Published in final edited form as:

PET Clin. 2013 July ; 8(3): . doi:10.1016/j.cpet.2013.04.004.

Towards non-invasive characterization of breast cancer and cancer metabolism with diffuse optics

David R. Busch^{a,b,*}, Regine Choe^c, Turgut Durduran^d, and Arjun G. Yodh^b

^aDept. of Pediatrics, Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA 19104 USA

^bDept. of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA 19104 USA

^cDept. of Biomedical Engineering, University of Rochester, Rochester, NY 14627 USA

^dICFO- Institut de Ciències Fotòniques, Castelldefels (Barcelona), Spain

Abstract

We review recent developments in diffuse optical imaging and monitoring of breast cancer, i.e. optical mammography. Optical mammography permits non-invasive, safe and frequent measurement of tissue hemodynamics oxygen metabolism and components (lipids, water, etc.), the development of new compound indices indicative of the risk and malignancy, and holds potential for frequent non-invasive longitudinal monitoring of therapy progression.

Keywords

Diffuse Optical Tomography; Diffuse Optical Spectroscopy; Metabolic Imaging; Blood Flow; Breast Cancer; Neo-adjuvant chemotherapy

1 Continuous, Non-invasive Monitoring of Blood Volume, Flow, and Oxygenation

Despite major advances in diagnosis and preventative medicine, breast cancer remains amongst the top causes of death in women. In the United States, for example, approximately 230,000 new cases of breast cancer were diagnosed and roughly 40,000 died from the disease in 2012 [1]. Currently, clinical recommendations are focused on the early detection of cancer and risk stratification, with screening techniques ranging from breast self exams and clinical palpation to serial X-Ray mammographic imaging to genetic testing. Ultrasound and MRI are frequently used in conjunction with the traditional techniques, e.g., to confirm a diagnosis. Modern breast cancer screening depends heavily on X-ray mammography, which is especially sensitive to micro-calcifications. However, this structural imaging modality can suffer from low sensitivity and specificity, especially in younger women [2].

Ultrasound breast imaging is highly effective in identifying classes of cysts [3,4], but it is not yet widely used for whole breast imaging. Contrast-enhanced MRI can be highly

© 2013 Elsevier Inc. All rights reserved.

*To whom correspondence should be addressed: drbusch@sdf.org (David R. Busch).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

sensitive and some studies have reported high specificity, but it is generally applied only to high-risk populations due to limited availability and cost [5–7]. Thus, the ideal screening modality has yet to be found, and multi-modal approaches, including genetic testing, are gaining popularity.

In this paper we are primarily concerned with the potential role of optics clinical breast cancer management and (possibly) screening based on the local tissue metabolism. Indeed, the enhanced glucose metabolism of cancer tissues is the basis for the success of FDG-PET cancer imaging which, in breast cancer, is primarily utilized to identify metastases and stage disease [8]. Measurements of the local metabolism provide insights into the cancer aggressiveness and the response to the treatments.

Significant recent breast cancer research has been oriented towards identifying and utilizing the potential of hemodynamic parameters such as blood flow, e.g., as a means to access tissue metabolism. This is based on the fact that the cancers are frequently hyper-metabolic and have associated angiogenic vessel formation, and therefore, blood flow is of considerable interest as a cancer contrast. Doppler ultrasound imaging of breast cancer blood flow has been extensively explored over the past three decades, both with and without contrast agents [4]. Gadolinium chelate contrast agent uptake and kinetics provide clinicians information on breast cancer perfusion and is part of the current clinical standard-of-care [5]. An alternative MRI method, Arterial-Spin-Labeling MRI, has proven very useful in probing brain blood flow without the injection of contrast agents. However, its use in the breast [9] is hampered by the low absolute blood flow in the breast tissue. Importantly, none of these techniques provide information about tumor blood oxygenation; this technical limitation makes it harder to gain insights about cancer oxidative metabolism.

Diffuse optical measurements can probe blood flow, oxygenation, and concentration, permitting calculation of tissue oxygen delivery, and perhaps offering a method to complement PET. Measurement of these important physiological parameters has been one of the driving reasons for the continued interest in diffuse optical mammography, despite its bumpy history from the 1920s [10]. These methods were attractive due to their non-invasive nature, relative low-cost and other features. The photons utilized for clinical diffuse optical measurements are non-ionizing (~ 1.5 eV), permitting repeated measurements without significant risk to the subjects [11, 12]. Indeed, pulse-oximeters utilizing similar wavelengths and light power are ubiquitous in hospital settings for long term care.

Diffuse optical spectroscopy (DOS) provides a localized measurement of optical properties. In the most basic form of imaging, an array of DOS measurements can be taken and the results projected onto a 2D map of tissue properties [13, 14]. More sophisticated imaging strategies rely on tomographic reconstruction, in which volumetric 3D maps of optical properties are reconstructed from light fluence measurements on the tissue surface. Human use of diffuse optical tomography (DOT) is most advanced in imaging of breast cancer, due in part to technical (low optical absorption and malleability of breast tissue) and logistical (public awareness of breast cancer, accessibility) factors. Note that DOS is also sometimes referred to as Near Infra-Red Spectroscopy (NIRS).

To date, both DOS and DOT technologies have been applied to quantify the optical properties of healthy human breast tissue [15–22] and its correlation with cancer risk prediction [23–26]. Several reviews of DOT breast cancer imaging and contrast are also available [20, 27, 28]; exogenous contrast agents can also be utilized and will be discussed in Sec. 5. DOT has been the focus of much effort in both academic [15, 29–35] and industrial [36–41] settings. More details about the DOT reconstruction process may be found in the recent review by Arridge and Schotland [42].

In Table 2, we attempt to illustrate the broad characteristics of current imaging techniques and the niche which we believe Diffuse Optics can fill, e.g., for providing continuous metabolic measurements without the use of ionizing radiation.

One of the obvious applications of optical metabolic monitoring of tumors arises during therapy. In particular, in the case of neoadjuvant chemotherapy which is an increasingly popular breast cancer treatment protocol. For neoadjuvant chemotherapy drugs are administered prior to surgical excision [43] to reduce the tumor size and eliminate or reduce micro-metastases prior to the surgery. Fig. 1 lays out a potential time-line for DOS/DOT to assist in judging the efficacy of therapy planning. This sequence permits the early observation of the clinical effects of specific drug regimens which, in turn, could potentially permit early determination of the chemotherapy effectiveness and inform clinical decisions on continuing a particular therapy. Monitoring the efficacy of neoadjuvant chemotherapy monitoring is an active area of research in clinical medicine [44–46], including optical imaging and monitoring [47–67].

We note that it is not the intention of this review to cover clinical applications of diffuse optics exhaustively; rather, we aim to provide a snapshot of recent diffuse optics-based efforts to non-invasively quantify oxygen metabolism in breast tissue, with an emphasis (though not exclusively) on research from our laboratories. For more extensive discussion about these issues, the reader is encouraged to peruse the primary papers we cite, as well as recent reviews about diffuse optical imaging/spectroscopy [68, 69] and diffuse correlation spectroscopy [61, 68, 70–72].

The review is organized as follows. Initially, we focus on use of statistical analysis of diffuse optical data to derive a probability of malignancy for cancer localization and therapy tracking. We then examine how diffuse optical data hold promise in metabolic imaging. We discuss a comparison study between endogenous DOT and FDG-PET imaging, an all-optical metabolism measurement, and the use of external perturbations to probe tissue metabolism. Finally, we review the use of exogenous contrast agents in optical breast cancer imaging.

2 The propagation of photons in tissues

2.1 Physics of Diffuse Optics

Light in the near-infrared (NIR) spectral window (650 - 950 nm) is weakly absorbed but strongly scattered by tissue. Fig. 2 depicts this scenario in a model turbid medium. Fig. 2(b) shows the propagation of laser light through a clear, non-scattering medium (tap water). Note that the light propagates straight across the bath, with minimal dispersion of the columnated beam. We then start adding scatterers to the medium; first the beam is broadened in a manner akin to the visibility on a “foggy day” (Fig. 2(c)), and finally the photon transport is diffusive as is NIR light in tissue (Fig. 2(d)). Two length scales are important: (1) a photon random walk step (l^*), and (2) a photon absorption length (l_a). The random walk step (l^*), or photon transport mean free path, corresponds to the typical distance traveled by a typical photon before its initial propagation direction becomes randomized ($l^* \sim 1\text{mm}$). For example, most of the photons shown in Fig. 2(c) have had their initial direction randomized and the directionality of the laser beam is lost; by contrast, in Fig. 2(b) many photons are still traveling in a straight line through the medium. A wavelength (λ) dependent reduced scattering coefficient ($\mu'_s(\lambda) = 1/l^*$) denotes the reciprocal of this photon transport mean free path and is often the term of choice to describe tissue scattering. The absorption length (l_a) corresponds to the typical distance traveled by a photon before it is absorbed; it is wavelength dependent. In the near-infrared range, this absorption length in tissue is typically much longer ($\sim 200\text{ mm}$) than scattering length. Its reciprocal is denoted by the absorption coefficient ($\mu_a(\lambda)$).

The first incarnation of optical mammography in 1920s [10, 73] simply relied on the transmission of lamp light through the breast and its observation in a dark room. Unfortunately, as the authors of the original papers admit, this technique was highly qualitative and unreliable. In the early 1980s the technique was revisited, this time using wide-beam transillumination; however, it was not successful clinically due (in part) to limited projection information and lack of separation of tissue absorption from tissue scattering effects [74–85].

The key to the reemergence of optical techniques for breast cancer imaging was the realization that light transport through tissue is a diffusive process and the subsequent development of accurate physical/mathematical models for photon propagation that separated tissue absorption from tissue scattering effects. This mathematical approach enabled researchers to accurately quantify both tissue absorber (or chromophore) concentrations and tissue scattering coefficients. The acceptance of this physical model, alongside the advances in the algorithms and technologies, re-ignited interest in the field by the mid-90s [86, 87].

Once a paradigm was developed to separate tissue absorption from tissue scattering, the use of multiple optical wavelengths enabled experimenters to quantitatively determine the concentrations of various tissue chromophores (and contrast agents). For example, since oxy-hemoglobin has a markedly different optical spectra than deoxy-hemoglobin in the NIR (i.e., see Fig. 3), it is readily possible to utilize absorption coefficient data at multiple wavelengths to recover absolute values of the concentrations of the hemoglobin species. Similar information about other chromophores, such as water and lipid content, and the scattering components of the tissue can be derived from the wavelength-dependent absorption and scattering data.

Fig. 4 illustrates two geometries frequently employed in optical mammography. Parallel plate transmission (left) and re-emission (right) geometries for measurements are shown. Other geometries include rings, and combinations of transmission and re-emission measurements. In all instruments, one or more light (e.g., laser) sources inject light into the tissue, and the light transmitted through the tissue is collected by one or more detectors. In the figure, representations of the most probable trajectories of the detected photons are super-imposed in gray scale; these trajectories are derived from analytic solutions of the photon diffusion equation in the relevant geometry.

3 Probability of Malignancy Optical Indices based on Diffuse Optical Tomography of Breast

Most current work in DOT focuses on the metabolically-dependent physiological variables, without explicitly calculating tissue metabolism. In this section, we discuss DOT imaging of total hemoglobin concentration (Hb_t), blood oxygen saturation (StO_2), and tissue scattering (μ'_s) and the subsequent development of malignancy indices. Several researchers have pushed diffuse optical methods in order to add additional specificity for diagnosis; in some cases, this research has led to the development of useful combinations of the measured physiological parameters or composite indices that better distinguish malignant from benign lesions [13, 35, 51, 88, 89]. Here we focus on a recently developed Computer Aided Detection (CAD) technique that builds on ideas from the X-ray community to improve radiological data interpretation [90]. These CAD techniques are quite versatile; for example, besides utilization of multi-dimensional optical data, they permit simultaneous use of multi-parameter data, for example, combining FDG uptake measured with PET and Gd-contrast agent kinetics with MRI [91].

Diffuse optical tomography offers a fertile testing ground to apply these ideas in cancer detection, diagnosis, and therapy monitoring. In general, each of the detected optical parameters is sensitive to tissue physiology, but interpretation of multiple 3D images is challenging. CAD offers a simple paradigm to develop composite indices, taking into account all parameters and their heterogeneities, while deriving a single “probability of malignancy” tomogram. To date, several groups have applied statistical analysis techniques to multi-parameter optical measurements to derive risk factors. Applications have included arthritic joints [92], high-risk [93] or high mammographic density [23, 24] breast tissue, and various ‘endoscopic’ measurements or excised tissues [94]. However, these data sets have limited spatial information and orders of magnitude fewer measurements per subject than, for example, the breast tomograms to be discussed below. Other researchers have implemented automated DOT image analysis techniques to identify lesions in a particular subject [95–97] however, this analysis neglects information about the common signatures of cancer across a population.

Here we will present illustrative results drawn from work conducted at the University of Pennsylvania [35, 66, 98]. The work is based on a set of 3D tomograms of total hemoglobin concentration (Hb_t), blood oxygen saturation (StO_2), and tissue scattering (μ'_s) that were obtained from 35 subjects with biopsy-confirmed lesions [34, 35]. This data set permitted exploration of the potential of DOT-CAD for both cancer localization and cancer therapy monitoring.

The CAD algorithm requires identification of a group of subjects with data from both the modality under consideration (DOT) and a “gold standard” diagnosis. Essentially, training set data from this group is used to derive a common malignancy signature, from the combination of optical parameters that best reproduces the gold standard diagnosis. As a test, the signature is then examined in additional subjects (the test set), who were not included in developing the diagnostic signature. Finally, the diagnostic results from the test set are compared to the gold standard diagnosis to evaluate the utility of the test.

Per diffuse optical properties of breast, we [35], and others [20, 51, 99], have observed that the inter-subject variation of physiological parameters (Hb_t , StO_2 , μ'_s) can be quite large. Therefore, the first technical strategy we introduced was to adopt an intra-subject normalization scheme which reduces this inter-subject variation. Specifically, we log-transform all data, and then we subtract the mean value of each parameter in healthy tissue *for each subject*; finally, we divide this result by the standard deviation of the parameter in healthy tissue. Thus we obtain a “Z-score” normalized variable for each optical parameter. This *intra-subject* normalization spectacularly reduces the *inter-subject* variation in all of the optically measured parameters, as shown in Fig. 5(a).

With these new “Z-score” normalized tissue parameters we can sensibly combine the Hb_t , StO_2 , and μ'_s data from the tissue volume elements (voxels) of multiple subjects (training set) in order to generate a single statistically derived “malignancy parameter” ($\mathcal{M}$). $\mathcal{M}$ is a weighted linear combination of physiological parameters from the training population of cancers. The weighting vectors, $\mathbf{w}$, are optimized using data from each voxel of each patient and logistic regression. The extracted weighting factors (or weighting vector) is then applied to an additional subject (test set); thus the $\mathcal{M}$ of each voxel in the subject is computed and a probability of malignancy ($P(\mathcal{M})$) for each volume element in the breast can be assigned. A threshold probability is then used to define a binary mask for cancer location (Fig. 5(b)). Example results are shown Fig. 6. Notice that the blood oxygen saturation (StO_2) images (Fig. 6(b)) have the least lesion contrast; the weighting factor of StO_2 is significantly smaller than Hb_t and μ'_s . Interestingly, one of our subjects with an *in situ* lesion (left column, Fig. 6)

exhibited a probability of malignancy that was significantly lower than the threshold cutoff we obtained for malignant tissue; this observation suggests the potential for future versions of the metric to distinguish between types of lesions, as well as between healthy and malignant tissue.

Finally, we have recently extended the DOT-CAD results to monitor the efficacy of neoadjuvant chemotherapy [66]. The $P(\mathcal{M})$ calculation brings together several physiological parameters to identify changes. In contrast to the composite probability of malignancy signature, significant differences were not observed in the responses of both absolute and relative values of the *individual* physiological parameters (Hbt, StO_2 , μ_s'). Although our pilot study was limited to three subjects, two of whom responded completely to chemotherapy and one who had a partial response, the dynamics of changes in $P(\mathcal{M})$ between these two groups were significantly different (Fig. 7). The observations shown in Figs. 6 and 7 demonstrate potential for extraction of a simple, clinically relevant metric for cancer staging and therapy monitoring from diffuse optical images. Promising early results [64] suggest that these optical techniques could be used to determine the efficacy of particular neoadjuvant chemotherapy regimes significantly prior to tumor changes revealed by structural imaging.

In total, the results of this study are encouraging, suggesting that statistical analysis of the metabolically-dependent optically-measured physiological parameters of cancer tissue can yield a useful signature of cancer location and response to treatment. We note that the study described here was derived from a limited population (35 subjects), and that our chemotherapy monitoring is in the pilot stage (3 subjects). A more expansive study is now needed.

4 Towards Metabolic Imaging

4.1 Endogenous DOT versus PET glucose imaging

It is difficult to validate the hemodynamic parameters measured by DOT directly against other diagnostic modalities, since none is able to non-invasively measure all of the same parameters. However, correlation of parameters such as the total hemoglobin concentration measured by DOT with microvessel density measured by histopathology has been carried out [54, 63, 101, 102]. To date, two major approaches have been explored for direct correlation/comparison of DOT with other imaging modalities: (1) Image correlation based on the stand-alone images taken independently, and (2) correlation based on concurrent image acquisition. The deformability of breast tissue makes the former approach challenging. Nonetheless, research along these lines has demonstrated that the high tumor-to-background ratio detected by DOT corresponds to the tumor locations identified by MRI [50, 52, 103] and by PET [89]. These and other works have led to the development of advanced software suites that permit researchers to transform the volumetric information between imaging modalities.

Per concurrent acquisition, it is challenging but possible to integrate DOT source- and detector-systems into other medical imaging modalities. This has led to interesting multi-modal imaging instrumentation, e.g., DOT and MRI [104–107], DOT and Tomosynthesis [108, 109], and, DOT and Ultrasound imaging [110–112]. The primary disadvantage of the multi-modal approach is that hardware constraints limit the total number of DOT measurements to values less than typical for stand-alone DOT systems. Most efforts to date have focused on utilization of anatomical information from MRI, Tomosynthesis, and Ultrasound to derive spatial priors for DOT reconstructions; this concept has been shown to improve DOT quantification. Furthermore, concurrent multi-modal imaging offers a great opportunity for DOT validation. In this vein, the combination of PET and DOT (and/or

DCS) is interesting for validation of mechanisms of oxidative metabolism, and for consideration of DOT as a surrogate to PET when frequent measurements are desirable.

While concurrent PET and DOT has not yet been demonstrated in humans, the co-registration and correlation of non-concurrent DOT and PET has been performed [89]. Figure 8(a) shows a qualitative correlation between FDG and DOT signals in axial, sagittal, and coronal views of patient breasts. Note, PET imaged the whole body in this comparison, whereas DOT imaged a single breast. The dotted square in PET indicates the same breast imaged by DOT. Since image co-registration between breast images taken in supine and prone positions is difficult, each 3D image was analyzed independently for quantifying tumor-to-background ratio for each parameter. Figure 8(b) shows a selected example from correlation between FDG and DOT-derived parameters. Positive correlations ($p < 0.05$) were found between FDG uptake and Hb_t and FDG uptake and μ'_s . The positive correlation between FDG and Hb_t might be expected, since increases in glucose metabolism of breast cancer require more blood for glucose and oxygen delivery which, in turn, is typically accompanied by an increased total hemoglobin concentration.

For three patients, PET imaging was performed using a dedicated breast imaging PET scanner [113, 114]. Then, a co-registration algorithm based on rigid body motion (translation and rotation), and linear scaling [103] was utilized to transform the DOT image space into the PET image space, i.e., since DOT was measured with axial compression whereas PET was measured without compression. A caudal-cranial slice from a 3D DOT image encompassing the suspicious mass for each parameter (Hb_t , StO_2 , μ'_s) is presented along with the FDG image slice in Fig. 9. Subject A had a ductal carcinoma in situ, subject B had a palpable mass which turned out to be a seroma due to biopsy-induced hemorrhage in the center, and subject C had invasive ductal carcinoma. Increases in Hb_t and μ'_s contrast exhibited correspondence with similar high FDG regions in the PET images. In total, these results demonstrate that DOT is indeed sensitive to the local metabolism and may provide complementary information to PET.

4.2 All optical measurement of oxygen extraction fraction and metabolism

Here we highlight another approach that utilizes diffuse light to non-invasively measure microvascular, local blood flow (BF). The measurement employs a technique referred to as diffuse correlation spectroscopy (DCS) in the biomedical optics community [61, 68, 115, 116]. DCS employs coherent laser sources and utilized the temporal statistics of light speckles emerging from tissue. These statistics reveal quantitative information about the motion of red blood cells (RBCs) in the interrogated tissues. Local blood flow (BF) is characterized by an index derived from the temporal decay rate of the diffusing light field temporal intensity autocorrelation function.

“Optical mammography” or breast tumor monitoring with DCS was first introduced in 2005 [117]. In that work, the authors observed a significant increase of the DCS BF index in tumors compared to the normal tissue. Later, the authors demonstrated that early changes in tumor physiology can also be discerned by DCS flow measurements and that the relative changes were comparable to relative changes measured by DOS/DOT [53]. Most recently, these approaches have exhibited potential to differentiate responders from non-responders amongst neoadjuvant chemotherapy patients, and potential for use in drug development schemes [61]. For recent reviews see references [61, 68].

Importantly, DCS information can readily be combined with information from DOS/DOT to gain access to tissue oxygen metabolism. This combination is attractive for two reasons: (1) DCS and DOS/DOT probe very similar tissue volumes for the same source-detector

positions and so the results are easily combined and compared; (2) the ability to collectively measure blood flow, blood volume and blood oxygenation enables researchers to estimate the oxygen extraction fraction (OE) and the tissue metabolic rate of oxygen extraction [53, 68, 118].

To illustrate this latter point, we introduce two composite “optical indices” which are indicative of the tissue physiology. The tissue optical index (*TOI*) suggested by the Tromberg group at University of California, Irvine, is a multiparameter contrast function created to maximize both the contrast and the specificity of the optical measurement. *TOI* is defined as [13, 51]

$$TOI = \frac{Hb \cdot H_2O}{Lipid}. \quad (1)$$

TOI is empirically constructed from the concentrations of deoxy-hemoglobin (*Hb*), water (H_2O), and lipids (*Lipid*). Increased *TOI* has been found to reflect a higher chance of tumor malignancy, and *TOI* is also related to metabolic activity, since increases in deoxy-hemoglobin are often a symptom of unmet metabolic demand.

Inclusion of DCS in the arsenal of diffuse optical tools for optical mammography enables us to derive a direct estimate of tissue oxygen metabolism by combining information about blood flow (delivery of oxygen) with the chromophore concentration information such as blood oxygen saturation (i.e. oxygen availability). This approach has most often been employed in neurological applications with hybrid diffuse optics [68], but these ideas are readily translated to other tissues, albeit without a robust validation. Here, we describe a simple model and index which offers a new means to define tumor contrast based on mammary metabolic rate of oxygen consumption (*MMRO*₂) following Zhou [53, 118]. We define

$$MMRO_2 = \gamma \cdot \frac{Hb}{Hb_t} \cdot BF \quad (2)$$

where $\gamma = \frac{Hb_v/Hb_{tv}}{Hb/Hb_t}$, the ratio of deoxy-hemoglobin (*Hb*) to total-hemoglobin (*Hb_t*) in the venous compartment () compared to the ratio of deoxy-hemoglobin to total-hemoglobin in the total vasculature. Here, we define the *relative* rates of oxygen metabolism between tumor

(“*T*”) and normal (“*N*”) breast tissues $\left(rMMRO_{2(T/N)} = \frac{MMRO_{2(T)}}{MMRO_{2(N)}} \right)$ as

$$rMMRO_{2(T/N)} = \frac{\gamma_T}{\gamma_N} \cdot \frac{Hb_{(T)}}{Hb_{(N)}} \cdot \left(\frac{Hb_{t(T)}}{Hb_{t(N)}} \right)^{-1} \cdot \frac{BF_{(T)}}{BF_{(N)}} \quad (3)$$

$$= \frac{\gamma_T}{\gamma_N} \cdot rHb_{(T/N)} \cdot \left(rHb_{t(T/N)} \right)^{-1} \cdot rBF_{(T/N)} \quad (4)$$

In Eqn. 4, we have abbreviated the relative values of a parameter between tumor and normal tissue as $rX_{(T/N)} = X_T/X_N$. If we assume that the ratio of γ_T/γ_N is unity and constant over time, Eqn. 4 reduces to

$$rMMRO_{2(T/N)} = \frac{rHb_{(T/N)}}{rHb_{t(T/N)}} \cdot rBF_{(T/N)} \quad (5)$$

or alternately

$$rMMRO_{2(T/N)} = \frac{1 - StO_{2(T)}}{1 - StO_{2(N)}} \cdot rBF_{(T/N)} \quad (6)$$

where StO_2 is the tissue oxygen saturation.

$rMMRO_{2(T/N)}$ is thus the relative oxygen extraction multiplied by the relative blood flow in the tumor compared to the normal tissue, i.e. $MMRO_2$ relates the local blood flow and the local oxygen extraction fraction to each other in a manner that is presumably proportional to the extraction of oxygen due to the local oxygen metabolism. $rMMRO_{2(T/N)}$ is an attempt to quantify the tumor oxygen metabolism with respect to the healthy surrounding tissue.

Figure 10 shows how one can use these data and these new indices in following cancer therapy. The figure shows data from a 45-year-old premenopausal woman undergoing neoadjuvant chemotherapy treatment for an invasive ductal carcinoma in the left breast [53, 118]. The patient was treated with neoadjuvant chemotherapy delivered in multiple stages. The initial treatment stage consisted of Doxorubicin and Cyclophosphamide. Further details are provided in References [53, 118].

The data shown in Figure 10 indicates the richness of the parameters measurable with the hybrid diffuse optical instrumentation. We access both hemodynamic information and structural information about the tumor over time. These parameters all show varying degrees of change as the therapy progresses at a very early stage, i.e., as early as three days after the start of the therapy. In particular, BF measured with DCS increased initially on day three after the start of therapy, and then decreased sharply and in a sustained manner during days four, five and seven. This type of behavior, in response to therapy, was found again in later studies and was suggested to be indicative of therapy response [61].

Figure 10 shows the evolution of the two composite indices; $rMMRO_{2(T/N)}$ and $rTOI_{(T/N)}$ ($rTOI_{(T/N)} = TOI_T / TOI_N$). Notice that they have diverged from one another by day three, wherein $rTOI_{(T/N)}$ exhibited an initial drop and $rMMRO_{2(T/N)}$ exhibited an initial increase. After day four, both metabolic indices dropped significantly and then stabilized until the end of the monitoring period. The authors of this work have speculated that although both $rTOI_{(T/N)}$ and $rMMRO_{2(T/N)}$ are related to tumor metabolic responses, $rTOI_{(T/N)}$ provides information about tumor cellular metabolic activities, while $rMMRO_{2(T/N)}$ provides information about tumor oxygen metabolic changes. At this time, it is too early to say whether these composite parameters will prove to be useful in the therapy monitoring or diagnostics in optical mammography, but they provide a new approach to a direct probing of the tissue physiology.

In principle, DCS can be used to carry out tomographic optical mammography of blood flow, i.e. diffuse correlation tomography (DCT). At this time, however, DCT has only been demonstrated in tissue phantoms and in rat brain [119]. We expect that with increased parallelization and improved technologies, DCT might soon be used for breast imaging. In fact, some spectroscopic results in the transmission geometry have been published [100];

these studies suggest that with a better dynamic range it should be possible to construct a standalone breast DCT system.

4.3 Dynamic imaging of physiological perturbations

In this section, we highlight a different approach to optical mammography which utilizes physiological perturbations to generate contrast in human breast tissue [100, 120–125]. For example, internal or external applied pressure can alter tissue optical properties in a predictable manner and thus provide the experimenter with additional metabolic/hemodynamic tissue information and contrast. Specifically, Carp and colleagues [126] have utilized mild cyclical perturbations of the applied load to derive an estimate for the baseline metabolism of breast tissue. Their experiment consisted of placing the breast between two parallel plates, i.e., similar to the geometry of an axial mammogram. Then frequency-domain optical data (DOS) was collected in the remission geometry from the inferior plate as a stepper motor applied force (up to 6 lbs) to the superior plate for 90 seconds (repeating the cycle three times). The underlying idea of this approach is that the changes in total hemoglobin concentration in response to partial vessel occlusion serves as a proxy for net, bulk blood flow. This is essentially borrowed from other areas such as venous cuff-occlusion photoplethysmography. The derived flow information is combined with measured changes in tissue oxygen saturation, in a similar manner to the above-mentioned $MMRO_2$, to calculate the bulk tissue oxygen consumption. When compared to values reported in literature (See Table 3), their measurements appear to underestimate these quantities. However, in the remission geometry one might also expect the measurements to be much more sensitive to the outer fatty region of breast rather than the central fibroglandular region. Thus the underestimation may be due to low blood flow and oxygen consumption in the fatty region. In spite of the limitation, this approach represents a step forward per extraction of absolute numbers for oxygen consumption and blood flow.

Similarly, while not explicitly extracting metabolic parameters, other scientists have utilized compression schemes to create differential images based on the variation of the compressibility and/or the vascular resistance of the tumors by comparison to surrounding healthy tissue [17, 38, 120–122]. Xu and colleagues have developed a hand-held pressure modulation device [124], and Dixit and colleagues have utilized inhaled gasses to generate vascular contrast [129–131]. Overall, these dynamic methods demonstrate the versatility of the diffuse optical techniques in seeking different physiological contrasts.

5 Exogenous Contrast Agents

Like other conventional imaging modalities, the capabilities of diffuse optics can be enhanced with the use of exogenous contrast agents. With the increase in contrast by use of a contrast agent image resolution and fidelity can be improved. In a different vein and perhaps more interestingly, DOT could be employed to access numerous parameters related to tumor pathology (e.g., pH, tumor-specific receptors, glucose metabolism, etc) with optimized tumor-targeting optical contrast agents [132]. Such agents are commonly utilized in animal models, and they are expected to become more widely available for human use in the near future.

Presently, the metabolic sensitivity and the spatial resolution of DOT are analogous to that of PET prior to the advent of PET/CT systems. As noted earlier, several research groups are exploring potential multi-modal combinations of DOT with other techniques that are sensitive to tissue structure. This multi-modal approach, analogous to that of PET/CT, could open a new chapter for the field. An interesting advantage to DOT, e.g., compared to some modalities, is its potential for *simultaneous* imaging of multiple contrast agents. This

capability provides opportunities for simultaneous monitoring of several tumor characteristics [107, 133].

Currently, Indocyanine Green (ICG, sold under the brand name Cardio-Green [134]) is the only FDA-approved agent that can be utilized as an absorption enhancing agent or a fluorescence agent in the near-infrared spectral region. In this case, the absorption/excitation (peak ~ 800 nm) and emission (peak ~ 820 nm) wavelengths have low absorption in tissue, permitting highly sensitive detection. ICG is used routinely in the clinic for measurement of cardiac output, in hepatology for liver function evaluation, in ophthalmology for choroidal angiography, and in neurology for cerebral artery infarction detection [135]. In plasma, rapid binding of ICG to macromolecules like lipoproteins and albumin makes ICG act as a blood-pooling agent. ICG is eliminated by the liver with a short half-life of a few minutes [136]. In malignant tumors, new blood vessels tend to be hyper-permeable, enabling large molecules such as albumin to extravasate into the interstitium [137]. Thus, there is a good probability that ICG bound to macromolecule could leak out of vasculature and therefore serve as a marker for vascular permeability.

The first uses of ICG in diffuse optics were as the absorption and fluorescence contrast agents for optical mammography. With a concurrent MRI/DOT device, **absorption** enhancement due to ICG at 830 nm was utilized to highlight different degrees of enhancement in three cases: one with an invasive ductal carcinoma, one with fibroadenoma, and another with no disease [104]. Highest tumor-to-normal contrast was found in the invasive ductalcarcinoma in 2D DOT, and its location was confirmed with the MRI taken simultaneously.

The first 3D reconstruction of ICG **fluorescence** in human breast has since been demonstrated in three subjects with malignant breast tumors [138]. High ICG concentration was found in the same region exhibiting high endogenous contrast (i.e., $Hb_b \mu'_s$); the fluorescence concentration contrast even for this blood pool agent was significantly higher than endogenous contrast based on absorption/scattering. Interestingly, the contrast enhancement due to ICG was even more dramatic in the heterogeneously dense breast than in the entirely fatty breast. In addition to static imaging, some attempts at differentiating benign from malignant tumors have been carried out based on the different pharmacokinetics of ICG-based on absorption contrast. These results have been mixed and to date have only been investigated with limited number of subjects [139–142]. The mixed results could be attributed to the fact that these measurements were taken within 10 minutes after bolus ICG injection when the signal was dominated by ICG contained in the blood, but not by ICG in the extravascular space.

Researchers at Physikalisch-Technische Bundesanstalt (PTB) and Charite' Hospital in Berlin have imaged ICG in vascular and extravascular phases using a combination of ICG bolus injection and continuous infusion (Fig. 11) [143]. The fluorescence-to-absorption ratio image taken during the extravascular phase showed enhancement corresponding to the location of an invasive ductal carcinoma. This lesion was detected utilizing contrast-enhanced MRI, but not detected in an X-ray mammogram. In the case of fibroadenoma, fluorescence-to-absorption ratio images were similar to that of surrounding healthy tissue. Charite' has extended this study to 20 women with 21 breast lesions (8 benign and 13 malignant lesions) yielding mean sensitivity of 92% and specificity of 75%. Compared to ~ 100% sensitivity and 25% specificity of conventional mammography alone, this fluorescence technique demonstrated improvement in specificity [144].

Recently, Bayer Schering Healthcare (Berlin, Germany) has attempted to translate a new NIR fluorescent optical contrast agent, “Omocyanine” to human use. Omocyanine is an ICG

derivative with a plasma half-life of approximately 15 hours in humans and with superior quantum efficiency compared to ICG (i.e., higher fluorescence). However, absorption and fluorescence measurements did not differentiate between benign and malignant tumors with this particular agent [135, 145].

In summary, several research groups have demonstrated that quantification of *in vivo* fluorescent dye concentration in breast is feasible in humans. One can simply enhance vascular contrast for DOT with bolus-ICG during vascular phase and/or access an additional parameter, vascular leakiness, with a carefully designed ICG injection protocol in extravascular phase. The latter parameter may be an especially powerful tool to differentiate benign and malignant lesions. Although Omocyanine failed to show improvement over ICG for breast cancer imaging, other researchers have found multiple applications for ICG fluorescent imaging in translational research [146]. When new fluorescent dyes with higher quantum efficiency than ICG, or moieties targeting particular cellular markers, finally get translated into the clinic, then the arsenal of optically available tumor characteristics will increase and optical imaging and monitoring should emerge as a powerful tool.

6 Summary and Outlook

In this contribution, we have discussed the recent trends in optical mammography as the field moves towards metabolic imaging and monitoring. Our discussion was oriented towards diffuse optics technologies, with attention to composite signatures of malignancy, metabolic information from blood flow and hemoglobin concentration parameters, exogenous contrast agents, and dynamic contrast. Indeed, the American Cancer Society 2003 guidelines for breast cancer screening recognized diffuse optical mammography as a promising technology, worthy of future study [147]. Since these guidelines were published a decade ago, the field has advanced significantly with several studies incorporating larger patient populations [20, 33, 148–150]. Researchers have focused on niche applications, for example, the neoadjuvant chemotherapy monitoring [61, 151] with perhaps the most exciting recent development, in terms of its translation to routine clinical use, being an ongoing trial sponsored by the American College of Radiology Imaging Network (ACRIN), in which neoadjuvant chemotherapy patients are enrolled in a seven-site trial with identical instrumentation (ACRIN 6691).

We note that in addition to the deep tissue applications of diffuse optics, there has been considerable effort focused on intra-operative measurements of cancer margins and biopsy samples [152–160]. Fluorescence planar imaging through thin tissue for sentinel lymph nodes may also offer an alternative to radioscinigraphy [161–163]. Other notable recent developments which we did not review here, include novel multi-modal instrumentation *combining* diffuse optics with ultrasound (photo-acoustic tomography) take advantage of hemoglobin absorption to selectively induce photothermal expansion. This rapid expansion produces a pressure wave detectable with ultrasound transducers [164], a technique that has been demonstrated in the breast [165–167].

We hope the reader can discern that the diffuse optical technologies offer potential applications over a wide range of areas in breast cancer screening, detection, staging and therapy monitoring. Indeed, in many ways the present stage of DOT development parallels the early stages of PET. More advances need time for demonstration, but the outlook is exciting.

Acknowledgments

We acknowledge fruitful collaborations and discussions about optics and clinical opportunities for optics over many years with numerous outstanding scientists including Britton Chance, Joseph P. Culver, Xingde Li, David A. Boas,

Guoqiang Yu, Chao Zhou, Ulas Sunar, Bruce Tromberg, Rickson Mesquita, Mitchell D. Schnall, Mark A. Rosen, Brian J. Czerniecki, Julia Tchou, Douglas L. Fraker, Angela DeMichele, Carolyn Mies, Michael D. Feldman, Mary E. Putt, Wensheng Guo, Simon R. Arridge, Martin Schweiger, John C. Schotland, Alper Corlu, Soren D. Konecky, So Hyun Chung, Wesley Baker, Ashwin Parthasarathy, Kijoon Lee, Saurav Pathak, Han Y. Ban, Malavika Chandra, Yu Chen, Jonathan Fisher, Joe Giammarco, Monica Holboke, Xiaer Intes, Xingde Li, Vasilis Ntziachristos, Maureen O'Leary, Yalin Ti, Hsing-Wen Wang, Guoqiang Yu, Udo Weigel, Peyman Zirak, Parisa Farzam, Johannes Johansson, Ki Won Jung and Leonid Zubkov.

This research would not have been possible without the generosity of the research subjects who participated in our studies.

AGY gratefully acknowledges partial support from the National Institutes of Health through grants CA087971, NS060653, EB002109, and EB015893.

TD gratefully acknowledges partial support by Fundació Cellex Barcelona, Marie Curie IRG (FP7, RPTAMON), Institute de Salut Carlos III (DOM-MON, FIS), Ministerio de Ciencia e Innovación (MICINN), Ministerio de Economía y Competitividad, Institució CERCA (DOCNEURO, PROVAT-002-11), Generalitat de Catalunya, European Regional Development Fund (FEDER/ERDF) and LASERLAB (FP7) and Photonics4Life (FP7) consortia.

RC gratefully acknowledges support from the National Institutes of Health through K99/R00-CA126187.

DRB gratefully acknowledges partial support from the Thrasher Research Fund and National Institutes of Health through grant NS072338.

The authors (TD, RC and AGY) are co-inventors in various patents about diffuse optical technologies, but they do not currently receive any royalties. There no other conflicting financial or ethical issues to disclose.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA-Cancer J Clin.* 2012; 2012; 62:1, 10–29. [PubMed: 22095204]
2. Kriege M, Brekelmans CT, Boetes C, et al. Efficacy of mri and mammography for breast-cancer screening in women with a familial or genetic predisposition. *N Engl J Med.* 2004; 351(5):427–437. [PubMed: 15282350]
3. Boyd, J.; Jellins, J.; Reeve, T., et al. chapter Doppler examination of the breast. John Wiley and Sons; New York: 1983. Ultrasound examination of the breast; p. 385–386.
4. Sehgal CM, Weinstein SP, Arger PH, et al. A review of breast ultrasound. *Journal of mammary gland biology and neoplasia.* 2006; 11(2):113–123. [PubMed: 17082996]
5. Saslow D, Boetes C, Burke W, et al. American cancer society guidelines for breast screening with MRI as an adjunct to mammography. *CA-Cancer J Clin.* 2007; 57(2):75–89. [PubMed: 17392385]
6. Lord S, Lei W, Craft P, et al. A systematic review of the effectiveness of magnetic resonance imaging (mri) as an addition to mammography and ultrasound in screening young women at high risk of breast cancer. *European journal of cancer.* 2007; 43(13):1905–1917. [PubMed: 17681781]
7. Weinstein SP, Localio AR, Conant EF, et al. Multimodality screening of high-risk women: a prospective cohort study. *J Clin Onc.* 2009; 27(36):6124–6128.
8. Rosen EL, Eubank WB, Mankoff DA. FDG PET, PET/CT, and breast cancer imaging1. *Radiographics.* 2007; 27(suppl 1):S215–S229. [PubMed: 18180228]
9. Wu, WC.; Englander, S.; S, MD., et al. Feasibility of arterial spin labeling in the measurement of breast perfusion. *Proceedings of the International Society for Magnetic Resonance in Medicine; Proceedings of the International Society of Magnetic Resonance in Medicine; 2007; p. 2801*
10. Cutler M. Transillumination as an aid in the diagnosis of breast lesions. *Surgery Gynecology and Obstetrics.* 1929; 48:721–29.
11. Laser Institute of America. American national standard for the safe use of lasers: Ansi z136.1-2007. Laser Institute of America; Suite 128, 13501 Ingenuity Drive, Orlando FL 32826: 2007.
12. Food and D. Administration. Code of federal regulations, title 21. Vol. 8. U. S. Government Printing Office via GPO Access; Apr. 2003 21CFR1040.10

13. Cerussi A, Shah N, Hsiang D, et al. In vivo absorption, scattering, and physiologic properties of 58 malignant breast tumors determined by broadband diffuse optical spectroscopy. *J Biomed Opt.* 2006; 11:044005(16pp)
14. Chance B, Nioka S, Zhang J, et al. Breast cancer detection based on incremental biochemical and physiological properties of breast cancers a six-year, two-site study. *Acad Rad.* 2005; 12(8):925–933.
15. Durduran T, Choe R, Culver JP, et al. Bulk optical properties of healthy female breast tissue. *PMB.* 2002; 47:2847–2861.
16. Spinelli L, Torricelli A, Pifferi A, et al. Bulk optical properties and tissue components in the female breast from multiwavelength time-resolved optical mammography. *J Biomed Opt.* 2004; 9(6):1137–1142. [PubMed: 15568933]
17. Fang Q, Carp SA, Selb J, et al. Combined optical imaging and mammography of the healthy breast: optical contrast derived from breast structure and compression. *IEEE Trans Med Imaging.* Jan; 2009 28(1):30–42. [PubMed: 19116186]
18. Taroni P, Bassi A, Comelli D, et al. Diffuse optical spectroscopy of breast tissue extended to 1100 nm. *J Biomed Opt.* 2009; 14(5):054030. [PubMed: 19895132]
19. Taroni P, Pifferi A, Salvagnini E, et al. Seven-wavelength time-resolved optical mammography extending beyond 1000 nm for breast collagen quantification. *Opt Exp.* 2009; 17(18):15932–15946.
20. Leff DR, Warren OJ, Enfield LC, et al. Diffuse optical imaging of the healthy and diseased breast: a systematic review. *Breast Cancer Res Tr.* Mar; 2008 108(1):9–22.
21. Srinivasan S, Pogue BW, Carpenter C, et al. Developments in quantitative oxygen-saturation imaging of breast tissue in vivo using multispectral near-infrared tomography. *Antiox Redox Sig.* Aug; 2007 9(8):1143–1156.
22. Shah N, Cerussi AE, Jakubowski D, et al. Spatial variations in optical and physiological properties of healthy breast tissue. *J Biomed Opt.* 2004; 9(3):534–540. [PubMed: 15189091]
23. Blyschak K, Simick M, Jong R, et al. Classification of breast tissue density by optical transillumination spectroscopy: optical and physiological effects governing predictive value. *Med Phys.* Jun; 2004 31(6):1398–1414. [PubMed: 15259643]
24. Blackmore KM, Knight JA, Jong R, et al. Assessing breast tissue density by transillumination breast spectroscopy (tibs): an intermediate indicator of cancer risk. *BJR.* Jul; 2007 80(955):545–556. [PubMed: 17537757]
25. Blackmore KM, Knight JA, Lilge L. Association between transillumination breast spectroscopy and quantitative mammographic features of the breast. *Cancer Epidem Biomar.* 2008; 17(5):1043.
26. Taroni P, Pifferi A, Quarto G, et al. Noninvasive assessment of breast cancer risk using time-resolved diffuse optical spectroscopy. *J Biomed Opt.* 2010; 15:060501. [PubMed: 21198142]
27. Nioka S, Chance B. NIR spectroscopic detection of breast cancer. *Technology in Cancer Research and Treatment.* Oct; 2005 4(5):497–512.
28. Ntziachristos V, Chance B. Probing physiology and molecular function using optical imaging applications to breast cancer. *BCR.* Nov.2001 3:41–46. [PubMed: 11250744]
29. Enfield LC, Gibson AP, Everdell NL, et al. Three-dimensional time-resolved optical mammography of the uncompressed breast. *App Opt.* Jun; 2007 46(17):3628–3638.
30. Poplack SP, Tosteson TD, Wells WA, et al. Electromagnetic breast imaging: results of a pilot study in women with abnormal mammograms. *Radiology.* May; 2007 243(2):350–359. [PubMed: 17400760]
31. Nioka S, Yung Y, Shnall M, et al. Optical imaging of breast tumor by means of continuous waves. *Adv Exp Med Bio.* 1997; 411:227–232. [PubMed: 9269431]
32. Grosenick D, Wabnitz H, Rinneberg HH, et al. Development of a time-domain optical mammograph and first in vivo applications. *App Opt.* May; 1999 38(13):2927–2943.
33. Spinelli L, Torricelli A, Pifferi A, et al. Characterization of female breast lesions from multi-wavelength time-resolved optical mammography. *PMB.* Jun; 2005 50(11):2489–2502.
34. Culver JP, Choe R, Holboke MJ, et al. Three-dimensional diffuse optical tomography in the parallel plane transmission geometry: Evaluation of a hybrid frequency domain/continuous wave clinical system for breast imaging. *Med Phys.* 2003; 30:235–247. [PubMed: 12607841]

35. Choe R, Konecky SD, Corlu A, et al. Differentiation of benign and malignant breast tumors by in-vivo three-dimensional parallel-plate diffuse optical tomography. *J Biomed Opt.* 2009; 14(2): 024020. 18pp. [PubMed: 19405750]
36. Hoogenraad JH, van der Mark MB, Colak SB, et al. First results from the Philips optical mammoscope. *Photon Propagation in Tissues III.* 1998
37. Nielsen T, Brendel B, Ziegler R, et al. Linear image reconstruction for a diffuse optical mammography system in a noncompressed geometry using scattering fluid. *App Opt.* 2009; 48(10):D1–D13.
38. Schmitz CH, Klemer DP, Hardin R, et al. Design and implementation of dynamic near-infrared optical tomographic imaging instrumentation for simultaneous dual-breast measurements. *App Opt.* Apr; 2005 44(11):2140–2153.
39. Intes X, Djaziri S, Ichalalene Z, et al. Time-domain optical mammography softscan: initial results. *Acad Rad.* Aug; 2005 12(8):934–947.
40. Athanasiou A, Vanel D, Fournier L, et al. Optical mammography: a new technique for visualizing breast lesions in women presenting non palpable birads 4–5 imaging findings: preliminary results with radiologic–pathologic correlation. *Canc Imag.* 2007; 7(1):34.
41. Fournier LS, Vanel D, Athanasiou A, et al. Dynamic optical breast imaging: A novel technique to detect and characterize tumor vessels. *Euro J Rad.* 2009; 69(1):43–49.
42. Arridge S, Schotland J. Optical tomography: forward and inverse problems. *Inverse Probl.* 2009; 25(12):123010. 59pp.
43. Kaufmann M, von Minckwitz G, Bear HD, et al. Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: new perspectives 2006. *Ann Oncol.* Nov; 2007 18(12):1927–34. [PubMed: 17998286]
44. Arlinghaus LR, Li X, Levy M, et al. Current and future trends in magnetic resonance imaging assessments of the response of breast tumors to neoadjuvant chemotherapy. *J Onc.* 2010; 919620:1–17.
45. Esserman L, Berry D, Cheang M, et al. Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). *Breast Cancer Res Tr.* 2011; 30:3242–3249.
46. McGuire K, Toro-Burguete J, Dang H, et al. MRI staging after neoadjuvant chemotherapy for breast cancer: Does tumor biology affect accuracy? *Ann Surg Oncol.* 2011; 18(11):3149–3154. [PubMed: 21947592]
47. Falou O, Soliman H, Sadeghi-Naini A, et al. Diffuse optical spectroscopy evaluation of treatment response in women with locally advanced breast cancer receiving neoadjuvant chemotherapy. *Trans Onc.* 2012; 5(4):238–246.
48. Tromberg, Bruce J.; Pogue, Brian W.; Paulsen, Keith D., et al. Assessing the future of diffuse optical imaging technologies for breast cancer management. *Med Phys.* 2008; 35(6):2443–2451. [PubMed: 18649477]
49. Jakubowski DB, Cerussi AE, Bevilacqua F, et al. Monitoring neoadjuvant chemotherapy in breast cancer using quantitative diffuse optical spectroscopy: a case study. *J Biomed Opt.* 2004; 9(1): 230–238. [PubMed: 14715078]
50. Shah N, Gibbs J, Wolverson D, et al. Combined diffuse optical spectroscopy and contrast-enhanced magnetic resonance imaging for monitoring breast cancer neoadjuvant chemotherapy: a case study. *J Biomed Opt.* 2005; 10(5):051503. [PubMed: 16292947]
51. Tromberg BJ, Cerussi A, Shah N, et al. Imaging in breast cancer: diffuse optics in breast cancer: detecting tumors in pre-menopausal women and monitoring neoadjuvant chemotherapy. *BCR.* 2005; 7(6):279–285. [PubMed: 16457705]
52. Choe R, Corlu A, Lee K, et al. Diffuse optical tomography of breast cancer during neoadjuvant chemotherapy: A case study with comparison to MRI. *Med Phys.* 2005; 32:1128–1139. [PubMed: 15895597]
53. Zhou C, Choe R, Shah N, et al. Diffuse optical monitoring of blood flow and oxygenation in human breast cancer during early stages of neoadjuvant chemotherapy. *J Biomed Opt.* 2007; 12:051903. [PubMed: 17994886]

54. Zhu Q, Tannenbaum S, Hegde P, et al. Noninvasive monitoring of breast cancer during neoadjuvant chemotherapy using optical tomography with ultrasound localization. *Neoplasia*. Oct; 2008 10(10):1028–1040. [PubMed: 18813360]
55. Cerussi A, Hsiang D, Shah N, et al. Predicting response to breast cancer neoadjuvant chemotherapy using diffuse optical spectroscopy. *Proc Nat Ac Sci*. Mar; 2007 104(10):4014–4019.
56. Tanamai W, Chen C, Siavoshi S, et al. Diffuse optical spectroscopy measurements of healing in breast tissue after core biopsy: case study. *J Biomed Opt*. 2009; 14(1):014024. [PubMed: 19256712]
57. Jiang S, Pogue BW, Carpenter CM, et al. Evaluation of breast tumor response to neoadjuvant chemotherapy with tomographic diffuse optical spectroscopy: Case studies of tumor region-of-interest changes. *Radiology*. Jun.2009 252:551–560. [PubMed: 19508985]
58. Cerussi AE, Tanamai VW, Mehta RS, et al. Frequent optical imaging during breast cancer neoadjuvant chemotherapy reveals dynamic tumor physiology in an individual patient. *Acad Rad*. Aug; 2010 17(8):1031–1039.
59. Soliman H, Gunasekara A, Rycroft M, et al. Functional imaging using diffuse optical spectroscopy of neoadjuvant chemotherapy response in women with locally advanced breast cancer. *Clin Cancer Res*. 2010; 16(9):2605–2614. [PubMed: 20406836]
60. Cerussi AE, Tanamai VW, Hsiang D, et al. Diffuse optical spectroscopic imaging correlates with final pathological response in breast cancer neoadjuvant chemotherapy. *Philos T Roy Soc A*. Nov; 2011 369(1955):4512–4530.
61. Choe R, Durduran T. Diffuse optical monitoring of the neoadjuvant breast cancer therapy. *IEEE J Sel Topics in Quantum Electron*. 2012; 18(99):1367–1386.
62. Enfield LC, Cantanhede G, Westbroek D, et al. Monitoring the response to primary medical therapy for breast cancer using three-dimensional time-resolved optical mammography. *Technol Cancer Res Treat*. Dec; 2011 10(6):533–547. [PubMed: 22066594]
63. Pakalniskis MG, Wells WA, Schwab MC, et al. Tumor angiogenesis change estimated by using diffuse optical spectroscopic tomography: demonstrated correlation in women undergoing neoadjuvant chemotherapy for invasive breast cancer? *Radiology*. May; 2011 259(2):365–374. [PubMed: 21406632]
64. Roblyer D, Ueda S, Cerussi A, et al. Optical imaging of breast cancer oxyhemoglobin flare correlates with neoadjuvant chemotherapy response one day after starting treatment. *Proc Nat Ac Sci*. Aug; 2011 108(35):14626–14631.
65. Santoro Y, Leproux A, Cerussi A, et al. Breast cancer spatial heterogeneity in near-infrared spectra and the prediction of neoadjuvant chemotherapy response. *J Biomed Opt*. 2011; 16:097007. [PubMed: 21950942]
66. Busch DR, Choe R, Rosen MA, et al. Optical malignancy parameters for monitoring progression of breast cancer neoadjuvant chemotherapy. *Biomed Opt Exp*. Jan; 2013 4(1):105–121.
67. Chung SH, Mehta R, Tromberg BJ, et al. Non-invasive measurement of deep tissue temperature changes caused by apoptosis during breast cancer neoadjuvant chemotherapy: a case study. *Journal of Innovative Optical Health Sciences*. 2011; 04(04):361–372. [PubMed: 22408653]
68. Durduran T, Choe R, Baker WB, et al. Diffuse optics for tissue monitoring and tomography. *Reports on Progress in Physics*. Jun.2010 73(7):076701.
69. Jacques SL, Pogue BW. Tutorial on diffuse light transport. *J Biomed Opt*. 2008; 13(4):041302–1:19. [PubMed: 19021310]
70. Mesquita R, Durduran T, Yu G, et al. Direct measurement of tissue blood flow and metabolism with diffuse optics. *Philos T Roy Soc A*. 2011; 369(1955):4390–4406.
71. Yu G. Near-infrared diffuse correlation spectroscopy in cancer diagnosis and therapy monitoring. *J Biomed Opt*. 2012; 17(1):010901–1. [PubMed: 22352633]
72. Yu G. Diffuse correlation spectroscopy (dcs): A diagnostic tool for assessing tissue blood flow in vascular-related diseases and therapies. *Current Medical Imaging Reviews*. 2012; 8(3):194–210.
73. Cutler M. Transillumination of the breast. *Annals of Surgery*. Jan; 1931 93(1):223–234. [PubMed: 17866468]
74. Homer MJ. Breast imaging: Pitfalls, controversies, and some practical thoughts. *Radiol. Clin North Am*. 1985; 23:459–472.

75. Watmough DJ. Transillumination of breast tissues: factors governing optimal imaging of lesions. *Radiology*. 1983; 147:89–92. [PubMed: 6828767]
76. Sickles EA. Breast cancer detection with transillumination and mammography. *Am J Roentgenol*. 1984; 142:841–844. [PubMed: 6608251]
77. Wallberg H, Alveryd A, Bergvall U, et al. Diaphanography in breast carcinoma. *Acta Radiol Diagn*. 1985; 26:33–44.
78. Profio AE, Navarro GA. Scientific basis of breast diaphanography. *Med Phys*. 1989; 16:60–65. [PubMed: 2921981]
79. Pera A, Freimanis AK. The choice of radiologic procedures in the diagnosis of breast disease. *Obstet. Gynecol. Clin North Am*. 1987; 14(3):635–650.
80. Carlsen EN. Transillumination light scanning. *Diagn Imaging*. 1982; 4:28–34.
81. Marshall V, Williams DC, Smith KD. Diaphanography as a means of detecting breast cancer. *Radiology*. 1984; 150:339–343. [PubMed: 6691086]
82. Monsees B, Destouet JM, Totty WG. Light scanning versus mammography in breast cancer detection. *Radiology*. 1987; 163:463–465. [PubMed: 3562828]
83. Gisvold JJ, Brown LR, Swee RG, et al. Comparison of mammography and transillumination light scanning in the detection of breast lesions. *Am J Roentgenol*. 1986; 147(1):191–4. [PubMed: 3487216]
84. Bartrum RJJ, Crow HC. Transillumination lightscanning to diagnose breast cancer: A feasibility study. *Am. J Roentgenol*. 1984; 142:409–414.
85. Alveryd A, Andersson I, Aspegren K, et al. Lightscanning versus mammography for the detection of breast cancer in screening and clinical practice. a Swedish multicenter study. *Cancer*. 1990; 65(8):1671–1677. [PubMed: 2180556]
86. Hebden J, Arridge S, Delpy D. Optical imaging in medicine: I. experimental techniques. *PMB*. 1997; 42:825–840.
87. Arridge SR, Hebden JC. Optical imaging in medicine: II. modelling and reconstruction. *PMB*. May; 1997 42(5):841–853.
88. Zhu Q, Hegde PU, Ricci A, et al. Early-stage invasive breast cancers: potential role of optical tomography with us localization in assisting diagnosis. *Radiology*. Aug; 2010 256(2):367–378. [PubMed: 20571122]
89. Konecky SD, Choe R, Corlu A, et al. Comparison of diffuse optical tomography of human breast with whole-body and breast-only positron emission tomography. *Med Phys*. Feb; 2008 35(2):446–455. [PubMed: 18383664]
90. Doi K. Computer-aided diagnosis in medical imaging: historical review, current status and future potential. *Computerized medical imaging and graphics: the official journal of the Computerized Medical Imaging Society*. 2007; 31(4-5):198. [PubMed: 17349778]
91. Pichler BJ, Kolb A, Nägele T, et al. Pet/mri: paving the way for the next generation of clinical multimodality imaging applications. *J Nuc Med*. 2010; 51(3):333–336.
92. Klose CD, Klose AD, Netz U, et al. Multiparameter classifications of optical tomographic images. *J Biomed Opt*. 2008; 13(5):050503. [PubMed: 19021375]
93. Simick MK, Jong R, Wilson B, et al. Non-ionizing near-infrared radiation transillumination spectroscopy for breast tissue density and assessment of breast cancer risk. *J Biomed Opt*. 2004; 9(4):794–803. [PubMed: 15250768]
94. Zhu C, Breslin TM, Harter J, et al. Model based and empirical spectral analysis for the diagnosis of breast cancer. *Opt Exp*. Sep; 2008 16(19):14961–14978.
95. Song X, Pogue BW, Jiang S, et al. Automated region detection based on the contrast-to-noise ratio in near-infrared tomography. *App Opt*. 2004; 43(5):1053–1062.
96. Pogue BW, Davis SC, Song X, et al. Image analysis methods for diffuse optical tomography. *J Biomed Opt*. 2006; 11(3):033001. 16pp.
97. Wang JZ, Liang X, Zhang Q, et al. Automated breast cancer classification using near-infrared optical tomographic images. *J Biomed Opt*. 2008; 13:044001. 10pp. [PubMed: 19021329]
98. Busch DR, Guo W, Choe R, et al. Computer aided automatic detection of malignant lesions in diffuse optical mammography. *Med Phys*. Apr; 2010 37(4):1840–1849. [PubMed: 20443506]

99. Pogue BW, Jiang S, Dehghani H, et al. Characterization of hemoglobin, water, and NIR scattering in breast tissue: analysis of intersubject variability and menstrual cycle changes. *J Biomed Opt.* 2004; 9:541. [PubMed: 15189092]
100. Busch, DR. Ph D thesis. University of Pennsylvania; 2011. Computer-Aided, Multi-Modal, and Compression Diffuse Optical Studies of Breast Tissue.
101. Pogue BW, Poplack SP, McBride TO, et al. Quantitative hemoglobin tomography with diffuse near-infrared spectroscopy: pilot results in the breast. *Radiology.* 2001; 218(1):261. [PubMed: 11152812]
102. Srinivasan S, Pogue BW, Brooksby B, et al. Near-infrared characterization of breast tumors in vivo using spectrally-constrained reconstruction. *Technology in Cancer Research and Treatment.* Oct; 2005 4(5):513–526.
103. Azar FS, Lee K, Khamene A, et al. Standardized platform for coregistration of nonconcurrent diffuse optical and magnetic resonance breast images obtained in different geometries. *J Biomed Opt.* 2007; 12(5):051902. [PubMed: 17994885]
104. Ntziachristos V, Yodh AG, Schnall M, et al. Concurrent MRI and diffuse optical tomography of breast after indocyanine green enhancement. *Proceedings of The National Academy of Sciences of the USA.* 2000; 97:2767–2772. [PubMed: 10706610]
105. Ntziachristos V, Yodh AG, Schnall MD, et al. MRI-guided diffuse optical spectroscopy of malignant and benign breast lesions. *Neoplasia.* 2002; 4:347–354. [PubMed: 12082551]
106. Carpenter CM, Srinivasan S, Pogue BW, et al. Methodology development for three-dimensional mr-guided near infrared spectroscopy of breast tumors. *Opt Exp.* Oct; 2008 16(22):17903–17914.
107. Pogue BW, Leblond F, Krishnaswamy V, et al. Radiologic and near-infrared/optical spectroscopic imaging: Where is the synergy? *Am J Roentgenol.* 2010; 195:321–332. [PubMed: 20651186]
108. Li A, Miller EL, Kilmer ME, et al. Tomographic optical breast imaging guided by three-dimensional mammography. *App Opt.* Sep; 2003 42(25):5181–5190.
109. Boverman G, Miller EL, Li A, et al. Quantitative spectroscopic diffuse optical tomography of the breast guided by imperfect a priori structural information. *PMB.* Sep; 2005 50(17):3941–3956.
110. Zhu Q, Durduran T, Ntziachristos V, et al. Imager that combines near-infrared diffusive light and ultrasound. *Opt Lett.* 1999; 24:1050–1052. [PubMed: 18073937]
111. Holboke MJ, Tromberg BJ, Li X, et al. Three-dimensional diffuse optical mammography with ultrasound localization in a human subject. *J Biomed Opt.* 2000; 5:237–247. [PubMed: 10938789]
112. Zhu Q, Tannenbaum S, Kurtzman SH. Optical tomography with ultrasound localization for breast cancer diagnosis and treatment monitoring. *Surg Oncol Clin N Am.* 2007; 16:307–321. [PubMed: 17560514]
113. Freifelder R, Karp JS. Dedicated PET scanners for breast imaging. *Phys Med Biol.* 1997; 42:2453–2480.
114. Freifelder R, Cardi C, Grigoras I, et al. First results of a dedicated breast PET imager, BPET, using NaI(Tl) curve plate detectors. *IEEE Nuclear Science Symposium Conference record.* 2001; 3:1241–1245.
115. Boas DA, Campbell LE, Yodh AG. Scattering and imaging with diffusing temporal field correlations. *Phys Rev Lett.* 1995; 75(9):1855–1858. [PubMed: 10060408]
116. Boas DA, Yodh AG. Spatially varying dynamical properties of turbid media probed with diffusing temporal light correlation. *JOSA-A.* 1997; 14(1):192–215.
117. Durduran T, Choe R, Yu GQ, et al. Diffuse optical measurement of blood flow in breast tumors. *Opt Lett.* 2005; 30:2915–2917. [PubMed: 16279468]
118. Zhou, C. Ph D thesis. University of Pennsylvania; Philadelphia, PA USA: 2007. In-Vivo Optical Imaging and Spectroscopy of Cerebral Hemodynamics.
119. Zhou C, Yu G, Daisuke F, et al. Diffuse optical correlation tomography of cerebral blood flow during cortical spreading depression in rat brain. *Opt Exp.* 2006; 14:1125–1144.
120. Flexman M, Khalil M, Al Abdi R, et al. Digital optical tomography system for dynamic breast imaging. *J Biomed Opt.* 2011; 16:076014. Compression, pressure, blood flow. [PubMed: 21806275]

121. Al abdi R, Graber HL, Xu Y. Optomechanical imaging system for breast cancer detection. *JOSA A*. 2011; 28(12):2473–2493. Compression, pressure, blood flow. [PubMed: 22193261]
122. Jiang S, Pogue BW, Paulsen KD, et al. In vivo near-infrared spectral detection of pressure-induced changes in breast tissue. *Opt Lett*. 2003; 28(14):1212–1214. [PubMed: 12885024]
123. Jiang SD, Pogue BW, Laughney AM. Measurement of pressure-displacement kinetics of hemoglobin in normal breast tissue with near-infrared spectral imaging. *App Opt*. 2009; 48(10)
124. Xu RX, Qiang B, Mao JJ, et al. Development of a handheld near-infrared imager for dynamic characterization of in vivo biological tissue systems. *App Opt*. 2007; 46(30):7442–7451.
125. Carp SA, Kauffman T, Fang Q, et al. Compression-induced changes in the physiological state of the breast as observed through frequency domain photon migration measurements. *J Biomed Opt*. 2006; 11(6):064016. [PubMed: 17212539]
126. Carp SA, Selb J, Fang Q, et al. Dynamic functional and mechanical response of breast tissue to compression. *Opt Exp*. Sep; 2008 16(20):16064–16078.
127. Beaney RP, Lammertsma AA, Jones T, et al. Positron emission tomography for in-vivo measurement of regional blood flow, oxygen utilisation, and blood volume in patients with breast carcinoma. *Lancet*. 1984; 1:131–134. [PubMed: 6140443]
128. Wilson BJH, Lammertsma AA, McKenzie CG, et al. Measurements of blood flow and exchanging water space in breast tumors using positron emission tomography: A rapid and noninvasive dynamic method. *Cancer Res*. 1992; 52:1592–97. [PubMed: 1540969]
129. Dixit SS, Kim H, Comstock C, et al. Near infrared transillumination imaging of breast cancer with vasoactive inhalation contrast. *Biomed Opt Exp*. Aug; 2010 1(1):295–309.
130. Dixit SS, Kim H, Visser B, et al. Development of a transillumination infrared modality for differential vasoactive optical imaging. *App Opt*. Jan.2009 :100949.
131. Kotz KT, Dixit SS, Gibbs AD, et al. Inspiratory contrast for in vivo optical imaging. *Opt Exp*. Jan; 2008 16(1):19–31.
132. Weissleder R, Ntziachristos V. Shedding light onto live molecular targets. *Nat Med*. 2003; 9(1): 123–128. [PubMed: 12514725]
133. Tichauer KM, Holt RW, El-Ghussein F, et al. Dual-tracer background subtraction approach for fluorescent molecular tomography. *J Biomed Opt*. 2013; 18(1):016003–016003.
134. Akorn Inc. IC-Green TM Sterile Indocyanine Green. Drug Packaging Insert, Version: 6-DCGN-01. 2006
135. Poellinger A. Near-infrared imaging of breast cancer using optical contrast agents. *J Biophotonics*. 2012; 11-12:815–826. [PubMed: 23132668]
136. Ott P. Hepatic elimination of Indocyanine Green with special reference to distribution kinetics and the influence of plasma protein binding. *Pharmacol Toxicol*. 1998; 83(S2):1–48. [PubMed: 9695126]
137. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000; 407:249–257. [PubMed: 11001068]
138. Corlu A, Choe R, Durduran T, et al. Three-dimensional in vivo fluorescence diffuse optical tomography of breast cancer in humans. *Opt Exp*. 2007; 15(11):6696–6716.
139. Intes X, Ripoll J, Chen Y, et al. In vivo continuous-wave optical breast imaging enhanced with indocyanine green. *Med Phys*. 2003; 30(6):1039–1047. [PubMed: 12852527]
140. Alacam B, Yazici B, Intes X, et al. Pharmacokinetic-rate images of indocyanine green for breast tumors using near-infrared optical methods. *Phys Med Biol*. 2008; 53:837–859. [PubMed: 18263944]
141. Rinneberg H, Grosenick D, Moesta KT, et al. Detection and characterization of breast tumours by time-domain scanning optical mammography. *Opto-Electronics Review*. 2008; 16(2):147–162.
142. Schneider P, Piper S, Schmitz CH, et al. Fast 3d near-infrared breast imaging using indocyanine green for detection and characterization of breast lesions. *Fortschr Röntgenstr*. Oct; 2011 183(10):956–963.
143. Hagen A, Grosenick D, Macdonald R, et al. Late-fluorescence mammography assesses tumor capillary permeability and differentiates malignant from benign lesions. *Opt Exp*. Sep; 2009 17(19):17016–17033.

144. Poellinger A, Burock S, Grosenick D, et al. Breast Cancer: Early-and Late-Fluorescence Near-Infrared Imaging with Indocyanine Green- Preliminary Study. *Radiology*. 2011; 258(2):409. [PubMed: 21177396]
145. Poellinger A, Persigehl T, Mahler M, et al. Near-infrared imaging of the breast using omocianine as a fluorescent dye: results of a placebo-controlled, clinical, multicenter trial. *Invest Radiol*. Nov; 2011 46(11):697–704. [PubMed: 21788905]
146. Sevic-Muraca E. Translation of near-infrared fluorescence imaging technologies: emerging clinical applications. *Annu Rev Med*. 2012; 63:217–231. [PubMed: 22034868]
147. Smith RA, Saslow D, Sawyer KA, et al. American cancer society guidelines for breast cancer screening: Update 2003. *CA-Cancer J Clin*. 2003; 53(3):141–169. [PubMed: 12809408]
148. Grosenick D, Wabnitz H, Moesta KT, et al. Time-domain scanning optical mammography: II. optical properties and tissue parameters of 87 carcinomas. *PMB*. Jun; 2005 50(11):2451–2468.
149. Grosenick D, Moesta KT, Mller M, et al. Time-domain scanning optical mammography: I. recording and assessment of mammograms of 154 patients. *PMB*. Jun; 2005 50(11):2429–2449.
150. Taroni P, Danesini G, Torricelli A, et al. Clinical trial of time-resolved scanning optical mammography at 4 wavelengths between 683 and 975 nm. *J Biomed Opt*. Mar.2004 9:464–473. [PubMed: 15189083]
151. Enfield LC, Gibson AP, Hebden JC, et al. Optical tomography of breast cancer - monitoring response to primary medical therapy. *Targ Oncol*. 2009; 4:219–233.
152. Bigio IJ, Brown SG, Briggs G, et al. Diagnosis of breast cancer using elastic-scattering spectroscopy: preliminary clinical results. *J Biomed Opt*. 2000; 5:221–228. [PubMed: 10938787]
153. Keller MD, Majumder SK, Mahadevan-Lansen A. Spatially offset Raman spectroscopy of layered soft tissues. *Opt Lett*. 2009; 34:926–928. [PubMed: 19340173]
154. Haka AS, Volynskaya Z, Gardecki JA, et al. Diagnosing breast cancer using raman spectroscopy: prospective analysis. *J Biomed Opt*. 2009; 14:054023. [PubMed: 19895125]
155. Nguyen FT, Zysk AM, Chaney EJ, et al. Intraoperative evaluation of breast tumor margins with optical coherence tomography. *Cancer Res*. 2009; 69:8790–8796. [PubMed: 19910294]
156. Nachabé R, Evers DJ, Hendriks BH, et al. Diagnosis of breast cancer using diffuse optical spectroscopy from 500 to 1600 nm: comparison of classification methods. *J Biomed Opt*. 2011; 16:087010. [PubMed: 21895337]
157. Wilke LG, Brown JQ, Bydlon TM, et al. Rapid noninvasive optical imaging of tissue composition in breast tumor margins. *Am J Surg*. 2009; 198:566–574. [PubMed: 19800470]
158. Brown JQ, Bydlon TM, Richards LM, et al. Optical assessment of tumor resection margins in the breast. *IEEE J Quantum Elect*. 2010; 16(3):530–544.
159. Bydlon TM, Barry WT, Kennedy SA, et al. Advancing optical imaging for breast margin assessment: An analysis of excisional time, cautery, and patent blue dye on underlying sources of contrast. *PLOS One*. 2012; 7:e51418. [PubMed: 23251526]
160. McLaughlin RA, Quirk BC, Kirk RW, et al. Imaging of breast cancer with optical coherence tomography needle probes: Feasibility and initial results. *IEEE J Sel Top Quantum Electron*. 2012; 18:1184–1191.
161. Tanaka E, Choi HS, Fujii H, et al. Image-guided oncologic surgery using invisible light: completed pre-clinical development for sentinel lymph node mapping. *Ann Surg Oncol*. 2006; 13:1671–1681. [PubMed: 17009138]
162. Sevic-Muraca EM, Sharma R, Rasmussen JC, et al. Imaging of lymph flow in breast cancer patients after microdose administration of a near-infrared fluorophore: feasibility study. *Radiology*. Mar; 2008 246(3):734–741. [PubMed: 18223125]
163. Hutteman M, Mieog JSD, van der Vorst JR, et al. Randomized, double-blind comparison of indocyanine green with or without albumin premixing for near-infrared fluorescence imaging of sentinel lymph nodes in breast cancer patients. *Breast Cancer Res Treat*. 2011; 127(1):163–170. [PubMed: 21360075]
164. Li C, Wang LV. Photoacoustic tomography and sensing in biomedicine. *PMB*. 2009; 54(19):R59.
165. Oraevsky AA, Jacques SL, Tittel FK. Measurement of tissue optical properties by time-resolved detection of laser-induced transient stress. *App Opt*. 1997; 36(1):402–415.

166. Ermilov SA, Khamapirad T, Conjusteau A, et al. Laser optoacoustic imaging system for detection of breast cancer. *J Biomed Opt.* 2009; 14(2):024007–024007. [PubMed: 19405737]
167. Kruger RA, Lam RB, Reinecke DR, et al. Photoacoustic angiography of the breast. *Med Phys.* 2010; 37(11):6096. [PubMed: 21158321]

Key Points

- Diffuse optical spectroscopies provide non-invasive, non-ionizing, serial measurements of tissue blood flow, oxygenation, and concentration.
- These physiological parameters provide a window into tissue metabolism without necessitating the use of ionizing radiation or transport to imaging suites.
- Current clinical investigations of diffuse optical mammography include applications of diffuse optical tomography and monitoring to neoadjuvant chemotherapy, contrast agent discovery, computer-aided detection, and measurement of breast oxygen metabolism.
- Diffuse optical measurements hold significant promise for commercialization and clinical integration.

Fig. 1.

A proposed timeline for including Diffuse Optical Spectroscopy (DOS) and Tomography (DOT) in neoadjuvant chemotherapy treatment planning (yellow box). DOS provides rapid measurements, integrable into each patient visit. More time consuming volumetric imaging with DOT would provide more detailed information. Together, these frequent measurements of tissue metabolic state may permit clinicians to more rapidly shift to more efficacious treatment regimes or even identify a complete response prior to completion of all scheduled doses.

Fig. 2. Illustration of photon migration in turbid media. (a) A schematic of the experiment. (b) Container filled with tap water, with minimal scattering from air bubbles and tank walls. The columnated laser beam propagates straight across the liquid bath. (c) Container filled with a low scattering liquid of a dilute mixture of soy emulsion and water. The light beam is somewhat dispersed. (d) Container filled with a high scattering liquid filled with a more concentrated soy emulsion mixture. These optical properties are similar to tissue in the NIR spectral region. Note that the light spreads almost isotropically from the point where the beam enters the scattering medium. Photos are in false color. (Images are courtesy of Han Ban.)

Fig. 3. Spectra of major tissue chromophores. (top) The visible and Near Infra-Red (NIR) windows overlap somewhat; note the much lower absorption in the NIR. (bottom) Expanded view of chromophores in the NIR spectral region at approximate concentrations found in human breast tissue.

Fig. 4.

Example transmission (left) and re-emission (right) geometry measurements in optical mammography. One or more lasers typically inject light into the tissue (maroon arrows), and the light transmitted through the tissue is collected by one or more detectors (red arrows). Typical representations of the most probable trajectories of the detected photons are superimposed in gray scale.

Fig. 5.

(a) Intra-subject data normalization brings inter-subject data distributions close to a normal distribution in the healthy tissue of 35 breasts with biopsy-confirmed malignant lesions [98]. The top row shows, for the full population, absolute values of Hb_t and the bottom row shows the population distribution of Z-transformed variables after intra-subject normalization (zHb_t). Each trace/color represents the healthy region of one subject. For clarity of presentation, the vertical axis is normalized to the total number of voxels in each subject. (b) Schematic of probability of malignancy calculation. Weighting coefficients (β) are calculated from normalized data in a population of known cancers (training set); this same weighting vector is then applied to data from an additional subject (test set) to calculate a probability of malignancy ($P(\mathcal{M})$) and a binary mask of tumor location [98].

Fig. 6.

Slices from 3D images of two subjects, showing total hemoglobin concentration (a, Hb_t), blood oxygen saturation (b, StO_2), reduced scattering coefficient (c, μ'_s), Probability of Malignancy (d, $P(M)$), and a binary cancer mask (e) using a cut-off of $P(M) = 0.95$. The DCIS & LCIS (Ductal and Lobular Carcinoma *In Situ*) lesion in the left column provides an interesting case study, with the $P(M)$ falling between the malignant lesions and the healthy regions. The invasive ductal carcinoma in the right column shows a typical result from invasive cancers. Figure from Busch [100].

Fig. 7. Pilot study of monitoring chemotherapy with DOT-CAD in three subjects. (a) Example images of $P(M)$ changing during the course of chemotherapy (subject 2). (b) Integrated probability of malignancy in tumor regions, normalized to first time point in subjects with complete (subjects 1 and 2) and partial (subject 3) response to chemotherapy. (For details, see Ref. [66].)

Fig. 8.

(a) Axial, sagittal, and coronal slices from the whole-body FDG-PET and DOT images of overall optical attenuation coefficient: μ_{eff} . Note the whole-body PET image is of the whole torso with both breasts, whereas DOT shows the left breast only. The rectangular dotted box in each image denotes the breast corresponding to the DOT image. (b) Correlation between contrast ratios in FDG uptake and Hb_t for the nine patients with tumors visible to both DOT and PET. (The figure is adapted from Ref. [89])

Fig. 9.

Images of the breasts of three females imaged with DOT and with a dedicated breast imaging PET. A representative caudal-cranial slice taken from the 3D reconstruction of a breast is presented for each parameter and patient. The DOT images have been transformed, using a co-registration algorithm, to be in the same uncompressed state as PET. The rows correspond to each patient with suspicious lesions. The columns correspond to Hb_t , StO_2 , μ'_s measured by DOT, and to FDG uptake measured by PET. The dashed white ellipses enclose regions with suspicious lesions. (Figures adapted from Ref. [89].)

Fig. 10.

Relative tumor/normal oxy-hemoglobin ($rHbO_{2(T/N)}$), blood flow index ($rBF_{(T/N)}$), optical index ($rTOI_{(T/N)}$), and mammary metabolic rate of oxygen consumption ($rMMRO_{2(T/N)}$) contrast in response to chemotherapy are shown. The optical indices were calculated as the ratio of the average tumor value to the average value of the normal tissue on the same side of the breast. These indices were then normalized to the pre-chemo values to reflect relative changes. (*) denotes statistically significant (at $p < 0.05$) difference from pre-chemo values. Figure courtesy of Chao Zhou [118].

Fig. 11.

(Top panel) Time course of molar ICG concentration in arterial blood, recorded by transcutaneous pulse densitometry. Optical mammogram measurements were performed during the native phase (i.e., before ICG injection, gray shading), vascular phase (i.e., after ICG bolus injection followed by continuous infusion, light salmon shading), and extravascular phase (i.e., more than 20 minutes after termination of infusion, blue shading). (Lower panels) Images of an invasive ductal carcinoma (malignant lesion, center column) and a fibroadenoma (benign lesion, right column). In the fluorescent/absorption ratio images, the malignant lesion lacks contrast in the vascular phase (top row), but is clearly visible in the extravascular phase (second row), while the benign lesion is much less distinct at both time points. Gadolinium enhanced MRI (third row) shows the malignant lesion clearly, although this lesion is difficult to locate in the X-ray mammogram (bottom row).

Table 1

Acronyms and symbols utilized in this manuscript.

ACRONYM	Description
ASL-MRI	Arterial-Spin Labeling MRI
BF	Blood Flow
CAD	Computer Aided Detection
CT	(X-Ray) Computed Tomography
DCS	Diffuse Correlation Spectroscopy
DCT	Diffuse Correlation Tomography
DOS	Diffuse Optical Spectroscopy
DOT	Diffuse Optical Tomography
DOT-CAD	Diffuse Optical Tomography - Computer Aided Detection
FDG-PET	Fluoro-deoxyglucose Positron Emission Tomography
Hb	Deoxygenated Hemoglobin Concentration
HbO_2	Oxygenated Hemoglobin Concentration
Hb_t	Total Hemoglobin Concentration
H_2O	Water Concentration
l^*	Photon Random Walk Step
l_a	Photon Absorption Length
$Lipid$	Lipid Concentration
$\mathcal{M}$	Malignancy Parameter
μ_a	Absorption Coefficient
μ_{eff}	Overall Optical Attenuation Coefficient
$MMRO_2$	Mammary Metabolic Rate of Oxygen consumption
μ_s'	Reduced Scattering Coefficient
MRI	Magnetic Resonance Imaging
NIR	Near Infra-Red Spectral Range, 650-950 nm
NIRS	Near Infra-Red Spectroscopy (a.k.a. DOS)
OEF	Oxygen Extraction Fraction
PET	Positron Emission Tomography
$P(\mathcal{M})$	Probability of Malignancy
RBCs	Red Blood Cells
StO ₂	Blood Oxygen Saturation
TOI	Tissue Optical Index

Characteristics of several imaging modalities. Note that Diffuse Optics permits convenient, non-ionizing, and frequent measurement of tissue metabolism.

Table 2

	X-Ray	PET	Ultrasound	MRI	Diffuse Optics
Clinician's Office	No	No	Yes	No	Yes
Tissue Structure	Yes	No	Yes	Yes	No
Tissue Metabolism	No	Yes	No	No	Yes
Ionizing Radiation	Yes	Yes	No	No	No
Frequent Measurements	No	No	Yes	No	Yes

Table 3

Comparison of metabolic parameters (oxygen consumption and blood flow) reported in the PET literature and estimated using DOS. PET literature included both tumor and normal breast values, but only normal values are presented in this table for comparison.

Method	Breast	N	Oxygen consumption ($\mu\text{mol}/100\text{ ml/min}$)	Blood flow (ml/100 ml/min)
PET, ^{15}O inhalation [127]	Normal	9	19.8	3.96
PET, ^{15}O inhalation [128]	Normal	20	N/A	5.6 ± 1.4
DOS [126]	Healthy	28	1.9 ± 1.3	2.8 ± 1.7