10-19-2007

Diffuse Optical Monitoring of Blood Flow and Oxygenation in Human Breast Cancer During Early Stages of Neoadjuvant Chemotherapy

Chao Zhou
University of Pennsylvania

Regine Choe
University of Pennsylvania

Natasha Shah
University of California - Irvine

Turgut Durduran
University of Pennsylvania, durduran@sas.upenn.edu

Guoqiang Yu
University of Pennsylvania, guoqiang@dept.physics.upenn.edu

See next page for additional authors

Suggested Citation:

Copyright 2007 Society of Photo-Optical Instrumentation Engineers. One print or electronic copy may be made for personal use only. Systematic reproduction and distribution, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper are prohibited.

http://dx.doi.org/10.1117/1.2798595

This paper is posted at ScholarlyCommons. http://repository.upenn.edu/physics_papers/143
For more information, please contact repository@pobox.upenn.edu.
Diffuse Optical Monitoring of Blood Flow and Oxygenation in Human Breast Cancer During Early Stages of Neoadjuvant Chemotherapy

Abstract
We combine diffuse optical spectroscopy (DOS) and diffuse correlation spectroscopy (DCS) to noninvasively monitor early hemodynamic response to neoadjuvant chemotherapy in a breast cancer patient. The potential for early treatment monitoring is demonstrated. Within the first week of treatment (day 7) DOS revealed significant changes in tumor/normal contrast compared to pretreatment (day 0) tissue concentrations of deoxyhemoglobin ($r_{cT/N}^{HbD}=69\pm21\%$), oxyhemoglobin ($r_{cT/N}^{O2Hb}=73\pm25\%$), total hemoglobin ($r_{cT/N}^{THb}=72\pm17\%$), and lipid concentration ($r_{cT/N}^{Lipid}=116\pm13\%$). Similarly, DCS found significant changes in tumor/normal blood flow contrast ($r_{BF}^{T/N}=75\pm7\%$ on day 7 with respect to day 0). Our observation suggest the combination of DCS and DOS enhances treatment monitoring compared to either technique alone. The hybrid approach also enables construction of indices reflecting tissue metabolic rate of oxygen, which may provide new insights about therapy mechanisms.

Keywords
breast cancer, diffuse correlation spectroscopy, blood flow, diffuse optical spectroscopy, neoadjuvant chemotherapy, early monitoring, oxygen metabolism

Disciplines
Physical Sciences and Mathematics | Physics

Comments
Suggested Citation:

Copyright 2007 Society of Photo-Optical Instrumentation Engineers. One print or electronic copy may be made for personal use only. Systematic reproduction and distribution, duplication of any material in this paper for a fee or for commercial purposes, or modification of the content of the paper are prohibited.

http://dx.doi.org/10.1117/1.2798595

Author(s)
Chao Zhou, Regine Choe, Natasha Shah, Turgut Durduran, Guoqiang Yu, Amanda Durkin, David Hsiang, Rita Mehta, John Butler, Albert Cerussi, Bruce J. Tromberg, and Arjun G. Yodh

This journal article is available at ScholarlyCommons: http://repository.upenn.edu/physics_papers/143
Diffuse optical monitoring of blood flow and oxygenation in human breast cancer during early stages of neoadjuvant chemotherapy

Chao Zhou
Regine Choe
University of Pennsylvania
Department of Physics & Astronomy
Philadelphia, Pennsylvania 19104

Natasha Shah
University of California
Beckman Laser Institute and Medical Clinic
Laser Microbeam and Medical Program
Irvine, California 92612

Turgut Durduran
University of Pennsylvania
Department of Physics & Astronomy and
Department of Radiology
Philadelphia, Pennsylvania 19104

Guoqiang Yu
University of Pennsylvania
Department of Physics & Astronomy
Philadelphia, Pennsylvania 19104

Amanda Durkin
University of California
Beckman Laser Institute and Medical Clinic
Laser Microbeam and Medical Program
Irvine, California 92612

David Hsiang
Rita Mehta
John Butler
University of California
Chao Family Comprehensive Cancer Center
Irvine, California 92612

Albert Cerussi
Bruce J. Tromberg
University of California
Beckman Laser Institute and Medical Clinic
Laser Microbeam and Medical Program
Irvine, California 92612

Arjun G. Yodh
University of Pennsylvania
Department of Physics & Astronomy
Philadelphia, Pennsylvania 19104

Abstract. We combine diffuse optical spectroscopy (DOS) and diffuse correlation spectroscopy (DCS) to noninvasively monitor early hemodynamic response to neoadjuvant chemotherapy in a breast cancer patient. The potential for early treatment monitoring is demonstrated. Within the first week of treatment (day 7) DOS revealed significant changes in tumor/normal contrast compared to pretreatment (day 0) tissue concentrations of deoxyhemoglobin (rctHHbT/N = 69 ± 21%), oxyhemoglobin (rctO2HbT/N = 73 ± 25%), total hemoglobin (rctTHbT/N = 72 ± 17%), and lipid concentration (rctLipidT/N = 116 ± 13%). Similarly, DCS found significant changes in tumor/normal blood flow contrast (rBF/T/N = 75 ± 7% on day 7 with respect to day 0). Our observations suggest the combination of DCS and DOS enhances treatment monitoring compared to either technique alone. The hybrid approach also enables construction of indices reflecting tissue metabolic rate of oxygen, which may provide new insights about therapy mechanisms.

Keywords: breast cancer; diffuse correlation spectroscopy; blood flow; diffuse optical spectroscopy; neoadjuvant chemotherapy; early monitoring; oxygen metabolism.

Paper 07037SSRR received Jan. 29, 2007; revised manuscript received Jul. 12, 2007; accepted for publication Jul. 12, 2007; published online Oct. 19, 2007.

1 Introduction

Near-infrared (NIR) diffuse optical spectroscopy (DOS) and tomography (DOT) have been employed quantitatively by several groups to characterize breast cancers. In clinical investigations, DOS and DOT have revealed tumor contrast in total hemoglobin concentration,1,4,6,9,11–17 hemoglobin oxygen saturation,2,12,14,17 water and lipid concentration,14,17,18 and tissue scattering.2,16,18 Furthermore, DOS and DOT have also shown promise for therapeutic monitoring of breast cancer patients.14–19,20 Studies during neoadjuvant chemotherapy (chemotherapy prior to surgery) have shown that tumor metabolic response can precede anatomical changes (e.g., size) accessible to traditional imaging and clinical palpation methods.21 Thus, new diagnostic methodologies focusing on physiological properties of the tumor, for example, hemodynamic response, may offer benefits beyond those of traditional imaging and palpation during the early course of chemotherapy. In a recent case study, for example, Jakubowski et al.14 used DOS and observed significant changes in total
hemoglobin concentration and water fraction within 5 days of the start of treatment.

Another important hemodynamic parameter is blood flow. Measurements of blood flow provide insight about oxygen delivery and the clearance of metabolic by-products, complementary to information about tissue chromophores and scattering available to DOS. In vivo diffuse optical measurement of blood flow has recently been explored using diffuse correlation spectroscopy (DCS) in brain, muscle, and in the tumor tissues of animal models and humans. A recent DCS study in human breast tumors showed increased blood flow contrast in tumor regions relative to adjacent healthy tissue, consistent with results from ultrasound, positron emission tomography (PET), and magnetic resonance imaging (MRI). In some cases, the DCS technique has been validated with ultrasound and MRI, and in a few cases the therapy monitoring capability of hybrid DCS/DOS instruments has been demonstrated. The latter instruments are particularly exciting because, in principle, the oxygen delivery information derived from blood flow can be used in combination with blood oxygenation measurements to estimate the metabolic rate of oxygen consumption in tissue.

In this paper, we describe a case study of a breast cancer patient during the early stages of neoadjuvant chemotherapy. In contrast to previous optical measurements of breast tumors, the current work used both DOS and DCS in the same patient to derive information about tumor chromophores, tumor scattering, and tumor blood flow. Significant changes in tissue hemodynamic parameters were detected as early as 3 days post-therapy. To date, several studies have focused on measurements of parameters related to cancer metabolism and blood flow after few cycles of chemotherapy, i.e., after several weeks. The present study has focused on daily measurements within the first week. The observations of blood flow changes are the first of their kind during early stage chemotherapy. The combined DOS/DCS information provides a more complete picture of tumor tissue hemodynamics and enables us to construct new indices reflecting changes in oxygen metabolism that require knowledge about flow and oxygenated/deoxygenated hemoglobin concentrations.

2 Materials and Methods

2.1 Protocol

The patient measurements were conducted at the Beckman Laser Institute of the University of California, Irvine (UCI), and were approved by the Internal Review Board at UCI. The patient was a 45-year-old premenopausal Caucasian woman undergoing neoadjuvant chemotherapy treatment; she provided informed written consent for Human Subjects protocol 95-563. The patient was diagnosed by core biopsy revealing invasive ductal carcinoma in the left breast. Clinical palpation identified an 11-cm × 5-cm area at 3 o’clock [Fig. 1(a)]. Dynamic contrast-enhanced MRI (DCE-MRI) detected several strongly enhanced masses within this area. The outer edge of the tumor was located 0.5 to 1 cm underneath the skin. In addition, the patient had multiple benign lesions at 1 o’clock (by ultrasound). The tumor position was identified by palpation and ultrasound and was marked with a surgical pen to ensure the scans on different days were performed at the same location. The surgical pen marks were visible after day 3, and they were redrawn every day afterward. The accuracy for redrawing was within 2 to 3 mm. The optical probe repositioning accuracy was of similar order (i.e., 2 to 3 mm). Measurements were made with the patient in the supine position. DOS and DCS measurements were acquired using handheld probes at 10 discrete points in a line along the curved surface at 1-cm intervals across the tumor and surrounding tissue (“line scan”). The same measurement pattern was used on the contralateral breast for comparison. Figures 1(a) and 1(b) show the tumor position and line-scan directions on the patient, where the tumor center was located at the 5-cm position. One line scan took about 10 (8) min per breast for DOS (DCS), respectively, resulting in a measurement time of about 40 min per session. Extra care was taken during the DCS measurement to gently press the probe on the patient’s breast; the operator attempted to keep that pressure relatively constant and the probe stable.

The patient was treated with neoadjuvant chemotherapy delivered in multiple stages. The initial treatment stage consisted of doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan, A/C) therapy. A/C treatments were administered once per cycle for four cycles, with each cycle lasting 2 weeks. Doxorubicin is a cytotoxic anthracycline antibiotic, and its mechanism of action is thought to prevent DNA and possibly RNA synthesis by intercalation. Doxorubicin was administered at a dose of 60 mg/m² once per cycle. Cyclophosph...
pharmaceuticals is biotransformed principally in the liver to activate alkylating metabolites, which cross-link to tumor cell DNA. Cyclophosphamide was administered at a dose of 600 mg/m² once per cycle. DOS and DCS measurements were performed on the same day prior to the therapy (day 0), and every day between 3 to 7 days after the first A/C cycle [day 3 to day 7, Fig. 1(c)]. Additional stages using other therapies were administered, but no DOS/DCS measurements were recorded during these treatment stages (DOS/DCS measurements ended on day 7). The most relevant radiologic assessment was the DCE-MRI study done 12 days after the first A/C therapy, which showed shrinkage of multiple lesions, the largest lesion of which shrunk by 1.7 cm (in diameter). Ultrasound measurements were performed after the completion of all four A/C cycles (8 weeks) and detected an irregular hypoechoic lesion of 1.3 cm in diameter, indicating a partial response to A/C therapy. At the end of chemotherapy, DCE-MRI showed several tiny enhanced foci. The surgical pathology from lumpectomy at the conclusion of chemotherapy revealed residual intraductal and invasive ductal carcinoma with extensive fibrosis.

2.2 Instrumentation

2.2.1 DOS

A broadband DOS system developed at UCI was utilized to determine tissue optical properties and tissue blood oxygenation in vivo. The system combined a steady state (SS) tungsten-halogen light source and spectrophotometer operating in the 600 to 1000-nm range and a frequency-domain photon migration (FDPM) component (based on a network analyzer) consisting of six laser diodes (operating at discrete wavelengths in the 660 to 850-nm range). The combination of the cw and discrete spectral components enabled determination of complete $\mu_a$ and $\mu'_s$ spectra from 600 to 1000 nm. Measurements were made using a handheld probe consisting of the SS and FDPM source fibers, one avalanche photodiode (APD) detector and one spectrometer detector fiber. The source-detector separation was 2.8 cm and the time to perform SS and FDPM measurements at a single position was typically about 30 to 45 s. A detailed description of the performance of this device in 58 breast cancer patients has recently been reported.

2.2.2 DCS

A portable two-channel DCS instrument (18 × 28 × 33 cm) was built at the University of Pennsylvania and transported to UCI for the measurements of blood flow. Briefly, a compact cw 785-nm long coherence laser (coherence length ~50 m, Crystalaser, Reno, Nevada) was used as the light source. Two fast-photon-counting APDs (Perkin-Elmer, Canada) were used to register the photons collected with single-mode fibers. A custom-built two-channel correlator board (www.correlator.com, New Jersey) took the transistor-to-transistor logic (TTL) output from the APDs and calculated normalized temporal intensity autocorrelation functions of the detected light, $g_2(\tau) = \langle (I(t)I(t+\tau)\rangle / \langle I(t) \rangle^2$, where $I(t)$ is the measured intensity at time $t$, $\tau$ is the correlation delay time, and the angle brackets $\langle \rangle$ denote an average over time. A single source fiber and two detector fibers were used in the handheld probe with 2.5-cm source-detector separations. Thus, the DCS measurement probed approximately the same volume of breast tissue as the DOS measurement and a typical photon count rate of ~30 kHz was obtained from the breast measurement. Each temporal autocorrelation curve was averaged for 2.5 s, and multiple DCS measurements (typically >10) were performed at a single position to ensure a good signal-to-noise ratio (SNR).

2.3 Data Analysis

2.3.1 DOS Measurements

The algorithm for analyzing the DOS data was described in detail by Bevilacqua et al. Briefly, the frequency domain measurements at six discrete wavelengths were used to extract the reduced scattering coefficient ($\mu'_s/\mu_a$) at each wavelength. A power law function, $\mu'_s(\lambda) = A\lambda^{-b}$, was then fit to the wavelength-dependent $\mu'_s$ to estimate the scattering information at all other wavelengths. With $\mu'_s(\lambda)$ input from the frequency-domain measurements, the data measured with the spectrometer system was employed to extract accurate absorption coefficients $\mu'_a(\lambda)$ over the whole spectral range. As a result, tissue concentrations of oxy-, deoxy-, and total hemoglobin (ctO2Hb, ctHHb and ctTHb), water (ctH2O) and lipid (ctLipid) were reconstructed by decomposing the absorption spectra. Note that our notation is designed to comply with Zander and Mertzlufft, where the prefix “ct” indicates tissue-level concentration (see Table 1).

2.3.2 DCS Measurements

We used the intensity temporal autocorrelation curves measured with the DOS instrument to extract tissue blood flow information. The decay rate of the correlation curves is correlated with the blood flow, e.g., faster decay reflects higher blood flow. In our analysis, the photon correlation diffusion equation was used to model the propagation of the electric field autocorrelation function, $G_1(r,\tau) = \langle E(r,t)E^\dagger(r,t+\tau) \rangle$, inside tissues,

$$\nabla[D \nabla G_1(r,\tau)] - \left[ v \mu'_s + \frac{1}{3} v \mu'_s k_0^2 \alpha (\Delta r^2(\tau)) \right] G_1(r,\tau) = -vS\delta(r-r_r). \tag{1}
$$

Here, $E(r,t)$ is the electric field at position $r$ and time $t$, $\tau$ is the correlation delay time, $v$ is the light speed in the media, $D \equiv v/3 \mu'_s$ is the light diffusion constant, $k_0$ is the optical wave vector, $\Delta r^2(\tau)$ is the mean-square displacement of the moving scatterers in time $\tau$, and $S\delta(r-r_r)$ is the point source term located at $r_r$. The normalized electric field autocorrelation function $g_1(r,\tau) = G_1(r,\tau)/G_1(r,0)$ is related to the measured intensity autocorrelation function $g_2(r,\tau)$ through the Siegert relation:

$$g_2(r,\tau) = 1 + \beta |g_1(r,\tau)|^2, \tag{2}
$$

where $\beta$ depends on the detection optics (e.g., it is inversely proportional to the number of detected speckles). We determined $\beta$ for each measurement. The decay of the correlation function depends on tissue $\mu_a$ and $\mu'_s$, the mean square displacement $\Delta r^2(\tau)$, of the moving scatterers inside the tissue, and a unitless factor $\alpha$ that represents the fraction of scatterers...
that are moving (i.e., red blood cells). A semi-infinite solution to Eq. (1) (Ref. 25) was used in our data analysis. We have observed that a diffusion model, i.e., \( (\Delta r^2(\tau)) = 6D_b \tau \), fits the autocorrelation curves rather well over a broad range of tissue types, including the present experiments.\(^{26,32,34,52}\) Here, \( D_b \) is the effective Brownian motion coefficient of the scatterers (distinct from the well known thermal Brownian motion coefficient due originally to Einstein\(^{53}\)). Although the units of \( aD_b \) (square centimeters per second) is different from the traditional blood perfusion unit (milliliters per minute per 100 g), we have verified that changes in \( aD_b \) are proportional to the changes in tissue blood flow in a wide range of measurements.\(^{32,34,52,54}\) Determination of \( a \) is difficult. Therefore in the current study, we report \( aD_b \) as the blood flow index (BFI=\( aD_b \)). Bulk optical properties of the breast, \( \mu_s \) and \( \mu'_s \), at 785 nm, were obtained from the DOS measurements and used in the DCS data analysis to minimize the influence of optical property changes on the accuracy of BFI. In our fitting process, the noise in the correlation curve \( \sigma(\tau) \) was estimated as a function of delay time \( \tau \), and was used in calculating \( \chi^2 \), e.g., \( \chi^2 = \| \hat{g}_{2m}(\tau) - g_{2n}(\tau)/\sigma(\tau) \| \), where \( g_{2m}(\tau) \) and \( g_{2n}(\tau) \) are the measured and calculated intensity autocorrelation curves, respectively. A more stable BFI is usually obtained from the fitting by minimizing \( \chi^2 \) as defined in this way.\(^{30}\)

### 2.3.3 Tissue Optical Index

The tissue optical index (TOI) is a multiparameter contrast function created to maximize both the contrast and the specificity of the optical measurement. The TOI is defined as\(^{10,19}\)

\[
\text{TOI} = \frac{\text{ctHHb} \cdot \text{ctH}_2\text{O}}{\text{ctLipid}}.
\]

The parameters in this contrast function were chosen based on a statistical study of 58 malignant breast lesions.\(^{10}\) In this study, deoxy-hemoglobin was identified as the single best discriminator between malignant tumors and normal tissue, and it was found that addition of water and lipid concentrations further improved malignant/normal discrimination. The simplest index combining these parameters used ctHHb and ct\(H_2\)O in the numerator and ctLipid in the denominator. As such, increased TOI reflects a higher chance of tumor malignancy. Conceptually, the TOI takes into account functional information (ctHHb and ct\(H_2\)O) and structural (ctLipid) information. TOI is also related to metabolic activity since increases in deoxy-hemoglobin are often a symptom of unmet metabolic demand.

### 2.3.4 Oxygen Metabolism

Another, and perhaps more direct, estimate of tissue oxygen metabolism can be calculated by combining information about blood flow (delivery of oxygen) measured by DCS with chromophore concentration information such as blood oxygen saturation (i.e., oxygen availability) measured by DOS. This approach is often employed in studies of cerebral metabolic rate of oxygen\(^{55–60}\) (CMRO\(_2\)). In the simplest model, CMRO\(_2\) is proportional to the tissue arterial-venous oxygenation difference or oxygen extraction fraction (OEF) as well as the rate of oxygen delivery (i.e., the cerebral blood flow, CBF). Several assumptions are typically made in the steady-state model, including constant vasculature compartmentalization. The CMRO\(_2\) model has been fairly well studied, validated and its limits examined.\(^{27,28,55–61}\)

In this paper, we construct a mammary oxygen metabolism model that closely follows the CMRO\(_2\) approach in brain (see the appendix). We define the tumor/normal contrast of the mammary metabolic rate of oxygen (MMRO\(_2(T/N)\)) as,

### Table 1 Table of abbreviations.

<table>
<thead>
<tr>
<th>Name</th>
<th>Symbol</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxy-hemoglobin concentration</td>
<td>ctHHb</td>
<td>( \mu \text{M} )</td>
</tr>
<tr>
<td>Oxy-hemoglobin concentration</td>
<td>ctO(_2)Hb</td>
<td>( \mu \text{M} )</td>
</tr>
<tr>
<td>Total hemoglobin concentration</td>
<td>ctTHb</td>
<td>( \mu \text{M} )</td>
</tr>
<tr>
<td>Hemoglobin oxygen saturation</td>
<td>sO(_2)</td>
<td>%</td>
</tr>
<tr>
<td>Water concentration</td>
<td>ct(H_2)O</td>
<td>% (with respect to pure substance)</td>
</tr>
<tr>
<td>Lipid concentration</td>
<td>ctLipid</td>
<td>% (with respect to pure substance)</td>
</tr>
<tr>
<td>Blood flow index</td>
<td>BFI</td>
<td>cm(^2)/s</td>
</tr>
<tr>
<td>Tissue optical index</td>
<td>TOI</td>
<td>( \mu \text{M} )</td>
</tr>
<tr>
<td>Mammary metabolic rate of oxygen</td>
<td>MMRO(_2)</td>
<td>( \mu \text{M}/100 \text{ g min}^{-1} )</td>
</tr>
<tr>
<td>Tumor/normal contrast</td>
<td>T/N</td>
<td>folds</td>
</tr>
<tr>
<td>Relative change compared to day 0</td>
<td>( r )</td>
<td>%</td>
</tr>
</tbody>
</table>

Notes on nomenclature: (1) a prefix ct indicates tissue-level concentration of the parameter; (2) subscripts T and N indicate values of tumor and normal tissues, respectively, where a subscript T/N indicates the tumor/normal contrast; (3) a prefix r indicates relative change compared to prechemotherapy value (day 0).
where T and N represent the values for tumor and normal breast tissue (i.e., normal tissues within the same breast), and \( \gamma_{TN} = \frac{\text{ctHHb}_{TN}}{\text{ctTHb}_{TN}} / \frac{\text{ctHHb}_{T/N}}{\text{ctTHb}_{T/N}} \) is the ratio of tumor (or normal) deoxy-to-total-hemoglobin in the venous compartment \( (v) \) compared to the ratio of deoxy-to-total-hemoglobin in the total vasculature (see the appendix). The simplest approximation, which we adopt here, assumes the ratio of \( \gamma_T / \gamma_N \) to be 1 and constant over time. The relative tumor/normal contrast of mammary metabolic rate of oxygen \( (r\text{MMRO}_{2(T/N)}) \) see Table 1) can now be estimated from our measurements. We note, however, that these assumptions are unverified. The precise quantification of \( \gamma_T \) and \( \gamma_N \) requires better understanding of the microcirculation in tumor and normal tissues, and such precision is beyond the scope of the current study.

### 2.3.5 Characterization of Responses

To quantify tumor response to chemotherapy, the tumor/normal contrast for the parameters at each time point was determined. Tumor/normal contrast was calculated as the ratio of the average tumor value (positions 3 to 7 in the line scans) to the average value of the normal tissue on the same side of the breast (i.e., positions 8 to 10), for example, \( \text{BFIT}_{TN} = \frac{\text{ctHHb}_{T/N}}{\text{ctTHb}_{T/N}} = \frac{\text{ctTHb}_{T}}{\text{ctTHb}_{N}} \), etc. The positions were chosen based on palpation, and positions 1 and 2 were excluded as normal tissue because they were too close to other benign lesions. This calculation effectively accounts for the global variations in response to chemotherapy by normalizing to the daily normal tissue values. Thus, the tumor specific responses of each parameter are “pulled” out of the measurement. The tumor/normal contrast for each time point was then normalized to prechemovalues (day 0) to reflect relative changes, indicated by a prefix “\( r \)” for each parameter (see Table 1). Wilcoxon rank-sum tests were conducted comparing the optically measured differences between tumor and normal on day 0. Significant changes were observed and marked in the figures with an “\*”, representing a significance level of \( p < 0.05 \) compared to pretherapy values.

### 3 Results and Discussion

Figure 2 exhibits representative DCS temporal autocorrelation curves measured on the tumor before and after the first A/C treatment (days 0, 3, and 7). The figure also shows data from normal tissue from both breasts on day 0. The symbols are plotted. The observed tumor/normal contrast of these parameters is consistent with previous reports, i.e., \( \text{ctHHb} \) had a contrast of 2.1±0.7; \( \text{ctO2Hb} \) had a contrast of 2.0±0.3, water had a contrast of 1.9±0.3, and lipid had a contrast of 0.8±0.05, and \( \mu’_i \) at 785 nm had a contrast of 0.96±0.03 prior to the A/C treatment.

Neo-adjuvant chemotherapy can induce hemodynamic changes in both tumor and normal tissues. To investigate the tumor specific changes in response to the chemotherapy, relative tumor/normal contrast of \( \text{ctHHb} \), \( \text{ctO2Hb} \), and \( \text{ctTHb} \) with respect to pretherapy values \( (r\text{ctHHb}_{T/N}, r\text{ctO2Hb}_{T/N}, \text{and } r\text{ctTHb}_{T/N}) \) were calculated and are plotted in Fig. 4. Notice that significant changes in these hemodynamic parameters were observed as early as 4 days after the start of A/C therapy (\( p < 0.05 \)), i.e. \( r\text{ctHHb}_{T/N} \) dropped to 68±12% of its original value (day 0); \( r\text{ctO2Hb}_{T/N} \) dropped to 63±10%; and \( r\text{ctTHb}_{T/N} \) dropped to 63±10%. By the end of the monitoring period (day 7), there were continued small hemodynamic reductions and all values remained substantially lower than pre-treatment levels (day 0); \( r\text{ctHHb}_{T/N} \) dropped to 69±21% (\( p < 0.05 \)); \( r\text{ctO2Hb}_{T/N} \) dropped to 73±25% (\( p < 0.05 \)); and \( r\text{ctTHb}_{T/N} \) dropped to 72±17% (\( p < 0.05 \)).

Relative tumor/normal contrast of blood flow \( (r\text{BF}_{T/N}) \), concentration of water \( (r\text{ctH}_2\text{O}_{T/N}) \) and lipid \( (r\text{ctLipid}_{T/N}) \), and reduced scattering coefficient \( (r\mu’_{v(T/N)}) \) in response to the chemotherapy are plotted in Fig. 5. Blood flow measured with DCS showed good SNR ratio and substantial tumor-to-normal contrast. \( r\text{BF}_{T/N} \) increased initially on day 3 after the A/C therapy (130±13% , \( p < 0.05 \)), followed by a sharp and sus-
tained drop on days 4 (72±7% \( p=0.13 \)), 5 (68±7% \( p<0.05 \)), and 7 (75±7% \( p=0.13 \)). 
\( r_{CtLipidN} \) increased significantly from day 5 (108±6% \( p<0.05 \)) to day 7 (116±13% \( p<0.05 \)). Since the lipid concentration in tumor was lower than in the normal breast tissues before the therapy, the increase in \( r_{CtLipidN} \) suggests that lipid concentration in the tumor is normalized over time. The \( r_{\mu'_N} \) at position 8 decreased to 68±7% \( p<0.01 \).

Note, the optical properties (\( \mu_s' \) and \( \mu'_N \)) of the breast tissue at 785 nm were measured by the DOS instrument. Measured absorption and scattering effects are incorporated into the DCS data analysis. As a result, the influence of oxygenation state of the breast should not influence our blood flow results. On the other hand, probe contact pressure and the movement of fiber optics could have an effect on our DCS measurements. In a previous experiment,\(^{32} \) we have carried out tests that suggest the movement of fiber optics do not influence the DCS measurements after the optical probe was secured in place. An indication that the pressure and motion effects are minimal can be seen from the relatively small variations measured on the contralateral side of the breast (Fig. 3), i.e., the
day-to-day tumor contrast and variation were large compared to the combination of measurement noise, physiological noise, motion artifact noise, and pressure related variations experienced on the contralateral breast.

Chemotherapy treatment alters tumor angiogenesis. Therefore, optical properties of the tumor are expected to change due to chemotherapy, which is observed in our measurements. Although chemotherapy may also induce changes of other kinds of microscopic motions inside the tumor, we believe such an effect is likely small compared to the changes in tumor vasculature and blood flow. In our previous study of photodynamic therapy treatment of mouse tumors, blood flow changes measured with both ultrasound Doppler and DCS showed comparable results, indicating the tumor responses measured with DCS are mainly due to blood flow. The relative tumor blood flow reduction in response to neoadjuvant chemotherapy observed in the current study (∼25% within the first week) is also consistent with results from PET studies, wherein a 30% to 50% blood flow decrease after 2 months of chemotherapy was reported. Yet blood flow changes exhibit distinct features compared to other hemodynamic parameters measured with DOS. For example, we have observed a ∼30% initial increase in rBF_T/N on day 3 in this patient (Fig. 5), accompanied by an ∼20% decrease in rctHHbT/N (Fig. 4). Although the mechanism is not clear, this may be due to an early tumor vascular response to cellular damage. The ∼20% decrease in rctHHbT/N on day 3 could be consistent with reduced oxygen extraction given that rctTHb_T/N remained roughly constant and flow was elevated. By day 4, A/C therapy has continued to damage both cells and vasculature; rBF_T/N, rctHHbT/N, and rctHHbT/N all decreased because of significant damage to tumor cells. A similar initial increase followed by a significant decrease of blood flow was observed during the first 3 days after radiation therapy in patients with breast tumors. In patients with head and neck tumors and rectal carcinoma, similar patterns of blood flow changes were also reported in the early weeks after radiation therapy. Further studies involving more patients are underway to confirm the observations in this case study.

As described in Sec. 2.3, the combination of DCS and DOS measurements enable oxygen metabolic changes in the tumor to be calculated using Eq. (4). Compared to flow, hemoglobin concentrations, and other tissue parameters alone, this relative metabolism index is a potentially more direct indicator of tumor metabolic activities that integrates many factors and may provide further insight about tumor physiological responses to therapy. Figure 6 displays the relative changes in tumor/normal contrast of mammary metabolic rate of oxygen contrast [rTOi_T/N and rMMRO2_T/N] in response to chemotherapy. The contrasts were calculated as the ratio of the average tumor value (positions 3 to 7 in the line scans) to the average value of the normal tissue on the same side of the breast (positions 8 to 10). The contrasts were then normalized to the pre-chemotherapy values to reflect relative changes. Significant decreases were observed in both rTOi_T/N and rMMRO2_T/N after day 4. Asterisks (*) denote the data points significantly different from the pre-chemotherapy values (day 0, p<0.05). Plots were slightly offset along the x axis for better illustration of the error bars.

![Fig. 6](image)

Relative tumor/normal tissue optical index and mammary metabolic rate of oxygen contrast [rTOi_T/N and rMMRO2_T/N] in response to chemotherapy. The contrasts were calculated as the ratio of the average tumor value (positions 3 to 7 in the line scans) to the average value of the normal tissue on the same side of the breast (positions 8 to 10). The contrasts were then normalized to the pre-chemotherapy values to reflect relative changes. Significant decreases were observed in both rTOi_T/N and rMMRO2_T/N after day 4. Asterisks (*) denote the data points significantly different from the pre-chemotherapy values (day 0, p<0.05). Plots were slightly offset along the x axis for better illustration of the error bars.

4 Conclusion

We reported a case study wherein we monitored hemodynamic and tissue optical property changes in a breast tumor patient undergoing neoadjuvant chemotherapy. Our approach employed a combination of DOS and DCS to measure simultaneously blood oxygenation, lipid, water, and blood flow. We demonstrated the feasibility of this methodology and detected significant changes in tissue parameters as early as day 3 following the first chemotherapy infusion. This study introduced...
Table 2  Tumor/normal contrast changes normalized to day 0 values of all parameters within the first week of chemotherapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>rctHHbT/N</td>
<td>1.00±0.09</td>
<td>0.81±0.12</td>
<td>0.68±0.12</td>
<td>0.71±0.17*</td>
<td>0.72±0.14*</td>
<td>0.69±0.21*</td>
</tr>
<tr>
<td>rctO2HbT/N</td>
<td>1.00±0.16</td>
<td>0.95±0.17</td>
<td>0.63±0.10</td>
<td>0.75±0.14*</td>
<td>1.00±0.28</td>
<td>0.73±0.25*</td>
</tr>
<tr>
<td>rctTHbT/N</td>
<td>1.00±0.15</td>
<td>0.92±0.16</td>
<td>0.63±0.10</td>
<td>0.75±0.15*</td>
<td>0.94±0.24</td>
<td>0.72±0.17*</td>
</tr>
<tr>
<td>rctO2/OT/N</td>
<td>1.00±0.16</td>
<td>0.97±0.14</td>
<td>1.02±0.15</td>
<td>1.04±0.16</td>
<td>1.01±0.19</td>
<td>1.04±0.22</td>
</tr>
<tr>
<td>rctlipidT/N</td>
<td>1.00±0.06</td>
<td>1.04±0.07</td>
<td>1.13±0.08</td>
<td>1.08±0.06*</td>
<td>1.19±0.07*</td>
<td>1.16±0.13*</td>
</tr>
<tr>
<td>rBFIT/N</td>
<td>1.00±0.17</td>
<td>1.30±0.13*</td>
<td>0.72±0.07</td>
<td>0.68±0.07*</td>
<td>0.91±0.10</td>
<td>0.75±0.07</td>
</tr>
<tr>
<td>rTHbT/N (N)</td>
<td>1.00±0.03</td>
<td>1.02±0.05</td>
<td>1.04±0.04</td>
<td>1.06±0.04*</td>
<td>1.06±0.03</td>
<td>1.07±0.04</td>
</tr>
<tr>
<td>rTOfIT/N</td>
<td>1.00±0.16</td>
<td>0.72±0.16</td>
<td>0.60±0.09*</td>
<td>0.65±0.23*</td>
<td>0.59±0.13*</td>
<td>0.59±0.18</td>
</tr>
<tr>
<td>rMMRO2(T/N)</td>
<td>1.00±0.17</td>
<td>1.18±0.13</td>
<td>0.78±0.07</td>
<td>0.66±0.08*</td>
<td>0.67±0.11*</td>
<td>0.73±0.11*</td>
</tr>
</tbody>
</table>

Asterisks (*) denote the data points significantly different from the prechemo therapy values (day 0, p<0.05).

Optical measurement of blood flow as a new parameter for therapy monitoring along with multiparameter indices that may provide further physiological insight, such as tissue metabolic rate of oxygen. Overall, the hybrid diffuse optical approach shows promise for therapy monitoring, optimization, and personalization by potentially detecting physiological changes that may precede anatomical changes accessible to traditional radiological diagnostics procedures.

Appendix

In this section, we derive the equation for the calculation of the tumor/normal contrast of the mammary metabolic rate of oxygen [MMRO$_{2(T/N)}$] following the steps in deriving the cerebral metabolic rate of oxygen (CMRO$_2$).

Using Fick’s law, the tumor metabolic rate of oxygen [MMRO$_2(T/N)$] can be calculated as

$$\text{MMRO}_{2(T/N)} = \text{OEF}_{T} \cdot \text{BF}_{T} \cdot [O_2]_{at(T/N)}.$$  \hspace{1cm} (5)

The tumor (or normal) oxygen extraction factor [OEF$_{T(N)}$] is by definition the fractional conversion of oxygen from arterioles to venules, i.e., OEF$_{T(N)}$ = ([O$_2$]$_{at(T/N)}$−[O$_2$]$_{at(T/N)}$)/([O$_2$]$_{at(T/N)}$), where [O$_2$]$_{at(T/N)}$ and [O$_2$]$_{at(T/N)}$ are the tumor (or normal) arteriolar and venous concentrations of oxygen. BF$_{T(N)}$ is the tumor (or normal) blood flow. In steady-state, assuming a balance between oxygen concentration and oxyhemoglobin saturation in the arteriolar and venous compartments [saO$_2$$_{2(T/N)}$ and svO$_2$$_{2(T/N)}$], we have

$$\text{OEF}_T \cdot [O_2]_{at} = \frac{[O_2]_{at} - [O_2]_{at}}{[O_2]_{at} - [O_2]_{at}} = \frac{\text{saO}_2 - \text{svO}_2}{\text{saO}_2 - \text{svO}_2}.$$ \hspace{1cm} (6)

As a result, the tumor/normal contrast of mammary metabolic rate of oxygen [MMRO$_{2(T/N)}$] can be expressed as

$$\text{MMRO}_{2(T/N)} = \frac{\text{saO}_2 - \text{svO}_2}{\text{saO}_2 - \text{svO}_2} \cdot \text{BF}_T = \frac{\text{saO}_2 - \text{svO}_2}{\text{saO}_2 - \text{svO}_2} \cdot \text{BF}_T,$$ \hspace{1cm} (7)

given the assumption that the changes of BFI are proportional to the changes of tissue blood flow (see Sec. 2.3). Assuming saO$_2$$_{2}=1$, we will have

$$\text{MMRO}_{2(T/N)} = \frac{1}{\text{BF}_T} \cdot \frac{\text{ctHHb}_T}{\text{ctHHb}_N}.$$ \hspace{1cm} (8)

The diffuse optical signal originates from the hemoglobin in the tissue within the view of the probe, and represents a mixture of arterial, capillary and venous blood. The mixed tissue compartment (MTC) viewed by the optical probe is a weighted average of the arterial (a), capillary (c), and venous (v) compartments, and the weight is proportional to the optical cross section of the corresponding compartment. For example, ctHHb$_a$=k$_1$·ctHHb$_a$+k$_2$·ctHHb$_c$+k$_3$·ctHHb$_v$, where k$_1$, k$_2$, and k$_3$ are the respective weights and k$_1$+k$_2$+k$_3$=1. If we further assume the quantities in the venous compartment are proportional to the MTC values, i.e.,

$$\text{ctHHb}_a = r_{1T} \cdot \text{ctHHb}_T, \text{ctTHb}_a = r_{2T} \cdot \text{ctTHb}_T,$$

$$\text{ctHHb}_c = r_{1N} \cdot \text{ctHHb}_N, \text{ctTHb}_c = r_{2N} \cdot \text{ctTHb}_N.$$ \hspace{1cm} (9)

We can further simplify by defining $\gamma_{T(N)} = r_{T(N)}/r_{T(N)} = \frac{\text{ctHHb}_T}{\text{ctHHb}_N}/[\text{ctTHb}_T]/[\text{ctTHb}_N]$, which is the ratio of deoxy to total hemoglobin in the venous com-
where $\gamma_T$ and $\gamma_N$ are two unknowns. The ratio of $\gamma_T / \gamma_N$ reflects the relative oxygen extraction ability of the vascular tissue. For example, a $\gamma_T / \gamma_N$ ratio larger than 1 means a higher tumor deoxyhemoglobin fraction in the venous compartment relative to the total mixed vasculature compared to the normal tissue, reflecting a larger portion of oxygen is extracted from the tumor tissue.

Acknowledgments

This work was supported by the National Institutes of Health (NIH) grants N010-1UI54CA105480 (BJT, AGY), LAMMP-P41-R001192 (BJT), and RO1-HL-077699 (AGY), and RO1-EB-002109 (AGY) and the California Breast Cancer Research Program (BJT). The authors thank Montana Compton and Tran D for their assistance in this study and the facilities and staff of the Chao Family Comprehensive Cancer Center (P30-CA-62203). The authors also thank Ulas Sunar and Britton Chance for useful discussions.

References

Zhou et al.: Diffuse optical monitoring of blood flow and oxygenation...


