2-nitroimidazole-ICG

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \lambda_{\text{abs}}^{\text{max}} ) (nm)</th>
<th>( \lambda_{\text{ems}}^{\text{max}} ) (nm)</th>
<th>Extinction Coefficient ( \epsilon ) (M(^{-1}) cm(^{-1}))</th>
<th>Quantum Yield (( \Phi ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICG from Sigma-Aldrich</td>
<td>780</td>
<td>807</td>
<td>115000</td>
<td>0.012</td>
</tr>
<tr>
<td>bis-carboxylic acid ICG</td>
<td>755</td>
<td>790</td>
<td>220920</td>
<td>0.0659</td>
</tr>
<tr>
<td>ethanolamine-2-nitroimidazole-ICG</td>
<td>760</td>
<td>790</td>
<td>159141</td>
<td>0.0590</td>
</tr>
<tr>
<td>piperazine-2-nitroimidazole-ICG</td>
<td>760</td>
<td>790</td>
<td>229543</td>
<td>0.0717</td>
</tr>
</tbody>
</table>
5.2.2 Murine Tumor Model

In vivo tumor imaging experiments were performed using a murine tumor model (4T1 Luc mouse mammary carcinoma cells grown in BALB/c mice). The animal protocol was approved by the Institutional Animal Care and Use Committee of University of Connecticut. 4T1 Luc cells were cultured at 37°C with 5% CO₂ in RPMI 1640 medium (Gibco, USA), supplemented with 10% FBS, 50 U/mL penicillin/streptomycin, 2mM l-glutamine, and 1 mM pyruvate. The 4T1 Luc cells were passaged 3 times at 70-80% confluence in a T75 flask (BD Biosciences, Bedford, MA) prior to injection. 1 x 10⁵ cells were injected into the lower right mammary fat-pad of 7 week old BALB/c female mice. The experiments were performed when the tumor sizes reached approximately 7-9 mm in diameter, 2-3 weeks post-inoculation. Because tumor hypoxia environment depends largely on tumor size, the hypoxia conditions for different groups of mice injected with different dyes should be statistically similar.

5.2.3 FDOT system and tumor imaging

The in vivo experiments were performed using a frequency domain fluorescence imaging system which consisted of 14 parallel detectors and 4 laser diodes of 690, 780, 808 830 nm. Each laser diode was sequentially switched to nine positions on a hand-held probe (see Figure 5-3). 690 nm was used as the excitation wavelength in this study. The 14-channel parallel detection system has two modes: fluorescence mode and absorption mode. The two modes can be easily switched by moving a mechanical handle. A stopper was designed in the system to ensure a precise optical collimation when switching between these two modes. Note that in the fluorescence mode, a bandpass filter was placed in the light path to remove the excitation and stray light; in the absorption mode,
the bandpass filter was moved out of the light path. 14 photomultiplier tubes were used as
detectors, and the received signals were amplified by preamplifiers, mixed by mixers,
low pass filtered, and further amplified before analog to digital converters. Two National
Instrument data acquisition cards of 8-channel each were used to acquire FDOT data.

![In vivo fluorescence imaging set-up](image)

One group of mice was injected intravenously through retroorbital injections with
100 µl of ICG (n=4 mice) at 50 and 25 µM concentrations, respectively, as control, and
the second group was injected with 100 µl of piperazine-2-nitroimidazole-ICG at 50
(n=2), 25 (n=3) and 15 (n=3) µM concentrations, respectively. The last group was
injected with 100 µl ethanolamine-2-nitroimidazole-ICG at 50µM concentration (n=2)
and 25 µM (n=2) to compare with results obtained from
piperazine-2-nitroimidazole-ICG.

Each mouse, anesthesized with inhalation of 1.5% isoflurane, was mounted on a
thin glass plate facing the probe with the lower mammary pads submerged in the
Intralipid solution with a typical soft tissue absorption coefficient $\mu_a = 0.02-0.03$ cm$^{-1}$.
and reduced scattering coefficient $\mu'_s = 6\text{--}8 \text{ cm}^{-1}$. The tumor was imaged for up to 1 minute post-injection and again at 15 minutes, 30 minutes, 1, 2, 3, 5 and 7 hours. The center of the probe was aligned to the center of the tumor and the separation between the tumor center and the probe surface was defined as the imaging depth for fluorescence images. Several imaging data sets were collected to compare the $in\ vivo$ imaging sensitivity of two hypoxia dye conjugates vs. ICG at different concentrations and tumors located at different depths.

### 5.2.4 Fluorescence imaging reconstruction

To reconstruct fluorescence images, the normalized Born approximation has been widely used.\textsuperscript{27,28} This normalization eliminates unknown system parameters, i.e., source strengths, gains of different detectors, background optical properties of the tissue, coupling efficiency to the tissue, etc. This normalized Born ratio was adopted in our early study\textsuperscript{15} and this study and is given as:

$$nB(r_s, r_d) = \frac{\phi(r_s, r_d)}{\phi_{exc}(r_s, r_d)},$$

where $nB$ is the normalized Born ratio, $\phi$ is the fluorescence measurement after subtraction of the system noise measurement without any fluorophores or targets in the background medium, and $\phi_{exc}$ is the excitation measurement at 690 nm. For inversion, a dual-zone mesh method was used to reconstruct the fluorophore concentrations at the target depth and the background regions described previously.\textsuperscript{15}
5.2.5 Immunohistochemistry (IHC)

Hypoxprobe™-1 plus kit from HPI Inc, was used to visualize the hypoxia tumor areas using an IHC technique. Based on the protocol recommended by the company, 45 minutes before the animal was euthanized, 1.5 mg of Hypoxprobe™-1 plus kit diluted in 100 μl of 0.9% saline solution was injected intravenously. Immediately after the animal was euthanized, the tumor was harvested. The tumor specimens were collected and directly frozen in liquid nitrogen until cryosectioned into 10 μm sections. The prepared sections were stored in -80 °C before staining. After thawing, the sections were fixed in cold acetone for 10 minutes. Sections were rinsed and incubated overnight at 4°C with rabbit anti-pimonidazol antisera PA2627 diluted 1:20 in PBS containing 0.1% bovine serum albumin and 0.1% Tween 20. The sections were then incubated for 60 minutes with FITC-conjugated goat anti-rabbit antibody. Between all steps of the staining procedure, the sections were rinsed three times with PBS for 5 minutes each.

Digital images of the stained sections were obtained using an optical microscope at 40× and 400× magnifications. The percentage of hypoxic areas was analyzed using the ImageJ program (National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/). For this purpose, obtained digital images were transferred to the ImageJ software and all color images were converted to the grayscale. Automated routine was used to threshold the images (same threshold for all mice images). The polygon selection tool was used to delineate the boundaries of the hypoxic areas. The percentage of hypoxia was defined as the number of pixels above the threshold in hypoxic areas over the total number of pixels of the total area analyzed.
5.2.6 Fluorescence images of tumor samples

To validate the in vivo FDOT imaging results, excised tumor samples were imaged using a commercially available Odyssey Imaging system (Li-COR Biosciences, Nebraska). 10 μm tumor sample sections were dried and imaged using this system at the highest scan resolution available (i.e. 21 μm). The excitation channel selected was 785 nm and emission was 820 nm with a bandwidth of 40 to 50 nm. The images were obtained using the analysis software provided by the company. The mean pixel value of the images including the entire tumor sample area was measured using Image J.

5.2.7 Experimental details on the synthesis of the dye-conjugates

All chemicals were used as received from Aldrich or Acros. All glassware was flame-dried under vacuum, and all reactions in organic solvents were performed under a nitrogen atmosphere, unless otherwise noted. All solvents were dried according to standard procedures. THF was distilled from sodium benzophenone ketyl, methylene chloride was distilled from calcium hydride, and dimethylformamide was vacuum distilled from calcium hydride. Thin-layer chromatography was done on Sorbent Technologies aluminum-backed TLC plates with fluorescent indicator and 0.2 mm silica gel layer thickness, and either p-anisaldehyde or phosphomolybdic acid were used as developing agents. Column chromatography was done using 60 Å porosity, 32-63 μm silica gel. Both 1H and 13C NMR spectra were collected on a Brüker Avance 300 (300.13 MHz 1H, 75.48 MHz 13C), Brüker DRX-400 (400.144 MHZ 1H, 100.65 MHz 13C) or a Brüker Avance 500 (500.13 MHz 1H, 125.65 MHZ 13C). Chemical shifts are given in ppm (δ), downfield from TMS in the following format: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet), coupling constant in Hz, and
integration. Mass spectroscopy data was collected on a HP 5870B GC/MSD mass spectrometer with an HP-1 column, and high resolution mass spectrometry was done on a Micromass VB-QTOF tandem mass spectrometer. IR spectra were taken on FT/IR-410/C031560585 JASCO and Nexus 670 FT-IR E.S.P under neat conditions unless and otherwise stated. Melting points were taken on a Uni-melt capillary melting point apparatus and Digimelt MPA160 and recorded to a maximum of 270 °C. For products described as waxy solid, melting points could not be obtained.

**tert-Butyl piperazine-1-carboxylate (compound 5):** Di-tert-butyl dicarbonate (2.54 g, 11.6 mmol) in CH₂Cl₂ (30 mL) was added to a stirring solution of piperazine (2 g, 23.2 mmol) in CH₂Cl₂ (60 mL) at room temperature, and stirred for 16 hours. Solvents were removed under reduced pressure and the residue dissolved in water (50 mL). The aqueous solution was extracted with methylene chloride (4 X 60 mL) and all the organic extractions were combined, dried over anhydrous magnesium sulfate and concentrated to get tert-butyl piperazine-1-carboxylate (5) as a colorless oil (1.5 g, 8.05 mmol, 34.7% based on piperazine); ¹H NMR (400 MHz, CDCl₃): δ 3.37 (t, J = 4 Hz, 2 H), 2.79 (t, J = 4 Hz, 2 H), 1.72 (bs, 1 H), 1.44 (s, 9 H) ppm; ¹³C NMR (100.6 Hz, CDCl₃): δ 154.3, 79.8, 45.6, 44.5, 28.8 ppm.

**tert-Butyl 4-(2-bromoacetyl) piperazine-1-carboxylate (compound 6):** Triethylamine (0.5 mL, 3.5 mmol) was added to a stirring solution of tert-butyl piperazine-1-carboxylate (5) (0.60 g, 3.22 mmol) in dry dichloromethane (25 mL) at 0°C and stirred for 15 minutes. Bromoacetyl bromide (0.3 mL, 3.5 mmol) was added dropwise at 0°C, and the resulting mixture was stirred at room temperature overnight. Reaction progress was monitored by TLC, and after disappearance of the starting
material, the mixture was concentrated and purified via column chromatography (petroleum ether: ethyl acetate 15-20%) to give tert-butyl 4-(2-bromoacetyl) piperazine-1-carboxylate (6) as a white solid (0.8 g, 2.6 mmol, 80.7%): Mp: 95-97 °C.

\( ^1H \text{ NMR} (400 \text{ MHz, CDCl}_3): \delta \) 3.85-3.84 (d, \( J = 4 \) Hz, 2 H), 3.59-3.57 (t, \( J = 6 \) Hz, 2 H), 3.49-3.48 (m, 4 H), 3.43-3.40 (m, 2 H), 1.45 (s, 9 H); \( ^{13} \text{C NMR} (100 \text{ MHz, CDCl}_3): \delta \) 165.7, 154.7, 80.7, 46.8, 42.2, 28.6, 25.8; HRMS Calcd C\(_{11}\)H\(_{19}\)BrN\(_2\)O\(_3\) 307.0657, found 307.0652

**tert-Butyl 4-(2-(2-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carboxylate (compound 7):** Sodium hydride (0.025 g, 1.06 mmol) was added to a stirring solution of commercially available 2-nitroimidazole (0.1 g, 0.88 mmol) in dry DMF (1 mL), under a \( \text{N}_2 \) atmosphere at 0 °C, and stirred at 0°C for 30 minutes. At this time, tert-butyl 4-(2-bromoacetyl) piperazine-1-carboxylate (6, 0.272 g, 0.88 mmol) was added slowly and the reaction mixture was stirred at room temperature overnight. The DMF was evaporated and water (5 mL) was added to give a white precipitate, which was filtered and dried in vacuo to give an amorphous white powder, identified as tert-butyl 4-(2-(2-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carboxylate (7) (0.28 g, 0.83 mmol, 94.3%). \( ^1H \text{ NMR} (400 \text{ MHz, CDCl}_3): \delta \) 7.19-7.18 (d, \( J = 4 \) Hz, 1 H), 7.05 (s, 1 H) 5.21 (s, 2 H), 3.60-3.57 (m, 4 H), 3.50 (m, 2 H), 3.46-3.45 (m, 2 H), 1.46 (s, 9 H); \( ^{13} \text{C NMR} (100 \text{ MHz, CDCl}_3): \delta \) 163.4, 154.6, 148.2, 137.4, 121.5, 81.0, 49.0, 45.0, 42.5, 28.6; HRMS Calcd C\(_{14}\)H\(_{22}\)N\(_5\)O\(_3\) 340.1621, found 340.1608.

2-(2-Nitro-1H-imidazol-1-yl)-1-(piperazin-1-yl)ethanone. TFA salt (8): Dropwise addition of trifluoroacetic acid (1 mL) to a stirred solution of tert-butyl 4-(2-(2-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carboxylate (7, 0.28 g, 0.83 mmol) in
dry chloroform (10 mL), was followed by stirring overnight at room temperature. The reaction mixture was concentrated and triturated with ethyl acetate to give 2-(2-nitro-1H-imidazol-1-yl)-1-(piperazin-1-yl)ethanone, TFA (8) as a white solid, which was filtered and dried in vacuo (0.25 g, 0.71 mmol, 85.5%); Mp: 95-96 °C, and used without further purification. \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 7.58 (s, 1 H), 7.40 (s, 1 H), 5.68 (s, 2 H), 4.08 (t, \(J = 4\) Hz, 2 H), 4.03-4.00 (m, 2 H), 3.59 (t, \(J = 4\) Hz, 2 H), 3.50-3.47 (m, 2 H); \(^13\)C NMR (100 MHz, D\(_2\)O) \(\delta\) 166.9, 163.3, 162.9, 162.6, 149.0, 129.2, 128.4, 121.0, 118.1, 115.2, 112.2, 51.3, 43.3, 42.2, 39.6; HRMS Calcd C\(_{10}\)H\(_{14}\)F\(_3\)N\(_5\)O\(_5\) 240.1096, found 240.1149.

Monosodium(II) mono(4-((Z)-2-((2E,4E,6E)-7-(3,3-dimethyl-5-(4-(2-(2-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carbonyl)-1-(4-sulfonatobutyl)-3H-indolium-2-yl)hepta-2,4,6-trienylidene)-3,3-dimethyl-5-(4-(2-(2-nitro-1H-imidazol-1-yl)acetyl)piperazine-1-carbonyl)indolin-1-yl)butane-1-sulfonate) (compound 9): A stirred solution of ICG bis(carboxylic acid) 3 (0.2 g, 0.26 mmol) in dry DMF (2 mL) at 0 °C was treated with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 0.30 g, 0.57 mmol), 1-hydroxybenzotriazole (HOBt, 0.08 g, 0.57 mmol), and diisopropylethylamine (DIPEA, 220 µL, 0.57 mmol) in that order, and stirred at 0°C for 15 minutes. At this time 2-(2-nitro-1H-imidazol-1-yl)-1-(piperazin-1-yl)ethanone. TFA salt (8) (0.204 g, 0.57 mmol) was added and the resulting mixture stirred at room temperature for 48 hours. The DMF was evaporated and the resulting solid was purified using C18 reverse phase column chromatography to give (9) as an amorphous green solid (0.065 g, 0.054 mmol, 20.7% based on 3). \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta\) 7.84 (t, \(J = 12\) Hz,
2 H), 7.57 (s, 2 H), 7.45 (bs, 5 H), 7.35-7.33 (m, 2 H), 7.27 (s, 2 H), 6.57 (t, \( J = 12 \) Hz, 2 H), 6.29 (d, \( J = 12 \) Hz, 2H), 5.57-5.51 (m, 4 H), 4.15 (bs, 4 H), 3.86-3.52 (m, 16 H), 2.97 (t, \( J = 8 \) Hz, 4 H), 1.99-1.97 (m, 4 H), 1.91-1.87 (m, 4 H), 1.61 (bs, 12 H); HRMS, Calcd protonated formula \( \text{C}_{55}\text{H}_{87}\text{N}_{12}\text{O}_{14}\text{S}_{2} \) 1183.4341, found 1183.4399.

5.3 Result

Figure 5-4 shows example fluorescence images obtained by FDOT with mice injected with 25 \( \mu \) M ICG (a), 25 \( \mu \) M ethanolamine-2-nitroimidazole-ICG (b), and 25 and 15 \( \mu \) M piperazine-2-nitroimidazole-ICG (c-d) obtained over 5 to 7 hour period. Each image is 9 cm by 9 cm in x and y spatial dimensions at the corresponding target depth and the color bar represents the reconstructed dye concentration in \( \mu \) M. ICG was completely washed out after 3 hours and the maximum fluorescence concentrations obtained from reconstructed images at 1, 15, 30, 60, 120, 180, and 300 minutes post-injection were 0.044, 0.066, 0.078, 0.033, 0.029 0.025, 0.000 \( \mu \) M, respectively, after subtraction of background value of 0.06 \( \mu \) M. However, for targeted conjugates, the maximum fluorescence concentrations measured post-injection of 25 \( \mu \) M ethanolamine-2-nitroimidazole-ICG were 0.028, 0.037, 0.041, 0.037, 0.036, 0.032, 0.026 and 0.025 \( \mu \) M above the background, and that of 25 \( \mu \) M piperazine-2-nitroimidazole-ICG post-injection were 0.064, 0.131, 0.111, 0.105, 0.072, 0.061, 0.044 and 0.044 \( \mu \) M above the background, obtained at 1,15, 30, 60, 120, 180, 300 and 420 minutes. The improvement of the second generation dye over the first is 2 to 3.5 times during the first 3 hour post-injection period and 1.8 times after 3 hour post-injection. The maximum fluorescence concentration with 15 \( \mu \) M
piperazine-2-nitroimidazole-ICG post-injection was approximately 0.040 \( \mu M \) higher than the background shown in the figure. The ICG fluorescence intensity completely washed out after 3 hours, while the targeted piperazine-2-nitroimidazole-ICG had a strong residue in the tumor after 7 hours. Figure 5-5 (a)-(d) show the corresponding fluorescence images obtained from Odyssey Imaging system of the tumors injected with ICG (a), ethanolamine-2-nitroimidazole-ICG (b), and piperazine-2-nitroimidazole-ICG (c-d). The signal intensity with injection of 2-nitroimidazole-ICG conjugates (b-d) is substantially higher than that of ICG (a), and the intensity of piperazine-2-nitroimidazole-ICG injection is substantially higher than that of ethanolamine-2-nitroimidazole-ICG. Two areas of higher and lower fluorescence intensity in each image obtained with the Odyssey system were selected for comparison to the corresponding tumor hypoxic areas in IHC images revealed with brown staining in 40x and 400x magnifications (middle column: lower hypoxic areas (e-l), right column: higher hypoxic areas (m-t)). Note that, the hypoxia conditions of these four excised tumor samples are statistically the same because the sizes of the tumors are similar.\(^{26}\) However, the sensitivity or signal intensity of different dye conjugates at different concentrations is different and can be seen in these images. Within each image, the higher and lower signal intensity areas correspond to higher (more brown stains) and lower (less brown stains) hypoxic areas. To quantitatively compare the high and low signal intensity areas within each image to hypoxia condition, four windows of size 1\( \times \)1 mm in the high signal intensity areas and four windows of same size in the low intensity areas are chosen and the computed percentage of hypoxic areas are shown in the bottom of Figure 5-5. The high and low signal intensity areas in each sample correlate with measured high and low
hypoxic percentages. In the case of ICG, the signal intensity was too low and the quantitatively comparison was not performed.
Figure 5-4: Fluorescence tomography images of four mice obtained over 5-7 hours. (a) images of mouse with tumor size of 10 mm injected with ICG and monitored over 5 hours. (b) images of mouse with tumor size of 8 mm injected with 25 μM ethanolamine-2-nitroimidazole-ICG and monitored for 7 hours. (c) images of mouse with tumor size of 8 mm injected with 25 μM piperazine-2-nitroimidazole-ICG and monitored for 7 hours. (d) images of mouse with tumor size 8 mm injected with 15 μM piperazine-2-nitroimidazole-ICG and monitored for 7 hours. The time points are marked on images. The background value is 0.06 μM in all images.
Figure 5-5 Top: The corresponding ex-vivo fluorescence images acquired from the Odyssey Imaging system of (a) mouse image injected with 25 \( \mu \text{M} \) ICG. (b) mouse image injected with 25 \( \mu \text{M} \) ethanolamine-2-nitroimidazole-ICG. (c-d) corresponding images of two mice injected with 25 \( \mu \text{M} \), and 15 \( \mu \text{M} \) piperazine-2-nitroimidazole-ICG, respectively. (e)-(h) corresponding IHC stains (40x, brown) at low tumor hypoxic area as marked at corresponding images, and (m-p) corresponding IHC stains (40x) at higher hypoxic area. (i-l) corresponding IHC stains at low hypoxic area (400x, brown) and (q-t) corresponding stains at higher hypoxic area. Scale bar is 1mm in images. Bottom: Percentage of hypoxic area from low fluorescence intensity area (green) and higher intensity area (red) of samples (b)-(d).

Figure 5-6 shows the comparison of average maximum fluorescence concentration of three groups of mice injected with ICG (n=2), ethanolamine-2-nitroimidazole-ICG (n=2) and piperazine-2-nitroimidazole-ICG (n=3) in 25 \( \mu \text{M} \) concentration. The statistical significance was achieved between piperazine-2-nitroimidazole-ICG and ethanolamine-2-nitroimidazole-ICG, and piperazine-2-nitroimidazole-ICG and untargeted ICG beyond 60 minutes post injection. The exponential fitting of the washout period of each dye is also shown in the figure. This figure demonstrates that the second generation dye conjugate had much higher fluorescence signal strength than that of the first generation dye. On average, the ratios of fluorescence concentration of piperazine-2-nitroimidazole-ICG injected tumors were 2.4 times higher within 3 hour post-injection period than that of ethanolamine-2-nitroimidazole-ICG injected tumors, and 1.7 times higher beyond 3 hour post-injection. The kinetics of the two conjugates is also different. The fluorescence signal from piperazine-2-nitroimidazole-ICG quickly reached maximum at 15 minutes post-injection and then declined and remained flat with approximately half-life \( (t_{1/2}) \) of 6–7 hours. While the signal of ethanolamine-2-nitroimidazole-ICG reached maximum at 30 minutes post-injection and then slowly reduced and remained flat with approximately \( t_{1/2} \) of 9-10 hours. The
fluorescence signal of ICG reached maximum at 30 minutes post-injection and then quickly washed out with approximately $t_{1/2}$ of 30 minutes. For both targeted dye conjugates, the optimal window to assess tumor hypoxia is beyond 3 hour post-injection when free untargeted ICG is washed out completely.

![Figure 5-6 Comparison of reconstructed fluorescence concentration (maximum) vs. time (minutes) of 25 μM ICG, ethanolamine-2-nitroimidazole-ICG and piperazine-2-nitroimidazole-ICG with tumor center located at 1.5 cm. The exponential fitting of the washout period of each dye is also shown.]

We conducted another set of experiment with three sets of mice injected with ICG ($n=2$), first generation ($n=2$) and second generation conjugate ($n=2$) in 50 μM concentration. On average, the ratios of fluorescence concentration of piperazine-2-nitroimidazole-ICG injected tumors were 2.4 times higher within 3 hour post-injection period than that of ethanolamine-2-nitroimidazole-ICG injected tumors,
and 1.6 times higher beyond 3 hour post-injection. The measured kinetics of each dye conjugate is the same as reported above for 25 $\mu$M injection.

One experiment was performed by imaging the piperazine-2-nitroimidazole-ICG injected mouse 24 hours later to evaluate how long the dye may remain in the tumor. Figure 5-7 shows the FDOT images obtained post-injection from one minute to 24 hours. The fluorescence signal strength after 3 hour post-injection remains at similar levels. Although our observation is limited, this example does suggest that the piperazine-2-nitroimidazole-ICG may remain for a longer period of time in the tumor which allows for in vivo targeting of tumor hypoxia.

![Fluorescence tomography images of one mouse obtained over 24 hours.](image)

Figure 5-7 Fluorescence tomography images of one mouse obtained over 24 hours. The mouse with tumor size 10 mm injected with 25 $\mu$M piperazine-2-nitroimidazole-ICG and monitored over 24 hours. The time points are marked on images.

To further evaluate the sensitivity of piperazine-2-nitroimidazole-ICG, we have conducted two sets of experiments with mice injected with 25 (n=3), and 15 $\mu$M (n=3) concentrations and tumors located at 1.5 and 2.0 cm depths, respectively. The average
fluorescence signals and the standard deviations are shown in Figure 5-8. For both concentrations at both depths, the targeted piperazine-2-nitroimidazole-ICG remains in tumor area beyond 3 hours. At a deeper depth of 2 cm, both concentrations yield same level of fluorescence signals beyond 3 hours. Thus, the tumor depth did not affect tumor imaging.

![Figure 5-8](image)

Figure 5-8 Average reconstructed fluorescence concentration (maximum) vs. time (minutes) of piperazine-2-nitroimidazole-ICG injection at 25 μM and 15 μM concentration with tumors located at depth of 1.5 cm (blue and red) and 2 cm (black and green).

To quantify the hypoxia conditions of two different groups of mice injected with ICG and dye conjugates, light microscopy (40x) images of the IHC samples were analyzed with Image J software. The mean hypoxia percentage for the piperazine-2-nitroimidazole-ICG, ethanolamine-2-nitroimidazole-ICG and ICG groups
was 2.29% (±0.72), 2.2% (±0.54), 1.98(±0.30), respectively. As expected, there is no
statistical difference in tumor hypoxia conditions between the three groups indicating the
tumor model is consistent. However, the tumor size measured from the excised sample
was found to strongly correlate with the percentage of hypoxia\(^2\) as shown in Figure 5-9
for all tumor samples (Pearson correlation = 0.888, p<0.001). Similarly, the fluorescence
images from the Odyssey system of the entire tumor sample were also analyzed by the
Image J software. Figure 5-10 shows the mean fluorescence signals and standard
deviations of the three sets of mice injected with ICG, first and second generation
conjugates in 25 (Figure 5-10 (a)) and 50 \(\mu\)M (Figure 5-10 (b)) concentrations. In
Figure 5-10 (a), the mean signal strengths were 3.0 (±0.98), 21.7(±3.82), and 37.7 (±2.22),
respectively. The signal strength of second generation dye over the first is 1.7 times, and
the second and first generation dyes over ICG are 12.6 and 7.2 times. In Figure 5-10 (b),
the mean fluorescence signals are 7.45 (±0.91), 29.6 (±11.38), and 48.6 (±1.55),
respectively. On average, the fluorescence signal strength of second generation dye over
the first is 1.6 times, and second and first generation dyes over ICG are 6.5 and 4.0 times,
respectively. These results from histological sections imaged with the Odyssey Imaging
system scanner support the in vivo findings.

5.4 Discussion and Summary

One method to improve tumor imaging is to improve the bioavailability\(^{30,31}\) of the
dye conjugate. A piperazine unit is common in many drugs\(^{31}\) so we incorporated a
piperazine linker with the goal of increasing bioavailability\(^{32}\) of the dye conjugate. We do
not believe that the piperazine per se exhibits any influence on the hypoxia targeting.
However, if the bioavailability of the dye-conjugate leads to a greater concentration in the
tumor, presumably enzymatic reduction of the nitro group will lead to an increased concentration of the dye-conjugate. We believe that the observed greater distribution of piperazine-2-nitroimidazole-ICG in tissue may be due to the larger number of carbon atoms and slightly diminished polarity which increases solubility in tissue when compared to the ethanolamine-2-nitroimidazole-ICG. Increased solubility would lead to a greater percentage of dye reaching the tumor, along with other tissues, however selective hypoxia binding of the dye should lead to a larger percentage of dye-conjugate in the tumor relative to other tissues, which would be measured as greater long-lasting fluorescence intensity. Indeed we have observed increased fluorescence intensity of excised mouse tissues, such as liver and kidney, injected with piperazine ICG as compared with the tissues injected with ethanolamine-2-nitroimidazole-ICG, as well as enhanced tumor imaging.

Figure 5-9 Correlation of measured tumor size vs. percentage hypoxia area for all tumor samples ($r=0.888$, $p<0.001$)
Figure 5-10 (a). The mean fluorescence signals and standard deviations of the three sets of mice injected with ICG, ethanolamine-2-nitroimidazole-ICG and piperazine-2-nitroimidazole-ICG in 25 µM concentrations. The mean signal strengths were 3.0(±0.98), 21.7(±3.82) and 37.7 (±2.22), respectively. (b). The mean fluorescence signals and standard deviations of the three sets of mice injected with ICG, ethanolamine-2-nitroimidazole-ICG and piperazine-2-nitroimidazole-ICG in 50 µM concentration. The mean fluorescence signals were 7.45(±0.91), 29.6(±11.38), and 48.6(±1.55), respectively.

References:

7. V. Ntziachristos, "Going deeper than microscopy: the optical imaging frontier in biology." Nat Meth. 7(8), 603-614 (2010).


6 Two-step imaging a two-layer tissue structure of breast lesions by optical tomography using genetic algorithm

6.1 Introduction

Diffuse optical tomography (DOT) in near-infrared (NIR) is a promising noninvasive approach for functional breast imaging.\textsuperscript{1-15} However, the resolution and lesion localization are poor due to the intense light scattering in tissue, the resolution and lesion localization are poor. Additionally, the inverse problem of DOT is ill-posed and the solution is not unique. To improve the quantification accuracy of DOT, a proved practical method is to provide a priori information provided by a coregistered modality, such as MRI, X-ray and ultrasound (US), has been investigated and proved to be a practical method for the clinical translation\textsuperscript{4, 5, 7-12} With the location information from the guidance, the DOT image reconstruction of a suspicious lesion in the semi-infinite turbid medium could acquire much higher accuracy. In general, this model is an accurate approximation for the reflection geometry when the patient in a supine position. But when the breast tissue thickness is less than 2 cm thickness, the contribution from the chest wall underneath to the total reflectance measurement could not be ignored. In this case, instead of the semi-infinite model, a layered structure instead of the semi-infinite model which could estimate the background properties more precisely is needed for better reconstruction.\textsuperscript{16-19}

In our group, we discussed several issues generated by chest wall issue and established different models and approaches to solve the inversion problem, such as the analytical solution for a two-layer tissue structure with a tilted interface\textsuperscript{20} and the
finite-element method to deal with the reference mismatch problem. In this paper, to get better estimation of the layered background properties, we introduced a small probe with short source-detector distance to calibrate the first layer parameters and then using the known values of the first layer to fit the second layer properties. In this way, the unknown variables reduced to half and the results were more accurate and robust. In addition, we develop a two-step reconstruction using genetic algorithm (GA) and conjugate gradient (CG) method to reconstruct the absorption and scattering distributions simultaneously from a two-layer tissue structure. Simulations and phantom experiments are were performed to establish the new reconstruction method, and similar clinical examples are were applied to validate the real utility of this new imaging method.

6.2 Methodology

6.2.1 Two-layer tissue model

Due to the shallow tissue upon the chest wall of the small breast patients in the reflectance geometry with a supine position, the chest wall effects for the lesion reconstruction could not be ignored. The semi-infinite model cannot produce accurate estimation of the lesions for such patients. Therefore, a two-layer tissue model was established to fit this need. Figure 6-1 shows the structure of the two-layer model. The cylinder with much bigger diameter compared with the lesion was modeled as the medium which had two layers of different absorption and scattering coefficients. The first layer mimicked the breast tissue of a small breast with less than 2.5 cm in thickness and the second layer accorded to the chest wall effect with 7 cm in depth as a semi-infinite layer. The lesion was embedded in the first layer.
6.2.2 Estimation of the background properties

To estimate the optical properties of the two-layer medium, a 4-parameter analytical fitting was developed in our previous work.\textsuperscript{17} Briefly, by solving the two-layer structured diffusion equations, the fluence rate at the tissue surface was related to the absorption and scattering coefficients of the two tissue layers:

\[ \phi(r, \omega) = f(\mu_{a1}, \mu_{s1}', \mu_{a2}, \mu_{s2}') , \]

where \( \phi(r, \omega) \) is the fluence rate, \( \mu_{a1}, \mu_{s1}', \mu_{a2} \) and \( \mu_{s2}' \) are absorption and reduced scattering coefficients of the two tissue layers. A nonlinear optimization method based on the Nelder-Mead simplex algorithm\textsuperscript{20, 23} was used to simultaneously fit the amplitude and phase to calculate the background properties of the two layers. To improve the accuracy of the estimation, we modified this method to a 2-parameter fitting by calibrating the \( \mu_{a1} \) and \( \mu_{s1}' \) of the first layer before fitting using a small probe with short source-detector distance (Figure 6-2 (c)). In this way, the first layer properties could be concerned as known, so that the variables to be fitted decreased to half (\( \mu_{a2} \) and \( \mu_{s2}' \)).
Figure 6-2 (a) Experimental setup of the new dual-probe imaging system. (b) and (c) Probe geometry used for simulations and phantom experiments. (b) the clinical probe of 1 cm thickness and the center slot is used for US transducer. (c) the small probe with short source-detector distance used for the estimation of the first layer parameters.

6.2.3 Two-step inversion with GA

A two-step reconstruction method using GA was developed for the lesion tomography in the semi-infinite medium recently in our previous work. In brief, GA was used to obtain the initial properties of the lesions for the reconstruction followed. First, an initial population is generated, formed by a certain individuals while each
member is a possible solution of the optimization problem and can be represented by a vector of real numbers. These individuals then undergo a set of genetic operations — selection, crossover and mutation — in order to promote the population evolution. The error of each individual is defined as:

\[ Er = \|U_{sc} - WX\|^2 \]  

\[ X = \begin{bmatrix} \int_v \delta \mu_a dv \\ \int_v \delta D dv \end{bmatrix} = \begin{bmatrix} \int_v (\mu_a - \mu_{a0}) dv \\ \int_v (\mu_s - \mu_{s0}) dv \end{bmatrix} \]  

where \( U_{sc} \) is the measurement, \( W \) is the weight matrix and \( \mu_{a0}, \mu_{s0} \) are the background absorption and reduced scattering coefficients, respectively. The fitness of each individual is defined as \( 1/Er \). When the improvement in the fitness of members is less than \( 10^{-6} \), or the generation number exceeds 100, the generation of the populations is stopped and the select values for the unknown parameters of the model are obtained. After that, CG reconstruction method with the initial guess from GA is applied to get the absorption and scattering distributions. To adjust this method to two-layer model, the weight matrix \( W \) in equation (2) is calculated as \( W_A = G(r_{ij}, r_{di}) \Phi_0(r_{ii}, r_{ij}) / D_I \) and \( W_S = -\nabla G(r_{ik}, r_{di}) \nabla \Phi_0(r_{ii}, r_{ik}) / D_I \), where \( W_A \) and \( W_S \) are weight matrices for the absorption and scattering, respectively; \( D_I \) equals \( D_1 \) or \( D_2 \), depending on the voxel location in the layered model; \( r_v \) represents the voxel location, \( r_s \) is the source position and \( r_d \) is the detector position. In equation (3), \( \mu_{a0}, \mu_{s0} \) are modified to \( \mu_{a01}, \mu_{s01} \) and \( \mu_{a02}, \mu_{s02} \), which are the background properties for the first and the second layer got from the 2-parameter fitting, respectively. After the initial values were obtained from
GA, they were applied to the CG reconstruction to get the final tomography for the lesion.

6.2.4 Simulation and experiments

In simulations, a commercial finite-element (FEM) package COMSOL, which is a powerful FEM analysis, solver and simulation software\textsuperscript{21, 24} was employed to solve the forward diffusion equation in frequency domain.\textsuperscript{21, 25} 140 MHz modulation frequency was used in all simulations. A cylinder of 20 cm diameter consisted of two layers, the first layer was 1.0 to 2.5 cm and the second layer was 7 cm was used to model the two-layer tissue medium (Figure 6-1), and 9 sources and 14 detectors were distributed on the surface in reflection geometry as shown in Figure 6-2 (b). A 1 cm diameter sphere represented the lesion was embedded in the first layer. To calculate the lesion perturbation, a corresponding reference data was generated with the same two-layer structure but with no target inside.

In phantom experiments, the frequency domain system we used consisted of 14 parallel detectors and 4 laser diodes of wavelength 740nm, 780nm, 808nm and 830nm, respectively. The experimental setup with the dual-probe system is shown in Figure 6-2 (a). Each laser diode was sequentially switched to 9 positions on the probe. The central slot on the probe shown in Figure 6-2 (b) was used to fit the ultrasound transducer, and the sources and detectors were distributed on both sides. Figure 6-2 (c) shows the small probe with center source used to estimate the first layer properties.

To make the two-layer structured phantom, the Indian ink and intralipid solution with controlled absorption and scattering coefficients of worked as the first layer were controlled by Indian ink and intralipid solution, and soft phantoms were placed under the
solution as the second layer. In order to validate the simulations, we tried to make the similar conditions for each layer. To control the properties of the first layer, the small probe (Figure 6-2 (c)) was merged into a big bottle of the solution to get quick calibration and adjust the amount of Indian ink and intralipid until reaching the required absorption and scattering coefficients we needed. Phantoms of the second layer were made using polyvinyl chloride-plastisol (PVCP) solution, which was a white opaque solution and became translucent when it was heated to a high temperature.\(^{26}\) When the solution was gradually heated, the Indian ink and titanium dioxide (TiO\(_2\)) powder were added to control the optical absorption and scattering coefficients of the phantom. The heated solution was poured into a 10 cm diameter bowl and solidified after cooling for several hours.

Clinical experiments were performed with the system of similar electronic and optical design as that used for phantom experiments. The study protocol was approved by the local Institution Review Board (IRB) committee. All patients who participated in our study signed the informed consent. The patients’ data were taken at the lesion area and the contralateral breast of the same quadrant as the lesion. Contralateral data set was used to estimate background optical properties for weight matrix computation. The perturbation used for imaging reconstruction was computed between lesion data and contralateral data and were used for imaging reconstruction.
6.3 Results

6.3.1 Optical property estimation of the two-layer structure

6.3.1.1 Simulations

In simulation, the structure showed in Figure 6-1 with two different homogenous layers but no target involved was considered as the background. The 2-parameter fitting described in section 6.2.2 was used to estimate the optical properties of the second layer (The first layer was assumed as known). Three conditions of the first layer and two conditions of the second layer (see Table 1) were simulated for 1.0, 1.5, 2.0, 2.5 cm thickness of the first layer, respectively. Figure 6-3 shows one fitting example of the first layer with 1.5 cm thickness in condition 1 and the second layer in condition 1. We used the true value of the first layer as initial value and the fitting results for the second layer were \( \mu_a = 0.12 \text{ cm}^{-1} \) and \( \mu_s' = 6.7 \text{ cm}^{-1} \). The fitting accuracy of the second layer in all the conditions is listed in Table 1, on average, the fitting error was lower than 5% for \( \mu_a \) and 23% for \( \mu_s' \) when the second layer in condition 1 and that was lower than 17% for \( \mu_a \) and 42% for \( \mu_s' \) when the second layer in condition 2, see Figure 6-4.

Table 1 The fitting accuracy of the second layer properties in all the conditions of the simulations.

<table>
<thead>
<tr>
<th>First layer (cm(^{-1}))</th>
<th>Second layer (cm(^{-1}))</th>
<th>1) ( \mu_a = 0.1, \mu_s' = 6.0 )</th>
<th>2) ( \mu_a = 0.2, \mu_s' = 6.0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) ( \mu_a = 0.02, \mu_s' = 4.0 )</td>
<td>( \mu_a )</td>
<td>97.9 %</td>
<td>85.0 %</td>
</tr>
<tr>
<td></td>
<td>( \mu_s' )</td>
<td>76.3 %</td>
<td>63.2 %</td>
</tr>
<tr>
<td>2) ( \mu_a = 0.05, \mu_s' = 6.0 )</td>
<td>( \mu_a )</td>
<td>97.5 %</td>
<td>86.3 %</td>
</tr>
<tr>
<td></td>
<td>( \mu_s' )</td>
<td>93.4 %</td>
<td>66.1 %</td>
</tr>
</tbody>
</table>
3) \( \mu_a = 0.05 \), \( \mu_s ' = 9.0 \)

<table>
<thead>
<tr>
<th>( \mu_a )</th>
<th>( \mu_s ' )</th>
<th>Absorption</th>
<th>Scattering</th>
</tr>
</thead>
<tbody>
<tr>
<td>90.0 %</td>
<td>61.3 %</td>
<td>77.7 %</td>
<td>43.4 %</td>
</tr>
</tbody>
</table>

Figure 6-3 Fitting curves of the second layer properties versus iteration in simulation. (a) fitted absorption coefficient versus iteration (b) fitted reduced scattering coefficient versus iteration for the second layer with 1.5 cm thick first layer of \( \mu_a = 0.02 \) cm\(^{-1} \), \( \mu_s ' = 4.0 \) cm\(^{-1} \).

Figure 6-4 The fitting error bars of the second layer properties versus different thickness of the first layer in all condition combinations for the simulations. (a) the fitted absorption coefficients (b) the fitted reduced scattering coefficients with the first layer thickness of 1.0, 1.5, 2.0 and 2.5 cm. The dashed lines are the true values of the fitted properties.
6.3.1.2 Phantom experiments

In phantom experiment, the calibrated intralipid solution (first layer) was poured onto the soft phantom (second layer) to construct the two-layer structure and the thickness of the first layer was defined by the US image. For better validation, the first layer had the same calibrated properties as the simulation and the two phantoms used for the second layer had similar values of \( \mu_a = 0.1 \text{ cm}^{-1}, \mu_s' = 9.0 \text{ cm}^{-1} \) and \( \mu_a = 0.2 \text{ cm}^{-1}, \mu_s' = 8.0 \text{ cm}^{-1} \), respectively. We tested all the conditions in simulations at all the depths from 1.0 cm to 2.5 cm. The results were consistent to our expectation that the thicker of the first layer was, the more accurate the estimation of the first layer is more accurate we got when the first layer was thicker (Figure 6-5). For instance, for the same combination of the first layer of \( \mu_a = 0.02 \text{ cm}^{-1}, \mu_s' = 4.0 \text{ cm}^{-1} \) and the second layer of \( \mu_a = 0.1 \text{ cm}^{-1}, \mu_s' = 9.0 \text{ cm}^{-1} \), the estimated \( \mu_a \) for the first layer were 0.06, 0.05, 0.03 and 0.023 cm\(^{-1}\) for the thickness of 1.0, 1.5, 2.0 and 2.5 cm, respectively. After the estimation of the first layer, the clinical probe with US guided was used to collect the data of the homogenous layers. The estimation from the small probe was set as the initial values for the first layer and the properties of the second layer could be fitted by the 2-parameter fitting. Figure 6-6 shows one fitting results for the second layer when the first layer of estimated \( \mu_a = 0.02 \text{ cm}^{-1}, \mu_s' = 4.0 \text{ cm}^{-1} \) and the true second-layer properties of \( \mu_a = 0.1 \text{ cm}^{-1}, \mu_s' = 9.0 \text{ cm}^{-1} \). The thickness of the first layer was 1.5 cm. The initial values of the first layer from the small probe estimation was \( \mu_a = 0.05 \text{ cm}^{-1}, \mu_s' = 4.1 \text{ cm}^{-1} \) and the fitting results for the second layer were \( \mu_a = 0.103 \text{ cm}^{-1} \) and \( \mu_s' = 9.2 \text{ cm}^{-1} \). The fitting accuracy of the second layer in all the conditions is listed in Table 2, on average, the
fitting error was lower than 15.7% for $\mu_a$ and 37.2% for $\mu_s'$ when the second layer of $\mu_a = 0.1$ cm$^{-1}$, $\mu_s' = 9.0$ cm$^{-1}$ and that was lower than 8% for $\mu_a$ and 47.9% for $\mu_s'$ when the second layer of $\mu_a = 0.2$ cm$^{-1}$, $\mu_s' = 8.0$ cm$^{-1}$, see Figure 6-7.

Table 2 The fitting accuracy of the second layer properties in all the conditions of the phantom experiments.

<table>
<thead>
<tr>
<th>First layer (cm$^{-1}$)</th>
<th>Second layer (cm$^{-1}$)</th>
<th>1) $\mu_a = 0.1$, $\mu_s' = 9.0$</th>
<th>2) $\mu_a = 0.2$, $\mu_s' = 8.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu_a$</td>
<td>88.3 %</td>
<td>80.0 %</td>
</tr>
<tr>
<td></td>
<td>$\mu_s'$</td>
<td>75.3 %</td>
<td>70.0 %</td>
</tr>
<tr>
<td>1) $\mu_a = 0.02$, $\mu_s' = 4.0$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) $\mu_a = 0.05$, $\mu_s' = 6.0$</td>
<td>$\mu_a$</td>
<td>80.0 %</td>
<td>83.3 %</td>
</tr>
<tr>
<td></td>
<td>$\mu_s'$</td>
<td>61.9 %</td>
<td>53.8 %</td>
</tr>
<tr>
<td>3) $\mu_a = 0.05$, $\mu_s' = 9.0$</td>
<td>$\mu_a$</td>
<td>73.3 %</td>
<td>82.5 %</td>
</tr>
<tr>
<td></td>
<td>$\mu_s'$</td>
<td>56.9 %</td>
<td>64.6 %</td>
</tr>
</tbody>
</table>

Figure 6-5 The estimated properties of the first layer versus layer thickness. (a) the estimated absorption coefficient (b) the estimated reduced scattering coefficient with the layer thickness of 1.0, 1.5, 2.0 and 2.5 cm, respectively. The dashed lines are the true values of first layer properties.
Figure 6-6 Fitting curves of the second layer properties versus iteration in phantom experiment. (a) fitted absorption coefficient versus iteration (b) fitted reduced scattering coefficient versus iteration for the second layer with 1.5 cm thick first layer of $\mu_a = 0.02 \text{ cm}^{-1}$, $\mu_s' = 4.0 \text{ cm}^{-1}$.

Figure 6-7 The fitting error bars of the second layer properties versus different thickness of the first layer in all condition combinations for phantom experiments. (a) the fitted absorption coefficients (b) the fitted reduced scattering coefficients with the first layer thickness of 1.0, 1.5, 2.0 and 2.5 cm. The dashed lines are the true values of the fitted properties.
6.3.2 Lesion imaging using two-step GA reconstruction method

6.3.2.1 Simulations

In simulation, the targets of 1.0 cm diameter were located at 1.0 cm center depth in the first layer with thickness of 1.5 cm and 2.0 cm. Since 1.0 cm thickness was too shallow to merge the target and the imaging probe together and 2.5 cm thickness was deep enough to ignore the chest wall effect, we did not include them into the simulation and phantom experiments of lesion imaging. In this set, we simulated the targets with absorption coefficient \( \mu_a = 0.16 \text{ cm}^{-1} \) and different scattering coefficients: low \( \mu_s' = 6.0 \text{ cm}^{-1} \) and high \( \mu_s' = 15.0 \text{ cm}^{-1} \), in the two-layer structure with all condition combinations listed in Table 1. The fitted maximum absorption and reduced scattering coefficients of the targets in all conditions by GA were plotted in . Then these

![Graph](image)

Figure 6-8 The fitted maximum (a) absorption coefficient (b) reduced scattering coefficient of the simulated targets located in 1.0 cm center depth using GA in all condition combinations of the two-layer structure with 1.5 and 2.0 cm first layer thickness.
Figure 6-9 simulated (a) absorption (b) reduced scattering coefficient distributions of the target with $\mu_a = 0.1 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$ located in the 1.0 cm center depth of the first layer. The first layer thickness is 1.5 cm and with $\mu_a = 0.02 \text{ cm}^{-1}$, $\mu_s' = 4.0 \text{ cm}^{-1}$ and the second layer is with $\mu_a = 0.1 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$.

Fitted parameters were applied as the initial values to the CG based two-layer reconstruction method. The target with $\mu_a = 0.16 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$ embedded in the two-layer medium with the first layer of $\mu_a = 0.02 \text{ cm}^{-1}$ and $\mu_s' = 4.0 \text{ cm}^{-1}$ and the second layer of $\mu_a = 0.1 \text{ cm}^{-1}$ and $\mu_s' = 6.0 \text{ cm}^{-1}$ was taken as an example. The target
center depth was 1.0 cm and the thickness of the first layer was 1.5 cm. The fitted initial parameters from GA were $\mu_a = 0.147$ cm$^{-1}$ and $\mu_s' = 4.86$ cm$^{-1}$ and the reconstructed values after CG were $\mu_a = 0.154$ cm$^{-1}$ (96%) and $\mu_s' = 6.5$ cm$^{-1}$ (108.3%). The absorption and scattering distributions are shown in . On average, the accuracy of the reconstructed $\mu_a$ for the targets was higher than 88.3% and that of the reconstructed $\mu_s'$ was 106% for true $\mu_s' = 6.0$ cm$^{-1}$ and 76.7% for true $\mu_s' = 15.0$ cm$^{-1}$. Using the new two-layer modeled reconstruction method with GA fitting, we could simultaneously reconstruct $\mu_a$ and $\mu_s'$ of the targets with decent accuracy.

6.3.2.2 Phantom experiments

Figure 6-10 The fitted maximum (a) absorption coefficient (b) reduced scattering coefficient of the phantom targets located in 1.0 cm center depth using GA in all condition combinations of the two-layer structure with 1.5 and 2.0 cm first layer thickness.
Figure 6-11 (a) coregisted US image. (b) absorption (c) reduced scattering coefficient distributions of the target with $\mu_a = 0.18 \text{ cm}^{-1}$ and $\mu'_s = 11.0 \text{ cm}^{-1}$ located in the 1.0 cm center depth of the first layer in the phantom experiment. The first layer thickness is 1.5 cm with $\mu_a = 0.02 \text{ cm}^{-1}$, $\mu'_s = 4.0 \text{ cm}^{-1}$ and the second layer is with $\mu_a = 0.2 \text{ cm}^{-1}$ and $\mu'_s = 8.0 \text{ cm}^{-1}$.

Phantom experiments were performed to validate the simulations. Two targets, one with $\mu_a = 0.18 \text{ cm}^{-1}$ and $\mu'_s = 11 \text{ cm}^{-1}$ and another one with $\mu_a = 0.14 \text{ cm}^{-1}$ and $\mu'_s = 15 \text{ cm}^{-1}$ were tested in the two-layer turbid medium. Same as simulation, the initial values of $\mu_a$ and $\mu'_s$ of the targets were fitted by GA algorithm, and then these parameters were applied into the two-layer modeled reconstruction method. The GA fitted parameters were shown in . shows the reconstruction images of an example that the target with $\mu_a = 0.18 \text{ cm}^{-1}$ and $\mu'_s = 11 \text{ cm}^{-1}$ embedded in a 1.5 cm thick intralipid
solution layer of $\mu_a = 0.02$ cm$^{-1}$ and $\mu_s' = 4.0$ cm$^{-1}$ with a phantom of $\mu_a = 0.2$ cm$^{-1}$ and $\mu_s' = 8.0$ cm$^{-1}$ as the second layer underneath. The fitted initial parameters from GA were $\mu_a = 0.21$ cm$^{-1}$ and $\mu_s' = 10.07$ cm$^{-1}$ and the reconstructed values after CG were $\mu_a = 0.204$ cm$^{-1}$ (113%) and $\mu_s' = 10.38$ cm$^{-1}$ (94.4%). (a) is the coregistered US image, (b) is the target absorption map and (c) is the scattering map. On average, the accuracy of the reconstructed $\mu_a$ for the target of $\mu_a = 0.18$ cm$^{-1}$ was 105.7% and for $\mu_a = 0.14$ cm$^{-1}$ was 95.6%, and that of the reconstructed $\mu_s'$ was 101.5% for true $\mu_s' = 11$ cm$^{-1}$ and 108.6% for true $\mu_s' = 15$ cm$^{-1}$.

### 6.3.3 Clinical example

A clinical example with chest wall effect is given in Figure 6-12. US image of the suspicious mass seating on the chest wall marked by the white arrows is shown in Figure 6-12 (a). The center of the lesion is 1.5 cm and the surface of the chest wall is 2.0 cm in depth approximately. First, the small probe was used to estimate the breast tissue (first layer) properties, which were $\mu_a = 0.04$ cm$^{-1}$ and $\mu_s' = 3.8$ cm$^{-1}$. And then, the properties of the chest wall (second layer) were fitted as $\mu_a = 0.26$ cm$^{-1}$ and $\mu_s' = 8.8$ cm$^{-1}$. After that, using the 2-layered structure and the estimated layer properties as background, the initial values of the lesion were fitted by GA, which were $\mu_a = 0.06$ cm$^{-1}$ and $\mu_s' = 10.2$ cm$^{-1}$. Finally, the initial guesses from GA were applied into the CG reconstruction method to obtain the absorption and scattering tomography of the lesion in 780 nm, as shown in Figure 6-12 (b) and (c). The maximum reconstructed $\mu_a$ is 0.08 cm$^{-1}$ and $\mu_s'$ is 8.3 cm$^{-1}$, which indicate a benign case.
Figure 6-12 A clinical example. (a) co-registered US image. The chest wall is pointed out by the white arrows. (b) absorption (c) reduced scattering coefficient distributions of the suspicious mass located in the 1.5 cm center depth in 780 nm.

6.4 Discussion and summary

A new two-step imaging model with the two-layer structure was established to improve the reconstruction accuracy of the small breast imaging with the chest wall effect. Simulations and the phantom experiments were performed to validate this method and the clinical example demonstrated its utility potential. A newly designed dual probe imaging system is used to obtain more accurate background properties from the breast tissue and the chest wall. A smaller probe with short source-detector distances is used to estimate the breast tissue properties (first layer) and then the regular clinical probe is used to acquire the data from both breast tissue (first layer) and chest wall (second layer). With the estimated first layer properties as known, the second layer properties could be fitted using the analytical solution with 2 parameters ($\mu_a$ and $\mu_s'$ of the second layer). To compare with
the previous fitting method from our group, see Ref. 17, 4 parameters ($\mu_a$ and $\mu_s'$ of the first layer and the second layer, respectively) are fitted in the same time from the data acquired from the regular clinical probe, the newly dual probe imaging method is faster and more accurate. We have an example to show the comparison. A same set of data from phantom experiment is used, which has the true values of $\mu_a = 0.02 \text{ cm}^{-1}$, $\mu_s' = 7.0 \text{ cm}^{-1}$ for the first layer and $\mu_a = 0.1 \text{ cm}^{-1}$, $\mu_s' = 9.0 \text{ cm}^{-1}$ for the second layer. In Figure 6-13, it is showed the fitting results using the previous 4-parameter fitting method. The fitted second layer properties are $\mu_a = 0.11 \text{ cm}^{-1}$ and $\mu_s' = 4.1 \text{ cm}^{-1}$. The absorption coefficient is reasonable but the scattering coefficient is far lower than the true value. When the new method is used, the first layer properties was first estimated by the small probe, which were $\mu_a = 0.025 \text{ cm}^{-1}$ and $\mu_s' = 7.2 \text{ cm}^{-1}$ (see Figure 6-14). Using this result as the known parameters, the second layer properties were fitted using the 2-parameter method as $\mu_a = 0.11 \text{ cm}^{-1}$ and $\mu_s' = 9.1 \text{ cm}^{-1}$. It is obvious that the new method could obtain much higher fitting accuracy for the scattering coefficient than the previous 4-parameter fitting. Additionally, the new method could converge within 70 iterations and that of the previous method is 250 iterations, which is about 3.5 times faster.

Two concerns in this paper we think are necessary to address here are the first layer thickness choosing and the interface distortion between breast tissue and the chest wall from the patients. First is the first layer thickness. For our experiment setup, the patients are in the supine position, so the breast is squashed by the imaging probe to be closer to the chest wall. For the small patients, the breast tissue layer is shallow, thus, the chest wall effect cannot be ignored. As the study for the thickness range which the chest wall
could affect the estimation of the first layer in Figure 6-5, the conclusion is when the first layer thickness is larger than 2.5 cm, no matter how absorbing the second layer is, the influence for the first layer is subtle. Therefore, we design the simulation and the phantom experiments with the first layer thickness no more than 2 cm. Second is the effect of the distortion between these two layers. The interface between the breast tissue and the chest wall is not always flat which may introduce some reconstruction error. In the Ref. 21, the possible distortion and mismatch were discussed sufficiently. Here, we concentrated on the new imaging method and model for the two-layer structure. Thus, we did not consider the distortion. In the future real clinical practice, we could simply apply the correction into our new two-step imaging model for the cases which needed.

Figure 6-13 The fitted properties of the two-layer versus iteration using the 4-parameter analytical solution fitting method. (a) and (c) fitted absorption coefficient versus iteration
of the first layer and the second layer, respectively (b) and (d) fitted reduced scattering coefficient versus iteration of the first layer and the second layer, respectively. The first layer thickness is 1.5 cm.

Figure 6-14 The fitted properties of the second layer versus iteration using the true values of the first layer. (a) fitted absorption coefficient versus iteration (b) fitted reduced scattering coefficient versus iteration for the second layer with 1.5 cm thick first layer of $\mu_a = 0.02 \text{ cm}^{-1}$, $\mu_s' = 7.0 \text{ cm}^{-1}$

In summary, a new two-step imaging model is set up for the two-layer tissue structure of breast imaging. A dual probe system is used to estimate the background properties of the two-layer structure, which could reach higher accuracy. The genetic algorithm is applied to fit the initial values of the absorption and reduced scattering coefficients of the lesion. After that the conjugate gradient method is used to reconstruct the distributions.

References:


7 Conclusion

Breast cancer is the most common cancer and the second major cause of cancer death for women. The screening tries to detect the breast cancer in early stage, which could save more lives. Diffuse optical tomography (DOT) in near-infrared (NIR) spectrum is a promising noninvasive approach for functional diagnostic breast imaging. Our group has explored the use of ultrasound (US)-guided DOT to improve the lesion localization and light quantification accuracy. In this dissertation, several special cases of breast cancer imaging are studied with the US guided DOT technique.

In the first study, a series simulation and phantom experiments was performed to systemically evaluate the effects of target parameters, target locations, and target optical properties on imaging periphery enhancement absorption distribution using reflection geometry. Large tumors were modeled as concentric semi-ellipsoidal targets of different outer shell and inner core optical properties. We have shown that larger targets of more than 3-4 cm diameter with outer shell thickness less than 1 cm can be resolved at depth less than 3 cm. A clinical example is given to show the complex vasculature distributions seen from an advanced cancer.

In the second study, the clustered breast lesions imaging was introduced. Clustered small breast lesions may be present in the neighboring areas which are difficult to resolve and quantify accurately in diffuse optical tomography. In addition, advanced breast cancers are often accompanied by clustered satellite lesions in the neighboring areas, which are also difficult to resolve and quantify. To improve the light quantification of clustered lesions, a multi-zone reconstruction algorithm guided by co-registered
ultrasound image was investigated using simulations and phantoms. The performance of the algorithm was demonstrated using clinical examples. This method separated one larger region of interest (ROI) into several ROIs based on the location information provided by co-registered ultrasound images. In general, the single ROI method cannot resolve two smaller targets when their separations were less than 2.5 cm and the target depth was greater than 2.0 cm. The highest reconstruction accuracy using a single ROI method was 52% for high contrast small dual targets. The multi-zone reconstruction method with the assistance of co-registered ultrasound improved both the resolving ability and reconstruction accuracy. As a result, two targets located at 2.5 cm depth with separation greater than 2.0 cm could be distinguished. With respect to reconstruction accuracy at all depths and separations investigated, the multi-zone method reached 91% highest accuracy for high contrast small dual targets.

In the third study, an US-guided DOT for mapping tumor deoxy-hemoglobin (deoxyHb) and oxy-hemoglobin (oxyHb) concentrations in blood phantoms and in in-vivo patients was presented. Because oxyHb and deoxyHb respond differently at different wavelengths, four laser diodes of wavelengths 740 nm, 780 nm, 808 nm and 830 nm were used in the study. Tumor model experiments were performed using phantoms of different hemoglobin oxygen saturations (14% - 89%) representing hemoglobin oxygenation in tissue. Targets of different sizes and located at different depths were used to validate the accuracy of oxygen saturation estimation. The absolute deviations between the estimated hemoglobin oxygen saturations obtained from reconstructed absorption maps and oxygen measurements obtained using a pO₂ electrode were less than 8% over the measured range of oxygen saturation. An inhomogeneous concentric blood phantom of deoxygenated
center core and oxygenated outer shell was imaged and deoxyHb and oxyHb maps revealed corresponding distributions which correlated well with inhomogeneous deoxy-
and oxy- distributions frequently seen in breast cancers. Clinical examples are given to demonstrate the utility of US-guided optical tomography in mapping heterogeneous
deoxyHb and oxyHb distributions in breast cancers.

In the fourth study, the synthesis of a 2-nitroimidazole-indocyanine green conjugate using a piperazine linker (piperazine-2-nitroimidazole-ICG) capable of robust fluorescent imaging of tumor hypoxia was described. In vivo mouse tumor imaging studies were completed and demonstrate an improved imaging capability of the new dye relative to an earlier version of the dye that was synthesized with an ethanolamine linker (ethanolamine-2-nitroimidazole-ICG). Mouse tumors located at imaging depths of 1.5 and 2.0 cm in a turbid medium were imaged at various time points after intravenous injection of the dyes. On average, the reconstructed maximum fluorescence concentration of the tumors injected with piperazine-2-nitroimidazole-ICG was two-fold higher than that injected with ethanolamine-2-nitroimidazole-ICG within 3 hour post-injection period and 1.6-1.7 times higher beyond 3 hour post-injection. The untargeted bis-carboxylic acid ICG completely washed out after 3 hour post-injection. Thus the optimal window to assess tumor hypoxia is beyond 3 hour post-injection. These findings were supported with fluorescence images of histological sections of tumor samples and an immunohistochemistry technique for identifying tumor hypoxia.

In the last study, a two-step imaging model is set up for the two-layer tissue structure of breast imaging. First, a small probe with short source-detector pairs is used to collect the diffuse light only from the first layer tissue of the regular breast; Then, a
clinical imaging probe is used to collect the perturbation data from lesion breast and the reference from the contralateral breast. With the relatively accurate information of the first layer, the optical properties of the second layer are fitted. After that, a two-step imaging reconstruction using genetic algorithm is performed for the two-layer model with the fitted bulk tissue properties. Absorption and scattering distributions of the lesions in the layered structure could be reconstructed simultaneously. Simulation and phantom experiments show promising accuracy and clinical examples are applied to demonstrate the utility of this imaging method.
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