DIFFUSE OPTICAL TOMOGRAPHY: IMAGING MULTIPLE STRUCTURAL AND FUNCTIONAL FEATURES

By

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To my dear mom
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<td>Blood Oxygen Level Dependent</td>
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<td>CBF</td>
<td>Cerebral Blood Flow</td>
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<td>CT</td>
<td>Computed Tomography</td>
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<td>EEG</td>
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<td>FEM</td>
<td>Finite Element Method</td>
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<tr>
<td>Hb</td>
<td>DeoxyHemoglobin</td>
</tr>
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<td>HbO2</td>
<td>OxyHemoglobin</td>
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<td>DOT</td>
<td>Diffuse Optical Tomography</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NIR</td>
<td>Near-Infrared</td>
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<td>PCDOT</td>
<td>Phase-contrast Diffuse Optical Tomography</td>
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<td>PET</td>
<td>Positron-emission Tomography</td>
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<tr>
<td>RI</td>
<td>Refractive Index</td>
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<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<td>SO2</td>
<td>Oxygen Saturation</td>
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Diffuse Optical Tomography has drawn more and more interests in the biomedical field over the recent couple of decades due to its ability to noninvasively recover not only tissue structural information but also functional and molecular properties. The contrasts that optical parameters could demonstrate in DOT are usually higher than those of the conventional methods. Based on these contrasts, different approaches had been developed applying DOT for imaging, and so far lots of efforts were spent on detecting breast cancer by imaging tissue absorption and scattering coefficients as well as hemoglobin concentration and oxygen saturation level.

In this work, we tried to expand the ability of DOT in breast cancer detection by introducing Phase-contrast diffuse optical tomography (PCDOT). PCDOT uses near-infrared diffusing light to non-invasively reconstruct tissue refractive index (RI) distribution. RI depends on the tissue’s physical and chemical properties and previous study revealed that it might serve as a promising imaging parameter in breast cancer detection. We’ve first developed a 2-step method to improve the PCDOT image both qualitatively and quantitatively at single-wavelength; then we’ve introduced a multispectral PCDOT algorithm to more efficiently reconstruct RI simultaneously with
other tissue functional parameters and attempted to improve this algorithm by different structural regularization methods.

Measuring hemodynamic changes, oxygen delivery and cerebral blood flow is important for locating and interpreting pathological variations associated with epileptic disorders. We then further expanded the application of DOT by presenting a method of dynamic, noninvasive and functional diffuse optical brain imaging that is conducted simultaneously with hippocampus CA1 local field potential recordings for anesthetized rats under resting conditions and during acute chemoconvulunt provoked seizures. By illuminating the scalp with near-infrared light and recovering, the backward scattered light were collected and three-dimensional (3D) absolute tissue optical absorption images with high temporal resolution were obtained using a finite-element based reconstruction algorithm. The measured tissue absorption changes were validated with optic-intrinsic-signals measurement. In the focal seizure model, the seizure focus could be identified using the technique denoted by local variations of tissue absorption level as well as hemoglobin and cerebral blood flow changes. The findings are consistent with general observations in seizures of significant local cerebral metabolism increase. Successive absorption images along with EEG signals demonstrated linearity relationships from the neurovascular coupling study, suggesting cerebral metabolism closely matches demand from neuronal changes. This preclinical study suggests that this technique is feasible to be applied to human study and can provide insights into brain function and mechanisms of seizure disorders.
CHAPTER 1
INTRODUCTION

1.1 History of Optical Imaging

Diffuse optical tomography (DOT) uses non-ionizing radiation to non-invasively reconstruct both structural and functional properties of tissue by applying tomographic measurements of near-infrared (NIR) diffusive light along the tissue boundary. Optical imaging was called transillumination or diaphanography at early time applied for breast imaging, in which the white light shine on a breast directly and the shadow of the breast was captured [1]. Later on, people use the red light and near infrared light (NIR, 600~800nm wavelength) for optical imaging since photons at these wavelengths could penetrate deeper into soft tissue [2].

Optical imaging made significant progress during the last two decades due to the advanced mathematical models of optical transportation (see section 1.4 for details of the models). Meanwhile, developments of modern computers provide an easy-accessed platform where the heavy computation of the mathematical models could be conducted, and advanced CPU and recent application of GPU further accelerated the computation process. Also, more advanced optical measurement tools become available in recent decades, such as photodiodes, photomultiplier tube, and CCD cameras. They are able to trace even single photons, detect the amplitude and phase of light, and thus provide accurate measurements that can lead to better quality imaging.

DOT, as a promising imaging technique, has been widely investigated in areas of breast cancer detection and functional brain imaging [3-7]. There are three major types of DOT systems, including time domain, frequency domain and continuous wave (CW) [8-11]. While time- and frequency-domain based systems may provide more optical
information than CW measurements, CW has been favored by some researchers due to its low cost and hardware simplicity and has been proven to be feasible in both phantom and in vivo clinical data [5, 11-15].

1.2 Breast Cancer and Optical Imaging

Breast cancer is the second most common type of cancer after lung cancer worldwide. Similar to other type of cancers, breast cancer is caused by the cancer cells that change and grow out of control, invading into normal tissue and spread throughout the body. Breast cancer begins in breast tissue, which is made up of lobules and the ducts that connect the lobules to the nipple. When constrained within the lobules and ducts where breast cancer initiated, the cancer stage is called in situ. The majority of in situ breast cancer is ductal carcinoma in situ (DCIS), which accounted for ~83% of local breast cancer diagnosed during 2004-2008, while lobule carcinoma in situ (LCIS) accounted for ~11% of female in situ breast cancer diagnosed [16]. The cancer cells may metastasize to other tissues of the body through lymphatics or blood as they develop. The seriousness of breast cancer is closely related to the stage of the disease. In clinical settings, the TNM system classifies tumors by tumor size and how far it spread within the breast and nearby organs (T), lymph node involvement (N), and the presence or absence of distant metastases (M). The earlier the cancer is detected, the less likely that the cancer cells would metastasize and the higher possibility that the cancer to be cured. According to the American Cancer Society, in 2011, an estimated 230,480 new cases of invasive breast cancer will be diagnosed among women and approximately 39,520 women are expected to die from breast cancer. In addition, about 2,140 cases of breast cancer are expected to occur among men [16].
A tumor or lump in breast could be palpable when grows to centimeter size but the specificity of lesion detection using the physical examination is only ~ 20% to 30%. As the predominant conventional approach for breast cancer detection, annual mammography screening had been introduced to public. Despite its advantages, X-ray mammography has low specificity and relatively lower sensitivity as the breast density increases [17, 18]. And mammography would put patients under risk with exposure to X-ray radiations. Thus other conventional techniques are developed to improve the specificity and sensitivity of mammography, among which magnetic resonance imaging (MRI) showing the most significant improvement but remaining costly [19] and therefore not being suitable for routine breast screening. Positron emission tomography (PET) has good specificity in detecting breast cancer but has low spatial resolution and high cost as well.

DOT is introduced as a noninvasive alternative for conventional breast detection methods which only depend on structural alterations. Unlike mammography where patients would be exposed to radiation, DOT has no side effects which make it possible to become a routine screening approach with its low cost. DOT is able to image both functional and morphological information of tissue, including the concentration of oxy-hemoglobin, deoxy-hemoglobin, and water based on the absorption spectra; particle size and density distribution based on the scattering spectra. The major imaging contrast of optical imaging of breast cancer resulted from the tumor-related angiogenesis [20], which triggers increased optical absorption [21, 22]. And metabolic imbalance of oxygen in tumors would also cause tissue hypoxia, leading to contrasts of oxygen saturation images [23]. The difference between abnormal and normal tissues
could reach 100% in the NIR region due to the increased hemoglobin concentration [24]. The blood volume in abnormal breast tissue can reach 400% compared to the normal tissue due to the increased blood vessels and dilations [25].

To date, there had been a lot of studies on imaging breast cancer using DOT and promising results had been reported [26-31]. While DOT shows high sensitivity towards breast cancer detection, it has a limited specificity [5, 12, 32-35]. In an effort to overcome this limitation, we exploited phase-contrast DOT (PCDOT) [36] by adding the possibility of reconstruction tissue refractive index (RI) due to the fact that phase contrast is generated by the spatial variation of tissue RI and tissue RI depends on the tissue’s physical and chemical properties. Yet phase contrast has been featured in optical microscopy [37, 38], phase-contrast computed tomography (CT) [39, 40], ultrasound tomography [41], and optical coherent tomography (OCT) [42]. From recent OCT measurements of RI in human-ductal-carcinoma-simulated animal cancer models, significantly differentiated RI is demonstrated between tumor and normal tissues [43]. Our multispectral PCDOT algorithm combined the RI recovery with other important functional parameters to further improve the quality of PCDOT and to attenuate the crosstalks between RI and absorption/scattering related coefficients, thus provided a more reliable criteria for the breast cancer detection.

1.3 Epilepsy and Optical Imaging

Epilepsy is a common chronic neurological disease involving recurrent, unprovoked seizures that affects approximately 3% of the human population during their lifetime[44]. Seizures are caused by the synchronous, rhythmic firing of a population of neurons, lasting from seconds to minutes. About 60-70% of patients experienced focal or partial seizures while another 30-40% of patients have generalized seizures [45].
Epilepsy in about 70% of patients could be controlled by medication [46]. While for medically refractory seizures, resection of the epileptogenic zone may be considered and thus a series of presurgical evaluation will be taken to assess brain structure abnormality and clinical feature of seizures.

For patient with new onset seizures, neuroimaging helps to determine whether the seizure is acute provoked or unprovoked and if any immediate treatment need to be taken [47]. Determination of whether there’s an underlying brain lesion is one of the primary steps in evaluating new-onset seizure disorders. Common causes of acute seizures are brain tumor, perinatal hypoxic or hypoxemic events and malformations of cortical development (MCDs) for young children, head trauma for young adults and stroke for elderly people [48]. Potentially epileptogenic lesion detected by neuroimaging would affirm focal seizure disorders and differentiate them from primary generalized seizure disorders, which are based on either genetic or idiopathic rather than a focal epileptogenic lesion. The distinction between these two types of seizure serves as the major standard for antiepileptic medication selection [49]. Medically intractable epilepsy is defined as unsatisfactory seizure control despite optimized sequential use of 2 or more appropriate antiepileptic drugs [47]. Epilepsy surgery is performed in patients with medically intractable epilepsy to improve seizure control and quality of life. A presurgical evaluation of patients with intractable epilepsy is applied to determine whether a patient has a single epileptogenic focus and to localize the epileptogenic zone. The epileptogenic zone is the cortical region that is indispensable for the generation of seizures and that has to be removed to render a patient seizure free. The epileptogenic zone is a theoretical construct, which is defined in terms of different cortical zones [50].
The seizure-onset zone is the region in which the seizures actually originate. The symptomatogenic zone is the (sub)cortical region producing ictal symptoms. The functional deficit zone is the part of cortex with an abnormal function in/between seizures, due to morphological or functional factors [51].

Acquiring brain structural and functional information with imaging is essential in the diagnosis and management of epileptic seizure disorders. As the primary structure imaging approach, magnetic resonance imaging (MRI) detects surgically relevant lesions in up to 80% of patients who undergo temporal lobectomy [52] and in about 60% of those who undergo frontal lobe surgery [53]. MRI could locate the lesion and surrounding structures accurately and identify structures known to serve critical brain functions. Another advantage of MRI over conventional computed tomography (CT) is that it allows 3D structural images. However, there are also many patients with active epilepsy in which structural imaging shows no abnormality. Thus advances have been made in functionally imaging the abnormal cerebral patho-physiology associated with epileptic seizures. Major functional imaging modalities for epilepsy include functional MRI (fMRI), positron emission tomography (PET) [54-57], ictal single-photon emission computed tomography (SPECT) [58] and magnetic resonance spectroscopy (MRS).

Along with hemoglobin changes, cerebral blood flow (CBF) and oxygen consumption (OC) changes resulting from functional activations are all important components of the hemodynamic response in epileptic seizure disorders. Studying the functional information in vivo would provide insights in to the seizure generation and propagation while contributing to clinical seizure localization and understanding of complex metabolic and neuronal changes which these functional variations are inner
related to. Knowledge of the coupling between neuronal activity and the associated hemodynamic response would also bring potential to predict seizure onset preceded its onset and thus benefit diagnosis and treatment of epilepsy [59]. Therefore, there has been a highly increasing interest in the subject of neurovascular coupling [60].

On the other hand, malformations of cortical development (MCD) have been considered significantly as a major cause of seizures [61], which would demonstrate imaging contrast in cellular morphology imaging. Thus simultaneous imaging of the spatiotemporal characteristics of particle size/density, hemoglobin change, OC, CBF, and the cerebral metabolic rate of oxygen (CMRO2) becomes important. As the primary functional image modality, fMRI detects the blood oxygenation level depend (BOLD) signal that is associated with neuronal activities noninvasively [62]. With the linear transform model, fMRI had been shown that the time course and amplitude of which could be predictable from the underlying neuronal responses and the fMRI variations could be colocalized with the underlying neuronal activity [63]. However, the fact that signal from BOLD fMRI depends on mixtures of blood flow, blood volume and blood oxygenation instead of a specific physiologic feature makes it hard to be quantified; the relatively low temporal resolution of fMRI would also disables it from detecting important temporal information during the fast-act brain activities in seizures; and moreover, ictal fMRI would not be routinely feasible clinically since the required cooperation level of fMRI scan might be hard to achieve for epileptic patients.

Optical imaging based on the changes in light absorption of active neural tissue has been applied to exposed cortex to provide in vivo mapping of clinically relevant epileptiform events, such as interictal spikes, ictal onsets, ictal spread and secondary
homotopic foci in animal models [64, 65]. In the near infrared (NIR) region, NIR spectroscopy (NIRS) uses the ability of light in the near-infrared region of 600 to 1000 nm wavelengths to penetrate tissue to depths of 8-10 cm, and could allow continuous and noninvasive monitoring of cerebral oxygen demand and supply by absorption spectra of oxyhemoglobin \((HbO2)\) and deoxyhemoglobin \((Hb)\) in cerebral blood vessels [66]. In recent years there has been an increased interest in using diffuse optical tomography (DOT) for noninvasively imaging the human head and brain. DOT uses near infrared light that can pass through structures such as skull, penetrating the brain and obtain images of the tissue optical properties and thus assess hemodynamic changes like regional blood volume and hemoglobin oxygen saturation. DOT can reconstruct 3D volumetric images of the head tomographically and hence can identify changes occurring in deeper tissues.

Cerebral changes have been imaged by DOT in animal models during hypercapnia [67], focal ischemia [68, 69] and fore-paw stimulation [70]. Infant brain images during passive motor activation have been reported [71, 72]. Recently, DOT was used to visualize the localized dynamics changes during seizure in rats that the seizure onset zone is localized by local dynamic changes of \(HbO2\), \(Hb\) and total hemoglobin \((HbT)\) concentrations during the ictal period [73]. Compared to fMRI which is only sensitive to changes in deoxyhemoglobin [74, 75], separate calculation of \(HbO2\) and \(Hb\) could be done from DOT measurements, and the sum of the changes in the concentrations of these two species additionally provides measurement of total hemoglobin concentration change, which is proportional to the cerebral blood volume change [76]. Besides, DOT is able to reach a temporal resolution <100ms, which gives
this technology advantage over fMRI [77]. Though the spatial resolution of DOT is low (but still comparable to that of fMRI), DOT has the advantage of being inexpensive and portable, and therefore it is possible to conduct DOT scanning bedside. The ability of DOT to retrieve 3D tissue functional and molecular properties noninvasively distinguishes it from intrinsic optical signal imaging where incision is required for imaging [78]. Epileptogenic foci induce a reduction of interictal glucose metabolism [79] while epileptic seizures increase cerebral metabolite dramatically coupled with cerebral vessels dilation [80]; change of glucose concentration would cause tissue RI variation and the level of contrast is high enough to be sensitively detected by diffuse optical tomography (DOT) [81]. Compared to 18F-fluorodeoxyglucose positron emission tomography (FDG-PET), which is the most commonly used technique for glucose-metabolism imaging, DOT is inexpensive and portable, so that it could possibly be conducted bed-side and used for continuous monitoring. Besides, the use of FDG-PET is limited to the interictal period due to the short half of the tracer and its relatively long period of cerebral uptake (cerebral uptake of FDG took place over 40 minutes after the injection [82]) whereas DOT won’t have such limitation.

In our previous work [73] we have reported in vivo 2D images of \textit{HbO2}, \textit{Hb}, and \textit{HbT} in the rat brain during seizure onset using DOT. This pilot study suggested that DOT may be useful for epilepsy imaging due to its ability to dynamically localize epileptic foci and to map functional activities. However, this previous work was based on a DOT system with low temporal resolution (min per frame) and thus could not provide detailed interpretation of neuronal activity. In this current study, we present in vivo 3D images of focal seizure obtained from a newly developed fast DOT system (700ms per
frame) in reflection-mode. The new DOT system also allows concurrent
electrophysiology (EEG) recordings during seizure onset. Moreover, the neurovascular
coupling study conducted demonstrates linearity of the tissue absorption (measured by
DOT) response to brain neuronal activities. The neurovascular coupling relationship
observed from DOT was cross-validated by optic-intrinsic-signal imaging. To our
knowledge, this is the first study considering quantitative neurovascular coupling during
the seizure ictal periods using DOT.

1.4 Optical Diffusion Theory

Boltzmann transport equation describes incoherent photon propagation through
highly scattering media. The time domain equation is described as following:

\[
\frac{n \, \partial L}{c \, \partial t} + \nabla \cdot \nabla L + \left( \nabla \cdot \Omega \right) L + \frac{1}{n} \left[ \nabla \cdot n - \left( \nabla \cdot \nabla \Omega \right) \right] \cdot \nabla L =
\]

\[-(\mu_a + \mu_s) L + \mu_s \int_{4\pi} f(\Omega, \Omega') L(\hat{r}, \Omega', t) d\Omega' + \epsilon(\hat{r}, \Omega, t) \quad (1-1)\]

where \( n(\hat{r}) \) is the spatially varying RI distribution, \( L(\hat{r}, \Omega, t) \) is the radiance which is
defined as the amount of energy perpendicular to the unit vector \( \Omega \) (\( L \) is measured in
unit \( \text{Wm}^{-2} \)), \( \mu_a \) is the absorption coefficient per unit length, \( \mu_s \) is the scattering
coefficient per unit length, \( f(\Omega, \Omega') \) is the scattering function which gives the probability
that an energy packet travelling in direction \( \Omega' \) travelling into direction \( \Omega \), \( f \) is normalized
according to \( \int_{4\pi} f(\Omega, \Omega') d\Omega' = 1 \), \( \epsilon(\hat{r}, \Omega, t) \) is a source distribution per unit volume.
However, the equation must be simplified to be mathematically manageable usually. By
expanding the equation with spherical harmonics and truncate the series at the \( N^{th} \)
term (\( P_N \) approximation), the quantities in Equation 1-1 could be expressed as:

\[
L(\hat{r}, \Omega, t) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \left( \frac{2l+1}{4\pi} \right)^{1/2} \psi_{l,m}(\hat{r}, t) Y_{l,m}(\hat{s}) \quad (1-2)
\]
\[
\varepsilon(\hat{r}, \hat{\Omega}, t) = \sum_{l=0}^{\infty} \sum_{m=-l}^{l} \left( \frac{2l+1}{4\pi} \right)^{1/2} \varepsilon_{l,m}(\hat{r}, t) Y_{l,m}(\hat{s}) \tag{1-3}
\]

\[
f(\hat{s}, \hat{s}') = \sum_{l=0}^{\infty} \left( \frac{2l+1}{4\pi} \right)^{1/2} f_{l} P_{l}(\cos \theta) \tag{1-4}
\]

where \(\left( \frac{2l+1}{4\pi} \right)^{1/2}\) is the normalization factor, \(Y_{l,m}\) is the spherical harmonic of order \(L\) at degree \(m\), \(P_{l}\) is the Legendre polynomial of order \(L\).

The \(P_{1}\) approximation is obtained and the following equations are generated:

\[
\left( \frac{1}{c} \frac{\partial}{\partial t} + (\mu_a + \mu_s)(\hat{r}) \right) \Phi(\hat{r}, t) + \nabla \cdot \hat{J}(\hat{r}, t) = \mu_s(\hat{r}) \Phi(\hat{r}, t) + f_0 \varepsilon_{0,0}(\hat{r}, t) \tag{1-5}
\]

\[
\left( \frac{1}{c} \frac{\partial}{\partial t} + (\mu_a + \mu_s)(\hat{r}) \right) \hat{J}(\hat{r}, t) + \frac{1}{3} \nabla \cdot \Phi(\hat{r}, t) = f_1 \mu_s(\hat{r}) \hat{J}(\hat{r}, t) + \varepsilon_1(\hat{r}, t) \tag{1-6}
\]

where \(\Phi(\hat{r}, t) = \psi_{0,0}(\hat{r}, t)\) is the photon fluence, \(\hat{J}(\hat{r}, t)\) is the photon flux.

The \(P_{1}\) approximation can be further simplified by making the diffuse approximations that \(\frac{\partial \hat{J}}{\partial t} = 0\), which is only justified when scattering coefficient is much larger than the absorption coefficient. Also assuming the photon source being isotropic yields \(\hat{\varepsilon}_1 = 0\). Thus the transport equation could be approximated to the following diffusion equations. In the time domain:

\[-\nabla \cdot D(\hat{r}) \nabla \Phi(\hat{r}, t) + \mu_a \Phi(\hat{r}, t) + \frac{1}{c} \frac{\partial \Phi(\hat{r}, t)}{\partial t} = \varepsilon_0(\hat{r}, t) \tag{1-7}\]

in the frequency domain:

\[-\nabla \cdot D(\hat{r}) \nabla \Phi(\hat{r}, \omega) + \mu_a \Phi(\hat{r}, \omega) + \frac{i\omega}{c} \Phi(\hat{r}, \omega) = \varepsilon_0(\hat{r}, \omega) \tag{1-8}\]

and in the continuous wave domain:

\[-\nabla \cdot D(\hat{r}) \nabla \Phi(\hat{r}) + \mu_a \Phi(\hat{r}, t) = \varepsilon_0(\hat{r}) \tag{1-9}\]
where $D(\hat{r}) = 1/3(\mu_a + \mu_s')$ is the diffusion coefficient, $\mu_s' = (1 - f_1) \mu_s$ is the reduced scattering coefficient and $\varepsilon_0(\hat{r}) = \varepsilon_{0,0}(\hat{r})$ is the isotropic source. For finite media, the boundary effects must also be taken into consideration.

### 1.5 DOT Reconstruction Algorithm: Forward solution and Inverse Solution Procedures

The process of DOT image reconstruction includes the forward solution and the inverse solution. In the forward solution, light distribution in the medium is predicted. Since it is impossible to acquire the analytical solution for the diffusion equation in the real case, finite element method (FEM) is applied to the study as it is able to solve the equation in heterogeneous medium with an arbitrary geometry. According to previous studies [83, 84] of FEM methods in continuous wave DOT, using the FEM discretization, coupling with type III boundary condition

$$-D \nabla \Phi \cdot \hat{n} = \alpha \Phi$$  \hspace{1cm} (1-10)

the steady-state diffusion equation could be transformed into

$$[A]\{\Phi\} = \{b\}$$  \hspace{1cm} (1-11)

where $\alpha$ is the boundary condition coefficient related to the internal reflection at the boundary; the elements of the matrix $[A]$ are

$$a_{ij} = \int_V (-D \nabla \phi_j \cdot \nabla \phi_i + \frac{2D}{n} \nabla n_k \cdot \nabla \phi_i - \mu_s \phi_j \phi_i) dV \quad \text{where the integrations are performed over}$$

the problem domain $V$; $D(\hat{r}) = 1/3(\mu_a + \mu_s')$ is the diffusion coefficient where $\mu_a$ and $\mu_s'$ are the absorption and reduced scattering coefficient; $\phi_i$ and $\phi_j$ are locally spatially varying Lagrangian basis functions at node $i$ and $j$, respectively; $\{b\} = -\int_V S \phi_i + \alpha \sum_{j=1}^M \Phi_j \dot{\phi}_i \phi_j ds$ is the source vector; $S = S_0 \delta(r - r_0)$ where $S_0$ is the source strength
and $\delta(r - r_0)$ is the Dirac delta function for a source at $r_0$; $M$ is the number of boundary nodes; \( \{\Phi\} = [\Phi_1, \Phi_2, \ldots, \Phi_N] \) is the photon density.

In the forward solution procedure, the boundary condition coefficient $\alpha$, the source strength $S_0$, and the initial value of absorption and diffusion coefficient should be determined by a preprocessing initial search optimization scheme.

\[
\Phi^{(m)} = (\Phi_1^{(m)}, \Phi_2^{(m)}, \ldots, \Phi_M^{(m)})^T \quad \text{and} \quad \Phi^{(c)} = (\Phi_1^{(c)}, \Phi_2^{(c)}, \ldots, \Phi_M^{(c)})^T, \]

where $\Phi_i^{(m)}$ and $\Phi_i^{(c)}$ are measured and calculated photon density at $i=1, 2, \ldots, M$ boundary locations. The squared difference of $\Phi_i^{(m)}$ and $\Phi_i^{(c)}$ are minimized as the function of the initial search parameters and the best initial values are generated based on the minimum squared difference.

The inverse solution is based on the Taylor expansion or Newton method. Assuming the photon density $\Phi$ are analytic functions of $\mu_a$ and $D$, and then $\Phi$ can be Taylor expanded over an assumed $(D, \mu_a)$ distribution, which is a perturbation away from some other distribution, $(\bar{D}, \bar{\mu}_a)$:

\[
\Phi(\bar{D}, \bar{\mu}_a) = \Phi(D, \mu_a) + \frac{\partial \Phi}{\partial D} \Delta D + \frac{\partial \Phi}{\partial \mu_a} \Delta \mu_a + \cdots
\]

(1-12)

where $\Delta D = \bar{D} - D$ and $\Delta \mu_a = \bar{\mu}_a - \mu_a$. If the assumed optical property distribution is close to the true one, the high order items in the expansion can be neglected and we acquire

\[
J \Delta x = \psi^m - \psi^c
\]

(1-13)

where
and are measured and calculated data at \(i=1,2,\ldots, M\) measurement sites, and \(\psi_i\) are the reconstructed optical parameters. Regularization method is used to make Equation 1-13 invertible:

\[
J = \begin{bmatrix}
\frac{\partial \psi_1}{\partial D_1} & \frac{\partial \psi_1}{\partial D_2} & \ldots & \frac{\partial \psi_1}{\partial D_K} & \frac{\partial \psi_1}{\partial \mu_{a1}} & \frac{\partial \psi_1}{\partial \mu_{a2}} & \ldots & \frac{\partial \psi_1}{\partial \mu_{aL}} \\
\frac{\partial \psi_2}{\partial D_1} & \frac{\partial \psi_2}{\partial D_2} & \ldots & \frac{\partial \psi_2}{\partial D_K} & \frac{\partial \psi_2}{\partial \mu_{a1}} & \frac{\partial \psi_2}{\partial \mu_{a2}} & \ldots & \frac{\partial \psi_2}{\partial \mu_{aL}} \\
\vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots \\
\frac{\partial \psi_M}{\partial D_1} & \frac{\partial \psi_M}{\partial D_2} & \ldots & \frac{\partial \psi_M}{\partial D_K} & \frac{\partial \psi_M}{\partial \mu_{a1}} & \frac{\partial \psi_M}{\partial \mu_{a2}} & \ldots & \frac{\partial \psi_M}{\partial \mu_{aL}}
\end{bmatrix}
\]

\(\lambda I\) is the identity matrix of the size \(2N \times 2N\), \(N\) is the node number of the finite element mesh, \(\lambda\) is the regularization parameter, \(J\) is the Jacobian matrix, and \(\Delta \chi\) is the updating vector.

\[
\Delta \chi = [\Delta D_1 \ \Delta D_2 \ \ldots \ \Delta D_K \ \Delta \mu_{a1} \ \Delta \mu_{a2} \ \ldots \ \Delta \mu_{aL}]^T
\]

\[
\psi^m = [\psi_1^m \ \psi_2^m \ \ldots \ \psi_M^m]^T
\]

\[
\psi^c = [\psi_1^c \ \psi_2^c \ \ldots \ \psi_M^c]^T
\]
CHAPTER 2
MULTISPECTRAL PHASE-CONTRAST DOT IMAGING: PHANTOM EXPERIMENTAL STUDIES

We have previously reported an in vivo study of 35 breast masses (11 malignant cases and 24 benign cases) from 33 patients with phase contrast DOT (PCDOT) [85]. The results obtained from this study showed that malignant lesions generally had a decreased RI while benign lesions exhibited an increased RI relative to the surrounding normal tissue. A sensitivity of 81.8% and a specificity of 70.8% were obtained from this study. While a significantly improved specificity was acquired by PCDOT compared to conventional DOT, the relatively noisy RI distribution recovered made it sometime difficult to identify the lesion, making the image inspection observer dependent. The primary goal of the current work is to develop a two step method for improving the quality of RI image reconstruction so that the image examination may be independent on the observer. In this two step method, a locally refined finite element mesh was created according to the reconstructed absorption/scattering images using conventional DOT, followed by incorporation of the structural prior information into the iterative process for RI reconstruction. We validate and evaluate this method using both phantom and in vivo data.

2.1 Measurement of Refractive Index Distribution and Concentration

2.1.1 Reconstruction Methods

A. Introduction to the Finite Element single wavelength PCDOT Reconstruction Algorithm

Our regularized nonlinear iterative reconstruction algorithm is based on the finite element solution to the following photon diffusion equation coupled with Type III boundary conditions [86, 87]:

\[
\frac{\partial}{\partial t} \phi = \nabla \cdot (\kappa \nabla \phi - \mu_0 \mu_r \kappa \phi) + \frac{\mu_0}{\mu_r} \nabla \cdot \mu_0 \nabla \phi + \kappa \nabla \cdot \left( \frac{\mu_0}{\mu_r} \nabla \phi \right) + \frac{\mu_0}{\mu_r} \nabla \cdot \mu_0 \nabla \phi + \frac{\mu_0}{\mu_r} \nabla \cdot \mu_0 \nabla \phi
\]

where \( \phi \) is the optical index of refraction, \( \kappa \) is the extinction coefficient, \( \mu_0 \) is the scattering coefficient, \( \mu_r \) is the reduced scattering coefficient, and \( t \) is time.
\[ \nabla \cdot D \nabla \Phi(r) + \frac{2D}{n} \nabla n \cdot \nabla \Phi(r) - \mu_a(r) \Phi(r) = -S \delta(r - r_0) \]  \hspace{2cm} (2-1)

\[-D \nabla \Phi \cdot \hat{n} = \alpha \Phi \]  \hspace{2cm} (2-2)

where \( \Phi(r) \) is the photon density; \( n \) is the refractive index; \( D \) is the diffusion coefficient;

\( \mu_a(r) \) is the absorption coefficient; \( \hat{n} \) is the unit normal vector for the boundary surface;

\( \alpha \) is a coefficient related to the internal reflection at the boundary and; \( S \) is the source strength and \( \delta(r - r_0) \) is the Dirac delta function for a source at \( r_0 \). The diffusion coefficient can be written as \( D = 1/(3(\mu_a + \mu_s')) \) where \( \mu_s' \) is the reduced scattering coefficient.

Through a finite-element discretization and other derived matrix relations by differentiation, a set of equations for inverse problem solution are obtained,

\[ [A] \{ \Phi \} = \{ b \} \]  \hspace{2cm} (2-3)

\[ [A] \{ \partial \Phi / \partial n \} = \{ \partial \Phi / \partial n \} - [\partial A / \partial n] \{ \Phi \} \]  \hspace{2cm} (2-4)

\[ (\mathcal{J}^T \mathcal{J} + \lambda \mathcal{L}^T \mathcal{L}) \Delta n = \mathcal{J}^T (\Phi^{(m)} - \Phi^{(c)}) \]  \hspace{2cm} (2-5)

in which the elements of the matrix \( [A] \) are \( a_{ij} = \int_V (-D \nabla \phi_i \cdot \nabla \phi_j + \frac{2D}{n} \nabla n_k \cdot \nabla \phi_i - \mu_a \phi_i \phi_j) dV \)

where the integrations are performed over the problem domain \( V \); \( \phi_i \) and \( \phi_j \) are locally spatially varying Lagrangian basis functions at node \( i \) and \( j \), respectively; \( \{ b \} \) is the source vector; \( \mathcal{J} \) is the Jacobian matrix formed by \( \partial \Phi / \partial n \) at the boundary measurement sites; \( \lambda \), a scalar and \( \mathcal{L} \), the regularization matrix or filter matrix, are used to realize the invertible system (Equation 2-5); \( \Delta n = (\Delta n_1, \Delta n_2, ..., \Delta n_N)^T \) is the update vector for RI,
where $N$ is the total node number of the finite-element mesh used;

$$
\Phi^{(m)} = (\Phi_1^{(m)}, \Phi_2^{(m)}, ..., \Phi_M^{(m)})^T \quad \text{and} \quad \Phi^{(c)} = (\Phi_1^{(c)}, \Phi_2^{(c)}, ..., \Phi_M^{(c)})^T,
$$

where $\Phi_i^{(m)}$ and $\Phi_i^{(c)}$ are measured and calculated photon density at $i=1, 2..., M$ boundary locations. To estimate the spatial distribution of $n$, this quantity needs to be expanded in a similar manner to $\phi$ as a finite sum of unknown coefficient multiplied by the locally defined Lagrangian basis function. The $n$ distribution is updated iteratively through Equations 2-3 to 2-5 so that a weighted sum of the squared difference between measured and calculated photon density can be minimized. Diffusion and absorption coefficients are assumed constant during the RI reconstruction process.

**B. Adaptive Meshing**

To generate a locally refined adaptive mesh, the absorption and scattering images are first reconstructed by conventional DOT where the lesion location/size can be confirmed in comparison with the x-ray mammography or ultrasound (US). From these images, the maximum values ($\text{max}$) of absorption/scattering coefficients in the lesion/target area and the minimum values ($\text{min}$) of absorption/scattering coefficients for the surroundings can be obtained. If the values of both absorption and scattering coefficients at a nodal location are larger than $\text{min} + \nu \cdot (\text{max} - \text{min})$, where $0 < \nu < 1$, then this node is labeled as part of $\text{Region I}$; otherwise, the node is identified as part of $\text{Region II}$. An element is split into four smaller elements (Figure 2-1(a)) if all three nodes associated with this element are part of $\text{Region I}$, while an element is divided into two smaller ones (Figure 2-1(b)) if only two nodes associated with this element are part of $\text{Region I}$.

**C. Incorporation of Structural a Priori Information**
The regularization matrix $L$ included in Equation 2-5 is commonly taken as the identity matrix and structural prior information is iteratively incorporated into the reconstruction process through the spatially varying regularization parameter $\lambda$ [88]. Here we use the Logarithm-type regularization matrix which is constructed according to the priors obtained when generating the adaptive mesh. This regularization matrix can relax the smoothness constraints at the interface of different regions so that the covariance of nodes within a region is basically realized [89]. The elements of matrix $L$ are:

$$L_{ij} = \begin{cases} 
1 & \text{if } i = j \\
-1/NN_1 & \text{if } i, j \subset \text{region I} \\
-1/NN_2 & \text{if } i, j \subset \text{region II} \\
0 & \text{if } i, j \subset \text{others}
\end{cases}$$

(2-6)

where $NN_1$ is the number of nodes within Region I and $NN_2$ is the number of nodes within Region II. The Jacobian matrix is reassembled according to the region or tissue type it is associated with structural a priors.

2.1.2 Development of Multispectral PCDOT Algorithm

In the single-wavelength PCDOT reconstruction algorithm, we made a first-order approximation that both absorption and scattering coefficients are being constant during the reconstruction process of RI. Though we’ve managed to improve the quality and quantity of the reconstructed RI images through a two-step method, it is still hard to retrieve the RI information at a lot of cases due to the highly heterogeneous distribution of absorption and scattering parameters and their big crosstalk on the RI image. Thus based on the general observation that the value of tissue RI does not have significant varies in the NIR region, we introduced more data from different wavelengths to the
reconstruction to simultaneously recover RI along with other absorption and scattering derived chromophores.

For breast tissue, the major absorption chromophores are oxyhemoglobin (HbO2), deoxyhemoglobin (Hb), water and lipid [90]. Previous studies have demonstrated that hemoglobin levels in tumors tended to be larger while oxygen saturation levels are found to be lower than normal tissue [91, 92]. Water content may also provide information for fiberadenoma or fibrocystic disease [93]. On the other hand, studies have shown that scattering spectra is correlated with tissue morphology [93, 94] and in pathology that tumor cells are significantly enlarged compared to normal ones [95].

Tissue absorption is contributed by \( L \) absorption chromophores with the concentration of \( C_i \) for the \( l \)th chromophore [96]. Thus the absorption spectra \( \mu_a(\lambda) \) could be written as

\[
\mu_a(\lambda) = \sum_{i=1}^{L} C_i e_i(\lambda)
\]  

(2-7)

where \( e_i(\lambda) \) indicates the absorption extinction coefficient of the \( l \)th chromophore at wavelength \( \lambda \).

The scattering spectra is correlated with particle size distribution and concentration under the Mie theory in the following relationship [96, 97]

\[
\mu_s'(\lambda) = \int_{0}^{\infty} \frac{3Q_{scat}(x,n,\lambda)[1-g(x,n,\lambda)]}{2x} \phi f(x) dx
\]  

(2-8)

where \( Q_{scat} \) is the scattering efficiency, \( g \) is the average cosine of scattering angles, \( x \) is the particle size, \( n \) is the RI of particles, \( \phi \) is the particle concentration/volume fraction, and \( f(x) \) is the particle size distribution. Assuming a Gaussian particle size distribution [96]

\[
f(x) = \frac{1}{\sqrt{2\pi}b^2} e^{-[(x-a)^2/2b^2]}
\]  

(2-9)
where \( \alpha \) is the average size of particles and \( b \) is the standard deviation. Assume there’re \( M \) kinds of particles with different sizes and fix \( b \) to be 1% as its impact is relatively small. The total scattering from all \( M \) kinds of particles can be expressed by

\[
\mu'_s(\lambda) = \sum_{m=1}^{M} \phi_m \mu'_{s_m}(\lambda)
\]  

(2-10)

where \( \phi_m \) is the volume fraction of the \( m \)th kind of particles. Thus \( c_i \) and \( \phi_m \), as well as the RI \( n \) could be reconstructed simultaneously with the following relationship

\[
\partial \Phi_\lambda = \mathcal{J}_{c_1,\lambda} \partial c_1 + \cdots + \mathcal{J}_{c_L,\lambda} \partial c_L + \mathcal{J}_{\phi_1,\lambda} \partial \phi_1 + \cdots + \mathcal{J}_{\phi_M,\lambda} \partial \phi_M + \mathcal{J}_n \partial n
\]  

(2-11)

where \( \Phi_\lambda \) is the wavelength-dependent photon density at boundary measurement sites.

The Jacobian matrix \( \mathcal{J} \) can be obtained by the following calculations

\[
\mathcal{J}_{c_l,\lambda} = \left( \frac{\partial \Phi_\lambda}{\partial \mu} \bigg|_{c_l} \right) = \left( \mathcal{J}_{c_{1},\lambda} \varepsilon_l \right), \text{ for } l = 1, \ldots, L,
\]  

\[
\mathcal{J}_{\phi_m,\lambda} = \left( \frac{\partial \Phi_\lambda}{\partial \mu} \bigg|_{\phi_m} \right) = \left( \mathcal{J}_{\phi_{1},\lambda} (-3k^2) \mu'_{s_m} \right), \text{ for } m = 1, \ldots, M.
\]  

(2-12)

(2-13)

while \( \mathcal{J}_n \) has the same expression as that of the single-wavelength reconstruction algorithm.

Substituting Equation 2-12 into Equation 2-11, the system equation at all wavelengths can be expressed as

\[
\begin{pmatrix}
\partial \Phi_{\lambda_1} \\
\partial \Phi_{\lambda_2} \\
\vdots \\
\partial \Phi_{\lambda_W}
\end{pmatrix}
= \begin{pmatrix}
\mathcal{J}_{c_1,\lambda_1} & \cdots & \mathcal{J}_{c_1,\lambda_1} & \mathcal{J}_{\phi_1,\lambda_1} & \cdots & \mathcal{J}_{\phi_1,\lambda_1} & \mathcal{J}_n \\
\mathcal{J}_{c_L,\lambda_1} & \cdots & \mathcal{J}_{c_L,\lambda_1} & \mathcal{J}_{\phi_L,\lambda_1} & \cdots & \mathcal{J}_{\phi_L,\lambda_1} & \mathcal{J}_n \\
\vdots & \ddots & \vdots & \vdots & \ddots & \vdots & \vdots \\
\mathcal{J}_{c_1,\lambda_W} & \cdots & \mathcal{J}_{c_1,\lambda_W} & \mathcal{J}_{\phi_1,\lambda_W} & \cdots & \mathcal{J}_{\phi_1,\lambda_W} & \mathcal{J}_n
\end{pmatrix}
\times
\begin{pmatrix}
\partial c_1 \\
\partial c_L \\
\vdots \\
\partial \phi_1 \\
\vdots \\
\partial \phi_M \\
\partial n
\end{pmatrix},
\]  

(2-14)

where \( W \) is the number of wavelengths used. A Tikhonov regularization based iterative Newton method was used to solve the above equation after the Jacobian matrix was obtained.
2.1.3 Numerical Simulations

For the following numerical simulation cases, the measured photon density used for reconstruction was calculated based on the finite element solution to Equation 2-1. The test geometry for case 1 is shown in Fig. 2-2, and the concentration of HbO2, Hb and water are listed in Table 2-3. Using measured data at five wavelengths (673, 733, 775, 840, and 922nm), the reconstructed concentration and RI images are shown in Fig. 2-3 without noise added in the data, and in Fig. 2-4 with 1% random noise added. These images implied that the absorption chromophore concentrations could be quantitatively recovered while RI could be qualitatively obtained both without and with noise. The crosstalk between the reconstructed parameters is not obvious without noise. Adding 1% random noise generated artifacts in the recovered images, especially for the water concentration image (Fig. 2-4(d)), and the crosstalk is also enlarged in this image. However, the reconstructed RI, as well as concentration of HbO2 and Hb images are not deteriorated by the impact of the random noise.

To see how much impact that different contrasts of RI and absorption chromophores have on each other, case 2 (a-d) was performed. In these cases, we assumed only 1 target (Fig. 2-5), but with different contrasts of RI and same contrasts of absorption chromophores. Values of RI and concentrations of HbO2, Hb and water are illustrated in Table 2-4. Using the same 5 wavelength as Case 1, the reconstructed images are shown in Fig. 2-6, where columns 1-4 represents recovered results for case 2 (a)-(d), respectively. The reconstructed images indicated that the distribution of HbO2, Hb and water concentrations could generally be quantitatively recovered in all 4 cases and the RI image could be qualitatively obtained in case 2(a) and 2(b) when the RI has a negative contrast compared to the background. However, the reconstructed RI target
has a small displacement in case 2(d) and in case 2(c) where the RI has a very low positive contrast (~1.5%).

2.2 Imaging of Refractive Index Distribution and Concentration in Heterogeneous Turbid Media

2.2.1 Phantom Study Methods

Phantom experiments were conducted with a multispectral, multichannel diffuse optical tomography system, which was previously described in detail [98]. For a 2D imaging experiment, light from a diode laser at 775 nm was transmitted sequentially to 16 source points at the phantom surface through an optical switch, and diffusing light was detected by 16 photodiodes. A set of 16x16 measured data was then input into the reconstruction algorithm to generate a 2D cross-section image of the phantom.

Four tissue-like phantom experiments were conducted with different contrasts in RI between the target and the background. Tissue absorption ($\mu_a = 0.007 \text{mm}^{-1}$) and scattering ($\mu_s' = 1.0 \text{mm}^{-1}$) were simulated for the background with India ink and Intralipid, respectively. Agar powder (2%) was used to solidify the mixed Intralipid-India ink solution. Thus RI of the background was close to that of water ($n=1.33$ at 775 nm). One 10 mm diameter target was placed off-center with various glucose concentrations to mimic different RI contrasts:

$$n = 0.2105 \cdot [C] + 1.3929$$  \hspace{1cm} (2-15)

where $[C]$ is the concentration of the glucose solution. Values of RI and their corresponding glucose concentrations used in this study are shown in Table 2-1. Geometry of the phantom is shown in Figure 2-7. A mesh of 717 nodes and 1368 triangular elements was applied in the reconstruction.
2.2.2 Results and Conclusions

Figures 2-8 and 2-9, respectively, present the reconstructed RI images for all four phantom cases without and with the two-step method. We see that the images shown in Figure 2-8 are qualitatively good in terms of target location and size, but have significantly overestimated RI values compared to the images shown in Figure 2-9. In addition, the images shown in Figure 2-9 also exhibit a better recovered target boundary relative to that shown in Fig. 2-8. To give a quantitative analysis of the two-step method, we calculated the relative errors of the recovered target RI value for the four cases and listed the results in Table 2-2. We found that the relative errors range from 0.067% to 0.540%, which are significantly improved compared to that from our previous study which used the hard-priori regional reconstruction [81].
Table 2-1. Values of RI and the Glucose Concentration used in the phantom study

<table>
<thead>
<tr>
<th>Glucose Concentration</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI</td>
<td>1.3312</td>
<td>1.3332</td>
<td>1.3353</td>
<td>1.3393</td>
</tr>
</tbody>
</table>

Table 2-2. Comparison of the actual and recovered values of RI of the target

<table>
<thead>
<tr>
<th>RI</th>
<th>Ideal</th>
<th>Calculated</th>
<th>Relative Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.3312</td>
<td>1.3354</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1.3332</td>
<td>1.3260</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>1.3353</td>
<td>1.3362</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>1.3393</td>
<td>1.3437</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Table 2-3. Concentration of absorption chromophores and RI in the background and the four targets used for numerical simulation case 1

<table>
<thead>
<tr>
<th>Regions</th>
<th>RI</th>
<th>Concentration of absorption chromophores (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HbO2</td>
</tr>
<tr>
<td>Background</td>
<td>1.33</td>
<td>40</td>
</tr>
<tr>
<td>Target 1</td>
<td>1.42</td>
<td>40</td>
</tr>
<tr>
<td>Target 2</td>
<td>1.33</td>
<td>80</td>
</tr>
<tr>
<td>Target 3</td>
<td>1.33</td>
<td>40</td>
</tr>
<tr>
<td>Target 4</td>
<td>1.33</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 2-4. Concentration of absorption chromophores and RI values in the background and the target used for numerical simulation case 2

<table>
<thead>
<tr>
<th>Regions</th>
<th>RI</th>
<th>Concentration of absorption chromophores (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HbO2</td>
</tr>
<tr>
<td>Background</td>
<td>1.33</td>
<td>40</td>
</tr>
<tr>
<td>Target in case</td>
<td>1.27</td>
<td>80</td>
</tr>
<tr>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target in case</td>
<td>1.30</td>
<td>80</td>
</tr>
<tr>
<td>2(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target in case</td>
<td>1.35</td>
<td>80</td>
</tr>
<tr>
<td>2(c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target in case</td>
<td>1.42</td>
<td>80</td>
</tr>
<tr>
<td>2(d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2-1. Geometry of split elements
Figure 2-2. Case 1 simulation geometry
Figure 2-3. Reconstructed images of RI (a), concentrations of Hbo2 (b), Hb (c), and water (d) for case 1.
Figure 2-4. Reconstructed images of RI (a), concentrations of Hbo2 (b), Hb (c), and water (d) for case 1 with 1% random noise.
Figure 2-5. Case 2 simulation geometry.
Figure 2-6. Reconstructed images of RI (a, e, i, m), concentrations of Hbo2 (b, f, j, n), Hb (c, g, k, o), and water (d, h, l, p) for case 2 (a-d).
Figure 2-7. Phantom geometry. $R1=50\text{mm}$, $R2=5\text{mm}$, $d=14\text{mm}$. 

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{phantom_geometry.png}
\caption{Phantom geometry. $R1=50\text{mm}$, $R2=5\text{mm}$, $d=14\text{mm}$.}
\end{figure}
Figure 2-8. RI images reconstructed from phantom measurements without the two-step method where the absorption and scattering coefficients were $\mu_a = 0.007\, \text{mm}^{-1}$ and $\mu_s' = 1.0\, \text{mm}^{-1}$ for both the background and the target. The target had (a) 1% glucose concentration ($n=1.3312$), (b) 2% glucose concentration ($n=1.3332$), (c) 3% glucose concentration ($n=1.3353$), and (d) 5% glucose concentration ($n=1.3393$).
Figure 2-9. RI images reconstructed from phantom measurements with the two-step method. The absorption and scattering coefficients were $\mu_a = 0.007 \text{mm}^{-1}$ and $\mu_s' = 1.0 \text{mm}^{-1}$ for both the background and the target. The target had (a) 1% glucose concentration ($n=1.3312$), (b) 2% glucose concentration ($n=1.3332$), (c) 3% glucose concentration ($n=1.3353$), and (d) 5% glucose concentration ($n=1.3393$).
CHAPTER 3
PHASE-CONTRAST DOT IMAGING: IN VIVO STUDY

3.1 Methods

The clinical study was approved by the institutional review board and was conducted in full compliance with the accepted standards for research involving human subjects. Signed informed consent from all study participants was obtained. In this study, 42 breasts from 42 different patients (mean age 59; range from 32-82) were screened. Biopsy reports demonstrated 21 invasive carcinoma and 21 benign lesions. 29 patients were imaged by the same multi-channel photodiodes based system used for the phantom experiments described earlier [98], 9 patients were examined by a multi-channel photomultiplier tubes (PMT) system [99] and 4 patients were screened by a single PMT based scanning system [100]. Data at one wavelength was used for the reconstruction, i.e., 775nm from the system described in [98] and 785nm from those described in [99, 100].

However, most of these patients were imaged by a 10-wavelength, 64 by 64 source/detector channels DOT system [98]. The schematic of the system is shown in Fig. 3-1(a). Generally, light beams from ten laser modules are transmitted to the optical switch, which sequentially passes one of the beams to 64 preselected points at the surface of the breast via source fiber bundles. The ring structure, which is detailed Fig. 3-1(c), holds the 64 source and 64 detection fiber bundles. Light from the bundles is sensed by the detection units, which convert the light intensity into voltage signals. The computer collects the signals though a data acquisition board. The DC motor near the ring is used to adjust the diameter of the ring. Two CCD cameras are mounted underneath the ring to monitor the contact between the breast and fiber optics. The
entire system is controlled by a LabVIEW program. During the examination, the patient would be positioned prone on the bed (Fig. 3-1(b)) and the breast imaged would be placed pendant through the opening. The bed could be moved vertically and the fiber-optic array could be moved in and out to ensure that the rings are all in gentle contact with the breast. All lights in the room would be turned off as the imaging went on and it took about 30 minutes for a 10-wavelength scan.

3.2 Results

3.2.1 Case studies: Invasive ductal carcinomas

Reconstructed images of absorption, scattering and RI from representative clinical cases are shown in Figs. 3-2 ~3-6. The first case was a 66 year-old woman who had an invasive ductal carcinoma that measured 1.9cm in maximal dimension. Both the absorption and scattering images exhibit marked increase in the region of tumor (indicated by arrow in Figs. 3-2a and 3-2b), whereas the RI image without the two step method (Fig. 3-2c) shows neither clear increase nor decrease in the tumor area corresponding to that of the absorption/scattering images. Fig. 3-2d gives the reconstructed RI image using the two step method where we see an identifiable decrease of RI at the tumor area (indicated by arrow).

The second case was a 39 year-old woman who had a palpable mass in the right breast (Fig. 3-3). Biopsy showed infiltrating ductal carcinoma and mammography imaged the size of the mass ~2cm in diameter. Both absorption and scattering images exhibit increased value in the region of tumor corresponding to the mammography result. The 2-step method showed decreased RI at the tumor, which is consistent with the one-step method.
The third case was a 50 year-old woman with palpable abnormality in the right breast (Fig. 3-4). Biopsy showed invasive ductal carcinoma with extensive ductal carcinoma in situ. Reconstructed absorption and scattering images showed marked increase at the tumor site. While the one-step showed a relative increased RI, the two-step method demonstrated decreased RI compared to the normal tissue.

The fourth case was a 58 year-old woman (Fig. 3-5). Biopsy showed the right breast had metastatic poorly differentiated carcinoma with ductal lobular features measuring 1.2cm. Absorption image showed a marked increased feature at the tumor site as indicated by arrow. RI recovered by the two-step method exhibited decreased RI at the tumor site.

The fifth case was a 62 year-old woman (Fig. 3-6). Biopsy showed infiltrating ductal carcinoma, moderately differentiated while mammography demonstrated a mass of 3cm at the biggest dimension. Both absorption and scattering images showed increased value at the abnormality compared to the background. The two-step method demonstrated decreased RI at the same area, which is consistent with the one-step method result.

3.2.2 Case studies: Benign modules

The first case was a 60 year-old woman with a 9mm diameter benign nodule. Increased absorption and scattering coefficients are noticed in the lesion area (Fig. 3-7a, 3-7b), while the RI image without the two step method (Fig. 3-7c) demonstrates moderate contrast in the lesion area. The RI image using the two step method (Fig. 3-7d) presents marked increase in the lesion.

The second case was a 22 year-old woman with a 1.2cm diameter benign nodule (Fig. 3-8). And the third case was a 46 year-old with a 1.9cm lobulated, solid benign
mass (Fig. 3-9). Absorption and scattering images showed marked increase at the lesion, while the RI image with the two-step method demonstrated marked increase at the same area.

3.3.3 Statistical Analysis

We also calculated the sensitivity and specificity for cancer detection for the 42 cases examined using a prediction rule revealed in previous clinical studies [85]. In this prediction rule, a lesion will be diagnosed as malignant if its RI value is smaller than that of its surroundings and its absorption and scattering coefficients are larger than its surroundings; otherwise it is a benign lesion. For the possible physiological reason behind this prediction rule, the glucose metabolism in tumor have been proved to be significantly increased compared to normal tissue in animal models [101], and as discussed in Ref. 102, we suspect the glucose consumption is higher in tumor and lower in benign lesions. Using this rule, we found that the sensitivity (81%) is similar to that obtained previously [85], but the specificity is significantly improved (81%) over the previous study (71%). We consider the PCDOT method to be a potential approach to provide glucose metabolism information. In the current single-wavelength PCDOT algorithm, we made a first-order approximation that both absorption and scattering coefficients are being constant during the reconstruction process of RI. In future studies, we plan to adopt multi-spectral reconstruction method to simultaneously recover RI along with other absorption derived chromophores such as oxy- and deoxy-hemoglobin concentrations, which allows the study of combining glucose metabolism with blood volume and oxygen metabolism for more complete diagnosis of breast cancer.
3.3.4 Conclusions

We have developed a two-step PCDOT method which is able to improve the RI reconstruction qualitatively and quantitatively, making PCDOT a potentially observer-independent approach for cancer diagnosis. This method has been confirmed by phantom experiments and 42 sets of clinical data. We expect to further evaluate this method using larger scale clinical data as well as applying this method for imaging other organs/diseases.
Figure 3-1. (a) Schematic of the 10-wavelength, 64 by 64 source/detector channels DOT system; (b) Overall look of the DOT system; (c) ring structure that holds the source/detector fiber bundles.
Figure 3-2. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for malignant case 1. Tumor is indicated by arrow.
Figure 3-3. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for malignant case 2. Tumor is indicated by arrow.
Figure 3-4. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for malignant case 3. Tumor is indicated by arrow.
Figure 3-5. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for malignant case 4. Tumor is indicated by arrow.
Figure 3-6. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for malignant case 5. Tumor is indicated by arrow.
Figure 3-7. Reconstructed absorption (a), scattering (b), and RI images with one-step method (c) or two-step method (d) for benign case 1. Lesion is indicated by arrow.
Figure 3-8. Reconstructed absorption (a), scattering (b), and RI images with two-step method (c) for benign case 2. Lesion is indicated by arrow.

Figure 3-9. Reconstructed absorption (a), scattering (b), and RI images with two-step method (c) for benign case 3. Lesion is indicated by arrow.
CHAPTER 4
FAST DOT IMAGING SYSTEM OF EPILEPSY AND ITS CALIBRATION

We have gained experience in using DOT for breast cancer detection and the results showed remarkably increased specificity compared to previous methods. The reason that DOT had been applied to breast cancer detection is mainly due to the fact that NIR light could penetrate soft tissue with low attenuations and thus optical properties could be retrieved. While DOT is able to recover different optical properties that are directly related to tissue biological features, brain imaging of epilepsy drew our attention because of the large optical contrasts that epilepsy could produce induced by intense neuronal activities. Current imaging modalities of brain are being either invasive or lack the ability of capturing fast dynamic information in the brain. However, the previous imaging system that we used for breast cancer detection could not be applied to the brain study due to its low temporal resolution. Imaging breast cancer is a relatively steady state process due to the slow evolvement of tissue angiogenesis, but brain imaging ought to be able to capture fast hemodynamic and molecular variations that are related to swift neuronal changes. Thus, in order to study epilepsy, we built a new DOT system for three-dimensional brain imaging that is able to reach a high temporal resolution.

4.1 DOT Imaging System

For our experiment, we used a reflection-mode-based continuous-wave (CW) DOT system (Fig. 4-1). In this system, light from a light-emitting diode (λ~780nm, radiated power ~1000mW, SMB780-1100-01-I, Epitex Inc.) was controlled by an isolated pulse stimulator (Model 2100, A-M Systems) and delivered through 2-axis galvanometers (XLR8 Open Frame Head QS-7, Nutfield Tech.) to multiple source points consequently
on the scanning surface. Two orthogonally placed linear polarizers were applied to eliminate specular reflections. The screening site was then imaged onto a 12-bit CCD camera (CoolSNAP EZ, Photometrics) and the whole system was controlled by a self-written LabView program. The individual components are detailed below.

4.1.1 Light Unit (LED)

The fiber-coupled LED unit at 780nm is used as CW light source. Compared to laser diode, LED is small and cheap, and the non-coherent light from LED is less likely to harm eyes and skins. According to the spectra of molar extinction coefficient (Fig. 4-2), tissue absorption towards hemoglobin and water would reach a relatively low level in the NIR region and thus light penetration can be enhanced. Each LED module is driven by a FPGA controller (CuteDigi Technologies, Inc.). The pigtail fiber is 100um in diameter and has a numerical aperture of 0.66. Figure 4-3 shows the NIR LED unit. The output power can be set from zero to maximum by adjusting the power supply control voltage from 0 to 10 volts. The lighting of the LED is triggered by a function generator.

4.1.2 Galvanometers

A set of programmable high performance open frame scan heads featuring galvanometers controlled by a LabView program is used to deliver light from LEDs to the scan surface (Fig. 4-4). The scan angle of the heads could reach +/- 22 degrees in each of the x/y direction and the small step could reach 270 usec with QD-3000 Servo Driver Board. The driver board is further connected to a 16bit daughter card and through digital interface cables and a connection box, receiving controls from the PC (Fig. 4-5).
4.1.3 CCD Camera

The 12 bit CCD camera is used as the detection unit in the experiment. The camera has a 1392x1040 imaging array with 6.45x6.45 um pixels. CoolSNAP LVDS cable connected the camera to the LVDS interface card on PC. TTL ouput is generated while exposing, providing temporal information of the data acquisition.

4.1.4 System Timing

For a regular frame during the animal experiment with 5ms exposure time, a 32x32 image would take ~27ms to be captured, delivered and saved to the PC. The galvanometers operate at 1ms per switching action. Thus for 5x5 illumination position array scanning, the data collection time adds up to 700ms.

4.2 DOT Imaging System Operations

Before the imaging experiment, the system should be powered for 15 minutes for warm up. Usually, the LED light power supply and function generator are powered first, and then the galvanometers. The CCD camera should be turned on the last and turned off the first to ensure system stability.

Since the region of interest (ROI) to be imaged is relatively small compared to the whole FOV of the camera (~250 pixels in each direction is taken in the 1392x1040 array), a PVCAMTEST program is applied to ensure the ROI selected on the camera covers the cortex region that need to be imaged (Fig. 4-7). In the animal experiments, 25 source points were evenly distributed on the flat region of the animal head extending approximately from 2mm anterior to 10mm posterior of the Bregma and 6mm to lateral of the midline. The whole field of view was divided into ~32x32 pixels on the CCD camera.
Figure 4-6 shows the LabView control panel of the imaging system. Operator changes the exposure time, ROI on the camera, and scanning area of the scan heads. At last, the system runs automatically for the data acquisition. The collected data will then be calibrated and reconstructed using the FEM based algorithm for DOT imaging.
Figure 4-1. Schematic diagram of the experiment setup. While the CCD camera and galvanometers were controlled by one computer, the EEG recordings were controlled by another computer. The two systems were correlated by the trigger signal from the CCD camera.
Figure 4-2. Molar extinction coefficient spectra of hemoglobin.

Figure 4-3. LED light bulbs applied in the study.
Figure 4-4. Galvanometers with 2-axis scanning heads.

Figure 4-5. Connection box for the 2 16bit daughter board.
Figure 4-6. Self-written LabView control panel for the system operation.
Figure 4-7. PVCAMTEST program inspects the ROI selected for imaging.
CHAPTER 5
FAST DOT IMAGING OF EPILEPSY: PHANTOM EXPERIMENTAL STUDIES

5.1 Measurement of Absorption Distribution in Heterogeneous Turbid Media

5.1.1 Materials and Methods

A regularized nonlinear iterative reconstruction algorithm was applied for image recovery and analysis, which is based on the finite element solution to the following photon diffusion equation coupled with Type III boundary conditions:

\[
\nabla \cdot D \nabla \Phi(r) - \mu_a \Phi(r) = -S_0 \delta(r - r_0)
\]

\[\nabla \cdot D \Phi \hat{n} = \alpha \Phi
\]

where \( \Phi(r) \) is the photon density; \( D \) is the diffusion coefficient; \( \mu_a(r) \) is the absorption coefficient; \( n \) is the unit normal vector for the boundary surface; \( \alpha \) is a coefficient related to the internal reflection at the boundary and; \( S_0 \) is the source strength and \( \delta(r - r_0) \) is the Dirac delta function for a source at \( r_0 \). The diffusion coefficient can be written as \( D = 1/(3(\mu_a + \mu_s')) \) where \( \mu_s' \) is the reduced scattering coefficient. In this algorithm, the 3D \( \mu_a \) and \( \mu_s' \) distributions are updated iteratively through a set of derived equations for the inverse problem so that a weighted sum of the squared difference between measured and calculated photon density can be minimized.

A time-series based calibration method for DOT was used to reduce systematic errors and enhance image reconstruction quality [103]. The average measurement data during the resting state was used as the reference medium to generate \( \Phi^{(m)} \) and divided by the mean measurement after the seizure drug injection, forming the calibration matrix.
which would then be applied to the data at a specific time point after the drug injection for image reconstruction.

5.1.2 Numerical Simulations

To validate the three-dimensional FEM based reconstruction methods, several sets of numerical simulations were conducted. Simulated data were generated using the forward solution model with absorption and scattering distribution and values given. A cylindrical target, 3 mm in diameter with 2mm length in the z direction, is placed 1mm, 2mm, 3mm, and 5mm under the surface to simulate variations at different depths of the brain. The optical values are set as $\mu_a = 0.007/0.028$ and $\mu'_s = 1.0/2.0$, for the background and target respectively. The forward data is then reconstructed with a 242x16 mesh with 25 source points and 242 detectors.

Results from the simulations are displayed in Figure 5-1. From these Y-Z plane cross sections, we can see that target at different depth were accurately recovered in the absorption images. It is validated for the reflection based three-dimensional system where source and detectors are on the same plane, target with depth variations could be recovered by the FEM method.

5.2 Imaging of Absorption Distribution in Heterogeneous Turbid Media

5.2.1 Methods and Materials

Two tissue-like phantom experiments were conducted to validate the DOT system. Tissue absorption ($\mu_{ab} = 0.01 \ mm^{-1}$, $\mu_{at} = 0.04 \ mm^{-1}$) and scattering ($\mu'_s = 1.0 \ mm^{-1}$, $\mu'_t = 4.0 \ mm^{-1}$) were simulated for the background and target respectively with india ink and intralipid; 2% agar powder was used to solidify the mixed intralipid-ink solution. One 3mm diameter, 2mm height target was placed with various depths in each of the
background phantoms. The geometry of the phantoms is shown in Fig. 5-2(a). The FOV of the camera was 12mm x 21mm and the FOV was divided into 24 x 44 detectors.

5.2.2 Results and Conclusions

We first tested the ability of the DOT system imaging targets at different depth with tissue-mimic phantoms. With 25 source points, there were 26400 source-detector pairs applied to the image reconstruction and the total reconstruction volume was 12mm x 21mm x 20mm. Fig. 5-2(b, c) shows that the embedded target could be reconstructed at the right location both on the X-Y plane and the Y-Z plane. Comparing the two Y-Z plane images, the difference of the reconstructed target depth position is significant, which shows that the DOT system can reasonably recover 3D volumetric objects.
Figure 5-1. Reconstructed cross section absorption images for the numerical simulation. Target is put (a) 1mm, (b) 2mm, (c) 3mm and (d) 5mm under the surface, respectively.
Figure 5-2. Phantom experiments: (a) Geometry of the phantom, target was located at 1 and 2 mm under the phantom surfaces respectively in the two cases; (b) Reconstructed DOT images of the single embedded target at 1mm depth in X-Y (z=2mm) and Y-Z (x=7mm) plane; (c) Reconstructed DOT images of the single embedded target at 2mm depth in X-Y and Y-Z plane.
6.1 Methods and Materials

6.1.1 Animal Preparation and Measurement Protocol

Six adult male Sprague-Dawley rats (4 rats for the DOT scanning, 2 rats for reference and validation), weighting 220 to 280g, were included in this study. All procedures were conducted according to protocols approved by the University of Florida Institutional Animal Care and Use Committee. During surgical procedures, animals were placed in a stereotaxic frame and anaesthetized with isoflurane (4%) and maintained with one third of the initial dose supplemented with 0.4 L/min oxygen. Body temperature was kept constant using a heating pad for each animal and the heart rate was continuously monitored. Generally, two incisions were made and three screw electrodes were implanted, two at 3mm anterior of the Bregma for electrophysiological (EEG) recording and another one posterior of the Lambda for ground reference. Another hole was then drilled on the skull over the left frontal lobe (with the same small piece of bone put back and covered the hole after the surgery) for later seizure drug injection (Fig. 6-1). Following the above surgical procedures, isoflurane was stopped and 1.2g/kg urethane was injected intraperitoneally instead. We allow at least 30 minutes for the anesthetic stability before the experiments started. To induce focal seizures, local injection of Bicuculline (BMI, 10µl, 1.9mM) by a mini syringe via the prepared hole was conducted. DOT measurements made before the BMI injection were used as calibration data and scans were conducted continuously for up to an hour after the BMI injection, along with whole course EEG monitoring.
6.1.2 Electrophysiological Recordings

Two channels of EEG data were collected simultaneously with DOT using a 16-channel digital amplifier system (R25 Bioamp processor, Tucker-Davis Tech.). 2-mm screw electrodes were applied to record the neuronal activities. In addition, two optical-electrical converters were connected to one channel on the amplifier system to deliver CCD shutter trigger signals to the EEG system so that optical signals captured by DOT could be synchronized with recorded electrical signals. EEG data were processed using self-written MATLAB programs. Low-pass filtered at a cut-off frequency at 50Hz, we calculated the integrated signal response during each frame of the DOT scan and applied it to off-line neurovascular coupling study.

6.1.3 Optical Recording of Intrinsic Signals

To validate the DOT images and neurovascular coupling study results, we applied intrinsic optical signal (OIS) measurement to the same seizure stimulation protocol. There have been several studies using OIS to investigate brain activities including neocortical seizures [64, 65] that this method had been able to image the seizure focus as well as to show the hemodynamic ‘initial dip’ associated with seizure activities. In our OIS measurement, with the same positions of EEG electrodes, the scalp of the animal was cut open and the skull was removed to expose the brain. 2% agar solution was poured and solidified on the brain to create a flat surface and eliminate specular reflections. Light from the LED source was directed and illuminated the whole brain and CCD was focused on the brain surface. Images of a 12x12mm area of the cortex were acquired every 33ms with 180x150 pixel resolution. For the data analysis, we consider the reflectance change from the mean pixel intensity as the inverse of the tissue absorption in order to qualitatively evaluate the neurovascular relationships.
6.1.4 Neurovascular Coupling Data Analysis

Numerous models had been applied to analyze the neural response in imaging by lots of groups. In fMRI, Statistical parametric mapping (SPM) had been widely applied [104]. The simple idea behind SPM is that images proceeded with voxel values that are, under the null hypothesis, distributed according to a known probability density function. The SPM method is usually tested for activation, or regression on some explanatory variable. As the basis for SPM, general linear model (GLM) acts as the foundations for the variant of most analysis methods [105], for instance, the simple t-tests on scans assigned to one condition to another, correlation coefficients between observed responses and boxcar stimulus functions in fMRI, or the evoked responses estimated using linear time invariant models. Bayesian inference would be applied where the null hypothesis based SPM method is rejected when the probability of obtaining the statistic is sufficiently small. Bayesian inference based upon the posterior distribution of the activation given the data, which could be computed from the probability that the activation exceeds some threshold. Dynamic models include convolution models and temporal basis function where the hemodynamic response function is applied, which is the cornerstone for making statistical inferences about activations in fMRI with the GLM; the hemodynamic response, induced by any given trail type, can be expressed as the linear combination of several basis functions of per-stimulus time. Biophysical models as modified convolution models are input-state-output systems that start with a causal dynamic model of how responses are generated and construct a general linear observation model that allows estimating and inferring things about the parameters of that model, where convolution models design matrices are not informed by a forward model of how data are caused.
To date there had been several studies on the neurovascular coupling in epilepsy. In PET and fMRI, most studies have been performed on interictal rather than ictal events, which may elicit such a brief focal increase in deoxygenated hemoglobin as to be undetectable without higher strength magnets. Some ictal fMRI studies have been done on generalized spike-and-wave events, which may not elicit an increase in HbR in the cortex, while some have been done without concurrent electrical recordings, rendering the timing of the imaging unclear with respect to the seizure onset. In optical spectroscopy, along with oxygen-sensitive electrodes, there has been studies of the ‘initial dip’, that whether cerebral blood flow is adequate to meet metabolic demand at the onset of epileptic event [65]. But the intrinsic optical spectroscopy is incapable of resolving subtle variations in the electrophysiology arising after seizure onset.

Hemodynamic response function (HRF) models have been used to model the dynamic BOLD signals in fMRI study [106, 107] that a linear and time invariant system is assumed. The basis of the analysis is the comparison between the actual fMRI signal and a model of the expected response given the knowledge of generalized spike and wave timing. Statistical maps were obtained, indicating at each voxel the level of correlation between the BOLD signal and the model, and among the results from the different models, the one yielding the highest t stat value was selected. The HRF is the impulse response function that the hemodynamic signal could be modeled as the convolution of HRF and input stimulus (neural activities):

\[ X(t) = S(t) \ast H(t) \]  

(6-1)

where \( X(t) \) is the measured hemodynamic signal, and \( S(t) \) is the measured neural activity and \( H(t) \) is the HRF.
In our neurovascular coupling study, in order to test the coupling between the measured tissue absorption changes and the neural activities, similar linear regression model was applied to predict the hemodynamic variations. Since the measured EEG signal (i.e., the input stimulus), has a much higher sampling rate than the recovered optical signals, EEG signal was integrated in each of the event related period. Here we used the integrated power of the neural impulse response as it shows robust linear relationship between it and the BOLD signal even if neurovascular coupling is nonlinear due to a purely vascular refractory effect [108].

6.2 Absorption Distributions of Epileptic Animals

6.2.1 Case Studies: Absorption Distribution

For the DOT animal seizure experiments, 17 seizures were imaged in four rats. Following the BMI injection, EEG recordings show that interictal spikes were generated and seizures occurred periodically for up to 1h. Fig. 6-2 shows a 100s EEG recording window for one animal at 200s after the drug injection. And Fig. 6-3 shows a typical 10s continuous spike-and-wave discharge piece during the seizure onset. In each animal, the first electrographic seizure onset occurred at 2-3 min following the BMI injection denoted by high frequency continuous spike-and-wave discharges (SWDs). Different amplitude of electrical signals could be observed between the two recording channels, which indicated the localized seizure onset.

For the first case, Absolute absorption coefficient $\mu_a (mm^{-1})$ images at different time points were recovered. Fig. 6-4(a) shows selected reconstruction absorption coefficient distribution of the brain at the depth of 2mm. Fig. 6-4(b) illustrates the 3D image at the 140s time point. Seizure focus (indicated by arrows) with increased
absorption coefficient could be seen in the reconstructed in vivo images despite the existence of boundary artifacts.

For the second case, absorption images at different time points were reconstructed. Fig. 6-5 shows five sets of reconstructed images at 35'', 1'43'', 4'01'', 6'19'', and 8'36'' after BMI injection. Fig. 6-6 illustrates the 3D image at the 8'36'' time point. Seizure focus (indicated by arrows) with increased absorption coefficient could be seen in the reconstructed in vivo images.

For the third case, the drug is injected at the primary somatosensory cortex and Fig. 6-7 shows selected reconstruction absorption coefficient distribution of the brain at the depth of 2mm. Seizure focus (indicated by arrows) could be observed with marked increasing of absorption compared to its surrounding regions.

6.2.2 Validation of Absorption Images

To confirm that changes of tissue absorption at the seizure focus were caused by epileptic activities, reference/control experiment was conducted where same volume of saline was injected instead of BMI. Fig. 6-8 shows selected time points images after the injection and no significant ‘focal’ change could be observed besides the unchanged boundary artifact.

To further validate the accuracy of the DOT images, Fig. 6-9 shows the OIS images from the reference experiment of the same stimulation protocol. As indicated by the OIS images, the position of the increased tissue absorption focus was consistent with the drug injection site and the size of the focus is very comparable with the DOT reconstruction images over time.
6.2.3 Results and Discussion

BMI is a well-established seizure model that had been used in many aspects and the EEG recordings from our study had been very similar to those from previous studies [109, 110]. Continuous high frequency SWDs from the EEG suggested seizure onset and it is also obvious that the closer the recording electrode to the BMI injection site, the more significant change of the EEG signal could be observed.

For this localized acute seizure onset model, the seizure foci could be detected in all cases with a marked increase of local tissue absorption. In the shown case (Fig. 6-4), there is a clear maximum around \((x=5.5, y=6, z=2\text{mm})\) relative to its surrounding basically throughout the 30 minute monitoring period after the drug injection. This is consistent with the general observation that epileptic seizures increase cerebral metabolism dramatically coupled with cerebral vessels dilations, and localized cerebral blood volume increase should be observed in localized seizure onset, which would result in local increase of tissue absorption levels. Both the size and absorption value of this focus could be observed to change significantly over time compared to the stable boundary artifacts. It is believed that it’s the seizure focus also because it is exactly where the BMI solution was injected. Ictal cerebral blood flow had been suggested to increase around 150%-200% in PET study [111], the scale of which is comparable to what we have observed in the absorption images (\(\mu_a \sim 0.4 \text{ mm}^{-1}\) at the focus and \(\sim 0.2 \text{ mm}^{-1}\) at the surrounding).
6.3 Coupling study

6.3.1 Case Studies: Linearity of Optical Signals and EEG

To quantitatively study the recovered 3D absorption images and to test the hypothesis of the system linearity, neurovascular coupling study were conducted using the linear regression model. In the current study, we only investigated the ictal periods that are denoted by fast SWDs. HRF was generated based on a single ictal period and generally, the HRF has an initial dip phase of 2s and delay of undershoot phase of 10s. Then the same response function was applied to all other event-related periods to predict the tissue absorption variations. The measured absorption value was calculated by the average of the seizure focus which usually measured ~2mm in diameter.

Fig. 6-10 shows the measured and predicted results in several trials from one animal. The duration of the ictal periods varied from 15s-30s. Fig. 6-11 shows the measured and predicted results from a second animal. The duration of the ictal periods varied from 25s-50s.

As indicated by the figures, the absorption variations could be qualitatively predicted by the input stimulus and the HRF that the predicted curve correlate with the actual change. Calculated correlation coefficient is ~0.20 for the given cases. This result would strongly suggest the linearity of the system and the linear regression model could be nicely applied to the study.

6.3.2 Validation of the Linearity Coupling

OIS was used to validate the DOT reconstructed results qualitatively. As indicated by the reflectance images over time after the BMI injection, both the size and the value of the focus varied over time after the BMI injection and the size of the increased absorption area was comparable to what had been recovered from DOT. Similar kind of
neurovascular coupling study had also been conducted and results are shown in Fig. 6-12. Here the change of reflectance could also be predicted using the same linear regression model that was used in the DOT study, which suggested consistent linearity of the system.

6.3.3 Discussions

So far there had been a number of studies, both on human and animals, on the quantitative coupling of hemodynamic and neural responses but had different conclusions. Local field potential from forepaw stimulation was observed to be linear with integration of BOLD and CBF generated from BOLD in animal subjects [119]; LFP from visual cortical response was found linear with amplitude of BOLD signal [120]; CBF from laser Doppler and optical intrinsic signal was found to be linear with summed neural activities in animal subjects [121]; in human subjects, somatosensory evoked potential was observed to be linear with BOLD signal amplitude [122]; deoxy-hemoglobin from diffuse optical imaging was also found to be linear with MEG peak amplitudes in human subjects [123]. Meanwhile, nonlinear cases had been found also. BOLD from visual stimulus was observed to be nonlinear with MEG in human subjects [124] and some studies claimed hemoglobin percentage change from spectroscopic optical measurements to be linear with LFP from somatosensory cortex stimulus from animal subjects [125]. In these studies, the quantification of neural and hemodynamic signals varied. Peak amplitude, steady-state amplitude, peak-to-peak difference, or time integral was used in different models. Lacking standard way of defining and quantifying neural and hemodynamic signals before analysis may lead to disagreement of linearity of the models.
In this study, we used the absolute value of reconstructed optical absorption coefficient as the hemodynamic response signal, and time integral of the EEG response as the neural response signal. Tissue optical absorption is directly related to hemoglobin concentration and tissue oxygen saturation, and EEG as the measurement of integrated synaptic activities, indicated the major utilization of cerebral energy. We found that tissue optical absorption is linearly related to EEG activities during the highly activated seizure onset period. This relationship implies close coupling between tissue oxygen metabolism and demand of neuronal energy in the intensive and comprehensive neuronal activities. This understanding of the coupling between hemodynamic responses and neuronal activities would also assist the interpretation of perfusion-based imaging techniques such as fMRI and PET.
Figure 6-1. (a) Locations of BMI injection site (solid dot) and scalp EEG electrodes (open circles), (b) DOT image domain.
Figure 6-2. Sample EEG signal piece where interictal spikes and high frequency SWD ictal onset could be observed.
Figure 6-3. EEG recording of a 10-second window with continuous SWDs.
Figure 6-4. (a-h) z=2mm cross-section images from the reconstructed 3D images at time points 70’, 140’, 210’, 280’, 350’, 420’, 490’ and 560’ respectively after BMI injection. Seizure focus indicated by arrows. (i) Three-dimensional image at the 140’ time point.
Figure 6-5. (a-e) \( z=2 \text{mm} \) cross-section images from the reconstructed 3D images at time points 35'', 1'43'', 4'01'', 6'19'', and 8'36'' respectively after BMI injection, (f-j) \( x=10 \text{mm} \) cross-section images from the reconstructed 3D images at time points 35'', 1'43'', 4'01'', 6'19'', and 8'36'' respectively after BMI injection.

Figure 6-6. Three-dimensional image at the 8'36'' time point during the ictal period.
Figure 6-7. z=2mm cross-section images from the reconstructed 3D images at successive different time points. Seizure focus is indicated by arrow.
Figure 6-8. z=3mm cross-section images from the reconstructed reference experiment at time points 70', 140', 210', and 280' after the saline injection.
Figure 6-9. OIS images for the validation experiment. (a) Brain images during the resting period; (b) Selected OIS images during 5~10 minutes after the BMI injection, seizure focus indicated by arrows.
Figure 6-10. System linearity study. (a) EEG signals from four trials of ictal periods; (b) Reconstructed tissue absorption value at the seizure focus; (c) predicted absorption coefficient generated by the input stimuli and HRF.
Figure 6-11. System linearity study. (a) EEG signals from two trials of ictal periods; (b) Reconstructed tissue absorption value at the seizure focus; (c) predicted absorption coefficient generated by the input stimuli and HRF.
Figure 6-12. Validation for the system linearity using OIS measurement. (a) EEG signals from three trials of ictal periods; (b) Measured reflectance change at the seizure focus; (c) predicted reflection generated by the input stimuli and HRF.
7.1 Methods and Materials

7.1.1 Multispectral DOT Imaging System

To further increase the temporal resolution of DOT imaging and to acquire functional information associated with seizure activities, a multispectral fast DOT imaging system was built. The details of the system are described elsewhere [112]. Generally, instead of the previous reflection-mode based imaging, the new system images the whole head. Two wavelengths of LED lights were chosen as the light source at 780nm and 850nm. Each wavelength contains 48 LEDs that are able to switch fast between one and another (50ns). FPGA is also used to swiftly and accurately control the LEDs. Two types of detectors, high sensitive APD (avalanche photodiode) and low sensitive SPD (silicon photodiode), are combined in the system to ensure a larger dynamic range. The highest sampling rate could reach 0.14 Hz after 100 times average of each detection time point, which is applied to reduce the system noises. All the source and detection fiber bundles are evenly placed on a ring holder within which the rat’s head goes through. The schematic of the system is illustrated in Fig. 7-1. Phantom studies have proved that the system has a better capability in imaging deeper targets compared to the reflection-mode based system.

7.1.2 Animal Preparation and Measurement Protocol

Four adult male Sprague-Dawley rats, weighting 220 to 280g, were included in this study. All procedures were conducted according to protocols approved by the University of Florida Institutional Animal Care and Use Committee. During surgical procedures, animals were placed in a stereotaxic frame and anaesthetized with isoflurane (4%) and
maintained with one third of the initial dose supplemented with 0.4 L/min oxygen. Body temperature was kept constant using a heating pad for each animal and the heart rate was continuously monitored. During the surgery, two incisions were made. One was made posterior of Lambda, and one screw electrode was implanted for ground reference. Another incision was made from 5mm anterior of the Bregma to 5mm posterior. As the EEG electrodes, two fine copper wires were implanted into the head 1mm left or right from the Bregma respectively. A small hole ~1mm in diameter was drilled 1mm posterior of the right electrode and a small tube was inserted into the hole to provide duct of later BMI drug introduction (Fig. 7-2). Following the above surgical procedures, isoflurane was stopped and 1.2g/kg urethane was injected intraperitoneally instead. We allow at least 30 minutes for the anesthetic stability before the experiments started. To fill the space between the holder ring and the animal head, liquid phantom above 40 degrees were infused into the space that it would cooled and solidified (Fig. 7-3). Bicuculline (BMI, 10µl, 1.9mM) injected into the brain by previously placed tube was used to induce focal seizures. DOT measurements made before the BMI injection were used as calibration data and scans were conducted continuously for up to an hour after the BMI injection, along with whole course EEG monitoring.

7.1.3 Multispectral DOT Data Analysis: Hemoglobin

For brain, the major absorption chromophores are also oxyhemoglobin, deoxyhemoglobin and water as in the breast tissue. Thus the absorption spectra $\mu_a(\lambda)$ could be written as

$$\mu_a(\lambda) = \sum_{i=1}^{I} c_i \varepsilon_i(\lambda)$$  \hspace{1cm} (7-1)
where $\varepsilon_i(\lambda)$ indicates the absorption extinction coefficient of the $i$th chromophore at wavelength $\lambda$, and $C_i$ is the concentration for the $i$th chromophore. Referring to the molar extinction coefficient spectra, the composition of water at 780nm and 850nm to the absorption is significantly low compared to those of Hb and HbO2. Thus the absorption spectra $\mu_a(\lambda)$ could be written as

$$\mu_a(\lambda) = C_{Hb}\varepsilon_{Hb}(\lambda) + C_{HbO2}\varepsilon_{HbO2}(\lambda) \quad (7-2)$$

given the above two wavelengths, the concentrations of Hb and HbO2 could be implied by solving Equation 7-1,

$$C_{HbO2} = \frac{\mu_a(780)\varepsilon_{HbO2}(850) - \mu_a(850)\varepsilon_{Hb}(780)}{\varepsilon_{HbO2}(780)\varepsilon_{Hb}(850) - \varepsilon_{HbO2}(850)\varepsilon_{Hb}(780)} \quad (7-3)$$

$$C_{Hb} = \frac{\mu_a(780)\varepsilon_{HbO2}(850) - \mu_a(850)\varepsilon_{HbO2}(780)}{\varepsilon_{Hb}(780)\varepsilon_{HbO2}(850) - \varepsilon_{Hb}(850)\varepsilon_{HbO2}(780)} \quad (7-4)$$

Tissue absorptions $\mu_a(780)$ and $\mu_a(850)$ were reconstructed independently under each wavelength using the FEM reconstruction method; $\varepsilon_{Hb}$ and $\varepsilon_{HbO2}$ were selected according to [113]. Thus oxyhemoglobin and deoxyhemoglobin maps could be generated based on the absorption distribution from the two wavelengths.

### 7.1.4 Multispectral DOT Data Analysis: Cerebral Blood Flow

So far different image modalities had been applied to study CBF, including PET, fMRI, laser Doppler, diffuse correlation spectroscopy, and laser speckle contrast imaging. However, among these image approaches, PET has limited spatiotemporal resolution[114]; fMRI is based on the BOLD signal thus requires careful selection of the scaling factor towards deoxyhemoglobin concentration[115]; laser Doppler flowmetry has limited penetration depth and is only able to measure limited points[116]; diffuse correlation spectroscopy may not be practical for continuous monitoring in humans although it had implied promising results[117]; laser speckle contrast imaging is able to
recover relative CBF with high spatiotemporal resolution but is not noninvasive. Thus there remains a demand for continuous and noninvasive imaging method of CBF with low cost and high spatiotemporal resolution.

DOT as a noninvasive, economical, portable imaging method had been proved to be able to recover continuous tissue absorption, scattering, hemoglobin concentration and oxygen saturation with a high spatiotemporal resolution in the previous studies. Yuan [118] developed the application of DOT by establishing a new mathematical model that connects changes in CBF to changes of hemoglobin and oxygen saturation (SO$_2$), and thus the model is able to indirectly measure neuron-induced vascular parameters including CBF.

To model oxygen transport in a blood vessel, consider a one-dimensional cylindrical vessel with $R_i$ and $R_o$ as the inner and outer radii, respectively, surrounded by other biological tissues. In addition, it is assumed that all the oxygen (O$_2$) diffusing out the segment is consumed in a tissue region. The law of mass conservation stipulates that the amount of O$_2$ lost from a vascular segment must be equal to the diffuse O$_2$ flux to the tissues, determined by the perivascular oxygen gradients. For a steady case,

\[
Q_{in} C_b[HbT]SO_{2,in} - Q_{out} C_b[HbT]SO_{2,out} = l_i \pi d_i J_i
\]

(7-5)

in which $Q_{in}$ (mL.$s^{-1}$) is the volumetric $BF$ into the $ith$ segment, $Q_{out}$ the volumetric $BF$ out the segment, $d_i$ is the diameter of the $ith$ segment, $l_i$ the length of the $ith$ segment, $HbT$ the total hemoglobin concentration in the blood (moles), $SO_{2,in}$ the hemoglobin oxygen saturation flowing in the segment, $SO_{2,out}$ oxygen saturation flowing out of the segment, $J_i$ the oxygen flux across the vessel wall (moles O$_2$ cm$^{-2}.s^{-1}$) and $C_b$ is the
oxygen binding capability of hemoglobin \((C_b=1.39\text{mLO}_2/\text{gmHb}; C_b =1.0\) if the concentration of \(\text{O}_2\) dissolved in plasma is considered).

Through a series of deductions \([\text{[]}]\), it could be concluded that,

\[
\frac{d\text{SO}_2}{dt} = -\frac{OC}{4V_{\text{tissue}}[HbT]} + \frac{Q}{V_{\text{tissue}}[HbT]}[HbT]_{\text{blood}} \left( \frac{\text{SO}_{2,\text{i}}}{1-f} - \frac{\text{SO}_2}{1-f} \right) - \frac{d[HbT]}{dt} \frac{\text{SO}_2}{[HbT]} \tag{7-6}
\]

where \(V_{\text{tissue}}\) is the tissue volume and is assumed constant here, \(\text{SO}_2\) is the oxygen saturation of tissue, \(Q\) is the mean \(\text{BF}\) for all the blood vessels inside the tissues and is specified as the mean \(\text{BF}\) of tissues, \([HbT]_{\text{blood}}\) is the mean total blood hemoglobin concentration in the blood circulating through the tissues, \(OC\) is the mean oxygen consumption for the whole tissue volume \(V_{\text{tissue}}\), and \(\text{SO}_{2,\text{i}}, \text{SO}_{2,\text{o}}\) is the averaged hemoglobin oxygen saturation at the inlet(artery) and outlet(vena)of the tissues, respectively.

This equation 7.6 is the developed mathematical model that connects changes in \(\text{CBF}\) known hemoglobin concentrations and oxygen saturations captured by DOT. Mean \(\text{CBF}\) can be recovered by fitting Equation 7-6 to time-resolved tissues oxygenation measurements. Equation 7-6 is an ordinary partial differential equation that can be solved iteratively by Runge-Kutta 4\textsuperscript{th} order method coupled with finite element method. With any given initial values for \(OC\) and \(BF\) within the specified range, the fitting method is to optimize the \(OC\) and \(BF\) parameters based on the solution of the minimal squared difference between measured and calculated oxygen saturation values from multiple discrete time points. To reconstruct \(BF\) and \(OC\), the initial parameters are given by:

\(HbT_{\text{blood}}=0.72\text{mM}, f=0.2, \text{SO}_{2,\text{i}}=0.98.\)
7.2 Results and Discussions: Multiple parameters

For the focus animal seizure experiments, multiple seizure activities were imaged in the four rats. Fig. 7-4 shows the EEG recording for one animal 8 minutes after the drug injection, and it could be observed that interictal spikes were generated and developed into periodic seizures. Using the collected data, tissue absorption, oxyhemoglobin, deoxyhemoglobin and CBF were recovered by the previously described methods. It is noted that since the images were averaged 100 times at each detection point in order to reduce system noise, the interval between each adjacent frames is 7s.

Figure 7-5 shows selected absolute absorption coefficient $\mu_a (mm^{-1})$ cross section images at different time points 2mm under the injection site of the brain, both under 780nm and 850nm wavelengths. Seizure focus (indicated by arrows) with increased absorption coefficient could be seen in the reconstructed *in vivo* images.

Based on the reconstructed absorption images, hemoglobin concentration distribution images were generated, which is illustrated in Figure 7-6. The increasing dynamics in blood volume and oxygenation in the scalp and brain caused the variations of DOT measured dynamics during seizures and the marked increase of hemoglobin concentration at the seizure focus is consistent with the general observations.

Then mean CBF is fitted for each linear segment based on the Hb and HbO2 images. For each segment, 2 discrete hemoglobin and oxygen saturation measurement points are used for fitting. Figure 7-7 presented the volume normalized CBF images over the 5 time points in Fig. 7-5&7-6. Increasing of CBF could be observed at the focus and the peak value of CBF (10-38ml/100ml/min) are in good agreement with reported CBF of rats (between 10 -120 ml/100ml/min) [126].
Simultaneous measurement of hemoglobin concentration, oxygen saturation, cerebral blood flow and EEG represented neuronal activities allowed a comprehensive assessment of quantified functional hemodynamic activities. In this study, we observed an increase in hemoglobin concentration and CBF across the animal’s epileptic foci under multiple trials. The results are consistent with the general observations of increased blood flow and oxygen consumption during the seizure activities and validated the multispectral imaging system and algorithms for reconstructing multiple optical parameters.
Figure 7-1. Schematic of the whole-head multispectral DOT imaging system.
Figure 7-2. Two incisions were made during the animal’s surgery to allow implants of EEG electrodes and tube for BMI drug infusion.

Figure 7-3. Agar solution was poured and solidified to fulfill the space between the animal's head and the ring interface.
Figure 7-4. EEG recording for one animal from 0 to 8 minutes after the drug injection. Discharges are generated and developed into continuous SWDs.
Figure 7-5. Selected absolute absorption coefficient $\mu_a (mm^{-1})$ cross section images at different time points 2mm under the injection site of the brain.
Figure 7-6. Selected Hb and HbO2 cross section images at same time points as in Fig. 7-4 2mm under the injection site of the brain.
Figure 7-7. Fitted volume normalized CBF cross section images at same time points as in Fig. 7-4 2mm under the injection site of the brain.
CHAPTER 8
ULTRA-FAST MULTISPECTRAL DOT IMAGING OF EPILEPSY: IN VIVO STUDY

8.1 Methods and Materials

The multispectral DOT system described in chapter 6 is able to image multiple optical parameters. However, averaging imaging 100 times to reduce the system noise greatly limited the temporal resolution of the system. Thus, by switching the data acquisition mechanism of the system and upgraded the data collection circuit, the temporal resolution of the system can reach its maximum performance at 14 Hz. The high sampling rate is crucial for the analysis of epilepsy especially the coupling study and could help to remove the noises from breathing and heart beating as well, thus providing better image qualities.

8.2 Results and Discussions

With the same BMI induced focus seizure model, three animal experiments were conducted. Figure 8-1 shows the recorded EEG signal from one animal extended to 8 minutes after the drug injection. This animal had intensive seizures that several continuous spike-and-wave discharges pieces could be observed from the EEG recording.

Figure 8-2 demonstrated 5 continuous cross section 780nm absorption images (1mm under the drug injection site) during the resting state. It is obvious that no focus-like targets could be observed from the images. This is consistent with the EEG observation that no intensive neuronal activities were induced when the animal is under anesthetization and resting.

Figure 8-3 shows 5 continuous cross section 780nm absorption images (1mm under the drug injection site) after the BMI drug injection. Marked increasing of
absorption could be seen at the seizure focus (indicated by arrow), which is markedly different from those of the resting state images. Figure 8-4 shows a 70s EEG signal piece that is corresponding to 1000 imaging frames of DOT. The average absorption of the seizure focus at two wavelengths is calculated. Total hemoglobin values were reconstructed based on the absorption distributions (Fig. 8-5). Dramatic change of HbT within 200-300ms had been observed in the curves, which could not be seen in previous relatively slow image systems.

It is noted by comparing the power spectra of EEG and the HbT curves that when spikes were observed, significant change of the HbT could be found. In Figure 8-5, the blue line (focus) represented the averaged HbT value in a r=2mm area around the drug injection point at the seizure focus level, while the red line (surrounding) represented averaged HbT value in a r=2mm area 3mm from the seizure focus plane. When spikes occur, hemoglobin at the focus and the surrounding would both drop to the same level and then increase. Hemoglobin at the focus increased much faster to 1.5 times more than the surrounding. The green line in Figure 8-5 indicated the average HbT value in an area >10mm distant from the focus, and no neural-activity related change could be observed. A detailed study of the EEG signal and optical response showed that the transient drop of HbT happened simultaneously with the neural abnormalities, and no significant advance or lag could be observed in the response (Figure 8-6). However, it should be noted that the EEG electrode placed is ~1mm distant from the drug injection site.

Our data indicates that, in a model of acute focus rat seizures, interictal spikes are accompanied by transient hypometabolism surrounding the seizure focus, followed by
clear increase of metabolism in the focus. In previous studies, the epileptic surround-
around inhibition induced by interictal spikes was considered to prevent interictal-to-
ictal transitions [127]. This phenomenon has been observed in several studies [128,
129], but yet it was not well studied during in vivo seizures. In this study, we show clear
evidence of interictal surround inhibition. The reason that focus area studied also went
through a transient inhibition is probably due to the spatial resolution of DOT itself that
the responses from the surrounding area could not be completely isolated from the
focus. However, the extent of hypermetabolism followed the initial suppression
differentiated the seizure focus from the surrounding. Previous studies hypothesized the
etiology of the surround inhibition to be a passive steal phenomenon or from active
shunting of blood initiated by vasoconstriction of vessels in the surrounding [130, 131].
Schwartz [132] reported preictal vasoconstriction followed by vasodilation in a recent
study, which is consistent with our observations. It was also reported in his study that
vessels start from ~1.5mm distance from the injection site would go through preictal
vasoconstrictions, which partially explained the drop of HbT in the focus area.
Figure 8-1. EEG recording for one animal from 0 to 8 minutes after the drug injection.
Figure 8-2. Selected continuous absolute absorption coefficient $\mu_s (mm^{-1})$ cross section images at different time points 1mm under the injection site of the brain during the resting state.

Figure 8-3. Selected continuous absolute absorption coefficient $\mu_s (mm^{-1})$ cross section images at different time points 1mm under the injection site of the brain after the drug injection.
Figure 8-4. (a) A 70s EEG signal piece that is corresponding to 1000 imaging frames of DOT. Significant changes of the spike frequencies could be observed in this piece. (b) Power spectra of the EEG signal.
Figure 8-5. Curves of the averaged HbT value over the same 70s as that of Fig. 8-4. Blue line represents the 2mm area of the injection site at the injection plane; red line represents the 2mm area on the plane 3mm from the injection level; green line represents the area 10mm from the injection site.
Figure 8-6. Detailed comparison of EEG abnormalities and corresponding optical signal response. (a-d) EEG signals, each represented a piece of 2s length; (e-h) corresponding response of optical signals
CHAPTER 9
CONCLUSIONS AND FUTURE STUDIES

9.1 Conclusions

This dissertation has explored the abilities of diffuse optical tomography to detect multiple spatial and functional features in breast cancer and epilepsy from both the hardware and software aspects. Generally, both tissue-mimicking phantom studies and in vivo studies show that DOT is capable of identifying breast lesions and epileptic abnormalities. The versatilities of DOT are tested and proved in this study, suggesting it to be a strong image modality.

We have developed a two-step PCDOT method which is able to improve the RI reconstruction qualitatively and quantitatively, making PCDOT a potentially observer-independent approach for cancer diagnosis. This method has been confirmed by phantom experiments and 42 sets of clinical data.

The reflection-mode based DOT system played an important role in this study. Using this system, preliminary study was conducted and we managed to prove that DOT is able to detect localized seizure onset and the measured signal shows a linear relationship with the input neural activities that similar linear system studies could be applied to analyze quantitative hemoglobin, oxygenation and blood flow information using multi-wavelength detections.

Using the multi-wavelength DOT system, multiple parameters including hemoglobin and CBF were recovered through a robust reconstruction algorithm. And DOT is proved to be able to locate the seizure focus under different functional parameters. Through the fast DOT imaging system, 14Hz image frames were recovered and the surround inhibition was observed, showing inner coupling of hemodynamic
response towards neural abnormal activities. The findings would serve as an important role in shaping the evolution of seizure.

**9.2 Future Studies**

For the breast cancer detection part, we expect to further evaluate this method using larger scale clinical data as well as applying this method for imaging other organs/diseases.

For brain imaging, based on the current observations, this system also has the potential to spatiotemporally detect the earliest neurovascular changes associated with seizure anticipation and onset, including providing a new tool for real-time seizure prediction based on the hemodynamic change before the seizure onset. Coupling study among the functional variables would bring insights into how these physiological features accompanied and interacted with each other during seizure activities, for instance the long-standing debate of if cerebral blood flow is able to meet the increased enormous metabolic demands during seizures; and coupling study between functional information and neural responses would help to understand mechanisms of how functional variations are associated with different aspects of neuronal activities including the average firing rate of all or partially of the neurons, the local field potential, the local synaptic activities and synchronized spiking activities.

The current epilepsy study is limited to the acute focus model only. While generalized seizures are more common in the actual situations, the next step of the study would be directed to the generalized seizure models. Current study have prepared hardware of data collection, reconstruction algorithm and interpretation methods. Should all these set as a robust foundation to investigate more complicated abnormal brain phenomenon. And last but not the least, DOT could be easily translated
to human and become a useful imaging modality to dynamically image seizure related activities.
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BIOGRAPHICAL SKETCH

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